JAST (Journal of Animal Science and Technology) TITLE PAGE

Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research Article
Article Title (within 20 words without abbreviations)	Evaluation of the Quality Characteristics of Nitrogen Gas-Stunned Chicken Meat and Small Intestine
Running Title (within 10 words)	Quality of chicken meat and small intestine by stunning method
Author	Muhammad Shahbubul Alam [first_author] 1,2, Dong-Heon Song [first_author] 1, Sun-Moon kang1, Inho Hwang2, Kuk-Hwan Seol1, Soo-Hyun Cho1, Jung-Hwan Jeon3, Hyoun Wook Kim1
Affiliation	1 Animal Products Utilization Division, National Institute of Animal Science, Rural Development Administration, Wanju-gun 55365, Korea, Republic of 2 Department of Animal Science, Jeonbuk National University, Jeonju 561756, Korea, Republic of 3 Animal Welfare Research Team, National Institute of Animal Science, Rural Development Administration, Wanju-gun 55365, Korea, Republic of
ORCID (for more information, please visit https://orcid.org)	Muhammad Shahbubul Alam (https://orcid.org/0000-0002-2697-1492) Dong-Heon Song (https://orcid.org/0000-0002-4670-3321) Sun-Moon kang (https://orcid.org/0000-0003-3947-4337) Inho Hwang (https://orcid.org/0000-0002-2474-2733) Kuk-Hwan Seol (https://orcid.org/0000-0002-0907-882X) Soo-Hyun Cho (https://orcid.org/0000-0002-8073-8771) Jung-Hwan Jeon (https://orcid.org/0000-0001-9725-547X) Hyoun Wook Kim (https://orcid.org/0000-0003-3947-4337)
Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	The study was funded by the “RDA Fellowship program” and “Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01621403 and PJ 01490102)”, NIAS, RDA, Republic of Korea.
Acknowledgements	
Availability of data and material	
Authors' contributions Please specify the authors’ role using this form.	Conceptualization: kang SM, Seol KH, Kim HW Data curation: Alam MS, Song DH Formal analysis: Alam MS, Song DH Methodology: Alam MS, Song DH, Hwang I, Jeon JH Software: Alam MS, Song DH Validation: Cho SH Investigation: Alam MS, Song DH Writing - original draft: Alam MS, Song DH Writing - review & editing: Alam MS, Song DH, kang SM, Hwang I, Seol KH, Cho SH, Jeon JH, Kim HW
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).

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	Hyoun Wook Kim
Email address – this is where your proofs will be sent	woogi78@korea.kr
Secondary Email address	
Address	
Cell phone number	
Office phone number	
Fax number	

Evaluation of the Quality Characteristics of Nitrogen Gas-Stunned Chicken Breast Meat and Small Intestine

Abstract

This study aimed to confirm the applicability of the new nitrogen (N_2) gas stunning method in the broiler slaughtering process by comparing the meat and small intestine quality following different stunning methods (electrical, carbon dioxide (CO_2), N_2, and halal). Four treatments were compared: (i) electrical stunning (Elec), (ii) 80% CO_2 gas stunning (CO_2-gas), (iii) 98% N_2 gas stunning (N_2-gas), and (iv) the non-stunning method (Halal). N_2 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_2 stunning were performed on the nearest commercial slaughter house. Meat pH_{24h}, 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_{24h}, 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_2-gas treatment showed high redness, low lightness, and low yellowness, and the coloration of meat from the N_2-gas treatment was intermediate between Elec and CO_2-gas. For other quality traits, the N_2-gas showed good results and was between Elec and CO_2-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_2-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_2 gas can be considered in broiler stunning.

Keywords: slaughtering process; gas stun; nitrogen-stunning; chicken breast meat; small intestine

Introduction

Livestock slaughterhouses in Korea use electricity and carbon dioxide (CO_2) stunning methods during slaughter. Chicken slaughterhouses have also introduced CO_2 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 slaughter houses [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_2 is commonly used in pre-slaughter stunning of broiler chickens [11] and pigs [12]. CO_2 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_2 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_2 stunning method [22], so the market value of CO_2 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_2 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_2), have been used in different proportions in conjunction with CO_2 in the stunning of animals to help reduce CO_2 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_2) in the atmosphere by a high concentration of inert gases can create hypoxic conditions with less than 2% O_2, 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_2 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_2 (70%-92%) can be mixed with CO_2 to maintain hypercapnic-hypoxia conditions for pig stunning [30,31]. Poultry showed less aversion to stunning with low atmospheric pressure and N_2 than CO_2, 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_2 gas stunning of pigs [22]. According to the syncope method, in broilers, fewer stress hormone changes occurred following N_2 stunning than with the CO_2 and halal methods [33]. Therefore, it is expected that N_2 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_2 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_2 (98%) stunning on the quality of meat and intestines in poultry, compared to conventional stunning methods such as electrical stunning, CO_2 stunning, and halal stunning. Additionally, the study aims to validate the industrial applicability of high-concentration N_2 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 National Institute of Animal Science (NIAS), RDA, Korea to assess the quality of hypoxic chicken breast meat and small intestine following N_2 gas-stunning and to compare it with meat stunned using standard electrical- and high-CO_2-based stunning and non-stunning (halal) methods. Four treatments were used: (i) electrical stunning (Elec-stun), (ii) 80% CO_2 gas stunning (CO_2-gas), (iii) 98% N_2 gas stunning (N_2-gas), and (iv) the non-stunning method (Halal). An electrical- and high-CO_2 -based stunning (80%) trial was conducted at the nearest contact commercial slaughter house; a high-N_2 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_2 and N_2) 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_2 and N_2 treatment groups were stunned using specially designed gas chamber. A modern digital gas chamber (length 220 cm × width 100 cm × height 135 cm, Thermal Instrument (STI), Dasa-eup, Dalseong-gun, Daegu, Korea) was used for gas stunning. The CO_2 or N_2 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_2, and in the case of N_2 (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 Ltd., 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℃. 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, 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 Co, 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* (lightness), CIE a* (redness), and CIE b* (yellowness).

Proximate components

The Proximate components were measured by using a Food Scan Lab 78810 (Foss Tecator Co., Ltd., 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 (WHC)

In measurement the water holding capacity (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℃ 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, USA). The ground samples were centrifuged for 10 min at a speed of 2000 rpm at 4℃. 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 (CL) and Warner-Bratzler Shear Force (WBSF)

To measure the cooking loss (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℃. The inner temperature of each sample was evaluated using a thermorecorder. When the core temperature reached 70℃, 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 cooking loss 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 cooking loss 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 Warner-Bratzler shear force (WSBF). 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 cooking loss 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_{24h} and surface color of chicken breast meat and small intestine under different stunning methods

Table 1 shows the pH_{24h} for chicken breast meat and small intestine with stunned and non-stunned. The results revealed significant differences in the pH_{24h} of the chicken breast meat and small intestine among different stunning methods. Among the stunning treatments, the pH_{24h} was comparatively high in the Elec-stun and low in the CO2-gas compared to other treatments ($p<0.05$). The pH_{24h} 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 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_2 for 70 s results in the production of large amounts of lactic acid in the blood

[39]. In pigs exposed to 80% CO_2 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_2 stunning. However, the pH of pork meat with N_2 stunning was higher than that with CO_2 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_2-stunned chickens than in CO_2-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 lightness (L*) and yellowness (b*) was significantly highest in the halal treatment, while it was the lowest in the CO_2-gas treatment ($p<0.05$). On the contrary, CO_2-gas treatment had the highest redness(a*), while halal had the lowest a* value ($p<0.05$). N_2-gas treatment showed higher L* and b* values and lower a* values compared to CO_2-gas treatment ($p<0.05$). Compared to N_2-gas treatment, Eelc-stun treatment showed similar L* valuse ($p>0.05$), 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_2-gas treatment exhibited the lowest L* and b* values ($p<0.05$). For a* values, CO_2-gas showed the highest, while halal had the lowest values. Elec-stun and N_2-gas treatments fell between CO_2-gas and halal treatments in terms of color. Elec-stun had higher L* and b* values compared to N_2-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 redness (a*) and yellowness (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 (WHC) and cooking loss (CL) 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±1.51) was higher than that of the stunning treatments ($p>0.05$). Among the stunning treatments, Elec-stun (59.21±2.06) had the highest WHC value and the lowest WHC value was observed in the CO_2-gas (56.22±0.99; $p>0.05$). The WHC value of N_2-gas (57.78±1.42) was intermediate between Elec-stun and CO_2-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, Can, and Warner [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_2, 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_2 than in those stunned with electrical stunning [22,55]. It has been reported that N_2-stunned (98%) pigs provide intermediate WHC values of meat, between those of electrical and CO_2 stunning [22]. Also in this study, the CO_2-gas treatment showed a lower WHC value and the Elec-stun demonstrated a higher value. The N_2-gas treatment provided values between Elec-stun and CO_2-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±1.17) than the all stunning treatment (p>0.05). Among the stunning treatments, Elec-stun showed the highest value (17.17±1.59) and CO_2-gas the lowest (13.81±1.06). The value of N_2-gas (15.84±0.32) was intermediate in Elec-stun and CO_2-gas (p>0.05).

The method of pre-slaughter stunning and slaughtering may have an impact on the cooking loss of meat. According to OnenC and Kaya [56], stunned meat shows lower cooking loss 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 cooking loss [57]. Higher CL was observed in more pale-colored broilers and turkey meat [58,59]. Electrically-stunned lambs showed significantly increased cooking loss at 72 h postmortem than CO_2-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_2-stunned pigs showed an intermediate value between that of electrical and CO_2 stunning [22]. The present study also supports these statements; Elec-stun provided a higher value of CL than CO_2-gas, and the value of N_2-gas was intermediate.

Warner-Bratzler shear force (WBSF) 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±0.07 kg/cm^2) was comparatively higher than the stunning treatments (p>0.05). The highest value was found in the Elec-stun (1.80±0.16

kg/cm^2) and lowest value was found in the CO_2-gas (1.35±0.41 kg/cm^2) amongst the different stunning treatments (p>0.05). The WBSF value of N_2-gas (1.67±0.08 kg/cm^2) was higher than CO_2-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. OnenC 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_2-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_2, a large amount of gas is absorbed in the 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_2 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_2-stunned pigs provided slightly higher WBSF values than CO_2-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_2-gas treatment and higher values in the Elec-stun, and N_2-gas was intermediate. Therefore, it is believed that the softening phenomenon of CO_2 can be improved by using N_2 gas.

Thickness and Warner-Bratzler shear force (WBSF) 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_2-gas (p<0.05). The thickness of N_2-gas treatment was higher than CO_2-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_2-stunned pigs' small intestine was higher than that of CO_2-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_2-stunning chicken small intestine (N_2-gas) was higher than those of CO_2-stunning chickens (CO_2-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 CO2-gas. The WBSF value of the N_2-gas treatment was between those of the Elec-stun and CO_2-gas.

The WBSF value of the small intestine can be influenced by pre-slaughter stunning. During exposure to high concentrations of CO_2 during stunning, an enormous amount of CO_2 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_2-stunned pork meat intestines was intermediate between those of electrically- and CO_2-stunned pigs [22]. Similar findings were observed in the present study; here CO_2-stunned broiler small intestine (CO_2-gas) showed a lower WBSF value than electrically-stunned (Elec-stun) small intestine, and N_2 stunning (N_2-gas) provided intermediate results.

Conclusion

The purpose of this study was to assess the quality attributes of N_2 gas (98%)-stunned broiler breast meat and small intestine compared with chicken breast meat stunned using conventional electrical, CO_2-gas and Halal stunning. Our research findings indicate that N_2 gas stunning results in less discoloration in meat and small intestines compared to CO_2 stunning and halal stunning. Additionally, N_2 stunning exhibits color properties similar to electrical stunning and inhibits the phenomenon of softening caused by CO_2 stunning method. Therefore, high-concentration N_2 gas stunning method can be considered for new industrial applications in poultry slaughter. However, for the adoption of N_2

stunning in the industry, further research is needed on factors such as changes in stress hormones and measures regarding the toxicity/safety of meat.

Acknowledgements

The study was funded by the "RDA Fellowship program" and "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01621403 and PJ 01490102)", NIAS, RDA, Republic of Korea.

References

1. Bilgili SF. Electrical stunning of broilers-Basic concepts and carcass quality implications: A review. J Appl Poult Res. 1992;1:35-146. https://doi.org/10.1093/japr.1.1.135

2. Turcsán ZS, Varga L, Szigeti J, Turcsán J, Csurák I, Szalai M. Effects of electrical stunning frequency and voltage combinations on the presence of engorged blood vessels in goose liver. Poult Sci. 2003;82:1816-1819. https://doi.org/10.1093/ps.82.11.1816

3. Gregory NG, Wotton SB. Effect of stunning on spontaneous physical activity and evoked activity in the brain. Br Poult Sci. 1990;31:215-220. https://doi.org/10.1080/00071669008417248

4. Hillebrand SJW, Lambooy E, Veerkamp CH. The effect of alternative electrical and mechanic stunning methods on haemorrhaging and meat quality of broiler breast and thigh muscles. Poult Sci. 1996;75:664-671. https://doi.org/10.3382/ps.0750664

5. Gregory NG, Wilkins LJ. Effect of stunning current on carcass quality in chickens. Vet Rec. 1989a;124:530-532. https://doi.org/10.1136/vr.124.20.530

6. Wilkins LJ, Scott CA. The effect of stunning on poultry carcass quality. Meat Hygien. 1987;31-32.

7. Anil MH. Effects of slaughter method on carcass and meat characteristics in the meat of cattle and sheep. EBLEX and AHDB-A Division of the Agriculture and Horticulture Development Board, UK, 2012;1-73

8. Berg C, Raj M. A review of different stunning methods for poultry-animal welfare aspects (stunning methods for poultry). Animals 2015;5:1207-19. https://doi.org/ 10.3390/ani5040407

9. Raj ABM, Grey TC, Audsely, Gregory NG. Effect of electrical and gaseous stunning on the carcass and meat quality of broilers. Br Poult Sci. 1990a;31:725-733. https://doi.org/ 10.1080/00071669008417303

10. Webster AB, Fletcher DL. Reactions of Laying Hens and Broilers to Different Gases Use. Poult Sci. 2001;80:1371-1377. https://doi.org/10.1093/ps.80.9.1371

11. Sandilands V, Raj A, Baker L, Sparks N. Aversion of chickens to various lethal gas mixtures. Anim Welf UFAW J. 2011;20:253. https://doi.org/10.1017/s0962728600002736

12. Verhoeven M, Gerritzen M, Velarde A, Hellebrekers L, Kemp B. Time to loss of consciousness and its relation to behavior in slaughter pigs during stunning with 80 or 95% carbon dioxide. Front Veter Sci. 2016;3:38. https://doi.org/10.3389/fvets.2016.00038

13. Gerritzen MA, Lambooij B, Reimert H, Stegeman A, Spruijt B. On-farm euthanasia of broiler chickens: Effects of different gas mixtures on behavior and brain activity. Poult Sci. 2004;83:1294-1301.

https://doi.org/10.1093/ps.83.8.1294

14. Raj A. Recent developments in stunning and slaughter of poultry. World Poult Sci J. 2006;62:467-484. https://doi.org/10.1079/WPS.2005109

15. Conlee KM, Stephens ML, Rowan AN, King LA. Carbon dioxide for euthanasia: Concerns regarding pain and distress, with special reference to mice and rats. Lab Anim. 2005;39:137-161. https://doi.org/10.1258/0023677053739747

16. Smith W, Harrap SB. Behavioural and cardiovascular responses of rats to euthanasia using carbon dioxide gas. Lab Anim. 1997;31:337-346. https://doi.org/10.1258/002367797780596130

17. Leach M, Bowell VA, Allan TF, Morton DB. Degrees of aversion shown by rats and mice to different concentrations of inhalational anaesthetics. Vet Rec. 2002;150:808-815. https://doi.org/10.1136/vr.150.26.808

18. Raj ABM, Gregory NG. Welfare implications of the gas stunning of pigs 1. Determination of aversion to the initial inhalation of carbon dioxide or argon. Anim Welf. 1995;4:273-280. https://doi.org/10.1017/s096272860001798X

19. Velarde A, Cruz J, Gispert M, Carrión D, Ruiz-de-la-Torre JL, Diestre A, Manteca X. Aversion to carbon dioxide stunning in pigs: Effect of the carbon dioxide concentration and the halothane genotype. Anim Welf. 2007;16:513-522. https://doi.org/10.1017/s0962728600027445

20. Dalmau A, Rodríguez P, Llonch P, Velarde A. Stunning pigs with different gas mixtures. Part 2: Aversion in pigs. Anim Welf. 2010;19:324-333. https://doi.org/10.1017/s096272860000172X

21. EFSA. 2004. Welfare aspects of animal stunning and killing method. In Scientific Report of the Scientific Panel of Animal Health and Welfare on a Request from the Commission. Brussels: European Food Safety Authority-EFSA; Switzerland, 2004. https://doi.org/10.2903/j.efsa.2004.4

22. Alam MS, Song DH, Lee JA, Hoa VB, Hwang I, Kim HW, Kang SM, Cho, SH, Seol KH. Effects of High Concentration Nitrogen Gas Stunning of Pigs on the Quality Traits of Meat and Small Intestine. Animals 2022;12:2249. https://doi.org/10.3390/ani12172249

23. KOSIS [Korean Statistical Information Service], Livestock Statistics Survey, Agriculture and Forestry statistical database. Daejeon, Korea 2022.

24. Ahn CH, Song S, Kim, Gap D. Technology for value-addition to animal by-products. Korean Soc for Food Sci of Anim Res. 2019;18:28-34.

25. Jeong OR, Ji HK. A study on the recognition and preference of a chicken menu in adults in the Jeonbuk area. J East Asian Soc Diet Life 2013;23:12-22. UCI : G704-001333.2013.23.1.001

26. Llonch P, Dalmau A, Rodríguez P, Manteca X, Velarde A. Aversion to nitrogen and carbon dioxide mixtures for stunning pigs. Anim. Welf. 2012;21:33-39. https://doi.org/10.7120/096272812799129475

27. Raj ABM. Behaviour of pigs exposed to mixtures of gases and the time required to stun and kill them: Welfare implications. Vet Rec. 1999;144:165-168. https://doi.org/10.1136/vr.144.7.165

28. Raj ABM, Johnson SP, Wotton SB, McInstry JL. Welfare implications of gas stunning of pigs 3. The time to loss of somatosensory evoked potentials and spontaneous electrocorticogram of pigs during exposure to gases. Vet Jour. 1997;153:329-340. https://doi.org/ 10.1016/s1090-0233(97)80067-6

29. Bager F, Braggins TJ, Devine CE, Graafhuis AE, Mellor DJ, Tavener A, Upsdell MP. Onset of insensibility at slaughter in calves: Effects of electroplectic seizure and exsanguination on spontaneous electrocortical activity and indices of cerebral metabolism. Res Vet Sci. 1992;52:162-173. https://doi.org/10.1016/0034-5288(92)90005-m

30. Llonch P, Andaluz A, Rodriguez P, Dalmau A, Jensen E, Manteca X, Velarde A. Assessment of consciousness during propofol anaesthesia in pigs. Vet Rec. 2011;169:496a. https://doi.org/10.1136/vr.d5643

31. Dalmau A, Llonch P, Rodrı´guez P, Ruı´z-de-la-Torre JL, Manteca X, Velarde A. Stunning pigs with different gas mixtures. Part 1: Gas stability. Anim Welf. 2010;19:315-323. https://doi.org/ 10.1017/S0962728600001718

32. Thomas CG, Sabine GH, Sarah-Lina AS, Abdulsatar AR, Michael JT. Evaluation of poultry stunning with low atmospheric pressure, carbon dioxide or nitrogen using a single aversion testing paradigm. Animals 2020;10:1308. https://doi.org/10.3390/ani10081308

33. Kim HW, Song DH, Seol KH, Kim CH, Joen JH. Changes in stress hormones concentration and meat quality according to stunning methods of broilers. In: Proceedings of the 19th AAAP (Asian-Australian Association of Animal Production) Animal Science Congress on the Animal Production for Human and Nature; 23-26 August 2022; Jeju, Korea, pp. 3-55.

34. Farouk MM, Al-Mazeedi HM, Sabow AB, Bekhit AED, Adeyemi KD, Sazili AQ, and Ghani A. Halal and kosher slaughter methods and meat quality: A review. Meat Sci. 2014;98:505-519. https://doi.org/10.1016/j.meatsci.2014.05.021

35. Hur SJ, Joo ST, Oh SH, Kim YJ, Kim YH, Lee JI, Park GB. Effects of packaging method and storage condition on meat shelf-life and water-holding capacity of pork loin. Korean J Animal Sci. 2001;43:121-130.

36. Khan AW, Nakamura R. Effects of pre- and postmortem glycolysis on poultry tenderness. J Food Sci. 2008;35:266-267. https://doi.org/ 10.1111/j.1365-2621.1970.tb12157.x

37. Ngoka DA, Froning GW. Effect of free struggle and pre-slaughter excitement on color of turkey breast muscle. Poult Sci. 1982;61:2291-2293. https://doi.org/10.3382/ps.0612291

38. Smolinksa T, Korzeniowska M. Evaluation of the PSE and DFD abnormalities occurrence in chicken meat. XVIIth European Symposium on the Quality of Poultry Meat; 2005; Doorwerth, The Netherlands, 193 p.

39. Nowak B, Mueffling TV, Hartung J. Effect of different carbon dioxide concentrations and exposure times in stunning of slaughter pigs: Impact on animal welfare and meat quality. Meat Sci. 2007;75:290-298. https://doi.org/10.1016/j.meatsci.2006.07.014

40. Becerril-Herrera M, Alonso-Spilsbury M, Lemus-Flores C, Guerrero-Legarreta, I, Ramírez-Necoechea R, Mota-Rojas D. CO_2 stunning may compromise swine welfare compared with electrical stunning. Meat Sci. 2009;81:233-237. https://doi.org/10.1016/j.meatsci.2008.07.025

41. Channon HAP, Payne AM, Warner RD. Comparison of CO_2 stunning with manual electrical stunning (50Hz) of pigs on carcass and meat quality. Meat Sci. 2002;60:63-68. https://doi.org/10.1016/S0309-1740(01)00107-3

42. Van Laack RLJM, Liu CH, Smith MO, Loveday HD. Characteristics of pale, soft, exudative broiler breast meat. Poult Sci. 2000;79:1057-61 https://doi.org/10.1093/ps/79.7.1057

43. Díaz O, Rodríguez L, Torres A, Cobos A. Chemical composition and physicochemical properties of meat from capons as affected by breed and age. Span J Agri Res. 2010;8:91-99. https://doi.org/ 10.5424/sjar/2010081-1147

44. Waritthitham A, Lambertz C, Langholz HJ, Wicke M, Gauly M. Assessment of beef production from Brahman x Thai native and Charolais x Thai native crossbred bulls slaughtered at different weights. II: Meat quality. Meat Sci. 2010;85:196-200. https://doi.org/10.1016/j.meatsci.2009.12.025

45. Bispo E, Monserrat L, González L, Franco D, Moreno T. Effect of weaning status on animal performance and meat quality of Rubia Gallega calves. Meat Sci. 2010;86:832-838. https://doi.org/ 10.1016/j.meatsci.2010.07.005

46. Alam MS, Song DH, Lee JA, Hoa VB, Kim HW, Kang SM, Cho SH, Hwang I, Seol KH. Effect of Different Gas-Stunning Conditions on Heme Pigment Solutions and on the Color of Blood, Meat, and Small Intestine of Rabbits. Animals 2022;12:3155. https://doi.org/10.3390/ani12223155

47. Banovi´c M, Grunert KG, Barreira MM, Fontes MA. Beef quality perception at the point of purchase: A study from Portugal. Food Qual Pref. 2009;20:335-342. https://doi.org/10.1016/j.foodqual.2009.02.009

48. Forrest JC, Aberle ED, Hedrick HB, Judge MD, Merkel RA. Principles of Meat Science; W.H. Freeman and Co.: Reading, UK; 1975.

49. Lawrie RA. Meat Science. 4th ed. Pergamon Press, Oxford: UK; Paris, France; 1985.

50. Joo ST, Kauffman RG, Kim BC, Park GB. The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in porcine longissimus muscle. Meat Sci. 1999;52:291-297. https://doi.org/10.1016/s0309-1740(99)00005-4

51. Grandin T. The effect of stress on livestock and meat quality prior to and during slaughter. Int J Study Anim Probl. 1980;1:313-337.

52. Gregory NG. Animal Welfare and Meat Science. CABI Publishing: New York, NY, USA; 2003.

53. Bond JJ, Can LA, Warner RD. The effect of exercise stress, adrenaline injection and electrical stimulation on changes in quality attributes and proteins in semi-membranous muscle of lamb. Meat Sci. 2004;68:469-477. https://doi.org/ 10.1016/j.meatsci.2004.05.001

54. Moore JV, Gill CO. The pH and display life of chilled lamb after prolonged storage under vacuum or under CO_2. NZJ Agric Res. 1987;30:449-452. https://doi.org/10.1080/00288233.1987.10417956

55. Linares MB, Bornez R, Vergara H. Effect of stunning systems on meat quality of Manchego suckling lamb packed under modified atmospheres. Meat Sci. 2008;78:279-287. https://doi.org/10.1016/j.meatsci.2007.06.009

56. OnenC A, Kaya A. The effect of electrical stunning and percussive captive bolt stunning on meat quality of cattle processed by Turkish slaughter procedures. Meat Sci. 2004;66:809-815. https://doi.org/10.1016/s0309-1740(03)00191-8

57. Lyon C, Buhr R. Biochemical basis of meat texture. In RI Richardson; Mead, G.C., Ed. Poultry Meat Science, Centre for Agriculture and Biosciences (CAB) International; New York, NY, USA; 1999: pp. 99-126.

58. Allen CD, Fletcher DL, Northcutt JK, Russell SM. The relationship of broiler breast color to meat quality and shelf-life. Poult Sci. 1998;77:361-366. https://doi.org/10.1093/ps.77.2.361

59. Mckee SR, Hargis BM, Sams AR. Pale, soft and exudative meat in turkeys treated with succinylcholine. Poult Sci. 1998;7:356-360. https://doi.org/10.1093/ps.77.2.356

60. Linares M, Bórnez R, Vergara H. Effect of different stunning systems on meat quality of light lamb. Meat Sci. 2007;76:675-681. https://doi.org/10.1016/j.meatsci.2007.02.007

61. Koch RM, Cundiff LV, Gregory KE. Heritabilities and genetic, environmental and phenotypic correlations of carcass traits in a population of diverse biological types and their implications in selection programs. J Anim Sci. 1982;55:1319-1329. https://doi.org/10.1016/j.meatsci.2007.02.007

62. Monsón F, Sañudo C, Sierra I. Influence of cattle breed and ageing time on textural meat quality. Meat Sci. 2004;68:595-602. https://doi.org/10.1016/j.meatsci.2004.05.011

63. Vergara H, Linares MB, Berruga MI. Meat quality in suckling lambs: effect of pre-slaughter handling. Meat Sci. 2005;68:595-602. https://doi.org/10.1016/j.meatsci.2004.09.002

64. Bórnez R, Linares MB, Vergara H. Effects of stunning with different carbon dioxide concentrations and exposure

times on suckling lamb meat quality. Meat Sci. 2009;81:493-498. https://doi.org/10.1016/j.meatsci.2008.10.004

65. Dransfeld E. Modelling post-mortem tenderization-V: Inactivation of calpains. Meat Sci. 1994;37:391-409. https://doi.org/10.1016/0309-1740(94)90055-8

66. Murachi T. Calpain and calpastatin. Trends Biochem Sci. 1983;8:167-169. https://doi.org/10.1016/0968-0004(83)90165-2

67. Gill CO. Controlled atmosphere packaging of chilled meat. Food Control 1990;1:74-78. https://doi.org/10.1016/0956-7135(90)90088-T

68. Vergara H, Linares MB, Berruga MI, Gallego L. Meat quality in suckling lambs: Effect of pre-slaughter handling. Meat Sci. 2005;69:473-478. https://doi.org/ 10.1016/j.meatsci.2004.09.002

69. Bruce HL, Wolfe FH, Jones SDM, Price MA. Porosity in cooked beef from controlled atmosphere packaging is caused by rapid CO_2 gas evolution. Food Res Int. 1996;29:189-193. https://doi.org/10.1016/0963-9969(96)00057-9

Table 1. Effect of stunning (with electric, CO_2 gas and N_2 gas) and non-stunning halal method on pH_{24h} and color value of chicken meat and small intestine

Items	Treatments[1)]			
	Elec-stun	**CO_2-gas**	**N_2-gas**	**Halal**
pH_{24h}				
Meat	6.10±0.08^{b}	5.88±0.08^{d}	6.04±0.03^{c}	6.26±0.06^{a}
Small intestine (Fresh)	6.38±0.06^{b}	6.20±0.06^{d}	6.28±0.04^{c}	6.62±0,06^{a}
Color value				
Meat				
L* (lightness)	55.17±3.02^{b}	51.45±2.26^{c}	54.62±1.24^{b}	58.63±0.98^{a}
a* (redness)	2.90±0.35^{c}	4.12±0.23^{a}	3.57±0.08^{b}	1.72±0.15^{d}
b* (yellowness)	5.89±1.45^{a}	4.55±0.74^{c}	5.46±0.17^{b}	6.19±0.06^{a}
Small Intestine (Fresh)				
L* (lightness)	60.11±1.19^{b}	54.94±0.86^{d}	58.29±0.62^{c}	62.66±0.73^{a}
a* (redness)	11.37±0.56^{c}	16.03±0.51^{a}	13.47±0.11^{b}	9.8±0.42^{d}
b* (yellowness)	13.44±0.68^{b}	8.58±0.56^{d}	12.55±0.61^{c}	14.24±0.18^{a}
Small Intestine (Cooked)				
L* (lightness)	69.93±1.30^{b}	63.78±0.75^{d}	67.64±0.99^{c}	71.56±0.71^{a}
a* (redness)	5.44±0.22^{c}	8.61±0.34^{a}	6.49±0.11^{b}	4.65±0.46^{d}
b* (yellowness)	15.27±0.49^{b}	10.62±0.78^{d}	14.57±0.28^{c}	16.11±0.19^{a}

[1)]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. $^{a-d}$ Different superscript letters in same row means significant differences ($p < 0.05$). SEM = Standard error of mean.

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

[1)]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. $^{a\text{-}d}$ Different superscript letters in same row means significant differences ($p < 0.05$). SEM = Standard error of mean.

Table 3. Effect of stunning (with electric, CO_2 gas and N_2 gas) and non-stunning halal method on thickness and Warner-Bratzler shear force (WBSF) of chicken small intestine

Items	**Treatments[1)]**			
	Elec-stun	**CO_2-gas**	**N_2-gas**	**Halal**
Thickness (mm)				
Fresh small intestine	0.94±0.06^b	0.69±0.06^d	0.80±0.07^c	1.09±0.06^a
Cooked small intestine	1.78±0.09^b	1.40±0.07^d	1.54±0.04^c	1.87±0.05^a
WBSF (kg/cm^2)				
Fresh small intestine	1.50±0.14^b	1.11±0.08^d	1.30±0.04^c	1.63±0.05^a
Cooked small intestine	0.39±0.05^b	0.15±0.03^d	0.27±0.02^c	0.47±0.03^a

[1)]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. $^{a-d}$ Different superscript letters in same row means significant differences ($p < 0.05$). SEM = Standard error of mean.

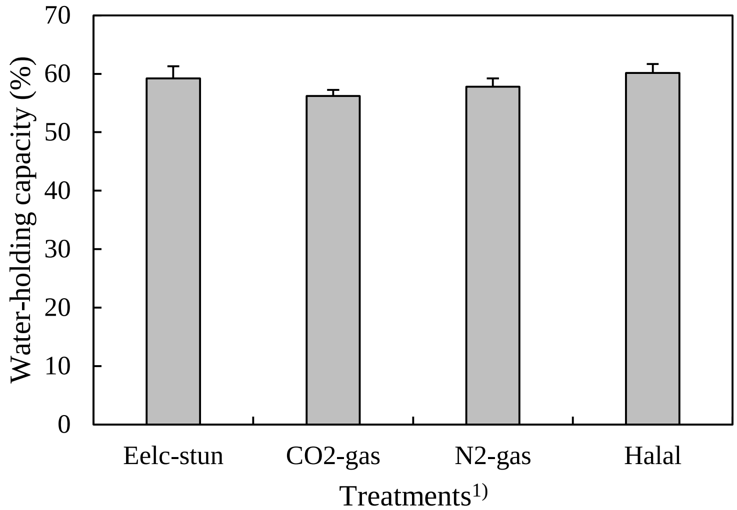

Fig. 1. WHC of stunning and non-stunning (halal) chicken meat. [1)]Treatments: Elec-stun, electrical stunning method; CO_2-gas, 80% CO_2 gas stunning method; N_2-gas, 98% N_2 gas stunning method; Halal, non-stunning method.

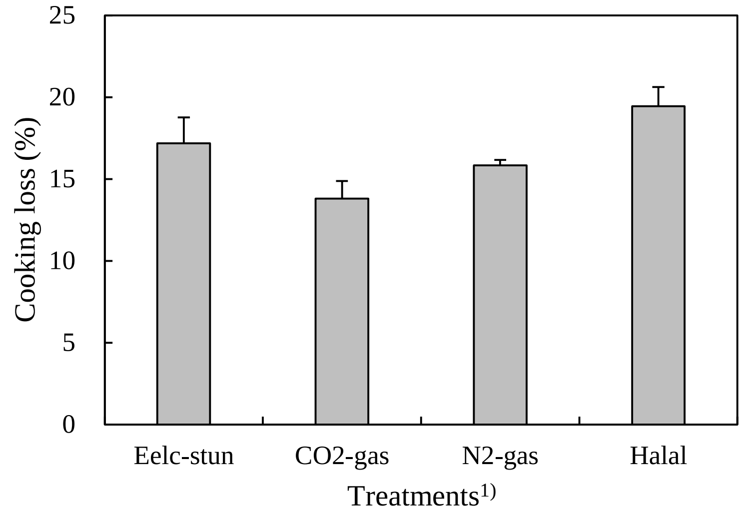

Fig. 2. Cooking loss of stunning and non-stunning (halal) chicken meat. [1)]Treatments: Elec-stun, electrical stunning method; CO_2-gas, 80% CO_2 gas stunning method; N_2-gas, 98% N_2 gas stunning method; Halal, non-stunning method.

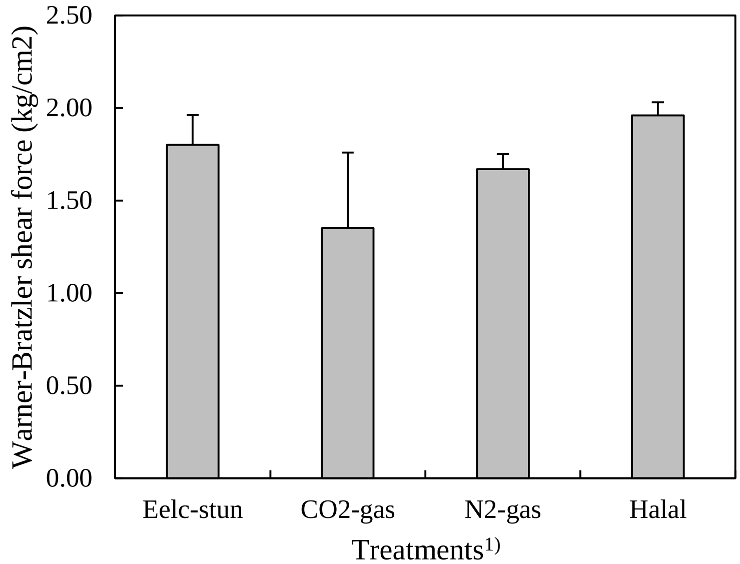

Fig. 3. Warner-Bratzler shear force (WBSF) of stunning and non-stunning (halal) chicken meat.

[1)]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..