An *in-situ* assessment of Dorsal Cranial Myopathy in broilers, approaching regarding meteorological influences in South Brazil, classification, and appearance of the lesions during industrial processing

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Primary Audience: Researchers, Nutricionists, Animal Science Professionals

SUMMARY

Broiler meat is a high-quality food for humans, and is worldwide consumed. Brazil plays an important role in the poultry industry and in its South region is situated the most important producer. Recently, alterations on the meat aspect have aroused the interest of researchers to clarify their causes. The objective of this investigation was to evaluate the relationship between environmental conditions and seasons on the prevalence of Dorsal Cranial Myopathy (**DCM**) and also, to generate a DCM classification in scores, for griller-type broilers. Carcasses were picked out from several stages of industrial processing from a slaughterhouse located in South Brazil. The prevalence of DCM was measured using the database of partial condemnations of the Official Inspection Service during 19 months. Meteorological data were obtained from the National Institute of Meteorology (in Portuguese *Instituto Nacional de Meteorologia*- INMET), of the Ministry of Agriculture, Livestock and Supply. In addition, microorganisms with public health importance were measured on samples taken from the muscles with DCM. The prevalence of DCM was compared among seasons using Analysis of Variance (**ANOVA**). The relation with other environmental conditions was accessed using a principal component analysis. Partial condemnations by DCM were higher ($P < 0.001$) during autumn (0.529%) and winter (0.547%). In the other extreme, summer presented the lowest percentage of condemned carcass (0.199%). The prevalence of DCM appeared to be inversely related to the temperatures. The association between relative humidity and the prevalence of DCM seemed to move in the same direction. No differences were observed in the qualitative and quantitative microbiological analysis between carcasses affected by DCM and the control group ($P > 0.05$). Visually, it could be noted that the hemorrhagic lesion of the *Anterior latissimus dorsi* (**ALD**) muscle diminished during the processing line, making the DCM imperceptible, even in those carcasses with severe DCM score at the beginning of the process. In conclusion, the climate features approached here could be correlated to this emerging myopathy. However, this lesion does

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not represent a food security issue for the consumers regarding evaluated microorganisms, as this kind of lesion tends to disappear in the final sellable product.

Key words: avian, condemn, muscular lesion, scores, weather

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DESCRIPTION OF PROBLEM

The poultry industry is a very important sector of the Brazilian agribusiness market and is mainly concentrated in its Southern region. In the second quarter of 2019, 1.43 billion of chicken were slaughtered and the South region accounts for 61% of the national slaughters (IBGE, 2020). This region comprises 7% of the country territory and is characterized by subtropical climate, seeing that it is situated between 22˚30'S and 33˚45'S latitude and 57˚59'W and 48˚00'W longitude (Wrege et al., 2012) consisting of four distinguishable seasons, furthermore, as can be seen, during the winter, frost and even snow may occur in some places (Embrapa, 2018). These features may challenge the poultry farming and require climatization systems, regarding the broiler's needs for welfare, health performance (Santos et al., 2017), and probably meat quality. However, climate influences on carcass quality seems to be hard to be established.

Winter seems to be related with an increase of carcasses affected by Dorsal Cranial Myopathy (**DCM**) incidence**,** especially having an impact on the economic losses due to carcass downgrading in the slaughterhouse. In 2009, this muscular lesion was found to be the main cause of downgrading in the winter season and the second cause in the entire year, with rates up to 6%. Independently of the climate, a study carried out in a company from South of Brazil reported that 0.55% of broilers processed in 2002 were monthly associated with carcass downgrading owing to the presence of DCM (Zimermann et al., 2012).

According to the regulations of Brazilian Ministry of Agriculture, Livestock and Supply by *Ministério da Agricultura, Pecuária e Abastecimento* (**MAPA**) (Brasil, 2010), the broiler carcasses presenting DCM should lead to carcass downgrading or be condemned. In general, carcasses with DCM are trimmed, with the removal of the skin (often from the neck to the cloaca), the affected and adjacent muscles, as well as the humerus and part of the thoracic muscles. Carcasses presenting extensive lesions that compromise their overall visual aspect are totally condemned (Amaral et al., 2017). It is noted that the griller-type chickens (~32 days of life and 1 kg of final processed product weight) are composed of whole carcass without viscera, and this type is one of the bestselling products for the foreign market (IBGE, 2018), whereas any change of carcass affected by any injury prevents the normal flow production of whole chicken. In this case, the remainder of the carcass may be used for the production of by-products or cuts of reduced value-added.

The production intensification to meet market demands, have contributed to metabolic alterations that resulted in damage to the cell structure of skeletal muscle, both morphologically and biochemically, with consequential meaningful losses in the poultry chain (Dransfield and Sosnicki, 1999; MacRae et al., 2006)

The specific origin of myopathy in broilers has not been well elucidated. Recent findings have linked their onset with hypoxia (Malila et al., 2019). Baldi et al., 2020 define this myopathy as a muscle with defective energy-generating pathways combined with a deficiency and/or dysfunction of tissue ATPases, with consequences in myodegeneration and on muscle fiber contraction degree. Genetics, factors related to early development, environment, and nutrition could be involved in the onset of the condition (Bailey et al., 2015; Montagna et al., 2019).

Regarding the characteristics of poultry industry, genetic, nutrition, management, it is possible to assume that this dorsal lesion may affect broilers worldwide (Zimermann et al., 2012). In addition to these animal features, the subtropical clime is part of important producing countries. However, the etiology and non-biological factors, which may participate in the development of this muscular disorder, have not yet been elucidated.

Thus, the objectives of this research were to determine the prevalence of DCM in a

slaughterhouse located in Southern Brazil and its relationship with the local meteorological data, in order to establish the relation between the weather conditions and occurrence of DCM in the four seasons of the year. In addition, the study intended to analyze the presence of public health related to microorganisms and to determine macroscopic aspects of the lesion, by grading the lesions through a score system, in the stages of the industrial processing of griller type chickens.

MATERIALS AND METHODS

All procedures were approved by the Ethics Committee on Animal Use from the Federal University of Rio Grande do Sul, Brazil (19.551).

The study was carried out in a slaughterhouse of griller-type chickens, under Federal Inspection, located in Southern Brazil. Male and female chicks, 30 days old, from Cobb 500 strain were slaughtered with an average weight of 1400 grams. The monthly prevalence of DCM was obtained from the database of partial or total rejection carcasses of the Brazilian Official Inspection Service from February 2012 to August 2013, totalizing 157,991,484 slaughtered broilers.

Meteorological conditions were obtained from the Climatological Station localized at -27.38 S Latitude, 51.2 W longitude, and at around 947 m Altitude, in the city of Campos Novos, in the state of Santa Catarina which were under the responsibility of the National Institute of Meteorology (*Instituto Nacional de Meteorologia,* INMET) of the MAPA. Relative humidity, insulation, cloudiness, compensated average temperature per month, as well as monthly minimum and maximum temperatures were considered. Meteorological data was included in the database containing the DCM information to indicate the conditions in which the chicks were reared. Thus, data of the month before the slaughter were considered for each line of the database.

Microbiological Analysis

The Official Inspection Service in Brazil performs the diagnosis of the DCM through visual inspection and diverts the affected carcasses to the Inspection Department of Final Inspection. During 1 month, 120 carcasses diagnosed with DCM were collected for microbiological analysis. For the control group, in that same period, 60 carcasses without DCM, were randomly collected in the slaughter line after the process of exposed viscera. Three carcasses were pooled to form one sample in order to increase the representability of the analysis. Thus, a total of 40 samples with DCM and 20 control samples were analyzed.

The carcasses were placed into a sterile polyethylene bag, placed in isothermal containers at a temperature of 4˚C and sent to the microbiology laboratory. Samples (25±0.2 g) were taken from the injured and adjacent muscles from each pool.

Qualitative analyses were performed for *Salmonella sp., Listeria monocytogenes,* and *Campylobacter sp*. and quantitative analyses were carried out for total coliforms, as well as *Staphylococcus aureus* and *Escherichia coli*. The procedures for sample collection and analysis followed the methodology described on the Normative Instruction 62/2003 of the MAPA (Brasil, 2003).

Lesion Classification and Macroscopic Lesion in the Industrial Processing Steps

In order to create a classification of the lesion, more than a 100 carcasses diagnosed with DCM were randomly collected in the slaughter line from the Department of Final Inspection. The DCM lesions were split into two scores according to the macroscopic changes observed in the cranial portion of the dorsum of the chicks with and without the skin. The appearance of the skin and subcutaneous volume was considered in the evaluation the following aspects: presence of exudate in the lesion and its color; presence of bleeding in the *Anterior latissimus dorsi* (**ALD**) muscle and odor. Four lesion scores were determined: normal (carcasses without apparent macroscopic changes), mild, moderate, and severe.

After classification, ten of these deviated carcasses, per different scores, were identified by a seal, which was placed back in the processing line in order to simulate the normal processing

steps. A macroscopic appearance and photographic record of the lesion were taken after each stage of the processing and after thawing.

The carcass firstly went through the pre-cooling stage, a two-stage operation (pre-chiller and chiller stages), performed in stainless steel tanks, using water immersion and a continuous screw system. The pre-chiller stage showed constant renewal of hyperchlorated water, in the countercurrent direction, with temperature at the beginning of the system at a maximum of 16 degrees, as recommended by Brazilian legislation. The chiller process took about one hour, throughout stage the tanks were fed with ice (Brasil, 1998). Afterwards, the carcasses were suspended in line and classified by weight and packaged. Thereafter, they went to a freezing tunnel where they reached the temperature of -18 °C.

Statistical Analysis

Initially, the values were submitted to descriptive statistical analysis. Analysis of Variance (**ANOVA**) was performed to evaluate the season effect on DCM and body weight using Glimmix procedure. Models included the effect of season and the flock age as a covariable ($P < 0.05$). Eventual differences ($P < 0.05$) among seasons were compared by Tukey's test. Quantitative microbiological results were also analyzed using Glimmix procedure considering the effect of presence/absence of DCM in the sample. Age effect was tested, but not included in final model due to its significance ($P > 0.05$). The normality of residual distribution was verified by Shapiro-Wilk test.

Complementarily, lesion frequency was classified in each repetition as low (≤ 0.5%) or high (> 0.5%). The association between this frequency classification and the season (both categorical variables) was studied using Pearson chi-square test for significant association at 5% level.

A principal component analysis was performed based on Gower's Similarity Index in order to study the relation among the variables that composed the database. Spearman correlations were used to relate the principal component with the studied variables. The Statistical Analysis System (SAS) (v. 9.3; SAS Inst. Inc., Cary, NC) and Minitab (v. 18, Minitab Inc., State College, PA) software programs were used for statistical analyses.

RESULTS AND DISCUSSION

Prevalence of DCM, Meteorological Data and Performance

The monthly meteorological condition, collected near the industrial slaughterhouse, and the prevalence of DCM during the studied period are shown in Figure 1. Table 1 shows the correlation between the meteorological variables and the percentages of DCM. The climate chart indicated that temperatures differed among the seasons. In autumn and winter, lower values of average compensated temperature and higher values of relative humidity were observed.

Relative humidity was one of the important factors in the multivariate analysis. This variable is closely related to the heat losses capacity in

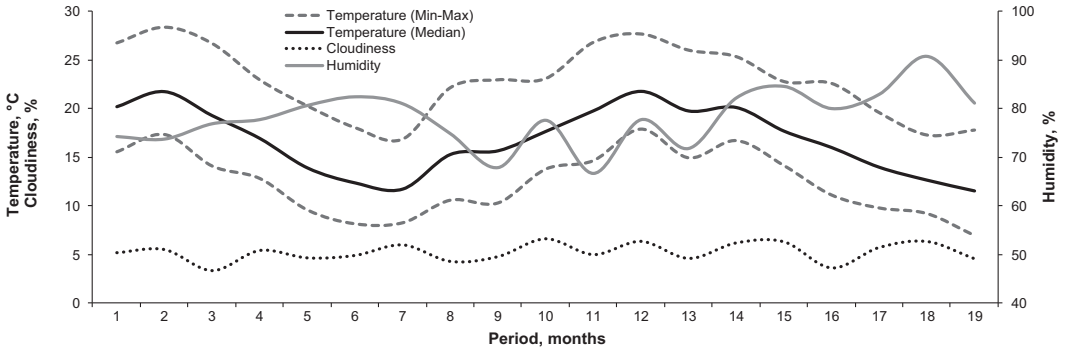

Figure 1. Meteorological condition collected per month near the industrial slaughterhouse located in Southern Brazil during the study period (from February, 2012 to August, 2013).

Table 1. Incidence of Dorsal Cranial Myopathy (DCM) and body weight of Cobb 500 broilers, 30 days old, slaughtered in an industrial process plant located in Southern Brazil and data evaluated by the 4 seasons.

Seasons	DCM, %	Body weight, g
Summer	0.199[c]	1.406[b]
Autumn	0.529[a]	1.446[a]
Winter	0.547[a]	1.413[b]
Spring	0.356[b]	1.426[b]
SEM	0.005	0.0001
P value	<0.001	<0.001

[a-c]Means with different superscripts in the same column are significantly different, by Tukey's test, at 5%.

chickens, because the higher factor is related to the humidity, while the lower are the heat losses. The respiratory thermoregulation pathway is well known and the most important for birds, where the body temperature drop by the water evaporation from the expired air (Bhadauria et al., 2014). Another humidity related aspect for being considered is the thermal sensation; both for cold, if there is wind current; and for heat, in an environment without minimal air renewing (Donald, 1998). Furthermore, relative humidity above 70% worsens litter quality and favors bacterial development, increasing sanitary challenges (Miragliotta et al., 2005), i.g increasing of ammonia production (Carvalho, 2010).

Strong association between the frequency of lesion classification and the season was found ($P < 0.001$), comprising 85% of the groups classified with high presence of lesions (>0.5% of the chicks) occurring during winter and autumn. The seasons which presented the highest rates of partial condemnations by DCM were autumn and winter. Summer was the season with lowest percentage of condemned chicks, in comparison with the other three seasons ($P < 0.001$). In 2012, autumn months (March, April and May) and winter months (June, July and August), expressed higher condemnations rates, as shown in Figure 2, where the third quartile presented a mean of 0.53 and 0.55%, respectively. However, in 2013, this value was higher, presenting mean values of 0.86 and 0.93%, confirming that the climate may influence the incidence ratio of this undesired myopathy.

The current results corroborated the study observed by Roso and Dickel (2011), in which these authors evaluated the prevalence of DCM in the condemned carcasses in a slaughterhouse located in the state of Rio Grande do Sul (RS) in Brazil. In this place, it could be observed higher percentages of condemnations in the months of autumn and winter, emphasizing months of July and August with 0.67 and 0.69% of partial condemnation, respectively. A gradual increase in carcass downgrading and condemnations due to DCM has been reported, particularly in winter, when its incidence may range from 1% (Amaral et al., 2017) to 6% (Zimermann et al., 2012).

The inclusion of DCM in the official condemnation reports is a recent additional information, as was noted on Oliveira et al., 2016 study. The condition is not mentioned as main cause of condemnation based on slaughtered broilers in Brazil from 2006 to 2011, considering the central database of the Federal Inspection Service Management Information System

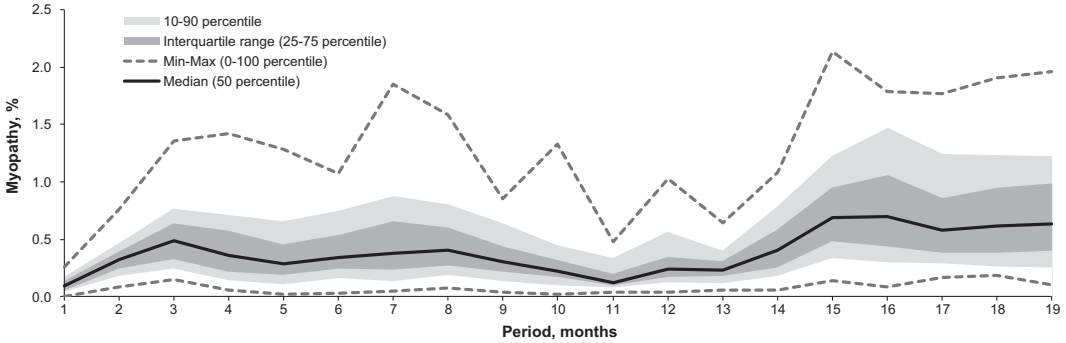

Figure 2. Monthly prevalence of Dorsal Cranial Myopathy (DCM) in broilers located in an industrial slaughterhouse located in Southern Brazil during the study period (157,991,484 slaughtered broilers were evaluated from February, 2012 to August, 2013).

(*Sistema de Informações Gerenciais do Serviço de Inspeção Federal* - SIGSIF). This absence of data was easy to be explained, because the compulsory specific registration of DCM in the notes of the inspection service in slaughterhouses of chicks in the RS began in October 2012. In other Brazilian States, this registration is not yet mandatory (Zimermann, 2011).

According to Carvalho (2010), local meteorological conditions, such as temperature and relative humidity, associated with management practices can influence the rates of ammonia production in the environment. High concentrations of ammonia may adversely affect feed conversion, carcass quality and chick mortality rate (Oliveira et al., 2006). Regarding the southern Brazilian conditions, some farmers reduce ventilation in winter to save energy and keep a warm environment, and this activity may increase ammonia levels and decrease oxygen levels inside the building. Meanwhile, burning wood or using natural gas to provide supplemental heat, consumes oxygen. Once oxygen concentration decreases from 21% to 17% in the air, the resulting is related to the reducing of fast and deep breathing in broilers performance. Nevertheless, lower levels of oxygen can threaten the life of these animals (Czarick and Fairchild, 2002). In addition, excessive ammonia concentrations can inflame the cornea of the chicken, burn the skin, and erode its tracheal lining which can lead to respiratory diseases, decreased weight gain, and high mortality (COBB, 2008). The association of low temperatures, high relative humidity, probably higher ammoniacal concentrations and lower oxygen availability for chickens, may contribute to the increase in the prevalence of DCM in the coldest seasons in subtropical climate regions.

The results obtained in the principal component analysis are shown in Figure 3. The first principal component represented 38% and second principal component comprised 29% of the total variation within the dataset. The

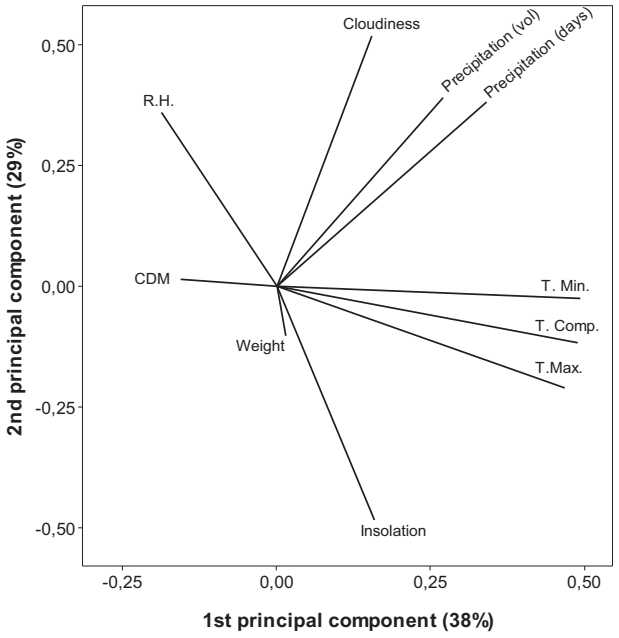

Figure 3. Scatterplot of the Principal Component Analysis (PCA) in which is indicated the explanatory variables[1] in the database (157,991,484 slaughtered broilers[2] were evaluated from February, 2012 to August, 2013). [1]DCM, prevalence of Dorsal Cranial Myopathy (mean: 0.44%, range: 0 to 2.13%); T. Comp., compensated temperature (mean: 16.7°C, range: 11.5 to 21.8°C); T. Max., maximum temperature (mean: 22.8°C, range: 16.8 to 28.4°C); T. Min., minimum temperature (mean: 12.4°C, range: 6.9 to 17.9°C); R.H., relative humidity (mean: 78.2%, range: 66.6 to 90.8%); Cloudiness (mean: 5.3%, range: 3.4 to 6.6%); Precipitation volume (mean: 159.2 mm, range: 67.9 to 224.9 mm) and duration (mean: 11.5 days, range: 5 to 22 days); Insolation (mean: 197.2 h, range: 128.6 to 270.9 h); Weight (mean: 1.424 kg, range: 1.012 to 1.698 kg). [2]Males and females, Cobb 500 strain, 30 days old.

prevalence of DCM appeared to be inversely related to the temperatures, indicating that those variables move in opposite directions. On the other hand, the association between relative humidity and the prevalence of DCM seemed to move in the same direction. Insulation, cloudiness, and precipitation were not associated to the myopathy. Surprisingly, the weight of the animals at slaughter were not related to the prevalence of DCM. Amaral et al. (2017) reported that DCM affects healthy heavy broilers more than the light ones. Researches have been shown an increase of the incidence of pectoral myopathy related to the fast growing and consequently high weight (Bauermeister et al., 2009; Kuttappan et al., 2013) and a recent study has showed that the muscles affected by DCM had alterations in the histomorphometric characteristics of fiber types, presenting multifocal degeneration and fibrosis, as well as alterations in muscles lesion related to serum enzymes (Sesterhenn et al., 2017). However, the main cause of these alterations has not been explained. The results of the current study indicated that some climatological factors might be more relevant than body weight in the understanding of the myopathy. However, the low weight at slaughter (griller-type) and the low heterogeneity on the weight data need to be considered in the interpretation of the results. More studies are necessary to better understand the relationship among these variables, particularly in conditions different from these studied here.

Microbiological Analysis

No differences were observed in the qualitative and quantitative microbiological populations between carcasses affected by DCM and the control group ($P > 0.05$) as shown in Table 2, which presented the quantitative analyzes of total coliforms, *S. aureus,* and *E. coli*. These values could meet the requirements of Collegiate Board Resolution (Resolução de Diretoria Colegiada - RDC) nº. 12/2001 for chilled or frozen *in natura* broilers meat. The qualitative research of *Salmonella* spp., *Listeria monocytogenes* and *Campylobacter* spp. showed an absence of these microorganisms, in compliance with the Brazilian legislation, which demands absence as a reference value in 25 g of cooled or frozen carcasses samples (RDC n° 12/2001).

Table 2. Quantitative microbiological analyzes in ALD muscle from griller-type broilers[1], diagnosed with DCM, by the Brazilian Official Inspection Department, in an industrial slaughterhouse in Southern Brazil.

Microorganism	Without DCM	With DCM	*P* value
Total coliforms, CFU/g	7.8×10^3	8.8×10^2	0.19
Staphylococcus aureus, CFU/g	1.5×10^2	5×10^1	0.45
Escherichia coli, CFU/g	6.4×10^3	3.3×10^2	0.35

[1]Observed in male and female broilers, Cobb 500 strain, 30 d old, average 1400 g BW.

Abbreviation: CFU, Colony Forming.

Current observations corroborate those found by Medina (2010), where Salmonella spp., C. jejuni subsp. jejuni, C. coli, L. monocytogenes, S. aureus, E. coli, Yersinia enterocolitica, and Pasteurella multocida were not isolated on 20 samples of muscles affected by DCM. However, on one of the samples Staphylococcus spp. non-hemolytic was isolated. Concerning the food security, Zimermann (2011) concluded that there is no risk of intoxication through the intake of the lesioned ALD muscle associated with the Public Health Interest Group (PHIG) on bacteria. According to Amaral et al. (2017), broilers with DCM did not show any abnormal hematological, biochemical or muscle pH values, suggesting that the presence of this lesion does not affect their overall health status, and, furthermore, any health risks for consumers.

DCM Lesion Classification

Selected carcasses to define the DCM scores presented macroscopic changes in the appearance of the skin, which cover the ALD muscle. This portion of skin had a yellowish color, sometimes greenish, with an increase in the subcutaneous volume when compared to the normal carcass. There was no presence of atypical odor in carcasses affected by DCM, as reported by Zimermann et al. (2012). After removing the skin from the dorsum, it could be observed the presence of "citrus" yellow exudate, and a gelatinous appearance near the ALD muscle was also exhibited. The ALD muscle, in general, presented an increase in volume and

hemorrhagic areas involving its inferior surface and the surface of the *Rhomboideus Superficialis* muscle, which is located under the ALD.

The ALD adjacent muscle was dissected and was observed that the DCM lesion is well located, in general not affecting the wings of the chickens. However, Roso and Dickel (2011) reported that wings are the main part reached by DCM.

The scores of DCM carcass were established based on these macroscopic features; nevertheless, the carcass without apparent lesion was considered as a normal score. When carcass shows a lesion, it was classified as mild; presence of a

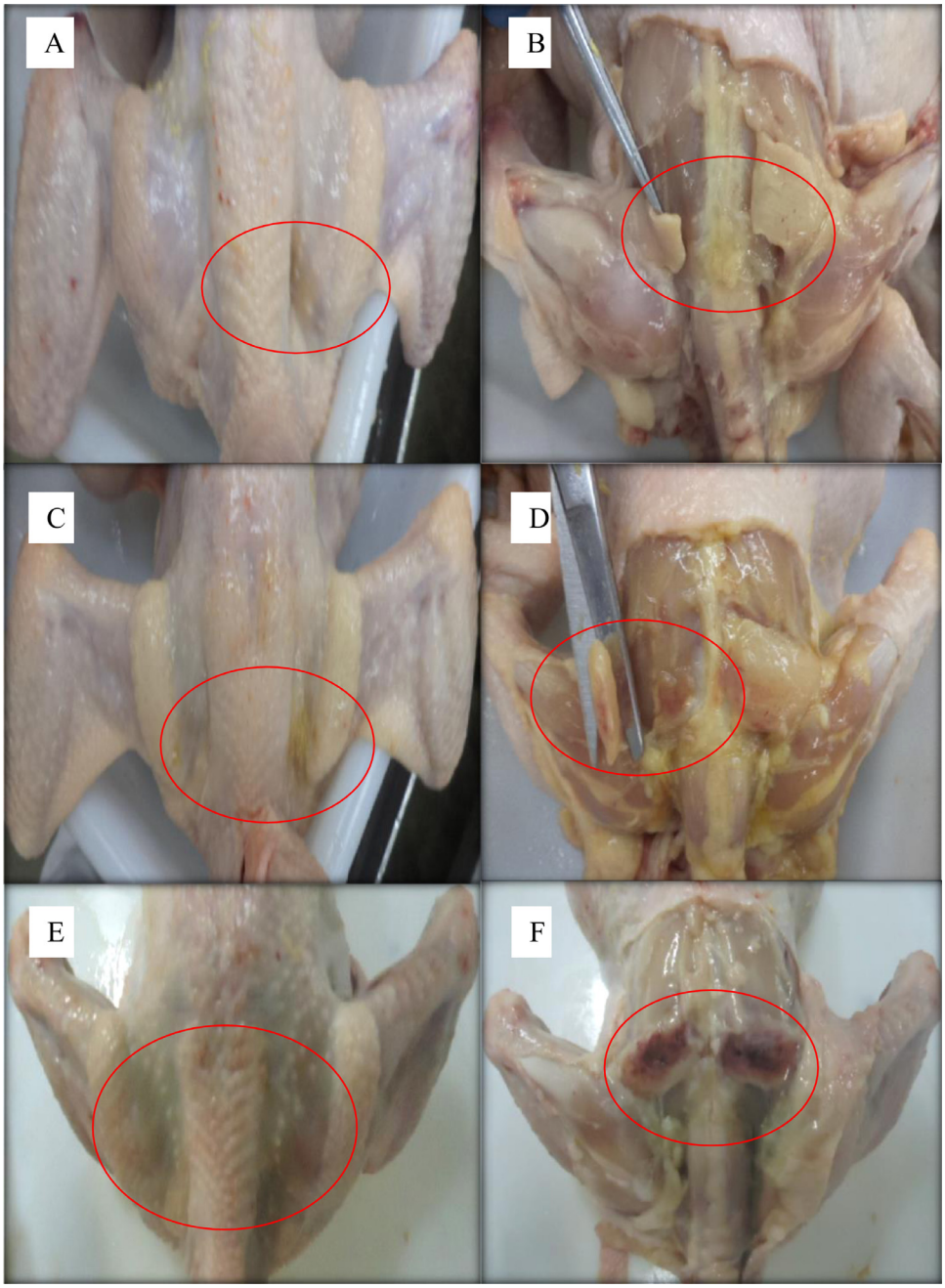

Figure 4. Broiler carcasses and *Anterior Latissimus Dorsi* (ALD) muscles. Mild score: external appearance (A), internal aspect (B). Moderate score: external aspect (C), internal aspect (D). Severe score: external aspect (E), internal aspect (F).

slightly yellowish skin color at the cranial region of dorsum (Figure 4A), and exhibiting internally the presence of exudate with a "citrus" yellow coloration and ALD without hemorrhage (Figure 4B). However, lesion classified as moderate had increased volume in the region between wings and neck, as external appearance, in addition to the skin with yellowish color (Figure 4C), exhibiting internally a large amount of exudate, "citrus" yellow colored attached to the skin in the region between wings and neck (Figure 4D). Lesion classified as severe presented increased volume in the region between the wings and neck as external appearance, a skin with yellowish green color and dark coloration spots in the humeral region of the wings due to the presence of hemorrhage in the ALD muscle (Figure 4E). Internally, it was noted a large amount of exudate with "citrus" yellow or greenish coloration, superficial hemorrhages and thickness increment of the ALD muscle (Figure 4F). These figures may be used as a step-by-step guide for classifying the lesions.

DCM at Industrial Processing Steps

Images from Figure 5 represent the lesion aspect of DCM classified as "severe" in the steps involved in frozen industrial processing of griller-type broilers. At the beginning of the process, there was an increase in volume and it could be detected a greenish appearance of the skin which covers the dorsal region (Figure 5A). Concerning aspect after removing the skin, it is showed in Figure 5B, where the muscle presented hemorrhage and greenish colored exudate. Figure 5C, shows the carcass which went through the immersion tank with cold water (pre-chiller stage). In addition, a reduction in the musculature hemorrhage and decrease of the exudate was observed at this step. After the cooling stage (chiller stage) the exudate is no longer observed and there is a depletion of the hemorrhagic aspect on ALD muscle (Figure 5D). When carcasses reached packing and freezing stages (Figure 5E), the hemorrhagic lesion of the ALD muscle becomes evident, becoming the DCM lesion imperceptible, even after thawing the product (Figure 5F). These findings corroborate those of James et al. (2005), which suggest that the cooling process improves the appearance of the carcass, increases the product's useful life and also reduces the growth of pathogenic bacteria.

Considering these industrial processing and the final sellable product, it is acceptable to

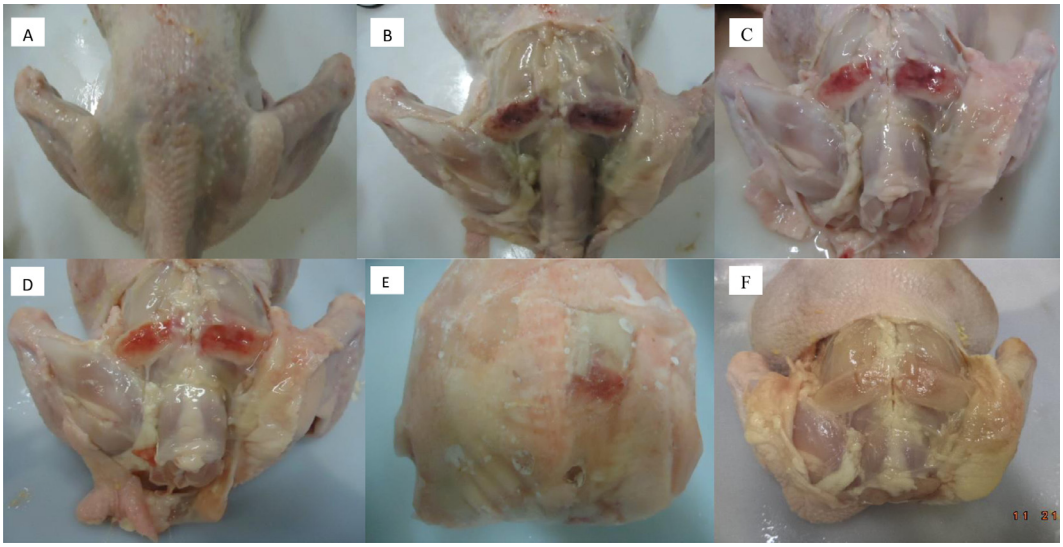

Figure 5. Aspect of Dorsal Cranial Myopathy (DCM) lesion[1] classified as severe score at industrial processing stages. (A) External appearance: carcass removed from the line of the Department of Final Inspection. (B) Internal aspect: carcass removed from the line of the Department of Final Inspection. (C) Carcass removed from the pre-chiller outlet. (D) Carcass removed from chiller output. (E) Carcass withdrawn from the exit of freezing tunnel. (F) Thawed product.

remove partially the affected muscles or not, because the visual with undesirable aspect disappears, and, furthermore, there are no implications for the public health. Otherwise, sensorial test should be done in the future studies to verify possible changes in taste and texture of the affected area, in order to determine if DCM should be neglected, in this way, it is possible to preserve the whole carcass of griller-type broilers.

CONCLUSIONS AND APPLICATIONS

1. The temperature of the region influences the prevalence of DCM lesion in the industry of the broiler production system. Furthermore, it was confirmed the absence of risks to consumers, regarding public health importance of microorganisms.
2. The macroscopic appearance of the final sellable carcasses with DCM reached an acceptable visual, suggesting that the condemnation of this high valuable product may be reviewed in further studies with larger sample size.
3. Considering this study approach, the economic impact, the natural resources used and the worldwide scale represented by the poultry industry, it is clear to note the importance of further studies to determine quantitatively the interference of "in house" environmental factors, such as ammonia, oxygen, temperature, and also humidity on synergy regarding natural climate features draw on the development of DCM.

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DISCLOSURES

The authors declare no conflicts of interest.

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