Gaping of pectoralis minor muscles: magnitude and characterization of an emerging quality issue in broilers

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ABSTRACT Recently, a certain number of broiler abattoirs located in different Countries around the World have signaled an emerging quality issue termed "gaping" because of the separation of the fiber bundles affecting the external portion of the bipinnate pectoralis minor muscle. Thus, after defining the criteria to classify the muscles as Normal (**NORM**), Moderate (**MOD**), or Severe (**SEV**) cases, the incidence of gaping under commercial conditions was assessed on a total of 8,600 P. minor obtained from broiler chickens belonging to 43 flocks during a 6-mo period. Then, a total of 180 P. minor were selected based on previously defined criteria to evaluate the main quality traits (pH, color, water-holding/-binding capacity and tenderness), proximate composition, water mobility, and thermal properties as well as metabolic profile through [1]H-Nuclear Magnetic Resonance spectroscopy. The average incidence of gaping defect was found to be 16.8% (8.8 and 8.0% MOD and SEV cases, respectively). As for the main quality traits, a reduction in ultimate pH was observed as the severity of the gaping defect increased, with SEV muscles displaying significantly lower values in comparison with NORM (5.96 vs. 6.02; $P < 0.01$), while MOD showed intermediate values (5.99). Concurrently, if compared with their NORM counterpart, MOD and SEV exhibited higher lightness (53.6 and 54.2 vs. 51.8; $P < 0.01$) coupled with higher ($P < 0.05$) cooking losses and longer ($P < 0.05$) transversal relaxation time of extra-myofibrillar water fraction. Overall, no significant differences were found concerning proximate composition and thermal properties. With regard to the metabolic profile, a significantly lower ($P < 0.001$) glutamine concentration was found in MOD and SEV muscles that, concurrently, revealed significant ($P < 0.05$) variations in the metabolites involved in energy-generating pathways. Overall, these findings evidenced that the gaping defect affecting broilers' P. minor muscles have strong similarities with the pale-soft-exudative condition previously described in poultry and likely results from the biochemical processes taking place during the post-mortem conversion of muscle to meat.

Key words: broiler, pectoralis minor, gaping, meat quality, NMR

INTRODUCTION

Demand for meat has been increasing during the past decades because of the population growth, the rising income and urbanization. In this context, being more affordable if compared with red meats, poultry meat is the primary driver of the growth in total meat production in response to expanding global demand (Godfray et al., 2018). Indeed, low production costs and lower product prices have contributed to making poultry the meat of choice both for producers and consumers in developing countries (Windhorst, 2017). On the other hand, in societies where people tend to live and work in urban areas, taking most meals away from home

and spending less and less time on home-meal preparation, together with increasing culinary ignorance, a greater proportion of poultry meat is processed before purchase (Swatland 2010; Godfray et al., 2018). In this case, there is a strong preference for white breast meat because of its low-fat content, higher tenderness and convenience (Wideman et al., 2016). In detail, consumer preference toward pectoralis minor muscles (**Pmin**) (commercially referred to tenderloin or tender) is currently increasing and many raw and cooked products (i.e., nuggets, grilled fillets, and pastrami) are manufactured by using these muscles (Graber, 2018).

During the past decades, in order to fulfil the increased demand for poultry meat, selection programs have been carried out to increase the growth rate and breast development in meat-type chickens and processors have been pushed to raise the live weight at slaughter (Petracci et al., 2015; Kuttappan et al., 2016). As

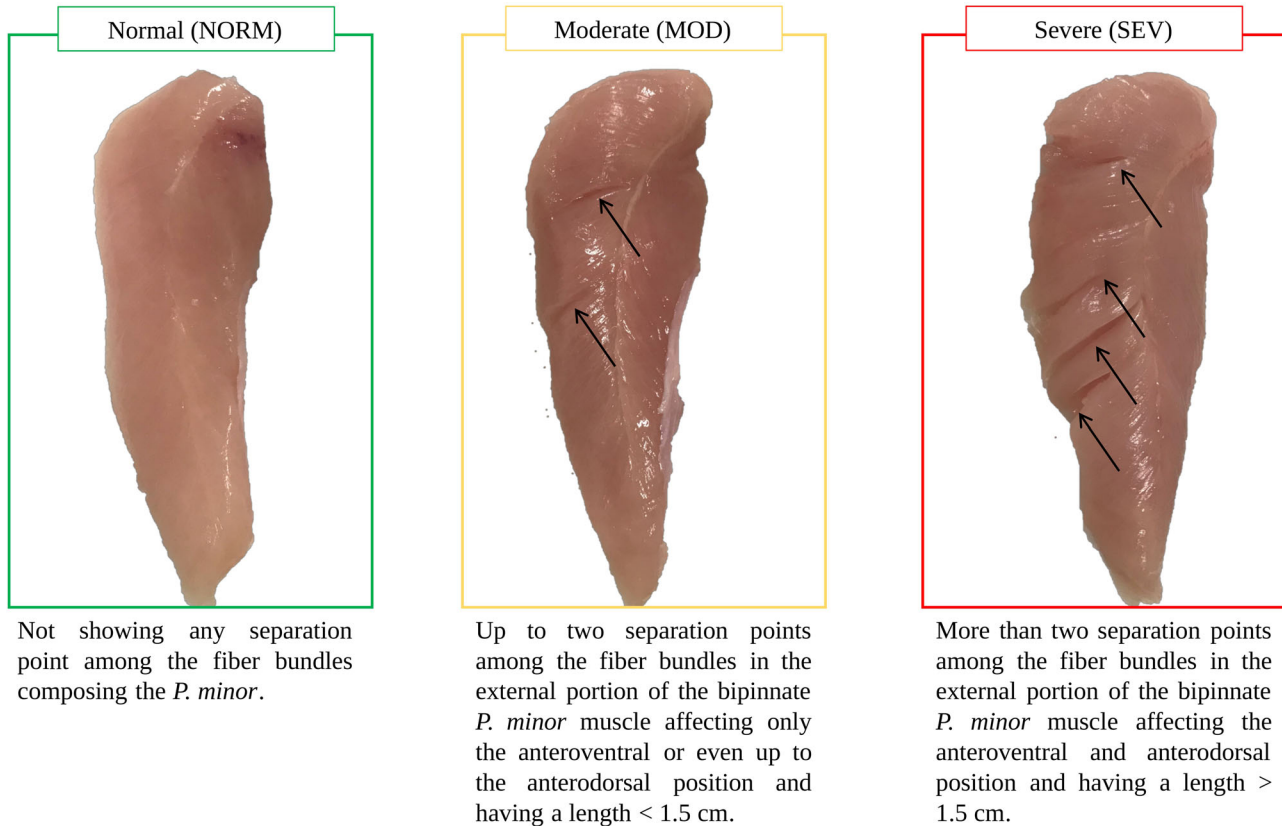| Normal (NORM)	Moderate (MOD)	Severe (SEV)
Not showing any separation point among the fiber bundles composing the *P. minor*.	Up to two separation points among the fiber bundles in the external portion of the bipinnate *P. minor* muscle affecting only the anteroventral or even up to the anterodorsal position and having a length < 1.5 cm.	More than two separation points among the fiber bundles in the external portion of the bipinnate *P. minor* muscle affecting the anteroventral and anterodorsal position and having a length > 1.5 cm.

Figure 1. Criteria adopted to classify the pectoralis minor muscles according to the presence and the severity of gaping defect.

a result, nowadays, pectoralis major and Pmin muscles reach a weight of about 300 and 60 g, respectively, in broilers slaughtered at 42 D of age (Griffin et al., 2018). However, these improved performances and production traits have resulted in an increased occurrence of breast meat downgrading rates due to blisters, bruises, and especially muscle abnormalities (Petracci et al., 2015). The main abnormality/quality issue affecting the Pmin muscle is the green muscle disease or deep pectoral myopathy (**DPM**) which was first described in fast-growing birds about 30 yr ago (Kijowski et al., 2014; Petracci et al., 2015). Despite the efforts exerted by poultry breeders and producers to reduce its occurrence, DPM still represents an important quality issue in poultry (Yalcin et al., 2018; Stangierski et al., 2019). In addition, pale soft and exudative (**PSE**)-like and white striping, which commonly affect P. major, muscles have also been observed in Pmin muscles (Kuttappan et al., 2016; Yalcin et al., 2018). More recently, the occurrence of a new quality defect affecting Pmin muscle have been signaled by a certain number of broiler abattoirs located in different Countries around the World (personnel communication). This defect is similar to the so-called "gaping" defect affecting fish fillets, where tearing of the intramuscular connective tissue during post-mortem rigor development results in splits, tears or holes (Jacobsen et al., 2017). Similarly, an increasing proportion of Pmin muscles display the occurrence of separation points along the fiber direction (as shown in Figure 1). Because

of their impaired visual appearance, Pmin muscles affected by gaping defect are normally downgraded thus resulting in economic losses for the industry. Intriguingly, similar quality issues were previously observed in porcine semimembranosus (Voutila et al., 2008, 2009; Théron et al., 2019) and adductor (Hugenschmidt et al., 2010) muscles as well as in broilers (Baldi et al., 2018). The latter, termed "spaghetti meat" abnormality, has been recently observed in chicken P. major in which a distinctive tendency towards the separation of fiber bundles in the cranial portion of the muscle has been found (Petracci et al., 2019). The histological analyses carried out on spaghetti meat affected muscles evidenced the occurrence of fiber degeneration and a progressive rarefaction of the endo- and peri-mysial connective tissue (Baldi et al., 2018). Similarly, destructured porcine muscles exhibit an evident tendency to lose their integrity as a consequence of both pre- and post-rigor handling operations, thus representing an important quality issue especially in case these muscles are processed to produce cooked ham (Hugenschmidt et al., 2010; Théron et al., 2019). In detail, the low ultimate pH and impaired water holding capacity frequently observed in destructured porcine muscles evidence many similarities with the PSE condition (Voutila et al., 2008; Laville et al., 2005; Théron et al., 2019). Within this context, after defining the criteria to classify the Pmin muscles displaying gaping, the present study aimed at assessing the incidence rate of this defect under
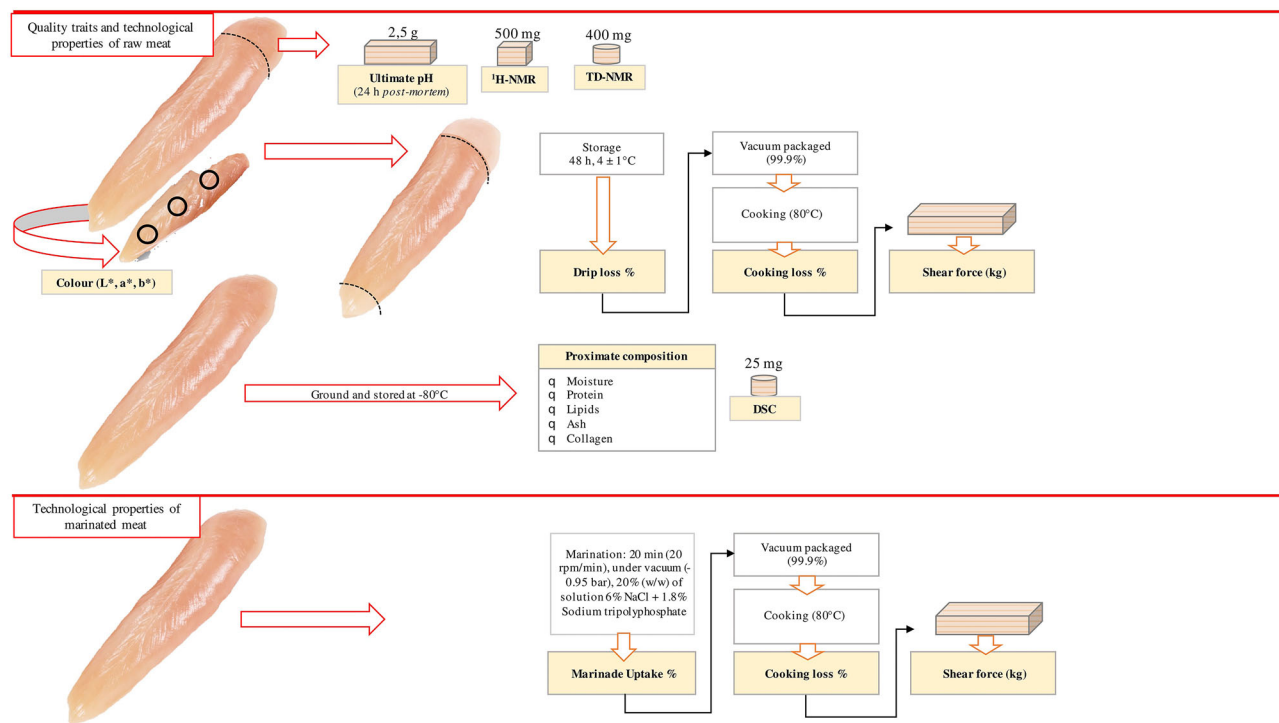
Figure 2. Sampling procedure used to evaluate the main quality traits of fresh meat (ultimate pH, drip and cooking losses, shear force), the technological properties of marinated meat (uptake, cooking loss, shear force), water mobility and distribution (TD-NMR), thermal properties (DSC) as well as to assess the concentration of free amino acids, histidine-containing dipeptides, and metabolites by using ^{1}H-NMR spectroscopy.

commercial conditions. In addition, quality traits and technological properties were assessed in order to characterize the affected meat and evaluate the implications of the occurrence of this defect.

MATERIAL AND METHODS

Experiment 1–Identification and Incidence of Gaping Defect in Pmin muscles

The survey was carried out in a major Italian processing plant on a total of 8,600 chickens randomly chosen from 43 flocks during a 6-mo period. Chickens of both sexes belonging to the main commercial hybrids used in U.S. and Europe were farmed under intensive conditions and slaughtered at an age from 42 to 54 D (average live weight: 2.84 kg). The birds were processed under commercial conditions: electrically stunned (150 mA/bird, 400 Hz), immediately killed by severing the jugular vein and carotid artery with an automatic device and allowed bleeding (180 s). Subsequently, birds were scalded at 51 to 52°C for 215 s, plucked, eviscerated, and air-chilled passing through a cold-air flow tunnel (−6°C for 150 min) until reaching 2 to 3°C at the core. After chilling, breasts were separated from the carcasses in-line, placed on cones and an opening cut was made at the shoulder joint on each side of the front half. Subsequently, 2 boneless skinless fillets (P. major) and 2 tenders (Pmin or Supracoracoideus muscle) were manually separated from each breast by employees. For each flock, a total of 200 tenders were

randomly collected and subsequently used to assess the presence/absence of the gaping defect. In detail, each muscle was classified as Normal (**NORM**) or for those displaying gaping, it was determined whether the Pmin was affected to a "moderate" (**MOD**) or "severe" (**SEV**) level by using the criteria described in Figure 1. All the aspects related to farming, handling, transportation, and slaughtering of the birds strictly accomplished with the European legislation (European Commission, 2005, 2007, 2009).

Experiment 2–Characterization of Quality Traits of Pmin muscles Affected by Gaping Defect

Two individual trials were conducted, using a total of 180 Pmin muscles (60 muscles/group) collected at 3 h post-mortem in the deboning area of the same processing plant and, in order to avoid any interference, muscles contextually affected by other defects (i.e., paleness, haemorrhages, etc.) were not considered. All Pmin muscles (N = 180) were weighed and used to assess color. Then, according to the protocol reported in Figure 2, 72 muscles (24 samples/group) were used to evaluate the main quality traits of fresh meat (ultimate pH, drip and cooking losses, shear force), water mobility and distribution through Time Domain Nuclear Magnetic Resonance (**TD–NMR**), as well as to assess the concentration of free amino acids, histidine-containing dipeptides, and metabolites by using

^{1}H-NMR spectroscopy. A total of 72 Pmin muscles (24 samples/group) were employed to evaluate the technological properties of marinated meat (uptake, cooking loss, and shear force) whereas other 36 samples (12 Pmin muscles/group) were analyzed to assess proximate composition (moisture, protein, fat, ash, and collagen) and thermal properties by differential scanning calorimetry (**DSC**). In order to minimize the variability resulting from the sampling position, sub-samples used to assess the same parameter were excised exactly in the same position of the Pmin muscle, immediately frozen and stored at −80°C until processing.

Analytical Methods

Quality Traits Color (CIE, L* = Lightness, a* = Redness and b* = Yellowness) was assessed in triplicate on the ventral surface of each Pmin muscle by using a Chroma Meter CR-400 (Konica Minolta Corp., Milan, Italy). Ultimate pH (**pHu**) was determined in the cranial portion of the muscle by homogenizing 2.5 g of meat in 25 mL of 150 mM KCl and 5 mM sodium iodoacetate solution (Jeacocke, 1977). Then, other 2 sub-samples of 500 and 400 mg were excised from the upper portion of the muscle and intended for ^{1}H-NMR and TD-NMR analyses, respectively. Subsequently, the remaining part of the Pmin muscles was placed in covered plastic boxes, over sieved plastic racks and, after 48 h of refrigerated storage (4 ± 1°C), individually weighed to determine drip loss. Then, after packing under vacuum, the samples were cooked in a water bath at 80°C until reaching the same temperature at the core of samples (in order to avoid any temperature gradient between the outer surface of the muscle and its inner part) and subsequently weighed to calculate cooking loss. Tenderness was assessed in triplicate on sub-samples ($3 \times 1 \times 1$ cm) excised in 3 different positions along the muscle (upper and lower part and central zone) by using a heavy-duty texture analyzer (Stable Micro Systems Ltd), equipped with a 5 kg-loading cell and a Warner-Bratzler shear blade set in order to shear the samples perpendicularly to the fiber direction. Water binding capacity was assessed on 72 Pmin muscles (24 muscles/group) individually labelled and vacuum-tumbled (−0.95 bar) for 20 min with the addition of 20% (w/w) marinade solution (6% sodium chloride and 1.8% sodium tripolyphosphate) in a small-scale tumbler (mod. MGH-20; Vakona Qualitat, Lienen, Germany). After tumbling, each Pmin muscle was weighed in order to evaluate the ability of the meat to bind the saline solution added. Then, each muscle was packed under vacuum and cooked in a water bath, by adopting the same conditions described for raw meat, to calculate cooking loss. Then, tenderness was assessed in triplicate on sub-samples ($3 \times 1 \times 1$ cm) following the same protocol used to determine shear force for raw meat.

NMR Measurements Water mobility and distribution was assessed by TD-NMR. Proton transverse relaxation (T_2) decay curves were recorded at the operating frequency of 20 MHz with a Bruker (Milan, Italy) Minispec PC/20 spectrometer using the Carr-Purcell-Meiboon-Gill (**CPMG**) pulse sequence previously described by Petracci et al. (2014). Briefly, measurements were performed at a constant temperature of 25°C on a meat sample weighing 400 mg and having a height not exceeding the active region of the radio frequency coil. The CPMG decays were normalized by the sample weight and transformed into relaxograms by using the program UPEN. Then, each relaxogram was interpreted in terms of proton populations ascribed to the bound, intra- and extra-myofibrillar water fraction. In detail, to separately observe these proton populations, relaxograms were fit to the sum of four exponential curves and the two with intermediate T_2, describing the behavior of intra-myofibrillar protons, were combined.

The ^{1}H-NMR analyses were performed on 500 mg of meat homogenized by Ultra-Turrax T25 (IKA®-Werke GmbH & Co., Germany) (20 s, 11,000 rpm) in 3 mL of distilled water. After centrifuging (10 min, 18,000 × g at 4°C) 1 mL of homogenate, 700 µL of the supernatant were transferred in a new tube in which 800 µL CHCl$_3$ were added. The samples were mixed by vortex (2 min) and then centrifuged (2 min, 18,000 × g at 4°C). An aliquot (500 µL) of supernatant was transferred to a new tube and 200 µL of potassium phosphate buffer (1 M, 2 mM sodium azide; pH 7.0) in D$_2$O and 10 mM 3-(Trimethylsilyl) propionic-2,2,3,3-d4 acid sodium salt (**TSP**) were added. Following centrifugation (10 min, 18,000 × g at 4°C), 700 µL of supernatant were transferred to NMR tube for analysis. The spectra were acquired using the *cpmgpr1d* pulse sequence with suppression of the solvent signal and the following parameters: size of fid: 32 k, number of scans: 16, number of dummy scans: 16, spectral width: 12 ppm, acquisition time: 2.28 s, delay d1: 5 s. NMR spectra were processed, adjusted and quantified with Topspin 3.1 and R software while elucidation and identification of the compounds was done with the help of the HMDB database (http://www.hmdb.ca/), Chenomx software and literature references. For quantification and calibration of the spectrum, the TSP was used (δ 0.00).

Proximate Composition Moisture, protein, fat, and ash contents were determined on finely minced Pmin muscles (12 samples/group) following standard methods. In detail, moisture and ash contents were calculated as the percentage of weight lost after drying (105°C for 16 h) and incinerating (at 525°C) samples weighing 5 g (AOAC, 1990). Nitrogen content, as directly related to the crude protein, was calculated according to Kjeldahl method by titrating the ammonia released after the digestion of a 0.5 g meat sample in the presence of copper sulphate as catalyst (AOAC, 1990). Lipids were quantified following the chloroform: methanol extraction procedure (Folch et al., 1957) whereas collagen content was calculated from the amount of hydroxyproline determined

according to the colorimetric method proposed by Kolar (1990).

Thermal Properties

Denaturation enthalpy was determined by using a differential scanning calorimeter Q20 (TA Instrument, Germany) equipped with a low-temperature cooling unit Intercooler II (Perkin-Elmer Corporation, Wellesley, USA). Temperature and melting enthalpy calibrations were performed with ion exchanged distilled water (mp 0.0°C) and indium (mp 156.60°C) while heat flow was calibrated using the heat of fusion of indium ($\Delta H = 28.71$ J/g). For the calibration, the same heating rate and dry nitrogen gas flux of 50 mL/min used for the analysis were applied. Each muscle sample (about 25 mg) was analysed in triplicate into a 50-µL aluminium pan, hermetically sealed and loaded into the instrument at room temperature. According to Wattanachant et al. (2005), the heating rate of DSC scans was 5°C/min over a range from 20 to 90°C. Empty aluminium pans were used as reference and for baseline corrections. The results were expressed as total enthalpy (J/g) and the relative denaturation enthalpies (%) for each protein fraction (sarcoplasmic, myofibrillar and collagen) were calculated through PeakFit Software (SeaSolve Software Inc. Framingham, MA, USA).

Statistical Analysis

Data from experiment 1 were presented through descriptive statistic as mean, SEM, minimum, and maximum values. The results obtained from experiment 2 were analyzed by 2-way ANOVA considering the occurrence of gaping defect with different levels of severity and replication as main factors, as well as the interaction term. Mean values were subsequently separated through the parametric Tukey HSD test. All statistical differences were considered significant at a level of $P \leq 0.05$. In addition, in order to provide a more comprehensive representation and interpretation of the results obtained through ^{1}H-NMR, Principal Component Analysis (**PCA**) was carried out by considering those variables that, having the highest discrimination power, resulted significantly different among the groups. All statistical analyses were carried out by using Statistica software (StatSoft Italy srl, Vigonza, Italy).

RESULTS

The incidence of the gaping defect affecting the Pmin muscle in broiler chickens evaluated under commercial conditions in the processing plant is shown in Table 1. The total incidence of tenders affected by gaping defect was found to be 16.8% (8.8 and 8.0% of MOD and SEV affected cases, respectively). Overall, the range of variation in the incidence was fairly large and varied from 7.5 to 29.5%. Considerable variations were also

Table 1. Incidence of gaping defect affecting pectoralis minor muscle in broiler chickens under commercial conditions.

Item	Severity of gaping defect (%)[1,2]		
	Moderate	Severe	Total
Mean	8.8	8.0	16.8
SEM	0.45	0.44	0.78
min-max	4.0–16.0	2.5–13.5	7.5–29.5

[1]Muscles displaying gaping were classified as according to criteria reported in Figure 1.

[2]No. of flocks considered in the present study: 43, corresponding to 8,600 broiler tenders examined.

Table 2. Impact of the occurrence of broilers' pectoralis minor muscles affected by gaping defect with different levels of severity on quality traits and technological properties of raw and marinated meat.

Parameter	Degree of gaping[1]			SEM	P-value
	Normal	Moderate	Severe		
Weight (g)[2]	60.7	57.2	58.8	0.89	ns
pHu[3]	6.02[a]	5.99[a,b]	5.96[b]	0.01	**
Lightness—L*[2]	51.8[b]	53.6[a]	54.2[a]	0.33	**
Redness—a*[2]	1.9	1.9	1.7	0.09	ns
Yellowness—b*[2]	2.2[b]	3.2[a]	3.2[a]	0.13	***
Technological properties of raw meat					
Drip loss (%)[3]	1.8	1.8	2.0	0.13	ns
Cooking loss (%)[3]	20.9[b]	21.9[a,b]	24.0[a]	0.50	*
Shear force (kg)[3]	1.58	1.55	1.78	0.06	ns
Technological properties of marinated meat					
Uptake (%)[3]	15.0[b]	16.6[b]	19.2[a]	0.46	***
Cooking loss (%)[3]	17.0[b]	18.1[a]	17.1[a,b]	0.18	*
Shear force (kg)[3]	1.07[a]	1.05[a,b]	0.95[b]	0.02	*

* $= P < 0.05$; ** $= P < 0.01$; *** $= P < 0.001$; ns $=$ not significant.
[a,b]$=$ Mean values followed by different letters significantly differ among the groups ($P < 0.05$).
[1]Muscles displaying gaping were classified as normal, moderate or severe according to criteria reported in Figure 1.
[2]N = 180; 60 muscles/group.
[3]N = 72; 24 muscles/group.

observed for MOD (range: 4.0 to 16.0%) and SEV (range: 2.5 to 13.5%).

The results concerning the impact of gaping defect, occurring with different levels of severity, on quality traits and technological properties of raw and marinated meat are shown in Table 2. With regard to color parameters, MOD and SEV muscles exhibited significantly higher lightness (L*, 53.6 and 54.2 vs. 51.8; $P < 0.01$) and yellowness (b*, 3.2 and 3.2 vs. 2.2; $P < 0.001$) in comparison with their NORM counterpart. Concurrently, a reduction in ultimate pH was observed as the severity of the gaping defect increased. In detail, if compared with NORM, SEV muscles displayed significantly lower pHu (5.96 vs. 6.02; $P < 0.01$) whereas MOD had intermediate values (5.99). On the other hand, no significant differences were found concerning redness (a*) and muscle weight.

As to the technological properties of raw meat, no significant variations were observed in the amount of fluid released after 48 h of storage as well as in the shear force. However, a remarkable increase in cooking loss was found as the severity of the gaping defect increased with SEV muscles displaying higher cooking losses in

Table 3. Impact of the occurrence of gaping defect with different levels of severity on the relative intensity (%) and T_2 relaxation time (ms) of the proton populations identified through TD-NMR in broilers' pectoralis minor muscles (N = 72; 24 muscles/group).

	Degree of gaping[1]				
	Normal	Moderate	Severe	SEM	*P*-value
Relative intensity (%) of each proton population					
Bound	3.4	3.4	3.4	0.03	ns
Intramyofibrillar	82.2	82.6	83.1	0.35	ns
Extramyofibrillar	14.4	13.9	13.6	0.35	ns
T_2 relaxation time (ms) of each proton population					
Bound	1.4	1.5	1.4	0.02	ns
Intramyofibrillar	40.3	40.1	40.8	0.27	ns
Extramyofibrillar	95.8[b]	97.3[a,b]	105.2[a]	1.66	*

*= $P < 0.05$; ns = not significant.
[a,b]= Mean values followed by different letters significantly differ among the groups ($P < 0.05$).
[1]Muscles displaying gaping were classified as normal, moderate or severe according to criteria reported in Figure 1.

Table 4. Impact of the occurrence of gaping defect with different levels of severity on proximate composition in broilers' pectoralis minor muscles (N = 36; 12 muscles/group).

	Degree of gaping[1]				
Parameter	Normal	Moderate	Severe	SEM	*P*-value
Moisture (%)	75.39	75.46	75.18	0.13	ns
Protein (%)	22.40	22.90	22.93	0.16	ns
Lipid (%)	1.16	1.36	1.20	0.05	ns
Ash (%)	1.26	1.27	1.30	0.01	ns
Collagen (%)	0.99	0.98	1.10	0.05	ns

ns = not significant.
[1]Muscles displaying gaping were classified as normal, moderate or severe according to criteria reported in Figure 1.

comparison with NORM (24.0 vs. 20.9%; $P < 0.05$). Slightly different results were found by evaluating the technological properties of marinated meat. Indeed, if compared with both NORM and MOD, SEV muscles exhibited a significantly higher uptake (15.0 and 16.6 vs. 19.2%; $P < 0.001$) and, concurrently, lower ($P < 0.05$) shear force values. In addition, if compared with their NORM counterpart, MOD exhibited significantly ($P < 0.05$) higher cooking losses whereas SEV had intermediate values.

The findings concerning the impact of gaping defect on the relative intensity (%) and T_2 relaxation time for the proton populations identified through TD-NMR and ascribed to the bound, intra- and extra-myofibrillar water fractions are reported in Table 3. Overall, the occurrence of gaping did not affect either the water distribution within the muscle tissue (relative intensity) or how each fraction is bound and interacts with the fibrillar structure (T_2), with the only exception being the relaxation time recorded for the water located in the extra-myofibrillar compartments. Indeed, for this fraction, if compared with NORM, significantly ($P < 0.05$) longer T_2 were measured as the severity of gaping increases: MOD displayed intermediate values whereas SEV exhibited significantly higher T_2.

Table 4 shows the impact of gaping defect on proximate composition of broiler Pmin muscles. The findings

Table 5. Peak temperature and enthalpies in broilers' pectoralis minor muscles displaying gaping defect with different levels of severity (N = 72; 24 muscles/group).

	Degree of gaping[1]			
	Normal	Moderate	Severe	*P-value*
Total enthalpy – ΔH (J/g)	2.95 ± 0.09[b]	3.44 ± 0.11[a]	3.13 ± 0.09[a,b]	*
Relative melting enthalpy[2] (%)				
peak 1	48.8 ± 0.49[a]	43.2 ± 0.47[b]	46.8 ± 0.55[a]	*
peak 2	17.7 ± 0.46[a,b]	19.4 ± 0.38[a]	17.4 ± 2.19[b]	*
peak 3	10.4 ± 0.34	10.3 ± 0.68	10.3 ± 1.84	ns
peak 4	10.9 ± 0.32	11.9 ± 0.15	11.4 ± 1.78	ns
peak 5	14.9 ± 0.24	14.7 ± 0.30	14.2 ± 2.82	ns

*= $P < 0.05$; ns = not significant.
[a,b]= Mean values followed by different letters significantly differ among the groups ($P < 0.05$).
[1]Muscles displaying gaping were classified as normal, moderate or severe according to criteria reported in Figure 1.
[2]The transition temperatures of the 5 endothermic peaks identified in the present study are: 58.7°C (sarcoplasmic proteins), 64.7°C (collagen), 68.7°C, 73.2°C and 78.1°C (myofibrillar proteins).

evidenced that the occurrence of gaping did not exert any detrimental effect on composition and, thus, on the nutritional value of the affected meats.

With regard to DSC analysis, the results, expressed in terms of total enthalpy (J/g) and relative melting enthalpy (%) are reported in Table 5. Five endothermic peaks were observed in the thermogram. Therefore, in order to attribute each one to the denaturation of specific components of the muscle, the myofibrillar and the sarcoplasmic proteins as well as the collagen composing the endo- and peri-mysial compartments, were extracted, lyophilised, and analysed adopting the same conditions previously described for the samples. The peak transition temperature recorded for the lyophilised protein fractions were 53°C (collagen), 54°C (sarcoplasmic protein), 61°C, 71°C and 76°C (myofibrillar proteins), which were a little lower, especially for collagen, than those assessed on the meat samples (58.7°C, 64.7°C, 68.7°C, 73.2°C, and 78.1°C) thus suggesting an effect of the matrix as well as of the extraction and lyophilisation procedure on the thermal properties of the proteins. Thus, according to these results, the five endothermic peaks observed in the present study were attributed to the denaturation of the sarcoplasmic proteins (58.7°C), of the collagen composing the endo- and peri-mysial compartments (64.7°C) and of the myofibrillar proteins such as myosin (68.7°C), other proteins (73.2°C) and actin (78.1°C). Overall, if compared with NORM, MOD exhibited a significantly higher total enthalpy (3.44 vs. 2.95 J/g; $P < 0.05$) whereas SEV displayed intermediate values (3.13 J/g). Significant differences among the groups concerning the relative melting enthalpies of peaks 1 and 2 (related to the denaturation of the sarcoplasmic proteins and collagen, respectively). In detail, if compared with NORM and SEV, MOD exhibited significantly lower relative melting enthalpy for the sarcoplasmic proteins (43.2 vs. 48.8 and 46.8%; $P < 0.05$) whereas the same muscles revealed a

Table 6. Impact of the occurrence of gaping defect with different levels of severity on the concentration (mg/100 g of meat) of free amino acids, histidine compounds, and main metabolites in broilers' pectoralis minor muscles (N = 36; 12 muscles/group).

	Degree of gaping[1]				
	Normal	Moderate	Severe	SEM	*P*-value
Free amino acids					
Threonine	30.6	31.1	29.9	0.42	ns
Serine	9.3[a]	8.2[b]	8.3[b]	0.17	**
Asparagine	9.9	9.0	9.9	0.27	ns
Glutamine	20.8[a]	14.8[b]	16.8[b]	0.69	***
Proline	7.7	6.9	7.1	0.22	ns
Glycine	23.4[b]	25.4[a]	24.0[a,b]	0.34	*
Alanine	38.7	38.3	36.1	0.55	ns
Valine	8.4[b]	9.2[a]	8.5[a,b]	0.16	*
Methionine	8.5[a]	7.9[a,b]	7.8[b]	0.12	*
Isoleucine	5.4	5.8	5.3	0.11	ns
Leucine	8.5[b]	9.4[a]	8.8[a,b]	0.15	*
Tyrosine	24.9	23.3	24.7	0.34	ns
Aspartato	3.6	3.8	3.9	0.17	ns
Beta-alanine	33.8	36.9	36.3	1.72	ns
Glutamate	49.4	48.9	46.2	1.02	ns
Phenylalanine	7.0	6.9	6.7	0.11	ns
Tryptophan	3.8	3.9	3.6	0.10	ns
Histidine-containing dipeptides					
Anserine	494.2	505.6	522.4	10.32	ns
Carnosine	182.1[b]	222.1[a]	177.3[b]	8.01	*
Metabolites—energetic metabolism					
IMP	179.9[b]	201.4[a]	193.8[a,b]	3.43	*
Creatine	439.4	431.9	415.8	6.18	ns
Lactate	672.7	669.2	648.9	8.01	ns
Fumarate	0.9[a,b]	1.1[a]	0.8[b]	0.04	**
Guanidoacetate	311.8	316.1	327.2	6.11	ns
Hypoxanthine	23.1[a]	20.7[a,b]	17.3[b]	0.69	**
Inosine	38.1	33.6	37.4	0.99	ns
Other compounds					
Niacinamide	6.8	6.7	6.6	0.11	ns
Uracil	0.7[a,b]	0.8[a]	0.6[b]	0.04	*
Arabinose	3.3	4.2	4.3	0.25	ns
myo-inositol	22.4	23.8	23.8	0.35	ns
Betaine	29.3	28.9	30.7	0.74	ns
N.N-dimethylglycine	9.3[b]	9.4[b]	10.5[a]	0.17	**
Acetate	3.3	3.2	3.1	0.06	ns

*= $P < 0.05$; ** = $P < 0.01$; ns = not significant.
[a,b]= Mean values followed by different letters significantly differ among the groups ($P < 0.05$).
[1]Muscles displaying gaping were classified as normal, moderate or severe according to criteria reported in Figure 1.

significantly higher ($P < 0.05$) relative enthalpy for the connective tissue composing the endo- and peri-mysial compartments.

The results concerning the effect of gaping, occurring with different levels of severity, on the concentration of free amino acids, histidine-containing dipeptides and metabolites in broilers' Pmin muscles are reported in Table 6. The concentration of some free amino acids was significantly affected by the occurrence of this defect. In detail, aside from the severity of the defect, the amount (mg/100 g of meat) of serine, glutamine and methionine was remarkably reduced ($P < 0.05$) within the Pmin muscles displaying gaping. On the other hand, the content of glycine, valine, and leucine was significantly higher ($P < 0.05$) within the Pmin affected by gaping with MOD exhibiting the highest values. A similar result was found by evaluating the amount of histidine-

containing dipeptides. Indeed, although no significant differences among the groups were found in the amount of anserine, a significantly higher carnosine content was observed in MOD in comparison with both NORM and SEV (222.1 vs. 182.1 and 177.3 mg/100 g of meat; $P < 0.05$). As to the metabolites, the occurrence of gaping significantly affected the concentration of IMP which was found to be significantly higher ($P < 0.05$) in MOD in respect to NORM, while SEV exhibited intermediate values. In addition, SEV exhibited significantly lower ($P < 0.01$) fumarate and hypoxanthine content coupled with a remarkably higher ($P < 0.01$) concentration of N, N-dimethylglycine. In order to provide a more comprehensive representation of the results obtained through ^{1}H-NMR, PCA was carried out by considering only those variables that resulted significantly different ($P < 0.05$) among the groups. The first two components explained about 60% of the total variance, being PC1 and PC2 30.8 and 27.9%, respectively. The resulting grouping of the Pmin belonging to NORM, MOD, and SEV groups is shown in Figure 3a. NORM muscles are grouped on the bottom part of the graph in which free amino acids (namely methionine, serine and glutamine) and N, N-dimethylglycine are found (Figure 3b).

DISCUSSION

During the past few years, Pmin gaping defect has been signaled by a certain number of processing plants located in different Countries around the World (personnel communication). However, few information is available concerning this defect on both technical and scientific journals. Tenders showing this condition are usually downgraded because of their impaired appearance and diverted for manufacturing chopped or ground processed products. Considering the increasing demand for both raw and cooked whole-muscle tenders (Graber, 2018), the occurrence of gaping defect can cause relevant economic losses to the poultry industry. Before assessing the incidence of gaping, it was necessary to establish proper criteria to evaluate the presence and the severity of this emerging defect that occurs as a separation of muscle fiber bundles in different positions of the Pmin. In fish fillets, gaps are slits between muscle blocks and their severity can vary within the range from a slight separation at the cut surface to a complete separation right down to the skin (Lavety et al., 1988; Jacobsen et al., 2017). Otherwise, in chicken tenders, the gaping defect almost exclusively affects the external portion of the bipinnate Pmin muscle. Therefore, the criteria selected to classify the occurrence of this defect have been based on both the number and length of fiber bundle separation points which occurred in the muscle (Figure 1). On the other hand, tenders showing damages and cuts clearly ascribable to the deboning operations (i.e., cuts not following the fiber direction) and not to the distinctive phenotype of the gaping defect were considered together with the normal samples showing no or minimal destructuration.
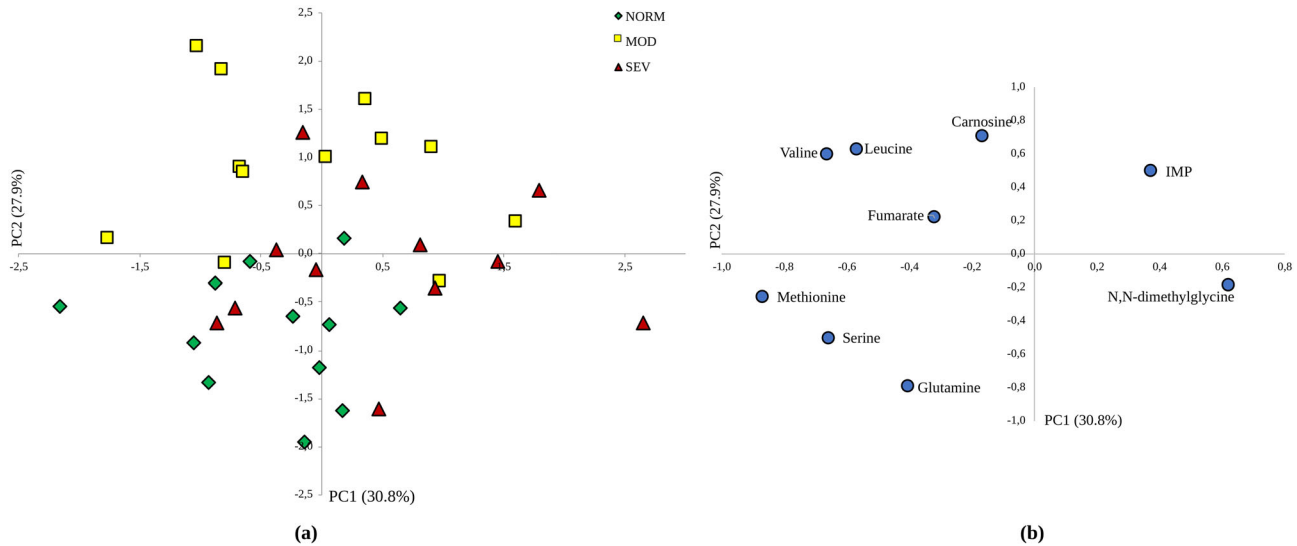
Figure 3. Principal component analysis of the metabolites (free amino acids, histidine-containing dipeptides and metabolites involved in energy-generating pathways) assessed through ^{1}H-NMR in broilers' pectoralis minor muscles displaying gaping defect. Plot of the first 2 principal components score vectors (**a**). Plot of the variables (free amino acids, histidine-containing dipeptides, and main metabolites) that resulted significantly different ($P < 0.05$) among the groups according to the two principal components loading vectors (**b**).

To evaluate the current incidence level of this defect, tenders were randomly selected among flocks of birds slaughtered from 42 to 54 D which are used to yield carcasses for the production of cut-up and further processed products. The total magnitude of tender gaping (16.8%) found in the present study demonstrated that this defect is an important quality issue for the poultry processors. Since no data are available on the occurrence levels of this condition in poultry, it was not possible to compare the data obtained in this study with those found in previous surveys. However, incidence levels are much higher in respect to those observed for the green muscle disease (from 0.02 up to 1.9%; Kijowski et al., 2014), even if the impact on product appearance is of lower extent. In their study, Voutila et al. (2008) reported that the incidence of destructuration in porcine semimembranosus muscles was approximately 20%. Slight differences among moderate and severe cases were found in the present survey thus supporting the hypothesis that the severity of this condition might not be due to an itself different predisposition of the muscle, but rather to different force tearing exerted during deboning procedures. Of course, it can be supposed that different primary chilling systems of the carcasses, deboning post-mortem time and principle (manual vs. automatic) can vary a lot the incidence of gaping defect. For this reason, it will be tricky to compare results obtained in different processing plants.

The analyses of the main quality traits and technological properties of Pmin muscles affected by gaping evidenced that its occurrence is associated with significantly higher lightness values coupled with remarkably lower pHu that accomplish the well-known relationship existing between these 2 parameters in meat: the lower

the pHu is, the higher the lightness values are (Fletcher, 2002; Barbut et al., 2008; Petracci et al., 2017), as a consequence of an increased scattering of the incident light (Mir et al., 2017). However, no significant differences in the concentration of lactate were found among the groups, thus evidencing that not only the accumulation of lactate but also other mechanisms, such as a fast ATP hydrolysis taking place early post-mortem (and resulting in the release of H^+) (Pösö and Puolanne, 2005), might be responsible for the lower pHu observed in SEV muscles. In addition, Pmin displaying gaping exhibited remarkably impaired water holding capacity (as evidenced by the higher cooking losses assessed on both raw and marinated meat). Overall, these quality traits (lower pHu, higher lightness, and reduced water holding capacity) assessed on MOD and SEV overlap with those previously found in raw and cooked pork semimembranosus muscles affected by destructuration (Voutila et al., 2008, 2009; Hugenschmidt et al., 2010) and have strong similarities with the PSE and PSE-like conditions formerly described in pork and chicken meat (Van Laack et al., 2000; Barbut et al., 2008; Petracci et al., 2017; Warner, 2017). The findings obtained through TD-NMR corroborate this hypothesis. Overall, the occurrence of gaping did not affect either the water distribution within the muscle tissue or how each fraction is bound and interacts with the fibrillar structure. In detail, the absence of significant differences in the relaxation times recorded for the bound water fraction might be ascribed to the protein content assessed in NORM as well as MOD and SEV muscles. Indeed, as the bound water refers to the molecules that, through electrostatic interactions, are tightly bound to the muscle proteins (Huff-Loneragn and Lonergan, 2005), the analogous protein content found in the Pmin belonging

to the different experimental groups likely implies that the same amount of water molecules interact with the charged hydrophilic groups of the proteins themselves. However, significantly longer relaxation times were recorded for the water located in the extra-myofibrillar compartments. This fraction, accounting for the water molecules outside the myofibrillar lattice and located within channels interspersed among the myofibrils, is only held by capillary forces and can be easily released during processing (Bowker, 2017; Warner, 2017). Thus, the significantly longer T_2 measured for the protons ascribed to the extra-myofibrillar water suggest that, in Pmin affected by gaping, this fraction is less tightly bound (Bertram et al., 2002). This phenomenon might be explained by considering that the pHu found in these muscles is close to the isoelectric point of their constituting proteins, and this is the primary cause for this kind of meat losing more juice during cooking (Bowker, 2017; Warner, 2017). A further possible explanation might rely in the partial denaturation of the sarcoplasmic proteins taking place during a fast-conversion of muscle-to-meat as previously found in chicken PSE-like breast meat (Van Laack et al., 2000). Similarly, Wilhelm et al. (2010) suggested that the significantly lower pHu observed in PSE-like fillets might be responsible for a shrinking of muscle cell and, concurrently, for a denaturation of proteins thus resulting in a water movement towards the extra-myofibrillar compartments. Otherwise, the significantly higher uptake found in those Pmin displaying gaping might be attributed to the phenotype associated to this defect. Indeed, marinade adsorption is largely affected by the surface area in contact with the solution (Smith and Acton, 2010; Yusop et al., 2011). Therefore, as a consequence of the fiber bundles separation, a higher surface can enter in contact with the marinade solution and, thus, an increased proportion of myofibrillar proteins can be solubilized likely resulting in a significantly higher ability of these meats to bind the saline solution added (Yusop et al., 2011).

With regard to proximate composition, in absolute terms, our results are in agreement with those reported in previous studies in which Pmin muscles were analyzed (Bianchi et al., 2007; Sirri et al., 2011). The absence of significant variations in the composition of muscles affected by gaping, coupled with their lower pHu, corroborates the hypothesis of a different underlying mechanism responsible for the development of this defect rather than that involved in the occurrence of spaghetti meat abnormality affecting broilers' P. major muscles. Indeed, although the phenotype associated to the gaping defect in Pmin is similar to the macroscopic features observed in P. major muscles affected by spaghetti meat abnormality (Petracci et al., 2019), a distinctively high pHu and profound modifications in the chemical composition (higher moisture and lower protein and ash content) were previously found in muscles affected by this condition (Baldi et al., 2018, 2019). On the other hand, if compared with their unaffected counterpart, the Pmin displaying gaping exhibited significantly lower pHu values and unchanged macronutrient contents. Thus, it might be reasonably hypothesized that the development of gaping is not promoted by the profound histological alterations previously observed in spaghetti meat affected muscles (Baldi et al., 2018), but likely results from the biochemical processes taking place during the post-mortem conversion of muscle to meat. Similarly, previous studies carried out on fish fillets evidenced that both pre- and post-mortem handling procedures can significantly impair the mechanical strength of the connective tissue that, once being overcome by the tension developed during rigor, result in the occurrence of gaps (Cheng et al., 2014).

With regard to the thermal properties assessed through DSC, both the number of endothermic peaks and their denaturation temperature are in agreement with those observed in a previous study carried out on broilers' Pmin muscles affected by DPM (Stangierski et al., 2019). In spite of the absence of significant differences in collagen content (see Table 4), MOD exhibited a higher enthalpy of thermal shrinkage of the intramuscular connective tissue. That means that a higher energy is spent in the transformation of collagen from a crystalline to an amorphous form (Voutila et al., 2009), and might be related to an increased number of cross-links in these muscles. However, this observation did not support the phenotype associated with the gaping defect, thus suggesting that other mechanisms and post-mortem factors rather than alterations in the development and histology of the muscle are likely responsible for this condition. Besides, in previous studies carried out on loose structured porcine semimembranosus muscle (Voutila et al., 2008, 2009) and fish fillets (Lavety et al., 1988) displaying gaping, both collagen content and solubility did not differ from their normal structured counterpart. However, the concentration of some free amino acids was remarkably affected by the occurrence of gaping defect (Table 6). Among them, the significantly lower glutamine concentration found in MOD and SEV muscles might be related to an altered and reduced collagen synthesis (Bellon et al., 1995) that could partially explain the phenotype associated to the development of this defect. Indeed, in an in vitro study carried out on human fibroblasts, an increased concentration of glutamine led to a higher collagen synthesis through both a direct stimulatory effect and a concurrent action as a precursor of proline and hydroxyproline (Bellon et al., 1987). In the same time, the significant variations observed in the metabolites involved in energy-generating pathways reinforce the hypothesis that the development of gaping is associated with alterations in post-mortem metabolism.

The distribution of the samples obtained through PCA analysis demonstrates that the occurrence of gaping in Pmin muscles negatively correlates with the

concentration of methionine, serine, and glutamine that are likely involved in collagen synthesis. In addition, although clustered in different regions of the graph, NORM, MOD, and SEV (and especially MOD and SEV) muscles cannot be clearly distinguished one from the others thus suggesting that the occurrence and the severity of gaping defect is not associated to a specific molecular fingerprinting. Within this context, it might be pointed out that, due to the selection practices carried out to develop high breast-yield broilers by inducing a hypertrophic growth of the P. major (main target of this process), a significant reduction in the perimysial spaces was previously observed by comparing P. major muscles of fast-growing hybrids with their random bred counterpart (Velleman, 2019). In addition, as a consequence of the selection for fast growth rate, broiler hybrids are slaughtered at always younger age when collagen has not gone through the post-translational modifications necessary to its maturation (McCormick, 1994; Velleman, 2019). Thus, a reduction in the thickness of the perimysial septa coupled with an overall immaturity of the connective tissue in modern broilers might result in Pmin to be more susceptible to the formation of gaps during the post-mortem deboning phase.

CONCLUSION

The results of this study evidenced that the gaping defect affects broilers' Pmin muscles with relevant incidence rates and negatively impact the quality traits of the tender meat thus representing an important issue for the broiler industry. In detail, the quality traits observed in Pmin muscles displaying gaping (lower pHu coupled with pale color and impaired water holding capacity) have strong similarities with the PSE-like condition previously described in poultry. Taken together, the findings of the present study suggest that peri-mortem factors as well as the slaughtering procedures might be responsible for the occurrence of gaping defect with different levels of severity. Thus, further studies are needed to more deeply investigate the underlying mechanisms resulting in the development of this quality defect and to identify proper processing strategies in order to reduce its prevalence.

ACKNOWLEDGMENTS

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