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Acute heat stress detrimental effects transpose high mortality rate and affecting broiler breast meat quality

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Abstract

Acute heat stress may affect the quality of broilers meat, however there are no reports considering thermal conditions commercially available in Brazil. In this way, the present work aimed to fill this gap of industrial relevance. Broilers of commercial strain (Cobb 500, n = 540) were randomly assigned to two thermal conditions: acute heat stress (AHS; 35°C; 75 – 85% relative humidity) and not-heat-stress (NS; 22°C; 83 ± 6.6% relative humidity), for 2 hours prior to slaughter. The mortality rate for AHS broilers reached 37%, which was greater than 5.2% verified for NS. According to the mean values, the broiler chickens were not totally affected in the parameters of pH_{24h}, lightness (L*), cooking loss, and shear force. However, the distributions of data show great variability in the values of pH_{24h}, L* and water holding capacity (WHC) for AHS broilers. It is suggested that AHS broilers, at severe conditions which result in increased mortality, present breast meat with greater incidence of higher pH_{24h}, and lower lightness and WHC values.

Keywords: broiler chickens; climate conditions; mortality rate; meat quality; breast meat

1. Introduction

The production of poultry meat is an important commercial sector around the world. In 2017, a production of 120.5 million tons was estimated, with a growth of 21.3 million tons between 2010 and 2017, being Brazil one of the world's largest chicken meat producer and exporter (FAO, 2018). At this high level of production, assuring meat quality by the poultry industry will be an ever growing demand. The competitiveness and leadership may be maintained by constant technological improvements, standardization and strict control of meat quality.

Broiler chickens in commercial settings may suffer stress at several points of the productive system as in the transport, lairage time prior to slaughter at the abattoirs, when birds may be submitted to extreme heat conditions. An ambient temperature above 30 °C is considered sufficient to induce heat stress in poultry, which negatively influences live weight, feed intake, and feed efficiency (Lu *et al.*, 2007; Quinteiro-Filho *et al.*, 2010), and induces high mortality (Quinteiro-Filho *et al.*, 2010; Azoulay *et al.*, 2011; Abdelqader and Al-Fataftah, 2014). Heat stress accelerates glycolysis (Wang *et al.*, 2017), decreases pH, increases L* and shear force of broiler breast muscle, and undergoes a significant deterioration in WHC properties (Sandercock *et al.*, 2001; Lu *et al.*, 2007; Wang *et al.*, 2009; Schneider *et al.*, 2012; Zhang *et al.*, 2012; Wang *et al.*, 2017). The problem may be worsened by the intensive genetic selection to obtain faster growing broiler chickens lines, which has been contributing for greater susceptibility to heat stress of the current strains (Lu *et al.*, 2007). On the other hand, the quality parameters of meat can also be affected by the age of the animal (Bianchi *et al.*, 2007; Schneider *et al.*, 2012).

The objective of the present study was to evaluate the effects of acute heat stress (AHS) prior to slaughter on mortality and quality parameters of breast meat of broiler chickens reared in commercial conditions in Brazil.

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2. Materials and methods

Broiler chicken husbandry

Five hundred and forty of 1-day-old male chicks, Cobb 500 strain, were allocated to the experimental broiler house at the Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo. They were housed in floor pens (rice hulls) at 0.083 m^2 per bird and reared under commercial conditions. Diets based in corn and soybean meal were formulated according to the requirements suggested by Rostagno *et al.* (2005) for each rearing stage (starter, grower and finisher). The animals were slaughtered at 42, 44 and 46 days of age, in an experimental abattoir, with average live weights of 2.8, 3.0 and 3.2 kg, respectively.

Thermal stress protocol

Since all birds were housed on the same day, there were three slaughter time points according to the age of the birds. For each of the slaughter time points, 180 birds were randomly selected, allocated to one of the pre-slaughter conditions and given a 6 hours fasting period. The birds were then placed in transport crates at a density of 10 birds per crate and taken to either a thermoneutral environment (22 °C; 83 ± 6.6% Relative Humidity) for 2 hours (not-heat-stress condition; NS) and to a controlled climate chamber at 35 °C and 75 – 85% Relative Humidity for 2 hours prior to slaughter (acute heat stress condition; AHS). After two hours at the different pre-slaughter environment conditions exposure, the birds travelled a very short distance (200 meters) to the abattoir inside a vehicle without ventilation. This procedure was scheduled to be repeated over 5 subsequent times at each day of slaughter. During the day, each group had 20 birds AHS and 20 NS slaughtered, except for the first group with 10 birds for each thermal condition. For the analyses, the experimental units were the breasts of chickens (*Pectoralis major*) that survived the acute heat stress, whereas the mortality rate was calculated based on the chickens that did not survive the procedures from selection and fasting to transportation to the abbatoir.

Breast muscle and meat quality measurements

Immediately upon arrival at the abattoir, the birds were slaughtered after electrical stunning followed by exsanguination. The eviscerated carcasses were chilled for 30 min at 17-20°C and 15 min at 4°C, which is similar to conditions of the commercial abattoirs in Brazil. After this time of chilling, the breast muscles were cut from the carcass.

Muscle pH was determined using a portable pH-meter with direct probe penetration (Oakton, pH 300 series, Eutech Instruments, Singapore) at 24 h postmortem (pH_{24h}). The samples were stored in self-sealed plastic bags for 24 h in 2 ± 1°C cooler chamber for pH_{24h} readings. For this measurement, a small incision 0.5 to 1 cm deep was made to allow insertion of the electrode. Four readings per sample were taken and the average was recorded. Lightness (L*) was measured at 24 h postmortem at 6°C on the left and right breast muscles using a Minolta Chroma Meter CR-400 (Konica Minolta Sensing Inc., Japan) colorimeter (aperture size: 8 mm; observation angle: 10 °C; light source: illuminant D65; bloom time: 15min) connected to a computer. The measurements were made on the internal cranial and central region of *Pectoralis major* muscle (four readings for sample) free from color defects. The water holding capacity (WHC) was determined 24 h postmortem according to Nakamura and Katok (1985), with small modification. The method consists in weighing one gram of raw muscle on filter paper, followed by centrifugation at 1500 × *g* for 4 min. After centrifugation, the sample is weighed and then placed in an oven at 70°C for 12 hours. The WHC was determined as the difference between the sample weight after centrifugation and the dry weight of the sample divided by the initial weight; the value was expressed as a percentage. Cooking loss was evaluated at 24 h postmortem by cooking at 163°C in an electrical grill until an internal temperature of 82°C was reached. The samples were cooled to room temperature and reweighed to determine cooking loss (expressed as a percentage of initial weight of the core sample). Shear force values were measured using an Instron Texture Test System model TP2 (FTC, Stable Micro Systems, Surrey, UK) equipped with Warner-Bratzler accessories. After cooking loss determination, breast samples were cut in slices of 2.0 x 1.0 x 1.0 cm according to Froning and Uijtteenboogaart (1988). The samples were oriented with muscle fibers perpendicular to the blade of the equipment at working velocity of 20 cm min^{-1}. The shear values were reported as kilograms of force per gram of sample.

Table 1
Total mortality and percentage of the chickens in three ages, according the thermal condition (AHS, NS) and slaughter group

Slaughter group	Age (days)						Mortality (Total and %)	
	42		44		46			
	AHS[1]	NS[1]	AHS[1]	NS[1]	AHS[1]	NS[1]	AHS	NS
1 (7:00h)	5	0	7	1	1	2	13 (43.3)	3 (10.0)
2 (9:00h)	3	2	7	0	9	1	19 (31.7)	3 (5.0)
3 (11:00)	5	2	4	1	11	0	20 (33.3)	3 (5.0)
4 (13:00)	9	2	10	0	11	1	30 (50.0)	3 (5.0)
5 (15:00)	6	2	8	0	4	0	18 (30.0)	2 (3.3)
Mortality (Total and %)	28 (31.1)	8 (8.9)	36 (40.0)	2 (2.2)	36 (40.0)	4 (4.4)	100 (37.0)	14 (5.2)

[1] Initial number of chickens per thermal condition and age: group 1=10, group 2=20, group 3=20, group 4=20, group 5=20. Total chickens AHS=270; NS=270.

Experimental design and statistical analysis
Three-way analysis of variance (ANOVA) ($P < 0.05$) was run at STATISTICA program, version 8.0 (2008) for a completely randomized design with a factorial arrangement (ages: 42, 44 and 46 days; slaughter group: 7:00h, 9:00h, 11:00h, 13:00h, 15:00h, and thermal condition: AHS, NS). Also, to compare the meat quality measurements distributions of both treatments (thermal condition), the Kolmogorov-Smirnov test ($P < 0.05$) was applied. This test is also sensitive to differences in the general shapes of the distributions (i.e., to differences in dispersion, skewness and kurtosis). Additionally, Levene's test for Homogeneity of Variances was applied ($P < 0.05$) to compare the treatments' variances.

3. Results and discussion

Mortality rate
The greater mortality rate ($P < 0.05$) of the broilers under AHS was confirmed compared to animals under thermoneutral environment (Table 1). The severity of heat stress resulted in a high number of deaths, which reached 37.0% compared to 5.2% in the thermoneutral condition. Similar results were obtained in chickens submitted to similar conditions of heat stress (Quinteiro-Filho *et al.*, 2010; Azoulay *et al.*, 2011).
The elevated mortality rate of birds under AHS is a result of heat stroke, which increases the body temperature (Borges *et al.*, 2004; Azoulay *et al.*, 2011). In addition, Santos (2007), using the same chickens used in this study, found that chickens exposed to thermal stress lose water in tissuees and organs caused by hyperventilation, which in turn caused hypovolemia due to the reduction of the hematocrit; consequently, these findings may be related to the higher mortality of chickens in the thermal stress group.

pH_{24h}
Among the thermal conditions, the mean pH_{24h} values of the AHS breasts were significantly higher compared to those of the NS group (Table 2). The impact of the differences observed may not be of practical importance, nonetheless it points to a controversial effect, since several studies reported an acceleration of the glycolysis in heat stressed birds resulting in lower pH values when compared to birds in thermoneutral environment (Wang *et al.*, 2009; Zhang *et al.*, 2012; Wang *et al.*, 2017). On the other hand, there are also findings indicating that high temperatures did not affect the pH_{24h} values of chicken breast meat (Petracci *et al.*, 2001; Schneider *et al.*, 2012). However, despite the similar mean values between the thermal conditions, the variability in their pH_{24h} values was different, which can be observed in the histograms (Figure 1). Broilers that were under AHS showed greater variability of breast pH values, which 22.4% had pH higher than 6.1 versus only 11.1% of the birds under NS. The greater proportion of breasts with $pH_{24h} > 6.1$ for broilers under AHS may indicate the possibility of increased occurrence of darker meat under such severe heat conditions, which could be harmful to the industry. Previous reports (Mallia *et al.*, 2000a,b) had indicated this possibility in poultry.

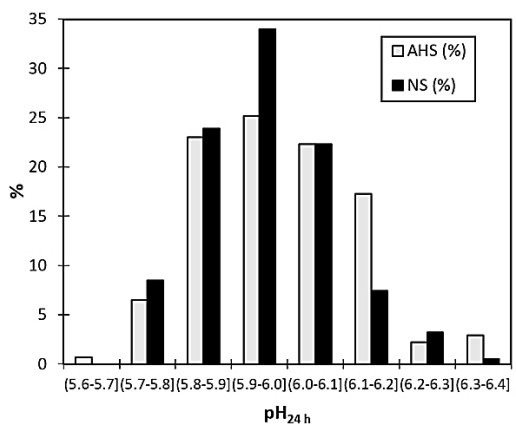

Figure 1. Histograms of pH_{24h} values of broilers under pre-slaughter acute heat stress (AHS) and without heat stress (NS).

Table 2
Breast muscle pH_{24h} of broiler chickens submitted to pre-slaughter heat stress in different ages at several day times intervals

		N	Mean[1]	SD	SE	-95%	+95%
Age	42	130	5.98a	0.129	0.011	5.95	6.00
	44	116	5.97a	0.109	0.010	5.95	5.99
	46	81	5.96a	0.140	0.016	5.93	5.99
Slaughter Group	1	43	6.01a	0.121	0.019	5.97	6.05
	2	73	5.98a	0.143	0.017	5.95	6.02
	3	74	5.98a	0.121	0.014	5.95	6.01
	4	66	5.98a	0.099	0.012	5.95	6.00
	5	71	5.92b	0.122	0.015	5.89	5.95
Thermal Condition	AHS	139	5.99a	0.136	0.012	5.97	6.01
	NS	188	5.96b	0.115	0.008	5.94	5.97

[1] Values with different letters among treatments (age, slaughter group and thermal condition) differ from each other ($P < 0.05$).

Table 3
Breast muscle L* of broiler chickens submitted to pre-slaughter heat stress in different ages at several day times intervals

		N	Mean[1]	SD	SE	-95%	+95%
Age	42	129	51.03c	2.613	0.230	50.57	51.48
	44	116	51.97a	2.255	0.209	51.55	52.38
	46	81	51.34b	2.050	0.228	50.89	51.79
Slaughter Group	1	43	51.20a	2.144	0.327	50.54	51.86
	2	73	51.35a	2.244	0.263	50.83	51.88
	3	73	51.30a	2.550	0.299	50.71	51.90
	4	66	51.52a	2.340	0.288	50.94	52.09
	5	71	51.74a	2.560	0.304	51.13	52.35
Thermal Condition	AHS	139	51.08b	2.447	0.208	50.67	51.49
	NS	188	51.70a	2.311	0.169	51.37	52.03

[1] Values with different letters among treatments (age, slaughter group and thermal condition) differ from each other ($P < 0.05$).

In relation to the age of slaughter, Sandercock *et al.* (2001) found no differences between ages in pH_{24h} values, similar to the result of this research. Analysing the slaughter groups, pH_{24h} of group 5 was significantly lower than the other groups (Table 2), but this difference would not have a great impact on the quality of the meat since the pH values are high.

Lightness (L*)

The broilers under AHS produced breasts with slightly smaller L* values ($P < 0.05$) than the NS counterparts (Table 3). The results do not corroborate neither the increased lightness with the heat stress (Bianchi *et al.*, 2007; Zhang *et al.*, 2012; Wang *et al.*, 2017) nor the lack of effect of heat stress on breast meat color (Petracci *et al.*, 2001; Sandercock *et al.*, 2001). The discrepant results may be consequence of differences in the strain or the type or condition of stress applied. On the other hand, Qiao *et al.* (2001) suggested that a normal breast meat lightness is in the range of 48 to 53. In the present study, the L* values of 51.08 and 51.70 for broiler under AHS and NS, respectively, can be considered normal, indicating that acute heat stress appeared to have no detrimental effect upon breast meat color. This is consistent with the findings for pH_{24h} values, which were not sufficiently low to affect the L* values. However, there were differences ($P < 0.05$) in the L* values distributions between AHS and NS broilers, which can be verified in the histograms (Figure 2). In the AHS group, 31.1% of the breasts showed L* values smaller than 50, while only 20.2% of those of the NS group fell in this category. On the other hand, only 38.4% of the breasts from broilers under AHS had L* values over 52 (or pale meat), while 51.6% of the breasts from broilers under NS were pale. These results indicate a trend towards darker breast color for AHS, the same hypothesis for the pH_{24h} values.

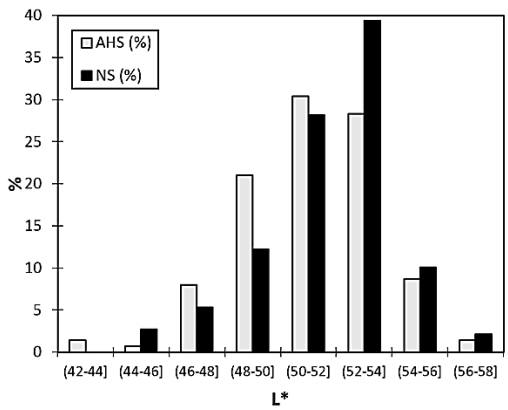

Figure 2. Histograms of L* values of broilers under acute heat stress (AHS) and without heat stress (NS).

There was no clear trend of decreasing or increasing L* values in relation to the age of the chickens (42, 44 or 46 days), which was expected given the range of 4 days.

Table 4
Breast muscle WHC of broiler chickens submitted to pre-slaughter heat stress in different ages at several day times intervals

		N	Mean[1]	SD	SE	-95%	+95%
Age	42	40	55.1b	3.75	0.59	53.9	56.3
	44	40	56.6a	2.46	0.39	55.8	57.4
	46	39	54.4b	3.02	0.48	53.4	55.4
Slaughter Group	1	24	54.6a	3.01	0.61	53.3	55.8
	2	24	56.0a	3.86	0.79	54.4	57.7
	3	24	55.8a	2.53	0.52	54.7	56.9
	4	23	55.5a	3.31	0.69	54.0	56.9
	5	24	55.0a	3.36	0.69	53.6	56.4
Thermal Condition	AHS	60	54.7b	3.50	0.45	53.7	55.6
	NS	59	56.1a	2.77	0.36	55.4	56.8

[1] Values with different letters among treatments (age, slaughter group and thermal condition) differ from each other ($P < 0.05$).

Table 5
Breast muscle cooking loss of broiler chickens submitted to pre-slaughter heat stress in different ages at several day times intervals

		N	Mean[1]	SD	SE	-95%	+95%
Age	42	39	24.9b	4.09	0.66	23.6	26.2
	44	40	25.6b	2.75	0.44	24.7	26.4
	46	38	28.7a	2.62	0.43	27.9	29.6
Slaughter Group	1	23	26.4a	3.95	0.82	24.7	28.2
	2	23	25.4a	2.82	0.59	24.2	26.6
	3	24	26.1a	3.09	0.63	24.8	27.4
	4	24	26.6a	3.78	0.77	25.0	28.2
	5	23	27.3a	4.25	0.89	25.5	29.2
Thermal Condition	AHS	59	25.6b	3.60	0.47	24.7	26.5
	NS	58	27.2a	3.46	0.45	26.3	28.1

[1] Values with different letters among treatments (age, slaughter group and thermal condition) differ from each other ($P < 0.05$).

Smith *et al.* (2002) found similar results for chickens between 42 and 52 days of age. However, in a wider range of age (from 28 to 56 days), the L* value increases with age (Schneider *et al.*, 2012).

Water Holding Capacity (WHC)

There were differences ($P < 0.05$) in the mean WHC values of breast muscle of chickens submitted to the two thermal conditions (56.1% vs. 54.7% for NS and AHS respectively, Table 4), which is in agreement with reports indicating that thermal stress decreases water retention of chicken breast meat (Sandercock *et al.*, 2001; Bianchi *et al.*, 2007; Wang *et al.*, 2009).

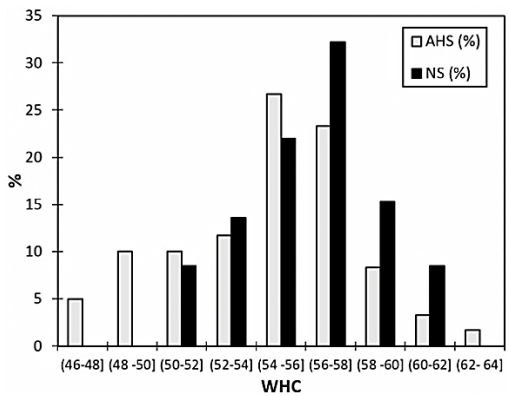

Figure 3. Histograms of WHC 24h values of broilers under acute heat stress (AHS) and without heat stress (NS).

Santos (2007) found that the WHC is associated with the loss of membrane integrity, since the hematocrit decreased and creatine kinase increased by the effect of the AHS. Similar results were reported by Sandercock *et al.* (2001), who observed that increase in creatine kinase activity was associated with higher drip loss in chicken breast. In addition, there were differences in the variances of the WHC values between the two thermal conditions ($P < 0.05$). This fact is demonstrated in the histograms (Figure 3), which show that for the AHS breasts, the values of WHC ranged between 46% and 64%, indicating greater variability, while in the NS group, these values varied from 50% to 62%. Moreover, the AHS group had 15% of the chickens with WHC values smaller than 50%, whereas in the control group all the birds showed WHC values greater than 50%.

Cooking loss

Broilers under AHS had breasts with less cooking loss ($P < 0.05$) than those from the NS animals (Table 5), despite the higher WHC of the NS breasts reported above. It is possible that the pH_{24h} values had influence in cooking loss, which were significantly greater in the AHS group, with a greater number of breasts with pH_{24h} higher than 6.1 (Table 2, Figure 1), thus maintaining the water retention of meat during cooking. This is supported by Wang *et al.* (2009) and Zhang *et al.* (2012), who found cooking loss values were related to lower pH values.

Table 6
Breast muscle shear force of broiler chickens submitted to pre-slaughter heat stress in different ages at several day times intervals

		N	Mean[1]	SD	SE	-95%	+95%
Age	42	40	3.72b	0.930	0.147	3.419	4.014
	44	40	5.23a	1.188	0.188	4.847	5.606
	46	38	5.62a	1.661	0.269	5.073	6.165
Slaughter Group	1	23	4.13a	1.147	0.239	3.629	4.621
	2	23	4.50a	1.655	0.345	3.787	5.218
	3	24	4.91a	1.378	0.281	4.327	5.491
	4	24	5.15b	1.521	0.310	4.504	5.788
	5	24	5.48b	1.586	0.324	4.808	6.147
Thermal Condition	AHS	60	4.87a	1.486	0.192	4.484	5.252
	NS	58	4.81a	1.567	0.206	4.401	5.225

[1] Values with different letters among treatments (age, slaughter group and thermal condition) differ from each other ($P < 0.05$).

On the other hand, there were no differences between variances ($P > 0.05$) and distributions ($P > 0.05$) of the thermal conditions, indicating that the changes were only in the mean values.

There were differences ($P < 0.05$) in the mean values of the cooking loss with the increasing age of broilers age (Table 5), similar to the results of Schneider *et al.* (2012), who found that cooking loss increased with age between 28 and 49 days. This can be concomitant with the increase in the chicken weights (from 2.8 to 3.2 kg, for 42 and 46 days old, respectively) as found by Bianchi *et al.* (2007), who reported that cooking loss was greater with increasing age and weight of broilers.

Shear force values

The mean shear force values were not different between broilers under AHS or NS environments (Table 6). These results corroborate the unchanged meat texture scores of broiler chickens under thermal conditions obtained by Petracci *et al.* (2001) and Sandercock *et al.* (2001), but other authors found that heat stress increased shear force of the *Pectoralis major* muscle (Schneider *et al.*, 2012; Zhang *et al.*, 2012). The shear force values are proportional to the myofibrillar fragmentation index (MFI); using the same breast meat of the present study, Santos *et al.* (2008) found no differences in the MFI values in the muscle of chickens subjected to both thermal conditions. In addition, in accordance with the mean values, there were no differences in the shear force variances ($P > 0.05$) and distributions ($P > 0.05$).

Broilers age ($P < 0.05$) and slaughter group ($P < 0.05$) were factors that interfered with shear force values (Table 6), which increased with age, as well as with time of slaughter. The influence of age was reported by Bianchi *et al.* (2007); it was shown that the increase of the weight and age of chickens decreased significantly the shear force values in the chicken breast, which was in a different trend as found in this study.

4. Conclusions

Broilers subjected to pre-slaughter heat stress (35 °C; 75 – 85% relative humidity) had a higher mortality rate than broilers subjected to thermoneutral conditions. The results of the breast meat quality parameters presented here show certain contradictions to results found in the literature, specifically, the pre-slaughter acute heat stress show a tendency towards producing meat with greater pH_{24h} and lower L*, characteristic of dark meat. In addition, despite applying the same conditions and using the same breed, there was variability in the results, which was greater in the stressed chickens than those observed in the themoneutral conditions; this demonstrates the individuality of the chickens in the physiological, physical, chemical and biochemical responses. Moreover, the identification of components associated with thermal stress may have a positive impact on breeding programs for tolerance to thermal stress. In this sense, it is suggested to conduct studies that determine cellular responses to identify biomarkers associated with thermal stress and their implications in meat quality. Finally, the age of the chickens was also a component of variability in the L* values, WHC, cooking loss, and shear force of the breast meat.

Acknowledgments

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