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Original Article

Evaluation of Heat Stress Through Temperature and Pupillary Dilatation of the Guinea Fowl (Numida Meleagris) in a Controlled Environment

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ABSTRACT

The commercial exploitation of guinea fowl (*Numidia meleagris*) is a viable alternative for small rural properties in arid and semi-arid regions, as they are rustic birds and capable of withstanding the climatic adversities of these regions. The objective of this study was to evaluate the pupillary temperature, smaller pupillary diameter, larger pupillary diameter and pupil area of guinea fowl housed in a controlled environment, under two air temperatures: 26 °C (thermal comfort zone) and 32 °C (above the thermal comfort zone). The birds were distributed in a completely randomized design, with two treatments (air temperatures) and four replications (experimental box), with twelve birds each. The increase in air temperature resulted in a significant average increase ($P < 0.05$) of 6.0, 17.9, 18.6 and 38.4%, respectively, in pupillary temperature, smaller pupillary diameter, larger pupillary diameter and pupillary area of birds, being these measurements accurate in evaluation of thermal stress in homeothermic animals.

INTRODUCTION

Maintaining animals in an environment within the thermal comfort zone is fundamental to the success of poultry farming, and among the causes of the low financial performance of broiler production, environmental issues stand out (Mendes *et al.*, 2014). Lamarca *et al*. (2018) analyzing the effect of heat waves on the climate change scenarios predicted by the IPCC (Intergovernmental Panel on Climate Change) on egg production in the Bastos region, SP, concluded that in all scenarios the average temperature and the number of extreme events, such as heat waves, should increase, which may reduce production in the region.

The condition of thermal stress can be identified through ethological, physiological and productive responses of birds, such as the elevation of surface temperature in birds (Baracho *et al*., 2011; Camerim *et al*., 2016). According to Marques *et al*. (2018) a tool to quantify thermal stress would be through temperature and pupillary dilation, since they react sensitively to external and internal stressors (Stewart *et al*., 2008; Laeng *et al*., 2012). The pupil may reflect the activities of the autonomic nervous system, and may expand (mydriasis) as a result of mental effort and exposure to stressful variants (Lempert *et al*., 2015), as well as raising its temperature in animals under thermal stress (Marques *et al*., 2018). The primary function of the pupil is to regulate the amount of light that enters the retina by dilating or constricting the iris. The pupil's reaction to light stimuli occurs to avoid over exposure and damage to the retina (Helene & Helene, 2011), which can also be affected by factors such as excitement, emotion and stress.

The dilation of the pupil also represents a state of being vigilant or excited, which indicates that the individual has detected an important situation in the environment in which he finds himself (Demos *et al*., 2008). Pupillometry can be a method used to assess the thermal comfort / stress situation of homeothermic animals, mainly because it is non-invasive, since there is no direct contact between the operator and the animal (Marques *et al*., 2018), as well as providing accurate information, enabling the development of mathematical models to describe the different levels of stress, from the dilated pupillary area (Pedrotti *et al*., 2014).

When assessing the pupillary area of Boer goats, Lopes Neto *et al*. (2018) found an increase in this area when the air temperatures of 26 and 33 °C were compared, showing the influence of this variable on the pupillary reactions of the animals. Similar results are found in humans, where stressful situations also interfere with pupil diameter, demonstrating that this method works as an indicator for recognizing and quantifying the levels of stress that the environment can provide to individuals (Pedrotti *et al*., 2014; Charier *et al*., 2017).

The objective of the work was to evaluate the pupillary temperature, smaller pupillary diameter, larger pupillary diameter and pupil area of guinea fowl housed in a controlled environment, under two air temperatures: 26 (thermal comfort zone) and 32 °C (above the thermal comfort zone).

MATERIAL AND METHODS

Experiment Location

The experiment was carried out in two climatic chambers belonging to the Rural Construction and Ambience Laboratory, at the Federal University of Campina Grande, Campina Grande *campus*, Paraíba (7° 13′ 51″ South, 35° 52′ 54″ West), the research was previously approved by the UFCG Ethics and Research Committee, process nº 020/2019.

Animals and Housing

For the experimentation, 96 chicks of guinea fowl hens of one-day-old were acquired, adopting a 21-day period of adaptation to environmental and management conditions, after this adaptation period, data collections began, extending to the 91days, totaling 13 weeks of bird life. The animals were individually marked with plastic rings with different colors, for their respective identification and, during the experimental period, the birds were exposed to a period of 23 consecutive hours of illumination with an hour in the dark.

The birds were housed in boxes of 1 m² of area, inside the climatic chambers, which had dimensions of 3.07 x 2.77 x 2.50 m in width, length and height, respectively, with housing density of 12 birds m-2, as recommended de Nahashon et al. (2009) for guinea fowl. The boxes were equipped with tubular feeders and bell-shaped drinkers and the floor was covered with wood-shavings. After the adaptation period of 21 days, the animals were subjected to cycles of 24 h at experimental air temperature (with the chamber turned on 24 h per day during the entire experimental period), and at 6:30 am and 5:00 pm the chambers were opened for the supply of feed and water. Upon reaching the third week of age, the birds were distributed in the experimental boxes in a completely randomized design, with two treatments (air temperatures) and 4 repetitions (experimental box) with 12 birds in each box.

The experimental period was subdivided into three phases, namely: F1 from the fourth to the sixth weeks of age; F2: from the seventh to the tenth weeks of age; and F3 from the eleventh to the thirteenth weeks of age. The adopted air temperatures were tenders based on the comfort and thermal stress ranges for birds proposed by Furlan & Macari (2002), namely: T26 = 26 °C (thermal comfort zone) and T32 = 32 °C (above the comfort zone), with average values of 70.2% and 0.5 m s^{-1} of relative humidity and air speed, respectively.

Experimental procedures

Throughout the experimental period, the birds received water and feed *ad libitum*. The diets were formulated based on the national research council (NRC, 1994) for broiler birds, where a diet of 3,200 kcal / kg of metabolizable energy and approximately 23, 20 and 18% of crude protein in the initial stages of growth is recommended and final, respectively, according to the formulation shown in Table 1.

The temperature and relative humidity of the air inside the climate chamber were controlled using a microcomputer with the aid of the free software SITRAD® connected to a controller of the type MT-530 PLUS from Full Gauge Controls®. Each climatic chamber was equipped with air conditioners, heaters and exhaust fans. The environmental variables collected were: air temperature, relative humidity, shown in Figure 1 and wind speed at the height of the birds' geometric center (with an average of 0.5 m/s). For the collection of climatic variables, hardware was installed inside the climatic chambers, consisting of temperature and air humidity sensors (DHT22), connected to a specific Arduino® microcontroller, model Mega 2560

Table 1 – Formulation for 100 kg of poultry feed, used in the experiment.

Macronutrients	Feed initial (kg)	Feed growth (kg)	Feed final (kg)
Ground corn 7.50/730	37	47	57
Ground Quirera rice	20	15	10
Soy flakes 45.0/80	38	32	26
Soybean oil	2	3	4
Premix CC[a] BC[b] pre-initial 30 kg/T	3	-	-
Premix CC BC growth 30 kg/T	-	3	-
Premix CC BC final 30 kg/T	-		3
Total	100	100	100
Nutritional levels			
Metabolizable energy (kCal/kg)	2988.62	3117.22	3235.55
Crude protein (%)	21.46	19.12	16.78
Crude fat (%)	4.20	5.40	6.60
Ashes (%)	5.74	5.46	5.24
calcium (%)	0.80	0.79	0.66
Phosphor (%)	0.45	0.43	0.38
Sodium (%)	0.18	0.19	0.17

[a]CC: concentrated core; [b]BC: Broiler Chicken

and for reading the wind speed, a digital anemometer was used Lutron®, model LM8000A.

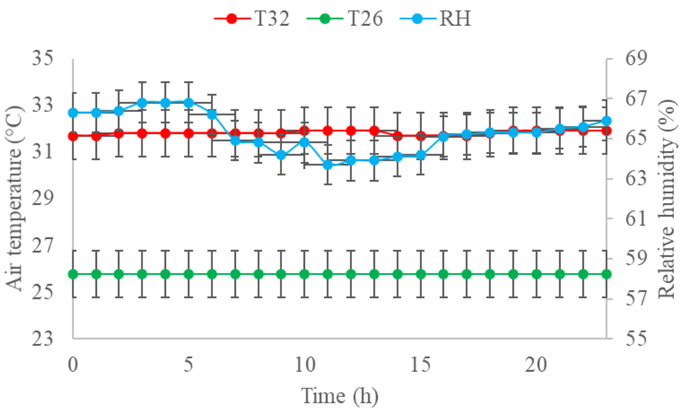

Figure 1 – Average behavior of bioclimatic variables within the climatic chamber: air temperature (TA), relative humidity (RH).

Data collection

The beginning of the collection of images for monitoring pupillary dilation of the animals occurred after the third week of age of the birds, measured weekly at experimental temperatures, during the period when the chambers remained closed. The images were captured by a Canon® model A2600 camera with 16 Mp of resolution, with flash disabled, positioned so as to capture the images of the animals' pupils.

Reference scales were fixed in the animals' beak (Figures 1 and 2), previously adapted to this situation, with 5 mm intervals to allow the conversion of the pupil dimensions that were collected in the images in pixel scale and later converted to millimeters in its real dimensions. The pupil temperature of the animals was monitored by capturing thermograms from the eyeball, using a thermographic camera Flir® model TG-165. The thermal images were collected weekly in each proposed treatment. The average pupil temperature of the animals was obtained from the thermograms in the two thermal conditions evaluated, adopting an emissivity of 0.98 (emissivity of biological tissue).

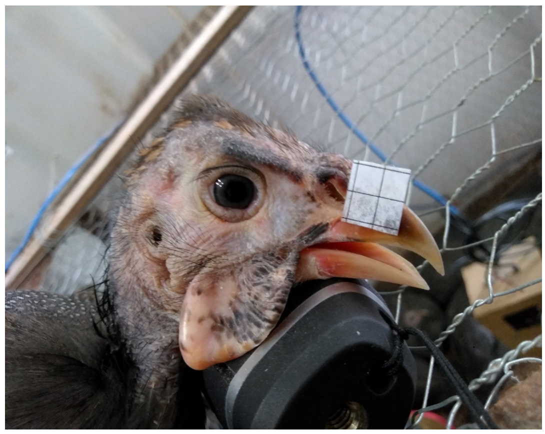

Figure 2 – Image of the pupil of one of the animals with a reference scale attached to the beak.

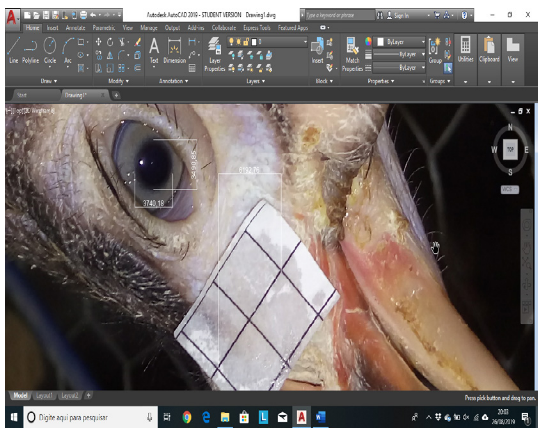

Figure 3 – Pupillary measurements of one of the animals evaluated.

Statistical analysis

The physiological responses, pupillary temperature (PT), smaller pupillary diameter (d), larger pupillary diameter (D) and pupillary area (PA) were presented in Tables. The normality of the data was verified, using the Shapiro-Wilk test ($p>0.05$) and, subsequently, the data were analyzed using the analysis of variance (ANOVA) and F test, using the ExpDes.pt package (Ferreira *et al*., 2013) of the statistical software R version 3.4.1., according to the statistical model described in Equation 1, in a completely randomized design, with two treatments (air temperatures) and 4 repetitions (experimental box) with 12 birds in each box, four birds from each experimental box were selected to obtain the physiological responses totaling 32 experimental plots. The Tukey test was used to compare the means, with a probability of error of 5% ($p<0.05$).

$$y_{ij} = \mu + \tau_i + \varepsilon_{ij} \quad (1)$$

Where,

y_{ij} - is the observed value for the response variable obtained for the ith treatment in its jth repetition;

μ - is the general average of the observations;

τ_i - is the effect of the ith treatment;

ε_{ij} - and the experimental error associated with the observed value y_{ij}.

RESULTS AND DISCUSSION

As it can be seen in Table 2, the rise in the air temperature significantly influenced ($p<0.05$) pupillary responses: pupillary temperature (PT), smaller pupil diameter (d), larger pupil diameter (D) and pupillary area (PA), regardless of the age of the birds evaluated.

The guinea fowl significantly increased ($p<0.05$) the pupillary temperature in all the evaluated experimental phases and also in the average between the phases, showing an average increase of 6.0% in the PT, due to the increase in air temperature and, this behavior occurs because the eye contains many innervated capillary channels, which increase blood flow due to the activation of sensitive heat loss mechanisms for the maintenance of homeothermia, and this increase in blood supply to the eyeball reflects the increase in vasodilation as a dissipation tool of sensitive heat from the animals' body nucleus, which intensifies due to the increase in air temperature (Marques *et al*., 2018).

The increase in eye temperature as a result of thermal stress, occurs due to the corresponding increase in activities in the hypothalamic-pituitary-adrenal axis (HPA), which constitute the central control system responsible for regulating body temperature (Stewart *et al*., 2008) and, that this measure may be accurate in the assessment of thermal stress in homeothermic animals, being obtained without interference from the skin or hair, proving to be a consistent measure of temperature change in response to stress when compared to other anatomical areas.

Camerim *et al.* (2016) cite that the increase in air temperature caused a significant increase in the temperature of the head of the laying hens, and the increase in the temperature of this part of the body of the birds occurred because this region is membranous and has a rich vascular network, making this an important region of thermolysis, where a great part of the sensitive heat exchanges of the animals occur due to the environment, a fact that can also be evidenced when the pupillary temperature of the birds that were

Table 2 – Means of pupillary temperature (PT), smaller pupillary diameter (d), larger pupillary diameter (D) and pupillary area (PA) of birds at both temperatures, in each experimental phase and in the general average.

Treatments	Responses	F1	F2	F3	Average
T26	PT (°C)	31.7±0.8b	31.4±0.8b	31.5±1.0b	31.5±0.8b
	d (mm)	3.8±0.6b	4.0±0.5b	4.0±0.8b	3.9±0.6b
	D (mm)	4.4±0.6b	4.2±0.5b	4.3±0.8b	4.3±0.6b
	PA (mm^2)	13.9±3.3b	13.5±2.8b	14.0±5.3b	13.8±4.5b
T32	PT (°C)	33.3±0.9a	33.5±0.7a	33.5±0.6a	33.4±0.7a
	d (mm)	4.2±0.4a	4.7±0.7a	4.9±0.7a	4.6±0.6a
	D (mm)	4.6±0.4a	5.3±1.0a	5.5±0.9a	5.1±0.8a
	PA (mm^2)	15.4±2.6a	20.0±6.7a	21.8±6.8a	19.1±5.4a
p-value	PT (°C)	0.01	0.01	0.01	0.01
	d (mm)	0.01	0.01	0.01	0.01
	D (mm)	0.01	0.01	0.01	0.01
	PA (mm^2)	0.03	0.01	0.01	0.01

F1: from the fourth to the sixth weeks of age; F2: from the seventh to the tenth weeks of age; F3: from the eleventh to the thirteenth weeks of age; T26: air temperature of 26 °C; T32: air temperature of 32 °C; PT: pupillary temperature; d: smaller pupillary diameter; and D: larger pupillary diameter. Means followed by the same letter in the column, do not differ by Tukey's test at 5% probability of error ($p<0.05$).

evaluated in the present research increased, according to the elevation of the air temperature.

There was a significant increase ($p<0.05$) average of 17.9, 18.6 and 38.4%, respectively, in the pupil dimensions d, D and PA, respectively, of the guinea fowls, as the air temperature increased, facts also observed by Lopes Neto *et al*. (2018) and Marques *et al*. (2018), when evaluating Boer goats submitted to different air temperatures. This possibly is due to the pupillary dilation of the animals being associated with the activation of the autonomic nervous system (sympathetic bundle) (Laeng *et al*., 2012), in response to thermal stress, since the pupil reflects the activities of the autonomic nervous system, expanding (mydriasis) as a consequence of exposure to high air temperatures (exposure to thermal stress) (Lempert *et al*., 2015). The increase in the pupillary diameter of birds can also be associated with the adrenaline discharge in the animals' body, which occur when the adrenal glands secrete an excessive amount of this hormone, in response to certain stimuli, such as thermal stress.

Thus, changes in the dimensions and pupillary temperature of animals, when subjected to different air temperatures, are intensely related to the responses of the thermoregulatory mechanisms triggered by the autonomic nervous system, reflecting changes in physiological responses, due to the effort made by the animals in an attempt to maintain body temperature within narrow limits of variation, even when subjected to stressful air temperature conditions.

CONCLUSION

With the increase in air temperature, there was an increase in pupillary dilation and pupillary temperature in the animals, and these measures can be used in the evaluation of the comfort or thermal stress condition of guinea fowl.

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REFERENCES

Camerini1 NL, Silva RC, Nascimento JWB, Oliveira DL, Souza BB. Variação da temperatura superficial de aves poedeiras criadas em dois sistemas de criação utilizando termografia. Agropecuária Científica no Semiárido 2016;12(2):145-152.

Charier DJ, Zantour D, Pichot V, Chouchou F, Barthelemy JCM, Molliex SB. Assessing pain using the variation coefficient of pupillary diameter. The Journal of Pain 2017;18(11):1346-1353.

Demos KE, Kelley WM, Ryan SL, Davis FC, Whalen PJ. Human amygdala sensitivity to the pupil size of others. Cerebral Cortex 2008;18(12):2729-2734.

Ferreira EB, Cavalcanti PP, Nogueira DA. ExpDes.pt: Experimental Designs pacakge (Portuguese). R package version 1.1.2; 2013.

Furlan RL, Macari M. Termorregulação. In: Macari M, Furlan RL, Gonzales E, editors. Fisiologia aviária aplicada a frangos de corte. Jaboticabal: Funep-Unesp; 2002. p.209-230.

Helene O, Helene AF. Some aspects of the optics of the human eye. Revista Brasileira de Ensino de Física 2011;33(3):1-8.

Laeng B, Sirois S, Gredebäck G. Pupillometry:a window to the preconscious? Perspectives Psychological Science 2012;7(1):18-27.

Lamarca DSF, Pereira DF, Magalhaes MM, Salgado DD. Climate change in layer poultry farming: impact of heat waves in region of Bastos, Brazil. Brazilian Journal of Poultry Science 2018;20(4):657-664.

Lempert KM, Chen YL, Fleming SM. Relating pupil dilation and metacognitive confidence during auditory decision-making. PLoS One 2015;10(5):1-12.

Lopes Neto JP, Marques JI, Furtado DA, Lopes FFM, Borges VP, Araújo TGP. Pupillary stress index: a new thermal comfort index for crossbred goats. Revista Brasileira de Engenharia Agrícola e Ambiental 2018;22(12):866-871.

Marques JI, Lopes Neto JP, Nascimento JWB, Talieri IC, Medeiros GR, Furtado DA. Pupillary dilation a thermal stress indicator in Boer crossbred goats maintained in a climate chamber. Small Ruminant Research 2018;158(1):26-29.

Mendes AS, Gudoski DC, Cargnelutti AF, Silva EJ, Carvalho EH, Morello GM. Factors that impact the financial performance of broiler production in southern states of Paraná, Brazil. Brazilian Journal of Poultry Science 2014;16(1):113-120.

Nahashon SN, Adefope N, Amenyenu A, Tyus J, Wright D. The effect of floor density on growth performance and carcass characteristics of French guinea broilers. Poultry Science 2009;88(11):2461–2467.

NRC - National Research Council. Nutrient requirements of poultry. 9th ed. Washington: National Academy Press; 1994.

Pedrotti M, Mirzaei MA, Tedesco A, Chardonnet, JR, Mérienne F, Benedetto S, et al. Automatic stress classification with pupil diameter analysis. International Journal of Human-Computer Interaction 2014;30(3):1-17.

Stewart M, Stafford KJ, Dowling SK, Schaefer AL, Webster JR. Eye temperature and heart rate variability of calves disbudded with or without local anaesthetic. Physiology & Behavior 2008;93(1):789-797.