Comparison of lipid oxidation, messenger ribonucleic acid levels of avian uncoupling protein, avian adenine nucleotide translocator, and avian peroxisome proliferator-activated receptor-γ coactivator-1α in skeletal muscles from electrical- and gas-stunned broilers


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ABSTRACT The aim of this study was to compare the effects of stunning methods [electrical stunning (ES) vs. gas stunning (GS)] on lipid oxidation in broiler meat and to investigate possible mechanisms of lipid oxidation by measuring plasma variables, muscle reactive oxygen species (ROS), and TBA reactive substance (TBARS) concentrations, muscle fiber ratios, and mRNA levels of avian uncoupling protein (avUCP), avian adenine nucleotide translocator, and avian peroxisome proliferator-activated receptor-γ coactivator-1α (avPGC-1α). Arbor Acres broilers (n = 36) were not stunned (control) or were exposed to the following stunning treatments: 40% CO2 + 21% O2 + N2; 60% CO2 + 21% O2 + N2; 35 V, 47 mA, 400 Hz; 50 V, 67 mA, 160 Hz; and 65 V, 86 mA, 1,000 Hz. The ROS level in tibialis anterior (TA; \( P < 0.05 \)) and the TBARS concentration in pectoralis major (PM; \( P < 0.01 \)) were decreased in the GS groups compared with the ES groups at 45 min postmortem. However, the TBARS concentrations at 24 h postmortem in the PM and TA groups were not affected by stunning method (ES or GS). Compared with ES, GS caused greater expression of avUCP mRNA (1.47-fold in PM, and 2.41-fold in TA) and avPGC-1α mRNA (1.42-fold in PM, and 2.08-fold in TA). In conclusion, the upregulation of avUCP and avPGC-1α reduced ROS accumulation and lipid oxidation at 45 min postmortem in the skeletal muscles of broilers stunned with hypercapnic moderate oxygenation GS. However, these changes were not sufficient to cause a difference in meat lipid oxidation at 24 h postmortem between broilers stunned with hypercapnic moderate oxygenation GS and those stunned with low-current, high-frequency ES.

Key words: broiler, electrical stunning, gas stunning, gene expression, lipid oxidation

INTRODUCTION

Recently, the traditional way of inducing unconsciousness in poultry by electrical stunning (ES) has been challenged by the newly emerging method of gas stunning (GS, including controlled-atmosphere stunning) in the industry. This has aroused much interest in comparing meat quality between poultry stunned with ES and GS. However, no conclusive results have yet been reached. Electrical stunning may impair meat quality by delaying rigor mortis (Raj et al., 1990, 1997, 1998; Raj and Nute, 1995). However, these studies have generally involved the use of high currents (e.g., 80, 105, or 150 mA) and low frequencies (50 or 60 Hz). High currents and low frequencies have been reported to have negative effects on carcass and meat quality (Xu et al., 2011c). Therefore, a low-current, high-frequency combination may be a good choice for ES. Recently, increased oxygen concentrations have been observed with hypercapnic GS, bringing advantages in welfare, carcass quality, and meat quality over hypercapnic anoxic GS (McKeegan et al. 2007). However, when the effects on meat quality of ES and GS are compared, previous studies have been concerned mostly with high-current, low-frequency ES and (hypercapnic) anoxic GS. Our previous studies indicated that meat quality (Xu et al., 2011a, c) and energy metabolism (Xu et al., 2011b) were essentially no different between ES and GS when low-current, high-frequency ES was compared with hypercapnic moderate oxygenation GS (\( Q_0 = 21\% \)). In the present study, we hypothesized that the lipid oxidation caused by ES would be no worse than that caused by GS if ES were based on...
a low current with a high frequency and GS were based on hypercapnic moderate oxygenation. If this were true, both ES and GS may be suitable for the broiler slaughter industry, and neither one would need to be rejected.

Lipid oxidation is one of the primary mechanisms of quality deterioration in meat products, especially in flavor, color, texture, nutritive value, and possibly the production of toxic compounds (Gray et al., 1996). Lipid oxidation is known to be affected by the stunning method and by the combination of stunning and meat packaging method in lambs (Linares et al., 2007; Börnez et al., 2009), or by the combination of stunning and bleeding method in chickens (Alvarado et al., 2007). However, only one stunning treatment was presented for ES and GS in a previous study (Alvarado et al., 2007), providing limited information on the effect of stunning method on lipid oxidation. Moreover, the molecular mechanism between lipid oxidation and pre-slaughter stunning is not clear.

Lipid oxidation occurs when the redox homeostasis is imbalanced. Usually it is initiated at the membrane level, with the intracellular phospholipid fractions attacked by reactive oxygen species (ROS). The production of ROS in skeletal muscles can be increased in cases of heat exposure (Mujahid et al., 2007; Lin et al., 2008), electrical stimulation (McArdle et al., 2001; Pattwell et al., 2004), exercise (Jackson, 2005), or other stresses in which a high metabolic rate is presented (Brookes et al., 1998). It is well known that intense movements (e.g., wing flapping, kicking) and muscle contractions (e.g., clonic or tonic contraction) happen in the process of stunning. Therefore, we suspect that stunning methods may affect ROS production and may cause changes in lipid oxidation in broilers.

The primary factor governing mitochondrial ROS generation is the redox state of the respiratory chain, which is inherently controlled by the transmembrane proton gradient (ΔpH) and membrane potential (Δψm; Brookes, 2005). Thus, one potential way to reduce ROS formation is to decrease the membrane potential by a proton (H+) leak (Brookes, 2005), which could be induced by the uncoupling proteins (Skulachev, 1996) and the adenosine monophosphate/adenine nucleotide translocator pathway (Brookes, 2005). Another broad and powerful regulator of ROS metabolism is PGC-1α (peroxisome proliferator-activated receptor γ coactivator-1α), which can protect cells from some serious oxidative stressors (St-Pierre et al., 2006). We hypothesized that these proteins could mediate ROS production and lipid oxidation in broilers stunned with ES and GS.

The aims of the present study were to compare the effects of stunning methods (low-current, high-frequency ES vs. hypercapnic moderate oxygenation GS) on lipid oxidation in broiler meat and to investigate the possible mechanism of lipid oxidation from the view of ROS production and mediation. This study is of importance to the broiler slaughter industry.

**MATERIALS AND METHODS**

**Birds and Housing**

Arbor Acres broilers (males, n = 200) were raised from 1 d of age until slaughter (49 d) in the chicken house of the Feed Research Institute. All the chickens were managed according to the Arbor Acres Broiler Commercial Management Guide (Aviagen Group, 2009). Water and feed were provided ad libitum until capture on the 49th day.

**Experimental Design**

The stunning (GS and water-bath ES) systems and stunning treatments were set up according to the procedure of Xu et al. (2011b). Birds (n = 36) weighing 2.50 ± 0.25 kg were selected and randomly allocated to the following treatments: without stunning (control); 40% CO2 + 21% O2 + N2 (G40%); 60% CO2 + 21% O2 + N2 (G60%); 35 V, 47 mA, 400 Hz (E35V); 50 V, 67 mA, 160 Hz (E50V); 65 V, 86 mA, 1,000 Hz (E65V). Each individual bird was stunned for 18 s with electricity or for 90 s with a gas mixture. Each treatment was replicated 6 times, with 1 bird as a replicate. All the experimental procedures were approved by the Animal Care and Use Committee of the Feed Research Institute of the Chinese Academy of Agricultural Sciences.

**Sampling**

Exsanguination was performed without stunning (control) or immediately after stunning (treatments) by severing the jugular vein and carotid artery on one side of the neck. Bleeding was allowed for 3 min. Blood (5 mL) was collected in heparinized centrifuge tubes during the bleeding and was cooled in ice water. Blood was centrifuged at 1,800 × g (4°C) for 10 min, and the supernatant was taken for the detection of plasma insulin, cholesterol, triglyceride, and nonesterified fatty acid (NEFA).

Carcasses were skinned and sampled within 45 min postmortem. Muscles (about 10 g each) of the pectoralis major (PM) and musculus iliobibularis (MI) were taken in duplicate from the left side of the carcass to measure TBA reactive substances (TBARS). One portion of the samples was immediately stored at −20°C, and the other portion was stored for 24 h at 4°C, and then transferred to storage at −20°C until analysis. Samples (approximately 0.2 g each) from the left PM and tibialis anterior (TA) muscles were also collected in duplicate and put into 0.5-mL deoxyribonuclease/ribonuclease-free tubes to detect gene expression. Samples from the left PM and TA were wrapped in aluminum foil for measurement of ROS levels (about 4 g) and fiber ratios (0.5 × 0.5 × 2.0 cm, in duplicates). The MI and TA belong to the hind muscles, and they are composed of similar fibers (fast-twitch glycolytic fibers,
slow-twitch oxidative fibers, and fast-twitch oxidative glycolytic fibers) that are distinct from breast muscles, which are composed predominantly of fast-twitch glycolytic fibers. Moreover, the fiber shape of the TA is regular. Therefore, the TA was measured for fiber type ratios and gene expression as representative of the hind muscle. Samples for gene expression, ROS, and fiber ratio analysis were immediately placed in liquid N, and subsequently stored at −80°C.

**Measurements**

**Plasma Variables.** Plasma concentrations of insulin were measured with an insulin RIA kit (RK-119, Institute of Radioimmunity, General Hospital of Chinese PLA, Beijing, China; Dong et al., 2003). Plasma NEFA, triglyceride, and cholesterol concentrations were determined with commercial diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) by using an automatic biochemical analyzer (CE-CX5, Beckman Corp., Fullerton, CA).

**Fiber Ratios.** Fiber ratios were analyzed by frozen sectioning and NADH tetrazolium reductase staining according to the procedures of Zhang et al. (2009), with the following modification. Section images were taken in the bright field of Spot RTke software (Spot Diagnostics, Sterling Heights, MI) after adjusting for white balance.

**TBARS.** Muscle TBARS concentrations were measured and expressed as malondialdehyde (MDA) concentrations with a colorimetric diagnostic kit (Nanjing Jiancheng Bioengineering Institute; Zhang et al., 2010), and muscle protein was measured with the biuret method using a total protein reagent (Nanjing Jiancheng Bioengineering Institute) according to the supplier’s manual. The TBARS concentration was calculated as micromoles of MDA per 100 g of protein.

**ROS.** Reactive oxygen species levels were detected with the electron spin resonance method according to the procedure of Zhang et al. (2010). A spin-trap solution was prepared with final concentrations of 50 mM of \(N\)-tert-butyl-\(\alpha\)-phenylnitrone (Sigma-Aldrich Inc., St. Louis, MO), 2 mM diethylenetriamine-pentacetic acid, 10 mM HEPES, 0.05% Tween-80, and 5 mM \(\beta\)-mercaptoethanol in PBS (pH 7.4). The sample (0.25 g) was homogenized in 1.0 mL of spin-trap solution in an ice bath, incubated in a 40°C water bath for 1.5 h, combined with ethyl acetate (400 μL), vortexed for 30 s, and centrifuged for 10 min at 13,200 × g at 4°C. The ethyl acetate phase was kept at 4°C. The ethyl acetate phase in the dark and detected with a Bruker ER200D-SRC spectrometer (Bruker, Billerica, MA) the following day. The relative intensity of ROS was expressed as the peak height (cm) of each electron spin resonance signal.

**Gene Expression.** The mRNA expression levels of \(\alpha\)vUCP (avian uncoupling protein), \(\alpha\)vANT (avian adenine nucleotide translocator), and \(\alpha\)vPGC-1\(\alpha\) (avian peroxisome proliferator-activated receptor \(\gamma\) coactivator-1\(\alpha\)) were determined using real-time quantitative reverse transcription PCR according to the procedures of Zhang et al. (2010). The mRNA levels were normalized as the ratio to \(\beta\)-actin mRNA in arbitrary units by using the \(2^{-\Delta\Delta C_T}\) method (Livak and Schmittgen, 2001). The SE of each treatment was calculated using data on the relative mRNA expression from a specific gene.

**Statistical Analysis**

All the data were analyzed with one-way ANOVA of SAS, version 8.02 (SAS Institute, 2001), with each chicken as a replicate. Means were separated by Fisher’s multiple range test. Results are presented as means ± SE. A level of \(P < 0.05\) was defined as significant unless otherwise stated.

**RESULTS**

**Plasma Variables**

The effects of stunning methods on the plasma variables of broilers are shown in Table 1. No consistent difference was observed in the plasma variables (cholesterol, triglyceride, insulin, and NEFA) between broilers stunned with ES and those stunned with GS. However, plasma cholesterol and triglyceride were affected by the stunning treatments. Compared with the control, plasma cholesterol was increased in all the stunning groups except for E65V \((P < 0.05)\), with the highest value appearing in G60% \((3.23 \text{ mmol/L})\), whereas plasma triglyceride was decreased only in E65V as compared with the control group \((P = 0.01)\). Plasma insulin and NEFA were not affected by the stunning treatment \((P > 0.05)\).

**ROS and TBARS**

The effect of stunning method on muscle ROS is shown in Figure 1. Muscle ROS was increased in the TA muscle of ES-stunned broilers as compared with GS-stunned birds \((P < 0.05)\). Reactive oxygen species production in the PM muscle was increased with a relatively high current (E65V) compared with ROS in the 2 GS groups \((P < 0.05)\), whereas the levels remained the same between broilers stunned with GS (G40% and G60%) and those stunned with low-current ES (E35V and E50V). A low frequency (E50V) resulted in the lowest level of ROS in both the PM and TA muscles \((2.07 \text{ and } 2.43 \text{ cm})\), respectively) compared with the high frequencies (E35V and E65V). Reactive oxygen species levels in the TA were decreased with G40% and increased with E35V and E65V \((P < 0.05)\) compared with the control, whereas the ROS level in the PM was increased only with E65V \((P < 0.05)\).

The effect of stunning method on muscle TBARS (expressed as MDA) is displayed in Table 2. At 45 min postmortem, the MDA in PM was increased in broilers stunned with ES (E35V, E50V, and E65V) compared...
with GS (G40%, and G60%, P < 0.01). At 45 min post-mortem, the TBARS in PM was increased in E50V and E65V (P < 0.01) compared with the control, whereas it was not affected by the other stunning treatments (P > 0.05). Concentration of MDA in the TA muscle at 24 h postmortem tended to increase (P = 0.08) with E50V compared with G60% and E65V. However, no significant difference was observed between GS and ES in the PM at 24 h postmortem or the MI at 45 min and 24 h postmortem.

In addition, the ratios of fiber types I, IIA, and IIB were not consistently different between broilers stunned with ES and those with GS (data not shown). Compared with the control, the ratio of fiber type I increased, whereas the ratio of fiber type IIB decreased in the stunning treatments (P < 0.05).

**Gene Expression**

The mRNA expression levels of avUCP, avPGC-1α, and avANT are displayed in Figures 2, 3, and 4, respectively. Expression levels of avUCP mRNA in both the PM and TA muscles were upregulated with G60% (P < 0.01) compared with the control, and avUCP levels in the PM were also increased with G40% and E50V (P < 0.01). However, mRNA levels of avUCP were generally increased in the PM (1.47-fold) and TA (2.41-fold) with the GS treatments as compared with the ES treatments. The avPGC-1α levels in the PM (P < 0.05) and TA (P < 0.01), and avANT levels in both the PM and TA (P < 0.01) were upregulated with G40%, G60%, and E50V compared with the control. The average mRNA levels of avPGC-1α in the PM and TA were, respectively, 1.42- and 2.08-fold higher with GS than with ES. The average mRNA levels of avANT were slightly higher with GS than with ES (1.32- and 1.23-fold in the PM and TA, respectively).

**DISCUSSION**

Stress-induced lipid peroxidation is related to the blood lipid profile, including concentrations of cholesterol and triglyceride (Lata et al., 2004). In the present study, stunning broilers with ES and GS had no significant effect on blood variables, suggesting that essentially no difference in the plasma lipid profile resulted from the stunning method (ES or GS). The E65V treatment resulted in the lowest plasma cholesterol (2.81 mmol/L) and triglyceride (0.84 mol/L) concentrations among the different stunning treatments. However, stunning with E65V may have accelerated the uptake of NEFA from the plasma, resulting in no influence on the homeostasis of plasma NEFA.

Myofibers are classified into types I (slow twitch-oxidative), IIA (fast twitch-oxidative-glycolytic), and IIB (fast twitch-glycolytic; Brooke and Kaiser, 1970; Larzul et al., 1997) after staining using the NADH tetrazolium reductase method. Fiber type composition is closely related to meat quality (Lefaucheur, 2010) because it affects the release and sequestration of Ca²⁺ in muscle, adenosine triphosphatase activities, adenine nucleotide content, stimulation of glycolysis, production of lactate, decrease in postmortem pH (Bowker et al., 2003; Lefaucheur, 2010), and concentrations of myoglobin and cytochromes, which affect meat color (Arany, 2008). Fiber ratios were not changed by ES or GS in the pres-
ent study, indicating no effect of stunning method on meat quality through a switch in the fiber type. Although the fiber ratios were different among the stunning groups, it was not possible that this was a result of a fiber type switch for the reason that avPGC-1α, the trigger of fiber type conversion, was not consistent with the observed fiber ratios. In addition, fiber type switches were reported to occur in response to long-term stresses, including long-duration hypercapnia gas stimulation (Kumagai et al., 2001), electrical stimulation (Leeuw and Pette, 1993), and cold exposure (Ueda et al., 2005). After short-term stunning, the mitochondrial biogenesis and NADH dehydrogenase (in complex I) activities may be augmented, causing increased stain precipitation in the sections and visual changes in the fiber ratios. The change in fiber ratios may indicate mitochondrial biogenesis caused by muscle contractions during the stunning procedures.

Lipid oxidation was assessed on the basis of TBARS and the ROS production in the present study. The ROS generally referred to are $\mathrm{H_2O_2}$ and $\mathrm{O_2}^\cdot$, along with the extremely reactive $\mathrm{OH}^\cdot$ radical (Grivennikova and Vinogradov, 2006). The formation of ROS in mitochondria requires high membrane potential and increases exponentially above 140 mV (Lee et al., 2002). The ES treatments supplied an electromotive force of 35 to 65 V (160 to 1,000 Hz), and this may have enhanced the mitochondrial membrane potential in the mitochondrial inner membrane and may have increased the generation of ROS in the hind muscle as compared with ROS in all the GS groups. The ROS level in the breast, being different from the hind muscle, was significantly increased only with the high-current, high-frequency treatment (E65V) as compared with the ROS level of the GS groups. Additionally, ROS level was increased in the stunning groups with a high frequency (E35V

### Table 2. Effects of stunning method on TBA reactive substances (TBARS) in broilers

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>G40%</th>
<th>G60%</th>
<th>E35V</th>
<th>E50V</th>
<th>E65V</th>
<th>SE</th>
<th>P-value</th>
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<tr>
<td>Pectoralis major</td>
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<td>MDA at 45 min, μmol/100 g</td>
<td>47.91bc</td>
<td>41.48c</td>
<td>39.12c</td>
<td>53.69b</td>
<td>63.65a</td>
<td>72.90a</td>
<td>2.40</td>
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<td>MDA at 24 h, μmol/100 g</td>
<td>43.8</td>
<td>43.4</td>
<td>45.7</td>
<td>47.1</td>
<td>39.7</td>
<td>41.6</td>
<td>1.03</td>
<td>0.35</td>
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<td>Musculus iliofibularis</td>
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<tr>
<td>MDA at 45 min, μmol/100 g</td>
<td>59.4</td>
<td>67.7</td>
<td>61.4</td>
<td>65.2</td>
<td>73.3</td>
<td>66.1</td>
<td>1.77</td>
<td>0.28</td>
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<tr>
<td>MDA at 24 h, μmol/100 g</td>
<td>48.4ab</td>
<td>49.1ab</td>
<td>44.1b</td>
<td>47.8ab</td>
<td>54.2a</td>
<td>41.7b</td>
<td>1.03</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*a*–cMeans within a row with no common superscripts differ significantly ($P < 0.05$).

1Control = without stunning; G40% = 40% CO$_2$ + 21% O$_2$ + N$_2$; G60% = 60% CO$_2$ + 21% O$_2$ + N$_2$; E35V = 35 V, 47 mA, 400 Hz; E50V = 50 V, 67 mA, 160 Hz; E65V = 65 V, 86 mA, 1,000 Hz.

2TBARS was measured and expressed by malondialdehyde (MDA; μmol of MDA per 100 g of protein) in the skeletal muscles.

**Figure 2.** Effect of stunning method on relative mRNA expression of avian uncoupling protein (avUCP) in the skeletal muscle of broilers. Control = without stunning; G40% = 40% CO$_2$ + 21% O$_2$ + N$_2$; G60% = 60% CO$_2$ + 21% O$_2$ + N$_2$; E35V = 35 V, 47 mA, 400 Hz; E50V = 50 V, 67 mA, 160 Hz; E65V = 65 V, 86 mA, 1,000 Hz. Results were normalized to β-actin mRNA levels. Values are expressed as means ± SE. Within the same muscle, data points lacking common letters (x–z; a, b) differ significantly ($P < 0.05$).

**Figure 3.** Effect of stunning method on the relative mRNA expression of avian peroxisome proliferator-activated receptor-γ coactivator-1α (avPGC-1α) in the skeletal muscle of broilers. Control = without stunning; G40% = 40% CO$_2$ + 21% O$_2$ + N$_2$; G60% = 60% CO$_2$ + 21% O$_2$ + N$_2$; E35V = 35 V, 47 mA, 400 Hz; E50V = 50 V, 67 mA, 160 Hz; E65V = 65 V, 86 mA, 1,000 Hz. Results were normalized to β-actin mRNA levels. Values are expressed as means ± SE. Within the same muscle, data points lacking common letters (x–z; a–c) differ significantly ($P < 0.05$).
and E65V) compared with the group with a low frequency (E50V) in both the breast and hind muscles. These changes agreed with a previous report indicating that increasing the frequency of electrical stimulation of a muscle cell culture increased the generation of NO and the hydroxyl radical activity (Pattwell et al., 2004). Consistent with the production of ROS, the production of MDA at 45 min was lower in the GS groups compared with the ES groups. However, the MDA concentration did not differ between GS and ES after 24 h of storage at 4°C. One possible reason was that electrical stimulation might induce a consequent increase in superoxide dismutase and catalase activities (McArdle et al., 2001), which may protect the muscle from damage by ROS in the ES groups. Similarly, ES and GS had no effect on lipid oxidation at 24 h in suckling lambs (Linares et al., 2007; Bórnez et al., 2009). In a study of a 15-min electrical stimulation of muscle fiber in vivo, McArdle et al. (2001) also observed no evidence of damage (reflected by representative hematoxylin- and cosin-stained histological sections) to skeletal muscle for up to 7 d postmortem. However, Alvarado et al. (2007) reported a decrease in TBARS in the PM muscle of ES-stunned broilers as compared with GS-stunned broilers. The discrepancy may have been due to differences in the experimental arrangement and in data processing. In the present study, this change in ROS and MDA concentrations at 45 min was not sufficient to cause a difference in meat lipid oxidation at 24 h postmortem.

The increased expression levels of avUCP and avPGC-1α in the breast and hind muscles led to lower ROS and MDA levels at 45 min postmortem with GS as compared with ES. One reason may be that PGC-1α induced the expression of some ROS-detoxifying enzymes such as glutathione peroxidase 1 and superoxide dismutase 2 (St-Pierre et al., 2006), which helped to scavenge the ROS in skeletal muscles. Another reason may be that the increase in avUCP eventually helped to reduce the formation of ROS in the PM muscle through uncoupling. The increased expression levels of avUCP and avPGC-1α also explained why the ROS level was lower in E50V compared with E35V and E65V. Similarly, ROS production was reported to be potentially downregulated by avUCP in fasted (Abe et al., 2006) and heat-stressed chickens (Mujahid et al., 2009). The increase in avANT mRNA was slight (1.32- and 1.23-fold) in the GS treatments and was not significantly different from the levels in most ES treatments. Hence, its function in preslaughter stunning is not clear. Some antioxidant systems may be activated by the stress of slaughter, leading to a decrease in MDA concentration at 24 h postmortem, as compared with 45 min postmortem, in both the breast and hind muscles. Although ROS and TBARS concentrations were higher in the ES treatments at 45 min postmortem, both ES and GS resulted in similar lipid oxidation in skeletal muscles at 24 h postmortem. This suggested that antioxidation was enhanced by ES during meat storage from 45 min to 24 h postmortem, but the reason remains unknown.

In conclusion, the upregulation of avUCP and avPGC-1α reduced ROS accumulation and lipid oxidation at 45 min postmortem in skeletal muscles of GS broilers stunned with hypercapnic moderate oxygenation gas. However, ES (low currents with high frequencies) and hypercapnic moderate oxygenation GS resulted in similar levels of meat lipid oxidation in the breast and hind muscles at 24 h postmortem. Further research is needed to study the influence of stunning method on the antioxidant system and its effect on prolonged meat storage.

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