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Pre-slaughter conditions influence skatole and androstenone in adipose tissue of boars

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ABSTRACT

Boar taint in carcasses may vary between farms and abattoirs, although the underlying mechanisms are not yet fully understood. In the present study, 169 boars from three farms were split into two groups and slaughtered at two abattoirs. Duration of transport and the time between arrival at the abattoir and unloading (pre-unloading time) were recorded. During slaughter, blood, feces, and urine were collected to measure testosterone and cortisol levels. Carcasses were classified according to the number of skin lesions, and fat samples were taken to determine skatole, indole and androstenone levels. Androstenone in fat and testosterone in blood, feces, and urine were mainly influenced by the duration of transport. Skatole and indole concentrations were increased by both pre-unloading time and duration of transport, but were also related to skin lesions. Thus it is concluded that androstenone and skatole concentrations in fat are significantly modified by pre-slaughter conditions.

1. Introduction

The number of boar carcasses with sex-specific off-odor varies considerably between studies (Bonneau, 1998). Several studies reveal differences in the numbers of tainted carcasses between abattoirs, although limited analytical data and a lack of standardized detection methods made it difficult to determine the causes (Moerlein, Grave, Sharifi, Buecking, & Wicke, 2012; Prusa et al., 2011). Although the reasons for such differences could not be determined so far, pre-slaughter conditions, especially transport and handling of the animals, have been discussed.

Androstenone and skatole are regarded as the main compounds leading to the sex-specific off-odor of boar carcasses (Claus, Weiler, & Herzog, 1994). Origin and regulation of formation differ between those substances. Androstenone is formed in the Leydig cells of boar testes, and the rates of formation and release are under endocrine control (Claus et al., 1994). It has been reported that several exogenous and endogenous factors influence androstenone formation and accumulation in adipose tissue. In addition to breed, age, and photoperiod, stimuli such as social rank, inter-male aggression, sexual activity and in particular the transfer to a new and unknown pen lead to changes in androstenone concentrations in blood and adipose tissue (Andersson, Rydhmer et al., 1998; Andersson, Wallgren et al., 1998; Claus & Alsing, 1976; Giersing, Lundström, & Andersson, 2000; Walstra et al., 1999).

Skatole and indole are microbial products of tryptophan degradation in the colon and their formation, resorption, and accumulation are mainly affected by nutritional factors and endocrine effects (for review see: Wesoly & Weiler, 2012). Earlier studies (Claus et al., 1994) also discussed, but did not conclusively prove, the effect of stress-related hormones on skatole and indole formation and accumulation. Quantification of hormones associated with stress like cortisol and cortisol metabolites in different physiological substrates such as blood, urine, and feces allows the monitoring of stressful conditions, because hormone concentrations in these substrates reflect the course of transient changes in adrenal activity. A stimulation of adrenal activity by a CRH (corticotropin-releasing hormone) injection in pigs led to an increase in urinary cortisol excretion after only 0.5 h. Maximum concentrations in urine were measured within 2–3 h (Hay, Meunier-Salaün, Brulaud, Monnier, & Mormède, 2000). An increase of cortisol metabolites in feces occurs later, as the diurnal pattern of cortisol secretion with high concentrations in blood in the morning is reflected with a delay of 12–36 h in fecal samples. Consequently, maximum concentrations in feces are found in samples collected during the night (Carlsson, Lyberg, Royo, & Hau, 2007). Similarly, measurements of testosterone in blood, urine, and feces allow monitoring changes in testicular activity (Palme, Fischer, Schildorfer, & Ismail, 1996).

The aim of this study was to compare skatole and androstenone concentrations in boar carcasses from boars raised on three different farms, which were slaughtered at two different abattoirs. Moreover, we sought to identify possible environmental factors and the underlying physiological mechanisms leading to differences in boar taint compounds by measuring the adrenal and testicular steroids in various substrates.

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2. Material and methods

2.1. Experimental strategy

The experiment had been designed to evaluate differences in concentrations of androstenone and skatole in adipose tissue of boars slaughtered at two abattoirs of the same company. The two abattaoirs that employed similar technical equipment for slaughter were similar in their technical equipment for slaughter but were assumed to differ in the amount of tainted boar carcasses according to human nose scoring at the slaughter line. In order to determine a possible contribution of different pre-slaughter conditions, animals from each farm were split into two groups, then transported to and slaughtered at the two abattoirs. As shown in Table 1, pre-slaughter conditions were evaluated for animals from three different farms. In order to analyze the physiological reactions of the boars during transport and pre-slaughter period, samples from different compartments (blood, feces, urine, and adipose tissue) were collected. These samples allow a determination of long-term parameters (measurements in liquid fat and feces), mid-term parameters (measurements in urine) and short term parameters (measurements in blood) of testicular and cortical activities.

2.2. Animals and sampling

A total of 207 boars from three farms with different genotypes (Farm: A Pietrain × BW Hybrids, Farms B and C: Duroc × Danbreed) were studied; each with a live weight of approximately 120 kg. All boars had been raised with commercial diets for growing boars, although feed composition and feeding regimen were not standardized between farms. The animals from each farm were delivered to the abattoirs on two consecutive days in separate vehicles, although animals from different pens of the same farm were mixed during transport. The duration of transport (transport time) and the time spent on the vehicle after arriving at the abattoir (pre-unloading time) were recorded. All animals had access to water after unloading during the standardized 60 min lairage period, before the animals were CO_2 stunned in a gondola–dip-lift system. After expulsion from the stunning pit, 27–38 animals per day were randomly fitted with ear tags to enable the identification of individual carcasses along the slaughter line. During exsanguination, 20 ml of blood from the jugular vein was collected into heparinized vials.

After scalding, dehairing, and flaming, the carcass was opened to remove the red offal. At this stage, distal colons and bladders from animals with ear tags were collected for further sampling of feces and urine. After cooling, each individual carcass was examined on one side for signs of fighting, especially skin lesions, and classified in a four-level system according to the following criteria: no skin lesions/side = score 0; 1–8 lesions/side = score 1; 8–25 lesions/side = score 2; and more than 25 lesions/side = score 3. From each tagged carcass an adipose tissue sample was taken from the neck area for further analysis. Due to mechanical influences, about 20% of the ear tags got lost at the slaughter line, so that samples could be obtained only from a reduced number of animals (maximum n = 169). Moreover, urine and feces could not be obtained from every animal due to empty bladders or colons. Details are provided in Table 1.

Table 1
Number of boars from each farm sampled at the two abattoirs and the corresponding duration of transport from farm to abattoir (min) and pre-unloading time (min).

Farm	Abattoir	Tissue samples				Transport time (min)	Pre-unloading time (min)
		Adipose tissue	Plasma	Urine	Feces		
A	1	29	28	19	16	60	480
	2	21	20	19	16	240	202
B	1	31	31	33	27	150	165
	2	33	32	25	22	270	93
C	1	28	27	28	20	300	260
	2	27	27	29	23	90	17

2.3. Treatment of samples

Blood samples were centrifuged within 1 h after collection and plasma was stored on ice until freezing. Feces were collected manually from the rectum and distal colon and stored on ice. Urine samples were aspirated with a syringe from the bladder and stored immediately on ice. All samples were then transferred on-ice to the lab within 12 h and stored frozen at −20 °C until assayed.

2.4. Determination of androstenone in adipose tissue

The androstenone concentrations in adipose tissue were measured after extraction with an in-house enzyme immunoassay as described earlier (Claus, Herbert, & Dehnhard, 1997; Weiler, Götz, Schmidt, Otto, & Müller, 2013). In short, 50 μl of melted fat was added to 450 μl warm methanol (55 °C). After mixing, samples were allowed to cool down to room temperature and centrifuged thereafter (200 rcf, 10 min, 4 °C). 100 μl of the supernatant was diluted with 900 μl assay buffer and 100 μl was used for further enzyme immunological tests as described above. All samples were measured in duplicate. Precision was determined using spiked fat samples with a mean recovery rate of 96.4%. Intra-assay variance and inter-assay variance were determined with biological samples and was below 10% and below 14%, respectively. A threshold of 0.5 μg/g fat for tainted samples was chosen in accordance with previous studies (Walstra et al., 1999). In this study, concentrations of androstenone, skatole and indole in adipose tissue refer to g melted fat.

2.5. Determination of skatole and indole in adipose tissue

Skatole and indole concentrations in adipose tissue were determined according to Dehnhard, Claus, Hillenbrand, and Herzog (1993), with slight modifications for UPLC systems. Samples of 100 μl liquid fat were pipetted in duplicate and the mass of each sample recorded (approximately 100 mg melted fat). One ml n-hexane and the internal standard 2-methyl-indole (IS; 30 ng/sample dissolved in 30 μl hexane) were added and mixed. Afterward, a solvent distribution was carried out against 1 ml acetonitrile: H_2O (4:1). The hexane layer was removed after centrifugation and the remaining sample was further analyzed with a Dionex UPLC system (pump: Ultimate 3000 RS; column oven: Ultimate 3000 RS Column Compartment; autosampler: Ultimate 3000 RS Autosampler WPS 3000RS; Thermo Scientific, Karlsruhe, Germany) combined with a fluorescence detector FLD (Ultimate 3000 RS Fluorescence Detector FLD-3400RS). Separation of the analytes was performed with an Acclaim 120 C18 2.1 × 100 mm 3 μm column (Thermo Scientific, Karlsruhe, Germany). The mobile phase was 0.011 M acetic acid in H_2O: acetonitrile: 2-propanol (55:30:15). A volume of 5.0 μl was injected at a flow rate of 0.4 ml/min and detection was performed with excitation wavelength of 275 nm and emission wavelength of 352 nm.

Precision was determined by measuring skatole- and indole-spiked fat samples. Mean recovery rate for skatole and indole was 97.3% and 101.2%, respectively. Intra-assay variability and inter-assay variability were determined for skatole and indole with biological samples and were below 5% each.

2.6. Determination of testosterone concentrations in plasma

Testosterone concentrations in plasma were determined in duplicate with a commercially available ELISA (EIA-1559, DRG Instrument GmbH, Marburg, Germany) which has been developed for testosterone measurements in human blood plasma. Measurements were carried out in pig plasma without modifications. Intra-assay variability and inter-assay variability were determined with pig plasma samples and was