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ORIGINAL PAPER

Impact of exposure time to harsh environments on physiology, mortality, and thermal comfort of day-old chickens in a simulated condition of transport

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Abstract

The aim of this study was to assess the variation of physiological responses and mortality of day-old chicks subjected to different thermal conditions and exposure times during simulated transport. For this purpose, day-old chicks ($n = 900$) were used and subjected to simulated conditions of transport in a climate chamber. The experimental design was a completely randomized block design, with the structure of the treatments in a 3 × 3 factorial scheme (thermal ranges and time intervals) and each level of containers considered a block. The physiological variables used in this trial were body weight, respiratory rate, cloacal temperature, average surface temperature, and gene expression of heat shock protein (HSP70). Regarding body weight, a small variation was observed between treatments ($P > 0.05$). The animals subjected to the heat treatment exhibited respiratory rates above 100 movements per minute ($P < 0.05$), average cloacal temperatures above 44.7 °C, surface temperatures above the comfort zone (greater than 39.6 °C; $P < 0.05$), and increased gene expression of HSP70 ($P < 0.001$), especially after 3 initial hours of exposure. In addition, the heat treatment lead to increased mortality of the animals (over 6%). Also in the cold treatment, despite the absence of mortality, the animals showed hypothermia from 3 h of exposure, based on the results of the average surface (28 °C) and cloacal temperatures (39.6 °C; $P < 0.05$). In this way, the results imply that the effects of thermal stress caused by heat as well as by cold in a simulated transport condition are increased when traveling for more than 3 h, indicating a trend of rising mortality after long-term transportation of day-old chickens.

Keywords Animal biometeorology · Poultry production · Transport of chicks · Physiological responses · HSP70

Introduction

Thermal conditions during transport to farms are considered a challenge to the thermal comfort of day-old chicks. Trucks that transport the animals have great thermal heterogeneity (Nazareno et al. 2015a, b), and this is unfavorable to the birds' comfort because newly hatched chicks do not have effective thermoregulatory systems (Mujahid and Furuse 2009a). Therefore, the maintenance of body temperature becomes dependent on the temperature of the environment. It has been indicated that the temperature supplied to the chicks should stay around 32–35 °C (Mujahid and Furuse 2009b; Nascimento et al. 2013) so that the comfort and health of the animals are preserved.

Day-old chickens kept at low temperatures have decreased live weight, and this is visible in the first week of life (Moraes et al. 2002). In addition, low temperatures can cause hypothermia, which is a triggering factor of pulmonary hypertension syndrome (ascites) in chickens (Wang et al. 2012; Zeng et al. 2016). Temperatures above the thermal comfort zone are also harmful to the animals. Heat stress may cause atrophy of lymphoid organs (Sahin et al. 2009), compromising the birds'

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immune systems. In addition, under heat the birds easily become dehydrated and changes in physiological parameters occur, such as increases in respiratory rate and in body temperature (Jacobs et al. 2017).

In relation to the molecular aspect, thermal stress has as its main result the accumulation of unfolded protein, which may generate incorrect interactions between molecules and great damage to the body (Staib et al. 2007). Therefore, after the exposure of cells to high temperatures, the expression of genes from the HSP (heat shock protein) family, which act as chaperones, generating proteostasis and thermal tolerance (Sahin et al. 2012). Among the HSPs, HSP70 is one of the more common proteins conserved and expressed during the period of stress (Roushdy et al. 2018).

Despite this, little is comprehensively known about the ideal conditions in relation to the commercial transport of young birds (Nazareno et al. 2015a). Few studies have investigated the heat exchanges of day-old chicks in conditions of transportation. Our hypothesis is that with increasing temperature and travel time in a simulated transport, the birds will exhibit changes in respiratory rate, average surface, and cloacal temperatures, as well as in the gene expression of HSP70, reduced body weight, and increased mortality. With this hypothesis, the aim of this study was to assess the variation of physiological responses and mortality of day-old chicks subjected to different thermal conditions and exposure times during simulated transport.

Methods

All procedures performed in this study were consistent with the Brazilian legislation on animal welfare and met the protocol requirements of animal research of the Institutional Animal Care and Use Committee of the University of São Paulo.

For the development of this research, day-old chicks (n = 900) from a local commercial hatchery were used. The animals belonged to the Cobb 500 strain, were properly vaccinated and sexed, and originated from 41-week-old arrays. The incubated eggs derived from the same batch of arrays, and were kept in the same incubator and hatcher in the hatchery. The birds were subjected to the treatments in a climate chamber simulating the thermal environment of a transport truck. Three evaluations were carried out, according to the thermal comfort and heat ranges described by Gustin (2003) and Lin et al. (2005) (Table 1).

In each treatment, the animals were exposed to three breaks of exposure time (0, 3, and 6 h), according to Dionello et al. (2002) and Shinder et al. (2007), and were kept in three containers (at different locations in a stack with five containers; Fig. 1a). Three-hundred chicks were used in each treatment, distributed in three stacked containers (top, middle, and bottom), totaling 100 birds per box (Fig. 1b), simulating what occurs in the transport truck.

Table 1 Thermal comfort and stress ranges used in the experiments

Thermal ranges	Temperature (°C)	Relative humidity (%)
Heat stress	38	75
Thermal comfort	35	60
Cold stress	20	75

To simulate the vibration of a truck's load, the containers were kept in a Marconi ® brand micromixers during exposure to the thermal environment. The acceleration adopted in the machine during all experiments was around 10 m s^{-2}, according to previous studies by Donofre et al. (2014).

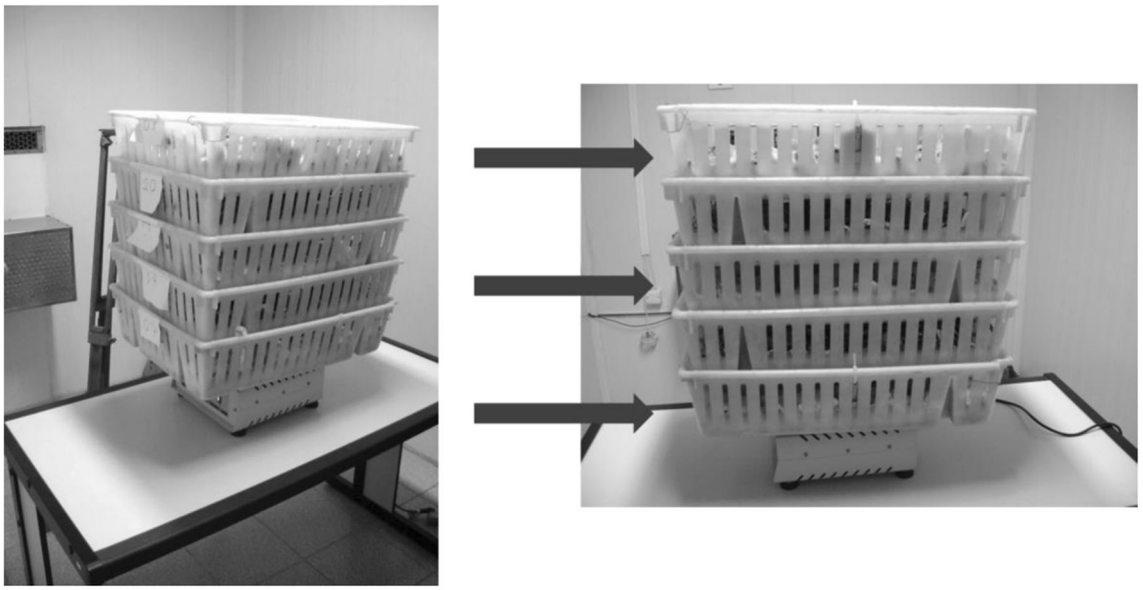
For the physiological assessment, measurements in 300 chicks were taken up to 10 min after exposure to the heat treatment of 0, 3, and 6 h. These measurements of the variables were taken in the same order each time so as not to influence the physiological responses, as described next: respiratory rate, weight, surface temperature, and cloacal temperature. The respiratory rate (RR; mov min^{-1}) was evaluated by counting the number of abdominal movements carried out by the chicks for 15 s, which was subsequently multiplied by 4 to obtain the measure in movements per minute. Body weight was monitored using a semi-analytical scale, with 0.1-g precision. Cloacal temperature (CT; °C) was measured with a type-T thermometer introduced in each chick's cloaca until its stabilization to obtain the temperature value. Surface temperature (°C) was assessed by measuring the temperature of the wing, head, leg, and back of each day-old chick using an infrared thermometer. From these points, it was possible to calculate the average surface temperature (MST; °C) through the equation proposed by Nascimento (2010) for broilers of the Cobb strain of 1 week old or less:

$$\mathrm{MST} = 3.47 + 0.11\mathrm{Tw} + 0.10\mathrm{Th} + 0.15\mathrm{Tl} + 0.56\mathrm{Tb}$$

where Tw is the temperature of the wing, Th is the temperature of the head, TL is the temperature of the leg, and Tb is the temperature of the back.

Mortality (%) was calculated as a descriptive analysis, through the number of dead birds in relation to the total of birds during the experiment and 1 day after the test, to verify the retrospective effects of the combination of treatments. This data was obtained separately for each treatment. To verify the mortality of the day after, the animals were moved to the farm in protection circles with heating, food and water ad libitum.

To confirm the answers obtained through other physiological variables, the gene expression of the HSP70 protein was studied. Various laboratory procedures were carried out to qualify this expression. For the analysis of gene expression in the treatment of thermal stress by heat, liver samples were



(a) (b)

Fig. 1 Containers stacked on the micromixer in the climate chamber (**a**) and arrangement of containers with chicks interspersed with empty containers in experiment (**b**)

extracted from five birds per treatment, totaling 45 chicks per experiment (thermal comfort and stress). The samples were immediately frozen in liquid nitrogen and stored at − 80 °C for subsequent quantification of the expression. Total RNA was isolated from the tissues according to the methodology described by Chomczynski and Sacchi (1987). The cDNA synthesis was carried out with the ImProm-II Reverse Transcription System (Promega) Kit, following the manufacturer's instructions.

Analysis of the expression of gene HSP70 and of the endogenous control genes (EEF and MRPS27) was carried out using the real-time quantitative RT-PCR methodology (qRT-PCR), in a LightCycler 480 (Roche). Each reaction was prepared with 2 μL cDNA in the dilution 1:10, 5.0 μL of SYBR Green I Master 2X, and 0.25 μL of each primer (10 μM μL^{-1}; Table 2) in a final volume corresponding to 10 μL. The conditions of the reaction for all pairs of primers were 95 °C incubation for 2 min; 40 cycles of 95 °C incubation for 15 s, 59 °C for 20 s, and 72 °C for 15 s; and final extension of 72 °C incubation for 5 s. Then, a PCR was carried out and 1.5% agarose gel was applied for visualization of the amplified product.

The analysis of the melting temperature for each of the genes was carried out using the LightCycler 480's software (Roche), allowing for verification of the reaction quality, as well as the absence of contaminants or the generation of non-specific products. The melting temperature indicates the dissociation of the double DNA strand with increasing temperature, and the product of the reaction is considered to be pure when only one peak or temperature of melting is raised. At the end of the reaction, the melting curve was determined to be between 75 and 95 °C for each gene. In this evaluation, the HSP70 protein gene exhibited specific amplification at 86 °C.

Gene expression results were generated and registered as Ct (cycle threshold) values. Ct refers to any PCR cycle in which the sample reached fluorescence above the initial level. That is, the higher the gene expression is, the earlier detection through fluorescence will occur and the lower the Ct value will be (Pfaffl 2001; Marchesin 2008). With this method, the measure evaluated through the mixed models was the Ct of HSP70's

Table 2 Gene, identification in the database, sequence of primers designed, and size of the flanked region

Gene	GenBank ID	Primer	Size (pb)
HSP70	423504	Sense: 5′AAGCGTAACACCACCATTCC3′ Anti-sense: 5′GCCCTCTCACCTTCATACACC 3′	101
EEF	373963	Sense: 5′TTCCACTGAGCCACCTTACA3′ Anti-sense: 5′GGTAACCTTCCATCCCTTGA3′	184
MRPS27	427216	Sense: 5′GCTTGTTCTTGCTCCCACTC3′ Anti-sense: 5′TAACCCTGACCACCCAACTC3′	102

gene expression. These values were analyzed using the REST 2008 program (Relative Expression Software Tool 2008).

Before the normalization of data, all Cts were corrected for 2.0 efficiency for subsequent correction of the Ct value of the target gene, using the geometric mean of constituent genes MRPS27 and EEF1. For the real-time PCR to be reliable and reproducible, the amplifications must be near 100% efficiency at each reaction cycle. This value ranges from 1.0 (minimum value) to 2.0 (maximum and optimal value), indicating the duplication of the region of interest at each amplification cycle and corresponding to 100% efficiency (Meijerink et al. 2001; Pfaffl 2001). In this study, the genes showed an efficiency value of 1.99. This process was done to correct possible variations in the data measured through the quantitative RT-PCR technique and generated during the extraction of total RNA, cDNA synthesis, or quantification (Ninov 2010).

A descriptive analysis was performed using means and standard deviations for the factors assessed, for visualizing the dispersion and central trend of the measures studied. A percentage approach was used for mortality data. The plot structure used in this work was completely randomized block design, with structure of the treatments in a 3 × 3 factorial scheme (thermal ranges and time intervals) and each level of containers considered a block. For the confirmatory statistical analysis, an analysis of variance followed by Tukey's test for multiple comparison of averages was conducted to find possible differences between the treatments in relation to body weight, respiratory rate, cloacal temperature, average surface temperature, and gene expression of protein HSP70. These analyses were performed using the SAS 9.2 statistical software (SAS Institute 2009).

Results

In Table 3, it is possible to observe the variation trend of the factors among the treatments throughout the duration of exposure, with the exception of the comfort treatment, which remained constant among the time intervals for most variables. In addition, the body weight of the day-old chicks varied very little.

During the heat treatment, there was significant increase in the first 3 h of stress for the respiratory rate, average surface temperature, and cloacal temperature variables. In the following 3 h there was stabilization of the animals' stress; however, the values of the factors involved in thermoregulation remained high. In the comfort treatment, despite the low expected variability over time, there was a change in the initial 3 h, a difference of about 1.5 °C in the cloacal temperature having been observed between 0 and 3 h. In the cold stress treatment, the greatest variation occurred in average surface temperature, which resulted in a 7.1 °C difference between time intervals 0 and 3.

Figure 2 illustrates the variation in cloacal temperatures among the different treatments and highlights the sharp increase after 3 h during the heat treatment, followed by the situation of comfort.

Even in the situation of comfort, cloacal temperature increased by approximately 1 °C, followed by stabilization of the curve after 6 h. During the cold treatment, cloacal temperature reduced about 1.7 °C below the comfort threshold (41.1 °C). In the heat, the birds exceeded the comfort threshold by 3 °C, which indicates a possible case of acute hyperthermia, from which significant increase in mortality was observed (Table 4).

In the heat stress treatment, 49 deaths were recorded in all, which corresponds to the highest concentrated number after 3 h of exposure to the thermal environment.

According to the analysis of variance for the variable respiratory rate, there was interaction between the heat treatments and time intervals ($P < 0.05$). Through the result of the interaction it may be noted that, in the heat, there was significant increase in respiratory rates after 3 h, followed by a sharp reduction, while in the cold, the rate remained constant after 3 and 6 h (Table 5).

Table 3 Mean and standard deviation for the variables: respiratory rate (RR), weight, mean surface temperature (MST), and cloacal temperature (CT) in relation to thermal treatments and exposure time

Ranges	Time (h)	Variables			
		RR (mov. min^{-1})	Weight (g)	MST (°C)	CT (°C)
Heat	0	50 ± 5	48.1 ± 2.6	34.7 ± 0.6	40.9 ± 0.5
	3	150 ± 12	47.8 ± 4.9	39.7 ± 0.5	44.7 ± 0.4
	6	107 ± 20	45.4 ± 3.3	39.5 ± 0.5	43.9 ± 0.6
Comfort	0	53 ± 5	46.5 ± 2.6	34.7 ± 0.7	41.3 ± 0.5
	3	54 ± 6	44.5 ± 4.5	37.6 ± 0.6	42.7 ± 0.6
	6	54 ± 7	45.1 ± 3.2	37.6 ± 0.5	42.7 ± 0.8
Cold	0	48 ± 3	45.7 ± 3.2	35.1 ± 0.5	41.4 ± 0.3
	3	38 ± 5	46.8 ± 3.3	28.0 ± 1.0	39.6 ± 0.8
	6	39 ± 5	45.5 ± 3.8	27.5 ± 0.9	39.4 ± 0.3

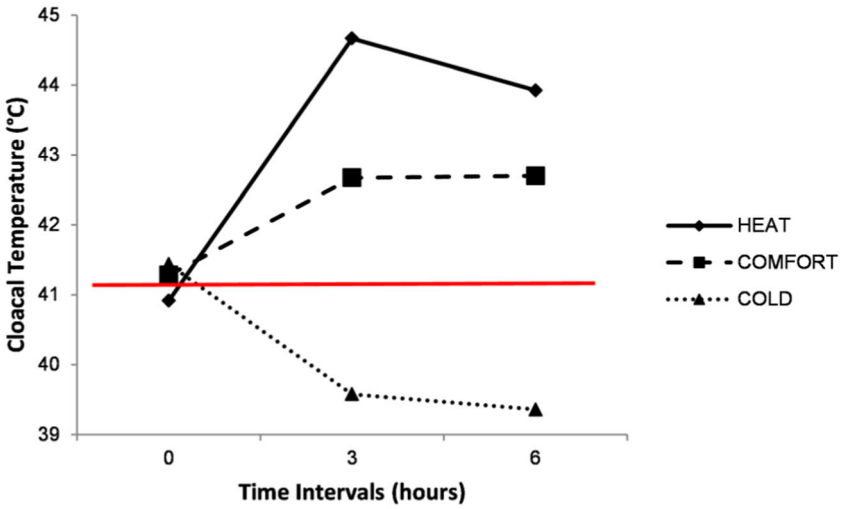


Fig. 2 Variations in cloacal temperature in relation to thermal treatments and exposure time intervals. Average value of comfort represented by the red line

In relation to the animals' body weight, there was no significant adjustment of the analysis of variance for this variable ($P > 0.05$), with little variability among treatments.

According to the analysis of variance adjusted to the average surface temperature, there was interaction between thermal ranges and time intervals ($P < 0.05$). With respect to the result of the interaction shown in Table 6, it is possible to note a significant change in stress after 3 h for all thermal ranges. However, the change between time intervals 3 and 6 was not significant. Despite the difference between time 0 and the other times in the position of comfort, the increase was mild compared to the cold treatment, which resulted in the greatest difference between the control situation and 3 h after exposure to the environment.

According to the analysis of variance for cloacal temperature, interaction between thermal ranges and time intervals was observed ($P > 0.05$). Through the result of the interaction between thermal treatments and time intervals in relation to cloacal temperature, it was found that there was significant difference between containers in all treatments. During the cold stress and comfort treatments, no differences between time intervals 3 and 6 were registered (Table 7).

In relation to the analysis of variance of HSP70's gene expression held in the comfort and heat stress experiments, it was possible to observe that there was an increase in the expression of gene HSP70 during the exposure time. In the thermal comfort treatment, there was a suggestive effect of time on Ct ($P < 0.1$), and in the thermal stress treatment there was a highly significative effect ($P < 0.05$) on the increase of gene expression.

When performing the Tukey-Kramer test within the time variable for the heat stress treatment, it was possible to observe that there was a significant difference in time intervals 3 and 6 in relation to time 0. However, there was no difference between time intervals 3 and 6 themselves (Table 8).

Table 4 Number of dead chicks (% per container) over time in the respective containers for each heat treatment performed in the climate chamber

Ranges	Heat			Comfort			Cold		
Time (h)	0	3	6	0	3	6	0	3	6
Container 1	0	1 (1%)	0	0	0	0	0	0	0
Container 2	0	9 (9%)	5 (6%)	0	0	0	0	0	0
Container 3	0	18 (18%)	16 (20%)	0	1 (1%)	0	0	0	0

Discussion

The respiratory rate of the birds is subject to more changes when they face a situation of heat stress, which forces the mechanism of heat loss through respiratory evaporation. However, according to the increase in the intensity of stress in relation to time, high respiratory rate ceases to be an efficient process due to its inability to keep the internal temperature within the physiological limits of stress long enough.

According to Yahav (2015), as the internal temperature of the bird rises, the respiratory rate also increases until it reaches

Table 5 Mean values of the interaction between time intervals and thermal ranges in relation to respiratory rate (mov min^{-1}) of day-old chicks

Time intervals	Thermal ranges		
	Heat	Comfort	Cold
0	50 aC	51 aA	48 aA
3	150 aA	54 bA	38 cB
6	107 aB	54 bA	39 cB

Means with different letters (uppercase in the columns and lowercase in the rows) differ among themselves by Tukey's test ($P < 0.05$)

Table 6 Mean values of the interaction between time intervals and thermal ranges in relation to the mean surface temperature (°C) of day-old chicks

Time intervals	Thermal ranges		
	Heat	Comfort	Cold
0	34.7 aB	34.7 aB	35.0 aA
3	39.6 aA	37.6 bA	28.0 cB
6	39.4 aA	37.6 bA	27.5 cB

Means with different letters (uppercase in the columns and lowercase in the rows) differ among themselves by Tukey's test ($P < 0.05$)

a peak, from which there is a decline. For this reason, in the heat treatment, the respiratory rate peaked after 3 h; however, after 6 h, this variable declined. According to the same authors, this sudden reduction is an effect of respiratory alkalosis caused by hypocapnia (reduction in the blood level of carbon dioxide) that causes vasoconstriction and reduced oxygen levels in the blood (hypoxia).

The body's response to it is the reduction of lung hyperventilation caused by high respiratory rate, followed by increased mortality due to the irreversibility of the process of acid-base imbalance. However, despite being the treatment with the highest mortality rates when compared with the cold and comfort treatments, most of the animals survived heat treatment. However, there is another possible explanation for this change in respiratory rate related to acclimatization, which will be discussed further through the analysis of cloacal temperature.

In the cold treatment, there was a decrease in respiratory rate after 3 h of exposure, the normal values for the species and age group being 48 mov min^{-1} according to Nascimento et al. (2012). The possible explanation for this is that neonatal chicks exposed to low room temperatures (20 °C) demonstrate a state of hypothermia, thus reducing their activity (Mujahid and Furuse 2009b). According to these authors, the chicks adopt, in cold environmental conditions, sleep posture (standing still with eyes closed). This can reduce the production of heat, making them even more sensitive to the cold.

Table 7 Mean values of the interaction between time intervals and thermal ranges in relation to the cloacal temperature (°C) of day-old chicks

Time intervals	Thermal ranges		
	Heat	Comfort	Cold
0	40.9 aC	41.3 aB	41.4 aA
3	44.7 aA	42.7 bA	39.6 cB
6	43.9 aB	42.7 bA	39.4 cB

Means with different letters (uppercase in the columns and lowercase in the rows) differ among themselves by Tukey's test ($P < 0.05$)

During the situation of cold, the surface temperature of the areas of the birds without thermal insulation decreases with the reduction in room temperature (Mujahid and Furuse 2009a). This explains the rapid decline between time intervals 0 and 3 highlighted in Table 5. However, the non-significative effect between time intervals 3 and 6 can be explained by the animals' behavior of huddling together, which substantially reduces the sensitive loss of heat to the environment. This increases the chances of maintaining the interior temperature of the animal's body at homeostasis levels.

In addition, the cold treatment resulted in the greatest surface temperature difference between the control situation and 3 h after exposure to the environment. This was due to these birds' thermoregulation capacity being ineffective. Also, day-old chicks are susceptible to losing heat to colder environments (Zeng et al. 2016).

In the heat, with the temperature rise, the birds try to dissipate thermal energy through sensitive means (conduction, convection, and radiation). However, when the room temperature approaches the birds' body temperature, which in this situation is between 41 and 43.5 °C, the efficiency of the sensitive means of heat exchange is reduced, and with it, the birds resort to the respiratory evaporation cooling mechanism of a high respiratory rate (Mujahid et al. 2007). This explains the stabilization of surface temperature between 3 and 6 h in this study. In addition, studies with chicks subjected to high temperatures tend to keep their surface temperatures similar to room temperature (Nazareno et al. 2016).

The variation in cloacal temperature was very similar to the average surface temperature, which was expected because the indicators of thermoregulatory mechanisms in the body vary according to internal temperature.

In relation to the heat treatment, cloacal temperature reached 44 °C after 6 h of exposure, indicating severe stress levels for these animals. In the cold, little change was observed over time.

In simulated transport conditions, Silva et al. (2007) reported increased mortality from a cloacal temperature of 46.3 °C. As the day-old chicks do not have developed thermoregulatory mechanisms, they become more vulnerable to thermal stress, easily surpassing the irreversibility zone. This in turn leads to increased mortality, found in the heat treatment, which had the highest rates.

Another important effect observed in the heat treatment between time intervals 3 and 6 refers to the reduction in cloacal temperature for the animals evaluated. According to Yahav (2015), acclimatization is the modification of physiological responses through induction for the control of specific bioclimatic factors. The same authors showed that this adaptive response to stress over time is characterized by an initial increase in body temperature, followed by a decline. In this way, the variations in cloacal temperature and respiratory rate can be explained by this adaptation of the chicks, as well as

Table 8 Tukey-Kramer's analysis to evaluate the difference between time intervals of heat exposure in the expression of gene HSP70

Comparison between time (h)		Estimate	Standard error	G.L.	*T* value	*P* value
0	3	5.19	1.12	38	4.62	0.0001
0	6	6.19	1.10	38	5.61	< 0.0001
3	6	1.00	1.10	38	0.91	0.6391

due to their being irreversible processes of hyperthermia associated with high mortality in this environment.

For the cold treatment, cloacal temperature decreased sharply between 0 and 3 h of exposure. Dahlke et al. (2005) found that chickens raised at temperatures below the thermal comfort zone had reduced cloacal temperatures. This is because the environment has a direct influence on the animals' body temperature, and chicks are susceptible to losing heat to cold environments through the sensitive loss of heat. In addition, chicks in their first days of life do not have an effective thermoregulatory system; that is, their thermal responses are not able to raise their body temperature. For this reason, according to Mujahid (2010), day-old chicks in the early stages can be considered poikilotherms; that is, their body temperature is largely dependent on the room temperature due to the immaturity of their thermoregulatory systems.

In the evaluation after 6 h of cold treatment, cloacal temperature remained low and similar to what was verified after 3 h. In this way, despite the animals having being subjected to thermal stress by cold, the physiological and behavioral changes were able to stabilize their body temperature after 6 h of exposure to this condition. Also, mortality rates were lower in this treatment than in the heat treatment, showing that for day-old chicks, the excess heat is more detrimental than cold.

As already reported, the chicks' behavior of huddling together is a factor that helps stabilize temperature after 6 h of exposure to the cold, since this behavior reduces heat exchanges with the environment.

Heat shock proteins (HSP) are used as physiological variables to measure the thermal condition of farm animals, i.e., to determine the animals' stress and comfort levels. These proteins are thus responsible for protecting the cells from damage caused by stress. Whenever the cells are subjected to high temperatures, there is rapid production of this class of proteins, with HSP70 having great importance in the cellular metabolism of stressed cells (Song et al. 2017).

The results of the samples in agarose gel (Fig. 3) of this study corroborate the results of Vinoth et al. (2015), the amplified product detected having been considered in a reasonable quantity for the analysis of the gene expression of HSP70.

In the heat stress group, when time intervals 3 and 6 were compared with time 0, a significant increase in the gene expression of HSP70 occurred. For this reason, it is possible to affirm that the heat environment increases the expression of this protein. Al-Zghould et al. (2015), according to whom the thermal manipulation of the environment increases the expression of HSP70 especially when thermal stress by heat is induced, also reported this.

Still in the heat treatment, the results are in line with the responses found through the respiratory rate, average surface temperature, and cloacal temperature. That is, between time intervals 3 and 6 there was no significant increase in gene expression, possibly due to a mechanism of adaptation to stress in the birds, as previously explained.

This piece of evidence contradicts the results obtained by Dionello et al. (2002), who concluded that there was no response in the HSP70 expression, representing a mechanism of adaptation to heat stress. However, the temperature ranges and exposure times used by the authors were different from those used in this study. Concomitant to this, Roushdy et al. (2018)

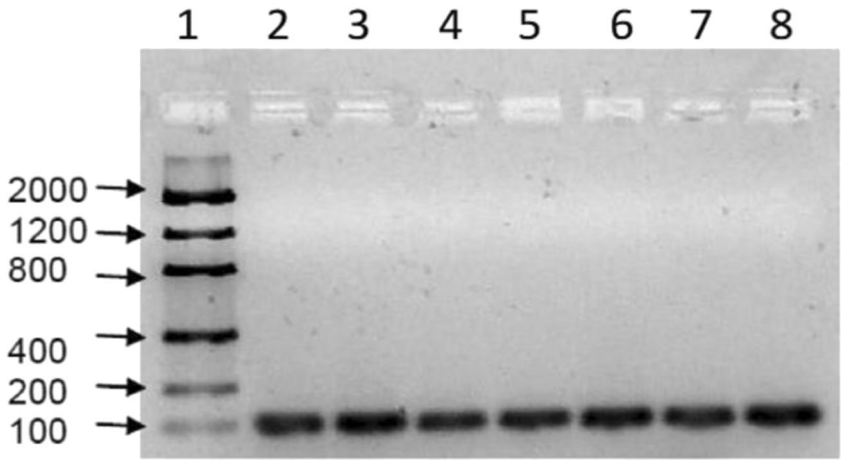


Fig. 3 The 1.5% agarose gel stained with GelRed. 1—Low DNA Mass Ladder Standard and 2 to 8—samples of amplified products of the HSP70 gene (101pb)

report that the role of HSP70 as a mechanism for the chicks to acquire thermotolerance or to adapt to a thermally manipulated environment is not fully understood. They also state that there are many contradictions associated with these mechanisms in the research conducted.

In the comfort treatment, the expression of gene HSP70 did not increase over the exposure time. That is to be expected, since this gene is expressed under adverse conditions of thermal stress by heat.

Typically, the concern with thermal stress caused by high temperatures is greater in the transportation of adult chickens, whereas the practical concern in the animals' first days of life is with lower temperatures. The results of this stage showed that the effects of thermal stress by heat are worrisome. It was also possible to see the influence of time, variations in the responses to this variable having occurred after 3 h of exposure.

Conclusion

In this way, the results imply that the effects of thermal stress caused by heat as well as by cold conditions in a simulated transport increase when traveling for more than 3 h, indicating a trend of rising mortality after long-term transportation of day-old chickens.

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Compliance with ethical standards

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the University of São Paulo.

References

Al-Zghould M-B, Dalab AES, Yahya IE, Althnaian TAA, Al-ramadan SY, Ali AM, Albokhadaim IF, El-Bahr AM, Busadah KAA, Hannon KM (2015) Thermal manipulation during broiler chicken embryogenesis: effect on mRNA expressions of Hsp108, Hsp70, Hsp47 and Hsf-3 during subsequent post-hatch thermal challenge. Res Vet Sci 103:211–217. https://doi.org/10.1016/j.rvsc.2015.10.015

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159. https://doi.org/10.1006/abio.1987.9999

Dahlke F, Gonzales E, Gadelha AC, Maiorka A, Borges AS, Rosa PS, Faria Filho DE, Furlan RL (2005) Empenamento, níveis hormonais de triiodotironina e tiroxina e temperatura corporal de frangos de corte de diferentes genótipos criados em diferentes condições de temperatura. Cienc Rural 35:664–667. https://doi.org/10.1590/S0103-84782005000300029(inPortuguese)

Dionello NJL, Macari M, Ferro JA, Rutz F, Ferro MIT, Furlan LR (2002) Respostas fisiológicas associadas à termotolerância em pintos de corte de duas linhagens por exposição a altas temperaturas. Rev Bras Zootec 31:79–85. https://doi.org/10.1590/S1516-35982002000100009 (in Portuguese)

Donofre AC, Silva IJO, Nazareno AC (2014) Vibrações mecânicas: Um agente estressor no transporte de pintos. Rev Bras Eng Agríc Ambient 18:454–458. https://doi.org/10.1590/S1415-43662014000400014 (in Portuguese)

Gustin PC (2003) Manejo dos pintos no incubatório, expedição, transporte e alojamento na granja. In: Macari M, Gonzales E (ed) Manejo da Incubação, FACTA, Jaboticabal, pp 537 (in Portuguese)

Jacobs L, Delezie E, Duchateau L, Goethals K, Ampe B, Buyse J, Tuyttens FAM (2017) Impact of transportation duration on stress responses in day-old chicks from young and old breeders. Res Vet Sci 112:172–176. https://doi.org/10.1016/j.rvsc.2017.04.015

Lin H, Zhang HF, Jiao HC, Zhao T, Sui SJ, Gu XH, Zhang ZY, Buyse J, Decuypere E (2005) Thermoregulation responses of broiler chickens to humidity at different ambient temperatures I one week of age. Poult Sci 84:1166–1172. https://doi.org/10.1093/ps/84.8.1173/2.

Marchesin ML (2008) Análise da expressão gênica de MyoD, MRF4, Miogenina e Miostatina nos músculos Bíceps femoris e Gastrocnemius lateralis em duas linhagens de *Gallus gallus* (corte e postura). Dissertation, Universidade Estadual Paulista "Júlio de Mesquita Filho" (in Portuguese)

Meijerink J, Mandigers C, Van de Locht L, Tonnissen E, Goodsai F, Raemaekers JA (2001) Novel method to compensate for diffent amplifications efficiencies between patient DNA samples in quantitative real-time PCR. J Mol Diagn 3:55–61. https://doi.org/10.1016/S1525-1578(10)60652-6.

Moraes VMB, Malheiros RD, Furlan RL, Bruno LDG, Malheiros EB, Macari M (2002) Effect of environmental temperature during the first week of brooding period on broiler chick body weight, viscera and bone development. Braz J Poultry Sci 4:1–8. https://doi.org/10.1590/S1516-635X2002000100003

Mujahid A, Pumford NR, Bottje W, Nakagawa K, Miyazawa T, Akiba Y, Toyomizu M (2007) Mitochondrial oxidative damage in chicken skeletal muscle induced by acute heat stress. J Poult Sci 44:439–445. https://doi.org/10.2141/jpsa.44.439

Mujahid A, Furuse M (2009a) Oxidative damage in different tissues of neonatal chicks exposed to low environmental temperature. Comp Biochem Physiol A Mol Integr Physiol 152:604–608. https://doi.org/10.1016/j.cbpa.2009.01.011

Mujahid A, Furuse M (2009b) Behavioral responses of neonatal chicks exposed to low environmental temperature. Poult Sci 88:917–922. https://doi.org/10.3382/ps.2008-00472

Mujahid A (2010) Acute cold-induced thermogenesis in neonatal chicks (*Gallus gallus*). Comp Biochem Physiol A Mol Integr Physiol 156:34–41. https://doi.org/10.1016/j.cbpa.2009.12.004

Nascimento ST (2010) Determinação do balanço de calor em frangos de corte por meio das temperaturas corporais. Dissertation, University of São Paulo (in Portuguese)

Nascimento ST, Silva IJO, Mourão GB, Castro AC (2012) Bands of respiratory rate and cloacal temperature for different broiler chicken strains. Rev Bras Zootec 41:1318–1324. https://doi.org/10.1590/S1516-35982012000500033

Nascimento ST, Silva IJO, Maia ASC, Castro AC, Vieira FMC (2013) Mean surface temperature prediction models for broiler chickens-a study of sensible heat flow. Int J Biometeorol 58:195–201. https://doi.org/10.1007/s00484-013-0702-7

Nazareno AC, Silva IJO, Vieira FMC, Santos RFS (2015a) One day-old chicks transport: assessment of thermal profile in a tropical region. Rev Bras Eng Agríc Ambient 19:663–667. https://doi.org/10.1590/1807-1929

Nazareno AC, Silva IJO, Vieira FMC, Santos RFS (2015b) Temperature mapping of trucks transporting fertile eggs and day-old chicks: efficiency and/or acclimatization? Rev Bras Eng Agríc Ambient 19:

134–139. https://doi.org/10.1590/1807-1929/agriambi.v19n2p134-139

Nazareno AC, Silva IJO, Donofre AC (2016) Thermal gradients of container and mean surface temperature of broiler chicks transported on different shipments. Eng agríc 36:581–592. https://doi.org/10.1590/1809-4430-Eng.Agric.v36n4p581-592/2016

Ninov N, Menezes-Cabral S, Prat-Rojo C, Manjón C, Weiss A, Pyrowolakis G, Affolter M, Martín-Blanco E (2010) Dpp signaling directs cell motility and invasiveness during epithelial morphogenesis. Curr Biol. 20(6): 513–520. https://doi.org/10.1016/j.cub.2010.01.063

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time PCR. Nucleic Acids Res 29:45

Roushdy EM, Zaglool AW, El-Tarabany MS (2018) Effects of chronic thermal stress on growth performance, carcass traits, antioxidant indices and the expression of HSP70, growth hormone and superoxide dismutase genes in two broiler strains. J Therm Biol 74:337–343. https://doi.org/10.1016/j.jtherbio.2018.04.009

Sahin K, Sahin N, Kucuk O, Hayirli A, Prasad A (2009) Role of dietary zinc in heat-stressed poultry: a review. Poult Sci 88:2176–2183. https://doi.org/10.3382/ps.2008-00560

Sahin K, Orhan C, Akdemir F, Tuzcu M, Iben C, Sahin N (2012) Resveratrol protects quail hepatocytes against heat stress: modulation of the Nrf2 transcription factor and heat shock proteins. J Anim Physiol Anim Nutr 96:66–74. https://doi.org/10.1111/j.1439-0396.2010.01123.x

SAS Institute (2009) SAS 9.2. SAS Institute Inc, Cary

Shinder D, Rusal M, Tanny J, Druyan S, Yahav S (2007) Thermoregulatory responses of chicks (*Gallus domesticus*) to low ambient temperatures at an early age. Poult Sci 86:2200–2209. https://doi.org/10.1093/ps/86.10.2200.

Silva MAN, Barbosa Filho JAD, Silva CJM, Rosário MF, Silva IJO, Coelho AAD, Savino VJM (2007) Avaliação do estresse térmico em condição simulada de transporte de frangos de corte. Rev Bras Zootec 36:1126–1130. https://doi.org/10.1590/S1516-35982007000500020 (in Portuguese)

Song Z, Cheng K, Zhang L, Wang T (2017) Dietary supplementation of enzymatically treated *Artemisia annua* could alleviate the intestinal inflammatory response in heat-stressed broilers. J Therm Biol 69: 184–190. https://doi.org/10.1016/j.jtherbio.2017.07.015

Staib JL, Quindry JC, French JP, Criswell DS, Powers SK (2007) Increased temperature, not cardiac load, activates heat shock transcription factor 1 and heat shock protein 72 expression in the heart. Am J Phys Regul Integr Comp Phys 292(1):R432–R439. https://doi.org/10.1152/ajpregu.00895.2005

Vinoth A, Thirunalasundari T, Tharian JA, Shanmugam M, Rajkumar U (2015) Effect of thermal manipulation during embryogenesis on liver heat shock protein expression in chronic heat stressed colored broiler chickens. J Therm Biol 53:162–171. https://doi.org/10.1016/j.jtherbio.2015.10.010

Wang Y, Guo Y, Ning D, Peng Y, Cai H, Tan J, Yang Y, Liu D (2012) Changes of hepatic biochemical parameters and proteomics in broilers with cold-induced ascites. J Anim Sci Technol 3:41. https://doi.org/10.1186/2049-1891-3-41

Yahav S (2015) Regulation of body temperature: strategies and mechanisms. In: Scanes C (ed) Sturkie's avian physiology. Academic Press, London, p 869

Zeng Q, Yang X, Zeng P, Zhang K, Luo Y, Ding X, Bai S, Wang J, Xuan Y, Su Z (2016) Effects of low ambient temperatures and dietary vitamin C supplementation on pulmonary vascular remodeling and hypoxic gene expression of 21-d-old broilers. J Integr Agric 15:183–190. https://doi.org/10.1016/S2095-3119(14)60968-0