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Influence of sampling procedure, sampling location and skin contamination on skatole and indole concentrations in adipose tissue of pigs

Raffael Wesoly, Volker Stefanski, Ulrike Weiler *

University of Hohenheim, Institute of Animal Science, Department of Behavioral Physiology of Farm Animals, Garbenstrasse 17, D-70593 Stuttgart, Germany

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ABSTRACT

Skatole leads to off-odor in pork and is influenced by several factors such as sex and management conditions of pigs, but the causal relationships have not yet been clarified. In the present study, physiological skatole concentrations along the carcass were monitored and the transdermal diffusion of skatole was experimentally studied with skatole-spiked feces. Additionally, the impact of different biopsy techniques on skatole in fat and blood was studied. Monitoring of skatole along the carcass revealed higher skatole concentrations in the belly than in dorsal cuts. Topical application of spiked feces increased skatole in fat strictly at the application site. In contrast to punch biopsies, surgical biopsies significantly affected skatole and cortisol levels in blood, but not in fat. We conclude that biopsies for skatole measurements should be taken without anesthesia from the dorsal side of the animals. Fecal contaminations on the ventral side are not likely to influence overall concentrations.

1. Introduction

The European pig production industry faces the challenge to end pig castration by 2018, even if the occurrence of boar taint, the main reason why piglet castration is undertaken has not yet been resolved. One approach to reduce the amount of tainted carcasses is the selection for low-tainted lines (Baes et al., 2013; Strathe et al., 2013). Measurements of androstenone and skatole in adipose tissue, the two main compounds leading to off-odors in boars, are therefore included in an increasing number of breeding programs (Frieden et al., 2012). Whereas in earlier studies such measurements were limited to samples from offspring after slaughter, more recent sampling is also carried out in order to assess the individual breeding value for boar taint for the breeding candidates (Baes et al., 2013). While detailed information about the variability of androstenone along the carcass of boars is available (Claus, 1978), similar data for skatole is scarce. Comparative measurements in adipose tissue from different anatomical locations of carcasses are limited to a small number of sampling sites such as neck, flare (visceral fat) and belly (Hawe, Moss, Walker & Porter, 1989; Lösel, Lacorn, Buttner & Claus, 2006). These studies revealed significantly higher skatole concentrations in flare and belly compared to dorsal fat samples. In contrast to measurements in carcasses, samples obtained by biopsies may be influenced by different levels of stress during the sampling procedure, which in turn may affect various physiological parameters. In previous studies, an influence of stress on skatole formation and accumulation in fat has been discussed, but the mechanisms have not been investigated in detail for pigs (Claus, Weiler & Herzog, 1994; Wesoly, Jungbluth, Stefanski & Weiler, 2015). A possible effect of stress on skatole levels and physiological parameters due to the choice of biopsy technique should therefore be quantified before routine application in breeding programs. So far, such an impact of biopsy technique and the effect of the sampling site has not been investigated in detail. Moreover, the contribution of fecal contamination of the skin to skatole levels in adipose tissue is not clear and has been subject to controversial debate for decades. Transdermal diffusion of ^{3}H-skatole has been described (Hansen, Larsen, Jensen, Hansen-Møller & Barton-Gade, 1994), but the relevance of this route of absorption on overall concentrations in adipose tissue has not yet been established. In some studies, this transdermal diffusion after soiling was used to explain elevated skatole concentrations in adipose tissue (Hansen et al., 1994; Hansen, 1998). In other studies such an effect could not be proven, even if daily cleaning of pigs was included as a control mechanism while the experimental group was anointed with its own feces twice daily (Aluwé et al., 2011; Bekaert et al., 2012). In this study, we therefore aimed at analyzing the effect of sampling locations and sampling techniques as well as fecal contamination of the skin on skatole concentrations in fat.

* Corresponding author.
E-mail address: weiler@uni-hohenheim.de (U. Weiler).

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2. Material & methods

2.1. Experimental strategy

Three different experiments were carried out to analyze the effect of sampling site, sampling technique, and fecal contamination of the skin on skatole concentrations in fat. In experiment 1, variations of skatole and indole concentrations were monitored along the carcasses of boars. In experiment 2, the influence of transdermal skatole diffusion was investigated, whereas experiment 3 compared the effects of two different biopsy techniques on endocrine stress parameters and skatole concentrations in blood and fat. For experiments 2 and 3, barrows were used to ensure low initial concentrations of skatole. All samples from each animal were measured either within the same assay or two consecutive assays within one week to avoid artifacts due to storage time or inter-assay variations.

2.1.1. Experiment 1: monitoring of skatole and indole concentrations along the carcass

Carcasses from 8 adult boars (German Landrace; 12–14 months; 200 kg live weight on average, range: 194–215 kg) were used for this study. In total, 36 samples per animal were collected from 18 anatomical locations on both left and right sides of the carcass as given in Fig. 1. The samples from the dorsal region were assigned to three cuts: neck (N1–N3; distance between samples: 6.5 cm), loin (L1–L6; distance between samples: 7.0 cm) and ham (H1–H4; distance between samples: 10.0 cm). Samples from the ventral region represent the belly (B1–B5; distance between samples: 7.0 cm). From each sampling site, subcutaneous adipose tissue (approximately 2 cm × 2 cm × 4 cm) was collected and stored at −20 °C until assayed.

2.1.2. Experiment 2: transdermal skatole diffusion

Six crossbred barrows (German Landrace × Pietrain) were individually housed in pens (2 m × 3 m) with concrete floors and fed 3 kg/day of a standard diet (13.4 MJ/kg; 17% crude protein; 1% total lysine). The average live weight at the beginning of the experiment was about 200 ± 10 kg. Feces with high skatole concentrations (HSF, high skatole feces) were prepared as follows. Two kilograms of fresh pooled normal feces (dry matter: 19.5%) was spiked with 1.0 g of skatole (Sigma Aldrich, M51458) which had been dissolved in 20 ml MeOH. After the addition of the dissolved skatole the feces were mixed thoroughly for 15 min with a commercial immersion blender. The even distribution was confirmed by measuring concentrations skatole and indole (not added) in four randomly collected samples from this mixture (coefficients of variation: skatole 19%, indole 9%). Analysis of skatole in HSF revealed 455 μg skatole/g fresh feces and 20 μg indole/g, respectively. High skatole feces were stored in portions of 25 g each at 4 °C until the start of the treatment.

In order to characterize the initial skatole and indole pretreatment concentrations in adipose tissue, a punch-biopsy of adipose tissue was taken as a control sample (CON1) from the neck region of each barrow before the first topical HSF application. In order to ensure standardized application of HSF, a 5 × 5 cm square was marked in the shoulder region on both sides of each animal as shown in Fig. 2. For seven days, the marked area on the left side was covered twice daily at 8:00 a.m. and 3:00 p.m. with 25 g of HSF (HSF-area). The same region on the right side was rinsed twice daily with 50% EtOH and served as post-treatment control (CON2). On day 7 all animals were euthanized and adipose tissue samples were collected. Samples of subcutaneous adipose tissue were taken from four locations as shown in Fig. 2. Two samples were collected from the HSF-area, one sample from the control area (CON2) and one sample each from cranial (CRAN) and caudal (CAUD), 5 cm from the HSF-area respectively. The post-treatment CON2 was taken from the marked area on the contra-lateral side, which had been cleaned twice daily during the treatment period. The samples from the HSF-area were split into a superficial (HSF-S) and a deep layer (HSF-D). The HSF-S samples represented the first cm of subcutaneous fat below the skin, while HSF-D comprised tissue from 1 to 2 cm below the skin.

To avoid contamination of subcutaneous adipose tissue with the topically applied HSF or other superficial skin contaminations during sampling, all areas were cleaned carefully with water and ethanol before the incision was made. The skin was carefully removed from the subcutaneous fat with a scalpel blade parallel to the surface. The adipose tissue was then divided into two layers (HSF-S and HSF-D), and samples were collected from each layer. All samples were immediately frozen and stored at −20 °C until assayed.

2.1.3. Experiment 3: influence of biopsy technique

A total of ten barrows (German Landrace × Pietrain) of 110 ± 6 kg live weight were included in this experiment. All animals had been fitted surgically with indwelling jugular vein catheters 6 days before the start of the experiment (Kraetzl & Weiler, 1998) and had been acquainted with the blood sampling procedure and the experimentators. Catheters were rinsed twice daily at 8 a.m. and 3 p.m. and plasma samples were collected at 3:00 p.m. throughout the experiment. Four animals were subject to repeated surgical fat biopsies (surgical biopsy group, SBG). A total of 5 surgical biopsies (SB) in weekly intervals (day 0, 7, 14, 21, and 28) were carried out in the neck area under total anesthesia (15 mg ketamine [Ursotamin®]/kg LW and 2 mg azaperon [Stresnil®]/kg LW i.v. via catheter). To ensure an undisturbed wound healing only each second biopsy was carried out on the same side.

In the SBG, an additional plasma sample was collected before the SB at 8:00 a.m. The surgical fat biopsies were then carried out between 09:00 a.m. and 10:00 a.m. Before the incision, the skin was cleaned thoroughly and rinsed with iodine. A 3 cm long incision was made and a 2 cm × 2 cm × 1 cm slice of adipose tissue excised and stored deep frozen (−20 °C) for further analysis. The wound was closed with a suture, and covered with iodine (Mundipharma, Limburg, Germany) and a

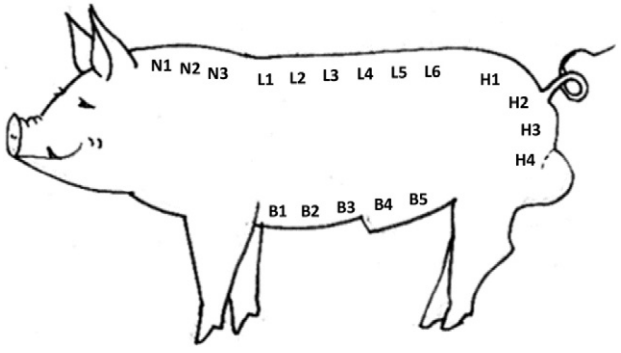

Fig. 1. Sampling procedure in different cuts along the carcass (N: neck; L: loin; H: ham; B: belly).

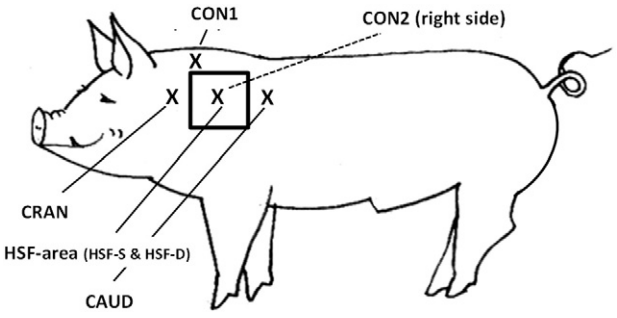

Fig. 2. Treatment site, site of punch biopsy on day 0 (CON1) and sampling procedure on day 7 (CON2, CRAN, CAUD, HSF-S, HSF-D).