# Factors involved in the development of boar taint

## Influence of breed, age, diet and raising conditions

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# Abstract

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Boar taint has been mainly associated with the presence of androstenone and skatole in adipose tissue of entire male pigs. This work was focused on the effects of breed, age, hormonal status, and diet composition on boar taint level.

The results showed that skatole levels in entire male pigs varied considerably with age, and the degree as well as the pattern of this variation differs between different breeds. The initial increase in skatole levels might occur at a relatively young age of 8-10 weeks. Later, the levels of skatole decreased and remained low until puberty. As puberty development progressed, skatole levels increased again reaching very high levels in some individuals. The increase in skatole levels followed the increase in levels of testicular steroids. These findings indicate that there is a relationship between the process of sexual development and high skatole levels, and that the increase in the levels of testicular steroids at puberty may affect skatole levels. The decreased activities of the main enzymes controlling skatole metabolism in the liver also contributed to increased skatole levels during this period. No direct relationship between skatole and thyroid hormones and IGF-1 was found.

The dietary supplement of raw potato starch or lower weight at slaughter reduced skatole levels in entire male pigs, but did not affect androstenone levels or the activities of cytochrome P4502E1 and cytochrome P4502A6. Raising entire male pigs in mixed pens resulted in increased skatole levels in plasma in pigs of 115 kg live weight fed raw potato starch. Androstenone levels were higher in pigs at 90 kg live weight raised in mixed pens, but not in heavier pigs.

A new method to determine free oestrone in porcine adipose tissue was developed and applied to investigate the relationship between boar taint compounds and hormonal status in entire male pigs. Both skatole and androstenone were positively correlated to free oestrone levels.

Overall, the results suggest that puberty is a main factor controlling androstenone levels. Skatole levels are regulated by a number of factors, including breed, age, hormonal status and diet.

*Keywords:* Androstenone, skatole, hormonal status, puberty, slaughter weight, raw potato starch, cytochrome P4502E1, cytochrome P4502A6

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Памяти моего отца, Гизатуллина Шамиля Шайхулловича, посвящается.
Ты всегда мечтал, что я стану писателем. Писателя из меня не вышло,
но по крайней мере, одну книгу я написала.

# Content

# Appendix

## Papers I-VI

The present thesis is based on the following papers, which will be referred to by their Roman numerals I-VI:

I. 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.

II. Zamaratskaia, G., Babol, J., Andersson, H. & Lundström, K. 2004. Plasma skatole and androstenone levels in entire male pigs and relationship between boar taint compounds, sex steroids and thyroxine at various ages. *Livestock Production Science* (In press, Corrected Proof, Available online 2 December 2003).

III. Zamaratskaia, G., Babol, J., Madej, A., Squires, E.J. & Lundström, K. 2004. Age-related variation of plasma concentrations of skatole, androstenone, testosterone, oestradiol-17b, oestrone sulphate, dehydroepiandrosterone sulphate, triiodothyronine and IGF-1 in six entire male pigs. *Reproduction in Domestic Animals* 39, 1-5.

IV. Zamaratskaia, G., Babol, J., Madej, A., Squires, E.J. & Lundström, K. 2004. Determination of free estrone in adipose tissue by radioimmunoassay and relationship between levels of free estrone, androstenone and skatole in fat of entire male pigs. *Submitted for publication.*

V. Zamaratskaia, G., Babol, J., Andersson, H.K., Andersson, K. & Lundström, K. 2004. Effect of live weight and dietary supplement of raw potato starch on the relationship between levels of skatole, androstenone, testosterone and estrone sulphate in entire male pigs. *Submitted for publication.*

VI. Zamaratskaia, G., Squires, E.J., Babol, J., Andersson, H.K., Andersson, K. & Lundström, K. 2004. Relationship between the activities of cytochromes P4502E1 and P4502A6 and skatole content in fat in entire male pigs fed a diet with and without raw potato starch. *Submitted for publication.*

# Introduction

Odour is a crucial sensory attribute that may determine whether consumers will accept a food product. Male piglets destined for meat production are castrated in most European countries to reduce the risk of boar taint, an unpleasant smell from heated pork products. Boar taint mainly occurs in meat from some entire male pigs and makes it undesirable for human consumption. Castration reduces boar taint, although the advantages of castration are disputable, since it ceases the synthesis of testicular steroids and therefore negatively affects the lean growth of the animal and reduces feed efficiency. Additionally, there is an expressed concern over effects of castration on pig welfare. Other methods to prevent boar taint are therefore desirable.

The current review outlines the present knowledge about chemical substances involved in the development of boar taint with special emphasis on skatole and androstenone.

## The origin of boar taint

There are a number of chemical substances responsible for boar taint, of which skatole and androstenone are judged to be the primary compounds (Hansson *et al*., 1980; Dijksterhuis *et al*., 2000). These two compounds, when present in fatty tissues separately or together, give rise to off-odour from meat of pigs. Skatole, 3-methylindole, first isolated from porcine adipose tissue by Vold (1970) and by Walstra & Maarse (1970), is perceived by most consumers as a faecal-like odour. Androstenone, isolated from adipose tissue of boars by Patterson (1968), contributes to urine-like odour. The sensitivity of consumers to androstenone is affected by various factors including genetics (Wysocki & Beauchamp, 1984; Pause *et al*., 1999; Araneda & Firestein, 2004; Wang, Chen & Jacob, 2004). Other chemicals might also contribute to off-odour in meat such as androstenol (Brennan *et al*., 1986; Brooks & Pearson, 1989), indole (García-Requeiro & Diaz, 1989; Moss, Hawe & Walker, 1993; Annor-Frempong *et al*., 1997a; Rius & García-Regueiro, 2001), and 4-phenyl-3-buten-2-one (Rius Sole & García-Regueiro, 2001). Nevertheless, their contribution seems to be of less importance because of relatively weak odour and/or weak lipophilic properties.

## Surgical castration in pigs – reasons and alternatives

Surgical castration of male pigs is commonly used to produce taint-free meat by reduction of the concentrations of skatole and androstenone in fat under the threshold level, 0.20-0.25 ppm for skatole and 0.5-1 ppm for androstenone. Castration also prevents unplanned breeding and reduces aggressive behaviour in entire male pigs. However, nowadays there is a tendency at least in European countries, to stop castration (Stevenson, 2000).

Raising entire male pigs is more profitable because of improved feed conversion and leaner carcasses of males (Hansson, 1974; Babol & Squires, 1995; Bonneau, 1998; Bañón *et al*., 2004). The higher protein content in carcasses from entire

males might indicate nutritional advantages of this meat compared to that from castrates (Wood *et al*., 1986; Naděje *et al*., 2000). Furthermore, surgical castration conflicts with principles of animal welfare and might adversely affect the health of the animal. The number of chronic inflammations is markedly higher in castrated male pigs compared to entire males and female pigs (Kruijf & Welling, 1988). It is generally believed that castration without anaesthetic is a painful process. However, the levels of pain that an animal is subjected to and the consequences of the pain are not well understood. The measurement of pain in animals is usually performed based on behavioural and physiological parameters. Castration induces alterations of a pig's behaviour in hours and even days after the procedure (McGlone *et al*., 1993; Hay *et al*., 2003). The increased level of vocalisation of piglets is also an indicator of the pain experienced by pigs during castration (Taylor *et al*., 2001; Marx *et al*., 2003). The levels of cortisol in urine (Hay *et al*., 2003) and plasma (Kattesh *et al*., 1996; Kohler *et al*., 1998) do not alter during castration. The response to castration is similar in piglets from 3 up to 17 days of age (Taylor *et al*., 2001), and analgesic treatment does not significantly affect post-castration behaviour (McGlone *et al*., 1993). Thus, both economic and ethical concerns make it necessary to re-evaluate the requirement for castration.

The use of local anaesthesia would reduce the pain during castration (Marx *et al*., 2003). Its provision will therefore improve the welfare status of piglets but increase the costs of the procedure.

Active immunization against gonadotropin-releasing hormone (GnRH; also referred to as luteinizing hormone-releasing hormone, LHRH), so called immunocastration, might be an appropriate alternative to surgical castration

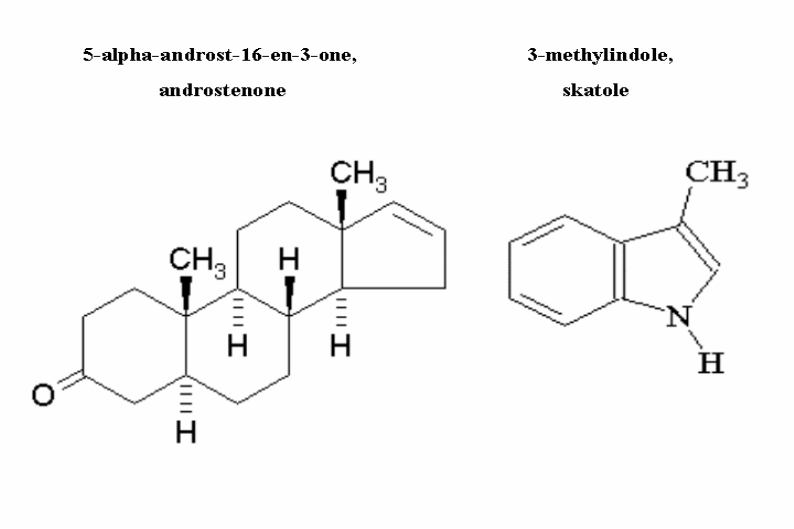


*Figure 1*. Chemical structure of androstenone and skatole.

(Bonneau *et al*., 1994; Dunshea *et al*., 2001; Turkstra *et al*., 2002; Metz & Claus, 2003; Oliver *et al*., 2003). GnRH is secreted by the hypothalamus to stimulate the secretion of pituitary luteinizing hormone (LH) and follicle stimulating hormone

(FSH), which subsequently controls sexual development and functions, including the production of testicular steroids. The principle of immunocastration is based on the immunological blocking of the signal from GnRH and thus decreasing the secretion of LH and testicular steroids. This approach therefore does not prevent androstenone formation selectively but also reduces the synthesis of anabolic steroids. The effect of immunocastration on the other boar taint compound, skatole, varies in different studies. Bonneau *et al*. (1994) and Schneider *et al.* (1998) found no differences in skatole levels in fat between immunocastrated and intact male pigs. However, it should be emphasized that skatole levels in the study of Bonneau *et al*. (1994) were low in all treatment groups. Other studies showed a significant reduction in skatole levels in immunocastrated male pigs (Hennessy *et al*., 1997; Dunshea *et al*., 2001; Metz *et al*., 2002). Some variability in the results is also likely due to variability in methods used in the studies (adjuvant, number of injections and age of vaccination) (reviewed in Bonneau & Enright, 1995). Growth performance of immunocastrated male pigs was similar (Bonneau *et al*., 1994) or even better (Dunshea *et al*., 2001) compared to that of control pigs. Immunocastration also reduced the level of aggressive behaviour and mountings (Cronin *et al*., 2003). More research is needed to reduce the variability in response to the vaccine and to determine the optimal age of vaccination. Reaction from consumers about whether immunocastration is a viable substitute to surgical castration is also a concern.

Genetic selection experiments reduced boar taint, particularly due to high androstenone levels (Jonsson & Andresen, 1979; Sellier & Bonneau, 1988; Willeke & Pirchner, 1989; Sellier *et al*., 2000). However, the results of selection against androstenone were not fully satisfactory due to the reduced levels of anabolic hormones and, therefore, negative effects on growth performance of entire male pigs and onset of puberty in male and female pigs (Sellier & Bonneau, 1988; Willeke & Pirchner, 1989). For such a selection process, boars with a low potential for androstenone production when sexual mature should be chosen. Therefore, genetic markers, which are related to both boar taint and age of sexual maturity, have to be identified to avoid undesirable effects of selection against boar taint. Research is needed to achieve this.

In recent years, gender selection has been discussed as a promising tool for the pork industry (Johnson, 2000; Seidel, 2003). This selection process sorts out male types of sperm cells and is followed by artificial insemination of the selected sperm. The separation of male and female sperm cells could allow the production of female-only herds, which do not express boar taint. At present, the technique for gender selection is not commercially available. Large quantities of material (sperm) are required for such a selection because of sperm losses and cell damage during selection. However, the technique might become a promising strategy in pork production if it is effective and precise, and costs of sperm separation are low.

Slaughter at a younger age and lower weight, before the onset of puberty, might reduce the risk of increased levels of boar taint compounds. In some countries, *e.g.* Ireland and the United Kingdom, male pigs are produced intact. This is acceptable only if pigs are slaughtered when they are immature, to minimise the incidence of

boar taint in the meat. This approach does not negatively affect animal welfare; however, from an economic point of view it is not an attractive alternative. Additionally, slaughter at lower weight does not entirely eliminate boar taint (Aldal *et al*., 2003).

The use of a technique that estimates boar taint on the slaughter line would permit sorting out tainted carcasses. The tainted meat could then be used for processed meat products. There are a number of methods developed for the measurement of concentrations of skatole (Dehngard *et al.*, 1993; García-Regueiro & Rius, 1998), androstenone (De Brabander & Verbeke, 1986; Mågård *et al.*, 1995; Claus, Herbert & Dehnhard, 1997; Tuomola, Hakala & Manninen, 1998) and both (Hansen-Møller, 1994) in adipose tissue (Table 1). Those methods, however, are not applicable on the slaughter line since they involve complicated sample preparation and purification steps and are usually time-consuming and labour-intensive. Radioimmunological assays (Claus, 1974; Andresen, 1975) are rapid and sensitive, but involve the use of radioisotopes and therefore the application of such methods for routine use at slaughterhouses is not possible. The colorimetric method for measuring skatole equivalents in adipose tissue was developed in Denmark (Mortensen & Sørensen, 1984) and has been used online in Danish slaughterhouses. The benefits of this method are rapidity and simplicity; however, it does not provide information about the levels of the other important boar taint compound, androstenone. A colorimetric method for total 16-androstenes was also developed (Squires, 1990) although it was never used at slaughterhouses. Annor-Frempong *et al.* (1998) developed a fast method to measure boar taint levels without discrimination between skatole and androstenone using an electronic nose. Recently, a method based on the thickness shear mode resonator technology (TSMR) was proposed for androstenone analysis in fat (Di Natale *et al*., 2003). These methods need to be validated in a slaughterhouse setting. The presence of boar taint in pork products might be evaluated by sensory analysis, but the use of sensory tests is not efficient due to high costs, high variability and low accuracy. Further investigations are required to develop a rapid and sensitive method for the systematic analysis of boar carcasses.

Summarising, the need for castration still exists since at present there is no valid alternative to prevent boar taint.

## Factors affecting the levels of skatole and androstenone in entire male pigs

Many aspects are important in the development of boar taint, and they can be divided into two classes: internal aspects directly related to the individual pig such as sex, breed and genetics, age, weight and liver metabolism, and external aspects such as season, raising condition and feeding system. The present review will focus on some of these aspects.

Table 1. *Some of the methods to measure boar taint compounds*

| Substance | Method | Reference |
|---|---|---|
| Androstenone | Radioimmunoassay (RIA) | Claus, 1974 |
| Androstenone | RIA | Andresen, 1975 |
| Androstenone | Gas chromatography (GC) with electron-capture detection | De Brabander & Verbeke 1986 |
| Androstenone | Microtiter plate enzyme-immunoassay (MTE) | Claus, Mahler & Münster, 1988 |
| Androstenone | Supercritical fluid extraction and gas chromatography-mass spectrometry (GC-MS) | Mågård *et al*., 1995 |
| Androstenone | Enzyme immunoassay (EIA) and High-performance liquid chromatography (HPLC) with fluorescence detector | Claus, Herbert & Dehnhard, 1997 |
| Androstenone | Enzyme-linked immunosorbent assay (ELISA) | Squires & Lundström, 1997 |
| Androstenone | Supercritical fluid chromatography-mass spectrometry | Tuomola, Hakala & Manninen, 1998 |
| Androstenone | ELISA | Sinclair *et al.*, 2001 |
| Androstenone | Thickness shear mode resonator technology (TSMR) | Di Natale *et al*., 2003 |
| Total 16-androstenes | Colorimetric method | Squires, 1990 |
| Skatole, indole | HPLC with fluorescence detector | Dehngard *et al.*, 1993 |
| Skatole, indole | HPLC with fluorescence detector (normal-phase) | Garcia Regueiro & Rius, 1998 |
| Skatole equivalent | Colorimetric method | Mortensen & Sørensen, 1984 |
| Androstenone, skatole, indole | HPLC with fluorescence detector | Hansen-Møller, 1994 |
| Boar taint level | Electronic nose | Annor-Frempong *et al.*, 1998 |

### *Androstenone biosynthesis and metabolism*

Androstenone (5-alpha-androst-16-en-3-one) is a steroid produced by the Leydig cells of the testis of entire male pigs in parallel with anabolic testicular hormones (Gower, 1972; Kwan, Orengo & Gower, 1985). The production of androstenone and other testicular steroids is controlled by the neuroendocrine system, particularly, by lutenizing hormone (LH). Androstenone is derived from the precursors pregnenolone and progesterone through the formation of androstadienone by the sequential action of a number of enzymes, particularly cytochrome P450C17 and cytochrome b5 (Meadus, Mason & Squires, 1993; Davis & Squires, 1999). Androstenone is metabolised in the liver with the production of alpha-androstenol and to a greater extent beta-androstenol (Bonneau & Terqui, 1983; Doran *et al.*, 2004).

Part of androstenone is transported to the submaxillary salivary gland where it binds to a specific binding protein pheromaxein (Booth, 1984; Booth & White, 1988; Booth & Von Glos, 1991). After being released in the saliva, it among other 16-androstene steroids serves as a pheromone to promote sexual behaviour in female pigs. The presence of mature boars stimulates puberty onset and induces a mating stance in female pigs (Pearce, Hughes & Booth, 1988). Due to its lipophilic nature, some androstenone is also accumulated in the adipose tissue and produces taint.

### *Skatole biosynthesis and metabolism*

Skatole is a by-product of the metabolism of the amino acid L-tryptophan in the large intestine of pigs. Biosynthesis of skatole occurs as a two-step procedure. Firstly, tryptophan is converted to 3-indoleacetic acid, which is subsequently converted to skatole (reviewed in Jensen & Jensen, 1998). These reactions are carried out by bacteria, particularly *Lactobacillius* sp. (Yokoyama, Carlson & Holdeman, 1977; Honeyfield & Carlson, 1990; Jensen & Jensen, 1998; Deslandes, Gariépy & Houde, 2001). Therefore, the production of skatole primarily depends on both the availability of tryptophan and the activity of intestinal bacteria. Tryptophan for skatole production originates mainly from gut-mucosa cell debris and not from dietary tryptophan (Claus, Weiler & Herzog, 1994; Claus & Raab, 1999); this might, however, depend on the diet (Jensen & Jensen, 1998). Intestinal cell turnover controlled by the growth factor IGF-I is essential in providing substrate for skatole synthesis (Claus & Raab, 1999). The pH in the large intestine also considerably affects tryptophan biotransformations, with low pH values being favourable for skatole, and high pH for indole biosynthesis (Jensen, Cox & Jensen, 1995a). Skatole is synthesised in the intestine of all genders of pigs but only some entire males accumulate skatole in the adipose tissue in high concentrations. A part of skatole is excreted with faeces and the remaining part is absorbed through the gut wall and released into the blood (Agergaard & Laue, 1998).

Skatole is metabolised in the liver to a variety of metabolites (Bæk *et al*., 1997; Diaz *et al*., 1999). The metabolism of skatole in the liver takes place in two

phases. Phase I is a chemical modification to add a hydroxyl group that can be used to attach a conjugate in Phase II. The conjugate makes the modified compound more hydrophilic so that it can be excreted in the urine or bile. Cytochromes P4502E1 and P4502A6 and aldehyde oxidase are involved in Phase I skatole metabolism (Babol, Squires & Lundström, 1998a, b; Diaz & Squires, 2000a, b). Seven Phase-I metabolites have been identified; 3-OH-3-methylindolenine, 3-methyloxindole and 3-OH-3-methyloxindole are the most common skatole metabolites in vitro (Diaz *et al.*, 1999). These metabolites serve then as the substrates for further modifications in Phase II, including the conjugation with either sulphate or glucuronic acid (Babol, Squires & Lundström, 1998b; Diaz & Squires, 2003). The enzyme accountable for sulphation was identified as thermostable phenol-sulphotransferase (Diaz & Squires, 2003).

High levels of the metabolite 6-sulphatoxyskatole in plasma were suggested as indicators of entire male pigs with the ability to rapidly metabolise and excrete skatole (Bæk *et al*., 1997).

No physiological function of skatole in pigs is currently known. Skatole has also been suggested to be toxic to various microorganisms, which might influence the endogenous intestinal microflora (Deslandes, Gariépy & Houde, 2001). In ruminant species, *e.g.* goats, production of skatole and its metabolites is associated with acute pulmonary oedema and emphysema (Carlson & Breeze, 1984; Nocerini, Carlson & Yost, 1984). Skatole was proposed to be a potential pneumotoxicant also in humans (Ruangyuttikarn, Appleton & Yost, 1991; Nichols *et al*., 2003). Moreover, skatole levels were increased in serum of patients with liver disease, hepatic encephalopathy (Suyama & Hirayama, 1988) and in the intestine of patients suffering from saccharo-butyric putrefaction (Herter, 1908). In healthy subjects, skatole is not detectable in serum (Suyama & Hirayama, 1988) and saliva (Cooke, Leeves & White, 2003). In the human intestine, skatole is also frequently undetectable or detectable only in traces (Rettger, 1906; Herter, 1908; Pierce, 1932; Smith & Macfarlane, 1997).

### *Genetics*

Genetic factors are important in the regulation of androstenone levels in entire male pigs (Fouilloux *et al*., 1997; De Vries *et al*., 1998). The heritability estimates of androstenone vary from 0.25 to 0.88 (Sellier, 1998). Moreover, the levels of androstenone vary considerably among breeds of pigs (Xue *et al*., 1996) providing additional confirmation of the importance of genetic factors. Several genomic regions were demonstrated to harbour QTL's for the variation of androstenone levels in fat (Quintanilla *et al*., 2003).

Genetics also affect the levels of skatole in adipose tissue. The heritability estimates ranged from 0.19 to 0.27 (Pedersen, 1998), and breed differences have also been described (Hortos *et al*., 2000; Doran *et al*., 2002a). A recessive gene has been suggested to be responsible for high skatole levels (Lundström *et al*., 1994). Polymorphisms of major enzymes affecting skatole metabolism may have an association with variations in skatole levels (Lin, Lou & Squires, 2003; Lin, Lou & Squires, 2004).

*Puberty and hormonal status*

Puberty is a complex process accompanied by dramatic changes in physiological and endocrinological characteristics of the organism, such as changed testis structure and increased secretion of androgens and oestrogens. Puberty is heralded by an increase in the secretion of LH and follicle-stimulating hormone (FSH) by the anterior pituitary. LH secretion is mainly regulated by gonadotrophin-releasing hormone (GnRH) produced by the hypothalamus. LH secretion is also controlled by some other hormones such as dopamine and prolactin, and most crucially by negative feedback from sex steroids. The binding of LH to the receptors on the surface of the Leydig cells results in the induction of steroidogenic enzymes and increased levels of testicular steroids including androstenone (Squires *et al*., 1993). Also, mature boars show an increase in average Leydig cell size and therefore an increase in steroidogenic capacity per Leydig cell (Lunstra *et al*., 1986). The biosynthesis of androstenone is low in young pigs and gradually increases simultaneously with other testicular steroids after sexual maturity has begun (Gower, 1972; Bonneau, 1982). Similar to other testicular steroids, the transient increase in androstenone levels also occurs at the age of approximately 2-4 weeks due to Leydig cell activity at that time (Bonneau, 1982; Schwarzenberger *et al*., 1993; Claus, Weiler & Herzog, 1994; Sinclair *et al*., 2001). The analogous age-related variations of androstenone and testicular hormones are due to the same regulatory system controlling the biosynthesis of all testicular steroids. Therefore, puberty is a central aspect regulating androstenone levels in entire male pigs by the maintenance of adult Leydig cell morphology and the stimulation of neuroendocrine system leading to increased biosynthesis of testicular steroids. In sexually mature boars, androstenone levels depend on the individual's ability to produce this steroid (Bonneau, 1987).

Skatole might also be affected by sexual maturity as indicated by positive correlations with both live weight (Babol, Squires & Gullett, 1996; Walstra *et al*., 1999) and the levels of testicular steroids (Bonneau *et al*., 1992; Annor-Frempong *et al*., 1997b; Babol, Squires & Lundström, 1999). The increased skatole levels in heavier/older pigs (Hansen *et al.*, 1997; Whittington *et al*., 2004) might also be explained by puberty-related factors. However, further evidence linking increased skatole levels with the puberty of entire male pigs is needed.

Changes in the pattern of non-testicular hormones that occur at puberty could also be involved in the regulation of androstenone and skatole levels. A variety of hormones influence the process of puberty. Thyroid hormones (Palmero, De Marco & Fugassa, 1995; Cooke, 1996; Manna, Tena-Sempere & Huhtaniemi, 1999; Manna *et al*., 2001) and insulin-like growth factor-1 (IGF-1) (Benton, Shan & Hardy, 1995; Gnessi, Fabbri & Spera, 1997) are involved in testicular development and thus might indirectly regulate the levels of androstenone and probably skatole. Furthermore, thyroid hormones and growth factors, particularly IGF-1, may affect skatole levels through affecting intestinal development. Thyroid hormone, triiodithyronine ($T_3$), is strongly involved in the regulation of growth

and differentiation of the intestine. Plateroti *et al.* (1999) described an effect of thyroid hormones through the TRa receptor on intestine development of mice. During puberty, the production rate of growth hormones including IGF-1 increases. The subsequent increased intestinal cell turnover and degree of mitosis due to increased levels of IGF-1 might explain the pubertal increase in skatole levels (Claus & Raab, 1999). Additionally, IGF-1 levels are higher in entire male pigs compared to that of castrates and female pigs (Clapper, Clark & Rempel, 2000).

### *Environment and feeding*

In the regulation of androstenone levels, environmental factors are probably only of minor importance unless they affect sexual maturity. Seasonal variation has been shown to affect androstenone levels; specifically, androstenone levels decrease when day length increases (Claus, Schopper & Wagner, 1983; Keller, Wicke & Von Lengerken, 1997). Androstenone levels might be affected by rank order, being higher in high-rank entire male pigs (Giersing, Lundström & Andersson, 2000). However, day length and rank order probably do not affect androstenone levels directly, but are both related to puberty which in turn affects androstenone levels. Androstenone levels in fat increased in entire male pigs fed ad libitum compared to those fed restrictively (Øverland, Berg & Matre, 1995) probably because a high-energy diet might accelerate puberty. Ad libitum feeding diet, however, did not accelerate pubertal development of boars, as estimated by sperm morphology and size of the reproductive organs (Einarsson *et al*., 1979), and Lundström *et al*. (1988) did not find any effect of nutrient density on androstenone levels. Feeding a fibre-rich diet did not affect androstenone levels in entire male pigs (Whittington *et al*., 2004).

In contrast to androstenone, skatole levels depend on environmental factors. The effect of day length on skatole levels has been demonstrated (Walstra *et al*., 1999). Skatole levels are highly affected by rearing conditions (Hansen *et al*., 1994; Hansen, Larsen & Hansen-Møller, 1995), being higher at high stocking density and in unclean conditions. Temperature might also affect skatole variations (Hansen, 1998).

Furthermore, the type of feeding system plays a crucial role in the regulation of skatole levels. There have been numerous attempts to manipulate skatole levels using feed additives. The results from those studies are summarised in Table 2. The inclusion of a high amount of low-digestible protein in the diet increases skatole levels (Jensen, Cox & Jensen, 1995b), and the use of casein as a source of protein in the diet decreases skatole levels. Addition of dietary carbohydrates, such as sugar beet pulp or raw potato starch, has been found to reduce skatole levels (Jensen, Cox & Jensen, 1995b; Claus *et al*., 2003; Whittington *et al*., 2004). The use of growth promoting antibiotics also prevents the risk of increased skatole levels (Hansen, 1998; Jensen & Jensen, 1998), but this approach has no practical application because the use of antibiotics as growth promoters is prohibited in Sweden and limited in the European Union.

Table 2. *Effect of different dietary supplements on skatole levels in entire male pigs*

| Source | Site of skatole measure-ment | Effect on skatole | Notes | Reference |
|---|---|---|---|---|
| | | **Proteins, amino acids** | | |
| High-protein diet (soybean meal) | Blood | Decrease | Skatole measured in boars at the age of 24, 32 and 40 weeks. | Lin *et al*., 1992 |
| High-protein diet | Fat | Decrease | Entire male pigs, Yorkshire, live weight (LW) 103 kg | Lundström *et al*., 1994 |
| Cristalline L-lysine HCl | Fat | No consistent effect | Skatole levels were low throughout the experiment | Andersson *et al*., 1997 |
| Yeast slurry | Fat | Increase | Entire male pigs, LW 112 kg | Jensen, Cox & Jensen, 1995b |
| Casein | Fat | Decrease compared to yeast slurry | | |
| | | **Carbohydrates** | | |
| Sugar beet pulp | Faeces | Decrease | Boars and gilts of Landrace x Large White, 90 kg LW | Hawe, Walker & Moss, 1992 |
| | Fat | No | Skatole levels were low in all diet groups | |
| Sugar beet pulp | Fat | No | Boars, Yorkshire x Norwegian Landrace | Øverland, Berg & Matre, 1995 |
| Sugar beet pulp + yeast slurry | Fat | Decrease | Entire male pigs, LW 112 kg | Jensen, Cox & Jensen, 1995b |
| Sugar beet pulp | Fat | No | Boars, Pietran x Seghers hybrid cross | Van Oeckel *et al*., 1998 |
| Sugar beet pulp + straw | Fat | Increase | This increase in skatole was due to one boar having very high skatole | Wiseman *et al*., 1999 |
| Sugar beet pulp | Blood, faeces | Decrease | Entire male pigs, age from 4 to 6 months | Knarreborg *et al*., 2002 |
| Sugar beet pulp | Fat | Decrease | Boars, Meishan x Large White | Whittington *et al*., 2004 |

Table 2. *Continued*

| | | | | |
|---|---|---|---|---|
| Jerusalem artichoke | Faeces | Decrease | Castrated pigs, 8 weeks of age | Farnworth, Modler & Mackie, 1995 |
| Soybean hulls | Fat | No | Boars, Pietran x Seghers hybrid cross | Van Oeckel *et al*., 1998 |
| Fructooligosac-charides (FOS) 0.75 g per pig per day | Faeces | No | Young pigs, 10 kg LW | Russell *et al*., 1998 |
| FOS, 1.5 g | Faeces | Decrease | | |
| Raw potato starch | Plasma, faeces, fat | Decrease | Castrated pigs, German Landrace x Pietrain | Claus *et al*., 2003 |
| | | **Other** | | |
| Low nutrient-density diet (oat, wheat bran, hay meal, yellow peas) | Fat | Increase | Entire male pigs, Swedish Yorkshire, 110 kg LW | Lundström *et al*., 1988 |
| High-energy diet and the purine supplementation | Blood | Increase | Castrated pigs, German Landrace | Claus, Raab & Röckle, 1996 |
| Linseed | Fat | Decrease | No data on sex of experimental pigs | Matthews *et al*., 2000 |
| Linseed | Fat | Decrease | Female pigs, Duroc x Landrace x Large White | Kouba *et al*., 2003 |

# Objectives

The long-term aim of this work is to develop methods that will enable the elimination of pigs expressing boar taint from the breeding stock. To facilitate the development of such methods, factors affecting boar taint level in entire male pigs need to be well understood. The project was focused on the effects of breed, age, hormonal status, and diet composition on boar taint level.

The specific objectives of the individual studies were:

- To investigate the distribution of skatole and indole levels in plasma from entire male pigs of four breeds; Landrace, Yorkshire, Hampshire and Duroc (Study I).

- To describe the age-related variations in skatole levels (Study II and III).

- To evaluate the relationships between the levels of skatole, androstenone, testicular hormones, thyroid hormones and IGF-1 in entire male pigs at various ages (Study II, III, IV and V).

- To develop a method for the analysis of free oestrone in porcine adipose tissue (Study IV).

- To evaluate the impact of slaughter weight, raising conditions (single-sex vs. mixed pens) and the dietary supplement of raw potato starch on the levels of skatole, androstenone, testosterone and oestrone sulphate and the activities of hepatic enzymes CYP2E1 and CYP2A6 in entire male pigs (Study V and VI).

# Materials and methods

## Animals

*Study I*

Purebred boars from four different breeds were included in the study. Plasma samples from 117 Yorkshire, 134 Landrace and 184 Hampshire entire male pigs were collected by Quality Genetics, Swedish Meats (Sweden) at different stations, and plasma from 75 Duroc boars were collected by Norsvin (Norway) at a single station. Skatole concentrations were determined in plasma of all boars. Indole concentrations were determined in all Yorkshire, 121 Landrace, 161 Hampshire and all Duroc boars. In addition, blood and fat samples were collected from 39 entire male pigs from a backcross (BC4) between European Wild Pig and Swedish Yorkshire (Andersson *et al*., 1994) to construct a regression equation between the levels of skatole in plasma and fat.

*Study II*

In this study, a total of 47 entire male pigs (Yorkshire×Hampshire), siblings from 13 litters were used. The pigs were part of a project on photoperiodic effects on pubertal maturation in crossbred boars (Andersson *et al.,* 1998a). The pigs were randomly divided into three groups with different light treatments: control group, spring/summer group and autumn/winter group. The control group was exposed to natural day length conditions. The spring/summer group was exposed to artificial conditions simulating the photoperiod from the vernal equinox, and the autumn/winter group from autumnal equinox. Blood samples were collected every 2 weeks from the average age of 8 weeks until slaughter at the age of 20–24 weeks, when live weight of boars equalled or exceeded 115 kg. Plasma samples were analysed for skatole, androstenone, testosterone, oestrone sulphate and thyroxine concentrations. Fat samples were taken at slaughter and analysed for skatole and androstenone.

*Study III*

Six crossbred entire male pigs (three pairs of littermates from three Yorkshire x Duroc dams and one Hampshire sire) were included in the study. Half of the pigs (one from each litter) were raised indoors and the remaining pigs outdoors on a field with grass. Blood samples were collected from the pigs at the age of 9 and 15 weeks, and thereafter at weekly intervals from the age of 20 to 32 weeks. The concentrations of skatole, androstenone, testosterone, oestradiol-17β, oestrone sulphate, dehydroepiandrosterone sulphate (DHEAS) and triiodothyronine ($T_3$) were measured in plasma. Fat samples were taken at slaughter and analysed for skatole and androstenone concentrations. The entire male pigs were slaughtered at the age of 33 weeks.

*Study IV*

Two groups of entire male pigs were used. The first group consisted of 33 entire crossbred male pigs (dam Yorkshire and sire backcross between Yorkshire x Wild boar, generation 7). From this group, plasma samples were used to analyse levels of oestrone sulphate and fat samples were used to analyse levels of skatole and androstenone. Plasma and fat samples were taken at slaughter. The second group included 194 entire male pigs of crossbreeds between Swedish Hampshire (H) and Finnish Landrace (L), LH x H, H x LH, LH x LH (dam x sire). Pigs were slaughtered at an age ranging from 20 to 29 weeks and live weights were from 80 to 115 kg. Fat samples from these pigs, taken at slaughter, were analysed for the levels of free oestrone, androstenone and skatole. Details of rearing of the pigs from the second group are given in Josell *et al*. (2003).

*Study V, VI*

A total of 111 entire male pigs of a crossbred between Swedish Yorkshire (dams) x Swedish Landrace (sires) were used. Pigs were raised either in mixed pens, with females and entire males, or single-sex pens with either 7 or 9 pigs in each. In the pens with 9 pigs, 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), and the remaining pigs were slaughtered at an average weight of 115 kg. In the pens with 7 pigs, all pigs were slaughtered at the average weight of 115 kg. All pigs were fed the same commercial diet according to the standard feeding regimen for finishing pigs in Sweden (restricted, 12 MJ ME per kg, digestible CP 13%) until the average pen weight reached 100 kg. Then, 33 out of 80 remaining pigs received additionally raw potato starch, 0.6 kg per day for two weeks prior to slaughter. Pigs were slaughtered when the average weight in the pen reached 115 kg.

Blood samples were taken from pigs at three occasions: first, the day prior to first slaughter occasion; second, the day prior to change in diet; and third, the day prior to second slaughter occasion. Plasma samples were analysed for skatole, androstenone, testosterone and oestrone sulphate concentrations (Study V). Fat samples were taken at slaughter and analysed for skatole (Study V and VI) and androstenone concentration (Study V). Liver samples were taken at slaughter and analysed for the activities of hepatic cytochromes P4502E1 (CYP2E1) and P4502A6 (CYP2A6) (Study VI).

## Biochemical analyses

All biochemical analyses were validated prior to their introduction into laboratory routine use. Validation included determining the linearity and range, precision, accuracy at different concentrations and limit of quantitation. Comparison of the concentrations obtained by different methods was performed when possible.

*Skatole and indole analyses*

In all studies, the analysis of skatole and indole in plasma was performed with a HPLC procedure. The extraction of indolic compounds used in Study I and III was

a slight modification of the method developed by Claus *et al.* (1993) and included the use of diethylether.

In Study II and V, skatole concentrations in plasma were determined with a new simplified method without extraction using acetonitrile to precipitate proteins (Blanchard, 1981). A total of 23 plasma samples with skatole concentrations ranging from 0.7 to 100.0 ng/ml were measured with two methods: (a) no extraction; (b) extraction of skatole was performed with diethylether. The indolic compounds were separated on a reversed-phase Li-Chrospher RP-18 column (5 μm) equipped with a guard column. The detection was performed using a fluorescence detector at an excitation wavelength of 285 nm and emission wavelength of 350 nm. The concentrations obtained using the two methods were closely correlated ($r = 0.98$). The equation of regression was $y = 0.96x - 1.95$, where x is the skatole level in plasma obtained by the method including the extraction step.

Skatole levels in fat were determined with a colorimetric method in all studies (Mortensen & Sørensen, 1984).

### *Androstenone analyses*

In Study II, androstenone concentrations in plasma were measured by a double antibody enhanced luminescence immunoassay after extraction with diethylether (Andersson *et al*., 1998b). In Study III and V, androstenone concentrations in plasma were determined with an ELISA method, using the rabbit anti-androstenone antibodies and androstenone peroxidase conjugate, which was kindly provided by Prof. Mats Forsberg, Department of Clinical Chemistry, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala. Androstenone in plasma samples was determined without the extraction procedure.

Androstenone concentrations in adipose tissue in Study II and III were measured by gas chromatography-mass spectrometry (GC/MS) using supercritical fluid extraction (Mågård *et al.*, 1995). In Study IV, androstenone concentrations in the adipose tissue from 194 entire male pigs of crossbreeds between Swedish Hampshire and Finnish Landrace were determined using the same method, and androstenone concentrations in the adipose tissue from 33 entire crossbred male pigs (dam Yorkshire and sire backcross between Yorkshire x Wild boar) were measured using an enzyme-linked immunosorbent assay (ELISA) as described by Sinclair *et al.* (2001) after extraction with methanol and defatting with hexane. Androstenone measurements in 12 samples obtained using the two methods were closely related ($r = 0.97$). The equation of regression was $y = 1.23x – 0.12$, where x is the androstenone concentration in fat obtained by gas chromatography using supercritical fluid extraction, and y is the androstenone concentrations obtained by ELISA. In Study V, analysis of androstenone in adipose tissue was performed with ELISA as above.

### *Hormone analyses*

In Study II, analysis of testosterone was performed with a radioimmunoassay (Diagnostic Product, Los Angeles, CA, USA, 1982) as described by Andersson *et al*. (1998a). Plasma oestrone sulphate (ES) and total thyroxine was analysed using a double antibody-enhanced luminescence immunoassay (Amerlite, Johnson and Johnson Clinical Diagnostics, Amersham, UK, 1994). The details of method validation criteria are given in Andersson *et al*. (1998a).

In Study III, total testosterone, oestradiol-17β (E17β), dehydroepiandrosterone sulphate (DHEAS) and triiodothyronine ($T_3$) concentrations were measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA), according to manufacturer's instructions. Serial dilutions of plasma with high concentrations of testosterone, E17β, DHEAS and $T_3$ produced displacement curves parallel to the standard curve. Detection limits for testosterone, E17β, DHEAS and $T_3$ were 0.2, 0.02, 54.7 and 0.2 ng/ml, respectively. The coefficients of variation of the methods for intra-assay and inter-assay were less than 10%. ES concentrations in this study were analysed by double antibody enhanced luminescence immunoassay (Andersson *et al.*, 1998a), and IGF-1 concentrations were determined by a non-competitive time-resolved immunofluorometric assay (TR-IFMA) of the sandwich type (Frystyk, Dinesen & Ørskov, 1995).

The levels of free oestrone in adipose tissue (Study IV) were measured with a new radioimmunoassay method developed by our research group. Free oestrone was extracted from fat with methanol and defatted with hexane. The extraction recovery was 97 ± 13.7%. The coefficients of variation were from 9.23 to 11.94% (intra-assay) and from 3.78 to 10.11% (inter-assay)

ES levels in plasma in Study IV and V were measured by a radioimmunoassay procedure (Diagnostic Systems Laboratories, Texas, USA), according to manufacturer's instruction. Testosterone concentrations in Study V were also measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA).

### *Enzymatic assays*

In Study VI, the catalytic activity of CYP2A6 was measured as the rate of formation of 7-hydroxycoumarin (Diaz & Squires, 2000b), and the activity of CYP2E1 as the rate of formation of p-nitrocatechol (Jiang *et al*., 1998). The formation of 7-hydroxycoumarin was linear between 5 and 25 min of incubation time with 0.8 mg of microsomal protein, and between 0.2 and 1.2 mg of microsomal protein with incubation time of 15 min. The formation of p-nitrocatechol was linear between 5 and 30 min of incubation time with 0.5 mg of microsomal protein for the samples with low and medium activity of CYP2E1 (range from 210 to 385 pmol/min/mg protein) and between 5 and 20 min of incubation time with 0.5 mg of microsomal protein for the samples with high activity of CYP2E1 (461 and 483 pmol/min/mg protein). The formation of p-nitrocatechol was linear between 0.25 and 0.7 mg of microsomal protein with an incubation time of 15 min.

### Statistical analyses

All statistical analyses were performed with the Statistical Analysis System, version 8.2 (SAS Institute, Cary, NC, USA). The following procedures were used: univariate (Study I), mixed (Study II, V and VI), Pearson correlation (Study II, IV, V and VI), partial correlation from procedure GLM (Study II, III and VI). A logarithmic transformation was applied on investigated variables when their distributions were skewed.

# Summary of presented investigations

## I. The effect of age on distribution of skatole and indole levels in entire male pigs in four breeds: Yorkshire, Landrace, Hampshire and Duroc

In this study, plasma samples from Yorkshire, Landrace, Hampshire and Duroc entire male pigs collected at different ages were analysed for skatole concentrations and slightly fewer samples were analysed for indole concentrations. For each breed, a distinct age-related distribution of plasma skatole levels was observed. Skatole levels were increased at approximately 180-200 days of age, reaching very high levels in some individuals. Afterwards, the levels decreased; in Yorkshire and Landrace at approximately 240-260 days of age, and in Hampshire and Duroc at 310-360 days of age. The decrease of skatole levels was also observed in samples collected from 15 Landrace boars at two different ages: 16.8 μg/L (SD = 17.36) vs. 5.9 μg/L (SD = 3.48, P = 0.016) in samples taken at the mean age of 210 and 314 days, respectively. Indole levels showed similar age- and breed-related variations to skatole levels. At the time of increased skatole concentrations (age from 180 to 360 days, depending on breed) 25.5% of Yorkshire, 31.6% of Landrace, 20.3% of Hampshire and 61.1% of Duroc entire male pigs had skatole levels above 12.6 μg/L of plasma, which corresponds to the threshold level of 0.20 μg/g of fat that is used for selecting tainted carcasses. The results indicate that increased skatole levels in entire male pigs are related to puberty and taking skatole measurements at that age could be advantageous when considering genetic selection to reduce boar taint level in entire male pigs. Breed differences should also be taken into account.

## II. Plasma skatole and androstenone levels in entire male pigs and relationship between boar taint compounds, sex steroids and thyroxine at various ages

The objectives of the study were to investigate the age-associated variations of skatole and androstenone levels in plasma and their relationship to testosterone, oestrone sulphate and thyroxine in plasma from entire male pigs. Effect of photoperiod on boar taint compounds in plasma was also investigated. Blood samples were collected every 2 weeks from 8 weeks of age until slaughter at the

age of 20-24 weeks (115 kg). Neither skatole nor androstenone levels in plasma were affected by photoperiod. Skatole concentrations in plasma varied with age and were high at the age of 8-10 weeks. The early increase of skatole levels was not related to other investigated compounds. At the older age, skatole levels in plasma were positively correlated to androstenone levels ($r = 0.30$; 20 weeks of age), testosterone and oestrone sulphate levels ($r = 0.43$ and 0.54, respectively; 22 weeks of age) and negatively to thyroxine levels ($r = -0.44$; 22 weeks of age, and $r = -0.72$; 24 weeks of age). Androstenone levels in plasma were positively correlated to levels of testosterone and oestrone sulphate from approximately 14 to 24 weeks of age, but not at younger age, with correlation coefficients ranging from 0.34 to 0.79. Negative correlations between androstenone and thyroxine levels in plasma were found at the ages of 16, 18 and 20 weeks ($r = -0.42$, -0.35 and -0.30, respectively). Skatole and androstenone levels in fat at slaughter, 20-24 weeks of age, were highly correlated ($r = 0.68$; $P < 0.001$).

## III. Age-related variation of plasma concentrations of skatole, androstenone, testosterone, oestradiol-17β, oestrone sulphate, dehydroepiandrosterone sulphate, triiodothyronine and IGF-1 in six entire male pigs

This study describes the age-related variation in skatole, androstenone, and testosterone, oestradiol-17β (E17β), oestrone sulphate (ES), dehydroepiandrosterone sulphate (DHEAS), triiodothyronine (T3) and insulin-like growth factor-1 (IGF-1) in six boars. Blood samples were taken at the age of 9 and 15 weeks and thereafter at weekly intervals from the age of 20 to 32 weeks. Plasma concentrations of skatole, androstenone, testosterone, E17β, ES, DHEAS, T3 and IGF-1 were measured. We found that skatole levels in boars increased at the age around puberty after an increase in the levels of testicular steroids. Skatole levels were not associated with the levels of sex steroids, T3 and IGF-1. However, the increased level of testicular steroids is probably the underlying factor needed for high skatole levels to occur although the specific mechanism leading to increased skatole levels remains unknown.

## IV. Determination of free oestrone in adipose tissue by radioimmunoassay and the relationship between levels of free oestrone, androstenone and skatole in fat of entire male pigs

A new method for the analysis of free oestrone in porcine adipose tissue was developed. Free oestrone was extracted from fat with methanol and defatted with hexane. After evaporation of the methanol phase, free oestrone was measured by radioimmunoassay. The extraction recovery was 97 ± 13.7%. The standard curve was linear for concentrations of free oestrone ranging from 0.13 to 5.10 ng/g. Intra-assay variations for samples with concentrations of free oestrone from 0.67 to 2.08 ng/g were from 9.23 to 11.94%. Inter-assay variations for the sample with concentrations of free oestrone from 0.89 to 2.96 ng/g were from 3.78 to 10.11%. The application of this method was demonstrated for the analysis of free oestrone in fat in two groups of entire male pigs of different breeds. Group A consisted of

entire crossbred male pigs between Yorkshire (dam) and backcross Yorkshire x Wild Boar (sire). Group B consisted of entire male pigs of crossbreeds between Swedish Hampshire and Finnish Landrace. The levels of free oestrone in fat from group A were well correlated with fat levels of androstenone ($r = 0.66$; $P < 0.001$) and levels of oestrone sulphate in peripheral plasma collected at the same time as the fat ($r = 0.74$, $P < 0.001$). Skatole levels in fat from group B were significantly correlated to free oestrone ($r = 0.28$, $P < 0.001$) and androstenone ($r = 0.32$, $P < 0.001$) levels in fat. This confirms a relationship between testicular steroids and skatole accumulation in fat from entire male pigs. The study indicates that free oestrone in fat may be used for evaluation of hormonal status; however, more research is needed to determine whether free oestrone is a suitable indicator of puberty.

## V. Effect of live weight and dietary supplement of raw potato starch on the relationship between levels of skatole, androstenone, testosterone and oestrone sulphate in entire male pigs

This study evaluated the effect of a supplement of raw potato starch (RPS) on the levels of skatole, androstenone, testosterone and oestrone sulphate in plasma from entire male pigs. The study also evaluated relationships between plasma levels of skatole and testicular steroids at three different live weights (LW) of approximately 90, 100 and 115 kg. Levels of skatole and testicular steroids in plasma were significantly higher in entire male pigs from the high-weight group fed no RPS compared to those from low- and middle-weight groups. Levels of the investigated compounds did not differ between low- and middle-weight groups ($P > 0.1$). The diet with RPS induced a decline in skatole levels in plasma and fat ($P < 0.001$), but not in plasma levels of testicular steroids and fat levels of androstenone ($P > 0.05$). Skatole levels were positively correlated to testosterone and estrone sulphate levels in the middle- and high-weight pigs fed no RPS as well as to testosterone in the low-weight group. In the high-weight group fed RPS, skatole levels were not correlated to any of the analysed compounds. Approximately 26 % of the entire male pigs (11 out of 43) from the high-weight group fed no RPS produced skatole levels in fat above 0.20 µg/g, whereas the pigs from the low- and high-weight group fed RPS did not produce skatole levels above 0.20 µg/g in fat. Androstenone levels in fat were high in all groups. In total 47% (52 out of 111) pigs expressed androstenone levels above the rejection levels in fat of 1.0 µg/g and 88% (98 out of 111) had androstenone levels above 0.5 µg/g. It was concluded that a lower slaughter weight and the supplement of raw potato starch to the diet could be used to reduce skatole levels in entire male pigs. Androstenone levels in fat, however, could not be reduced by either a lower weight at slaughter or dietary manipulation.

## VI. Relationship between the activities of cytochromes P4502E1 and P4502A6 and skatole content in fat in entire male pigs fed a diet with and without raw potato starch

The objective of this study was to evaluate the potential impact of the dietary supplement of raw potato starch (RPS) on the relationship between skatole in fat and the activities of cytochromes P4502E1 (CYP2E1) and P4502A6 (CYP2A6) in entire male pigs. Activities of hepatic CYP2E1 and CYP2A6 were higher in low-weight males ($P < 0.001$). Enzyme activities did not differ between groups slaughtered at 115 kg that were fed diets with and without RPS. The age-related increase in skatole levels might be due to the decreased activities of cytochromes CYP2E1 and CYP2A6. Skatole levels did not induce either CYP2E1 or CYP2A6 in vivo. Addition of RPS to the diet significantly reduced skatole levels in fat. Cytochromes CYP2E1 and CYP2A6 are not involved in the mechanism of this reduction.

# Discussion of main findings

## Factors affecting androstenone levels

*Age, weight, puberty, hormonal status*

In Study II, III and V, age-related changes in androstenone levels were investigated. Androstenone levels in plasma increased simultaneously with other testicular steroids at the age of puberty (Study II, III and V) and positive correlations were obtained between androstenone and all measured steroid levels (Study II, IV and V). The similarity in age-related changes of testicular steroids was expected due to linked biosynthesis and metabolic pathways (Gower, 1972; Bonneau, 1982; Brooks & Pearson, 1986). Sinclair *et al*. (2001) showed a positive correlation between androstenone, oestrone sulphate and testosterone in plasma from entire male pigs at slaughter weight. Several investigations showed positive correlations between the levels of testosterone and androstenone in plasma (Carlström *et al*., 1975; Andresen, 1976; Bonneau *et al*., 1982). The levels of testicular steroids are mostly regulated by developmental changes in Leydig cells and by sensitivity of the Leydig cells to gonadotropin stimulation. In pigs, Leydig cell development might be described as triphasic, with the first phase occurring in foetal life, the second during prepubertal development and the third at puberty onset (Allrich *et al*., 1982; Schwarzenberger *et al*., 1993; Lunstra *et al*., 1997; Geiger *et al*., 1999; França *et al*., 2000; Sinclair *et al*., 2001). Subsequently, the elevated level of testicular steroids is due to increased steroidogenic activity of Leydig cells. The presented work was focused on the pubertal increase in testicular steroid levels because it often coincides with slaughter age/weight of pigs (20-24 weeks of age, 100-110 kg of live weight). The large variation in androstenone levels at the age of 20-24 weeks is due to differences in age at puberty and individual potential for androstenone production. The relationship between androstenone levels and size of reproductive organs as puberty estimators, such as length of bulbourethral glands, and testes size, has been demonstrated by several researchers (Førland, Lundström & Andresen, 1980; Bonneau & Russeil, 1985; Babol, Squires & Gullett, 1996; Sellier *et al*., 2000). There are additional factors, which may influence puberty onset. Einarsson *et al*.

(1979) suggested that puberty is more closely related to boar age than live weight. Nutrition might also be important in puberty onset (Hughes & Varley, 1980).

In Study V, androstenone levels in fat did not differ between groups of 90 and 115 kg of live weight, whereas plasma androstenone levels were significantly lower in low-weight pigs. The absence of a weight effect on androstenone levels in fat was not expected and therefore difficult to explain. Perhaps the pigs at 90 kg have already reached maximum concentration of androstenone in fat; in that case an additional increase at 115 kg might not be observed. However, this does not explain the increase in plasma androstenone levels. It appears that androstenone accumulation in fat does not ultimately depend on plasma androstenone levels. This suggestion is in agreement with a study of Lundström *et al*. (1978) where no correlation between plasma and fat androstenone levels was found. The nature of the mechanism for androstenone accumulation in fat remains to be investigated.

### *Nutrition and raising conditions (single-sex vs. mixed pens)*

Androstenone levels in entire male pigs fed raw potato starch (RPS) were similar to those in pigs fed only the commercial diet. Similarly, Whittington *et al*. (2004) observed no changes in androstenone levels in pigs fed sugar beet pulp. However, some studies suggested that diet might influence androstenone levels in entire male pigs. Androstenone levels were higher in entire male pigs of the same age fed ad libitum compared to those fed restrictively (Bonneau, 1987). Øverland, Berg & Matre (1995) obtained similar results. As indicated by these studies, feeding intensity affects androstenone levels more than specific ingredients in the diet.

Androstenone levels in fat and plasma in the low-weight pigs (90 kg) were higher in the pigs raised in mixed pens compared to those in single-sex pens. The effect of social factors on androstenone levels of entire male pigs has received little attention. The introduction of a mature boar to gilts is known to induce the attainment of puberty (Patterson *et al*., 2002). In the same way, the presence of female pigs might accelerate onset of puberty in entire male pigs. The absence of the effect of mixing sexes on the levels of androstenone in the middle- and high-weight pigs (100 and 115 kg) suggests that exposing males to female pigs might affect the differences in the age of pigs when levels of testicular steroids increase, but not the intensity of this increase afterwards.

## Factors affecting skatole levels

### *Genetics*

In Study I, an attempt was made to investigate the possible presence of a major gene (Lundström *et al*., 1994) affecting skatole levels. For this purpose, the frequency distribution of skatole levels in purebred boars was evaluated. Distributions may have one or more peaks (unimodal with one peak, bimodal with two peaks, polymodal with many peaks). A normal distribution of skatole levels would indicate the effect of several factors. A polymodal distribution can indicate involvement of a major gene in the regulation of skatole levels. The samples for construction of distribution graphs were taken from boars at the age of increased

levels and/or high variation in skatole levels. No clear polymodal distribution was observed for any of the investigated breeds and, therefore, the major gene influencing skatole levels could not be detected. However, the observed breed differences confirm the existence of genetic effects on skatole levels in entire male pigs. The age and the level of skatole increase varied considerably between breeds. These differences may be related to the differences in puberty onset between breeds, which are under genetic control (Wilson, Johnson & Wettermann, 1977; Hughes & Varley, 1980; Rothschild, 1996). The populations of Hampshire, Yorkshire and Landrace pigs used in this study were also evaluated for sperm morphology and significant differences in percentage of mature sperm were observed (Wallgren, 2000).

### *Age, weight, puberty*

Age-related variations in skatole levels were evaluated in Study I, II, III and V. Surprisingly, high skatole levels in some pigs were observed at a young age, from 8 to 10 weeks (Study II). The nature of this increase is not clear. Leydig cells of entire male pigs undergo a transient period of activation during the period of 2-4 weeks, which results in an increased secretion of testicular steroids, including androstenone (Schwarzenberger *et al*., 1993; Sinclair *et al*., 2001). This increase in testicular steroid level might subsequently initiate an increase in skatole levels at a young age, either by inhibiting the liver metabolism of skatole directly (Babol, Squires & Lundström, 1999), or by repressing the expression of enzymes involved in skatole metabolism in the liver (Doran *et al*., 2002b). The fact that increased levels of testicular steroids, particularly androstenone preceded the rise in skatole levels (Study II and III) suggests that the latter hypothesis is more probable.

Other factors might be also involved in the occurrence of the pre-pubertal increase in skatole levels, such as age-related variations in IGF-1 levels. IGF-1 is involved in stimulation of gut cell growth and apoptosis, and, therefore, regulates the availability of the substrate for skatole formation (Claus & Raab, 1999). IGF-1 levels in pigs increase during the postnatal period (Louveau *et al*., 1996) and might thus contribute to increased skatole levels at a young age. Postnatal maturation of the intestine is characterized by significant modifications of enzyme activities (Jensen, Jensen & Jakobsen, 1997), microbial populations (Franklin *et al*., 2002) and transport properties. These developmental changes in enzyme and microbial status and intestinal transport are particularly remarkable during the period shortly after weaning and might also be important for skatole formation in young pigs. The subsequent decrease in skatole levels from 12 to 18 weeks of age may be due to a balance between skatole production and an ability of growing pigs to metabolise skatole. At puberty, this balance is probably disturbed leading to the puberty-related increase of skatole levels, observed in Study I, II, III and V.

There were numerous attempts to explain the puberty-related increase in skatole levels. Firstly, it was suggested an adduct-formation between skatole and androstenone in blood (Singh *et al*., 1988). Secondly, at puberty, the turnover of intestinal cells is increased, thus providing more tryptophan for skatole formation. In this process, growth factors and glucocorticoids are involved (Claus, Weiler & Herzog, 1994; Claus & Raab, 1999). Babol, Squires & Lundström (1999)

suggested that skatole and androstenone may share a common metabolic pathway. Later, in vitro studies in the UK demonstrated that androstenone inhibits the skatole-induced expression of the enzyme CYP2E1 (Doran *et al*., 2002b). In study II and III, increased testicular steroid levels preceded increased skatole levels. The rise in the levels of testicular steroids occurred from the age of 16 weeks in Study II, and 24-25 in Study III, and skatole levels increased approximately 4 weeks afterwards (20 weeks in Study II and 28 weeks in Study III). These observations suggest that if testicular steroids inhibit the expression of enzymes involved in skatole metabolism as it was shown in the in vitro study by Doran *et al*. (2002b), in vivo inhibition does not occur immediately after the increase in steroid levels but may be delayed for up to 4 weeks. The subsequent decrease in skatole levels at older ages suggested that the inhibitory effects of testicular steroids could be reversed, although this could also be due to decreased production of skatole. The decrease in the levels of testicular steroids in older pigs (Gray *et al*., 1971; Tan & Raeside, 1980; Study III) may also contribute to decreased skatole levels at an older age.

Summarising, the age-related pattern of plasma skatole levels in entire male pigs can be described as follows. Skatole levels undergo a temporary increase at the younger age of 8-10 weeks (Study II). Then, they decrease and remain relatively low up to approximately 20-26 weeks of age; in some pigs, they then increase again (Study I, II, III and V). The time and the level of this second increase appeared to be breed-dependent (Study I). The second increase is also temporary and skatole levels, possibly after reaching a certain maximum level, decrease at older ages (Study I and III). The mechanism(s) responsible for the age-related variations in skatole levels in entire male pigs is not known. The fact that skatole levels increased at the age of puberty suggests that changes which occur during puberty, e.g. the increased production of testicular steroids, could be involved in the regulation of skatole levels. The positive correlation between skatole levels and testicular steroid levels (Bonneau *et al*., 1992; Annor-Frempong *et al*., 1997b; Babol, Squires & Lundström, 1999; Cameron *et al*., 2000), and live weight (Babol, Squires & Gullett, 1996) confirms the role of puberty in affecting skatole levels. The increase of skatole levels with increasing live weight of entire male pigs has also been demonstrated (Hansen *et al*., 1997; Walstra *et al*., 1999; Whittington *et al*., 2004; Study V).

Knowledge of the time of puberty onset and the age at which entire male pigs express maximum skatole levels is needed to detect pigs with a low capability for skatole synthesis at puberty. The genetic and nutritional factors affecting skatole levels should be strictly controlled in such studies.

### *Hormonal status*

Overall, skatole levels in plasma were not significantly correlated to levels of either steroid or thyroid hormones or IGF-1 (Study II and III). A positive correlation between plasma levels of skatole and androstenone in Study II, although statistically significant, was too low ($r = 0.13$) to be of importance. However, as indicated by results from Study II, the relationships between plasma levels of skatole, testicular steroids and thyroxine vary between ages. Skatole

levels were positively correlated to testicular steroids from approximately 20 weeks. At this age, high variability in the levels of all substances was observed. The absence of correlations at younger ages suggests that testicular steroids need to reach a certain physiological level to perform their action on skatole metabolism. These findings indicate that the increase in levels of testicular steroids during the process of sexual development may affect skatole accumulation. The relationship between skatole and androstenone in fat in pigs at slaughter age has been extensively studied and most reports demonstrated a positive but moderate relationship; the correlation coefficients varied from $r = 0.30$ up to $r = 0.73$ (reviewed in Walstra *et al*., 1999). In Study II, the correlation coefficient between levels of skatole and androstenone in fat was 0.68. In crossbred entire male pigs, skatole levels in fat were positively correlated to both free oestrone and androstenone levels ($r = 0.28$ and 0.32, respectively) indicating the importance of testicular steroids in the control of skatole levels (Study IV).

In Study V, positive correlations between skatole, testosterone and oestrone sulphate, but not androstenone were observed only in the groups that expressed high variations in skatole levels. As discussed above, a certain physiological level of testicular steroids is probably needed to affect skatole metabolism. This level remains to be determined. The absence of a relationship between skatole and androstenone in any of the groups supports the suggestion that other testicular steroids might be more important in the regulation of skatole levels.

The possibility to alter boar taint levels by exogenous steroids in entire male pigs has been the subject of several investigations. Results obtained in such studies ranged widely. Lopez-Bote & Ventanas (1988) found a reduction in boar taint levels in male pigs at slaughter age as a result of neonatal administration of testosterone. It is not clear from their study whether injection with testosterone affected skatole or androstenone or both because the level of boar taint was assessed by sensory evaluation. It is however likely that testosterone administration mainly affected androstenone levels because testicular weight and endogenous testosterone levels were also reduced in treated pigs. No effect of oestradiol-17β or combination of oestradiol-17β and testosterone treatment (implantation at the base of the ear) on skatole levels in fat was found, whereas androstenone levels were significantly reduced by both treatments (De Brabander *et al*., 1991). The implantation of anabolic hormones into the neck muscle also suppressed androstenone accumulation in fat (Daxenberger *et al*., 2001). Testosterone as a feed additive, 20 mg per pig per day for 8 weeks, increased skatole levels in fat (Mortensen, 1991). Dragoeva *et al*. (2000) obtained a reduction in total indole levels in plasma from 4 entire male pigs injected with diethylstilbestrol dipropionate (synthetic oestrogen) 5 days prior to slaughter. These studies are worthy of note because they investigate the effect of testicular steroids on skatole and androstenone levels. None of these results, however, are of practical significance because the use of anabolic hormones in animal production is banned in the European Union.

*Nutrition*

The production of skatole in the intestine depends on dietary factors (Jensen & Jensen, 1998; Claus *et al*., 2003). Dietary supplement of raw potato starch (RPS) reduced skatole levels in entire male pigs in both plasma and fat (Study V). Pigs fed RPS did not have skatole levels in fat above 0.20 μg/g, which is the rejection level for skatole in fat in Sweden.

The role of nutrition in skatole levels has been well established (Table 2); however, the mode of action of dietary additives on skatole levels is still being studied. Previous studies have shown that including certain types carbohydrates into the diet affects the microflora of the gastrointestinal tract and influences intestinal functions (Drochner, 1993; Jensen, Cox & Jensen, 1995b; Kleessen *et al*., 1997; Claus *et al*., 2003; Le Blay *et al*., 2003; Wang *et al*., 2004). This might subsequently induce changes in intestinal production of skatole. The absorption of skatole from the intestine might also be affected by diet. Undigested carbohydrates increase faecal wet and dry weight (Wang *et al*., 2004) and decrease intestinal transit time (Drochner, 1993), which might reduce the rate of skatole absorption from the large intestine. Little is known about the mechanism of skatole uptake from the intestine. The amount of skatole absorbed through the intestinal wall depends to a great extent on water content and intestinal transit time (Jensen & Jensen, 1998). It is likely that skatole is absorbed from the intestine by passive diffusion, in which absorption occurs because of concentration gradient across the intestinal mucosa. No skatole-binding carrier is known.

Claus *et al*. (2003) explained the effect of RPS on skatole levels as follows. RPS, similarly to dietary fibre, is not fully digestible in the upper gastrointestinal tract. Resistant starch, comprising a significant part of raw potato starch, undergoes bacterial fermentation in the large intestine producing gases ($CO_2$, $CH_4$, $H_2$) and short-chain fatty acids (SCFA), mainly acetate, propionate and butyrate. Butyrate has been shown to reduce colonocyte apoptosis (Mentschel & Claus, 2003) and thus would reduce the availability of cell debris, which provide a substrate for skatole formation (Claus *et al*., 2003). The production of SCFA also caused a reduction in pH of the colon (Mentschel & Claus, 2003; Claus *et al*., 2003). Claus *et al*. (2003) demonstrated a significant reduction in skatole levels in fat and plasma from castrated male pigs fed RPS. There is evidence that diet affects the hepatic cytochrome P450 activity, which may consecutively affect the metabolism of substrates for those enzymes (Sonawane *et al*., 1983). Whittington *et al*. (2004) suggested that a fibre-rich diet is related to the increased expression of hepatic cytochrome P4502E1 (CYP2E1), an enzyme involved in skatole metabolism. In their study, supplement of sugar beet in the diet decreased skatole levels, whereas increased the expression of CYP2E1. However, the mechanism of induction of CYP2E1 by diet is not known.

The impact of carbohydrate-rich diets on skatole levels obtained from different studies is variable (Table 2). The variability may reflect either the effect of quantity or differences between carbohydrate types. Carbohydrates from various sources differ in chemical structure, rate of digestion and fermentation (Theander, Westerlund & Åman, 1993; Bednar *et al*., 2001). Within one source, the effect might be dose-dependent. The addition of 0.75 g fructooligosaccharides per pig

per day did not affect skatole levels in faeces of young pigs at approximately 10 kg live weight, whereas increasing the amount of fructooligosaccharide to1.5 g per pig and day successfully decreased skatole levels (Russell *et al*., 1998). Processing and storage might also differentially affect the chemical characteristics of different carbohydrates leading to inconsistent dietary effects (Niba, 2003).

### *Metabolism*

The results from Study VI confirm the involvement of enzymes CYP2E1 and CYP2A6 in skatole metabolism (Babol *et al*., 1998a; Diaz & Squires, 2000). Increased skatole levels in fat from entire male pigs at a live weight of 115 kg might be linked to decreased activity of CYP2E1 and CYP2A6. Also, skatole levels were negatively correlated to the activities of CYP2E1 and CYP2A6 in the group of high-weight boars fed no RPS. If the enzyme activity is low, skatole is not entirely metabolised in the liver but can accumulate in adipose tissue. All entire male pigs with high skatole levels showed a low activity of CYP2A6, whereas the activity of CYP2E1 in these males varied from low to intermediate, indicating that CYP2A6 might be more important in skatole metabolic clearance than CYP2E1. However, these influences are important only in pigs with high skatole production. If the production is low the skatole levels in the fat will be low regardless of slow liver metabolism (Study V).

In an in vitro study, the expression of CYP2E1 was induced by skatole and this induction was inhibited by androstenone (Doran *et al*., 2002b). In Study VI, it was concluded that enzyme activities varied independently of androstenone levels in fat, and thus the decreased enzymes' activities in high-weight pigs were not a result of an inhibition by androstenone. However, this conclusion was based on androstenone levels in fat. Plasma analysis in the same animals revealed significantly increased levels of androstenone, testosterone and oestrone sulphate in pigs at 115 kg live weight (Study V). Probably, higher activities of CYP2E1 and CYP2A6 in low-weight pigs were due to low levels of steroids in plasma. Increased levels of androstenone, testosterone and oestrone sulphate in pigs of the high-weight group could inhibit the expression and activities of these enzymes. No correlations between testicular steroid levels in plasma and activities of CYP2E1 and CYP2A6 were found when calculated within groups, except for testosterone, which was negatively correlated to activity of CYP2A6 in the group of high-weight pigs fed no RPS ($r = -0.34$). Between groups, activities of CYP2E1 and CYP2A6 were negatively correlated to testicular steroid levels (Table 3).

Table 3. *Pearson correlation coefficients between levels of androstenone (AND), testosterone (T), oestrone sulphate (ES) in plasma and activities of hepatic cytochromes P4502E1 (CYP2E1) and P4502A6 (CYP2A6) in entire male pigs (n = 111) (unpublished data)*

| | AND | T | ES | CYP2E1 |
|---|---|---|---|---|
| T | $0.49^{***}$ | | | |
| ES | $0.63^{***}$ | $0.74^{***}$ | | |
| CYP2E1 | - 0.15 | $-0.25^{**}$ | $-0.29^{**}$ | |
| CYP2A6 | $-0.29^{**}$ | $-0.35^{***}$ | $-0.33^{***}$ | $0.73^{***}$ |

Levels of significance: $^{**}P < 0.01$; $^{***}P < 0.001$.

Thus, results from Study VI suggest an inhibiting effect of testicular steroids on the activities of enzymes involved in skatole metabolism.

*Raising conditions (single-sex vs. mixed pens)*

Raising entire male pigs in mixed pens resulted in increased skatole levels in plasma in the high-weight group fed RPS (Study V). It should be emphasised that skatole levels in this group were very low. In contrast, Andersson *et al*. (1997) observed slightly increased skatole levels in fat from entire male pigs raised in single-sex pens at 105 kg live weight. Practical significance and a mechanism responsible for these variations remain to be determined.

## Free oestrone levels as a potential estimator of puberty stage

Knowledge about puberty status of entire male pigs is needed to define the mechanism of a relationship between stage of sexual development and expression of boar taint. This knowledge is also needed to identify pigs that may express low androstenone and skatole levels at sexual maturity. Then, boar taint and maturity could be separated during the selection procedure.

Adipose tissue plays an important role in the accumulation, storage and metabolism of sex steroids (Mizutani *et al*., 1994; Bélanger *et al*., 2002; Kamat *et al*., 2002) and steroid levels in fat are less likely to be affected by diurnal variations than those in plasma. The measurement of oestrogen levels in fat might give reliable information regarding puberty stage of entire male pigs.

Considering this, Study IV was performed to develop a method to measure levels of free oestrone in fat and to investigate the relationship between the levels of free oestrone and boar taint compounds, skatole and androstenone, in fat. In Sweden, samples of adipose tissue of entire male pigs are routinely collected at slaughter to determine skatole levels and sort out tainted carcasses. These samples could also be used to measure oestrogens in fat. Concentrations of oestrogens in adipose tissue have mostly been determined in bovine and human tissues (Hoffmann, 1983; Hoffmann & Blietz, 1983; Szymczak *et al*., 1998) and there is limited information on the levels of free oestrone in porcine adipose tissue (Claus *et al*., 1989). Sample preparation often includes the steps of homogenisation, extraction and purification. In the present method, direct extraction of oestrone with methanol followed by defatting with hexane does not require further purification and could be applied for analysis of free oestrone in laboratory conditions. However, more research is needed to evaluate this method as a puberty indicator since the level of free oestrone needs to be related to other measurements of sexual maturity such as sperm maturity and development of sex glands.

# General conclusions

- The maximum variation and increase in androstenone and skatole levels in entire male pigs occurred at puberty.
- Androstenone levels were not affected by including raw potato starch in the diet.
- Raising entire males in mixed pens in the presence of female pigs results in increased androstenone levels in entire male pigs at 90 kg of live weight.
- Skatole levels in entire male pigs differed between breeds.
- The increase in skatole levels occurred at a young age, from 8 to 10 weeks. This increase might be a consequence of pre-pubertal increase of testicular steroids.
- The second increase in skatole levels occurred at puberty after the increase in testicular steroid levels.
- The relationships between skatole and testicular steroid levels differed at different ages. However, skatole levels were generally positively correlated to the levels of testicular steroids at slaughter age.
- No direct relationship between skatole, thyroid hormones and IGF-1 was found.
- Relatively low skatole levels in fat and plasma could be achieved by slaughter at low weight (90 kg).
- Hepatic CYP2E1 and CYP2A6 were both involved in skatole metabolism, CYP2A6 being more important.
- The dietary supplement of raw potato starch reduced skatole levels in fat and plasma but did not affect activities of hepatic CYP2E1 and CYP2A6.
- Raising entire male pigs in mixed pens resulted in increased skatole levels in plasma in entire male pigs at 115 kg of live weight fed raw potato starch.
- Measurements of free oestrone in adipose tissue may be potentially used to evaluate puberty stage in entire male pigs.

# Recommendations and future perspectives

The results presented in the current review provide important information on the factors involved in age-related variations in skatole and androstenone levels. However, they are insufficient to determine a method that eliminates boar taint from entire male pig carcasses. Indeed, instead, they bring up new lines of investigation, which are summarized in Table 4.

The general goal, i.e. to find a satisfactory solution to the boar taint problem, remains to be achieved. There are some important aspects, which need to be definitively clarified. To determine a biomarker related to boar taint and age of puberty is still a central requirement so that genetic selections against boar taint can be performed without side-effects. Management factors that can affect boar taint level, such as nutrition, rearing conditions, lighting periods, have to be further examined. Future efforts should focus on the optimisation of methods for on-line detection of boar taint. Meat with high skatole and/or androstenone levels can be used through processing; however, further research is needed on the acceptance of such products by consumers. Each country or region has its specific demands for pork products, as a result of differences in breeds and slaughter weight of pigs for pork production, and cooking habits. Thus, the acceptability of meat in relation to the boar taint problem varies between different countries. Most studies are focused on skatole and androstenone as the main contributors to boar taint; however, the possible role of other contributors also requires investigation.

Special attention should be paid to the communication with consumers. An understanding of the factors that determine consumer perceptions of a product's value has to be determined.

Table 4. *Some specific questions intended for further investigations*

| Aspects | Achieved aims | Question raised | Application |
|---|---|---|---|
| Genetic | Skatole levels in entire male pigs differ between breeds | Which breeds have low potential to accumulate skatole? | Breeds with lowest skatole levels can be used in pork production |
| | | Is a major gene involved? | Genetic selection against only those genes that cause boar taint |
| Metabolism | CYP2A6 activity is likely more important in metabolic clearance of skatole than CYP2E1 | Do steroids affect the expression of CYP2A6 in skatole metabolism in the same way as was shown for androstenone and CYP2E1 (Doran *et al*., 2002b)? | An explanation for the related increase in the levels of skatole and testicular steroids |
| Puberty and hormonal status | The increase in skatole and androstenone levels mainly occurs in sexual mature boars | Which factor(s) should be taken into account when estimating puberty? | The determination of potential biomarker of puberty can enable separation of boar taint and puberty during the selection procedure |
| | Skatole levels are related to the levels of testicular steroids rather than puberty itself | Which steroid is most important in the regulation of skatole levels and which mechanisms are involved? | The elucidation of the factors regulating skatole levels is important |
| Nutrition | Skatole levels are reduced by addition of raw potato starch (RPS) to a diet | Are there any negative effects of RPS on animal health? | The portion of RPS that can reduce skatole levels without affecting animal health and can minimize costs should be determined |
| | Androstenone levels are not affected by RPS in the diet | What will happen if the portion of RPS in the diet is higher than 20% as used in Study V? | Increased portion of digestible starch which is also present in RPS might accelerate growth performance and probably onset of puberty |

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