
Effects of chrono-nutritional feeding (timed feeding) strategies on thermoregulation, respiratory rate and oxidative stress status of broiler chickens raised under humid tropical conditions

Ifeanyichukwu C. Egbuniwe * and Esohe S. Felix

Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Benin, Benin City, Nigeria.

=====

Abstract

Broiler production in humid tropical environments is challenged by heat stress and daily fluctuations in ambient temperature, relative humidity and temperature-humidity index (THI), which disrupt physiological stability. This study evaluated the effects of chrono-nutrition (timed feeding) on thermoregulation, respiratory responses and oxidative stress status of broiler chickens raised under humid tropical environmental condition. A total of 100 Arbor Acre broilers were used for the study. They were randomly assigned to four feeding regimens: Ad libitum (Control), Morning (06.00 – 08.00 h), Afternoon (12.00 – 14.00 h), and Evening feeding (17.00 – 19.00 h) groups. Environmental parameters (ambient temperature, relative humidity and THI) were monitored to capture diurnal variation. Rectal temperature, respiratory rate, serum levels of malondialdehyde (MDA) and antioxidant enzymes (superoxide dismutase [SOD] and catalase [CAT]) were measured at days 14 (baseline), 28 and 42 of the feeding experiment. Results showed that Evening-fed birds had significantly lower ($p < 0.05$) mean rectal temperatures at days 28, 35 and 42, and lower respiratory rates at days 35 and 42, indicating improved thermoregulation under peak environmental load. Morning- and Afternoon-fed groups also showed moderate lower rectal temperatures and respiratory rates, when compared with the Control. Time-restricted feeding significantly reduced ($p < 0.05$) MDA levels at day 28, with the greatest decline in Evening-fed birds. Serum SOD activity was higher in Morning-fed birds at day 14, and CAT activity was higher in time-restricted feeding groups at day 28 ($p < 0.05$). The reduction in MDA at 28 days highlights improved oxidative stability during peak growth, making evening feeding a simple, cost-effective strategy to enhance poultry welfare and productivity. Feeding time produced stage-specific differences in feed intake, with Afternoon-fed birds showing lower intake than controls at days 21 and 28, whereas Morning- and Evening-fed birds showed higher ($p < 0.05$) intake at selected periods. Initial live-weight did not differ significantly among groups, but by day 42, Afternoon-fed birds had significantly lower ($p < 0.05$) live-weight than all other groups. Weight gain was significantly lower ($p < 0.05$) in Afternoon-fed birds at days 28 and 42 compared with the Control group. It was concluded that aligning feeding time with cooler periods led to significantly less thermal and oxidative stress, and greater weight gain per feed consumed in the broilers chickens.

Keywords: Chrono-nutrition (timed feeding); Circadian entrainment; Heat load; Redox homeostasis; Temporal feeding strategy; Weight gain.

* **Correspondence:** Ifeanyichukwu C. Egbuniwe; Email: ifeanyi.egbuniwe@uniben.edu; Phone: +2348065308980

Article History: Initial manuscript submission received – April 11, 2026; Final revised form received – August 31, 2026;

Accepted for publication – September 03, 2026; Published – September 09, 2026.

Introduction

Over the past few decades, intensive genetic selection has produced broiler chickens that grow rapidly and convert feed with remarkable efficiency. While these advances have boosted productivity, they have also introduced physiological and welfare challenges, including metabolic imbalance, excessive fat deposition, skeletal disorders and reduced resilience to stress (Nureldin *et al*, 2017). These concerns have prompted growing interest in feeding strategies that go beyond maximizing growth to supporting overall physiological stability and welfare.

One promising approach is manipulating feeding patterns, particularly, the timing of feed delivery. Feeding is not merely a source of nutrients; it is a complex, coordinated process involving behavioral, metabolic and hormonal regulation. Restricting feeding to specific periods requires birds to adapt both their behavior and internal physiology to maintain homeostasis (Neves *et al*, 2014). These adaptive responses are largely governed by the circadian system, which orchestrates daily rhythms in metabolism, thermoregulation and endocrine function.

Circadian rhythms in poultry are reportedly synchronized by environmental cues such as light, temperature and feeding schedules. Feeding time, as a non-photic zeitgeber, can powerfully entrain metabolic rhythms even when other cues are inconsistent (Challet, 2022a). This principle forms the basis of chrono-nutrition – the strategic alignment of feeding with biological rhythms to optimize physiological function. Evidence across various animal systems show that time-restricted feeding can improve metabolic efficiency, regulate oxidative stress and enhance health by synchronizing peripheral clocks (Wang *et al*, 2023).

Live-weight, weight gain, and feed intake are fundamental indicators of broiler productivity and provide complementary measures of the

response to feeding management. Live-weight reflect the cumulative outcome of nutrient intake and utilization, while weight gain quantifies growth over a defined period, and feed intake indicates nutrient consumed and available for maintenance and tissue deposition. Recent studies demonstrate that these traits can respond differently to altered feeding schedules. Quantitative feed restriction has been associated with reduced weight gain but improved feed conversion, while the duration of restriction can determine the extent of growth response (Gobane *et al.*, 2021; Pizolotto *et al.*, 2024). Conversely, feeding frequency and meal distribution may improve body weight and weight gain, although feed intake can differ among feeding regimens (Aziz and Masoud, 2021). Earlier reports have shown that under tropical heat stress, daytime feed withdrawal has also reduced feed intake while improving feed utilisation without compromising final body weight or weight gain (Oke *et al.*, 2026). Collectively, these findings indicate that productive responses to altered feed availability are context-dependent and may vary with feeding duration, frequency, age, genotype, diet and environmental conditions (Qui and Linh, 2026).

Simultaneous assessment of live-weight, weight gain and feed intake is therefore important when evaluating feeding-time interventions because changes in growth must be interpreted alongside changes in feed consumption. This is particularly relevant under environmental heat challenge, where reduced feed intake may represent an adaptive response to limit the metabolic heat associated with nutrient ingestion, yet may also compromise growth if prolonged. The finding that strategic daytime feed withdrawal can improve feed utilisation without reducing final growth under tropical heat stress further suggests that the productive consequences of altered feeding schedules cannot be inferred from feed intake alone (Oke *et al.*, 2026).

Beyond its nutritional role, feeding provides temporal information to the biological system. Poultry exhibit daily variation in metabolic processes and muscle protein deposition, supporting consideration of circadian organisation in precision nutrition (Moss *et al.*, 2023). Broader chrononutrition research indicates that meal timing can influence peripheral-clock synchronisation, clock-gene expression, circadian hormones, and metabolic function (BaHammam and Pirezada, 2023; Pickel and Sung, 2020). Experimental studies had further demonstrated that feeding can coordinate rhythmicity across peripheral tissues through interactions between the liver clock and other tissue clocks (Manella *et al.*, 2021; Zhang *et al.*, 2021). Although these findings provide a plausible physiological basis for timed feeding, much of the mechanistic evidence originates from controlled experimental systems and non-avian models, limiting direct extrapolation to broilers maintained under naturally fluctuating environmental conditions.

Importantly, feeding time should not be regarded as producing a universally optimal production response. Poultry responses to feeding time and frequency depend on bird characteristics, production objectives, diet and environmental conditions (Qui and Linh, 2026). Moreover, existing studies have predominantly examined restriction duration, feeding frequency or daytime withdrawal rather than comparing distinct morning, midday and evening feeding periods under naturally fluctuating tropical conditions. The extent to which the timing of feed availability is associated with coordinated changes in feed intake, growth performance and physiological adaptation in broiler chickens under such conditions therefore remains insufficiently resolved.

The importance of chrono-nutrition is heightened in humid tropical environments, where daily fluctuations in ambient temperature, relative humidity and

temperature-humidity index (THI) create shifting thermal loads. In regions such as southern Nigeria, mornings are relatively cool, midday brings peak heat and humidity, and evenings provide partial thermal relief (Kpomasse *et al.*, 2021). High THI exacerbates heat stress by reducing evaporative cooling, increasing respiratory effort and promoting oxidative stress through excessive ROS production, which can impair growth, immunity and endocrine stability (Oke *et al.*, 2024; Kim *et al.*, 2024). Concurrently, reduced light during cloudy or rainy periods can weaken photic entrainment of circadian rhythms, increasing the risk of internal desynchronization (Jiang *et al.*, 2023).

Malondialdehyde (MDA) is a key biomarker of lipid peroxidation and oxidative stress, reflecting cellular damage induced by reactive oxygen species (ROS). Monitoring serum MDA together with serum activity of antioxidant enzymes provide complementary information on lipid peroxidation and enzymatic antioxidant defence, helping to characterise oxidative status and stress adaptation in broilers (Habashy *et al.*, 2019).

Despite growing research on heat stress management, the combined effects of temperature, humidity and THI under natural tropical conditions remain poorly explored, particularly during the rainy season when environmental oscillations are most pronounced (Masi *et al.*, 2023; Odiano and Idahosa, 2023). Feeding time, as a controllable management tool, has the potential to restore circadian alignment, regulate metabolic rhythms and mitigate oxidative stress (Dixon *et al.*, 2022a; Rodrigues *et al.*, 2019; Saibaba *et al.*, 2021).

Against this backdrop, the present study investigated how different feeding schedules (*Ad libitum*, Morning, Mid-day, and Evening feeding) interact with naturally fluctuating tropical conditions to influence thermoregulation, respiratory dynamics and

oxidative stress in broiler chickens. The study aimed to provide practical insights into using chrono-nutrition to enhance physiological resilience, welfare and productivity in climate-sensitive poultry systems.

Materials and Methods

Study area and Environmental conditions:

The study was conducted over 28 days at a poultry research facility in Benin City, Edo State, Nigeria, under natural tropical conditions. The site, located in Ekosodin, is near the University of Benin (6° 24' 29.92" N and 5° 37' 7.89" E). The study was conducted under the warm, humid tropical conditions characteristic of Benin City, Nigeria, where mean annual temperature is approximately 28.6°C, typical daytime maximum temperatures are around 32°C and may reach approximately 34°C in the hottest months, and relative humidity averages about 73.5% and may approach 82% (World Meteorological Organization, 2021; Nigerian Meteorological Agency, 2022). Typically, mornings are relatively cool, followed by peak heat and humidity around mid-day, and milder conditions towards evening. These daily fluctuations in ambient temperature (AT, °C), relative humidity (RH, %), and light intensity (lux) are important environmental cues that interact with the birds' circadian rhythms, influencing metabolism, stress responses and nutrient utilization.

Environmental parameters were recorded at 08.00, 13.00 and 17.00 h using calibrated digital thermometers (°C) and hygrometers (% RH). Light intensity was measured in lux using a digital light meter. To assess combined thermal stress, the temperature-humidity index (THI) was calculated as: $THI = T_{db} - [(0.31 - 0.31 \times RH) \times (T_{db} - 14.4)]$, where T_{db} = dry-bulb temperature in °C and RH = relative humidity in decimal form (e.g., 70% RH = 0.70) (Panda, 2023; Morris *et al*, 2023). THI values are dimensionless.

Experimental birds, Ethical approval, Housing and Feeding management:

A total of 100-day-old Arbor Acres broiler chicks were procured and brooded for 14 days under standard management practices. During this period, the chicks received supplemental heat to maintain optimal brooding temperature, *ad libitum* access to water, and a nutritionally balanced starter diet formulated according to NRC (2020) guidelines. Birds were monitored daily for health status and uniform growth. At 14 days of age, the chicks were weighed, and body weights were used to randomly assign them to four treatment groups, ensuring homogeneity in initial body weight across groups.

All the experimental procedures followed internationally accepted guidelines for animal care and use, and were approved by the Faculty of Veterinary Medicine Ethical and Research Compliance Committee (ERCC/FVM/045). Handling and sampling were conducted to minimize stress and discomfort.

The broiler chicks were housed in open-sided pens measuring 1.5 × 1.5 m, with 8 – 9 birds per pen to promote adequate ventilation, reduce stress from overcrowding and maintain thermal comfort (Scanen, 2020a). Pens were roofed with corrugated iron to provide shade, floored with wood shavings to facilitate bedding management and litter hygiene, and equipped with centrally placed feeders and drinkers for equal access. Bedding material was replaced weekly, and pens were cleaned regularly to prevent accumulation of pathogens and maintain sanitary conditions.

A randomized complete block design (RCBD) with three replicates per treatment was used to evaluate the four feeding regimens, reflecting a chrono-nutrition approach that synchronizes feed delivery with environmental conditions and birds' circadian physiology, as follows:

- a. Ad libitum fed group (Control): Birds that had continuous access to feed throughout the day.
- b. Morning fed (06.00 – 08.00 h) group: Feed was provided for this group during cooler early-morning conditions (Challet, 2022b).
- c. Mid-day fed (12.00 – 14.00 h) group: Feed was provided for this group during the hottest period of the day (Panda, 2023).
- d. Evening fed (17.00 – 19.00 h) group: Feed was provided for this group during the cooler evening periods (Challet, 2022b).

Restricted feeding (Morning, Mid-day and Evening fed) groups received feed only within their allocated time windows, and leftover feed was collected and weighed to calculate actual feed intake. Water was provided *ad libitum* throughout the study. This chrononutrition strategy was designed to investigate

how aligning feed availability with both internal circadian rhythms and environmental oscillations, particularly temperature and humidity fluctuations, can influence thermoregulation, respiratory activities and stress resilience in broilers.

Diet composition: The starter diet (days 1 – 21) was formulated to provide approximately 23% crude protein and 3200 kcal/kg metabolizable energy (ME), while the grower diet (days 22 – 42) contained about 20% crude protein and 3100 kcal/kg ME. Both diets were based mainly on maize and soybean meal and were designed to meet the nutrient requirements recommended by NRC (2020). The feed was offered in mash form to promote uniform intake and minimize selective feeding. The detailed composition and proximate analysis results of the diets are presented in Tables 1 and 2. Feed was offered at 80, 100 and 120 g/bird/day during days 0 – 14, 15 – 28 and 29 – 42, respectively. Actual feed intake for each group was measured.

Table 1. Nutrient composition of the diets used in feeding the broiler chickens during the study.

Parameters	Starter Feed (Day 0 - 28)	Finisher Feed (Day 29 – 42)
Crude Protein (%)	23	20
Metabolizable Energy (kcal/kg)	3200	3100
Fat (%)	5 – 7	6–10
Calcium (%)	0.9 – 1.0	0.8 – 0.9
Phosphorus (%)	0.45	0.45
Crude Fibre (%)	5 – 10	5 – 10
Ash (%)	7 – 13	7 – 13
Nitrogen-Free Extract (%)	39 – 49	39 – 49
Lysine (%)	1.22	1.3
Methionine (%)	0.53	0.56
Cystine (%)	0.36	0.3
Dry matter (%)	93.84	94.14
Ether extract (%)	3	3

Table 2. Ingredients used in formulating the diets used in feeding the broiler chickens during the study.

Ingredients (%)	Starter Feed (Day 0 - 28)	Finisher Feed (Day 29 – 42)
Maize	30	34
Sweet potato meal	30	26
Blood meal	5	5
Groundnut cake	29.7	29.7
Wheat offal	1	1
Bone meal	3.25	3.25
dl-Methionine	0.25	0.25
Lysine	0.25	0.25
Vitamin Premix	0.3	0.25
Salt	0.25	0.25

Vitamin premix supplied per kg diet: Vitamin A – 10,000IU; Vitamin D3 – 2,000 IU; Vitamin E – 51 mg; Vitamin K – 2.34 mg; Riboflavin – 5.5 mg; Calcium pantothenate – 10 mg; Niacin – 25 mg; Sodium chloride – 250 mg; Folic acid – 1 mg; Manganese – 56 mg; Zinc – 50 mg; Copper – 10 mg; Iron - 20 mg; and Cobalt – 1.25 mg.

Baseline measurements: At day 14 (08.00 h), baseline body weight, rectal temperature, respiratory rate, tonic immobility and blood parameters were recorded. Blood (1.5 ml) was collected from six birds per treatment into plain test tubes, centrifuged at 3,000 revolutions per minute for 10 minutes, and serum was aspirated and stored at –20 °C for the assay of biochemical/oxidative stress markers (Scanes, 2020a).

Measurement of Physiological parameters: Rectal temperature (RT) was measured using a digital thermometer (°C) inserted 2 – 3 cm into the cloaca, three times daily (06.00, 12.00, 17.00 h) to capture diurnal variation (Ayo *et al*, 2022). Respiratory rate (RR) was recorded as flank movements per minute (breaths/minute) at the same time points, providing a rapid indicator of heat stress response.

Malondialdehyde (MDA) and antioxidant enzyme analysis: Serum malondialdehyde (MDA) concentration, expressed as nmol/mL, was determined as an index of lipid peroxidation using the thiobarbituric acid-reactive substances (TBARS) assay, according

to the method described by Aguilar Díaz de León and Borges (2020). Antioxidant enzymes status was evaluated using superoxide dismutase (SOD, U/mL) and catalase (CAT, U/mL) activity assay, using standard spectrophotometric methods (Wu *et al*, 2023; Oke *et al*, 2024). Blood samples for these assays were collected at 08.00 h to minimize circadian variation.

Measurement of Live-weight: Individual live-weight was measured using a digital weighing scale with a precision of ± 10 g, following standard poultry weighing procedures (Scanes, 2020b). Baseline measurements were obtained at 08:00 h before commencement of the feeding-time intervention, and subsequently at 08:00 h on weekly basis. Each bird was weighed individually and the body weight recorded in grammes.

Determination of Weight Gain: Weight gain was calculated from successive live-weight measurements as described for poultry growth-performance assessment (Scanes, 2020b): Weight gain (g) = Live-weight_{t2} – Live-weight_{t1}; where t1 represents the preceding

weight and t2 the subsequent weight. Thus, interval weight gain was calculated for each measurement period.

Determination of Feed Intake: Feed intake was determined gravimetrically from the quantity of feed offered and the quantity remaining after each scheduled feeding period. For the timed-feeding groups, feed was provided during the designated 2-h feeding windows, after which unconsumed feed was weighed (Scanes, 2020b). Feed intake was calculated as: Feed intake (g) = Feed offered (g) – Feed remaining (g). Where feed intake was expressed on a per-bird basis, it was calculated as: Feed intake/bird (g) = Feed consumed by experimental unit (g) / Number of birds in experimental unit. Feeders were cleaned daily to maintain feeding hygiene.

Statistical analysis: Data were analyzed using two-way ANOVA to examine effects of feeding regimen, measurement time, and their interaction. Tukey's post hoc test was used to separate means where significant differences were observed. Results were presented as mean $\pm$ SEM, with $p < 0.05$ considered significant. Analyses were done using the GraphPad Prism 10 software. This approach allowed assessment of both treatment and time-dependent effects, critical in chrononutrition studies, linking feeding, circadian rhythms and environmental oscillation (Challet, 2022a; Morris *et al.* 2023).

Results

Environmental patterns and daily oscillations:

Diurnal variations in environmental parameters are presented in Tables 3, 4 and 5. Continuous environmental monitoring revealed clear and consistent daily fluctuations in ambient temperature (AT), relative humidity (RH), temperature-humidity index (THI) and light intensity, which are characteristic of humid tropical systems. Ambient temperature ranged from 19.5 to 33.0 °C, while THI followed a similar trend (24.1 – 32.8), indicating that birds were exposed to variable thermal loads throughout the day rather than constant stress. Relative humidity remained high, ranging from 64% to 99%, potentially limiting evaporative cooling efficiency. Light intensity fluctuated widely between 0 and 92.8 lux, reflecting the frequent cloud cover typical of the rainy season, and suggesting a relatively weak photic signal for circadian entrainment. These environmental patterns highlight the potential importance of feeding time as a non-photocue for regulating physiological rhythms.

Diurnal patterns were pronounced: mornings at 08.00 h were the coolest and most humid (AT 25.58 °C, THI 25.60, RH 97.65%), while midday at 13.00 h experienced peak thermal load (AT 29.63 °C, THI 28.65, RH 78.00%), and evenings at 17.00 h presented intermediate values (Table 5).

Table 3. Ambient temperature, relative humidity and temperature-humidity index in the study location during the study period.

Experimental period (days)	Ambient temperature (°C)	Relative humidity (%)	Temperature-humidity index
21	28.90 $\pm$ 0.63	82.00 $\pm$ 2.88	27.03 $\pm$ 1.17
28	28.86 $\pm$ 0.65	81.52 $\pm$ 2.67	26.99 $\pm$ 1.19
35	27.06 $\pm$ 0.43	88.71 $\pm$ 2.21	25.66 $\pm$ 1.06
42	26.88 $\pm$ 0.50	89.27 $\pm$ 2.57	19.50 $\pm$ 2.26

Table 4. Light intensity, minimum and maximum light intensity during the study period.

Experimental period (days)	Light intensity (Lux)	Minimum light intensity (Lux)	Maximum light intensity (Lux)
21	38.79 ± 5.94	19.81 ± 2.56	57.15 ± 9.50
28	31.29 ± 5.56	26.25 ± 4.54	56.35 ± 9.29
35	33.03 ± 5.90	27.20 ± 5.76	74.45 ± 12.58
42	43.63 ± 2.00	35.19 ± 5.47	92.79 ± 12.70

Table 5. Diurnal variation of micro-environmental conditions prevailing the study period.

Environmental variables	Hour of Day		
	08.00 h	13.00 h	17.00 h
Ambient Temperature (°C)	25.58 ± 0.33	29.63 ± 0.44	28.81 ± 0.41
Relative Humidity (%)	97.65 ± 0.51	78.00 ± 1.86	79.58 ± 1.88
Temperature-humidity index	25.60 ± 0.32	28.65 ± 0.33	27.95 ± 0.32
Light intensity (Lux)	47.04 ± 8.54	48.29 ± 5.69	47.08 ± 8.67
Minimum Light intensity (Lux)	28.20 ± 5.68	43.88 ± 9.15	24.86 ± 4.19
Maximum Light intensity (Lux)	69.42 ± 11.20	66.02 ± 6.91	69.91 ± 10.85

Rectal temperature: Rectal temperature measurements obtained during the study are presented in Tables 6 and 7. Rectal temperature (RT) measurements reflected both environmental oscillations and feeding schedules. Evening-fed birds consistently maintained lower RT across all ages, declining progressively from 41.58 ± 0.14 °C at 28 days to 41.00 ± 0.11 °C at 42 days, compared with relatively stable higher RT values in Ad libitum-

fed Control birds ($41.74 - 41.78$ °C). Morning- and Afternoon-fed birds also exhibited reductions in RT, although the magnitude varied depending on age and prevailing thermal conditions. Diurnal measurements further highlighted these trends: Evening-fed birds maintained lower RTs at all recorded times, particularly during mid-day, when environmental stress peaked (13.00 h: 41.40 ± 0.12 °C versus Control 42.04 ± 0.01 °C).

Table 6. Mean rectal temperature (°C) of the broiler chicken groups fed at different times during the study period (n = 15)

Age of birds (days)	Means ± standard error			
	Ad lib fed Control group	Morning fed group	Afternoon fed group	Evening fed group
21	41.72 ± 0.08 ^a	41.45 ± 0.07 ^b	41.58 ± 0.07 ^a	41.56 ± 0.05 ^a
28	41.74 ± 0.19 ^a	42.13 ± 0.19 ^a	42.20 ± 0.17 ^a	41.58 ± 0.14 ^b
35	41.83 ± 0.08 ^a	41.47 ± 0.10 ^b	41.08 ± 0.15 ^b	41.02 ± 0.10 ^b
42	41.78 ± 0.12 ^a	41.59 ± 0.17 ^a	41.22 ± 0.13 ^b	41.00 ± 0.11 ^b

^{a, b} Different superscripts in a row indicate significant difference ($p < 0.05$) between the groups.

Table 7. Means of diurnal variations of rectal temperature (°C) of broiler chicken groups fed at different times during the study period (n = 15)

Hour of day (h)	Means ± standard error			
	Ad lib fed Control group	Morning fed group	Afternoon fed group	Evening fed group
8.00	41.51 ± 0.10 ^a	41.38 ± 0.09 ^a	41.32 ± 0.09 ^a	41.20 ± 0.08 ^b
13.00	42.04 ± 0.01 ^a	41.95 ± 0.08 ^a	41.63 ± 0.12 ^b	41.40 ± 0.12 ^b
17.00	41.71 ± 0.09 ^a	41.65 ± 0.12 ^a	41.70 ± 0.17 ^a	41.37 ± 0.09 ^b

^{a, b} Different superscripts in a row indicate significant difference (p < 0.05) between the groups.

Table 8. Mean respiratory rates (cycle/minute) of broiler chicken groups fed at different times during the study period (n = 10)

Age of birds (days)	Means ± standard error			
	Ad lib fed Control group	Morning fed group	Afternoon fed group	Evening fed group
21	80.35 ± 3.11 ^a	81.56 ± 3.69 ^a	89.69 ± 3.18 ^a	79.43 ± 4.49 ^a
28	76.0 ± 6.66 ^a	83.62 ± 5.41 ^a	82.67 ± 3.89 ^a	76.10 ± 5.84 ^a
35	57.11 ± 1.88 ^a	60.48 ± 2.51 ^a	54.86 ± 2.31 ^a	50.67 ± 2.16 ^b
42	64.0 ± 3.28 ^a	59.81 ± 2.47 ^a	61.52 ± 3.24 ^a	49.81 ± 3.31 ^b

^{a, b} Different superscripts in a row indicate significant difference (p < 0.05) between the groups.

Table 9. Diurnal variations of respiratory rates (cycle/minute) of broiler chicken groups fed at different times during the study period (n = 10)

Hour of the day	Means ± standard error			
	Ad lib fed Control group	Morning fed group	Afternoon fed group	Evening fed group
8.00 h	71.92 ± 3.02 ^a	61.63 ± 2.14 ^b	66.27 ± 2.97 ^a	62.85 ± 3.81 ^b
13.00 h	81.79 ± 4.32 ^a	79.41 ± 4.60 ^a	77.87 ± 4.67 ^a	69.33 ± 5.27 ^b
17.00 h	73.35 ± 3.82 ^a	61.70 ± 2.73 ^b	74.43 ± 3.48 ^a	61.77 ± 2.52 ^b

^{a, b} Different superscripts in a row indicate significant difference (p < 0.05) between the groups.

Respiratory rate: Tables 8 and 9 shows the variations in respiratory rate observed throughout the study period. Respiratory rate (RR) followed a pattern similar to RT, with Evening-fed birds consistently recording lower values than Ad libitum Controls, especially during later stages of growth when thermal susceptibility was greatest. At day 35, Evening-fed birds had RR of 50.7 ± 2.2 cycles/minute

compared with 57.1 ± 1.9 in Controls, and at day 42, 49.8 ± 3.3 versus 64.0 ± 3.3 cycles/minute. RR rose during midday in response to higher THI and declined in the evening, yet birds on time-restricted feeding maintained consistently lower RR, reflecting variations associated with both growth stage and environmental load.

Oxidative stress markers/antioxidant enzymes: The serum malondialdehyde concentrations and antioxidant enzyme activities in broiler chickens are presented in Tables 10, 11 and 12. Serum malondialdehyde (MDA) concentrations, which indicate lipid peroxidation and oxidative stress, showed clear age- and feeding-dependent patterns (Table 10). At day 28, birds fed in the morning or evening had significantly lower ($p < 0.05$) MDA than *ad libitum* Controls, with Evening-fed birds having the lowest values. No

significant variations ($p > 0.05$) in MDA values were recorded at days 14 or 42 (Table 10).

Antioxidant enzyme activity also varied with age and feeding schedule. Morning, Afternoon and Evening-fed birds had significantly higher ($p < 0.05$) superoxide dismutase (SOD) activity at days 14 and 42, when compared with the *ad libitum* fed Controls (Table 11). Catalase (CAT) activity was significantly higher ($p < 0.05$) at day 28 in Morning- and Afternoon-fed birds, while no significant variations were observed at days 14 or 42 (Table 12).

Table 10. Variations of serum levels of serum malondialdehyde levels (nmol/ml) of broiler chicken groups fed at different times during the study period (n = 6)

Age of birds (days)	Means $\pm$ standard error			
	Ad lib fed Control group	Morning fed group	Afternoon fed group	Evening fed group
14	9.62 $\pm$ 0.67	9.62 $\pm$ 0.64	8.30 $\pm$ 0.39	8.55 $\pm$ 0.11
28	23.92 $\pm$ 9.09 ^a	13.82 $\pm$ 3.07 ^b	10.34 $\pm$ 1.09 ^a	9.09 $\pm$ 1.50 ^b
42	25.98 $\pm$ 1.10	23.28 $\pm$ 2.31	22.19 $\pm$ 2.11	26.57 $\pm$ 1.86

^{a, b} Different superscripts in a row indicate significant difference ($p < 0.05$) between the groups.

Table 11. Variations in superoxide dismutase (SOD; U/mL) activity of broiler chicken groups fed at different times during the study period.

Age of birds (days)	Means $\pm$ standard error			
	Ad lib fed Control group	Morning fed group	Afternoon fed group	Evening fed group
14	23.45 $\pm$ 0.73 ^a	42.97 $\pm$ 0.82 ^b	41.31 $\pm$ 2.07 ^b	43.68 $\pm$ 0.85 ^b
28	61.08 $\pm$ 2.25 ^a	65.35 $\pm$ 0.85 ^a	63.14 $\pm$ 0.77 ^a	63.99 $\pm$ 1.82 ^a
42	138.40 $\pm$ 4.44 ^a	153.90 $\pm$ 5.40 ^b	163.00 $\pm$ 4.91 ^b	158.30 $\pm$ 7.71 ^b

^{a, b} Different superscripts in a row indicate significant difference ($p < 0.05$) between the groups.

Table 12. Mean serum catalase activity (U/ml) of broiler chicken groups fed at different times during the study period.

Age of birds (days)	Means $\pm$ standard error			
	Ad lib fed Control group	Morning fed group	Afternoon fed group	Evening fed group
14	11.23 $\pm$ 0.22 ^a	11.31 $\pm$ 0.0026 ^a	10.86 $\pm$ 0.55 ^a	11.53 $\pm$ 0.12 ^a
28	19.11 $\pm$ 2.18 ^a	23.54 $\pm$ 2.32 ^b	24.59 $\pm$ 2.69 ^b	19.20 $\pm$ 1.80 ^a
42	71.58 $\pm$ 5.40 ^a	66.09 $\pm$ 3.41 ^a	71.83 $\pm$ 3.65 ^a	72.45 $\pm$ 3.88 ^a

^{a, b} Different superscripts in a row indicate significant difference ($p < 0.05$) between the groups.

Live-weight: The initial and final live-weights of the birds are presented in Table 13. Initial live-weight at day 14 ranged from 141.5 to 292.2 g across the experimental groups. There were no significant variations ($p > 0.05$) in the baseline live-weight among the groups. By day 42, however, birds fed in the afternoon had significantly lower ($p < 0.05$) live-weight than all other groups.

Weight gain: Weight gain varied significantly ($p < 0.05$) among the groups at day 28 and 42 (Table 14). At day 28, birds fed in the afternoon had significantly lower weight gain than birds in the Control, Morning-fed, and Evening-fed groups, while at day 42,

Afternoon-fed birds also recorded significantly lower weight gain (Table 14).

Feed intake: Feed intake also varied significantly ($p < 0.05$) among feeding-time groups during the study (Table 15). Afternoon-fed birds had significantly lower feed intake than Control birds on days 21 and 28. Morning-fed birds had significantly higher feed intake than Control birds on days 21 and 35, while Evening-fed birds also recorded significantly higher feed intake than the control at days 35 and 42. These differences indicate that feeding time was associated with period-specific variation in feed intake, with the afternoon treatment consistently showing lower intake at some measurement points.

Table 13. Means of initial and final live-weight of broiler chicken groups fed at different times during the study period.

Age of birds (days)	Means $\pm$ standard error			
	Ad lib fed Control group	Morning fed group	Afternoon fed group	Evening fed group
14	228.20 $\pm$ 14.92 ^a	212.10 $\pm$ 7.70 ^a	225.10 $\pm$ 6.76 ^a	206.70 $\pm$ 7.87 ^a
42	1852.0 $\pm$ 60.58 ^a	1753.0 $\pm$ 50.79 ^a	1342.0 $\pm$ 40.93 ^b	1785.0 $\pm$ 41.54 ^a

^{a, b} Different superscripts in a row indicate significant difference ($p < 0.05$) between the groups.

Table 14. Means of weekly weight gain of broiler chicken groups fed at different times during the study period.

Age of birds (days)	Means $\pm$ standard error			
	Ad lib fed Control group	Morning fed group	Afternoon fed group	Evening fed group
21	383.2 $\pm$ 46.81	348.0 $\pm$ 6.97	313.9 $\pm$ 15.48	394.3 $\pm$ 40.77
28	311.3 $\pm$ 30.14 ^a	511.0 $\pm$ 68.01 ^b	269.0 $\pm$ 19.27 ^b	589.4 $\pm$ 57.53 ^b
35	380.0 $\pm$ 38.93 ^a	420.9 $\pm$ 30.45 ^a	332.1 $\pm$ 19.26 ^a	433.8 $\pm$ 27.99 ^a
42	316.7 $\pm$ 25.80 ^a	307.2 $\pm$ 25.90 ^a	203.0 $\pm$ 15.90 ^b	296.6 $\pm$ 22.76 ^a

^{a, b} Different superscripts in a row indicate significant difference ($p < 0.05$) between the groups.

Table 15. Mean daily individual feed intake of broiler chicken groups fed at different times during the study period.

Age of birds (days)	Means $\pm$ standard error			
	Ad lib fed Control group	Morning fed group	Afternoon fed group	Evening fed group
21	70.30 $\pm$ 1.80 ^a	90.63 $\pm$ 3.47 ^b	59.85 $\pm$ 6.50 ^b	74.04 $\pm$ 6.92 ^a
28	98.70 $\pm$ 2.40 ^a	83.66 $\pm$ 4.37 ^a	80.18 $\pm$ 2.58 ^c	80.94 $\pm$ 2.75 ^a
35	81.40 $\pm$ 1.20 ^a	91.80 $\pm$ 3.60 ^b	80.30 $\pm$ 1.69 ^a	90.33 $\pm$ 0.59 ^b
42	83.60 $\pm$ 4.80 ^a	82.88 $\pm$ 0.24 ^a	79.95 $\pm$ 2.65 ^a	90.88 $\pm$ 0.42 ^b

^{a, b, c} Different superscripts in a row indicate significant difference ($p < 0.05$) between the groups.

Discussion and Conclusion

Rainy-season microclimate and chrono-nutrition: The present study highlights a key but often overlooked reality in tropical poultry production: during the rainy season, environmental stress arises not only from temperature but from the combined and fluctuating effects of ambient temperature, relative humidity, temperature-humidity index (THI) and light intensity. These interacting factors create a dynamic microclimate that imposes physiological strain even when absolute temperatures are moderate (Refinetti, 2016; Jiang *et al*, 2023).

Across the study period, environmental variables exhibited clear diurnal rhythms. Mornings were cooler and highly humid, mid-day represented peak temperature, light intensity and THI, while evenings offered partial thermal relief. Although light intensity fluctuated unpredictably under cloudy conditions, this variability suggested weaker photic cues for circadian entrainment, emphasizing the potential of feeding time as a non-photoc Zeitgeber (Challet, 2022b; Jiang *et al*, 2023).

A central finding in this study is the importance of THI as a more meaningful stress indicator than temperature alone. Despite temperatures within the thermoneutral range (24 – 33 °C), high morning humidity (up to

99%) likely limited evaporative cooling, elevating effective heat load. Mid-day THI frequently approached or exceeded thresholds associated with heat stress, reinforcing that humidity-driven stress is a defining feature of humid tropical systems (Wasti *et al*, 2020; Oke *et al*, 2024). The inverse temperature-humidity relationship (higher humidity in the morning and lower at midday) did not reduce overall stress; rather, it maintained elevated THI during critical periods.

From a chrono-nutritional perspective, these environmental oscillations directly influence metabolic demand and internal rhythms. Feeding time acts as a potent non-photoc cue that can either align or disrupt physiological processes with external cycles (Challet, 2022b; Manoogian *et al*, 2022). The present study demonstrated that time-restricted feeding during cooler periods (morning or evening) reduced thermal strain and supported more efficient nutrient utilization, as evidenced by lower rectal temperature and respiratory rate, and decreased serum malondialdehyde (MDA) at day 28. Evening feeding produced the most pronounced benefits, suggesting stronger synchronization with endogenous circadian rhythms governing metabolism, thermoregulation, and antioxidant enzyme activity (Onagbesan *et al*, 2023; Son *et al*, 2023).

Light variability further complicates this interaction. Because light is a primary circadian regulator in poultry, inconsistent photic cues may desynchronize internal clocks. Under such conditions, feeding schedules serve as a stabilizing Zeitgeber, supporting circadian organization and metabolic homeostasis. These findings align with previous reports that chrono-nutrition enhances thermotolerance, metabolic efficiency and oxidative resilience (Rodrigues *et al*, 2019; Dixon *et al*, 2022b).

The physiological mechanisms underlying these observations likely involve reduced metabolic heat production during cooler feeding periods, improved thermoregulation and enhanced antioxidant defense. Lower MDA concentrations indicate diminished lipid peroxidation, reflecting reduced reactive oxygen species (ROS) formation when feeding aligns with environmental rhythms. Synchronized feeding may also optimize the activity of endogenous antioxidant enzymes such as superoxide dismutase and catalase, mitigate oxidative damage and support cellular integrity.

The implications of our findings for poultry production are substantial: By incorporating feeding-time manipulation into management practices, farmers can reduce thermal and oxidative stress, enhance welfare, improve growth performance and optimize nutrient utilization through a simple, cost-effective timed-feeding intervention. Monitoring environmental oscillations, particularly THI, rather than relying solely on ambient temperature, is critical for implementing effective chrono-nutrition strategies.

The study is limited by the lack of detailed endocrine and molecular assessments. Future research should integrate hormonal profiling, circadian gene expression and additional oxidative stress markers to elucidate mechanistic pathways. Combining chrono-nutrition with improved ventilation, controlled

lighting or other management interventions could provide a holistic approach to mitigating environmental stress in tropical broiler systems.

It can be concluded from the study that rainy-season environmental oscillations are significant drivers of physiological stress in broilers. Strategically timed feeding represents a biologically grounded, practical strategy to mitigate these effects, enhance resilience and support productive and welfare-friendly poultry management in humid tropical environments.

Feeding time, thermoregulation and tropical microclimates: The results of the present study demonstrate a clear and practical insight: when broilers are fed can be as important as what they are fed, particularly in climates characterized by fluctuating ambient temperature, relative humidity, and temperature–humidity index (THI). Environmental monitoring confirmed predictable daily cycles: mornings were cooler and more humid, midday represented peak temperature, light intensity, and THI, while evenings provided partial thermal relief. These fluctuations created periods where birds were more or less able to cope with metabolic heat. Under these conditions, feeding time determined whether nutrient intake coincided with thermal comfort or compounded heat stress.

Birds on time-restricted feeding, especially Evening-fed group, consistently maintained lower rectal temperatures across all ages and times of day, supporting the hypothesis that temporal feeding mitigates thermal strain. In contrast, *Ad libitum*-fed birds experienced sustained metabolic heat production that overlapped with peak environmental heat, resulting in higher rectal temperatures and respiratory rates. Evening-fed birds exhibited lower respiratory rates, particularly at midday, indicating reduced reliance on evaporative

cooling – a primary mechanism for heat dissipation (Kim *et al*, 2024; Wu *et al*, 2023).

The underlying mechanism involves the heat increment of feeding. Digesting feed generates metabolic heat; when this occurs during periods of high THI, it adds to the external heat load. Shifting feeding to cooler windows, such as evening, reduces this overlap, lowering cumulative thermal stress. This aligns with chrono-nutrition principles, where feeding acts as a strong non-photic Zeitgeber, synchronizing metabolism with predictable environmental cycles (Challet, 2022a; Wang *et al*, 2023; Petridi *et al*, 2024).

Although temperatures remained mostly moderate (24 – 33 °C), high humidity (up to 99%) restricted evaporative heat loss, elevating THI to stressful levels even without extreme heat. This underscores that tropical heat stress is often humidity-driven, and integrative measures like THI better reflect cumulative thermal strain than temperature alone (Jongbo *et al*, 2026). High temperature-humidity conditions increase the thermal load imposed on broilers, and birds commonly reduce feed intake to limit the heat generated during digestion, absorption, and nutrient metabolism; these responses can impair performance and physiological homeostasis (Teyssier *et al.*, 2022).

The findings in the present study also highlight why chrono-nutrition benefits may be more pronounced in tropical than temperate environments. Under stable climates, timing has limited effect; in regions with strong daily oscillations, it becomes a critical determinant of physiological outcomes. Practically, evening feeding emerges as a low-cost strategy to improve thermoregulation, reduce stress, and potentially enhance growth performance and welfare.

Feeding time and respiratory rate in tropical conditions: Results of the study clearly show that respiratory rate closely mirrors fluctuations in ambient temperature, relative

humidity, and temperature–humidity index (THI). In these environments, where evaporative cooling is limited, panting becomes the bird’s primary thermoregulatory strategy. Importantly, feeding time significantly affected the intensity of this respiratory response.

Birds fed in the evening consistently maintained lower respiratory rates compared with those on ad libitum feeding. This indicates that aligning feeding with cooler periods reduces the need for excessive panting. Continuous feeding likely sustained metabolic heat production throughout the day, overlapping with peak midday thermal stress, thereby elevating overall respiratory burden. This reduction was consistent across growth stages and throughout diurnal cycles, suggesting improved and stable thermoregulation in Evening-fed birds. Since respiratory rate is a sensitive indicator of heat stress, lower rates reflected reduced cumulative thermal load and lower physiological strain (Oluwagbenga and Fraley, 2024).

Mechanistically, these differences arise from the heat increment of feeding. Digesting feed generates metabolic heat, which, when coinciding with high midday THI, compounds environmental heat stress. Shifting feeding to evening hours reduces this overlap, effectively lowering total thermal load – a core principle of chrono-nutrition, where feeding schedules are synchronized with circadian rhythms and environmental cycles to optimize physiological efficiency (Challet, 2022a). Diurnal patterns in the present study reinforce this: Ad libitum feeding of birds led to peak respiratory rate at midday, while evening feeding led to lower rates even during environmental stress peaks.

These findings align with previous reports demonstrating that feeding schedules influence heat tolerance and respiratory effort in poultry (Wasti *et al*, 2020). They also highlight a critical nuance: benefits are most

pronounced under humid tropical conditions, where high humidity limits evaporative cooling and amplifies thermal strain (Oke *et al*, 2024). Physiologically, reduced panting lowers energy expenditure, maintains acid–base balance, reduces stress hormone activation, and may support immune function and growth (Wasti *et al.*, 2020).

From a management perspective, adjusting feeding to cooler periods (particularly evenings) offers a low-cost strategy to improve thermal comfort, reduce heat stress and support productivity. By considering environmental oscillations in THI, temperature and humidity, chrono-nutrition can be leveraged as a practical tool for tropical poultry production.

Chrono-nutrition and age-dependent lipid peroxidation in broiler chickens:

Malondialdehyde (MDA) serves as a widely recognized biomarker of lipid peroxidation and a proxy for oxidative damage to cell membranes. Results of the present study indicate that time-restricted feeding, especially in the evening, led to significantly lower MDA levels at day 28, suggesting that aligning feed intake with the birds' biological rhythms can mitigate oxidative stress. Essentially, Evening-fed birds either generated relatively lower levels of reactive oxygen species (ROS) or had enhanced capacity to neutralize them, which is particularly critical during rapid growth phases when metabolic demand is high and birds are more susceptible to oxidative stress under fluctuating temperature and humidity (Estévez, 2015).

The lower MDA levels observed in Evening-fed birds at day 28 highlights the interplay between chrono-nutrition and circadian regulation. Feeding later in the day may synchronize nutrient intake with peak antioxidant enzyme activity, including superoxide dismutase (SOD) and glutathione peroxidase, which are known to follow daily rhythmicity (Feng *et al*, 2021; Yang *et al*,

2022). In tropical systems, evening feeding also coincides with cooler ambient temperatures, reducing thermal load and mitochondrial ROS generation. The combined effect of circadian alignment and thermal relief likely explains the pronounced decrease in oxidative damage in this group. These findings are consistent with Olanrewaju *et al* (2019) and Attia *et al* (2021), who reported that strategic feeding times and heat mitigation strategies improve oxidative stress status and reduce cellular damage in poultry.

Interestingly, MDA differences were not significant at 14 and 42 days, suggesting that the effects of chrono-nutrition on MDA were age-dependent and transient. It is thought that at 14 days, antioxidant systems are still maturing, limiting responsiveness to environmental or nutritional interventions (Surai, 2016). By 42 days, adaptive physiological mechanisms – including acclimatization to oxidative stress – may stabilize MDA levels across groups, masking the impact of feeding time. This age-dependent response aligns with previous observations that prolonged exposure to environmental stress can induce compensatory antioxidant adjustments in poultry (Lara and Rostagno, 2013).

Mechanistically, the lower MDA levels in the Evening fed group can be attributed to several interacting factors. First, synchronizing feed intake with cooler periods improves metabolic efficiency and reduces unnecessary substrate oxidation, thereby limiting mitochondrial ROS production (Lara and Rostagno, 2013). Second, alignment with circadian rhythms may enhance antioxidant enzyme expression and activity, boosting the birds' capacity to neutralize free radicals (Akbarian *et al.*, 2016). Third, reduced heat stress during evening feeding likely lowers corticosterone release, further minimizing oxidative damage (Zhang *et al.*, 2023).

From a practical perspective, these findings have clear implications for tropical poultry production. Lower MDA levels reflect lower level of oxidative stress, which supports improved immune function, better growth performance, enhanced meat quality, and decreased mortality (Estévez, 2015). For producers, simply adjusting feeding schedules – shifting a larger proportion of feed provision to the cooler evening hours – offers a low-cost, management-based intervention to reduce oxidative stress and enhance overall flock welfare.

Feeding time, environmental stress and antioxidant enzyme markers: The findings in the present study demonstrated that feeding time interacted closely with daily oscillations in temperature, relative humidity and temperature–humidity index (THI), shaping the birds' capacity to cope with oxidative stress. Morning feeding coincided with cooler conditions and lower THI, likely reducing metabolic strain and allowing antioxidant enzymes to function efficiently. Afternoon feeding, occurring closer to peak thermal stress, appeared to trigger adaptive responses, reflected in higher superoxide dismutase (SOD) and catalase (CAT) activity. Birds on continuous (*ad libitum*) feeding experienced sustained metabolic activity across fluctuating environmental conditions, which may have limited their ability to mount targeted antioxidant defenses. Overall, aligning feeding with favorable environmental windows improved redox balance.

The pattern of antioxidant enzyme responses was dynamic and age-dependent. Early in the growth period, morning-fed birds exhibited higher SOD activity, suggesting early activation of defenses during a vulnerable developmental stage. As birds matured, both morning and afternoon feeding led to higher SOD activity compared to continuous feeding, while CAT activity was higher mainly at mid-growth. At later stages, differences between feeding groups diminished, likely reflecting

physiological adaptation or a shift toward other components of the antioxidant system. These findings highlight the complexity of redox regulation during rapid growth and the importance of timing feeding to match both circadian rhythms and environmental conditions (Surai *et al*, 2021; Coudert *et al*, 2023).

These results are consistent with recent studies demonstrating that elevated THI disrupts redox balance by increasing ROS production in broiler chickens (Oke *et al*, 2024; Kim *et al*, 2025). In humid environments, high relative humidity limits evaporative cooling, increasing metabolic pressure and ROS generation. Antioxidant enzymes such as SOD and CAT act as the first line of defense, scavenging free radicals (Surai, 2016). Our findings extend this understanding by showing that feeding time alone can significantly modulate these defenses when aligned with environmental rhythms.

Feeding time and productive performance in broiler chickens: Variation in feed intake, weight gain and live-weight: The present study demonstrated that feeding time was associated with stage-specific variations in feed intake, weight gain and terminal live-weight in the broiler chickens. This suggests that feeding time may modify productive performance through changes in the timing of feed consumption and nutrient availability rather than through a uniform effect throughout growth. The finding that at day 42, Afternoon-fed birds had significantly lower live-weight than both control and morning-fed birds, broadly agrees with evidence that the temporal organisation of feed availability can modify broiler growth, although responses vary with the feeding schedule. Azis *et al*. (2019) reported early reductions in body weight under restricted feeding that were no longer evident later in growth, while Silambarasan *et al*. (2012) and Ballay *et al*. (1992) similarly demonstrated that the duration and organisation of feed availability

influence growth and recovery. These differences suggest that growth responses depend on the specific feeding regimen rather than feeding time alone.

The lower terminal live-weight of Afternoon-fed birds does not necessarily indicate that afternoon feeding suppresses growth. Feeding time may alter the distribution of nutrient intake relative to endogenous patterns of feeding, digestion and utilisation. Feeding can act as a metabolic timing cue and influence peripheral metabolic rhythms (Challet, 2019; Peek *et al.*, 2013). In chickens, Decuypere and Kühn (1984) demonstrated that changing meal time altered rhythms of glucose, rectal temperature and circulating T3 and T4. Nevertheless, these mechanisms were not assessed in the present study, hence, there is need for further study to ascertain the role of thyroid hormones in relation to timed feeding in broiler chickens.

A particularly notable finding was the stage-specific reduction in weight gain among Afternoon-fed birds. Their weight gain was significantly lower at days 28 and 42, whereas no significant differences occurred at days 14 and 35. This indicates that the feeding-time response was not constant throughout the growing period. A similar temporal pattern was reported by Azis *et al.* (2019), although the persistence of reduced weight gain to day 42 in the present study suggests later divergence or incomplete compensation. Nutritional and environmental conditions may also contribute. Previous studies indicate that the consequences of scheduled feeding can differ between thermoneutral and hot conditions under controlled study, while dietary energy and protein can modify growth responses to feeding schedules (Brown and McCartney, 1982).

Feed intake showed a corresponding treatment-dependent pattern. Afternoon-fed birds consumed less feed than controls on days 21 and 28, whereas morning-fed birds

consumed more on days 21 and 35, and evening-fed birds also showed higher intake during some periods. The lower feed intake of afternoon-fed birds corresponded temporally with their lower weight gain, suggesting that reduced nutrient intake may have contributed to poorer growth. However, feed intake and feeding time should not be equated with feed restriction. Dixon *et al.* (2022a) showed that feeding schedule, feeding history and feed quantity are distinct biological variables, while Adeyemo *et al.* (2017) demonstrated that feeding regime can also affect nutrient utilisation. Thus, the present data cannot establish whether poorer growth resulted primarily from reduced intake, altered nutrient utilisation, or both.

Several physiological pathways may plausibly link feeding time with productive performance. Meal timing can synchronise peripheral metabolic processes through nutrient, hormonal and metabolic signals (Challet, 2019; Tahara and Shibata, 2013), while postprandial changes in nutrient availability and insulin signaling may influence tissue metabolism. Feeding may also modify thermogenesis. Wilson *et al.* (1989) showed that body temperature increased following feeding and that the magnitude and timing of this response varied with meal time. These findings indicate that the physiological consequences of feeding depend partly on when the meal is consumed, but they did not demonstrate that altered thermogenesis caused the lower growth observed in the present study.

The thermal environment may be an important modifier of these responses. Samara *et al.* (1996) demonstrated that high temperature reduced feed consumption and altered body weight, while feeding schedule and temperature jointly affected production responses. Thus, the lower afternoon feed intake observed in the present study could plausibly coincide with greater environmental

thermal challenge under naturally varying tropical conditions.

Overall, the findings are consistent with previous evidence that scheduled feeding can modify feed intake and growth (Ballay *et al.*, 1992; Silambarasan *et al.*, 2012; Azis *et al.*, 2019) and that meal timing can alter physiological rhythms in chickens (Decuypere and Kühn, 1984; Wilson *et al.*, 1989). However, productive outcomes may depend on the interaction among feeding time, duration of feed availability, nutritional composition, developmental stage and environmental conditions.

The biological significance of the present findings is therefore that the temporal placement of feed availability may be an important component of poultry production management, even when birds begin with comparable body weight. The findings support the broader concept of chrono-nutrition in poultry, in which nutrient provision is considered in relation to biological time as well as quantity and composition (Challet, 2019; Iiams *et al.*, 2026). Nutrient digestibility, glucose, insulin, thyroid hormones, corticosterone, oxidative status, gastrointestinal function and circadian markers were not assessed. Consequently, mechanisms involving endocrine resetting, metabolic entrainment, nutrient partitioning or circadian synchronisation remain hypotheses. Future studies should integrate cumulative feed intake and feed conversion with nutrient digestibility and retention, environmental thermal measurements, and postprandial glucose, insulin, T3, T4 and corticosterone profiles. Such studies could clarify whether feeding time can be optimised by matching nutrient availability with endogenous physiological rhythms and predictable environmental conditions.

Chrono-feeding and thermal stress in humid tropics: Broiler production during the rainy season in humid tropical environments

imposes subtle but meaningful thermal stress, driven largely by high relative humidity and elevated THI, especially around mid-day peaks. Within this daily oscillation, feeding time plays a pivotal role in shaping physiological responses.

Results of the present study show that chrono-nutritional management (particularly evening feeding) can reduce core body temperature, respiratory effort and minimize oxidative stress by shifting metabolic activity to cooler periods. This temporal alignment minimizes the overlap between internally generated heat and peak environmental stress, improving thermoregulation, reducing panting, and supporting antioxidant defenses.

These findings underscore the fact that feeding time is not merely a management convenience but a practical tool for enhancing physiological stability. By synchronizing feeding with environmental oscillations, producers can improve bird welfare, reduce thermal and oxidative stress, and support better productivity without additional cost.

Conclusion: Rainy-season conditions in humid tropical environments create daily fluctuations in temperature, humidity, and THI, which impose thermal and oxidative stress on broilers. The present study showed that timing feeding to cooler periods (especially in the evening) reduces core body temperature, lowers respiratory effort and strengthens antioxidant defenses. By shifting metabolic heat away from peak environmental stress, evening feeding enhanced thermoregulation, physiological stability and overall resilience. Feeding time is therefore a simple, low-cost strategy to improve welfare and productivity, and integrating it with environmental monitoring provides a practical framework for tropical poultry management. Ultimately, aligning feeding with natural environmental rhythms is a powerful physiological tool that reduces stress and supports efficient, resilient production.

Acknowledgement

This research was funded by TETFund Institutional Based Research Fund 2025. I also acknowledge the effort of Gift Osayuki Agbonteng, who assisted with some of the data collected.

Conflict of Interest

The authors declare that there is no conflict of interest arising from this study.

References

- Adeyemo GO, Badmus RT, Longe OG, Ologhobo AD (2017). Effect of Ad-libitum, split and restricted feeding on performance, digestibility and welfare of broiler chicken. *Biotechnology Journal International*, 18(2): 1 – 7.
- Aguilar Díaz de León JS and Borges C (2020). Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. *Journal of Visualized Experiments*, 159: e61082.
- Akbarian A, Michiels J, Degroote J, Majdeddin M, Golian A, De Smet S (2016). Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. *Journal of Animal Science and Biotechnology*, 7: 37. <https://doi.org/10.1186/s40104-016-0097-5>
- Attia YA, Alagawany M and Farag MR (2021). Environmental management and nutritional strategies to reduce heat stress in poultry. *Journal of Thermal Biology*, 98: 102915.
- Ayo JO, Sinkalu VO and Hambolu JO (2022). Thermoregulation and physiological responses of poultry to heat stress in the tropics. *Journal of Thermal Biology*, 103: 103172.
- Azis A, Berliana S and Afriani (2019). Effects of feeding time restriction during the whole rearing period on the growth performance of broiler chickens. *International Journal of Poultry Science*, 18: 14 – 20. <https://doi.org/10.3923/ijps.2019.14.20>.
- Aziz EK and Masoud SR (2021). The effect of feeding frequency and amount on performance, behavior and physiological responses of broilers. *Journal of Applied Veterinary Sciences*, 6(3); 76 – 85. doi.org/10.21608/JAVS.2021.80537.1086.
- BaHamam A and Pirzada A (2023). Timing matters: The interplay between early mealtime, circadian rhythms, gene expression, circadian hormones, and metabolism—A narrative review. *Clocks and Sleep*, 5(3): 34. doi.org/10.3390/clockssleep5030034.
- Ballay M, Dunnington E A, Gross, W B and Siegel P B (1992). Restricted feeding and broiler performance: Age at initiation and length of restriction. *Poultry Science*, 71: 440 – 447. <https://doi.org/10.3382/ps.0710440>.
- Brown HB and McCartney MG (1982). Effects of dietary energy and protein and feeding time on broiler performance. *Poultry Science*, 61: 304.
- Challet E (2019). The circadian regulation of food intake. *Nature Reviews Endocrinology*, 15: 393 – 405. <https://doi.org/10.1038/s41574-019-0210-x>.
- Challet E. (2022a). Circadian rhythms and feeding time in poultry physiology. *Frontiers in Physiology*, 13: 834123. <https://doi.org/10.3389/fphys.2022.834123>
- Challet E. (2022b). Feeding as a non-photic cue: Synchronizing metabolism with circadian rhythms. *Frontiers in Endocrinology*, 13: 834567.
- Coudert E, Baéza E, Chartrin P, Jimenez J, Cailleau-Audouin E, Bordeau T and Berri C (2023). Slow and fast-growing chickens use

- different antioxidant pathways to maintain their redox balance during postnatal growth. *Animals*, 13(7): 1160. <https://doi.org/10.3390/ani13071160>.
- Decuypere E and Kühn ER (1984). Effect of fasting and feeding time on circadian rhythms of serum thyroid hormone concentrations, glucose, liver monodeiodinase activity and rectal temperature in growing chickens. *Domestic Animal Endocrinology*, 1: 251 – 262. [https://doi.org/10.1016/0739-7240\(84\)90005-5](https://doi.org/10.1016/0739-7240(84)90005-5).
- Dixon BR, Edwards J and O'Neill R (2022a). Time-restricted feeding and thermoregulation in poultry. *Poultry Science*, 101(6): 102091.
- Dixon LM, Dunn I C, Brocklehurst, S and Baker L (2022b). The effects of feed restriction, time of day, and time since feeding on behavioral and physiological indicators of hunger in broiler breeder hens. *Poultry Science*, 101: 101838. <https://doi.org/10.1016/j.psj.2022.101838>.
- Estévez I. (2015). Oxidative stress in poultry: Implications for health and productivity. *World's Poultry Science Journal*, 71(3): 429 – 441.
- Feng D, Liu T, Su Z and Wang Z (2021). Circadian regulation of oxidative stress and metabolism in animals. *Frontiers in Physiology*, 12: 689492.
- Gobane Z, Goni S, Chikwanda D and Zhou L (2021). The effect of quantitative feed restriction duration on growth performance and carcass characteristics of broiler chickens. *Open Journal of Animal Sciences*, 11(04): 635 – 645. doi.org/10.4236/ojas.2021.114043.
- Habashy WS, Milfort MC, Rekaya R and Aggrey SE (2019). Cellular antioxidant enzyme activity and biomarkers for oxidative stress are affected by heat stress. *International Journal of Biometeorology*, 63(12): 1569 – 1584.
- Iiams SE, Skinner NJ, Green CB and Takahashi JS (2026). Circadian alignment through time-restricted feeding: Implications for health and longevity. *Annual Review of Nutrition*, 46: 435 – 462. <https://doi.org/10.1146/annurev-nutr-112525-011241>.
- Jiang N, Zhao Z and Liu L (2023). Light intensity and circadian rhythm regulation in poultry production systems. *Poultry Science*, 102(4): 102456.
- Jongbo AO, de Borba LP, Pereira RMM, Bell QO, Gregoratto LL, de Souza DP, Adeyeye OA and Vieira FMC (2026). Heat stress in livestock under tropical climates: Impacts and mitigation strategies. *Tropical Animal Health and Production*, 58(2): 65.
- Kim H, Park J and Lee S (2024). Temperature–humidity index and oxidative stress in poultry. *Animal Science Journal*, 95(1): 45 – 54.
- Kpomasse CC, Oke OE, Houndonougbo FM and Tona K (2021). Broiler production challenges in the tropics: A review. *Tropical Animal Health and Production*, 53: 36.
- Lara LJ and Rostagno MH (2013). Impact of heat stress on poultry production. *Animals*, 3(2): 356 – 369.
- Manella G, Sabath E and Aviram R (2021). The liver-clock coordinates rhythmicity of peripheral tissues in response to feeding. *Nature Metabolism*, 3(6): 829 – 842. <https://doi.org/10.1038/s42255-021-00395-7>
- Manoogian ENC, Chow LS and Panda S (2022). Circadian rhythms, time-restricted feeding, and metabolic health. *Cell Metabolism*, 34(6): 773 – 789.
- Masi A, Adejuwon J and Oloruntade A. (2023). Rainfall variability and climate trends in southern Nigeria. *Climate Dynamics*, 61(2): 1123 – 1137.
- Morris AA, Ko YA, Hutcheson SH, Quyyumi AA and Vaccarino V (2023). Biomarkers and

- metabolic profiling in animal physiology research. *Journal of Applied Physiology*, 134(2): 345 – 356.
- Moss A, Dao TH, Crowley T and Wilkinson S (2023). Interactions of diet and circadian rhythm to achieve precision nutrition of poultry. *Animal Production Science*, 63(18): 1926 – 1932. doi.org/10.1071/AN23075.
- Neves DP, Banhazi TM and Nääs IA (2014). Feeding behavior and biomechanics in poultry. *Brazilian Journal of Poultry Science*, 16(1): 1 – 7.
- Nigerian Meteorological Agency (2022). Annual Climate Review Bulletin. Abuja, Nigeria.
- NRC (National Research Council) (2020). Nutrient requirements of poultry (9th rev. ed.). *National Academies Press*. <https://doi.org/10.17226/2114>.
- Nureldin AE, Abudabos AM and Aljumaah RS (2017). Effects of feed restriction on broiler performance. *Revista Brasileira de Ciência Avícola*, 19(3): 449 – 456.
- Odiano IO and Idahosa LO (2023). Climate variability and agricultural implications in southern Nigeria. *Environmental Research Letters*, 18(5): 054012.
- Oke O, Oni A and Lawal AA (2026). Optimizing daytime feed withdrawal duration to modulate thermophysiological and metabolic responses of broiler chickens under tropical heat stress. *Journal of Applied Poultry Research*, 35(3): 100753. doi.org/10.1016/j.japr.2026.100753.
- Oke OE, Uyanga VA, Iyasere OS and Daramola JO (2024). Thermal stress and poultry adaptation in tropical climates. *Poultry Science*, 103(1): 103210.
- Olanrewaju HA, Purswell JL, Collier SD and Branton SL (2019). Effect of feeding strategies on broiler performance under heat stress. *Poultry Science*, 98(11): 5465 – 5472.
- Oluwagbenga EM and Fraley GS (2024). Heat stress and poultry production: A comprehensive review. *Poultry Science*, 102(12): 103141.
- Onagbesan OM, Uyanga VA, Oso OO, Tona K and Oke OE (2023). Alleviating heat stress effects in poultry: Updates on methods and mechanisms of actions. *Frontiers in Veterinary Science*, 10: 1255520.
- Panda S (2023). Thermal stress and management in poultry production. *Journal of Thermal Biology*, 110: 103405.
- Peek, CB, Moynihan AK, Kuo R, Ilkayeva SO, Yasukazu BM, Bass KJ (2013). Circadian clock interaction with HNF4 α modulates the circadian transcriptional program in the liver. *Cell Metabolism*, 17: 783 – 793.
- Petridi F, Geurts JMW, Nyakayiru J, Schaafsma A, Schaafsma D, Meex RCR and Singh Povel CM (2024). Effects of early and late time restricted feeding on parameters of metabolic health: An explorative literature assessment. *Nutrients*, 16(11): 1721.
- Pickel L and Sung H. (2020). Feeding rhythms and the circadian regulation of metabolism. *Frontiers in Nutrition*, 7: 39. doi.org/10.3389/fnut.2020.00039.
- Pizolotto W, Costa MM and Gasperin NZ (2024). Economic and productive performance of broilers subjected to quantitative feed restriction. *Acta Veterinaria Brasilica*, 18(2): 173 – 178. doi.org/10.21708/avb.2024.18.2.12410.
- Qui N and Linh N (2026). Feeding time and feeding frequency: Mechanism and effect on poultry production. *Open Veterinary Journal*, 16(6): 3304. doi.org/10.5455/ovj.2026.v16.i6.
- Refinetti R (2016). Circadian physiology, 3rd edition, CRC Press.
- Rodrigues DF, Cruz VA and Boleli IC (2019). Feeding time and circadian rhythms in poultry. *Journal of Animal Physiology and Animal Nutrition*, 103(5): 1342 – 1350.

- Saibaba P, Jeganathan KB and Venkatesh G (2021). Circadian clock genes and metabolic regulation in poultry. *Frontiers in Physiology*, 12: 678345.
- Samara M, Robbins KR, Smith, MO (1996). Interaction of feeding time and temperature and their relationship to performance of the broiler breeder hen. *Poultry Science*, 75(1), 34-41.
- Scanes CG (2020a). Poultry physiology and welfare under tropical conditions. *Avian Biology Research*, 13(2): 101 – 114.
- Scanes CG (Ed.) (2020b). *Sturkie's Avian Physiology*, 7th ed, Academic Press.
- Silambarasan, P, Samanta, R and Das TK (2012). Production performance of broiler chickens influenced by feed restriction systems. *Indian Journal of Animal Sciences*, 82(11): 1451 – 1455.
- Son J, Lee WD, Kim H, Hong EC, Kim HJ, Yun YS and Kang HK (2023). A comparative study on feeding timing and additive types of broilers in a high-temperature environment. *Journal of Animal Science*, 101: skad290. <https://doi.org/10.1093/jas/skad290>.
- Surai PF (2016). Antioxidant systems in poultry biology: Superoxide dismutase. *Animal Nutrition*, 2(1): 1 – 11.
- Surai PF, Kochish II and Kidd MT (2021). Redox homeostasis in poultry: Regulatory roles of NF- κ B. *Antioxidants*, 10(2): 186.
- Tahara Y and Shibata S (2013). Chronobiology and food intake. *Neuroscience*, 253, 78–88.
- Teyssier, J., Brugaletta, G., Sirri, S., Dridi, S., & Rochell, S. (2022). A review of heat stress in chickens. Part II: Insights into protein and energy utilization and feeding. *Frontiers in Physiology*, 13, 943612.
- Wang L, Zhang X and Sun Y (2023). Chrono-nutrition: Aligning feeding with biological rhythms to optimize metabolism. *Frontiers in Nutrition*, 10: 115412.
- Wasti S, Sah N and Mishra B (2020). Impact of heat stress on poultry health and performance. *Animals*, 10(8): 1266. <https://doi.org/10.3390/ani10081266>.
- Wilson HR, Mather FB, Brigmon RL, Besch EL, Dugan VP and Boulos NZ (1989). Feeding time and body temperature interactions in broiler breeders. *Poultry Science*, 68: 608–616. <https://doi.org/10.3382/ps.0680608>.
- World Meteorological Organization (2021). *Guide to Climatological Practices* (WMO-No. 100). Geneva, Switzerland.
- Wu XY, Wang FY, Chen HX, Dong HL, Zhao ZQ and Si LF (2023). Chronic heat stress induces physiological and oxidative responses in broiler chickens. *Poultry Science*, 102(12): 103066.
- Yang X, Li F, Zhang Y and Li Y (2022). Circadian rhythms regulate antioxidant defense systems in poultry. *Poultry Science*, 101(3): 101678.
- Zhang Z, Shui G and Li MD (2021). Time to eat reveals the hierarchy of peripheral clocks. *Trends in Cell Biology*, 31(11): 869 – 872. <https://doi.org/10.1016/j.tcb.2021.08.003>