

EFFECTS OF HABITUATION ON TONIC IMMOBILITY AND CLOACAL TEMPERATURE RESPONSES IN BROILER CHICKENS DURING THE HOT-DRY SEASON

^{1*}OGUNDEJI, T. & ²AYO, J. O.

¹Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Benin, Benin City, Nigeria

²Department of Veterinary Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria

*Correspondence: tunde.ogundeji@uniben.edu Telephone: 07066509471

ABSTRACT

This study aimed to determine the effects of habituation on tonic immobility (TI) and cloacal temperature responses in broiler chickens during the hot-dry season. A total of 60 *Arbor Acres* broilers were randomly assigned to control and habituated groups (n = 30 each). The habituated group underwent a 4-day habituation training before behavioural and physiological assessments. Thermal environmental conditions confirmed exposure to heat stress conditions (temperature-humidity index = $23.83 \pm 0.66 - 37.02 \pm 0.98$). Habituated group showed significantly shorter TI durations and lower ($P < 0.05$) midday cloacal temperatures (41.36 ± 0.04 °C vs. 41.63 ± 0.06 °C) than the control, indicating reduced fear and improved thermoregulatory stability. In conclusion, the findings demonstrate that habituation enhances thermal stability and behavioural calmness, reflecting improved animal welfare under heat stress. The study highlights behavioural conditioning as a simple, low-cost, and non-invasive strategy to improve poultry welfare and resilience in hot climates.

Keywords: Animal Welfare, Handling Stress, Habituation, Heat Stress, Stress Adaptation, Thermoregulation

INTRODUCTION

Heat stress is a major global challenge in poultry production, adversely affecting welfare, productivity, and profitability. Rising global temperatures and more frequent heat waves continue to impose severe thermal burdens on commercial flocks (Lara & Rostagno, 2013; Wasti *et al.*, 2020). Birds are highly susceptible to heat stress due to their feather covering and lack of sweat glands, which hinder heat dissipation. Consequently, heat stress disrupts homeostasis, reduces feed intake and growth, and increases mortality, particularly in tropical and subtropical regions (Ayo *et al.*, 2022). Broiler chickens are especially vulnerable during the hot-dry season, when elevated dry-bulb temperature, relative humidity, and temperature-humidity index values exceed the thermoneutral range, activating the hypothalamic-pituitary-adrenal (HPA) axis and elevating corticosterone levels.

Conventional mitigation strategies mainly address environmental or nutritional aspects, with limited focus on

behavioural adaptation. Habituation, a basic form of non-associative learning, offers a promising but underexplored approach. Through repeated, controlled exposure to non-threatening stimuli, animals gradually reduce behavioural reactivity and stress responses (Rankin *et al.*, 2009). Habituation represents a form of stress adaptation, enhancing an animal's capacity to cope with routine handling and environmental challenges. In poultry, habituation training can attenuate fear responses, enhance behavioural stability, and promote physiological resilience (Jones, 1996; Tamagi *et al.*, 2024). Habituation training designed to reduce fear and handling reactivity, rather than direct thermal conditioning, has been shown to attenuate fear-related responses and physiological stress under thermal challenge (Ogundeji & Ayo, 2025). Behavioural desensitization may indirectly improve heat tolerance, as calmer birds exhibit reduced hyperthermic responses and more stable physiological control under heat stress conditions. By reducing the novelty or

perceived threat of heat or handling stimuli, habituation may lessen HPA axis activation and corticosterone release, supporting improved thermoregulation and calmer behavioural responses (Nakamura and Morrison, 2022; Jackson *et al.*, 2025).

Behavioural indicators such as tonic immobility (TI) and physiological measures like cloacal temperature provide complementary insight into stress and welfare (Forkman *et al.*, 2007; Campbell *et al.*, 2019). Shorter TI duration and stable cloacal temperature reflect better adaptability. This study therefore evaluated the effects of habituation on TI duration and cloacal temperature in broiler chickens during the hot-dry season. It was hypothesised that habituated broilers would exhibit reduced TI duration and more stable cloacal temperature, reflecting enhanced adaptation and welfare under heat stress. The findings are expected to advance understanding of behavioural conditioning as a heat stress mitigation tool and highlight the practical potential of habituation for improving poultry welfare and productivity under climate stress conditions.

MATERIALS AND METHODS

EXPERIMENTAL SITE AND LOCATION

The experiment was performed in the poultry house of the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria (11°10'N, 07°38'E), located in the Northern Guinea Savannah zone of Nigeria. It was carried out from March to April, during the hot-dry season (Ayo *et al.*, 2022). The dimensions of the broiler chicken house were 7.5 x 4.0 x 2.0 metres. The broiler chicken house from the ground to a height of about 0.5 m was made of cement blocks, whereas wire mesh covered from that point to the zinc roof to allow for proper ventilation.

EXPERIMENTAL FLOCK AND MANAGEMENT

A total of sixty day-old, male, broiler chicks (*Arbor Acres*) were used for the experiment. They were raised on a deep litter system and identified using masking tape on the legs. Stocking density of 10 birds/m² was used (Olanrewaju *et al.*, 2018). They were fed a standard diet from day one to day 21. The experimental house was naturally ventilated. The lighting consisted of natural day/night cycle. The broiler chickens were given access to water and feed ad libitum. Biosecurity measures were followed throughout the experiment. This study with reference number ABUCAUC/2021/025 was approved by the Ethical Committee on Animal Use and Care of Ahmadu Bello University, Zaria.

THERMAL MICROENVIRONMENTAL PARAMETERS

To monitor heat stress, the dry- and wet-bulb temperatures of the microenvironment of the poultry pen were measured three

times daily using a calibrated dry- and wet-bulb thermometer (Aura Labtech, India) with an accuracy of ± 0.1 °C at 07:00 h, 13:00 h, and 19:00 h (GMT+1) from day 8 to 21. Calibration was performed in an ice–water bath and adjusted until a stable reading of 0 °C was obtained. Measurements were taken inside the pen at bird level. Data collection began on day 8 to allow for acclimatization of the chicks and stabilization of environmental conditions before heat stress assessment. The RH was calculated using Osmon's hydrometric table (Narindra Scientific Industries, Haryana, India). The THI on each day was determined using the formula as described by Tao and Xin (2003):

$$\text{THI} = 0.85 \times \text{DBT} + 0.15 \times \text{WBT}$$

(Where DBT = dry-bulb temperature, and WBT = wet-bulb temperature).

EXPERIMENTAL DESIGN AND GROUPINGS

The sample size of the study was determined using power analysis conducted in G*Power version 3.1.9.7 (Faul *et al.*, 2009), cloacal temperature as the primary outcome variable. Assuming a medium effect size (Cohen's $d = 0.5$), a significance level (α) of 0.05, a mixed-effects model test type and a desired power ($1 - \beta$) of 0.80, the analysis indicated that a minimum of $n = 30$ birds per group was required to detect meaningful differences in the mixed-effects model design. The calculation assumed independence at the individual bird level, and the resulting sample size was considered sufficient to achieve the study's objectives. A total of 60 day-old broiler chicks were divided into two groups of 30 chickens each. Using simple random sampling, the birds were assigned to control and habituated groups. Each broiler chicken in the habituated group was subjected to 4 days of habituation training from days 17 to 20, as described by Ogundeji & Ayo (2024). The habituation protocol was based on brief exposure to a mild aversive stimulus (dorsal restraint) to evaluate whether repeated handling of this nature could reduce fear responses to similar stimuli. Briefly, habituation training consists of manually restraining a bird on dorsal recumbency for 15 seconds in a U-shaped cradle covered with cloth by trained personnel. The duration of each resulting immobility episode was terminated after about 15 seconds by gentle prodding. The control group was not subjected to handling beyond standard husbandry routines (feeding, cleaning, and inspection). On day 21, all broiler chickens in both groups were subjected to the TI test on day 21, with birds selected one at a time from each group in an alternating manner. The habituation training and TI test were conducted in a separate test area, with each bird tested once on its designated day between 08:00 and 13:00 h (GMT+1). Cloacal temperature was measured in each broiler chicken from the control and habituated groups on day 21 only at 07:00, 13:00, and 19:00

h (GMT+1), representing low, peak, and declining environmental heat loads, respectively. All measurements at each time point were taken at each pen and completed within a 15-minute window to ensure consistency.

TONIC IMMOBILITY TEST

The TI test was carried out as described by Ogundeji & Ayo (2024). Briefly, each broiler chicken was gently caught with both hands and prevented from having auditory and visual contact with other birds. TI was induced as soon as the broiler chicken arrived in the separate room by gently restraining it in dorsal recumbency for 15 seconds in a U-shaped cradle covered with foam and cloth. The birds were then observed from a position about one metre away without making any unnecessary noise or movement. Direct eye contact between the observer and the broiler chicken was avoided, as it may prolong TI duration. A stopwatch was started to record latencies until the bird righted itself. If it was righted in less than 10 seconds, the restraining procedure was repeated. When TI was not induced after three attempts, the duration of TI was considered zero. The maximum duration of TI allowed was 600 seconds. It was assumed that the catching and returning of the birds did not disturb the other flock members.

CLOACAL TEMPERATURE MEASUREMENT

A digital clinical thermometer (Hartmann Digital Thermometer, Paul Hartman AG, Heidenheim, Germany) was used to take the cloacal temperature. It was placed into the cloaca about 2 cm and in direct contact with the mucosal wall. After the thermometer sounded an alert to signal that the reading had stabilised, the value was recorded. The thermometer was disinfected with 70% alcohol and air-dried between birds.

DATA ANALYSES

The data obtained were expressed as mean \pm standard error of the mean (mean \pm SEM). Cloacal temperatures measured at 07:00, 13:00, and 19:00 h on day 21 were analysed using a linear mixed-effects model - treatment and time were fixed effects, and bird identity a random intercept. Student's t-test was used to compare the diurnal range of fluctuations in cloacal temperature. Pearson's correlation analysis was used to assess the relationship between cloacal temperature and DBT, RH and THI. Tonic immobility (TI) duration, which did not meet the assumption of normality (Shapiro-Wilk test, $P < 0.05$), was analysed using the Mann-Whitney U-test. Values of $P < 0.05$ were considered significant. The analyses were performed using GraphPad 8.02 for Windows (San Diego, CA, USA).

RESULTS

THERMAL MICROENVIRONMENT PARAMETERS DURING THE STUDY PERIOD

The thermal environment parameters inside the broiler chickens' house during the study period are presented in Table I. The DBT ranged from 24.14 ± 0.66 °C at 7:00 h to a peak of 38.29 ± 0.97 °C at 13:00 h, with an overall average of 31.38 ± 0.61 °C. The RH fluctuated between $55.71 \pm 5.57\%$ and $82.97 \pm 1.84\%$, and the THI ranged from 23.83 ± 0.66 °C at 7:00 h to 37.02 ± 0.98 °C at 13:00 h. The overall average daily RH and THI during the study period were $64.29 \pm 2.84\%$, and 30.50 ± 0.61 °C, respectively.

Table I: Thermal environment parameters inside the broiler chickens' house during the study period.

Parameter	Hour of the Day			Average Daily
	7:00 h	13:00 h	19:00 h	
DBT (°C)	24.14 ± 0.66 (19 – 28)	38.29 ± 0.97 (32 – 43)	31.71 ± 0.90 (27 – 38)	31.38 ± 0.61 (27.33 – 33.67)
RH (%)	82.97 ± 1.84 (68.50 – 92.00)	54.50 ± 3.51 (35.30 – 93.80)	55.71 ± 5.57 (16.70 – 79.90)	64.29 ± 2.84 (43.20 – 78.90)
THI	23.83 ± 0.66 (18.85 – 27.70)	37.02 ± 0.98 (31.10 – 41.65)	30.64 ± 0.80 (25.95 – 35.75)	30.50 ± 0.61 (26.68 – 32.97)

Values in parentheses are the minimum and maximum. DBT: Dry-bulb temperature; RH: Relative humidity; THI: Temperature-humidity index.

TONIC IMMOBILITY DURATION OF THE BROILER CHICKENS

The control group exhibited a longer ($P < 0.05$) TI duration of 81.42 ± 12.80 seconds compared to the habituated group, which had a TI duration of 40.61 ± 4.56 seconds (Figure I).

CLOACAL TEMPERATURE

At 7:00 h, the control group recorded a cloacal temperature which was slightly lower ($P > 0.05$) than that of the habituated group (Table II). However, the most pronounced difference was observed at 13:00 h where the control group exhibited a higher ($P < 0.05$) cloacal temperature value of 41.63 ± 0.06 °C compared to 41.36 ± 0.04 °C recorded in the habituated group. By 19:00 h, both groups returned to similar values (40.90 ± 0.05 °C, $P > 0.05$). The daily range of fluctuation in cloacal temperature was notably greater ($P < 0.05$) in the

control group (1.16 ± 0.08 °C) than in the habituated group (0.77 ± 0.06 °C). Positive but non-significant ($P > 0.05$) relationship was obtained between the DBT and THI at day 21 versus the cloacal temperature in both groups ($r = 0.99$, $P < 0.05$, $n = 3$) (Table III).

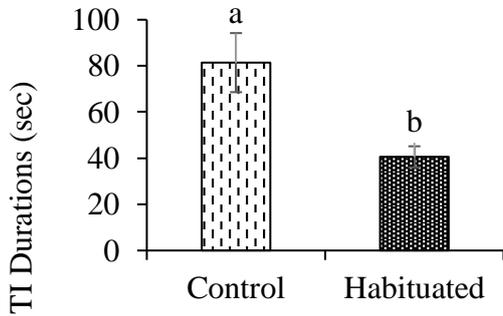


Figure I: Effects of habituation on tonic immobility (TI) duration in broiler chickens during the study period

Table II: Effects of habituation on cloacal temperature (°C) responses in broiler during the study period.

Groups	Hour of the day			Range
	07:00 h	13:00 h	19:00 h	
Control	40.49 ± 0.07^c	41.63 ± 0.06^{a1}	40.90 ± 0.05^b	1.16 ± 0.08^1 (39.4 – 42.2)
Habituated	40.63 ± 0.07^b	41.36 ± 0.04^{a2}	40.90 ± 0.05^b	0.77 ± 0.06^2 (40.0 – 41.6)

^{ab12} Means for the same parameter having different superscript numbers along the column and letters across the row are significantly ($P < 0.05$) different; Values in parenthesis represent minimum and maximum temperature.

Table III: Effects of habituation on the relationship between thermal microenvironmental parameters and cloacal temperatures in broiler during the hot-dry season.

Parameters	Control	Habituated
DBT	0.984	0.978
RH	-0.810	-0.794
THI	0.987	0.983

$n = 3$

DISCUSSION

A critical aspect of understanding the physiological responses of broilers to heat stress is characterising the thermal microenvironment within the rearing facility. In the present study, the DBT, RH and THI values indicate that the birds are exposed to moderate to severe heat stress. These align with previous studies which reported that broilers in similar climates experience considerable physiological stress (Ogundeji *et al.*, 2025). The spike in DBT and corresponding THI at 13:00 h indicates a critical period when the birds are most at risk of heat-induced stress. High DBT coupled with a high RH at 13:00 h suggests that evaporative cooling might be less effective during these hours, exacerbating thermal strain. This is particularly concerning given that optimal performance and welfare in broilers require maintenance within a narrow thermoneutral zone (Yahav *et al.*, 1995). As such, interventions that reduce the physiological impact of these harsh conditions are of utmost importance.

The markedly shorter TI duration observed in habituated birds compared with controls demonstrates a clear reduction in fear and stress sensitivity. This aligns with earlier findings that repeated, controlled exposure to benign stimuli can attenuate fear responses through habituation (Nash & Gallup, 1976; Stingo-Hirmas *et al.*, 2022; Tiemann *et al.*, 2023). Habituation likely promotes adaptive desensitisation, allowing birds to cope more effectively with environmental stressors. The reduced TI duration in the habituated group suggests a diminished behavioural stress response, possibly mediated by reduced activation of the hypothalamic–pituitary–adrenal (HPA) axis. Habituation likely reduces activation of the HPA axis and sympathetic outflow, lowering stress-induced metabolic heat and facilitating more effective heat-loss behaviours (Herman, 2013; Calefi *et al.*, 2017). Additionally, repeated mild stress may enhance central autonomic networks to thermoregulatory control (Nakamura & Morrison, 2022), contributing to the observed stabilization of cloacal temperature. Although HPA axis activity was not directly assessed in this study, the pattern of results suggest that habituation or repeated stress exposure down-regulate HPA reactivity (Ericsson *et al.*, 2014; Ericsson *et al.*, 2016). A limitation of this study is that HPA activity was inferred from behavioural and thermal indices rather than directly measured. Future research should incorporate HPA activity or corticosterone assays to clarify the neuroendocrine basis of the observed behavioural and thermal adaptations.

The deliberate use of a mild aversive conditioning protocol in this study allowed assessment of how repeated exposure to a common handling stressor influences behavioural adaptation and thermal stability. Although brief dorsal restraint may initially elicit stress, repeated exposure under controlled, non-harmful conditions can promote desensitization and improved coping, reflecting adaptive learning rather than chronic

distress (Ogundeji & Ayo, 2025). Such findings are consistent with the concept of stress inoculation, whereby manageable stress exposure enhances resilience to subsequent challenges through modulation of central and autonomic stress pathways (Nakamura & Morrison, 2022). Nevertheless, the aversive nature of the procedure warrants ethical caution. Future studies should explore whether comparable adaptive outcomes can be achieved using gentler, positively reinforced handling techniques to optimize welfare while preserving the benefits of habituation-based resilience training. Although the procedure involved brief restraint, the absence of distress indicators or postural recovery delay suggests that the birds did not experience significant welfare compromise (Jones, 1996; EFSA AHAW, 2023).

These behavioural improvements were paralleled by physiological adaptations indicative of stress adaptation, suggesting that habituation exerts a systemic influence on stress regulation (Koolhaas *et al.*, 2011). Habituated birds maintained lower and more stable cloacal temperatures during the hottest period of the day, indicating enhanced thermoregulatory control under heat stress. The relationship between cloacal temperature and THI suggests a general trend consistent with increased thermal strain, with the absence of a clear association likely attributable to limited statistical power or sample sensitivity. Reduced fluctuations in cloacal temperature, together with shorter TI durations, collectively reflect improved welfare. Repeated, non-threatening exposures have been shown to lower fear responses and associated behavioural arousal in poultry, supporting the interpretation that habituation reduces stress reactivity (Jackson *et al.*, 2024). The welfare implications of these findings are notable. By lowering behavioural reactivity and stabilising body temperature, habituation could improve resilience to thermal stress, potentially reducing mortality, enhancing feed efficiency, and supporting better overall productivity in hot climates. Such benefits align with the broader goal of promoting sustainable poultry production under increasingly variable environmental conditions. In commercial settings, habituation could be implemented by incorporating short, daily handling or environmental exposure sessions during early life stages. These interactions can be performed by farm staff during routine management (e.g., feeding or inspection), requiring no specialized equipment. Over time, such conditioning may reduce panic-related injuries, improve feed conversion during heat waves, and enhance worker–bird interactions, especially in open-sided tropical housing systems. These insights highlight habituation as a feasible, welfare-oriented management tool that can be integrated into existing production systems without significant cost.

CONCLUSION

This study demonstrates that habituation significantly reduces heat stress in broiler chickens during the hot-dry season by enhancing both behavioural and physiological adaptations. The shorter TI duration in the habituated group suggests a reduction in fear and stress sensitivity, indicating improved coping mechanisms. Furthermore, the stabilization of cloacal temperature fluctuations highlights the role of habituation in promoting thermal homeostasis. These findings have direct implications for poultry management, particularly in hot climates where heat stress can severely impact broiler welfare and productivity. By integrating habituation techniques into routine handling practices, poultry producers may enhance birds' resilience to environmental stressors, ultimately enhancing welfare and performance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Apalowo, O. O., Ekunseitan, D. A. & Fasina, Y. O. (2024). Impact of heat stress on broiler chicken production. *Poultry*, 3(2), 107–128.
- Ayo, J. O., Ogbuagu, N. E., Toluh, C. A., Abdullahi, U. S. & Ramon-Yusuf, S. B. (2022). Diurnal and seasonal fluctuations in cloacal temperature in helmeted guinea fowl (*Numida meleagris*) and the effect of ascorbic acid. *Biological Rhythm Research*, 53(10), 1550–1561.
- Calefi, A. S., Quinteiro-Filho, W. M., Ferreira, A. J. P. & Palermo-Neto, J. (2017). Neuroimmunomodulation and heat stress in poultry. *World's Poultry Science Journal*, 73(3), 493–504.
- Campbell, D. L., Dickson, E. J. & Lee, C. (2019). Application of open field, tonic immobility, and attention bias tests to hens with different ranging patterns. *PeerJ*, 7, e8122.
- EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), Nielsen, S. S., Alvarez, J., Bicout, D. J., Calistri, P., Canali, E. & Michel, V. (2023). Welfare of broilers on farm. *EFSA Journal*, 21(2), e07788.
- Ericsson, M., Henriksen, R., Bélteky, J., Sundman, A. S., Shionoya, K. & Jensen, P. (2016). Long-term and transgenerational effects of stress experienced during different life phases in chickens (*Gallus gallus*). *PLoS One*, 11(4), e0153879.
- Ericsson, M., Fallahsharoudi, A., Bergquist, J., Kushnir, M. M. & Jensen, P. (2014). Domestication effects on behavioural and hormonal responses to acute stress in chickens. *Physiology & Behavior*, 133, 161–169.
- Faul, F., Erdfelder, E., Lang, A.-G. & Buchner, A. (2007). *G*Power 3: A flexible statistical power analysis*

- program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, 39, 175–191.
- Forkman, B., Boissy, A., Meunier-Salaün, M. C., Canali, E. & Jones, R. B. (2007). A critical review of fear tests used on cattle, pigs, sheep, poultry, and horses. *Physiology & Behavior*, 92(3), 340–374.
- Herman, J. P. (2013). Neural control of chronic stress adaptation. *Frontiers in Behavioral Neuroscience*, 7, 61.
- Jackson, A., Quino, M., Gautam, A., Gilpin, M., Still, K., Landers, D. & Baker-Cook, B. (2025). The impact of multiple exposures and movement on the fear response of poultry. *Poultry Science*, 104(1), 104594.
- Jones, R. B. (1996). Fear and adaptability in poultry: Insights, implications, and imperatives. *World's Poultry Science Journal*, 52(2), 131–174.
- Jones, R. B. (1986). The tonic immobility reaction of the domestic fowl: A review. *World's Poultry Science Journal*, 42(1), 82–96.
- Kim, H. R., Seong, P., Seol, K. H., Park, J. E., Kim, H., Park, W., Cho, J. H. & Lee, S. D. (2024). Effects of heat stress on growth performance, physiological responses, and carcass traits in broilers. *Journal of Thermal Biology*, 127, 103994.
- Koolhaas, J. M., Bartolomucci, A., Buwalda, B., de Boer, S. F., Flügge, G., Korte, S. M., Meerlo, P., Murison, R., Olivier, B., Palanza, P., Richter-Levin, G., Sgoifo, A., Steimer, T., Stiedl, O., van Dijk, G., Wöhr, M. & Fuchs, E. (2011). Stress revisited: a critical evaluation of the stress concept. *Neuroscience & Biobehavioral Reviews*, 35(5), 1291–1301.
- Lagadic, H., Faure, J. M., Mills, A. D. & Williams, J. B. (1990). Effects of blood sampling on plasma concentrations of corticosterone and glucose in laying hens caged in groups. *British Poultry Science*, 31(4), 823–829.
- Lara, L. J. & Rostagno, M. H. (2013). Impact of heat stress on poultry production. *Animals*, 3(2), 356–369.
- Masuda, Y., Sakai, R., Kato, I. & Nagashima, K. (2022). Thermoregulatory heat-escape/cold-seeking behavior in mice and the influence of TRPV1 channels. *PLoS One*, 17(11), e0276748.
- Nakamura, K. & Morrison, S. F. (2022). Central sympathetic network for thermoregulatory responses to psychological stress. *Autonomic Neuroscience*, 237, 102918.
- Nash, R. F. & Gallup, G. G. (1976). Habituation and tonic immobility in domestic chickens. *Journal of Comparative and Physiological Psychology*, 90(9), 870–876.
- Ogundeji, T. & Ayo, J. O. (2024). Open field and tonic immobility responses in broiler chickens administered lycopene. *Veterinary Integrative Sciences*, 23(1), 1–10.
- Ogundeji, T., Ayo, J. O., Aluwong, T. & Mohammed, A. (2023). Physiological responses in broiler chickens administered lycopene during the hot-dry season. *Folia Veterinaria*, 67(4), 10–18.
- Olanrewaju, H. A., Purswell, J. L., Collier, S. D. & Branton, S. L. (2018). Influence of light sources and photoperiod on growth performance, carcass characteristics, and health indices of broilers grown to heavy weights. *Poultry Science*, 97, 1109–1116.
- Rankin, C. H., Abrams, T., Barry, R. J., Bhatnagar, S., Clayton, D. F., Colombo, J., Coppola, G., Geyer, M. A., Glanzman, D. L., Marsland, S., McSweeney, F. K., Wilson, D. A., Wu, C. F. & Thompson, R. F. (2009). Habituation revisited: An updated and revised description of the behavioural characteristics of habituation. *Neurobiology of Learning and Memory*, 92(2), 135–138.
- Stingo-Hirmas, D., Cunha, F., Cardoso, R. F., Carra, L. G., Rönnegård, L., Wright, D. & Henriksen, R. (2022). Proportional cerebellum size predicts fear habituation in chickens. *Frontiers in Physiology*, 13, 826178.
- Tamagi, H. M., Idrus, Z., Farjam, A. S., Awad, E. A. & Hussein, A. N. (2024). Effects of auditory enrichment and regular human contact on stress response, underlying fearfulness, and growth performance in broiler chickens. *European Poultry Science/Archiv für Geflügelkunde*, 88, 1–10.
- Tao, X. & Xin, H. (2003). Acute synergistic effects of air temperature, humidity, and velocity on homeostasis of market-size broilers. *ASABE*, 46(2), 491–497.
- Tiemann, I., Becker, S., Fournier, J., Damiran, D., Büscher, W. & Hillemacher, S. (2023). Differences among domestic chicken breeds in tonic immobility responses as a measure of fearfulness. *PeerJ*, 11, e14703.
- Wasti, S., Sah, N. & Mishra, B. (2020). Impact of heat stress on poultry health and performance, and potential mitigation strategies. *Animals*, 10(8), 1266.
- Yahav, S., Goldfeld, S., Plavnik, I. & Hurwitz, S. (1995). Physiological responses of chickens and turkeys to relative humidity during exposure to high ambient temperature. *Journal of Thermal Biology*, 20(3), 245–253.