



# **“WELFARE ASPECTS OF THE CASTRATION OF PIGLETS”**

**Scientific Report of the Scientific Panel for Animal Health  
and Welfare on a request from the Commission related to  
welfare aspects of the castration of Piglets**

**(Question N° EFSA-Q-2003-091)**

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## 1. GLOSSARY

*NB: The (terms) are synonyms found in the literature*

**A:** Androgens.

**ACTH:** Adrenal Corticotrophin Releasing Hormone.

**Active immunisation (AI):** Production of antibodies by the target animal, elicited by the injection of an immunogen.

**Anaesthesia (general/regional/local):** Entire or partial loss or absence of feeling or sensation; a state of general or local insensibility produced by inhalation or application of an anaesthetic; general anaesthesia causes loss of consciousness, local/regional anaesthesia causes loss of feeling or sensation only to a specific area.

**Analgesia/Pre-emptive analgesia:** Absence of pain in response to stimulation which would normally be painful (IASP). Usually analgesia is accompanied by sedation without loss of consciousness. Pre-emptive analgesia involves the administration of analgesics before painful stimuli. This prevents the establishment of a hypersensitized state and, thus, the amplification of postoperative pain (Lascelles *et al.*, 1994a and 1994b).

**Androstenone:** 5 $\alpha$ -androst-16-ene-3-one, a steroid of the 16-androsten family, one of the major contributors to boar taint (synonyms: 5 $\alpha$ -androstenone, 5 alpha androstenone).

**(Androst-16-enes):** see 16-androstenes.

**BC:** Before Christ.

**(5 $\alpha$ -androstenone, 5 alpha androstenone):** see Androstenone.

**(C19 $\Delta$ 16, C19 delta-16 (steroids)):** see 16-androstenes.

**$\Delta$ 16 steroids:** see 16-androstenes.

**Castration:** + females.

**CY:** Cytochrome.

**DFD:** dark, firm, dry - an abnormal condition of meat caused by reduced glycolysis resulting from a prolonged period of stress before slaughter.

**(Delta-16 steroids):** see 16-androstenes.

**Dimorphic:** existing in two forms.

**Dressing percentage:** Hot carcass weight as a percentage of live weight immediately before slaughter.

**EU:** European Union.

**FSH:** Follicle Stimulating Hormone.

**Funiculum:** Spermatic chord. Hence intrafunicular – into the spermatic chord.

**Genetic correlation:** The genetic correlation between traits caused by pleiotropic action of genes or close linkage between genes.

**Gilt:** female pig prior to parturition.

**GnRH tandem:** Higher molecular weight molecules with enhanced antigenicity, obtained by coupling GnRH molecules.

**GnRH, Gn-RH:** Gonadotrophin Releasing Hormone, a hypothalamic hormone stimulating the secretion of LH and FSH by the pituitary (synonyms: Gonadoliberin, GnRF, LHRH, LHRF).

**(GnRF, Gn-RF):** Gonadotrophin Releasing Factor; see GnRH.

**(Gonadoliberin):** see GnRH.

**(Gonadotrophin):** see LH.

**(Gonadotrophin-releasing hormone):** see GnRH.

**Gubernaculum:** Part of the testicle.

**HCG:** human chorionic gonadotrophin.

**IASP:** International Association for the Study of Pain.

**Kill out percentage (KO%):** See dressing percentage.

**LH (Luteinizing Hormone):** a pituitary hormone stimulating the production of steroids in the gonads (synonym: Gonadotrophin).

**LHRH:** Luteinizing Hormone Releasing Hormone = GnRH.

**(LHRF):** Luteinizing Hormone Releasing Factor, see GnRH.

**Major gene:** a single gene with a large effect.

**MLC:** Meat and Livestock Commission, United Kingdom (UK).

**MRL:** Maximum Residue Limits. Maximum concentrations of chemicals or pharmaceuticals in tissues to be used as human food or animal feed.

**Nociceptor:** A receptor preferentially sensitive to a noxious stimulus or to a stimulus that would become noxious if prolonged (IASP).

**Nociception:** Reception, conduction and central nervous processing of nerve signals generated by the stimulation of nociceptors result in perception of pain when an animal is conscious. Unconsciousness prevents perception of pain, but not the nociception, which can be harmful by inducing a stress response during surgery and increasing postoperative pain when conscious.

**NSAID:** Non-Steroidal Anti-Inflammatory Drug.

**O:** Oestrogens.

**Pain:** Animal pain is an aversive sensory experience representing awareness by the animal of damage or threat to the integrity of its tissues; it changes the animal's physiology and behaviour to reduce or avoid damage, to reduce the likelihood of recurrence and to promote recovery (Molony and Kent, 1997).

**PSE:** pale, soft, exudative – an abnormal condition of meat caused by accelerated glycolysis resulting from stress at or shortly before slaughter.

**Passive immunisation:** Administration to the target animal of antibodies produced by another animal submitted to active immunisation.

**p.c.:** post-conception.

**QTL:** Quantitative Trait Loci.

**16-androstenes, 16 androstene steroids:** A family of steroids with 19 carbons and a double bond in position 16 (synonyms: C19 $\Delta$ 16, C19 Delta-16, Androst-16-enes, 16-unsaturated steroids,  $\alpha$ -16 steroids, Delta-16 steroids).

**16-unsaturated steroids:** see 16-androstenes.

**Sexually dimorphic:** having the different properties of both sexes.

**Skatole:** 3-methyl indole, one of the major contributors to boar taint, originating from the degradation of the amino acid tryptophan in the hind-gut.

**Sedatives:** An agent that calms nervousness, irritability and excitement by depressing the central nervous system.

**Selection index:** A mathematical formula by which different traits are weighted for selection purposes.

**Soft fat:** Pig backfat which feels soft rather than firm. It reduces the visual and handling quality of the meat. Usually caused by an increase in the concentrations of unsaturated fatty acids.

**Stress:** A broad concept to describe the state or response, including behavioural, endocrinological, and physiological reactions, by means of which the animal adjusts to and copes with situations which it perceives as challenging or threatening.

**Taint:** Boar taint is a distinctive and unpleasant taint perceived through a combination of sensory odour, flavour and taste in pork and pork products during cooking and eating. It has been described as 'animal', 'urine', 'fecal' and/or 'sweat' like in character.

## **2. MANDATE/ BACKGROUND**

### **2.1. BACKGROUND**

Council Directive 2001/88/EC amended Council Directive 91/630/EEC laying down minimum standards for the protection of pigs. In particular it requires the Commission to submit to the Council a report, based on a scientific opinion, concerning the castration of piglets. The scientific opinion should also consider the development of techniques and systems of pig production and meat processing which would be likely to reduce the need to resort to surgical castration.

The Commission's report based on this scientific opinion is required to be submitted to the Council preferably before 1<sup>st</sup> January 2005 and in any event by 1<sup>st</sup> July 2005. The Commission's report will be drawn up also taking into account socio-economic consequences, sanitary consequences, environmental effects and different climatic conditions concerning this issue.

Commission Directive 2001/93/EC also amended Council Directive 91/630/EEC and provides that the castration of male pigs may only be performed by other means than tearing of tissues. In addition, when carried out after the seventh day of life it shall only be performed under anaesthetic and additional prolonged analgesia by a veterinarian.



## **2.2. TERMS OF REFERENCE**

In view of the above, the Commission asks the European Food Safety Authority to issue a scientific opinion on the welfare aspects of the castration of piglets. The scientific opinion should describe:

- Welfare aspects of various methods for the castration of piglets, including methods of analgesia and anaesthesia and consequences for animal health,
- The state of art concerning techniques and systems of pig production and meat processing which would be likely to reduce the need to resort to surgical castration, and the impact of the castration, or other alternative methods, on the organoleptic characteristics / quality of the meat.

## **3. GENERAL INTRODUCTION**

### **3.1. BASIS FOR THE REPORT**

Over two hundred million pigs are reared annually in the European Union for meat production. Improvements in diets, housing conditions and genetic selection to enhance commercial efficiencies have been such that modern pigs and pig production systems are very different to when the pig was first domesticated. The amount of feed consumed per kg of meat produced and age at slaughter are major criteria of production efficiency. Criteria of safety and quality dictate market demand and have major commercial implications. It is more profitable to produce meat from entire males due to their enhanced feed conversion and higher proportion of meat on their carcasses. It is recognised that the quality of meat from some entire males is influenced by its odour and taste, or taint, which many individual consumers find objectionable. Even among castrates the proportion of carcasses having the sensory perception of taint is significant. Although, there are practical difficulties in detecting taint in pig carcasses on slaughter lines, carcasses with pronounced taint should not be considered fit for human consumption, according to the current legislation. Slaughtering of pigs earlier in life and at a lower live weight has been associated with a reduction in detectible taint in entire males. Castrating male pigs reduces, if not eliminates taint, and if castrated when young, farmers have noted for centuries that such pigs are less aggressive and are easier to manage. In this report castration of pigs generally refers to surgical castration of males pigs. It is recognised that the risks associated with castration of female pigs are much greater than those associated with the castration of males. Available information indicates that while castration of female members of “local breeds” may occur in localised areas of the EU, the practice is not widespread. It is estimated that 100 million male pigs are surgically castrated annually in the EU. Studies on the effects of immunocastration of fattening pigs indicate that the benefits of entire pigs may be experienced for longer in the pigs life while also reducing the levels of boar taint. Altering the components of pig diets has also been shown to reduce boar taint.

Traditionally the practice of surgical castration has been carried out on piglets by producers who may have little appreciation of the associated welfare implications. The consideration of the welfare of pigs has raised the issue of the necessity of the practice. However it is also recognised that entire male pigs express their natural instincts by attempting to mate and also to socially dominate littermates with associated welfare implications and difficulties in management especially in confined environments.

The report highlights the dilemma of a potential “welfare balance” between the poor welfare associated with the effects of castration and the results of the “social” effects of non-castration of pigs farmed in confined spaces. Surgical castration is associated with pain and risk to the health of animals. However, non-castration is associated with social stress and fighting, resulting in skin lesions and ultimately carcass damage. While potentially painful effects of castration can be temporarily ameliorated by anaesthetics and analgesics, management processes may reduce the undesirable features of male dominance among non-castrates.

This report reviews the methods of castration, their welfare implications, and anaesthesia currently practised in Europe, the factors associated with “boar taint”, and considers how genetic selection, feeding, management practices and meat processing may influence taint.

According to the mandate of the Panel, ethical, socio-economic, cultural and religious aspects are outside the scope of this report.

### **3.2. REVIEW OF THE PIG INDUSTRY**

The 25 EU countries produce slightly more than 240 million pigs per year (Table 3-1). Weights at slaughter differ markedly in different countries. Italy has a tradition of high carcass weights, in connection with the production of dry meat products. On the contrary, UK, Ireland, Denmark, Greece and Portugal slaughter much lighter pigs. In the remaining countries, including most of the new EU Member States, carcass weights are in the range of 80-90 kg, corresponding to a live weight of 105-115 kg.

Over the last 15 years, there was a general tendency for increasing carcass weights in most countries, including those slaughtering light pigs. This elevation in slaughter weight is likely to result in increased incidence of boar taint in entire males (see chapter 7). Slaughter weights in the new Member States tend to converge towards the average slaughter weight in the EU15 countries. Slaughter weight in Switzerland is very similar to the average slaughter weight in the EU15 countries, while it is slightly lower in Norway.

**Table 3-1 Statistics on number of pigs slaughtered and weights at slaughter in EU, and selected other countries (Numbers are millions of heads; adult boars and culled entire males are not considered)**

	Total 2002 (Millions)	Approximate numbers		% males left entires (2)	Mean carcass weight (kg)		Live weight at slaughter (kg) (calculated)
		Castrates	Entires				
Austria	5.4	2.7	0.0	0%	93	(2)	121
Belgium	11.2	5.6	0.0	0%	90	(2)	117
Denmark	22.4	10.7	0.5	5%	78	(4)	103
Finland	2.1	1.1	0.0	0%	82	(2)	108
France	26.6	13.3	0.0	0%	90	(5)	117
Germany	44.3	22.1	0.0	0%	93	(6)	121
Greece (3)	2.2	1.1	0.0	0%	64	(8)	86
Ireland	3.1	0.0	1.6	100%	71	(2)	95
Italy	13.3	6.6	0.0	0%	109	(2)	139
Luxemburg	0.2	0.1	0.0	0%	74	(8)	98
Netherlands	15.4	7.7	0.0	0%	89	(7)	116
Portugal (3)	5.0	1.1	1.5	58%	64	(8)	86
Spain	37.6	7.9	10.9	58%	79	(2)	104
Sweden	3.3	1.6	0.0	0%	89	(2)	116
UK	10.6	0.0	5.3	100%	74	(9)	98
Cyprus	0.7	0.3	0.0	0%	75	(2)	99
Czech Republic	4.5	2.3	0.0	0%	92	(2)	119
Estonia (3)	0.5	0.3	0.0	0%	77	(2)	102
Hungary (3)	6.6	3.3	0.0	0%	90	(2)	117
Latvia (3)	0.5	0.2	0.0	0%	79	(8)	104
Lithuania (3)	1.1	0.6	0.0	0%	88	(2)	115
Malta (3)	0.1	0.1	0.0	0%	82	(8)	108
Poland	23.0	11.5	0.0	0%	80	(2)	105
Slovak Republic	2.1	1.0	0.0	0%	90	(2)	117
Slovenia	0.7	0.4	0.0	0%	83	(2)	109
<b>EU 25</b>	<b>242.5</b>	<b>101.5</b>	<b>19.7</b>	<b>16%</b>	<b>84</b>		<b>110</b>
Norway	1.3	0.7	0.0	0%	78	(10)	103
Switzerland (3)	2.7	1.4	0.0	0%	86	(11)	112

Sources :

- (1) 2002; EU15 countries: EUROSTAT; Accession countries, Norway and Switzerland: FAOSTAT
- (2) 2000; Estimated proportion of males left entire: "A description of the European slaughtering populations and their classification", an internal report of the EUPIGCLASS Project (GRD1-1999-10914), G Daumas, Institut Technique du Porc, France. For Denmark source = (4)
- (3) In the absence of information, the percentage of males left as entires has been assumed to be 0 (Greece, Estonia, Hungary, Latvia, Lithuania, Malta and Switzerland), or the same as in Spain (Portugal)
- (4) 2003; J. Larsen, Danish Slaughterhouses
- (5) 2003; Uniporc Ouest
- (6) 2003; K. Schulz, Zentralverband der Deutschen Schweineproduktion e.v.
- (7) 2002; EUROSTAT-MLC
- (8) 2004; FAOSTAT
- (9) 2003; MLC-BPEX
- (10) 2002; Annual report: classification and weight results 2002. Norwegian Meat Research Centre
- (11) 2002; Federal Office of Swiss Statistics

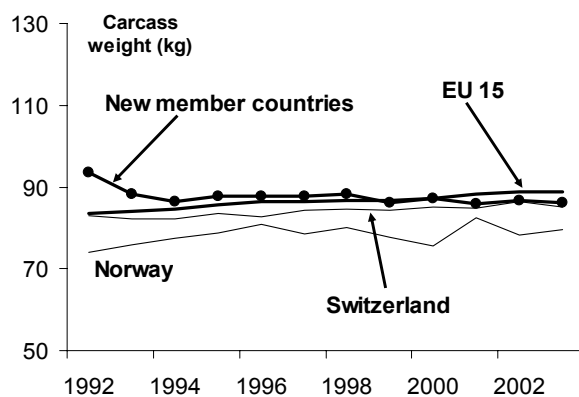
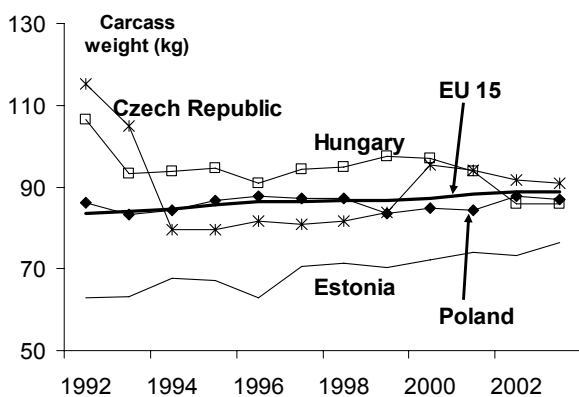
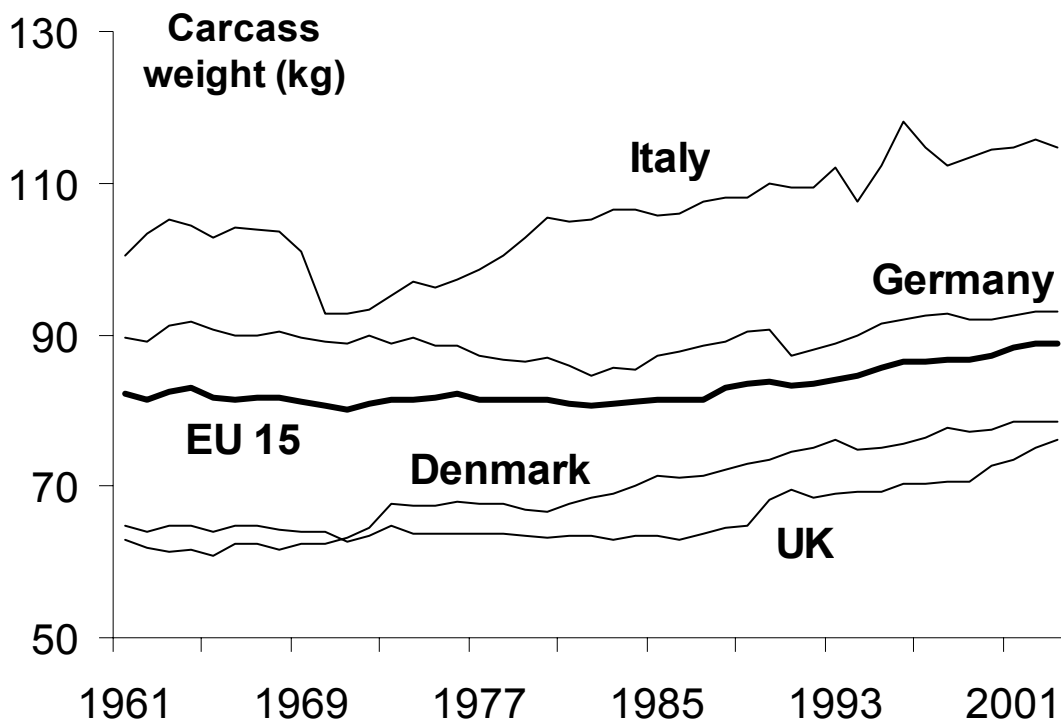


Figure 3-1 Time-related changes in carcass weights (source: FAOSTAT)

### 3.3. HUSBANDRY OF PIGS IN EUROPE

Although many pigs are reared in extensive outdoor facilities particularly when neonates, most pigs in the EU are now raised indoors under intensive farming conditions, which itself has implications for the local environment of intensive pig farms and also raises concerns for control of diseases in such units. In intensive systems three separate phases of production (farrowing (birth and neonatal period), weaning and finishing) are recognized and in many instances necessitate different feeding and housing conditions. The gestation length of the sow is approximately 112 to 115 days. The average litter size in the EU is 11. After birth piglets are nursed by their dams for approximately 21 to 28 days. During this phase of production in

most member states male piglets that will not be used for breeding are surgically castrated. In some countries this phase of life is spent outdoors. After weaning piglets are generally moved to, and mixed with members of other litters in, specially designed housing systems for weaners. This phase presents the greatest management challenge as dietetic changes are frequently associated with disease outbreaks. After about 5 weeks, when the piglets reach approximately 30 kg liveweight the weaned pigs are moved on to further accommodation to finish their growth prior to slaughter. It is now rare that weaning and fattening phases of a pig's life take place in outdoor facilities in the EU. As selection of individuals to fill pens in the fattening sheds is based on liveweight, members of different litters may become penmates in the fattening pens. This mixing will provoke the establishment of new social hierarchies resulting in dominating and submissive behaviour. If entire males are becoming sexually mature at this stage, aggressive behaviour may be prolonged.

The design of the pens, temperatures, and ventilation, will determine if pigs lie in their excrement or not. The length of time that pigs spend in the fattening sheds will be determined by their growth rate as in most systems liveweight determines time of slaughter. The weight of carcasses will depend on the demand for meat cuts.

### **3.4. RELEVANT PHYSIOLOGY OF PIGS**

#### **3.4.1. Hormonal control of gonadal activity**

##### **3.4.1.1. In males**

Release of GnRH by the hypothalamus allows synthesis and release of FSH and LH by the pituitary gland (Figure 3-2). These two hormones are necessary for the production of steroids by the Leydig cells (LH especially) and for spermatogenesis in the seminiferous tubules of the testes (FSH especially). The main steroids produced by boar testes are androgens, oestrogens and androstenes (androstenone and related steroids). Androgens and oestrogens are necessary for spermatogenesis and sexual behaviour, they influence metabolism and favour the development of lean tissue. They also act on the liver metabolism and especially on the enzyme systems responsible for the breakdown of skatole (Babol *et al.*, 1998a and b). Skatole is produced in the hind gut from the microbial degradation of the amino acid tryptophan. Androstenes are pheromones that play an important role in the recognition of the boar by the female pigs and may stimulate the sexual development of young gilts. Among androstenes, androstenone is the most important taking into account its level of production and its storage in fat tissues and salivary glands.

##### **3.4.1.2. In females**

The gonadotrophins, FSH and LH, are also necessary for the control of the reproductive function (growth and maturation of ovarian follicles, ovulation, establishment and maintenance of pregnancy) of female pigs. Under this hormonal stimulation, especially LH, ovaries synthesize and secrete oestradiol and progesterone. Antral follicles are the main source of oestradiol and corpora lutea of progesterone. Oestradiol stimulates oestrous behaviour and may influence nutrient metabolism and appetite whereas progesterone is essential for the establishment and maintenance of pregnancy.

#### **3.4.2. Pubertal development**

##### **3.4.2.1. In males**

Sexual differentiation occurs early in the prenatal life of the pig and seminiferous cords (which will differentiate later into seminiferous tubules), Sertoli and Leydig cells can be identified as early as the 30<sup>th</sup> day post-conception (p.c.) (for reviews see Ford, 1990; Goxe,

Salesse and Prunier, 1995). Testosterone production starts very early and two peaks of production occur before puberty, one around 35 days post conception (p.c.) and the other during the first month of postnatal life. These variations in testosterone production are paralleled by two waves of development of the Leydig cells. At birth, the testes are very small (around 100 mg each) and are usually already in an inguinal position. Testicular mass increases slowly during the first weeks of life. There is a typical rapid pubertal increase during the following weeks. After that, testes grow again slowly until about 3-4 years of age (Figure 3-3).

The pubertal phase starts with the occurrence of the first stages of spermatogenesis and is completed when the first mature spermatozoa are produced. Pubertal development is mainly under the control of the pituitary hormones, LH and FSH, whose levels of secretion are increased (FlorCruz and Lapwood, 1978; Schinckel, Johnson and Kittok, 1984). In parallel to the pubertal development of the testes, androgen and androstene secretion increases (Martin *et al.*, 1984; Schinckel, Johnson and Kittok, 1984) and as a consequence, androstenone accumulates in fat (Figure 3-4).

Behaviour, especially social, aggressive and sexual behaviour (see section 5.2), is influenced by gonadal steroids (mainly oestrogens and androgens) due to their organizational/morphogenic and activational effects. Therefore, variations of testicular hormones during pubertal development will influence the behaviour of animals. Behaviour in pigs is sexually dimorphic by 1 month of age. (Ford, 1990; Berry and Signoret, 1984). The effects of castration on behaviour are analysed in section 5.2.

Age and weight at puberty are highly dependent on genetic and environmental factors. For instance, onset of spermatogenesis occurs around 8 weeks of age (around 20 kg live weight) in Chinese Meishan boars, and around 18 weeks of age (around 60 kg live weight) in conventional boars from European breeds. Nutrition, social and physical environments (type of housing, temperature, light...) may influence testicular development and hence, androstenone patterns (see sections 7.2 and 7.3).

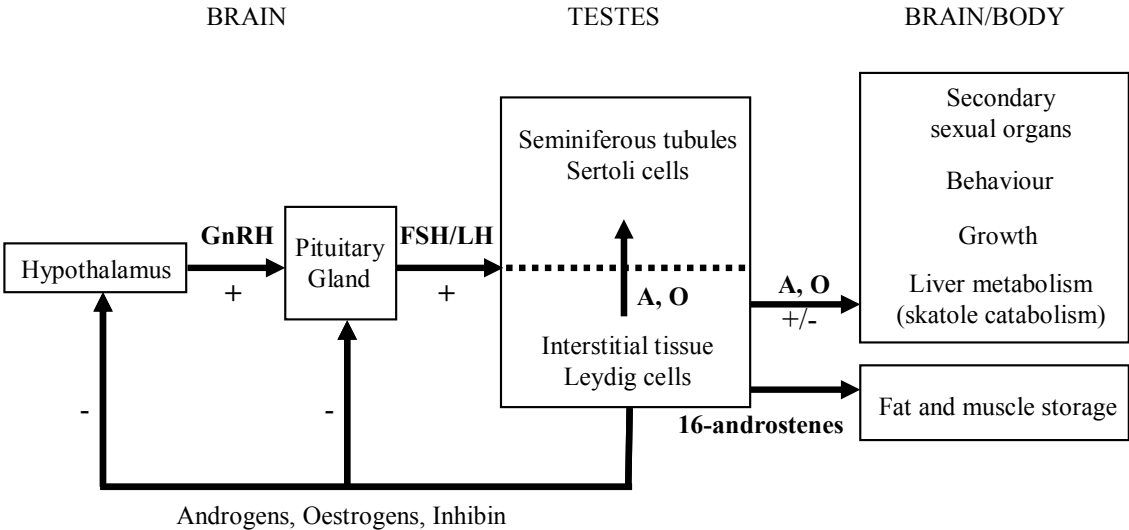
#### 3.4.2.2. In females

Foetal pig gonads differentiate into ovaries which contain egg follicles at about 31-32 days p.c. Primordial follicles (ovocytes surrounded by a single layer of cells) can be detected 20 days later, and they form a stock of primordial follicles that is completed within the first 15 days of postnatal life. These follicles may remain quiescent for several weeks or years. Growing follicles appear at 15 days of age and first antral follicles (= follicles with an antrum, differentiated granulosa and theca cells) at about 2-3 months of age in European breeds of pigs. An increase in LH release probably leads to the appearance and multiplication of these antral follicles around 2-4 months of age (= juvenile phase) as shown by Camous, Prunier and Pelletier (1985) and Prunier *et al.* (1993). The first ovulation occurs simultaneously with first oestrus (= puberty) at about 5-7 months of age in European breeds. Female pigs can become pregnant at this first oestrus for this reason if females are to be castrated this will generally be carried out before puberty.

Age and weight at puberty vary greatly between breeds of pigs. For instance, the mean age at puberty is about 190 days (around 120 kg live weight) in Large White females and around 90 days (around 30 kg live weight) in Meishan females (Martinat-Botté *et al.*, 1996). Within a breed, genotype plays an important role (heritability of age at first oestrus is around 0.3) together with nutritional and environmental factors (Hughes, 1982; Martinat-Botté *et al.*, 1996). Severe feed restriction (energy intake < 70% of *ad libitum* intake) may delay onset of puberty whereas moderate restriction only reduces live weight at puberty. Season, light

duration, presence of other females may also influence onset of puberty but the most important factors are acute stress and boar stimulation (Hughes, 1982; Martinat-Botté *et al.*, 1996). Indeed, acute stress such as transport with relocation and mixing of the animals is able to induce puberty within 4-7 days in a majority of females when it is applied during the prepubertal phase of development (i.e. when females have numerous 2-5 mm ovarian follicles). Regular contacts of females with a mature boar may reduce onset of puberty by a couple of weeks.

In commercial piggeries, where females are reared for meat production and often slaughtered around 110 kg live weight, a significant percentage of them may have reached puberty. For instance, Stern *et al.* (2003) observed that 6% of females reared indoors and in groups with castrates were pubertal at slaughter (107 kg live weight in average) in one replicate and 27% in the second one.



**Figure 3-2 Main sexual regulations in the mature boar (+: stimulation, -: inhibition)**

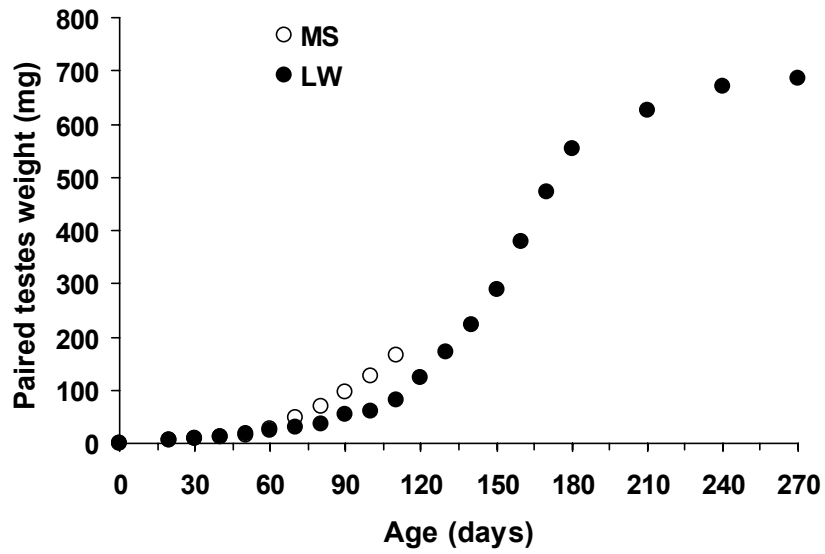


Figure 3-3 Schematic age-related patterns of growth in weight of the testes in one European breed (Large White = LW) and in one Chinese breed (Meishan = MS), (redrawn from Godinho, Cardoso and Nogueira 1979; Prunier, Caritez and Bonneau, 1987)

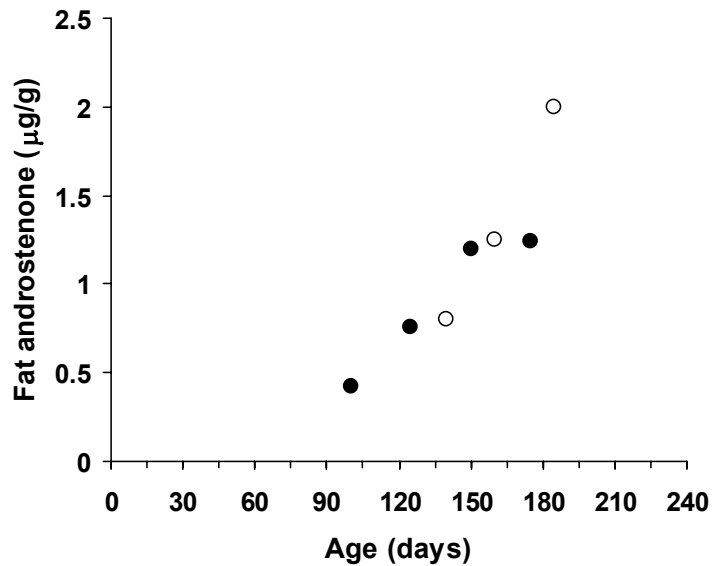


Figure 3-4 Variation in fat androstenone during sexual development in Large White entire male pigs (redrawn from Bonneau, 1987: ●; Bonneau et al., 1987: ○)



## 4. CASTRATION OF PIGLETS

Since the testes and the scrotal skin are innervated with nociceptors (for details see below in section 4.3.2), it is highly likely that castration induces pain and is, therefore, both a painful and a stressful event when it is performed without anaesthesia and post-operative analgesia. To identify all the advantages and drawbacks of the different methods of castration, it is necessary to evaluate pain related to castration in addition to the physiological, behavioural and health consequences which may derive from castration. These consequences may be due to the process of castration itself (handling and surgery) but also to the deprivation of testicular hormones.

### 4.1. DEFINITION OF CASTRATION

The word "castration" is derived from the Latin "*castrare*", meaning to cut, or to prune, and may derive from the ancient Sanskrit "sastrum," or "knife". Nowadays, castration means to deprive an animal of its gonads and thus make it incapable of reproduction. It can apply to both males (e.g. to geld horses) and females (spay bitches and queens). Castration in males may be achieved in several different ways:

- Traditional methods: surgical removal of the two testicles (surgical castration) or by the use of rubber rings or crushing methods, such as "Burdizzo", to interrupt the blood supply and produce an ischemic atrophy of the testes. The commonest and most practical method of castration of male pigs is surgical removal of the testes during the first days or weeks of life.
- Alternative methods of castration which are not approved in the EU:
  - arrest of the testicular function by inducing an immune response against hypothalamic or pituitary hormones (immunological castration e.g. GnRH);
  - arrest of the testicular function by the use of other hormones (e.g. progesterone), thereby inhibiting sperm production; and
  - destruction of testicular tissue by the use of chemical agents (e.g. formalin or lactic acid).

### 4.2. HISTORY AND EXTENT OF THE CURRENT PRACTICE

Castration of male pigs is a very old custom and was probably carried out in order to get both calmer and fatter pigs. Archaeological evidence of the castration of pigs has been found dating from Neolithic times, i.e. as early as 4000-3000 BC (Steen, 2004, personal communication).

Castration of male animals used for meat production has been widely practised for centuries, mainly for easier control of their behaviour (entire males tend to be more aggressive), but also because of the higher propensity of castrates to deposit fat, a commodity that had been in high demand until quite recently. Consumers currently have a greater demand for lean meat and this, together with the lower production costs associated with the production of entire males, have led to the cessation of castration in cattle and sheep in most countries. The rearing of entire male pigs is avoided in most countries because of its association with boar taint. However, animal welfare concerns are increasing the pressure on pig producers to stop castration. Castration of male pigs has been generally abandoned in a number of countries including Australia, Ireland and the UK, and has been partially abandoned in Portugal and Spain (see data in section 3-2). In Denmark, about 5% of males are left entire. In the

remaining countries, all males except those retained for breeding are castrated. According to the data presented in Table 3-1, about 100 million pigs are castrated each year in the 25 EU countries, representing 83% of the EU male pig population.

### **4.3. PAIN: PHYSIOLOGY AND IDENTIFICATION**

#### **4.3.1. Physiology of pain and innervation of the testes**

Different types of pain exist that are induced by various causes and involve different types of neural mechanisms. Only nociceptive pain, which results from the activation of primary afferent nociceptors by mechanical, thermal or chemical stimuli will be considered in this report, since these stimuli are those ones encountered during surgical and chemical castration methods. In general the mechanism for detecting pain starts in nociceptors that are found in the skin and organs of the body and then electrical impulses are relayed from those pain perceiving organs, through nerves that pass from the periphery to the brain, where they may be interpreted and felt as pain.

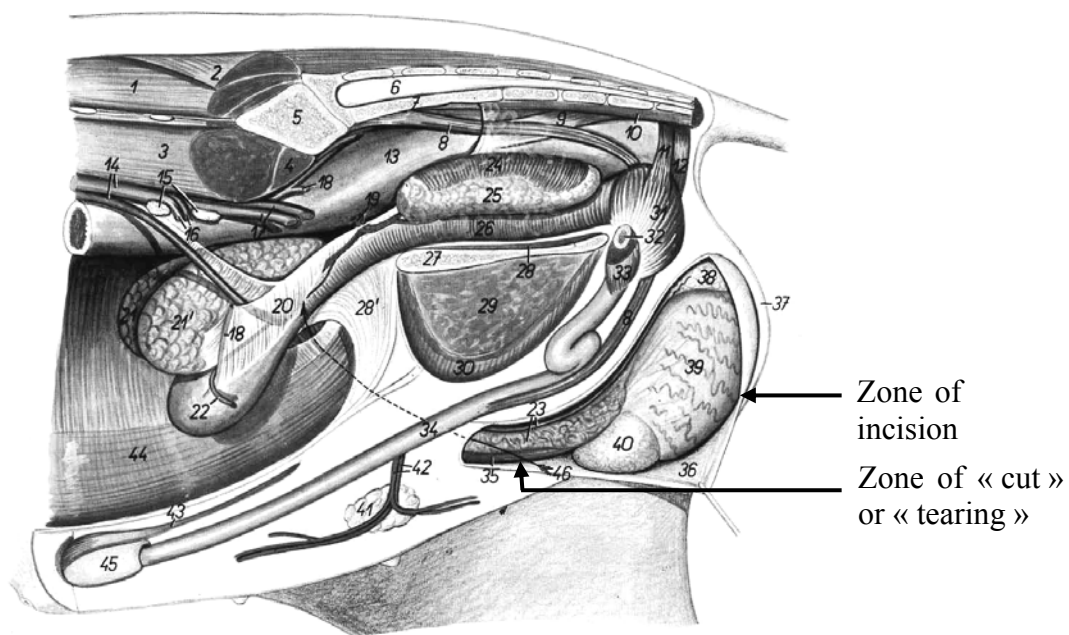
The first step of the pain process is transduction, which is the conversion of the stimulus into an action potential at nociceptors from A $\delta$  and C fibres (for review see Lamont *et al.*, 2000). These fibres traverse the dorsal root ganglia along with the A $\alpha$ , A $\beta$  and sympathetic afferent fibres, into the dorsal horn of the spinal cord, where various connections (synapses) are made. A second-order neuron is then activated and transmits the information along the spinal cord to the level of the thalamus. Finally, a third-order neuron transmits the modified stimulus to higher brain centres, notably the cerebral cortex. Nociceptive information will provoke numerous responses, which may modify the behaviour and the physiology of the animal and its perception of pain. For instance, descending neural pathways may be activated and inhibit the rostral (upwards) transmission of nociceptive information. There are also synapses linking to other areas of the brain involved with memory and other emotional states. Each step of the pain process can be a target of endogenous mechanisms of control or of exogenous analgesic agents.

The innervation of the scrotum and testes is as complex as the tissues that contribute to those organs and associated structures (skin, testes, epididymes, ductus deferens, fascial and muscular contributions from the abdominal wall and skin such as tunica and fascial sheaths, blood vessels, lymphatics, and so on, see Fig 4.1). Sensory and motor innervations are supplied to the skin of the scrotum and the tissues it contains (sacral and lumbar nerves). There are also sensory sympathetic nerves that can detect pain from the testes and associated structures, and that innervate the superficial muscle of the scrotum (tunica dartos) and the blood vessels. Again these innervations stem from both lumbar and sacral nerves and nerve plexi (nerve groupings as an identifiable structure). All the tissues associated with castration (Figure 4.1) are innervated by these nerves and the tissue damage that is inevitably caused during castration could be detected as painful. In the case of chemical castration, nociceptive stimuli are likely to originate mainly from the testes (section 4.7).

Castration is usually performed in young piglets. For a long time, it has been believed that neonates do not suffer from pain because of the immaturity of their neural development (e.g. incomplete myelination of the nerve fibres). However, recent data on humans and rodents have clearly demonstrated that neonates can suffer from pain and may even experience exacerbated pain since the endogenous mechanisms of pain control are not functional (Anand, 1990; Fitzgerald, 1994; Andrews and Fitzgerald, 1994).

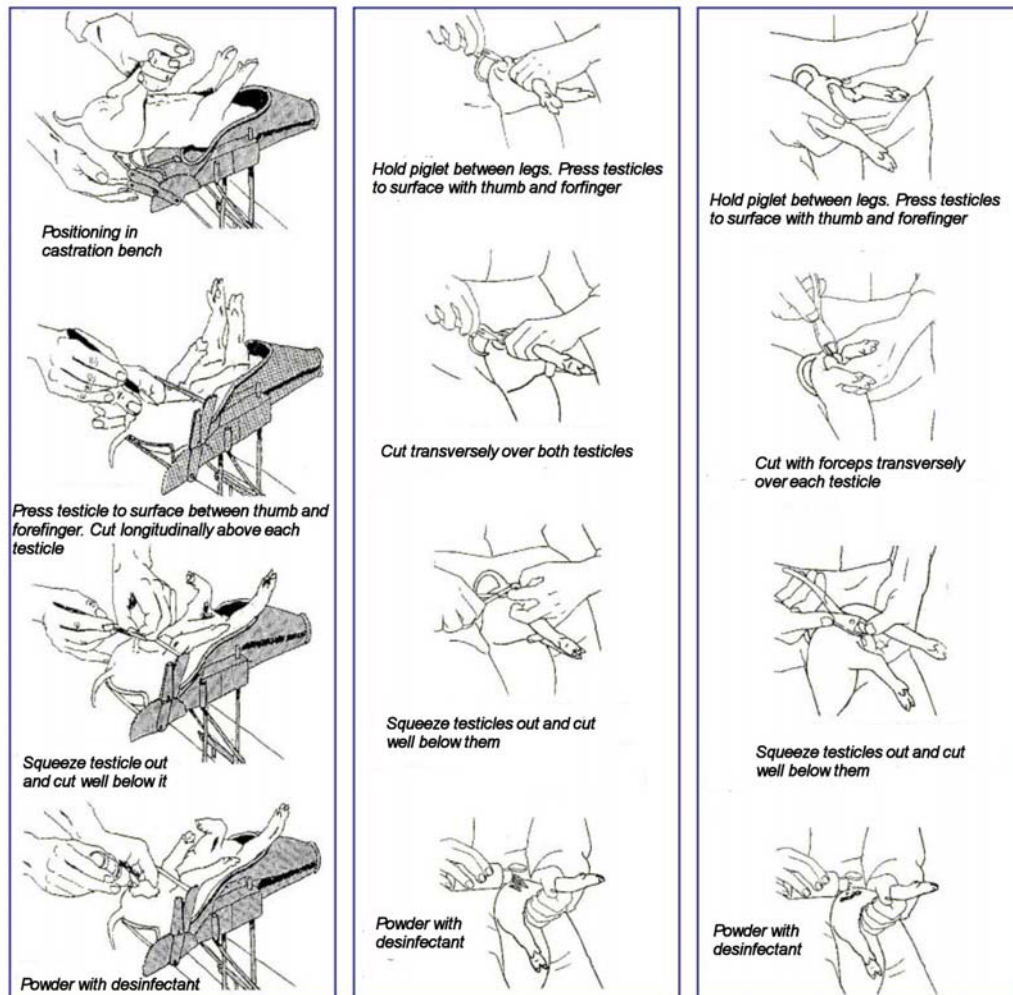
To our knowledge, there are no data concerning the age-related variations of the endogenous mechanisms controlling pain in pigs or of the innervation of the porcine testes by A $\alpha$  or C

fibres. There are, however, no strong *a priori* reasons to expect there would be any differences between pigs and other mammalian species concerning pain perception in neonates.



21 seminal vesicle, 23 spermatic cord (funiculus spermaticus), 25 bulbourethral gland,  
 35 cremaster muscle, 36 tunica vaginalis, 37 scrotum, 38 cauda epididymidis 39 testis,  
 40 caput epididymidis

**Figure 4-1 Anatomy of the genital tract of males piglets and localisation of incisions during surgical castration (Adapted from Popesko, 1980)**



*According to current EU-rules piglets may be castrated without anaesthesia within the first week of life. Recommended instruments are scalpel or sharp castration forceps and scissors. Piglets can be held firmly head down between the legs of the operator. However, a commercially available castration bench is recommended, as it leaves both hands free. The spermatic cords may not be torn, but must be cut well below the testicles. Care should be taken not to castrate piglets that may have an inguinal hernia. Before making the incision, the instruments and the skin area should be disinfected.*

**Figure 4-2 Methods of surgical castration of male piglets (Christiansen, 2004).**

### 4.3.2. Identification of pain

In humans, pain is seen as a subjective perception that is very difficult to communicate and evaluate. It is even more difficult to identify and measure pain in animals, as their communication capacities are much less. Numerous physiological and behavioural indices can be used to assess pain (Molony and Kent, 1997; Mellor, Cook and Stafford, 2000; Table 4-1). The use of these signs is justified taking into account (i) their analogy with those observed in humans experiencing pain and (ii) their reduction or suppression when analgesic substances are used.

Most animals in pain show adrenal cortex reactions but these are not necessarily specific to pain. Numerous signs are indicative of stress and correspond to physiological adaptations of the animal to stop the cause or reduce the consequences of the nociceptive stimulus which threatens the individual's integrity. Application of nociceptive stimuli generally stimulates the adrenal and sympathetic axes which, in turn, induce numerous reactions such as acceleration of heart rate, release of energetic nutrients and glucocorticoid hormones. These reactions can be used as indices of pain (Table 4-1). Behaviour is also modified in order to increase an individual's chance of survival. Adaptative behaviours to pain can be classified in four types according to their purposes: (i) automatic responses that protect the whole animal or a part of it (e.g. withdrawal reflexes); (ii) those that minimize pain and favour healing (e.g. avoidance of movement or antalgic postures); (iii) those that are designed to elicit, help or stop infliction of more pain (e.g. vocalizations, isolation, aggressiveness); and (iv) those that induce learning (Molony and Kent, 1997). These variations in behaviour can be used as indicators of pain with the advantage of (i) being non-invasive and (ii) not inflicting any additional disturbance to the nociceptive situation that is under study.

In order to identify and measure pain after castration, most of the indices described in Table 4-1 have been used in pigs.

**Table 4-1 Signs of pain which can be used in pigs (adapted from Mellor *et al.*, 2000; Hay *et al.*, 2003)**

Physiological indices	Behavioural indices
Hormone concentrations (in blood, urine or saliva): Adrenal axis: CRH, ACTH, cortisol Sympathetic axis: adrenaline, noradrenaline Blood energetic nutrients (metabolites in blood): Glucose, lactate Free fatty acids Activity of the sympathetic axis: Heart rate Respiratory rate Blood pressure Internal temperature Immune system (in blood): Immunoglobulins Number, phenotype and activity of immune cells C-fos expression in neurons of the spinal cord	Calls: Number and duration Intensity Spectral composition Postures: Antalgic Tonic immobility, stiffness Locomotion Flight Specific pain-related behaviours Trembling, spasms Scratching Withdrawal reflex General behaviour: Agitation, restlessness Prostration and immobility Isolation, social desynchronization Aggressiveness Loss of appetite

#### 4.4. SURGICAL METHODS OF MALE CASTRATION

Castration is usually carried out by surgical means on young male piglets during the first days or weeks of age. The procedures described below apply to surgical castration with or without analgesia or anaesthesia. Methods of analgesia and anaesthesia will be described later (section 4.6).

Directive 2001/93/EC stipulates that, "if castration is practised after the seventh day of life, it shall only be performed under anaesthetic and additional prolonged analgesia by a veterinarian". Some pig producers perform castration on the day of birth or the day after, together with tail docking, iron injection and, in many cases, tooth resection. Surgery at that age requires great dexterity since the testes are very small. Moreover, the risk of an incomplete castration is increased since one or both testes may not be fully descended and so may be retained within the abdomen of an animal. It seems that some producers may carry out castration of piglets later than the first week of life, most often, without any anaesthesia/analgesia for practical reasons: the testes are bigger, planning the work is easier, to reduce the risk of cryptorchidism and to avoid prolapse of the intestine as inguinal hernia are more obvious as the animals are larger (but there is no scientific literature available on this last point). When piglets with an inguinal hernia are castrated, they have to be surgically sutured to close the inguinal channel.

Castration is carried out very rapidly (the actual process of castration of young piglets, without including the time for catching animals, may take less than 30 seconds without anaesthesia) and involves cutting and/or tearing of tissue (Figure 4-1). However, some differences exist between the methods that are used. Piglets are restrained during castration (which takes various length of time) to minimize any movement. They may be held between the handler's legs with the head down, held on a flat bench, restrained in a v-trough or in a commercially available device (Figure 4-2). The scrotum is incised with a sharp scalpel (Figure 4-1). Some producers make a single incision while others make two, one on each side of the scrotum. The incision(s) in the scrotum is approximately 2cm in length, depending on the size of testes. Additional tissue separation is performed to free each testicle from the surrounding tissue, especially the gubernaculum. It is recommended to make the incision(s) as low as possible in the scrotum to facilitate drainage of wound fluids and hence, reduce the risk of wound infections (no scientific literature is available but it is a standard and accepted surgical protocol). The testes are extracted and removed either by cutting the cord (Figure 4-1) or by pulling the cord so that it breaks somewhere along its length. Cutting is carried out with a scalpel and scraping the cord to sever it with minimal haemorrhage, or with an emasculator that clamps and crimps the cord for several seconds again to limit bleeding. An antiseptic is usually applied to the open wound and piglets are rapidly returned to their pen. It is recommended that scalpels and emasculators should be dipped in an appropriate antiseptic (alcohol, chlorhexidine...) between operations on piglets. Directive 2001/93/EC stipulates that castration of males must be done by means other than tearing tissues. However, the practicality of castrating piglets without tearing tissues is questionable.

Females are occasionally castrated and the procedure is described in section 4.8.

## 4.5. EFFECTS OF SURGICAL CASTRATION WITHOUT ANAESTHESIA AND WITHOUT ANALGESIA ON WELFARE AND HEALTH OF MALE PIGLETS

### 4.5.1. General welfare consequences of castration

The consequences of castration on welfare may be due to surgery itself as well as to deprivation of the testicular hormones. Indeed, testicular hormones influence behaviour and hence may influence welfare of male pigs. These latter consequences will be developed in section 5.2.

Catching and handling the animals are likely to be stressful. However, comparison between non-handled animals and sham-castrated ones after sham-castration shows very few differences in hormonal profiles (Prunier, Mounier and Hay, 2004) and in their behaviour (Hay *et al.*, 2003).

Experiments carried out in pigs clearly indicate that surgical castration induces endocrine and behavioural responses (Wemelsfelder and van Putten, 1985; Mc Glone and Hellman, 1988; Mc Glone *et al.*, 1993, White *et al.*, 1995; Weary, Braithwaite and Fraser, 1998; Taylor and Weary, 2000; Tuytens, 2002; Hay *et al.*, 2003; Prunier *et al.*, 2001; Llamas Moya *et al.*, 2004), which are accepted as indicators of pain (section 4.3.2.).

#### 4.5.1.1. During castration

The high frequency calls (> 1000 Hz) are due, at least in part, to the surgery of the animals since they are more frequent, of higher intensity and longer duration in castrated than in sham-manipulated pigs (Weary, Braithwaite and Fraser, 1998; Taylor and Weary, 2000; Prunier *et al.*, 2002; Marx *et al.*, 2003). More precisely, Marx *et al.* (2003) identified three call types during the castration of pigs: grunts, squeals and screams. The number of screams per animal was almost doubled in piglets that were castrated without local anaesthesia compared with piglets castrated with anaesthesia. These calls are accompanied by physical resistance movements and an activation of the sympathetic system, as demonstrated by an increase in heart rate (White *et al.*, 1995). Analysis of the calls during the overall procedure of castration suggests that pain is most acute during extraction of the testes and severing the spermatic cords (Taylor and Weary, 2000). This is further supported by the observation that local anaesthesia is most effective by reducing behavioural resistance when the cords are cut (Horn, Marx and von Borell, 1999).

#### 4.5.1.2. Immediately after surgical castration

Measurement of hormones in plasma clearly indicates an activation of the adrenal and sympathetic axes (Prunier, Mounier and Hay, 2004). A 40-fold increase in plasma ACTH, peaking 5 minutes after surgery, is followed by a 3-fold increase in plasma cortisol, peaking 15 to 30 minutes after surgery (Table 4-2). A very rapid and transient increase in plasma adrenaline is followed by a longer lasting increase in plasma noradrenaline. Adrenaline is probably of adrenal medullary origin and noradrenaline from peripheral sources. As a consequence of the catecholamine stimulation, glycogen is mobilized, leading to a transient increase in lactate from muscles.

The expression of the protein c-fos in neurons of the spinal cord, which are likely to transmit the nociceptive stimuli originating from the perineal region to the brain, has been studied in pigs after castration (Nyborg *et al.*, 2000). It was shown that the number of activated neurons

was 3 times lower in pigs that were treated with local anaesthetic before castration than in pigs that only received an injection of saline.

In parallel with these physiological reactions, behaviour is modified. Castrated pigs spend less time at the mammary glands (massaging and/or suckling) (McGlone and Hellman, 1988; McGlone *et al.*, 1993; Hay *et al.*, 2003). They remain more inactive while awake, they show more pain related behaviours (prostration, stiffness, trembling) and tail wagging (Table 4-3). However, postures (ventral and lateral lying, sitting and standing) and location in the crate (at the sow's udder or sow's back, at heat lamp) are not altered. Finally, they frequently seek solitude and their behaviour is more frequently desynchronized compared with the remaining piglets in the litter (Hay *et al.*, 2003).

#### 4.5.1.3. *During the days following castration*

There are fewer data related to this time. Measurements of corticosteroids and catecholamines in urine suggest that the adrenal and sympathetic axes are no longer stimulated (Hay *et al.*, 2003). Behavioural observations (increased abnormal behaviours, reduced play behaviour and overall activity) by Wemelsfelder and van Putten (1985) suggest that piglets experience pain for up to 5 days after castration. Hay *et al.* confirmed that some behavioural alterations persist beyond 24 hours (Hay *et al.*, 2003). For instance, tail wagging is more frequently observed in castrated pigs during the four days after castration, even though the difference is not always significant (Table 4-3). Scratching the rump reaches a peak 24 hours after castration but is still present on the fourth night following castration.

Data from calves clearly indicate that surgical castration induces an inflammatory reaction as measured by an increased release of acute phase proteins and of fibrinogen (Fisher *et al.*, 1997; Earley and Crowe, 2002).

In the papers cited previously dealing with the influence of castration on behaviour, growth and welfare of piglets, mortality rate is rarely mentioned, suggesting that there is no obvious effect. In one of these studies, death loss between birth and 29 days of age was compared in males castrated either at 1 (n = 191) or 14 days (n = 214) and in 339 females (McGlone *et al.*, 1993). There was no difference between groups. However, data from commercial herds have suggested that poor hygiene at castration could favour the occurrence of arthritis which itself may result in death of the piglets (Strom, 1996).

#### 4.5.1.4. *Long term effects*

There are some indications that surgical castration may compromise the health of pigs. Tielen (1974) reported that the prevalence of pneumonia is higher in castrates than in gilts. This was confirmed by an investigation of 18,000 pigs at a Dutch abattoir (de Kruijf and Welling, 1988). The incidence of chronic inflammation as in pericarditis, pleurisy, pneumonia, inflammation of the tail and inflammation of the feet was significantly higher in castrates than in gilts. In a smaller sample of 395 gilts, 425 castrates and 348 entire males, de Kruijf and Welling (1988) discovered that pneumonia, chronic pleurisy and chronic pericarditis were also found less frequently among entire males compared with castrates, whereas the prevalence of these diseases was similar between gilts and entire males. The causes for these differences are not clear. The higher prevalence of inflammations of the tail in castrates than in gilts might be explained by differences in behaviour or in immune response. In fact, the tails of castrates are more often bitten than those of gilts (Penny and Hill, 1974) and it could be speculated that this is due to a more "passive" attitude (i.e. castrates are less aggressive than gilts and will more easily allow other animals to bite them). It was also suggested that immunosuppression occurs in castrated males (De Kruijf and Welling, 1988), which itself



may influence the immune response to wounds such as those due to tail biting. The influence of castration on the immune system was demonstrated in suckled pigs by data from Lessard *et al.* (2002). They observed lower antibody responses in piglets castrated either at 10 or 17 days of age and challenged twice with an antigen. Immunosuppressive effects of castration could be related to the stress reaction, especially cortisol and catecholamine release, or to the lack of testicular androgens since these hormones are known to promote immunocompetence (De Kruijf and Welling, 1988; da Silva, 1999).

In addition to these effects on the immune system, it should be noted that castration has long term effects on behaviour and growth performance. It reduces undesirable behaviours such as injurious and mounting behaviours (see section 5.2), it stimulates fat deposition and has a negative effect on feed conversion (see section 5.3).

Finally, it is not known if there may be longer term effects of such a painful process as has been shown in humans (circumcision of young boys is associated with greater pain perception at vaccination at 6 months old than in the control group, Taddio *et al.*, 1995) or whether the cut nerve ends may lead to neuromata and neuropathic pain at a later time as in hens after debeaking (Gentle *et al.*, 1986).

#### **4.5.2. Effects of method**

Comparison of two methods of severing the cord (pulling and tearing vs. cutting) does not show any difference in the calls recorded during this procedure (Taylor and Weary, 2000). This suggests either that both methods are equally painful or that both methods evoke the piglets' maximal vocal response. The technique of pulling/tearing is believed to reduce bleeding due to the recoil of the testicular artery and consequent narrowing of the lumen of the artery, but also probably results in more ragged edges that disrupt platelets. However, it may also be more difficult to heal but published data are not available. Informal observations support the assertion that pulling/tearing results in less bleeding (Taylor and Weary, 2000). Tearing the cord is not allowed by Directive 2001/93/EC.

Comparison between methods of restraining (piglets held on a flat bench vs. piglets suspended by the legs vs. piglets restrained in a v-trough, see Figure 4-2) does not show any difference in the number and duration of "low" calls (frequency < 1000 Hz) as well as in the number, duration and frequency of "high" calls (frequency > 1000 Hz) (Weary, Braithwaite and Fraser, 1998).

#### **4.5.3. Effects of age**

In Directive 2001/93/EC, castration without anaesthesia and prolonged analgesia is not allowed in animals above 7 days of age. This Directive is based on limited scientific data as studies comparing the pain inflicted on piglets according to age at castration are very scarce (McGlone *et al.*, 1993; Taylor *et al.*, 2001). Comparing the time spent suckling in intact and castrated piglets during the 6 hours following castration, McGlone *et al.* (1993) observed a similar reduction in piglets castrated at 1, 5, 10, 15 and 20 days of age. Comparing the calls during castration (numbers of low frequency, high frequency and total calls) and sham-castration, at 3, 10 and 17 days of age, Taylor *et al.* (2001) obtained very ambiguous results. The treatment (surgery vs. sham castration) and the age had significant effects but the interaction between age and treatment was not significant. Indeed, the increase with age that was observed for high-frequency calls (more calls at 10 and 17 days of age) in castrated pigs was also observed in sham-castrated pigs. Similarly, Marx *et al.* (2003) observed age-related variations in the characteristics of piglets' calls. Therefore, it can be assumed that the effect of age on calls at castration is merely due to an age-related increased response to stressful situations regardless of pain. Moreover, comparing the time of arrival at the udder and the

number of missed nursings in the hours following castration, Taylor *et al.* (2001) did not find any effect of age.

When growth rate of the piglets was measured in the days following castration, a decrease was observed when surgery was performed, without anaesthesia, shortly after birth (1 to 3 days, McGlone *et al.*, 1993; Kielly, Dewey and Cochran, 1999). This decrease may reflect a more stressful and painful situation when castration is performed early or may be the result of castrated piglets being disadvantaged when competing for teats in early lactation. Indeed, teat order is established in the days following birth and any lack of suckling at that age may have more consequences on it than at an older age.

In sheep undergoing surgical castration, data show that the amplitude of the castration-related peak of cortisol decreases between 5 and 25 days of age (comparison of castration + tail docking at 5, 25 and 42 days of age; Kent, Molony and Robertson, 1993). A similar decrease was observed in calves between 5 and 21 days of age followed by an increase between 21 and 42 days of age (Roberston, Kent and Molony, 1994). Endogenous mechanisms that inhibit nociception are probably not fully mature in neonates, rendering them more sensitive to nociceptive stimuli (see section 4.3.1).

It was claimed by Lessard *et al.* (2002) that castration had a more pronounced immunosuppressive effect when piglets were castrated at 10 and 17 days of age instead of 3 days of age. However, the immune response was similarly low in control pigs “treated” in parallel with those castrated at 3 days. Therefore, the influence of the age at castration on the immune system is doubtful.

**Table 4-2. Comparison of Endocrine Responses to Surgical Castration without Anaesthesia, Sham-castration and No-handling in Pigs of Seven to Eight Days of Age (Prunier, Mounier and Hay, 2004, n = 5 or 6/group)**

	Castrated	Sham-castrated	No handling
Time to reach maximum values (min from the beginning of the sampling)			
ACTH	5	5	30
Cortisol	30	5	30
Lactate	5	5	15
% of increase between maximum and pre-treatment levels			
ACTH	4198 <sup>b</sup>	150 <sup>a</sup>	36 <sup>a</sup>
Cortisol	269 <sup>b</sup>	60 <sup>a</sup>	18 <sup>a</sup>
Lactate	235 <sup>b</sup>	71 <sup>a</sup>	13 <sup>a</sup>

<sup>a,b</sup> Within a line, means with different superscripts differ at  $P < 0.05$ .

**Table 4-3 Comparison of Behaviour between Surgical-castrated (c) and Non-castrated Piglets (nc) of Five days of Age at Different Periods Following Castration or Not (percentage of observations, Means + SEM; Hay *et al.*, 2003). Castration was performed without anesthesia.**

T <sup>1</sup>		0-2,5 hours	24-26 hours	48-50 hours	72-74 hours	Overall
Suckling/udder massage	C	11.9 ±1.8***	20.5±2.1	24.7±1.9	21.5±2.2	21.7±0.8
	NC	20.6±1.4	17.6±1.6	25.7±2.1	25.5±2.3	22.7±0.8
Awake inactive	C	20.8±1.9***	6.7±1.3**	10.3±2.1	11.2±1.5	10.2±0.6
	NC	10.2±1.3	13.4±1.6	11.3±1.9	10.1±1.7	9.3±0.5
Prostration	C	5.0±1.0***	0.0±0.0	0.0±0.0	0.0±0.0	0.7±0.2***
	NC	0.2±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.1
Huddled up	C	23.6±2.6	15.7±1.8	17.6±1.8	18.6±2.3	18.7±0.9***
	NC	14.6±1.5	14.1±2.0	13.5±2.0	14.7±2.2	14.9±0.7
Stiffness	C	2.8±0.9***	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.1***
	NC	0.2±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Trembling	C	4.2±1.1**	0.0±0.0	0.0±0.0	0.3±0.3	0.7±0.2**
	NC	0.6±0.3	0.0±0.0	0.2±0.2	0.0±0.0	0.2±0.1
Scratching	C	0.6±0.4†	3.8±0.8***	0.6±0.4†	0.0±0.0	0.9±0.2***
	NC	0.0±0.0	0.5±0.3	0.0±0.0	0.0±0.0	0.1±0.1
Tail wagging	C	3.3±1.1***	7.4±1.6***	2.9±0.8**	1.9±0.8†	3.0±0.4***
	NC	0.0±0.0	0.5±0.3	0.7±0.4	0.5±0.3	0.5±0.1
Isolated	C	9.4±2.0***	2.9±0.9	3.8±1.5	2.2±1.3	3.7±0.5
	NC	3.1±1.1	2.0±0.7	5.0±1.4	1.9±0.8	2.8±0.4
Desyn- chronized	C	2.8±1.1†	2.2±0.9*	0.6±0.4	0.0±0.0*	1.1±0.2
	NC	0.8±0.4	0.2±0.2	1.4±0.7	1.9±0.8	1.2±0.2

\*\*\*, \*\*, \*, †: Comparison between experimental treatments within periods indicates a statistical difference at  $P < 0.001$ ,  $P < 0.01$ ,  $P < 0.05$  and  $P < 0.1$ , respectively.

T<sup>1</sup> = Treatment regime.

## 4.6. EFFECTS OF SURGICAL CASTRATION WITH ANAESTHESIA ON WELFARE AND HEALTH OF MALE PIGLETS

### 4.6.1. Methods of anaesthesia/analgesia

Surgical castration of male piglets can be performed with either general or local anaesthesia. The method of choice should result in a significant reduction or elimination of pain, discomfort and stress for the piglets. However, it has to be considered that most anaesthetic procedures may induce stress due to the additional handling and to the recovery associated with the anaesthesia itself. It may also induce pain during the injection process. The behaviour of the piglets after castration should be affected as little as possible. Furthermore, the method of choice must be practical at the farm level, without requiring expensive equipment.

It should be emphasized that castration is performed in young pigs that have proportionally more body water and less body fat than adult animals. This may affect the distribution of drugs in the body and the required effective dose. In addition, the metabolic and excretory capacities of the liver and kidneys are not fully developed in these young animals (Baggot, 2001).

In EU countries and Norway, the use of anaesthetics is limited to veterinarians. Moreover, veterinary medicinal products used in animals reared for human consumption are subjected to the MRL regulation (Council Regulation No 2377/90). They can be used only if they belong to the list of products for which maximum residue limits have been fixed (List I) in the species (this the case for azaperone and flunixin in pigs) or to the list of substances not subjected to maximum residue limits (List II) in the species (this is the case for aspirin (i.e. acetylsalicylic acid, ketamine, ketoprofen, xylazine and adrenaline (i.e. epinephrine in pigs)). Therefore, anaesthetics such as halothane and isoflurane are not licensed for use in pigs reared for meat production. The local anaesthetic lidocaine belongs to List II but only for the equine species. Similarly, xylazine belongs to List II for bovine and equidae. However, it is possible to use products licensed for one food producing species in another food producing species if no appropriate medicine is licenced for that species. Moreover, in some countries (as in France and Norway), it is possible to use lidocaine under the control of a veterinarian if a delay of 28 days before slaughter is respected.

While efforts are made in some countries to find a method that can be performed by the producers, in Norway anaesthesia and castration of piglets are regulated by a law that restricts the practice to veterinarians, and the use of an anaesthetic is mandatory. There is no documentation about the current practice but it seems that the most current procedure is to perform local anaesthesia on piglets in Norway at castration (Fredriksen, personal communication).

#### 4.6.1.1. Sedation

Occasionally, sedatives (e.g. acepromazine or azaperone (this latter drug is no longer available)) have been used for piglet castration. However, even if sedation makes the piglets easier to handle during castration, it is not very effective in relieving pain. Therefore, in order to prevent post castration pain analgesia must be used in conjunction with local anaesthesia

#### General anaesthesia

Performing general anaesthesia for castrating pigs in commercial herds has numerous drawbacks: it is time consuming, anaesthetics may represent a risk both for people and piglets (mortality rate of piglets may reach 28% according to McGlone and Hellman, 1988) and their availability is restricted to veterinarians. Moreover, neonatal animals are more vulnerable to

hypothermia than adults, as their temperature regulation capacity is poor (Sjaastad *et al.*, 2003) and their natural homeostatic mechanisms are impaired under anaesthesia.

### ***Injection***

Some anaesthetics used alone (e.g. ketamine or tiletamine) or in combination (e.g. ketamine + xylazine) have been described for pigs. The effects on piglets have not been investigated in depth. In most cases, general anaesthesia induced by injection also affects the behaviour of the piglets after castration, as it is associated with a period of sedation, which makes them more vulnerable to injury by the sow (e.g. getting laid on), and prevents them from suckling. This risk may be mitigated at castration at an older age.

### ***Inhalation***

Several inhalation anaesthetics such as isoflurane, halothane and CO<sub>2</sub> have been tested in pigs. The use of isoflurane and halothane is not recommended without gas evacuation systems. In addition, such anaesthetics are capable of inducing malignant hyperthermia in certain breeds of pigs.

The economical and practical aspects of halothane anaesthesia for piglet castration are under investigation in Switzerland (Jäggin *et al.*, 2001). The amount of time required per piglet castration was slightly higher with anaesthesia than without (2.3 + 0.3 min vs. 1.3 + 0.4 min). Halothane contamination of the environment was usually below 5 ppm.

In a recent study, Walker *et al.* (2004) tested a modified special anaesthetic delivery system on farm with a respiratory bag and a special mask to prevent the loss of gas. Isoflurane and a combination of isoflurane and nitrous oxide were chosen to induce general anaesthesia. Surplus gas was scavenged by a vacuum ventilator. The palpebral reflex disappeared after a mean of 36.5 seconds and mean anaesthesia induction time was 123 seconds for isoflurane/N<sub>2</sub>O. The authors suggested that the use of isoflurane or a combination of isoflurane and nitrous oxide is a safe, quick and reliable method for piglets undergoing castration.

Reactions of discomfort such as restlessness and hyperventilation are observed during induction of anaesthesia with CO<sub>2</sub> (Kohler *et al.*, 1998; Schonreiter *et al.*, 2000). It was concluded that CO<sub>2</sub> does little to alleviate stress at castration. CO<sub>2</sub> has also been shown to be aversive to pigs (Raj and Gregory, 1995). However, the method has the advantage of not needing an evacuation system for excess of gas and may be easily used at the farm level. A Danish project on the topic is on-going (Svendsen, personal communication).

#### ***4.6.1.2. Regional anaesthesia***

It is possible to use epidural anaesthesia on piglets but the method is labour-intensive and not suitable for large numbers of animals.

#### ***4.6.1.3. Local anaesthesia.***

Local anaesthesia is the most common method of anaesthesia used in experiments designed to relieve pain in piglets at castration. Both intratesticular and intrafunicular administrations have been used. Subcutaneous administration of the anaesthetic at the site of incision may sometimes be performed but not always. A 0.5, 1.0 or 2 % solution of lidocaine (= lignocaine) hydrochloride is most commonly injected. The toxic dose for lidocaine is 6-10 mg/kg. This dose can be easily exceeded, especially if the highest concentration is used (for a 2 kg piglet, the toxic dose is reached by injecting 1.2 ml of the 2% solution). However, the toxicity is

reduced if adrenaline is added to the solution. Lidocaine injection into the testes or into the testes and the scrotal sac reduces the pain-related calls (White *et al.*, 1995; Marx *et al.*, 2003) as well as ACTH and cortisol responses to castration (Prunier, Hay and Servièrè, 2002). It was demonstrated that lidocaine was efficient at reducing the number of high frequency calls and the heart rate during pulling and severing the spermatic cords (White *et al.*, 1995) which seem to be the most painful parts of the operation (see section 4.5.1). The analgesic effect of lidocaine was also evaluated by the increase in blood pressure during and after the operation. Intratesticular or intrafunicular administration (both combined with subcutaneous administration), of 4 mg lidocaine/kg (1% solution with adrenaline) was shown to reduce the antinociceptive input from skin, testis and spermatic cord (Haga, Ranheim and Andresen, 2003). Moreover, it was shown that lidocaine with adrenaline injected intratesticularly diffuses proximally into the spermatic cord in 10 minutes (Ranheim *et al.*, 2003). Comparison between sites of lidocaine injection was also carried out (Prunier, Hay and Servièrè, 2002). It seems that applying the dose of lidocaine into the testes and into the scrotum around the funicular area is more efficient in reducing calls during castration compared with injecting the entire dose of lidocaine into the testes.

Bupivacaine has been tried as an alternative to lidocaine, since it has a longer effect, but the induction of analgesia is slower, and there may be a complication in that the remnant of the spermatic cord may be prominent in the wound and so, the risk of post-operative infection is increased (Nyborg *et al.*, 2000). In addition, currently, there are no MRL values for the drug and hence it is not licenced for use in the EU.

Pain associated responses to local anaesthesia alone has been examined. It was observed that ACTH and cortisol peaks occurred after intratesticular lidocaine injection but they were of much lower amplitude than those observed after castration without local anaesthesia (Prunier, Mounier and Hay, 2004). Pain-related behaviour has been observed when local anaesthetics have been administered, and has been associated with the low pH of the solution (Waldmann, Otto and Bollwahn, 1994). Therefore, a pH buffered vehicle has been recommended in order to avoid additional pain (Waldmann *et al.*, 1994; Horn, Marx and von Borell, 1999).

#### 4.6.1.4. Prolonged analgesia

Castration of piglets older than 7 days may only be performed by a veterinarian using an anaesthetic and additional prolonged analgesia (European Directive 2001/93/EC). While several NSAIDs are licenced for use in pigs, there is no documentation available in regard to their efficacy, toxicity and side-effects as for example bleeding in piglets. NSAIDs are the only group of "long-lasting" analgesics currently available for pigs due to MRL regulations.

Few data are available concerning the effects of pre-emptive analgesics. A preliminary experiment in 6-7 day-old pigs suggests that injecting the NSAID flunixin 15 minutes before surgical castration and the day after amplifies the reduction in ACTH and cortisol release caused by lidocaine treatment (Prunier, personal communication). Oral administration of aspirin or intravenous injection of the opioid butorphanol before castration (30 minutes) had no effect on the reduction of weight gain (50%) observed the day after castration of 8-week old pigs (McGlone *et al.*, 1993). In 5.5-month old calves, intravenous injection of the NSAID ketoprofen before castration (20 minutes) reduced cortisol release after castration down to control levels. However, a combination of ketoprofen with local anaesthesia (lidocaine) was not more efficient in reducing the glucocorticoid response but some of that response may indicate tissue damage rather than pain (Earley and Crowe, 2002).

## 4.7. NON-SURGICAL METHODS OF CASTRATION

A number of alternatives to surgical castration have been considered. A first approach is based on the local destruction of testicular tissue by various chemical compounds. Alternatively, testis development can be inhibited through a reduction of the production of the stimulatory hormones of the hypothalamic-pituitary-gonadal axis (see section 2.4.1). Such a reduction can be obtained either from the application of exogenous hormones which down-regulate the hypothalamic-pituitary-gonadal axis or from the neutralisation of the stimulatory hormones by specific antibodies (immunocastration).

### 4.7.1. Local destruction of testicular tissue by chemical compounds

Various chemical substances have been used in different species to induce destruction of spermatogenic and hormone-producing testicular cells: formaldehyde, acids (lactic and acetic) and salts (silver and zinc) (Table 4-4). The advantages which are claimed by authors for the use of acids and salts are: they are easy to administer, safe for the animals and people who administer them, not expensive, produce no haemorrhage and only little pain, and have little side-effects (the risk of post-operative infection is low). However, when data are carefully examined, it appears that (i) swelling of the testes or of the scrotum was very common suggesting an inflammatory painful reaction, (ii) evaluation of pain-related reactions was very limited and not sufficient to conclude on this aspect (Table 4-4). Most of the products that have been tested are not subject to maximum residue limits (i.e. zinc acetate, lactic acid, formaldehyde).

### 4.7.2. Down-regulation of the hypothalamic-pituitary-gonadal axis by exogenous hormones

Down-regulation of the hypothalamic-pituitary-gonadal axis can be achieved through the administration of steroid agonists or antagonists (Busch *et al.*, 1979; Daxenberger *et al.*, 2001; Hagelschuer *et al.*, 1978; Lopez-Bote and Ventanas J., 1988; Denzer *et al.*, 1986). It can also be achieved via the continuous administration of GnRH, which then has a negative feed back on LH release, contrary to its usual stimulatory effect when it is secreted in a pulsatile manner. (Ziecik, Esbenshade and Britt, 1989; Xue *et al.*, 1994; Reid, Dufour and Sirard, 1996; Schneider *et al.*, 1998).

Currently the use of hormones in meat producing animals is considered unfavourably by consumers in the EU.

### 4.7.3. Immunocastration

The aim of immunocastration is to inhibit testicular development and functions via the neutralisation of the hormones of the hypothalamic-pituitary-gonadal axis by specific antibodies. Immunisation can be directed against either the pituitary hormone LH or the hypothalamic hormone GnRH.

Immunisation against LH is less effective than immunisation against GnRH (Falvo *et al.*, 1986).

Most immunocastration studies in pigs have used active immunisation against GnRH, although the possibility of using passive immunisation has also been considered (Van der Lende, Kruijt and Tieman, 1993). Immunisation of young intact male pigs against GnRH is effective at inhibiting genital tract development and reducing plasma LH, FSH and testosterone concentrations (Table 4-5 and Table 4-6).

Immunisation against a GnRH dimer instead of native GnRH is more effective in that much less variation is observed between animals in their response to the immunisation (Meloan *et al.*, 1994).

Earlier studies have involved the use of Freund's adjuvant and/or repeated administration of the vaccine preparation. Freund's adjuvant is unacceptable for use because it is not licenced in a commercial vaccine and repeated administrations are too laborious and expensive and can cause repeated stress to the animals. Therefore, alternative anti-GnRH immunisation methods were developed, using an acceptable adjuvant and fewer injections (Table 4-5 and Table 4-6). A maximum of two injections is likely to be acceptable.

There are two possible schedules of immunisation:

- The first one emphasises the need to cause complete castration with unambiguous results on testes weight, making differentiation on the slaughter line very easy (Oonk *et al.*, 1995). This is obtained via an immunisation schedule that ensures early castration of the animals. However, most of the economic advantages of the entire males are lost in immunised animals (Early castration studies in Table 4-6).
- The second alternative concentrates on maintaining most of the performance advantages of intact male pigs in immunised animals. The disadvantage is that some measurements would have to be performed on the carcasses in order to check the effectiveness of the treatment. In this procedure, an optimum time interval between the booster injection and slaughter needs to be established. The challenge is to keep testicular secretion of anabolic steroids at a high level until as late as possible and still allow enough time for immunocastration to decrease fat skatole and androstenone concentrations to acceptable levels before slaughter. Compared with entire males, immunised pigs grow faster (once the castration effect is achieved), have a similar feed efficiency and exhibit higher fat contents (although lower than in surgical early castrates) in their carcass (see Table 4-6 for late castration studies)

Once the immunocastration is totally effective, the behaviour of immunocastrated male pigs is similar to that of the surgically castrated ones (Cronin *et al.*, 2003). Both exhibit reduced aggressive and mounting behaviours and increased duration of feeding behaviour compared with entire males.

Immunocastration has been perceived as a potential market by a number of companies. In Australia, the Animal Health division of CSL (Commonwealth Serum Laboratories) developed a GnRH vaccine which has been approved and is commercially available under the brand name Improvac in Australia, and New Zealand. The vaccine seems to have been quite successful in Australia where about 25% of the boars are currently being treated with Improvac (Hennessy, 2004, personal communication). Individual meat processors have encouraged its use to differentiate their product in the marketplace, by paying a significant percentage of the cost of the vaccine (Brennan, 2003). Improvac technology is now under American control, since Pfizer Animal Health acquired CSL Animal Health in March 2004 (Phillips, 2004; Hennessy, 2004, personal communication).

Other companies are having, or had, GnRH vaccines under investigation:

- MetaMorphix Canada (formerly Biostar) is developing a product under the brand name "Clean" (Manns and Robbins, 1997; Agwest.sk.ca, 1997; Business Communications Company, 2000).
- Merial and United Biomedical entered into a licence agreement for the development, manufacturing, marketing, and distribution of a LHRH vaccine for boar taint (United Biomedical, 1998).



- The Institute for Animal Science and Health, in the Netherlands, is collaborating with Pepsican Systems B.V. on the development of a GnRH vaccine (Turkstra *et al.*, 2001), using a fully synthetic peptide-carrier construct (Beekman *et al.*, 1999).
- Protherics PLC has GnRH vaccines under clinical trial, one of them being aimed at avoiding the boar taint problem (Protherics, 2004).

However not all companies are still active in this field. One of them has confirmed that it is no longer currently active in the field of immunocastration of male pigs, having stopped its development programme in approximately 2000 (Bonneau, personal communication).

Possible drawbacks of immunocastration, that may hamper its commercial development include:

- The cost of the treatment. The cost of the treatment with Improvac is in the range of 3 € per pig (4.70 Australian dollars; Moore, 2000). This cost has to be compared with the economic gains obtained from discontinuing castration of male pigs.
- The possibility and cost of control on the slaughter line. When immunocastration is performed early, testis size is reduced so dramatically that it is easy for a buyer to be confident of the absence of boar taint in the carcass (Oonk *et al.*, 1995). When it is performed only a few weeks before slaughter, in order to benefit from the advantages of the entire male status, testicle shrinkage in some immunocastrates does not occur to the point of being easily distinguishable from the testicles of some entire males (Brennan, 2003; see late castration studies in section 7.5). In such cases, the buyer cannot be confident of the absence of boar taint unless some kind of control of the efficacy of immunocastration is performed, with a method that has still to be developed.
- Possible low acceptability. Consumers may be reluctant to accept immunocastration, because they are hormonal vaccines (residues issue).
- Safety concerns for the humans. Because the immunogen is not species-specific, it may be active in humans in case of accidental self-injection to the person who is vaccinating the pigs. Although a special device has been developed to reduce the risk of self-injection (Phillips, 2004), this hazard cannot be totally controlled. It is outlined in the Material Safety Data Sheet of Improvac.
- Pain for the treated animal. To our knowledge, this aspect has not been directly investigated. In the case of Improvac, because the vaccine preparation is aqueous, there is little reaction on the site of injection (Dunshea *et al.*, 2001). However, because the GnRH vaccines are directed against hormones produced by tissues of the animal, they may induce cellular damages away from the injection site or testicular areas. Molenaar *et al.* (1993) found that anti-GnRH immunisation in the pig resulted in lesions of the hypothalamus. Whether this induces pain to the animal remains unknown. In any case, the pain resulting from immunisation should be compared with that inflicted by surgical castration.

There is no marketing authorisation on the EU market for such products at present time.

**Table 4-4 A summary of various methods used for chemical castration and their effects in pigs and other species**

Protocol				Efficiency		Daily growth	Welfare			References
Chemical compounds	Where?	Species	Age*	Testicular tissue	Steroid production		Behaviour	Stress hormones	Others	
Potassium permanganate + acetic acid	Intratestes	Pig		Disappearance of germ cells		long term: increase <sup>1</sup>	no difference <sup>1</sup>		swelling of testes mild pain	Giri, Yadav and Panda, 2002
Lactic acid	Intratestes	Dog Rat				1st wk: increase <sup>1</sup> > 1st wk: no effect <sup>1</sup>			less scrotal edema <sup>1</sup>	Nishumara <i>et al.</i> , 1992
Lactic acid (Chem-Cast®)	Intratestes	Bov.	15 d	75% bilateral castration rate						Hill <i>et al.</i> , 1985
Lactic acid (Chem-Cast®)	Intratestes	Bov.	7-9 m	palpable remnants testicular tissue	75% lower plasma T2	1st wk: increase <sup>1</sup> no effect <sup>2</sup>		lower cortisol peak <sup>1</sup>	swelling of testes at d 6	Cohen <i>et al.</i> , 1990
Lactic acid (Chem-Cast®)	Intratestes	Bov.	2-6 w	95% lower testes weight		1st wk: increase <sup>1</sup> , no effect <sup>2</sup> 2nd wk: no effect <sup>1, 2</sup>			swelling of testes at d 7, 14, 28	Cohen <i>et al.</i> , 1991
Lactic acid (Chem-Cast®)	Intratestes	Bov.	2-6 w 5-6 m	85% lower testes weight		1st wk: lower <sup>1</sup> , lower <sup>2</sup> 2nd wk: no effect <sup>1, 2</sup>			swelling of testes at d 7, 14, 28	Cohen <i>et al.</i> , 1991
Lactic acid	Intratestes	Bov.	2-4 m?	18% calves with 1 functional testis		no effect <sup>1</sup>	male sexual behaviour in some calves		necrosis, longer healing duration <sup>1</sup>	Fordyce <i>et al.</i> , 1989
Formaldehyde	Epididymus	Bov.	13 m	73% males without spz at d 85					epididymitis	Gardner, 1980
Formaldehyde	Intratestes	Sheep		more connective tissue						Kang, Park and Chung, 1993
Silver nitrate lactic acid	Intratestes	Pig	some w	full atrophy of testicular tissue		no effect <sup>1</sup>				Ljaz, Abkakhail and Khamas, 2000
Zinc acetate	Intratestes	Pig	15 d		75% lower plasma T2 48% lower fat skatole	less fat <sup>1</sup>				Fahim, 1994

<sup>1</sup>comparison with surgical-castrated males, <sup>2</sup>comparison with entire males, \*: d: days, m: months, w: weeks

**Table 4-5 Effect of immunocastration (anti-GnRH vaccine) of male pigs on performance, hormone levels and sexual development. Small scale studies (Results are expressed as % of the control entire males).**

“Immunogen”	Adjuvant	Number of injections	Number of anim. per treatment	LH	Testosterone	Testes	Acess. sex glands	Growth rate	Feed efficiency	Fat	Reference
GnRH	FCA	5	9	32	-	-	-	-	-	-	Caraty and Bonneau, 1986
GnRH	FCA-FIA	3	5	ND	ND	32	11	111	-	159	Falvo <i>et al.</i> , 1986
GnRH	PEP	3	5	ND	ND	27	10	95	-	106	Falvo <i>et al.</i> , 1986
GnRH	FCA-FIA	3	10	ND	3	34	25				Awoniyi <i>et al.</i> , 1988
GnRH	FCA-FIA	3	6	-	32	-	-				Hagen <i>et al.</i> , 1988
GnRH	FCA-FIA	2	5	-	30	39	-				Meloen <i>et al.</i> , 1994
GnRHT	FCA-FIA	2	5	-	ND	8	-				Meloen <i>et al.</i> , 1994
GnRHT	?	2	11	-	ND	-	-	-	-	-	Manns and Robbins, 1997
GnRH	?	2	11	-	ND	< 100	< 100	115	101	128	Liu <i>et al.</i> , 2001
Improvac (anti-GnRH)		3	10	-	-	11	-	92	78	123	Metz <i>et al.</i> , 2002
GnRHT	Specol	2	8	42	ND	21	-	-	-	-	Zeng <i>et al.</i> , 2002c
Improvac (anti-GnRH)		2	6	-	-	-	-	90 (\$)	86 (\$)		Mc Cauley <i>et al.</i> , 2003

GnRH = gonadotrophin-releasing hormone; GnRHT = GnRH tandem; Improvac = Brand name for the CSL vaccine; FCA = Freund’s complete adjuvant; FCA-FIA = Freund’s complete adjuvant for the primary immunisation, Freund’s incomplete adjuvant for boosters; PEP = muramyldipeptide.

ND = non detectable; - = not determined.

(\$): Performance measured during the last 4 weeks before slaughter.

**Table 4-6 Effect of immunocastration (anti-GnRH vaccine) of male pigs on performance, hormone levels and sexual development. Larger scale studies (Results are expressed as % of the control entire males).**

“Immunogen”	Adjuvant	Number of injections	Number of anim. per treatment	LH	Testosterone	Testes	Acess. sex glands	Growth rate	Feed efficiency	Fat	Reference
Late castration studies											
GnRH	Oil-SAP	2	20	-	15	84	51	104	103	105	Bonneau <i>et al.</i> , 1994
Improvac (anti-GnRH)		2	100	-	9	47	45	121 (\$)?	103 (\$)	114	Dunshea <i>et al.</i> , 2001
Improvac (anti-GnRH)		2	60	-	2	-	-	106	-	-	Cronin <i>et al.</i> , 2003
Improvac (anti-GnRH)		2	28	-	-	44	-	109 (\$)?	96 (\$)	116	Oliver <i>et al.</i> , 2003
Improvac (anti-GnRH)		2	270	-	-	30	-	-	-	-	Thun <i>et al.</i> , 2003
Early castration studies											
GnRHT	FCA-FIA	2	16	55	4	18	-	96	95	103	Turkstra <i>et al.</i> , 2002
GnRHT	Specol	2	20	-	1	9		110	94	124	Zeng <i>et al.</i> , 2002a

GnRH = gonadotrophin-releasing hormone;  $\alpha$ GB = Alpha Globulin; Improvac = Brand name for the CSL vaccine; GnRHT = GnRH tandem; OVA = ovalbumin; Oil-SAP = mineral oil for the primary immunisation, saponin in aqueous solution for the booster; FCA-FIA = Freund’s complete adjuvant for the primary immunisation, Freund’s incomplete adjuvant for boosters.

- = not determined.

(\$) : Performance measured during the last 4 weeks before slaughter.

## **4.8. CASTRATION OF FEMALE PIGLETS**

Ovariectomy is defined as the surgical removal of the ovaries. It is not allowed by Directive 2001/93/EC which stipulates: “All procedures intended as an intervention carried out for other than therapeutic or diagnostic purposes or the identification of the pigs in accordance with relevant legislation and resulting in damage to or the loss of a sensitive part of the body or the alteration of bone structure shall be prohibited” with exceptions limited to tooth resection, tail docking and male castration.

Castration of females is an uncommon practice in commercial farms. There are no available data regarding the practice of ovariectomy in Europe. Unlike in males where castration is used as a method to control boar taint in millions of pigs castration of females is limited to the farming of some local breeds that are slaughtered at an heavy live weight (around 150 kg) after the onset of puberty. The purpose of castration of females is to (i) avoid management difficulties due to oestrous behaviour, (ii) avoid pregnant females at slaughter and (iii) improve growth performance. Indeed, it was shown that sexual behaviour of mature female pigs reduces growth rate and increases management difficulties (Zeng *et al.*, 2002b; Ramis *et al.*, 2001 ). Cristea and Stanescu (1969) concluded that the yields of ovariectomized sows were 4.1% higher than non-ovariectomized sows and 1.6% higher than castrated boars. It was shown that the decrease in growth of non-castrated females results in a loss of 10 kg or a delay of 20 days compared with castrated males in Iberian pigs production (Ramis *et al.*, 2001).

For ovariectomy, female pigs are restrained on a table in lateral position, an incision is made in the lateral flank and both ovaries are removed through that incision (Cinotti, 1952; Ramis *et al.*, 2001). Ovariectomy is performed by some producers during the first days of life together with tail docking, iron injection and tooth resection. However, at that age, the localization and removal of the ovaries are difficult taking into account their very small size. Other producers may perform ovariectomy when the animals are between 20 and 40 kg (Ramis *et al.*, 2001). It is believed that ovariectomy is performed without anaesthesia.

Immunization against GnRH has been carried out under experimental conditions in Chinese female pigs, as a practical alternative to surgical castration (Zeng *et al.*, 2002b). The administration of a GnRH-tandem dimer peptide (Oonk *et al.*, 1998) at 10-13 weeks and 8 weeks later results in a decrease of serum LH levels and in a suppression of reproductive function. However, not all the animals responded to immunization.

## **5. PRODUCTION OF ENTIRE PIGS**

### **5.1. INTRODUCTION**

As described in chapter 4.2 pigs were originally castrated to promote docility, and fatness. More recently the focus has been on leanness, nutritional quality, high growth rate and efficiency of feed conversion, as well as environmentally friendly production and animal welfare – all factors which are to some degree improved by the production of entire males. Recently, however, castration has been practised primarily to avoid the problem of boar taint, despite the changes in consumer demand towards leaner meat. Nevertheless, some countries do not castrate some or all of their male pigs (see Table 3-1, sections 3.2 and 3.3) to exploit the more efficient growth and greater carcass lean content and thereby, reduce the overall cost of producing a kilogram of lean meat. Apparently, boar taint is not a serious problem in those

countries due either to the production methods, for instance slaughtering at low weights (well before sexual maturity), or to the relative insensitivity of consumers in those countries to boar taint. In other countries intensive selection for leanness in the modern pig, changing costs of feed, costs of screening carcasses for boar taint etc., continually change the assumptions on which the calculated advantages of entire males over castrates are based. There are also factors that do not favour entire male production. Entires display aggressive and sexual behaviour, which can pose an animal welfare problem – the severity of which must be weighed against the welfare problems connected to piglet castration. In addition, some meat quality and carcass attributes are negatively affected. If the production of entire male pigs is to become economically viable in the pig-producing (and especially exporting) countries, it is absolutely necessary to find ways of controlling the frequency of unacceptably tainted carcasses, otherwise the cost of removing tainted carcasses from the fresh pork supply chain will be too high. Thus, although there are many good arguments for the cessation of piglet castration, the production of entire male pigs creates a dilemma with regard to both animal welfare and meat product quality. This chapter describes the factors involved in this dilemma.

## **5.2. WELFARE AND MANAGEMENT ASPECTS OF NON-CASTRATION**

### **5.2.1. The behaviour and development of castrates versus non-castrates**

#### *5.2.1.1. Seasonal differences, age differences, sexual development and differentiation*

Studies of domestic pigs in semi-natural or natural environments have shown that the behaviour and physiology of these animals hardly differ from that of the wild boar (Stolba and Wood-Gush, 1989). In the wild, sows generally produce one litter per year in early spring, and mating thus takes place in late autumn to early winter – a time with declining day length, but in years where food is plentiful there may be two reproductive periods (Mauget, 1982). Consequently, the seasonality of reproduction is under the influence of the availability of food and primarily, of photoperiod. This applies also to domestic pigs under production conditions, where periods of lower reproductive efficiency can arise during the summer and early autumn months (Love, Evans and Klupiec, 1993; Dial, 1984). Prunier *et al.* (1996) conclude that in domestic pigs these effects are probably due primarily to high ambient temperatures and loss of appetite, i.e. undernutrition. Although males may be willing to mate at any time, seasonal variation has been found in spermatogenesis and in the quality of ejaculates (Claus and Weiler, 1985), as well as in the attainment of puberty in both sexes (Paterson and Pearce, 1990; Andersson *et al.*, 1998) so that reproductive success was found to be highest during times of decreasing photoperiod. Levels of sex steroids, including androstenone, in boars fluctuate accordingly, being highest in the short-day mating season (Claus *et al.*, 1983) and it has been shown, that these effects can in some cases be simulated or counteracted by artificially manipulating day-length (photoperiod).

Entire males show significantly more mounting behaviour than females and a peak in this behaviour is reached at 2 months of age (Ford, 1990 ; Berry and Signoret, 1984), but they also perform more social play fighting than females, each sex interacting more frequently with its own sex than with the opposite sex. Castration at birth reduces these behaviours to the levels seen in females. After castration at 30 days of age, mounting behaviour persists, as in intact males, for another month after castration - despite the lack of testes - but then declines. Castration at 60 days of age does not influence subsequent mounting activity. A reduction of sexual and aggressive activity is seen after immunocastration (Cronin *et al.*, 2003). Male pigs immunocastrated at 14 and again at 18 weeks of age, still show activity comparable with

entire males at 17 weeks of age, whereas at 21 weeks (three weeks after the second immunisation) activity is reduced to the level of pigs surgically castrated at 2 weeks of age.

Prepubertal males show bisexual behaviour. When presented with an oestrous sow, they will mount and thrust, and when presented with a mature boar they assume the immobile mating stance and erect ears characteristic of receptive females (Signoret, 1989). This is not seen in castrates or prepubertal females. Males are behaviourally sensitive to both male and female sex hormones.

An increase in plasma testosterone and androstenone can be induced in boars of only 30kg by HCG injection (Lundström *et al.*, 1978), but it is not known whether androstenone at this stage is stored in fat or salivary glands. The steroid levels in these young boars were not significantly correlated with levels at 85 kg, reflecting individual differences in sexual maturation rates, and can therefore not predict later androstenone levels. Moreover, it was found that unprovoked testosterone and androstenone levels follow an episodic secretory pattern, which should be taken into consideration when basing research results on plasma levels of the hormones.

The salivary glands of the pig are sexually dimorphic, with males having larger glands containing specific binding proteins for circulating testicular pheromones. Salivation and champing - a behaviour in males which releases the pheromones from the salivary glands – occur in connection with courtship and mating and when boars meet in aggressive encounters (Hafez and Signoret, 1969).

#### *5.2.1.2. Social behaviour*

Social behaviour occurs whenever individuals meet. Pigs are a social species and have well developed social behaviour and organisation. Only the aspects relevant to the production of entire males, i.e. dominance, aggressive behaviour and sexual behaviour will be discussed. Behaviour and hormones interact. Male hormones can initiate behaviour; on the other hand behaviour can affect the secretion of hormones, as will be seen in the following sections.

#### ***Dominance and aggression***

Entire males are more aggressive than gilts and castrates (Lundström *et al.*, 1987; Giersing, 1998; Cronin *et al.*, 2003). It was mentioned above that entire males have a high frequency of play fighting and mounting at a young age. As they grow older, play decreases and fighting becomes more serious.

Fighting associated with the mixing of unfamiliar pigs, hierarchy formation and competition lead to skin lesions (Ellis *et al.*, 1983; Warriss, 2000; Fredriksen *et al.*, 2003; Andersson *et al.*, 2003) and can lead to reduced growth rate. Fighting also causes an increase in plasma testosterone in males, which according to Claus *et al.* (1994) should result in a concurrent increase in androstenone. Booth (1980) measured high levels of androstenone in boars showing aggressive behaviour. In support of this, boars with high aggression scores were also found to have high levels of androstenone (Giersing *et al.*, 2000). However, lairage of boars and gilts for 2 hours before slaughter did not increase the androstenone level or boar taint in fat, compared with no lairage time, in spite of fighting and mounting behaviour (Lundström *et al.*, 1987).

In all groups of pigs, within litters or in groups mixed at any age, a dominance order will be established to determine the priority of access to resources. In males, this rank order affects

the levels of testosterone, androstenone, skatole and the size of the accessory bulbourethral gland in that high rank is associated with high values (Jonsson, 1985, Giersing *et al.*, 2000). In several mammalian species, e.g. rodents, the presence of a dominant male can suppress the sexual development of other males in the group. This is not the case in pigs: high androstenone level (implying high rank) in a boar does not inhibit sexual development and androstenone secretion in other boar penmates – on the contrary it has a stimulating effect (Giersing *et al.*, 2000). This may help to explain why higher levels of androstenone are found in group-reared boars compared with boars reared individually (Bonneau and Desmoulin, 1980; Narendran *et al.*, 1980). Other pheromonal effects of androstenone have been demonstrated by McGlone and Morrow (1988), who found that androstenone in a single, but not repeated, application to the snouts of juvenile pigs, reduced the level of aggression. However it had no such effect on aggression between sows (Stansbury and McGlone, 1987) or between boars and gilts (Parrott *et al.*, 1985). The presence of a mature boar reduced aggression in groups of fattening pigs (Grandin and Bruning, 1992), but this may have been a result of the boar's much greater body size. Dorries *et al.* (1991) found that adult male pigs are not particularly sensitive to the odour of androstenone. These different and inconsistent results concerning the effects of androstenone, especially in relation to entire males, suggests that sensitivity to androstenone may vary according to age or stage of sexual development and also raises the question of whether other androstenes may function as pheromones.

Skatole is excreted in the faeces. In many mammals, including ungulates, the deposition of faeces serves some communicative function e.g., in the marking of territory, for group- or individual identification, or to communicate social or reproductive status. No communicative function of skatole has been demonstrated in pigs (Giersing, 1998).

### ***Sexual behaviour***

Defeminisation of sexual behaviour in boars takes place slowly and progressively (Tuytens, 2002) with boars showing increased mounting behaviour as they reach sexual maturity. Aggression and mating/copulation increase testosterone and androstenone levels (Andresen, 1976; Lundström *et al.*, 1978; Narendran *et al.*, 1981/82; Parrott *et al.*, 1985) and cortisol levels (Liptrap and Raeside, 1978). A single mating causes a rise in plasma levels of testosterone and androstenone, whereas it seems to require repeated copulations before the androstenone increase can be measured in boar fat. Lundström *et al.* (1978) found that 120 kg boars that observed other boars mating, also experienced an increase in plasma testosterone and androstenone, but not in fat levels.

Claus, Weiler and Herzog (1994) argue that testicular hormones, including testosterone and androstenone, will always increase in parallel. However, Lundström *et al.* (1978) found this only to be the case after HCG stimulation. Maximum levels in plasma of both 30 kg and 85 kg boars were reached after approximately 1 day, whereas maximum levels in fat of 85 kg boars were reached after 2-3 days. They also found that the correlation between testosterone and androstenone was only significant after this challenge. It is therefore conceivable, that levels of androstenone in fat only increase after substantial challenge. Levels in older/heavier boars will of course be higher and it is possible that more mature boars do not need as much challenge or stimulation as younger boars.

Gilts in oestrus show interest in, and will seek out, boars, whereas when not in oestrus, they do not distinguish between boars and other pigs (Signoret *et al.*, 1975). Boars, however, are not able to distinguish between a receptive and non-receptive female by odour, which is why boars readily mount gilts, sows, other boars and dummies for sperm collection. This extensive mounting behaviour by boars may cause leg problems/lameness (Rydhmer *et al.*, 2003), in



addition to unrest and irritable aggression because victims of the behaviour are unable to avoid the harasser.

At high slaughter weights (over 100 kg) there is a risk that females become pregnant before slaughter (Rydhmer *et al.*, 2003; Andersson *et al.*, 1999), especially if they are slow-growing, e.g. from out-door systems.

Boar taint, and of course sexual behaviour increase in parallel with sexual maturity. Delay or suppression of sexual maturity would therefore be advantageous in the production of entire males. In the wild, males are ousted from the group by the females as they reach maturity. It is not known whether it would have an inhibitory effect on sexual development if males were to stay in sibling or family groups. (See Chapter 7 for possible practical applications of this.)

#### 5.2.1.3. *Feeding behaviour*

Coinciding with sexual maturity entire males spend less time feeding and more time in mounting and aggressive behaviour than castrates. Accordingly, the growth rate of entire males stagnates at the end of the growing period compared with immuno- and surgically-castrated males (Cronin *et al.*, 2003). A similar result was found by Giersing (1998) comparing entire males with females. Although entire males are more aggressive than the other sexes, as they reach maturity and spend less time feeding, they displace other pigs from the feeder less frequently than castrates do (Cronin *et al.*, 2003). It is not known how the higher aggressiveness of prepubertal males affects feeding behaviour and competition, compared with castrates.

#### 5.2.2. **Effects of stress on level of boar taint substances**

Stress is a broad concept with many definitions (Toates, 1995; Borell, 1995). It can be characterised as a condition in the pig where it is not able to respond adequately and therefore finds it difficult to adjust to, or cope with, certain factors, challenges or threats in its surroundings, i.e. it does not feel in control. The stressful state includes behavioural, endocrinological and physiological responses.

In many mammalian species different types of social stress, e.g. the stress of being subordinate, of being crowded, or of being bullied (i.e. being the recipient of much aggression) can result in delay or even inhibition of sexual maturation, in reduced immune response or in poorer feed conversion rate and growth rate. These effects are mediated through changes in the neuroendocrine system. Stress can be measured or evaluated through behavioural, physiological, neuroendocrinological, developmental, health and production measures. Since none of these parameters alone can be used as an indicator of stress, it is recommended that several measures be used in parallel (Pedersen, 1996; Dawkins, 1997; Borell, 1995). For example, behavioural measures include tests for fearfulness and aggressiveness, physiological measures include assessment of 'stress hormones' (typically cortisol) and sexual maturation.

Since boar taint is closely related on the one hand to sexual maturity (androstenedione), and on the other hand to digestion and gut microflora (skatole), (see chapter 7) it is easy to imagine that severe stressors could have an effect on boar taint. One could also imagine, that the production of entire males for slaughter could introduce additional stressors to the rearing environment of males as well as females. In an experiment containing several regroupings of entire males and females, and measuring aggression, sexual maturation, boar taint substances, growth rate and cortisol response, no indications were found of stress-effects on boar taint (Giersing, 1998). In pubertal boars, plasma cortisol was found to be positively related to

social rank at a first sampling (88 kg liveweight), but not a second sampling (107 kg liveweight), whereas no relationship was found between rank and cortisol level in females.

High levels of received aggression have been found to be correlated with low levels of androstenone and low levels of skatole, which is probably related to the low dominance rank of individuals that are the recipients of much aggression (Giersing, 1998). In an on-farm and abattoir study (Kaminder, 1991) no consistent connections were found between aggressive behaviour and skatole levels.

Warriss and Brown (1985) found a positive correlation between carcass damage as a result of fighting and the level of cortisol in post-slaughter blood plasma, whereas Moss and Robb (1978) found no difference in plasma cortisol between males and females slaughtered after different lairage times. However, measures of free and total thyroxin could indicate that entire males are more sensitive to stressors than females or castrates. Giersing (1998) did find that entire males responded differently from females to repeated challenges consisting of restraint in a nose sling and procedures for obtaining fat and blood samples. Males had a higher initial plasma cortisol response, but lower response to an additional stressor, compared with females – which indicated greater sensitivity/emotionality towards the procedure.

Measurement of plasma constituents for the assessment of pre-slaughter and slaughter treatments of livestock has been reviewed by Shaw and Tume (1992). They conclude that certain plasma constituents, including cortisol, can be useful indicators of pre-slaughter stress, but could not be related to meat quality.

The interpretation of changes in cortisol levels are often the subject of much discussion. Cortisol increases in response to a multitude of challenges of the animal, including fighting, mating, physical activity and psychological stressors – and it interacts with many other hormones. It is therefore difficult to specifically relate it to negative stress, especially chronic stress. The same applies to many other physiological measures of stress and therefore, in stress research, the search continues for reliable physiological indicators.

### **5.2.3. Welfare and management in relation to transport, lairage and slaughter procedures**

As mentioned previously, entire males are more aggressive towards other pigs than are castrates and females. There is no scientific documentation to support the opinion that entire males at normal slaughter weight (90-120 kg liveweight) are more aggressive towards humans and therefore more difficult to handle. The higher occurrence of aggressive and mounting behaviour of entire males has consequences for how they should be mixed and/or grouped during rearing, transport and slaughter (see also Chapter 7.) The major welfare and management issues with entire males are the potential for increased fighting when mixed on farms, during transport, and lairage at abattoirs leading to carcass damage and the development of dark, firm and dry (DFD) meat. Fighting sometimes results in only superficial skin blemishes (Sather *et al.*, 1995). In other cases, however, it can cause major carcass bruising and consequently financial losses to the meat industry (Warriss, 1984). The incidence of carcass and skin damages varies considerably between slaughter plants (Warriss, 1984; Moss and Trimble, 1988) and major welfare problems are likely to occur where good pre-slaughter practices are not exercised. Fighting during loading/unloading and penning can also cause a higher frequency of carcasses with pale soft and exudative (PSE) meat, caused by rapid glycolysis post slaughter. Such meat has a lower economic value and is a problem for the industry.

### 5.3. ADVANTAGES AND DISADVANTAGES ASSOCIATED WITH THE PRODUCTION OF ENTIRE MALE PIGS

#### 5.3.1. Efficiency of growth and carcass composition

Several authors have reviewed the literature on the production traits of entire boars compared with castrates (Walstra, 1974; Walstra and Vermeer, 1993; Brooks and Pearson, 1986; Moss *et al.*, 1992; Vahlun, 1993; Xue *et al.*, 1997). All conclude that entire boars grow faster, eat less food, convert food to liveweight gain more efficiently and produce leaner carcasses than castrates. The size of the differences is not consistent from trial to trial and this is likely to be due to differences in factors such as breed, feeding system, diet, weight at slaughter etc. The superior growth rate of boars can be up to 13%. Entire boars may eat up to 9% less feed and convert this to liveweight gain up to 14% more efficiently. Boars are generally leaner than castrates by up to 20% with an exceptional difference of 40% being recorded in one trial (Nadeje *et al.*, 2000). This leads to improved grading and higher payments to the producer, though current grading methods may underestimate the difference between the sexes (Andersson *et al.*, 1997). When the difference in carcass composition is taken into account, the greater efficiency of entire boars over castrates increases. For example, in an MLC trial (Meat and Livestock Commission, 1989) *ad libitum* fed boars grew 4.5% faster than castrates but their lean tissue growth rate was 13% higher. Similarly, *ad libitum* fed boars converted feed into liveweight gain 11% more efficiently than castrates, but they were 22% more efficient at converting feed into lean tissue. Comparisons between restricted fed boars and castrates showed similar trends. At the feed prices prevailing at that time the cost of producing a kg of lean meat was 12.5% lower and 11.2% lower for entires for ad lib and restricted fed animals respectively. However, the advantage of entires in lean growth rate has decreased due to selection for leaner pig breeds and decreasing feed prices in EU has also decreased the profitability of raising entires (Udesen, 1998).

**Table 5-1 Summary of differences between boars and castrates in production traits**

Reference	Growth rate: Boars grow faster	Feed Consumed: Boars eat less	Feed Conversion Efficiency: Boars more efficient	Carcass composition: Boars leaner
Casteels <i>et al.</i> , 1974	-	-	Yes	Yes
Allen <i>et al.</i> 1981	Yes = 6.4%	No	Yes = 7.7%	Yes = 8.7% less backfat
MLC, 1982	Yes = 11.3% (restricted), = 4.5% (ad lib)	Yes = 8.7% (ad lib only)	Yes = 13.7% (restricted), = 11.1% (ad lib)	Yes = 20.5% (ad lib), = 16.4% (restricted)
Paschma <i>et al.</i> , 1989	-	-	-	Yes
Campbell <i>et al.</i> , 1989	Yes	Yes	Yes	Yes
Dunshea <i>et al.</i> , 1993	Yes	-	Yes	Yes
Oeckel <i>et al.</i> , 1996	-	-	Yes	Yes = 4-5%
Park <i>et al.</i> , 1999	Yes	-	-	Yes = 18-37%
Nadeje <i>et al.</i> , 2000	Yes = 13%	-	Yes = 9%	Yes = 39.8%
Turkstra <i>et al.</i> , 2002	Yes	-	Yes	-
Lawlor <i>et al.</i> , 2003	No (Expt 1) Yes (Expt 2) = 4.8%	Yes = 9.2% (Expt 1), = 6.6% (Expt 2)	Yes = 8.4% (Expt 1), No (Expt 2)	-

### 5.3.2. Carcass and meat quality

Entire males are generally found to be considerably leaner than castrates raised under identical conditions. Allen *et al.* (1981) found that the backfat of entire males was 8.7% less than that of castrates. In the 1982 MLC trial the difference was greater at 20.5% for ad lib and 16.4% for restricted feeding. As indicated above, with producers being paid for lean meat content, this difference in leanness in favour of entire males increases the economic benefit to producers of non-castration. There are, however, some negative carcass quality factors that make entire males less profitable to processors. These include a lower kill out percentage (carcass weight as a percentage of live weight), due to the developing genital tract in the entire male, lower bacon yield and less favourable joint proportions, though the latter two differences are rather small. Depending on conditions before slaughter (e.g. mixing during transport and/or lairage before slaughter), the proportion of DFD meat may be higher in entire males as they are more active, more aggressive, and mount more frequently (Moss and Robb, 1978; Ellis *et al.*, 1983; Andersson *et al.*, 2003).

The decreased adipose tissue content of meat cuts from entire males makes them more appealing to the consumer. The characteristics of muscle and adipose tissue differ between entire males and castrates (e.g. Malmfors and Nilsson, 1978; Wood and Enser, 1982; Ellis *et al.*, 1983; Desmoulin *et al.*, 1983; Barton-Gade, 1987). The lower lipid content and the higher content of unsaturated fatty acids in adipose tissues of entire males may be regarded as favourable from the human dietetic point of view. However, for very lean genotypes, the decreased amount of adipose tissue in entire males may be a disadvantage. Indeed, the processing industry requires a minimum quantity of good quality fat and extreme leanness results in a lack of cohesion between backfat and the underlying muscle (Wood, 1984a). The lower intramuscular lipid content of entire males may cause the meat to be less tender than that of castrates (Martin, Fredeen and Stohart, 1968; Bonneau *et al.*, 1979; Barton-Gade, 1987).

A common complaint from the meat industry concerning handling and butchery of boar carcasses is soft fat. This is a potential problem in lean carcasses, generally because in leaner carcasses, the concentration of the main polyunsaturated fatty acid linoleic acid (C18:2) increases as does the concentration of water in adipose tissue. Together these cause "soft fat" (Wood, 1984b). A series of studies on boars and castrates concluded that even at the same fat thickness as castrates, boars had softer fat because both C18:2 and water concentrations were higher (Wood *et al.*, 1986). In other words, the pattern of fat metabolism and deposition is different in boars, perhaps caused by sex hormones. Thus, the processing quality of this soft fat from entire males is poorer than from castrates and it is also less resistant to oxidation. The problems associated with fat quality in entire males are exacerbated with the use of lean genotypes and by the use of diets with a high content of unsaturated fatty acids, typically used in organic pig production (Hansen *et al.*, 2001). It can be argued that differences in fat quality between boars and castrates at the same fat thickness are quite small, nevertheless they constitute another negative aspect of meat quality in boars. The development of soft fat can, however, be avoided by changing the fatty acid composition of the diet.

Although the above-mentioned problems with meat quality may be important, particularly in lean strains of pigs and in organic pig production, the most important limitation to the use of entire males is the existence of boar taint.

### 5.3.3. Waste reduction and environmental effects

As entire males grow more quickly and are more efficient converters of food to weight gain, they produce less waste per kilogram of lean meat - reducing the volume of manure and,

especially, the nitrogen content of urine. This is due to the higher feed conversion ratio and higher nitrogen retention rate of boars compared with castrates (Eeckhout, Bekaert and Casteels, 1971; Desmoulin, Bonneau and Bourdon, 1974).

#### **5.3.4. Other factors / costs**

There are additional cost savings due to eliminating the labour involved in castrating piglets and avoiding any losses. On the other hand, the production of entire males in many countries, and for many export markets, will necessitate the screening and sorting of male carcasses for boar taint. The costs of these procedures, including analyses, must be included in calculations of the economical viability of producing entire males.

## **6. BOAR TAIN**

### **6.1. BOAR TAIN, GENERAL DESCRIPTION AND INCIDENCE**

Boar taint is a distinctive and unpleasant taint perceived through a combination of sensory odour, flavour and taste in pork and pork products during cooking and eating. It has been described as 'animal', 'urine', 'fecal' and/or 'sweat' like in character. At commercial slaughter weights, the incidence of boar taint is very variable, ranging from 10 to 75% according to different studies (e.g. Williams, Pearson and Webb, 1963; Desmoulin, Dumont and Jacquet, 1971; Rhodes and Patterson, 1971; Malmfors and Hansson, 1974; Squires and Lou, 1995; Xue *et al.*, 1996).

It is considered that two compounds are largely causal in boar taint namely, androstenone and skatole (Patterson, 1968; Vold, 1970; Walstra and Maarse, 1970). However, their level of contribution and interaction in contributing to boar taint is a subject of contention. Moreover, there are additional compounds that have been proposed as involved, e.g. indole. In general, the androstenone aspect of the taint phenomenon is associated with the presence of normally functioning testes in the sexually mature male pig. Without testes, boar taint is rarely found present in males or females except when the pigs are kept in heavily fouled surroundings with their own faeces and urine (Hansen *et al.*, 1994) or when fed with certain carbohydrate based dietary components such as yeast from breweries (Jensen and Jensen, 1998). Gibis (1994) found a large difference in elevated skatole levels between castrated male pigs and female pigs. As many as 6.4% of the castrated male pigs (7 out of 110) had skatole levels above 0.19 ppm, whereas 1 out of 130 of the female pigs had elevated skatole values. Both sexes had a similar seasonal influence with highest levels during March and June and July.

### **6.2. CONTRIBUTION OF COMPOUNDS TO BOAR TAIN**

Two main compounds, androstenone a male sex pheromone, produced in the Leydig cells of the testes and of similar chemical structure to testosterone, is associated with a urine and perspiration odour (5 $\alpha$ -androst-16-ene-3-one; Patterson, 1968) and skatole a metabolite of the amino acid tryptophan, produced in the lower gut by intestinal bacterial flora, associated with naphthalene and faecal odour (3-methyl indole; Vold, 1970; Walstra and Maarse, 1970), mainly associated with fat, are considered largely responsible for boar taint in entire male pigs.

Both androstenone and skatole are highly fat-soluble. Thus, their concentration in some porcine animals can be high. These are the boars that consumers detect as tainted. As the boar reaches sexual maturity, at approximately 14-15 weeks of age, there is a surge of testosterone

and androstenone production. This is quickly followed by an increase in the concentration of androstenone in fat. The physiology and importance of skatole may be less well defined. While androstenone is only produced in entire males, skatole is produced in both entire, castrate male and female pigs. However, the fat levels of skatole are higher in some genetically predisposed boars than in castrates or gilts. The reasons for this have been unclear until recently. Androstenone or other testicular steroids might be involved in the regulation of skatole by affecting skatole metabolism (Babol, Squires and Lundström, 1999; Doran *et al.*, 2002). If the sex steroid metabolism is related to skatole metabolism, as was shown for androstenone (Doran *et al.*, 2002) it might be due to the inhibition effect of testicular steroids on the expression of enzymes involved in skatole metabolism.

Androstenone belongs to  $\Delta$ -16-steroids and other substances in this group may also contribute to boar taint as reviewed by Claus (1979). The androstenones with a 3-keto-binding have a urinary odour (5 $\alpha$ -androst-16-en-3 $\alpha$ -one, androstenone and 5 $\beta$ -androst-16-en-3 $\alpha$ -one, 5 $\beta$ -androstenone) whereas the androstenols with 3-hydroxy-bindings have a musky odour (5 $\alpha$ -androst-16-en-3 $\alpha$ -ol, An- $\alpha$  and 5 $\alpha$ -androst-16-en-3 $\beta$ -ol, An- $\beta$ ). Also the configuration of the molecule is important and the  $\beta$ -configuration has a weaker odour than the  $\alpha$ -configuration. There are few studies where more  $\Delta$ -16-steroids than androstenone have been analysed and the results vary. Claus (1979) found an approximately 15 times higher androstenone concentration in fat compared with An- $\alpha$  and a 30 times higher concentration compared with An- $\beta$ . Also Garcia-Regueiro and Diaz (1989) found on average 15 times higher androstenone levels compared with An- $\alpha$ , but higher levels of An- $\beta$  than An- $\alpha$  and with a large variation between individuals. Brennan *et al.* (1986) found a ratio between androstenone and androstenol (sums of  $\alpha$ - and  $\beta$ -configurations) of 2.2:1. Even if the related  $\Delta$ -16-steroids might have a certain influence, androstenone has without doubt the largest influence.

While androstenone and skatole are the primary contributors to boar taint there are several other compounds (indole, p-cresol) that may in some carcasses make a contribution to the perception of boar taint. Indole, like skatole is a breakdown product of tryptophan. However, the contribution to boar taint is probably low, as the threshold was calculated to be 50 times lower than skatole (Moss, Hawe and Walker, 1993). Also 4-phenyl-3-buten-2-one (Rius Solé and Garcia Regueiro, 2001) has recently been suggested to be of importance for boar taint. Even though these compounds have milder unpleasant odours relative to androstenone and skatole, they may nevertheless in certain instances contribute significantly to the overall sensory perception of boar taint. Thus, any boar taint control solutions should address all compounds suspected of involvement in the phenomenon.

Overall, it can be stated that androstenone and skatole are very strong contributors to boar taint in an individual sense. However, skatole has been demonstrated to enhance the sensory perception of androstenone. Unpleasant boar taint odours and flavours associated with androstenone can be magnified when high skatole levels are also present. It is clear that the most objectionable odours and flavours associated with boar taint are associated with meat from animals that have elevated concentrations of both compounds.

### 6.3. SENSORY PERCEPTION OF BOAR TAIN

Boar taint *per se* has been described by various researchers as having distinctive sensory characteristics described as urine-like, animal-like, sweat-like and faecal-like (Dijksterhuis *et al.*, 2000). Trained sensory panels can distinguish between androstenone and skatole in boar meat, and between different levels of these substances. In a recent international study involving trained sensory panels in six European countries, it was found that androstenone

related mostly to an "urine" attribute whereas skatole was mostly associated with "manure" and, to a lesser extent to "naphthalene" (Disjksterhuis *et al.*, 2000).

Although both skatole and androstenone contribute to boar taint, there is some evidence that they cannot completely account for the occurrence of boar taint as determined by a trained sensory panel (Babol, Squires and Gullet, 1996). Using entire males that were near sexual maturity as judged by the length of the bulbourethral glands, this study reported that there was still significantly higher boar taint odour and flavour scores from entire males with low levels of androstenone and skatole. It may be that other factors related to sexual maturity also contribute to boar taint, but these factors have not been identified.

Overall, the sensory effect may be considered one of repulsion and rejection of the cooked meat, however, there are populations that consider the taint to be an integral characteristic of pork meat. The majority of the variation in the sensory description of boar taint results from individual variation in the meat consumer and their sensitivity to the compounds (skatole and androstenone) that are causal in boar taint, and by variation between porcine carcasses in their levels of boar taint compounds (Weiler *et al.*, 1997; Wysocki and Beauchamp, 1984).

#### **6.4. STUDIES WITH LABORATORY PANELS**

The contributions of androstenone and skatole to boar taint have been investigated in many studies. Coefficients of correlation between fat androstenone or skatole levels and boar taint intensity, assessed by laboratory panels, range between 0.4 and 0.8 (Bonneau, 1993). Taking into account the lack of precision inherent in the subjective assessment of odour intensity, such relationships suggest that both compounds make a significant contribution to boar taint.

According to Berg *et al.* (1993), androstenone and skatole have similar contributions to boar taint. In a number of studies (Lundström *et al.*, 1984; Mortensen and Sorensen, 1984; Walstra, Engel and Mateman, 1986; Lundström *et al.*, 1988; Andresen *et al.*, 1993; Bejerholm and Barton Gade, 1993), skatole has been reported to have a higher contribution to boar taint than androstenone. In some cases, this can be explained by the fact that few animals in these studies exhibited high androstenone levels, therefore leaving skatole as the main contributor to boar taint off-odours. However, Bejerholm and Barton Gade (1993) demonstrated that, although androstenone alone resulted in a significantly decreased off-odour score, the contribution of skatole to boar taint was more important. On the contrary, Bonneau *et al.* (1992) demonstrated that the contribution of androstenone to boar taint was larger than that of skatole. Moreover, androstenone and skatole odours may strengthen each other synergistically (Lundström *et al.*, 1980; Walstra, Engel and Mateman, 1986; Bonneau *et al.*, 1992).

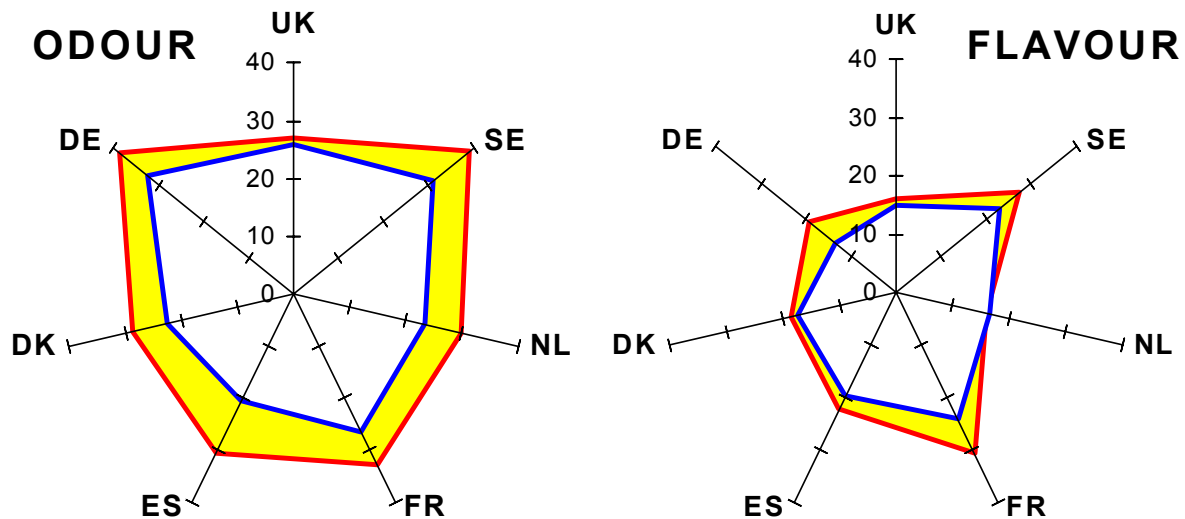
The lack of consistency between the results obtained in the various studies may result from differences in the androstenone and skatole characteristics of the animal populations from which the samples were taken. Differences in the methodology used for the sensory assessment of odours (Bonneau, 1993), including selection and training of the panel members and preparation and presentation of the samples may also be important.

#### **6.5. IMPACT ON CONSUMER ACCEPTABILITY**

Because of the large variation in the incidence of boar taint, and because of the variety of culinary habits between countries, the acceptability of boar meat, as measured in consumer surveys, can be quite inconsistent between studies (Malmfors and Lundström, 1983).

From the results of a recent international study on boar taint (Bonneau *et al.*, 2000a, Matthews *et al.*, 2000) the proportion of consumers that would be dissatisfied with the odour or with the

flavour of entire male pork has been calculated (Bonneau *et al.*, 2000b; Fig. 6-1) under the assumption that they consumed meat from entire male pigs produced from all over Europe and exhibiting the currently observed levels of androstenone and skatole (Walstra *et al.*, 1999).



**Figure 6-1 Proportion of consumers dissatisfied with the odour or flavour of entire male pork (outer line), compared with gilt pork (inner line) adapted from Bonneau *et al.*, 2000b. UK: United Kingdom; SE: Sweden; NL: Netherlands; FR: France; ES: Spain; DK: Denmark; DE: Germany; The shaded surface represents the difference between entire male and gilt pork**

Overall, 6.5% more consumers would be dissatisfied with the odour of entire male pork than with that of gilt pork. These results did not include comparison of entire and castrated pigs. The corresponding difference for flavour would be 3.0%. Large variations were observed between countries. The difference in acceptability between entire male and gilt pork was very small in Great Britain for both odour and flavour and in Denmark and the Netherlands for flavour. In all other cases, substantially more consumers would be dissatisfied with entire male than with gilt pork, the difference ranging from 6.1 to 10.2% for odour and from 2.4 to 6.3% for flavour.

With respect to the main boar taint compounds, skatole is perceived by the majority of consumers, as high as 99%, (Weiler *et al.*, 1997; Matthews *et al.*, 2000) while a variable percentage of individuals are insensitive or anosmic to androstenone. This anosmia is genetically determined (Wysocki and Beauchamp, 1984) and depends on the gender of the individual (Griffiths and Patterson, 1970) and the individuals' origin (Gilbert and Wysocki, 1987). Women have been shown to be more sensitive to androstenone than men in the majority of studies carried out (Font I Furnols *et al.*, 2004). The percentage of anosmic women to men has been reported as 15.8 vs. 24.1% in Europe, 10.9 vs. 30.0% in United Kingdom, 29.5 vs. 37.2% in USA and 17.2 vs. 25.5% in Asia (Gilbert and Wysocki, 1987). In a more recent study the percentage of insensitive persons in Germany was determined to be 66% of women and 70% of men and in Spain 48% and 60%, respectively (Weiler *et al.*, 2000). The lack of agreement with respect to this aspect of the boar-taint phenomenon needs to be resolved as it is critical to the understanding of boar taints negative impact on consumer pork acceptability. Overall, the sensitivity of consumers to androstenone has been



demonstrated as an important aspect to be considered with respect to the boar taint issue and pork acceptability. It must be noted that it has also been demonstrated that certain individuals appear to consider the odours a positive aspect in pork (Pause *et al.*, 1999).

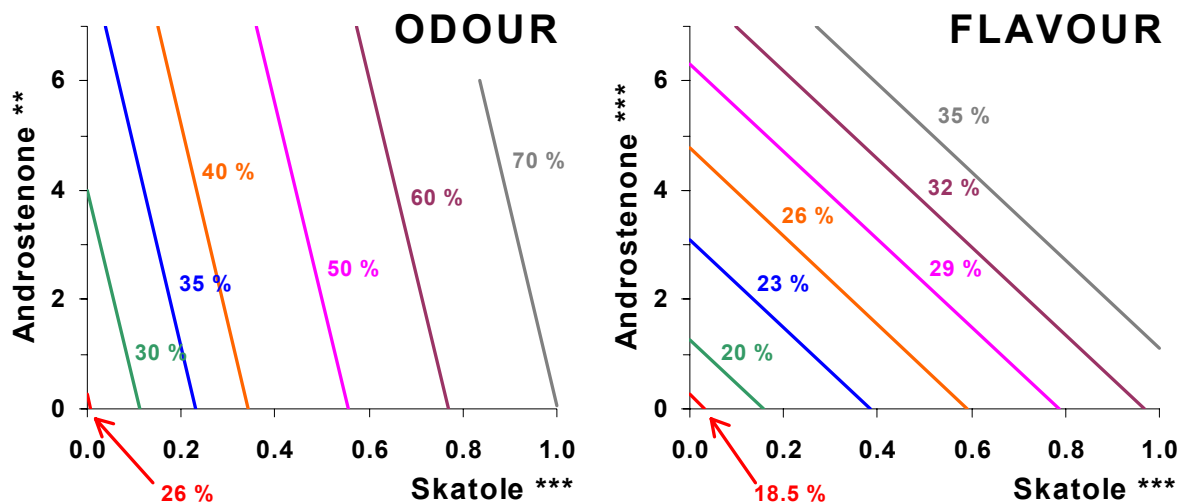
Moreover, as boar taint is mainly evidenced from a sensory perspective during cooking and consumption of pork meat, consumers who prepare the meal are most likely to detect boar taint. If these individuals are also the main meat purchasers then negative experiences with boar tainted pork will influence their repeat purchasing decisions (Bryhni *et al.*, 2002; Bryhni *et al.*, 2003).

## **6.6. STUDIES BASED ON CONSUMER SURVEYS**

Very little published information is available regarding the influence of skatole and androstenone on the consumer perception of the odour and flavour of meat from entire male pigs. In addition to the differences in the level of malodorous compounds in meat samples and in the methodology used for sensory assessment, consumer reactions to boar taint are affected by differences among consumers in the ability to detect compounds and in their culinary habits, which may vary widely between countries.

An international study aimed at resolving the controversy surrounding the respective contributions of androstenone and skatole to boar taint has been conducted (Bonneau *et al.*, 2000a,b, Matthews *et al.*, 2000). A pool of entire male pigs with known concentrations of androstenone and skatole was selected from a larger population of entire male pigs raised in 6 European countries (Walstra *et al.*, 1999). Meat samples from the selected animals were used for consumer surveys conducted in 7 European countries (Matthews *et al.*, 2000). The results of the overall study, including data from all seven consumer surveys, show that skatole contributes more than androstenone to the proportion of consumers which are dissatisfied with the odour of entire male pork (Fig. 6-2). Concerning flavour, skatole and androstenone have a similar contribution, which is additive. The higher contribution of skatole than androstenone to unpleasant odours perceived by consumers can be related to the observation that a rather high proportion of people are unable to smell androstenone (Griffiths and Patterson, 1970; Gilbert and Wysocki, 1987) whereas no such anosmia is observed for skatole (Weiler *et al.*, 2000).

Unfortunately, only a small proportion of the entire male pigs had low levels of both skatole and androstenone. It was therefore not possible to determine the proportion of dissatisfied consumers for meat from entire males with low levels of both compounds. It seems unlikely that entire males will be used for pig production in most countries without sorting out the tainted boars on the slaughter line. Moreover, for the production of entire males to be economically feasible, the proportion of rejected animals would have to be relatively small. This means that the incidence of animals with high levels of skatole and androstenone would have to be reduced quite dramatically if castration were to cease.



**Figure 6-2 Isoresponse curves for the proportion of dissatisfied consumers for odour (26 to 70%) or flavour (18.5 to 35%) according to skatole and androstenone levels in entire male pork. The proportion of consumers dissatisfied with gilt pork was 26% for odour and 18.5% for flavour. The significance of the effects of skatole and androstenone on the proportion of dissatisfied consumers is given as \*\* for  $P < 0.01$  and \*\*\* for  $P < 0.001$ . Overall results from a consumer study performed in 7 European countries. Adapted from Matthews *et al.*, 2000.**

## 7. CONTROL OF TAINT: ANTE MORTEM

### 7.1. INTRODUCTION

Currently, there are several possible methods to reduce the incidence of boar taint in slaughter pigs. Some methods have significant effects, others seem to have only marginal effects, and the two main substances responsible for boar taint do not always respond to the same measures. Skatole levels can be reduced by modulating nutrition, feeding, rearing and management (including hygienic) conditions, whereas genetic selection is more efficient at lowering androstenone content (see Bonneau and Squires, 2000). Both compounds can be reduced by measures that delay or suppress sexual development (Babol and Squires, 1995; Babol, Squires and Gullett, 1996; Bonneau and Squires, 2000; Doran *et al.*, 2003). Measures to delay sexual development includes restricted feeding or nutrient supply, limited social interaction and conflict to avoid aggressive and sexual behaviour and, probably to a small degree, increasing daylength. Suppression of sexual development can be achieved through surgical or non-surgical castration or immunisation methods. Immunisation against GnRH, however is an effective method (see section 7.5.4). Finally, a solution could be to produce only female pigs by means of sperm-sorting and deep artificial insemination, but several of the latter methods are far from ready for commercial use. In the following sections the different possibilities for reducing the level of boar taint in the live animal, under present or future practical pig production conditions are reviewed.

## 7.2. REARING AND MANAGEMENT PROCEDURES AND ENVIRONMENTS

### 7.2.1. The social environment

#### 7.2.1.1. *Single versus mixed sex rearing*

The higher level of aggression and mounting behaviour in groups containing entire males lead in most cases to increased levels of boar taint. The effect does not seem to be conditional on the presence of females, as all male groups often do not differ in androstenone levels from mixed sex-groups. It seems to be primarily the group rearing that has a stimulatory effect, compared with individual rearing (Bonneau and Desmoulin, 1980; Narendran *et al.*, 1980). Moreover, the effect on boar taint depends on slaughter weight. Males from mixed-sex groups slaughtered at over 100 kg live weight have increased levels of boar taint (Walker, 1978; Patterson, 1982; Patterson and Lightfoot, 1984). However, Andersson *et al.* (1999) found that males raised in mixed sex groups to a slaughter weight of 107 kg were more sexually mature (heavier epididymis weights) than males from single-sex groups, but there were no differences in either androstenone or skatole backfat concentrations, perhaps because sexual maturity had been reached in 90% of all animals.

Puberty in females is stimulated by social contact with other females (Christenson, 1984) and can also be accelerated by exposure to a mature boar from around 160 days of age. Continuous boar exposure does not have this stimulating effect - possibly due to habituation (Hemsworth *et al.*, 1988; Paterson Hughes and Pearce, 1989). This suggests that the rearing of entire males and females together from birth to slaughter will not have a stimulatory effect on female puberty. Andersson *et al.* (1999), in a study comprising 550 pigs each of entire males and females, compared mixed and single-sex rearing from 26 – 107 kg live weight and found that females from mixed-sex groups reached sexual maturity significantly earlier than all-female groups in one trial, but not in the other. In this study some females from mixed groups were pregnant at slaughter.

In summary, experiments on the rearing of entire males in mixed or single-sex groups show no consistent effects on boar taint in fat. The different involved factors, social as well as non-social, probably interact. Single sex rearing will prevent harassment of females by entire males and single sex rearing has also in some cases resulted in lower boar taint levels in entire males at high slaughter weights. This complex problem is a subject for further research.

#### 7.2.1.2. *Competition level after mixing pigs and group formation*

Mixing of pigs unavoidably leads to fighting (Hafez and Signoret, 1969; Rydhmer *et al.* 2003; Andersson *et al.*, 2003). Removal of the dominant (largest) individuals in a group likewise can lead to renewed fighting, even if the remainder of the group are left intact. Regrouping of pigs during production has been a common procedure in order to maintain uniform groups of similar age and weight, so that pigs can be slaughtered pen-wise (the all-in, all-out system). This has been practised in spite of the fact that pigs of uniform weight fight more than pigs of different weight in order to establish a hierarchy (Rushen, 1987). Moreover, the degree of unfamiliarity (number of unacquainted litters that make up the group) also affects the level of aggression (Rundgren and Löfquist, 1985; Arey and Franklin, 1995). Fighting, mounting behaviour, skin damage and exhaustion have been shown to be increased in entire males (Ellis *et al.*, 1983; Fredriksen *et al.*, 2003; Andersson *et al.*, 2003; Moss and Trimble, 1988). Fighting and exhaustion lead to deterioration of meat quality measured by higher frequency of dark, firm and dry (DFD) meat (Warriss, 2000; Walker, 1978).

It is likely that aggravated aggression will lead not only to poor welfare, but also to increased androstenone levels. It is also likely that limiting factors in the rearing environment, that create competitive conditions, become more critical due to higher boar aggression. As puberty is reached, boars become less interested in feed, but social and sexual activity increases, so that competition for other resources than feed increase.

As described in Chapter 5, dominant entire males have significantly higher androstenone levels than subordinates, and high levels in a group have a stimulating effect on others in that group. Initial studies to test whether the inclusion of one substantially larger female animal in a group of pigs would inhibit or delay sexual development of males in the group, have shown no effect (Fredriksen, personal communication).

#### 7.2.1.3. 'Birth to slaughter' systems

'Birth to slaughter' systems – where pigs are kept in their litter groups from birth to slaughter, and are not mixed with other pigs - seem to result in lower levels of androstenone (Fredriksen *et al.*, 2003) and lower incidence of skin damage. Mixing and moving animals will necessitate establishment of new hierarchies through aggressive behaviour, and this behaviour will accelerate the initiation of puberty and provoke testicular activity. By keeping littermates together in stable groups, the level of aggression is low, and the initiation of puberty may moreover be inhibited in sibling groups. Consequently the levels of androstenone, and probably also skatole, will remain at a lower level.

#### 7.2.2. The physical production environment (space, floors, transport, etc.)

As mentioned above, the increased aggressive and sexual behaviour of entire males may require improved standards for floor space, floor quality, feeder space, substrate/bedding and pen lay-out. Restrictions in these factors may exacerbate effects of behaviour on skin and leg problems, as well as increase the general stress-level if pigs cannot escape or avoid harassment by particularly active males. Effects of these factors on boar taint levels are not documented, and will also depend on slaughter weight.

During loading on the farm, transport and lairage at the abattoir, special consideration should be given to the social and physical environments. Pig from different farms and pens should not be mixed and floors should not be slippery. Mixing with unfamiliar pigs prior to slaughter has been shown to result in more damaged skin and darker meat, compared with pigs handled pen-wise (Andersson *et al.*, 2003). Loading, transport and lairage procedures are under all circumstances very stressful for the pigs. The floor area and type of flooring in a pig pen have particular consequences for the development and levels of skatole and indole, as described in the next section.

Most of the experimental work on rearing environments and procedures, as well as transport and slaughter procedures, for pigs has been done with castrates and females. There is not enough knowledge concerning special requirements for the rearing and slaughter of entire males.

#### 7.2.3. Deposition and handling of excreta

In warm weather (approx. 20°C, but lower for large pigs), pigs are strongly motivated to wallow. In intensive production systems, the only possibility for this is in the dunging area, which leads to increased levels of skatole and indole in fat. It has been shown that fat skatole and indole levels can be increased in both entires, females and castrates by keeping the pigs on heavily soiled concrete floors with a mixture of faeces and urine, compared with rearing pigs on clean concrete or clean slatted floors (Kjeldsen, 1993; Hansen *et al.*, 1994, 1995 ). Furthermore, it was feasible during the week before slaughter to increase or lower the skatole

and indole levels according to the treatment of the pigs. The experiments showed proportional treatment differences in all three sexes (Hansen *et al.*, 1995). This is especially a problem in warm summer periods when the temperature of the faeces and urine on the piggery floor may be higher than the ambient temperature, particularly when the pigs were lying in the excreta (Hansen *et al.*, 1994). Spoelstra (1977) demonstrated that protein degradation followed by transformation of tyrosine and tryptophan contributed to the accumulation of the aromatic compounds phenol, p-cresol, indole and skatole during anaerobic storage of piggery waste. Faeces alone as well as faeces and urine produced considerable amounts of p-cresol and skatole when incubated anaerobically at 25°C, but only small quantities at 15°C. Indole production was further stimulated by adding urine to the faeces when compared with faeces only, however, the temperature was not as important as for skatole production (Spoelstra, 1977). Friis (1993a) demonstrated that around 40% of 14 C-skatole used experimentally was absorbed through the skin in the belly region of the pigs. This means that pen floors should be kept clean, or that floors should be well drained, and that pigs should have other means of thermoregulation than wallowing in excreta.

The way pigs choose to use the pen area for resting, activity, dunging etc. depends on the ambient temperature, ventilation, air movement, stocking rate, floor types and position of the drinking valves, etc. Pedersen and Strager (1979) found that a shower in the excretory area, wetting the area occasionally during day and night, diminished soiling of the resting area.

In Denmark, the law stipulates that pigs must have access to a shower, or other cooling, system from a live weight of 20 kg (Act n°104 on indoor keeping of piglets and pigs for breeding or slaughter, 2000). Highly active pigs such as entire males will have a greater need for thermoregulation than less active pigs.

#### **7.2.4. Slaughter at low live weight**

Most studies show that the concentrations of boar taint compounds increase with carcass weight, increasing the proportion of abnormal odours detected by taste panellists or consumers. However, this relationship between taint compounds and carcass weight across the commercial slaughter weight range in the EU is quite small and correlations between skatole and androstenone concentrations on the one hand and carcass weight on the other are low. For example in the coordinated EU study, overall correlations were about 0.1 for both compounds (Walstra *et al.*, 1999). There were small differences between countries, the highest correlation being 0.20 for androstenone and 0.13 for skatole. The range of carcass weight in that study was 49-104 kg (average 76 kg). In a comparison of 65 and 80 kg carcass weights by MLC in UK (Meat and Livestock Commission, 1998), skatole did not increase as weight increased and only 5% of samples exceeded the threshold of 0.25 ppm at both weights. However, androstenone concentrations exceeding the threshold of 1.0 ppm increased from 3% at the lighter weight to 8% at the heavier weight. In a subsequent MLC study, skatole concentration did not increase between 70 and 100 kg carcass weight and overall, 11.4% of carcasses exceeded 0.25 ppm.

Nonetheless, the slaughter of entire males at a lower weight, i.e. before sexual maturity, has been considered as a possibility for avoiding boar taint. A Norwegian investigation, where entire males were slaughtered at average live weights of 53 to 62 kg. did not, however, show consistent results. For the four different farms included in the study, 5, 10, 30 and 36% respectively, of the entire male pigs had skatole levels greater than 0.20 µg/g (ppm) and/or androstenone levels greater or equal to 1.0 µg/g (ppm) and were thus above the set threshold limits (Aldal *et al.*, 2003).

In a Swedish experiment skatole levels in fat from entire males slaughtered at 90 kg liveweight were all below the threshold level of 0.20 µg/g (Zamaratskaia *et al.*, 2003). The difference between the Norwegian and the Swedish results may be related to the pig breeds used.

#### **7.2.5. Other management procedures**

**Photoperiod:** As described in Chapter 5, androstenone levels fluctuate with the ‘natural’ mating season (Claus *et al.*, 1983; Claus *et al.*, 1985; Patterson, 1982; Walstra and Garssen, 1995, Keller, 2000). Manipulating photoperiod (day length) has an effect on sexual development (Andersson, 2000; Andersson *et al.*, 1998; Neupert *et al.*, 1995) as decreasing day length results in earlier sexual maturity and a tendency to higher levels of boar taint (androstenone and skatole). However, the effect seems minor and artificial day length seems difficult to apply under practical conditions. For instance, it is not known for how long a period or within which photoperiod limits, day length should be controlled.

**Water intake:** Field test data of Kjeldsen (1993) suggested that increased intake of water could reduce skatole concentration in back fat. However, further field test results and experimentation (Sloth, Ruby, and Udesen, 1994; Larsen and Hansen, 1997) did not confirm this assumption.

**Feeding before slaughter:** Although restricted feeding has the potential to delay sexual development and thereby reduce the level of taint at slaughter weight, the withholding of feed immediately (26 hrs) prior to slaughter can reduce the level of skatole produced (Kjeldsen, 1993), whereas the level of androstenone was found to be increased (Andersson *et al.*, 1999).

#### **7.2.6. Specific immunisation against 16-androstene steroids**

A number of studies have investigated the possibility to immunise entire male pigs against androstenone (Shenoy, Daniel and Box, 1982; Williamson and Patterson, 1982; Williamson *et al.*, 1985) or immediate precursors of androstenone (Brooks *et al.*, 1986) although, in most cases, with little success.

### **7.3. EFFECT OF NUTRITIONAL LEVEL AND DIETARY COMPOSITION ON TAIN**

High energy feeding has been shown to increase levels of both androstenone and skatole (Malmfors *et al.*, 1989; Claus *et al.*, 1994; Øverland *et al.*, 1995; Zeng *et al.*, 2002a). A high energy diet is associated with acceleration of pubertal development in entire male pigs, which may in turn result in an earlier increase in the levels of skatole and androstenone (Einarsson *et al.*, 1979).

An explanation for the influence of high-energy diets on skatole levels was suggested by Claus and Raab (1999). They propose that if energy supply in the diet is high, the concentrations of IGF-I increase and stimulate gut cell mitosis and apoptosis, providing substrate for bacterial degradation and skatole formation and leading to increased blood plasma and fat skatole levels. The effect could be further enhanced by addition of high amounts of purines from brewers yeast in combination with the high- energy diet.

Skatole is a product of bacterial activity in the large intestine. Its levels in fat are influenced by diet, possibly through altering the bacterial activity or availability of the substrate, tryptophan (Hansen *et al.*, 1997; Hawe, Walker and Moss, 1992; Jensen *et al.*, 1997). The level of tryptophan in the feed per se, however, does not influence skatole production (Pedersen *et al.*, 1986; Gibis, 1994), as tryptophan is easily absorbed in the small intestine and thus does not reach the caecum and colon where skatole is produced (e.g. Jensen and Jensen,

1998). Dietary changes may have quantitative and qualitative effects on intestinal microflora and may therefore influence the rate of skatole synthesis. The effect of feeding on skatole production has been extensively reviewed by Jensen and Jensen (1998) and Zamaratskaia (2004).

Carbohydrates with low digestibility in the small intestine have been shown to decrease skatole levels in blood and fat in pigs (Jensen *et al.*, 1997), with most pronounced effects from the use of fructo-oligosaccharides, lupines and potato starch. With the use of a high-fibre diet consisting of 10% sugar beet pulp added to the control feed, Agergaard *et al.* (1998) found a much lower conversion rate to skatole from infused tryptophan (6 vs. 26%), in comparison with the control diet. This was followed by a proportional decrease in skatole absorption. In a recent study by Claus *et al.* (2003), resistant starch (potato starch) fed to castrated male pigs increased the production of butyrate in the colon and led to a decreased formation of skatole. The effect was suggested to be due to a butyrate dependent inhibition of apoptosis in the colon and thus a reduced availability of cell debris for microbial formation of skatole (Claus, Weiler and Herzog, 1994; Claus *et al.*, 2003). Butyric acid is the main energy source for the colonic epithelium, accounting for up to 70% of the total energy consumption for this cell type (as reviewed by Smith, Yokoyama and German, 1998). When raw potato starch was fed to entire male pigs during the last two weeks before slaughter in addition to the commercial feed, skatole levels in back fat were significantly less than in the control group, where 25% of the male pigs had skatole levels above 0.20 ppm (Zamaratskaia *et al.*, 2003). The effect of diet varied between individual pigs, as also pointed out by Jensen and Jensen (1998). This can depend on variation in the individual's ability to respond to a feed additive, leading to differences in the synthesis of skatole, but also on genetic variation in the liver metabolism (see section 7.4).

According to Jensen and Jensen (1998), the concentration of skatole can be reduced in several ways. One way is to reduce protein fermentation, either by lowering the amount of protein reaching the hindgut, or by changing the microbial metabolism by using carbohydrates, which are preferentially fermented by the microbiota. Another way to reduce the concentration of skatole is to increase the bulk of digesta in the hindgut, by the use of water holding dietary fibres such as lignin or pectin. This could also lead to an increase in the gastro-intestinal transit time, leaving less skatole for absorption in the hindgut.

Changing the composition of the microbiota in the hindgut, stimulating production of indole instead of skatole, could also be feasible. As both skatole and indole are metabolites of tryptophan, the relative proportion of these substances will change depending on e.g. diet. Liquid feed instead of dry feed (Jensen *et al.*, 1998), or a higher intake of dietary fibres (Agergaard *et al.*, 1998) will lead to a relatively higher amount of indole production. Increasing the pH-value in the intestine by addition of bicarbonate has also been shown to increase the proportion of indole and skatole. In an *in vitro* study, Jensen and Jensen (1993) showed that the proportion of indole vs. skatole was 20, 60 and 85% at pH 5.0, 6.5 and 8.0, respectively. Increasing the pH-value in the intestine by addition of bicarbonate has been shown to increase the proportion of indole. (Claus, Weiler and Herzog, 1994).

Other ways could be to block skatole production by certain chemicals or by fasting the animals for the day prior to slaughter (Kjeldsen, 1993). Use of antibiotics may also prevent skatole production, but the effect is not consistent as pointed out by Hansen (1998). However, this is not an acceptable method to prevent skatole production. The most promising method appears to be reducing the availability of cell debris (see above, Claus *et al.*, 2003). Investigations concerning the effects of diet on skatole content in pigs are summarised in Table 7-1.

**Table 7-1 Investigations of the effect of diet on skatole levels in pigs**

Source	Active substance	Effect on skatole	Organ	Notes	Authors
Beet pulp	Pectin	No effect	Fat		Øverland, Berg and Matre, 1995
Beet pulp	Fibre (Pectin)	No effect	Fat	Pietran x Seghers hybrid	Van Oeckel <i>et al.</i> , 1998
Bicarbonate	Bicarbonate	Decrease	Fat, Faeces		Claus, Weiler and Herzog, 1994
Brewers yeast	Low-digestible protein	Increase	Fat, Faeces		Jensen and Jensen, 1998
Brewers yeast + dig. carbohyd.	Digestable carbohydrates + purines	Increase			Claus and Raab, 1999
Brewers yeast + beet pulp	Low-digestible protein + pectin	Decrease	Fat	In vitro, in vivo	Jensen, Cox and Jensen, 1995
Casein	High-digestible protein	Decrease	Fat	In vitro, in vivo	Jensen, Cox and Jensen, 1995
De-Odorase	Yucca schidigera extract (sarsaponin )	Decrease	Fat	No consistent reduction, best effect around 95kg	Ender, Kuhn and Nürnberg, 1995
Inulin	Inulin	Decrease	Fat, Faeces		Claus Weiler and Herzog, 1994
Fermented liquid food		Decrease or no effect	Caecum, colon, blood, fat		Jensen <i>et al.</i> , 1998
???	Fructooligosaccharides	Decrease	Blood, Faeces	10% addition	Jensen <i>et al.</i> , 1997
Lactose	Lactose	Decrease	Fat		Hawe Walker and Moss, 1992
Lupines		Decrease	Blood, Faeces	10% addition	Jensen <i>et al.</i> , 1997
Raw potato starch	Resistant starch	Decrease	Blood, Faeces	10% addition	Jensen <i>et al.</i> , 1997
Raw potato starch	Resistant starch	Decrease	Fat, blood plasma, faeces	Only castrated male pigs, effect of buturate	Claus <i>et al.</i> , 2003
Raw potato starch	Resistant starch	Decrease	Fat, blood plasma	All skatole levels in fat < 0.20ppm, 20% suppl.	Zamaratskaia <i>et al.</i> , 2003
Wheat bran	Fibre	No effect	Fat	Pietran x Seghers hybrid	Van Oeckel <i>et al.</i> , 1998
Soybean hulls	Fibre	No effect	Fat	Pietran x Seghers hybrid	Van Oeckel <i>et al.</i> , 1998



## 7.4. GENETICS OF BOAR TAIN AND RELATIONSHIP TO POSSIBLE CONTROL

### 7.4.1. Skatole

There are indications of genetic influence on skatole levels in pigs. These include differences in fat skatole levels between breeds (Squires and Lou, 1995; Xue *et al.*, 1996; Pedersen, 1998; Hortos *et al.*, 2000; Doran *et al.*, 2002), significant heritability estimates for skatole levels in fat around 0.3 (Pedersen, 1998; Lundeheim, personal communication), as well as indications of the presence of a major gene affecting boar taint level due to skatole (Lundström *et al.*, 1994). Pigs from the Landrace and Meishan breeds usually have the highest levels of skatole in back fat and Yorkshire/Large White together with Hampshire the lowest (Pedersen, 1998; Hortos *et al.*, 2000; Doran *et al.*, 2002). In a Danish study, skatole levels in fat from Duroc pigs were in the same range as Large White and Hampshire (Pedersen, 1998). A possible explanation for the genetic background of high skatole levels could be a polymorphism in some of the enzymes involved in the liver metabolism of skatole, with a variation in gene frequencies between breeds (Lundström, personal communication).

Skatole is generally metabolised in the liver, however metabolism of skatole is low in some intact male pigs (Friis, 1995; Squires and Lundström, 1997; Babol, Squires and Lundström, 1998a,b). The enzymes CYP2E1 (Squires and Lundström, 1997), CYP2A6 (Diaz and Squires, 2000a) and aldehyde oxidase (Diaz and Squires, 2000b) are the key enzymes in the Phase I metabolism of skatole. The Phase II metabolism of skatole metabolites occurs via thermostable phenol sulfotransferase (TS-PST) (Diaz and Squires, 2003), the product of the *SULT1A1* gene. The activities of these key enzymes are negatively correlated with skatole accumulation in fat. Thus, pigs with high levels of these liver enzymes have low levels of skatole in the fat, since skatole is rapidly metabolised and cleared from the body. Pigs with low levels of these enzymes can have high levels of skatole in the fat if the amount of skatole absorbed from the gut or the environment is high. If androstenone or other testicular steroids are involved in the regulation of skatole as suggested (Babol, Squires and Lundström, 1999; Doran *et al.*, 2002), this might also imply a genetic relationship between skatole and androstenone. As the levels of both skatole and androstenone increase at puberty, genes affecting age at puberty could also lead to a genetic correlation between the substances.

The molecular cloning and functional characterization of the pig CYP2A6 and *SULT1A1* genes has been reported (Lin, Lou and Squires, 2003 and 2004). Porcine CYP2A6 cDNA was isolated from liver and a deletion was found in the coding region of CYP2A6, which resulted in a complete lack of the enzymatic activity. For *SULT1A1* a substitution was found at nucleotide 546 bp within the coding region that caused a change in amino acid sequence and a significant decrease in the catalytic activity (Lin, Lou and Squires, 2003).

Such polymorphisms may be at least partially responsible for a higher level of skatole in some pigs. However, the gene frequencies for these polymorphisms have not yet been determined. These findings are an important step toward making it possible to predict the status of skatole metabolism in the pig and towards the development of genetic markers for boar taint from skatole.

### 7.4.2. Androstenone

Androstenone is produced in pig testes predominantly from pregnenolone along with the sex steroids, androgens and estrogens. This reaction is catalyzed by andien- $\beta$  synthase enzymatic system that includes cytochrome P450c17 and cytochrome b5 (CYB5) (Meadus *et al.*, 1993).

The major reason for high levels of androstenone in fat in some entire male pigs is the increase in androstenone testicular synthesis at puberty (Claus *et al.*, 1994). However, there is a high variation in fat androstenone levels among male pigs, and at slaughter weight, only a certain percentage of entire male pigs express increased levels of androstenone in fat. Cytochrome b5 has been cloned from a number of species including pig. Levels of cytochrome b5 protein and total cytochrome b5 mRNA in testis are correlated with fat androstenone concentrations (Davis and Squires, 1999). This suggests that levels of cytochrome b5 in the testis could be used as a marker to reduce boar taint without reducing the biosynthesis of the androgens. Also, a specific 5 $\alpha$ -reductase enzyme, involved in the synthesis of androstenone, has been identified in pig testis. It may therefore be possible to reduce boar taint from androstenone by inhibition of this enzyme without affecting the anabolic effect of androgens (Cooke *et al.*, 1997).

The androstenone levels in fat, compared with those of skatole, are more clearly under genetic control with heritabilities around 0.5 (0.25 - 0.88), (Jonsson and Andresen, 1979; Willeke *et al.*, 1980; Sellier and Bonneau, 1988; Fouilloux *et al.*, 1997).

There are also large differences in androstenone levels between breeds, with entire male pigs from Meishan and Duroc breeds having a high frequency of animals having high androstenone concentrations (Hortos *et al.*, 2000; Doran *et al.*, 2002). A large variation in levels between groups of off-spring from boars used for artificial insemination was found by Keller (2000). Selection experiments for high or low levels of androstenone have been successful but was associated with delayed puberty in litter-mate gilts (Willeke *et al.*, 1987; Sellier and Bonneau, 1988). In a French selection experiment, index selection for a reduction in androstenone content without any adverse effects on reproduction - by controlling the size of the bulbo-urethral glands - was only partly successful, due to deviations of the estimated genetic parameters from the expected ones and thus the use of erroneous weights in the selection index (Sellier *et al.*, 2000).

Since androstenone levels are affected by the degree of sexual maturity at slaughter as well as the genetic potential for androstenone production, androstenone can potentially be reduced by controlling either of these factors. The genetic predisposition for early sexual maturity and the possible genetic polymorphism of the enzymes responsible for androstenone testicular synthesis or liver metabolism may be important. Studies have indicated a presence of dominant genes affecting fat androstenone levels. One dominant gene of unknown location on the genome has been described by Fouilloux *et al.*, (1997). In a recent study by Quintanilla *et al.* (2003), several quantitative trait loci (QTL) were discovered for androstenone levels in pigs, one of them on chromosome 7, close to the major histocompatibility complex of the pig (swine leucocyte antigen system, SLA). Fat androstenone levels were measured from 100 days of age every 20 days until slaughter at 180 days or 85 kg. Some of the QTLs were only significant at younger ages, indicating a strong connection with sexual maturity. The largest effects were seen for the SLA region on chromosome 7. Many other QTLs affecting growth, carcass composition, reproduction and meat quality traits have been detected on the same chromosome (Bidanel and Rotschild, 2002).

## **7.5. IMPACT OF SURGICAL METHODS AT DIFFERENT AGES AND NON-SURGICAL CASTRATION METHODS ON TAINT IN MEAT**

### **7.5.1. Taint levels in pigs castrated via local destruction of testicular tissue by chemical compounds**

Fahim (1994) reported that local destruction of testicular tissue, using zinc acetate, resulted in a 48% reduction in fat skatole, compared with intact males. Because they observed a 75% decrease in plasma testosterone, it is very likely that fat androstenone levels were also reduced substantially.

### **7.5.2. Taint levels in pigs castrated via down-regulation of the hypothalamic-pituitary-gonadal axis by exogenous hormones**

With the exception of zeranol (Denzer *et al.*, 1986), exogenous steroids or steroid agonists are effective in reducing androstenone in fat (Daxenberger *et al.*, 2001) and boar taint levels (Busch *et al.*, 1979; Lopez-Bote and Ventanas, 1988). Similarly treatment with GnRH agonists result in a significant reduction in androstenone (Xue *et al.*, 1994; Schneider *et al.*, 1998) and boar taint (Reid *et al.*, 1996). The effect is not long lasting, since treatment of young pigs does not decrease boar taint at maturity (Ziecik, Esbenshade and Britt, 1989).

### **7.5.3. Taint levels in pigs surgically castrated at a late age**

Surgical castration of entire male pigs towards the end of the fattening period results in a sharp reduction in their levels of androstenone and skatole while the beneficial anabolic effects of androgens and estrogens is expressed during most of the productive life of the animal (Texier, Desmoulin and Dumont, 1970; Newell *et al.*, 1973). Indeed it has been demonstrated that fat androstenone levels drop sharply after late surgical castration of male pigs (Claus, 1976), its half life ranging from 4 to 10 days (Bonneau *et al.*, 1982). Fat skatole levels is also very likely to decrease after castration, as shown in immunocastration studies (see section 7.5.4), as skatole in pig tissues requires the presence of intact testes and the half life of skatole in fat is short (Friis, 1993b). Therefore, castrating pigs a few weeks before slaughter is sufficient for the androstenone and skatole concentrations in fat to decrease to levels similar to those observed in castrates and gilts. However, late surgical castration is not a realistic option on commercial farms for welfare reasons, performance losses, high cost and intense labour requested. Late castration can be more easily achieved via immunocastration (see sections 4.7.3 and 7.5.4).

### **7.5.4. Taint levels in immunocastrated pigs**

Immunisation of young intact male pigs against GnRH is effective by decreasing fat androstenone and skatole levels and thereby, the incidence of boar taint (Table 4-5 and Table 4-6). Both the mean levels and the variability of androstenone and skatole levels are sharply reduced in immunocastrated pigs, compared with entire males. The levels of both compounds, as well as boar taint intensity, are similar in immuno- and surgical castrates.

One concern is the variability of the immune response of the animals, resulting in some pigs not being neutered (e.g. the results of Bonneau *et al.*, 1994 and Turkstra *et al.*, 2002). Larger scale studies show that the number of non-respondent pigs is low. However, the number of pigs tested so far, which are representative of the EU pig populations, is very low. Further to other uncertainties referred to in section 4.7, immunocastration cannot be recommended until the proportion of non-respondent pigs is determined in large scale, on-farm studies involving all the major genotypes used in the EU.

Because of the very high variability of androstenone and skatole levels, the non-respondent pigs do not necessarily exhibit high levels of boar taint-related compounds. Therefore, the proportion of non-respondent pigs that do exhibit boar taint (= percentage of non-respondents multiplied by frequency of tainted pigs in the population) is lower than the total proportion of non-respondent pigs.

As non-respondent pigs are a feature of immunocastration, the absence of boar taint in immunocastrates cannot be guaranteed, unless the effectiveness of the procedure in reducing boar taint is measured in individual animals, preferably on the slaughterline. When immunocastration is performed at an early age, this can be easily achieved, using testis size as a marker of sexual development (see section 4.7). However, when it is performed only a few weeks before slaughter, the level of boar taint should be determined.

The absence of boar taint in all pigs from herds which practice surgical castration can not be guaranteed. In every pig population, there is a proportion of cryptorchids and intersex animals (in the range of 0.1-0.6% for intersex and 0.3-0.8% for cryptorchids; Albertsen, 1951; Koch, 1963; Backstrom and Henricson, 1971; Krishnamurthy, Macpherson and King, 1971; Nador, 1990; Bellot and Vogt, 1994), which are known to exhibit high levels of boar taint. High levels of androstenone (Pailhoux *et al.*, 1995) or 16-androstens (Booth and Polge, 1976) have been observed in such animals. It may be expected that a significant proportion of them also exhibit high skatole levels, however, to our knowledge, this has not been investigated. It may be hypothesised that they would respond to anti-GnRH immunisation in the same way as normal entire male pigs.

Due to the absence of a satisfactory method for the assessment of boar taint on the slaughter line, it is presently not possible to guarantee the absence of boar taint in all individual immunocastrated pigs. However, the absence of boar taint cannot be guaranteed either when all males in a herd are surgically castrated (see above). Therefore, a realistic and acceptable goal of immunocastration could be to aim at a level of boar taint similar to that observed when all male pigs in a herd are surgically castrated. To achieve that, the proportion of immunocastrates that exhibit boar taint should be similar to the proportion of cryptorchids and intersex pigs which exhibit boar taint. However, the frequency of such animals in EU pig populations is not known, nor is the the proportion of them which exhibit boar taint.

**Table 7-2 Effects of immunocastration on taint levels in male pigs: small scale studies (Results are given as mean within treatment group; standard deviation is given between brackets)**

Immunogen	Adjuvant	Number of injections	Number of animals per treatment	Androstenone in fat (ppm)			Skatole in fat (ppm)			Boar taint or off-odour (\$)			Reference
				Entire males	Immun. males	Surgical castrates	Entire males	Immun. males	Surgical castrates	Entire males	Immun. males	Surgical castrates	
GnRH	FCA	5	9	1.95 (1.35)	0.25 (0.03)	-	-	-	-	-	-	-	Caraty and Bonneau, 1986
GnRH	FCA-FIA	3	5	-	-	-	-	-	-	1.90 (0.2)	0.67 (0.1)	1.00 (0.1)	Falvo <i>et al.</i> , 1986
GnRH	PEP	3	5	-	-	-	-	-	-	1.90 (0.2)	1.10 (0.2)	1.00 (0.1)	Falvo <i>et al.</i> , 1986
GnRH	FCA-FIA	2	5	1.32 (0.57)	0.34 (0.48)	-	-	-	-	-	-	-	Meloen <i>et al.</i> , 1994
GnRHT	FCA-FIA	2	5	1.32 (0.57)	0.10 (0.00)	-	-	-	-	-	-	-	Meloen <i>et al.</i> , 1994
GnRHT	?	2	11	1.40 (0.69)	0.24 (0.17)	0.20 (0.15)	(\$\$)	(\$\$)	(\$\$)	-	-	-	Manns and Robbins, 1997
GnRHT	Specol	2	5	2.26 (0.87)	0.05 (0.01)	-	-	-	-	-	-	-	Zeng <i>et al.</i> , 2001
GnRH	?	2	11	-	-	-	-	-	-	(\$\$\$)	(\$\$\$)	-	Liu <i>et al.</i> , 2001
Improvac (anti-GnRH)		3	10	1.31 (0.88)	0.09 (0.01)	-	0.11	0.01	-	-	-	-	Metz <i>et al.</i> , 2002
GnRHT	Specol	2	8	2.80 (1.36)	ND	-	-	-	-	-	-	-	Zeng <i>et al.</i> , 2002 a
Improvac (anti-GnRH)		2	6	2.47	0.10	-	0.17	0.05	-	-	-	-	McCauley <i>et al.</i> , 2003

GnRH = gonadotrophin-releasing hormone; GnRHT = GnRH tandem; Improvac = Brand name for the CSL vaccine; FCA = Freund's complete adjuvant; FCA-FIA = Freund's complete adjuvant for the primary immunisation, Freund's incomplete adjuvant for boosters; PEP = muramyl dipeptide.

ND = non detectable; - = not determined.

(\$\$) Boar taint or off-odour measured by a sensory panel.

(\$\$) Skatole levels were similar in immuno- and surgical castrates and significantly lower than in entire males.

(\$\$\$) Off odour was significantly lower in immunocastrates than in entire males.

**Table 7-3 Effects of immunocastration on taint levels in male pigs: larger scale studies (Results are given as mean within treatment group; standard deviation is given between brackets)**

Immunogen	Adjuvant	Number of injections	Number of animals per treatment	Androstenone in fat (ppm)			Skatole in fat (ppm)			Boar taint or off-odour (\$)			Reference
				Entire males	Immun. males	Surgical castrates	Entire males	Immun. males	Surgical castrates	Entire males	Immun. males	Surgical castrates	
Late castration studies													
GnRH	Oil-SAP	2	20	0.66 (0.31)	0.21 (0.05)	-	-	-	-	-	-	-	Bonneau <i>et al.</i> , 1994
Improvac (anti-GnRH)		2	100	1.13 (0.77)	0.14 (0.13)	0.10 (0.03)	0.11 (0.09)	0.06 (0.03)	0.05 (0.02)	-	-	-	Dunshea <i>et al.</i> , 2001
Improvac (anti-GnRH)		2	270	-	0.03 (0.01)	0.01 (0.00)	-	-	-	-	-	-	Thun <i>et al.</i> , 2003
Early castration studies													
GnRHT	FCA-FIA	2	16	0.48	ND	-	-	-	-	-	-	-	Turkstra <i>et al.</i> , 2002
GnRHT	Specol	2	20	0.45	ND	-	-	-	-	-	-	-	Zeng <i>et al.</i> , 2002b

GnRH = gonadotrophin-releasing hormone; Improvac = Brand name for the CSL vaccine; GnRHT = GnRH tandem; Oil-SAP = mineral oil for the primary immunisation, saponin in aqueous solution for the booster; FCA-FIA = Freund's complete adjuvant for the primary immunisation, Freund's incomplete adjuvant for boosters.

ND = undetectable; - = not determined.

## 7.6. SEXING OF SPERM AND INSEMINATION METHODS

Sperm sexing techniques, which are continuously being refined, have the potential to resolve some welfare and ethical problems in animal husbandry. For example, there are attempts to exclusively produce female laying hens that are usually only produced for breeding purposes in order to avoid mass killing of males. Sexing boar sperm would offer the opportunity to produce only female pigs that would not need to undergo castration.

Research on sperm sexing from boar semen was initiated in the early 1990s. The first report on live piglets as a result of surgical insemination of a sow with sexed sperm was published by Johnson in 1991. Sexed sperm is commercially available for cattle but not for pigs, as huge number of sperm are needed for insemination of sows with the intracervical insemination technique (2.5 billion sperm per dose). The high number of sperm needed for a satisfactory result is partly a consequence of the anatomy of the reproduction organs of the sow (i.e. having long uterine horns), and partly because of large losses of living sperm during storage and transport as well as after insemination. Because of this, new insemination techniques for sows are needed as well as the development of more efficient methods for sexing sperm.

The first results of separation of X (female)- and Y (male)-bearing pig-sperm was published in the late eighties (Johnson *et al.*, 1987, Johnson and Clark, 1998). Different methods have been tested, but flow cytometry is the only commercially available method at the moment. The method is based on differences in size between the X- and Y-chromosomes. Because of different amount of DNA, the X- and Y-bearing sperms are given different electric loading, which is used to separate them. Only four labs in the world (USA, Australia, Germany and Italy) are equipped with a sperm separator that presently reaches an accuracy of about 95%. The licence for the procedure was initially filed by USDA and is now controlled worldwide by one US company. Currently, the technique only allows the sorting 10 to 15 million sperm per hour. As a consequence, one sperm separator would take up to 5 hours to produce only one dose of sperm, as a 5 ml portion with 50 million sperm is currently considered as the minimal amount needed for successful insemination with a specific catheter that places the sperm at the base of uterus (Rath, 2002). The production time would be several days when using ordinary insemination techniques. In order to become a practical tool for daily use, this technology would need to be improved for higher efficiency, mainly by increasing the speed of separation and reduction of cost.

Sorting of spermatozoa by immunological methods has been tried. Immunological methods would probably be more suitable for production of large amounts of sexed sperm than spermatozoa deriving from flow cytometry. However, more than 1000 surface proteins (antigens) from sexed boar sperm have been identified, without success in using these for sorting sperm (Hendriksen *et al.*, 1996, Hendriksen, 1999).

The ordinary technique for insemination of sows places the semen in the cervix. New insemination techniques place semen closer to the ovaries, either in the uterus or in the uterine horns. A successful production of a normal sized litter of pigs through AI, using a highly reduced number of spermatozoa which was sorted by flow cytometry and the semen deposited deeply into the uterine horn, was recently reported by Rath, Ruiz and Sieg, 2003. Modified catheters have been developed, also including fibre-optic methods. However, intra-uterine insemination of sows with inappropriate (non-flexible) catheters often causes cervical bleeding, and will consequently raise the question of whether this method is acceptable from an animal welfare point of view (because of severe discomfort to the sow and bleeding. Intra-uterine insemination by the methods presently used is prohibited in Denmark since 2001 by a statement from the Veterinary Health Council; Giersing, personal communication).

Cryopreservation has been successfully applied to sexed sperm resulting in the production of piglets; however, freezing and processing protocols in combination with sex-sorted sperm are not yet optimized for routine use (Rath, 2003, personal communication). Alternative methods for the transfer of sorted spermatozoa are intracytoplasmic sperm injection (ICSI, Probst and Rath, 2003) and *in vitro* fertilization (IVF; Rath *et al.*, 1997) in combination with embryo transfer. These methods are still at an experimental stage.

## **8. CONTROL OF TAINT: POST-MORTEM**

### **8.1. PUBLIC HEALTH ASPECTS OF THE BOAR TAINT COMPONENTS: SKATOLE AND ANDROSTENONE**

There are very few data on the toxicological effects of the boar taint compounds, skatole and androstenone. According to Sigma (2002), the toxicological properties of androstenone have not been investigated. Like other steroids it could have carcinogenic effects in high concentrations, much greater than would be possible by eating pork. The minimum lethal dose quoted for skatole when given by subcutaneous injection to frogs was 1g/kg body weight (Spector, 1956) which is again much higher than could be achieved by pork consumption.

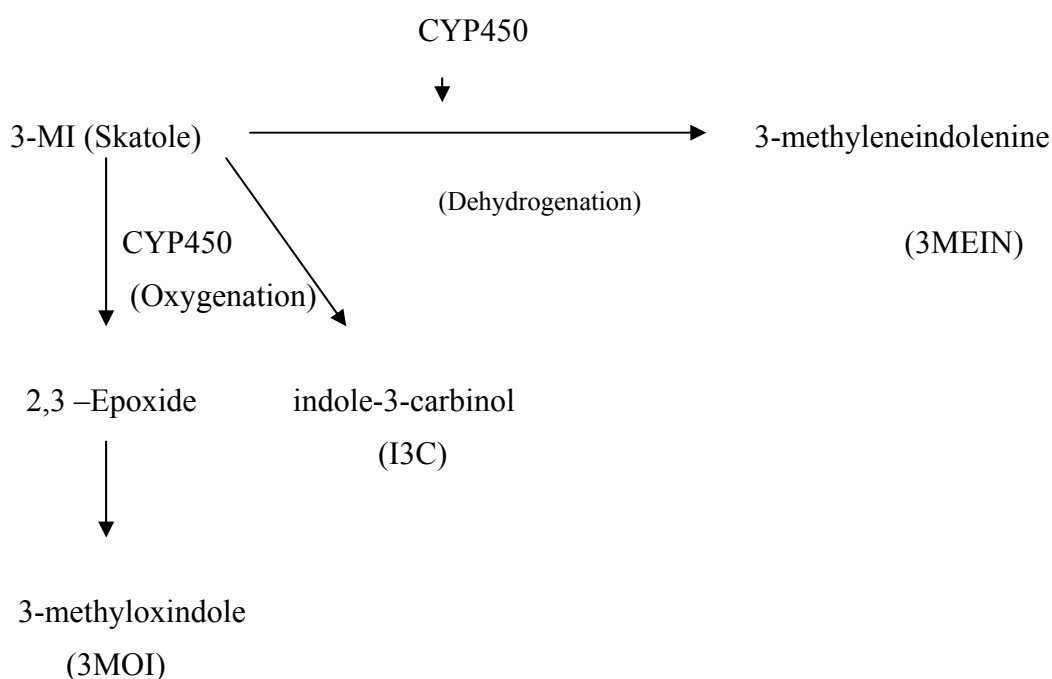
Many studies have speculated whether the emissions from intensive pig production facilities, which include skatole, could have harmful effects in man. Most attention has been given to the major pollutants, ammonia and hydrogen sulphide, and the conclusion is that concentrations of these are much lower than toxicity levels (O'Neill and Phillips, 1992).

Most of the research carried out has been done on skatole as a pneumotoxin and its primary effects on lung tissue in animals. The physiological response to skatole has varying effects according to the species, ranging from fatal pneumonia in cattle to transient mucosal injury in mice, some examples of which will be discussed later. The toxic effects of skatole are caused by the compound 3-methyleneindolenine (3MEIN), a product of skatole metabolism, whose formation is shown in Figure 8-1.

3MEIN is electrophilic and therefore unstable, forming stable adducts with cellular macromolecules such as glutathione and proteins (Lanza and Yost, 2001). A study by Regal *et al.* (2001), indicates that it may be a potential mutagenic and/or carcinogenic compound. This metabolite is considered responsible for the toxic effects of skatole in tissues, in particular pulmonary tissue and also in liver. It induces early cell death in human bronchial epithelium (Nichols *et al.*, 2003). Skatole is only toxic when bioactivated by certain isoforms of Cytochrome P450, for example CYP2F1 and CYP2F3 according to Lanza and Yost, (2001) and CYP4B2 in a study reported by Carr *et al.* (2003).

Animal studies show that the toxic effects of skatole vary according to species. It appears to have its highest toxicity in goats and cattle, followed by rodents, with rabbits being relatively resistant.





**Figure 8-1 Scheme depicting the formation of dehydrogenated and oxygenated products of skatole by Cytochrome P450 enzymes (Lanza and Yost, 2001)**

Acute bovine pulmonary edema (ABPE) is a condition in cattle, often fatal if not quickly identified and treated, that results from sudden high physiological levels of skatole. This can occur when the animals (2-3 years of age) are suddenly fed lush green grass/forages after a long period of dry feeding (sometimes known as ‘fog fever’). The disease is caused by 3MEIN as shown in the diagram above, skatole itself being derived from high levels of protein in the fresh pasture.

Bronchiolar epithelial injury in mice is transient, but olfactory mucosal injury with scarring and metaplasia persists. In contrast, Miller *et al.* (2003) found that 3 days of 100mg skatole/kg in horses resulted in persistent damage (necrosis, scarring and metaplasia) to the bronchiolar epithelium but only transient injury occurred in olfactory mucosa.

Variations in response to skatole by different species have also been shown by Nocerini *et al.* (1985). This study found that the highest rates of 3-MEIN-adduct formation were in pulmonary microsomes from goat, followed in decreasing order by horse, monkey, mouse and rat. In contrast, 3MEIN-adduct formation was greatest in hepatic microsomes from the rat, followed by mouse, monkey, goat and horse.

Variations in sensitivity to skatole were shown by Smith *et al.* (1993) who demonstrated the difference in dosage required in order to induce lung lesions in goats given 15mg/kg (i.v.) and in mice given 400mg/kg (i.p.), possibly due to differences in enzyme expression.

There have been very few studies in humans with which to compare these data. It is known that skatole is a component of cigarette smoke (Hoffman and Hecht, 1989) but whether this is a factor in lung disease is unknown. An *in vitro* study by Nicholls *et al.* (2003) in cell lines of human bronchial epithelial cells showed that apoptosis could be induced at a concentration of 10µM skatole due to the formation of 3-MEIN. Skatole was bioactivated by CYP2FI.

For people consuming very high levels of heavily tainted meat the intake of skatole could be around 15µg per day. This is the level found in a single cigarette (Hoffmann and Hecht, 1989) and it assumes that skatole is efficiently metabolised following passage through the stomach. There are no data on concentrations of skatole in lung tissue or other tissues in relation to skatole ingestion.

## 8.2. ONLINE DETECTION OF BOAR TAIN IN PORK CARCASSES

On-line detection of carcasses with unacceptable levels of boar taint compounds, which may make them unsuitable for production of high quality products, would be one solution to the boar taint problem. A large EU study found that the proportion of carcasses with androstenone and skatole concentrations above the commonly-used thresholds (1.0 ppm androstenone and 0.25 ppm skatole) were 30% and 11% respectively (Walstra *et al.*, 1999). This was the average for 6 countries although there was wide variation, presumably caused by breed, carcass weight and production system differences between countries as well as differences in analytical techniques.

Under current EU legislation (Directive 64/433 as amended), pork with pronounced boar taint is considered unfit for human consumption. However, male carcasses over 80 kg may be passed fit for human consumption provided they bear a special mark and undergo treatment i.e. processing before entering the food chain. Member States may establish their acceptability criteria and recognize a test method to ensure that carcasses with pronounced boar taint will be detected. At present, in the EU, there is no harmonized method for detecting boar taint, but some Member States have established an appropriate test system, for example, in the UK the “boiling test” is used for this purpose.

An alternative to the boiling test is a soldering iron applied to the exposed backfat of the carcass. This also causes volatilisation of androstenone and skatole which can be detected by an operator (Jarmoluk, Martin and Fredeen, 1970). This has proved successful in some situations but effective detection differs between operators and fatigue of the sensory response develops quickly.

The most successful on-line method used so far is the spectrophotometric method for skatole used in Danish slaughter plants (Mortensen and Sorensen, 1984). A backfat sample is physically removed from the carcass, analysed in a laboratory and the results used for controls later down the production line. The limitation is that androstenone is not measured and no more than 180 samples per hour can be tested.

More recently, the advent of chemical sensors and sensor systems (electronic noses) has opened up the possibility of their use in the meat industry. Chemical sensor array technology when combined with multi-variate data-processing systems, has been shown to have the potential for rapid non-destructive analysis of food quality. The detection methods used can be based on heat generation, conductivity, electrical polarisation, electrochemical activity, ionisation, optical, dielectric, and magnetic properties. A recent review of these systems by Haugen (2003) and a book entitled ‘Electronic noses and sensor array based systems’, with relevance to the food industry, by Hurst (1999) are useful information sources.

Gas sensor devices work in a variety of different ways and can be based on physical or chemical adsorption and desorption, optical adsorption, or chemical reactions of a compound in the gas phase that take place on the surface or in the gas phase. These interactions cause characteristic physical changes in the sensor that can be detected in a variety of ways (see above). Gas sensor devices have been used on pork fat by Santos *et al.* (2004), Van Dijk (1995), Bourrounet, Talou and Gaset (1995) and Berdagüe and Talou (1993).

Immunosensors work by coupling antibody-antigen reactions to an electronic signal generated by a transducer. In the Surface Plasmon Resonance immunosensor (SPR), this reaction causes a change in the refractive index at the metal-liquid interface, which is detected by a change in the intensity of a reflected laser beam.

Other chemical electronic sensors are composed of organic semiconductors whose characteristics change when a particular substance is absorbed onto the surface. This type of sensor / e-nose was used on truffles (Persaud, 1990), swine manure odour (Persaud *et al.*, 1996), and pork backfat (Annor-Frempong *et al.*, 1998). In the study of Annor-Frempong *et al.* (1998), an e-nose with a 12-conducting polymer array was used to discriminate between different intensities of boar taint caused by different concentrations of both boar taint compounds in a model system. The e-nose responses were compared with those given by a trained taste panel. The correlation between the e-nose and the taste panel was high (correlation 0.78). In subsequent work, the e-nose was able to identify 'abnormal' samples in relation to odour but wrongly identified some normal samples as abnormal. Nevertheless, these preliminary results were promising.

There are a number of projects currently being undertaken to develop sensors for odours and taste which may have implications for the determination of boar taint. In 1995 Alpha MOS, based in Toulouse, France, received financial aid from EC for 2 years (EC CRAFT project) to develop its e-nose. This instrument has a combination of metal oxide-coated sensors and quartz microbalance sensors (varies from 6 to 24), which respond using changes in conductivity and oscillation frequency respectively. The project targeted online odour assessment of meat as well as the processes of roasting coffee and manufacturing oak barrels for the wine industry.

In 2001, Van Dijk applied for a patent on his work on odour analysis of heated pork fat using ion mobility spectrophotometry, and its possible use on the slaughter line (Van Dijk, 2001).

More recently, Di Natale *et al.* (2003) have developed thickness shear mode resonators for the detection of androstenone in pork fat.

In 2003, Dr. Edward Elson of Opto-gene Inc. Maryland, USA, was awarded an SBIR Phase1 grant for his proposal "A novel biosensor for electrical-optical detection of boar taint". This work involves the use of an optical fiber sensor as described by Pilevar, Davis and Portugal (1998).

These various developments show that the development of e-noses for on-line assessment of food quality has great potential. However, progress towards developing an e-nose specifically for boar taint has been limited.

The DNA-based tests discussed in chapter 7 are not suitable for on-line application at present but lend themselves to detection of potentially-tainted or already-tainted live animals on the farm or destined for slaughter.

### **8.3. EFFECTS OF MEAT PROCESSING ON THE PERCEPTION OF BOAR TAIN**

Since research on boar taint in pigmeat began, it has been speculated that the negative impact in processed products would be less than in fresh meat because processing in various ways may mask the effects of skatole and androstenone. In a review, Malmfors and Lundstrom (1983) concluded that cooking of meat could reduce the concentrations of both boar taint volatile compounds so that taste panel responses to 'control' and tainted meat became similar.

It was thought that boar meat could also be used for meats eaten cold, and where spices, onion and garlic were used in the preparation.

A large European-wide study showed that consumers in different countries had different attitudes to the taste of boar meat (Bonneau *et al.*, 2000b). British consumers were not critical of pork from boars whereas consumers in France, Germany, Spain and Sweden were critical. Consumers in Denmark and Holland objected to the odour but not the flavour of fresh pork from boars. In a companion paper, Matthews *et al.* (2000) speculated that in countries where a high proportion of pigmeat is eaten in the form of products, the reaction to boar taint compounds is reduced because processing reduces boar taint compounds.

An example of the masking effect of processing on boar taint is provided by a study of polyphosphates.

The use of polyphosphates or solutions with low or high pH to increase the water-holding capacity of pork affects its flavour. In one study, Sheard *et al.* (1999) showed that the abnormal flavour score given by the taste panel increased when a 5% polyphosphate solution was injected. However, this increase was greater for gilts than boars and at the 5% level, the abnormal flavour and also the pork flavour score were similar in the 2 sexes. At the 0% level, boars had higher abnormal flavour and lower pork flavour as expected. Thus there was evidence that polyphosphate masked boar taint.

There is evidence that cooking conditions affect the perception of boar taint. For example taste panel scores for pork flavour in loin steaks increased and abnormal flavour declined as final internal temperature increased from 65 to 80°C (Wood *et al.*, 1995). Thus, boar taint compounds could be masked by other chemical reactions occurring during cooking.

Despite these reports, which suggest masking of taints by processing, others show that boar taint is a problem in all products. Production of bacon by injecting or immersing in salt solutions seems to have only a minor effect on the concentration of taint compounds, and is likely to increase them as the water content of the fat tissue declines (Mottram, Wood and Patterson, 1982). In that study, the correlation between fresh and cured fat androstenone concentration was 0.95. In 2 studies conducted by Meat and Livestock Commission (MLC, 1989 and MLC, 1992), scores given by taste panellists to fresh pork and cured bacon from the same pigs were very similar. In the 1992 MLC study (MLC, 1992) the difference in abnormal odour and flavour scores for boars, and for gilts and castrates combined, was actually higher in bacon (Table 8-1). Nevertheless, a common meat industry view in Britain, where castration of male pigs ceased in the 1980s, is that boar taint is not a problem in bacon.

A series of studies in Germany in the 1990s, summarised by Honikel (1993) concluded that boar taint was a major issue for the German pig industry and would have deleterious effects in both fresh meat and meat products. 95% of raw or cooked products produced from fat containing detectable amounts of skatole or androstenone were identified and scored lower by taste panellists.

**Table 8-1 Taste panel scores for abnormal flavour and odour in pork and bacon given as the difference between boars, and castrates and gilts combined**

	Pork	Bacon	Score for boars (approx)	
			Pork	Bacon
Abnormal flavour lean	+0.09	+0.12	2.2	2.5
Abnormal flavour fat	+0.07	+0.21	2.2	2.5
Abnormal odour fat	+0.11	+0.25	2.5	3.0

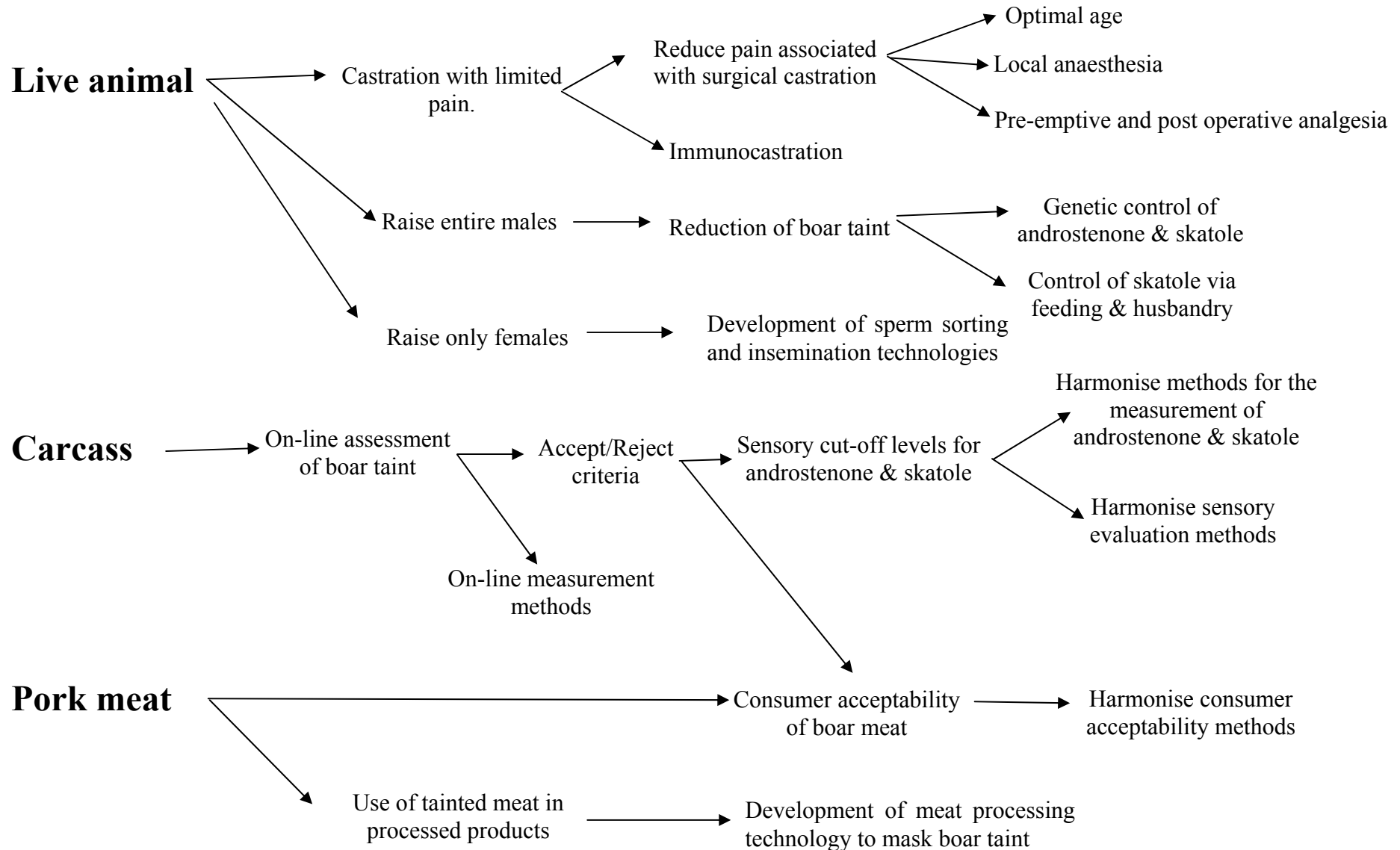
In studies on Spanish dry cured ham (Arnau *et al.*, 1986, Banon, Gill and Garrido, 2003) it has been concluded that boar taint is not masked by the curing and drying process and the product produced from boars is less acceptable than that from castrates.

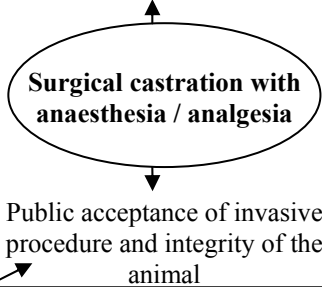
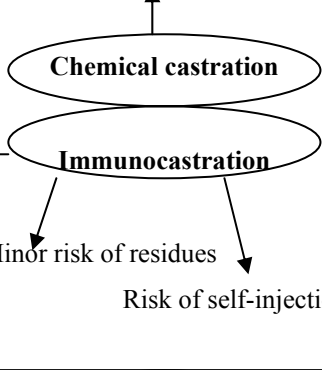
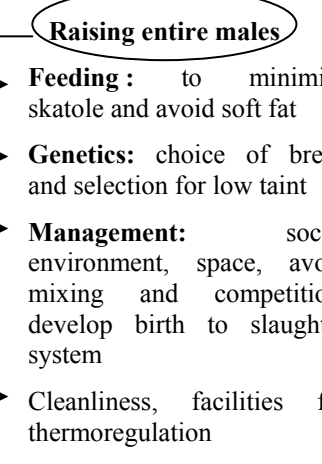
In a study of the taste panel responses to meat containing different concentrations of boar taint compounds, McCauley *et al.* (1997), showed that boar taint was detected equally in oven roasted pork, marinated pork, and bacon. There was no evidence that these treatments masked taint. On the other hand, ham and salami made from tainted and non-tainted pork elicited similar responses from the taste panel. These results confirm that products eaten cold are less likely to be adversely affected by the presence of tainted meat.

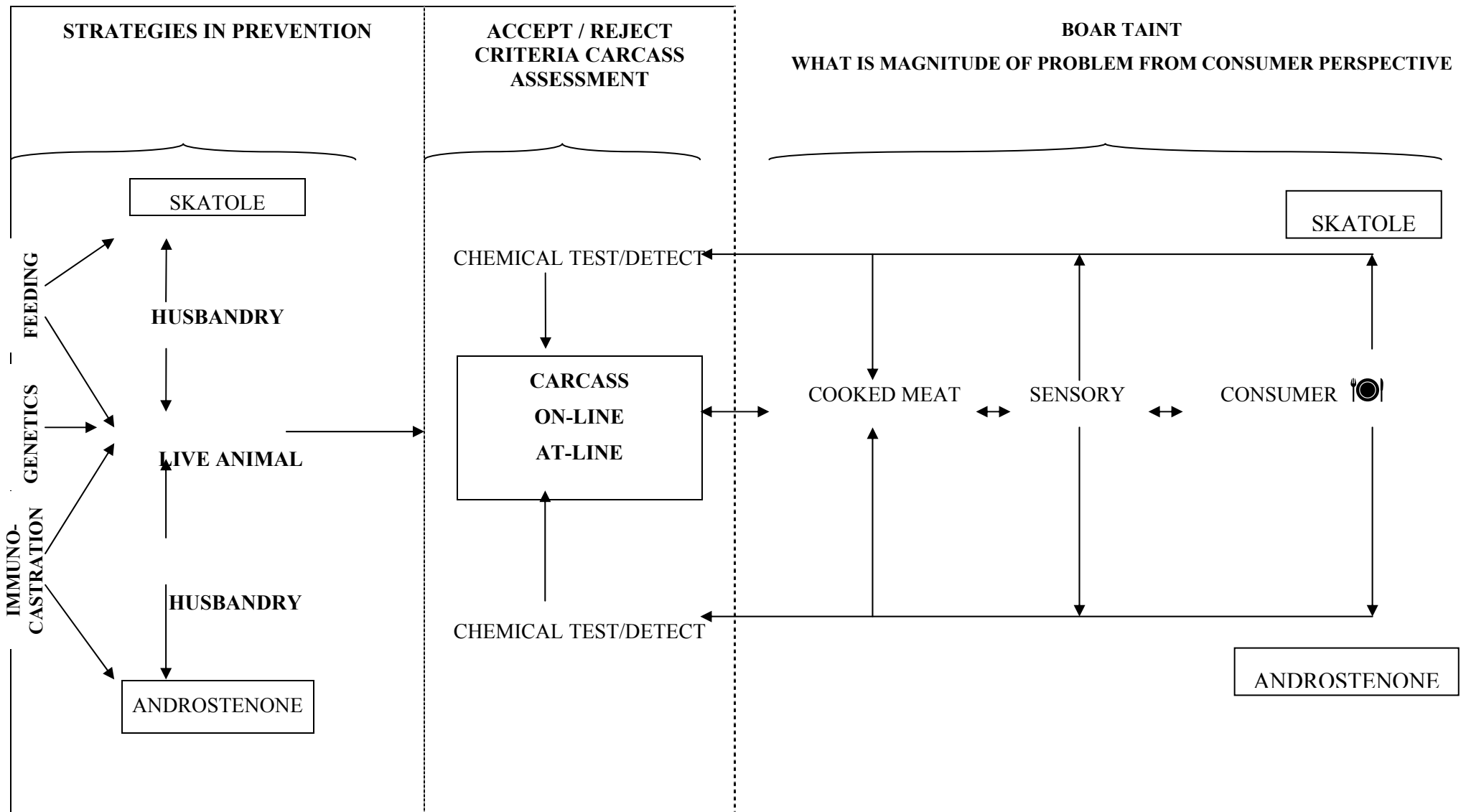
Finally, it should be noted that in the early review by Malmfors and Lundstrom (1983) that concluded that processing could mask boar taint, some papers had contradictory conclusions. Thus in smoked sausages consumed cold, strongly tainted boar meat could only contribute 25% of the total sausage content without adverse reactions (Walstra, 1974)

It seems reasonable to conclude from these studies that incorporation of small amounts of tainted boar meat into products along with meat from castrates and gilts will go undetected by consumers in many countries. However, in some countries where no boars have been used, and especially for the production of specialist products, the use of boar meat may cause dissatisfaction. Marinades, spices and treatments such as polyphosphates may mask boar taint but not conclusively.

# STRATEGIES TO PREVENT THE DEVELOPMENT OF BOAR TAIN



WELFARE	BOAR TAIN	PROCEDURES, CONDITIONS, ACCEPTIBILITY AND RISKS	PRODUCTION, MEAT QUALITY	COST
<p>General anaesthesia : risk for animals, discomfort at induction and awakening</p> <p>Local anaesthesia: transient pain at injection</p> <p>Handling stress</p>	<p>Effective against boar taint</p>	<p>General anaesthesia: no licensed anaesthetic, risk for people doing anaesthesia</p> 	<p>No production advantages of entire males (growth, leanness, efficiency)</p> <p>Easier management than for entire</p>	<p>Price of anaesthetics and analgesics</p> <p>Anaesthesia: cost of veterinary assistance</p>
<p>Chemical castration : pain and discomfort ?</p> <p>Immunocastration: minor pain at injections, discomfort?, hypothalamic lesions?</p> <p>Handling stress</p>	<p>Chemical castration : effectiveness to be ascertained</p> <p>Immunocastration: largely effective against boar taint, some animals do not respond</p>		<p>Chemical castration: presumably advantages and drawbacks of castrates</p> <p>Early immunocastration: advantages and drawbacks of castrates</p> <p>Late immunocastration: production's advantages of entire males, management as for castrates?</p>	<p>Price of vaccine</p> <p>Workload of double vaccination</p> <p>Cost of screening /analysis of carcasses</p>
<p>No invasive procedures</p> <p>More fighting and sexual harassment</p>	<p>Unacceptable high frequency of tainted carcasses which must be reduced by various means</p>	 <ul style="list-style-type: none"> <li>→ <b>Feeding :</b> to minimise skatole and avoid soft fat</li> <li>→ <b>Genetics:</b> choice of breed and selection for low taint</li> <li>→ <b>Management:</b> social environment, space, avoid mixing and competition, develop birth to slaughter system</li> <li>→ Cleanliness, facilities for thermoregulation</li> </ul>	<p>Poduction's advantages of entire males</p> <p>More complex management than for castrates</p> <p>Risk of carcass damage, inferior meat quality and soft fat</p> <p>Risk of taint</p> <p>Necessity to develop new products for use of tainted meat</p>	<p>Cost of analyses for skatole and androstenone on slaughter line</p> <p>Reduced value of tainted carcasses</p> <p>Cost of marking system if consumers are to have choice between meat from different types of carcasses</p>





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