# DETERMINATION OF SKATOLE AND INDOLE LEVELS IN PIG SERUM, SUBCUTANEOUS FAT AND SUBMAXILLARY SALIVARY GLANDS

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## INTRODUCTION

Intact (non-castrated) male pigs possess superior fattening performance and carcass traits compared with castrates and gilts. 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 been associated with androstenone (5α-androst-16-en-3-one) and skatole (3-methylindole), but also 5α-androst-16-en-3α-ol and indole may contribute to the taint. 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.

## METHODS

The animals for investigation comprised 272 Finnish Landrace, Yorkshire and Landrace-Yorkshire crossbred pigs (91 boars, 96 barrows, and 85 gilts; average live weight 78.4 kg). The animals had been fasting for 12 hours before delivery to the abattoir, and they were held in lairage for about two hours prior to slaughter. Blood samples were collected during exsanguination and allowed to clot for 1 h. Serum was isolated by centrifugation and stored at -20 °C. Fat samples were taken from the neck region 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.

**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). The homogenate was cooled for 30 min at -20 °C and centrifuged (1200*g*, 10 min). The supernatant was then passed through an activated Sep-Pak $C_{18}$ column (Waters, Milford, MA). An aliquot of 20 µl was analyzed by HPLC. Salivary glands were treated in the same manner with the exception that 10 µl of internal standard was added. Serum samples were prepared as described by Claus *et al.* (1993) with minor modifications and a 100 µl aliquot was analyzed.

**High-performance liquid chromatography:** A Shimadzu HPLC system (Kyoto, Japan) was used, including an SIL-6A autosampler and an RF-530 fluorescence monitor (excitation 270 nm, emission 350 nm). The column used was a Superspher 100 RP-18 (125 x 4 mm i.d., particle size 4 µm) fitted with a LiChrospher RP-18 precolumn (Merck, Darmstadt, Germany). The mobile phase was water:acetonitrile (60:40 v/v) at a flow rate of 1 ml/min at 30 °C. 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.

## RESULTS AND DISCUSSION

The sensitivity of the method (s/n-ratio = 3) for both compounds was 1 ng/g in fat and 0.1 ng/ml in serum. 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 (C.V.) 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 C.V. values were 6.7/5.6% for skatole and 3.7/5.5% for indole.

### Fat samples

As expected, boars had higher concentrations of skatole and indole in fat samples than barrows or gilts (Table 1). Most samples contained skatole less than 100 ng/g (Figure 1) but 9.9% of boars exceeded the sorting limit of 200 ng/g. The results are in good agreement with studies from other countries (Andresen *et al.*, 1993; Dehnhard *et al.*, 1993; Lundström *et al.*, 1994; Hansen-Møller, 1994). High concentrations of skatole were usually accompanied with elevated indole levels and a significant correlation was found between skatole and indole concentrations in the adipose tissue ($r=0.71$, $p<0.001$). The relative distribution of indole concentrations was similar to skatole.

### Submaxillary salivary gland samples

On average, skatole concentrations were eight-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) (Table 2). The corresponding decrease in indole was only four-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 concentrations in boar salivary glands were significantly correlated with respective fat ($r=0.78$, $p<0.001$) and serum levels ($r=0.92$, $p<0.001$). 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 serum levels of skatole and indole in were significantly higher in boars than in barrows or gilts (Table 1). 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, the general frequency distribution of concentrations was very similar in all sexes. 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). 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 ($r=0.90$, $p<0.001$) and indole ($r=0.65$, $p<0.001$). The relationship between serum and fat skatole concentrations in all pig samples is presented in Figure 2. 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). 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).

**Table 1.** Concentrations of skatole and indole in fat and serum samples.

| | boars | barrows | gilts |
|---|---|---|---|
| number 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[a] | 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 ($p<0.001$)

* marked groups are statistically different at $p=0.012$

## CONCLUSIONS

The terminal half-life of skatole in the blood has been reported to be approximately one hour (Agergaard and Laue, 1993; 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). 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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**Table 2.** Concentrations of skatole and indole in salivary gland samples.(*groups different, $p<0.001$)

| | boars | barrows | gilts |
|---|---|---|---|
| number of animals | 35 | 34 | 3 |
| salivary gland skatole (ng/g) | | | |
| median | 8 | 7 | 6 |
| range | 2 - 96 | 2 - 16 | 3 - 6 |
| salivary gland indole (ng/g) | | | |
| median | 6* | 3* | 5 |
| range | 2 - 115 | 1 - 9 | 3 - 8 |

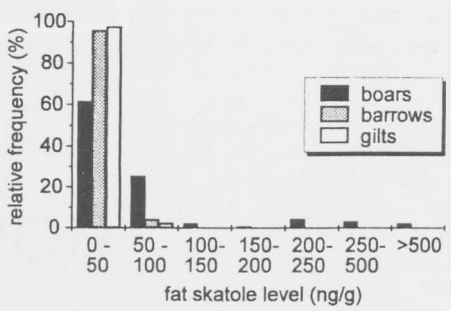


**Figure 1** Distribution of skatole levels in fat samples

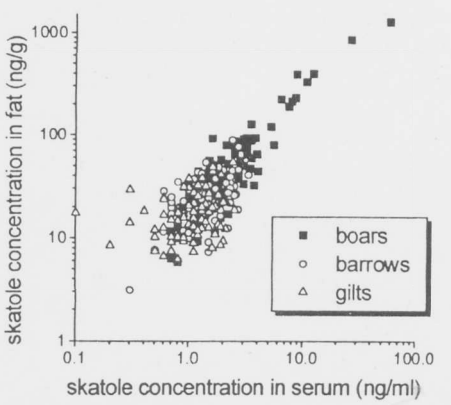


**Figure 2** The relationship between serum and fat skatole concentration