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Analytical Methods

Development of a quantitative method for the simultaneous analysis of the boar taint compounds androstenone, skatole and indole in porcine serum and plasma by means of ultra-high performance liquid chromatography coupled to high resolution mass spectrometry

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ABSTRACT

Boar taint is an off-odour occurring while heating meat or fat from boars. A method detecting the three compounds (androstenone, skatole and indole) simultaneously in blood would offer substantial advantages since it would allow monitoring the impact of rearing strategies.

Therefore, a UHPLC-HR-Orbitrap-MS analysis method is optimized and validated for the quantification of these compounds in plasma or serum. Sample pre-treatment involved an extraction with diethylether followed by a centrifugal filtration (30 kDa).

Limits of detection and quantification varied between 0.5 and 1 μg L^{-1} and 2 and 3 μg L^{-1} for the three compounds, respectively. Besides, an excellent repeatability (RSD < 7.6%), within-laboratory reproducibility (RSD < 10.5%), recovery (87–97%) and linearity (R^2 > 0.99) were recorded.

Correlations between serum/plasma and fat levels of the boar taint compounds were positive for skatole (r_{serum} = 0.39 and r_{plasma} = 0.84) and androstenone (r_{serum} = 0.73–0.78 and r_{plasma} = 0.32–0.80).

1. Introduction

The benefits of castration of male piglets have been strongly questioned, not only because of the animals' distress, but also due to substantial economic losses and ecological issues related with raising barrows instead of boars.

Hence, the cessation of castration was recently publically announced and will be obligatory by 2018 (EFSA., 2004). Rearing entire boars has, however, a major disadvantage that is up to now difficult to control. Indeed, boar taint, an off-odour potentially released while heating the meat, is disliked by most consumers who may therefore eventually prefer alternative meat products (Aluwe et al., 2009).

To meet the consumers' expectations, governments, farmers, meat companies and research groups have been and are currently working together to elucidate the physiological mechanisms that are responsible for this taint specific for non-castrated pigs.

A major compound associated with boar taint is the steroidal pig pheromone 5α-androst-16-en-3-one (AEON), which has an unpleasant urine-like and sweaty odour. Upon synthesis in the testis, AEON is released into the bloodstream via the testicular vein and subsequently released in boar saliva or accumulated in adipose tissue due to its lipophilic character (Brooks & Pearson, 1989; Patterson, 1968). Furthermore, predominantly skatole (SK) but also indole (IND), which are both microbial degradation products of tryptophan formed in the intestine by specific bacteria, contribute to boar taint (Claus, Dehnhard, Herzog, Bernalbarragan, & Gimenez, 1993; Deslandes, Gariepy, & Houde, 2001). Their odour perceptions are often described as musty and faecal-like (Claus, Weiler, &

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Herzog, 1994). SK and IND are partly absorbed by the intestinal mucosa, distributed via the bloodstream, and finally enriched in adipose tissue and as such also contribute to boar taint (Zamaratskaia & Squires, 2009).

The level of AEON is demonstrated to alter during sexual maturation and is consequently also age and body weight dependent (Zamaratskaia, Babol, Madej, Squires, & Lundström, 2004b; Zamaratskaia & Squires, 2009). These maturation factors are substantially influenced by genetic background. One approach to control boar taint may thus be based on gene identification and manipulation. The SK level, on the other hand, is not only depending on genetic factors, but can also be influenced by diet and environmental factors, which provide another interesting research field possibly allowing to reduce boar taint in a more natural way, e.g. by management measures (Aluwe et al., 2009).

Up to now, the consequences of these interacting factors on the boar taint compounds in fat tissue or blood are studied with a variety or a combination of techniques since only a limited number of authors describe methods for the simultaneous analysis of the indolic compounds and the steroid compound in adipose tissue (Bekaert et al., 2012; Buttinger, Karasek, Verlinde, & Wenzl, 2014; Hansen-Moller, 1994; Rius & Garcia-Regueiro, 2001; Verheyden et al., 2007), and no previous reports on simultaneous measurements of the boar taint compounds in serum or plasma are available. AEON is often determined by ELISA (Claus, Herbert, & Dehnhard, 1997) or gas chromatography coupled to electron-capture detection (De Brabander & Verbeke, 1986), flame ionisation detection (Rius & Garcia-Regueiro, 2001) or mass spectrometry (Berdagué, Viallon, Bonneau, & Ledenmat, 1993), while the indolic compounds skatole and indole are determined by colorimetric methods (Babol, Zamaratskaia, Juneja, & Lundström, 2004) or liquid chromatography coupled to fluorescence detection (Garcia-Regueiro & Rius, 1998). Analysis of the three boar taint compounds simultaneously has been reported using ultra-high performance liquid chromatography coupled to linear trap liquid chromatography or high-resolution mass spectrometry (Bekaert et al., 2012; Buttinger et al., 2014; Verheyden et al., 2007), but also by means of (stable isotope dilution analysis – headspace solid-phase microextraction –) gas chromatography/mass spectrometry (Fischer et al., 2011, 2014; Sørensen & Balling Engelsen, 2014). For liquid matrices such as plasma or serum, which allow generic extraction, liquid chromatography is however the method of choice for separation (Wu, 2001). Besides, the lower concentrations of the boar taint compounds ($\mu g\,L^{-1}$ level) in plasma or serum as compared to adipose tissue ($mg\,kg^{-1}$ level), limit the application of conventional equipment such as single quadrupole mass analysers.

The objective of this study was to develop a quantitative, accurate, robust and fast UHPLC-HRMS-based method that is capable of quantifying the three known boar taint compounds simultaneously in serum or plasma, which would be highly valuable for routine analysis of boar taint at the pre-slaughter stage, e.g. for studies that aim to establish a boar taint decreasing management on the farm. This method was validated according to the guidelines of 2002/657/EC (European Commission Decision, 2002) and ISO 17025 (ISO, 2005).

2. Experimental

2.1. Reagents and chemicals

The reference standards IND (2,3-benzopyrrole), SK (3-methylindole) and AEON (5α-androst-16-ene-3-one) and the internal standards 2-methylindole (2-MID) and androstadienedione (1,4-androstadiene-3,17-dione, ADD) were obtained from Sigma Aldrich (St. Louis, MO, USA). For each compound a stock solution was prepared in methanol at a concentration of $1\,mg\,mL^{-1}$. Working solutions were made for each compound in methanol at a range of 5–100 $ng\,\mu L^{-1}$. Solutions were stored in dark glass bottles at −20 °C.

Reagents were of analytical grade when used for extraction purposes and of MS-grade for UHPLC-MS applications. They were obtained from VWR International (Merck, Darmstadt, Germany) and Fisher Scientific (Leicestershire, VS), respectively.

Centrifugal filter devices and solid phase extraction (SPE) columns were purchased from Millipore Corporation (Bilerica, US) and Waters Corporation (Milford, US), respectively.

2.2. Samples

2.2.1. Serum samples (study group 1)

The serum samples of entire boars ($n = 32$) were taken from experimental animals at ILVO (Melle, Belgium) after obtaining approval of the ethical committee (EC 2012/180). Samples were acquired by venopunction of the vena jugularis with a Venoject system (Terumo Europe NV, Belgium) coupled to serum collection tubes. Sampling occurred in the morning, one to 13 days before slaughter in a time range of two months (January–February). The boars' ages, all hybrids of Piétrain boars × Rattlerow Seghers sows, varied between 22 and 26 weeks at the time of slaughter. After arrival in the lab (transport on ice), the tubes were centrifuged at 3000*g* during 10 min at 4 °C. The harvested serum was stored at −80 °C until analysis.

In order to obtain reference material, blood of barrows was collected in serum collection tubes during exsanguination at the slaughterhouse and processed as described previously.

2.2.2. Plasma samples (study group 2)

Plasma samples ($n = 79$) were taken from food producing boars of various breeds (Piétrain, Large White, Landrace) which were slaughtered between January 2010 and October 2011 at 18–39 weeks old. Blood was collected into EDTA plasma collection tubes during exsanguination. After arrival in the lab (transport on ice), the tubes were centrifuged at 3000*g* during 10 min at 4 °C. The harvested plasma was stored at −20 °C until analysis.

2.2.3. Paired serum and plasma samples (study group 3)

Serum and plasma samples ($n = 25$) were taken from food producing boars (Landrace; 26–36 weeks old) slaughtered in April 2014. Blood was collected during exsanguination in serum and EDTA plasma collection tubes. Upon arrival at the lab (cooled transport) plasma tubes were centrifuged during 10 min at 1811*g* and 4 °C, while serum tubes were centrifuged at identical speed and temperature but during 30 min. Plasma and serum aliquots were stored at −20 °C until analysis.

2.2.4. Neck fat samples

Back fat samples of the neck region were taken during the slaughter process, packed in plastic bags with (study group 1) or without (study group 2 and 3) vacuum treatment, transported on ice or liquid nitrogen and stored at −80 °C (study group 1 and 3) or −20 °C (study group 2), until analysis. For both study groups, blood samples and fat samples were correctly matched with the help of an additional ear tag attached during exsanguination followed by writing the matching non-erasable pig number on the carcass with a special pencil once the pigs had left the hot baths and scalder prior to evisceration.