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Microbiological, Physicochemical, and Histological Analyses of Broiler Carcasses with Cachexia

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ABSTRACT

Broiler carcasses presenting cachexia, as determined by the federal inspection agency, were submitted to microbiological, physicochemical, and histological analyses. Over a 2-month period, 278 carcasses from straight-run flocks of 43- to 48-d-old Cobb broilers from two different farms were condemned due to cachexia and subjected to gross examination in the final inspection sector. Out of the 278 carcasses, 131 did not present any gross signs of infection, and were submitted to microbiological analyses (n=83), physicochemical analyses (n=28), and viscera and muscle samples of 20 carcasses were evaluated by histology. The microbiological results of cachectic carcasses complied with the current legislation on the consumption of fresh poultry meat. Lower lipid and higher protein, moisture, and volatile matter contents, and higher pH ($p<0.05$) were determined in the cachectic than in the control carcasses from a same flock, but no ash content differences. All carcasses were negative for hydrogen sulfide. The histological analysis showed that65% of the cachectic carcasses did not have any muscle injury, 20% showed mild hyalinization and 15% moderate proliferation of fibrous connective tissue. The findings of this study indicate the importance of further technical and scientific studies on the utilization of cachectic broiler carcasses for the manufacture of edible products, rather than whole carcasses rendering, thereby preventing the resulting economic losses.

INTRODUCTION

Cachexia is one of the main causes for the condemnation of whole carcasses in the inspection lines broiler processing plants. In Brazil, in processing plants under federal inspection, 2,565,411 broiler carcasses were condemned for cachexia in 2014, accounting for 0.05% of birds processed. In 2015, this volume was 2,886,829 and amounted to 896,995 between January and June of 2016, representing an average of 0.03% of the total number of broilers slaughtered in Brazil during these two years (Brasil, 2016), causing economic losses of USD 2,116,908 for the poultry industry in 2016.

Cachexia is characterized by muscle malformation and muscle mass reduction, purplish meat color, protrusion of the sternum keel due to the loss of muscle cover, and the almost complete absence of fat in the carcass (Rio Grande do Sul, 2015). According to Olivo (2006), cachexia is caused by poor flock management and it is believed to be associated with chick quality, poor early management, environmental temperature, health management, water and feed consumption, stocking density, and runting-stunting syndrome during the first week of age.

Whole cachectic carcasses are removed from the processing line by the inspection services at pre-inspection stage and destined to rendering (Rio Grande do Sul, 2015). Therefore, whole condemned carcasses and offal are no submitted to thorough gross examination, which does not allow to determine if the etiology of cachexia is infectious or not. Some carcasses are condemned for non-infectious causes; however, these should be further investigated to determine if they actually need to be totally condemned.

In the present study, whole cachectic carcasses identified by the federal inspection service and which presented no gross findings associated with infectious processes, such as abscesses, fibrin deposition and adhesions, were evaluated. Microbiological, physicochemical, and histological analyses of cachectic or non-cachectic carcasses of broilers of a same flock and of the viscera and muscles of broilers with cachexia were conducted to determine the possible presence of infectious agents.

MATERIALS AND METHODS

Location and sampling

The study was performed with carcasses collected in a commercial processing plant under federal inspection in the State of Rio Grande do Sul, southern Brazil, where broilers from a broiler farm located in the State of Rio Grande do Sul (Farm 1 = F1) and another located in the State of Santa Catarina (Farm 2 = F2), both contracted by two different companies, are slaughtered.

Over a 2-month period, 278 cachectic carcasses from straight-run flocks of 43- to 48-d-old Cobb broilers were identified by the federal inspection service during pre-inspection, and collected. Cachexia was identified by properly trained inspection officials, according to the guidelines for postmortem poultry inspection (Rio Grande do Sul, 2015). Cachectic carcasses were then submitted to the Department of Final Inspection for thorough gross examination by the official veterinarian of the federal inspection agency in order to rule out any infection in the viscera or carcasses (Brasil, 1998).

Out of the 278 carcasses condemned for cachexia, 147 carcasses presented visceral lesions presumably caused by an infectious agent and were discarded, and the remaining 131 carcasses with no lesions suggesting an infectious condition were included in the study, out of which 83 were submitted to microbiological, 28 to physicochemical, and 20 to histological analyses. The carcasses were weighed on a calibrated digital scale.

Microbiological analysis

Out of the 131 carcasses selected for evaluation, 83 were aseptically collected using gloves, placed in sterile plastic bags, identified with numbered seals, and submitted to a laboratory (Allabor, Toledo, PR, Brazil) accredited by the Brazilian Ministry of Agriculture (MAPA) in coolers containing reusable ice packs. Carcasses were analyzed for *Clostridium perfringens*, thermotolerant coliforms, *Staphylococcus aureus*, and *Salmonella* spp. enumeration, expressed inCFU/g, according to the guidelines of the Brazilian Ministry of Agriculture (Brasil, 2016).

Physicochemical analysis

Out of a same processed flock, 28 healthy and uniform carcasses considered suitable for human consumption and 28 cachectic carcasses were selected for physicochemical analyses. Carcasses were collected and shipped to the laboratory as described for the microbiological analysis. Protein, lipid, ash, moisture and volatile matter contents, expressed ing/100g of carcass, and pH and hydrogen sulfide reaction, as an indication of meat spoilage, were determined in the above-mentioned laboratory, according to the guidelines of the Brazilian Ministry of Agriculture (Brasil, 1999).

Histological analysis

Twenty of the 131 collected cachectic carcasses and their respective viscera were submitted to histological analysis. Fragments of the *pectoralis major* muscle, liver, heart, kidneys, and pancreas were collected, fixed in 10% formaldehyde, and submitted to an animal pathology laboratory (Mercolab, Chapecó, State of Santa Catarina, Brazil). Tissue samples were dehydrated in graded ethanol series, cleared in xylol, and embedded in paraffin. The paraffin blocks were then sectioned in a microtome, mounted on glass slides, stained with hematoxylin-eosin (HE), and examined under a light microscope.

Statistical analysis

Quantitative data were subjected to analysis of variance, and means were compared by Student's t-test at 5% significance level ($p<0.05$). Statistical analyzes were performed using SPSS® 23 software.

RESULTS AND DISCUSSION

The average weights of the cachectic carcasses were 0.6475±0.1142kg (F1) and 0.6586±0.1167kg (F2), and not statistically different ($p=0.6638$), indicating that the broilers showed similar responses the stressors that triggered cachexia, regardless flock management on the farms, and also because they belonged to the same genetic line.

Microbiological analysis

Thermotolerant coliform counts did not exceed 10^4CFU/g (Table 1), in compliance with the Technical Regulations on Microbiological Standards for Food Products issued by the Brazilian Agency of Health Surveillance (ANVISA; Brasil, 2001), of the maximum tolerance level for indicative samples (M) of $\leq 10^4$ CFU/g for refrigerated or frozen poultry meats (whole carcasses, cut-up parts, or meat cuts)for human consumption.

Table 1 – Total counts of thermotolerant coliforms, *Clostridium perfringens* and *Staphylococcus aureus*in cachectic carcasses with no lesions suggestive of infection.

Microorganism	Acceptance criteria[1]	Total samples (F1+F2)	Total samples F1	Total samples F2	Number of samples between m and M (c)	Results Samples F1 (c)	Results Samples F2 (c)
Coliforms at 45°C/g	n^2=5, c^3=3; m=5x10^3 CFU/g M^*=10^4	83 (100%)	41 (49.40%)	42 (50.60%)	1 (1.20%)	1 (2.44%)	0
Clostridium perfringens	n^2=5, c^3=2; m=1x10^2 CFU/g; M^* = 1x10^3 CFU/g	83 (100%)	41 (49.40%)	42 (50.60%)	4 (4.82%)	2 (4.87%)	2 (4.76%)
Staphylococcus aureus	n^2=5, c^3=2; m=5x10^2 CFU/g M^* = 5x10^3 CFU/g	83 (100%)	41 (49.40%)	42 (50.60%)	0	0	0

[1]Acceptance criterion established by the current regulations, where M is the maximum tolerance level (Brasil, 2000; Brasil, 2001);

[2]Number of samples randomly collected from the same flock;

[3]Maximum acceptable number of samples which microbial counts are within the limits of m and M for representative samples. Values greater than M are not acceptable; *Tolerance level for the indicative sample.

Clostridium perfringens and *Staphylococcus aureus* (Table 1) counts determined in the evaluated carcasses were also below the maximum tolerance levels of 1x10^3 CFU/g and 5x10^3 CFU/g for *Clostridium perfringens* and *Staphylococcus aureus*, respectively, established by the Technical Regulation on Identification and Quality of Mechanically Separated Meat - MSM (Brasil, 2000).

Out of the 83 cachectic carcasses tested for the presence of *Salmonella* spp. (absence in 25g), 8 were positive (Table 2). This result complies with the official criteria established by the national program for the control and monitoring of *Salmonella* spp. in commercial broiler and turkey farms and in broiler and turkey processing plants under federal inspection (Brasil, 2016), which allows two carcasses (25%) in each cycle of 8 samples (n=8), collected after dripping and before packing to be positive.

Table 2 – Enumeration of *Salmonella* spp. in cachectic carcasses without injury suggestive of infectious condition.

Microorganism	Acceptance criteria	Total number of samples (F1+F2)	Number of samples F1	Number of samples F2	Positive for *Salmonella* spp.	Positive for *Salmonella* spp. in F1	Positive for *Salmonella* spp. in F2
Salmonella spp.[1]	n=8; c=2 or 25%	83	41	42	8 (9.64%)	3 (7.32%)	5 (11.90%)

[1]Carcass evaluation according to the criteria established by Normative Instruction no. 20 (Brasil, 2016)

In the present study, the presence of *Salmonella* spp. was detected in flocks reared on the farms contracted by two different broilers companies, which may have different rearing systems, i.e., may apply different management and biosecurity practices. The control of this pathogen in Brazil is regulated by Normative Instruction no. 56 (IN 56) issued by MAPA, and includes the registration, inspection, and control of commercial and breeding poultry farms (Brasil, 2007).

Physicochemical analysis

The protein and lipid contents of the cachectic and the control carcasses (Table 3) were significantly different (p<0.05).

The higher protein and lower lipid contents of the cachectic carcasses (p<0.05) relative to the healthy carcasses This is probably due to the increase in lipid catabolism and the reduction in basal metabolism as a physiological adaptation to chronic low feed intake (Vieira, 2012). According to Vieira (2014), nutritional deficiencies immediately after hatch can

Table 3 – Physicochemical parameters (mean ± standard deviation) measured in cachectic and healthy broiler carcasses.

	Number of samples	Average weight (kg)	Protein (g/100g)	Lipids (g/100g)	Ashes (g/100g)	Moisture and volatile matter (g/100g)	pH	Hydrogen sulfide reaction
Cachectic carcasses	28	0.62±0.14	19.69±2.28	2.65±2.14	0.97±0.16	75.32±2.71	6.16±0.19	Negative
Healthy carcasses	28	1.68±0.21	17.66±1.66	8.75±3.26	1.04±0.12	70.33±2.48	5.97±0.16	Negative
p value[1]	-	<0.0001	0.0003	<0.0001	0.0661	<0.0001	0.0001	-

[1]Statistical analysis made using Student's t test for two independent samples at a significance level of 5% (0.05).

cause permanent carcass yield damage. These results indicate that cachectic carcasses may be used for the manufacture of MSM in compliance with the Technical Regulation of Identification and Quality (Brasil, 2000), which establishes12% minimum protein content and 30%maximum fat content.

A significant difference (*p*<0.05) was also observed in pH, moisture and volatile matter between cachectic and control carcasses. These characteristics are closely related, as the formation of lactic acid from glycogen, with consequent meat pH reduction postmortem pH, allows the meat to hold water (Roque-Specht, 2009). Factors such prolonged stress due to physical exertion, exhaustion during transport, fasting, and aggressive behavior or fear cause muscle glycogen depletion before slaughter. This prevents the formation of lactic acid in the muscles, hindering muscle pH decline and slowing down the establishment of *rigor mortis*. The ultimate meat pH in such cases remains relatively high, generally greater than 6.0 or even close to physiological values (Miller, 2002). Normal chicken breast ultimate pH (24 h postmortem) ranges between 5.7 and 5.9, and it is greater than 6.2, breast meat water-holding capacity increases, resulting in short preservation time and dark meat color (Venturini, 2007), which was also described by Olivo (2006).

Ash content was not significantly different between healthy and cachectic carcasses (*p*>0.05).

Hydrogen sulfide is an indication of meat spoilage due to the decomposition of sulfur amino acids (Brasil, 1981). The negative results determined both in the cachectic and the control carcasses may be attributed to the time interval of less than 24 hours between sample shipping to the laboratory and hydrogen sulfide analysis.

Histological analysis

The microscopic findings did not indicate the presence of infectious agents or any significant microscopic lesions in the liver, kidney, pancreas, or heart.

Out of the 20 *pectoralis major* muscle samples examined, 13 did not present microscopic lesions. Mild and moderate levels of hyalinization of muscle fibers and proliferation of fibrous connective tissue were determined in four and three samples, respectively. Such lesions are observed in cases of capture myopathy, degenerative disorders caused by vitamin E and/or selenium deficiency, and ionophore toxicity. The immune system is directly influenced by the animal's nutritional status. Vitamin E is an example of that, as it maintains the integrity of the macrophage membrane and modulates prostaglandin synthesis (Qureshi & Gore, 1997). No histological muscle fiber differences between broilers with high or low feed conversion ratio were observed by Remignon (1996).

Howard & Senior (1999) state that the diagnosis of cachexia should be reserved for those animals with chronic or infectious diseases, and should be differentiated from emaciation due to malnourishment. Therefore, the evaluated carcasses should be considered as emaciated, and not as cachectic. However, Kotler (2000) warns that infectious diseases can emerge due to immune dysfunction induced by malnutrition. Therefore, these concepts are closely related (Palmeira-Borges, 2006). The results obtained in the present study indicate that the broiler carcasses currently condemned in Brazil due to cachexia do not pose any infectious hazard, and therefore, could be considered as "lean meat", complying with article 161 of the Industrial and Health Inspection Regulation for Products of Animal Origin (RIISPOA) (Brasil, 2017), which allows the conditional utilization of the carcass of emaciated animals free from any pathology.

Cachectic broiler carcasses are condemned based on their gross examination by the federal inspection services at pre-inspection, regardless the cause of cachexia. However, in the evaluated carcasses, the microbiological parameters complied with the official regulations and no histological lesions, including those indicative of infection, were detected. This suggests that cachectic broiler carcasses should not be condemned at pre-inspection, but rather sent to final inspection, after which they should be disposed if any gross findings of infection (abscesses, fibrin deposition, adhesions, changes in color, smell and consistency, among others) are observed.

CONCLUSION

The microbiological, physicochemical, and histological parameters determined in the cachectic broiler carcasses evaluated in the present experiment indicate that these carcasses may be utilized for the manufacture of edible products, such as mechanically-separated meat, and to not need to be completely rendered, which results in significant economic losses in the poultry industry.

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