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Characterization of hemorrhages in the ham topsides and tenderloins of slaughter pigs

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ABSTRACT

The aim of the study was to characterize the different types of muscle hemorrhages in the ham and tenderloin of CO_2-stunned slaughter pigs. The distinct types of hemorrhages were characterized according to their distribution and size. The hemorrhages in the ham were multiple, pinpoint hemorrhages predominantly distributed in the caudal part of the muscle. The hemorrhages in the tenderloin were single and circular, located either at the tip or the head. Histologically, three distinct types of hemorrhages were observed. Type 1, which was peracute (<4 h old), and present in both the ham and the tenderloin. Type 2, which was acute (>4 h old), and restricted to the ham. Type 3 contained bone marrow and cartilage, was peracute (< 4 h old) and restricted to the tenderloin.

1. Introduction

Muscle hemorrhages ("blood splash") are regularly observed in the carcasses of slaughter pigs (Grandin & Smith, 2004). The presence of these lesions reduces product quality and the value of the meat (Velarde, Gispert, Faucitano, Manteca, & Diestre, 2000). The distribution and types of the hemorrhages have not been systemically examined, but it has been speculated that they are a consequence of physical strain of the pigs around the time of slaughtering, as this has been settled to be the cause of muscle hemorrhages in broiler chickens (Kranen, Lambooy, Veerkamp, Van Kuppevelt, & Veerkamp, 2000). At major slaughterhouses in Denmark the presence of blood splash in the topsides and in the tenderloins of CO_2 stunned pigs is on average 10% and 13%, respectively. However, for the presence of bleeding in both muscles there is a high degree of variation between slaughterhouses and within the same slaughterhouse (Danish Meat Research Institute, Taastrup, Denmark, unpublished case-confidential information).

Electrical stunning commonly causes hemorrhages in the muscles due to intense generalized muscle contraction, which causes rupture of the small capillaries while the circulatory system still is intact (Gregory, 2005). A similar pathogenesis is thought to be the reason for blood splashes in lambs following a shot in the head with a captive-bolt pistol (Leet, Devine, & Gavey, 1977). Muscle contraction results in compression of the veins, which leads to increased venous pressure. The venules in the capillary bed probably burst where they are weakest, or where the venous pressure is particularly high. This leads to distant petechial hemorrhages (Gregory, 2005). Stunning with carbon dioxide (CO_2) generally results in less muscle hemorrhage (Gregory, 2005). However, during CO_2-stunning a stage of excitation develops where some of the animals have vigorous muscle movements (Velarde, Gispert, Faucitano, Manteca, & Diestre, 2000). Excessive muscle contraction has shown to increase the incidence of petechial hemorrhages in CO_2-stunned pigs (Velarde et al., 2000).

The aim of the present study was to characterize muscle hemorrhages, both macro- and microscopically, in the ham and tenderloins of CO_2-stunned slaughter pigs.

2. Materials and methods

A total of 124 muscles with hemorrhage, 62 ham topsides (*M. semimembranosus*) and 62 tenderloins (*M. psoas major*), from slaughter pigs, were collected at an abattoir using CO_2-stunning. In this cross-sectional study, the live weight of the animals (cross-bread LYD (Danish Landrace, Yorkshire and Duroc)) was 104–107 kg, they were under transport to the slaughter house for 1.7 to 2.3 h, and were in lairage for 50 to 70 min. at the slaughterhouse in groups of 14 animals. In the driveway to the CO_2-pit, animals were moved in groups of 7 or 8 by a Backloader XL7 with a pre-divider (Butina A/S, Holbæk, Denmark). They were stunned for 180 s in the pit, and the time from stunning until shackling just prior to bleeding was 80–90 s. In the CO_2-pit, the concentration of CO_2 at 1 m below the flooring was 90.2–91.5% and at the lowest level it increased to 94.6–95.3% CO_2. The bleeding procedure was measured automatically by a sensor detecting that blood was drained from the animal. Moreover, the area of shackling up animals and the process of bleeding was under constant video surveillance.

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The muscles were collected at the meat inspection platform at the slaughter line, immediately after evisceration, and at the cutting platform during the deboning process of chilled carcasses or cuts. The muscles were examined in order to characterize the hemorrhages according to their distribution on the surface of the individual muscles and their number and size. The muscles were afterwards dissected, in order to characterize the hemorrhages throughout the whole muscle.

Controls included 10 ham topsides and 10 tenderloins without hemorrhage.

All muscles were photographed before being sampled for histology and in each muscle the hemorrhages were measured with a slide caliper.

2.1. Histology

From each muscle with hemorrhage and without hemorrhage (controls), a single tissue sample (approximately 1 × 1 × 1 cm) was taken for histological evaluation. The tissue was immersion fixed in 10% neutral buffered formalin for 3–5 days at 20 °C. After fixation, the tissue was processed through graded concentrations of alcohols and xylene, and finally embedded in paraffin wax. Sections of 4–5 μm were cut and stained with hematoxylin and eosin (HE). Selected tissue sections were stained with phosphotungstic acid hematoxylin (PTAH) for identification of fibrin (Bancroft & Gamble, 2008).

For histological analysis, a quantitative assessment was carried out on all samples, based on the following histological parameters: 1) hemorrhage (present (+) or absent (−)), 2) the type and location of inflammatory cells, and 3) bone marrow elements and cartilage (present (+) or absent (−)).

The type and location of inflammatory cells (leukocytes) in injured tissue can assist with estimating the age of the lesion (Raekallio, 1980). As the age of muscle hemorrhages in slaughter pigs has not previously been investigated, the estimation of the age was based on knowledge gained from studies on wound healing in general and on bruises in pigs (Fisher, Baracos, Shnitka, Mendryk, & Reid, 1990; Randeberg et al., 2007; Saukko & Knight, 2004; Velnar, Bailey, & Smrkolj, 2009). Barington and Jensen (2016) created a porcine model for determining the timing of bruises in pigs. They showed that through histological evaluation of bruises, these could be grouped as being either <4 h old or between 4 and 10 h of age.

2.2. Immunohistochemistry

Selected sections were immunohistochemically stained for leukocytes, blood clot formation (by visualizing fibrinogen), platelets (by detecting thromboxane synthase) and endothelial cells (by visualizing Von Willebrand factor expression). The immunostaining was performed by application of the UltraVision LP Detection System HRP (Thermo Scientific, Lab Vision Corporation, Fermont, CA, USA) (Isling, Aalbæk, Birck, Heegaard, & Leifsson, 2011).

To confirm the presence of leukocytes a monoclonal mouse antibody (Serotec MCA874G) raised towards an intracytoplasmic antigen (the calprotectin or L1 leukocyte protein) expressed by granulocytes, monocytes and by tissue macrophages was used at a dilution of 1:500.

For visualization of endothelial cells, a polyclonal rabbit anti-human antibody to Von Willebrand factor (DAKO A0082) was used at a dilution of 1:500.

For visualization of fibrinogen, a polyclonal rabbit antibody to fibrinogen (DAKO A0080) was used at a dilution of 1:500. This immunostaining was performed without antigen retrieval. In order to avoid unspecific binding of the primary antibody and to improve penetration of immunoreagents, TBS with 0.05% tween 20 detergent was added before blocking by Ultra V Block.

For detection of thromboxane synthase, a polyclonal rabbit antibody to thromboxane synthase (anti-thromboxane synthase antibody, Abcam 39362) was applied at a dilution of 1:100. The immunostaining was performed by application of the UltraVision One Detection System HRP (Thermo Scientific, Lab Vision Corporation, Fermont, CA, USA). This indirect technique involved only two layers, as the Primary Antibody Enhancer is left out. Antigen retrieval was carried out by treatment with Protease solution (Sigma-Aldrich Denmark A/S, Vallensbæk Strand, Denmark).

All negative controls were performed with normal rabbit immunoglobulin (DAKO 0903) substituting the primary reagent and at similar concentration as in the different protocols.

Table 1
Characterization of muscle bleedings in 124 muscles (62 ham topsides and 62 tenderloins) according to their pattern of distribution on the surface of each muscle and their average size (mean ± S.D.).

Muscles	Hemorrhages on the surface	Number of muscles
Ham topside	Multiple, pinpoint	62
	5-30 per muscle	59 (95.2 %)
	>30 per muscle	3 (4.8 %)
Tenderloin	Single circular	62
	At the tip	51 (82.3 %)
	At the head	11 (17.7 %)

The hemorrhages in the ham topside and tenderloin measured 0.3 ± 0.29 cm and 2.2 ± 0.98 cm, respectively.

2.3. Statistical analyses

The data from the histological quantitative assessment were analyzed (SAS 9.4) by comparing the type of hemorrhages in relation to muscle type (ham or tenderloin). Chi-square tests were used to determine whether these variables were independent from one another. A p-value <0.05 was considered significant.

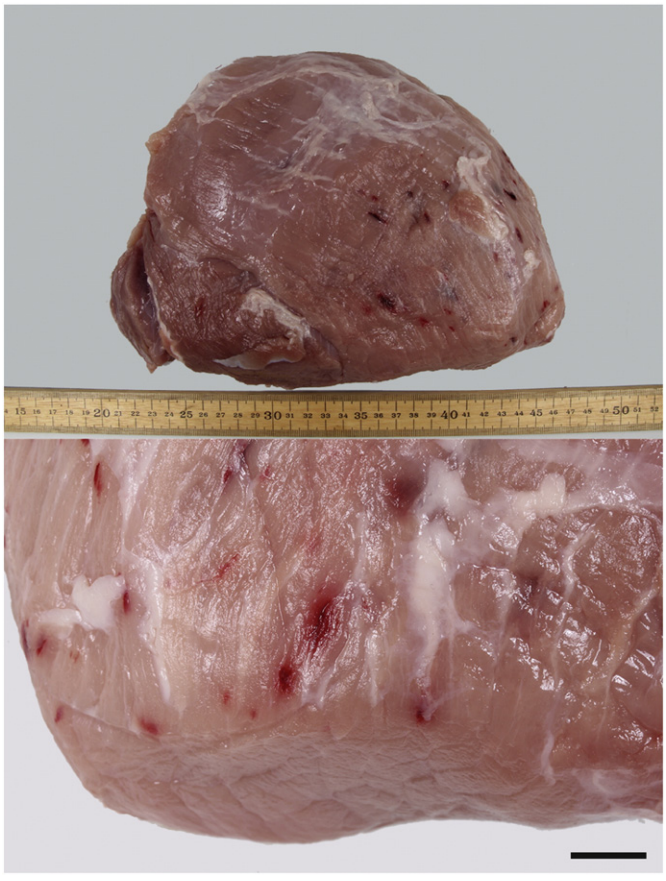

Fig. 1. Ham topside with pinpoint hemorrhages in the caudal part of the muscle. Upper figure: ruler in cm. Lower figure: bar = 4 cm.