# Physiological and Biochemical Factors Responsible for Boar Taint

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# Abstract

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We used entire male pigs to: 1) compare different methods to analyse boar taint compounds; 2) evaluate the effects of raw potato starch (RPS), high amylose barley cultivar (Karmosé), sire selection and live weight on the levels of boar taint compounds; 3) investigate the relationship between indolic compounds and testicular steroids by using an hCG injection model; 4) evaluate the effect of incubation with steroids and indolic compounds on CYP2A6 protein expression in hepatocytes; 5) investigate the relationship between testicular steroids and gene expression of hydroxysteroid dehydrogenases (3βHSD and 17βHSD) in entire male and castrated male pigs.

The results showed that HPLC method and GC-MS method for fat androstenone analyses were comparable. HPLC and ELISA methods for fat androstenone analyses were well correlated (r = 0.90), but the result from the ELISA method was overestimated. HPLC and colorimetric method for fat skatole analyses were well correlated (r = 0.94) even though the two methods used different sample matrix. Plasma androstenone results determined by ELISA method with and without sample extraction were moderately correlated (r = 0.70). The results from analyses without sample extraction were 4 times higher than the results from analyses with sample extraction.

Feeding RPS reduced fat skatole levels, but not fat androstenone and indole levels of entire male pigs. High amylose barley did not influence any of the boar taint compounds analyzed in fat (skatole, androstenone and indole). Androstenone levels in fat were higher at live weight 115 kg than at 90 kg, whereas fat skatole and indole levels did not differ between the two live weights. Sire selection based on plasma skatole levels significantly influenced skatole, androstenone and indole levels in fat in the progenies.

Indole levels in plasma and in fat were significantly increased after hCG injection, and skatole levels in fat tended to be increased. This increase was associated with the abrupt increase of testicular steroids by hCG injection.

An *in vitro* study showed that both skatole and indole at low concentrations (0-100 nM) induced cytochrome P450 2A6 (CYP2A6) protein expression in cultured hepatocytes, with a more prominent effect for indole. Androstenone at low concentration (0-100 nM) inhibited CYP2A6 protein expression. Testosterone and estrone sulfate (E1S) did not affect CYP2A6 protein expression.

High fat androstenone levels were negatively associated with low 3βHSD gene expression in entire male pigs. Fat androstenone levels were also negatively associated with 17βHSD gene expression in liver but not in testis. The correlation between hepatic 17βHSD gene expression and plasma E1S was high. In addition, gene expression of 3βHSD and 17βHSD was highly correlated. Gene expressions of 3βHSD and 17βHSD in liver were much higher in surgically castrated and immunocastrated pigs than in entire male pigs with high fat androstenone levels. The gene expression of 3βHSD was generally much higher than 17βHSD both in liver and in testis.

*Keywords:* Pig, boar taint compounds, steroids, HPLC, ELISA, real-time PCR, cell culture, western blot, raw potato starch, hydroxysteroid dehydrogenases (3βHSD and 17βHSD), cytochrome P450 2A6

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# List of abbreviations

| | |
|---|---|
| CYP2E1 | cytochrome enzyme P450 2E1 |
| CYP2A6 | cytochrome enzyme P450 2A6 |
| AO | aldehyde oxidase |
| CYP17 | cytochrome P450 C17 |
| CYB5 | cytochrome b5 |
| 3βHSD | 3β-hydroxysteroid dehydrogenase |
| 17βHSD | 17β-hydroxysteroid dehydrogenase |
| hCG | human chorionic gonadotropin |
| RS | resistant starch |
| RPS | raw potato starch |
| NSP | non-starch polysaccharides |
| HPLC | high performance liquid chromatography |
| ELISA | enzyme-linked immunosorbent assay |
| E1S | estrone sulfate |
| DHEA | dehydroepiandrosterone |
| DHEAS | dehydroepiandrosterone sulfate |
| GnRH | gonadotropin-releasing hormone |
| FSH | follicle stimulating hormone |
| LH | luteinizing hormone |
| QTL | quantitive trait loci |
| PCR | polymorphase chain reaction |
| SFE | supercritical fluid extraction |
| MIP | molecularly imprinted polymers |
| SPR | surface plasmon resonance |
| COUP-TF1 | chick ovalbumin upstream promoter transcription factor 1 |
| SDS-PAGE | sodium dodecyl sulfate polyacrylamide gel electrophoresis |

# Contents

# Appendix

The thesis is based on the following papers, which are referred to by Roman numerals. Reprints were published with kind permission of respective publisher concerned:

**Papers I-V**

I Chen, G., Zamaratskaia, G., Andersson, H. K. & Lundström, K. 2007. 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. *Food Chemistry 101,* 439-448.

II Chen, G., Andersson, K., Andersson, R. & Zamaratskaia, G. 2007. Effect of a high amylose barley cultivar (Karmosé) on boar taint and performance of entire male pigs. (Submitted).

III Chen, G., Zamaratskaia, G., Madej, A. & Lundström, K. 2006. Effect of hCG administration of the levels of androstenone, skatole and indole in entire male pigs. *Meat Science 72,* 339-347.

IV Chen, G., Cue, R.-A., Lundstrom, K., Wood, J.D. & Doran, O. 2007. Regulation of cytochrome P450 2A6 protein expression by skatole, indole and testicular steroids in primary cultured pig hepatocytes. (*Drug Metabolism and Disposition* doi:10.1124/dmd.107.017285 in press).

V Chen, G., Bourneuf, E., Marklund, S., Zamaratskaia, G., Madej, A. & Lundström, K. 2007. Gene expression of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase in relation to androstenone, testosterone, and estrone sulphate in gonadally-intact male and castrated pigs. *Journal of Animal science* 85, 2457–2463.

**Author's main contributions to the papers:**
Laboratory analyses, data evaluation and manuscript preparation in **Paper I** to **III**, and **V** were mainly done by Gang Chen. The laboratory analyses in **Paper IV** were mainly done by Gang Chen.

# 1 Introduction

## 1.1 Pig domestication and surgical castration

Archaeological evidence suggests the pig was first domesticated 9,000 years ago in Eastern Turkey. They were also domesticated in China at around the same time (Larson *et al*., 2005). Raising domestic pigs for meat production is a great asset from our ancestors. Untill now, pork has been the favorite meat in many countries, and production of pork is a large industry. Traditionally, male piglets are surgically castrated. The main reason for this is to remove boar taint (see review by Claus *et al*., 1994, and Prunier *et al*., 2006), the offensive odour existing in pork from boar pigs. In fact this odour has been perceived by humans since the Middle Ages, and the history of castration can be traced back to 4,500 years ago.

The main advantage of performing surgical castration is that meat quality is improved from sensory aspects. Castrated pigs are also easier to manage. However, surgical castration still causes many disadvantages. Raising surgically castrated pigs loses the benefit of better feed efficiency (Walstra, 1974; see review by Squires *et al*., 1993) and leaner meat production (Hansson *et al*., 1975; Knudson *et al*., 1985), compared with entire male pigs. Meat from castrated pigs contains more fat, which is not desirable by consumers. Another disadvantage is that surgical castration causes pain and distress to piglets and definitely violates the welfare of animals (see review by Prunier *et al*., 2006). This has prompted the impetus to abandon castration in many countries, especially in Europe, where animal welfare is of great concern (see review by Bonneau, 1998).

## 1.2 Boar taint

Boar taint is defined as an offensive odour in pork from entire male pigs. It is perceived by the human nose when the meat or fat is heated, and makes the pork less acceptable for eating. Boar taint is described as a urine-like or perspiration-like odour, and it can be differentiated from odours of other origin. For example, when pigs are fed with fish meal, their pork is perceived to have a fishy odour. Boar taint is a problem mainly in male pigs, and it causes much more attention than other odours. It is known that boar taint is caused primarily by two compounds: androstenone (5-alpha-androst-16-en-3-one) and skatole (3-methyl-indole). Indole might intensify the off-flavour in meat, but skatole has a more pronounced odour than indole (Hansson *et al*., 1980) even though they have similar smells (own observation).

## 1.3 Boar taint compound: androstenone

### *1.3.1 Androstenone origin and pheromonal function*

In 1968, a substance with typical urine-like odour was found in adipose tissue of boars but not in that of barrows (Patterson, 1968). This substance was identified as the 16-unsaturated steroid 5α-androst-16-en-3-one (androstenone). Androstenone is derived from cholesterol and has a main structure (4-ring and 17-carbon skeleton) in common with other cholesterol-derivate steroids (**Figure 1**). Due to

the high lipophilic property, androstenone can be easily accumulated in fat of boar pigs causing boar taint. Androstenone can be detected in boar serum with high amounts (up to 215 ng/ml) (Tuomola *et al*., 2002).

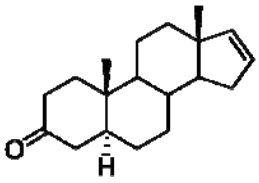


Figure 1. Chemical structure of androstenone

Androstenone is a naturally occurring $C_{19}$-$\Delta^{16}$-steroid (16-androstene steroid) pheromone in male mammals, which differs from sex hormones in that it has no hormonal function (see review by Claus *et al*., 1994). Androstenone is the first mammalian pheromone identified. Androstenone can be found in man's sweat. In male pigs, androstenone can be transported to the salivary gland and be presented together with other 16-androstene steroids, *e.g.* 5α-androst-16-en-3α-ol (3α-androstenol) and 5α-androst-16-en-3β-ol (3β-androstenol). In the salivary gland, 16-androstene steroids are bound to the binding protein pheromaxein and can be excreted into boar saliva during sexual excitement. The odour of the salivary 16-androstene steroids is detected by the estrous female pig and their pheromonal action results in the characteristic mating stance (see review by Brooks and Pearson, 1986). Melrose *et al*. (1971) found that spraying androstenone or 3α-androstenol towards the snouts of estrus females also induces the mating stance.

The smell of androstenone has been described as sweaty, ammonia, dirty, parsnip, silage, prickling sensation and acrid, but only a sweaty smell has been noted as the most significant (Annor-Frempong *et al*., 1997). However, pork with high androstenone level was perceived by human nose as "perspiration-like" or "urine-like" odour when heated (see review by Malmfors & Lundström, 1983; Dijksterhuis *et al*., 2000). Androstenone is perceived differently by different sexes. Women are more sensitive than men, with the perceptional population ratio 76% in women compared with 54% in men (Kloek, 1961). However, androstenone perceptional ability can be learned after repeated exposure of androstenone to a training person (Wysocki *et al*., 1989). There is discrepancy among studies about the androstenone sensory threshold in pork. However the most commonly used androstenone threshold is between 0.5 to 1.0 μg/g of adipose tissue (Bonneau *et al*., 2000a), and this threshold has been used successfully in a large EU project for comparing and harmonizing the experimental results among countries (Walstra *et al*., 1999; Bonneau *et al*., 2000a; Dijksterhuis *et al*., 2000; Matthews *et al*., 2000; Bonneau *et al*., 2000b)

### *1.3.2 Androstenone biosynthesis*

Androstenone is produced from testicular Leydig cells together with other testicular steroids (see review by Claus *et al*., 1994). However, the biosynthetic pathway of androstenone differs from that of androgens and estrogens (see review by Brooks and Pearson, 1986). Pregnenolone is the precursor for androstenone biosynthesis. Pregnenolone is hydroxylised at C20 position to form 20β-

dihydropregnenolone. 20β-Dihydropregnenolone is further cleaved at C17-C20 bond to form 5,16-androstadien-3β-ol. The 5,16-androstadien-3β-ol synthetase (andien-β synthetase) is the key enzyme for this conversion (Loke and Gower, 1971). A review by Brooks and Pearson (1986) stated that andien-β synthetase is the different enzyme with 17α-hydroxylase, which is responsible for androgen synthesis, and therefore concluded that androgen and $C_{19}$-$\Delta^{16}$-steroid (16-androstene steroid) were synthesized separately. This might explain the low correlation between plasma androstenone and testosterone found by Lundström *et al*. (1978). However, a more recent study showed that the andien-β synthetase function was also performed by cytochrome P450 C17 (CYP17), and cytochrome b5 (CYB5) strongly enhanced CYP17 andien-β synthetase function (Soucy *et al*., 2003). Androstenone can also be synthesized from progesterone but to a much less extent (see review by Brooks and Pearson, 1986).

### *1.3.3 Androgen biosynthesis*

Productions of all testicular steroids were controlled by hypothalamic-pituitary-gonadal axis. All of the testicular steroid hormones, as well as 16-androstenes were synthesized from a common precursor pregnenolone (3β-hydroxypregn-5-en-20-one) ($\Delta^5$ steroid). Testosterone (17β-hydroxy-4-androsten-3-one) is synthesized in Leydig cells of the testis utilizing the precursor pregnenolone, and there are two pathways ($\Delta^4$ or $\Delta^5$) for testosterone biosynthesis (see review by Brooks and Pearson, 1986). Testosterone is the primary and most potent androgens in males. Testosterone has anabolic and virilizing effects. Testosterone anabolic effects include growth of muscle mass and strength, increased bone density and strength, and stimulation of linear growth and bone maturation. Testosterone promotes protein synthesis by increasing RNA transcription (see review by Brooks and Pearson, 1986). Testosterone virilizing effects mainly develop male secondary sex characteristics.

### *1.3.4 Estrogen biosynthesis*

Estrogens are C18 steroids, differing from androgens (C19 steroids) in that they lack a methyl group at C10 position. Moreover, estrogens contain a phenolic A ring, which is distinct from other natural steroids (see review by Brooks and Pearson, 1986). Estradiol (17β-estradiol) represents the most active estrogen, Estradiol is the female hormone but also present in males, which might prevent apoptosis of male germ cells. Estradiol in plasma is largely bound to binding protein and a fraction is free to have bioactive function. Deactivation includes conversion to less active estrogens such as estrone and estriol (see review by Brooks and Pearson, 1986). Estrone sulfate (E1S) exists in large amount in plasma of the male human (Ruder *et al*., 1972) and entire male pigs (Schwarzenberger *et al*., 1993; Zamaratskaia *et al*., 2004a), and this sulfoconjugation process mainly occurs in liver. E1S might be a storage form of estrogens. Santner *et al*. (1990) demonstrated that E1S can be converted to estradiol *in vivo* by estrone sulfatase enzyme. Biosynthesis of estradiol from cholesterol utilizes the same $\Delta^4$ and the $\Delta^5$ metabolic pathway with testosterone, and androstenedione is the key intermediate (see review by Payne and Hales, 2004).

### *1.3.5 Enzymes involved in androstenone metabolism*

#### 1.3.5.1 3β-hydroxysteroid dehydrogenase/$\Delta^5$-$\Delta^4$-isomerase (3βHSD)

The enzyme 3β-hydroxysteroid dehydrogenase/$\Delta^5$-$\Delta^4$-isomerase (3βHSD) plays an essential role in the formation of all classes of active steroid hormones. It is responsible for the oxidation and isomerization of $\Delta^5$-3β-hydroxysteroid precursors into $\Delta^4$-ketosteroids (see review by Payne and Hales, 2004). The 3βHSDs are membrane-bound enzymes and are distributed to both mitochondrial and microsomal membranes depending on the type of cell in which they are expressed. In different animals, the isoforms of 3βHSDs are also different. In humans, 3βHSD I and II are the two distinct isoforms involved in the biosynthesis of all active steroid hormones (see review by Payne and Hales, 2004). Until now, only one type of 3βHSD was found in pig, and the sequence was obtained using a cDNA library from adipose tissue (von Teichman *et al*., 2001).

The 3βHSDs are expressed in different tissues, and the expressions of 3βHSD isoforms show a cell- and tissue-specific manner. In humans, hepatic 3βHSD expression is presumed to be important in the metabolism and inactivation of steroids (see review by Simard *et al*., 2005). The 3βHSD activity in human liver microsomes was shown to be three times higher for hydroxylation of DHT (dihydrotestosterone) (3-keto steroid) to 3β-DIOL (androst-5-ene-3β,17β-diol) (3-hydro steroid) (Pirog & Collins, 1999). Immunohistochemical studies showed that 3βHSD was mainly present in bile duct epithelium and microsomal fraction in liver of human and pig (Furster, 1999).

More recently, the research about 3βHSD in entire male pigs has gained more attention. This is due to the finding that 3βHSD might be involved in androstenone biotransformation (von Teichman *et al*., 2001) or metabolism (Doran *et al*., 2004). Androstenone in liver microsomes can be metabolized to 3β-androstenol, and the metabolizing rate depends on 3βHSD protein expression (Doran *et al*., 2004). Furthermore, other studies showed that high fat androstenone was negatively correlated with hepatic 3βHSD protein expression (Nicolau-Solano *et al*., 2006). The 3βHSD mRNA levels in liver determined by RT-PCR method also negatively correlated with androstenone levels of back fat (Doran *et al*., 2004). All of the studies strongly suggested the importance of 3βHSD enzyme in androstenone metabolism in liver of entire male pigs.

#### 1.3.5.2 17β-hydroxysteroid dehydrogenases (17βHSD)

The 17β-hydroxysteroid dehydrogenases (17βHSD) belong to the same phylogenetic protein family as 3βHSD, called the short-chain alcohol dehydrogenase reductase superfamily (see review by Payne and Hales, 2004). These enzymes are involved in the reduction and oxidation of steroid hormones. The 17βHSDs convert inactive 17-ketosteroids into their active 17β-hydroxy forms. Therefore they are responsible for catalyzing the final step in the biosynthesis of active gonadal steroid hormones, estradiol and testosterone. In total 11 different 17βHSD isozymes have been identified, and they show little homology. Only three 17βHSD isotypes (type I, III and VII) were reported to have the active steroid hormones biosynthetic function (see review by Payne and Hales,

2004). The gene 17βHSD7 was positioned at human chromosome 1q23 (Törn *et al*., 2003), which was comparatively mapped to porcine chromosome 4q1.5 (SSC4q1.5) (Moller *et al*., 2004). The study on human and mouse found that 17βHSD type 7 (17βHSD7) also catalyzes the reduction of keto group in either 17- or 3- position of the substrate, and presents minor 3βHSD-like activity (Törn *et al*., 2003). Therefore 17βHSD7 might also be involved in androstenone metabolism in entire male pigs.

1.3.5.3 Hydroxysteroid Sulfotransferase

Hydroxysteroid Sulfotransferase (HST, gene name SULT2A1) catalyzes the sulfation of steroids and bile acids in the liver and adrenal glands. It is the primary enzyme for DHEA sulfation to DHEAS in human. The position of SULT2A1 has been mapped to human chromosome 19q13.32 (http://www.ebi.uniprot.org/entry/Q06520). More recently, SULT2A1 was shown to be responsible for the formation of androstenone-sulfate and consequently decreased androstenone accumulation in fat of entire male pigs (Sinclair *et al*., 2005a,b,c). Sulfation of androstenone includes two steps; the first step is to convert androstenone 3-keto group to 3-enol form, and the second step is to change the 3-ol group with sulfate group to form the sulfoconjugated androstenone (Sinclair *et al*., 2005c). Androstenone sulfoconjugation increased polarity and the hydrophilic property, and therefore androstenone sulfoconjugate can easily be metabolised and excreted.

### *1.3.6 Ways of suppressing androstenone accumulation*

Over decades, ways to prevent high fat androstenone accumulations have been pursued in many projects, with EU countries the most actively involved. It has been suggested that fat androstenone levels differ between breeds, and that Asian pig breeds, *e.g.* Meishan, accumulate more fat androstenone than European or North American breeds. This might be because of the early puberty in Meishan pigs compared with other pig breeds (Ellis *et al*., 1995). Androstenone production is related to both puberty development and anabolic steroids biosynthesis; therefore, the selective suppression of androstenone without affecting other factors is not achievable (see review by Claus *et al*., 1994). Genetic selection of low androstenone pigs resulted in a delayed pubertal development and the postponed appearance of anabolic hormones as well (Willeke *et al*., 1987; see review by Claus *et al*., 1994). Photoperiod (Andersson *et al*., 1998), as well as the social rank order (Giersing *et al*., 2000) influences androstenone production through altering sex maturation. Slaughter entire male pigs at low live weight (≤ 90 kg) partly reduced the frequency of high androstenone levels (Andresen, 1976). Feeding regimen was reported to have no effect on altering fat androstenone levels unless sexual maturity was affected (see review by Claus *et al*., 1994). Immunization against androstenone biosynthetic pathway is efficient to block androstenone production. The successful performances of vaccination include anti-androstenone vaccine (Shenoy *et al*., 1982; Williamson & Patterson, 1982) and anti-GnRH (LH-RH) vaccine (Meloen *et al*., 1994; Bonneau *et al*., 1994). The effect of anti-GnRH vaccine on reducing boar taint compounds has been observed in many studies (Dunshea *et al*., 2001; Jaros *et al*., 2005) and such a vaccine is commercially available (Improvac™, Pfizer). More recently, the effect of synthesized anti-

chicken GnRH vaccine on the testicular function of boar pigs has also been evaluated (Bowen *et al*., 2006), and the biosynthetic function of steroids in Leydig cells of boar testis was significantly inhibited after vaccination. The current problems of applying vaccination on entire male pigs are that the procedures need to be performed twice during the pig finishing period, which is practically difficult and time consuming. In addition, in some countries, reactions of consumers against the vaccination utilization are also of concern.

## 1.4 Boar taint compound: skatole

### *1.4.1 Skatole origin*

Skatole originates from degradation of L-tryptophan in hind gut (see review by Yokoyama and Carlson, 1979). L-tryptophan is either degraded to indole, or converted to indole-3-acetic acid and then metabolized to skatole (see review by Deslandes *et al*., 2001). In pigs, a wide range of bacteria species are involved in tryptophan degradation to indole. Whereas the microflora for producing skatole are very specific, only six species have been found. Skatole is speculated to be produced by a decarboxylation enzyme in a specific bacterium, but this enzyme has not been identified (see review by Deslandes *et al*., 2001).

Skatole in ruminants cause acute disease in lung, called acute bovine pulmonary edema and emphysema (ABPE) (see review by Deslandes *et al*., 2001). In contrast, indole did not cause lung damage in ruminants, suggesting the key role of skatole 3-methyl group in the pneumotoxicity problem (see review by Yokoyama and Carlson, 1979). Skatole is not as toxic to pigs as to ruminants since high amounts of skatole can be found both in plasma and in fat of entire male pigs without adversely affecting health. This suggests the pigs have higher tolerance to skatole levels, and it seems the unique problem caused by skatole is the sensory quality deterioration to pork. Skatole has bacteriostatic effect on gram-negative enterobacteria including some pathogenic bacteria *e.g. Escherichia* and *Salmonella*. In diluted solutions, skatole can also inhibit the growth and fermentation of *Lactobacillus acidophilus* (see review by Yokoyama and Carlson, 1979).

The skatole produced from large intestine can be reabsorbed by the intestinal mucosa (see review by Claus *et al*., 1994). Its absorption is very rapid and its half-life in blood is approximately 1 h (Agergaard and Laue, 1993). Skatole passes through the liver where most of it is immediately metabolized at a rate of 1 l/h/kg (Friis, 1993). The metabolized products are excreted in the urine (Baek *et al*., 1997). Skatole that is not metabolized mainly accumulates in adipose tissue due to its lipophilic properties (Squires and Lundström, 1997; Babol *et al*., 1998), and no traces of skatole have been detected in urine (Friis, 1993).

### *1.4.2 Skatole metabolism*

Skatole is metabolized in liver tissue by cytochrome enzymes P450 2E1 (CYP2E1) (Squires and Lundström, 1997), P450 2A6 (CYP2A6) (Diaz *et al*., 1999), aldehyde oxidase (AO) and phenol sulfotransferase (SULT1A1). Inhibition of CYP2A6 and CYP2E1 by specific inhibitors reduced skatole metabolites

production in porcine hepatic microsomes (Diaz and Squires, 2000). High skatole accumulation in fat of entire male pigs were associated with low enzyme activity of both CYP2E1 (Squires and Lundström, 1997) and CYP2A6 (Zamaratskaia *et al*., 2005a). Skatole accumulation expressed a sex-dependent pattern, and entire male pigs had much higher fat skatole levels than gilts and castrates. Moreover, entire male pigs had low CYP enzyme expressions compared with females and castrates (Skaanild & Friis, 1999). The low enzyme expressions in entire male pigs reduced skatole metabolic rate in liver and consequently caused high skatole accumulation in fat (Diaz and Squires, 2000; Zamaratskaia *et al*., 2006). Liver is the main tissue for CYP2E1 and CYP2A6 expression (Lin *et al*., 2004a; 2006), suggesting the dominance of liver as responsible for skatole metabolism.

Skatole metabolism includes two phases: phase I, oxidation of skatole into hydroxyl compounds, which is mainly activated through the enzymes CYP2E1 and CYP2A6; phase II, hydroxyl compounds further react with other groups, *e.g.* sulfate group or glucuronic acid group to form more hydrophilic conjugated form (Baek *et al*., 1997; Babol *et al*., 1998; Diaz & Squires, 2003; Lin *et al*., 2004a). Seven skatole metabolites have been identified in an *in vitro* study (Diaz *et al*., 1999). Baek *et al*. (1997) reported that high amount of 6-sulfatoxyskatole was found in plasma of entire male pigs, suggesting rapid metabolism and clearance of skatole *in vivo*. SULT1A1 is involved in this metabolism.

### *1.4.3 Skatole modification by feed regimen*

Human studies have shown that some carbohydrates are not hydrolyzed by pancreatic amylases in the upper gastrointestinal tract, but can be metabolized by bacterially produced enzymes in the colon, *e.g.*, from *saccharolytic clostridia*, *bacteroides* and *bifidobacteria* (see review by Roberfroid *et al*., 1998). The metabolism process produces a variety of products such as short-chain fatty acids (*e.g.*, acetate, propionate and butyrate) and organic acids (*e.g.*, lactate, succinate and pyruvate), which may beneficially affect host health (see review by Roberfroid *et al*., 1998). In pigs, this process has a particular function, and skatole accumulation in fat of entire male pigs can be reduced by dietary carbohydrates (see review by Claus *et al*., 1994). These dietary carbohydrates include resistant starch (RS), non-digestible oligosaccharides, and non-starch polysaccharides such as celluloses and hemicelluloses. Other sugars such as lactose, raffinose and stachyose and certain sugar alcohols, *e.g.* sorbitol and xylitol, also contribute to the fermentable carbohydrate load (Cummings and Englyst, 1995).

#### 1.4.3.1 Raw potato starch

Raw potato starch is one of the well studied carbohydrates having skatole-reducing function in pigs (Claus *et al*., 2003; Zamaratskaia *et al*., 2005b). Raw potato starch contains high amount of resistant starch (RS), which exists in discrete granular form (Hizukuri, 1985). The RS granule has a very complicated structure. Its property is not only decided by first class chemical structure, but also by spatial physical structure. One of the most distinct phenomenons of RS granules is gelatinization, when RS granules are heated in the presence of water. Gelatinization is a process of serial irreversible reactions including swelling of the

starch granules, leakage of amylase from starch granules and loss of crystallinity (Colonna *et al*., 1992).

#### 1.4.3.2 Other non-digestible carbohydrates

The effect of chicory inulin on reducing skatole levels in entire male pigs has also been evaluated (Rideout *et al*., 2004; Hansen *et al*., 2006). Inulin is one kind of linear fructan, which is a polymer of fructose molecules linked by β (2→1) glycosidic bonds (see review by Roberfroid *et al*., 1998). Inulin belongs to non-digestible oligosaccharides, and it has been authorized as functional food ingredients for humans, and was classified as prebiotic due to its health benefit to host intestine (Gibson and Roberfroid, 1995). Inulin is a selective substrate for one or a limited number of potentially beneficial bacteria commensal to the colon in humans, *e.g.*, *bifidobacteria* and *lactobacilli*, which are stimulated to grow. As a consequence, colonic microflora are altered toward a potentially more healthy composition (Gibson & Roberfroid, 1995). Alteration of microbial fermentation by inulin in the large intestine of entire male pigs explains the reduction of skatole formation (Rideout *et al*., 2004). Feeding sugar beet also has skatole-reducing effect in entire male pigs (Knarreborg *et al*., 2002; Whittington *et al*., 2004). This was achieved by non-starch polysaccharides (NSP) in the feed (Knarreborg *et al*., 2002). No skatole-reducing effect by feeding sugar beet has also been reported in some studies (Øverland *et al*., 1995; Van Oeckel *et al*., 1998). This might be due to the overall low fat skatole levels in the experimental entire male pigs (Van Oeckel *et al*., 1998).

### *1.4.4 Intestinal cell apoptosis and skatole modification*

Modification of L-tryptophan availability in large intestine may alter the skatole production (see review by Yokoyama & Carlson, 1979), and this might further influence its fat accumulation (see review by Claus *et al*., 1994). Claus *et al*. (1996) demonstrated that L-tryptophan was obtained from intestinal mucosa cells apoptosis due to the fact that dietary supplementation of L-tryptophan did not increase skatole production in large intestine (Bonneau & Desmoulin, 1981). Anabolic hormones, *e.g.* testosterone, regulate cell renewal and apoptosis in intestine and they therefore contribute to the sex difference of skatole accumulation (Claus *et al*., 1994). High energy feed was reported to increase skatole formation (Claus *et al*., 1996) because high energy supply also increased plasma IGF-1 levels, which resulted in the concomitant increase of mitotic and apoptotic index in the gut mucosa (Claus & Raab, 1999). This explains the energy-dependent increase of skatole levels. Butyrate inhibits colon cell apoptosis and can therefore reduce tryptophan availability. Moreover butyrate is the main product of resistant starch (RS) fermentation in the large intestine (Claus *et al*., 2003). Immunocytochemical analyses showed that after feeding raw potato starch, colon crypt cells apoptosis decreased from 2.06 to 0.90 cells/crypt; meanwhile the mitosis rate was not changed (Claus *et al*., 2003). From this, the authors concluded that the skatole reduction by RS was due to inhibition of cell apoptosis in the colon.

### *1.4.5 Microflora adaptation in large intestine is related to skatole formation*

Katouli *et al*. (1997) have previously reported that the fecal microflora was changed after weaning and that the microbial populations were stabilized at 5 to 6 wk post weaning. This shift coincided with a decreased fermentative capacity in the intestine (Katouli *et al*., 1997). Zamaratskaia *et al*. (2004c) reported elevated plasma skatole levels after weaning, which decreased gradually afterwards. More recently, a study performed on post weaning entire male pigs showed that the increase in skatole production after weaning was not due to the intestinal cell turnover, but probably due to the post weaning adaptation of the intestinal microflora (Lanthier *et al*., 2006). In an *in vitro* study, addition of fructooligosaccharide in pig slurries with L-tryptophan significantly changed the microflora population, and the transformation of L-tryptophan to skatole was also reduced (Xu *et al*., 2002). In humans, oligosaccharide (inulin) can selectively stimulate the increase of beneficial bacteria and alter the microflora in large intestine towards a potentially more healthy composition (see review by Roberfroid *et al*., 1998). It is possible that the microflora adaptation in the large intestine after feeding non-digestive polysaccharides contributes to skatole reduction (Lanthier *et al*., 2006), and this is due to the increased fermentation changing the colonial environment in the intestine (Claus *et al*., 2003), or selective stimulation of certain beneficial bacteria growth, or both.

## 1.5 Genetic effect on boar taint

### *1.5.1 QTLs and candidate genes for androstenone*

Important genetic variation exists both between and within breeds for fat androstenone levels. Heritability estimates have ranged from 0.25 to 0.88 (Sellier, 1998). Breed differences of androstenone levels have also been described (Bonneau *et al*., 1979; Xue *et al*., 1996a). QTLs for androstenone have been the focus of many studies. Quintanilla *et al*. (2003) reported major QTLs on pig chromosome (SSC) 3, 7, 14, but in general the QTLs seem scattered from this study. Two candidate genes (CYP21, CYP11A) were selected from the main QTL region of SSC 7 and genotyped, but no polymorphisms were responsible for androstenone variation. From the available information about biological functions of CYP21 and CYP11A, it is assumed that they might not be candidates specific for androstenone, because they are involved in the common steroids biosynthetic pathway. Moreover, Lee *et al*. (2005) used the same pig breeds (Large White x Meishan) as Quintanilla *et al*. (2003), and analysed QTLs on SSC 2, 4, 6, 7, 9. However, only a QTL on SSC 6 was identified for back fat androstenone. It should be mentioned that the effect of age on androstenone levels was not considered in this analysis, and this might mask some other QTLs, because the result from Quintanilla *et al*. (2003) showed that QTL effect on androstenone is highly significant in young animals but not in the animals reaching maturity. Conversely, Bayesian analysis of a Landrace outbred population found no QTL for fat androstenone levels (Varona *et al*., 2005). The inconsistent results of different studies suggest that other factors than genetic effect might have stronger influence on fat androstenone accumulation.

Candidate genes responsible for androstenone metabolism have been sought. Sinclair *et al*. (2005 a, b, c) demonstrated that a large amount of sulfoconjugated form of androstenone existed in plasma of boar pigs and was negatively correlated to fat androstenone levels. Another study stressed the importance of hydroxysteroid sulfotransferase (SULT2A1) on the formation of sulfated androstenone (Sinclair *et al*., 2006). A polymorphism was found in SULT2A1 coding sequence. However, this did not affect the amino acid sequence. The possible mutation carried by SULT2A1 regulatory region remains to be investigated.

In human (Auchus *et al*., 1998) and in rat (see review by Payne and Hales *et al*., 2004), cytochrome b5 (CYB5) enzyme is an accessory protein that acts as an allosteric effector of the CYP17-oxidoreductase complex (P450c17.OR). CYP17 is the key enzyme determining the C19 sex steroids biosynthesis (as mentioned above). CYB5 interacts primarily with the P450c17.OR complex to stimulate the cleavage of $C_{17}$-$C_{20}$ bond (17, 20-lyase activity) (Auchus *et al*., 1998). In pigs, CYB5 was reported to be related with 16-androstene production (Davis and Squires, 1999). The gene CYB5 was positioned on SSC 1, which is not a QTL region of androstenone (Quintanilla *et al*., 2003). A causative mutation in the CYB5 5'-untranslated region in relation to fat androstenone levels has been reported (Lin *et al*., 2005). Future work might be necessary to elucidate the relationship between CYB5 polymorphism and fat androstenone accumulation.

### *1.5.2 Genetic effects on skatole*

There is evidence showing a genetic influence on skatole accumulation in entire male pigs. Heritability ($h^2$) for skatole equivalents (all indolic compounds) has been determined to be from fairly high (0.34±0.04) to low (0.19) (Pedersen, 1998). A difference between breeds in skatole levels has also been reported (Xue *et al*., 1996a; Babol *et al*., 2004). Lundström *et al*. (1994) suggested that a major recessive gene was responsible for high skatole levels. QTL for skatole has been detected. The QTL with largest effect has been mapped on pig chromosome 14 (SSC14) at 61 cM, spanning the markers between S0007 and SW761 (Lee *et al*., 2005). However, no candidate genes have been selected within this QTL. Another QTL for skatole has been mapped on pig chromosome 6 (SSC6) (Lee *et al*., 2005).

Polymorphisms in candidate genes responsible for skatole metabolism have been the focus of many studies. A causative mutation of a SNP has been found on CYP2E1 coding region, which changed CYP2E1 enzymatic activity and therefore the skatole metabolizing activity (Lin *et al*., 2006). Moreover, in another study using a large population of commercial pigs, one SNP in the CYP2E1 promoter region was found, which tended to be associated with fat skatole levels (Skinner *et al*., 2005). A single base deletion in CYP2A6 coding region has been identified, which resulted in the inactiveness of CYP2A6 enzyme and high skatole accumulation (Lin *et al*., 2004a). However, Skinner *et al*. (2006) demonstrated that none of the CYP2A6 polymorphisms were associated with fat skatole levels when genotyping a large commercial pig population, suggesting that variation of CYP2A6 skatole metabolizing function was not caused by a regulatory mutation on the gene, but by regulation of transcription factors. When genotyping other

candidate genes, phenol sulfotransferase (SULT1A1) and CYP2C18, no polymorphism was found to be responsible for skatole variation (Skinner *et al*., 2006).

## 1.6 Detection of boar taint

The methods for measuring fat androstenone and skatole levels have been well established. Liquid fat samples are more commonly used for analyses, but in some studies total fat tissue is also used.

### *1.6.1 Classical methods for analysing boar taint*

Gas chromatography was the first method applied for fat androstenone analysis (Patterson, 1968). Furthermore, GC method has been optimized in other studies (De Brabander & Verbeke, 1986; Brennan *et al*., 1986). The method has very high sensitivity, and compounds similar to androstenone, *e.g.* 3α-androstenol and 3β-androstenol, can also be identified in the analysis. The drawback of this method is that the fat samples extractions are very complicated; saponification must be done first, followed by solvent extraction, evaporation and the derivatisation step. Similar to androstenone, fat skatole levels were also first identified using the GC method by Vold (1970) and Walstra & Maarse (1970).

Hansen-Møller (1994) developed a simultaneous analysis for androstenone, skatole and indole in fat by HPLC method. This method includes simpler sample extraction compared with the GC method, and the pre-column derivatisation for androstenone can be performed automatically. The limit of quantification for androstenone is 0.2 µg/g fat, and for skatole 0.03 µg/g fat (Hansen-Møller, 1994). This method has been adapted in many different labs (Claus, 1997a; Dunshea *et al*., 2001) and the large EU project (Bonneau *et al*., 2000a). Besides this method, the analyses of only fat skatole and indole levels by HPLC with fluorescence detector has also been established (Dehngard *et al*., 1993; Gibis, 1994; García-Regueiro & Rius, 1998).

A method of supercritical fluid extraction (SFE) by carbon dioxide combined with GC-MS for fat androstenone analysis has also been developed (Mågård *et al*., 1995; Tuomola *et al*., 1998). The SFE extraction method is rather fast and efficient and the matrix effect on the analysis is significantly reduced. Using the mass detector allows the analysis to obtain high specificity and low detection limit (0.05 µg/g). Androstenone and other 16-androstene steroids can be clearly separated and identified. The drawback of this method is that it is more expensive than other methods, and the equipment needed is rather expensive and not commonly used.

Immunoassay based methods for fat androstenone analyses have also been well established. The methods include radio-immunoassay (Claus, 1974; Andresen, 1975), enzymatic immunoassay (Claus, 1988; Squires & Lundström, 1997) and fluoroimmunoassay (Tuomola *et al*., 1997). The sensitivity of the methods is high enough for research purpose or sorting tainted carcasses. Before analysis, fat androstenone has to be extracted by organic solvent, a manual and time consuming

step. Another drawback of immunoassay method is that the sensitivity and specificity of analysis relies much on androstenone antibody. Therefore analyses by different antibodies from different laboratories may give different results. In comparison with androstenone, applications of immunological methods for skatole analyses are few. This might be due to the low molecular weight of the antigen and the unavailability of specific antibody. Tuomola *et al*. (2000) successfully developed a monoclonal antibody which can specifically recognize skatole, but further information about using the antibody for method development is scarce.

### *1.6.2 Methods with potential for online sorting of tainted carcass*

Due to the importance of online sorting tainted carcasses at slaughterhouse, rapid methods for boar taint analyses have been pursued broadly. This is particular important to Norway where castration will be stopped by 2009. The main criterion for a rapid method for androstenone analysis in Norway is ≤ 45 min/sample (Haugen, personal communication). There is no current method reaching this criterion. Screening method for on-line fat skatole analysis at slaughter is available (Mortensen & Sørensen, 1984; Hansen-Møller & Kjeldsen, 1998). This method is based on a colour reaction with dimethylaminobenzaldehyde after extraction with a mixture of acetone and aqueous buffer (Mortensen & Sørensen, 1984). This is a fast and fully automatic method. The equipment for on-line analysis was invented in Danmark (Hansen-Møller, 1998).

The following section summarizes recent development of methods with potential for rapid analyses of fat androstenone and skatole.

#### 1.6.2.1 Electronic nose (e-nose)

Annor-Frempong *et al*. (1998) applied an electronic nose (e-nose) with a 12-conducting-polymer sensor array to distinguish pork fat from high or low boar taint. The study showed that there was significant correlation between the results from e-nose and sensory panel, and the e-nose could successfully sort out tainted fat samples. Recently, an e-nose based on ion mobility spectrometry (MGD-1, Environics Ltd., Finland) was used for measuring boar taint levels in fat samples with different androstenone and skatole levels (0.09–0.88 μg/g fat and 0.01–0.26 μg/g fat, respectively) (Vestergaard *et al*., 2006). The authors suggest that the e-nose technology based on ion mobility spectrometry has a potential for future rapid sorting of carcasses at the slaughter line. Fat samples do not need to be extracted by organic solvent; thus the time and cost for analyses can be significantly reduced. It should be noted that e-nose is for identifying total odour compounds and is not specific for androstenone or skatole. It seems that the current drawback when using the e-nose is that the sensors are not specific and the sensitivity needs to be improved (Vestergaard *et al*., 2006).

#### 1.6.2.2 Functional polymers for molecular recognition

Molecularly imprinted polymers (MIPs) can be considered as mimics of biological receptors, *e.g.* antibodies. Imprinted recognition sites have been exploited for chiral separation of pharmaceutical compounds, used as antibody mimics for development of assays and biomimetic sensors (see review by Takeuchia & Haginakab, 1999). Molecular recognition nanostructures can be created in

synthetic polymers, and therefore polymers for recognizing boar taint molecules are possible. Ideally, these polymers can be coupled with other sensors in the equipment and function as artificial antibody. However, the progress of technology regarding boar taint analysis is limited.

#### 1.6.2.3 Surface plasmon resonance (SPR)

SPR technique is a label-free, real-time technology, which has been widely used for monitoring reversible interactions of different molecules, *e.g.* proteins, nucleic acids, as well as for screening small molecules (Säfsten *et al*., 2006). A commercial equipment (Biacore, Biacore AB, Uppsala, Sweden) is available and the development of a chip specific for androstenone and skatole should not be a problem. However, similar to immunoassay, sample extraction with this technology is necessary at present. This technology cannot be used for online sorting unless innovative progress has been made.

## 1.7 Skatole and androstenone interaction

Skatole accumulations in entire male pigs increase after puberty (Zamaratskaia *et al*., 2004c), and this coincides with the increase in fat androstenone accumulation (Claus *et al*., 1994). Moderate correlation between fat skatole and androstenone were also observed (Walstra *et al*., 1999). Babol *et al*. (2004) suggested that increased steroid levels after puberty might regulate skatole levels, and this might be achieved through regulating the rate of skatole metabolism. Doran *et al*. (2002) found that skatole as substrate induced CYP2E1 protein expression in primary cultured hepatic cells from entire male pigs. Incubation with androstenone could inhibit CYP2E1 protein expression to basal level (*i.e.* did not differ with control). Tambyrajah *et al*. (2004) reported that androstenone could block the binding of one of the CYP2E1 gene transcription factor (chick ovalbumin upstream promoter transcription factor 1, COUP-TF1) to the promoter region; consequently, CYP2E1 enzyme expression was inhibited by androstenone through inhibiting CYP2E1 gene expression. These *in vitro* studies clearly show that androstenone could stimulate skatole increase by inhibiting skatole metabolizing enzyme. Castration of entire male pigs not only reduces androstenone levels but also skatole levels (Whittington *et al*., 2004). High CYP2E1 enzyme expression was found in castrated pigs (Skaanild and Friis, 1999), which results in clearance of skatole after castration.

## 1.8 Other boar taint compounds

Besides skatole and androstenone, other compounds might also be responsible for boar taint. Indole can be detected in boar fat. Claus *et al*. (1997b) reported that indole amounts to approximately 40% of skatole levels in the fat of boar pigs given normal feed. However, this percentage might change depending on other factors. Dirty pens result in high fat indole accumulation (Hansen *et al*., 1995). Other indolic compounds *e.g.* indolemethanol (indole-3-methanol), indolepropionic acid (indole-3-propionic acid), indoleacetonitril (indole-3-acetonitril) and indolethanol (indole-3-ethanol) were also found in fat of boar pigs (Hansen-Møller, 1998), and might contribute to boar taint.

Brooks & Pearson (1989) reported that in total five different kinds of 16-androstene steroid have odour smell, and androstenone has the lowest threshold easily to be perceived (**Table 1**). 5α-Androst-16-en-3α-ol and 5α-androst-16-en-3β-ol can be detected in the fat of boar pigs with the range of concentration 0.05 – 0.81 μg/g (Mågård *et al*., 1995). Lower accumulation of these compounds in fat compared with androstenone might be due to the 3-hydroxyl group of their structure. The existence of androstadienone or androstadienol in boar fat has not been reported, and they might have much less importance to boar taint because of their high odour threshold (**Table 1**).

Table 1: *Perception threshold for 16-androstene steroids in cottonseed oil* *.

| Compounds | Odour threshold (μg/g) |
|---|---|
| 5α-Androst-16-en-3-one | 0.6 |
| 5α-Androst-16-en-3α-ol | 0.9 |
| 5α-Androst-16-en-3β-ol | 1.2 |
| 4,16-Androstadien-3-one | 7.8 |
| 5,16-Androstadien-3β-ol | 8.9 |

* *Source: Brooks & Pearson, 1989*

The existence of p-cresol (4-methylphenol) (Patterson, 1967) and 4-phenyl-3-buten-2-one (Rius & García-Regueiro, 2001) in fat of boar pigs has also been reported. They might either contribute to boar taint or enhance the perception of other odour compounds. Rius & García-Regueiro (2001) reported that the fat samples classified to contain boar taint by sensory panels also contained higher amount of p-cresol and 4-phenyl-3-buten-2-one than the fat samples classified without boar taint. More recently, analyses by GC-MS method showed that other volatile compounds (aldehydes and short chain fatty acids) in the fat samples with low skatole and androstenone levels also contribute to boar taint odour (Rius *et al*., 2005).

## 1.9 Objectives

- To compare different methods for skatole and androstenone analyses.
- To further study the effects of dietary supplement of raw potato starch (RPS), slaughter weight, raising system and sire on skatole and androstenone levels.
- To evaluate the effect of a new high-amylose barley cultivar (Karmosé) on reducing skatole and indole levels in entire male pigs, and its effect on performance.
- To evaluate pigs injected with human chorionic gonadotropin (hCG) as a potential model to study the effect of increased levels of testicular steroids on skatole and indole levels.
- To investigate effects of testicular steroids (androstenone, testosterone and E1S) and skatole and indole on the expression of CYP2A6 protein using primary cultured pig hepatocytes as a model system.
- To investigate the expression of 3βHSD and 17βHSD mRNA in relation to accumulation of fat androstenone and other testicular steroids and to determine how the variation of 3βHSD and 17βHSD gene expression was affected by immunocastration and surgical castration, respectively.

# 2 Materials and methods

## 2.1 Animals, treatments and sampling

*Study I: Effect of raw potato starch and live weight on variations of boar taint compounds in fat and plasma determined by different methods*

A total of 96 crossbred entire male pigs (4 Landrace sires × 13 Swedish Yorkshire dams) were included in the study. The pigs were slaughtered at two occasions per pen. The most fast-growing three pigs were slaughtered when they reached approximately 90 kg live weight (LW; n=27). The remaining pigs were slaughtered when the average LW in each pen reached 115 kg (n=69). When the average LW of entire male pigs reached 100 kg, 34 out of the 69 pigs were additionally fed 0.6 kg of raw potato starch (RPS, Lyckeby Culminar; Karlshamn, Sweden) per day for two weeks prior to slaughter. Blood samples were taken at three occasions: i) the day before first slaughter (90 kg LW), ii) the day before changing diets (100 kg LW), and iii) the day before the second slaughter (115 kg LW). Back fat was taken after slaughter.

*Study II: Effect of a high amylose barley cultivar (Karmosé) on boar taint and performance of entire male pigs*

A total of 72 crossbred entire male pigs (Swedish Yorkshire dams × Swedish Landrace sires) were included. The piglets within litter at birth were randomly assigned to two groups: a control group and a treatment group. The study included two trials, with 40 pigs in trial 1 and 32 pigs in trial 2. When the average LW in the pen reached 100 ± 11.8 kg, 16 out of 40 (trial 1) and 16 out of 32 (trial 2) pigs received an experimental diet containing 17% high amylose barley (Karmosé). Half of these pigs received this diet for 14 days and the other half for 28 days, due to the applied slaughter routines. The rest of the pigs were given commercial feed. Live weights of the pigs were recorded at start of the experiment, thereafter every second week until their final weighing one day prior to slaughter. Feed consumption was recorded on a daily basis and feed conversion ratio was calculated pen wise. The pigs were slaughtered at 122.3 ± 8.6 kg LW (age 167.2 ± 7.5 days). Blood samples were taken one day prior to slaughter and back fat was taken after slaughter.

*Study III: Investigating the relationship between testicular steroids and indolic compounds in entire male pigs using an hCG injection modeling system*

A total of 34 entire male pigs of a crossbreed (2 Landrace sires × 13 Swedish Yorkshire dams) were raised. The sires were pre-selected according to their plasma skatole values, one with lower concentration (0.6 ng/ml) and one with higher (12.4 ng/ml). The entire male pigs from the two sires were randomly distributed within litter into two groups of 17 pigs: control and hCG group. Each group had 8 pigs from the high skatole level sire and 9 pigs from low skatole level sire. Four days prior to slaughter, the pigs in the hCG group were injected with

hCG (30 IU/kg LW, Pregnyl®, Organon International pharmaceutical company, Netherlands). Pigs in the control group were injected with sterile saline. Blood samples were taken from all pigs twice: just before injection and the day before slaughter. The time interval between samplings was three days. Before the main study, a pilot study with 3 entire male pigs was done. hCG injection was performed 14 d prior to slaughter, the treatment was the same as described in the main study. Blood samples were taken 5 times: the same day as hCG injection, each day during three days after injection and the day before slaughter. All pigs were slaughtered at the average LW of 114 kg.

### *Study IV: Regulation of cytochrome P450 2A6 protein expression by skatole, indole and testicular steroids in primary cultured pig hepatocytes*

Entire male pigs of a commercial Large White crossbreed (40% Large White × 40% Landrace × 20% Duroc) were used in the study. Animals were reared on a commercial standard pelleted diet (ABN, Peterborough, UK) and slaughtered in the EU-approved abattoir of the Department of Clinical Veterinary Science, University of Bristol, UK in compliance with regulations for humane care and slaughter. Samples of liver from the left lateral lobe were collected within 5 min after slaughter and were used immediately for hepatocyte isolation.

### *Study V: Gene expression of 3βHSD and 17βHSD in relation to androstenone, testosterone and E1S in entire male and castrated pigs*

Pigs from a Landrace sire × Yorkshire dam cross were used. Two separate experiments were studied. In Exp. 1, fat and testis samples from 22 entire male pigs were included. The pigs were classified in 2 groups based on androstenone concentration. The median androstenone concentration (0.74 μg/g) in the samples was arbitrarily chosen as a level below which pigs were included in the low androstenone group (LA), and above which they were included in the high androstenone group (HA).

In Exp. 2, a total of 20 pigs were included in 4 groups. One group comprised 5 male pigs surgically castrated before 1 wk of age. A second group comprised 5 male pigs treated with Improvac$^{TM}$ (Pfizer Ltd, formerly CSL Ltd, Parkville, Victoria, Australia). The remaining 10 entire pigs were classified in 2 groups, one with 5 pigs with the 5 lowest fat androstenone concentrations and the other with 5 pigs with the greatest fat androstenone concentrations. All pigs in both experiments were slaughtered at 24 wk of age (120 kg LW) in a commercial slaughterhouse.

Through out the studies (Study I to III and V), control pigs were fed a commercial diet according to the standard feeding regimen for growing pigs in Sweden (restricted, 12 MJ ME per kg, digestible CP 13%). The experimental pigs were fed with treatment diet in addition to commercial diet. The care of the pigs and the experimental design of the studies were approved by the Local Animal Ethics Committee in Tierp, Sweden, and the pigs were treated according to accepted standards for the humane treatment of animals. Blood samples were taken via jugular venipuncture into heparinised vacutainer tube. Plasma was separated by centrifugation at 2000 × g for 15 min at 4 °C and kept at -80 °C for

steroid analyses. In connection with slaughter, pigs were withheld from feed overnight but had free access to water. They were transported 5 km to the slaughterhouse and kept in lairage for 2 h. Pigs were immobilized by $CO_2$ and killed by exsanguination. Fat samples from the neck region were taken and kept at -20 °C until analyses. Testis and liver samples were taken approx. 20 min after exsanguination, frozen in liquid nitrogen, and then stored at -80 °C until analyses.

## 2.2 Skatole, indole and androstenone analyses

Androstenone, skatole and indole in fat were analysed by an HPLC method. 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 in –20 °C for 60 min and centrifuged 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 analyses.

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 (3µm, 60*4.6mm, Hewlett-Packard) with a guard column and a Stand-Alone holder. The column was operated at 40 °C.

Mobile phase for androstenone analyses was tetrahydrofurane: 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 derivatized according to the method of Hansen-Møller (1994). Spiked fat samples were prepared to construct standard curve. Linear range of the assay was from 0.1 to 2.5 µg/g (**Figure 2A**). Samples containing high amounts of androstenone (> 2.5 µg/g) were diluted with methanol to the ratio of 1:5.

Mobile phase for skatole and indole analyses was tetrahydrofurane: 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 standard curve. Linear range of the assay was from 0.002 to 0.5 µg/g for both compounds (**Figure 2B**).

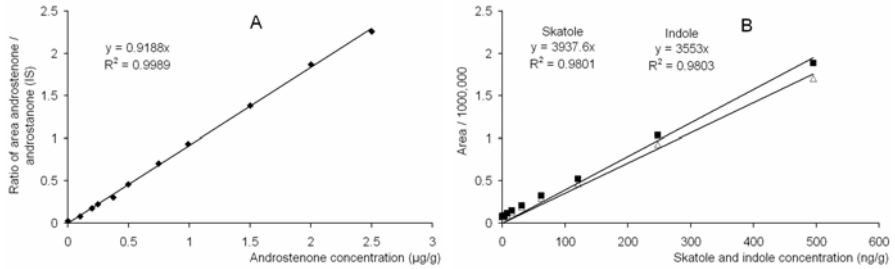


Figure 2. Standard curves of androstenone (A), skatole and indole (B) spiked in liquid fat.

Skatole equivalents in fat were measured by the colorimetric method (Mortensen & Sørensen, 1984). Skatole and indole in plasma were measured by a HPLC method described by Zamaratskaia *et al*. (2004c). Androstenone in plasma was measured by ELISA method described by Squires and Lundström (1997).

In Study I, different methods for analyzing boar taint compounds were compared, i) comparison between colorimetric and HPLC method for fat skatole analyses; ii) HPLC and ELISA for fat androstenone analyses; in addition, in order to validate HPLC method performed in our lab, the HPLC method was compared with GC-MS method which was developed previously by Mågård *et al*. (1995); iii) comparison of androstenone levels from plasma samples with and without sample extraction. For the plasma extraction procedure, ethyl acetate was used.

## 2.3 Steroids analyses

Testosterone in plasma was measured using a radioimmunoassay (RIA) kit (Coat-A-Count Total Testosterone, PITKTT-1, Diagnostic Products Corporation, Los Angeles, US). Dehydroepiandrosterone-sulfate (DHEAS) in plasma was measured by a commercial RIA kit (Coat-A-Count DHEA-SO4, PITKDS-3, Diagnostic Products Corporation, Los Angeles, US). E1S in plasma was measured by a commercial RIA kit (DSL-5400, DSL UK Ltd, Cherwell Innovation Centre, Upper Heyford, UK). Free estrone in fat was measured by an RIA method as described by Zamaratskaia *et al*., (2005).

## 2.4 Total RNA isolation, reverse transcription and real-time PCR

Total RNA was extracted from testis and liver tissues by TRIzol reagent (Invitrogen/Life technologies, USA) according to the manufacturer's protocol. The concentration of total RNA was quantified at 260 nm using the NanoDrop spectrophotometer (ND-1000, NanoDrop Technologies, Wilmington, Delaware). The quality of total RNA was assessed using Agilent's 2100 Bioanalyzer (Agilent Technologies, Palo Alto, California) with RNA chip kits (for nanogram assay; Caliper Technologies, Mountain View, California), and samples showing good RNA quality (*i.e.* low degradation and presence of 2 ribosomal peaks) were selected for further reverse transcription (**Figure 3**). Reverse transcription was performed using the First-Strand cDNA Synthesis Kit (Amersham Biosciences UK Limited, Little Chalfont, Buckinghamshire) according to the manufacturer's protocol. A total of 3 µg total RNA in 20 µl was used for reverse transcription and pd(N)6 was used for cDNA synthesis. The cDNAs were further purified using QIAquick PCR purification columns (Qiagen, Valencia, California).

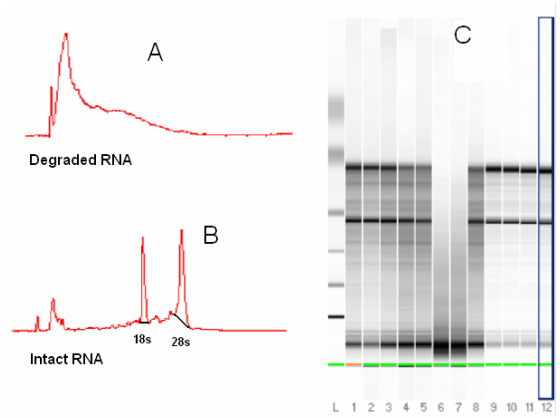


Figure 3. RNA integrity check. Degraded RNA (A) vs. Intact RNA (B). The 18S and 28S peaks are clearly visible at plot B, but not in plot A. Plot C stands for the gel electrophoresis. Lane 6-7 is totally degraded RNA. Lane 9-12 is intact RNA, with clear 28S (up) and 18S (down) rRNA bands (ratio 28S:18S approx. 2:1). The smears in Lane 1-5 show RNA which is partly degraded.

Amplification efficiency measurement: Three cDNA samples chosen randomly were used to make a serial dilution (1/5, 1/10, 1/20, 1/40, 1/80, 1/160) to determine the efficiency of specific amplification. For each sample, a plot of threshold cycle (Ct) vs. log10 of the sample dilution series was produced. The slope of this graph was used to determine the reaction efficiency by Ct slope method (Application Note TaqMan Gene Expression Assays, Applied Biosystems). For all of the sample analyses, a cDNA dilution of 1/40 was chosen, giving amplification efficiency above 95% (**Figure 4**).

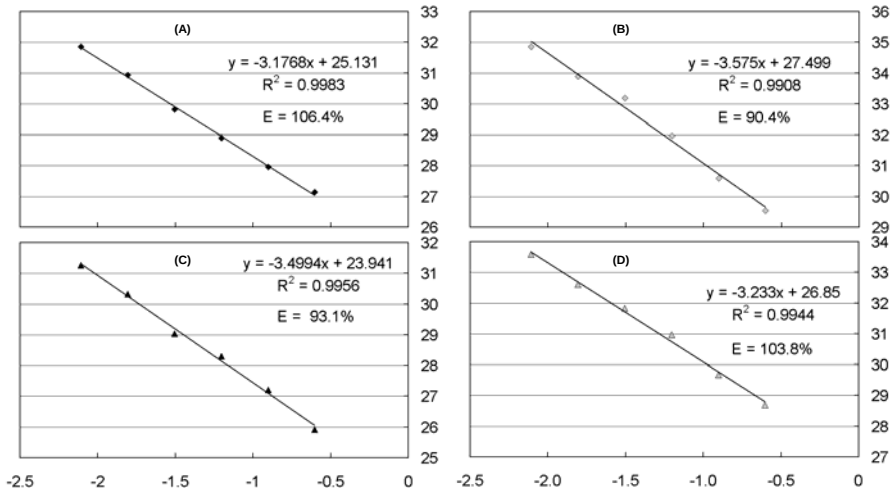


Figure 4. Amplification efficiency standard curve of gene 3βHSD (A), 17βHSD (B), TFR (C) and HPRT (D). Figures were plotted with logarithm of dilution ratio (in X-coordinate) against threshold cycle (Ct, in Y-coordinate). Each point represents the mean Ct value from three samples against the same dilution ratio. E-value stands for the efficiency of target amplification of each gene.

Fragments of 3βHSD and 17βHSD genes as well as 2 housekeeping genes [transferrin receptor (TFR) and hypoxanthine phosphoribosyltransferase (HPRT)] were amplified by real-time PCR using TaqMan system (Applied Biosystems, USA). Each reaction well was loaded with 0.7 µM primers (forward and reverse primer), 0.25 µM probe, 2.5 µl Taqman buffer A (10X), 3.5 mM $MgCl_2$, 0.2 mM dNTP, 0.025 U/µl Ampli Taq GOLD, and 200 pg/µl cDNA. The final reaction volume was 25 µl. All samples were run in triplicate. The following PCR conditions were used: 40 cycles of 15 s at 95 °C followed by 1 min at 60 °C. PCR product was analysed by 2% agarose gel (NuSieve/SeaKem, FMC Bioproducts, Rockland, Maine) electrophoresis to evaluate the amplification specificity. TFR gene in testis, HPRT gene in liver was used for data normalization. Comparative Ct method (2-ΔΔCt method, ABI User Bulletin #2) was used for 3βHSD and 17βHSD mRNA relative quantification.

## 2.5 Hepatocyte isolation and culture

Method for porcine hepatocyte isolation was described by Doran *et al*. (2002). Around 300 g of liver from left lateral lobe was collected immediately after slaughter. Liver was perfused with ice-cold buffer I (142 mM NaCl, 6.7 mM KCl, 10 mM HEPES, 0.5 mM EGTA, pH=7.4), and then transported on ice to the lab. The liver was perfused further in the lab with buffer I at 37 °C, and then changed to buffer II (buffer I without EGTA). After this, the liver was recirculating perfused with buffer III (142 mM NaCl, 6.7 mM KCl, 10 mM HEPES, 4.75 mM $CaCl_2$ and 75 mg of collagenase type IV (Sigma, Dorset, UK), pH=7.4) for 30 min. After perfusion, the liver was lightly chopped with scissors and gently agitated in ice-cold HEPES-buffered Hank's balanced salt solution (pH=7.65). The cell suspension was filtered twice through a nylon mesh, sedimented at 100 g for 5 min at 4 °C and washed twice with the same solution. Cells were suspended in Medium 199 supplemented with 10% of foetal bovine serum (Gibco BRL, Paisley, UK), 2 mM L-Glutamine (Gibco BRL, Paisley, UK), 100 U/ml penicillin and 100 µg/ml streptomycin (Sigma, Dorset, UK). Cell viability was assessed by trypan blue exclusion and routinely greater than 90%.

Approximately $6 \times 10^6$ cells were plated with 10 ml of Medium 199 on each of collagen-coated petri dishes (100 mm diameter, Appleton Woods, Birmingham, UK). Hepatocytes were cultured in a humidified atmosphere of air (95%) and $CO_2$ (5%) at 37 °C. After 24 h of pre-incubation, the medium were replaced by fresh medium with or without treatment, and the cells were further incubated for 24 h under the same conditions. No obvious suspension of hepatocytes was observed during incubation. Plates with hepatocytes were washed twice with phosphate buffered saline (PBS), scraped into 0.3 ml of PBS with added inhibitors of proteolytic enzymes antipain + pepstatin + leupeptine (1 µg/ml), and snap-frozen in liquid nitrogen. The frozen cells were stored at -80 °C until further analyses. The protein level in isolated hepatocytes was determined by the Bradford method with bovine serum albumin (BSA) as reference standard (Bradford, 1976) (**Figure 5**).

Treatments for cultured cells included testosterone, androstenone, E1S, skatole and indole with final concentrations at 1, 10, 50, 100, 500 and 1000 nM. All of the stock solutions were prepared in methanol, except that testosterone was prepared in ethanol. A volume of 50 µl of ethanol (or methanol) was added into control sample. The final concentration of methanol or ethanol added to the cells was not higher than 5%. Adding of methanol or ethanol alone at the final concentration of 5% has been reported to have no effect on cultured cells (Doran *et al*., 2002). Triplicates were performed for each concentration.

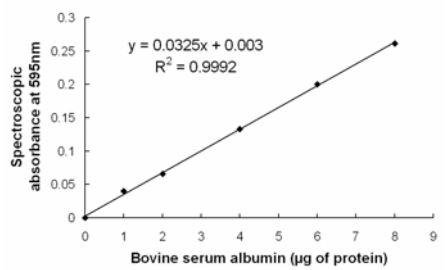


Figure 5. Bovine serum albumin (BSA) standard curve for protein quantification. Each point represents the average value of three absorbances at the same protein concentration. One microgram of protein gives the absorbance value 0.0355 at 595 nm wavelength.

## 2.6 Western blotting

Procedure for western blotting was described by Doran *et al*. (2002). In brief, a total amount 6 µg of protein per sample were loaded to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and separated at 120 V for 1 h. After electrophoresis, the proteins were transferred onto nitrocellulose membrane. The transferred membrane was blocked by fat-free powder milk first, then probed with commercial CYP2A6 polyclonal antibody. The membrane was further probed with a commercial secondary antibody (ECL horseradish peroxidase linked donkey anti-rabbit IgG; Amersham Bioscience, Bucks, UK). Two times of washing with PBST were included for each step. The membrane was developed by Enhanced Chemiluminescence (ECL) reagent following the manufacturer's instructions (Amersham Bioscience, Bucks, UK). The luminal signals were captured using KODAK processing chemicals products system (Hyperfilm MP, Kodak, UK). The film was scanned and the CYP2A6 signal was quantified using ImageQuant programme (Molecular Dynamics, GE Healthcare, Buckinghamshire, UK)

## 2.7 Statistical analyses

Statistical Analysis System (SAS Institute, Cary, NC, USA) was used throughout studies. The following procedures were used: mixed (Study I, II, III, V), Pearson correlation (Study I, III, V), and Student t-test (Study IV). Data with skewed distribution were logarithmic transformed for normalization distribution. Confidence interval and least square means were obtained after transforming back from logarithmized data.

# 3 Results

## 3.1 Methods comparison and further evaluation of the effect of live weight, sire and resistant starch on boar taint compounds (Paper I)

Validation of an HPLC method for fat androstenone analyses in our lab in comparison with a GC-MS method showed that they were highly correlated (y=0.994x + 0.200; r = 0.98; x = HPLC and y = GC-MS results). Androstenone analyses on 96 fat samples by both HPLC and ELISA method showed that the two methods were well correlated; however, results by the ELISA method were overestimated (y = 1.003x + 0.784; r = 0.90; x = HPLC and y = ELISA results). Sample extraction procedure significantly influenced plasma androstenone results, and androstenone values obtained by direct ELISA (without extraction) were about four times higher compared to those after extraction (y = 2.611x + 7.392; r = 0.70; x = ELISA with sample extraction and y = ELISA without sample extraction).

In this study, the effect of live weight (LW) at slaughter, raising system and sire selection on boar taint compounds were evaluated. In addition, feeding raw potato starch (RPS) on boar taint was also evaluated. There was a significant difference in fat androstenone levels between low LW (90 kg) and high LW (115 kg) (P = 0.02), whereas fat skatole and indole levels did not differ between LW. Raising system only influenced plasma indole levels. Sire selection based on plasma skatole levels significantly influenced skatole, androstenone and indole levels in fat in the progenies. Feeding RPS significantly reduced skatole levels in plasma and in fat, whereas indole levels were not affected. Fat androstenone levels analysed by HPLC method did not differ at 115 kg LW between control and RPS-feeding group (0.90 vs. 0.64 μg/g; P = 0.238), but plasma androstenone levels analysed by ELISA method with extraction procedure were significantly lower in RPS-feeding group at 115 kg LW than control group at the same LW (2.20 vs. 4.46 ng/ml; P < 0.001).

## 3.2 Evaluation of the effect of high amylose barley on the variation in indolic compounds and growth performance of entire male pigs (Paper II)

Skatole levels in plasma were significantly lower in the pigs fed the diet with high amylose barley (P = 0.037) although skatole levels in fat did not differ between groups (P = 0.169). Neither indole nor androstenone levels were affected by high amylose barley feed. Puberty status was not affected by dietary composition, as demonstrated by similar levels of E1S, testes weight and bulbourethral gland length in both groups. No differences were detected in daily weight gain, carcass and meat quality among the pigs fed the different diets. Analyses of commercial and high amylose barley diets showed that the resistant starch (RS) content in both feeds were similar (commercial vs. high amylose barley diet: 0.21 vs. 0.28%, respectively). RS content in pure high amylose barley and raw potato starch were 0.43% and 45.52%, respectively.

## 3.3 The effect of hCG injection on the relationship between testicular steroids and indolic compounds (Paper III)

In the pilot study, we found a rapid increase of plasma androstenone levels after hCG injection in all of the three experimental pigs. The plasma androstenone levels reached a maximum on the second day and then gradually decreased. For plasma skatole and indole levels, a pronounced increase was only observed in one of the three experimental pigs.

In the main study, an hCG injection model for regulating steroids was successfully established. In plasma, levels of androstenone, testosterone, dehydroepiandrosterone sulfate (DHEAS) and E1S were significantly increased after hCG injection. In fat, androstenone and estrone were significantly increased. For indolic compounds, plasma indole levels were increased in the hCG injected group ($P = 0.026$), whereas plasma skatole levels were not changed ($P = 0.292$). Fat indole levels were significantly increased after hCG injection ($P = 0.035$); moreover, fat skatole levels were slightly but not significantly increased after hCG injection ($P = 0.107$). Comparison of individuals over skatole sensory threshold ($\geq$ 0.20 μg/g in fat) showed that in the hCG injection group, 4 out of 17 individuals had skatole levels over threshold, whereas 1 out of 17 individuals had skatole levels over the threshold in the control group.

Testicular steroids in plasma and in fat were correlated both in control and hCG-treated pigs. Correlation between fat skatole and androstenone was not significant in controls ($r = 0.32$; $P > 0.05$), but was significant after hCG injection ($r = 0.54$; $P < 0.05$). Correlation between fat androstenone and indole was high in control pigs ($r = 0.67$; $P < 0.01$), but lower in hCG-treated pigs ($r = 0.48$; $P = 0.054$).

Pre-selected sires significantly influenced androstenone, estrone, skatole and indole levels in fat, as well as androstenone, skatole and E1S in plasma. On the contrary, main androgens (testosterone and DHEAS) did not differ between the progenies of selected sires. This suggested a strong influence of genetic background on boar taint compounds, which were independent of androgen status in our animals.

## 3.4 Evaluation of hepatocytes CYP2A6 protein expression by incubation with steroids and indolic compounds (Paper IV)

Our results showed that skatole at the final concentrations of 1, 10 and 100 nM induced CYP2A6 protein expression in cultured primary hepatocytes by 70%, 33% and 63% respectively compared with controls. There were no statistically significant differences of CYP2A6 protein levels at skatole concentrations of 1, 10 and 100 nM. Further increases in skatole concentration to 500 nM and 1000 nM resulted in decrease of CYP2A6 protein expression, which returned to the control values in the presence of 1000 nM of skatole. Similarly to skatole, incubating the hepatocytes with indole also resulted in an increase in CYP2A6 protein expression. However, induction of CYP2A6 expression by indole was more

pronounced than by skatole when comparing at similar concentrations. Expression of CYP2A6 protein in the presence of 1, 10 and 100 nM of indole was 105%, 131% and 145% higher respectively than CYP2A6 protein level in the control. Further increase of indole concentration to 500 and 1000 nM, similarly to skatole, resulted in a gradual decrease in CYP2A6 protein expression and return to the control level.

Androstenone at the final concentrations of 1, 10 and 100 nM, resulted in a significant inhibition of CYP2A6 expression by 55%, 37% and 44% respectively in hepatocytes compared with controls. Further increase of androstenone concentration to 500 and 1000 nM resulted in CYP2A6 protein expression return to control level or even higher than control. In contrast to androstenone, incubation with testosterone and E1S had no inhibitory effects on CYP2A6 protein expression at any of the concentrations studied. Moreover CYP2A6 protein expression tended to increase in the presence of 1 to 100 nM E1S, as well as in the presence of 500 and 1000 nM of testosterone. However, this increase did not reach significance.

## 3.5 Gene expression of 3βHSD and 17βHSD and the relationship with testicular steroids (Paper V)

Entire male pigs and castrated pigs were used in this study. Gene expressions of 3βHSD and 17βHSD were analyzed by real-time PCR and associated with fat androstenone levels that were predetermined by HPLC method. Association between gene expression and other testicular steroids in plasma (testosterone and E1S) were also evaluated. The male pigs with high fat androstenone concentrations had low 3βHSD gene expression in both liver and testis tissue. The gene expression of 17βHSD in liver, but not in testis varied negatively with fat androstenone concentrations. Immunocastrated and surgically castrated male pigs had nondetectable levels of androstenone in fat, and testosterone and E1S in plasma. The castration procedure induced a significant increase of both 3βHSD and 17βHSD gene expression. The mRNA levels of the 3βHSD gene were generally much higher than the 17βHSD gene both in liver and in testis. Fat androstenone was negatively correlated with liver 3βHSD gene expression ($r = -0.69$; $P < 0.05$). The 17βHSD gene expression in liver was negatively correlated with plasma E1S ($r = -0.95$; $P < 0.001$). In addition, the 3βHSD and 17βHSD gene expression in liver of entire male pigs were strongly correlated ($r = 0.86$; $P < 0.01$).

# 4 Discussion

## 4.1 Comparing different methods for analyses of boar taint compounds

A HPLC method for fat androstenone, skatole and indole were developed in our laboratory. This method followed the principle described by Hansen-Møller (1994). Different mobile phase, as well as manual derivatisation for androstenone analysis was performed in order to adapt to our own system. The accuracy of the HPLC method for androstenone was additionally evaluated by analyzing 20 fat samples by HPLC and GC-MS (Mågård *et al*., 1995), and the results from the two methods were well correlated. HPLC and ELISA methods were also compared for fat androstenone analyses. In our study, the results from the ELISA method were overestimated, which is inconsistent with a previous report by Claus *et al*. (1997). However, at low concentrations (below 1.0-1.5 μg/g) of androstenone levels, variation between the two methods was large in their study. It should be noted that different androstenone antibodies were used between labs, and also that the optimized ELISA procedures were different. It is likely that the difference of specificity of these antibodies caused the variation in the results. In addition, the possibility of the presence of some unknown androstenone metabolites in the adipose tissue could not be excluded. Our results suggest that the HPLC method is a more accurate way to measure androstenone concentrations in fat. Androstenone in plasma were measured by ELISA method including sample extraction with ethyl acetate or without extraction. This was done to evaluate the extraction procedure on plasma androstenone analyses. Plasma androstenone levels from analyses without sample extraction were much higher compared with the analyses with sample extraction, even though the correlation between these two methods was high (r=0.70). We assume this might be due to non-specificity of antibodies for androstenone measurements. Cross-reactivity of the antibodies against androstenone metabolites cannot be excluded. A recent study showed that most androstenone in plasma exists in sulfated form (Sinclair & Squires, 2005a). 3β-androstenol is another androstenone metabolite (Doran *et al*., 2004; Nicolau-Solano *et al*., 2006). In addition, the binding of androstenone to plasma components might inhibit the efficiency of sample extraction by organic solvent. Moreover, 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.

In our study, results from the colorimetric method and the HPLC method for fat skatole analyses were highly correlated. The colorimetric method measures the total amount of indolic compounds *e.g.* skatole and indole, whereas the HPLC method determine skatole and indole levels separately. The colorimetric method is based on measurements in homogenized fat tissue, whereas we use liquid fat in the HPLC analysis. Gibis (1994) reported that skatole values measured in liquid fat were approximately 34% higher than those in homogenized fat. On the other hand, 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 and

highly correlated. A previous study also reported high correlations between these two methods (r = 0.973; n = 137; Hansen-Møller, 1994). However, moderate correlations between the two methods (r = 0.50) were reported by Gibis (1994).

## 4.2 Influence of live weight and feeding raw potato starch on boar taint compounds

Feeding raw potato starch (RPS) on reducing fat skatole accumulation in entire male pigs has been well illustrated (Jensen *et al*., 1995a; Losel & Claus, 2005; Willig *et al*., 2005; Zamaratskaia *et al*., 2005b), and this effect was further supported by our study. Claus *et al*. (2003) explained that RPS fermentation in the large intestine produced short chain fatty acids, mainly butyric acid, which can inhibit colonocyte apoptosis and thus lead to decreased amount of cell debris, the key source of tryptophan for skatole synthesis. However, there is one question remaining, as indole is produced from L-tryptophan, the same origin as skatole. If tryptophan availability was reduced due to a low rate of cell apoptosis, indole production should also be reduced. Due to the fact that RPS did not affect indole levels, we assume there might be other mechanisms explaining the skatole reduction by RPS. Indole synthesis in the large intestine is mediated by many types of bacteria, whereas skatole production requires the presence of highly specific bacteria, *e.g. Lactobacillus* and *Clostridium* (Jensen *et al*., 1995b). Microflora adaptation towards more beneficial growth might contribute to skatole reduction. An *in vitro* study by Xu *et al*. (2002) showed that fructooligosaccharide (FOS) can considerably suppress the growth of pathogenic bacteria, *E. coli* and *Clostridium spp*. Moreover, incubation of FOS with pig faecal slurries significantly reduced skatole production from tryptophan, whereas indole production was not affected (Xu *et al*., 2002). Rideout *et al*. (2004) reported that feeding pigs with chicory inulin did not change indole levels in faeces, a possible indicator of intestinal indole production. Raw potato starch, similar to FOS and inulin, is not digestible in the small intestine and is fermented by the intestinal bacteria to yield short chain fatty acids (Claus *et al*., 2003). It is possible that RPS fermentation products inhibit skatole-producing bacteria in the large intestine, whereas indole-producing bacteria remain unaffected. Zamaratskaia *et al*. (2005a; 2006) reported that feeding RPS did not affect CYP enzymes expression, suggesting that RPS reduce fat skatole levels only by reducing its formation *in vivo* and not by influencing its metabolism.

In our study, neither skatole nor indole levels differed between 90 and 115 kg LW. This is inconsistent with our previous study where 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*., 2004a). This inconsistent result needs to be further checked. Fat androstenone levels were significantly higher at 115 kg than at 90 kg LW, which could be expected. Androstenone levels increase simultaneously with other testicular steroids at puberty. No effect of LW on plasma androstenone levels was found in the present study. As well, RPS did not significantly change androstenone accumulation in fat, but reduced androstenone levels in plasma when analysed by ELISA method with sample extraction procedure.

## 4.3 Evaluation of a high amylose barley cultivar (Karmosé) on boar taint and performance of entire male pigs

The composition of barley starch is highly variable due to different genetic background and growing conditions. Amylose content, which is important to starch resistant character (Xue *et al*., 1996b), varies between 0 to 45% in different barleys (Li *et al*., 2001). More recently, a novel barley cultivar (Himalaya 292) was introduced (Morell *et al*., 2003), which contains higher proportion of amylose and higher total and soluble non-starch polysaccharides (NSP) (Bird *et al*., 2004a). Feeding pigs with Himalaya 292 increased large-bowel fermentation and short chain fatty acid (SCFA) concentrations (Bird *et al*., 2004b). Because increasing fermentation might result in skatole reduction, the barley cultivar may be potentially used as a skatole-reducing means. In the present study, we evaluated the effect of a high amylose barley cultivar (Karmosé) (HAB) diet on skatole accumulation in entire male pigs. Skatole levels were significantly reduced in plasma in the pigs fed the HAB diet. The levels of skatole in fat were numerically lower in the pigs fed HAB, although not statistically significant. The inconsistency between the effect of HAB diet on skatole levels in plasma and fat might be due to overall low skatole levels in fat in the present study. Only 7 out of 69 pigs had fat skatole levels over 0.2 μg/g, the threshold used to detect tainted carcasses in Sweden; 5 of those 7 pigs were from the control group, and 2 from the HAB group. Besides, Lösel and Claus (2005) demonstrated that the magnitude of skatole reduction in fat by RPS was dose-dependent. In the present study, 17% of HAB in diet was used, and the RS content in HAB was 0.43%, which is very low compared with the RS in RPS (45.52%). In addition, the feeding period that we used *i.e.* either 14 or 28 d before slaughter may not be long enough to reduce skatole levels. Further research is needed to evaluate the effect of HAB on fat skatole levels. In our study, growth performance and meat quality were not changed by feeding the HAB diet, which is consistent with another study (Bird *et al*., 2004b). Additionally, HAB may have much broader implications than just altering boar taint level, considering its potential beneficial effect on animal health, *e.g.* reduced plasma cholesterol and increased large-bowel SCFA production, as reported by Bird *et al*. (2004b).

## 4.4 hCG injection on the relationship between testicular steroids and indolic compounds

The effect of hCG injection on stimulating androstenone as well as on other testicular steroids has been well reported. Plasma androstenone can reach maxium between 24-30 h (Andresen, 1975; Carlström *et al*., 1975; Malmfors *et al*., 1976; Lundström *et al*., 1978), or between 24-48 h (Bonneau *et al*., 1982) after hCG injection. Whereas the increase of fat androstenone levels usually occurs later than in plasma (Malmfors *et al*., 1976; Bonneau *et al*., 1982), the increase of plasma testosterone occurrs in parallel with plasma androstenone (Carlström *et al*., 1975). Our results further showed that the maximum of plasma androstenone levels appeared at the 2nd day after hCG injection and subsequently decreased. Other testicular steroids in plasma, *i.e.* testosterone, DHEAS, E1S were significantly increased at the sampling 3 d after hCG injection. Both androstenone and estrone

in fat also significantly increased 4 d after hCG injection. At 14 d after hCG injection, the plasma androstenone declined to the same level as before hCG injection.

Accumulation of high fat skatole levels occur more frequently in entire male than in female and castrated pigs, and an association between puberty development and plasma skatole escalation has also been suggested (Zamaratskaia *et al*., 2004c). Our study evaluated the changes in indole and skatole levels after hCG injection. Indole levels in plasma and in fat were significantly increased, and skatole levels in fat were also slightly increased. More recently, a study showed that hCG injection induced a significant increase in fat skatole levels in two pig breeds (Duroc and Landrace), and this induction was associated with a reduced CYP2E1 and CYP2A enzyme activity after hCG injection (Zamaratskaia *et al*., 2007b). Babol *et al*. (1999) suggested that androstenone might be involved in skatole metabolism. Doran *et al*. (2002) showed that androstenone inhibited induction of CYP2E1 by skatole in hepatocytes. Tambyrajah *et al*. (2004) found that androstenone inhibited CYP2E1 enzyme expression through inhibitory binding to COUP-TF1, one of the transcription factors for CYP2E1. Moreover, Cue *et al*. (2007) reported a concurrent low protein expression of both CYP2E1 and COUP-TF1 in Duroc entire male pigs in comparison with Large White pigs, and concluded that a low level of CYP2E1 protein might be due to a defective expression of COUP-TF1. The low protein expressions of CYP2E1 and COUP-TF1 can be an explanation for the high skatole levels in the Duroc breed observed by Babol *et al*. (2004). However, other studies reported low fat skatole levels in the Duroc breed in comparison with Hampshire breed (Squires & Lou, 1995; Xue *et al*., 1996a) or Landrace breed (Zamaratskaia *et al*., 2007b). Previous studies show that Duroc breed has earlier puberty development and higher fat androstenone accumulation (Christenson & Ford, 1979; Xue *et al*., 1996a). It might be assumed that the high androstenone levels can inhibit CYP2E1 protein expression through inhibiting COUP-TF1, by reducing skatole metabolism and causing high skatole accumulation. Besides skatole metabolism, skatole production and uptake are also important in regulating skatole levels in entire male pigs. Anabolic hormones, *e.g.* testosterone, accelerate the cell renewal and apoptosis in the intestine (Claus *et al*., 1994), which increase the L-tryptophan availability and result in more skatole production. The disturbed balance between skatole production and metabolism due to increased steroid levels by hCG injection might explain the increase in fat skatole accumulation (Zamaratskaia *et al*., 2007b), and also the indole increase in both plasma and in fat. The exact mechanism behind the effect of hCG stimulation on the levels of skatole and indole accumulation remains to be elucidated.

## 4.5 Effect of sire selection on accumulation of boar taint compounds in progeny

The effect of sire selection based on plasma skatole levels on influencing fat skatole levels of progenies has been evaluated. Our results from both Study I and III showed significant differences in skatole concentrations in plasma and fat between the offspring of different sires. This confirms that genetic background is an important factor regulating skatole levels (Lundström *et al*., 1994).

Additionally, progenies with low fat skatole levels also had low levels of androstenone, free estrone and indole in fat, whereas the plasma testosterone and DHEAS did not differ between groups. The similar pattern between androstenone, skatole and indole in association with pre-sire selection is difficult to explain. We assume that androstenone levels *in vivo* might influence skatole metabolism.

## 4.6 Regulation of cytochrome P450 2A6 protein expression by skatole, indole and testicular steroids in primary cultured pig hepatocytes

The present study used primary cultured hepatocytes as a model system, and investigated whether pig hepatic CYP2A6 protein expression is regulated by the testicular steroids testosterone, androstenone or E1S, as well as the two indolic compounds, skatole and indole. The study showed that CYP2A6 expression was significantly inhibited by low concentrations of androstenone; this is consistent with the previous observation that androstenone downregulates the skatole-induced expression of CYP2E1 in cultured pig hepatocytes (Doran *et al*., 2002). Another study also showed that CYP2A6 activity was inhibited by androstenone (Zamaratskaia *et al*., 2007a). However, the effect of androstenone on CYP2A6 activity (Zamaratskaia *et al*., 2007a) was smaller than the effect of androstenone on CYP2A6 protein expression (the present study). This might be partially explained by the use of different experimental systems (isolated microsomes and isolated hepatocytes respectively) and different ranges of androstenone concentrations. The levels of steroid treatment used in this study are similar to steroid concentrations used by other authors for cell culture experiments (Schwenk and Del Pino, 1980; Sinclair *et al*., 2005b). It should be noted that the inhibitory effect of androstenone on CYP2A6 protein expression was only observed at low androstenone concentrations (1, 10 and 100 nM). The effect was abolished when androstenone concentrations were increased to 500 nM and 1000 nM. The mechanism of this response is unknown. In contrast to androstenone, testosterone did not significantly affect CYP2A6 protein expression in our experiments. This finding is consistent with the results of Zamaratskaia *et al*. (2007a) who demonstrated that testosterone does not alter CYP2A6 enzyme activity in hepatic microsomes isolated from entire male pigs. Moreover, the present study did not show any significant effect of E1S on the expression of CYP2A6 protein in primary cell hepatocytes. Previous studies reported that an increase in skatole level in pig adipose tissue is accompanied by an elevated level of E1S in plasma and fat (Babol *et al*., 1999, Zamaratskaia *et al*., 2005c). We assume that E1S may directly affect CYP2A6 activity without influencing CYP2A6 expression, and this needs to be further researched.

Our study showed that CYP2A6 protein expression in primary cultured hepatocytes can be induced by its substrates, skatole and indole. These results are consistent with the previous finding that skatole induces expression of the other skatole-metabolising enzyme, CYP2E1, in isolated pig hepatocytes (Doran at al., 2002). Cytochrome P450 system (CYP450), in particular the isoforms CYP2E1 and CYP2A6 are involved in the first stage of hepatic skatole metabolism (Babol *et al*., 1998; Diaz & Squires, 2000). Liver is the main tissue responsible for skatole metabolism in entire male pigs (Squires and Lundström, 1997; Lin *et al*., 2004a;

2006). The mechanisms regulating the expression of CYP450 isoforms in pig liver have not been completely understood. We also observed a biphasic response of CYP2A6 protein expression to skatole and indole treatments, *i.e.* an increase in the CYP2A6 level at low (physiological) concentrations of the treatments and a decline in the CYP2A6 protein level at high concentrations. The reason for the biphasic response is not clear. One possible explanation could be a cytotoxic effect at high concentrations. The biphasic response of CYP450 enzyme was also shown in another study (Donato *et al*., 2000). An interesting observation in the present study is that the effect of indole on CYP2A6 protein expression was higher than the effect of skatole (on average 127% versus 55%). It is well known that enzyme expression can be induced by its substrates; therefore, we speculate that indole might be a more preferable substrate for the porcine CYP2A6. This hypothesis is consistent with observations of Gillam *et al*. (2000) showing that CYP2A6 is the most active cytochrome in the formation of at least two products of oxidative indole metabolism and that CYP2E1 has less input in indole metabolism in this system than CYP2A6.

## 4.7 3βHSD and 17βHSD gene expression in entire male pigs

Accumulation of fat androstenone levels is mainly determined by androstenone production in testis and metabolism in liver. Recently, more studies were done in relation to androstenone metabolism. Doran *et al*. (2004) established that the enzyme 3βHSD in liver can catalyze the transformation of androstenone to 3β-androstenol. Moreover, sulfated and glucuronidated androstenone have also been determined (Sinclair *et al*., 2005a; 2006). In our study, we determined 3βHSD mRNA levels in entire male pigs by real-time PCR. The results showed that high 3βHSD gene expressions in liver and in testis were associated with low fat androstenone accumulation. In addition, the negative correlation between liver 3βHSD and fat androstenone was also strong. This further confirmed 3βHSD enzyme as a likely candidate in catalyzing androstenone metabolism. In contrast to 3βHSD gene expression, the expression of 17βHSD gene in testis was not different between entire male pigs with high or low fat androstenone levels. However, the levels of 17βHSD mRNA in liver varied negatively with fat androstenone. The different pattern of 17βHSD gene expression suggests that the 17βHSD enzyme catalytic activity might vary between tissues. It is known that 17βHSD in human is involved in transformation of estrone to active estradiol (see review by Payne and Hales, 2004), but its function in pigs has not been determined. Our study of the entire male pigs revealed a strong negative correlation between liver 17βHSD gene expression and plasma E1S, suggesting an important role of the 17βHSD enzyme in the metabolism of pig estrogens. Our study showed that E1S concentration in plasma of entire male pigs at 120 kg LW was greater than the main anabolic steroid hormone, testosterone. This increased amount of E1S in the blood circulation in entire male pigs has been reported elsewhere (Schwarzenberger *et al*., 1993), and this is possibly because the boar requires estrogens as well as androgens for normal activity (Joshi and Raeside, 1973).

## 4.8 3βHSD and 17βHSD gene expression in castrated pigs

In our study, both surgical castration and immunocastration caused a significantly higher 3βHSD gene expression in liver compared with entire male pigs with high fat androstenone concentrations. To the best of our knowledge, this has not been reported before, and the mechanism is not obvious. However, it is well known that steroid hormones are synthesised via LH stimulating testicular Leydig cells, and that castration completely blocks this pathway. Thus, the induction of 3βHSD gene expression might be caused by the absence of steroids after castration. Indeed, there is evidence showing that androgens can downregulate human type II 3βHSD transcription in the cortical cells and in testicular Leydig cells (see Simard *et al*., 2005 for review). Like 3βHSD, 17βHSD gene expression in liver was also significantly higher after castration, and the increase in immunocastrated pigs was more prominent than in surgically castrated pigs. This induction of both 3βHSD and 17βHSD gene expression in the castrated pigs suggests that the absence of testicular steroids not only affects 3βHSD transcription regulation, but also the 17βHSD transcription. However, 17βHSD mRNA levels in liver did not differ between either immunocastrated pigs and low androstenone entire male pigs, or surgically castrated pigs and low androstenone entire male pigs. Our study showed that immunocastrated pigs and entire male pigs with low fat androstenone concentrations differed significantly in 3βHSD gene expression in testis, but not in liver. We assume that immunocastration induces more 3βHSD gene expression in testis than in liver because this procedure directly changes the testicular physiological properties.

# 5 Main conclusions

The findings of this thesis can be summarized as follow:

- The HPLC method for fat androstenone, skatole and indole analyses was well applied in our laboratory. Fat androstenone results analyzed by HPLC were comparable with those analyzed by GC-MS method. However, in our laboratory, fat androstenone results analyzed by ELISA method were overestimated.

- Fat skatole results analyzed by HPLC and colorimetric method were comparable even though different sample matrix were used.

- We further confirmed previous findings that fat skatole levels were significantly reduced by feeding raw potato starch (RPS) to entire male pigs. RPS did not change fat androstenone and indole levels. Entire male pigs had lower fat androstenone levels at 90 kg live weight than at 115 kg live weight, but fat skatole and indole levels did not differ between different live weights.

- Sire selection based on plasma skatole levels significantly influenced fat skatole accumulation in the progenies. In addition, variation of fat androstenone and indole accumulation by sire selection showed a similar pattern with fat skatole accumulation.

- hCG injection influenced indolic compounds levels in entire male pigs. Indole levels in fat and plasma were significantly increased after hCG injection; skatole levels in fat tended to be increased, but skatole levels in plasma were not affected.

- Androstenone significantly inhibited cytochrome P450 2A6 (CYP2A6) protein expression in cultured hepatocytes. Skatole and indole significantly induced increase of CYP2A6 protein expression, and the effect by indole was more prominent. Testosterone and E1S did not affect hepatic CYP2A6 protein expression.

- High gene expression of 3βHSD was negatively associated with low fat androstenone accumulation in entire male pigs. The 17βHSD gene expression in liver, but not that in testis, was negatively associated with fat androstenone levels. Castration procedure (surgical castration and immunocastration) induced a significant increase of both 3βHSD and 17βHSD gene expression in liver. Moreover, the gene expression of 3βHSD was generally much higher than 17βHSD.

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# 7 References


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

Andersson, H., Wallgren, M., Rydhmer, L., Lundström, K., Andersson, K. & Forsberg, M. 1998. Photoperiodic effects on pubertal maturation of spermatogenesis, pituitary responsiveness to exogenous GnRH, and expression of boar taint in crossbred boars. *Animal Reproduction Science* 54, 121-137.

Andresen, Ø. 1975. 5α-Androstenone in peripheral plasma of pigs, diurnal variation in boars, effects of intravenous hCG administration and castration. *Acta Endocrinologiga* 78, 385-391.

Andresen, Ø. 1976. Concentrations of fat and plasma 5 alpha-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.

Annor-Frempong, I.E., Nute, G.R., Whittington, F.W. & Wood, J.D. 1997. The problem of taint in pork: 1. Detection thresholds and odour profiles of androstenone and skatole in a model system. *Meat Science* 46, 45-55.

Annor-Frempong, I.E., Nute, G.R., Wood, J.D., Whittington, F.W. & West, A. 1998. The measurement of the responses to different odour intensities of boar taint using a sensory panel and an electronic nose. *Meat Science* 50, 139-151.

Auchus, R.J., Lee, T.C. & Miller, W.L. 1998. Cytochrome b5 augments the 17,20-lyase activity of human P450c17 without direct electron transfer. *The Journal of Biological Chemistry* 273, 3158–3165.

Babol, J., Squires, E.J. & Lundström, K. 1998. Relationship between oxidation and conjugation metabolism of skatole in pig liver and concentrations of skatole in fat. *Journal of Animal Science* 76, 829-838.

Babol, J., Squires, E.J. & Lundström, K. 1999. Relationship between metabolism of skatole and androstenone in intact male pigs. *Journal of Animal Science* 77, 84-92.

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, 351-358.

Baek, C.E., Mansen-Møller, J., Friis, C., Cornett, C. & Hansen, S.H. 1997. Identification of selected metabolites of skatole in plasma and urine from pigs. *Journal of Agricultural and Food Chemistry* 45, 2332-2340.

Bird, A.R., Flory, C., Davies, D.A., Usher, S. & Topping, D.L. 2004a. A novel barley cultivar (Himalaya 292) with a specific gene mutation in starch synthase IIa raises large bowel starch and short-chain fatty acids in rats. *Journal of Nutrition* 134, 831-835.

Bird, A.R., Jackson, M., King, R.A., Davies, D.A., Usher, S. & Topping D.L. 2004b. A novel high-amylose barley cultivar (Hordeum vulgare var. Himalaya 292) lowers plasma cholesterol and alters indices of large-bowel fermentation in pigs. *British Journal of Nutrition* 92, 607-615.

Bonneau, M., Desmoulin, B. & Dumont, B.L. 1979. Production de viande de porcs mâles entiers ou castrés: efficacité alimentaire et composition corporelle chez les races hypermusclées. *Ann. Zootech*. 28, 53–72.

Bonneau, M. & Desmoulin, B. 1981. Influence de l'exces de tryptophane et des conditions d'elevage sur la frequence des odeurs sexuelles des jeune porcs males entiers: relation avec le developpement de l'appareil genital. *Journées de la Recherche Porcine en France*. 13, 329-334.

Bonneau, M., Meusy-Dessolle, N., Léglise, P.C. & Claus, R. 1982. Relationships between fat and plasma androstenone and plasma testosterone in fatty and lean young boars during growth and after hCG stimulation. *Acta endocrinologica* 101, 119-128.

Bonneau, M., Dufour, R., Chouvet, C., Roulet, C., Meadus, W. & Squires, E.J. 1994. The effects of immunization against luteinising hormone-releasing hormone on performance,

sexual development, and levels of boar taint-related compounds in intact male pigs. *Journal of Animal Science* 72, 14-20.
Bonneau, M. 1998. Use of entire males for pig meat in the European Union. *Meat Science* 49, S257-272.
Bonneau, M., Kempster, A.J., Claus, R., Claudi-Magnussen, C., Diestre, A., Tornberg, E., Walstra, P., Chevillon, P., Weiler, U. & Cook, G.L. 2000a. 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, 251-259.
Bonneau, M., Walstra, P., Claudi-Magnussen, C., Kempster, A.J., Tornberg, E., Fischer, K., Diestre, A., Siret, F., Chevillon, P., Claus, R., Dijksterhuis, G., Punter, P., Matthews, K.R., Agerhem, H., Béague, M.P., Oliver, M.A., Gispert, M., Weiler, U., von Seth, G., Leask, H., Font i Furnols, M., Homer, D.B. & Cook, G.L. 2000b. An international study on the importance of androstenone and skatole for boar taint: IV. Simulation studies on consumer dissatisfaction with entire male pork and the effect of sorting carcasses on the slaughter line, main conclusions and recommendations. *Meat Science* 54, 285-295.
Bowen, A., Khan, S., Berghman, L., Kirby, J.D., Wettemann, R.P. & Vizcarra, J.A. 2006. Immunization of pigs against chicken gonadotropin-releasing hormone-II and lamprey gonadotropin-releasing hormone-III: effects on gonadotropin secretion and testicular function. *Journal of Animal Science* 84, 2990-2999.
Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72,** 248-254.
Brennan, J.J., Shand, P.J., Fenton, M., Nicholls, L.L. & Aherne, F.X. 1986. Androstenone, androstenol and odour intensity in backfat of 100- and 130-kg boars and gilts. *Canadian Journal of Animal Science* 66, 615-624.
Brooks, R.I. & Pearson, A.M. 1986. Steroid hormone pathways in the pig, with special emphasis on boar odour: A review. *Journal of Animal Science* 62, 632-645.
Brooks, R.I. & Pearson, A.M. 1989. Odour thresholds of the C19-Δ16-steroids responsible for boar odour in pork. Meat Science 25, 11-19.
Carlström, K., Malmfors, B., Lundström, K., Edqvist, L.E. & Gahne, B. 1975. The effect of HCG on blood plasma levels of 5α-androstenone and testosterone in the boar. *Swedish journal of agricultural research* 5, 15-21.
Christenson, R.K. & Ford, J.J. 1979. Puberty and estrus in confinement-reared gilts. *Journal of Animal Science* 49, 743-751.
Chung, B.C., Matteson, K.J., Voutilainen, R., Mohandas, T.K. & Miller, W.L. 1986. Human cholesterol side-chain cleavage enzyme, P450scc: cDNA cloning, assignment of the gene to chromosome 15, and expression in the placenta. *Proceedings of the National Academy of Sciences 83*, 8962–8966.
Claus, R. 1974. Radioimmunoassay of 5-alpha-androst-16-en-3-one, steroid responsible for boar taint, in the adipose tissue of swine. *Comptes Rendus Hebdomadaires Des Seances De l'Academie Des Sciences. Serie D: Sciences Naturelles* 278, 299-302 (French).
Claus, R., Mahler, G. & Münster, E. 1988. Determination of boar taint steroid 5-alpha-androst-16-en-3-one in adipose tissue of pigs with rapid microtiter plate enzyme-immunoassay (MTE). *Archiv für Lebensmittelhygiene* 39, 87-90.
Claus, R., Weiler, U. & Herzog, A. 1994. Physiological aspects of androstenone and skatole formation in the boar-a review with experimental data. Meat Science 38, 289-305.
Claus, R., Raab, S. & Röckle, S. 1996. Skatole concentrations in blood plasma of pigs as influenced by the effects of dietary factors on gut mucosa proliferation. Journal of Animal Physiology and Animal Nutrition 76, 170-179.
Claus, R., Herbert, E. & Dehnhard, M. 1997a. 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. & Raab, S. 1999. Influences on skatole formation from tryptophan in the pig colon. *Advances in Experimental Medicine and Biology* 467, 679-684.

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, 239-248.

Colonna, P., Leloup, V. & Buleón, A. 1992. Limiting factors of starch hydrolysis. *European Journal of Clinical Nutrition* 46, S17-S32.

Cue, R.A., McGivan, J.D., Wood, J.D. & Doran, O. 2007. Variations in expression of CYP2E1 and COUP-TF1 proteins between Large White and Duroc cross-breeds. In: *Proceedings of 53rd International Congress of Meat Science and Technology*. China Agricultural University Press. Pp. 77-78.

Cummings, J.H. & Englyst, H.N. 1995. Gastrointestinal effects of food carbohydrate. *American Journal of Clinical Nutrition* 61, 938S–945S.

Davis, S.M. & Squires, E.J. 1999. Association of cytochrome b5 with 16-androstene steroid synthesis in the testis and accumulation in the fat of male pigs. *Journal of Animal Science* 77, 1230-1235.

De Brabander, H.F. & Verbeke, R. 1986. Quantitative determination of androstenone in pig adipose tissue. *Journal of Chromatography A* 363, 293-302.

Dehngard, M., Claus, R., Hillenbrand, M. & Herzog, A. 1993. High-performance liquid chromatographic method for the determination of 3-methylindole (skatole) and indole in adipose tissue of pigs. *Journal of Chromatography A* 616, 205-209.

Deslandes, B., Gariépy, C. & Houde, A. 2001. Review of microbiological and biochemical effects of skatole on animal production. *Livestock Production Science* 71, 193-200.

Diaz, G.J., Skordos, K.W., Yost, G.S. & Squires, E.J. 1999. Identification of phase I metabolites of 3-methylindole produced by pig liver microsomes. *Drug Metabolism and Disposition* 27, 1150-1156.

Diaz, G.J., Squires, E.J. 2000. Metabolism of 3-methylindole by porcine liver microsomes: responsible cytochrome P450 enzymes. Toxicological Science 55, 284-292.

Diaz, G.J. & Squires, E.J. 2003. Phase II in vitro metabolism of 3-methylindole metabolites in porcine liver. *Xenobiotica* 33, 485-498.

Dijksterhuis, G.B., Engel, B., Walstra, P., Font i Furnols, M., Agerhem, H., Fischer, K., Oliver, M.A., Claudi-Magnussen, C., Siret, F., Béague, M.P., Homer, D.B. & Bonneau, M. 2000. An international study on the importance of androstenone and skatole for boar taint: II. Sensory evaluation by trained panels in seven European countries. Meat Science 54, 261-269.

Donato, M.T., Viitala, P., Rodriguez-Antona, C., Lindfors, A., Castell, J.V., Raunio, H., Gomez-Lechon, M.J. & Pelkonen, O. 2000. CYP2A5/CYP2A6 expression in mouse and human hepatocytes treated with various in vivo inducers. *Drug Metabolism and Disposition* 28, 1321-1326.

Doran, E., Whittington, F.W., Wood, J.D. & McGivan, J.D. 2002. Cytochrome P450IIE1 (CYP2E1) is induced by skatole and this induction is blocked by androstenone in isolated pig hepatocytes. *Chemico-Biological Interactions* 140, 81-92.

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.

Dunshea, F.R., Colantoni, C., Howard, K., McCauley, I., Jackson, P., Long, K.A., Lopaticki, S., Nugent, E.A., Simons, J.A., Walker, J. & Hennessy, D.P. 2001. Vaccination of boars with a GnRH vaccine (Improvac) eliminates boar taint and increases growth performance. *Journal of Animal Science* 79, 2524-2535.

Ellis, M., Lympany, C., Haley, C.S., Brown, I. & Warkup, C.C. 1995. The eating quality of pork from Meishan and large white pigs and their reciprocal crosses. *Animal Science* (Pencaitland) 60, 125-131.

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

Furster, C. 1999. Hepatic and extrahepatic dehydrogenation/isomerisation of 5-cholestene-3β,7α-diol: localization of 3β -hydroxy-Δ5-C27-steroid dehydrogenase in pig tissues and subcellular fractions. *Biochimica et Biophysica Acta-Molecular and Cell Biology of Lipids* 1436, 343–353.

García-Regueiro, J.A. & Rius, M.A. 1998. Rapid determination of skatole and indole in pig back fat by normal-phase liquid chromatography. *Journal of Chromatography A* 809, 246-251.
Gibis, M. 1994. Influence of substance indole and skatole on meat quality. *Doctoral Thesis*, Allgemeine und Angewandte Naturwissenschaften der Universität Hohenheim, Germany.
Gibson, G. R. & Roberfroid, M. B. 1995. Dietary modulation of the human colonic microbiota—introducing the concept of prebiotics. *Journal of Nutrtion* 125, 1401–1412.
Giersing, M., Lundström, K. & Andersson, A. 2000. Social effects and boar taint: significance for production of slaughter boars (Sus scrofa). *Journal of Animal Science* 78, 296-305.
Gillam, E.M., Notley, L.M., Cai, H., De Voss, J.J. & Guengerich, F.P. 2000. Oxidation of indole by cytochrome P450 enzymes. *Biochemistry* 39, 13817–13824.
Grover, P.K. & Odell, W.D. 1975. Correlation of in vivo and in vitro activities of some naturally occurring androgens using a radioreceptor assay for 5α-dihydrotestosterone with rat prostate cytosol receptor protein. *Journal of Steroids and Biochemistry* 6, 1373-1379.
Hall, P.F. 1970. Endocrinology of the testis. In: *The testis. Vol. II. Biochemistry*. Academic Press, New York. Pp. 1-71.
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 Agriculturae Scandinavica, Section A, Animal science*, 45, 178-185.
Hansen, L.L., Mejer, H., Thamsborg, S.M., Byrne, D.V., Roepstorff, A., Karlsson, A.H., Hansen-Møller, J., Jensen, M.T. & Tuomola. M. 2006. Influence of chicory roots (Cichorium intybus L) on boar taint in entire male and female pigs. *Animal Science* 82, 359-368.
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, 219-230.
Hansen-Møller, J. & Kjeldsen, N.J. 1998. Introduction to Danish research on boar taint. In: *Skatole and boar taint (ed W.K. Jensen)*. Danish Meat Research Institute, Roskilde, Denmark. Pp 13-20.
Hansen-Møller, J. 1998. Analytical methods for determination of boar taint compounds. In: *Skatole and boar taint (ed W.K. Jensen)*. Danish Meat Research Institute, Roskilde, Denmark. Pp 21-40.
Hansson, I., Lundström, K. & Malmfors, B. 1975. Effect of sex and weight on growth, feed efficiency and carcass characteristics of pigs. 2. Carcass characteristics of boars, barrows and gilts, slaughtered at four different weights. *Swedish Journal of Agricultural Research* 5, 69-80.
Hansson, K.-E., Lundström, K., Fjelkner-Modig, S. & Persson, J. 1980. The importance of androstenone and skatole for boar taint. *Swedish Journal of Agricultural Research* 10, 167-183.
Hizukuri, S. 1985. Relationship between the distribution of the chain length of amylopectin and the crystalline structure of starch granules. *Carbohydrate Research* 141, 295-306.
Jaros, P., Bürgi, E., Stärk, K.D.C., Claus, R., Hennessy, D. & Thun, R. 2005. Effect of active immunization against GnRH on androstenone concentration, growth performance and carcass quality in intact male pigs. *Livestock Production Science* 92, 31-38.
Joshi, H.S. & Raeside, J.I. 1973. Synergistic effects of testosterone and oestrogens on accessory sex glands and sexual behaviour of the boar. *Journal of Reproduction and Fertility* 33, 411-423.
Jensen, M.T., Cox, R.P. & Jensen, B.B. 1995a. 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.
Jensen, M.T., Cox, R.P. & Jensen, B.B. 1995b. 3-methylindole (skatole) and indole production by mixed population of pig fecal bacteria. *Applied and Environmental Microbiology* 61, 3180-3184.

Katouli, M., Lund, A., Wallgren, P., Kühn, I., Söderlind, O. & Möllby, R. 1997. Metabolic fingerprinting and fermentative capacity of the intestinal flora of pigs during pre- and post-weaning periods. *Jounal of Applied Microbiology* 83, 147–154.

Kloek, J. 1961. The smell of some steroid sex-hormones and their metabolites. Reflections and experiments concerning the significances of smell for the mutual relation of the sexes. Psychiat. Neur. Neurochir. 64: 309.

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

Knudson, B.K., Hogberg, M.G., Merkel, R.A., Allen, R.E. & Magee, W.T. 1985. Developmental comparison of boars and barrows: II. Body composition and bone development. *Journal of Animal Science* 61, 797-801.

Lanthier, F., Lou, Y., Terner, M.A. & Squires, E.J. 2006. Characterizing developmental changes in plasma and tissue skatole concentrations in the prepubescent intact male pig. *Journal of Animal Science* 84, 1699-1708.

Larson G, Dobney K, Albarella U, Fang M, Matisoo-Smith E, Robins J, Lowden S, Finlayson H, Brand T, Willerslev E, Rowley-Conwy P, Andersson L, Cooper A 2005. Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. *Science* 307, 1618-1621.

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.

Li, J.H., Vasanthan, T., Rossnagel, B. & Hoover, R. 2001. Starch from hull-less barley: I. Granule morphology, composition and amylopectin structure. *Food Chemistry* 74, 395-405.

Lin, Z., Lou, Y. & Squires, E.J. 2004a. Molecular cloning, expression and functional characterisation of the cytochrome P450 2A6 gene in pig liver. *Animal Genetics* **35,** 314-316.

Lin, Z., Lou, Y., Peacock, J. & Squires, E.J. 2005. A novel polymorphism in the 5' untranslated region of the porcine cytochrome b5 (CYB5) gene is associated with decreased fat androstenone level. *Mammalian Genome* 16, 367-373.

Lin, Z., Lou, Y. & Squires, E.J. 2006. Functional polymorphism in porcine CYP2E1 gene: Its association with skatole levels. *Journal of Steroid Biochemistry and Molecular Biology* 99, 231-237.

Loke, K.H. & Gower, D.B. 1971. Further studies on the biosynthesis of androsta-5,16-dien-3β-ol and the subcellular location of the site of biosynthesis. *Biochemical Journal* 122, 27p.

Lösel, 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 Agricultural Research* 8, 171-178.

Lundström, K., Malmfors, B., Stern, S., Rydhmer, L., Elaisson-Selling, L., Mortensen, A.B. & Mortensen, H.P. 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., Hansson, I. & Gahne, B. 1976. The effect of hCG and LH-RH on 5-alpha-androstenone levels in plasma and adipose tissue of boars. *Swedish Journal of Agricultural Research* 6, 73-79.

Malmfors, B. & Lundström, K. 1983. Consumer reactions to boar meat -- a review. *Livestock Production Science* 10, 187-196.

Matthews, K.R., Homer, D.B., Punter, P., Béague, M.-P., Gispert, M., Kempster, A.J., Agerhem, H., Claudi-Magnussen, C., Fischer, K., Siret, F., Leask, H., Font i Furnols, M. & Bonneau, M. 2000. An international study on the importance of androstenone and skatole for boar taint: III. Consumer survey in seven European countries. *Meat Science* 54, 271-283.

Meloen, R.H., Turkstra, J.A., Lankhof, H., Puijk, W.C., Schaaper, W.M., Dijkstra, G., Wensing, C.J. & Oonk, R.B. 1994. Efficient immunocastration of male piglets by immunoneutralization of GnRH using a new GnRH-like peptide. *Vaccine* 12, 741-746.

Melrose, D.R., Reed, H.C.B. & Patterson, R.L.S. 1971. Androgen steroids associated with boar odour as an aid to the detection of oestrus in pig artificial insemination. *British Veterinary Journal* 127, 497-502.

Moller, M., Berg, F., Riquet, J., Pomp, D., Archibald, A., Anderson, S., Feve, K., Zhang, Y., Rothschild, M., Milan, D., Andersson, L. & Tuggle, C.K. 2004. High-resolution comparative mapping of pig Chromosome 4, emphasizing the FAT1 region. Mammalian Genome 15, 717-731.

Morell, M.K., Kosar-Hashemi, B., Cmiel, M., Samuel, M.S., Chandler, P., Rahman, S., Buleon, A., Batey, I.L. & Li, Z. 2003. Barley sex6 mutants lack starch synthase IIa activity and contain a starch with novel properties. *Plant Journal* 34, 173-185.

Morohashi, K., Fujii-Kuriyama, Y., Okada, Y., Sogawa, K., Hirose, T., Inayama, S. & Omura, T. 1984. Molecular cloning and nucleotide sequence of cDNA for mRNA of mitochondrial cytochrome P-450(SCC) of bovine adrenal cortex. *Procceedings of the National Academy of Sciences* 81, 4647–4651.

Morohashi, K., Sogawa, K., Omura, T. & Fujii-Kuriyama, Y. 1987. Gene structure of human cytochrome P-450(SCC), cholesterol desmolase. *Journal of Biochemistry (Tokyo)* 101, 879–887.

Mortensen, A.B. & Sørensen, S.E. 1984. Relationship between boar taint and skatole determined with a new analytical method. In *Proc. 30th European Meeting of Meat Research Workers*, Bristol, England. 394-396.

Mågård, M., Berg, H., Tagesson, V., Järemo, M., Karlsson, L., Mathiasson, L., Bonneau, M. & Hansen-Möller, J. 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.

Nicolau-Solano, S.I., McGivan, J.D. Whittington, F.M., Nieuwhof, G.J., Wood, J.D. & Doran, O. 2006. Relationship between the expression of hepatic but not testicular 3ß-hydroxysteroid dehydrogenase with androstenone deposition in pig adipose tissue. *Journal of Animal Science* 84, 2809-2817.

Oonk, R.B., Krasnow, J.S., Beattie, W.G. & Richards, J.S. 1989. Cyclic AMP dependent and -independent regulation of cholesterol side chain cleavage cytochrome P-450 (P-450scc) in rat ovarian granulosa cells and corpora lutea. cDNA and deduced amino acid sequence of rat P-450scc. *Journal of Biological Chemistry* 264, 21934–21942.

Patterson, R.L.S. 1967. A possible contribution of phenolic components to boar odour. *Journal of Science of Food and Agriculture* 18, 8-10.

Patterson, R.L.S. 1968. 5-androst-16-en-3-one, compound responsible for taint in boar fat. *Journal of the Science of Food and Agriculture* 19, 31-38.

Payne, A.H. & Hales, D.B. 2004. Overview of Steroidogenic Enzymes in the Pathway from Cholesterol to Active Steroid Hormones. *Endocrine Reviews* 25, 947-970.

Pedersen, B. 1998. Heritability of skatole in back fat. In *Skatole and boar taint*. Danish Meat Research Institute, Roskilde, Denmark. Pp 129-135.

Pirog, E.C. & Collins, D.C. 1999. Metabolism of dihydrotestosterone in human liver: importance of 3α- and 3β-hydroxysteroid dehydrogenase. *Journal of Clinical Endocrinology and Metabolism* 84, 3217–3221.

Prunier, A., Bonneau, M., von Borell, EH, Cinotti, S., Gunn, M., Fredriksen, B., Giersing, M., Morton, DB, Tuyttens, FAM & Velarde, A. 2006. A review of the welfare consequences of surgical castration in piglets and the evaluation of non-surgical methods. *Animal Welfare* 15, 277-289.

Quintanilla, R., Demeure, O., Bidanel, J.P., Milan, D., Iannuccelli, N., Amigues, Y., Gruand, J., Renard, C., Chevalet, C. & Bonneau M. 2003. Detection of quantitative trait loci for fat androstenone levels in pigs. *Journal of Animal Science* 81, 385-394.

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

Rius, M.A. & García-Regueiro, J.A. 2001. Role of 4-phenyl-3-buten-2-one in boar taint: identification of new compounds related to sensorial descriptors in pig fat. *Journal of Agricultural and Food Chemistry* 49, 5303-5309.

Rius, M.A. Hortós, M. & García-Regueiro, J.A. 2005. Influence of volatile compounds on the development of off-flavours in pig back fat samples classified with boar taint by a test panel. *Meat Science* 71, 595-602.

Roberfroid M.B., Van Loo J.A., & Gibson G.R. 1998. The bifidogenic nature of chicory inulin and its hydrolysis products. *Journal of Nutrition* 128, 11-19. Review.

Ruder, H.J., Lorlaux, L. & Lipsett, M.B. 1972. Estrone Sulfate: Production Rate and Metabolism in Man. *The Journal of Clinical Investigation* 51, 1020-1033.

Santner, S.J., Levin, M.C. & Santen, R.J. 1990. Estrone sulfate stimulates growth of nitrosomethylurea-induced breast carcinoma in vivo in the rat. *International Journal of Cancer* 46, 73-78.

Schwarzenberger, F., Toole, G.S., Christie, H.L. & Raeside, J.I. 1993. Plasma levels of several androgens and estrogens from birth to puberty in male domestic pigs. *Acta Endocrinologica (Copenhagen)* 128, 173-177.

Schwenk, M. & del Pino, V.L. 1980. Uptake of estrogen sulfate by isolated rat liver cells. *Journal of Steroid Biochemistry* 13, 669-673.

Sellier, P. 1998. Genetics of meat and carcass traits. In: *The Genetics of the Pig*. CAB International, Wallingford, Oxon, UK, 463-510.

Shenoy, E.V.B., Daniel, M.J. & Box, P.G. 1982. The 'boar taint' steroid 5 alpha -androst-16-en-3-one: an immunisation trial. *Acta Endocrinologica (Copenhagen)* 100, 131-136.

Simard, J., Ricketts, M.L., Gingras, S., Soucy, P., Feltus, F.A. & Melner, M.H. 2005. Molecular biology of the 3β-Hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase gene family. Endocrine Reviews. 26, 525-582.

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

Sinclair, P.A., Hancock, S., Gilmore, W.J. & Squires, E.J. 2005b. Metabolism of the 16-androstene steroids in primary cultured porcine hepatocytes. *The Journal of steroid biochemistry and molecular biology* 96, 79-87.

Sinclair, P.A., Squires, E.J., Raeside, J.I. & Renaud, R. 2005c. 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, 217-228.

Sinclair, P.A., Gilmore, W.J., Lin, Z., Lou, Y. & Squires, E.J. 2006. Molecular cloning and regulation of porcine SULT2A1: relationship between SULT2A1 expression and sulfoconjugation of androstenone. *Journal of Molecular Endocrinology* 36, 301-311.

Skaanild, M.T. & C. Friis. 1999. Cytochrome P450 sex differences in minipigs and conventional pigs. *Pharmacology & Toxicology* 85, 174-180.

Skinner, T.M., Doran, E., McGivan, J.D., Haley, C.S. & Archibald, A.L. 2005. Cloning and mapping of the porcine cytochrome-p450 2E1 gene and its association with skatole levels in the domestic pig. *Animal Genetics* 36, 417-422.

Skinner, T.M., Anderson, J.A., Haley, C.S. & Archibald, A.L. 2006. Assessment of SULT1A1, CYP2A6 and CYP2C18 as candidate genes for elevated backfat skatole levels in commercial and experimental pig populations. *Animal Genetics* 37, 521-522.

Soucy, P., Lacoste, L., & Luu-The V. 2003. Assessment of porcine and human 16-ene-synthase, a third activity of P450c17, in the formation of an androstenol precursor Role of recombinant cytochrome b5 and P450 reductase. *European Journal of Biochemistry* 270, 1349–1355.

Squires, E.J., Adeola, O., Young, L.G. & Hacker, R.R. 1993. The role of growth hormones, β-adrenergic agents and intact males in pork production: A review. *Canadian Journal of Animal Science* 73, 1-23.

Squires, E.J. & Lou, Y. 1995. Level of boar taint in purebred entire male pigs in Canada. In: *Proceedings of a meeting of the production and utilization of entire male pigs*. Session III, Milton Keynes, UK. pp. 5.

Squires, E.J. & Lundström, K. 1997. Relationship between cytochrome P450 2E1 in liver and levels of skatole and its metabolites in entire male pigs. *Journal of Animal Science*, 75, 2506-2511.

Säfsten, P., Klakamp, S.L., Drake, A.W., Karlsson, R. & Myszka, D.G. 2006. Screening antibody-antigen interactions in parallel using Biacore A100. *Analytical Biochemistry* 353, 181-190.

Takeuchia, T. & Haginakab, J. 1999. Separation and sensing based on molecular recognition using molecularly imprinted polymers. *Journal of Chromatography B* 728, 1–20.

Tambyrajah, W.S., Doran, E., Wood, J.D. & McGivan, J.D. 2004. The pig CYP2E1 promoter is activated by COUP-TF1 and HNF-1 and is inhibited by androstenone. *Archives of Biochemistry and Biophysics* 431, 252-260.

Tuomola, M., Harpio, R., Knuuttila, P., Mikola, H., Lövgren, T. 1997. Time-resolved fluoroimmunoassay for the measurement of androstenone in porcine serum and fat samples. *Journal of Agricultural and Food Chemistry* 45, 3529-3534.

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* 719, 25-30.

Tuomola, M., Harpio, R., Mikola, H., Knuuttila, P., Lindström, M., Mukkala, V.M., Matikainen, M.T. & Lövgren T. 2000. Production and characterisation of monoclonal antibodies against a very small hapten, 3-methylindole. *Journal of Immunological Methods* 240, 111-124.

Tuomola, M., Harpio, R., Wirta, E.R. & Lövgren, T. 2002. Monitoring androstenone levels in boars by direct immunochemical analysis of serum samples. *Meat Science* 61, 193-197.

Törn, S., Nokelainen, P., Kurkela, R., Pulkka, A., Menjivar, M., Ghosh, S., Coca-Prados, M., Peltoketo, H., Isomaa, V. & Vihko, P. 2003. Production, purification, and functional analysis of recombinant human and mouse 17β-hydroxysteroid dehydrogenase type 7. *Biochemical and Biophysical Research Communications* 305, 37-45.

User Bulletin No. 2. 2001. ABI PRISM 7700 Sequence Detection System. Warrington, UK: Applied Biosystems.

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: EAAP Working Group, "*Production and Utilisation of Meat from Entire Male Pigs*", Milton Keynes, UK, pp. 6.

Van Oeckel, M.J., Warnants, N., Paepe, M.De., Casteels, M. & Boucqué, Ch.V. 1998. Effect of fibre-rich diets on the backfat skatole content of entire male pigs. *Livestock Production Science* 56, 173-180.

Varona, L., Vidal, O., Quintanilla, R., Gil, M., Sanchez, A., Folch, J.M., Hortos, M., Rius, M.A., Amills, M. & Noguera, J.L. 2005. Bayesian analysis of quantitative trait loci for boar taint in a Landrace outbred population. *Journal of Animal Science* 83, 301-307.

Vestergaard, J.S., Haugenc, J.-E. & Byrneb, D.V. 2006. Application of an electronic nose for measurements of boar taint in entire male pigs. *Meat Science* 74, 564-577.

Vold, E. 1970. Fleishproduktionseigenschaften bei Ebern und Kastraten. IV. Organoleptische und gaschromatografische Untersuchungen wasserdampfflüchtiger Stoffe des Rückenspeckes von Ebern. *Meldinger fra Norges Landbrukshøgskole* 49, 1-25.

Von Teichman, A., Joerg, H., Werner, P., Brenig, B. & Stranzinger, G. 2001. cDNA cloning and physical mapping of porcine 3β-hydroxysteroid dehydrogenase/Δ5–Δ4 isomerase. *Animal Genetics* 32, 298–302.

Walstra, P. & Maarse, G. 1970. Onderzoek gestachlengen van mannelijke mestvarkens. *Researchgroep voor Vlees en Vleeswaren TNO, IVO-rapport C-147, Rapport 2.* p 30.

Walstra, P. 1974. Fattening of young boars: quantification of negative and positive aspects. *Livestock Production Science* 1, 187-196.

Walstra, P., Claudi-Magnussen, C., Chevillon, P., von Seth, G., Diestre, A., Matthews, K.R., Homer, D.B. & Bonneau, M. 1999. An international study on the importance of androstenone and skatole for boar taint: levels of androstenone and skatole by country and season. *Livestock Production Science* 62, 15-28.

Whittington, F.M., Nute, G.R., Hughes, S.I., McGivan, J.D., Lean, I.J., Wood, J.D. & Doran, E. 2004. Relationships between skatole and androstenone accumulation, and cytochrome P4502E1 expression in Meishan x Large White pigs. *Meat Science* 67, 569-576.

Willeke, H., Claus, R., Muller, E., Pirchner, F. & Karg, H. 1987. Selection for high and low-level of 5-alpha-androst-16-en-3-one in boars. 1. Direct and correlated response of endocrinoligic traits. *Journal of Animal Breeding and Genetics* 104, 64-73.

Williamson, E.D. & Patterson, R.L.S. 1982. A selective immunization procedure against 5 alpha -androstenone in boars. *Animal Production* 35, 353-360.

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

Wysocki, C.J., Dorries, K.M. & Beauchamp, G.K. 1989. Ability to perceive androstenone can be acquired by ostensibly anosmic people. *Proceedings of the National Academy of Sciences* 86, 7976-7978.

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. *The Journal of General and Applied Microbiology* 48, 83-89.

Xue, J., Dial, G.D., Holton, E.E., Vickers, Z., Squires, E.J., Lou, Y., Godbout, D. & Morel, N. 1996a. Breed differences in boar taint: relationship between tissue levels boar taint compounds and sensory analysis of taint. *Journal of Animal Science* 74, 2170–2177.

Xue, Q., Newman, R.K. & Newman, C.W. 1996b. Effects of heat treatment of barley starches on in vitro digestibility and glucose responses in rats. *Cereal Chemistry* 73, 588-592.

Yokoyama, M.T. & Carlson, J.R. 1979. Microbial metabolites of tryptophan in the intestinal tract with special reference to skatole. *American Journal of Clinical Nutrition* 32, 173-178.

Zamaratskaia, G., Babol, J., Madej, A., Squires, E.J. & Lundstrom K. 2004a. Age-related variation of plasma concentrations of skatole, androstenone, testosterone, oestradiol-17 beta, oestrone sulphate, dehydroepiandrosterone sulphate, triiodothyronine and IGF-1 in six entire male pigs. *Reproduction in Domestic Animals* 39, 168-172.

Zamaratskaia, G. 2004b. Factors involved in the development of boar taint: Influence of breed, age, diet and raising conditions. *Doctoral thesis*, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Zamaratskaia, G., Babol, J., Andersson, H. & Lundström, K. 2004c. 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, 91–98.

Zamaratskaia, G., Squires, E.J., Babol, J., Andersson, H.K., Andersson, K. and Lundström, K. 2005a. 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, 83-88.

Zamaratskaia, G., Babol, J., Andersson, H.K., Andersson, K. and Lundström K. 2005b. 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, 235-243.

Zamaratskaia, G., Rydhmer, L., Chen, G., Madej, A., Andersson, H.K. & Lundström, K. 2005c. Boar taint is related to endocrine and anatomical changes at puberty but not to aggressive behaviour in entire male pigs. *Reproduction in Domestic Animals* 40, 500-506.

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, 331-338.

Zamaratskaia, G., Gilmore, W.J., Lundström, K. & Squires, E.J. 2007a. Effect of testicular steroids on catalytic activities of cytochrome P450 enzymes in porcine liver microsomes. *Food Chemistry and Toxicology* 45, 676-681.

Zamaratskaia, G., Oskam, I.C., Ropstad, E., Tajet, H., Dahl, E. & Andresen, Ø. 2007b. Effects of hCG stimulation on hepatic activities of cytochromes P4502E1 and P4502A in

pubertal male pigs. *Reproduction in Domestic Animals* doi: 10.1111/j.1439-0531.2007.00867.x , in press.