High-Performance Liquid Chromatography Determination of Skatole and Indole Levels in Pig Serum, Subcutaneous Fat, and Submaxillary Salivary Glands

Mika Tuomola,* Merja Vahva, and Heikki Kallio

Department of Biochemistry and Food Chemistry, University of Turku, FIN-20014 University of Turku, Finland

A sensitive high-performance liquid chromatographic method was developed for the analysis of skatole and indole in porcine tissue and serum samples. The sensitivity of the method for tissue and serum samples was 1 ng/g and 0.1 ng/mL, respectively. The skatole content in adipose tissue (fat) was higher ($p < 0.0001$) in boars (median 39 ng/g, $n = 91$) than in barrows (24 ng/g, $n = 96$) or gilts (17 ng/g, $n = 85$). The corresponding levels of skatole in serum were 1.9, 1.4, and 1.1 ng/mL, respectively. Significant correlations ($p < 0.0001$) were found between boar serum and fat levels of skatole ($r = 0.90$) and indole ($r = 0.65$). Elevated skatole levels were found exclusively in boars, of which 9.9% exceeded the 200 ng/g limit in fat. Skatole concentrations were 8-fold lower in the salivary glands than in the fat samples, and they were ($p < 0.0001$) correlated with both fat and serum concentrations. On the basis of the close correlation between fat and serum skatole levels, it is proposed that serum measurements could be used for routine assessment of skatole in pork meat, a factor contributing to boar taint.

Keywords: *HPLC; skatole; indole; boar taint; pigs*

INTRODUCTION

Intact (noncastrated) male pigs possess superior fattening performance and carcass traits compared with castrates and gilts due to the anabolic effects of testicular androgens. Thus, the production of boars instead of castrates is an attractive approach for improving the efficiency of pork meat production. However, castration of male piglets is still a common practice in many countries because the meat and fat from a small proportion of intact male pigs emit an unpleasant taint during cooking.

Boar taint has mainly been associated with androstenone (5α-androst-16-en-3-one) (Patterson, 1968) and skatole (3-methylindole) (Vold, 1970; Walstra and Maarse, 1970; Hansson *et al.*, 1980), but also 5α-androst-16-en-3α-ol and indole may contribute to the taint (Hansson *et al.*, 1980; Brennan *et al.*, 1986; Brooks and Pearson, 1989; Bejerholm and Barton-Gade, 1993). 16-Androstene steroids are synthesized in the testes and released into the circulation, where they are taken up by the salivary glands and adipose tissue. In contrast skatole and indole are produced by microbial degradation of tryptophan in the intestinal tract. Intestinal production occurs in both sexes, but for some unknown reason only intact male pigs accumulate these indolic substances in adipose tissue.

The relative importance of skatole and androstenone to taint appears to vary between different production systems in different countries. Both androstenone and skatole levels are influenced by genetic factors, but unlike skatole, androstenone production is highly dependent on the sexual maturity of the pig (Bonneau, 1982, 1987). Skatole levels have been shown to be more susceptible to dietary and environmental conditions (Lundström and Malmfors, 1993; Claus *et al.*, 1994; Hansen *et al.*, 1994, 1995; Lundström *et al.*, 1994). Recent studies have indicated that skatole is the main taint contributor when slaughter weights are low (Andresen *et al.*, 1993; Bejerholm and Barton-Gade, 1993; Berg *et al.*, 1993; Le Denmat *et al.*, 1993; Støier, 1994).

Skatole has been primarily analyzed from adipose tissue samples. Cutoff levels of 250 and 200 ng/g have been used as threshold values for sorting out tainted carcasses, the latter being more prevalent in the recent studies. Gas chromatographic (GC) methods have been used for fat analysis (Hansson *et al.*, 1980; Peleran and Bories, 1985; Porter *et al.*, 1989), but they require considerable amount of sample preparation. A colorimetric method has been developed by Mortensen and Sørensen (1984) for routine analysis of skatole in fat samples, but the reaction is not skatole-specific. More specific methods have been developed using high-performance liquid chromatography (HPLC) with UV detection (García-Regueiro *et al.*, 1986; García-Reguiero and Diaz, 1989) and, more recently, fluorescence detection (Suortti and Lehtonen, 1989; Gibis *et al.*, 1991; Nonboe, 1991; Dehnhard *et al.*, 1993; Gibis and Fischer, 1993; Hansen-Møller, 1992, 1994). However, processing of fat samples is tedious, and additional purification steps are needed before chromatography.

Submaxillary salivary glands may offer an alternative site for the estimation of skatole as these glands are easily sampled and extracted. However, no data are available on skatole concentrations in these tissues. Boar submaxillary salivary glands are known to concentrate specifically 16-androstene steroids, and a good correlation has been found between the salivary glands and fat 16-androstene levels (Booth *et al.*, 1986; Squires *et al.*, 1991). Preliminary trials carried out in our laboratory showed that existing methods developed for fat samples had insufficient sensitivity to measure skatole levels in salivary glands.

* Author to whom correspondence should be addressed (telephone +358-21-3338089; fax +358-21-3338050; e-mail mika.tuomola@utu.fi).

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The levels of skatole and indole in the circulating blood have been given little consideration, and the suitability of blood analysis for skatole screening has not been extensively studied. Singh *et al*. (1988) used an enzyme-linked immunoassay (ELISA) to measure skatole in porcine serum, but the method lacked specificity. GC methods (Bradley and Carlson, 1974; Mackenzie *et al*., 1977; Norheim and Rygge, 1978; Williams *et al*., 1979; Lin *et al*., 1991) and HPLC methods (Lin *et al*., 1991) have been used for determination of the bovine, ovine, and porcine blood plasma skatole concentrations, but only the HPLC method of Claus *et al*. (1993) has been sensitive enough to measure concentrations of skatole and indole found in the peripheral serum of pigs.

The aim of the current study was to investigate the possibility of using serum or salivary glands as a sample material in skatole analysis and to obtain information regarding the level of skatole and indole in Finnish pigs. Concentrations were determined in adipose tissue, salivary gland, and peripheral serum samples from boars, barrows, and gilts with a sensitive and reliable HPLC method.

MATERIALS AND METHODS

Material. The animals for investigation comprised 272 Finnish Landrace, Yorkshire, and Landrace-Yorkshire crossbred pigs (91 boars, 96 barrows, and 85 gilts; average live weights 78.2, 78.6, and 78.4 kg, respectively). The animals had been fasting for 12 h before delivery to the commercial abattoir, and they were held in lairage for about 2 h prior to slaughter. Blood samples (*ca*. 10 mL) were collected during exsanguination and allowed to clot for 1 h. Serum was then isolated by centrifugation and stored at −20 °C. Fat samples (*ca*. 4 × 4 cm) consisting of the whole fat layer were collected from the neck region between the third and fourth cervical vertebra and stored at −20 °C. Submaxillary salivary glands were collected from an additional 72 pigs (35 boars, 34 barrows, and 3 gilts) and stored at −20 °C.

Reagents. Reference compounds indole (BDH, Poole, England), skatole (Aldrich, Steinheim, Germany), and the internal standard 2-methylindole (Fluka Chemie, Buchs, Switzerland) were of analytical reagent grade. Dried GR-quality diethyl ether was obtained from Merck (Darmstadt, Germany). Methanol and acetonitrile were HPLC grade (Rathburn Chemicals, Walkerburn, Scotland).

Fat Sample Preparation. A fat sample (2.0−2.5 g) was homogenized in methanol (5.0 mL) together with a 50 μL volume of 2-methylindole used as an internal standard (10 μg/mL in methanol) by means of a Ultra Turrax T25 homogenizer (Janke & Kunkel, Straufen, Germany). The homogenate was cooled for 30 min at −20 °C and centrifuged (1200*g*, 10 min). The supernatant was then passed through an activated and chilled Sep-Pak C_{18} column (Waters, Milford, MA). The first 1 mL of the eluate was discarded and the next 2 mL collected. An aliquot of 20 μL was analyzed by HPLC.

Salivary Gland Sample Preparation. The treatment of submaxillary salivary gland sample was identical with the fat sample preparation with the exception that 10 μL of internal standard was added.

Serum Sample Preparation. Serum samples were prepared as described by Claus *et al*. (1993) with minor modifications. In brief, a 1.0 mL serum sample spiked with 50 μL of internal standard (0.1 μg/mL in methanol) was extracted with 2 mL of diethyl ether, centrifuged, and frozen. The ether phase was decanted into a tube containing 1.0 mL of HPLC mobile phase, and ether was removed by evaporating at 47 °C. A 100 μL aliquot of the resulting sample was analyzed by HPLC.

HPLC. A Shimadzu (Kyoto, Japan) HPLC system was used, consisting of an SCL-6A system controller, two model LC-6A pumps, an SIL-6A autosampler, and an RF-530 fluorescence monitor. The column used was a Superspher 100 RP-18 (125 × 4 mm i.d., particle size 4 μm) fitted with a LiChrospher RP-18 precolumn (4 × 4 mm i.d., particle size 5 μm) (LiChroCART system, Merck, Darmstadt, Germany). The column was eluted with a mobile phase consisting of water: acetonitrile (60:40, v/v) at a flow rate of 1 mL/min at 30 °C. The native fluorescence of indolic compounds was monitored by using excitation wavelength of 270 nm and emission wavelength of 350 nm.

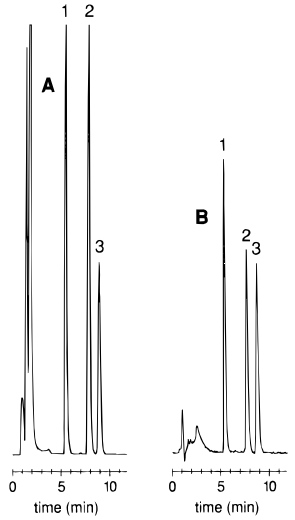

Figure 1. Typical HPLC chromatograms of fat (A) and serum (B) samples. Peaks: 1 = indole (5.5 min), 2 = 2-methylindole (7.8 min), and 3 = skatole (8.9 min).

Quantitative Analysis and Reliability. The linearity of the analysis method was investigated with standard solutions of skatole and indole. For tissue analysis, concentrations of 0.4, 2, 4, 20, 40, 80, 200, and 400 ng/mL were prepared in methanol, corresponding to a range of 1−1000 ng/g in tissue samples. The standard solutions used for serum analysis were prepared with concentrations of 0.1, 1, 5, 10, 50, and 100 ng/mL in 12 mM phosphate-buffered saline (150 mM NaCl, pH 7.4). Both series of standard solutions were spiked with internal standard and taken through the same sample preparation steps as the biological samples. The linearity of the calibration curves was evaluated with Pearson product−moment correlation averaged from six different determinations. The sensitivity of the method was defined as the concentration of standard at which the peak height exceeded the base-line noise by at least 3-fold. In routine measurements a single-point calibration was used for quantitation over the working range, and two identical calibration standards were included in every sample batch, containing equal amounts of skatole, indole, and 2-methylindole. The peak areas of the internal standard and other indoles were determined, and the area ratios were used to determine the levels of analyte in the biological samples. The accuracy of the method was determined from the recovery of added amounts of skatole and indole to serum or adipose tissue samples with a low known content of these compounds. The precision was determined by repeated within-day (intra-assay variation, $n = 5$) and between-day (inter-assay variation, $n = 5$) measurements of the same samples.

Statistical Analysis. The data was analyzed using Statistica Program version 4.5 (StatSoft Inc., Tulsa, OK). The skewed distributions of skatole and indole levels were normalized by transforming the data to natural logarithms, and transformed data were used for correlation studies. Significant differences among nontransformed data were identified using nonparametric Kruskall−Wallis ANOVA and Mann−Whitney U-tests.

RESULTS AND DISCUSSION

HPLC Analysis. Satisfactory chromatographic separation was achieved between the indolic compounds using a simple isocratic HPLC method (Figure 1). All

Table 1. Precision and Accuracy of the Skatole and Indole Determinations ($n = 5$)

added	measured mean ± SD	coefficient of variation (%) within-day	coefficient of variation (%) between-day
skatole in fat (ng/g)			
100	99 ± 4	3.3	3.7
200	199 ± 6	4.5	3.2
400	394 ± 9	2.3	3.3
indole in fat (ng/g)			
100	96 ± 4	5.3	4.4
200	199 ± 8	3.5	4.0
400	415 ± 11	2.7	3.0
skatole in serum (ng/mL)			
1.0	1.0 ± 0.1	13.0	9.3
5.0	5.1 ± 0.2	3.2	1.3
25.0	23.0 ± 1.4	4.0	6.2
indole in serum (ng/mL)			
1.0	0.9 ± 0.1	4.6	7.7
5.0	5.0 ± 0.2	2.7	2.6
25.0	23.0 ± 1.4	3.8	6.3

calibration curves obtained were linear with correlation coefficients >0.999 over the whole range studied. The slope of the regression line was close to 1 in all plots, and the confidence intervals of the intercepts included the origin at the 95% significance level, enabling the use of single-point calibration for the quantitation. The sensitivity for both compounds was 1 ng/g in fat and 0.1 ng/mL in serum. Assay precision and accuracy are given in Table 1. The use of an internal standard compensated effectively for procedural losses, as 96−104% of skatole (mean 99%) and indole (mean 100%) added to fat samples were detected. The mean within- and between-day coefficients of variation (CV) were 3.4% for skatole and 3.8% for indole, showing good analysis reproducibility. The accuracy of the spiked serum analysis was on average 98% for skatole (range 92−102%) and 94% for indole (range 90−100%). The mean within-/between-day CV values for skatole and indole were 6.7/5.6% for skatole and 3.7/5.5% for indole.

Fat Samples. Skatole is known to be nonuniformly distributed in the pig adipose tissue with highest concentrations in the vicinity of lean meat and the levels decreasing toward the skin (Nonboe, 1991; Hansen *et al*., 1994). Sample uniformity was ensured in this study by using the whole depth of the fat layer in the samples, excluding only the first few millimeters from the skin. Acetonitrile and methanol were originally tested for the fat extraction, but the use of acetonitrile was discontinued because the residual fat in the samples caused late eluting peaks in the chromatograms and increased the column back-pressure (data not shown). Solid-phase extraction with a stationary C_{18} phase was used to remove the interfering lipid material from extracts as indolic compounds were not retained by the column (Hansen-Møller, 1992).

As expected, boars had higher concentrations of skatole and indole in fat samples than barrows or gilts (Table 2). Most samples contained skatole <100 ng/g (Figure 2), but 9.9% of boars still exceeded the sorting limit of 200 ng/g. The results were in good agreement with recent studies from several countries (Andresen *et al*., 1993; Dehnhard *et al*., 1993; Lundström *et al*., 1994; Hansen-Møller, 1994). The relative distribution of indole concentrations was similar to that of skatole. The concentration range of both compounds was rather narrow in barrows and gilts, and no high levels were observed. Elevated levels of skatole have sometimes been found in barrows and gilts (Bonneau *et al*., 1992;

Table 2. Concentrations of Skatole and Indole in Fat and Serum Samples

	boars	barrows	gilts
no. of animals	91	96	85
fat skatole (ng/g)			
median[a]	39	24	17
95% conf interval	33−50	22−27	14−20
range	6−1269	3−88	6−54
fat indole (ng/g)			
median[a]	21	16	13
95% conf interval	18−25	15−18	12−14
range	7−242	5−68	6−43
serum skatole (ng/mL)			
median[a]	1.9	1.4	1.1
95% conf interval	1.7−2.5	1.3−1.5	1.0−1.3
range	0.7−58.3	0.3−3.3	0.1−2.5
serum indole (ng/mL)			
median	2.3	1.7*	1.4*
95% conf interval	1.9−2.5	1.5−1.8	1.2−1.6
range	0.9−14.8	0.6−6.7	0.2−3.3

[a] Groups are statistically different ($p < 0.001$). Groups marked with an asterisk (*) are statistically different at $p = 0.012$.

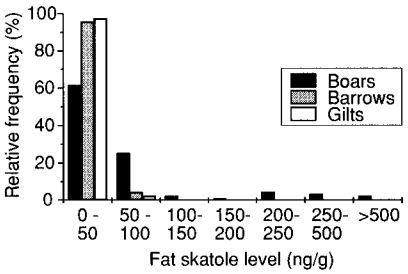

Figure 2. Distribution of skatole levels in fat samples.

Table 3. Correlation Coefficients between Boar Fat and Serum Measurements (Transformed Data, $n = 91$, $p < 0.0001$)

	fat skatole	fat indole	serum skatole
fat indole	0.71		
serum skatole	0.90	0.60	
serum indole	0.53	0.65	0.66

Bejerholm and Barton-Gade, 1993; Berg *et al*., 1993; Stamer *et al*., 1993; Hansen *et al*., 1994, 1995), but these results have been based on spectrophotometric measurements, and other compounds in addition to skatole may have affected the results (Stolle *et al*., 1994).

In this study, high concentrations of skatole were usually accompanied with elevated indole levels, and a significant positive correlation was found between skatole and indole concentrations in the adipose tissue (Table 3). This observation is in good agreement with previous studies (Bejerholm and Barton-Gade, 1992; Dehnhard *et al*., 1993; Herzog *et al*., 1993; Hansen *et al*., 1994, 1995). The close relationship can be explained by the competition for tryptophan, which is a common precursor for both skatole- and indole-producing microbes. Boars exceeding the skatole limit of 0.2 μg/g had a lower average indole/skatole ratio (0.16) than the rest of the group (0.54), and the screening result was almost identical whether based on the skatole level of 200 ng/g or on the sum of skatole and indole concentrations of 230 ng/g. Thus, similar screening results could be expected with the less specific spectrophotometric method that cross-reacts mainly with indole (Stolle *et al*., 1994). According to Hansen-Møller (1994), it is possible to predict the spectrophotometric skatole equivalent from the content of skatole and indole obtained by HPLC, and good correlations have been established between these methods in Danish studies (Hansen-Møller, 1992, 1994;

Table 4. Concentrations of Skatole and Indole in Salivary Gland Samples[a]

	boars	barrows	gilts
no. of animals	35	34	3
salivary gland skatole (ng/g)			
median	8	7	6
95% conf interval	5−13	5−9	
range	2−96	2−16	3−6
salivary gland indole (ng/g)			
median	6*	3*	5
95% conf interval	5−11	2−3	
range	2−115	1−9	3−8

[a] Groups marked with an asterisk (*) are statistically different ($p < 0.0001$).

Table 5. Correlation Coefficients between Boar Salivary Gland, Fat, and Serum Measurements (Transformed Data)

	serum ($n = 22$)		fat ($n = 35$)		salivary gland
	skatole	indole	skatole	indole	skatole
salivary gland skatole	0.92***	0.75***	0.78***	0.74***	
salivary gland indole	0.61*	0.90***	0.52*	0.85***	0.80***

*** Significance level $p < 0.0001$; *$p < 0.01$.

Hansen *et al.*, 1994, 1995). However, contradicting results occur when high levels of indole exceeding those of skatole are found (García-Regueiro *et al.*, 1986; Gibis *et al.*, 1991; Gibis and Fischer, 1993). Boar live weights did not correlate with the concentrations of skatole or indole in the 65.5−88.0 kg weight range studied (data not shown), which is consistent with other reports (Le Denmat *et al.*, 1993; Lundström and Malmfors, 1993; Støier, 1994).

Submaxillary Salivary Gland Samples. Results of salivary gland measurements are presented in Table 4. One cryptorchid pig with a retained abdominal testis was found among barrows in the salivary gland sample group, and it was excluded from the results. On average, skatole concentrations were 8-fold lower in salivary glands than in fat samples, and the concentration range was similar to that reported for lean meat samples by Gibis *et al.* (1991). The corresponding decrease in indole was only 4-fold, which is probably due to the fact that indole is less lipophilic than skatole. As in fat samples, the high contents of skatole and indole were found in boars only. Skatole and indole levels in boar salivary glands were significantly correlated with respective fat and serum levels (Table 5). This close relationship and the fact that glandular tissue was easier to homogenize and extract than adipose tissue suggest that skatole levels could also be screened by salivary gland analysis. However, the average skatole level is below the detection limit of most analytical methods reported.

Serum Samples. The levels of skatole and indole in peripheral serum were significantly higher in boars than in barrows or gilts (Table 2). The concentration ranges were considerably wider in boar samples than in barrows or gilts, but with the few cases in the high-level classes omitted (>5.0 ng/mL), the general frequency distribution of concentrations was very similar in all sexes (Figure 3). Elevated skatole levels were found in boars only, which is in contrast to the findings of Singh *et al.* (1988) who found no differences between gilts and boars. The serum skatole concentrations of gilts were in the same range as reported for peripheral plasma of ovariectomized sows (Claus *et al.*, 1993). Exceptionally high peripheral levels of indole as observed by Claus *et al.* (1993) were not found, which may

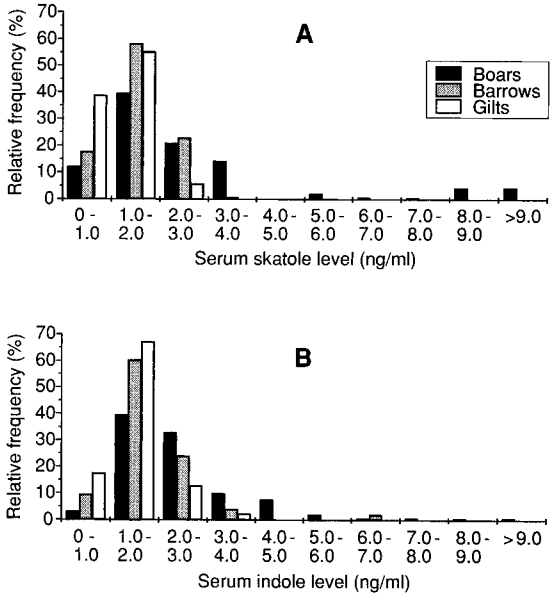

Figure 3. Distribution of skatole and indole levels in serum samples.

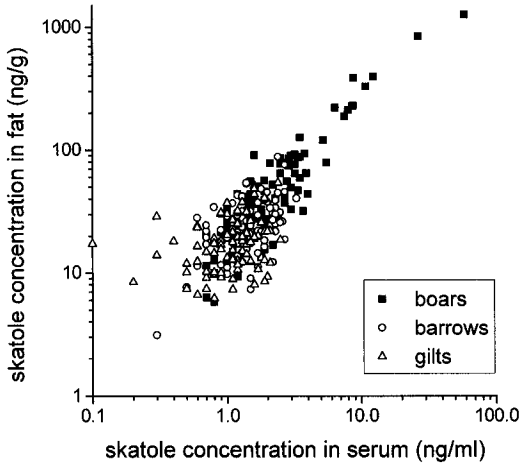

Figure 4. Relationship between serum and fat skatole concentration in boars ($n = 91$), barrows ($n = 96$), and gilts ($n = 85$).

be due to the effects of ovariectomy and differences in pig material and the intestinal flora. In this study elevated levels of serum indole were found in boar samples only.

High skatole and indole concentrations in serum samples were accompanied by high values in the corresponding fat samples, and significant correlations were found between boar serum and fat levels of skatole and indole (Table 3). The relationship between serum and fat skatole concentrations in all pig samples is presented in Figure 4. It is evident that the same group of high-skatole boars is identified whether based on the fat or serum analyses. The serum/fat correlations were higher with skatole than with indole, and they were in good agreement with the results of Herzog *et al.* (1993) (skatole $r = 0.91$, indole $r = 0.68$). The first reported study on skatole levels in serum and fat samples (Singh *et al.*, 1988) did not find any relationship between them, probably due to the inherent limits of specificity of methods used for the determinations (ELISA and colorimetry).

Skatole is known to be quickly absorbed from the intestine, and the level of skatole in hepatic vein blood has been shown to be <50% of that in the portal vein

at any given time, indicating high liver clearance (Agergaard and Laue, 1993). The terminal half-life of skatole in the blood has been reported to be *ca*. 1 h (Agergaard and Laue, 1993; Friis, 1993). Close correlation has been demonstrated between intravenously administered skatole doses and concentration of skatole in fat (Friis, 1993), and it has been suggested that high skatole concentrations in the fat result from reduced skatole degradation in the liver (Agergaard and Laue, 1993; Friis, 1993; Lundström *et al*., 1994; Hansen *et al*., 1995). The results of the current study support this hypothesis, as high skatole levels in fat were always associated with elevated levels of peripheral serum skatole. The close relationship suggests that a peripheral blood skatole measurement can be a suitable method for assessment of skatole in pork meat.

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LITERATURE CITED

Agergaard, N.; Laue, A. Absorption from the gastrointestinal tract and liver turnover of skatole. In *Measurement and Prevention of Boar Taint in Entire Male Pigs*; Bonneau, M., Ed.; INRA: Paris, 1993; pp 107−111.

Andresen, Ø.; Frøystein, T.; Rødbotten, M.; Mortensen, H. P.; Eik-Nes, O.; Lea, P. Sensoric evaluation of boar meat with different levels of androstenone and skatole. In *Measurement and Prevention of Boar Taint in Entire Male Pigs*; Bonneau, M., Ed.; INRA: Paris, 1993; pp 69−74.

Bejerholm, C.; Barton-Gade, P. The relationship between skatole/androstenone and odour/flavour of meat from entire male pigs. In *Measurement and Prevention of Boar Taint in Entire Male Pigs*; Bonneau, M., Ed.; INRA: Paris, 1993; pp 75−79.

Berg, H.; Agerhem, H.; von Seth, G.; Tornberg, E.; Andresen, Ø. The relationship between skatole and androstenone content and sensory off-odour in entire male pigs. In *Measurement and Prevention of Boar Taint in Entire Male Pigs*; Bonneau, M., Ed.; INRA: Paris, 1993; pp 55−61.

Bonneau, M. Compounds responsible for boar taint, with special emphasis on androstenone: a review. *Livest. Prod. Sci*. **1982**, *9*, 687−705.

Bonneau, M. Effects of age and live weight on fat 5α-androstenone levels in young boars fed two planes of nutrition. *Reprod. Nutr. Dev*. **1987**, *27*, 413−422.

Bonneau, M.; Le Denmat, M.; Vaudelet, J. C.; Veloso Nunes, J. R.; Mortensen, A. B.; Mortensen, H. P. Contributions of fat androstenone and skatole to boar taint: I. Sensory attributes of fat and pork meat. *Livest. Prod. Sci*. **1992**, *32*, 63−80.

Booth, W. D.; Williamson, E. D.; Patterson, R. L. S. 16-androstene steroids in the submaxillary salivary gland of the boar in relation to measures of boar taint in carcasses. *Anim. Prod*. **1986**, *42*, 145−152.

Bradley, B. J.; Carlson, J. R. A gas-liquid chromatographic procedure for the determination of indole and 3-methylindole in bovine plasma. *Anal. Biochem*. **1974**, *59*, 214−219.

Brennan, J. J.; Shand, P. J.; Fenton, M.; Nicholls, L. L.; Aherne, F. X. Androstenone, androstenol and odor intensity in backfat of 100- and 130-kg boars and gilts. *Can. J. Anim. Sci*. **1986**, *66*, 615−624.

Brooks, R. I.; Pearson, A. M. Odor thresholds of the C_{19}-Δ^{16}-steroids responsible for boar odor in pork. *Meat Sci*. **1989**, *24*, 11−19.

Claus, R.; Dehnhard, M.; Herzog, A.; Bernal-Barragan, H.; Giménez, T. Parallel measurements of indole and skatole (3-methylindole) in feces and blood plasma of pigs by HPLC. *Livest. Prod. Sci*. **1993**, *34*, 115−126.

Claus, R.; Weiler, U.; Herzog, A. Physiological aspects of androstenone and skatole formation in the boar—a review with experimental data. *Meat Sci*. **1994**, *38*, 289−305.

Dehnhard, M.; Claus, R.; Hillenbrand, M.; Herzog, A. High-performance liquid chromatographic method for the determination of 3-methylindole (skatole) and indole in adipose tissue of pigs. *J. Chromatogr*. **1993**, *616*, 205−209.

Friis, C. Distribution, metabolic fate and elimination of skatole in the pig. In *Measurement and Prevention of Boar Taint in Entire Male Pigs*; Bonneau, M., Ed.; INRA: Paris, 1993; pp 113−115.

García-Regueiro, J. A.; Diaz, I. Evaluation of the contribution of skatole, indole, androstenone and androstenols to boar-taint in back fat of pigs by HPLC and capillary gas chromatography (CGC). *Meat Sci*. **1989**, *25*, 307−316.

García-Regueiro, J. A.; Hortós, M.; Arnau, C.; Monfort, J. M. Determination of skatole and indole in back fat of pigs by HPLC. *J. High Resolut. Chromatogr. Chromatogr. Commun*. **1986**, *9*, 362−363.

Gibis, M.; Fischer, A. HPLC method for the rapid determination of indole and skatole in the adipose tissue of swine. *Dtsch. Lebensm.-Rundsch*. **1993**, *89*, 313−316.

Gibis, M.; Dehnhard, M.; Fischer, A. Determination of skatole and indole in backfat and lean meat of pigs by HPLC with fluorimetric detection. *Z. Lebensm. Unters. Forsch*. **1991**, *193*, 220−223.

Hansen, L. L.; Larsen, A. E.; Jensen, B. B.; Hansen-Møller, J.; Barton-Gade, P. Influence of stocking rate and faeces deposition in the pen at different temperatures on skatole concentration (boar taint) in subcutaneous fat. *Anim. Prod*. **1994**, *59*, 99−110.

Hansen, L. L.; Larsen, A. E.; Hansen-Møller, J. Influence of keeping pigs heavily fouled with faeces plus urine on skatole and indole concentration (boar taint) in subcutaneous fat. *Acta Agric. Scand. Sect. A, Anim. Sci*. **1995**, *45*, 178−185.

Hansen-Møller, J. Determination of indolic compounds in pig back fat by solid-phase extraction and gradient high-performance liquid chromatography with special emphasis on the boar taint compound skatole. *J. Chromatogr*. **1992**, *624*, 479−490.

Hansen-Møller, J. Rapid high-performance liquid chromatographic method for simultaneous determination of androstenone, skatole and indole in back fat from pigs. *J. Chromatogr*. **1994**, *661*, 219−230.

Hansson, K.-E.; Lundström, K.; Fjelkner-Modig, S.; Persson, J. The importance of androstenone and skatole for boar taint. *Swed. J. Agric. Res*. **1980**, *10*, 167−173.

Herzog, A.; Herbert, E.; Dehnhard, M.; Claus, R. Comparative studies on androstenone and skatole in various tissues. *Mitt. Bundesanst. Fleischforsch. Kulmbach* **1993**, *120*, 116−124.

Le Denmat, M.; Hervo, N.; Vaudelet, J. C.; Bonneau, M. The effect of slaughter weight on fat androstenone and skatole levels and on the assessment of boar taint in entire male pigs. In *Measurement and Prevention of Boar Taint in Entire Male Pigs*; Bonneau, M., Ed.; INRA: Paris, 1993; pp 167−171.

Lin, R. S.; Orcutt, M. W.; Patterson, J. A.; Judge, M. D. Serum skatole detection using gas chromatography and high performance liquid chromatography. *Meat Sci*. **1991**, *30*, 33−40.

Lundström, K.; Malmfors, B. Skatole levels as affected by inheritance and season. Proceedings of the 39th International Congress on Meat Science and Technology, Calgary, Canada, 1993; Paper S2P13.

Lundström, K.; Malmfors, B.; Stern, S.; Rydhmer, L.; Eliasson-Selling, L.; Mortensen, A. B.; Mortensen, H. P. Skatole levels in pigs selected for high lean tissue growth rate on different dietary protein levels. *Livest. Prod. Sci*. **1994**, *38*, 125−132.

Mackenzie, A.; Heaney, R. K.; Fenwick, G. R. Determination of indole and 3-methylindole in plasma and rumen fluid from cattle with fog fever or after L-tryptophan administration. *Res. Vet. Sci*. **1977**, *23*, 47−50.

Mortensen, A. B.; Sørensen, S. F. Relationship between boar taint and skatole determined with a new analysis method.

Proceedings of the 30th European Meeting of Meat Research Workers, Bristol, U.K., 1984; pp 394−396.

Nonboe, U. Licentiate thesis, Royal Danish Veterinary College, Copenhagen, 1991.

Norheim, G.; Rygge, J. Gas chromatographic determination of indole and 3-methylindole in ovine plasma and rumen fluid using a nitrogen-phophorous selective detector. *J. Chromatogr.* **1978**, *154*, 291−294.

Patterson, R. L. S. 5α-Androst-16-ene-3-one: Compound responsible for taint in boar fat. *J. Sci. Food Agric.* **1968**, *19*, 31−38.

Peleran, J. C.; Bories, G. F. Gas chromatographic determination and mass spectrometric confirmation of traces of indole and 3-methylindole (skatole) in pig back fat. *J. Chromatogr.* **1985**, *324*, 469−474.

Porter, M. G.; Hawe, S. M.; Walker, N. Method for the determination of indole and skatole in pig fat. *J. Sci. Food Agric.* **1989**, *49*, 203−209.

Singh, P.; Sinha, A.; Afzal, R.; Brock, T. K. A practical enzyme-linked immunoassay for quantitation of skatole. Proceedings of the 34th International Congress on Meat Science and Technology, Brisbane, Australia, 1988; pp 692−694.

Squires, E. J.; Gullett, E. A.; Fisher, K. R. S.; Partlow, G. D. Comparison of androst-16-ene steroid levels determined by a colorimetric assay with boar taint estimated by a trained sensory panel. *J. Anim. Sci.* **1991**, *69*, 1092−1100.

Stamer, S.; Nürnberg, K.; Kanitz, W.; Kalm, E. Assessment of boar taint by androstenone and skatole levels and organoleptic tests. In *Measurement and Prevention of Boar Taint in Entire Male Pigs*; Bonneau, M., Ed.; INRA: Paris, 1993; pp 87−92.

Støier, S. Deviating smell in Danish entire male pigs - relative to skatole and androstenone contents. Proceedings of the 40th International Congress on Meat Science and Technology, The Hague, The Netherlands, 1994; Paper W-3.12.

Stolle, A.; Sedlmeier, H.; Wirrer, B. On the efficiency of photometric determination of skatole in slaughtered pigs. *Z. Lebensm. Unters. Forsch.* **1994**, *199*, 301−306.

Suortti, T.; Lehtonen, T. HPLC determination of indole and skatole. *Proceedings of Euro Food Chem V.*; Institut National de la Recherche Agronomique: Paris, 1989; Vol. 2, pp 698−702.

Vold, E. Meat production from boars and castrates. IV. Organoleptic and gaschromatographic studies on the steam distillate of back fat from boars. Report No. 238; Institute of Animal Genetics and Breeding, NLH: Vollebekk, Norway, 1970.

Walstra, P.; Maarse, H. IVO-Rapport C-147 and Rapport No. 2; Researchgroep Vlees en Vleeswaren, TNO: Zeist, 1970.

Williams, G. D.; Rippon, P.; Chen, P.-M.; Camp, B. J. An improved gas-liquid chromatographic procedure for the determination of 3-methylindole in rumen liquor, plasma, and tissue of ruminants. *Anal. Biochem.* **1979**, *99*, 324−331.

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