Physicochemical and biochemical parameters of chicken breast meat influenced by stunning methods

Rosana Aparecida da Silva-Buzanello,∗†,1 Alexia Francielli Schuch,† Diego Ricardo Nunes Nogues,† Priscila Falcão de Melo,† André Wilhan Gasparin,§ Alex Sanches Torquato,# Cristiane Canan,† and Adriana Lourenço Soares∗

∗Departamento de Ciência e Tecnologia de Alimentos, Centro de Ciências Agrárias, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, Km 6, ZIP 86.057-970, Londrina, PR, Brazil; †Programa de Pós-graduação em Tecnologia de Alimentos, Universidade Tecnológica Federal do Paraná, Avenida Brasil 4232, Parque Independência, ZIP 85884-000, Medianeira, PR, Brazil; ‡Departamento Acadêmico de Alimentos, Universidade Tecnológica Federal do Paraná, Avenida Brasil 4232, Parque Independência, ZIP 85884-000, Medianeira, PR, Brazil; §Cooperativa Agroindustrial Lar, Slaughter Supervision, BR-277, ZIP 85887-000, Matelândia, PR, Brazil; and #Departamento Acadêmico de Química, Universidade Tecnológica Federal do Paraná, Avenida Brasil 4232, Parque Independência, ZIP 85884-000, Medianeira, PR, Brazil

ABSTRACT The influence of stunning methods on the physicochemical and biochemical parameters of chicken breast meat, as indicators of bird stress, was investigated. A total of 200 Cobb broiler chickens aged from 42 to 48 d were submitted to gas or electrical stunning and slaughtered according to the standard industry practice. Pectoralis major muscles (24 h post-mortem) from broilers stunned by electronarcosis exhibited a higher L∗ and R-value and lower pH45min than did those from gas stunning, indicating modification of the glycolytic rate. Protease activity, measured as the myofibril fragmentation index, and the sarcoplasmic Ca2+ concentration were highest in samples from broilers stunned by electronarcosis, suggesting the greatest activity of the calpain system. In the fatty acid profile, a higher ratio of polyunsaturated fatty acids was observed in samples from broilers stunned by electronarcosis. These characteristics are related to phospholipase A2 activity, which is higher in animal stress conditions. These results indicated that the gas-stunning method produced less bird stress than electrical stunning.

Key words: gas stunning, electronarcosis, fatty acid profile, sarcoplasmic Ca2+, myofibril fragmentation index

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INTRODUCTION

Stunning is the first stage of slaughter and is considered essential to the attendance of animal welfare. Stunning methods are regulated by Brazilian and international legislation with the aim to prevent unnecessary suffering of the animal (European Commission, 1993; Brazil, 2000; European Food Safety Authority, 2009).

Electrical stunning by an electronarcosis water bath is commercially more common for inducing broiler chicken insensibility. The method consists of the application of electrical current (high voltage and low amperage) through the animal’s brain and causes instantaneous unconsciousness before slaughter (Kettlewell and Hallworth, 1990; Raj, 2004; Petracci et al., 2010). Previous research has demonstrated the low efficiency of the electronarcosis stunning method in broiler chickens and greater animal stress. Changes in carcass appearance, such as broken bones, hemorrhages, and blood pooling, have been correlated to electronarcosis, mainly due to the use of high electrical amperage. These alterations have also been attributed to the hanging and manipulation of conscious birds (Craig et al., 1999; Hoen and Lankhaar, 1999; Fletcher, 2002; Savenije et al., 2002).

Therefore, governments and animal protection organizations have suggested the gas-stunning method as a substitute to electrical stunning (Petracci et al., 2010; Gerritzen et al., 2013). In gas stunning, the animal is taken into a closed chamber containing an anesthetic gas, usually carbon dioxide or argon, that is applied in isolation or in combination with other gases, and stunning is delivered via cerebral anoxia (Raj et al., 1990; Raj, 1998).

The 2-phased CO2 system is a practical alternative to the electronarcosis method. In this method, the broiler is kept under narcosis first, with a 40% maximum CO2 concentration and decreasing O2 levels. The increase in CO2 concentration (up to 80%) in the second phase
induces a deeper state of unconsciousness until slaughter (Gerritzen et al., 2013).

Few studies have reported the influence of the stunning method on chicken meat quality. Raj et al. (1990) observed that a gas-stunning method using argon and carbon dioxide produced relatively better-quality carcasses with low muscle hemorrhage, suitable pH decline, and more tender breast meat than an electrical method. However, more physical, chemical, and biochemical parameters are necessary to deduce the real effect of stunning method on chicken meat quality.

Animal stress can change the sequence of post-mortem biochemical events and alter physicochemical and biochemical parameters in chicken breast meat, as reported by Soares et al. (2003, 2009), Santos et al. (2012), and Wilhelm et al. (2010), who studied PSE (pale, soft, and exudative) meat. These authors reported the greatest values of sarcoplasmic Ca$^{2+}$, phospholipase $\text{A}_2$ (PLA$_2$) activity, myofibril fragmentation index (MFI), and unsaturated fatty acid amounts in PSE chicken breast meat. These alterations to chicken breast meat can be attributed an altered sequence of biochemical events, preceded by liberation of sarcoplasmic Ca$^{2+}$. The presence of the ion activates the calpain system responsible for meat tenderness (Koohmaraie, 1992) and PLA$_2$ activity, which catalyzes the hydrolysis of the phospholipid membrane. This hydrolysis results in the release of fatty acids, increasing the ratio of unsaturated fatty acids in meat (Murakami and Kudo, 2002). Thus, these physicochemical and biochemical properties could be used to evaluate the relationship between stunning method and animal stress.

In this study, the influence of stunning method on the physicochemical and biochemical parameters of chicken breast meat was evaluated as an indicator of animal stress.

**MATERIALS AND METHODS**

**Sample Preparation**

The experiment was performed in the winter of 2016 at a commercial processing plant located in the state of Paraná, Brazil. This processing plant slaughters 340,000 broilers/day in 2 lines at a rate of 12,000 broilers/hour, operating in 3 shifts. The birds of the strains Cobb Fast and Cobb Slow of both genders (44 to 48 d old) and with an average weight of 2.84 ± 0.14 kg were slaughtered according to routine industrial slaughtering practices: electrical or gas stunning, bleeding, scalding, defeathering, evisceration, and carcass water cooling. The Ethical Committee of Animal Research of the State University of Londrina, Brazil, approved this research (protocol number: 3158.2016.57).

**Experimental Design**

The study was performed using a randomized block design applied in 5 lots of broiler chickens, and each lot came from the same farm and truck cargo. Half of the birds in each lot were stunned by electronarcosis or a gas-stunning method, and 20 birds/treatment/block were collected ($n = 200$).

Electronarcosis was the electrical-stunning method used in this study and is the usual method in a commercial processing plant. A 3-m-long water-bath stunning with the capacity to stun 19 birds at once was used. A water-bath electrified saline solution, 180 V, 400 Hz, and approximately 150 mA per broiler in 5.7 s were the experimental conditions applied.

Multistage carbon dioxide gas stunning was applied using a CO$_2$ supply tank (White Martins, Praxair Inc.) and a test chamber with the outer dimensions 2,600 x 2,200 x 2,500 mm (length x width x height). The broiler chickens were taken to the test chamber inside their transport containers immediately after unloading the truck. Thirty transport containers containing 8 birds each were added into the chamber test, for a total of 240 stunned birds per batch. The stunning method was performed in a 2-phase CO$_2$ system in a 5-stage scheme and took 6 min to complete. The broilers were exposed at CO$_2$ gradual concentrations of 20, 30, 35, 40, and 60% by 60 s at each stage, except for the last batch, which lasted 120 s. They were immediately suspended from shackles after stunning and subsequently followed routine industrial slaughtering practices.

After evisceration (45 min post-mortem), 25 carcasses per treatment ($n = 50$) were collected and the pH was determined under the upper extremity of chicken breasts on the right side in triplicate. This analysis permits evaluation of the post-mortem decrease in pH.

After water cooling, the carcasses were deboned manually, and the chicken breast fillets (Pectoralis major m.) were collected. The chicken breast left sides were stored in a cold room at 5°C and submitted to analysis of color ($L^*$, $a^*$, and $b^*$), pH, Warner-Bratzler (WB) shear force, and water holding capacity (WHC) at 24 h post-mortem. The chicken breast right sides were frozen using tunnel freezing (−35°C) and maintained at −18°C until determination of the MFI, sarcoplasmic Ca$^{2+}$ content, R-value, and fatty acid profile.

**Physicochemical and Biochemical Parameters**

**Color Measurements** The color $L^*$ (lightness), $a^*$ and $b^*$ (CIELAB color system) were evaluated using a Minolta CR400 colorimeter (Osaka, Japan) with a D65 illuminant and a 10° standard observer on chicken breast meat 24 h post-mortem. The determination was performed on the underside surface of the intact skinless fillets to avoid scalding effects and measured on 3 different sides—proximal and distal extremity of the muscle and the medial side at the halfway point between extremities (Olivo et al., 2001).

**pH Measurements** For each sample, the pH was measured in triplicate at 45 min and at 24 h
post-mortem by inserting electrodes into the breast muscle using a pH meter (Hanna, HI 99,163), as reported in Ólivo et al. (2001).

**Warner-Batzler (WB) Shear Force** Raw samples of chicken breast fillets were cut into pieces of 1 cm × 1 cm × 2 cm (height × width × length), and the length followed the fiber direction. The samples were sheared in triplicate using a WB shear-type blade with a 4 mm s⁻¹ test speed coupled in a TA-HD plus texture analyzer (Stable Micro Systems, Surrey, UK) fitted with a 5-kg load cell. The blade cut the fibers across, and the maximum force measured to cut was expressed in Newtons (N).

**Water Holding Capacity (WHC)** The WHC was determined according to Hamm (1960) with modifications in triplicate. The samples were weighed (2.0 ± 0.10 g), placed between 2 filter papers and left under a 10.0 kg weight for 5 min. The samples were weighed again, and the WHC was determined via exudated water weight using Equation 1.

\[
\text{WHC} = 100 - \left( \frac{i - f}{i} \times 100 \right)
\]

where \(i\) is the initial weight and \(f\) is the final weight.

**Myofibril Fragmentation Index (MFI)** The MFI was determined via indirect measurement of calpain activity according to Culler et al. (1978). Frozen chicken breast meat free of external fat and visible connective tissue was cut off (4 g) and homogenized in 20 mL of cold MFI buffer (100 mM KCl, 20 mM potassium phosphate, 1 mM EDTA, 1 mM MgCl₂, and 1 mM Na₃) at pH 7.0 for 1 min. The homogenate was centrifuged at 6,000 rpm for 25 min at 2°C. The supernatant was collected and stored in an ice bath. The sediment was resuspended in 20 mL of cold MFI buffer and centrifuged at 6,000 rpm for 25 min at 2°C. The supernatant was added to the first fraction, and the sediment was resuspended in 10 mL of cold MFI buffer, homogenized, and filtered. The filtered solution was added to the collected supernatants.

The myofibril suspension was strained, and the protein concentration was determined by the Biuret method. The absorbance of suspension aliquots in MFI buffer with a final protein concentration of 0.5 mg mL⁻¹ was determined at 540 nm in an ultraviolet-visible spectrophotometer (Perkin Elmer, Lambda XLS Beaconsfield, UK). The MFI was expressed as \(A_{540 \text{ nm}} \times 200\).

**Sarcoplasmic Ca²⁺ Concentration** The sarcoplasmic Ca²⁺ levels in chicken breast meat (\(n = 50\)) were determined in duplicate using 10 g of samples in 25 mL of 150 mM KCl according to Cheah et al. (1986). The samples were homogenized in a vortex mixer for 2 min and centrifuged at 6,000 rpm for 4 min. The supernatant was collected and centrifuged at 26,000 g for 4 min. Then, 1 mL of supernatant was collected and added to 4 mL of 0.5% lanthanum solution. Ca²⁺ was quantitatively analyzed by atomic absorption spectrophotometry at 422.7 nm (Varian, AA240FS, USA) using calcium carbonate as a standard.

**R-value** The R-value was determined according to Honikel and Fisher (1977) with modifications. One aliquot of 3 g of frozen chicken breast meat (\(n = 30\)) was homogenized with 5 mL of 1 M perchloric acid in a vortex mixer for 30 s. The homogenate was filtered, and 0.1 mL of the filtrate was diluted into 4.9 mL of phosphate buffer, pH 7.0. The absorptions at 250 and 260 nm were measured using phosphate buffer as a reference. The R-value was calculated from the \(A_{250 \text{ nm}}/A_{260 \text{ nm}}\) ratio, which represents the ratio between inosine 5’-monophosphate (IMP) and adenosine 5’-monophosphate (ATP).

**Fatty Acid Profile** Lipids were extracted according to the method of Bligh and Dyer (1959) with modifications. Chicken breast meat samples were crushed using a food processor (Philco, 800 w, Brazil), and 15 g of samples were used in lipid extraction with moisture correction to 80%. The samples were homogenized in methanol (30 mL) and chloroform (15 mL) for 5 min. Chloroform (15 mL) was added to the mixture, and the homogenization continued for 2 min. Distilled water (15 mL) was added to the mixture, and the homogenization continued for 5 min. The homogenate obtained was filtered and transferred to a separation funnel. A saturated solution of NaCl equivalent to 1/5 of the filtrate was added to a separation funnel. After phase separation, the lower phase containing chloroform and fatty matter was collected, and the solvent was evaporated in a rotatory evaporator (801, Fisatom, Brazil) with a bath at 33 ± 2°C.

Transesterification of fatty acids was achieved according to method 5509 of the International Organization for Standardization (International Organization for Standardization, 1978). First, 200 mg of fatty matter extracted were transferred to 10-mL tubes with a screw cap, 2 mL of n-heptane was added, and the mixture was stirred until complete dissolution of fatty matter. Then, 2 mL of 2 M NaOH in methanol were added to the mixture, and it was submitted to vigorous agitation to obtain a slightly turbid solution. After phase separation, the superior phase containing n-heptane and fatty acid methyl esters (FAME) was collected, transferred to 2-mL vials, and stored in a freezer (–18°C) for later chromatographic analyses.

FAMEs were analyzed by gas chromatography (Perkin Elmer, Clarus 680 GC, Waltham, MA) with flame ionization detection and a fused silica capillary column (100 m × 0.25 mm) with 0.25 μm of a cyanopropyl polysiloxane CP 7420 stationary phase. The carrier gas was helium (1.1 mL min⁻¹), and the flame gases were hydrogen and synthetic air (40 and 400 mL min⁻¹, respectively). The split was 1:150, and the column temperature was set to 80°C for 1 min; ramped at 20°C min⁻¹ to 160°C, at 1°C min⁻¹ to 198°C, and at 5°C min⁻¹ to 250°C; and held at 250°C for 1.6 min. The injector and detector temperatures were set at 240 and 250°C, respectively. For peak area...
Table 1. Comparison of physicochemical and biochemical parameters between chicken breast meat (Pectoralis major m.) samples from broilers stunned by the gas or electrical method.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Stunng method</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH(_{45\text{min}})</td>
<td>Gas</td>
<td>Electrical</td>
</tr>
<tr>
<td>pH(_{24\text{h}})</td>
<td>6.62 ± 0.16</td>
<td>6.50 ± 0.16</td>
</tr>
<tr>
<td>L*</td>
<td>5.96 ± 0.12</td>
<td>5.95 ± 0.12</td>
</tr>
<tr>
<td>b*</td>
<td>55.12 ± 2.46</td>
<td>55.88 ± 2.37</td>
</tr>
<tr>
<td>WHC (%)</td>
<td>11.06 ± 1.63</td>
<td>11.29 ± 1.56</td>
</tr>
<tr>
<td>WHB (N)</td>
<td>67.82 ± 2.96</td>
<td>67.47 ± 2.04</td>
</tr>
<tr>
<td>Ca(^{2+}) (µg g(^{-1}))</td>
<td>9.10 ± 1.47</td>
<td>9.23 ± 1.44</td>
</tr>
<tr>
<td>MFI</td>
<td>78.64 ± 7.06</td>
<td>100.35 ± 6.47</td>
</tr>
<tr>
<td>R-value</td>
<td>1.30 ± 0.03</td>
<td>1.36 ± 0.03</td>
</tr>
</tbody>
</table>

WHC: water holding capacity; Ca\(^{2+}\): sarcoplasmic calcium concentration; MFI: myofibril fragmentation index; R-value: inosine 5’-monophosphate (IMP)/adenosine 5’-triphosphate (ATP) ratio.

Means ± standard deviation.

*P-values ≤ 0.05 indicate significant difference by the F test (ANOVA).

Results and Discussion

The values of pH\(_{45\text{min}}\) and L* were significantly different (P ≤ 0.05) between the stunning methods (Table 1). The pH\(_{45\text{min}}\) was higher in chicken breast from broilers stunned by the gas method than by the electrical method, and the inverse was observed for L* values. These results suggested that electrical stunning might decrease the pH in the initial post-mortem phase until 45 min but not affect the final pH\(_{24\text{h}}\). Lower pH\(_{45\text{min}}\) and higher L* values are related to conditions of animal stress before or during slaughter (Guarnieri et al., 2004).

However, there was a greater incidence of pH\(_{24\text{h}}\) ≤ 5.8 in chicken breast meat from broilers stunned by the electrical method (15%) than the gas (8%) method. This observation can be visualized in Figure 1, which shows histograms of pH\(_{24\text{h}}\) from broilers stunned by both methods. According to Kato et al. (2013), pH ≤ 5.8 is indicative of PSE meat that is correlated with animal stress before and during slaughter. Nevertheless, the correlation between the values of L* and pH\(_{24\text{h}}\) post-mortem might be considered in meat classification (Li et al., 2014; Zhao et al., 2016).

L* value has been used to classify chicken breast meat color as pale (L* > 53), dark (L* < 46), and normal (46 < L* < 53) (Qiao et al., 2001). Figure 2 shows histograms of L* values of chicken breast meat, where a normal distribution of the data was observed. The L* values varied from 46 to 60 in samples from broilers stunned by gas and from 48 to 60 in samples from broilers stunned by the electrical method. Mean values of L* > 53 were obtained in both experimental conditions, indicating a predominance of pale chicken breast meat, and the incidence of L* > 53 values was greater in samples from broilers stunned by the electrical method (81%) compared with gas (63%) method. The literature uses L* and pH\(_{24\text{h}}\) values to classify chicken breast meat as normal (pH > 5.80 and L* > 53), PSE (pH ≤ 5.80 and L* ≤ 53) (Soares et al., 2003), and pale (pH > 5.80 and L* > 53) (Almeida et al., 2016). In the present study, a high incidence of pale chicken breast meat with pH > 5.8 was observed: in 70 and 72% of samples from broilers stunned by the gas and electrical methods, respectively. Recently, many Brazilian slaughterhouses have correlated a high incidence of pale chicken breast to normal pH. A new category of broiler breast meat quality has been suggested, but the cause of this variation has not yet been fully elucidated (Almeida et al., 2016).

Therefore, L* values were not used to classify PSE chicken breast meat in this study; only pH\(_{24\text{h}}\) was used (pH ≤ 5.80). In samples from broilers stunned by the gas method, 8% were characterized as PSE, while in samples from broilers stunned by electrical method, 15% were characterized as PSE. Thus, there was a greater incidence of chicken breast meat PSE from broilers stunned by the electrical method than the gas method.

The physicochemical parameters a*, b*, WHC, and WB shear force were not different (P > 0.05) between the treatments (Table 1). The values of a* and b* varied, respectively, from –2.57 to –2.42 and from 11.5 to 11.29, in samples from broilers stunned by the gas and electrical methods. Similar results were reported by Zhuang et al. (2010), where a* and b* values of –0.4 and 11.5, respectively, were obtained in chicken breast meat deboned 2 h post-mortem. The WHC values varied from 67.82 to 67.47% and were intermediate between those reported by Wilhelm et al. (2010), who obtained WHC values of 69.12 and 65.66% in chicken breast meat characterized as control and PSE, respectively.

Nevertheless, the biochemical parameters of Ca\(^{2+}\) and MFI differed (P ≤ 0.05) and were higher in samples from broilers stunned by the electrical method than the gas method. These biochemical parameters are indirect determinations of post-mortem enzymatic activity, as the calpain systems are activated in the presence of Ca\(^{2+}\) (Koolhmaare, 1992). A concentration of 10 µM Ca\(^{2+}\) is sufficient to enhance the activity of the endogenous muscle protease system. The µ-calpain activity is the first to increase at higher ion concentrations.

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until 6 h post-mortem (Lee et al., 2008). During maturation, \( \mu \)-calpain activity is ceased due to autolysis, and the \( \mu / m \) calpain proteolytic system begins (Lee et al., 2007). The actuation of the calpain system results in proteolysis of the sarcomere structure (Koohmaraie and Geesink, 2006), which can be visualized using the MFI. Conditions of animal stress can induce greater sarcoplasmic \( Ca^{2+} \) (Soares et al., 2003), thus affecting the MFI values. These considerations explain the results obtained in the present study.

Although the MFI varies between the treatments, the WB shear force does not \( (P > 0.05) \), varying from 9.10 to 9.23 N for gas and electrical stunning, respectively, which indicates tender meat.

The R-value also differed between treatments \( (P \leq 0.05) \) and was higher in samples from chickens stunned by electronarcosis than the gas method. This biochemical parameter represents the ratio between IMP and ATP. Low concentrations of ATP and high levels of IMP result in high R-values, which could suggest an accelerated rate of post-mortem metabolism (Alanini et al., 2013). Therefore, the results obtained in the present study indicated that glycolytic rate might have been more accelerated by electronarcosis than the gas-stunning method, corroborating with previous reports.

### Table 2. Pearson correlation between post-mortem biochemical parameters of chicken breast meat \( (Pectoralis major m.) \) from broilers stunned by the gas or electrical method.

<table>
<thead>
<tr>
<th></th>
<th>R-value</th>
<th>MFI</th>
<th>( Ca^{2+} )</th>
<th>( pH_{24, h} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( pH_{45, min} )</td>
<td>0.07</td>
<td>-0.44**</td>
<td>-0.07</td>
<td>0.20</td>
</tr>
<tr>
<td>( pH_{24, h} )</td>
<td>-0.63**</td>
<td>-0.33**</td>
<td>-0.43**</td>
<td></td>
</tr>
<tr>
<td>( Ca^{2+} )</td>
<td>0.46**</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFI</td>
<td>0.14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-value</td>
<td></td>
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</tbody>
</table>

\( Ca^{2+} \): sarcoplasmic calcium concentration, MFI: myofibril fragmentation index, R-value: inosine 5'-monophosphate (IMP)/adenosine 5'-triphosphate (ATP) ratio.  

\*\* \( P < 0.05 \).  
\* \( P < 0.01 \).  

Pearson’s correlations between the measurements of \( pH \) at 45 min and 24 h post-mortem, \( Ca^{2+} \), MFI, and R-value are listed in Table 2.

A significant negative correlation was observed between \( pH_{45\, min} \) and MFI \( (P < 0.001) \), \( pH_{24\, h} \) and R-value \( (P < 0.001) \), \( pH_{24\, h} \) and MFI \( (P < 0.05) \), and \( pH_{24\, h} \) and \( Ca^{2+} \) concentration \( (P < 0.001) \). The sarcoplasmic \( Ca^{2+} \) concentration regulates the proteolysis and, consequently, the lactic acid produced (Koohmaraie, 1992), resulting in decreased \( pH \). Thus, this sequence of biochemical events influences the glycolytic rate and consequently the MFI, \( pH \), and R-values, justifying the

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Histograms of the \( pH_{24\, h} \) values of chicken breast meat from broilers stunned by the gas (a) or electrical (b) method.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Histograms of the \( L^* \) values of chicken breast meat from broilers stunned by the gas (a) or electrical (b) method.
negative correlations observed. Additionally, a significant positive correlation ($P < 0.001$) was observed between the Ca$^{2+}$ concentration and R-value, demonstrating the significant influence of calcium ions on the glycolytic rate.

The levels of palmitoleic acid (16:1n-7) and oleic acid (18:1n-9c) were lowest ($P \leq 0.05$) in samples from broilers stunned by the electrical method (Table 3), which can be related to the lipid oxidation that develops after lipolysis (Chen et al., 2010).

The levels of eicosapentaenoic acid (20:5n-3) and arachidonic acid (20:4n-6) were greater ($P \leq 0.05$) in chicken breast meat from broilers stunned by the gas rather than electrical method, according to quality parameters observed in chicken breast meat.

**CONCLUSIONS**

The stunning methods changed the physicochemical and biochemical parameters of chicken breast meat. The gas-stunning method produced less bird stress than electrical stunning, according to parameters observed to quality parameters observed in chicken breast meat.

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