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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

32

33 Livestock slaughterhouses in Korea use electricity and carbon dioxide (CO₂) stunning methods during slaughter.
34 Chicken slaughterhouses have also introduced CO₂ stunning methods due to improved animal welfare compared with
35 the traditional electric method. Electrical stunning is the most common pre-slaughter stunning method used for animals
36 in the meat industry. Among the different types of electrical stunning systems, electrical water bath stunning is
37 commonly practiced in commercial poultry plants [1]. It is expedient, inexpensive, has few space users, and can be
38 readily tailored to commercial processing plants [2]. To induce unconsciousness in a water bath system, the EU
39 recommends a minimum current of 120 mA per chicken [3]. This recommended minimum current causes muscle
40 contraction, broken bones, and breast muscle hemorrhage in broilers [4-6]. On the other hand, Islamic countries
41 perform slaughtering without stunning, following religious requirements, using the halal method [7].

42 Gas stunning can be considered an alternative method to the pre-slaughter stunning system to maintain animal
43 welfare and meat quality, and is widely used in traditional electric-based slaughter houses [8]. According to Raj et al.
44 [9], gas use as a novel method for stunning animals has increased daily. Minimizing the stressful handling of awake
45 broilers using gas stunning prior to electrical stunning in a commercial slaughterhouse increases animal welfare and
46 reduces the need for workers [10]. CO₂ is commonly used in pre-slaughter stunning of broiler chickens [11] and pigs
47 [12]. CO₂ has been demonstrated to be aversive to chickens and induces suffering and pain [17, 18]. Mice, rats, and
48 pigs also have also shown an aversion to CO₂ stunning [19-24], including irritation of the nasal mucosal membranes
49 [22], severe respiratory distress [24], and strong head shaking [25]. Additionally, meat and small intestine
50 discoloration (dark-red colored) occurs following the CO₂ stunning method [13], so the market value of CO₂ stunned
51 meat and by-products, especially the intestine, is low in Korea. According to KOSIS [26], ~77,000 cows, 1.5 million
52 pigs, and 84 million chickens are slaughtered each month in Korea. The intestines of animals have been widely utilized
53 globally for the food ingredients and production of casings [27, 28]. Therefore, any deterioration in the quality of
54 intestines due to gas stunning can render them unsuitable for sale, leading to potential economic losses. Consequently,
55 it is necessary to study an alternative to the CO₂ stunning method that does not affect the quality of meat or animal
56 welfare.

57 On the other hand, inert gases, such as argon (Ar) or nitrogen (N₂), have been used in different proportions in
58 conjunction with CO₂ in the stunning of animals to help reduce CO₂ aversion [15,24]. Exposure to high concentrations
59 of inert gases has been found to induce hypoxic conditions in animals [22, 29]. The displacement of oxygen (O₂) in

60 the atmosphere by a high concentration of inert gases can create hypoxic conditions with less than 2% O₂, leading to
61 a decrease in blood oxygen levels of animals [30]. This, in turn, affects the central nervous system (CNS) and induces
62 unconsciousness to animal [31]. N₂ is the most abundant gas in the air (78%), and can be used widely for commercial
63 purposes due to its low price. Different concentrations of N₂ (70%-92%) can be mixed with CO₂ to maintain
64 hypercapnic-hypoxia conditions for pig stunning [32,33]. Poultry showed less aversion to stunning with low
65 atmospheric pressure and N₂ than CO₂, which may offer a significant welfare refinement [34]. No adverse effects on
66 the quality traits of meat or the small intestine were observed using the high-concentration N₂ gas stunning of pigs
67 [13]. According to the syncope method, in broilers, fewer stress hormone changes occurred following N₂ stunning
68 than with the CO₂ and halal methods [37]. Therefore, it is expected that N₂ gas could be effective in preventing
69 excessive discoloration and softening compared to conventional stunning methods. However, the feasibility of using
70 only high concentrations of N₂ gas (98%) in the stunning of birds (broiler chickens) and its effects on the quality traits
71 of meat and the small intestine have not yet been investigated.

72 The objective of this study is to evaluate the impact of high-concentration N₂ (98%) stunning on the quality of meat
73 and intestines in poultry, compared to conventional stunning methods such as electrical stunning, CO₂ stunning, and
74 halal stunning. Additionally, the study aims to validate the industrial applicability of high-concentration N₂ stunning.

76 **Materials and Methods**

78 **Animal care**

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

83 **Experimental design and facilities**

84 An experimental trial was conducted at the National Institute of Animal Science (NIAS), RDA, Korea to assess the
85 quality of hypoxic chicken breast meat and small intestine following N₂ gas-stunning and to compare it with meat
86 stunned using standard electrical- and high-CO₂-based stunning and non-stunning (halal) methods. Four treatments

87 were used: (i) electrical stunning (Elec-stun), (ii) 80% CO₂ gas stunning (CO₂-gas), (iii) 98% N₂ gas stunning (N₂-
88 gas), and (iv) the non-stunning method (Halal). An electrical- and high-CO₂-based stunning (80%) trial was conducted
89 at the nearest contact commercial slaughter house; a high-N₂ gas stunning (98%) and a non-stunning (halal) method
90 trial was conducted at the NIAS pilot plant abattoir. Forty Cobb-500 commercial broiler chickens were assigned to
91 each treatment (n = 40). The chickens were collected from the nearest commercial farm. Just before the day of the
92 experiment, broilers were transported to the institutional abattoir and stored in a commercial slaughterhouse. The
93 chickens rested for 12 h in pens with *ad-libitum* water and finisher diet. Fasting conditions were maintained overnight
94 before slaughter. Body weight was measured during the evening of slaughter. The body weight range was 2.51–2.68
95 kg at 42 days of age.

96 In the electrical stunning and non-stunning (halal) methods, single broilers were used, whereas gas stunning (both
97 CO₂ and N₂) was performed on a crate containing eight broilers. Electrical stunning was performed within the contact
98 commercial slaughterhouse, using a water bath stunner delivering a constant current of 120 mA (50 Hz in the form of
99 sinusoidal waveform) for 4–5 s. In the case of the halal method, according to the rules of Islam, broilers were
100 slaughtered without stunning [35]. The chickens in the CO₂ and N₂ treatment groups were stunned using specially
101 designed gas chamber. A modern digital gas chamber (length 220 cm × width 100 cm × height 135 cm, Thermal
102 Instrument (STI), Dasa-eup, Dalseong-gun, Daegu, Korea) was used for gas stunning. The CO₂ or N₂ gases flow was
103 continued into the pit of the chamber until the desired concentration (80% or 98%) was reached. After reaching the
104 desired concentration level, the gas flow was stopped, and the time (s) counting was started until the stunned state was
105 reached. It was found that within 70 s, broilers were completely stunned by 80% CO₂, and in the case of N₂ (98%), it
106 required approximately 110 s for complete stunning.

107

108 **Slaughtering and sample collection**

109 After gas stunning, each crate of broilers was removed from the gas chamber as quickly as possible and heart
110 activity was measured using an electrocardiogram (ECG) with two adhesive patches (Unilect, 5 cm in diameter;
111 Unomedical Ltd., Stonehouse, UK) placed on the left and right pectoralis muscles under the wing base, and then
112 slaughtered within 30 s. In electrical stunning, each stunned chicken was kept on a movable table, heart activity was
113 measured using ECG, and then the chicken was slaughtered within 15–20 s. After slaughter, for proper bleeding, one
114 leg (hock joint) of each broiler was tied with an iron chain and hung for 2–3 min. This process was observed in all

115 treatments, including those treated with the halal method. Subsequently, chickens from the different treatments were
116 inserted separately in a pluckier machine for feather plucking. After 7–8 min, the chickens were removed from the
117 pluckier and eviscerated manually. Individual carcasses were cleaned, washed with tap water, and stored in polythene
118 bags. The small intestine (100 cm) was collected from each broiler in every treatment (n = 40). After cleaning and
119 washing, each small intestine was placed in a polythene bag. All carcasses and small intestines were stored in a cold
120 room overnight at 2°C. After chilling, all carcasses and small intestine samples were transported (after slaughter at 18
121 h) to the laboratory, and then the breast muscle of all carcasses were obtained for further study.

122

123 **pH measurement**

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

128

129 **Surface color values**

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

138

139 **Proximate components**

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

143

144 **Water-Holding Capacity (WHC)**

145 In measurement the water holding capacity (WHC) of a raw chicken breast meat, the sample was ground, and
146 subsequently, any fat, fibers, and coarse particles were removed. The fine meat (0.5 g) was inserted into an ultra-
147 centrifugal tube. These tubes were heated at 80°C for 20 min using water-bath. The next step in determining the WHC
148 of the meat sample involved using a centrifugal machine (Avanti(R) J-E, Beckman coulter, USA). The ground samples
149 were centrifuged for 10 min at a speed of 2000 rpm at 4°C. After centrifugation, the tubes were allowed to stand for
150 10 min at room temperature. The samples were weighed, and the WHC of the chicken breast meat was calculated
151 using a formula. The above method was performed twice for each sample, and the average value was used for the
152 study [36].

153

154 **Cooking Loss (CL) and Warner-Bratzler Shear Force (WBSF)**

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

161 The WBSF were measured using an Instron Universal Testing Machine (5543, Instron Corp., USA). The WBSF
162 for chicken breast meat was prepared using a sample with completed cooking loss measurements. From cooked
163 chicken breast meat, five samples (length 2.0-2.5 cm, diameter 1.25 cm) were made using a metal corer according to
164 the muscle fiber direction. In the small intestine, WBSF was determined in both fresh and cooked samples. For samples
165 of fresh small intestine, each 5-7 cm of samples from the small intestine was taken and directly cut using an Instron
166 machine. To measure the WBSF of the cooked small intestine, a clean sample of 50 cm in length was taken from each
167 small intestine. Also, in case of the cooked sample, the fresh small intestine were cooked under the same heating
168 conditions as meat (cooking time, ~40 min) and were then used to measure the Warner-Bratzler shear force (WBSF).
169 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.

170

171 **Thickness of small intestine**

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

177

178 **Statistical analysis**

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

182

183 **Results and Discussion**

184

185 **pH_{24h} and surface color of chicken breast meat and small intestine under different stunning methods**

186 Table 1 shows the pH_{24h} for chicken breast meat and small intestine with stunned and non-stunned. The results
187 revealed significant differences in the pH_{24h} of the chicken breast meat and small intestine among different stunning
188 methods. Among the stunning treatments, the pH_{24h} was comparatively high in the Elec-stun and low in the CO₂-gas
189 compared to other treatments (*p*<0.05). The pH_{24h} of Halal was also significantly higher than that of all other stunning
190 treatments in both the chicken breast meat and the small intestine.

191 The pH level of the chicken breast meat and small intestine is one of the indicators that change according to
192 stunning methods and reflects the biochemical state of the muscle following the development of rigor mortis [1,5].
193 Several reports imply that during stunning and exsanguination, muscular activity is elevated and glycolysis is
194 increased, resulting in the accretion of lactic acid in at the muscle decreased the pH level [9,38,39]. The accumulation
195 of lactic acid in the cells of chicken muscle is closely associated with pre-slaughter stress [40]. The detection of
196 stunning stress in animals has been attempted by some researchers through the use of blood samples [2,37]. Exposing
197 hogs to an 80% concentration of CO₂ for 70 s results in the production of large amounts of lactic acid in the blood

198 [41]. In pigs exposed to 80% CO₂ for 60 s, lactic acidosis, hyperglycemia, hypercapnia, and increased hematocrit
199 levels were observed [42]. According to Channon et al. [43], the pH decline of pork loin was faster in electrically-
200 stunned pig meat than after CO₂ stunning. However, the pH of pork meat with N₂ stunning was higher than that with
201 CO₂ stunning, which was slightly lower than that with electrical stunning [13]. Similar to a previous study, the pH in
202 chicken breast and small intestine was higher in N₂-stunned chickens than in CO₂-stunned chickens, and lower than
203 in electrically-stunned chickens.

204 The surface color of chicken breast meat and small intestines is presented in Table 1. In chicken breast meat, the
205 lightness (L*) and yellowness (b*) was significantly highest in the halal treatment, while it was the lowest in the CO₂-
206 gas treatment ($p < 0.05$). On the contrary, CO₂-gas treatment had the highest redness (a*), while halal had the lowest a*
207 value ($p < 0.05$). N₂-gas treatment showed higher L* and b* values and lower a* values compared to CO₂-gas treatment
208 ($p < 0.05$). Compared to N₂-gas treatment, Elec-stun treatment showed similar L* value ($p > 0.05$), lower a* value
209 ($p < 0.05$), and higher b* value ($p < 0.05$). In both raw and cooked intestines, halal treatment had the highest L* and b*
210 values, while CO₂-gas treatment exhibited the lowest L* and b* values ($p < 0.05$). For a* values, CO₂-gas showed the
211 highest, while halal had the lowest values. Elec-stun and N₂-gas treatments fell between CO₂-gas and halal treatments
212 in terms of color. Elec-stun had higher L* and b* values compared to N₂-gas treatment ($p < 0.05$), while its a* value
213 was lower ($p < 0.05$). The surface color of the chicken breast meat and intestines is a factor that can influence the
214 purchasing decision of consumers. When purchasing meat and meat products, consumers prioritize physical
215 appearance, especially color [44,45]. Discolored or dark-red meat and intestines are usually disliked by consumers,
216 and they tend to prefer bright-red fresh meat and intestines [44,45]. The redness (a*) and yellowness (b*) are related
217 to the pigment concentration, oxidation, and redox conditions [46,47]. The stunning method influences the color
218 characteristic of chicken breast meat and the small intestines. It has been reported that bright-red colored meat and
219 small intestine were observed in highly concentrated (98%) N₂-stunned pigs and rabbits, and dark-red colored meat
220 was found in highly concentrated (80%) CO₂-stunned pigs and rabbits [13,48]. Electrical stunning showed a higher
221 L* value in the *Longissimus thoracis* muscle of pigs than CO₂ stunning [13,43]. Also, CO₂-stunned pigs were observed
222 to have high a* values and low b* values compared to electrically stunned pigs [13]. In the case of N₂-stunned pigs,
223 the color characteristics was intermediate between electrically- and CO₂-stunned pigs [13]. Additionally, in rabbits,
224 the N₂ stunning method resulted in higher L* and b* values and lower a* values compared to CO₂ and Ar stunning
225 method [48]. In this study, the Elec-stun showed high L* and b*, and low a* values of chicken breast meat and small

226 intestine, implying a pale color, and CO₂-gas showed lower L* and b* values and higher a*, indicating a dark-red
227 color. The color characteristics of N₂-gas treatment was intermediate between that of Elec-stun and CO₂-gas
228 treatments, which implies bright-red chicken breast meat and small intestine.

229

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

231 The proximate components of the stunned and non-stunned chicken breast meat are presented in Table 2. The
232 moisture content showed a significant difference among all treatments ($p < 0.05$). In proteins content, among the
233 stunning treatments, a noteworthy dissimilarity was found ($p < 0.05$). The Elec-stun and N₂-gas treatments were not
234 significantly affected by fat content ($p > 0.05$). In terms of ash and collagen content, Elec-stun treatment showed
235 significant higher than the other stunning treatments ($p < 0.05$). In this study, although there was a significant difference,
236 each component showed a small difference of ~1% among all treatments. Several factors can influence the nutrient
237 composition of meat, including feeding, genetics, age, and gender [49]. In a previous study, it was shown that the
238 stunning method did not have an effect on the proximate composition of the meat, except for moisture [13].

239

240 **Water-holding capacity (WHC) and cooking loss (CL) of chicken breast meat and small intestine under** 241 **different stunning methods**

242 Fig. 1 shows the WHC for chicken breast meat and small intestine with stunned and non-stunned. There were no
243 significant differences observed in WHC among all treatments. The WHC value of Halal (60.14 ± 1.51) was higher
244 than that of the stunning treatments ($p > 0.05$). Among the stunning treatments, Elec-stun (59.21 ± 2.06) had the highest
245 WHC value and the lowest WHC value was observed in the CO₂-gas (56.22 ± 0.99 ; $p > 0.05$). The WHC value of N₂-
246 gas (57.78 ± 1.42) was intermediate between Elec-stun and CO₂-gas ($p > 0.05$).

247 Meat industries are concerned with the improved WHC of meat and meat products. Meats with a lower WHC
248 percentage may not fulfill export quality regulations, resulting in huge economic losses [51,52]. The decrease in pH
249 causes the meat to become more acidic, which in shift causes the protein filaments in the meat to tighten and reduces
250 the WHC of the meat [53]. Previous studies have shown that because of pre-slaughter stunning, muscle glycogen
251 quickly breaks down and produces large amounts of lactate in the blood, resulting in a low pH and decreased WHC
252 [54,55]. Our present study is consistent with this, where the Halal stunning observed a higher WHC value than any
253 other treatment. According to Bond, Can, and Warner [56] increased amounts of adrenaline were observed in the

254 blood due to pre-slaughter stress, which can potentially lead to water loss from meat. During exposure to CO₂, lambs
255 inhaled a large amount of gas that severely influenced the breakdown of cells, resulting in decreased WHC of the lamb
256 meat [57]. In previous studies, a lower WHC value was found in pigs and lambs stunned with 80% CO₂ than in those
257 stunned with electrical stunning [13,58]. It has been reported that N₂-stunned (98%) pigs provide intermediate WHC
258 values of meat, between those of electrical and CO₂ stunning [13]. Also in this study, the CO₂-gas treatment showed
259 a lower WHC value and the Elec-stun demonstrated a higher value. The N₂-gas treatment provided values between
260 Elec-stun and CO₂-gas treatments.

261 The CL values of the stunned and non-stunned chicken breast meat are shown in Fig. 2. There were no significant
262 differences observed in CL among all treatments. The figure indicates that the Halal treatment provided a higher value
263 (19.45±1.17) than the all stunning treatment ($p>0.05$). Among the stunning treatments, Elec-stun showed the highest
264 value (17.17±1.59) and CO₂-gas the lowest (13.81±1.06). The value of N₂-gas (15.84±0.32) was intermediate in Elec-
265 stun and CO₂-gas ($p>0.05$).

266 The method of pre-slaughter stunning and slaughtering may have an impact on the cooking loss of meat. According
267 to OnenC and Kaya [59], stunned meat shows lower cooking loss than non-stunned meat. Our research supports this
268 statement, where the Halal provided a higher CL than that of the stunning treatments. The quicker pH decline in
269 electrically-stunned animals resulted in higher cooking loss [60]. Higher CL was observed in more pale-colored
270 broilers and turkey meat [61,62]. Electrically-stunned lambs showed significantly increased cooking loss at 72 h
271 postmortem than CO₂-stunned lambs [63]. Elevated CL was also found in electrically-stunned cattle [59] and lambs
272 [63] at the 1st and 2nd weeks postmortem. The CL value of N₂-stunned pigs showed an intermediate value between
273 that of electrical and CO₂ stunning [13]. The present study also supports these statements; Elec-stun provided a higher
274 value of CL than CO₂-gas, and the value of N₂-gas was intermediate.

275

276 **Warner-Bratzler shear force (WBSF) of chicken breast meat under different stunning methods**

277 The WBSF values of the stunned and non-stunned broiler meat are shown in Fig. 3. There were no significant
278 differences observed in WBSF among all treatments. The WBSF value of the Halal treatment (1.96±0.07 kg/cm²) was
279 comparatively higher than the stunning treatments ($p>0.05$). The highest value was found in the Elec-stun (1.80±0.16

280 kg/cm²) and lowest value was found in the CO₂-gas (1.35±0.41 kg/cm²) amongst the different stunning treatments
281 ($p>0.05$). The WBSF value of N₂-gas (1.67±0.08 kg/cm²) was higher than CO₂-gas but lower than Elec-stun.

282 The WBSF of chicken breast meat depends on several factors such as stress, genes, chilling system, and cooking
283 situation [64,65]. The method of stunning is a factor that affects the WBSF value of meat. OnenC and Kaya [59] and
284 Vergara et al. [66] found that stunned meat provides a lower WBSF value (more tendered) than non-stunned meat.
285 We also found a higher WBSF value in non-stunned meat compared with any stunning treatment animals in our study.
286 An elevated WBSF value was found in electrically-stunned meat compared with CO₂-stunned suckling lamb meat
287 [67]. Electrically-stunned animals demonstrated higher WBSF values owing to less calpain activity [68]. The muscle
288 pH and number of enzymes present controlled the activity of calpain [69]. When animals are exposed to high levels
289 of CO₂, a large amount of gas is absorbed in the animal body, which is greatly soluble in muscle tissue and remains
290 at prominent levels in the muscle tissues as residue, resulting in a lower WBSF value [70]. CO₂ stunned pigs and
291 lambs showed lower WBSF values than electrically-stunned meat [13,71]. One possible reason for these findings is
292 that the gas stunning method may result in higher calpain activity compared to electrical stunning. This is a speculative
293 hypothesis, and further analysis on the muscle calpain activity based on the stunning methods should be conducted in
294 future studies to clarify this relationship. N₂-stunned pigs provided slightly higher WBSF values than CO₂-stunned
295 pigs, but no statistically significant difference was found between them [13]. The findings of this study concur with
296 these statements. We found lower WBSF values in the CO₂-gas treatment and higher values in the Elec-stun, and N₂-
297 gas was intermediate. Therefore, it is believed that the softening phenomenon of CO₂ can be improved by using N₂
298 gas.

299 **Thickness and Warner-Bratzler shear force (WBSF) of chicken's small intestine under different stunning** 300 **methods**

302 Table 3 shows the thickness and WBSF value for chicken small intestines with stunned and non-stunned. The
303 thickness of both fresh and cooked small intestines was significantly higher in the Halal treatment compared to the
304 stunning treatments ($p<0.05$). Among the stunning treatments, Elec-stun had the highest thickness value and the
305 lowest value was found in CO₂-gas ($p<0.05$). The thickness of N₂-gas treatment was higher than CO₂-gas but lower
306 than Elec-stun.

307 The thickness value of small intestine is influenced by various factors including genetics, sex, feeding habits,
308 disease, nutrition, age, and bacterial load *etc.* The stunning method and pre-slaughter stress may affect the thickness
309 value of the small intestine in chickens post-mortem. The thickness value of N₂-stunned pigs' small intestine was
310 higher than that of CO₂-stunned pigs, and lower than that of electrically-stunned pigs under both fresh and cooked
311 conditions [13]. The present study demonstrates similar findings. The electrically-stunned (Elec-stun) broiler small
312 intestine showed higher thickness values than the other treatments. The thickness value of N₂-stunning chicken small
313 intestine (N₂-gas) was higher than those of CO₂-stunning chickens (CO₂-gas).

314 In both fresh and cooked conditions, the WBSF in small intestine of thickness differed significantly between all
315 treatments. The Halal treatment provided higher values of WBSF than all the stunning treatments. Among the stunning
316 treatments in both fresh and cooked conditions, the Elec-stun treatment showed the highest values of WBSF and the
317 lowest values of WBSF were found in the CO₂-gas. The WBSF value of the N₂-gas treatment was between those of
318 the Elec-stun and CO₂-gas.

319 The WBSF value of the small intestine can be influenced by pre-slaughter stunning. During exposure to high
320 concentrations of CO₂ during stunning, an enormous amount of CO₂ is absorbed in the body. This gas was highly
321 dissolved in the chicken breast-meat and small intestine of the animals and remained elevated as a residue [70,73]. It
322 was reported that the WBSF value of N₂-stunned pork meat intestines was intermediate between those of electrically-
323 and CO₂-stunned pigs [13]. Similar findings were observed in the present study; here CO₂-stunned broiler small
324 intestine (CO₂-gas) showed a lower WBSF value than electrically-stunned (Elec-stun) small intestine, and N₂
325 stunning (N₂-gas) provided intermediate results.

326

327

Conclusion

328 The purpose of this study was to assess the quality attributes of N₂ gas (98%)-stunned broiler breast meat and small
329 intestine compared with chicken breast meat stunned using conventional electrical, CO₂-gas and Halal stunning. Our
330 research findings indicate that N₂ gas stunning results in less discoloration in meat and small intestines compared to
331 CO₂ stunning and halal stunning. Additionally, N₂ stunning exhibits color properties similar to electrical stunning and
332 inhibits the phenomenon of softening caused by CO₂ stunning method. Therefore, high-concentration N₂ gas stunning
333 method can be considered for new industrial applications in poultry slaughter. However, for the adoption of N₂

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

336

337

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514 **Table 1.** Effect of stunning (with electric, CO₂ gas and N₂ gas) and non-stunning halal method on pH_{24h} and
 515 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

516 ¹⁾Treatments: Elec-stun, electrical stunning method; CO₂-gas, 80% CO₂ gas stunning method; N₂-gas, 98% N₂ gas
 517 stunning method; Halal, non-stunning method. n = 40. ^{a-d} Different superscript letters in same row means significant
 518 differences (*p* < 0.05). SEM = Standard error of mean.

519 **Table 2.** Effect of stunning (with electric, CO₂ gas and N₂ gas) and non-stunning halal method on proximate
 520 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

521 ¹⁾Treatments: Elec-stun, electrical stunning method; CO₂-gas, 80% CO₂ gas stunning method; N₂-gas, 98% N₂ gas
 522 stunning method; Halal, non-stunning method. n = 40. ^{a-d} Different superscript letters in same row means significant
 523 differences (*p* < 0.05). SEM = Standard error of mean.

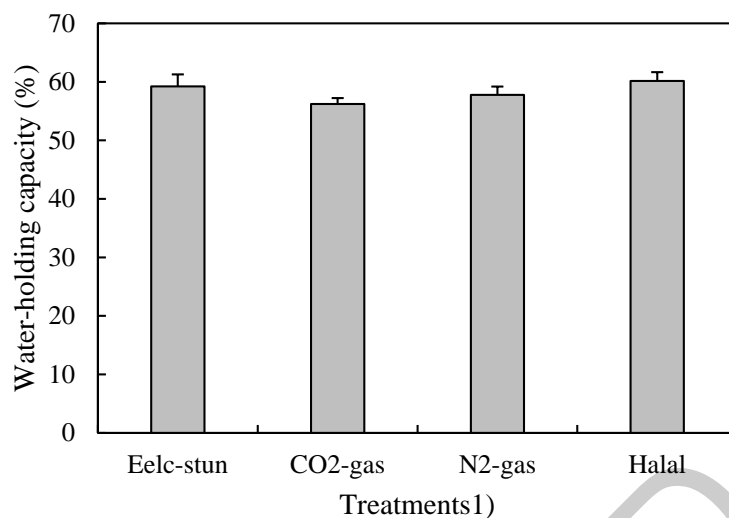
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524 **Table 3.** Effect of stunning (with electric, CO₂ gas and N₂ gas) and non-stunning halal method on thickness
 525 and Warner-Bratzler shear force (WBSF) of chicken small intestine

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/cm²)				
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

526 ¹⁾Treatments: Elec-stun, electrical stunning method; CO₂-gas, 80% CO₂ gas stunning method; N₂-gas, 98% N₂ gas
 527 stunning method; Halal, non-stunning method. n = 40. ^{a-d} Different superscript letters in same row means significant
 528 differences (*p* < 0.05). SEM = Standard error of mean.

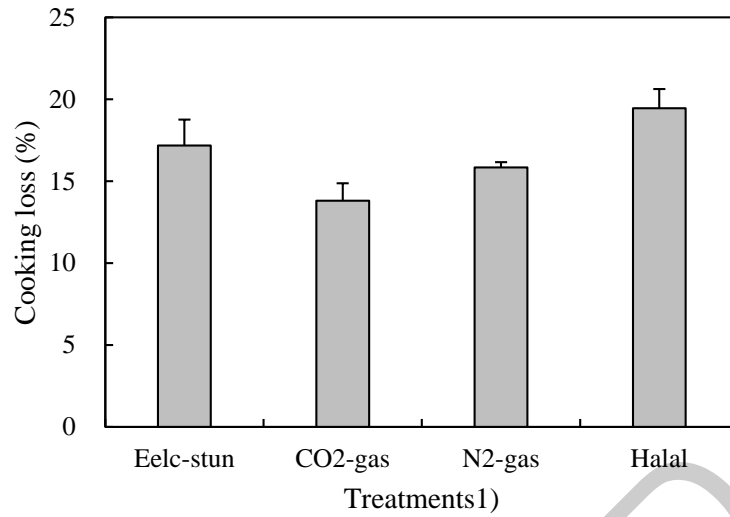
ACCEPTED



529

530 **Fig. 1. WHC of stunning and non-stunning (halal) chicken meat.** ¹⁾Treatments: Elec-stun, electrical
 531 stunning method; CO₂-gas, 80% CO₂ gas stunning method; N₂-gas, 98% N₂ gas stunning method; Halal, non-
 532 stunning method.

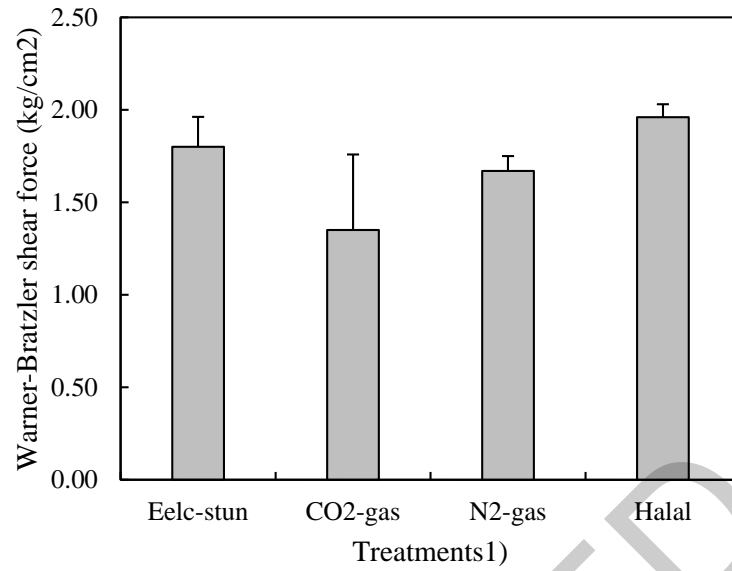
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533

534 **Fig. 2. Cooking loss of stunning and non-stunning (halal) chicken meat.** ¹⁾Treatments: Elec-stun, electrical
 535 stunning method; CO₂-gas, 80% CO₂ gas stunning method; N₂-gas, 98% N₂ gas stunning method; Halal, non-stunning
 536 method.

ACCEPTED



537

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

539 ¹⁾Treatments: Elec-stun, electrical stunning method; CO₂-gas, 80% CO₂ gas stunning method; N₂-gas, 98% N₂ gas
 540 stunning method; Halal, non-stunning method..