

# Evaluation of the quality characteristics of nitrogen gas-stunned chicken meat and small intestine

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## Abstract

This study aimed to confirm the applicability of the new nitrogen (N<sub>2</sub>) gas stunning method in the broiler slaughtering process by comparing the meat and small intestine quality following different stunning methods (electrical, carbon dioxide (CO<sub>2</sub>), N<sub>2</sub>, and halal). Four treatments were compared: (i) electrical stunning (Elec), (ii) 80% CO<sub>2</sub> gas stunning (CO<sub>2</sub>-gas), (iii) 98% N<sub>2</sub> gas stunning (N<sub>2</sub>-gas), and (iv) the non-stunning method (halal). N<sub>2</sub> gas stunning (98%) and the halal method were conducted at the pilot plant abattoir of the national institute of animal science, Korea, and electrical and 80% CO<sub>2</sub> stunning were performed on the nearest commercial slaughter house. Meat pH<sub>24h</sub>, color (lightness, redness and yellowness), proximate composition, water holding capacity (WHC), cooking loss, and Warner-Bratzler shear force (WBSF) were measured, and in the small intestine, pH<sub>24h</sub>, color, thickness, and WBSF were measured. The Elec treatment showed high lightness, yellowness, and low redness in both meat and the small intestine, indicated by a pale color; the CO<sub>2</sub>-gas treatment showed high redness, low lightness, and low yellowness, and the coloration of meat from the N<sub>2</sub>-gas treatment was intermediate between Elec and CO<sub>2</sub>-gas. For other quality traits, the N<sub>2</sub>-gas showed good results and was between Elec and CO<sub>2</sub>-gas. Additionally, severe stress (low pH in both meats), low WHC in meat, and cracked small intestine with numerous apertures were observed in the CO<sub>2</sub>-gas, and pale colored hemorrhagic breast meat was found in the Elec. Therefore, in view of animal welfare and quality traits of meat and the small intestine, 98% N<sub>2</sub> gas can be considered in broiler stunning.

**Keywords:** Slaughtering process, Gas stun, Nitrogen-stunning, Chicken breast meat, Small intestine

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#### Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

#### Authors' contributions

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#### Ethics approval and consent to participate

The experiment was performed at the research of the National Institute of Animal Science of Korea (NIAS). The animal care and use committee of NIAS reviewed and approved the protocol for this study (Approval Number: NIAS20191536).

## INTRODUCTION

Livestock slaughterhouses in Korea use electricity and carbon dioxide (CO<sub>2</sub>) stunning methods during slaughter. Chicken slaughterhouses have also introduced CO<sub>2</sub> stunning methods due to improved animal welfare compared with the traditional electric method. Electrical stunning is the most common pre-slaughter stunning method used for animals in the meat industry. Among the different types of electrical stunning systems, electrical water bath stunning is commonly practiced in commercial poultry plants [1]. It is expedient, inexpensive, has few space users, and can be readily tailored to commercial processing plants [2]. To induce unconsciousness in a water bath system, the EU recommends a minimum current of 120 mA per chicken [3]. This recommended minimum current causes muscle contraction, broken bones, and breast muscle hemorrhage in broilers [4–6]. On the other hand, Islamic countries perform slaughtering without stunning, following religious requirements, using the halal method [7].

Gas stunning can be considered an alternative method to the pre-slaughter stunning system to maintain animal welfare and meat quality, and is widely used in traditional electric-based slaughterhouses [8]. According to Raj et al. [9], gas use as a novel method for stunning animals has increased daily. Minimizing the stressful handling of awake broilers using gas stunning prior to electrical stunning in a commercial slaughterhouse increases animal welfare and reduces the need for workers [10]. CO<sub>2</sub> is commonly used in pre-slaughter stunning of broiler chickens [11] and pigs [12]. CO<sub>2</sub> has been demonstrated to be aversive to chickens and induces suffering and pain [13,14]. Mice, rats, and pigs also have also shown an aversion to CO<sub>2</sub> stunning [15–20], including irritation of the nasal mucosal membranes [18], severe respiratory distress [20], and strong head shaking [21]. Additionally, meat and small intestine discoloration (dark-red colored) occurs following the CO<sub>2</sub> stunning method [22], so the market value of CO<sub>2</sub> stunned meat and by-products, especially the intestine, is low in Korea. According to KOSIS [23], ~77,000 cows, 1.5 million pigs, and 84 million chickens are slaughtered each month in Korea. The intestines of animals have been widely utilized globally for the food ingredients and production of casings [24,25]. Therefore, any deterioration in the quality of intestines due to gas stunning can render them unsuitable for sale, leading to potential economic losses. Consequently, it is necessary to study an alternative to the CO<sub>2</sub> stunning method that does not affect the quality of meat or animal welfare.

On the other hand, inert gases, such as argon (Ar) or nitrogen (N<sub>2</sub>), have been used in different proportions in conjunction with CO<sub>2</sub> in the stunning of animals to help reduce CO<sub>2</sub> aversion [20,26]. Exposure to high concentrations of inert gases has been found to induce hypoxic conditions in animals [18,27]. The displacement of oxygen (O<sub>2</sub>) in the atmosphere by a high concentration of inert gases can create hypoxic conditions with less than 2% O<sub>2</sub>, leading to a decrease in blood oxygen levels of animals [28]. This, in turn, affects the central nervous system (CNS) and induces unconsciousness to animal [29]. N<sub>2</sub> is the most abundant gas in the air (78%), and can be used widely for commercial purposes due to its low price. Different concentrations of N<sub>2</sub> (70%–92%) can be mixed with CO<sub>2</sub> to maintain hypercapnic-hypoxia conditions for pig stunning [30,31]. Poultry showed less aversion to stunning with low atmospheric pressure and N<sub>2</sub> than CO<sub>2</sub>, which may offer a significant welfare refinement [32]. No adverse effects on the quality traits of meat or the small intestine were observed using the high-concentration N<sub>2</sub> gas stunning of pigs [22]. According to the syncope method, in broilers, fewer stress hormone changes occurred following N<sub>2</sub> stunning than with the CO<sub>2</sub> and halal methods [33]. Therefore, it is expected that N<sub>2</sub> gas could be effective in preventing excessive discoloration and softening compared to conventional stunning methods. However, the feasibility of using only high concentrations of N<sub>2</sub> gas (98%) in the stunning of birds (broiler chickens) and its effects on the quality traits of meat and the small intestine have not yet

been investigated.

The objective of this study is to evaluate the impact of high-concentration N<sub>2</sub> (98%) stunning on the quality of meat and intestines in poultry, compared to conventional stunning methods such as electrical stunning, CO<sub>2</sub> stunning, and halal stunning. Additionally, the study aims to validate the industrial applicability of high-concentration N<sub>2</sub> stunning.

## MATERIALS AND METHODS

### Animal care

The experiment was performed at the research of the National Institute of Animal Science of Korea (NIAS). The animal care and use committee of NIAS reviewed and approved the protocol for this study (Approval Number: NIAS20191536).

### Experimental design and facilities

An experimental trial was conducted at the NIAS, RDA, Korea to assess the quality of hypoxic chicken breast meat and small intestine following N<sub>2</sub> gas-stunning and to compare it with meat stunned using standard electrical- and high-CO<sub>2</sub>-based stunning and non-stunning (halal) methods. Four treatments were used: (i) electrical stunning (Elec-stun), (ii) 80% CO<sub>2</sub> gas stunning (CO<sub>2</sub>-gas), (iii) 98% N<sub>2</sub> gas stunning (N<sub>2</sub>-gas), and (iv) the non-stunning method (halal). An electrical- and high-CO<sub>2</sub>-based stunning (80%) trial was conducted at the nearest contact commercial slaughter house; a high-N<sub>2</sub> gas stunning (98%) and a non-stunning (halal) method trial was conducted at the NIAS pilot plant abattoir. Forty Cobb-500 commercial broiler chickens were assigned to each treatment (n = 40). The chickens were collected from the nearest commercial farm. Just before the day of the experiment, broilers were transported to the institutional abattoir and stored in a commercial slaughterhouse. The chickens rested for 12 h in pens with *ad-libitum* water and finisher diet. Fasting conditions were maintained overnight before slaughter. Body weight was measured during the evening of slaughter. The body weight range was 2.51–2.68 kg at 42 days of age.

In the electrical stunning and non-stunning (halal) methods, single broilers were used, whereas gas stunning (both CO<sub>2</sub> and N<sub>2</sub>) was performed on a crate containing eight broilers. Electrical stunning was performed within the contact commercial slaughterhouse, using a water bath stunner delivering a constant current of 120 mA (50 Hz in the form of sinusoidal waveform) for 4–5 s. In the case of the halal method, according to the rules of Islam, broilers were slaughtered without stunning [34]. The chickens in the CO<sub>2</sub> and N<sub>2</sub> treatment groups were stunned using specially designed gas chamber. A modern digital gas chamber (length 220 cm × width 100 cm × height 135 cm, Supreme Thermal Instrument [STI], Daegu, Korea) was used for gas stunning. The CO<sub>2</sub> or N<sub>2</sub> gases flow was continued into the pit of the chamber until the desired concentration (80% or 98%) was reached. After reaching the desired concentration level, the gas flow was stopped, and the time (s) counting was started until the stunned state was reached. It was found that within 70 s, broilers were completely stunned by 80% CO<sub>2</sub>, and in the case of N<sub>2</sub> (98%), it required approximately 110 s for complete stunning.

### Slaughtering and sample collection

After gas stunning, each crate of broilers was removed from the gas chamber as quickly as possible and heart activity was measured using an electrocardiogram (ECG) with two adhesive patches (Unilect, 5 cm in diameter; Unomedical, Stonehouse, UK) placed on the left and right pectoralis muscles under the wing base, and then slaughtered within 30 s. In electrical stunning, each

stunned chicken was kept on a movable table, heart activity was measured using ECG, and then the chicken was slaughtered within 15–20 s. After slaughter, for proper bleeding, one leg (hock joint) of each broiler was tied with an iron chain and hung for 2–3 min. This process was observed in all treatments, including those treated with the halal method. Subsequently, chickens from the different treatments were inserted separately in a pluckier machine for feather plucking. After 7–8 min, the chickens were removed from the pluckier and eviscerated manually. Individual carcasses were cleaned, washed with tap water, and stored in polythene bags. The small intestine (100 cm) was collected from each broiler in every treatment ( $n = 40$ ). After cleaning and washing, each small intestine was placed in a polythene bag. All carcasses and small intestines were stored in a cold room overnight at 2°C. After chilling, all carcasses and small intestine samples were transported (after slaughter at 18 h) to the laboratory, and then the breast muscle of all carcasses were obtained for further study.

### pH measurement

To determine the pH of the raw meat, 5 g of the sample was mixed with 50 ml of distilled water using a hand blender. For the small intestine, 3 g of sample was mixed with 30 ml of distilled water. The resulting sample solutions were then measured using a digital pH meter (FP 20, Mettler Toledo, Greifensee, Switzerland). The pH readings were taken four times for every sample of chicken breast meat and small intestine.

### Surface color values

The measurements of the surface color was carried out samples of the raw chicken breast meat and the raw and cooked small intestine were bloomed in air at room temperature for ~40 min. The surface color were measured using a chroma meter (CR-400, Minolta Camera, Osaka, Japan). First, the chroma meter calibrated using a white plate ( $Y = 86.32$ ,  $X = 0.3165$ , and  $y = 0.3242$ ). In the chicken breast meat, for each rectangular sample (size 12 × 5 cm), color values were measured from 8 different sections on the surface and bone-side. For the small intestine, 100 cm of a freshly cut clean small intestine was measured from 8 different sections, and there were intervals of 10 cm minimum between different locations. The color values were reported as CIE L\*, CIE a\*, and CIE b\*.

### Proximate components

The Proximate components were measured by using a Food Scan Lab 78810 (Foss Tecator Hillerød, Denmark). Approximately 100 g of raw chicken breast meat was blended using a hand mixer and used as a sample. The sample was measured in triplicates.

### Water-holding capacity

In measurement the WHC of a raw chicken breast meat, the sample was ground, and subsequently, any fat, fibers, and coarse particles were removed. The fine meat (0.5 g) was inserted into an ultra-centrifugal tube. These tubes were heated at 80°C for 20 min using water-bath. The next step in determining the WHC of the meat sample involved using a centrifugal machine (Avanti(R) J-E, Beckman coulter, Brea, CA, USA). The ground samples were centrifuged for 10 min at a speed of 470×g at 4°C. After centrifugation, the tubes were allowed to stand for 10 min at room temperature. The samples were weighed, and the WHC of the chicken breast meat was calculated using a formula. The above method was performed twice for each sample, and the average value was used for the study [35].

### Cooking loss and Warner-Bratzler shear force

To measure the CL of the chicken breast meat, they were weighed and placed in heat-durable plastic bags. The mouths of the bags were folded and blocked using steel clips. Then, these sample was heated using a water bath at 71.3°C. The inner temperature of each sample was evaluated using a thermorecorder. When the core temperature reached 70°C, the samples were transferred an ice water for cooling for 30 min. Next step, the surface of cooked samples remove moisture with absorbant tissue paper. The cooked samples were weighed, and the CL was determined using a formula.

The WBSF were measured using an Instron Universal Testing Machine (5543, Instron Corp., USA). The WBSF for chicken breast meat was prepared using a sample with completed CL measurements. From cooked chicken breast meat, five samples (length 2.0–2.5 cm, diameter 1.25 cm) were made using a metal corer according to the muscle fiber direction. In the small intestine, WBSF was determined in both fresh and cooked samples. For samples of fresh small intestine, each 5–7 cm of samples from the small intestine was taken and directly cut using an Instron machine. To measure the WBSF of the cooked small intestine, a clean sample of 50 cm in length was taken from each small intestine. Also, in case of the cooked sample, the fresh small intestine were cooked under the same heating conditions as meat (cooking time, ~40 min) and were then used to measure the WBSF. Machine with a speed of 200 mm/min and a load cell of 40 N; a 1 cm distance was maintained from one cut to another.

### Thickness of small intestine

The thickness was measured using a digital caliper scale of both fresh and cooked small intestines. The sample was longitudinally spread on a white plastic board. Readings were obtained from six different locations of the small intestine. The minimum interval distance from one location to another was 5–7 cm. The cooking conditions used to prepare the cooked samples were equivalent to the CL of chicken breast meat, however, the cooking time was ~40 min.

### Statistical analysis

The Statistical Analysis System (SAS) package was used for data analysis. The mean, standard deviation, and *p*-value were calculated for all treatments. Duncan's multiple range test was used. Differences were considered statistically significant at a *p* value of < 0.05.

## RESULTS AND DISCUSSION

### pH<sub>24h</sub> and surface color of chicken breast meat and small intestine under different stunning methods

Table 1 shows the pH<sub>24h</sub> for chicken breast meat and small intestine with stunned and non-stunned. The results revealed significant differences in the pH<sub>24h</sub> of the chicken breast meat and small intestine among different stunning methods. Among the stunning treatments, the pH<sub>24h</sub> was comparatively high in the Elec-stun and low in the CO<sub>2</sub>-gas compared to other treatments (*p* < 0.05). The pH<sub>24h</sub> of halal was also significantly higher than that of all other stunning treatments in both the chicken breast meat and the small intestine.

The pH level of the chicken breast meat and small intestine is one of the indicators that change according to stunning methods and reflects the biochemical state of the muscle following the development of rigor mortis [1,5]. Several reports imply that during stunning and exsanguination, muscular activity is elevated and glycolysis is increased, resulting in the accretion of lactic acid in at the muscle decreased the pH level [9,36,37]. The accumulation of lactic acid in the cells

**Table 1.** Effect of stunning (with electric, CO<sub>2</sub> gas and N<sub>2</sub> gas) and non-stunning halal method on pH<sub>24h</sub> and color value of chicken meat and small intestine

Items	Treatments <sup>1)</sup>			
	Elec-stun	CO <sub>2</sub> -gas	N <sub>2</sub> -gas	Halal
pH <sub>24h</sub>				
Meat	6.10 ± 0.08 <sup>b</sup>	5.88 ± 0.08 <sup>d</sup>	6.04 ± 0.03 <sup>c</sup>	6.26 ± 0.06 <sup>a</sup>
Small intestine (Fresh)	6.38 ± 0.06 <sup>b</sup>	6.20 ± 0.06 <sup>d</sup>	6.28 ± 0.04 <sup>c</sup>	6.62 ± 0.06 <sup>a</sup>
Color value				
Meat				
L*	55.17 ± 3.02 <sup>b</sup>	51.45 ± 2.26 <sup>c</sup>	54.62 ± 1.24 <sup>b</sup>	58.63 ± 0.98 <sup>a</sup>
a*	2.90 ± 0.35 <sup>c</sup>	4.12 ± 0.23 <sup>a</sup>	3.57 ± 0.08 <sup>b</sup>	1.72 ± 0.15 <sup>d</sup>
b*	5.89 ± 1.45 <sup>a</sup>	4.55 ± 0.74 <sup>c</sup>	5.46 ± 0.17 <sup>b</sup>	6.19 ± 0.06 <sup>a</sup>
Small intestine (Fresh)				
L*	60.11 ± 1.19 <sup>b</sup>	54.94 ± 0.86 <sup>d</sup>	58.29 ± 0.62 <sup>c</sup>	62.66 ± 0.73 <sup>a</sup>
a*	11.37 ± 0.56 <sup>c</sup>	16.03 ± 0.51 <sup>a</sup>	13.47 ± 0.11 <sup>b</sup>	9.8 ± 0.42 <sup>d</sup>
b*	13.44 ± 0.68 <sup>b</sup>	8.58 ± 0.56 <sup>d</sup>	12.55 ± 0.61 <sup>c</sup>	14.24 ± 0.18 <sup>a</sup>
Small intestine (Cooked)				
L*	69.93 ± 1.30 <sup>b</sup>	63.78 ± 0.75 <sup>d</sup>	67.64 ± 0.99 <sup>c</sup>	71.56 ± 0.71 <sup>a</sup>
a*	5.44 ± 0.22 <sup>c</sup>	8.61 ± 0.34 <sup>a</sup>	6.49 ± 0.11 <sup>b</sup>	4.65 ± 0.46 <sup>d</sup>
b*	15.27 ± 0.49 <sup>b</sup>	10.62 ± 0.78 <sup>d</sup>	14.57 ± 0.28 <sup>c</sup>	16.11 ± 0.19 <sup>a</sup>

<sup>1)</sup>Treatments: Elec-stun, electrical stunning method; CO<sub>2</sub>-gas, 80% CO<sub>2</sub> gas stunning method; N<sub>2</sub>-gas, 98% N<sub>2</sub> gas stunning method; Halal, non-stunning method. n = 40.

<sup>a-d</sup>Different superscript letters in same row means significant differences ( $p < 0.05$ ).

of chicken muscle is closely associated with pre-slaughter stress [38]. The detection of stunning stress in animals has been attempted by some researchers through the use of blood samples [2,33]. Exposing hogs to an 80% concentration of CO<sub>2</sub> for 70 s results in the production of large amounts of lactic acid in the blood [39]. In pigs exposed to 80% CO<sub>2</sub> for 60 s, lactic acidosis, hyperglycemia, hypercapnia, and increased hematocrit levels were observed [40]. According to Channon et al. [41], the pH decline of pork loin was faster in electrically-stunned pig meat than after CO<sub>2</sub> stunning. However, the pH of pork meat with N<sub>2</sub> stunning was higher than that with CO<sub>2</sub> stunning, which was slightly lower than that with electrical stunning [22]. Similar to a previous study, the pH in chicken breast and small intestine was higher in N<sub>2</sub>-stunned chickens than in CO<sub>2</sub>-stunned chickens, and lower than in electrically-stunned chickens.

The surface color of chicken breast meat and small intestines is presented in Table 1. In chicken breast meat, the L\* and b\* was significantly highest in the halal treatment, while it was the lowest in the CO<sub>2</sub>-gas treatment ( $p < 0.05$ ). On the contrary, CO<sub>2</sub>-gas treatment had the highest a\*, while halal had the lowest a\* value ( $p < 0.05$ ). N<sub>2</sub>-gas treatment showed higher L\* and b\* values and lower a\* values compared to CO<sub>2</sub>-gas treatment ( $p < 0.05$ ). Compared to N<sub>2</sub>-gas treatment, Elec-stun treatment showed similar L\* value ( $p > 0.05$ ), lower a\* value ( $p < 0.05$ ), and higher b\* value ( $p < 0.05$ ). In both raw and cooked intestines, halal treatment had the highest L\* and b\* values, while CO<sub>2</sub>-gas treatment exhibited the lowest L\* and b\* values ( $p < 0.05$ ). For a\* values, CO<sub>2</sub>-gas showed the highest, while halal had the lowest values. Elec-stun and N<sub>2</sub>-gas treatments fell between CO<sub>2</sub>-gas and halal treatments in terms of color. Elec-stun had higher L\* and b\* values compared to N<sub>2</sub>-gas treatment ( $p < 0.05$ ), while its a\* value was lower ( $p < 0.05$ ). The surface color of the chicken breast meat and intestines is a factor that can influence the purchasing decision of consumers. When purchasing meat and meat products, consumers prioritize physical appearance, especially color [42,43]. Discolored or dark-red meat and intestines are usually disliked by consumers, and

they tend to prefer bright-red fresh meat and intestines [42,43]. The  $a^*$  and  $b^*$  are related to the pigment concentration, oxidation, and redox conditions [44,45]. The stunning method influences the color characteristic of chicken breast meat and the small intestines. It has been reported that bright-red colored meat and small intestine were observed in highly concentrated (98%)  $N_2$ -stunned pigs and rabbits, and dark-red colored meat was found in highly concentrated (80%)  $CO_2$ -stunned pigs and rabbits [22,46]. Electrical stunning showed a higher  $L^*$  value in the *Longissimus thoracis* muscle of pigs than  $CO_2$  stunning [22,41]. Also,  $CO_2$ -stunned pigs were observed to have high  $a^*$  values and low  $b^*$  values compared to electrically stunned pigs [22]. In the case of  $N_2$ -stunned pigs, the color characteristics was intermediate between electrically- and  $CO_2$ -stunned pigs [22]. Additionally, in rabbits, the  $N_2$  stunning method resulted in higher  $L^*$  and  $b^*$  values and lower  $a^*$  values compared to  $CO_2$  and Ar stunning method [46]. In this study, the Elec-stun showed high  $L^*$  and  $b^*$ , and low  $a^*$  values of chicken breast meat and small intestine, implying a pale color, and  $CO_2$ -gas showed lower  $L^*$  and  $b^*$  values and higher  $a^*$ , indicating a dark-red color. The color characteristics of  $N_2$ -gas treatment was intermediate between that of Elec-stun and  $CO_2$ -gas treatments, which implies bright-red chicken breast meat and small intestine.

### Proximate components of chicken breast meat and small intestine under different stunning methods

The proximate components of the stunned and non-stunned chicken breast meat are presented in Table 2. The moisture content showed a significant difference among all treatments ( $p < 0.05$ ). In proteins content, among the stunning treatments, a noteworthy dissimilarity was found ( $p < 0.05$ ). The Elec-stun and  $N_2$ -gas treatments were not significantly affected by fat content ( $p > 0.05$ ). In terms of ash and collagen content, Elec-stun treatment showed significant higher than the other stunning treatments ( $p < 0.05$ ). In this study, although there was a significant difference, each component showed a small difference of ~1% among all treatments. Several factors can influence the nutrient composition of meat, including feeding, genetics, age, and gender [47]. In a previous study, it was shown that the stunning method did not have an effect on the proximate composition of the meat, except for moisture [22].

### Water-holding capacity and cooking loss of chicken breast meat and small intestine under different stunning methods

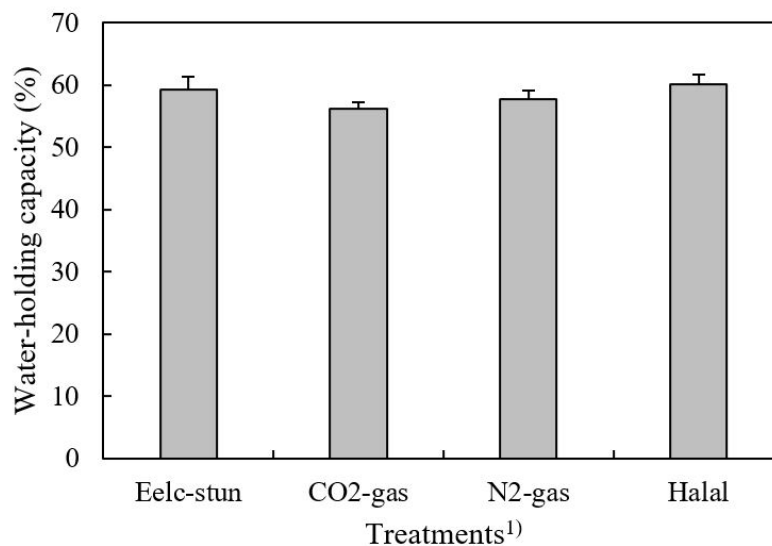
Fig. 1 shows the WHC for chicken breast meat and small intestine with stunned and non-stunned. There were no significant differences observed in WHC among all treatments. The WHC value of halal ( $60.14 \pm 1.51$ ) was higher than that of the stunning treatments ( $p > 0.05$ ). Among the stunning

**Table 2.** Effect of stunning (with electric,  $CO_2$  gas and  $N_2$  gas) and non-stunning halal method on proximate components of chicken meat

Items	Treatments <sup>1)</sup>			
	Elec-stun	$CO_2$ -gas	$N_2$ -gas	Halal
Moisture (%)	$75.23 \pm 0.68^c$	$75.53 \pm 0.47^b$	$74.90 \pm 0.24^d$	$76.02 \pm 0.56^a$
Protein (%)	$22.79 \pm 0.66^b$	$22.34 \pm 0.56^c$	$23.02 \pm 0.37^a$	$22.67 \pm 0.17^b$
Fat (%)	$1.34 \pm 0.35^b$	$1.21 \pm 0.28^c$	$1.36 \pm 0.11^b$	$1.49 \pm 0.13^a$
Ash (%)	$1.34 \pm 0.51^a$	$1.04 \pm 0.08^b$	$1.16 \pm 0.05^b$	$1.11 \pm 0.08^b$
Collagen (%)	$1.25 \pm 0.24^a$	$1.17 \pm 0.17^b$	$1.13 \pm 0.09^{bc}$	$1.08 \pm 0.06^c$

<sup>1)</sup>Treatments: Elec-stun, electrical stunning method;  $CO_2$ -gas, 80%  $CO_2$  gas stunning method;  $N_2$ -gas, 98%  $N_2$  gas stunning method; Halal, non-stunning method. n = 40.

<sup>a-d</sup>Different superscript letters in same row means significant differences ( $p < 0.05$ ).



**Fig. 1. WHC of stunning and non-stunning (halal) chicken meat.** <sup>1)</sup>Treatments: Elec-stun, electrical stunning method; CO<sub>2</sub>-gas, 80% CO<sub>2</sub> gas stunning method; N<sub>2</sub>-gas, 98% N<sub>2</sub> gas stunning method; Halal, non-stunning method.

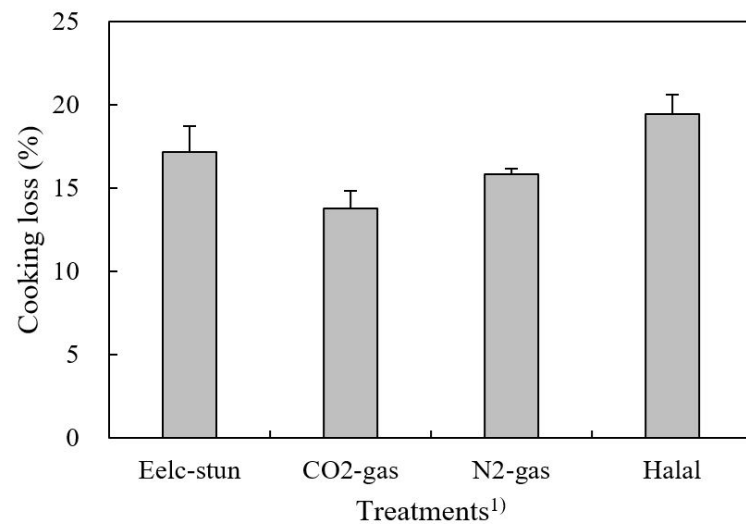
treatments, Elec-stun ( $59.21 \pm 2.06$ ) had the highest WHC value and the lowest WHC value was observed in the CO<sub>2</sub>-gas ( $56.22 \pm 0.99$ ;  $p > 0.05$ ). The WHC value of N<sub>2</sub>-gas ( $57.78 \pm 1.42$ ) was intermediate between Elec-stun and CO<sub>2</sub>-gas ( $p > 0.05$ ).

Meat industries are concerned with the improved WHC of meat and meat products. Meats with a lower WHC percentage may not fulfill export quality regulations, resulting in huge economic losses [48,49]. The decrease in pH causes the meat to become more acidic, which in shift causes the protein filaments in the meat to tighten and reduces the WHC of the meat [50]. Previous studies have shown that because of pre-slaughter stunning, muscle glycogen quickly breaks down and produces large amounts of lactate in the blood, resulting in a low pH and decreased WHC [51,52]. Our present study is consistent with this, where the halal stunning observed a higher WHC value than any other treatment. According to Bond et al. [53] increased amounts of adrenaline were observed in the blood due to pre-slaughter stress, which can potentially lead to water loss from meat. During exposure to CO<sub>2</sub>, lambs inhaled a large amount of gas that severely influenced the breakdown of cells, resulting in decreased WHC of the lamb meat [54]. In previous studies, a lower WHC value was found in pigs and lambs stunned with 80% CO<sub>2</sub> than in those stunned with electrical stunning [22,55]. It has been reported that N<sub>2</sub>-stunned (98%) pigs provide intermediate WHC values of meat, between those of electrical and CO<sub>2</sub> stunning [22]. Also in this study, the CO<sub>2</sub>-gas treatment showed a lower WHC value and the Elec-stun demonstrated a higher value. The N<sub>2</sub>-gas treatment provided values between Elec-stun and CO<sub>2</sub>-gas treatments.

The CL values of the stunned and non-stunned chicken breast meat are shown in Fig. 2. There were no significant differences observed in CL among all treatments. The figure indicates that the halal treatment provided a higher value ( $19.45 \pm 1.17$ ) than the all stunning treatment ( $p > 0.05$ ). Among the stunning treatments, Elec-stun showed the highest value ( $17.17 \pm 1.59$ ) and CO<sub>2</sub>-gas the lowest ( $13.81 \pm 1.06$ ). The value of N<sub>2</sub>-gas ( $15.84 \pm 0.32$ ) was intermediate in Elec-stun and CO<sub>2</sub>-gas ( $p > 0.05$ ).

The method of pre-slaughter stunning and slaughtering may have an impact on the CL of meat.





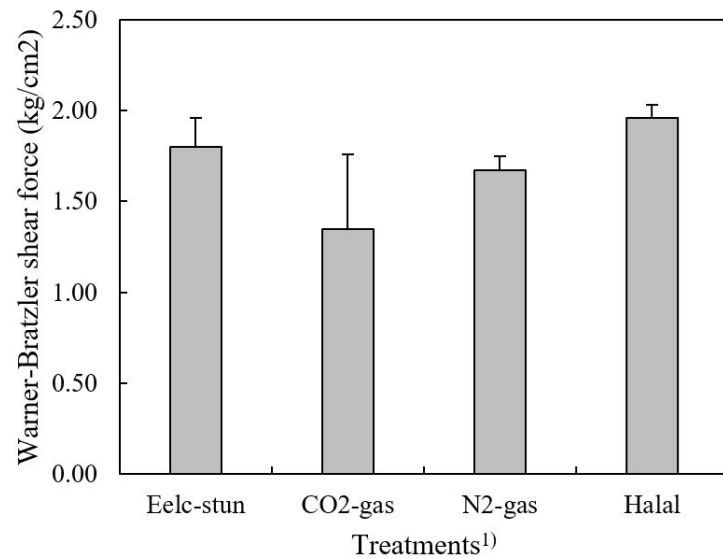
**Fig. 2. Cooking loss of stunning and non-stunning (halal) chicken meat.** <sup>1)</sup>Treatments: Elec-stun, electrical stunning method; CO<sub>2</sub>-gas, 80% CO<sub>2</sub> gas stunning method; N<sub>2</sub>-gas, 98% N<sub>2</sub> gas stunning method; Halal, non-stunning method.

According to Önenç and Kaya [56], stunned meat shows lower CL than non-stunned meat. Our research supports this statement, where the halal provided a higher CL than that of the stunning treatments. The quicker pH decline in electrically-stunned animals resulted in higher CL [57]. Higher CL was observed in more pale-colored broilers and turkey meat [58,59]. Electrically-stunned lambs showed significantly increased CL at 72 h postmortem than CO<sub>2</sub>-stunned lambs [60]. Elevated CL was also found in electrically-stunned cattle [56] and lambs [60] at the 1st and 2nd weeks postmortem. The CL value of N<sub>2</sub>-stunned pigs showed an intermediate value between that of electrical and CO<sub>2</sub> stunning [22]. The present study also supports these statements; Elec-stun provided a higher value of CL than CO<sub>2</sub>-gas, and the value of N<sub>2</sub>-gas was intermediate.

#### Warner-Bratzler shear force of chicken breast meat under different stunning methods

The WBSF values of the stunned and non-stunned broiler meat are shown in Fig. 3. There were no significant differences observed in WBSF among all treatments. The WBSF value of the halal treatment ( $1.96 \pm 0.07$  kg/cm<sup>2</sup>) was comparatively higher than the stunning treatments ( $p > 0.05$ ). The highest value was found in the Elec-stun ( $1.80 \pm 0.16$  kg/cm<sup>2</sup>) and lowest value was found in the CO<sub>2</sub>-gas ( $1.35 \pm 0.41$  kg/cm<sup>2</sup>) amongst the different stunning treatments ( $p > 0.05$ ). The WBSF value of N<sub>2</sub>-gas ( $1.67 \pm 0.08$  kg/cm<sup>2</sup>) was higher than CO<sub>2</sub>-gas but lower than Elec-stun.

The WBSF of chicken breast meat depends on several factors such as stress, genes, chilling system, and cooking situation [61,62]. The method of stunning is a factor that affects the WBSF value of meat. Önenç and Kaya [56] and Vergara et al. [63] found that stunned meat provides a lower WBSF value (more tendered) than non-stunned meat. We also found a higher WBSF value in non-stunned meat compared with any stunning treatment animals in our study. An elevated WBSF value was found in electrically-stunned meat compared with CO<sub>2</sub>-stunned suckling lamb meat [64]. Electrically-stunned animals demonstrated higher WBSF values owing to less calpain activity [65]. The muscle pH and number of enzymes present controlled the activity of calpain [66]. When animals are exposed to high levels of CO<sub>2</sub>, a large amount of gas is absorbed in the



**Fig. 3. Warner-Bratzler shear force (WBSF) of stunning and non-stunning (halal) chicken meat.** <sup>1)</sup> Treatments: Elec-stun, electrical stunning method; CO<sub>2</sub>-gas, 80% CO<sub>2</sub> gas stunning method; N<sub>2</sub>-gas, 98% N<sub>2</sub> gas stunning method.

animal body, which is greatly soluble in muscle tissue and remains at prominent levels in the muscle tissues as residue, resulting in a lower WBSF value [67]. CO<sub>2</sub> stunned pigs and lambs showed lower WBSF values than electrically-stunned meat [22,68]. One possible reason for these findings is that the gas stunning method may result in higher calpain activity compared to electrical stunning. This is a speculative hypothesis, and further analysis on the muscle calpain activity based on the stunning methods should be conducted in future studies to clarify this relationship. N<sub>2</sub>-stunned pigs provided slightly higher WBSF values than CO<sub>2</sub>-stunned pigs, but no statistically significant difference was found between them [22]. The findings of this study concur with these statements. We found lower WBSF values in the CO<sub>2</sub>-gas treatment and higher values in the Elec-stun, and N<sub>2</sub>-gas was intermediate. Therefore, it is believed that the softening phenomenon of CO<sub>2</sub> can be improved by using N<sub>2</sub> gas.

### Thickness and Warner-Bratzler shear force of chicken's small intestine under different stunning methods

Table 3 shows the thickness and WBSF value for chicken small intestines with stunned and non-stunned. The thickness of both fresh and cooked small intestines was significantly higher in the halal treatment compared to the stunning treatments ( $p < 0.05$ ). Among the stunning treatments, Elec-stun had the highest thickness value and the lowest value was found in CO<sub>2</sub>-gas ( $p < 0.05$ ). The thickness of N<sub>2</sub>-gas treatment was higher than CO<sub>2</sub>-gas but lower than Elec-stun.

The thickness value of small intestine is influenced by various factors including genetics, sex, feeding habits, disease, nutrition, age, and bacterial load etc. The stunning method and pre-slaughter stress may affect the thickness value of the small intestine in chickens post-mortem. The thickness value of N<sub>2</sub>-stunned pigs' small intestine was higher than that of CO<sub>2</sub>-stunned pigs, and lower than that of electrically-stunned pigs under both fresh and cooked conditions [22]. The present study demonstrates similar findings. The electrically-stunned (Elec-stun) broiler small intestine showed higher thickness values than the other treatments. The thickness value of N<sub>2</sub>-stunning chicken

**Table 3.** Effect of stunning (with electric, CO<sub>2</sub> gas and N<sub>2</sub> gas) and non-stunning halal method on thickness and Warner-Bratzler shear force (WBSF) of chicken small intestine

Items	Treatments <sup>1)</sup>			
	Elec-stun	CO <sub>2</sub> -gas	N <sub>2</sub> -gas	Halal
Thickness (mm)				
Fresh small intestine	0.94 ± 0.06 <sup>b</sup>	0.69 ± 0.06 <sup>d</sup>	0.80 ± 0.07 <sup>c</sup>	1.09 ± 0.06 <sup>a</sup>
Cooked small intestine	1.78 ± 0.09 <sup>b</sup>	1.40 ± 0.07 <sup>d</sup>	1.54 ± 0.04 <sup>c</sup>	1.87 ± 0.05 <sup>a</sup>
WBSF (kg/cm <sup>2</sup> )				
Fresh small intestine	1.50 ± 0.14 <sup>b</sup>	1.11 ± 0.08 <sup>d</sup>	1.30 ± 0.04 <sup>c</sup>	1.63 ± 0.05 <sup>a</sup>
Cooked small intestine	0.39 ± 0.05 <sup>b</sup>	0.15 ± 0.03 <sup>d</sup>	0.27 ± 0.02 <sup>c</sup>	0.47 ± 0.03 <sup>a</sup>

<sup>1)</sup>Treatments: Elec-stun, electrical stunning method; CO<sub>2</sub>-gas, 80% CO<sub>2</sub> gas stunning method; N<sub>2</sub>-gas, 98% N<sub>2</sub> gas stunning method; Halal, non-stunning method. n = 40.

<sup>a-d</sup>Different superscript letters in same row means significant differences ( $p < 0.05$ ).

small intestine (N<sub>2</sub>-gas) was higher than those of CO<sub>2</sub>-stunning chickens (CO<sub>2</sub>-gas).

In both fresh and cooked conditions, the WBSF in small intestine of thickness differed significantly between all treatments. The halal treatment provided higher values of WBSF than all the stunning treatments. Among the stunning treatments in both fresh and cooked conditions, the Elec-stun treatment showed the highest values of WBSF and the lowest values of WBSF were found in the CO<sub>2</sub>-gas. The WBSF value of the N<sub>2</sub>-gas treatment was between those of the Elec-stun and CO<sub>2</sub>-gas.

The WBSF value of the small intestine can be influenced by pre-slaughter stunning. During exposure to high concentrations of CO<sub>2</sub> during stunning, an enormous amount of CO<sub>2</sub> is absorbed in the body. This gas was highly dissolved in the chicken breast-meat and small intestine of the animals and remained elevated as a residue [67,69]. It was reported that the WBSF value of N<sub>2</sub>-stunned pork meat intestines was intermediate between those of electrically- and CO<sub>2</sub>-stunned pigs [22]. Similar findings were observed in the present study; here CO<sub>2</sub>-stunned broiler small intestine (CO<sub>2</sub>-gas) showed a lower WBSF value than electrically-stunned (Elec-stun) small intestine, and N<sub>2</sub> stunning (N<sub>2</sub>-gas) provided intermediate results.

## CONCLUSION

The purpose of this study was to assess the quality attributes of N<sub>2</sub> gas (98%)-stunned broiler breast meat and small intestine compared with chicken breast meat stunned using conventional electrical, CO<sub>2</sub>-gas and halal stunning. Our research findings indicate that N<sub>2</sub> gas stunning results in less discoloration in meat and small intestines compared to CO<sub>2</sub> stunning and halal stunning. Additionally, N<sub>2</sub> stunning exhibits color properties similar to electrical stunning and inhibits the phenomenon of softening caused by CO<sub>2</sub> stunning method. Therefore, high-concentration N<sub>2</sub> gas stunning method can be considered for new industrial applications in poultry slaughter. However, for the adoption of N<sub>2</sub> stunning in the industry, further research is needed on factors such as changes in stress hormones and measures regarding the toxicity/safety of meat.

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