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# Effects of raw potato starch and live weight on fat and plasma skatole, indole and androstenone levels measured by different methods in entire male pigs

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**Abstract**

A total of 96 entire male pigs were used to evaluate the effects of feeding raw potato starch (RPS) and live weight (LW; 90, 100 or 115 kg) on skatole, indole and androstenone levels in fat and plasma. Different methods to analyse the compounds were compared. Fat levels of skatole analysed by HPLC and colorimetric methods, and androstenone analysed by HPLC and ELISA, were highly correlated. However, androstenone values obtained by ELISA were overestimated. Feeding RPS reduced skatole levels in plasma and fat but did not affect indole levels. Androstenone levels in plasma measured by direct ELISA were not altered by diet, whereas the levels measured by ELISA with extraction were lower in the pigs fed RPS. Feeding RPS did not reduce androstenone levels in fat. Androstenone levels in fat were higher at 115 than at 90 kg, whereas skatole and indole levels were not affected by LW.


*Keywords:* Skatole; Indole; Androstenone; Analytical methods; Raw potato starch; Live weight

## 1. Introduction

Castration of male piglets in many countries is a common practice. The aim of castration is to prevent boar taint, an objectionable odour in meat from some entire male pigs. However, entire male pigs have superior performance compared with barrows and gilts, in terms of better carcass traits, lean meat percentage and feed efficiency. Castration of male piglets not only diminishes the benefits of entire male pigs, but also causes increased debate about animal welfare. Nowadays, surgical castration is not performed in some countries, e.g., Australia, United Kingdom and Ireland. In Norway, a total ban on castration will start from January 2009. However, if entire male pigs are to be used for pork production, the problem of boar taint must be substantially reduced.

High levels of skatole (3-methylindole) and androstenone (5α-androst-16-en-3-one) in fat are the major contributors to boar taint (Malmfors & Lundström, 1983). Indole also causes unpleasant odour in meat, but to a lesser degree (Moss, Hawe, & Walker, 1993). In pigs, both skatole and indole are produced by microbial degradation of the amino acid L-tryptophan in the caecum and colon (Jensen, Cox, & Jensen, 1995a). Skatole and indole are partly absorbed by intestinal mucosa and metabolised in the liver by cytochromes P450 (Squires & Lundström, 1997). The unmetabolised part of skatole and indole can accumulate in adipose tissue, causing boar taint. Skatole levels are considerably affected by dietary composition (Claus, Weiler, & Herzog, 1994). The effect of diet on indole levels has not been extensively studied. There is, however, evidence that indole synthesis in the intestine is also dependent on dietary ingredients (Knarreborg et al., 2002; Willig, Lösel, & Claus, 2005).

Androstenone is synthesised by the Leydig cells of the testis near sexual maturity, and its biosynthesis is

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associated with that of other testicular steroids (Claus et al., 1994). Since androstenone is related to puberty, slaughter at a lower weight might reduce the risk of high androstenone levels. Androstenone is highly lipophilic and accumulates in adipose tissue in much higher concentrations than other testicular steroids (Claus et al., 1994). In the regulation of androstenone levels, dietary factors are less important, unless they affect puberty.

Genetic background is important in the regulation of androstenone levels (Lee et al., 2005; Sellier, Le Roy, Fouilloux, Gruand, & Bonneau, 2000) and skatole levels (Lee et al., 2005; Lundström et al., 1994; Pedersen, 1998).

Numerous methods based on chemical or immunological techniques have been developed to analyse skatole, indole and androstenone in porcine tissues (Bonneau et al., 2000). The methods for determining skatole and indole levels include colorimetric method (for skatole equivalents), high performance liquid chromatography (HPLC) with fluorescence detection and gas chromatography (GC). The methods developed for androstenone analysis include HPLC, GC, GC–MS (gas chromatography–mass spectrometry) and ELISA (enzyme-linked immunosorbent assay). However, these methods either lack the necessary specificity (e.g., colorimetric method for skatole equivalent), or require tedious derivatisation procedures in the sample preparation (e.g., HPLC for androstenone). Additionally, the results obtained by different methods are not always consistent throughout the literature. It is important, therefore, to choose an optimal method to measure boar taint compounds, which should be cross-validated between different laboratories.

This study was a part of a larger project on effects of diet, raising system and slaughter weight on boar taint and aggression level. This project was performed as two replicates over two years. The results of the first replicate are presented elsewhere (Zamaratskaia, Babol, Andersson, Andersson, & Lundström, 2005).

The aim of our present study (second replicate) was to verify the findings of Zamaratskaia, Babol et al. (2005) on effects of dietary supplement of raw potato starch (RPS), slaughter weight and raising system on skatole and androstenone levels. Additionally, the effect of sire was evaluated. Different methods to measure skatole and androstenone in fat (HPLC and colorimetric methods for skatole, and HPLC and ELISA for androstenone) were compared. Direct ELISA to measure androstenone in plasma was compared with ELISA after extraction.

## 2. Materials and methods

### 2.1. Animals and sampling

A total of 96 entire male pigs of a crossbreed (4 Landrace sires × 13 Swedish Yorkshire dams) were included in the study. Two of the sires were pre-selected based on their plasma skatole values, one with low concentration (0.6 ng/ml) and one with high (12.4 ng/ml), whereas the other sires were randomly selected from the available sires used for artificial insemination. The pigs were raised either in mixed pens, with females and entire males, or single-sex pens. Each pen contained either seven or nine pigs. In the pens with nine pigs, the pigs were slaughtered on two occasions per pen. The three fastest-growing pigs were slaughtered when they reached an average live weight (LW) of 95.4 kg (standard deviation 7.0 kg; $n = 27$). The remaining pigs were slaughtered when the average weight in each pen reached 115.0 kg (standard deviation 9.9 kg; $n = 69$). All pigs were fed the same commercial diet according to the standard feeding regimen for growing and finishing pigs in Sweden (restricted, 12 MJ ME per kg, digestible CP 13%) (Andersson et al., 1997) until the average pen weight reached 100 kg. Then, 34 out of the 69 entire male pigs were additionally fed 0.6 kg of raw potato starch (RPS, Lyckeby Culminar; Karlshamn, Sweden) per day for two weeks prior to slaughter. The care of the pigs and the experimental design of this study were approved by the Local Animal Ethics Committee in Tierp, Sweden.

Blood samples were taken on three occasions: the day before the first slaughter (mean LW ± standard deviation; 88.4 ± 9.9 kg), the day before changing diets (101.4 ± 8.3 kg) and the day before the second slaughter (115.0 ± 9.9 kg). These three occasions will be labelled as 90, 100 and 115 kg groups throughout the text. Plasma was separated by centrifugation at 2000*g* for 15 min at 4 °C and stored at −80 °C until analysis. Back fat samples were taken after slaughter and kept at −20 °C until analysis.

### 2.2. Chemical analyses

#### 2.2.1. Chemicals

Androstenone, skatole, indole, androstanone (5α-androstan-3-one), dansylhydrazine, $BF_3$ solution in methanol (20%) and goat-anti-rabbit antibody were obtained from Sigma–Aldrich (Steinheim, Germany). Acetonitrile, methanol, tetrahydrofuran and ethyl acetate of analytical grade were obtained from Merck (Darmstadt, Germany). Androstenone antibody and enzyme-conjugate were kindly provided by Professor Mats Forsberg (Department of Biomedical Sciences and Veterinary Public Health, SLU, Uppsala, Sweden).

#### 2.2.2. Analysis of androstenone, skatole and indole by HPLC

Androstenone, skatole and indole in fat were analysed as described by Hansen-Møller (1994) with the following modifications. Fat samples were liquefied in a microwave oven (300 W) for 3 min. Then, 150 μl tissue-free liquid fat was pipetted into 1.5-ml centrifuge tubes in duplicates. Next, 750 μl methanol containing 0.33 μg/ml androstanone as internal standard were added into the tubes and incubated for 5 min at 60 °C in a water bath. After vortexing for 30 s, the tubes were kept at −20 °C for 60 min and cen-

trifuged for 5 min at 4500*g* at 4 °C. Lastly, 140 µl of the supernatant was transferred into HPLC vials for androstenone, skatole and indole analysis.

The HPLC system from Merck-Hitachi included a pump (L-6200A), auto sampler (AS2000), fluorescence detector (L-7480), column oven (L-5025) and D-6000A interface operated by D-6000 HPLC Manager software. Column for separation was Hypersil ODS (3 µm, 60 × 4.6 mm, Hewlett–Packard) with a guard column and a Stand-Alone holder. The column was operated at 40 °C.

Mobile phase for androstenone analyses was tetrahydrofuran:acetonitrile:sodium phosphate buffer (25 mM): acetic acid (34:23.8:41.4:0.8), flow rate was 1.5 ml/min, and injection volume was 20 µl. Fluorescence detection was performed with excitation at 346 nm and emission at 521 nm. The samples were derivatised according to the method of Hansen-Møller (1994). Recovery was calculated by comparing responses of spiked fat samples subjected to the complete extraction procedure with the concentrations of a standard solution. The spiking levels selected to establish the recovery values were 0.1, 0.495 and 2.0 µg/g. Recovery averaged 100.6 ± standard deviation 4.0%, with a range from 96.0% to 103.0%. Intra- and inter-assay variations did not exceed 10%. Spiked fat samples were prepared to construct a standard curve. Linear range of the assay was from 0.1 to 2.5 µg/g. Samples containing high amounts of androstenone (>2.5 µg/g) were diluted and re-analysed. The accuracy of the HPLC method for androstenone was additionally evaluated by analysing 20 fat samples by two methods, HPLC and GC–MS (Mågård et al., 1995) (Fig. 1).

Mobile phase for skatole and indole analyses was tetrahydrofuran:sodium phosphate buffer (25 mM):acetic acid (31:67.6:1.4), flow rate was 1.5 ml/min, and injection volume was 20 µl. Fluorescence detection was performed at an excitation of 285 nm and emission of 340 nm. Spiked fat samples were used to construct a standard curve. Linear range of the assay was from 0.002 to 0.5 µg/g for both compounds.

Skatole and indole in plasma were analysed by HPLC (Zamaratskaia, Babol, Andersson, & Lundström, 2004).

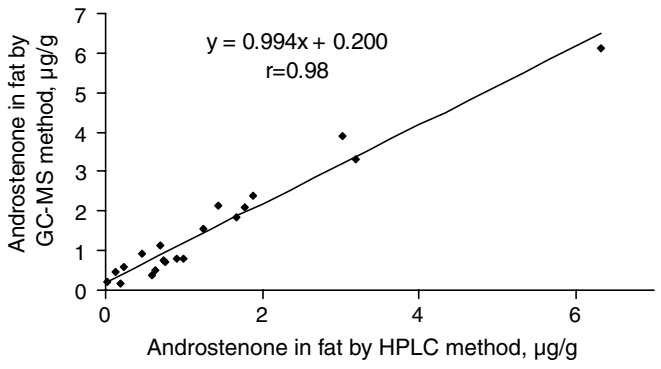


Fig. 1. Relationship between androstenone levels in fat by HPLC and GC–MS.

In brief, 400-µl plasma samples were mixed with 400 µl 100% acetonitrile by vortexing in a plastic tube, and then kept at −20 °C for 15 min. After centrifugation at 4800*g* for 10 min at 4 °C, supernatants were placed in HPLC vials. The HPLC system was the same as for androstenone, skatole and indole analysis in fat. Separation of skatole and indole was performed on RP-C18 (5 µm, 250 × 4 mm, Merck) column with a guard column. Two solvents were used in the analysis: solvent A: 10 µM potassium dihydrogen phosphate, pH 3.9:acetonitrile (9:1); solvent B: acetonitrile:water (9:1). Gradient profile was: 0–5 min, 75% A; 5–7 min, 20% A; 7–12 min, 0% A; 12–17 min, 75% A. The flow rate was 1.2 ml/min and the injection volume was 50 µl. The fluorescence detection was performed at an excitation wavelength of 285 nm and emission wavelength of 350 nm.

In addition, skatole equivalents in fat were analysed by the colorimetric method (Mortensen & Sørensen, 1984).

### 2.2.3. Analysis of androstenone by ELISA

Androstenone levels in fat were analysed by ELISA, as described by Squires and Lundström (1997), with slight modifications. Fat samples were liquefied as above and 25 µl of the liquid fat was pipetted into 1.5-ml centrifuge tubes. Samples were extracted with 0.5 ml of methanol, the tubes were kept at −20 °C for 60 min and then centrifuged for 5 min at 4500*g* at 4 °C. Then, 50 µl of the extract were transferred into 0.5 ml of PBS buffer (containing 0.1% BSA and 0.05% Tween 80). Recovery averaged 79.3 ± 2.8%, with a range from 77.3% to 85.9%. To increase the accuracy of measured concentrations, calibration standards for quantitative analysis of androstenone were spiked with blank fat and subjected to the same extraction procedure. Intra-assay variations were 8.1% and 9.6%, and inter-assay variations were 9.5% and 7.7% for the samples with high (2.2 µg/g) and low (0.4 µg/g) concentrations of androstenone, respectively.

Androstenone levels in plasma were analysed by ELISA method with and without sample extraction. The extraction was performed as follows: 250-µl plasma samples were extracted with 2.5 ml ethyl acetate. The solvent was removed by evaporation under nitrogen, and the residue was dissolved in 250 µl of phosphate buffer (containing 0.1% BSA and 0.05% Tween 80) in a glass vial and kept at 4 °C overnight for equilibration. Next day, the extract was analysed by ELISA (Squires & Lundström, 1997). Intra- and inter-assay variations did not exceed 15%. Plasma samples for the analysis without extraction procedure were directly applied on ELISA plates and analysed the same day. Recovery for direct measurements was 111.2 ± 13.0%. Intra-assay variations were 10.8% and 8.9%, and inter-assay variations were 2.1% and 5.9% for the samples with high (141.1 ng/ml) and low (14.9 ng/ml) concentrations of androstenone, respectively. All chemical analyses were performed in duplicate.

Table 1
Experimental design of the study

| | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| *Plasma* | | | | |
| RPS | No | No | No | Yes |
| Body weight (kg) | 90 | 100 | 115 | 115 |
| Number of animals | 92 | 69 | 34 | 35 |
| *Fat* | | | | |
| RPS | No | No | Yes | |
| Body weight (kg) | 90 | 115 | 115 | |
| Number of animals | 27 | 34 | 35 | |

### 2.3. Statistical analysis

All data were analysed with the Statistical Analysis System, version 9.1 (SAS Institute, Cary, NC, USA). Pigs were divided into four groups to analyse the combined effect of diet and slaughter weight on skatole, indole and androstenone in plasma, and into three groups to analyse the effects on the same substances in fat. The experimental design is presented in Table 1. Mixed procedure was used to evaluate the results. For the analysis of skatole, indole and androstenone in plasma, the model included sire, group, raising system (mixed or single-sex) and two-way interactions as fixed factors, dam within sire, and individual pig within sire and dam as random factors. For the analysis of the compounds in fat, the model included sire, group and raising system (mixed or single-sex) as fixed factors, and dam within sire as random factor. Raising system and the interaction between group and raising system were excluded from the final models when $p > 0.10$. A logarithmic transformation was applied to normalise the distributions of skatole, indole and androstenone in plasma and fat. Results are presented after back transformation. Values for skatole, indole and androstenone obtained by different methods were compared using regression analysis and Pearson correlation analysis.

## 3. Results

### 3.1. Comparison of different methods

Skatole levels in fat were determined by colorimetric and HPLC methods. The correlation between the two methods was 0.94, and the regression equation was $y = 0.590x + 0.083$ ($x$: HPLC, $y$: colorimetric method; Fig. 2). The threshold value of 0.20 μg/g for skatole measured by HPLC corresponded to 0.202 μg/g by the colorimetric method. However, the HPLC analyses revealed that some fat samples contained high indole levels, which considerably contributed to the levels of skatole equivalents obtained by the colorimetric method.

Androstenone levels in fat were determined by HPLC and ELISA. The correlation between these two methods was 0.90, and the regression equation was $y = 1.003x + 0.784$ ($x$: HPLC, $y$: ELISA; Fig. 3). Results by the ELISA method overestimated androstenone levels compared to the HPLC method, and, e.g., 1.0 μg/g analysed by HPLC corresponded to 1.8 μg/g when analysed by ELISA.

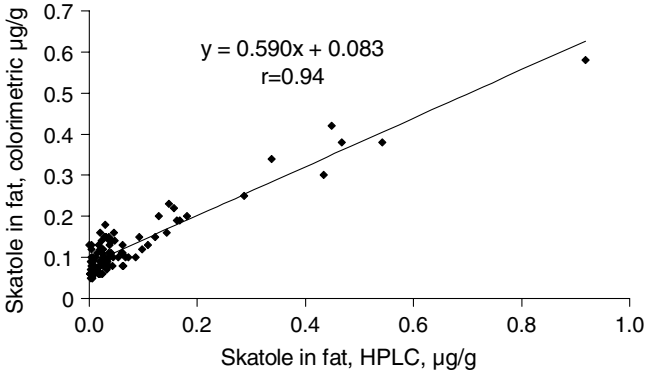


Fig. 2. Relationship between skatole levels in fat by HPLC and colorimetric method.

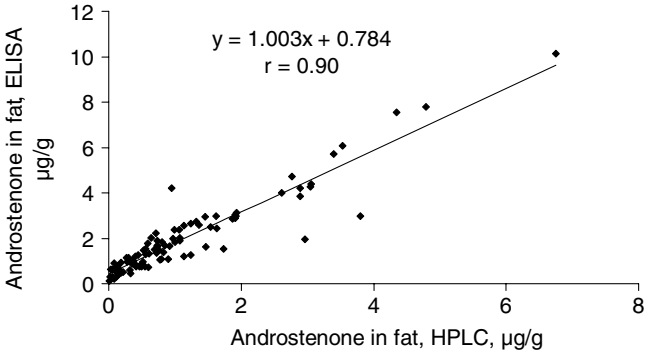


Fig. 3. Relationship between androstenone levels in fat by HPLC and ELISA.

Androstenone levels in plasma were determined by ELISA with and without extraction with ethyl acetate. The correlation between the extracted and non-extracted values was 0.70 (Fig. 4). Androstenone values obtained by direct ELISA were about four times higher compared to those after extraction (see Table 2).

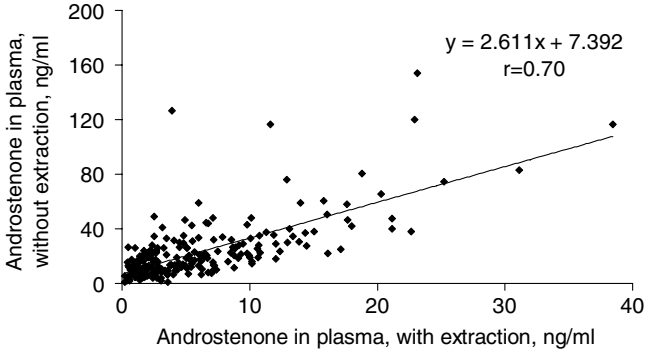


Fig. 4. Relationship between androstenone levels in plasma by ELISA with and without extraction.

Table 2
Least-squares means and 95% confidence intervals (within brackets) of skatole, androstenone and indole levels in plasma from entire male pigs with different live weights and diets

| Plasma (ng/ml) | Live weight and diet | | | | *P*-value | |
|---|---|---|---|---|---|---|
| | 90 kg | 100 kg | 115 kg no RPS | 115 kg + RPS | Weight and diet | Sire |
| Skatole | 4.67[a] (3.90–5.59) | 4.50[a] (3.70–5.48) | 3.75[a] (2.93–4.79) | 0.70[b] (0.55–0.89) | 0.001 | 0.004 |
| Indole | 1.41 (1.21–1.64) | 1.50 (1.27–1.77) | 1.62 (1.31–2.00) | 1.67 (1.36–2.05) | 0.303 | 0.001 |
| Androstenone (direct ELISA) | 16.00 (13.46–19.02) | 18.10 (14.85–22.07) | 16.58 (12.96–21.21) | 18.55 (14.63–23.52) | 0.479 | 0.107 |
| Androstenone (after extraction) | 4.29[a] (3.49–5.27) | 4.16[a] (3.25–5.33) | 4.46[a] (3.24–6.12) | 2.20[b] (1.62–2.98) | 0.001 | 0.122 |

Least-squares means with different superscripts differ ($p < 0.05$).

### 3.2. Effect of live weight at slaughter, raising system and sire on skatole, indole and androstenone levels in plasma and fat

Androstenone levels in plasma did not differ between pigs at 90, 100 and 115 kg LW ($p > 0.05$, Table 2), whereas androstenone levels in fat increased with higher slaughter weight without RPS-feeding ($p = 0.02$, Table 3). Slaughter at lower weight (90 kg) did not affect the levels of skatole and indole in either plasma or in fat (Tables 2 and 3). The raising system used significantly affected indole levels in plasma at 90 and 100 kg. Pigs raised in mixed pens with nine pigs had lower indole levels in plasma than pigs raised in mixed pens with seven or single-sex pens with nine pigs ($p < 0.01$). Raising system did not affect indole levels at 115 kg, in either pigs fed RPS or not.

Sire significantly affected skatole and indole in plasma and all the analysed compounds in fat (Tables 2 and 3). The sire pre-selected for low skatole concentration in plasma had lower levels of both skatole and indole in plasma and fat, and androstenone in fat.

### 3.3. Effect of RPS on skatole, indole and androstenone levels in plasma and fat

Dietary supplement of RPS significantly reduced skatole levels both in plasma and fat ($p < 0.001$), whereas indole levels were not affected (Tables 2 and 3). The androstenone values measured by ELISA with extraction (free androstenone) were significantly lower in the pigs fed RPS, compared to those only fed the commercial diet at 115 kg LW ($p < 0.001$). On the other hand, plasma androstenone levels did not differ between the RPS and control group at 115 kg when measured by direct ELISA (total androstenone) (Table 2). Fat androstenone levels, measured by HPLC, did not significantly differ between the RPS group and the control group at the same weight (0.64 vs. 0.90 μg/g; $p = 0.238$; Table 3).

Table 4
Frequency of skatole and androstenone levels in fat above threshold

| | Skatole, ⩾0.20 μg/g | | | Androstenone, ⩾1.0 μg/g | | |
|---|---|---|---|---|---|---|
| Weight at slaughter (kg) | 90 | 115 | | 90 | 115 | |
| Potato starch | No | No | Yes | No | No | Yes |
| Total number of animals | 27 | 34 | 35 | 27 | 34 | 35 |
| Number above threshold | 5 | 2 | 0 | 6 | 15 | 13 |
| Percentage | 19 | 6 | 0 | 22 | 44 | 37 |

### 3.4. Frequency of boar taint compounds in fat above threshold

Frequency of the boar taint compounds in fat above threshold levels is shown in Table 4. For skatole, 19% of the pigs in the 90 kg group and 6% of the pigs in the 115 kg group without RPS feeding had skatole levels exceeding the threshold 0.20 μg/g, whereas none of the pigs fed RPS had skatole levels above the threshold. When the colorimetric method was used, one pig with a high indole level (0.29 μg/g) but low skatole level (0.13 μg/g) after RPS feeding was misclassified, as the skatole concentration was 0.20 μg/g with the colorimetric method. For androstenone, 22% of the pigs at 90 kg had levels exceeding the threshold 1.0 μg/g; 44% of pigs in the 115 kg group without RPS feeding exceeded the threshold, and 37% of pigs in the 115 kg group fed RPS exceeded the threshold.

## 4. Discussion

### 4.1. Comparison of different methods

Many methods to measure the level of boar taint have been developed. These methods range from sensory analysis evaluating the taint in pork products to specific chemical methods measuring skatole, indole and androstenone in

Table 3
Least-squares means and 95% confidence intervals (within brackets) of skatole, androstenone and indole levels in fat from entire male pigs with different live weights and diets

| Fat (μg/g) | Live weight and diet | | | *P*-value | |
|---|---|---|---|---|---|
| | 90 kg | 115 kg no RPS | 115 kg + RPS | Weight and diet | Sire |
| Skatole | 0.06[a] (0.04–0.09) | 0.05[a] (0.04–0.08) | 0.01[b] (0.01–0.01) | 0.001 | 0.026 |
| Indole | 0.02 (0.01–0.03) | 0.02 (0.01–0.03) | 0.02 (0.01–0.03) | 0.644 | 0.003 |
| Androstenone | 0.44[a] (0.27–0.71) | 0.90[b] (0.59–1.38) | 0.64[ab] (0.42–0.96) | 0.073 | 0.006 |

Least-squares means with different superscripts differ ($p < 0.05$).

porcine tissues (HPLC, GC–MS, RIA, ELISA). Our study compared some of those chemical methods.

The levels of skatole equivalents obtained by the colorimetric method were highly correlated to skatole concentrations measured by HPLC. These results are in agreement with results by Hansen-Møller (1994). However, Gibis (1994) found only moderate correlation between these two methods ($r = 0.58$). The colorimetric method is based on the measurements in homogenised fat tissue, whereas liquid fat has been used for HPLC analysis in our study. According to Gibis (1994), skatole values measured in liquid fat are approximately 34% higher than those in homogenised fat. The colorimetric method measures the total amount of indolic compounds, whereas the HPLC method is designed to separate skatole and indole levels. Our results showed that the obtained skatole equivalents measured by the colorimetric method included approximately 30% indole. Therefore, the concentrations obtained by the colorimetric and HPLC methods were close. The colorimetric method is advantageous because it is rapid and simple, which makes it suitable for estimating skatole equivalents on the slaughter line. However, it does not provide information about the individual level of skatole and indole in single samples and thus is not an optimal method for research purposes. To evaluate the effect of RPS and slaughter weight, skatole and indole levels obtained by HPLC were used in this study.

Androstenone levels in plasma were measured by direct ELISA and after extraction with ethyl acetate. This was done to determine the appropriacy of a direct method compared to more conventional methods with sample extraction. Due to removal of a sample preparation step, the direct ELISA was simpler and less time-consuming. However, direct ELISA overestimated androstenone values, even if the correlation coefficient between these two methods was high ($r = 0.70$). This difference might be due to non-specificity of antibodies for androstenone measurements. Androstenone is a highly lipophilic molecule with a keto-group in C-3 position. A recent study reported that most androstenone in plasma exists in sulphated form (Sinclair & Squires, 2005), which probably cannot be completely extracted by organic solvent. Sulphated androstenone may cross-react to a substantial degree with the antibodies. The cross-reactivity of the antibodies against androstenone sulphate has never been determined since it was believed that androstenone does not form sulpho-conjugates because of lack of hydroxyl groups. We assume that direct ELISA measures total androstenone, whereas extraction separates, at least partly, free and sulphated forms. The matrix difference between standard solution and plasma measured by direct ELISA might also cause some minor differences in the results obtained with and without extraction.

A number of methods to determine androstenone in fat have been developed. Some of these methods are currently in use in different laboratories. Hansen-Møller (1994) demonstrated a good agreement between the results obtained by HPLC and other methods used for the determination of androstenone (RIA and GC–MS). They found a correlation coefficient of 0.946 and a regression line of $y = 1.15x - 0.047$ between HPLC and RIA methods. Tuomola, Hakala, and Manninen (1998) successfully employed packed column supercritical fluid chromatography (SFC), in combination with mass spectrometry, to measure androstenone in fat. As part of the validation procedure for fat androstenone measurement by HPLC in the present study, fat samples from 20 boars, previously analysed by GC–MS (Mågård et al., 1995) were selected for HPLC analysis. The results from the methods agreed, as demonstrated by a correlation coefficient of 0.98 and a regression line of $y = 0.9936x + 0.2002$. The slight ''underestimation'' of androstenone by HPLC might be a result of inter-laboratory variation. The correlation between androstenone levels in fat obtained by HPLC and ELISA was high. However, the ELISA results overestimated fat androstenone levels compared to the HPLC results, in contrast to a previous investigation (Claus, Herbert, & Dehnhard, 1997). It is also worth mentioning that below 1.0–1.5 μg/g the results vary to some extent, when comparing all three methods (Figs. 1 and 3). The reason for the overestimation of androstenone in fat by ELISA in our study is unknown. The antibodies against androstenone used in the study of Claus et al. (1997) and our present study have been raised in different laboratories. It is likely that specificity of these antibodies differed, causing disagreement in the results. In addition to the different specificity of antibodies and the measurement precision, there are several factors that can contribute to the total uncertainty of androstenone analysis, in particular when working with concentrations below 1.5 μg/g. One of the uncertainty sources might be the variations in fat androstenone levels within the animal. Additionally, the possibility of the presence of some unknown androstenone metabolites in the adipose tissue has not been studied and can not be excluded. Androstenone metabolism has so far been mainly studied in the liver. However, adipose tissue plays an important role in the metabolism of sex steroids and was shown to exhibit 3β-hydroxysteroid dehydrogenase activity (Kershaw & Flier, 2004), the major enzyme of androstenone metabolism (Doran, Whittington, Wood, & McGivan, 2004). This implies that androstenone can be metabolised in adipose tissue to produce metabolites which can also bind to the antibodies. Our results suggest that the HPLC method is a more accurate way to measure androstenone concentrations in fat. If ELISA is used to measure androstenone, the threshold level for androstenone in fat, e.g., 1.0 ppm, needs to be re-evaluated.

### *4.2. Effect of dietary RPS on the levels of skatole, indole and androstenone*

The metabolism of tryptophan by the intestinal microflora is an important process affecting the production of skatole and indole. Given that indole is a minor contributor to boar taint, most studies have focused on the reduc-

tion of skatole levels and paid no or little attention to indole. The present study investigated the effect of a dietary supplement of RPS on the levels of both skatole and indole. Skatole levels in fat and plasma were significantly lower in the pigs fed RPS, compared to levels in the pigs fed only a commercial diet. The effect of RPS on skatole production in pigs is well known (Claus, Losel, Lacorn, Mentschel, & Schenkel, 2003; Jensen, Cox, & Jensen, 1995b; Losel & Claus, 2005; Willig et al., 2005) and is mainly attributed to the inhibition of colonocyte apoptosis and thus decreased amount of cell debris, the key source of tryptophan for skatole synthesis (Claus et al., 2003). However, if skatole reduction in the pigs fed RPS is due to decreased tryptophan availability, indole reduction could also be expected. Our study found that RPS did not affect fat and plasma indole levels, suggesting that factors other than apoptotic rate might be modified by RPS in the diet. These factors would be specific towards skatole formation and would not affect indole. It is likely that RPS or products of its fermentation inhibit skatole-producing bacteria in the large intestine, whereas indole-producing bacteria remain unaffected. Indole synthesis in the large intestine is mediated by many types of bacteria, whereas skatole production requires the presence of highly specific bacteria. Initially, tryptophan is transformed to indole-3-acetic acid, which is subsequently converted to skatole. *Escherichia coli (E. coli)* and *Clostridium spp*. are involved in the production of indole-3-acetic acid, and then *Lactobacillus* and *Clostridium* convert indole-3-acetic acid to skatole (Jensen et al., 1995a). The growth of *E. coli* and *Clostridium spp*. was considerably suppressed in the presence of fructooligosaccharide (FOS) in an in vitro study (Xu, Hu, & Wang, 2002). It was also shown that addition of FOS to incubated pig faecal slurries significantly reduced skatole production from tryptophan, whereas indole production was not affected (Xu et al., 2002). In vivo, Rideout, Fan, Cant, Wagner-Riddle, and Stonehouse (2004) found no significant changes in indole levels in faeces after feeding pigs with chicory inulin. Raw potato starch, similar to FOS and inulin, is not digestible in the small intestine and is fermented by intestinal bacteria to yield short chain fatty acids (Claus et al., 2003) and might therefore express an analogous effect on tryptophan biotransformation. However, this suggestion conflicts with the results obtained by Willig et al. (2005), where pigs fed RPS had lower indole concentrations in faeces. Additionally, our recent study has demonstrated a significant decrease in indole levels in the liver when male pigs were fed RPS (Zamaratskaia, Chen, & Lundström, 2006). The reasons for the conflicting results between studies investigating the response of indole to RPS need to be elucidated. In these studies qualitative and quantitative evaluations of intestinal microflora should be included. Nevertheless, the inclusion of RPS to the diet repeatedly decreases skatole levels, independent of sites of measurements – plasma, fat, liver and intestinal content (Claus et al., 2003; Losel & Claus, 2005; Zamaratskaia, Babol et al., 2005; Zamaratskaia et al., 2006).

Androstenone levels measured by direct ELISA were not affected by diet. This is in agreement with our results from the first replicate where plasma androstenone was measured without extraction and no effect of RPS on androstenone levels was demonstrated (Zamaratskaia, Babol et al., 2005). Conversely, the levels measured after extraction were significantly lower in the pigs fed RPS. As mentioned above, most androstenone in plasma is present as sulphoconjugates (Sinclair & Squires, 2005), and direct measurements in plasma might provide information about total androstenone, whereas extraction separates free and sulphated forms. It is likely that besides androstenone sulphation occurring in the testes (Sinclair, Squires, Raeside, & Renaud, 2005), it also occurs in other tissues, e.g., the intestine. The presence of phase II enzymes, including sulphotransferase, in the intestine has been demonstrated (Falany, 1997), and these enzymes can be either up-regulated or inhibited by components of the diet. Dietary changes may accelerate intestinal sulphation of androstenone through the activation of the enzyme sulphotransferase and change the ratio of free/conjugated androstenone, whereas the levels of total androstenone remain unaffected. So far, no relationship between dietary composition and intestinal androstenone sulphation has been reported. There is a major interest in understanding the endocrinological processes involved in this biotransformation to find a way to reduce androstenone levels through the diet. Interestingly, androstenone levels in fat in the present study slightly decreased after feeding RPS, although this decrease did not reach statistical significance. Investigations of an effect of different dietary content of RPS and various feeding periods on free and total androstenone levels should provide a better understanding of the role of intestinal androstenone metabolism in the development of boar taint.

### *4.3. Effects of live weight, raising system and sire on the levels of skatole, indole and androstenone*

The effect of age and slaughter weight on skatole levels has been well documented (Babol, Zamaratskaia, Juneja, & Lundström, 2004; Whittington et al., 2004; Zamaratskaia et al., 2004). However, in the present study neither skatole nor indole levels differed between pigs slaughtered at 90 and 115 kg LW. Skatole and indole levels usually increase at the age close to puberty (Babol et al., 2004), and this increase was often explained by the puberty-related production of testicular steroids (Zamaratskaia et al., 2004). Recently, it was demonstrated that increased skatole levels are linked to decreased activity of CYP2E1 and CYP2A6 (Zamaratskaia, Squires et al., 2005). In the present study, the activities of CYP2E1 and CYP2A6, measured in the liver microsomes from the same animals, did not differ between pigs at 90 and 115 kg LW, causing no increase in skatole levels in heavier pigs (Zamaratskaia et al., 2006). This is further evidence of the important role of liver metabolism in the regulation of skatole levels.

At puberty, the synthesis of testicular steroids dramatically increases as a result of increased secretion of gonadotrophin-releasing hormone (GnRH) by the hypothalamus, and lutenizing hormone (LH) and follicle-stimulating hormone (FSH) by the anterior pituitary. LH binds to the receptors on the surface of the Leydig cells and induces steroidogenic enzymes. Androstenone levels increase simultaneously with other testicular steroids at puberty. No effect of LW on plasma androstenone levels was demonstrated in the present study. The three fastest-growing pigs in each pen were slaughtered at 90 kg, which probably was related to a higher feed intake close to ad libitum feeding in those pigs. It is known that a high feeding intensity increases levels of both androstenone and skatole (Øverland, Berg, & Matre, 1995). Perhaps the pigs at 90 kg had already reached sexual maturity and maximum concentration of androstenone in plasma; in that case an additional increase at 115 kg might not be observed. However, this does not explain the increase in androstenone levels in fat. It appears that androstenone accumulation in fat does not entirely depend on plasma androstenone levels, despite the strong positive correlation between fat and plasma androstenone levels observed in the present study (data not shown), as well as other studies (Andresen, 1976; Sinclair, Squires, Raeside, Britt, & Hedgpeth, 2001; Zamaratskaia et al., 2004). In contrast, Lundström, Malmfors, Hansson, Edqvist, and Gahne (1978) found no correlation between androstenone levels in plasma and fat. A number of factors might be important here, including genetic factors, variation in chemical analysis and the period between fat and plasma sampling. Sinclair et al. (2001) suggested that androstenone accumulation might depend on the fat composition of the pig. Zamaratskaia, Babol et al. (2005) proposed that variation in the relationship between fat and plasma androstenone levels might be due to individual ability of pigs to reach a balance between androstenone release into the blood and androstenone accumulation in fat. The mechanism of androstenone accumulation in fat remains unknown.

Raising system did not affect the levels of skatole and androstenone. Unexpectedly, raising entire male pigs in mixed pens with nine pigs resulted in lower indole levels in plasma in the pigs at 90 and 100 kg LW. Despite an incomplete understanding of the mechanism responsible for occurrence of high indole levels, there is evidence that raising conditions are important in indole regulation (Hansen, Larsen, & Hansen-Møller, 1995; Hansen, Larsen, Jensen, & Hansen-Møller, 1997). However, practical implications of this finding remains to be determined.

Significant differences in skatole concentrations in plasma and fat were found between the offspring of different sires. This confirms that genetic background is an important factor regulating skatole levels. Lundström et al. (1994) suggested the presence of a major gene affecting skatole levels. The heritability estimates of skatole vary from 0.19 to 0.27 (Pedersen, 1998), and a genomic region to harbour QTL for the variation of skatole and indole levels in fat has recently been identified (Lee et al., 2005). The sire with a low skatole level in plasma produced offspring with low indole levels, which is not surprising given that skatole and indole levels are often highly correlated (Tuomola, Vahva, & Kallio, 1996). Additionally, androstenone levels in fat were also low in the offspring from the sire with low skatole concentration, confirming the relationship between androstenone and skatole

## 5. Conclusions

Different androstenone and skatole analyses were highly correlated, but the overestimation of androstenone levels in fat by ELISA should be considered. Thus, the currently suggested threshold level for androstenone in fat, 1.0 ppm, needs to be re-evaluated, if ELISA is used to measure androstenone.

Dietary supplement of RPS significantly reduced skatole levels in both fat and plasma, and androstenone levels in plasma, measured after ethyl acetate extraction. Diet did not affect indole levels in either fat or plasma. Pigs slaughtered at 90 kg LW had lower androstenone levels in fat, compared to those slaughtered at 115 kg. The frequency of samples over the threshold 1 μg/g was lower at 90 kg than at 115 kg. Skatole and indole levels were not affected by LW. Overall, our results confirmed that dietary composition and genetic background are important determinants of skatole levels in fat and plasma. Future research should therefore address the possibility of reducing other boar taint compounds, especially androstenone.

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## References

Andersson, H. K., Schaub, A., Andersson, K., Lundström, K., Thomke, S., & Hansson, I. (1997). The effects of feeding system, lysine level and gilt contact on performance, skatole levels and economy of entire male pigs. *Livestock Production Science, 51*, 131–140.

Andresen, Ø. (1976). Concentrations of fat and plasma 5alpha-androstenone and plasma testosterone in boars selected for rate of body weight gain and thickness of back fat during growth, sexual maturation and after mating. *Journal of Reproduction and Fertility, 48*, 51–59.

Babol, J., Zamaratskaia, G., Juneja, R. K., & Lundström, K. (2004). The effect of age on distribution of skatole and indole levels in entire male pigs in four breeds: Yorkshire, Landrace, Hampshire and Duroc. *Meat Science, 67*(2), 351–358.

Bonneau, M., Kempster, A. J., Claus, R., Claudi-Magnussen, C., Diestre, A., Tornberg, E., et al. (2000). An international study on the importance of androstenone and skatole for boar taint: I. Presentation of the programme and measurement of boar taint compounds with different analytical procedures. *Meat Science, 54*(3), 251–259.

Claus, R., Herbert, E., & Dehnhard, M. (1997). Comparative determination of the boar taint steroid androstenone in pig adipose tissue by a rapid enzyme immunoassay and an HPLC-method. *Archiv für Lebensmittelhygiene, 48*, 25–48.

Claus, R., Losel, D., Lacorn, M., Mentschel, J., & Schenkel, H. (2003). Effects of butyrate on apoptosis in the pig colon and its consequences for skatole formation and tissue accumulation. *Journal of Animal Science, 81*(1), 239–248.

Claus, R., Weiler, U., & Herzog, A. (1994). Physiological aspects of androstenone and skatole formation in the boars – a review with experimental data. *Meat Science, 38*, 289–305.

Doran, E., Whittington, F. M., Wood, J. D., & McGivan, J. D. (2004). Characterisation of androstenone metabolism in pig liver microsomes. *Chemico-Biological Interactions, 147*, 141–149.

Falany, C. N. (1997). Enzymology of human cytosolic sulfotransferases. *FASEB Journal, 11*, 206–216.

Gibis, M. (1994). *Einfluss der Substanzen Indol und Skatol auf die Schweinefleischqualität (Influence of substance indole and skatole on meat quality)*. Doctoral thesis, Allgemeine und Angewandte Naturwissenschaften der Universität Hohenheim, Germany.

Hansen, L. L., Larsen, A. E., & Hansen-Møller, J. (1995). Influence of keeping pigs heavily fouled with faeces plus urine on skatole and indole concentration (boar taint) in subcutaneous fat. *Acta Agriculturæ Scandinavica Section A, Animal Science, 45*, 178–185.

Hansen, L. L., Larsen, A. E., Jensen, B. B., & Hansen-Møller, J. (1997). Short time effect of zink bacitracin and heavy fouling with faeces plus urine on boar taint. *Animal Science, 64*, 351–363.

Hansen-Møller, J. (1994). Rapid high-performance liquid chromatographic method for simultaneous determination of androstenone, skatole and indole in back fat from pigs. *Journal of Chromatography B: Biomedical Sciences and Applications, 661*(2), 219–230.

Jensen, M. T., Cox, R. P., & Jensen, B. B. (1995a). 3-Methylindole (skatole) and indole production by mixed population of pig fecal bacteria. *Applied and Environmental Microbiology, 61*, 3180–3184.

Jensen, M. T., Cox, R. P., & Jensen, B. B. (1995b). Microbial production of skatole in the hind-gut of pigs given different diets and its relationship to skatole deposition in backfat. *Animal Science, 61*, 293–304.

Kershaw, E. E., & Flier, J. S. (2004). Adipose tissue as an endocrine organ. *The Journal of Clinical Endocrinology and Metabolism, 89*, 2548–2556.

Knarreborg, A., Beck, J., Jensen, M. T., Laue, A., Agergaard, N., & Jensen, B. B. (2002). Effect of non-starch polysaccharides on production and adsorption of indolic compounds in entire male pigs. *Animal Science, 74*, 445–453.

Lee, G. J., Archibald, A. L., Law, A. S., Lloyd, S., Wood, J., & Haley, C. S. (2005). Detection of quantitative trait loci for androstenone, skatole and boar taint in a cross between Large White and Meishan pigs. *Animal Genetics, 36*, 14–22.

Losel, D., & Claus, R. (2005). Dose-dependent effects of resistant potato starch in the diet on intestinal skatole formation and adipose tissue accumulation in the pig. *Journal of Veterinary Medicine Series A, 52*, 209–212.

Lundström, K., Malmfors, B., Hansson, I., Edqvist, L.-E., & Gahne, B. (1978). 5-Androstenone and testosterone in boars. *Swedish Journal of Agricultural Research, 8*, 171–178.

Lundström, K., Malmfors, B., Stern, S., Rydhmer, L., Elaisson-Selling, L., Mortensen, A. B., et al. (1994). Skatole levels in pigs selected for high lean tissue growth rate on different dietary protein levels. *Livestock Production Science, 38*, 125–132.

Malmfors, B., & Lundström, K. (1983). Consumer reaction to boar meat – a review. *Livestock Production Science, 10*, 187–196.

Mågård, M., Berg, H., Tagesson, V., Järemo, M., Karlsson, L., Mathiasson, L., et al. (1995). Determination of androstenone in pig fat using supercritical fluid extraction and gas chromatography–mass spectrometry. *Journal of Agricultural and Food Chemistry, 43*, 114–120.

Mortensen, A. B., & Sørensen, S. E. (1984). Relationship between boar taint and skatole determination with a new analysis method. In *Proceedings of 30th European meeting meat research workers*, Bristol, UK, pp. 394–396.

Moss, B. W., Hawe, S. M., & Walker, N. (1993). Sensory thresholds for skatole and indole. In M. Bonneau (Ed.), *Measurement and prevention of boar taint in entire male pigs* (pp. 63–68). Paris: INRA Editions.

Øverland, M., Berg, J., & Matre, T. (1995). The effect of feed and feeding regime on skatole and androstenone levels and on sensory attributes of entire male and female pigs. In *Proceeding of meeting EAAP working group, production and utilisation of meat from entire male pigs*. Milton Keynes, UK, 6pp.

Pedersen, B. (1998). Heritability of skatole in back fat. In W. K. Jensen (Ed.), *Skatole and boar taint* (pp. 129–136). Roskilde, Denmark: Danish Meat Research Institute.

Rideout, T. C., Fan, M. Z., Cant, J. P., Wagner-Riddle, C., & Stonehouse, P. (2004). Excretion of major odor-causing and acidifying compounds in response to dietary supplementation of chicory inulin in growing pigs. *Journal of Animal Science, 82*, 678–684.

Sellier, P., Le Roy, P., Fouilloux, M. N., Gruand, J., & Bonneau, M. (2000). Responses to restricted index selection and genetic parameters for fat androstenone level and sexual maturity status of young boars. *Livestock Production Science, 63*, 265–274.

Sinclair, P. A., & Squires, E. J. (2005). Testicular sulfoconjugation of the 16-androstene steroids by hydroxysteroid sulfotransferase: its effect on the concentrations of 5{alpha}-androstenone in plasma and fat of the mature domestic boar. *Journal of Animal Science, 83*(2), 358–365.

Sinclair, P. A., Squires, E. J., Raeside, J. I., Britt, J. H., & Hedgpeth, V. G. (2001). The effect of early postnatal treatment with a gonadotropin-releasing hormone agonist on the developmental profiles of testicular steroid hormones in the intact male pig. *Journal of Animal Science, 79*, 1003–1010.

Sinclair, P. A., Squires, E. J., Raeside, J. I., & Renaud, R. (2005). Synthesis of free and sulphoconjugated 16-androstene steroids by the Leydig cells of the mature domestic boar. *The Journal of Steroid Biochemistry and Molecular Biology,* 96(2), 217–228.

Squires, E. J., & Lundström, K. (1997). Relationship between cytochrome P450IIE1 in liver and levels of skatole and its metabolites in entire male pigs. *Journal of Animal Science, 75*, 2506–2511.

Tuomola, M., Hakala, M., & Manninen, P. (1998). Determination of androstenone in pig fat using packed column supercritical fluid chromatography–mass spectrometry. *Journal of Chromatography B: Biomedical Sciences and Applications, 719*, 25–30.

Tuomola, M., Vahva, M., & Kallio, H. (1996). High-performance liquid chromatography determination of skatole and indole levels in pig serum, subcutaneous fat, and submaxillary salivary glands. *Journal of Agricultural and Food Chemistry, 44*, 1265–1270.

Willig, S., Lösel, D., & Claus, R. (2005). Effects of resistant potato starch on odor emission from feces in swine production units. *Journal of Agricultural and Food Chemistry, 53*, 1173–1178.

Whittington, F. M., Nute, G. R., Hughes, S. I., McGivan, J. D., Lean, I. J., Wood, J. D., et al. (2004). Relationships between skatole and androstenone accumulation, and cytochrome P4502E1 expression in Meishan × Large White pigs. *Meat Science, 67*, 569–576.

Xu, Z. R., Hu, C. H., & Wang, M. Q. (2002). Effects of fructooligosaccharide on conversion of L-tryptophan to skatole and indole by mixed populations of pig fecal bacteria. *Journal of general and applied microbiology, 48*, 83–90.

Zamaratskaia, G., Babol, J., Andersson, H. K., Andersson, K., & Lundström, K. (2005). Effect of live weight and dietary supplement of raw potato starch on the levels of skatole, androstenone, testosterone and oestrone sulphate in entire male pigs. *Livestock Production Science, 93*(3), 235–243.

Zamaratskaia, G., Babol, J., Andersson, H., & Lundström, K. (2004). Plasma skatole and androstenone levels in entire male pigs and relationship between boar taint compounds, sex steroids and thyroxine at various ages. *Livestock Production Science, 87*(2–3), 91–98.

Zamaratskaia, G., Chen, G., & Lundström, K. (2006). Effects of sex, weight, diet and hCG administration on levels of skatole and indole in the liver and hepatic activities of cytochromes P4502E1 and P4502A6 in pigs. *Meat Science, 72*(2), 331–338.

Zamaratskaia, G., Squires, E. J., Babol, J., Andersson, H. K., Andersson, K., & Lundström, K. (2005). Relationship between the activities of cytochromes P4502E1 and P4502A6 and skatole content in fat in entire male pigs fed with and without raw potato starch. *Livestock Production Science, 95*(1–2), 83–88.