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Melanosis Contributing to Downgrading in Processed Broiler Chickens in Argentina: Preliminary Diagnostic Findings

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Abstract: Melanosis is scarcely reported as a potential cause of carcass downgrading in broilers worldwide. We described the preliminary diagnostic findings of dermal and celomic fat tissue melanosis in a total of four episodes of increased downgrading in 31-to-50-day-old male Cobb500^{TM} broiler chicken carcasses at a processing plant in Argentina from November 2016 to March 2022. Veterinary inspectors evaluated and sampled a total of 10 carcasses. Gross findings included the gray discoloration of subcutaneous tissue in wings and legs, celomic fat tissue and peritoneum. Microscopically, melanocytes infiltrated the connective tissue and adipose cells of subcutaneous tissue in the skin of legs and wings as well as the celomic fat tissue of all 10 birds. The Fontana–Masson stain revealed black melanin granules in subcutaneous tissue and celomic fat tissue, demonstrating its value as a histologic tool to confirm grossly compatible melanosis in all of the evaluated tissues sampled at the broiler processing plant. We propose to add melanosis as one of the less frequently and rare diagnosed cutaneous darkening conditions which potentially contributes to downgrading in chicken processing plants globally.

Keywords: melanosis; hyperpigmentation of visceral peritoneum; subcutaneous tissue; Fontana–Masson stain; broiler chicken carcasses; processing plant

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1. Introduction

Melanin, which etymologically comes from the Greek word "mélās" ("black" or "dark [in color]"), is an autochthonous, brown–black intracellular pigment produced by melanocytes [1,2]. Melanocytes are generally limited to the epidermal follicles of feathers, pigmenting plumage, and iris, producing pigmentation of the eyes [3]. Chinese black Silkie, Sumatra, Ayam Cemani, and hybrid-related chickens are an exception with melanin present in the integument, musculoskeletal, central nervous, respiratory, gastrointestinal, and reproductive systems [3,4].

Melanosis is a disorder in which an anomalous storage of melanin occurs [1,2]. In commercial chickens, the presence of melanin was selected out in broilers with white-to-yellow skin due to consumer preferences in Western chicken meat markets [5]. Hence, melanosis of the skin, abdominal fascia and adipose tissue has been rarely described in processing plants of broilers with white-to-yellow skin, being only previously reported in processed and downgraded 45-to-46-day-old Cobb broiler chickens in Spain [5] and as multicentric melanosis in a processed and condemned 48-to-49-day Cobb broiler chicken in Brazil [6].

Melanosis was previously documented in chickens as "black abdomen", recently known as hyperpigmentation of the visceral peritoneum (HVP), in Plymouth Rock, Leghorn, and Cobb commercial genetic lines [5,7,8], and Chinese meat-type breed (Huiyang Bearded) [9]

and related cross-breed chickens [10]. HVP has been reported in higher occurrence in females than males [7,8]. The commercial yellow-feathered chicken industry in China is negatively impacted by melanosis affecting the quality of processed chicken carcasses nowadays [10]. Therefore, the genetic mapping for HVP in an F_2 cross-breed population originated from Huiyang Bearded chicken and a commercial broiler breed named "High Quality Chicken A Line" was carried out to elucidate potential gene candidates for HVP development [10]. Indeed, pigmentation of the skin in chickens is known to be controlled by the loci *Yellow skin* (W/BCO2), *Fibromelanosis* (FM/EDN3) and *Inhibition of dermal melanin* (ID), in addition to the epidermal melanosis observed in chicken carrying the most dominant alleles *Extended black* (E) and *Birchen* (R) [11].

The aim of this work is to describe the preliminary diagnostic findings of dermal and celomic fat tissue melanosis as a rare condition in processed male Cobb500^{TM} broiler chickens not significantly contributing to downgrading at a broiler processing plant during the period studied in Argentina.

2. Materials and Methods

Four episodes of increased downgrading and condemnation in 31-to-50-day-old, male Cobb500^{TM} broiler chicken carcasses were noticed at a processing plant in Entre Ríos province, Argentina, from November 2016 to January 2022. A total of 10 processed carcasses were examined for the presence of gray to black discolored areas in tissues, and the location of the lesions was recorded (Table 1). Affected tissues were sampled and fixed by immersion in 10% buffered formalin (pH 7.2) for 48–72 h and processed to produce 4 µm thick sections that were stained with hematoxylin and eosin (H&E) and Fontana–Masson (Fontana–Masson Stain Kit, StatLab^{TM}, McKinney, TX, USA), following standardized methodology from human histology [12]. Veterinary inspectors and technicians declared no such lesions in broiler chicken carcasses during the 2016–2019 and 2019–2022 periods at this processing plant. We studied the sampled carcasses with the permission of sanitary authorities from the slaughterhouse, and followed standard procedures of the Argentinian commercial processing plants.

Table 1. Dermal and celomic fat tissue melanosis in downgraded male Cobb500^{TM} broiler chicken processed carcasses at a processing plant in Entre Ríos province, Argentina, from November 2016 to March 2022.

Farm n°/Age/n° Downgraded Carcasses/Year	Farm Geolocation	Average Weight	Total of Processed Carcasses	Affected Organs
1. 50-day-old/3/Nov 2016	−32.24471993 Lat −58.26843195 Long	3.23 kg	26,890	ST W, CFT
2. 47-day-old/2/Apr 2019	−32.25155806 Lat −58.21081044 Long	3.06 kg	16,765	ST L, CFT
3. 47-day-old/2/Jan 2022	−32.33049322 Lat −58.20971761 Long	2.97 kg	115,233	ST L-W, CFT
4. 31-day-old/3/Mar 2022	−32.19587809 Lat −58.28020526 Long	2.84 kg	117,308	ST L-W, CFT

Lat = latitude/Long = longitude. ST = subcutaneous tissue/L = Legs/W = wings/CFT = celomic fat tissue.

Available data from the studied chicken slaughterhouse showed a total of 500,154 (farm 1); 466,737 (2); 1,484,201 (3); and 1,425,878 (4) processed chicken carcasses from 2016 to 2024 (1); 2019 to 2024 (2); and 2022 to 2024 (3 and 4), respectively.

3. Results

Grossly, extensive gray discoloration of subcutaneous tissue of wings and legs, celomic fat tissue, and visceral peritoneum were noticed in all 10 chicken carcasses included in this study (Figure 1a–d). Veterinary inspectors and technicians declared no other organs affected by gray discoloration or darkening gross lesions after evisceration of the studied broiler chicken processed carcasses.

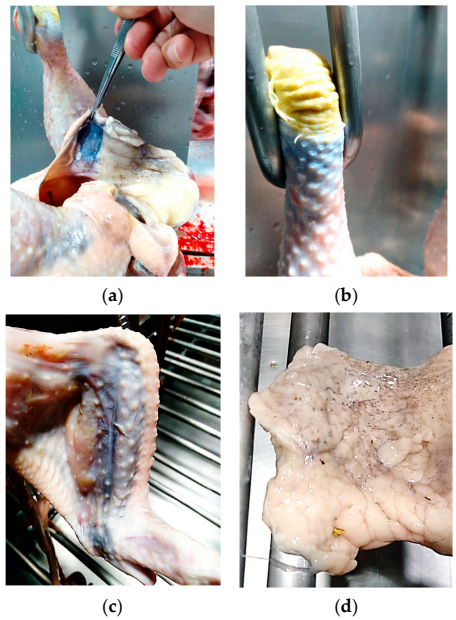

Figure 1. (**a**–**d**). Gross findings of dermal and celomic fat tissue melanosis from downgraded 31-to-50-day-old, male Cobb500^{TM} broiler chicken carcasses of cases 3 and 4 in Argentina. Diffuse gray discoloration of subcutaneous tissue of legs (**a**,**b**), wings (**c**), and celomic fat tissue (**d**) are noted.

Microscopically, a moderate number of melanocytes were randomly distributed within the connective tissue and surrounding adipose cells of subcutaneous tissue in the skin of legs and wings in H&E sections (Figure 2a). Celomic fat tissue showed a mild to moderate number of melanocytes randomly distributed in between the adipocytes in H&E sections (Figure 2b). No mitotic figures in melanocytes, no melanocytes infiltrating blood vessel walls, and no inflammatory or neoplastic cell infiltration in any of the studied sections were noted. There was no evidence of intralesional infectious agents such as intralesional bacterial colonies, viral inclusions, fungal hyphae or protozoal organisms in the studied skin and fat celomic tissue sections. The Fontana–Masson stain revealed higher amounts of black melanin granules, multifocally to coalescent distributed and surrounding numerous adjacent adipocytes and within connective tissue in subcutaneous tissue, rather than fewer black melanin granules multifocally surrounding adipocytes in celomic fat tissue (Figure 2c).

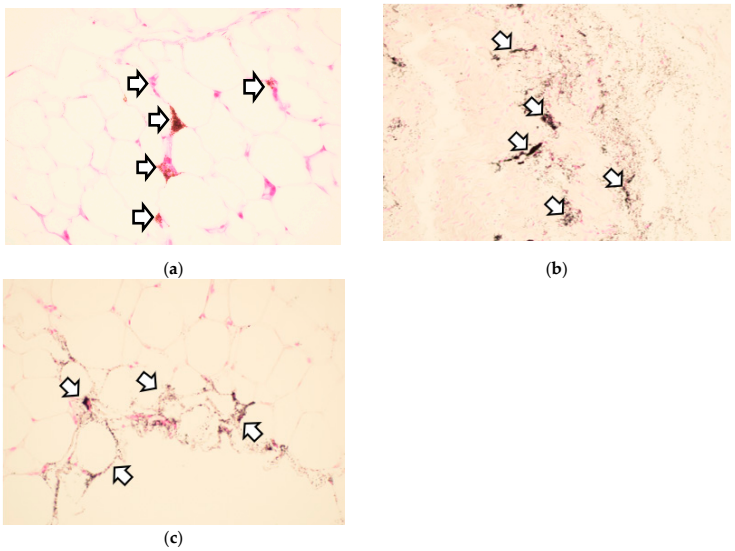

Figure 2. (**a**–**c**). Histologic findings of melanosis in dermal and celomic fat tissue from downgraded 31-to-50-day-old, male Cobb500^{TM} broiler chicken carcasses of cases 3 and 4 in Argentina. (**a**). Scattered

melanocytes randomly distributed in between the adipocytes of celomic fat tissue (white arrows). H&E (objective 40×). (**b**,**c**). Back melanin granules, diffusely distributed within subcutaneous tissue, and multifocally to coalescent surrounding adipocytes (white arrows). Fontana–Masson (objectives 20× and 40×).

4. Discussion

The current literature shows a scarcity of cases reporting the negative economic impact of melanosis in worldwide chicken processing plants over a period of 66 years [5–11]. Moreover, a lack of melanosis evidence was noticed among other numerous cutaneous conditions at chicken slaughterhouses and processing plants in North and South American, European, and Middle Eastern previously published surveillance studies [13–19]. Melanosis, which is differentiated in the congenital and acquired form in livestock [1,20–23], was rarely reported as melanosis maculosa in beef cattle [20] and melanosis of the skin, melanosis uberis and melanosis coli in pigs [21,22,24,25] at slaughterhouses.

The Fontana–Masson stain kit used in our work allowed us to identify black melanin granules of melanocytes over the routine H&E stain in all evaluated sampled tissues from chickens, providing a valuable histologic tool for the microscopic evaluation of grossly compatible melanosis, being a human histologic method successfully transpolated to poultry in this diagnostic study in chickens [12].

This is the first report of dermal and celomic fat tissue melanosis of Cobb500^{TM} male broilers at a chicken processing plant in Argentina. In the studied cases, the extensive deposition of melanin in the subcutaneous tissue of legs and wings and HVP, over a 7-year period, contributing to downgrading in broiler chicken carcasses. In our study, we report that dermal and celomic melanosis had a low prevalence in the four episodes studied: 0.0006% (farm 1); 0.0004% (2); 0.0001% (3); and 0.0003% (4), representing a rare condition not significantly contributing to downgrading during the study period in a chicken slaughterhouse in Argentina.

HVP is currently rampant in the Chinese yellow-feathered chicken industry [10], negatively affecting one of the top-three global producers of broilers (20 M tons), which added to the production of the United States (23 M tons) and Brazil (16 M tons), providing 45% of the global broiler production [22]. Zhou et al. [10] carried out a mapping of genome-wide association studies on an F_2 cross-breed population to explore genetic variations and genome segments controlling HVP and to find related primary candidate genes, such as the melanoma-associated gene named CYP2D6. The medium heritability of HVP in the studied F_2 cross-breed population of chickens highlighted potential further improvements by molecular breeding selection in order to reduce the impact of HVP in the Chinese chicken industry [10].

Females from the Plymouth Rock and Leghorn genetic commercial chicken lines were previously identified with a higher occurrence of melanosis in the abdomen or HVP [7,8]; although we observed gross and microscopic preliminary diagnostic HVP findings in ten processed and downgraded male Cobb500^{TM} broiler chickens, we cannot speculate on the possibility the incidence being higher in more males rather than females because of the few HVP cases included in this study. Further studies including a larger number of processed carcasses might be necessary to provide robust information about correlation between sex and melanosis in chickens from this genetic commercial line.

In the pork market, melanosis uberis in bacon shows no food safety concerns for consumers, being able to be marketed and consumed by humans [25]. Therefore, if a similar conclusion is considered for chicken meat from processed carcasses with melanosis, the downgrading and related negative economic impact affecting chicken producers can be reduced. In any case, further studies on the chemical parameters of processed broiler carcasses with melanosis could add valuable information to the chicken meat market.

In poultry, traumatic injuries in legs and wings due to inappropriate handling, transport and stunning potentially producing subcutaneous hematomas and subsequent darkening of the skin could be firstly considered as a differential diagnosis of melanosis [13–15]. Secondly, malignant melanoma, a pigmented neoplasia rarely reported but present in commercial chickens affecting the skin, liver, lungs, bone marrow, celomic wall, intestinal

serosa, and ovary, is one of the two neoplasia to differentiate from melanosis [4,23,26–30]. Less commonly, hemangioma and hemangiosarcoma affecting skin as "blood blisters" and numerous organs leading to hemorrhages if ruptured in chickens of all ages, being spontaneously produced by the leukosis-sarcoma group of avian retroviruses, should be listed thirdly as the second neoplasia to be differentiated here [4,15,27,28,31,32]. Both necropsy and histopathologic postmortem studies represent unique diagnostic tools to identify and characterize causes potentially related to the downgrading and condemnation at chicken processing plants, as performed in this work.

Veterinarians and technicians play a key role in the identification of gross lesions on carcasses at slaughterhouses and processing plants dealing with potential food safety and public health threats, and animal health and welfare issues, as seen in our work with a rarely reported entity. Accordingly, continuous education is crucial to properly train the personnel in this area. The Davis–Thompson Foundation provides outstanding veterinary anatomopathological databases and online and in-person events, among which poultry pathology and diagnostic are covered [33]. The Servei de suport a escorxadors (*Slaughterhouse Support Network*, SESC) also offers a valuable online, open-access database as well as hands-on courses on the identification of condemnation and downgrading causes on slaughterhouses and processing plants in the Catalonian chicken and livestock industries [24,25].

5. Conclusions

Melanosis should be included as a low prevalent and rare condition potentially contributing to the downgrading of processed chicken carcasses with white-to-yellow skin to avoid being potentially overlooked, underdiagnosed or misdiagnosed at most chicken processing plants globally. Diagnosing and characterizing melanosis could help to identify and reduce the potential negative economic impact in markets with increasing high demand of chicken meat, alerting and providing input for improvement in parent and grandparent stocks. The Fontana–Masson stain offered a rapid, simple and accurate histologic method to identify melanin black granules of melanosis in our study. Additionally, melanosis should be grossly and microscopically differentiated from traumatic injuries in legs and wings, as well as neoplastic entities such as malignant melanoma and hemangioma/hemangiosarcoma which cause focal to diffuse darkening lesions of the integument and numerous organs from other systems in chickens, without being involved in any food safety concern for chicken meat consumers in relation to the entities named here. These preliminary diagnostic findings represent the first step to design and carry out a large-scale study identifying the real dimension of HVP affecting processed broiler chickens in Argentina.

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