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## Research Article

# Simultaneous Occurrence of Broiler Chicken Hyper and Hypothermia In-transit and Lairage and Dead on Arrival (DOA) Index Under Tropical Climate

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## Abstract

**Background:** The pre-slaughter management conditions and transport of poultry from the farm to the slaughterhouse could represent significant economic losses compromising the animal welfare affecting consequently the meat quality if the good practices are not followed. Moreover, studies on broiler chicken journey under Brazilian tropical climate zone are scarce. **Objective:** The objective of this study was therefore to evaluate the influence of broiler microclimates formed in a lorry container during journey from farm to the slaughterhouse associated to lairage under commercial conditions on breast-meat quality in a tropical environment. Also, DOA index, dead on arrival was determined. **Material and Methods:** The broilers of 47 day-old ( $n = 1,400$ ) were monitored from farm to slaughterhouse over a distance of 289 km under rainy conditions and 5 h of lairage. Anemometers were placed at 3 lorry container positions: Front, middle and rear to measure Temperature (T), Air Velocity (AV), Relative Humidity (RH) and Heat Index (HI) throughout the journey and lairage. Hyper and hypothermia were evaluated by determining the occurrence of PSE (pale, soft and exudative) and DFD (dark, firm and dry) meat. The mortality of chickens was quantified at the time the birds were hung on the receiving platform. The Tukey's test ( $p \leq 0.05$ ) was applied to compare means of micro-environmental data, PSE, DFD and DOA at 3 different lorry container positions. **Results:** The recorded AV was highest in the front and the other variables also altered depending on the container compartments location. The RH values were also the highest at the rear and lowest at the front throughout the experiment. The rainfall occurred in every journey, which might have influenced the variables, as the lorries had open roofs. The results were 10.14 and 3.59%, PSE and DFD, respectively and the DOA index was 0.45%. **Conclusion:** The presence of PSE and DFD meat samples indicated that the birds were micro-environmentally exposed to heat and cold stress, respectively, leading to a relatively high DOA index. All of these factors are controllable and it is advisable to encourage poultry-processing plant companies to follow good pre-slaughter management practices in order to preserve animal welfare and thus the meat quality.

**Key words:** Animal welfare, DFD meat, microclimate profile, PSE meat

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Brazil ranks as the world's largest exporter (4.304 million tons) and the second-largest producer of broiler chicken meat (13.146 million tons), after the USA (17.966 million tons). From the total quantity of chicken produced, 67.3% was designated for domestic consumption and 32.7% for export, according to the ABPA<sup>1</sup>. This position has been made possible by the sophisticated technology applied throughout the broiler meat production chain. However, the poultry industry in Brazil involves several factors that can be detrimental to maintaining the meat quality, especially in tropical and subtropical zones under several variety of weather conditions faced during transport. Appropriate management systems are necessary to be adopted in order to promote the welfare of these birds preventing stressful conditions which are able to the development of meat-colour abnormalities therefore becoming a major problem in the poultry industry<sup>2-5</sup> leading eventually to dead on arrival (DOA)<sup>6,7</sup>.

The PSE (pale, soft and exudative) meat is caused by a rapid decline in pH while the muscle is still warm during the completion of glycolysis promoting a denaturation of myofibril proteins and compromising their functional properties. The PSE meat is pale in colour, soft and exudative<sup>3,7</sup> while DFD (dark, firm and dry) defect results from stress prior to slaughter that causes depletion of muscle glycogen<sup>4</sup>. This phenomenon results in higher post-mortem muscle pH because full glycolysis is prevented through elimination of its substrate. The DFD meat is dark in colour, firm in texture and has a dry appearance or high water-holding capacity<sup>8</sup>.

The PSE meat is estimated to generate costs in the poultry industry of over US \$200 million in the USA<sup>9</sup> and over US \$55 million in Brazil<sup>7</sup>. Detailed evaluations by our own research group in a subtropical climate in Southern Brazil have revealed that with few exceptions, every management activity step in the production of broiler meat presents a potential for thermal or physiological stress to the birds. Stress was observed on the farm in the housing systems<sup>5</sup>, in the water shower before leaving the farm<sup>2</sup>, in transit<sup>3,4,6,7</sup>, at the processing plant during lairage under nebulization<sup>2</sup> and finally during and after slaughter, including the carcass management<sup>8,10</sup>.

Stress is a common term for a set of physiological reactions to situational physical, psychological or other types of stimuli capable of disturbing homeostasis<sup>11</sup>. Thermal stress is one of the most important of these factors and there is evidence that the more intense the thermal stress is, the higher the DOA index. Transportation conditions thereby influence the development of PSE abnormalities and thus the

DOA index<sup>6</sup>. Moreover, appropriate management systems are needed to promote poultry welfare and thus preventing the heat stress that leads to the development of meat-colour abnormalities in PSE meat<sup>2,4</sup>. This point is particularly important because the Brazilian North-eastern region, located within a tropical zone, has only dry and rainy seasons thus resulting in a unique problem for animal welfare therefore for meat quality. In lorries in which the birds receive less ventilation and/or longer exposure to harmful conditions during transport, the animals show signs of stress, as seen in the increase in PSE meat<sup>3,6,7</sup>.

The DOA index obviously measures directly the animal welfare and most studies focused on the effect of one particular processing step, such as catching<sup>12,13</sup>, transport<sup>14,15</sup>, on-farm characteristics<sup>16,17</sup> or even the effect of the season<sup>18</sup>.

In this study, the effect of in-transit and lairage periods under commercial conditions over broiler breast meat quality and DOA index in geographical tropical zone as the North-eastern region in Brazil during the rainy season.

## MATERIALS AND METHODS

**Commercial farm location:** This experiment was carried out in Brazilian North-eastern region. The broiler industry is a cooperative integrated system located around Guarabira Municipality, Paraíba State, subjected to Federal Veterinary inspection. The farm is located in Touros municipality, Rio Grande do Norte State, within 289 km of the processing plant, or approximately 6 h driving time. Figure 1 shows the map of the region with the main motorway transport route. The experiments were carried out during the rainy season (from May-July) in May 2015 to observe the effect of transportation conditions from the farm to the processing plant (Fig. 2) on the bird's welfare thereby on meat quality. Birds were handled in accordance with the principles and procedures outlined by the Londrina State University Animal Care and Use Ethical Committee (No. 021/2014).

**Assessment of container microenvironments:** A total of 13,440 broiler chickens was transported in three lorries and 1,400 birds were separated for analysis from each vehicle. Six portable weather meters with bidirectional Kestrel anemometers and data logging were placed in the container front, middle and rear positions to take measurements of Temperature (T), Air Velocity (AV), Relative Humidity (RH) and Heat Index (HI) at 30 sec intervals throughout the journey<sup>7</sup> as shown in Fig. 3. The weather conditions in this region were characterized by an average temperature of 25.5°C and a relative humidity of 80.43%, as measured using

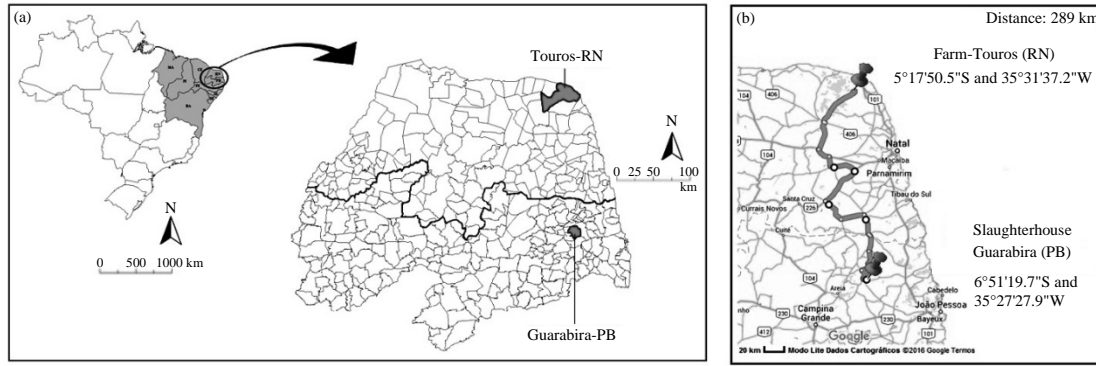


Fig. 1(a-b): (a) Map of tropical North-eastern Brazil, Paraiba (PB) and Rio Grande do Norte (RN) States, where the experiment was carried out and (b) The main highway on which the birds were transported over a distance of 289 km from the farm to the commercial processing plant

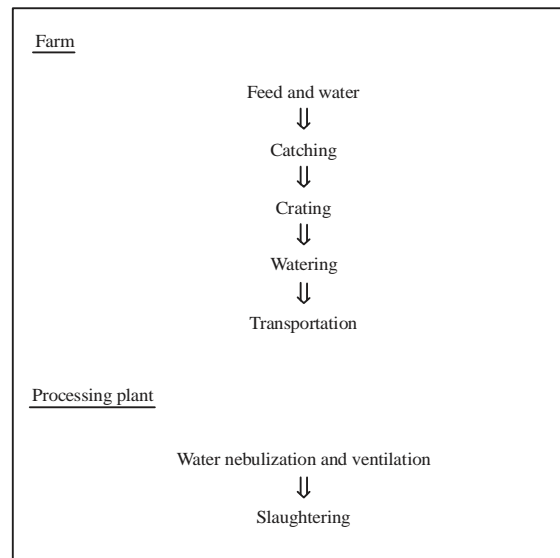


Fig. 2: Flowchart of broiler handling for transportation from farm to processing plant<sup>4</sup>

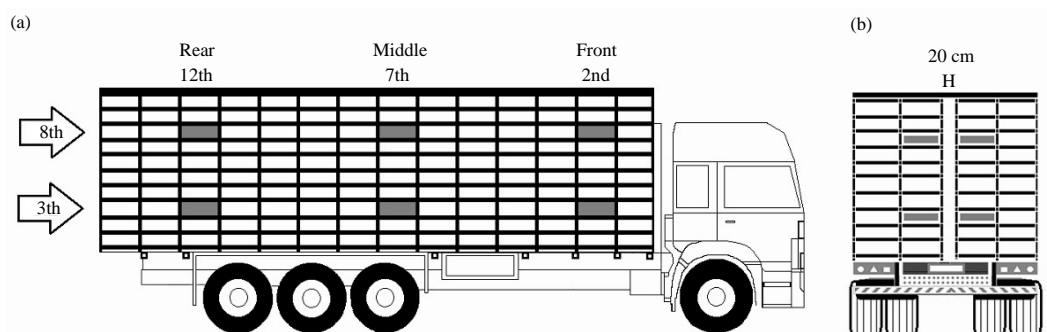


Fig. 3(a-b): Side and back views of the lorry container. (a) Side view showing the positions of the data loggers (dark-grey crates): Horizontally at 1.5, 5.4 and 9.2 m (2nd, 7th and 12th crate columns, respectively), from the front and vertically at 0.84 and 2.24 m (3rd and 8th crate, respectively) from the lorry floor and (b) Back view showing the position of data loggers (dark grey) in the two central lanes and a 20 cm empty space<sup>7</sup>

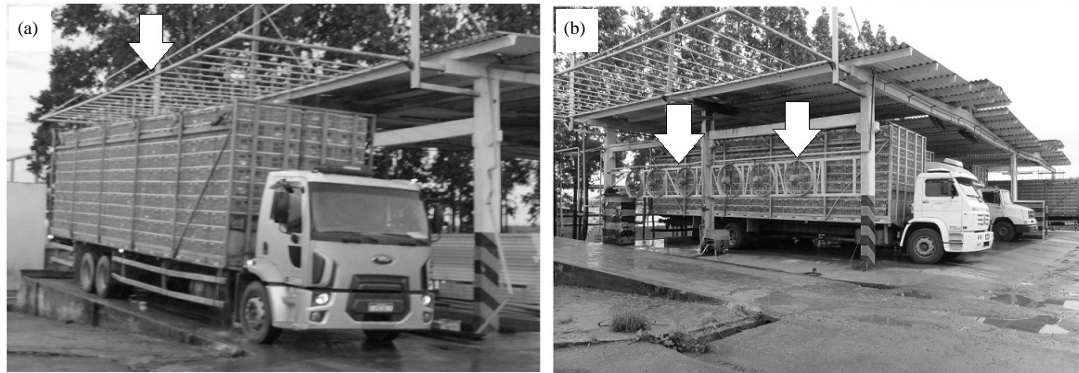


Fig. 4(a-b): (a) Birds under a 5 min shower just after arrival at the commercial precinct slaughterhouse and processing plant and (b) Birds under forced ventilation

a Kestrel 4000 series weather and environmental meters instrument (Nielsen Kellerman Chester, PA). Moreover, the rain fell during most of the three journeys and the experiment was conducted at night (from 00:00 to 06:00 h) and treated with a bath shower just before leaving the farm.

Upon arrival at the slaughterhouse, the animals were left under nebulization for approximately 5 min (Fig. 4a) and left for 300 min as lairage under forced ventilation before sacrificing (Fig. 4b). The birds were slaughtered according to the standard industry practices which consisted in sequence on hanging, electrically stunning, bleeding, scalding, defeathering, evisceration, water chilling and deboning<sup>7</sup>. Subsequently, the breast meat (pectoralis major muscle) samples were collected and pH, colour and water holding capacity were measured for PSE, DFD and normal samples classification after 24 h post-mortem and stored at  $2 \pm 2^\circ\text{C}$ .

**PSE, DFD and normal meat samples classification:** The meat samples were classified according to pH and  $L^*$  values based by Soares *et al.*<sup>19</sup> and Langer *et al.*<sup>4</sup> as PSE meat with  $\text{pH} < 5.8$  and  $L^* \geq 51.0$ , DFD meat with  $\text{pH} > 6.2$  and  $L^* \leq 45.0$  and Normal meat with  $5.9 < \text{pH} < 6.2$  and  $45.0 < L^* < 51.0$ . The pH was measured (in duplicate) by inserting electrodes into the pectoralis major muscle. The colour was evaluated using a Minolta CR-400 colorimeter, taking five different reading points per sample for colour determination ( $L^*$ ,  $a^*$  and  $b^*$ ).

**Water holding capacity (WHC) measurement:** The WHC was determined based on a technique described by Hamm<sup>20</sup>. After 24 h post-mortem, samples were collected from the cranial side of the breast fillets and cut into cubes  $2.0 \pm 0.10$  g. They were carefully placed between two pieces of filter paper (Whatman No. 2) on acrylic plates and then left under a 10 kg weight for 5 min and analyzed in duplicate. The samples

were weighed and WHC was determined by the exudated water weight via the following formula:

$$\left[ \left( \frac{W_i - W_f}{W_i} \right) \times 100 \right] - 100$$

where,  $W_i$  and  $W_f$  are the initial and final sample weights.

**Dead on arrival (DOA):** The mortality of chickens was quantified at the time the birds were hung on the receiving platform and designated dead on arrival (DOA)<sup>7</sup>.

**Statistical analysis:** The Tukey's test ( $p \leq 0.05$ ) was performed using the statistical software Statistica 7.0 (StatSoft, Tulsa, USA) and was applied to compare means of micro-environmental data, PSE, DFD and DOA.

## RESULTS

### Evaluation of micro-environments in the lorry container:

Table 1 shows AV, T, HI and RH throughout the 6 h journey and also during lairage for a further 5 h at the slaughterhouse and commercial plant. The microclimate data were significantly different ( $p \leq 0.05$ ) among the three compartments (front, middle and rear). The front compartment has a significantly lower T compared to the middle and rear compartments, respectively ( $1.7$  and  $4.1^\circ\text{C}$ ), RH ( $4.85$  and  $8.76\%$ ) and HI ( $2.8$  and  $5.9^\circ\text{C}$ ). Figure 5 shows the details of variations in these variables throughout the journey from the farm to the slaughterhouse and during lairage.

**Evaluation of meat quality:** Table 2 shows the values of  $L^*$ ,  $a^*$ ,  $b^*$ , pH and characterizing the meat-colour abnormalities

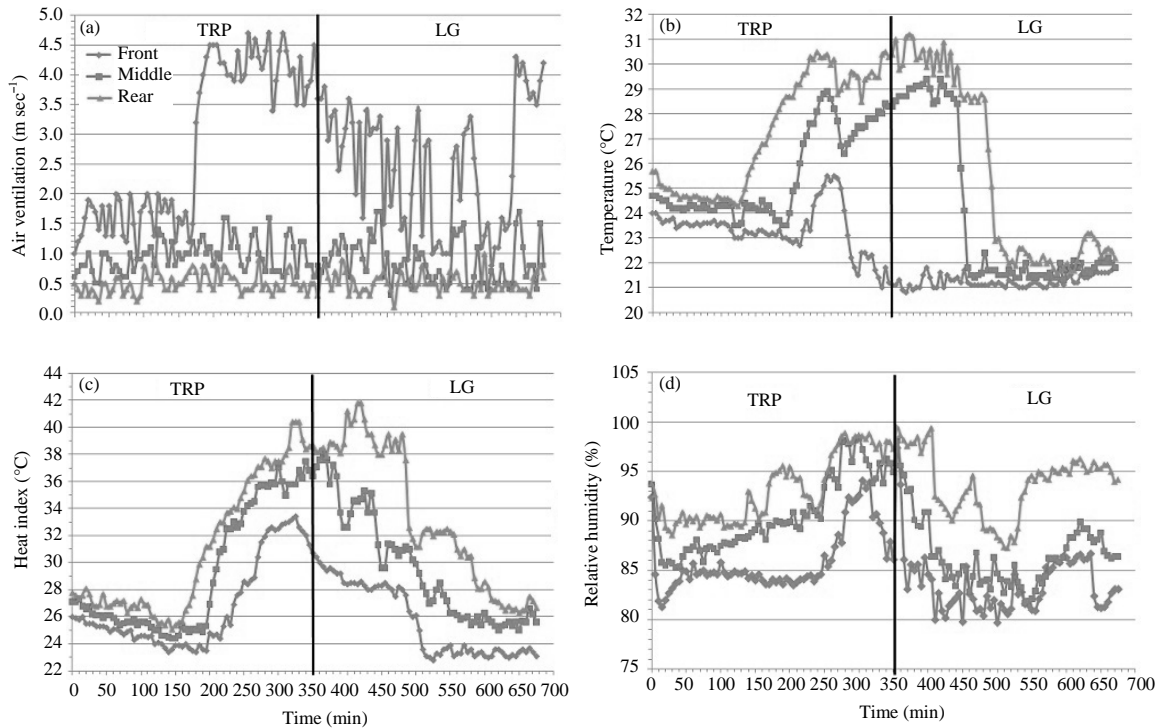


Fig. 5(a-d): Variations in (a) Air ventilation, (b) Temperature, (c) Heat index and (d) Relative humidity at the front, middle and rear of the lorry container during a transport (TRP) journey of 350 min (T) and lairage (LG) of 300 min at the slaughterhouse holding space (n = 3 journeys)

Table 1: Mean values of Air Ventilation (AV), Temperature (T), Relative Humidity (RH) and Heat Index (HI) performed in a 289 km journey and at lairage for 5 h before birds slaughtering measured at the lorry container front, middle and rear positions

Treatments	AV (m sec <sup>-1</sup> )	T (°C)	RH (%)	HI (°C)
Front	2.69±1.20 <sup>a</sup>	22.32±1.26 <sup>c</sup>	84.77±3.29 <sup>c</sup>	26.27±2.05 <sup>c</sup>
Middle	0.91±0.33 <sup>b</sup>	24.77±2.74 <sup>b</sup>	88.68±4.11 <sup>b</sup>	29.45±3.39 <sup>b</sup>
Rear	0.51±0.16 <sup>c</sup>	26.47±3.20 <sup>a</sup>	93.53±3.28 <sup>a</sup>	32.26±4.24 <sup>a</sup>

Means±Standard Deviation on the same column with no common superscripts are significantly different by Tukey's test ( $p \leq 0.05$ )

Table 2: Values of L\*, a\*, b\*, pH and WHC (water holding capacity) of breast fillets classified as normal, PSE and DFD meat

Parameters	Normal	PSE	DFD
L*	49.63±2.61 <sup>b</sup>	55.49±1.35 <sup>a</sup>	42.68±0.89 <sup>c</sup>
a*	1.73±0.70 <sup>a</sup>	1.73±0.68 <sup>a</sup>	1.79±0.57 <sup>a</sup>
b*	4.67±1.34 <sup>b</sup>	5.95±1.46 <sup>a</sup>	2.25±0.91 <sup>c</sup>
pH	6.09±3.10 <sup>a</sup>	5.70±0.07 <sup>a</sup>	6.15±0.13 <sup>a</sup>
WHC (%)	71.46±3.04 <sup>a</sup>	69.47±2.79 <sup>b</sup>	73.88±2.03 <sup>a</sup>

Means±Standard Deviation on the same line with no common superscripts are significantly different by Tukey's test ( $p < 0.05$ )

Table 3: Occurrence (%) of PSE and DFD abnormalities and normal breast meat samples taken from birds located in lorry container positions of front, middle and rear after 6 h journey and 5 h lairage

Meat classification	Front	Middle	Rear
Normal	96.60±0.45 <sup>a</sup>	95.20±0.15 <sup>b</sup>	94.16±0.84 <sup>b</sup>
PSE	2.75±0.38 <sup>b</sup>	4.40±0.18 <sup>a</sup>	5.62±1.17 <sup>a</sup>
DFD	0.65±0.08 <sup>a</sup>	0.41±0.08 <sup>b</sup>	0.22±0.09 <sup>b</sup>

Means±Standard Deviation on the same line with no common superscripts are significantly different by Tukey's test ( $p < 0.05$ )

corroborating by the WHC (%) results. Table 3 shows the occurrence (%) of PSE and DFD and normal samples in the breast meat.

**DOA index:** The DOA index determined was 0.45%. The simultaneous detection of PSE and DFD meat of 10.14 and 3.59%, respectively (Table 3) reveals that the birds were under

heat and cold stress, depending on their location in the lorry container. These results were unexpected because only heat stress would be expected in a tropical zone. In fact, this relatively high DOA value found suggested that the animals were under heat stress (middle and rear) or cold stress (front) depending on their location in the container as observed in Fig. 5b.

## DISCUSSION

There were significant variations in the microclimate during transport; T, RH and HI values increased gradually from the front to the rear as the result of decreasing the air velocity. The average AV value in the front was approximately 3 and 5-fold higher than those in the middle and rear, respectively.

Figure 5 clearly shows the influence of AV (5A) on the T (5B), HI (5C) and RH (5D) values at these three different locations within the lorry during approximately 11 h of routine activity before slaughtering. Because of the rough conditions of the farm roads, in the first 150 min of the journey, the vehicle velocity barely reached 10-20 km h<sup>-1</sup> and the AV was approximately 1-2 m sec<sup>-1</sup> at the front, 0.6-1.0 m sec<sup>-1</sup> at the middle and 0.5-0.3 m sec<sup>-1</sup> at the rear compartments and the T, RH and HI values were relatively constant. Upon reaching the asphalt road, the average lorry speed was approximately 68 km h<sup>-1</sup>, with a strong wind blowing at the front of the vehicle and increasing AV in every compartment: Front ( $\approx 4.5$  m sec<sup>-1</sup>), middle ( $\approx 1.6$  m sec<sup>-1</sup>) and rear ( $\approx 0.9$  m sec<sup>-1</sup>). The recorded AV was highest at the front and the other variables also varied depending on the container compartment. Similar conditions were obtained after the lorry arrived at the processing plant under the immediate water nebulization (Fig. 4a) and forced ventilation (Fig. 4b) for 5 h. During lairage, the AV dropped down gradually but irregularly at the front and at other compartments, it remained constant despite the forced ventilation.

After approximately 3 h of lairage, the T values dropped from 30-20°C at the rear, followed by the middle compartment remaining constant at the front compartment (Fig. 5b). The RH values were also the highest at the rear and the lowest at the front throughout the experiment (Fig. 5d). During this 5-6 h journey, there were other sources of variation; for example, the 3-4 lorry stops caused the AV to reach minimum values, as depicted in Fig. 5a. In addition, rainfall occurred throughout every journey, which might have influenced the variables, as the lorries had open roofs. Therefore, under these conditions, the recorded RH values were irregular, although they still followed the rear>middle>front pattern (Fig. 5d). Thus, the resultant HI (Fig. 5c), which is the actual temperature experienced by the birds was high at those located at the rear of the container. This fact is particularly important as the birds with the highest HI values were subjected to heat stress, resulting in the highest PSE values being detected in samples from birds transported at the rear (Table 3). The T values followed similarly the pattern of HI as seen in Fig 5b and c, respectively.

These results were expected as lower on-board temperatures were recorded at the front and middle, where the birds were exposed air circulation and rain, besides the feathers might not exercise the thermal insulating function because they were wet. Thus, birds located at these regions were exposed to the most wet and cold weather conditions, while birds at the rear were exposed to higher heat values (Fig. 5b), subjecting them to different welfare conditions. The results of this harsh thermal treatment included the development of DFD meat in samples from the front (under hypothermia) and PSE meat from birds at the rear (under hyperthermia) as observed previously in other experiment within Brazilian subtropical zone<sup>4</sup> and other temperate countries<sup>21</sup>.

As expected, WHC presented the significantly lower value in PSE (Table 2) about 5-6% in relation to normal and DFD samples as a consequence of the protein denaturation promoted by warm conditions and relatively low pH and higher value was from birds located at the rear. Corroborating this result, the Table 2 also shows the highest b\* value because a yellow colour is predominant in PSE meat, typical from heat-stressed animals<sup>22</sup>. As observed in Table 3, the total occurrence of PSE meat was relatively lower and conversely, the occurrence of DFD was higher both in relation the results previously obtained by our group in the sub-tropical climate of southern region of the country<sup>4</sup>.

As birds do not have sweat glands, the conditions of high T and RH may severely affected their heat exchange systems therefore, the best way for heat dissipation used by birds is evaporation by hyperventilation (increased respiratory rate) and such exchange is impaired by high values of relative humidity. The risk of heat stress was increased when RH rose at the centre towards rear planes of the vehicle. The reduced air circulation, increase in respiratory rate and heat generated from the birds and from the voided faeces, apparently, increased the moisture content at those locations giving rise to the heat stress development. Similar heterogeneous thermal conditions have been reported during transportation of broilers during winter in temperate countries<sup>21,23,24</sup>.

The RH values recorded at the end of the journey and beginning of the lairage were higher than the ideal ambient temperature values of 18-28°C and RH values of 50-70% established for birds in the tropical zone<sup>25</sup> and may have severely affected the bird's heat exchange system. This situation has the potential to become catastrophic, as birds are homoeothermic they increase their respiratory rate to dissipate the heat. The reduced air circulation (Table 1, Fig. 5a) made to increase this respiratory rate and heat generated from the birds increased the RH values, especially at the rear

area (Table 1, Fig. 5d) corroborated by the resulting highest value of HI (Table 1, Fig. 5d).

Physiologically, the birds are strongly affected by the variables observed in this experiment, especially over long-distance journeys and long periods in lairage without food. It is therefore possible that hidden effects influence the animal welfare and hence the meat qualities. The relative high value of DOA indicated these dramatic conditions. As observed previously, DOA seems not to be closely related to heat stress; in this experiment, the rate of PSE, which is associated with heat stress, was lower than is normally observed in Southern Brazil<sup>3,4,7</sup>. Under conditions similar to those reported herein, the DOA index value found in the south was 0.20, much lower than the 0.45% reported herein. Similarly heterogeneous on-board thermal conditions have been reported during transportation of broilers in other temperate<sup>21,26</sup> and tropical climates<sup>27</sup>.

### CONCLUSION

The development of DFD and PSE meat indicates that in a tropical climate, birds in transit and lairage are placed under cold and hot stress. This situation led to a high DOA index, the highest so far evaluated in this country. Several potential causes were identified, including the relatively long period on a rough farm road, as there was virtually no AV, increasing the heat stress on-board and inducing PSE. Once the lorry reached the asphalt road and the chickens were exposed to wind and rain, the cold and wet conditions produced DFD meat. All of these abnormalities were exacerbated during lairage and in both situations, heat and cold, along with elevated RH, resulted in an increase in the DOA index. Nevertheless, all of these factors are controllable and it is advisable to encourage poultry-processing plant companies to follow good pre-slaughter management practices in order to preserve animal welfare and thus meat quality.

### SIGNIFICANCE STATEMENTS

This study was conducted in order to characterize the microclimatic profile of broiler in-transit within the lorry container during the rainy season under geographical tropical climate zone.

The combination of time period and the journey distance from the broiler farm to the slaughterhouse affected the animal welfare thus the meat quality.

The main stress factors developed during the birds transportation were related to the formed microclimate and the high temperature and relative humidity values were the

main factors responsible for the heat index consequently on the death of arrival index.

It is advisable to encourage poultry-processing plant companies to follow good pre-slaughter management practices in order to preserve the animal welfare thus meat quality.

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