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Broiler Meat Quality Evaluation Created in Simulated Conditions of Heat

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Abstract

The effect of different periods (0, 24, 48 and 72 h) of condition heat on the physical and chemical qualities of broiler meat was evaluated. Five hundred Cobb 500® chicks were used, of which 100 were reared at a thermoneutral temperature, ideal for every rearing stage, constituting the control group. The other 400 animals were reared in a climate chamber at 32 ± 2°C, simulated conditions heat for birds. The physical and chemical qualities of the meat was evaluated at 21, 35, and 42 days. This experiment was carried out using a completely randomized design with a factorial of 2 × 4 (temperature and periods of conditions heat, respectively) and four replicates. The means were compared by Tukey`s test at the 5% significance level. It was found that the heat affected the qualitative properties of the meat, particularly its lipid oxidation, water retention capacity, shear force, r value, and pH. Microbiological assessment was carried out on days 21, 35, and 42. The temperature treatments were not found to be associated with the occurrence of associated with the occurrence of any of the microbial species considered.

Keywords: High temperature; Physiological alterations; Qualitative properties

Introduction

Carcass quality is related to variations in conviction rate in the slaughterhouse, and primarily by factors such as age, sex, nutrition, handling, transportation, ambient temperature, time of fasting, and the method of harvesting birds on the farm [1]. Birds are homeothermic animals that have a thermoregulatory center located in their hypothalamus, which is constituted of neurons that are activated in response to temperature changes, triggering behavioral reactions and adaptive mechanisms for thermoregulation. This center is responsible for maintaining and controlling homeothermy through heat exchange with the environment [2]. When in high environmental temperatures, the optimum strategies adopted by the bird for heat dissipation are: increased respiratory rate hyperventilation and peripheral vasodilation, which do not promote evaporative heat loss [3].

Climatic aspects are the major limiting factor in the development of industrial production system in warm regions, because most modern commercial broiler strains were genetically improved for breeding in temperate countries. Therefore, heat stress is a particularly serious concern for the poultry industry in the tropics and during the summer in temperate countries, since it causes stunted growth, immunosuppression, and high mortality rates, resulting in significant economic losses in production [4].

The higher susceptibility of birds to heat stress is directly related to the relative humidity of the air and the environmental temperature because, when exposed to heat, compensatory physiological responses occur to enable a return to the thermal comfort zone [5].

The problems arising from increased susceptibility to heat stress causes changes in the chicken meat, seriously damaging the industrialization of meat products as well as causing increased rejection by the consumer due to the resulting physico chemical and organoleptic changes to the meat. An animal's defense against foreign agents such as viruses, bacteria, protozoa, and other parasites occurs due to the fundamental functioning of the immune system. The balance between immune system function and environmental challenges is a determining factor for animal health [6].

The main factors that negatively interfere with the immune system are stress caused by management, mycotoxins, and low levels of vitamins and minerals in the diet. Ensuring that these factors are controlled may improve animal health and productivity, as well as directly affect the cost of animal production. Over the past few years, the influence of stress on neuroimmune function has been widely studied, due to the notoriously damaging effects on individuals. Animals exposed to adverse situations suffer the effects of stress, including delays in growth, reproductive damage, and even death in animals grown for meat production [7]. It is also important to note that thermal stress, over a prolonged period, causes lesions to the mucosa of the gastrointestinal tract and hinders the absorption of nutrients [8,9].

Salak-Johnson and McGlone [10] review studies of the effect of stress on immunity and particularly the suppression of the cellular and humoral immune responses. These authors concluded that stressful conditions trigger the release of glucocorticoids and this makes animals more susceptible to infectious diseases.

Thaxton and Siegel [11] and Miller and Quershi [12] showed that birds exposed to different types of environmental stress show reduced immune functions. When hens are exposed to temperatures varying from 32.2°C to 43°C for short periods, or cycles of constant high temperatures, the immune response is significantly reduced. Heckert et al. [13] showed that broilers subjected to overcrowding (20 birds/m^2), show reduced immune activity.

According to Fuller [14], any factor that causes an imbalance in

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intestinal microbiota may allow pathogenic microorganisms to invade and multiply; therefore, an imbalance of intestinal microbiota directly reflects the health of the host. According to Courrier [15], acute infections caused by *Salmonella* serotype Enteritidis usually occur in young birds or birds reared under stressful conditions.

The most significant pathogenic bacteria in aviculture are *Salmonella* spp.; *Campylobacter jejuni,* involved in gastroenteritis outbreaks; and Listeria monocytogenes, associated with meningitis and meningoencephalitis [16]**.** In addition to affecting animal productivity, these bacteria are hugely important to public health, since they are intimately related to infections from food. Bacteria such as *Salmonella* spp. are more frequent in human cases of food-borne illnesses from meat and chicken products. These bacteria are found in the intestinal tracts of humans and other animals and can multiply in culture and produce visible colonies at 37°C in 24 h. The optimal pH for the development of *Salmonella* spp. is approximately neutral, with values above 9.0 and below 4.0 considered bactericidal Gast [17].

Another important group of microorganisms are *Campylobacter* spp., especially C. jejuni, which acts as a pathogenic agent or forms the normal microbiota of the gastrointestinal tract of animals such as cattle, birds, sheep, dogs, and cats [18]. Hence, meat is the greatest source of intestinal campylobacteriosis. *L. monocytogenes* is the only species of the genus Listeria that is pathogenic to humans. Listeriosis is the food-borne disease that causes the greatest number of deaths and hospitalizations (91% of cases), particularly in pregnant women, newborns, and immunocompromised individuals [19].

Thus, conditions of heat is relevant in aviculture, since high temperatures may compromise bird immune systems resulting in increased proliferation of pathogenic bacteria and decreased populations of beneficial bacteria in the gastrointestinal tract. In this sense, stress may interfere with the behavior of intestinal microbiota, which protects the host against pathogens and opportunistic infections. Due to the issues described above, the aim of this work was to evaluate the impact of heat condition on the physical, chemical and microbiological qualities of chicken meat.

Materials and Methods

Animals, initial management, and experimental conditions

The work was submitted to the Ethics Committee on the Use of Animals of the Faculty of Agrarian and Veterinary Sciences, University Estadual Paulista and was approved under protocol number 4207/2010. The experiment was conducted in climate chambers in the experimental aviary facility at Faculty of Agrarian and Veterinary Sciences, University Estadual Paulista - UNESP, Câmpus de Jaboticabal – Brazil.

For the experiment, 500 male Cobb strain 500 broilers were used. The animals received water and feed ad libitum during the 45 days of husbandry. The diets were formulated according to the ages of the birds and were based on their nutritional requirements Rostagno et al. [20] The animals were vaccinated against Gumboro disease (intermediate strain Lukert) for 7 and 19 day old birds, and against New castle disease (strain Ulster) for 12 and 24 day old birds.

Two climatic chambers were used to house the birds, with a density of 10 birds/m^2, distributed in boxes of 2.5 × 1 m dimensions, containing 25 birds each. One hundred birds were kept in a chamber at a thermoneutral temperature, ideal for every stage of husbandry, according to the recommendations of Cobb. The other 400 birds were housed in another climatic chamber with heating and cooling systems, and were subjected to different periods of heat (0 h, 24 h, 48 h and 72 h), at which the camera's internal temperature rose to 32 ± 2°C. A 24 h light period was used throughout the experimental period in both chambers.

At 21 days of age the birds were subjected to different periods of conditions simulated heat. After maintaining condition heat for 72 h the heaters were turned off and the animals were kept in a thermoneutral environment until the next phase of simulated condition heat, at 35 and 42 days. After each condition heat simulated period, 12 birds from each chamber were slaughtered in the experimental abattoir in the aviary sector at FCAV / UNESP using conventional slaughtering procedures. The carcasses were then packed in plastic bags and placed in boxes with crushed ice until the time of analysis. The remaining birds stayed in their boxes until the next period of simulated conditions of heat.

Evaluation of meat quality

The carcasses were sent to the laboratory of Animal Products Technology at FCAV/UNESP, where physical and chemical analysis of the pectoralis major muscle was performed. The pH in the muscle was measures 24 h after slaughter using a Testo 205-digital pH meter, coupled to a Digimed glass probe.

The color was assessed in three parts of the muscle sample using a Minolta Chroma Meter CR-300 colorimeter, and the CIELab system to evaluate the L* (lightness) parameters, ranging from black (0) to white (100), a* (red content) ranging from green (- 60) to red (60), and b* (yellow content), ranging from blue (- 60) to yellow (60). The water holding capacity (whc) was evaluated according to the methodology described by Hamm [21,22], by measurement of the water released when a 10 kg pressure is applied for five minutes to 0.50 g samples of muscle tissue. The percentage of water lost was calculated from the difference in sample weight before and after undergoing pressurization. To determine the weight loss by cooking (wlc). The samples were weighed and packed in plastic bags and cooked in a water bath at 85°C for 30 min until the internal temperature reached 75°C. Next, the samples were removed from the bags, left at ambient temperature, and reweighed to calculate the weight loss [23].

The softness was evaluated from the shear strength of the samples after firing, taken perpendicular to the muscle fiber orientation, using Warner-Bratzler blade adapted with a Stable Micro Systems TA-XT2i texturometer, and the results were expressed as the maximum shear force in kgf/cm^2 [23]. The thiobarbituric acid reactive substances (tbars), resulting from lipid oxidation of the chicken meat samples, were determined according to the method described by Pikul et al. [24]. Triplicate samples, weighing around 10 g were homogenized with 50 mL of 7.5% trichloroacetic acid solution (tca) The supernatant was filtered and 4 mL aliquots of the filtrate were treated with 5 mL of thiobarbituric acid solution (tba), placed in boiling water, cooled, and analyzed with a spectrophotometer at 538 nm. The results were expressed in milligrams of the tbars per 1 kg of sample. The value of r was determined according to the methodology described by Honikel and Fischer [25]. This assessment was based on the extraction of nucleotides through homogenization with 1 M perchloric acid using a ratio of, 1:10 v / m. After filtering, a 0.1 mL aliquot was diluted with 4.9 mL of 0.1M phosphate buffer at pH 7.0 and was analyzed at 250 nm (inosine monophosphate) and 260 nm (adenosine triphosphate) in a spectrophotometer. The r value was determined from the ratio of the two absorbance wavelengths.

Preparation of swab dilutions

Swab dilutions were prepared according to the methods of Apha (2001). From each sample, 1 mL of the swab transport solution was removed aseptically and placed into 9 mL of 0.1% sterilized peptone water, which was then homogenized in a Stomacher for one minute. An initial dilution of 10:1 was obtained, and decimal dilutions were prepared (up to 10:5) using the same dilutant.

Detection of *Campylobacter* spp.

To detect *Campylobacter* spp., the SimPlate method was used according the manufacturer specifications Biocontrol [26,27]. Initially, 1 mL of the sample, 9 mL sterile distilled water, 0.025 mL of rifampicin and 0.04 mL of Hemin were added to each tube containing the substrate to hydrate it. This mixture was then added to the center of the plates and distributed over all cavities using circular movements. The plates were incubated in an inverted position for 48 h at 42°C in the dark under microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2), in anaerobiosis jars. After incubation, the cavities were observed and those with color changes from yellow to red were considered presumptive positive samples. These plates that showed colored cavities were observed using a fluorescence camera under 365 nm UV light and the colored cavities that did not fluoresce were considered to be positive for *Campylobacter*. To confirm the positive results, the difference between the numbers of red and fluorescent cavities was estimated and the SimPlate conversion table was used to obtain the total *Campylobacter* count per plate.

Detection of *Escherichia coli*

For the *Escherichia coli* assay, the most probable number method described by Hunt and Rice [27] was used. A set of three tubes containing lauryl sulphate tryptose broth with 4-methylumbelliferyl-beta-D-glucuronide (LST-MUG) by dilution were used, and 1 mL of the dilution was added to each tube of 10 mL LST-MUG. Next, the tubes were incubated at 35°C ± 0.5°C for 24 h to observe the development and/or production of gas. In the samples with a positive result, i.e., those with growth and/or gas production, the tubes were observed under ultraviolet (6 W) and long-wave (365 nm) lamps. Those with blue fluorescence were confirmed as positive for *E. coli*. The result was based on the most probable number (MPN) table.

Determination of the total enterobacteria

For the total enterobacteria assays, the plate count method described by Kornacki and Johnson [28] was used. Each dilution (1 mL) was inoculated onto empty sterile petri dishes and violet red bile glucose agar was added. After complete solidification of the medium, it was covered with an additional layer of the same medium. The plates were incubated in an inverted position at 35°C ± 1°C for 24 h. After the incubation period, the typical colonies of total enterobacteria were counted.

Detection of lactic bacteria

For the lactic bacteria assay, the plate count method of Hall and Yousef Hall and Yousef [29] was used. Each dilution (1 mL) was inoculated onto empty sterile petri dishes, and the Man, Rogosa and Sharpe culture media was subsequently added. The plates were incubated in an inverted position at 35°C ± 1°C for 72 h. After the incubation period, the plates with colonies were counted and those with at least five colonies present were selected for subsequent Gram staining and the catalase test. Isolation, selection, and identification of Listeria monocytogenes and *Salmonella* spp. by real time polymerase chain reaction (RT-PCR).

To detect the presence of the pathogenic *Salmonella* spp. and *L. monocytogenes*, the real-time polymerase chain reaction was used (RT-PCR) Biocontrol [26], which currently is accepted for use by the Ministry of Agriculture, Livestock and Supply (MAPA) due to its specificity.

RT-PCR was performed using SYBR Green dye following the manufacturer's guidelines using Assurance GDS Rotor-Gene®. If *Salmonella* spp. were confirmed, samples were subjected to a pre-enrichment, selective enrichment, differential plating, preliminary confirmation of typical colonies and a polyvalent somatic serological test.

Pre-enrichment

A portion of the 1 mL cloacal swab sample was homogenized in 9 mL of 0.1% buffered peptone water and incubated at 37°C for 18 h.

Selective enrichment

The pre-enrichment tube was carefully shaken and 0.1 mL was transferred to 10 mL of Rappaport Vassiliadis broth and between 1 mL and 10 mL tetrathionate broth. The Rappaport Vassiliadis broth was incubated at 41.5°C ± 1°C for 24 h and the tetrathionate broth at 37°C ± 1°C for 24 h.

Differential plating

The selective enrichment tubes were shaken in a "vortex" agitator and a sample of the tetrathionate broth was streaked onto bismuth sulfite agar and xylose lysine deoxycholate agar. The same procedure was repeated using the Rappaport Vassiliadis broth. The plates were incubated in an inverted position at 35°C for 24 h to verify the development of typical colonies of *Salmonella* spp.

Preliminary confirmation of typical colonies of *Salmonella* spp.

When a suggestive colony was found, part of the cell mass was removed from the center of the typical colony using an inoculation needle and inoculated in inclined tubes containing iron lysine agar and triple sugar iron (TSI) Agar. Two typical colonies from each plate were selected, and inoculation was performed by stabbing and streaking on the slant, using the same loop to inoculate both tubes. The tubes were incubated at 35°C ± 1°C for 24 h and the typical reaction for *Salmonella* spp. was observed.

Polyvalent somatic serological test

Two squares of approximately 2 cm^2 were marked onto a glass slide, using a glass hydrophobic marker pen. A sample from the culture grown for 24 h in TSI was transferred with a loop to each of the squares, placing the culture sample in the upper part of each square. A drop of physiological saline solution was added to the lower part of one of the squares and the culture was thoroughly emulsified. A drop of polyvalent somatic serum anti-*Salmonella* spp. was added to the lower part of the other square. Holding the slide against a well-lit dark background, the slide was gently inclined and rotated in order to agitate the emulsion to observe the occurrence of agglutination in the square containing the serum. This result was compared with the result of the emulsion performed with the saline solution on the other square (the negative control), so that there was no confusion between cloudy appearance and an agglutination reaction.

Statistical analysis

The experimental design was completely randomized in a factorial scheme of 2 x 4 (temperatures thermoneutral and simulated conditions of heat) duration of heat (0, 24, 48, and 72 h) respectively, with four replications. The averages were compared by Tukey`s test at a 5% significance level, using the SAS [30] statistical program.

Results and Discussion

The results obtained for the thiobarbituric acid reactive substances (tbars), water holding capacity (whc), shear force (sf) and weight loss by cooking (wlc) of the broiler meat at 21 days are shown in Table 1.

There was a significant correlation between the temperature (thermoneutral and heat) and the duration of heat with the tbars ($p<0.01$), whose breakdown is shown in Table 2. Through the breakdown of this correlation, it is found that the lipid oxidation degree of the meat from birds that were subjected to heat was higher. This is expected because a high body temperature can cause the release of steroids, initiating the peroxidation of membrane lipids, and thus oxidation of the meat from the birds that were subjected to heat at 32 ± 2ºC is increased. Heat stress can also cause disturbances in the balance between oxidant and antioxidant defense systems, causing lipid peroxidation and oxidative injury to proteins and DNA [31].

With regards to the results of the whc and sf, it is noteworthy that the duration of heat significantly alters these characteristics ($p<0.01$). The whc of meat was lower for birds that were subjected to 24 h of heat, as indicated by statistical difference ($p<0.01$) with those that experienced 0 h of heat. However, the lower whc is consistent with the notion that the pH drops during maturation, for birds that were subjected to 24 h of heat, resulting in denaturation of myofibrillar proteins. Thus, this type of meat can cause irreparable disorder during industrialization providing income disabled during processing due to its difficulty in retaining water compared to regular meat. These results are similar to those reported by Fischer et al. [32] who found there was a lower whc in the breast meat of broilers reared under heat conditions.

Smoothness is a very important factor in the consumer's perception of meat quality. It is closely related to the amount of intramuscular water and, therefore, to the whc of the meat, so that the higher the water content in the muscle set, the greater the tenderness of the meat. According to the results obtained, it appears that the 72 h duration of heat caused more tenderness in the meat compared to other periods of 0-24 h, coinciding with a high whc value.

Table 3 shows the results obtained for the analysis of lightness (L*), redness (a*), yellow intensity (b*), pH, and r value of broiler chicken meats at 21 days of age. It can be observed that there was only a significant correlation between the temperature (thermoneutral and heat) and the duration of heat with the pH variable ($p<0.01$) the breakdown of wich is shown in Table 4. Notably, the pH value of the meat at 21 days after heat for 24 h was significantly lower ($p<0.05$) than that for the control group. The low pH value in animals that were subjected to heat may be related to the acceleration of glycolysis reactions post mortem. Muscle tissues with pH values below 5.8 about 15 min after slaughter, when the carcass is still at near-physiologic temperatures, usually undergoes partial protein denaturation, impairing its functional properties by Tankson et al. [33].

The lightness (l*), intensity of yellow (b*), and redness (a*) of broiler chicken meat at 21 days, showed no significant differences between the temperatures (thermoneutral and heat) tested and were not influenced by the period of exposure to high temperature ($p>0.05$). Regarding the red content (a*), a significant increase ($p<0.05$) in the chest of the birds that remained in a hot environment for 48-72 h compared to birds that were not exposed to heat is observed. Most authors found a higher red content in the muscles of birds reared in cold weather, reporting that the environmental temperature has a greater influence on the development of muscle fiber type.

The r value differed ($p<0.05$) between temperature (thermoneutral and heat) and duration of the exposure. This value represents an indirect measure of the depletion of ATP in the muscle during the development of rigor mortis, with r becoming greater as the ATP is

	Tbars (mg TMP/Kg am)	whc (%)	sf (kgf/cm²)	wlc (%)
Temperature (T)				
Thermoneutral	0.021	70.90A	0.90A	30.44A
Heat	0.266	69.86A	0.99A	31.12A
F test	190.00**	1.78^{NS}	0.88^{NS}	0.80^{NS}
Duration in hours of heat (D)				
0	0.077	73.71A	1.17A	30.97A
24	0.109	68.37B	1.02A	31.44A
48	0.148	68.49AB	0.86AB	31.27A
72	0.243	70.95AB	0.74B	29.44A
F test	34.04**	10.62**	3.38**	1.45^{NS}
F Int. TxD	30.39**	0.72^{NS}	0.35^{NS}	0.18^{NS}
CV (%)	11.37	3.77	16.71	8.55

Averages in the same column followed by the same letter do not differ by Tukey`s test (5%); **(p<0.01); CV = Coefficient of Variation; NS = Not Significant.

Table 1: Averages obtained for thiobarbituric acid reactive substances (tbars), water holding capacity (whc), shear force (sf), and weight loss by cooking (wlc) of broiler meat at 21 days of age.

Temperature (T)	Duration in hours of heat (D) – tbars (mg TMP/kg am)			
	0 h	24 h	48 h	72 h
Thermoneutral	0.026Ba	0.027Ba	0.009Aa	0.019Ba
Heat	0.085Ac	0.538Aa	0.163Ab	0.277Ab

Averages in the same column followed by the same capital letter do not differ by Tukey`s test (5%); averages in the same row followed by the same lowercase letter do not differ by Tukey`s test (5%).

Table 2: Breakdown of the interaction between temperature and duration (in hours) of heat for the means of the thiobarbituric acid reactive substances (tbars) of broiler meat at 21 days of age.

	l*	a*	b*	pH	r value
Temperature (T)					
Thermoneutral	50.19A	3.64A	2.76A	5.71	0.86B
Heat	49.54A	3.39A	2.69A	5.58	0.96A
F-test	0.56^{NS}	1.13^{NS}	0.07^{NS}	13.69**	9.37**
Duration in hours of heat (D)					
0	48.85A	2.91B	2.67A	5.79	0.73B
24	50.26A	3.22AB	2.87A	5.57	0.88A
48	50.96A	4.01A	2.53A	5.62	0.95A
72	49.44A	3.92A	2.82A	5.61	1.08A
F test	1.10^{NS}	5.21**	0.33^{NS}	7.00**	18.74**
F Int. TxD	0.17^{NS}	0.14^{NS}	0.25^{NS}	6.62**	1.69^{NS}
CV (%)	6.08	18.22	10.45	2.17	12.35

Averages in the same column followed by the same letter do not differ by Tukey`s test (5 %); ** (p < 0.01); CV = Coefficient of Variation; NS = Not Significant.

Table 3: Averages obtained for lightness (l*), redness (a*), yellow intensity (b*), pH, and r value of broiler meat at 21 days of age.

Temperature (T)	Duration in hours of heat (D) – pH			
	0	24	48	72
Thermoneutral	5.58Aa	5.99Ab	5.63Aa	5.63Aa
Heat	5.54Aa	5.61Ba	5.60Aa	5.60Aa

Averages in the same column followed by the same capital letter do not differ by Tukey's test (5%); averages in the same row followed by the same lowercase letter do not differ by Tukey's test (5%).

Table 4: Breakdown of the interaction between temperature and duration of heat (in hours) for the average pH of broiler meat at 21 days of age.

	tbars (mg TMP/kg am)	whc (%)	sf (kgf/cm²)	wlc (%)
Temperature (T)				
Thermoneutral	0.008	73.18A	1.63A	27.94A
Heat	0.065	70.07B	1.28B	27.87A
F test	179.01**	12.50**	17.72**	0.01^{NS}
Duration in hours of heat (D)				
0	0.021	72.08A	1.54AB	27.65A
24	0.031	71.83A	1.79A	27.98A
48	0.041	71.47A	1.29BC	29.08A
72	0.059	71.15A	1.19C	26.89A
F test	19.67**	0.23^{NS}	10.07**	1.76^{NS}
F Int. TxD	19.37**	1.96^{NS}	0.42^{NS}	1.24^{NS}
CV (%)	14.35	4.21	19.67	8.08

Averages in the same column followed by the same letter do not differ by Tukey's test (5%); **(p < 0.01); CV = Coefficient of Variation; NS = Not Significant.

Table 5: Averages obtained for thiobarbituric acid reactive substances (tbars), water holding capacity (whc), shear force (sf), and weight loss by cooking (wlc) of broiler meat at 35 days of age.

consumed. According to these results, it can be concluded that birds subjected to heat for 24, 48, and 72 h showed a faster decrease of ATP in the muscle, resulting in a higher r value and accelerated rigor mortis. These results were similar to those reported by Marchi et al [34] who found higher r values, for birds subjected to heat at 35°C.

The tbars, whc, sf and wlc results for broiler meats at 35 days of age are presented in Table 5. It can be seen that there is a significant correlation between temperature (thermoneutral and heat) and the duration of the heat with the tbars ($p<0.01$), whose breakdown is described in Table 6. In analyzing the breakdown of this correlation, an increased lipidoxidation is observed in the meat of the birds that were subjected to heat. These data are consistent with those found by other authors, who report that heat leads to increased generation of free radicals, resulting in higher rates of lipid oxidation in the meat. Lin et al. [35] investigated the exposure of chickens to simulated conditions heat (32°C for 6 h) and the results of this study suggest that elevated body temperature can induce metabolic disorders, causing oxidative stress and raising the tbars levels in the plasma and liver compared with the control chickens.

There was no correlation ($p>0.05$) between temperature (thermoneutral and heat) and the duration of heat for the whc, sf, and wlc at 35 days of age. There was a statistical difference ($p<0.05$) between the whc values obtained for birds at different temperatures (thermoneutral and heat).

The ability to retain water, one of the main contributors to the yield during product processing, was lower in birds stressed by heat, wich causes a reduced juiciness Fletcher [36] and lower product life Barbut [37]. The data obtained are consistent with those in the literature Woefel et al. [38] which identified a trend of reduced whc in meat originating from animals that were stressed before slaughter. The results show that the shear strength at the different temperatures (thermoneutral and heat) and the duration of heat influence the tenderness of the meat ($p<0.05$).

The softness increased in birds that were exposed to high temperatures for a heat period of 72 h, differing significantly from that at 0-24 h. The wlc of broiler meats at 35 days, showed no significant difference ($p>0.05$) for all temperatures (thermoneutral and heat) and was not affected by different periods of exposure to high temperature. The results of this study agree with the data obtained by Oba et al. [39], where the husbandry temperatures of 32 (hot), 26 (thermoneutral), and 18°C for animals at 47 days did not affect this characteristic.

Table 7 show no significant correlation ($p>0.05$) between the temperature (thermoneutral and heat) and the duration of heat for the color (l*, a*, and b*), pH and r value of chicken meat at 35 days.

The lightness (l*) was influenced ($p<0.05$) by the temperature (thermoneutral and heat) with a lower l* value being observed after heat. The length, 24 and 72 h, for which the birds were subjected to high temperature, significantly affected the yellow intensity (b*) of the meat compared to the period of 0 h heat ($p<0.05$). As seen from Table 7, there was no statistical difference ($p>0.05$) for the pH and r value, for the birds at different temperatures (thermoneutral and heat), in contrast to what was seen for the animal meats at 21 days. According to the data obtained, a lower incidence of pale breast meat was found in birds reared under stressful conditions. These results are similar to those previously described in the literature. Fischer et al. [15] found darker coloration reduced l* and less yellow reduced b*) in the meat of birds subjected to heat stress at 35°C. Bianchi et al. [40] when studying the influence of the seasons on the quality of broiler meat, observed that birds slaughtered during the summer also exhibited a pale chest which contradicts the results obtained in the present work.

Temperature (T)	Duration in hours of heat (D) – tbars (mg TMP/kg am)			
	0	24	48	72
Thermoneutral	0.004Ba	0.013Ba	0.007Ba	0.007Ba
Heat	0.021Ac	0.061Ab	0.069Ab	0.111Aa

Averages in the same column followed by the same capital letter do not differ by Tukey's test (5%); averages in the same row followed by the same lowercase letter do not differ by Tukey's test (5%).

Table 6: Breakdown of the interaction between temperature and duration of heat in hours for the average of the thiobarbituric acid reactive substances (tbars) of broiler meat at 35 days of age.

	l*	a*	b*	pH	r value
Temperature (T)					
Thermoneutral	49.92A	3.23A	0.71A	5.53A	0.97A
Heat	48.27B	3.48A	0.68A	5.59A	0.96A
F test	7.73**	0.91^{NS}	0.06^{NS}	1.69^{NS}	0.00^{NS}
Duration in hours of heat (D)					
0	49.17A	3.22A	1.38A	5.51A	1.05A
24	48.98A	3.24A	0.35B	5.66A	0.99A
48	49.05A	3.21A	0.77AB	5.56A	0.94A
72	49.18A	3.76A	0.29B	5.52A	0.88A
F test	0.03^{NS}	1.10^{NS}	8.62**	2.77^{NS}	1.73^{NS}
F Int. TxD	0.39^{NS}	0.04^{NS}	0.16^{NS}	0.47^{NS}	1.72^{NS}
CV (%)	4.07	14.05	9.03	2.53	13.49

Averages in the same column followed by the same letter do not differ by Tukey's test (5%); ** (p <0.01); CV = Coefficient of Variation; NS = Not Significant.

Table 7: Average obtained for lightness (l*), redness (a*), yellow intensity (b*), pH, and r value of broiler meat at 35 days of age.

The results obtained for tbars, whc, sf, and wlc for broiler meat at 42 days of age are shown in Table 8. There was a significant correlation ($p<0.01$) between the temperature (thermoneutral and heat) and the duration of heat for tbars, the breakdown of wich is shown in Table 9. The breakdown of this correlation, shows higher lipid oxidation in meat from birds that were subjected to heat for periods of 24, 48, and 72 h of heat compared to that of 0 h. These results suggest that the high temperature induced metabolic disorders involving oxidative stress, as was seen previously at 21 and 35 days.

There was no correlation ($p>0.05$) between temperature (thermoneutral and heat) and the duration of heat for the whc, sf, and wlc at 42 days of age (Table 8). Of the evaluated parameters only the whc was not significantly different ($p>0.05$) at all temperatures (thermoneutral and heat) and durations of heat, contrary to what occurred at 21 and 35 days.

It is observed that the meat of the birds that remained in high temperatures for 24, 48, and 72 h was stiffer, than that at 0 h ($p<0.05$), resulting in a decreased quality for this type of meat. However, higher rates of sf indicate that there was less post mortem proteolytic potential, leading to a decrease in softness.

The results indicate that the wlc in the breast muscle was affected ($p<0.05$) by the duration of the heat. Greater wlc was observed in the breasts of birds kept at 32 ± 2°C for 48 h, averaging 30.64%, than in the breasts of birds subjected to 32 ± 2°C for 0 h with an average of 26.81%. Some authors report that a faster rigor mortis leads to greater weight losses in cooking, but this hypothesis is not consistent with the results obtained in this work since the value of r was not statistically different ($p>0.05$) for the duration of heat (Table 10). Bressan and Beraquet [41] reported that the wlc in the chest muscles was influenced by the preslaughter environmental temperatures. The birds that were maintained at 30°C provided meat with wlc values (28.7%) greater than birds reared at 17°C (27.2%).

There was no correlation ($p>0.05$) between temperature (thermoneutral and heat) and the duration of heat for the color variables (l*, a*, and b*), pH, and r at 42 days of age (Table 10). The results of color (l*, a*, and b*) and r, show that the temperature (thermoneutral and heat) and the duration of the heat does not significantly alter these features ($p>0.05$). A significant increase in the pH of meat from birds that remained in the heat for 72 h, compared to periods of 0, 24, and 48 h ($p<0.05$) was observed. The pH of the muscle has been associated with other meat quality attributes, including softness, whc, wlc, juiciness, and microbial stability. The final pH of the meat is intimately connected with the glycogen concentration in the muscle moments before slaughter, as this will significantly affect the pH reduction Roça [42]. According to the results, the highest pH value results from a depletion of glycogen *in vivo*, preventing reduction of the pH post mortem. However, it can be concluded that the rigor of the muscle was not sufficient for meat processing.

	tbars (mg TMP/kg am)	whc (%)	sf (kgf/cm²)	wlc (%)
Temperature (T)				
Thermoneutral	0.010	72.32A	2.15B	28.88A
Heat	0.081	73.92A	2.74A	29.15A
F test	145.94**	1.67^{NS}	16.88**	0.11^{NS}
Duration in hours of heat (D)				
0	0.023	73.47A	1.87B	26.81B
24	0.047	72.69A	2.90A	29.60AB
48	0.049	73.63A	2.49A	30.64A
72	0.063	72.68A	2.52A	26.98AB
F test	8.26**	0.16^{NS}	8.94**	3.87*
F Int. TxD	5.19**	2.00^{NS}	2.54^{NS}	1.49^{NS}
CV (%)	10.52	5.80	15.05	9.80

Averages in the same column followed by the same letter do not differ by Tukey's test (5%); * ($p < 0.05$); ** ($p < 0.01$); CV = coefficient of variation; NS = Not significant

Table 8: Averages obtained for thiobarbituric acid reactive substances (tbars), water holding capacity (whc), shear force (sf), and weight loss by cooking (wlc) of broiler meat at 42 days of age.

Temperature (T)	Duration in hours of heat (D) – tbars (mg TMP/Kg am)			
	0	24	48	72
Thermoneutral	0.015Ba	0.005Ba	0.011Ba	0.009Ba
Heat	0.041Ab	0.083Aa	0.089Aa	0.112Aa

Averages in the same column followed by the same capital letter do not differ by Tukey's test (5%); averages in the same row followed by the same lowercase letter do not differ by Tukey's test (5%).

Table 9: Breakdown of the interaction between temperature and duration (in hours) of heat for the average of the thiobarbituric acid reactive substances (tbars) of broiler meat at 42 days old.

	l*	a*	b*	pH	r value
Temperature (T)					
Thermoneutral	49.48A	2.86A	0.59A	5.59A	0.91A
Heat	48.84A	2.57A	0.89A	5.64A	0.94A
F test	0.61^{NS}	2.19^{NS}	1.89^{NS}	0.72^{NS}	0.88^{NS}
Duration in hours of heat (D)					
0	48.92A	2.62A	0.86A	5.45B	0.94A
24	48.83A	2.55A	0.84A	5.48B	0.96A
48	48.07A	3.12A	0.80A	5.52B	0.87A
72	49.83A	2.57A	0.76A	6.01A	0.94A
F test	1.08^{NS}	1.95^{NS}	0.61^{NS}	30.51**	1.49^{NS}
F Int. TxD	0.14^{NS}	0.81^{NS}	2.64^{NS}	0.26^{NS}	0.18^{NS}
CV (%)	5.73	15.01	9.57	2.96	11.66

Averages in the same column followed by the same letter do not differ by Tukey's test (5%); ** ($p < 0.01$); CV = Coefficient of Variation; NS = Not Significant.

Table 10: Averages obtained for lightness (l*), redness (a*), yellow intensity (b*), pH, and r value of broiler meat at 42 days of age.

The results for *Salmonella* spp., *L. monocytogenes*, and *Campylobacter* spp. in the bird cloacal samples are shown in Table 1. Note that all results were negative during all experimental phases.

The results for the lactic bacteria, *E. coli,* and total enterobacteria counts on the cloacal swab samples are shown in Figures 1-3. The assay results of the 16 cloacal samples obtained using a swab from birds during their first few days of life were used to monitor the microbiological conditions that precede thermal heat in birds.

Note that there was no significant difference (Figures 1-3) in the incidence of lactic bacteria, *E. coli*, and total enterobacteria between the samples from animals that were grown at a thermoneutral temperature and those that were subjected to heat at 32°C ± 2°C. In all samples, there was a significant increase in log UFC/mL or log NMP/mL over the 21 days; however, a correlation with acute heat could not be confirmed, since both the control samples and the samples with treatment showed the same profile.

Conclusion

Although determining the population of total enterobacteria was important, as it is a group of microorganisms frequently present in the gastrointestinal tract of birds. Lactic bacteria are associated with

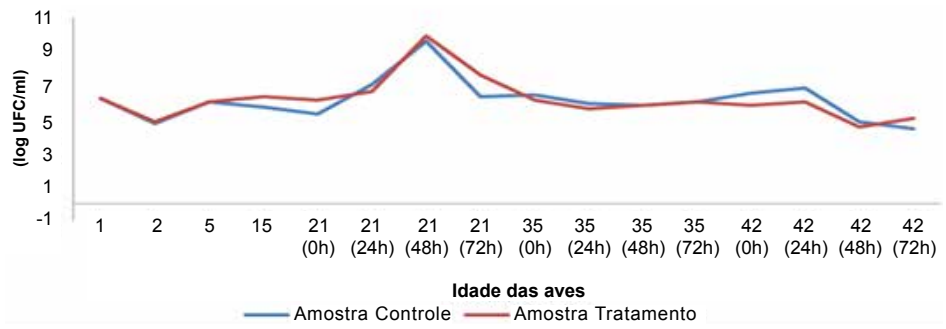

Figure 1: Lactic bacteria counts in cloacal swab samples obtained from the broilers on the following experimental days: 1, 2, 5, 15, 21, 35, and 42 (at 0 h, 24 h, 48 h, and 72 h).

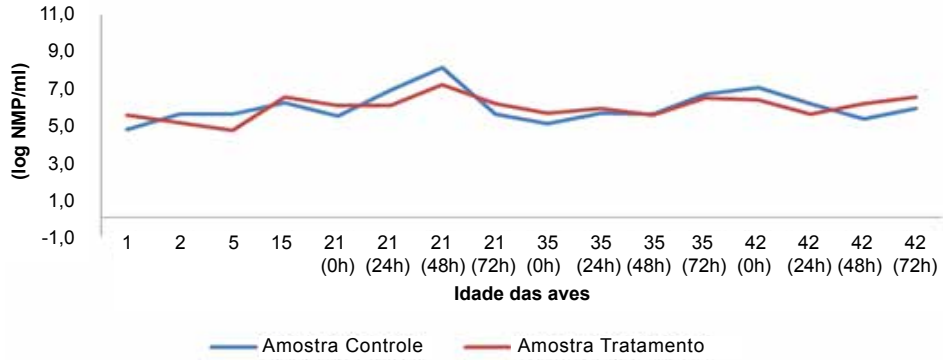

Figure 2: Results obtained for the Escherichia coli screening in cloacal swab samples from birds on the following experimental days: 1, 2, 5, 15, 21, 35, and 42 (at 0 h, 24 h, 48 h, and 72 h).

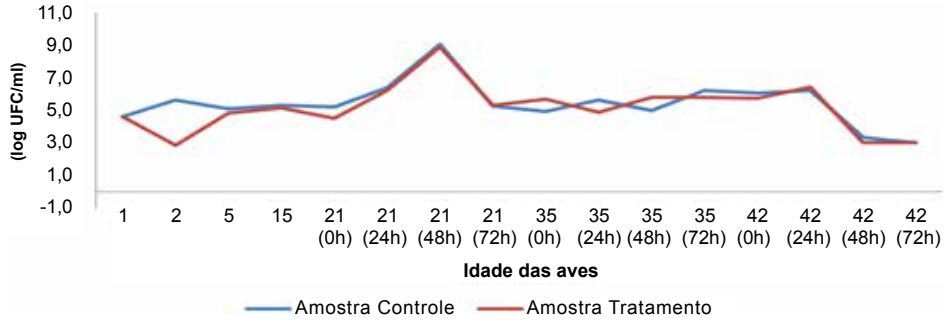

Figure 3: Results obtained from the total *Enterobacteriaceae* screening in cloacal swab samples from the broilers on the following experimental days: 1, 2, 5, 15, 21, 35, and 42 (at 0 h, 24 h, 48 h, and 72 h).

protection against pathogenic microorganisms, and determining this population was important since in conditions of heat, the number of lactic bacteria may decrease, which contributes to an increase in pathogenic bacteria in the gastrointestinal flora. Thus, decreases in the zootechnic and physiological indices of animals are expected, but this was not observed in our study using heat.

In the available literature, we did not find articles assessing the relationship between heat and the incidence of lactic bacteria, *Campylobacter* spp., *E. coli*, total enterobacteria, and *L. monocytogenes*. However, some authors suggest that after heat, there may be a greater incidence of pathogenic microorganisms, since the increase in temperature decreases the immune response of birds, leading to a greater colonization of the intestine by pathogens in these animals. Quinteiro-Filho et al. [43] verified that heat is capable of increasing *Salmonella* spp. colony forming units (UFC/g) in the spleen of animals and causes a decrease in weight gain and food consumption and an increase in mortality [44-48].

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