

## Physiological response of broilers raised under simulated conditions of heat waves

Malagoli de Mello, J.L.<sup>1</sup>®; Manente Boiago, M.<sup>2</sup>; Giampietro-Ganeco, A.<sup>1</sup>; Piatto Berton, M.<sup>1</sup>; Alves de Souza, R.<sup>1</sup>; Borba Ferrari, F.<sup>1</sup>; Alves de Souza, P.<sup>1</sup> and Borba, H.<sup>1</sup>

<sup>1</sup>São Paulo State University. UNESP. Brasil.

<sup>2</sup>University of State of Santa Catarina. UDESC. Brasil.

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Correspondencia a los autores/Contact e-mail:  
[julianalolli@zootecnista.com.br](mailto:julianalolli@zootecnista.com.br)

### SUMMARY

To evaluate the influence of heat stress for up to 72 hours, and the possible cumulative effect of heat stress on the physiological responses of broilers raised under simulated conditions of heat waves, 500 male Cobb broilers were raised in two climatic chambers equipped with heating and cooling mechanisms, for up to 45 days. Half of the birds were raised in thermal comfort and the remainder broilers were submitted to heat waves ( $32 \text{ }^{\circ}\text{C} \pm 0.5 \text{ }^{\circ}\text{C}$  for up to 72 hours) in three ages (starting at 21, 35 and 42 days of age). In the beginning of each heat wave simulation and after 24, 48 and 72 hours of exposure to heat stress, physiological parameters as rectal temperature and respiratory frequency were measured, and blood samples were collected for determining hormonal levels (T3 and T4) and hematocrit. The increase of the birds' respiratory frequency was verified after 24 hours of exposure to high temperatures in three simulated heat waves. During the third heat wave there was no variation of the rectal temperature. The environmental temperature did not influence the hematocrit value, and T3 and T4 thyroid hormone levels. Submit birds to periods of intermittent acute heat stress, like during the simulation of heat waves, influences the broilers' respiratory frequency and rectal temperature, showing the consequent effect of heat stress on the birds' metabolism and body thermal balance. Heat waves doesn't affect the secretion of triiodothyronine and thyroxine, and the percentage of red blood cells.

### Respostas de frangos de corte criados sob estresse por calor

### RESUMO

Para avaliar a influência do estresse por calor por até 72 horas, e o possível efeito acumulativo do estresse sobre as respostas fisiológicas de frangos de corte criados sob condições simuladas de ondas de calor, 500 frangos de corte machos, da linhagem Cobb, foram criados em duas câmaras climáticas equipadas com sistemas de aquecimento e refrigeração, por até 45 dias. Metade das aves foram criadas sob conforto térmico (ajustado de acordo com o ideal para cada idade) e as demais aves foram submetidas a ondas de calor ( $32 \text{ }^{\circ}\text{C} \pm 0,5 \text{ }^{\circ}\text{C}$  por até 72 horas) em três idades (com início aos 21, 35 e 42 dias de idade). No início de cada simulação de onda de calor e após 24, 48 e 72 horas de exposição ao calor, foram avaliados parâmetros fisiológicos como temperatura retal e frequência respiratória. Além disso foram coletadas amostras de sangue para determinação de níveis hormonais (T3 e T4) e hematócrito. O aumento da frequência respiratória das aves foi verificado após 24 horas de exposição a altas temperaturas nas três ondas de calor simuladas. Durante a terceira onda de calor não houve variação da temperatura retal. A temperatura ambiente não influenciou o valor do hematócrito e os níveis de hormônios tireoidianos T3 e T4. Submeter aves a períodos de estresse por calor agudo intermitente, como durante a simulação de ondas de calor, influencia a frequência respiratória e a temperatura retal dos frangos de corte, evidenciando o consequente efeito do estresse por calor sobre o metabolismo das aves e o equilíbrio térmico corporal. As ondas de calor não afetam a secreção de tri-iodotironina e tiroxina, e a porcentagem de glóbulos vermelhos.

### INTRODUCTION

Changes in the poultry sector in recent years have transformed Brazil into an important producer and supplier of cuts and derived products from chicken. Brazil currently is the second largest producer (13.13 million tons) and the world's largest exporter (4.304 million tons) of chicken meat (ABPA, 2016).

Due to the great climatic variations and temperature fluctuations caused by heat waves, in Brazil the conditions of thermal comfort inside the poultry sheds

are hardly obtained, directly affecting animal production. The high temperature and high humidity, characteristic of tropical regions, are limiting to the optimal productivity (Zhang et al., 2012), and the strains with high growth rate are more sensitive to heat than others (Dahlke et al., 2005).

The ideal temperature for optimum performance of growing chickens varies from 18 to 22 °C (Lin et al., 2006). When the environmental temperature is higher than thermal comfort temperature, the animal is subjected to heat stress conditions characterized by in-

creased body temperature, whose main consequences include high mortality, reduced feed consumption and less body weight gain (Quinteiro-Filho et al., 2010). Birds are homeothermic animals that keep their body temperature through adaptive behavioral and mechanical reactions of thermoregulation, which are responsible for the maintenance and control of homeothermy by exchanging heat with the environment (Furlan and Macari, 2002).

The main mechanisms activated to maintaining homeostasis in chickens are thermal radiation, convection and evaporation but, in heat stress situations, the main mechanism of heat loss used by the animal is respiratory evaporation (Brossi et al., 2009). When a fowl is exposed to stress, the sympathetic nervous system is activated, increasing the respiratory and cardiac frequencies and the redistribution of blood supply to vital organs (to reduce the peripheral resistance of the circulatory system and facilitate heat dissipation through body extremities), also increasing rectal temperature and causing variation in hematocrit values (Furlan and Macari, 2002).

The increase of respiratory rate involves great muscle effort, results in a greater use of energy, and produces more heat with consequent reduction of the bird's productive efficiency. In these cases, death by exhaustion may occur quickly, mainly in heavier birds (Santos, 2007). The circulatory system is also sensitive to tempe-

rature variations and represents an important indicator of the animal's physiological responses to stressing agents (Borges et al., 2003). Thus, the aim of this study was to evaluate the influence of heat stress for up to 72 hours, in different phases of growing, and the possible cumulative effect of heat stress, on the physiological responses of broilers raised under simulated conditions of heat waves.

## MATERIAL AND METHODS

This study was performed at Department of Animal Science of the São Paulo State University / UNESP, Jaboticabal, São Paulo, Brazil (21°08' S, 48°11' W, 583 m altitude). The experiment was reviewed and approved by the Ethics Committee for the Use of Animals from São Paulo State University (Jaboticabal, São Paulo, Brazil) by protocol number 4207/2010.

The experiment was carried out in two experimental climatic chambers with five hundred one-day-old Cobb male broilers that were raised until 45-d-old and fed with three different kinds of diet (starting, growing and finishing diets as described in **Table I**), formulated according to the birds' age and their nutritional demands (Rostagno et al., 2005).

**Table I.** Composition and calculated nutrient content of diets (Composição e conteúdo calculado dos nutrientes contidos nas dietas).

Ingredient (%)	Starter (1 to 21-day-old)	Growing (22 to 35-day-old)	Finisher (36 to 45-day-old)
Corn grain	57.20	63.89	65.04
Soybean meal	36.94	30.26	27.86
Soybean oil	1.81	2.47	3.87
Dicalcium phosphate	1.83	1.63	1.38
Calcitic limestone	1.30	0.85	0.95
Salt	0.30	0.30	0.30
Vitamin and mineral mix*	0.50	0.50	0.50
DL- methionine (98%)	0.12	0.10	0.10
Calculated nutrient composition			
Crude protein (%)	21.5	19.00	18.00
ME (kcal/kg)	3000	3121	3225
Available phosphorus (%)	0.45	0.40	0.35
Calcium (%)	0.95	0.84	0.80
Total M+C (%)	0.85	0.78	0.75
Total methionine (%)	0.50	0.46	0.45
Total Lysine (%)	1.20	1.06	1.00

ME: Metabolizable energy; M+C: methionine + cysteine.

\*Product composition (per kg) – Starter: A vitamin: 176.000 UI, D3 vitamin: 40.000 UI, E vitamin: 500 mg, K vitamin: 120 mg, B2 vitamin: 200 mg, B6 vitamin: 70 mg, B12 vitamin: 700 mcg, B3 vitamin: 750 mg, biotin: 3 mg, pantothenic acid: 600 mg, folic acid: 30 mg, C vitamin: 20 mg, Fe: 1.100 mg, Cu: 300 mg, I: 24 mg, methionine: 32 mg, Ca: 180 mg, P: 66 mg, Na: 23 mg, Cl: 36 mg, growth promoter: 2 mg, coccidiostatic: 10g, BHT: 1 mg, Mg: 5 g, S: 4 g, inert vehicle: 1.000 g. Growing: A vitamin: 150.000 UI, D3 vitamin: 35.000 UI, E vitamin: 480 mg, K vitamin: 110 mg, B2 vitamin: 170 mg, B6 vitamin: 70 mg, B12 vitamin: 650 mcg, B3 vitamin: 700 mg, biotin: 3 mg, pantothenic acid: 500 mg, folic acid: 25 mg, C vitamin: 12 mg, Fe: 1.100 mg, Cu: 300 mg, I: 24 mg, methionine: 20 mg, Ca: 176 mg, P: 60 mg, Na: 23 mg, Cl: 36 mg, growth promoter: 2 mg, coccidiostatic: 10g, BHT: 1 mg, Mg: 5 g, S: 4 g, inert vehicle: 1.000 g. Finisher: A vitamin: 150.000 UI, D3 vitamin: 35.000 UI, E vitamin: 450 mg, K vitamin: 100 mg, B2 vitamin: 160 mg, B6 vitamin: 70 mg, B12 vitamin: 650 mcg, B3 vitamin: 700 mg, biotin: 3 mg, pantothenic acid: 500 mg, folic acid: 25 mg, C vitamin: 12 mg, Fe: 1.100 mg, Cu: 300 mg, I: 24 mg, methionine: 18 mg, Ca: 176 mg, P: 58 mg, Na: 23 mg, Cl: 36 mg, BHT: 1 mg, Mg: 5 g, S: 4 g, inert vehicle: 1.000 g.

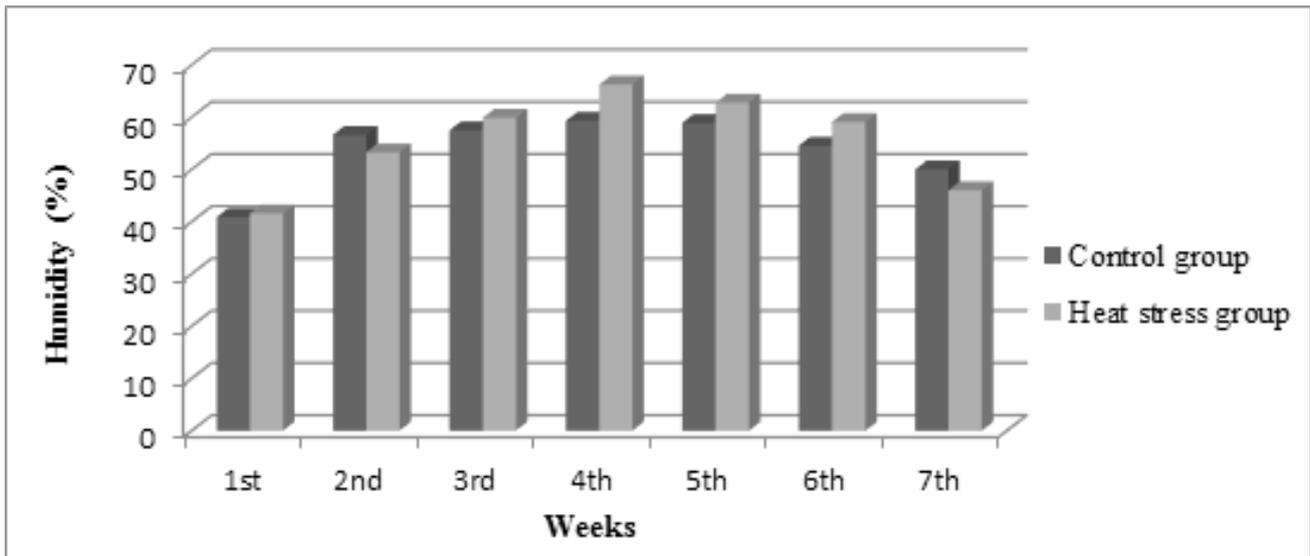


Figure 1. Relative humidity inside of each chamber throughout the experiment (Umidade relativa no interior de cada câmara climática durante o experimento).

Chicks were distributed in two climatic chambers being one equipped with heating and cooling systems (to provide heat during the heat waves and comfort temperature between the heat waves) and other one with coolers (to provide thermal comfort to control group). The rearing density was 10 birds / m<sup>2</sup>, the floor was covered with wood-shavings poultry litter (layer thickness of 10 cm) and water and food offered ad libitum. Water was provided using pressure drinkers until 8-d-old, and later bell drinkers. Feed was provided through tray feeders from 1-d-old until 8-d-old and then substituted by tube feeders whose height was adjusted according to the birds' growth. The used light program was continuous light throughout the experiment, using incandescent 100-watt lamps. Infrared lamps (250 W / 127 V) were used as heaters in order to control the temperature inside the chambers; heaters and coolers were controlled by digital thermostats and thermos-hygrometers. Three thermos-hygrometers were used to register the average temperatures and relative humidity inside

of each chamber three times a day (morning, afternoon and night), and were placed at the extremities and at the middle of the sheds. The temperature and relative air humidity averages recorded inside each chamber during the experimental period are shown on **Figures 1 and 2**.

Half of the birds was used as the control group and was raised in a chamber with ideal internal thermoneutral temperature for each age, according to the recommendations of the Cobb Breeder Management Guide (Cobb, 2013) during the whole experimental period. The remainder birds were raised in another climatic chamber, and submitted to different periods of heat stress (heat waves), characterized by the increase of the climatic chamber internal temperature to 32°C with variation of  $\pm 0.5^\circ\text{C}$ . The stress on the animals started at three ages (21, 35 and 42-d-old) to simulate three 72-hour long heat waves. After 72 hours, the heaters were turned off and the animals submitted to heat stress were also kept under

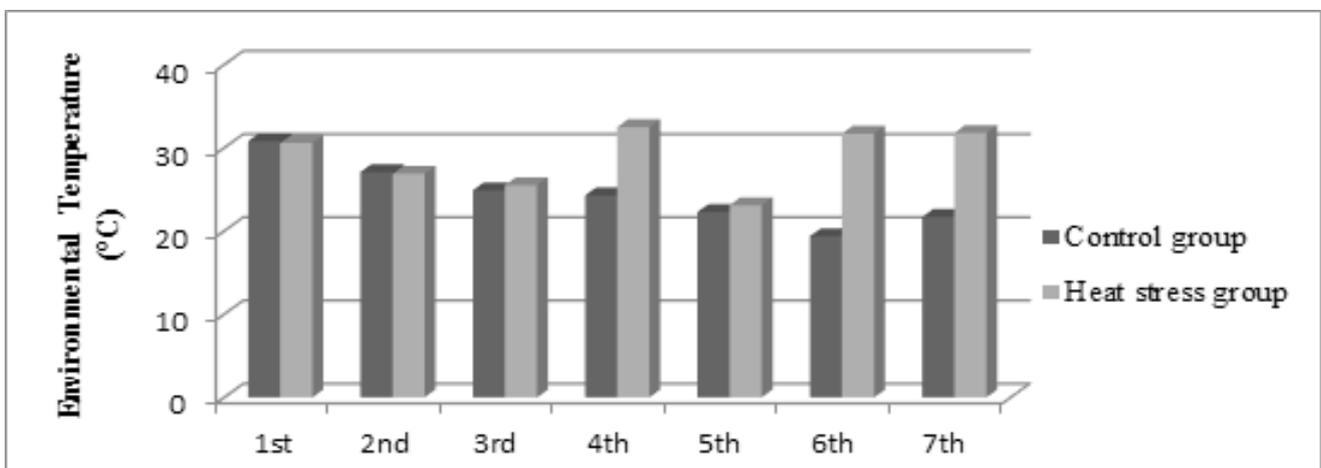


Figure 2. Environmental temperature inside of each chamber throughout the experiment (Temperatura ambiental média no interior de cada câmara climática durante o experimento).

**Table II. Respiratory frequency (RF) and rectal temperature (RT) of broilers submitted to heat stress during the simulation of three heat waves (HW), started at 21, 35 and 42-d-old, respectively.** (Frequência respiratória (RF) e temperatura retal (RT) de frangos de corte submetidos ao estresse térmico durante a simulação de três ondas de calor (HW), iniciadas aos 21, 35 e 42 dias de idade, respectivamente).

	RF (resp.min <sup>-1</sup> )			RT (°C)		
	1st HW	2nd HW	3rd HW	1st HW	2nd HW	3rd HW
Treatments (T)						
Heat stress group	88.08	108.58	107.17	40.8	41.9	41.6 <sup>A</sup>
Control group	47.54	52.92	48.71	40.5	40.6	40.7 <sup>B</sup>
Stress Duration (SD)						
Start	39.17	36.83	50.67	40.3	40.6	41.2
After 24 hours	80.92	100.00	93.17	40.8	41.3	41.1
After 48 hours	80.66	92.33	83.92	41.0	41.5	41.1
After 72 hours	70.50	93.83	84.00	40.6	41.7	41.1
P-value						
Treatments	<0.0001	<0.0001	<0.0001	0.0004	<0.0001	<0.0001
Stress Duration	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.9330
Int. TxSD	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.2552

<sup>A,B</sup> Means in the same column followed by distinct letters differ significantly according to Tukey's test ( $P < 0.05$ ).

thermoneutral conditions until the beginning of the next heat wave, when the heaters were turned on again.

In the beginning of each heat wave simulation and after 24, 48 and 72 hours of exposure to heat stress, physiological parameters as rectal temperature and respiratory frequency were measured, and blood samples were collected for determining hormonal levels (T3 and T4) and hematocrit. The rectal temperature was determined by a clinical veterinarian thermometer, 0.1 °C precision, inserted in the bird's cloaca until reaching constant temperature. The respiratory frequency monitoring was visually evaluated, considering the number of times the birds inhaled air in a minute.

The birds' blood samples (5 mL) were collected by puncturing the jugular vein with heparinized syringes and disposable needles. Blood samples were divided into two aliquots, one for determining hormonal levels (T3 and T4) and the other one for determining hematocrit. Immediately after the blood collection, hematocrit was determined by microhematocrit technique, according to Rosário et al. (2000), using heparinized micro tubes filled with blood by capillarity until 2/3 of the volume. Two micro tubes were used per dosed samples, centrifuged at 11,500 rpm for 5 minutes. Each micro tube was read with a reading card (FANEM Ltda.), and the percentage of cells in relation to the total volume of collected blood was obtained. Aliquots intended to T3 and T4 levels were centrifuged at 3,000 rpm for five minutes. The obtained plasma was stored in tubes and frozen at -20 °C for later analysis. Plasma samples were sent to the Department of Animal Reproduction, UNESP, Botucatu, São Paulo, Brazil. Following the thawing (room temperature), T3 and T4 plasma levels were determined by solid-phase radioimmunoassay using commercial kits containing labelled antibodies (radioactive I-125) according to the procedures recommended by the manufacturer.

The experiment followed a 2x4 completely randomized factorial design (two treatments - heat stress

group and control group; and four periods of heat stress - 0, 24, 48 and 72 hours), with ten replicates. Results were analyzed by the General Linear Models procedure of the Statistical Analysis System (SAS Institute Inc, Cary, NC), data were tested by analysis of variance and compared by Tukey's test with a significance level of  $P < 0.05$ .

## RESULTS AND DISCUSSION

There was an interaction between treatments (submission to heat stress) and stress duration for respiratory frequency ( $P < 0.01$ ) during the simulation of three heat waves, and for rectal temperature ( $P < 0.01$ ) during the first and second heat waves (Table II). Those results are described on Tables II and III, respectively. Broilers exposed to three heat waves showed higher average rectal temperature (41.6 °C) than birds raised under thermoneutrality conditions (40.7 °C).

The birds initiated the exposure to the third heat wave with internal body temperature equal to 41.2 °C. It is not possible to affirm that the environment temperature had cumulative effect on the birds' organism, once the results obtained during 72 hours under high environment temperature were statistically the same ( $P > 0.05$ ). Values higher than 41.1 °C characterize broilers stressed by environmental heat (Macari et al., 2004), but it is not possible to affirm that the birds started the third heat wave already under heat stress condition, we consider this difference (0.1 °C) much low to characterize a possible cumulative thermal stress. It is possible that chickens have undergone an adaptation during the first two heat waves, which would have caused the absence of the effect of heat on the chicken's rectal temperature during the third heat wave simulation.

At the beginning of the simulation of three heat waves, the birds from both groups showed the same physiological conditions, that is, respiratory frequency and

**Table III. Breakdown of the interaction between treatments and stress duration for respiratory frequency (resp.min<sup>-1</sup>) during the heat waves simulation** (Desdobramento da interação entre tratamentos e duração do estresse para a variável frequência respiratória (resp.min<sup>-1</sup>) durante a simulação de ondas de calor).

	1st H			
	Start	After 24 hours	After 48 hours	After 72 hours
Heat stress group	34.30 <sup>Ab</sup>	104.70 <sup>Aa</sup>	113.00 <sup>Aa</sup>	100.30 <sup>Aa</sup>
Control group	44.00 <sup>Aa</sup>	57.20 <sup>Ba</sup>	48.30 <sup>Ba</sup>	40.70 <sup>Ba</sup>
2nd Heat Wave				
	Start	After 24 hours	After 48 hours	After 72 hours
Heat stress group	36.00 <sup>Ab</sup>	140.70 <sup>Aa</sup>	128.00 <sup>Aa</sup>	131.30 <sup>Aa</sup>
Control group	39.33 <sup>Aa</sup>	59.30 <sup>Ba</sup>	56.70 <sup>Ba</sup>	56.30 <sup>Ba</sup>
3rd Heat Wave				
	Start	After 24 hours	After 48 hours	After 72 hours
Heat stress group	50.33 <sup>Ab</sup>	135.00 <sup>Aa</sup>	123.00 <sup>Aa</sup>	120.30 <sup>Aa</sup>
Control group	51.00 <sup>Aa</sup>	51.30 <sup>Ba</sup>	44.80 <sup>Ba</sup>	47.70 <sup>Ba</sup>

Means followed by distinct uppercase letters in each column are different according to Tukey's test ( $P < 0.05$ ). Means followed by distinct lowercase letters in each line are different according to Tukey's test ( $P < 0.05$ ). 1st heat wave: started at 21-d-old; 2nd heat wave: started at 35-d-old; 3rd heat wave: started at 42-d-old.

rectal temperature statistically equivalent (**Tables III and IV**). It is noteworthy to mention that in the beginning of the first heat wave, both evaluated groups had not been exposed to any heat stress condition before, differently from posterior simulations. The increase ( $P < 0.05$ ) of the birds' respiratory frequency was verified after 24 hours of exposure to high temperature ( $32\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$ ) and was kept high until 72 hours under heat stress conditions, when the fowls were again submitted to thermoneutral temperature until the beginning of the next heat wave.

During the first heat wave, the birds' rectal temperature was high after 24 hours of exposure to the environmental heat, but, after 72 hours, this parameter reached normality again, and did not differ ( $P > 0.05$ ) from the average showed by the control group (**Table IV**). After 72 hours of heat stress, it was verified that the maintenance of high respiratory frequency (100.3 resp.min<sup>-1</sup>) was efficient in the reduction of the birds' average rectal temperature from  $41.3^{\circ}\text{C}$  (after 24 hours) to  $40.4^{\circ}\text{C}$ , and re-established the animals' body thermal balance. During the second heat wave, the rectal tem-

perature was also higher after 24 hours and it was kept high until the end of the evaluated period.

The increase of the rectal temperature is a physiological response to the high temperature and humidity conditions resulting of the storage of metabolic heat (Silva et al., 2003), which may increase the normal rectal temperature (on average it varies from  $41^{\circ}\text{C}$  to  $42^{\circ}\text{C}$  in adult broilers (Oliveira et al., 2006b) in  $4.7^{\circ}\text{C}$  (Menten et al., 2006). Consequently, the increase of respiratory frequency stimulates heat loss by panting and tries to eliminate internal heat by airways to maintain homeothermy. The birds have the ability to increase the respiratory frequency 10 times (which is usually, on average, 40 breaths per minute (Nazareno et al., 2009)), and, consequently, lose heat through the respiratory system (Furlan and Macari, 2002). These responses result from direct stimuli in the hypothalamus that sends impulses to the cardiorespiratory system to attempt to eliminate heat (Macari et al., 2004).

Heat responsive neurons are activated when the temperature increases, and induce the animal to respond to heat loss. This temperature control acts as

**Table IV. Breakdown of the interaction between treatments and stress duration for rectal temperature ( $^{\circ}\text{C}$ ) during the heat waves simulation** (Desdobramento da interação entre tratamentos e duração do estresse para a variável temperatura retal ( $^{\circ}\text{C}$ ) durante a simulação de ondas de calor).

	1st Heat Wave			
	Start	After 24 hours	After 48 hours	After 72 hours
Heat stress group	40.3 <sup>Ab</sup>	41.3 <sup>Aa</sup>	41.3 <sup>Aa</sup>	40.4 <sup>Ab</sup>
Control group	40.3 <sup>Aa</sup>	40.4 <sup>Ba</sup>	40.6 <sup>Ba</sup>	40.7 <sup>Aa</sup>
2nd Heat Wave				
	Start	After 24 hours	After 48 hours	After 72 hours
Heat stress group	40.6 <sup>Ac</sup>	41.9 <sup>Ab</sup>	42.3 <sup>Aab</sup>	42.7 <sup>Aa</sup>
Control group	40.5 <sup>Aa</sup>	40.6 <sup>Ba</sup>	40.7 <sup>Ba</sup>	40.7 <sup>Ba</sup>

Means followed by distinct uppercase letters in each column are different according to Tukey's test ( $P < 0.05$ ). Means followed by distinct lowercase letters in each line are different according to Tukey's test ( $P < 0.05$ ). 1st heat wave: started at 21-d-old; 2nd heat wave: started at 35-d-old.

**Table V. Hematocrit of broilers submitted to heat stress during the simulation of three heat waves (HW), started at 21, 35 and 42-d-old, respectively.** (Hematócrito de frangos de corte submetidos ao estresse térmico durante a simulação de três ondas de calor (HW), iniciadas aos 21, 35 e 42 dias de idade, respectivamente).

	1st HW	2nd HW	3rd HW
<b>Treatments (T)</b>			
Heat stress group	29.28	30.56	29.97
Control group	30.28	30.53	30.34
<b>Stress Duration (SD)</b>			
Start	29.12	30.19	30.62
After 24 hours	29.56	30.56	30.12
After 48 hours	29.75	29.62	29.81
After 72 hours	30.69	29.81	30.06
<b>P-value</b>			
Treatments	0.0721	0.9687	0.6030
Stress Duration	0.2312	0.1158	0.8756
Int. TxSD	0.5991	0.9894	0.1981

a set-point thermoregulator, approximately 41.1°C (Furlan and Macari, 2002), which resists to any environmental alteration so that the body temperature be kept within tolerable limits, allowing internal environmental consistency, perfect system functioning, better performance and greater production.

The higher the air relative humidity, more difficult is the heat dissipation by evaporation, making higher the respiratory frequency (Oliveira et al., 2006a). It is believed that the air humidity (an average of 57%) and the high temperature of heat stress (32°C ± 0.5°C) imposed in this study have made the maintenance of the animals' body homeothermy more difficult during the simulation of the three studied heat waves. The increase of body temperature could be even greater as a consequence of the energy generated by the muscu-

**Table VI. Plasmatic levels of triiodothyronine (T3) (ng.mL<sup>-1</sup>) of broilers submitted to heat stress during the simulation of three heat waves (HW), started at 21, 35 and 42-d-old, respectively** (Níveis plasmáticos de tri-iodotironina (T3) (ng.mL<sup>-1</sup>) de frangos de corte submetidos ao estresse térmico durante a simulação de três ondas de calor (HW), iniciadas aos 21, 35 e 42 dias de idade, respectivamente).

	1st HW	2nd HW	3rd HW
<b>Treatments (T)</b>			
Heat stress group	1.42	1.50	0.98
Control group	1.39	1.31	1.14
<b>Stress Duration (SD)</b>			
Start	1.45	1.11	0.77
After 24 hours	1.31	1.41	0.80
After 48 hours	1.34	1.66	1.36
After 72 hours	1.53	1.61	1.04
<b>P-value</b>			
Treatments	0.9160	0.2657	0.9124
Stress Duration	0.9306	0.0924	0.1359
Int. TxSD	0.7417	0.0819	0.1766

lar contraction during panting that produces heat and causes hyperthermia in the bird.

It should be considered that the metabolic heat production increases as the bird ages, making it more susceptible to heat stress, whereas its capacity to dissipate it decreases (Marchini et al., 2007) which may justify the increase of the birds' rectal temperature observed at 35 and 42-d-old, when compared to the averages obtained during the simulation of the first heat wave. When the effects of heat stress on 35-d-old Hubbard broilers were evaluated, Salvador et al. (1999) verified that after 6 hours under 34 °C stress, the birds had rectal temperature of 43.4°C, which was even higher than the values found in this study.

The hematocrit value did not show significant difference between the tested treatments and was not influenced by the periods of high temperature exposure (Table V). The obtained results for the hematocrit analysis corroborate Silva et al. (2007) who, when studying the effect of heat stress in a simulation of broilers' transportation until the slaughterhouse, did not find significant differences between the hematocrit values that allowed to associate the blood cell variation percentage to other birds' heat stress condition. On the other hand, Yahav et al. (1998) found variations in the hematocrit values of broilers exposed to heat and it was higher after the birds' exposure to 35°C for 6 hours.

Due the metabolic and physiological disorders, and the deficiency of oxygen supply caused by the bird's fast growth, a greater number of blood cells are produced and there is a consequent increase of blood viscosity and hematocrit (Rosário et al., 2004). The hematocrit value is also directly related to the dehydration degree suffered by the bird during the heat stress (Cardoso and Tessari, 2003). It is possible that a greater water intake may have reduced the birds' dehydration during the heat wave simulation and, thus, hematocrit was not influenced ( $P > 0.05$ ) by the environmental temperature. The hematocrit values obtained in this study ranged from 29.12 to 30.69% like the results found by Silva et al. (2003) that varied from 29 to 31% (which are considered intermediate and normal values). However, in the present study there were no differences related to the bird's blood hematocrit attributed to the thermal stress.

In order to keep the body homeothermy birds reduce the endogenous heat production by physical, behavioral and endocrine mechanisms (Dahlke et al., 2005). The main endocrine mechanism is the reduction of circulating thyroid hormones in the body. The levels of T3 and T4 in the blood of broilers were not influenced by the exposure to heat stress during the heat waves simulation (Tables VI and VII).

Birds raised under thermal comfort and heat stress conditions showed statistically similar results for T3 and T4 levels, different from results obtained by Urbano (2006), that found levels 34.4 and 36.1% higher, respectively for T3 and T4, in birds raised in a thermoneutral environment (22 °C) at 21 days of age, and by Tao et al. (2006) that confirmed the decrease of T3 and

**Table VII. Plasmatic levels of thyroxine (T4) (ng.mL<sup>-1</sup>) of broilers submitted to heat stress during the simulation of three heat waves (HW), started at 21, 35 and 42-d-old, respectively (Níveis plasmáticos de tiroxina (T4) (ng.mL<sup>-1</sup>) de frangos de corte submetidos ao estresse térmico durante a simulação de três ondas de calor (HW), iniciadas aos 21, 35 e 42 dias de idade, respectivamente).**

Treatments (T)	1st HW	2nd HW	3rd HW
Heat stress group	4.52	5.95	5.81
Control group	4.76	7.18	6.48
Stress Duration (SD)			
Start	5.02	6.83	5.16
After 24 hours	4.04	6.01	5.78
After 48 hours	5.20	6.19	5.79
After 72 hours	6.86	6.39	7.32
P-value			
Treatments	0.1641	0.7052	0.4171
Stress Duration	0.8267	0.3090	0.6722
Int. TxSD	0.1567	0.2186	0.3664

T4 levels (45.9 and 45.6%, respectively) in broilers after 24 hours of exposure to thermal stress (32 °C).

The decreased secretion of thyroid hormones is due to the characteristic thermogenic effect of this kind of hormone. According to Dahlke et al. (2005) in heat stress situations, with the hypothalamus heating occurs the reduction of thyroid activation which results in lower secretion of T4 and also T3, and birds have an increased tolerance to heat. The decreased secretion of thyroid hormones was not verified in this study. Other control mechanisms of body heat were effective to the dissipation of endogenous heat, and there was no influence by the heat stress on the thyroid gland activity.

## CONCLUSIONS

Submit birds to periods of acute heat stress, like during the simulation of heat waves, influences the broilers' respiratory frequency and rectal temperature, showing the consequent effect of heat stress on the birds' metabolism and body thermal balance. Heat waves don't affect the secretion of triiodothyronine and thyroxine, and the percentage of red blood cells.

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