SUMMARY

The present report, requested by the Danish Ministry of Food, Agriculture and Fisheries, summarizes the current knowledge on alternatives to surgical castration based on the scientific literature supplemented with results from Danish field trials. Besides animal science the review deals with production economy, consumer attitudes and the time horizon upon implementation of alternatives. Furthermore, the relevance of the alternatives in Danish pig production is evaluated on the basis of cost-benefit analyses of alternatives that are implementable within a time scale of 2 to 5 years. The report was prepared in an interdisciplinary collaboration between researchers in meat quality, nutrition, reproduction, ethology, immunology, management and decision support and genetics at Aarhus University.

ALTERNATIVES TO SURGICAL CASTRATION IN DANISH PIG PRODUCTION - A POSITION REVIEW

B.B. JENSEN, A.B. KUDAHL, R. THOMSEN, M.K. RASMUSSEN, A.G. KONGSTED, V.R. GREGERSEN, H. CALLESEN, C. BENDIXEN, B. EKSTRAND, K.H. JENSEN

DCA REPORT NO. 042 • MAY 2014



AARHUS UNIVERSITY DCA - DANISH CENTRE FOR FOOD AND AGRICULTURE



ALTERNATIVES TO SURGICAL CASTRATION IN DANISH PIG PRODUCTION - A POSITION REVIEW

B.B. JENSEN, A.B. KUDAHL, R. THOMSEN, M.K. RASMUSSEN, A.G. KONGSTED, V.R. GREGERSEN, H. CALLESEN, C. BENDIXEN, B. EKSTRAND, K.H. JENSEN

DCA REPORT NO. 042 · MAY 2014



B.B. Jensen, A.B. Kudahl, R. Thomsen, H. Callesen, K.H. Jensen¹
M.K. Rasmussen, B. Ekstrand²
A.G. Kongsted³
V.R. Gregersen, C. Bendixen⁴

Aarhus University

Department of Animal Science¹ Department of Food Science² Department of Agroecology³ Department of Molecular Biology and Genetics⁴

Blichers Allé 20 PO Box 50 DK-8830 Tjele

ALTERNATIVES TO SURGICAL CASTRATION IN DANISH PIG PRODUCTION - A POSITION REVIEW

Series:	DCA report
No.:	042
Authors:	B.B. Jensen, A.B. Kudahl, R. Thomsen, M.K. Rasmussen, A.G. Kong- sted, V.R. Gregersen, H. Callesen, C. Bendixen, B. Ekstrand and K.H. Jensen
Publisher:	DCA - Danish Centre for Food and Agriculture, Blichers Allé 20, PO box 50, DK-8830 Tjele. Tlf. 8715 1248, e-mail: dca@au.dk Web: www.dca.au.dk
Commissioned	
by:	Ministry of Food, Agriculture and Fisheries
Photo:	Rikke Thomsen
Print:	www.digisource.dk
Year of issue:	2014
	Copying permitted with proper citing of source
ISBN:	978-87-93176-11-9
ISSN:	2245-1684

Reports can be freely downloaded from www.dca.au.dk

Scientific report

The reports contain mainly the final reportings of research projects, scientific reviews, knowledge syntheses, commissioned work for authorities, technical assessments, guidelines, etc.

Contents

Preface	6
Summary	8
Chapter 1 - Introduction	17
Chapter 2 - An introduction to boar taint	17
2.1 Perception of tainted meat	18
2. 2 Skatole	19
2.2.1. Intestinal production of skatole	20
2.2.2 Metabolism of skatole	21
2.2.3 Skatole in plasma and fat accumulation	24
2.3 Androstenone	24
2.3.1 Biosynthesis of androstenone	24
2.3.2 Salivary glands and pheromone action	25
2.3.3 Metabolism of androstenone	26
2.3.4 Androstenone in plasma and fat accumulation	27
2.4 Summary	27
Chapter 3 – Alternatives to surgical castration	29
3.1 Production of entire males	29
3.1.1 Common practice in entire male pig production	30
3.1.2 Potential tools to reduce boar taint	30
3.1.2.1 Effect of management and housing on boar taint	30
3.1.2.1.1 Effect of slaughter weight on boar taint	
3.1.2.1.2 Effect of social environment on boar taint	32
3.1.2.1.3 Effect of hygiene on boar taint	34
3.1.2.2 Effect of feeding on boar taint	35
3.1.2.2.1 Feeding techniques	35
3.1.2.2.2 Diet composition	36
3.1.2.2.3 Feed additives	45
3.1.2.2.4 Effective feeding strategies	49
3.1.2.3 Breeds	49
3.1.3 Mode of operation at production level	51
3.1.3.1 Effect of entire male production on animal welfare	51
3.1.3.1.1 Effect of the social environment on the welfare of entire male pigs	53
3.1.3.1.2 Effect of housing on the welfare of entire male pigs	55
3.1.3.2 Performance and carcass characteristics of entire male pigs	56
3.1.3.3 Meat quality (excluding boar taint) of entire male pigs	57
3.1.3.4 Working environment in production of entire male pigs	58
3.1.5 Concluding remarks	59
3.2 Alternative methods of castration – immunocastration	60

3.2.1 Mode of action by immunocastration	61
3.2.2 Practice by immunocastration	64
3.2.3 The effect of immunocastration on boar taint	64
3.2.4 Mode of operation of immunocastration at production level	66
3.2.4.1 Effect of immunocastration on animal welfare	66
3.2.4.2 Effect of immunocastration on performance and carcass traits	68
3.2.4.3 Effect of immunocastration on meat quality and safety	70
3.2.4.4 Effect of immunocastration on working environment	72
3.2.5 Concluding remarks	72
3.3 Sperm sexing	73
3.3.1 Methods for sperm sexing	73
3.3.2 State of the development of the methods	74
3.3.3 Unsolved problems, research needs and time horizon	75
3.3.4 Concluding remarks	75
3.4 Breeding as an alternative to surgical castration	75
3.4.1 State of the art	76
3.4.1.1 Concerns in relation to the selection index	77
3.4.1.2 Conventional selection	78
3.4.1.3 Molecular genetics approaches	79
3.4.2 Concluding remarks	80
Chapter 4 - Needs and methods for assessing boar taint at the slaughter line	81
4.1 Methods for rapid detection of boar taint	82
4.1.1 Sensory tests	82
4.1.1.1 Human nose methods	82
4.1.1.2 Biosensors	83
4.1.2 Chemical tests	83
4.1.2.1 Colorimetric methods	84
4.1.2.2 Electronic nose methods (gas sensor arrays)	84
4.1.2.3 Direct mass spectrometry	84
4.1.2.4 Fast gas chromatography	84
4.1.2.5 Gas-phase spectrometry	85
4.2 Methods used to quantify boar taint compounds	85
4.2.1 Gas chromatography	85
4.2.2 Liquid chromatography	85
4.2.3 Mass spectrometry	85
4.2.4 Immunological methods	86
4.3 Concluding remarks	86
Chapter 5 - Time horizon, stakeholder attitudes and economic feasibility of alternatives to surgical castration	88
5.1 Stakeholders - attitudes and interests	89
5.1.1 Consumers	89

5.1.2 Pig producers	91
5.1.3 Pig Industry	91
5.2 Economic aspects at farm level and cost-benefit analyses of non-breeding alternatives	93
5.2.1 Introduction	93
5.2.2 Method and assumptions behind cost-benefit analysis	94
5.2.2.1 Production of entire male pigs	95
5.2.2.1.1 Production of entire male pigs with a carcass weight of 83 kg and managed like surgically castrates	95
5.2.2.1.2 Production of entire male pigs with a carcass weight of 83 kg and using skatole- reducing feeding strategies	96
5.2.2.1.3 Production of entire male pigs with a carcass weight of 75 kg and use of skatole- reducing feeding strategies	97
5.2.2.2 Immunocastrated males	98
5.2.2.2.1 IC scenario 1: standard vaccination, ad libitum feeding	98
5.2.2.2.2 IC scenario 2: standard vaccination, restricted feeding after second vaccination	98
5.2.2.2.3 IC scenario 3: Early second vaccination, restricted feeding after second vaccination.	99
5.2.2.2.4 IC scenario 4: Early second vaccination, ad libitum feeding	99
5.2.3 Results and discussion of the cost-benefit analyses	99
5.2.3.1 Profitability	101
5.2.3.1.1 Profitability of entire male production	101
5.2.3.1.2 Profitability of immunocastration	106
5.2.3.2 Economic latitude for improving animal welfare	108
5.3 Concluding remarks	109
Chapter 6 – Current relevance of alternatives to surgical castration in Danish pig production	113
References –according to the order of citation	118
References – alphabetic ordered	145
Glossary	146
Appendix	152

Preface

The European Commission and representatives of the European pig producers, pork industry, researchers, retailers, veterinarians and animal welfare organizations have joined a voluntary declaration of ceasing surgical castration of pigs by 1 January 2018.

As part of the contract between Aarhus University and the Ministry of Food, Agriculture and Fisheries on the provision of research-based public-sector services, etc., at DCA – Danish Centre for Food and Agriculture for the period 2013-2016, the Danish Ministry of Food, Agriculture and Fisheries therefore requested an updated review of current knowledge on alternatives to surgical castration based on the scientific literature supplemented with results from Danish field trials.

Besides animal science the review should deal with production economy, consumer attitudes and the time horizon upon implementation of alternatives. Furthermore, an evaluation of the relevance of the alternatives in Danish pig production was desired on the basis of cost-benefit analyses of alternatives that are implementable within a time scale of 2 to 5 years. As surgical castration with pain relief is not considered a suitable alternative to surgical castration in Denmark, this alternative to prevent boar taint has been excluded from the review.

The present report was prepared in an interdisciplinary collaboration between researchers in meat quality, nutrition, reproduction, ethology, immunology, management and decision support and genetics from the Departments of Food Science, Animal Science, Agroecology and Molecular Biology and Genetics at Aarhus University.

Senior scientist Bent Borg Jensen and Senior scientist Karin Hjelholt Jensen wrote Chapter 1.

Post.doc Martin Krøyer Rasmussen was in charge of Chapter 2 with contributions from Senior scientist Bent Borg Jensen and Senior scientist Karin Hjelholt Jensen.

PhD fellow Rikke Thomsen and Senior Scientist Anne Grete Kongsted were responsible for the sections concerning Production of entire males (Chapter 3.1) with contribution to the section about Feeding (Chapter 3.1.3.2) from Senior scientist Bent Borg Jensen, Post.doc Martin Krøyer Rasmussen, Senior scientist Bo Ekstrand and Senior scientist Karin Hjelholt Jensen, and contribution to the section concerning Breeds (Chapter 3.1.3.3) from Post.doc Vivi Raundahl Gregersen. The section on Immunocastration (Chapter 3.2) was written by Senior scientist Karin Hjelholt Jensen with contributions from Post.doc Martin Krøyer Rasmussen. Professor Henrik Callesen carried out the section on Sperm sexing (Chapter 3.3), and Postdoc Vivi Raundahl Gregersen and Professor Christian Bendixen are responsible for the section on Breeding as an alternative to surgical castration (Chapter 3.4).

Senior scientist Bent Borg Jensen summarized the state-of-the-art with respect to Needs and methods for assessing boar taint at the slaughter line (Chapter 4).

Senior advisor Anne Braad Kudahl did the cost-benefit analyses and is responsible for the writing of Chapter 5 on the basis of contributions from all authors, whereas Senior scientist Bent Borg Jensen wrote Chapter 6 on the basis of contributions from all authors.

Senior scientist Bent Borg Jensen, Professor Knud Erik Bach Knudsen, and Senior scientist Karin Hjelholt Jensen edited the review.

In addition, Senior scientist Niels Oksbjerg and senior scientist Mette S. Herskin commented on the report, Tina Albertsen and Karin Vikkelsø Østergaard proofread, and Mette Graves Madsen was in charge of setting up the report.

Finally, Anni Øyan Pedersen, Lisbeth Jørgensen, Hanne Maribo and Nicolaj Christoffersen, Danish Agriculture and Food Council, Lars Ole Blaabjerg, Danish Meat research Institute, Tove Serup and Birgit Ingvorsen, The Knowledge Centre for Agriculture, Jens Christian Eskjær Jensen, Orion Pharma Animal Health, Denmark, Gitte Hansen, GEFION, Marianne Kjær Bonde, Friland – Organic and Free range meat, Kai Grevsen, Aarhus University, Department of Food Science, Kent Nedergaard, Danish Crown and Bjarne Nygaard, Dan-Cikorie, contributed to procure material that was not readily available to the public.

The report was peer-reviewed by Professor Michel Bonneau, French National Institute for Agricultural Research, INRA, France, Dr. Marijke Aluwé, Institute for Agricultural and Fisheries Research, ILVO, Belgium and Professor Kerstin Lundström, Swedish University of Agricultural Science, SLU, Sweden.

Bent Borg Jensen and Karin Hjelholt Jensen, Aarhus University, May 2014.

Summary

Currently, surgical castration of male piglets is a common practice in the pig production in many countries to prevent boar taint. However, due to animal welfare concerns there is an increased desire to stop surgical castration at least in the European countries. The European Commission and representatives of European pig farmers, meat industry, retailers, scientists, veterinarians and animal welfare NGOs have recently committed themselves to voluntarily end surgical castration of pigs in the European Union by January 1, 2018. Therefore, realistic alternatives to surgical castration are needed.

Boar taint is an offensive odour and flavour released upon heating and eating meat from some pubertal or sexually mature male pigs and is disliked by many consumers. It is mainly caused by accumulation of skatole and androstenone in fat, but the relationship between boar taint, the perceived odour and flavour of the meat and fat levels of skatole and androstenone is not straightforward and acceptable thresholds of skatole and androstenone that takes the interdependence between skatole and androstenone into consideration are still lacking.

Skatole is a microbial degradation product originating from microbial fermentation of feed not digested in the small intestine and cell debris in the large intestine. It is absorbed into the blood and may accumulate in fat. Thus, the main determinants for the amount of skatole in fat are considered to be the amount produced by the microbiota in the large intestine and the degradation capacity of the liver, leaving factors affecting these processes as potential tools to reduce fat skatole and boar taint. Absorption through the skin into the blood has, however, also been suggested. A major factor likely to affect the intestinal produced skatole is the diet of the pigs but also stress and presence of enteric diseases may affect the production. Only skatole that fails to be metabolized in the liver accumulates in fat with the risk of causing boar taint. As testicular steroids affect the hepatic skatole metabolism fat skatole may be affected by the same factors that regulate androstenone. Furthermore, bioactive feed components may affect hepatic enzyme activity and the amount of fat skatole. Based on the high clearing rate of skatole in adipose tissue skatole reducing steps at farm level are likely to reduce boar taint within few days.

Androstenone is an endogenously produced pheromone synthesized in the testis in entire male pigs from onset of puberty that elicits the mating posture of mature female pigs. The time of sexual maturation in pigs is age-dependent, but varies between breeds. In addition, it is likely to be influenced by seasonal variation and maybe the availability of feed as well as the social and physical environment. Thus, apart from castration the steps at farm level that may influence androstenone may be age at slaughter, choice of breeds and breeding, availability of feed as well as the social and physical environment. Based on the low clearing rate of androstenone in adipose tissue androstenone reducing steps at farm level have to be implemented at least 2 to 3 weeks before slaughtering of the pigs. **Entire male pig production** is production of intact male pigs, with focus on other initiatives than castration to reduce the existence of boar taint. The efforts to reduce boar taint may include management, housing, nutrition and genetics.

At present, reasonable strategies for reducing fat skatole may include an improved hygiene of the rearing environment and consequently less fouled animals, but some inconsistency of results exists. However, different alternative skatole-reducing feeding strategies seem available. Thus, withdrawel of feed for more than 6 hours before slaughter reduces skatole and the perceived boar taint. In addition, sources of protein with low ileal digestibility, such as products based on blood, meat and bone meal, yeast slurry from breweries or yellow peas, should be minimized as they increase intestinal skatole production. Likewise, a reduction of protein intake by feeding grain only for 4 days before slaughter may reduce the skatole problem of boar taint although more studies on this are needed. Furthermore, supplementation of easily fermentable carbohydrates with low ileal digestibility reduces intestinal skatole production. The most promising sources seem to be feed components rich in inulin, such as chicory root or Jerusalem artichoke. Addition of these feed components to a diet in an amount resulting in an inulin concentration above 50g/kg feed and given in the diet for 7 days reduces the skatole content in back fat by two thirds. Chicory root also contains bioactive compounds that may increase the metabolism of skatole in the liver. Other carbohydrate sources such as raw potato starch have also been shown to reduce skatole but are unable to stand heating and pelleting of the feed.

Strategies that are likely to reduce the level of androstenone include slaughter at a lower weight but it has to be taken into account that the effectiveness of this strategy varies between breeds. Although the social and physical environment as well as stress are likely to affect time of onset of puberty and fat androstenone the literature is not consistent with respect to applicable initiatives, probably due to a complex interaction with factors like weight, age, genetics, nutrition and stress or a combination of some of these. In addition, the feed interventions investigated show only marginal or no effect on fat androstenone deposition in adipose tissue.

Several other methods of reducing fat levels of boar taint compounds have been investigated, including liquid feeding, dietary supplementation of lupines, sugar-beet pulp, slowly fermentable dietary fibres, non-starch polysaccharide degrading enzymes, antibiotics, organic acids, bioactive components in plant product, or adsorbent materials; changing in the dietary ratio of different grain types or the fat ratio and its composition; and choice of breeds. However, at present these methods seem either insufficiently elucidated or inappropriate to use at farm level.

From a welfare point of view, production of entire male pigs improves the animal welfare in early life due to the omission of the painful surgical castration procedure. However, the increased aggression and mounting behaviour compared to female and surgical castrated pigs may compromise animal welfare. Whether this in practice will lead to an impaired animal welfare is likely to depend on the social environment and the housing conditions. There are indications that aggression can be reduced by single-sex rather than mixed-sex housing and by reduction of the frequency of mixing of unfamiliar pigs combined with a general enrichment of the environment (e.g. access to rooting material, low stocking density and unlimited access to feeding space). This would reduce the risk of serious welfare problems in entire male pig production.

With respect to performance, carcass and meat quality, production of entire male pigs is characterised by improved feed conversion ratio, higher lean meat percentages and lower intramuscular fat content compared to surgical castrated pigs. The latter may ultimately compromise the tenderness and juiciness of the meat.

Immunocastration is the only non-surgical method of castration that at present is available in EU and only one commercial vaccine, Improvac[®], exists. The vaccine is injected subcutaneously in the neck twice at least 4 weeks apart with the second vaccination given within 4 to 6 weeks before slaughter. The second vaccination results in a functional castration that works through an active immunisation by vaccination against gonadotropin-releasing hormone (GnRH), thereby interrupting the hypothalamic-pituitary-gonadal -axis, which is responsible for reproductive function and production of testicular steroids involved in boar taint.

In relation to boar taint, the vaccine generally seems to abolish the problem of boar taint efficiently with respect to the content of androstenone and skatole in fat, but one concern is the occurrence of the so-called non-responders that may have too high fat levels of one or both boar taint compounds and have to be pre-sorted at farm level or sorted at the slaughter line. In research context, non-responders account about 1 %, but un-successful castration may be more frequent with use of the method in large scale where several management related sources of error may act, such as vaccination of diseased animals, some animals escaping vaccination and inadequate storage and handling of the vaccine. Furthermore, an additional small part of the meat may be perceived as tainted by a sensory panel although the thresholds for androstenone and skatole in fat are not exceeded, but data on this issue are scarce.

Overall, immunocastration eliminates the pain in relation to the surgical procedure of surgical castration, but the entire male-like behaviour of immunocastrated pigs until the second vaccination means an increased risk of aggression and mounting with the risk of causing social stress and injuries to pen mates in 60 to 75 % of the growth period if they are housed and managed in the same way as surgical castrated pigs, which may compromise animal welfare. Improvement of the social and physical environment as well as earlier vaccination will improve animal welfare.

In addition, immunocastration has the potential of improving feed conversion, increasing lean meat percentage and reducing back fat thickness compared to surgical castration if an appropriate feeding strategy is used, but it reduces the carcass yield. A short duration of the period from second vaccination to slaughtering, restrictive feeding for the last 4 weeks before slaughtering and selection of specific breeds favour performance and carcass quality. Apparently, immunocastration neither seems to compromise technological meat quality traits or sensory quality compared to surgical castration and meat quality traits other than boar taint may be rated higher than in meat from entire males. There is no risk for vaccine residues in the meat, but the vaccine works in humans as well which means that there is a risk of adverse effects on the reproductive system of the farmer if two times of self-injection should happen. It is therefore dissuaded to carry out vaccination after an accidental first self-injection and none lasting and severe symptoms after exposure to self-injection have been reported. The majority of previous studies have only included few animals, and the experience of immunocastration in practise within EU with respect to the amount of tainted meat, performance, animal welfare and working environment is restricted.

The use of **sex sorted boar semen** is not expected to be practically useful at least within the next 2 to 5 years. More research is needed in the area, and some new and major breakthroughs must be made to exploit the potentials of the methods.

Breeding provides a sustainable approach to reducing the level of boar taint. Numerous QTL have been identified in relation to both skatole and androstenone, and relevant candidate genes involved in either biosynthesis or metabolism have been identified for many of the regions. Analyses in relation to the selection index indicate small changes in breeding values, however, long term changes, e.g. morphological changes due to hormonal changes needs careful surveillance during implementation. Depending of the time frame different approaches can be made in relation to breeding strategy where conventional selection in the sire line has the longest timeframe and the candidate SNP test the shortest. We propose an additional strategy combining data from genomic selection with reported QTL information in order to evaluate the effects on additional traits to ensure an effective and sustainable implementation as described below.

Genomic selection is now being applied in many countries to achieve breeding goals faster and at a lower cost than traditional breeding. The implementation of genomic selection only partly considers complex and non-additive interactions between "major genes" (genetic variants with a relatively large effect upon the phenotype). The expected results are likely to be gains on the short term, but there are difficulties with harvesting the full genetic potential, which comes from identifying and exploiting the best combination(s) of genetic variants. For a trait like boar taint numerous haplotypes having a strong association to the trait have already been identified in the Danish pig breeds and more will be identified during integration of the boar taint trait through the genomic selection program. These haplotypes will in many cases be supported by the presence of relevant candidate genes within the chromosomal region encompassing the haplotype. A procedure, where these haplotypes are evaluated in relation to the data obtained from genomics selection, are likely to be efficient in exploring and

exploiting complex and non-additive interactions in relation to boar taint QTL. This procedure would increase the knowledge of the QTL regions affecting skatole and androstenone and provide the opportunity to restrict selection pressure to haplotypes showing little or no adverse effects on other traits.

Most alternatives to surgical castration have to be accompanied by **reliable methods for detection of boar taint** at the abattoir. There is so far no international accepted and validated on-line method available for the measurements of boar taint in carcasses that throughout fulfils the requirement for a highly streamlined industry at the slaughterhouses.

There is a need for a method that 1) measures both skatole and other boar taint compounds including androstenone, 2) is simple and does not require highly qualified personal to operate it, 3) is rapid enough to handle up to several hundred carcasses per hour, 4) is accurate and gives a high sensitivity as well as specificity of the classification and 5) has low costs.

A number of chemical methods exist for quantification of androstenone and skatole in adipose tissue. However, these methods are not applicable on the slaughter line since they involve complicated sample preparation steps and are usually labour intensive, but they permit quantification of androstenone and skatole which is essential for research purposes.

Several rapid chemical boar taint detection methods have been tested. Apart from the spectrophotometric method that has been used with success for skatole equivalent analysis in Danish slaughterhouses, none of these methods has been properly evaluated. The limitation with the Danish skatole equivalent method is that it is not up to date, androstenone is not measured and only 180 samples per hour can be tested.

Sensory test has been postulated not to be efficient due to high cost, high variability and low accuracy. In addition, there is to date still no established internationally approved definition of the sensory perception of boar taint. Of the human nose methods tested, the hot iron method used in the Netherlands seems to be the best validated method and it seems to be promising.

In spite of the variety of analytical protocols available for measurements of boar taint, a harmonized, approved method for detecting boar taint at the slaughter line in the EU is still lacking. Until a reliable on-line method that both measure skatole and androstenone is developed a reasonable approach may be to use an up to date modification of the Danish colorimetric method for measuring skatole equivalents, which has been shown to be valid as an at-line method, combined with the hot iron method used in the Netherlands, which seems to be valid as an on-line method. The combination of these two methods will address the problem with skatole measured by the colorimetric method, which seems to be the most important compound for boar taint, as well as the fact that some samples will

have an odour even though skatole level is low, either due to high concentrations of androstenone or other compounds.

Time horizon and stakeholder attitudes of the alternatives to surgical castration vary. A number of strategies have the potential of reducing boar taint and improving animal welfare. However, sperm sexing is not applicable within 2 to 5 years, and evaluation of the feasibility of molecular genetic selection approaches rely on an impact analysis by the pig industry followed by a focused effort if the initial analysis gives reasons for it. Production of entire male and immunocastrated pigs may, theoretically, be implementable within a short time, but the methods require reliable methods for online detection of boar taint. Another major barrier in implementation of these alternatives is the uncertainty on whether the consumers will accept the methods and resulting pork quality, hence continuing to purchase pork, as this is the key factor for maintaining the profitability in the whole production chain. In addition, to ensure a future production of meat an alternative to surgical castration has to be profitable for the farmer.

The risk of tainted meat in production of entire male pigs and the consumers' uncertainty of whether there are risks to food safety by immunocastration are issues that should be taken into account in the decisions on which alternatives to surgical castration that should be implemented. The consumers' knowledge about castration methods are limited and the provided information about alternative methods, especially on whether they imply risks for the consumers, is important for consumer acceptance of immunocastration, whereas acceptance of production of meat from entire male pigs demands a focused effort on avoiding tainted meat.

Based on the literature, the producers seem in general to have a less positive attitude than the consumers towards implementation of alternatives to surgical castration and mainly concern on labour implications, the risk of boar taint and the expected profit of the alternatives, but experience with the alternatives can change their attitude. The attitude of Danish producers has not been surveyed.

The Danish pig industry is positive to the ban on surgical castration and they regard production of entire males to be the best alternative to surgical castration. Therefore, one of the main issues for the slaughterhouses is the development of fast and reliable on-line detection of boar taint. In addition, development of methods for reducing the amount of tainted meat has high priority as full-scale production of entire male pigs implies an increase in the total amount of meat sorted out, which has to be used for processed products. The market for processed products will, however, easily be saturated.

The cost-benefit analyses of different scenarios at herd level of entire male pig production and immunocastration indicate that production of entire males with the current sorting methods is very profitable if using the usual carcass weight of 83 kg. In case of full-scale production, however, the amount of boar tainted meat will constitute a big problem and a reduction in the payment per kg carcass may occur. This problem will be further enlarged if sorting is also based on androstenone, which is suggested to be implemented in order to protect market shares. However, with decreasing levels of skatole, higher levels of androstenone may be acceptable. Therefore, there will be an urgent need to reduce skatole, among others by special end-feeding. By feeding pure grain the last days before slaughter, profitability is still better with production of entire male pigs at a carcass weight of 83 kg than by producing surgical castrated pigs, while it is a little lower when adding chicory to endfeed instead as long as the current sorting methods are used.

In case of new sorting methods including the level of fat androstenone are implemented, a reduction in slaughter weight may also contribute to a reduction in sorting out if combined with skatole-reducing feeding. But with the current prices and sorting methods, the profitability of slaughtering at a reduced weight is only at the same level as SC production if the farmers are paid DKK 0.1 - 0.27 more per kg carcass when combined with feeding pure grain or chicory, respectively. In case a new and more sensitive sorting procedure the meat price has to be increased by DKK 0.48 to 0.67 per kg to pay these extra costs. These increases in payment to the farmer are less than the variation in the listed meat price during the year.

Supplying the feeding of male pigs with chicory or pure grain for the last 4 to 7 days before slaughter is able to reduce the amount of skatole significantly, but with the current price-level only end-feed with pure grain is profitable, and only in case of normal slaughter weight. Feeding with pure grain will be easy to implement, but the effect is less documented than the effect of inuline-containing feeds like chicory and Jerusalem artichoke. If the profitability of using inuline-based feed is improved, e.g. through a minor increase in meat prices or reduced price of the crops, both crops can be grown and processed in Denmark with good outcomes.

At the current prices, immunocastration will in general only be profitable provided that less than 1 % of the meat is tainted, and the control for boar taint at the abattoir therefore is redundant. Only vaccination in accordance with the manufacturer's recommendation and restrictive feeding after the second vaccination, which is the most profitable scenario of immunocastration in the present analyses, will be able to pay for control for boar taint but this practise is only implementable at about half of the Danish farms.

In comparable environment, immunocastrated pigs are likely to have better welfare than entire male pigs due to the reduction of the duration of the period with increased levels of mounting and aggression as well as the reduced risk of aggression at the time of delivery. Furthermore, in the condition of restrictive feeding immunocastration without the need for control for boar taint seems to possess the economic latitude to further improve animal welfare compared with production of entire male pigs by increasing the space allowance and earlier application of the second vaccination. However, only a small increase in the meat price has to occur (DKK 0.05 per kg), which is very low compared to the usual variation in payment over a year, to pay the costs for the farmer to increase space allowance by 10 % in production of entire male pigs at a carcass weight of 83 kg if no skatole-reducing feed is added. Thus, there is considerable economic latitude for less expensive initiatives to improve animal welfare in this production. This, in combination with the fact that the economic latitude for reduction of aggression and mounting in immunocastrated pigs only is achievable in 50 % of the Danish farms with facilities for synchronous restrictive feeding, entails that animal welfare in practise may not generally be better in immunocastrated pigs than in entire male pigs.

The cost-benefit analyses did not include organic farming due to lack of several parameter estimates. However, based on previous Danish research projects, organic farms might face larger problems with boar taint than conventional production, and methods to reduce the risk of boar taint may, therefore, be even more necessary in organic production. The grain feeding method is easy to implement in organic pig production as organic wheat and barley are already available. Reduced slaughter weight is expected to have the same effects in organic as in conventional production. The organic pig production might, however, have better possibilities to market meat from smaller pigs as this consumer group is supposed to be more open to alternative products and willing to pay a little more for specialized products. Immunocastration is not regarded to be a realistic alternative within organic pig production.

Future research and activities should primary focus on acceptable thresholds of skatole, androstenone and potential other relevant compounds that take the interdependence between individual boar taint compounds into consideration as well as elaboration of an international accepted, reliable on-line method for sorting that fulfils the requirement for a highly streamlined industry at the slaughterhouses, as this is a precondition for implementation of all alternatives to surgical castration that may be relevant within 2 to 5 years.

If entire male production is intended to be implemented in traditional as well as organic pig production further research in strategies for reducing boar taint may improve the profitability considerable and should preferential include refinement of the most reliable strategies influencing boar taint (e.g. the optimal duration of grain-feeding or adding chicory before slaughter or guidelines with respect to hygiene) as well as identification of new, potential reliable strategies (e.g. availability of feed; dietary supplementation of organic acids, bioactive components or adsorbent materials; changing in the dietary ratio of different grain types or the fat ratio and its composition; optimal slaughter weight; choice of breeds and the social and physical environment).

In case immunocastration is preferred it should be taken into account that the experience in practise within EU is limited and large scale studies informing on the amount of tainted meat, performance, animal welfare and working environment is restricted.

As breeding seems to be a sustainable approach reducing the level of boar taint, an evaluation of the feasibility of molecular genetic selection approaches should preferentially be done by the pig industry followed by a focused breeding effort if the initial analysis gives reasons for it.

Furthermore, although all alternatives are based on omission of surgical castration and as such improve animal welfare in the early rearing period, the welfare consequences of the alternatives in relation to surgical castration taking the whole growing period into consideration are poorly elucidated. Presumed that decisions on alternatives to surgical castration should be based on knowledge, further studies on animal welfare and strategies for improving it have to be performed.

Finally, the effect of a change towards production of entire male pigs or immunocastrated pigs on overall resource-use and environmental impact may be relevant but was without the scope of this report. Due to the improved feed conversion ratio in entire male, and partly in immunocastrated pigs, compared to surgical castrated pigs a reduction in environmental effects is expected compared to the present pig production.

In conclusion, production of entire male pigs as well as immunocastration may be implemented within 2 to 5 years and improve animal welfare in early life compared to surgical castration but both alternatives may compromise welfare due to increased aggression and mounting behaviour in the growing period if no initiatives to improve animal welfare is taken. At present, it is not possible to evaluate whether the alternatives overall improve welfare compared to surgical castration. In addition, ranking of the possibilities of animal welfare in the respective alternatives is not possible. Both alternatives may be economical profitable at farm level depending on the specific conditions but imply new challenges for the pig industry, especially concerning the potential increased amounts of tainted meat requiring improved sorting methods and only usable for processing as well as consumers' attitudes on export markets. The national economic consequences of this have to be evaluated. Implementation of sperm sexing or selection against boar taint are promising alternatives as well but need further development or a focused decision based on analyses by the pig industry.

For all alternatives an evaluation of the environmental impact will be highly relevant. In addition, future research on entire male pig production should focus on identification of acceptable thresholds for boar taint compounds; elaboration of reliable and international accepted on-line method for sorting; refinement of the most reliable strategies influencing boar taint and identification of new, potential reliable strategies. In case immunocastration is preferred large scale studies informing on the amount of tainted meat, performance, animal welfare and working environment is desirable. Furthermore, the welfare consequences of the alternatives in relation to surgical castration taking the whole growing period into consideration is essential for decision making and elaboration of guidelines for optimizing animal welfare in the specific productions are needed.

Chapter 1 – Introduction

Currently, surgical castration of male piglets is a common practice in the pig production in many countries to prevent boar taint mainly caused by skatole and androstenone. Castration of entire male pigs also prevents unplanned breeding and reduces aggressive behaviour (Chapter 3). However, due to animal welfare concerns there is an increased desire to stop surgical castration at least in the European countries. The European Commission and representatives of European pig farmers, meat industry, retailers, scientists, veterinarians and animal welfare NGOs have recently committed themselves to voluntarily end surgical castration of pigs in the European Union by January 1, 2018. Therefore, realistic alternatives to surgical castration are needed.

The present report presents initially an introduction to boar taint (Chapter 2) and subsequently reviews of the current knowledge on alternatives to surgical castration (Chapter 3) except surgical castration with pain relief, which is both considered unsuitable on farms in Danish pig production, due to the lack of effective methods that are cost-effective and possible to use at farm level (1-6), and may be irrelevant in the long-term. In addition, the state-of-the-art with respect to methods for detection of boar taint at the slaughter line is summarized (Chapter 4). In Chapter 5 we present and discuss cost-benefit analyses of alternatives that may be implementable within a time horizon of 2 to 5 years. Finally, the relevance of potential alternatives to surgical castration is outlined with focus on the Danish pig production in the conclusion (Chapter 6).



Photo: Rikke Thomsen

Chapter 2 - An introduction to boar taint

Boar taint is an offensive odour and flavour released upon heating (odour) and eating (flavour) meat from some pubertal or sexually mature male pigs. The odour of tainted meat seems more unpleasant than the flavour, which means that it is perceived more during cooking than eating and more in hot and fresh meat products than in cold or processed products (7). According to an international study in seven European countries (UK, DK, FR, SE, NL, ES, DE) the odour and flavour of meat from entire male pigs are disliked in 6.5 and 3 % more cases, respectively, than meat from female pigs (8). The disliking of meat from entire male pigs is considered mainly to be caused by a higher presence of two compounds: skatole and androstenone (e.g. 9).

Skatole is a microbial degradation product originating from microbial fermentation of the feed in the large intestine that is absorbed into the blood and may accumulate in fat. It is described to have a faecal-like or "naphthalene/mothballs"-like odour (10). In contrast, androstenone is an endogenously produced compound with a urine-like odour (10) serving as a pheromone that elicits the mating posture of sows (11). It is mainly synthesized in the testis of entire male pigs along with the steroid sex hormones at onset of puberty, although minor amounts are suggested to be produced in the adrenal cortex or the ovaries as low levels are detectable in females as well (9).

2.1 Perception of tainted meat

The relationship between boar taint, odour and flavour, and fat levels of skatole and androstenone is not straightforward. Based on all consumers an increase in flavour-based disliking of meat from entire male pigs is almost equally dependent on high levels of androstenone and skatole (8, 12), whereas high skatole is the most important component with respect to the odour-based disliking (8, 12), which may be related to its higher volatility (7). In addition, more consumers have a negative perception of skatole than androstenone (13). The contribution of each compound to the consumer response to odour, flavour or both seems, however, to depend on the level of the other compound, and the effect may not be linear (14, 15). In a European study, extrapolating the relationship between consumer response and fat content of boar taint compounds to fat skatole and androstenone measurements from 4291 entire male pigs, the highest improvement of the overall acceptability of meat from entire male pigs was obtained if sorting out of carcasses was done on the basis of an index where the acceptable level of each compound depended on the level of the other and vice versa (8). However, a subset of the same data showed that for consumers who are highly sensitive to androstenone the main determinant for disliking of odour as well as flavour is the amount of androstenone (14).

The consumers' perception of androstenone is highly individual ranging from anosmia to high sensitivity and is related to genetic variation in the human odorant receptor OR7D4 (7, 16), whereas 82

to 99 % of consumers are sensitive to skatole (7, 13). The percentage of consumers sensitive to androstenone ranges from 18 to 74 % in different studies (13, 14, 17), but may be considered to be approximately 45 % if taking methodological differences into account (17). The androstenone sensitivity varies according to country, region, age and gender with older consumers and men being less sensitive, respectively (14, 17, 18). This may partially explain that the same factors affect the consumer rating of overall acceptability of meat along with the culinary habits (12, 15, 17, 18). Moreover, androstenone may be perceived differently by the consumers with 13 to 24 % of the total population liking the smell of androstenone and 21 to 50 % disliking it (13, 19). The varying sensitivity to androstenone as well as the different appreciation of the odour affect the overall acceptance of meat from entire male pigs with the more sensitive consumers rejecting meat with high levels of androstenone if they find androstenone aversive (13, 14, 17, 19). In a recent study carried out in Denmark (20) approximately 1/3 of the consumers were classified as sensitive towards androstenone and 5 to 12 % were classified as highly sensitive, but no effects of age and sex were evident.

The thresholds for sensory perception of boar taint mentioned in the literature are usually 0.20 to 0.25 μ g/g for skatole and 0.5 to 1.0 μ g/g for androstenone (7). However, in the European study extrapolating consumer responses to a larger scale, it was demonstrated that sorting out carcasses of entire males on the basis of skatole and androstenone would decrease, but not eliminate, the difference in consumer acceptability of meat from entire male and female pigs even if using thresholds of 0.2 μ g/g were used for both compounds, which corresponds to the levels of the compounds in castrates and female pigs (8). This result could be due to other compounds contributing to the presence of boar taint, such as indole, androstenols, *p*-cresol, 4-phenyl-3-buten-2-one as well as aldehydes and short chain fatty acids as previously suggested (9, 21-24). However, recent experimental documentation shows that meat from female and entire male pigs with equal levels of skatole and androstenone may not differ in consumer ratings (19) suggesting that the content of skatole and androstenone explains the occurrence of tainted meat. Furthermore, if the level of skatole is very low androstenone levels up to 2 to 3 μ g/g are accepted by the consumers, irrespectively of their androstenone sensitivity and perception of liking/disliking androstenone (13, 16, 19).

Thus, although it is well-documented that boar taint is associated with skatole and androstenone, there is a need for further studies on the acceptability thresholds of skatole and androstenone taking the interdependence between skatole and androstenone into consideration. As tainted meat is disliked by the consumers, alternative steps to reduce the level of skatole and androstenone in the pigs are necessary if surgical castration is banned.

2.2 Skatole

The main source of skatole (3-methylindole) and other indolic compounds is the bacterial breakdown in the large intestine of the amino acid, tryptophan, originating from dietary protein and cell debris (25, 26). Approximately 70 % of the skatole in the large intestine of male pigs are absorbed into the portal vein (27, 28), probably by passive diffusion as no skatole carrier protein is known (29). Via the portal vein, skatole is transported to the liver where more than half of the amount absorbed is degraded (30). Skatole that fails to undergo hepatic degradation accumulates in peripheral tissues, mainly in adipose tissue, due to its lipophilic characteristics, and may cause boar taint (Figure 1).

Additional sources of skatole in fat have been suggested, including absorption from faeces or urine through the skin of the belly, absorption through the lungs and coprophagy (31). However, the main determinants for the amount of skatole in fat are considered to be the amount produced by the microbiota in the large intestine and the degradation capacity of the liver, leaving factors affecting these processes as potential tools to reduce fat skatole and boar taint.

2.2.1. Intestinal production of skatole

Skatole is an indolic compound. The initial step in the production of indolic compounds in the large intestine involves conversion of tryptophan to either indole acetic acid or to indole, which is performed by a variety of bacteria. Further degradation of indole acetic acid to skatole is, however, known for bacteria strains of a few genera (26) and to our knowledge only one bacteria strain capable to produce skatole has been isolated from the gastrointestinal tract of pigs (26, 32). The bacterium seems to be a so far uncultured species belonging to the genus *Olsenella* (33). Skatole producing bacteria have been estimated to account for less than 0.01 % of the total microbiota in the large intestine of male pigs (26) and the concentration of skatole in the large intestine is similar among males, females and castrates (34, 35). It is, however, not known whether the microbial production or the absorption of skatole into the portal vein differs between sexes.

In general, the microbial activity depends on the substrates, which in the case of the large intestine microbiota are the diet, intestinal cell debris and gastrointestinal secretions. Consequently, the diet may be a tool for reducing fat skatole. Previous studies show that especially the availability of protein and carbohydrates in the large intestine seems to be important as protein is the source of tryptophan, whereas carbohydrates provide energy for bacterial growth (Chapter 3). In accordance with this it has been shown that diets containing proteins with low ileal digestibility stimulate skatole production in the hindgut (25, 35, 36), whereas diets with high concentrations of carbohydrates with low ileal digestibility reduce skatole production (Chapter 3). Taken together a low availability of carbohydrates, limiting the energy for bacterial growth, and a high availability of protein, providing tryptophan, favour microbial degradation of tryptophan to skatole in the large intestine, whereas a state of sufficient carbohydrates leads to an enhanced incorporation of tryptophan into the microbial biomass, leaving less substrate for skatole production. In addition, the diet may affect mucosa turnover and release of cell debris that is a major source of the skatole precursor, tryptophan (37) as well as the intestinal transit time that affects the absorption rate (29).

Furthermore, high levels of glucocorticoids, such as cortisol, have been suggested to increase skatole formation through stimulation of mucosal cell degradation (38), thus indicating that stress from housing and management may affect skatole levels as well.

In addition, the intestinal microbiota is markedly affected by infectious, enteric diseases. Recently, it was observed that an outbreak of dysentery, caused by *Lawsonia intracellularis* and *Brachyspira hyodysenteria*, was accompanied by increased levels of skatole in fat (39). The authors suggested that the increased fat skatole was a consequence of an increased intestinal skatole production due to increased mucosal damage and intestinal tryptophan as well as of an inflammation-induced reduction in the expression of hepatic enzymes involved in the metabolism of skatole (39).

Thus, the intestinal produced skatole, which is crucial for the amount of fat skatole and boar taint, depends to a large extent on the diet for the pigs but seems also influenced by stress and enteric diseases.

2.2.2 Metabolism of skatole

Only skatole that fails to be metabolized in the liver accumulates in fat with the risk of causing boar taint (Figure 1). The main metabolic pathway of skatole is considered to be hepatic clearance, although some of the enzymes involved in the skatole metabolism (40) have been identified, but not further investigated in the intestinal walls, kidneys and lungs of pigs. As skatole is an extrinsic compound, the metabolism is comparable with general hepatic detoxification of xenobiotics and is conducted in two phases: Phase I and II. Phase I is usually conducted by enzymes performing hydrolysis, reduction or oxidation, while Phase II enzymes perform conjugations. The conjugated compounds can be excreted through bile or urine (41). Thus, in Phase I a functional group, making the compound more hydrophilic, is usually added or exposed, whereas in Phase II the hydrophilicity of the Phase I metabolites is further increased thereby significantly promoting their excretion.

Among the enzymes responsible for the Phase I reactions, the cytochrome P450 monooxygenases (CYP) are the most important. The overall reaction is as follows: the substrate (RH) is oxidized by incorporation of one oxygen atom to generate the product (ROH), using NADPH as a co-factor:

$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

Members of the CYP1A, 2A, 2C, 2E and 3A families have been studied in order to establish their importance for hepatic skatole clearance (42-46). Taken together, these studies show that CYP2A19 and 2E1 are the most important ones, but that CYP1A2, 2C33 and 3A are capable of metabolizing skatole *in vitro*. The importance of high CYP2A19 and 2E1 activity for the existence of boar-tainted meat is further supported by studies showing a negative correlation between enzyme activity and fat

skatole concentrations (47-50). In addition, aldehyde oxidase that may catalyse the oxidation of intermediate CYP products has been suggested to be of importance for skatole clearance (51).



Figure 1. Skatole metabolism. Skatole is produced in the gastro-intestinal tract by microbial breakdown of the amino acid, tryptophan, and enters the bloodstream after absorption trough the intestinal wall. The hepatic clearance of skatole is conducted in two steps, ultimately resulting in excretion. If the hepatic clearance is insufficient, skatole accumulates in the adipose tissue. CYP, cytochrome P450; AO, aldehyde oxidase; SULT, sulfotransferase; UGT, uridine diphosphate glucuronosyltranferase.

Seven Phase I metabolites of skatole have been identified, the major ones being 3-OH-3methylindolenine, 3-methyloxindole and 3-OH-3-methyloxindole (46, 52), several of them being substrates for the Phase II conjugation. The Phase II conjugation with sulphate or glucuronic acid is conducted by sulfotransferase 1A1 (SULT1A1) (53, 54). The importance of Phase II in the clearance of skatole is, however, not fully elucidated given the fact that the Phase I metabolism is often the rate limiting step (41).

The hepatic skatole metabolism depends on the enzyme activity and the gene and protein expression of the enzymes (9). In general, the expression of individual CYPs is controlled by intracellular ligand binding receptors and has been shown to adapt to the challenges that the liver is presented with. At least three receptors, termed the xenobiotic receptors (XR), regulate CYP expression: aryl hydrocarbon

receptor (AhR), constitutive androstane receptor (CAR; NR1I2) and pregnane X receptor (PXR; NR1I3) (55, 56). In addition, some CYPs are suggested to be regulated trough protein stabilization, e.g. CYP2E1 (56, 57).

There is strong support for an inhibitory effect of testicular steroids on hepatic CYP expression (Figure 2). CYP1A, 2A and 2E expression and/or activity have been shown to be low in entire male pigs compared to females (49, 58, 59) and surgically castrated pigs (58-63). Moreover, artificial reduction of the level of testicular steroids by immunocastration (Chapter 3.2) may increase CYP expression and/or activity (57, 61) whereas injection with human chorionic gonadotropin (64-66) or steroids (59, 67) has the reverse effect. Additionally, *in vitro* studies, treating primary porcine hepatocytes with steroids have shown decreased CYP expression in the presence of androstenone (68, 69). Moreover, androstanol and androstenol that are derivates of androstenone have been identified as inverse agonists of CAR, repressing the constitutive activity of this ligand binding receptor (70), which is believed to initiate gene transcription of the CYP2A family. In addition, it has been suggested that testicular steroids have a direct inhibitory effect on CYP-dependent activity (61, 71-73).

The influence of testicular steroids on enzymes in the hepatic skatole metabolism implies that the hepatic clearance of skatole may be affected by the same factors that regulate androstenone. Furthermore, bioactive feed components may affect enzyme activity (Chapter 3.1.2.2.3).



Figure 2. The impact of androstenone on skatole concentrations in entire and castrated male pigs. Increased androstenone concentrations in sexual mature male pigs lower the CYPs and thereby the CYP activity, giving rise to large skatole concentrations. Additionally, androstenone has a direct inhibitory effect on CYP activity. In castrated pigs, the production site of androstenone is removed, thereby abolishing CYP inhibition, resulting in low skatole concentrations.

2.2.3 Skatole in plasma and fat accumulation

Plasma skatole levels vary with age showing a peak 2 to 3 weeks post weaning (74, 75) followed by low levels until puberty at which time it increases (74). The causal relationship of the early increase is not known, but has been suggested to be related to microbial adaptation to the dietary changes at weaning (75). The increase at puberty is likely to be linked to inhibition of the hepatic clearance of skatole due to the concomitant increase of testicular steroids, especially oestrogen and androstenone (9, 74).

Skatole has a high lipophilicity and accumulates in adipose tissue if the plasma level is increased for a longer period. Likewise, prolonged decreased levels in plasma result in reduced fat levels within a few days (29, 38). The accumulation seems to depend on the specific adipose tissue and the amount of saturated fatty acids (29). However, skatole reducing steps are likely to reduce boar taint within 4 to 7 days.

2.3 Androstenone

Androstenone is predominantly produced in the Leydig cells of the testis with cholesterol as a precursor (76). From the testis androstenone enters the bloodstream, where it either enters the salivary gland, acting as a pheromone, or the liver, where it is metabolized. Like skatole, the even more lipophilic non-metabolised androstenone accumulates in adipose tissues (Figure 3) and thus factors affecting the synthesis or hepatic metabolism are in focus in relation to finding alternatives to surgical castration.

2.3.1 Biosynthesis of androstenone

The production of androstenone is controlled by the hypothalamic-pituitary-gonadal (HPG) axis and increases from onset of puberty as other testicular steroids, such as testosterone and oestrogen (7).

The time of sexual maturation in pigs is age-dependent, but varies between breeds (7). In addition, it is likely to be influenced by seasonal variation and maybe the availability of feed, which seems to interact with the reproductive hormones as the mating season of the wild boar is in the late autumn to early winter, but sows will give birth to 2 litters in years with high availability of feed (77). Thus, it would be expected that a declining day length will stimulate the achievement of sexual maturity. This hypothesis was partly confirmed by the studies of Andersson et al. (1998a and 1998b) (78, 79), whereas other studies did not find any effect of season (74, 80) or obtained contradicting results on fat levels of the boar taint compounds (81). Furthermore, the social environment, the physiological stress responses including the hypothalamic-pituitary-adrenal (HPA) axis and the HPG axis interact (82-84), which implies that the social and physical environment in general may affect time of sexual maturation.

Thus, apart from castration the steps at farm level that may influence androstenone may be age at slaughter, choice of breeds and breeding, availability of feed as well as the social and physical environment.



Figure 3. Androstenone metabolism. Androstenone is produced in the Leydig cells of the testis from where it enters the bloodstream. Via the bloodstream androstenone can enter the salivary gland, where it can be released as a pheromone. Androstenone is metabolised in the liver in two steps; if the hepatic clearance is insufficient, it accumulates in the adipose tissue. HSD, hydroxysteroid dehydrogenase; SULT, sulfotransferase, UGT, uridine diphosphate glucuronosyltransferase.

2.3.2 Salivary glands and pheromone action

16-Androstenens produced in the testis are secreted into the blood and transported to the submaxillary glands where they are bound to a specific binding protein called "pheromaxien". It is assumed that pheromaxein is vital for the transportation of the very hydrophobic androstenens in the aqueous medium of saliva, generating high concentrations in submaxillary glands and saliva of pigs (85). There are discrepancies with respect to the magnitude of boars' saliva 16-androstene concentrations. It has been found that salivary androstenol concentrations were 10 to 20 times higher than androstenone and that boars may contain up to 50 ug/mL androstenol (85). The pheromone effect of 16-androstenes is mediated by the free steroid, this suggests that the pheromonal steroid dissociate from their binding

protein in voided saliva and become volatile as air-born pheromones, and it has been shown that about 21 % of the pheromones are in the free form in freshly voided saliva at 37°C (85).

2.3.3 Metabolism of androstenone

The hepatic metabolism of androstenone is, like the skatole metabolism, conducted in two phases. The Phase I metabolizing enzymes 3α -hydroxysteroid dehydrogenase (HSD) and 3β -HSD androstenone to 3α - and 3β -androstenol, respectively. It has been shown that the major part of androstenone is converted to 3β -androstenol (86, 87). This is further indicated by the finding that low hepatic 3β -HSD expression and activity are accompanied by high concentrations of androstenone in adipose tissue (88-90). Other HSD isoforms like 17β -HSD have also been suggested to be involved in the metabolism of androstenone (88), but a recent study indicates that the effect on the overall androstenone clearance is limited (91).

During the Phase II metabolism, the androstenols are further converted to sulfoconjugated and glucuronidated metabolites (87). SULT2A1 and 2B1 together with UGT enzymes have been suggested to be involved in the Phase II metabolism - the involvement of SULT2B1 was shown to be breed dependent, however (89, 92). The hepatic expression and activity of SULT2A1 have been shown to be higher in pigs with low androstenone concentrations (92).

The regulation of porcine hepatic 3β -HSD expression is complex and not fully elucidated. The removal of testicular steroids by immunocastration has been shown to increase the expression of hepatic 3β -HSD in comparison to entire male pigs (93), whereas *in vitro* studies show no effect or increased expression of 3β -HSD in the presence of steroids (94, 95). The molecular regulation of the transcription of the porcine 3β -HSD gene has recently started to be elucidated (96), but is basically unknown. Like for 3β -HSD, knowledge about the molecular regulation of porcine SULT2A1 transcription is limited, though one study identified ligands of CAR, PXR and PXR/FXR as inducers of SULT2A1 activity *in vitro* (92).

The presence in the testis's of both Phase I and II enzymes, involved in androstenone metabolism, suggests that this compound can be metabolized at its production site. In fact, the isolated leydig cells to produce sulfoconjugated metabolites from androstenone and the conversion of androstenone to 3β -androstenol have been shown (97). Apart from its localization in liver, testis and salivary gland, 3β -HSD has been detected in the adrenal gland of humans and rats (98).

As for skatole, the Phase II metabolites of androstenone, the sulfoconjugated or glucuronidated androstenones are suggested to be excreted through the bile and urine. However, recently it has been suggested that androstenone can also undergo enterohepatic circulation (99, 100), which implies that conjugated androstenone secreted into the bile may be bacterially deconjugated, reabsorbed by the enterocytes, and released to the bloodstream once more, thereby prolonging the biological activity of

the compounds. Biliary excretion of the chemically related, sulfoconjugated and glucuronidated oestrogens, progesterone and testosterone has been demonstrated in humans (99, 101). In addition, oestrogens and progesterone may be bacterially deconjugated, absorbed as free steroids by enterocytes, and released as free or conjugated steroids into the portal vein (99, 101). Enterohepathic circulation of oestrogens seems to occur in pigs as well (102) and according to Roberts et al. (2002) all steroids may be expected to be excreted through the bile and undergo enterohepatic circulation to some extent (99).

The potential existence of this pathway in the metabolism of androstenone opens the possibility of reducing boar taint by blocking reuptake of androstenone in the intestine (Chapter 3.1.2.2.3).

2.3.4 Androstenone in plasma and fat accumulation

Androstenone is even more lipophilic than skatole and the water solubility is approximately 2000 times less (7, 29), which means that androstenone in fat is more slowly released after a drop in plasma level. Thus, after castration of male pigs plasma androstenone is reduced to levels comparable to what is found in female pigs within four days (103), whereas the half-life of fat androstenone is ranging from 4 to 14 days (104) or even more (105). This means that androstenone reducing steps at farm level have to be implemented at least 2 to 3 weeks slaughtering of the pigs.

2.4 Summary

In summary, boar taint is an offensive odour and flavour released upon heating and eating meat from some pubertal or sexually mature male pigs that is disliked by many consumers. It is mainly caused by accumulation of skatole and androstenone in fat but the relationship between boar taint, the perceived odour and flavour of the meat, and fat levels of skatole and androstenone is not straightforward and acceptability thresholds of skatole and androstenone that takes the interdependence between skatole and androstenone into consideration are still lacking. If surgical castration is stopped alternative steps to reduce the level of skatole and androstenone in the pigs are necessary.

Skatole is a microbial degradation product originating from microbial fermentation of the feed and cell debris in the large intestine that is absorbed into the blood and may accumulate in fat. Thus, the main determinants for the amount of skatole in fat are considered to be the amount produced by the microbiota in the large intestine and the degradation capacity of the liver, leaving factors affecting these processes as potential tools to reduce fat skatole and boar taint. Absorption through the skin into the blood has, however, also been suggested. A major factor likely to affect the intestinal produced skatole is the diet of the pigs but also stress and presence of enteric diseases may affect the production. Only skatole that fails to be metabolized in the liver accumulates in fat with the risk of causing boar taint. As testicular steroids affect the hepatic skatole metabolism this may be affected by the same

factors that regulate androstenone. Furthermore, bioactive feed components may affect enzyme activity. Skatole reducing steps at farm level are likely to reduce boar taint within few days.

Androstenone is an endogenously produced pheromone in entire male pigs that elicits the mating posture of mature female pigs and it is mainly synthesized in the testis of entire male pigs along with the steroid sex hormones from onset of puberty. The time of sexual maturation in pigs is age-dependent, but varies between breeds. In addition, it is likely to be influenced by seasonal variation and maybe the availability of feed as well as the social and physical environment. Thus, apart from castration the steps at farm level that may influence androstenone may be age at slaughter, choice of breeds and breeding, availability of feed as well as the social and physical environment. Androstenone reducing steps at farm level have to be implemented at least 2 to 3 weeks before slaughtering of the pigs to clear fat androstenone.



Photo: DCA – Danish Centre for Food and Agriculture

Chapter 3 – Alternatives to surgical castration

The present chapter addresses the effect on boar taint, animal welfare, performance and carcass traits, meat quality and safety, and working environment of four fundamentally different alternatives to surgical castration (SC):

- 1. production of entire males pigs (EM),
- 2. non-surgical castration including immunocastration (IC),
- 3. sperm sexing to produce female pigs (FE) primary, and
- 4. breeding.

3.1 Production of entire males

Entire male pig production is production of intact male pigs, with focus on other initiatives than castration to reduce the existence of boar taint. These efforts may include management, housing, nutrition and genetics.

Of the approximately 19 million pigs slaughtered in Denmark in 2012, 1.8 % was classified as EM (106) corresponding to approximately 6,000 male pigs slaughtered every week. In organic production, the proportion of EM was 4 % of all slaughtered organic pigs in 2012, corresponding to 3,600 EM in total (107). In the beginning of the nineties, when the number of EM was at its maximum in Denmark, approximately 100,000 EM were slaughtered every week (108). However, during the nineties, the number of EM declined dramatically. This was mainly due to a German stop for import of pork from EM produced in Denmark. Since 1997, the proportion of EM has varied between 1.6 and 2.5 % of all slaughtered pigs (106, 108).

Systematic and updated information of the exact extent of the production of EM is lacking for many countries. However, it is clear that the extent of EM production varies a lot across European countries. A survey conducted in 2006 across European countries indicated that of the 125 million male pigs slaughtered in Europe each year, approximately 20 % were left entire with varying percentages in the different countries (109). The highest proportion was found in the UK and Ireland where castration was hardly performed at all followed by the Southern European countries Cyprus, Portugal, Spain and Greece where the proportion of EM varied from 60 to 89 %. In Finland, Norway and Sweden 1 to 5 % of all male pigs were left entire. In some countries there has been a profound increase in the production of EM during the last few years. In the Netherlands, the proportion of males left entire is currently estimated to approximately 50 % of all male pigs (110), and in Germany, one of the largest slaughter house slaughtered between 10,000 and 14,000 EM every week in the beginning of 2011 (111).

3.1.1 Common practice in entire male pig production

There are no specified management or housing requirements for producers of EM pigs in Denmark. Stocking densities and feeding strategies resemble to a large degree the practice from production of FE and SC pigs (112). This means e.g. stocking densities of not less than 0.65 m² per pig in conventional production and not less than 2.3 m² per pig in organic production. However, systematic information on housing and management of EM in practice is generally lacking. In the Netherlands, the majority of EM producers aim to keep the FE and EM in separate groups with 0.8 to 1 m² per EM, but the feeding and management are in general not different from the production of castrates (110, 113). In Germany, producers of EM are advised to use 10 % more space per animal than required according to the legislation and to keep EM in small groups (114). No distinctive practice regarding slaughter weight seems to exist.

In Denmark, the average carcass weight of EM slaughtered in 2012 in the conventional (106) as well as organic (107) production was comparable with the carcass weight of SC pigs and FE, i.e. 80.6 kg, which corresponds to a live weight of approximately 105 kg. Data from 3,506 slaughtered EM pigs collected from six countries (UK, FR, DK, SE, NL, ES) in 1995 showed that the mean carcass weight differed markedly between countries with the lowest carcass weight in the UK (66 kg) and the highest in the Netherlands (84 kg) (80) corresponding to a calculated live weight of approximately 87 and 110 kg, respectively. In the UK, the average carcass weight in general has increased during the last 20 years and is presently 78.2 kg (115). Likewise, the carcass weight in the Netherlands has increased to around 93 kg for EM (113).

3.1.2 Potential tools to reduce boar taint

When leaving the male pig intact, increased levels of skatole and androstenone are likely (Chapter 2). If all conventional pig producers in Denmark have to produce EM it is suggested that about 8 to 10 % of EM would have fat skatole above the threshold of 0.25 μ g/g (116). For fat androstenone an international study found 30.8 % with a level above 1 μ g/g (80). In the Danish organic pig production it has been shown that for skatole about 20 (107) to 26 % (117) of EM are sorted out on the basis of the human nose method only (107) or the human nose method combined with fat skatole above 0.25 μ g/g (117). Sixty eight percent was found to have fat androstenone above 1 μ g/g (117).

As mentioned in Chapter 2 the levels may, however, be reduced by management, housing and feeding that may either reduce the production/biosynthesis of skatole or androstenone or increase their metabolism. Further, choosing of breeds with low levels of boar taint compounds may reduce the percentage sorted out.

3.1.2.1 Effect of management and housing on boar taint

Although the management and housing of EM to a large degree resemble the management and housing of FE and SC pigs in practice, several attempts have been made to identify factors related to

management and housing that reduce the risk of boar taint in EM. In this chapter, focus will be on the effect of slaughter weight, social environment and hygiene on the boar taint compounds.

3.1.2.1.1 Effect of slaughter weight on boar taint

Slaughter weight which reflects the age of a pig has been shown to be positively correlated with boar taint measured as androstenone (118-120). Based on this and the fact that the probability of reaching sexual maturity increases by age, a reduced weight at slaughter is expected to be accompanied by lower androstenone levels. As can be seen from Table 1, summarizing the results from six experiments on the effect of slaughter weight on boar taint compounds, this has been demonstrated in several studies (119-124), but does not always reach significance (124). Also in studies dealing with organic male pigs an increase in androstenone with increasing weight was found for EM (125), however, no exact data on androstenone levels were given. The level of fat androstenone at slaughter may vary considerably between herds (126) and in accordance to Aluwè et al. (2011a) breed differences could be a responsible factor (119).

The increase in androstenone by increasing slaughter weight from 75 to 95 kg resulted in an increase in tainted meat (>1 μ g/g fat androstenone) of 20 % in a Danish field study (121). However, a huge variation in the androstenone levels among pigs of the same slaughter weight exists, and a reduction of weight is no guarantee of keeping fat androstenone below 1 μ g/g. High androstenone levels are found also for low slaughter weights (Table 1).

To the extent that a reduced slaughter weight lowers plasma androstenone it may in addition increase the hepatic clearance of skatole (Chapter 2.2.2) which in turn is expected to reduce fat skatole. In accordance with this, the level of plasma skatole has been shown to increase with increasing age (127). However, the results in Table 1 do not indicate a direct effect of slaughter weight on skatole.

Overall, a reduction of the slaughter weight is considered a reasonable strategy for reduction of fat androstenone. However, for crossbred pigs of the same slaughter weight a variation in androstenone levels exists. The effectiveness of a lower slaughter weight as a strategy to reduce androstenone in back fat is also likely to be influenced by the breeds used, partly due to the difference in time of onset of puberty between breeds. Regarding skatole, reducing slaughter weight is not considered an effective strategy.

Reference	Number of entire male pigs	Breed	Age (days)	Live weight (kg)	Mean androstenone level in fat (μg/g)	Mean skatole level in fat
						(µg/g)
Andersson et al. 2005	204	Swedish Landrace X	140	90	1.05	0.11
(Sweden) (124)		Swedish Yorkshire	172	115	1.31	0.13
Fàbrega et al. 2011	80	(Large White x	155	105	0.6 ^a	-
(Spain) (120)	Spain) (120)		175	130	1.02 ^b	
Aluwé et al. 2011a	288	BN: Belgian	-	50	0.06 ^{bc}	0.08
(Belgium) (119)	(96 per breed)	Landrace		70	0.13 ^{abc}	0.04
				90	0.42 ^{ad}	0.05
				110	0.28 ^{abcd}	0.05
		LW: Large White		50	0.21 ^{abcd}	0.09
				70	0.27 ^{acd}	0.07
				90	0.21 ^{abcd}	0.04
				110	0.48 ^d	0.05
		P: Piétrain		50	0.06 ^b	0.03
				70	0.13 ^{abc}	0.03
				90	0.32 ^{ad}	0.03
				110	0.16 ^{abc}	0.03
Maribo 2013	468	Crossbreds (LYxD)	168	75 [*]	1.23 ^a	0.06
(Denmark) (121)				95 [*]	1.72 ^b	0.07
Jensen 2013	12	Crossbreds		62.3	0.80 ^a	0.109
(Denmark) (122)		(LYxD)		98.2	1.14 ^a	0.059
				118	1.99 ^b	0.125
Thomsen 2013	342	Organic LYxD	-	80	0.88 ^a	0.16
(Denmark) (123)				100	2.15 ^b	0.18
				120	3.24 ^c	0.17

Table 1. Overview of the relationship between weight at slaughter and fat levels of androstenone and skatole.

*slaughter weight

Different superscripts are significantly different within each study.

3.1.2.1.2 Effect of social environment on boar taint

The social environment in which the pigs are reared is likely to affect boar taint compounds through several mechanisms. One example is that high ranking EM pigs have higher plasma androstenone levels than lower ranking EM pigs and a high level of plasma androstenone was associated with a high level of aggression, but not the opposite, meaning that aggression did not increase the level of androstenone (128).

Group composition

Within the Danish production system, SC pigs and FE are generally reared in mixed sex groups. This could pose challenges in relation to boar taint in EM as the presence of FE may affect the time of onset of puberty in the males.

As suggested by Zamaratskaia et al. (2005a) the presence of FE may accelerate the onset of male puberty and thus the time of the increase in androstenone but not the magnitude of the increase of androstenone (129). Consistently with this, an increase was found in both skatole and androstenone levels in mixed sex groups compared to single sex groups, although for androstenone the effect was only significant in low-weight pigs (129). This was partly confirmed in the study by Patterson et al. (1984) but in this study the effect was only significant in heavy weight EM (130).

However, Salmon (2007) found a delayed maturation, based on a delayed physiological development, with lower levels of fat androstenone and skatole in EM reared with fence-line contact to FE than in EM without contact to FE (131), whereas no difference in androstenone levels was found between groups with visual contact to FE or no contact (120). In accordance with these results, a Danish study with organic pigs showed lower levels of skatole and androstenone in mixed sex groups compared to single sex groups of EM (107). Courboulay et al. (2013) found lower androstenone levels of EM in mixed sex groups (132), whereas Allen et al. (1997) did not find differences relating to group composition in boar taint levels measured as androstenone or by sensory evaluation (133). The effect of female contact on development of boar taint compounds is thus not clear.

Group dynamics

To reduce the variation in weight between pigs within the pen and to target a certain slaughter weight, mixing of unfamiliar pigs are usually performed both after weaning when moving the pigs to the fattening unit and before slaughter. Mixing of unfamiliar pigs is a significant stressor and mixing may therefore affect boar taint compounds through the potential interactions between the glucocorticoid, cortisol, and skatole production that are likely to increase the skatole production during stress (Chapter 2.2.1), or through an interaction between the hypothalamic-pituitary-adrenal (HPA) axis and the HPG axis, which may affect time of onset of puberty and androstenone synthesis (Chapter 2.3.1) as well as hepatic clearance of skatole (Chapter 2.2.2). Thus, lower levels of fat androstenone have been demonstrated in groups of mixed sex pigs reared in farrow-to-finish pens (with no mixing of litters) compared to groups of mixed sex pigs coming from different litters (81). In addition, Salmon et al. (2007) found that EM held in groups of mixed litters were more sexually mature at slaughter (based on the physiological development) than EM from stable litter groups (131). However, an effect of mixing unfamiliar pigs vs. litter-wise grouping on androstenone was not found by Rydhmer et al. (2013) (134).The social effects of mixing unfamiliar pigs on production of boar taint compounds remain to be further investigated.

Procedures related to slaughter

The procedures around slaughter would be another aspect affecting the social environment and stress of the pigs (e.g. mixing of animals) and potentially the likelihood of tainted meat. However, knowledge of this topic is very limited. A recent study reports that the duration of transport to the slaughterhouse influenced androstenone in fat and plasma, whereas pre-unloading time (after arrival at the slaughter) influenced skatole and was correlated with cortisol in faeces (135), thus suggesting an influence of stress.

In summary, no clear effect of the social environment in which the EM are reared (mixed sex vs. single sex, mixing strategy, female contact and slaughter procedure) is found on levels of androstenone and skatole at slaughter. The very complex process of puberty and its regulation that is influenced by many different factors is believed to play a role in this divergence. Factors like weight, age, genetics, nutrition and stress or a combination of some of these are suggested to influence the regulation of puberty and in this way influence the different results. More research is needed on the area, but based on the research already conducted the effect of the social environment on boar taint is not presumed to hinder a future production of EM.

3.1.2.1.3 Effect of hygiene on boar taint

It is suggested that skatole, besides being absorbed from the gut, can also be absorbed from faeces plus urine either through the skin or by inhalation in a gaseous form through the lungs (Chapter 2.2). Based on this, boar taint levels coming from skatole could be affected by the hygiene level of the rearing environment and of the animals, when hygiene is defined as fouling with manure.

Hansen et al. (1994 and 1995) showed that keeping pigs heavily fouled with manure (dirty) at a high stocking rate for at least a week prior to slaughter, compared to keeping the pigs in a clean environment (clean), increases the skatole concentration in the back fat of the pigs (31, 136). The skatole concentration in faeces was not different between treatments (dirty vs. clean), gender and temperature and there was no correlation between the concentration in faeces and fat. The authors therefore hypothesize that increased skatole concentration in the back fat of dirty pigs could either be caused by absorption through the skin or the lungs or a combination of both. A recent study partly confirms that dirtiness increases boar taint as EM from a dirty group with extra soiling (rubbed with faeces daily) compared to a clean group (washed daily) had elevated boar taint as evaluated by a standardized consumer panel, although the chemical analyses of skatole in fat and serum did not differ between dirty and clean pigs (137). However, decreased fat skatole with increased amount of soiling in 18-week-old EM and lack of association between soiling and skatole at other ages have been reported as well (138). Furthermore, the latter study showed a positive correlation between fat androstenone level and soiling (138) indicating that the effect of hygiene on boar taint may not simply be explained by absorption of skatole through the skin or by inhalation through the lungs.

In summary, there are indications that copious amounts of manure in pig pens resulting in heavily fouled pigs can increase the skatole concentration in fat, maybe due to absorption of skatole through skin and/or lungs. The research on this topic shows somewhat inconsistent results, but focusing on hygiene levels in future EM production could be a favourable management strategy regarding skatole deposition.

3.1.2.2 Effect of feeding on boar taint

Various feeding strategies are known to influence the concentration of skatole in adipose tissue, whereas androstenone is only moderately influenced by nutrition. In the present section we will address the efficacy of various feeding intervention on production, absorption and degradation with special focus on the effect on deposition of skatole and androstenone in adipose tissues.

3.1.2.2.1 Feeding techniques

In general, the gastrointestinal ecosystem is markedly affected by aspects related to the feeding strategy, such as whether liquid feeding is used and the structure of the feed (pelleted vs. meal/fine vs. coarse), thereby having a potential of affecting skatole production. However, the potential of liquid feeding in decreasing skatole production seems to be limited as summarized in Table 2 (26, 139-141). Results from the entire male pig data base of the Federation of Danish Pig Producers and Slaughterhouses indicate that EM from herds using liquid feed have lower fat skatole than EM from herds using dry feed (140). However, according to Jensen et al. (1998) and Hansen et al. (2000) the effect of liquid feed on skatole production, absorption and deposition in adipose tissue were inconsistent, mostly indicating no or only minor effects (26, 141). The effect of liquid feed on androstenone has not been studied. Likewise, we are not aware of studies on the effect of feed structure on the level of boar taint compounds.

Table 2. Effect of liquid vs. dry feeding on skatole in colon digesta, faeces, plasma and back fat and on androstenone in plasma and back fat. Results are presented as results from control group /results from experimental group. Significant values are given in bold and yellow.

Туре	Number of pigs	Duration (days)	Weight of pigs (kg)	Skatole				Andro	stenone	Reference
				Colon µg/kg	Faeces	Plasma	Fat	Plasma	Fat	
					µg/kg	ng/ml	ng/g	ng/ml	µg/g	
Dry/FLF	24/45						<mark>130/80</mark>			Jensen et al., 1998 (26)
Dry/LF	24/24			<mark>27.0/7.0</mark>			130/110			Jensen et al., 1998 (26)
Dry/FLF	35/36					5.4/5.4	140/140			Jensen et al., 1998 (26)
Dry/FLF	18/18						180/240			Hansen et al., 2000 (141)
Dry/LF	566/651						<mark>230/140</mark>			Kjeldsen and Udesen, 1998 (140)
Dry/LF	538/577						100/100			Kjeldsen and Udesen, 1998 (140)
DRY/LF	118/66						87/82			Anderson et al., 1997 (139)

LF: liquid feed

FLF: fermented liquid feed
In general, the amount of feed intake has no effect on the skatole level but 12 hours withdrawal of feed from the pigs prior to slaughter has been shown to reduce the skatole level (140), although Anderson et al. (1997) were unable to confirm this. However, a recent Dutch study shows that the prevalence for boar taint (human nose scoring system) was lower if the fasting period before slaughter exceeded 6 hours (110). The reduced flow of digesta to the large intestine after withdrawal of feed is likely to result in a reduced fermentation of non-digested protein and thereby reduces the skatole production and deposition in adipose tissue.

In summary, liquid feeding has only minor effect on skatole, whereas withdrawal of feed for more than 6 hours before slaughter reduces skatole and perceived boar taint.

3.1.2.2.2 Diet composition

With respect to diet composition, especially the availability of protein and carbohydrates in the large intestine seems to be important for the production of skatole as protein is the source of tryptophan, whereas carbohydrates provides energy for bacterial growth (Chapter 2).

Protein

The availability of tryptophan is the limiting factor for the skatole production in the large intestine of pigs (28, 142, 143) and thereby for the skatole concentration in blood and fat.

As shown by Jensen (2006) infusion of tryptophan into the cecum of pigs increases the skatole concentration in the portal vein. The increase started 2h after infusion, reached a maximum value up to five fold higher than in the control pigs after 6 to 10 hours with a decreasing pattern thereafter. The total conversion of infused tryptophan to skatole was estimated based on portal blood concentrations. It ranged from 26 % for animals on a low fibre diet to 6 % for animals on a high fibre diet (10 % sugar beet pulp). Most of the infused tryptophan was converted to indole (69 % and 35 % on the low and high fiber diet, respectively).

Apparently, free tryptophan and tryptophan bound in easily digestible protein sources are absorbed in the small intestine and therefore are not available for microbial fermentation in the colon, and fat skatole is neither affected by increased protein content in the diet nor by supplementing the diets with synthetic L-tryptophan above the nutritional requirements (29). Thus, the type rather than the amount of protein is important for the production and deposition of skatole. Consistent with this, skatole production and deposition are increased by diets with low ileal protein digestibility, such as products based on blood, meat, or bone meal (36), yeast slurry from breweries (25, 35, 144) and yellow peas (145, 146).

However, recent studies carried out in Denmark has shown that reducing the daily protein intake in pigs by feeding grain only (50 % wheat, 50 % barley) for 4 days before slaughter significantly reduced

skatole deposition in back fat (0.080 μ g/g in pigs fed compound feed vs. 0.060 μ g/g in pigs fed only grain; 121). However, a more moderate reduction in protein level obtained by replacing 40 % of soybean with barley for the whole growth period from 25 to 100 kg resulted in a significant increase in the skatole content in back fat (270 vs. 110 ng/g for ad libitum fed pigs and 280 vs. 140 for restrictively fed pigs; 147).

To our knowledge the effect of protein content and type on androstenone production, degradation and deposition has never been investigated.

In summary, skatole production and deposition are increased by diets with low ileal protein digestibility, such as products based on blood, meat, or bone meal, yeast slurry from breweries and yellow peas. In addition, feeding grain only for 4 days before slaughter may be a feeding strategy that in practical pig production can be used to reduce the boar taint problem.

Carbohydrates

Carbohydrates are a very diverse group of compounds, which chemically can be classified according to their degree of polymerisation into sugars, oligosaccharides and polysaccharides. The latter fraction can further be divided in starch and non-starch polysaccharides based on the linkage (α or β) between the monomers. Almost all α -linked carbohydrates can potentially be broken down to monosaccharaides by endogenous enzymes in the small intestine (digestible carbohydrates). In contrast, compounds linked together with β -linkage cannot be broken down by endogenous enzymes (non-digestible carbohydrates), and most of it will reach the large intestine where it potentially can be fermented by the microbiota. However, although all α -linked starch potentially can be broken down to glucose in the small intestine a certain fraction may be resistant either because of encapsulation in cell walls (e.g. peas or other legumes), the chemical structure (crystallization) or because of recrystallization, which may occur during cooling at high water activity after being gelatinized. Non-digestible carbohydrates consist of three main categories of carbohydrates: 1) starch resistant to enzymatic breakdown (resistent starch), 2) Non-digestible oligosaccharides and 3) Non-starch polysaccharides. Non-starch polysaccharides together with lignin make up the fiber fraction of the diet.

In general, the addition of different sources of non-digestible carbohydrates to the diet of growing pigs has been shown to reduce skatole formation in the large intestine, the absorption to the portal vein, and the deposition in adipose tissue. Claus et al. (1994) were first to show that addition of non-digestible carbohydrates to pig diets was effective in reducing skatole concentration in the colon and in back fat. By addition of inulin (the amount not given) to a pig diet for 7 days, a significant reduction in faecal and back fat skatole concentrations was found (38). This finding was later supported by the study of Jensen and Jensen (1998), who showed that addition for two weeks of 10 % of either: raw potato starch (resistent starch), inulin (non-digestible oligosaccharide), lupins (non-starch

polysaccharides) or barley hull meal (non-starch polysaccharides) significantly reduced skatole concentrations in the blood when compared to a control diet (barley/soya) (26). In contrast, the addition of either: palm cake (non-starch polysaccharides), coconut cake (non-starch polysaccharides) or sugar beet pulp (non-starch polysaccharides) at the same concentration had no effect. The effect of non-digestible carbohydrates may, however, depend on the content of fibres in the diet as the rate of conversion to skatole of tryptophan infused into the caecum differed significantly according to whether pigs were fed a low fibre diet (barley/soya) or a high fibre diet containing 10 % sugar beet pulp (28). Low fibre diet resulted in a higher production of skatole due to a higher degradation of tryptophan to indole in the large intestine, whereas the high fibre diet decreased the conversion of tryptophan to indole and the skatole formation in the large intestine (28). It may be assumed that in the case of the high fibre diet, the remaining tryptophan was incorporated in bacterial protein as has been found after application of fructooligosaccharides to faecal samples (148).

Resistant starch

Raw potato starch. Raw potato starch is high in resistant starch, with approximately 60 % of the starch reaching the large intestine and being available for microbial fermentation (149). As evident from Table 3, summarizing the effect of adding raw potato starch, non-pelleted diets supplemented with 20 % raw potato starch (200g/kg) for 7 to 21 days proved to be effective in reducing skatole in the large intestine and adipose tissue (129, 150-156); whereas lower concentrations of raw potato starch seem to be insufficient (157). Moreover, if the raw potato starch is added to the diet before pelleting it has no effect on skatole (156) most likely because the high temperature during the pelleting process gelatinize the starch resulting in increased ileal digestibility of the starch (149).

Although the effect of raw potato starch on the androstenone concentration in plasma or back fat was not measured in all experiments referred to in Table 3, there is no consistent indication that raw potato starch affect androstenone in back fat. Only Chen et al. (2007) found that feeding pigs 600 g raw potato starch/day/pig reduced androstenone in plasma if androstenone was measured by ELISA with extraction (153). No effect was found on androstenone in fat or on androstenone in plasma if it was measured by direct ELISA.

Table 3. Effect of potato starch on skatole in colon digesta, faeces, plasma and back fat and on androstenone in plasma and back fat. Results are presented as results from control group /results from experimental group. Significant values are given in bold and yellow.

Туре	Amount (g/kg WW)	Num- ber of pigs	Duration (days)	Weight of pigs (kg)		Ska	tole		Andros	stenone	Reference
					Colon	Faeces	Plasma	Fat	Plasma	Fat	
					µg/g	µg/g	ng/ml	ng/g	ng/ml	µg/g	
Raw	580	12	19			99.0/1.9	<mark>1.62/0.19</mark>	<mark>240/0</mark>			Claus et al., 2003 (150)
Raw	200	8ª	14-21	109		134/76		<mark>159/67</mark>			Lösel and Claus 2005 (151)
Raw	300	8ª	14-21	109		<mark>134/86</mark>		<mark>159/26</mark>			Lösel and Claus 2005 (151)
Raw	400	8ª	14-21	109		<mark>134/48</mark>		<mark>159/20</mark>			Lösel and Claus 2005 (151)
Raw	600 g/day	16	14	115			<mark>2.0/0.4</mark>	<mark>150/90</mark>	5.4/5.0	1.0/0.9	Zamaratskaia et al., 2005a (129)
Raw	300	20				39.8/0	<mark>0.77/42</mark>				Lösel et al., 2006 (152)
Raw	600 g/day	35	14	115			<mark>3.75/0.7</mark>	<mark>50/10</mark>	<mark>4.46/2.2</mark>	0.9/0.64	Chen et al., 2007 (153)
Raw	300	12	7	106			<mark>20/7</mark>	<mark>850/220</mark>		1.7/2.0	Pauly et al., 2008 (154)
Raw	100	18	28-42	107				111/130		0.29/0.25	Aluwe et al. <i>,</i> 2009 (157)
Raw	300	12	7	103				<mark>140/60</mark>		0.9/11	Pauly et al., 2010 (155)
Raw	200	11	14	112	<mark>37.6/4.7</mark>	<mark>35.5/14.7</mark>		<mark>170/20</mark>		3.08/1.72	Øverland et al., 2011 (156)
Pelleted	200	11	14	112	37.6/64.7	35.5/30.3		170/110		3.08/1.81	Øverland et al., 2011 (156)

Oligosaccharides

The monomers in inulin and frugtooligosaccarides (FOS) are linked together by β -linkage, and therefore they are not susceptible to endogenous enzyme breakdown (158). Inulin is present as storage carbohydrates in chicory root and artichokes, and in smaller quantities in wheat, rye, asparagus, onion, garlic and leeks. Three sources of inulin have been investigated for their effect on prevention of boar taint: purified inulin, chicory root and Jerusalem artichoke.

Purified inulin. The results of previous studies on purified dietary inulin (156, 157, 159-161) are summarized in Table 4 and show that a dietary concentration above 50 g/kg significantly reduces skatole in faeces, plasma as well as in back fat. On the contrary, no significant effect on androstenone was found in any of the investigations.

Table 4. Effect of purified inulin on skatole in colon digesta, faeces, plasma and back fat and on androstenone in plasma and back fat. Results are presented as results from control group /results from experimental group. Significant values are given in bold and yellow.

Туре	Amount	Inulin	Num-	Duration	Weight		Skat	ole		Andros	tenone	Reference
	(g/kg	(g/kg	ber of	(days)	of pigs							
	WW)	DW)	pigs		(kg)							
						Colon	Faeces	Plasma	Fat	Plasma	Fat	
						µg/g	µg/g	ng/ml	ng/g	ng/ml	µg/g	
77.5%	50	39					<mark>183/79</mark>					Rideout et al.,
												2004 (159)
Pure	163	172	8	42	124			<mark>3.49/0.68</mark>	<mark>80/30</mark>	27.0/27.0		Hansen et al.,
												2006 (160)
60%	50	30							110/90		0.29/0.36	Aluwe et al.,
												2009 (157)
70%	30	21	11	30	100	36.2/33.2	53.1/38.4		40/60		1.59/1.99	Øverland et
												al., 2011 (156)
70%	60	42	12	30	100	36.2/15.9	53.1/24.9		40/20		1.59/1.87	Øverland et
												al., 2011 (156)
70%	90	63	12	30	100	<mark>36.2/5.4</mark>	<mark>53.1/6.8</mark>		40/10		1.59/2.64	Øverland et
												al., 2011 (156)
75%	90	68	11			<mark>4.6/1.3</mark>	13/9.7		37/17		1.8/1.3	Vhile et al.,
												2012 (161)

Chicory root. Hansen et al. (2006) were the first to investigate if dietary chicory root was effective in preventing boar taint (160). It was hypothesised that chicory would have an effect on the taste of pork meat due to the presence of high concentration of inulin (known to affect hind gut fermentation) and to a lesser extent to the presence of sesquiterperne lactones, which are bioactive component expected to affect liver metabolism of skatole and androstenone. Hansen et al. (2006) added crude and dried chicory root and chicory-inulin in diets for pigs (25 % of total energy intake) during different periods before slaughter from 1 to 9 weeks. It was concluded that dried chicory for as little as 3 days reduced skatole in plasma (160). Since the first study by Hansen et al. (2006), several studies with chicory roots have been conducted (121, 122, 160, 162-166 - summarised in Table 5).

As evident from Table 5, the use of 10 to 15 % chicory root in pig feed resulting in a final concentration of inulin in the diet above 50 g/kg seems to be an effective way to prevent the skatole problem of boar taint. The data also indicate that the chicory root only had to be given in the diet for 7 days to be able to prevent skatole accumulating in back fat. Further, in contrast to raw potato starch inulin can stand heating and pelleting of the feed (121, 163).

Table 5. Effect of chicory root on skatole in colon digesta, faeces, plasma and back fat and on androstenone in plasma and back fat. Results are presented as results from control group /results from experimental group. Significant values are given in bold and yellow.

Туре	Amount (g/kg WW)	Inulin (g/kg DW)	Number of pigs	Duration (days)	Weight of pigs (kg)		SI	katole		Andro	stenone	Reference
						Colon	Faeces	Plasma	Fat	Plasma	Fat	
						µg/g	µg/g	ng/ml	ng/g	ng/ml	µg/g	
Crude	564	139	4	28	118			<mark>1.82/0.13</mark>	<mark>130/0</mark>	17.7/13.7		Hansen et al., 2006 (160)
Crude	564	139	4	63	118			<mark>1.82/0.08</mark>	<mark>130/30</mark>	<mark>17.7/9.1</mark>		Hansen et al., 2006 (160)
Crude	563	139	8	42	124			<mark>3.20/0.32</mark>	<mark>80/30</mark>	27.0/27.1		Hansen et al., 2006 (160)
Dry	276	155	8	42	124			<mark>3.20/0.11</mark>	<mark>80/30</mark>	27.0/23.2		Hansen et al., 2006 (160)
Dry	276	155	8	14	110			<mark>4.30/0.00</mark>		20.0/30.0		Hansen et al., 2006 (160)
Dry	276	155	8	7	110			<mark>3.20/0.00</mark>		34.0/25.0		Hansen et al., 2006 (160)
Dry	90	40	24	8-15				2.43/1.95	160/140			Hansen et al., 2008 (162)
Dry	150	90	50	14		<mark>26.3/6.0</mark>			<mark>110/40</mark>		1.14/1.08	Maribo et al., 2010 (163)
Extract	30	18	30	14					240/130			Zammerini et al., 2012 (165)
Dry	60	36	30	14					240/120			Zammerini et al., 2012 (165)
Dry	90	54	30	14					<mark>240/50</mark>			Zammerini et al., 2012 (165)
Dry	100	47	6/16	16	130			4.1/5.5	49/42		<mark>2.3/0.9</mark>	Rasmussen et al., 2012a (164)
Dry	250	120	8		64				<mark>110/0</mark>		0.82/0.64	Jensen et al., 2013 (122)
Dry	250	120	8		98				<mark>49/0</mark>		1.52/1.04	Jensen et al., 2013 (122)
Dry	250	120	8		118				<mark>125/0</mark>		1.45/2.24	Jensen et al., 2013 (122)
Dry	150	ххх	60	14	98				<mark>80/60</mark>		1.13/1.35	Maribo, 2013 (121)
Dry	150	ххх	96	14	124				<mark>110/70</mark>		1.78/1.76	Maribo 2013 (121)
Dry	50	30	52/47	10	93 (car)				<mark>36/12</mark>		0.86/0.97	Aluwe et al., 2013 (166)

In most of the studies shown in Table 5 use of 50 to 100 g inulin/kg feed reduced skatole in back fat to approximately 1/3 of the value without inulin. Since most of the studies carried out with Chicory roots were conducted with few animals it is difficult to say anything about the effect on the sorting out range. But one may assume that it is in the same range as the reduction in the skatole value. The data in Table 5 also indicate that use of higher amount of chicory root (inulin content above 150 g/kg feed) may completely eliminate skatole from back fat. This may be interesting since it has been shown, as discussed in Chapter 2, that skatole at high concentration enhances the perception of androstenone and as such chicory root may have an indirect effect on the androstenone problem of boar taint.

Taking the size of previous experiments into account there is no clear indication of a direct effect of chicory root on androstenone in back fat (Table 5). Hansen et al. (2006) found a significant effect in 1

of the 6 treatments investigated (160). However, the data are based on an experiment with only four pigs (160). Rasmussen et al. (2012a and 2013) found that chicory root at a low concentration affected both global androstenone levels in male pigs and resulted in an increase in the levels of the androstenone degrading enzyme 3β -HSD expression in the liver although a methanol extract of chicory root decreased the expression of 3β -HSD *in vitro* using porcine primary hepatocytes (95, 164).

Jerusalem artichoke. Only one investigation has been published on the effect of Jerusalem artichoke on boar taint (161). The authors concluded that adding dried Jerusalem artichoke to diets for entire male pigs 1 week before slaughter resulted in a dose-dependent decrease in skatole levels in the hindgut and back fat. As for purified inulin and chicory root, Jerusalem artichoke seems to be effective in preventing accumulation of skatole if used at an amount equivalent to an inulin content in the feed above 50 g/kg. No significant effect of adding Jerusalem artichoke to the diet was found on androstenone levels in back fat.

Dietary fibre (non-starch polysaccharides and lignin)

Dietary fibre reaches the large intestine almost undigested thereby providing substrate for microbial fermentation in the hind gut (158). Moreover, non-starch polysaccharides are the most diverse group of carbohydrates with different properties, which affect the gastrointestinal tract in different ways (158). It has been proposed that dietary fibre because of its physicochemical properties contributes with an increased endogenous loss from the gut epithelial cells thereby increasing the amount of protein including L-tryptophan reaching the large intestine. More L-tryptophan will therefore be available for skatole production in the large intestine.

Sugar-beet pulp. Sugar-beet pulp contains a high level of pectin containing polysaccharides in the non-starch polysaccharide fraction, which have low digestibility in the small intestine but are relatively easily fermented in the large intestine, thus potentially reducing the skatole production.

As shown in Table 6, however, the results with sugar-beet pulp varies as several investigations have been shown to decrease skatole concentrations in back fat (25, 27, 140, 167), whereas other studies (34, 168) have not shown any significant effect. One explanation for the variable results could be that SBP both stimulates skatole production by increasing the amount of tryptophan available for skatole production due to its effect on the endogenous loss of epithelial cells and on the other hand decreases skatole production due to its non-starch polysaccharides content.

Table 6. Effect of Sugar Beet Pulp (SBP) on skatole in colon digesta, faeces, plasma and back fat and on androstenone in plasma and back fat. Results are presented as results from control group /results from experimental group. Significant values are given in bold and yellow.

Туре	Amount	Number	Duration	Weight		Ska	atole		Androstenone		Reference
	(g/kg	of pigs	(days)	of pigs							
	WW)			(kg)							
					Colon	Faeces	Plasma	Fat	Plasma	Fat	
					µg/g	µg/g	ng/ml	ng/g	ng/ml	µg/g	
SBP	400	8	17	90				43/51			Hawe et al., 1993 (34)
SBP	400	24	(25-110	90-110				<mark>23/9</mark>			Gill et al., 1993 (167)
			kg)								
SBP	90	70	21-35					<mark>(170/150)</mark>			Kjeldsen and Udesen,
											1998 (140)
SBP	200	7	(60-112		<mark>26.5/8.1</mark>			<mark>150/100</mark>			Jensen et al., 1995b
			kg)								(25)
SBP	150	15	(57-111	111				65/37			Van Oeckel et al., 1998
			kg)								(168)
SBP	100	6	30		33.4/13.1	<mark>45.9/19.8</mark>	<mark>3.5/1.3</mark>				Knarreborg et al., 2002 (27)

Lupins. Blue lupins have a high content of soluble non-starch polysaccharides and non-digestible oligosaccharides which are readily fermentable substrates for the microflora in the large intestine thereby potentially reducing the fermentation of protein and the production of skatole. The effect of lupine on skatole is summarized in Table 7. Inclusion of 25 % blue lupins in pig diets reduced skatole in backfat in SC and FE pigs (162, 169). Jensen and Jensen (1998) found similar results with entire male pigs using 10 % lupins (26). However, these results were not confirmed in later studies using 10 to 15 % lupines (121, 157). The strong effect of the lupine diet on skatole found by Hansen et al. (2008) (162) may not be an effect of lupines as the lupine and control diets also differed with respect to the content of yellow peas, which as mentioned above may increase skatole production.

Table 7. Effect of lupine on skatole in colon digesta, faeces, plasma and back fat and on androstenone in plasma and back fat. Results are presented as results from control group /results from experimental group. Significant values are given in bold and yellow.

Туре	Amount (g/kg WW)	Num- ber of pigs	Duration (days)	Weight of pigs (kg)	Skatole Androstenone				Reference		
					Colon	Faeces	Plasma	Fat	Plasma	Fat	
					µg/kg	µg/kg	ng/ml	ng/g	ng/ml	µg/g	
Blue											Hansen and Claudi-
lupine											Magnussen, 2004 (169)
Blue	250	18	28-42				<mark>2.43/0.43</mark>	<mark>160/40</mark>			Hansen et al.,
lupine											2008 (162)
	100	24	8-15	108				110/60		0.29/0.15	Aluwe et al.,
											2009 (157)
	150	60	14	98				88/80		1.13/1.23	Maribo,
											2013 (121)
	150	96	14	124				110/11		1.78/1.61	Maribo,
								0			2013 (121)

Interestingly, in the study of Aluwe et al. (2009) with lupines a significant reduction of androstenone in back fat was identified (157). However, Maribo (2013) was unable to confirm this finding (121). It

has been speculated that the reducing effect of lupines on androstenone could be explained by the presence of bioactive compounds in lupines (Chapter 3.1.2.2.3).

Other non-starch polysaccharides. As shown in Table 8, wheat bran, which consists of dietary fibre rich in non-starch polysaccharides and lignin and is slowly digested by the microbiota in the large intestine of pigs (158), is unable to reduce skatole in adipose tissue (25, 168). The same was the case for the fibre rich feed ingredient, sugar-beet hulls (168), whereas the pectin rich feedstuffs, molassed sugar-beet and sugar-beet fibre, reduced skatole in adipose tissue (62, 170 - Table 8). No effect on androstenone was found by sugar-beet fibre.

Table 8. Effect of other non-starch polysaccharides on skatole in colon digesta, faeces, plasma and back fat and on androstenone in plasma and back fat. Results are presented as results from control group /results from experimental group. Significant values are given in bold and yellow.

Туре	Amount (g/kg WW)	Number of pigs	Duration (days)	Weight of pigs (kg)	Skatole			Androstenone		Reference	
					Colon	Faeces	Plasma	Fat	Plasma	Fat	
					µg/g	µg/g	ng/ml	ng/g	ng/ml	µg/g	
MSB	200	12	25-85 kg	85				<mark>66/33</mark>			Wood et al.,
											1994 (170)
WB	200	7	Nd					150/200			Jensen et al.,
											1995b (25)
WB	300	15	(57-111 kg)					65/126			Van Oeckel et al.,
											1998 (168)
SBH	150	15	(57-111 kg)					65/55			Van Oeckel et al.,
											1998 (168)
SBF	200	10 ^a	30	174				<mark>590/190</mark>		1.9/1.2	Whittington et al.,
				davs							2004 (62)

MSB: Molassed sugar-beet, WB: wheat bran, SBH: Sugar beet hulls, SBF: Sugar beet fiber.

Grain type and non-starch polysaccharides degrading enzymes

It has been hypothesized (171) that the type of cereal and addition of exogeneous non-starch polysaccharides degrading enzymes to pig diets may affect the production of skatole in the gastrointestinal tract as it will affect the availability of fermentable substrates for the microbiota in the large intestine. However, no significant effect of cereal type or enzymes has been found on skatole (147, 171), although the concentration of indole was significantly lower both in the hindgut tract and in adipose tissue in barley fed pigs (171).

Surprisingly, addition of non-starch polysaccharides degrading enzymes resulted in a significant reduction of androstenone in adipose tissue in the pigs fed the oat-based diet (171). The reason for this decrease is not clear but it should be kept in mind that the number of animals was too low to evaluate the effect on a parameter like androstenone, which shows large variation in the weight range at which the animals were slaughtered.

Summary

In summary, supplementation of raw potato starch to the feed the last 7 days before slaughter seems to be a possible solution to the skatole problem of boar taint. However, it has to be added to the diet at high concentrations (> 200g/kg diet) and in raw forms as it cannot stand pelleting. Raw potato starch does not seem to be able to solve the androstenone problem of boar taint.

The most promising carbohydrate seems to be inulin either as purified inulin or as the inulin rich feed components Chicory root or Jerusalem artichoke. Addition of these feed components to a diet at concentrations so the amount of inulin is above 50g/kg reduces the skatole content in back fat to 1/3 of the control diet. As for raw potato starch it only had to be given in the diet for 7 days to be able to prevent skatole accumulating. Further, in contrast to raw potato starch inulin can stand heating and pelleting of the feed. As for raw potato starch there is no clear indication of a direct effect of chicory root on androstenone in back fat.

At present, the data on use of lupines are inconclusive. Use of 25 % had a significant effect on skatole but these amounts of lupines had a negative effect on animal production parameters showing that the importance of sensory effect versus production results has to be investigated further before implementation of this feeding strategy in practice.

The results with sugar-beet pulp vary as several investigations have been shown to decrease skatole concentrations in back fat, whereas other studies have not shown any significant effect.

Slowly fermentable dietary fibres as wheat bran have no effect on skatole production and accumulation. Grain type (barley versus oat) has no effect on skatole nor has the addition of non-starch polysaccharides degrading enzymes.

Fat

Dietary manipulations of skatole and androstenone concentrations caused by increased fat turnover seem to have been neglected. Only a few data are available on the effect of dietary fat or its composition on skatole and androstenone in adipose tissue and no effects that clearly could be ascribed to the amount of dietary fat have been found (25, 146, 172). Thus, further investigations are needed.

3.1.2.2.3 Feed additives

Since skatole is a product of microbial activity in the gastrointestinal tract several feed additives documented to influence the microbial ecosystem in the gut of pigs have been investigated for their possible effect on skatole production and deposition. Such additives include compounds with antimicrobial activity and compounds that change the physicochemical conditions in the gut.

Furthermore, feed additives may function as bioactive components that may affect the degradation and deposition of skatole and the production, degradation and deposition of androstenone.

Antibiotics

The most radical approach to influence the composition and the activity of the microbiota in the gastrointestinal tract is to apply antibiotics. When used in appropriate concentrations several antibiotics have been shown to reduce the concentration of skatole in the gastrointestinal tract and in adipose tissue (26, 141, 173), whereas lower doses were without effect (174, 175). The most effective antibiotics seem to be those acting against gram positive bacteria like virginiamycin (26) and bacitracin (141, 173). As shown by Hansen et al. (1997), it is sufficient to use the antibiotics for 3 to 7 days before slaughter in order to reduce skatole in adipose tissue (173). To our knowledge it has never been investigated if in-feed antibiotics have any effect on the androstenone level in adipose tissue. Most of the studies with antibiotics were conducted in the late 90s where it still was allowed within EU to use antibiotics as in-feed growth promoters. The risk for developing resistant bacteria makes the use of antibiotics to reduce skatole unacceptable.

Organic acids

Due to the profound effect of some organic acids on the composition and activity of the microbiota in the gastrointestinal tract, ileal digestion of amino acids, and growth performance (176), it is expected that skatole production, plasma skatole and the deposition of skatole in adipose tissue are affected. As summarized in Table 9, it has been shown that formic and benzoic acid have the potential of reducing plasma skatole in entire male pigs. However, skatole in adipose tissue and colon content were not significantly affected (176).

Table 9. Effect of organic acids added at a level corresponding to a final concentration of 0.85 % pure acid on
skatole in colon digesta, faeces, plasma and back fat and on androstenone in plasma and back fat. Results are
presented as results from control group /results from experimental group. Significant values are given in bold
and yellow.

Acid	Amount (%)	Number of pigs	Duration	Weight og pigs (kg)	Skatole Androstenone						Reference
					Colon	Faeces	Plasma	Fat	Plasma	Fat	
					µg/g	µg/g	ng/ml	ng/g	ng/ml	µg/g	
Formic	0.85	10	32-113 kg	113	58.7/48.5		<mark>1.76/0.18</mark>	130/30		1.63/0.84	Øverland et al., 2008 (176)
Benzoic	0.85	9	32-114 kg	114	58,7/50.2		<mark>1.76/0.15</mark>	130/20		1.63/2.34	Øverland et al., 2008 (176)
Sorbic	0.85	9	32-111 kg	111	58.7/42.9		1.76/1.90	130/20		1.63/1.38	Øverland et al., 2008 (176)
Butyrate (fat coated)	0.85	9	32-115 kg	115	58.7/62.6		1.76/0.85	130/50		1.63/1.96	Øverland et al., 2008 (176)
Butyrate (inulin coated)	0.85	9	31-112 kg	112	58.7/42.5		1.76/0.52	130/40		1.63/2.40	Øverland et al., 2008 (176)

However, entire male pigs fed formic acid had significantly lower androstenone levels in adipose tissue compared to pigs fed benzoic acid and coated butyrate, whereas the level did not differ significantly compared to the control group. The numerically lower level of fat androstenone and the marked lower level of skatole in plasma in the pigs feed formic acid compared to the control group make this acid very interesting and its effect on skatole and androstenone deposition in adipose tissue deserves further investigation.

Bioactive compounds/plant extracts

Bioactive plant compounds can be used to increase the hepatic activity of the skatole and androstenone metabolizing enzymes, and thereby increase the hepatic clearance of the boar taint compounds. Alternatively, bioactive compounds can be directed towards lowering the androstenone producing enzymes in the Leydig cells. Further approaches to control skatole formation in the large intestine by bioactive components/plant extracts may also be attractive, as they reveal antimicrobial properties at very low concentrations. While the effect of bioactive components on the metabolism of skatole and androstenone in the liver has been intensively studied, almost no studies have been carried out elucidating the effect of bioactive compounds on the production of androstenone in the Leydig cells or on skatole production in the large intestine.

Several studies have shown that the expression or activity of CYPs involved in the skatole metabolism is changed if plant materials or herbal based medicine containing a diverse range of bioactive compounds is added to the feed. One example is artemisinin from *Artemisia* which induces CYP expression in pigs, mice, rats and human hepatocytes (177, 178) and is able to decrease skatole accumulation in fat within 8 days in pigs (179).

Interestingly, artemisinin belongs to a class of secondary plant metabolites called sesquiterpene lactones, which are also present in e.g. chicory root. Additionally, a number of different sesquiterpene lactones have been isolated in chicory, the major ones being lactucin, 8-deoxylactucin and lactucopicrin (180-182). It has been shown that, feeding chicory for 16 days increases the expression and activity of CYP1A, 2A and 3A involved in the skatole metabolism (183, 183) as well as the hepatic expression of 3β -HSD involved in androstenone metabolism and consequently reduced androstenone accumulation in fat (164). Recent studies suggest that the effect of chicory specific sesquiterpene lactones was due to a lactucin-induced increase in the mRNA expression of CYP2A and 2E (185) as well as of 3β -HSD and SULT2A1 (95). Furthermore, the compound esculetin, belonging to another group of secondary plant metabolites called coumarines, increased the CYP1A mRNA expression in the hepatocytes (95). Chicory has also been shown to modulate the kinetic properties of the hepatic CYPs by affecting the inhibitory potential of testicular steroids or directly affecting enzyme activity (72, 183). However, in spite of the effect of feeding chicory root on the enzymes of the androstenone metabolism, no significant effect on fat androstenone was found the majority of studies with chicory root (Table 4).

Another potential bioactive plant is the legume, lupine, that as shown in Table 8, in some studies has been shown to decrease the accumulation of skatole (26, 162) or androstenone (157) while no effect was found in other studies (121). Similarly, linseed has been shown to lower the skatole concentration in back fat of gilts (172), which may be ascribed to the compound, myristicin, that increases the expression and activity of a number of hepatic CYPs (186).

It is not given that bioactive components have a positive effect on the occurrence of boar taint. Thus garlic has been shown to increase fat skatole concentration in female pigs in a dose dependent manner (36) and both up- and down-regulation of several CYP isoforms by bioactive components found in garlic have been reported (187-189).

In summary, some bioactive components in plant products may have beneficial effects on the skatole and androstenone metabolism and may contribute to the effect of these products on accumulation of boar taint compounds in back fat. However, further research on the significance and the potential of using bioactive components in practise is needed.

Adsorbants

Recent studies indicate that it is possible to reduce plasma androstenone by oral administration for 21 to 28 days of 5 % non-nutritive adsorbent materials, such as activated charcoal or Tween-60 (polyoxyethylene sorbitan monostearate), without affecting growth rate (100). Activated charcoal was most effective in reducing fat androstenone as none of the EM fed activated charcoal had fat androstenone above the sensory threshold (1 μ g/g). In contrast, 45.5 % of EM not fed adsorbents and 23.0 % of EM fed Tween-60 had androstenone levels above 1 μ g/g (100).

The effect is suggested to be caused by blocking of an enterohepatic circulation of androstenone (Chapter 2) due to binding of androstenone to the adsorbants in the small intestine (100, 190). A comparable effect of activated charcoal on plasma oestrogens, which undergoes enterohepatic circulation (Chapter 2), has been shown (191). However, it remains to be demonstrated whether enterohepatic circulation of androstenone occurs in pigs. Furthermore, additional mechanisms of action of the adsorbents on fat androstenone may be unspecific binding of the adsorbents which potentially may block the enterohepatic circulation of other intestinal compounds, such as oestrogens (100, 191). Oestrogens along with other testicular hormones seem to affect the enzymatic activity of the hepatic androstenone metabolism (94). In fact, *in vitro* studies showed that activated charcoal had a higher binding efficiency for oestrogens than for androstenone (190).

The adsorbents also bind to skatole *in vitro* (190) but no effect of adsorbents on fat skatole has been demonstrated *in vivo* (100, 192).

Addition of adsorbent materials to the feed may constitute an alternative to surgical castration with respect to androstenone but further research is needed. Firstly, the effect has to be confirmed in further studies addressing relevant breeds, age of the pigs and different aspects of commercial conditions. Secondly, the existence and significance of the enterohepatic circulation of androstenone in relation to additional mechanisms of actions have to be elucidated in order to find or develop adsorbents with optimal binding characteristics in relation to reduction of boar taint. At present, activated charcoal is too expensive to be a competitive alternative in practise and probably has a low security of supply at present. Thirdly, knowledge of the required dose and duration of supplementation of adsorbents do not have any adverse effects on the pig due to binding of essential nutrients as well as to study whether development or other aspects of meat quality may be affected.

3.1.2.2.4 Effective feeding strategies

Of the feeding intervention investigate two approaches seem possible to solve the skatole problem of boar taint. One is to reduce the protein intake by feeding grain only for 4 days before slaughter; the other is to increase the intake of easily fermentable fibre by feeding pigs with inulin supplemented diets the last 7 days before slaughter. In general, all the feed intervention investigated shows only marginal effect on androstenone deposition in adipose tissue and at present it seems not to be possible to reduce androstenone in adipose tissue by feeding.

3.1.2.3 Breeds

The heritability of boar taint is high as reviewed in Chapter 3.4. The level of boar taint compounds in adipose tissue varies considerable within breeds, among breeds and between the European countries as evident from the studies summarized in Table 10 (145, 193-196). Although some of the variation may be attributed to varying methods for chemical analysis, differences between studies in slaughter weight as well as differences in the influence of the weight among breeds (137), it is obvious that at present no single breed, among the high valued Danish production breeds (Duroc, Landrace, and Yorkshire), is well suited for entire male pig production with respect to boar taint.

1								
Trait (μg/g in fat)	Country	Breed	N	Mean	SD	Min	Max	Reference
Skatole	the Nederlands	D	942	0.091	0.097	0.06	0.928	Duijvesteijn et al 2010 (196)
Androstenone		D	943	1.88	1.67	0.07	10.1	
Skatole	Denmark	D	179	0.064	0.099	0	0.745	Gregersen et al. 2012 (193)
Androstenone		D	180	3.53	2.51	0.07	19.83	
Skatole		L	258	0.254	0.203	0	2.551	
Androstenone		L	259	1.318	1.114	0.16	8.83	
Skatole		Y	132	0.045	0.049	0	0.32	
Androstenone		Y	132	0.952	0.914	0.13	6.9	
Skatole	Norway	D	1236	0.06	0.11	0.01	1.51	Grindflek et al. 2011 (194)
Androstenone		D	1266	3.28	2.7	0.05	20.52	
Skatole		L	1878	0.1	0.16	0.01	1.95	
Androstenone		L	1930	1.14	1.1	0.05	13.4	
Skatole	Spain	L	217	0.068	0.114			Varona et al. 2005 (195)
Androstenone		L	217	0.216	0.232			
Skatole	Sweden	Y	143	0.074	0.095	0	0.69	Lundström et al. 1994 (145)
Androstenone		Y	143	1.26	0.94	0.01	4.8	

Table 10. Descriptive statistics of the boar taint compounds, skatole and androstenone, in adipose tissue measured within production cross breeds in different European countries.

N: number of animals; SD: standard deviation; Min, Max: minimum, maximum; D, L, Y: Duroc, Landrace, Yorkshire.

Traditionally, selection has been used as a tool to shape different porcine breeds and this has led to differences in the genetic background. Some breeds are valued for their reproduction or nursing skills while others are valued for their meat related characteristics. The traditional production cross in Denmark takes all of the above into account, but boar taint has not been included in the selection index. In 2006 and 2007 the Yorkshire breed had the lowest level of both androstenone and skatole among the three pig breeds used for Danish swine production, whereas the Duroc breed had the highest level of androstenone and the Danish Landrace had the highest level of skatole. Alternative breeds that may be included in the European pig production are Hampshire or Pietrain. Hampshire has been found to have high levels of androstenone similar to Duroc in the Nederlands, but also high levels of skatole somewhere between the levels found in Danish and Norwegian Landrace entire male pigs (197), although the high level of skatole in Hampshire was not confirmed in a Swedish study (127). In contrast, the Pietrain breed has levels of androstenone comparable to the sow line breeds and skatole levels below the other boar line breeds used in production crosses in Europe (137).

Overall, none of the present breeds is able to abolish the problem of boar taint. As reviewed in Chapter 3.4 the heritability of boar taint is, however, high, although complex, and breeding against boar taint has a high potential in future control of boar taint.

3.1.3 Mode of operation at production level

3.1.3.1 Effect of entire male production on animal welfare

The welfare of EM compared to SC will be improved early in life as the animals do not experience the pain during and after surgical castration procedure. However, the increased level of testicular steroid hormones in EM compared to SC and FE has a number of behavioural effects (198). The most pronounced are increased aggression and sexual behaviour, such as mounting (199). As these types of behaviour may cause social stress and injuries at pen mates, the general animal welfare might be compromised when EM are produced.

The effect of gender on the aggression and sexual behaviour of pigs has been investigated in numerous studies. In Table 11, the results from 11 experiments presented in this chapter are summarised.

In the majority of studies, EM were observed to perform more aggression compared to both FE (128, 200-204) and SC (203, 205-207). However, the effect of gender seems to be less pronounced in more extensive systems. In a study by Thomsen et al. (2012), EM did not display more aggression than FE in two organic herds, and no significant difference between EM and FE in the number of skin lesions was found (208). In a study by Jensen et al. (2005), where EM were raised litter-wise in outdoor tents with access to pasture, the results showed slightly more aggression (nudging) in EM compared to FE, but the level in both sexes was very low (125). The enriched environment, including more space as well as access to straw and roughage in the extensive production systems, could be contributing factors for the apparently reduced effect of gender. This is discussed in further details in Chapter 3.1.3.1.2.



Photo: Rikke Thomsen

Table 11. Overview of studies investigating aggression and sexual behaviour in entire male (RES: restricted feeding, LIB: Ad libitum feeding, MIX: mixed-sex groups, SINGLE: single-sex groups, FTF: Farrow-to-Finish (litter-wise), LW: Live weight, w: week)

REF	Feeding	Group size	Space	Group	Measurements	LW (kg)	Sex	Aggression	Mounts
			allowance	composition		or age		frequency	frequency
			ḿpig⁻¹			(w)			
а	RES	10	0.86	MIX	All interactions,	67 kg²	EM ²	5.91	
					20 min ¹⁾ per pig		FE ²	4.01	
					day⁻¹	2	2		
						94 kg²	EM	5.51	
							FE ⁴	3.80	
b	LIB	15	1.3	SINGLE	All interactions	17 w	EM	27.4°	9.4 ^ª
					/		SC	4.5	0.1
					mounts per pig			2	2
					for 24 h	21 w	EM	27.9°	7.2 ^ª
							SC	9.5°	0.1
с	RES	9	1.2	SINGLE	All interactions ¹	90 kg	MIX	34 ^{ab}	0.6ª
-				MIX	/mounts per 9		EM	24 ^a	2.1 ^a
					pigs for 20 min		FE	19 ^b	
d	RES	14	-	SINGLE	All interactions	75 kg	FE	10.8 ^a	0.35ª
				MIX	per pig hour ⁻¹	0	EM	15.0 ^a	1.77 ^b
					Mounts per 10		MIX	11.8 ^a	1.91 ^c
					min per pig				
					Interactions per		FE ¹⁾	10.3 ^a	
					pig for 9 min ¹⁾ .		EM ¹⁾	14.5 ^b	
							MIX ¹⁾	10.5 ^a	
е	-	-	-	SINGLE	All interactions	5-21 w	EM	More in	More in EM ⁵⁾
							SC	EM ⁶⁾	
f	LIB	11 mean	0.8	FTF,	All interactions	10)	FTF	1.5	0
			min	MIX, SC	per animal for		MIX	2.0	0
					55 min		SC	1.0	0
					(median) ¹⁾			P<0.01	
a	DEC	6		SINGLE	All interactions	11)	ENA	2 6ª	4 0 ^a
б	NL5	Ū	_	SINGLE	ner nig ner h		EF	2.0 1.0 ^b	4.0 0 ^b
h	LIB	12	11	SINGLE	All interactions/	8 ₩	EM	1.0 4.0 ^a	More in FM
	LID	12	1.1	SINGLE	mounts per pig	0 W	EF	1.25 ^b	compared to
					ner 10 min			1.25	EF and SC ⁷⁾
1 ⁸⁾	LIB	15	15	SINGLE	Interactions/	14 w	FM	1 7 ^a	0.3ª
	2.0	10	1.0	0	mounts per pig		FF	2.0 ^a	0.01 ^b
					h ⁻¹			2.0	0.01
J ⁸⁾	LIB	-		MIX	All interactions/	9 w	EM	2.9 ^ª	0.25 ^ª
					mounts per pig		FE	1.5 ^ª	0.03 ^b
					per 24 min ¹⁾ .				
k	LIB	11-12	-	SINGLE	Scan samples	18-24 w	EM	0.015 ^a	0.015 ^a
					between 14-		FE	0.004 ^b	0.001 ^b
		1			16:00 h,				
		1			interactions per				
		<u> </u>			pig day ⁻¹				
I	LIB	20	0.7	SINGLE	Not reported	15-20 w	EM	-	19.7
	1	1		MIX			FE		5.3
	1	1					MIX		11.7
	1	1							P<0.05

References (REF): a) Giersing et al, 2000 (128); b) Cronin et al, 2003 (205), c) Rydhmer et al., 2006 (200), d) Boyle and Björklund, 2007 (201), e) Tuyttens et al., 2008 (206), f) Fredriksen et al., 2008 (207), g) Fredriksen & Hexeberg, 2009 (202), h) Fàbrega et al., 2010 (203), i) Thomsen et al., 2012 (208), j) Jensen et al., 2005 (125), k) Vanheukelom et al., 2012 (204), l) Hintze et al., 2013 (210). 1) during feeding, 2) After third regrouping, 3) After fourth regrouping, 4) only significant at 5 w of age, 5) only significant at 21 w of age, 6) all behavioural data performed 1 to 3 weeks before slaughter and the pigs were slaughtered at 75 kg carcass weight, 7) During the days prior to slaughter, 8) Organic production.

abc: Different superscripts are significantly different (P≤0.05)

It is difficult to evaluate whether the reported incidences of aggressive behaviour are high enough to impair animal welfare if EM pigs are produced. In all of the studies presented in Table 11, the reported incidences of aggressions include mild aggressions like threat, push, lift and head knock. If only more serious aggressions like bites and chasing are considered, 0.7 and 1 interaction per pig per 20 minutes during routine feeding were observed in single-sex pens with EM and mixed-sex pens, respectively (200). Although the prevalence may seem small, the general increase in aggression in EM compared to SC pigs and FE represents a risk of reduced animal welfare in EM production.

The sexual behaviour of EM compared to FE and SC pigs has been investigated in several studies, mainly with focus on mounting behaviour. Mounting events are found to be both more frequent and longer-lasting in pens with EM compared to pens with FE and SC pigs (200, 201, 205, 209, 210). More frequent mounting behaviour of EM compared to FE (125, 208) and SC pigs (107) has also been observed in extensive production systems. Mounting is part of the pigs' normal sexual behavioural repertoire (211) but is also used as dominance (212) and play behaviour (210). Sexual mounts last longer and provoke more screaming by the recipient than non-sexual mounts (210) and may therefore be considered to have a greater impact on animal welfare compared to other mounting categories. However, more research is needed to better understand the impacts of mounting on the recipient as well as on the rest of the pigs within the pen.

Although no direct relationship between sexual behaviour and leg problems has been found (200, 210), frequent mounting is presumed to increase the risk of locomotory problems. Numerous leg problems in addition to increased sexual behaviour have been observed in single-sex EM pens in one study (200), but not in others (209). The risk of leg problems due to increased mounting behaviour is likely to depend on the housing conditions, especially floor type and floor hygiene.

In summary, EM pigs show more aggression and perform more mounting behaviour compared to FE and SC pigs. The prevalence reported may be considered small but the general increase in undesired behaviour represents a risk of reduced animal welfare in EM production. More studies are needed before it is possible to conclude whether production of EM will reduce animal welfare, especially studies that reflect the conditions seen in commercial practice with regard to space allowances, group sizes and group dynamic. Further, it is important to investigate short and long term consequences for the individual pig to be mounted.

3.1.3.1.1 Effect of the social environment on the welfare of entire male pigs

Aggression and sexual behaviour of pigs are presumed to be affected by both the social environment (e.g. group composition and dynamics) and the housing (e.g. space allowance and group size) (213, 214).

Effect of group composition

Mixed housing of EM and FE may lead to pregnant gilts delivered at the abattoir and thus raise some ethical concerns (215). Further, there are indications that the welfare of FE is improved in single-sex housing as the majority of aggressions and mountings are performed by EM (200, 201, 216). It is less clear how single-sex housing affects the welfare of EM. Boyle & Björklund (2007) and Courboulay et al. (2013) concluded that EM in single-sex groups were exposed to more aggression than EM in mixed-sex groups (201, 132), but Rydhmer et al. (2006) found no significant difference in received attacks between EM in single-sex and mixed pens (200). Likewise, it is not clear whether single-sex rearing of EM is an advantage for EM with regard to mounting behaviour. Fewer problems with mounts in single-sex EM groups compared to mixed groups are reported from organic indoor practice (107), but more mounting behaviour has also been reported in single-sex EM groups than in mixed groups (200) and in pens without contact to FE in adjacent pens than in pens with contact (131). The difference between studies in the effect of mixing the sexes on mounting seems not simply to be due to differences in whether the presence of FE accelerates the onset of puberty in EM and thereby increases mounting. In the study of Bonde (2012) androstenone, measured by the human nose test, was lower in mixed-sex groups, indicating a later onset of puberty, but problems with mounts were larger (107). However, in accordance with the suggested influence of onset of puberty, the effect of group composition on mounting frequency may depend on the weight and age of the pigs. Boyle & Björklund (2007) observed significantly fewer mountings in single-sex EM groups than in mixed-sex groups, but only at 75 kg, whereas the opposite was observed at 95 kg and at 100 kg live-weight (201). Another factor able to influence the amount of mounting behaviour is the aggressiveness induced by the actual experimental set-up in different studies as mounting may be a dominance behaviour (212).

Effect of group dynamics

Mixing of unfamiliar pigs is found to increase the aggression level in the initial hours after mixing until a dominance hierarchy has been established (216, 217). As EM in general show more aggression, mixing could have an even more pronounced and undesirable effect in EM than in SC and FE. A Farrow-to-Finish system where pigs are reared litter-wise without mixing is one way of reducing the negative consequences of mixing unfamiliar pigs. Entire males reared in this system showed less aggression 3 weeks before slaughter and had lower skin lesion scores at slaughter compared to EM reared in groups mixed with unfamiliar pigs once after weaning (207). A similar effect may be obtained by allowing two or more litters to socialise from two weeks of age until weaning and thereafter keeping entire males from these litters in stable Wean-to-Finish groups until slaughter (134, 218). Even though Farrow-to-Finish systems may delay the initiation of puberty as discussed in chapter 3.1.2.1.2 this system does not seem to reduce the frequency of mountings compared to a conventional system with mixing (134).

The handling of EM on the farm before slaughter and later at the abattoir may potentially affect the welfare of EM. Split-marketing where pigs from the same pen are sent to slaughter at different times

over a period of time is a very common on-farm procedure to target the planned slaughter weight. This strategy compared to all-out marketing increased the level of aggression more in groups of EM pigs than in groups of mixed sex (201) and groups of FE pigs in some studies (202), but had the opposite effect in a recent study where aggressive interactions were reduced both in groups of EM and in groups of FE pigs (219), maybe due to an interference with space allowance that is increased concurrently with the number of pigs sent to the abattoir. Mixing of unfamiliar EM during rearing and at slaughter increased the frequency of skin lesions compared to no mixing (134). When comparing EM and FE pigs transported to the abattoir together with pen-mates, more skin blemishes were observed on the carcasses of EM (220). Finally, a long pre-loading time may compromise animal welfare as indicated by a positive correlation between time spent on the vehicle after arrival at the abattoir and number of skin lesions in entire male pigs (135).

In summary, the results regarding the effect of the social environment on aggressive and sexual behaviour in EM are few and inconsistent. More knowledge is needed before it is possible to identify the most appropriate group compositions and dynamics. However, there are indications that single-sex rather than mixed-sex housing is beneficial for animal welfare. Further, mixing of unfamiliar pigs should be kept at a minimum from birth to slaughter.

3.1.3.1.2 Effect of housing on the welfare of entire male pigs

Besides the aggression associated with mixing of unfamiliar pigs discussed in 3.1.3.1.1, aggression in pigs is primarily associated with competition for resources of high priority (221). Improved access to resources like space (221, 222), enrichment material such as straw (223-226), roughage (227, 228) and feeder space (229) seems generally to improve animal welfare. It is presumable that these factors will have a similar or perhaps even a more pronounced effect in EM due to the increased agonistic behaviour in EM compared to SC and FE. In accordance, Prunier et al. (2013) found significantly less skin lesions in EM housed in an enriched system (2.5 m² pig⁻¹, straw bedding, outdoor run) compared to a conventional system (1 m²/pig, slatted floor) (230). Furthermore, Thomsen et al. (2012) observed only very few aggressions in organically produced EM with access to an outdoor run, 2.5 m² pig⁻¹ and access to roughage, compared to previous studies with EM housed conventionally (208).

There may, however, be specific drawbacks of an enriched environment on the animal welfare of EM. Salmon et al. (2008) found more aggression in EM housed in a straw yard accommodation compared to un-bedded housing (231). Likewise, Prunier et al. (2013) found a tendency for more mounting behaviour in an enriched environment compared to a conventional environment, with a higher number of received mountings in the enriched environment (230). In both studies, the effects were hypothesized to be caused by an overall higher activity level of the animals in the enriched environment stimulated by the increased space available and the presence of straw.

In summary, a general enrichment of the environment such as access to rooting material, low stocking density and unlimited access to feeding space is presumed to reduce agonistic behaviour and increase the welfare of EM. However, there may be drawbacks of enriched systems with regards to undesired sexual behaviour. There is a need for more knowledge of the effect of specific housing strategies on the agonistic and sexual behaviour of EM.

3.1.3.2 Performance and carcass characteristics of entire male pigs

The production of EM pigs may increase the performance due to increased gonadal steroids synthesis in the EM (232).

Performance

The performance of EM pigs compared to CM pigs has been reviewed thoroughly by EFSA (2004) (199), Lundström et al. (2009) (7) and Millet et al. (2011) (233). In the majority of the reviewed studies, EM were superior in daily gain and feed conversion ratio compared to SC pigs. Entire males had 5 to 13 % higher growth rates and 8 to 14 % improved feed to live weight conversion efficiency. In other studies, however, the daily gain of EM was similar or even lower than the daily gain of SC pigs (e.g. 203, 234-236). The variation in performance differences between EM and SC pigs between studies is probably due to differences in breed, slaughter weight, housing and feeding. The difference between EM and SC pigs in performance traits depends, among other things, on feeding strategy. There are indications that *ad libitum* feeding reduces the difference between EM and SC pigs in growth rate, because the lower feed intake by EM is compensated by a better feed efficiency (233). When comparing the performance of EM and SC pigs under Danish commercial conditions, the differences in performance traits are generally smaller. In Table 12, the results from five experiments conducted in Danish commercial herds and one experiment conducted on a Danish research station are presented (237-239). Regarding daily gain, there seems to be none or only minor differences between EM and SC pigs, in favour of SC pigs. However, in all studies, the EM had better feed conversion ratios compared to SC pigs varying from 4 to 10 % because the lean accretion is larger and their fat accretion smaller.

Carcass characteristics

A recent meta-analysis study including results from 28 studies conducted from 1990 until 2010 concluded that if boar taint was excluded the most marked carcass quality trait differences between EM and SC pigs were related to higher lean meat percentages where the means of weighted differences between EM and SC pigs were 3 percentage units (240). A similar effect was found in a later study where EM had 2.6 percentage units higher lean meat percentage than SC (166). In comparison, seven Danish studies not included in the meta-analysis showed improved meat percentages of EM compared to SC pigs from 0.7 to 3.7 percentage units (Table 12). Consistently, in the majority of studies, the back fat depth of EM was lower compared with the back fat depth of SC pigs (7, 166, 199, 241), although this may depend on breed combination (242).

Another characteristic of entire males is a lower carcass yield (carcass weight as a percentage of live weight) (154, 241, 243, 244). This is partly due to the higher genitalia weight (120). Compared with SC pigs, the carcass of EM is characterised by larger yields of fore-ends but lower yields of middles and legs (245-247). The lower yield of the middle is mainly due to a smaller yield of back bacon (247) as also reported in Kempster & Lowe (1993) (248).

Poforonco			114/	Cov		Daily gain g	ECD	Moot %
Reference			LVV,	Sex		Daliy galii, g	FUN	Wedt 70
	RES	WEI	kg				Feed:gain	
Udesen, 1988 ¹ (237)	RES	DRY	27-sl ³	EM	747 ²	747	2.73	56.1
				SC	746 ²	746	2.83	55.4
	RES	WET	27.5-sl ³	EM	1,780	791	2.54 ^ª	56.5
				SC	1,572	769	2.70 ^b	54.7
	LIB	DRY	30.5-sl ³	EM	1,290	724	2.86	56.4
				SC	1,111	743	3.04	54.1
Andersen & Pedersen,	LIB	DRY	-	EM	48	902 ^ª	2.48 ^ª	60.3 ^ª
1993 (238)				SC	48	913 ^ª	2.76 ^b	58.5 ^b
Maribo & Christiansen	RES	WET	30-110	EM	960	920 ^ª	2.81 ^ª	60.5ª
(2013)				SC	928	929 ^a	2.95 ^b	59.3 ^b
(239)	LIB	DRY	30-110	EM	478	908 ^ª	2.53 ^ª	60.5 ^ª
				SC	449	942 ^b	2.77 ^b	59.2 ^b

Table 12. The performance of entire males (EM) compared to castrated males (SC) found in Danish studies.

¹ Review of three experiments on commercial farms

 2 Half of them female pigs, mixed groups of females and males, performance results average for both females and males

³ Slaughtered (sl) at around 100 kg

^{a, b, c} Figures with different superscripts are significantly different

RES: restricted feeding, LIB: Ad libitum feeding, DRY: dry feeding, WET: Liquid feeding, LW: live weight, n: number of animals, FCR: feed conversion ratio.

In summary, the most profound characteristics of EM in relation to performance and carcass characteristics are improved feed conversion ratios, higher lean meat percentages and lower back fat depths compared to SC.

3.1.3.3 Meat quality (excluding boar taint) of entire male pigs

There is no doubt that boar taint is by far the most obvious disadvantage of the EM production regarding meat quality (Chapter 3.1.2). Although the results are much less pronounced, there are, however, other meat quality differences between EM and SC pigs reported in the literature. In accordance with a meta-analysis including technological and sensory quality traits based on the results from 13 studies, EM have significantly less intramuscular fat (0.55 % points) in the *longissimus dorsi* than SC pigs (240). Less intramuscular fat may ultimately compromise the sensory quality of the meat especially with respect to tenderness and juiciness (243, 249). Consistently, the most pronounced characteristic of EM compared to SC in sensory traits in the meta-analysis (based on 11 studies) was higher shear force values, which is an objective measurement of tenderness, the higher the value the lesser the tenderness (240). The difference in shear force values between EM and SC pigs could, however, not be confirmed in a later study, although home consumers evaluated meat from EM as significantly less tender compared with meat from SC pigs (166). The sensory quality of the pork can

be affected by the protein content of the diet (250-252). Thus, a reduction in the crude protein content from 17 % to 14 % increased intramuscular fat by 0.9 percentage points and improved pork tenderness and juiciness in EM (249).

Entire males are generally considered to be more susceptible to the development of dark, firm and dry (DFD) meat (243, 253, 254). The extreme meat quality condition of DFD occurs if the ultimate pH is larger than 6.0. Prolonged stress and increased activity before slaughter may reduce the glycogen depots and thereby increase the risk of high post mortem pH and consequently DFD meat. Long transport or lairage time before slaughter increases the risk of DFD meat more in EM than in SC pigs, probably due to a higher activity and aggression level in EM (243, 245, 255).

The adipose tissue of EM is characterised by higher concentrations of polyunsaturated fatty acids (7, 199, 240). The higher content of unsaturated fatty acids may be considered positive from a human nutritional perspective but may cause problems with soft fat, especially in lean genotypes and in the organic production where more oil-rich ingredients are commonly used for amino acid supply (254, 256). Soft fat may cause challenges in meat processing and in product appearance when packaged (257).

There seem to be no clear and consistent differences between EM and SC pigs regarding meat colour neither assessed objectively (240) or subjectively (243).

In summary, low intramuscular fat is a well-documented meat quality characteristic of EM. This may in some circumstances compromise the tenderness of the meat. Entire males are more susceptible to the development of dark, firm and dry meat when exposed to stressful conditions before slaughter. Finally, EM have a higher content of polyunsaturated fatty acids than SC. This may cause problems with soft fat in very lean genotypes and in the organic production where more oil-rich ingredients are used for amino acid supply.

3.1.3.4 Working environment in production of entire male pigs

Not much literature exists on the working environment in the production of entire male pigs. In a study by Patterson et al. (1984) the raising of entire male pigs in either single-sex groups or mixed with gilts did not cause management problems of any significance (130). Neither farmers producing EM and FE until the weight of 115 kg did experience more problems with raising EM up to this weight than with pig production based on SC (258). Studies made in Denmark with the production of organic EM did not get any reactions from the farmers concerning problems with the daily management and handling of EM compared to SC pigs (personal experience). Thus, famer's perception of the handling of pigs does not seem to be affected when rearing EM compared to FE or SC.

3.1.5 Concluding remarks

Entire male pig production is production of intact male pigs, with focus on other initiatives than castration to reduce the occurrence of boar taint. The efforts to reduce boar taint may include management, housing, nutrition and genetics.

At present, reasonable strategies for reducing fat skatole may include an improved hygiene of the rearing environment and consequently less fouled animals, but some inconsistency of results exists. However, more alternative skatole-reducing feeding strategies seem available. Thus, withdrawal of feed for more than 6 hours before slaughter reduces skatole and perceives boar taint. In addition, sources of protein with low ileal protein digestibility, such as products based on blood, meat, bone meal, yeast slurry from breweries, or yellow peas, should be minimized as they increase the substrate for intestinal skatole production. Likewise, a reduction of protein intake by feeding grain only for four days before slaughter may reduce the skatole problem of boar taint although more studies on this are needed. Furthermore, supplementation of easily fermentable carbohydrates with low ileal digestibility reduces intestinal skatole production, and the most promising sources seem to be feed components rich in inulin, such as chicory root or Jerusalem artichoke. Addition of these feed components to a diet in an amount resulting in an inulin allocation above 50 g/kg and given in the diet for 7 days reduces the skatole content in back fat with two thirds. It remains to be studied whether addition of these feed components will affect fat skatole if given for a shorter period. Interestingly, chicory root also contains bioactive compounds that increase the metabolism of skatole in the liver. Other carbohydrate sources as raw potato starch have also been shown to reduce skatole but is unable to stand heating and pelleting of the feed.

Strategies that are likely to reduce the level of androstenone include slaughter at a lower weight but it has to be taken into account that the effectiveness of this strategy varies between breeds. Although the social and physical environment as well as stress are likely to affect time of onset of puberty and fat androstenone the literature is not consistent with respect to applicable initiatives, probably due to a complex interaction with factors like weight, age, genetics, nutrition and stress or a combination of some of these. In addition, the feed interventions investigated show only marginal effect on fat androstenone deposition in adipose tissue.

Several other methods of reducing fat levels of boar taint compounds have been investigated, including liquid feeding; dietary supplementation of lupines, sugar-beet pulp, slowly fermentable dietary fibres, non-starch polysaccharide degrading enzymes, antibiotics, organic acids, bioactive components in plant product or adsorbent materials; changing in the dietary ratio of different grain types or the fat ratio and its composition; and choice of breeds. However, at present these methods seem either insufficiently elucidated or inappropriate to use at farm level due to low effectiveness in reducing boar taint compounds.

From a welfare point of view, production of entire male pigs improves the animal welfare in early life due to the omission of the painful surgical castration procedure. However, the increased aggression and mounting behaviour compared to FE and SC pigs may compromise animal welfare as these types of behaviour have the risk of causing social stress and injuries on pen mates. Whether this in practice will lead to an impaired animal welfare is likely to depend on the social environment and the housing conditions. There are indications that aggression can be reduced by single-sex rather than mixed-sex housing and by reduction of the frequency of mixing of unfamiliar pigs combined with a general enrichment of the environment (e.g. access to rooting material, low stocking density and unlimited access to feeding space). This would reduce the risk of serious welfare problems in EM production. However, there may be drawbacks of enriched systems with regards to undesired sexual behaviour.

With respect to performance and carcass quality, production of entire male pigs is characterised by an improved feed conversion ratio, higher lean meat percentages and lower back fat depths compared to SC. However, EM have low intramuscular fat compared to SC, which may ultimately compromise the sensory quality of the meat, especially with respect to tenderness and juiciness. In addition, entire males seem more susceptible to the development of dark, firm and dry meat when exposed to stressful conditions before slaughter.

However, it is difficult to extrapolate the results presented in the current chapter into practice as the experimental circumstances do not always reflect the conditions in commercial herds and a considerable effect of management may be expected, which demands studies in large scale. Therefore, further studies and monitoring activities are needed to elucidate practical applicable strategies to reduce the risk of boar taint and improve animal welfare as well as the general acceptability of meat from entire male pigs. In addition, future research should focus on gaining more basic knowledge on the influence on boar taint, animal behaviour and welfare, as well as meat quality in relation to age and weight at slaughter, different aspects of the social and physical environment, the procedures related to slaughtering and the influence of management and nutrition.

3.2 Alternative methods of castration – immunocastration

The close relationship between boar taint and sexual maturity of male pigs means that non-surgical interventions, making the sexual glands non-functional and suppressing their hormonal production, are likely to reduce the risk of boar taint.

Non-surgical castration of male pigs may be achieved by injection of chemicals, such as acids and salts, into the testis, thereby destructing the testicular tissue. Alternatively, the hormonal activity of the sexual organs may be suppressed by treatments that affect the regulatory hormones of the HPG-axis, which is responsible for reproductive function. This includes treatment with hormones (e.g. prolactin

or gonadotropin-releasing hormone, GnRH) or vaccines that induce an immune response against regulatory hormones of the HPG-axis thereby neutralizing the hormones as in immunocastration (1, 199). However, use of chemicals may be associated with pain to the animal and continued risk of boar taint, and use of drugs with hormonal activity in meat producing animals is considered unacceptable in the EU (1, 199). Therefore, at present only immunocastration (IC) is an available alternative method of non-surgical castration in EU.

More experimental vaccines have been reported but only one of these, Improvac[®] (Pfizer Ltd.), is commercially available and approved for use in EU (259). Overall, the different vaccines work in the same way but the time course of functional castration and the biological consequences vary according to dose, immunogenicity of the GnRH antigen and the adjuvant used (260-263). Therefore, at the level of the consequences of vaccination, the main focus in the following review is on the effects of IC with Improvac[®].

3.2.1 Mode of action by immunocastration

Immunocastration is a functional castration that works through an active immunisation by vaccination against GnRH. This triggers an antibody formation that subsequently neutralizes endogenous GnRH; thereby interrupting the HPG-axis (Figure 4).

Entire males

Immunocastrated males



Figure 4. Following immunocastration antibodies are produced, which obscure the signal from hypothalamus to the pituitary gland by blocking gonadotropin-realeasing hormone (GnRH). This results in an interruption of the hypothalamic-pituitary-gonadal (HPG) axis, responsible for reproductive function, and causes functional castration.

GnRH is a hormone that is released to the pituitary portal system from the hypothalamus and initiates the HPG-axis in the unvaccinated animal by triggering the release of luteinising hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary. In the male, LH stimulates Leydig cell production of steroids (Chapter 2.3), whereas FSH stimulates spermatogenesis in the seminiferous tubuli (264).

It is well documented that the release of LH is almost ceased with time by IC and, consequently, the size and weight of testis and the accessory reproductive glands as well as the production of testosterone, oestrogens and androstenone (steroidogenesis) are markedly reduced and the spermatogenesis is impaired (57, 232, 235, 236, 241, 260-263, 265-292).

Successful IC depends on an adequate formation of antibodies to the vaccine with affinity to endogenous GnRH. This is ensured by a sufficient immunogenic vaccine and provision of a booster vaccination at least 4 weeks after the initial vaccination. The general principle for construction of the vaccine is to create an antigen that contains a GnRH-like peptide that is hormonally inactive but still causes formation of specific antibodies to GnRH. This may be ensured by modification of the amino acid sequence in the region responsible for activation of the GnRH receptor or by polymerization of the GnRH sequence (264). However, the small and self-like antigen is poorly immunogenic. Therefore, the antigen is increased in molecular size and complexity by conjugation to an immunogenic carrier molecule, an adjuvant is added to the vaccine, and the immunogenicity may be further increased by repetition and polymerization of the GnRH sequence (260, 261, 266, 280). Recently, a vaccine based on a recombinant fusion protein has been shown to be highly immunogenic and effective in impairing spermatogenesis and steroidogenesis as well (286, 287). The commercial vaccine, Improvac[®], consists of a synthetic GnRH analogue-protein conjugated to a diphteria toxoid carrier protein in an aqueous dextran-based adjuvant (259).

Studies on vaccination with Improvac[®] have shown that the first vaccination induces a low but detectable antibody response to GnRH (236, 275-278). After the second vaccination with Improvac[®] the antibody response is markedly enhanced within the first week, reaches a maximum at days 8 to 11 and then gradually decreases to a low level at 7 to 8 weeks (275, 276, 278, 279, 288) followed by a further slow decrease over several weeks (276, 277). The first vaccination with Improvac[®] induces a slight decrease in plasma level of LH and FSH (276) that may reach a magnitude able to induce functional castration in some animals within 8 weeks if apparently more immunogenic vaccines are used (293). However, in general a second vaccine is needed to ensure that the majority of animals respond. The second vaccination with Improvac[®] induces a marked decrease in LH and steroidogenesis within the first 1 to 2 weeks (275, 276, 278, 279, 288, 292). At slaughter, 2 to 6 weeks after the second vaccination, the systemic level of testosterone, androstenone and oestrogens is at the same level as in surgical castrates in the majority of pigs (236, 288, 292), and a progressive reduction in size, number and function of the steroid-producing Leidig cells in testes is evident from 2 until 8 weeks after the second vaccination (292).

Signs of a gradual restoration in the release of gonadal steroids may be evident as early as 6 weeks after the second vaccination with Improvac[®] (294). However, in most studies re-establishment of steroidogenesis occurred considerably later with large variation between individuals. Thus, in the study of Claus et al. (2008) return of steroidogenesis, defined as plasma testosterone higher than 0.5 ng/ml, varied between 10 and 24 weeks after the second vaccination, whereas other studies have shown that the effect on steroidogenesis may persist at least until 16 weeks (295) or 22 weeks (277) after the second vaccination.

Histomorphological restitution required at least another 13 weeks (296) and may be evident within 22 weeks after a second vaccination at 18 to 19 weeks of age (280). The ability to restore testicular function probably depends on the timing of vaccination in relation to puberty and sexual maturity. Pre-pubertal or early pubertal vaccination starting at an age of 10 weeks affects testicular size, structure and function more than later vaccination, and complete recovery of testicular structure and function in early vaccinated pigs has been questioned (281, 288). One case of complete restitution and fertility resulting in successful mating has, however, been reported 60 weeks after five vaccinations with Improvac[®] performed 4 to 5 weeks apart from an age of 10 to 16 weeks (274).

Several studies report that the antibody response varies considerably between individuals (e.g. 263, 265, 267, 275, 276, 278, 293), and some animals may not respond sufficiently to the vaccine to reach functional castration within a time course comparable to the majority of individuals (e.g. 263, 273). Based on the available scientific publications on the effect of IC on antibody response, steroidogenesis or size of the reproductive organs, the frequency of non-responders to two vaccinations with Improvac[®] may range from 0 to 2 pigs in small scale experiments comprising 9 to 48 animals (236, 269, 290-292, 297). However, the low number of animals in these studies does not allow assessment of the occurrence of non-responders at production level. In a large scale study 2 of 270 IC pigs, corresponding to less than 1 %, were non-responders (273), but further studies on this issue are needed. The frequency of 'non-responders' may, besides the intrinsic individual responsiveness, depend on health status and ability to mount an immune response at the time of vaccination as suggested by Zeng et al. (2002b), who reported that 2 of 4 non-responders had serious diarrhoea at the time of the second vaccination (263). In addition, some individuals may escape vaccination in field condition (298), and inadequate storage and handling are likely sources of error as the vaccine should be kept refrigerated and protected from light (259).

An effective suppression of the HPG-axis of growing male pigs has been demonstrated with booster vaccination with Improvac[®] from an age of 14 weeks (288) and upward (e.g. 277, 292) as well as in sexually matured male pigs (299, 300). As usual with booster vaccination, an interval of 4 weeks between the first and the second injection is considered optimal and in most studies it has been 4 to 6 weeks. However, based on testis weight and steroidogenesis at slaughter, successful suppression of the

HPG-axis by Improvac[®] can be obtained with an interval between vaccination of up to 9 to 10 weeks (e.g. 284, 285, 289, 290, 292, 301).

3.2.2 Practice by immunocastration

The practical experience with immunocastration in Europe is limited (Chapter 3.2.4). According to the manufacturer's instruction, Improvac[®] shall be injected subcutaneously in the neck behind the ear at least twice in a dose of 2 ml and may be given as early as 8 weeks of age. To ensure sufficient time for clearance of boar taint components in all individuals, without risk of partly restoration of testicular function, it is recommended that the second vaccination is given within 4 to 6 weeks before slaughter, but the risk of boar taint is stated to be minimal up to 10 weeks after the second injection (259). Furthermore, the manufacturer advises that only healthy animals should be immunized (259), presumably to ensure optimal immunological responsiveness.

Due to the risk of self-injection by the farmer and needle stick injury (Chapter 3.2.4.4), which may cause similar effects in humans as in pigs, the vaccine must be given by a safety vaccinator with a dual safety system providing both a needle guard and a mechanism to prevent accidental operation of the trigger. Use of a short needle giving 12 to 15 mm penetration is recommended (259).

3.2.3 The effect of immunocastration on boar taint

The functional castration by IC implies a markedly reduced plasma level of androstenone and, consequently, a progressive elimination of accumulated androstenone in the adipose tissue within approximately 2 to 3 weeks (Chapter 2). In addition, the suppressed testicular steroidogenesis cancels the inhibitory effect of testicular steroids on hepatic skatole metabolism, thereby increasing the skatole metabolism and indirectly reducing fat skatole (Chapter 2). The effect on hepatic skatole metabolism is more pronounced at immunization at 10 to 14 weeks of age than at 16 to 20 weeks of age (57).

Recent studies show that fat androstenone and skatole in IC pigs are reduced below the sensory detection thresholds within 2 weeks after the second vaccination with Improvac[®] with no further decrease until 6 or 8 weeks after the second vaccination (292, 294). In addition, avoidance of boar taint requires that the pigs are slaughtered before the above mentioned gradual restoration of the HPG-axis resulting in an increase of boar taint components in fat above the sensory detection limit. As return of steroidogenesis in Improvac[®] vaccinated pigs may start 10 weeks after the second vaccination in some animals (276), slaughter before 10 weeks after the second vaccination should be safe with respect to boar taint. This seems to be true at least in early vaccinated IC pigs that receive the first vaccination at 10 to 11 weeks of age (288, 295), whereas an interval from the second vaccination to slaughter on 16 weeks increases the number of animals with potential boar taint (295).

According to a meta-analysis on the effect of GnRH vaccines in general on boar taint compounds, based on reports published before July 2011, IC leads to slightly higher skatole levels and tends to

cause slightly higher androstenone levels than SC (includes results from 11 reports; 302), but compared to production of EM, IC results in considerably lowered levels of boar taint compounds in adipose tissue, especially with respect to androstenone (includes results from 15 reports; 302). However, the majority of reports on IC with Improvac[®] published later than July 2011 or for other reasons not included in the meta-analysis, show that IC and SC have comparable levels of fat skatole (288, 291, 303-305) and fat androstenone (288, 290, 291), although slightly higher fat androstenone in IC than in SC pigs has been found as well (305). Compared with EM, IC with Improvac[®] effectively reduces fat androstenone and fat skatole (269, 288, 290-292, 304, 305).

In most studies on Improvac[®] IC pigs had androstenone and skatole below 1 $\mu g/g$ and 0.25 $\mu g/g$, respectively (232, 236, 267, 269, 277, 282, 285, 288-290, 292, 294, 301, 304-306). However, two onfarm studies have shown that the content of fat and rostenone may be above $1 \mu g/g$ in some pigs (2 of 270 pigs in reference no. 273; 2 of 50 in reference no. 303), and in an international study conducted by the manufacturer of the vaccine, including Spain, Germany, Denmark, Hungary and United Kingdom, 3 of 639 IC pigs, i.e. about 0.5 %, exceeded 0.2 $\mu g/g$ of skatole, whereas none exceeded 1 $\mu g/g$ of androstenone (283). The majority of animals with androstenone levels about the threshold were considered to be non-responders to the vaccine (Chapter 3.2.1) but at farm level there is also a risk that some pigs escape vaccination and therefore have a risk of developing boar taint. With early IC, nonresponders and pigs that have escaped the second vaccination are possible to identify by behaviour as well as testis size (266, 288, 307) and may therefore be re-vaccinated before slaughter (307). Alternatively, they may be sorted at the slaughter line on basis of the size of reproductive organs responding fast to IC, such as the seminal vesicles (288, 291, 298), but the efficiency of the method as well as the possibility for implementation on the slaughter line remain to be tested. With late vaccination, atrophy of reproductive organs is less (288), and assessment of boar taint or concentration of boar taint compounds has to be performed at the slaughter line (1, 199).

According to sensory tests of boar taint including the hot iron method, most studies confirm that meat from Improvac[®] vaccinated pigs is rated as acceptable and comparable to meat from SC pigs (155, 166, 284, 301, 306, 308), whereas other studies indicate that 3 to 10 % of group housed IC pigs may be perceived as having slight to strong boar taint as opposed to none or only a very small proportion of SC pigs (273, 282, 303). In the study of Jaros et al. (2005), none of the tainted IC pigs (10 %) would, however, have been rejected in Switzerland (273), and in the study of Fuchs et al. (2009), none of the pigs perceived as tainted (3 %) would have been rejected on basis of chemical analysis (282). Andreasen & Maribo (2012) did not inform the percentage of tainted meat but the negative ratings could, in general, not be explained by exceeded threshold for androstenone (303). This lack of consistency between chemical measures of boar taint compounds seems to be a general problem in relation to boar taint (Chapter 2.1) and needs further investigation to be clarified.

In relation to boar taint, the concerns associated to IC seem to be the occurrence of the so-called, nonresponders, that have too high fat levels of one or both boar taint compounds and have to be presorted at farm level or sorted at the slaughter line. In research context, non-responders account about 1 % but they may be more frequent with use of the method in large scale where several sources of error may act. Furthermore, as in general an additional small part of the meat may be perceived as tainted by a sensory panel, but data on this issue are scarce.

3.2.4 Mode of operation of immunocastration at production level

Improvac[®] is approved for use in pigs at many markets, including Australia, Brazil, Chile, China, Costa Rica, El Salvador, EU, Guatemala, Japan, Korea, Mexico, New Zealand, Norway, Panama, Philippines, Russia, South Africa, Switzerland, Thailand and Venezuela, but the practical use of the vaccine is still limited (109, 264) as the consumers are expected to be sceptical to the method. The experience at the level of farm, abattoir, food processing and retailer is therefore also restricted.

Immunocastration has been performed since 1998 in Australia (109) and about 40 % of the male pigs in Australia (approximately 0.9 million per year in 2013) are estimated to be immunocastrated (309). In addition, IC has been used in New Zealand, Brazil and Mexico (310), and the largest Belgian retailer has decided exclusively to sell meat from vaccinated pigs from 2011 (311).

3.2.4.1 Effect of immunocastration on animal welfare

From a welfare point of view, IC has obvious advantages compared with SC due to the avoidance of the surgical procedure. The local reaction at the injection site is reported to be limited and is not considered to threaten welfare if the vaccine is provided in an aqueous adjuvant (232, 265, 267, 270, 283) as is the case with Improvac[®] (259). As the vaccine is directed against hormones produced by the animal, there may be a risk of tissue damage away from the injection site or testis. Lesions in the hypothalamus were demonstrated in the study of Molenaar et al. (1993) using another vaccine than Improvac[®] (312) but this side effect was not verified in a second study on the same vaccine (266). Nor lesions in major organs, pituitary or brain were evident in the study on Improvac[®] by Hilbe et al. (2006) (274).

However, IC entails that the pigs behave as EM until approximately 1 week after the second vaccination (205, 209, 313, 314) which is recommended to be performed 4 to 6 weeks before slaughter by use of Improvac[®]. In single-sex groups this means increased behaviour directed at pen mates (209, 313, 315), increased aggression (205, 313, 315, 316), increased skin lesions (209, 317) and increased mounting (205, 209, 313, 315, 316) compared with SC pigs during 60 to 70 % of the growth period from 30 to 110 kg. In addition, IC pigs may be more active (205, 316) and spend less time feeding before the second vaccination than SC pigs if feed is offered *ad libitum* (305, 317). Within 1 to 2 weeks after the second vaccination, activity, behaviour directed at pen mates, aggression, mounting and skin lesions decrease to a level corresponding to SC pigs (203, 205, 209, 277, 313, 315-317). The lowered

aggression of the IC pigs after the second vaccination is also reflected in a lower level of skin lesions at slaughter compared with EM (203, 236, 267, 290, 291, 315, 318). In addition, in *ad libitum* fed IC pigs, the time spent feeding increases after the second vaccination to the same or an even higher level than in SC pigs (205, 305, 317).

From a welfare point of view, it is obvious that the second vaccination should be done as early as possible to decrease aggression, mounting behaviour and the concomitant risk of skin and leg injuries on pen mates and social stress (Chapter 3.1.3.1). As mentioned above, a Swedish study showed that the desired effects on boar taint can be achieved by allocation of the second vaccination about 10 weeks before slaughter (288), which limits the period with increased aggression compared to SC to about 30 % of the growing period (30 to 110 kg). However, an early second vaccination compromises the potential spin-off benefits of IC compared to SC, such as lowering of abdominal fat, which may be obtained by giving the second vaccination 4 to 6 weeks before slaughtering (315).

Furthermore, the negative effect on welfare of the 'male behaviour' before the second vaccination is likely to be ameliorated by optimizing the social and physical environment as described for EM production (Chapter 3.1.3.1). Specifically for IC pigs, it has been shown that skin lesions are reduced by *ad libitum* feeding if the pigs only have access to a single space feeder (319). In addition, skin lesion score has been shown to be lower in IC pigs housed in mixed-sex groups compared to IC pigs housed in single-sex groups in the period before the second vaccination (317), but generally, the effect on aggression of group composition with respect to sexes is obscure as described for EM production (Chapter 3.1.3.1.1).

The importance of space allowance, which in general has a profound effect on aggression (e.g. 320, 321), has not been elucidated in relation to sex in the part of the rearing period relevant for IC. In the studies on IC that informed on space allowance and documented the negative effects before the second vaccination on the level of aggression, space allowances were from 0.87 to 2.45 m² per pig (205, 313, 314, 316, 317). The legal space requirement and practise in Denmark are 0.55 m² for pigs at 50 to 85 kg, 0.65 m² for pigs at 85 to 110 kg and 1 m² for pigs with a live weight above 110 kg. Thus, the negative effect of IC on aggression may be magnified under the typical conditions in Danish production herds. However, the relationship between aggression and space allocation is not linear (321), and the overall welfare impact of IC under production conditions has to be experimentally assessed.

Overall, IC eliminates the pain in relation to the surgical procedure of SC, but the EM-like behaviour of IC pigs until the second vaccination means increased aggression and mounting in 60 to 75 % of the growth period compared to SC if they are housed and managed in the same way as SC pigs, which may compromise animal welfare. Improvement of the social and physical environment as well as earlier vaccination may considerably improve animal welfare; the latter by decreasing the period with increased aggression to about 30 % of the growth period from 30 to 110 kg. However, studies

comparing the overall costs for animal welfare of IC during different conditions and of SC are lacking. In addition, there is a need for field trials elucidating the function of IC during farm conditions.

3.2.4.2 Effect of immunocastration on performance and carcass traits

Performance

Immunocastrated pigs perform as EM until the functional castration is established shortly after the second vaccination. This means that IC pigs have lower feed intake and better feed conversion than SC pigs until this time as reviewed by Millet et al. (2011) (233) and recently confirmed by most other studies on Improvac[®] (203, 285, 289, 291, 319, 322,) as well as by a meta-analysis on the effect of GnRH vaccines in general, based on data from 24 to 26 reports published before July 2011 (291). The magnitude of the effect may, however, differ between breeds (322, 323).

After the second vaccination with Improvac[®] the feed intake of *ad libitum* fed IC pigs increases to a level that is comparable with SC pigs (233, 285, 289, 291, 319, 322-324) or is even higher (203, 318, 325), and the feed intake is higher than in EM (233, 285, 289, 291, 318, 319, 326) and in FE (285, 322, 326). The increased feed intake is considered to be due to elimination of the inhibitory influence of testicular hormones on appetite (233). The average daily feed intake in IC pigs increases from 2 to 6 weeks after the second vaccination (294).

The EM-like metabolism gradually changes following the second vaccination. Thus, plasma urea nitrogen gradually increases even before the feed intake starts to increase (275, 279), indicating that the rate of lean tissue deposition decreases. This trait is, however, only slightly decreased for approximately 4 weeks after the second vaccination (327) and the feed conversion is therefore higher than in SC. Thus, the feed conversion ratio is improved compared to SC pigs for at least 4 to 6 weeks after the second vaccination (233) as confirmed in a recent meta-analysis of the effect of Improvac[®] in group housed pigs (327) as well as by other recent studies on Improvac[®] not included in the analysis or the review by Millet et al. (2011) (291, 322, 324), although the difference did not reach significance in all studies (285, 289). Feed conversion ratio was neither significantly affected by the time interval from the second vaccination to slaughter on 2, 3, 4 or 6 weeks (294) nor by the age at vaccination (267, 315) and at slaughter (267). In general, but with few exceptions (305, 315), IC with Improvac[®] improves feed conversion ratio during the fattening period compared to SC pigs (203, 232, 233, 285, 289, 291, 303, 322, 323) although it may not reach significance (236). With a single exception (305), feed conversion ratio in IC pigs is comparable (285, 303, 323, 326) or even better (285) than in FE.

The improved feed conversion ratio, in spite of ceased steroidogenesis after second vaccination in IC compared to SC pigs, may be related to an increase in growth hormone before functional castration, which may persist for 5 weeks after the second vaccination, although not accompanied by an increase in Insulin-like Growth Factor-1 (IGF-1) (271). However, in the study by Batorek et al. (2012b), IGF-1 in

IC pigs was higher than in SC pigs but lower than in EM (291). Furthermore, the different state of IC pigs and SC pigs at the time of the second vaccination with respect to body composition and feed intake has been suggested to affect feed conversion (233).

Frequently, the EM-like feed conversion ratio of IC pigs combined with a feed intake comparable with SC pigs in *ad libitum* fed IC pigs results in an increased growth of *ad libitum* fed IC pigs after second vaccination compared with SC pigs (e.g. 233, 291, 297, 318, 322-324) as well as with EM (e.g. 233, 291, 318). In addition, the second vaccination has been reported to reduce the variation in live weight of IC pigs compared to EM and SC pigs (318).

Carcass characteristics

In the majority of studies, back fat thickness and lean meat percentage are numerically intermediate in IC pigs compared to the more fatty SC pigs and leaner EM as reviewed by Millet et al. (2011) (233). This has been confirmed by a meta-analysis on the effect of GnRH vaccines in general, based on 22 to 24 reports published before July 2011 (302) and on group housed pigs vaccinated with Improvac[®] (327). This relationship is also consistent with most studies on the effect of Improvac[®] not included in the meta-analysis or the review (166, 291, 328). According to Millet et al. (2011), the differences between IC pigs, SC pigs and EM in back fat thickness and lean meat percentage are mainly due to the increased feed intake in IC pigs but EM-like maximal protein deposition rate and feed conversion (233). In agreement with this interpretation, restrictively fed IC pigs, vaccinated according to the manufacturer's recommendation, have a numerical or statistical higher lean meat percentage and lower back fat thickness than *ad libitum* fed IC pigs (291, 319) and restrictively fed SC pigs in the majority of recent studies with Improvac[®] (273, 284, 315, 329), and they have a lean meat percentage that is comparable with restrictively fed EM (315). However, if vaccinated at an early age, 6 weeks before the standard time of vaccination, the lean meat percentage was lower in restrictively fed IC pigs than in EM (315).

In studies including FE, the back fat thickness of IC pigs is higher (326) or comparable with FE (241, 285, 297, 322, 323), and the lean meat percentage is lower (241) or comparable with FE (285, 297, 322, 328).

In addition to restrictive feeding back fat thickness may be lowered in IC pigs by decreasing the time from the second vaccination to slaughter from 6 to 4 weeks (294). Consistent with this, restrictively fed IC pigs vaccinated at the standard time 5 to 6 weeks before slaughter did not differ in lean meat percentage from restrictively fed EM, whereas restrictively fed IC pigs vaccinated 4 weeks earlier than standard had lower lean meat percentage than restrictively fed EM (315).

In most studies on Improvac[®], the carcass yield of IC pigs equals the carcass yield of EM (285, 315, 318, 330) or may even be lower than in EM (241, 267, 236). Thus, the carcass yield of IC pigs is lower

than that of SC pigs as reviewed by Millet et al. (2011) (233), demonstrated in a meta-analyses on the effect of GnRH vaccines in general (302) and on group housed Improvac[®] vaccinated pigs (327), and confirmed in studies on Improvac[®] not included in the analyses and the review (285, 327). The low carcass yield of IC pigs is suggested to be due to factors such as increased gut fill (267, 327), intermediary weight of testis and accessory reproductive glands or reminiscence (e.g. 265, 35, 241, 288), intermediary or high EM-like muscle mass and weight of head, shoulder, feet and liver (232, 241, 265) and intermediary (315) or high abdominal fat (236). Some of these factors are negatively influenced by increasing time from the second vaccination to slaughter, e.g. abdominal fat (315), whereas other factors are positively affected by time as is the case with the size of the reproductive glands (e.g. 292, 294). Thus, the carcass yield is unaffected by the time from second vaccination to slaughter within a time span of 2 to 6 weeks (294, 329).

In general, the overall performance is affected by the feeding level (*ad libitum* versus restrictive), the level of energy and the nutrient supply in relation to requirements (233). Due to the different level of testicular steroids and related differences in metabolism, the nutrient requirements of IC pigs differ from EM and SC pigs, and dose-response studies on nutrient requirements and determination of growth curves are needed for optimizing the performance of IC pigs (233). Based on meta-analyses of previous studies, Dunshea et al. (2013) suggest that the lysine level of the feed would be optimized by increasing the lysine level for IC pigs compared to FE by 5 % from 25 to 50 kg and by 8 % from 50 to 95 kg live weight but decreasing it by 6 % from 95 kg to slaughter (327).

However, performance and carcass quality are improved by IC compared to SC, especially if the pigs are restrictively fed after the second vaccination. Obviously, the overall performance, as well as carcass quality, depends on the time of the second vaccination, as a late second vaccination extends the period of achieving advantage of the anabolic effect of the testicular hormones. The desired effect on boar taint can be obtained by carrying out the second vaccination as late as 2 weeks before slaughter (Chapter 3.2.3), but late vaccination extends the period with EM-like behaviour with the risk of reducing the welfare (Chapter 3.2.4.1).

Thus, the impact of IC on performance and carcass traits indicates that IC has the potential of improving feed conversion, increasing lean meat percentage and reducing back fat thickness compared to SC if an appropriate feeding strategy is used, but it reduces the carcass yield. A short duration of the period from second vaccination to slaughtering, specific feeding strategies and selection of specific breeds favour performance and carcass quality.

3.2.4.3 Effect of immunocastration on meat quality and safety

Immunocastrated pigs do not differ consistently from SC pigs in meat quality measures such as intramuscular fat, drip loss, shear force, ultimate pH or colorimetric parameters according to a metaanalysis on the effect of GnRH vaccines in general, based on 12 reports published before July 2011 (302) and later studies on Improvac[®] (290, 291, 308, 318, 329). However, Aluwé et al. (2013) found increased redness, drip loss and cooking loss in IC pigs compared with SC pigs (166). Differences between studies may to some extend be due to differences between breeds (323).

Only few reports concern the consumers' sensory evaluation of meat quality of IC pigs. The acceptability of pig meat from IC pigs depends on country, gender and age of the consumers (306). In studies from Spain and Korea, the sensory evaluation was comparable for meat from IC pigs, SC pigs and FE (301, 306, 308), whereas Belgian and Swiss tasters perceived IC meat as less juicy than SC meat (155, 166), and the Belgian tasters tended to prefer meat from SC pigs over meat from IC pigs (166). According to the study of D'Souza & Mullan (2003), the effect of the eating quality assessed by Australian consumers may depend on the genotype of the pigs and for some genotypes, SC pigs are preferred over IC pigs, whereas the reverse is the case for other genotypes (331).

Compared with EM the meat from IC pigs may have higher intramuscular fat content and drip loss and lower shear force according to a meta-analysis of data from five to seven reports on the effect of GnRH vaccines in general (302). This is, however, not confirmed by later reports on Improvac[®] (166, 290, 291, 308). In the Korean study, the overall acceptability of meat from IC pigs was also comparable with meat from EM (308), whereas IC meat was rated higher than EM meat in Spanish studies (301, 306) as well as for one of two Australian genotypes (331). The lower acceptance of meat from EM in the Spanish studies was not simply due to a higher content of boar taint compounds in fat (306). The meat of IC pigs was scored as being more juicy and tender than meat from EM (301), which was also confirmed by the Belgian study (166) but not in a Swiss study (155).

The fatty acid composition of the meat seems not regularly affected by IC (232, 297, 329, 332, 333).

There is no risk for human health by eating meat from IC due to potential vaccine residues in the meat. The vaccine has no hormonal activity in itself as demonstrated in a sheep model (334) but acts only through the induction of an anti-GnRH vaccine response. Furthermore, the vaccine is not able to pass the intestine, as pigs and rats orally fed with the vaccine did neither show a vaccine response, a changed serum testosterone nor any other abnormal signs (334). Thus, the withdrawal period after vaccination with Improvac[®] is 0 days (259). In addition, the reduced level of sex hormones after IC does not seem to affect hepatic enzymes related to the metabolism of drugs which indicates an unchanged detoxifying ability and unaltered risk of drug residues in the meat, but further studies in this area are needed (335).

Overall IC neither seems to compromise technological meat quality traits or sensory quality compared to SC. Compared to EM, meat from IC pigs may be rated higher with respect to quality aspects. There is no risk for vaccine residues in the meat.
3.2.4.4 Effect of immunocastration on working environment

As GnRH is conserved across most species of mammals (336), the vaccine does not only work in pigs but also in humans, and IC can therefore be problematic from a safety point of view. It is recommended to use a safety vaccinator, which is a safety device including a needle guard and a mechanism to prevent accidental operation of the trigger (259). In addition, it is dissuaded to carry out vaccination after an accidental first self-injection, during pregnancy or by women who may be pregnant (259). However, according to Campbell (2007), self-injection is a rare event with any compound, and no cases of self-injection by GnRH vaccine were reported in Australia in 2007 after use of the vaccine in pig production for 7 years (307). According to the Danish distributor of the vaccine reported cases of self-injection is less than 0.5 per million doses administered; most of the reported symptoms after exposure are transient and similar to symptoms reported with other injectable products and none have resulted in lasting or severe adverse effects (337).

3.2.5 Concluding remarks

Immunocastration is the only non-surgical method of castration that at present is available in EU and only one commercial vaccine, Improvac[®], exists. The vaccine is injected subcutaneously in the neck twice at least 4 weeks apart with the second vaccination given within 4 to 6 weeks before slaughter. The vaccination results in a functional castration that works through an active immunisation by vaccination against GnRH, thereby interrupting the HPG-axis, which is responsible for reproductive function and production of testicular steroids involved in boar taint.

In relation to boar taint, the vaccine generally seems to abolish the problem of boar taint efficiently with respect to the content of androstenone and skatole in fat, but one concern is the occurrence of the so-called non-responders that may have too high fat levels of one or both boar taint compounds and have to be pre-sorted at farm level or sorted at the slaughter line. In research context, non-responders account about 1 %, but un-successful castration may be more frequent with use of the method in large scale where several management related sources of error may act, such as vaccination of diseased animals, some animals escaping vaccination and inadequate storage and handling of the vaccine. Furthermore, an additional small part of the meat may be perceived as tainted by a sensory panel although the thresholds for androstenone and skatole in fat are not exceeded, but data on this issue are scarce.

Overall, IC eliminates the pain in relation to the surgical procedure of SC, but the entire male-like behaviour of IC pigs until the second vaccination means an increased risk of aggression and mounting with the risk of causing social stress and injuries to pen mates in 60 to 75 % of the growth period if they are housed and managed in the same way as SC pigs, which may compromise animal welfare. Improvement of the social and physical environment as well as earlier vaccination may considerably improve animal welfare. Studies comparing the overall costs for animal welfare of IC during different conditions and of SC are lacking.

In addition, IC has the potential of improving feed conversion, increasing lean meat percentage and reducing back fat thickness compared to SC if an appropriate feeding strategy is used, but it reduces the carcass yield. A short duration of the period from second vaccination to slaughtering, restrictive feeding for the last 4 weeks before slaughtering and selection of specific breeds favour performance and carcass quality. Apparently, IC neither seems to compromise technological meat quality traits or sensory quality compared to SC and meat quality traits other than boar taint may be rated higher than in meat from EM. There is no risk for vaccine residues in the meat, but the vaccine works in humans as well as which means that there is a risk of adverse effects on the reproductive system for the farmers if two times of self-injection should happen. It is therefore dissuaded to carry out vaccination after an accidental first self-injection and none lasting and severe symptoms after exposure to self-injection have been reported.

The majority of previous studies have, however, only included few animals, and the experience within EU of IC at farm level is restricted. Although IC seems acceptable in relation to boar taint, only few published data independent of the vaccine manufacturer on use in large scale confirm that this is likely to be the case in European or Danish pig production. Therefore, the effect of IC on especially boar taint but also performance, carcass quality and animal welfare, at large scale needs to be assessed in field testing independent of the vaccine manufacturer with sufficient animal numbers and including more commercial herds to evaluate the impact of variation in management. These studies should also take differences in practice of production into consideration in order to refine the production environment and working environment. To improve the profitability of IC at farm level, making IC a reliable economic alternative to SC at the same time as securing the economic latitude of improving animal welfare by reducing the risk of increased aggression and mounting compared to SC, and to improve the efficiency of utilisation of resources, dose-response studies on nutrient requirements and determination of growth curves are desirable for optimizing the performance of IC pigs. Preferentially, these studies should also take meat quality traits into consideration.

3.3 Sperm sexing

Sexing of sperm before insemination to produce only female pigs can be an attractive alternative to avoid boar taint as it does not compromise animal welfare, although some may consider it to be an unacceptable manipulation from an ethical point of view.

3.3.1 Methods for sperm sexing

Biological background

Each spermatozoon is carrying either an X or a Y sex chromosome, while the oocyte always carries an X sex chromosome. Sorting the spermatozoa in an ejaculate into those carrying either the X- or the Y-

spermatozoa will therefore be a way to decide the sex of the offspring at insemination through use of either X- (giving females = XX) or Y-spermatozoa (giving males = XY).

The two methods

Different methods have been tried to sort the spermatozoa into X or Y. However, currently only two still seem to be realistic: (a) immunological or (b) physical.

Ad (a). This is based on an expectation that on the cell surface there are protein markers specific for males and for females (e.g. 338). Therefore, specific antibodies to the protein markers can be bound to the surface of the spermatozoa in an ejaculate, so the spermatozoa afterwards can be sorted according to which antibody it has been coupled to, for example using separation by centrifugation (338). The method is a fairly simple technical approach because it is the whole ejaculate that is handled.

Ad (b). This is based on the difference in DNA-content between X- and Y-spermatozoa, that is around 3.6 % (e.g. 339). The chromosomes in each spermatozoon are first stained with a fluorescence marker that emits light when stimulated by a laser beam. The sperm sample is then passed into a sorting machine, where each spermatozoon is lying in its own media droplet in a long narrow line. These droplets are then passed through a laser beam, so the sperm's chromosome is sending out light. This light amount is measured and the result is used to turn the droplet to one side or the other side depending on the amount of light; the more light, the more chromosomal DNA - so most light comes from the X-spermatozoa (339).

The method is a fairly complex technical approach because it is each single spermatozoon that is handled during sorting. Furthermore, the method is a mechanical and physical stress for the spermatozoa, and it can result in a reduced motility or viability of the sorted sperm (339, 340).

3.3.2 State of the development of the methods

Ad (a). Different attempts have been made over the last approx. 30 years (e.g. 338), and also several companies have been working on the method for shorter periods. One recent example was the company Ovasort Ltd. (based in Wales, UK - 341) that was also collaborating with the Danish Agriculture and Food Council. However, in 2012 this project was stopped as no significant progress was expected within a reasonable future (342).

Ad (b). This sorting method has been possible for approx. 30 years, and today it has been proven to work in several species, also with boar semen (339). The present sorting speed is approx. 8,000 spermatozoa/second with a precision around 90 % (343, 344). Piglets have been born after use of sorted semen, but both pregnancy rates and litter size are reduced to approx. 70 to 90 % of the level after use of non-sorted sperm. The lifespan of sorted sperm is also reduced, and this is even more pronounced after cooling, which is a necessary process in relation to semen delivery in practice.

Because of the sorting speed - that is limited in relation to the number of spermatozoa needed in a normal insemination dose (approx. 2 billion) - a parallel development has been made to inseminate with reduced number of spermatozoa (345). Equipment has been developed to perform a so-called deep insemination where the sperm is deposited almost at the end of the uterine horn (e.g. 346). Such equipment is a very long (more than $1\frac{1}{2}$ m) and flexible catheter, and reports are available where acceptable pregnancy rates and litter sizes have been obtained with this equipment using reduced semen doses (approx. $\frac{1}{2}$ million).

3.3.3 Unsolved problems, research needs and time horizon

Ad (a): It seems from the literature that at present no further attempts are on-going to use this method for sperm sorting, and certainly not in commercial practice. New, basic knowledge is needed related to the differences in presence of surface markers on X- versus Y-spermatozoa.

Ad (b): This sorting method does work, also in pig, but the main challenge seems to be the limited sorting speed in relation to the high number of spermatozoa needed for insemination in pigs (340, 345). Research therefore continues to increase the sorting speed in parallel with further development of equipment that makes insemination possible with reduced sperm doses as there is a need to establish more convincing results with use of deep insemination under practical conditions with focus on the resulting pregnancy rates and litter size together with stability of results and safety for the animal.

It can be expected that it will take at least 2 to 5 years before acceptable results in relation to use of deep insemination with sex-sorted boar semen may be available in practice.

3.3.4 Concluding remarks

The use of sex sorted boar semen is not expected to be practically useful at least within the next 2 to 5 years. More research is needed in the area, and some new and major breakthroughs must be made to exploit the potentials of the methods.

3.4 Breeding as an alternative to surgical castration

As the level of boar taint in entire males generally shows a high heritability it should be possible to reduce boar taint through breeding. However, an effective breeding strategy has to take the complex nature of the genetic traits underlying boar taint as well as associated traits into account.

3.4.1 State of the art

Genetic factors significantly affect the level of boar taint. This is most clearly realized by population differences in subcutaneous fat levels of skatole and androstenone as well as their different heritability estimates. The heritability for skatole has been found to vary between 0.23 to 0.89 among different breeds of Landrace, Duroc and commercial sire lines (194, 195, 347-351), although most values are estimated within the range of moderate heritability. The heritability for this trait (194, 195, 247-251). These levels of heritability strongly suggest that a reduction of both compounds is possible by selection. Moreover, the two compounds have been found to be moderately correlated in several studies. Hence it has been suggested that it is preferable to put the breeding emphasis on both compounds (350, 351).

In 1994, it was suggested that boar taint was influenced genetically by a major gene (145). However, additional studies of genetics in relation to boar taint identified the trait to be controlled by more genes, which makes selection of the trait more challenging. Several studies have used genetic markers to identify chromosomal regions associated to skatole and androstenone levels, the so-called quantitative trait loci (QTL) (193, 195, 196, 353-354). In addition, a commonly used measure that provides a skatole-equivalent, containing both indole and skatole, has been studied, and some of the QTL reported overlap those previously identified for skatole (193). Numerous chromosomal regions affecting skatole (Sus scrofa chromosome (SSC) 1, 3, 5, 6, 7, 8, 9, 10, 11, 13 and 14) and androstenone (SSC 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15 and 18) have been identified, but only little overlap between breeds is observed and great differences in the effect of the QTL exist. However, as the effect of the QTL is often reported as a log value of the compound level, it is difficult to evaluate the actual effect on the phenotype. In the study by Duijvesteijn et al. (2010), the QTL identified on SSC6 showed a high difference in androstenone level between two homozygous groups of a commercial Duroc-based sire line of pigs (0.66 μ g/g), and there was a clear difference in the number of animals having high level of androstenone within the two groups as well (196). Gregersen et al. (2012) also reported the effects in relation to the non-log-transformed values (193). In this study of three Danish breeds, Duroc, Danish Landrace and Yorkshire, the most frequent haplotype was contrasted to all other haplotypes identified for a certain QTL. The largest effect on androstenone and the skatole-equivalent in Danish Landrace was found within the QTL regions on SSC1 (1.28 μ g/g) and SSC14 (-0.038 μ g/g), respectively, whereas the largest effect on androstenone and skatole in Duroc was found by SSC12 $(1.29 \ \mu g/g)$ and SSC3 $(0.02 \ \mu g/g)$, respectively. In Yorkshire, the largest effects were found by SSC11 $(0.84 \ \mu g/g)$ and SSC6 $(0.02 \ \mu g/g)$ for and rostenone and the skatole-equivalent, respectively (193). These effect values, which might be overestimated due to small sample populations, indicate that the level of boar taint is highly affected by the allele composition within the QTL regions. However, if almost all animals have the favourable haplotype, the total gain might be lower. Analyses with the purpose of estimating how much of the phenotypic variance can be affected by a change in allele frequency indicate how much a given QTL contributes to the total phenotypic variance. In relation to

skatole and the skatole-equivalent, the identified QTL explained from 3 % to 12 % of the phenotypic variation (193, 352, 354), whereas for androstenone the values range from 1 % to 16.8 % (193, 196, 352-354). This indicates that there is great difference between how much individual QTL contributes and which QTL is most suitable for use in marker assisted selection.

Candidate genes have been suggested for some of the QTL identified in relation to boar taint. The genes CYP11A1, CYB5, CYP21 and SRD5A are all genes related to the biosynthesis of androstenone in the testis (355) and have been identified within QTL regions affecting androstenone level (193, 352, 354). In relation to skatole, some of the identified QTL regions' harbour genes related to the hepatic clearance of the compound (CYP2E1, CYP2A1 and SULT1A1) (193, 352, 353).

Lately, various differential expression studies using testis or liver tissues have added genes to the list (356-359). Moreover, Gunawan et al. (2013) related the differentially expressed candidate genes to the previously described QTL studies and suggested nine new possible candidate genes for the DNA regions (HBA2, LOC100516362, IRG6, ARG2, IFIT2, KRT18, CDKN1A, SDS and MBL2) (356). Three of the genes contained mutations that could explain part of the differences in androstenone level by an association analysis (IRG6, IFIT2 and KRT18), and two of these were contained by QTL identified in Danish breeds (193). Furthermore, a large study of 121 genes, predicted to have an effect on either skatole or androstenone level, was conducted by Moe et al. (2009) (360). They showed clear breed differences between Duroc and Norwegian Landrace, both in locations of mutations having an impact on the traits and in the effect of the mutations.

The number of genes and DNA regions associated to skatole and androstenone levels clearly illustrates the complexity of the traits, and it emphasizes the necessity to look for different genes in different breeds to overcome the boar taint problem. However, given the relatively large QTL effects, it appears realistic to markedly reduce the level by selection.

3.4.1.1 Concerns in relation to the selection index

When considering the introduction of additional traits into selection indices, their potential effects on index traits should be taken into account. In relation to the pig breeding index reproduction, production and meat traits are of high value, and there is an obvious risk of an undesirable effect of selection against androstenone on these traits due to genetic correlations.

Although the serum sex hormones have been identified to be highly correlated to the level of androstenone (194), the important production and litter size traits do not seem to be affected. This was investigated in a study by Strathe et al. (2013b) where seven traits of reproductive performance showed low or favourable genetic correlations to the level of androstenone, at least in Danish Landrace (361). However, due to the risk of introducing morphological and functional changes by hormonal

alterations (362, 363), additional studies, taking libido and sexual maturity into account, should be performed.

With respect to the trait, lean meat percentage, a favourable, negative genetic correlation has been found with skatole (361, 364), but even though the lean meat percentage has improved, the level of skatole in Landrace has not been reduced. This is most likely due to an increase in carcass slaughter weight over the last 10 years. In agreement with this, Windig et al. (2012) reported a low, although non-significant, positive genetic correlation between skatole and back fat thickness, which is the opposite of lean meat percentage (350). In addition, lower slaughter weight has been associated with animals having haplotypes associated with low skatole and indole levels (193) increasing the risk of a lower growth rate after applying selection. However, as EM are known to be more feed efficient than SC castrated male pigs, the time delay until slaughter (same average weight) is not an issue economically.

According to the study of Strathe et al. (2013a), a low, unfavourable genetic correlation was found between gain from birth to 30 kg BW and boar taint compounds, whereas the genetic correlations between boar taint compounds and gain from 30 to 100 kg BW or feed efficiency were non-significant (351). In accordance with this, Windig et al. (2012) did not find genetic correlations between overall gain and boar taint compounds (350).

These investigations indicate that a selection process can be performed without changing the existing index markedly; however, additional analyses are needed for confirmation.

3.4.1.2 Conventional selection

A possible approach to reducing the level of boar taint is to include the traits into the ongoing selection program removing boars having high level of either androstenone or skatole. For this method to work, a biopsy of back fat is needed from boars in the artificial insemination (AI) stations. The back fat sample can then be examined chemically for boar taint compounds, and this has been shown to be a good way to do the performance test for these traits (365). The timeframe for this approach is relatively long as only the boars can be tested and excluded. Frieden et al. (2011) have simulated a number of possible scenarios modelling androstenone in back fat as a proportional selection index (366). Provided that 80 % of the conventional reproductive and carcass traits should be retained, their model suggests that at least four to six generations or 8 to 12 years would be required for the level to reduce from 20 % of the boars having fat androstenone above the consumer's threshold of androstenone to only 5 % being in this category. This is in agreement with another simulation study (367).

3.4.1.3 Molecular genetics approaches

Within molecular genetics, mainly three approaches are used in relation to breeding, namely, Marker Assisted Selection, causative SNP tests or genomic selection. Pros and cons for these molecular selection methods as well as their time estimates in relation to reduction of boar taint are described in the following.

Marker Assisted Selection

For complex traits like boar taint, the causative single nucleotide polymorphism (SNP) for every single gene associated to the trait might not be possible to identify why Marker Assisted Selection (MAS) can be of value. A clear advantage by applying MAS, instead of identifying the actual causative SNP for all single genes having an effect on the trait, is that a result is often more quickly achieved. However, a disadvantage is that future examinations are needed to ensure that the haplotype marker is still tightly linked to the true marker, the causative SNP. The implementation of MAS can be added as an initial step in selection as exemplified by a Danish project aiming at reducing post weaning diarrhoea in piglets caused by Enterotoxigenic Escherichia coli (ETEC) expressing F4ab and F4ac fimbriae (368). The F4 receptor causing diarrhoea is inherited as an autosomal dominant Mendelian trait (369). Due to the large DNA region associated to the phenotype and the difficulties in finding the actual causative SNP, MAS was used to reduce the number of animals having the F4 receptor haplotype. The actual selection has been ongoing since 2003 in parallel with the regular best linear unbiased prediction (BLUP) selection in Landrace, Large White and Duroc. Boars that are relevant for breeding are F4 receptor tested upon transfer to AI quarantine, i.e. their F4 receptor status is known when they enter the AI station. The result of the breeding strategy was that in 2010 the frequency of the F4 Coli resistance among all performance tested young animals of the nucleus populations was close to 1 (370). As only the male line has been considered piglets can still receive the F4 receptor causing diarrhoea from their mother and hence develop the disease.

Causative SNP test

As the genomic technologies are advancing rapidly, we now have more sophisticated options in the search of candidate genes. By combining QTL data with whole genome sequences data and transcriptome data of specific tissues, we can in many cases identify causative SNP. A number of advantages follow if the actual causative SNPs are identified. For instance, simple genetic tests can be made to determine whether both the sires and dams carry an un-favourable allele. As boar taint is a complex trait involving many genes, numerous causative SNPs should be genotyped and several generations will likely be required to exclude most boars having unwanted alleles. However, it can be used for QTL having large effects and in combination with other selection strategies. But as mentioned above, the SNPs need to be investigated in relation to the other traits in the selection index. One of the advantages of molecular genetic approaches over conventional selection is that here only a DNA sample is needed and not a biopsy of the back fat. In addition, the timeframe of this approach is much

faster than MAS because selection may be applied to the sows as well. The biggest challenge in relation to SNP tests is in identifying the causative SNPs. This process can be complex and time consuming.

Genomic selection

Genomic selection is a novel approach to traditional marker-assisted selection where selection is made based on a large number of markers covering the whole genome. In most cases the markers are SNPs. Rather than seeking to identify individual loci significantly associated with a trait, genomic selection uses a set of marker data as predictors of performance. In order to increase the accuracy of the prediction of breeding and genotypic values, genomic prediction uses a combination of marker data and phenotypic traits as well as pedigree data when available (371). This is done by using the associations between markers and traits in a background reference population. The models are traditionally based on additive genetic variance of the markers. However, models considering the nonadditive genetic variance have recently been developed (372). Additionally, genomic selection also identifies novel QTL regions that can be used in order to identify the underlying causative genes. Identification of causative SNPs from these regions will likely strengthen selection opportunities.

In cattle it has been shown that genomic selection is powerful in single breed populations, however, there are a number of issues concerning the estimation of the genomic estimated breeding values (GEBV) as well as the genetic effect over time (373). When multiple populations are considered, it is essential to include enough animals of each breed into the reference population or alternatively to use a multi-bred population (374). In genomic selection, haplotypes or SNP effects in linkage disequilibrium with the traits are used. In Denmark, genomic selection is presently being implemented to evaluate porcine GEBVs of pure breed populations based upon evaluation of both pure line populations as well as production crossbreeds.

In order to keep the desired effects over time, genomic selection needs to be continuously re-evaluated and additional animals and phenotypic registration should be entered into the background population for the effects to be maintained (375). Overall, the timeframe for implementation through genomic selection might be quite fast depending on the weighted selection pressure applied.

No matter which selection strategy is chosen, including the selection on an additional trait will have economic consequences since animals with a high selection index for other traits are in risk of being selected against. However, no matter which method is applied the slaughtered carcasses need to be continuously monitored for elevated levels of boar taint.

3.4.2 Concluding remarks

Altogether, breeding provides a sustainable approach to reducing the level of boar taint. Numerous QTL have been identified in relation to both skatole and androstenone, and relevant candidate genes involved in either biosynthesis or metabolism have been identified for many of the regions. Analysis in

relation to the selection index indicate small changes in breeding values, however, long term changes, e.g., morphological changes due to hormonal changes needs careful surveillance during implementation. Depending of the time frame different approaches can be made in relation to breeding strategy where conventional selection in the sire line has the longest timeframe and the candidate SNP test the shortest. We propose an additional strategy combining data from genomic selection with QTL information in order to evaluate the effects on additional traits to ensure an effective and sustainable implementation as described below.

Genomic selection is now being applied in many countries to achieve breeding goals faster and at a lower cost than traditional breeding. The implementation of genomic selection only partly considers complex and non-additive interactions between "major genes" (genetic variants with a relatively large effect upon the phenotype) (372). The expected results are likely to be gains on the short term, but there are difficulties with harvesting the full genetic potential, which comes from identifying and exploiting the best combination(s) of genetic variants. For a trait like boar taint numerous haplotypes having a strong association to the trait have already been identified in the Danish pig breeds and more will be identified during integration of the boar taint trait through the genomic selection program. These haplotypes will in many cases be supported by the presence of relevant candidate genes within the chromosomal region encompassing the haplotype. A procedure, where these haplotypes are evaluated in relation to the data obtained from genomic selection, are likely to be efficient in exploring and exploiting complex and non-additive interactions in relation to boar taint QTL. This procedure would increase the knowledge of the QTL regions affecting skatole and androstenone and provide the opportunity to restrict selection pressure to haplotypes showing little or no adverse effects on other traits.



Photo: Jesper Adler

Chapter 4 - Needs and methods for assessing boar taint at the slaughter line

Currently, none of the suggested alternatives to surgical castration discussed in chapter 3 initially guarantees complete elimination of boar taint. Thus, a specific, reliable and sensitive method to assess boar taint at the slaughter line to sort out tainted carcasses is fundamental.

Traditionally, analysis of boar taint has been performed by a trained sensory panel and has been related to the levels of skatole and androstenone in adipose tissue (7).

4.1 Methods for rapid detection of boar taint

4.1.1 Sensory tests

Within the EU, the Meat Hygiene Regulation 842/2004/EC states that meat manifesting a "pronounced sexual odour" has to be declared unfit for human consumption. Such odours from meat can be evaluated by sensory test at the abattoir after specific heating methods (boiling in water, microwave, melting of fat and heating with a hot iron).

The most reliable heating methods have been shown to be the microwave method, the hot wire method and the boiling at 75° C method (376). The correlation between abnormal score and skatole and androstenone concentration was higher for skatole than for androstenone suggesting that skatole is the more important boar taint compound. However, skatole at high concentration enhances the perception of androstenone, which indicates that there is a synergistic effect of skatole on androstenone (Chapter 2). Taking all factors into consideration, including the ease of use, the authors concluded that the microwave method seems to be the most suitable for use at the abattoir, but individual assessors differ in their response to boar taint, so careful choice of the assessors is important (376). However, in contrast to the hot iron method which can be used online this is not possible for the microwave method.

4.1.1.1 Human nose methods

A hot iron method termed "Human nose scoring (HNS)" has been evaluated in fat samples from 6,574 entire males (377). Back fat samples were heated by a soldering iron heated to 370° C. The hot iron tip (6 x 20 mm) was applied to the sample for 2 to 3 s. The panellists then recorded the intensity of boar taint on a scale of 0 to 4. Each sample was recorded by three panellists and it was shown that the method was related to the boar taint compounds, androstenone (correlations between 0.22 and 0.52) and skatole (correlation between 0.31 and 0.89), but in addition also captured variation not accounted for by androstenone and skatole (377). Furthermore, the estimated cost (<1€) and time requirements

(one panellist may core up to more than 600 samples per hour at the processing line) were acceptable, and it was concluded that HNS was suitable for large scale evaluations of boar taint (377). The method is currently used by slaughter companies in the Netherlands (110) where about half of the male pigs raised in 2012 were entire males. Results from a major Dutch slaughter company using HNS detection have shown that the prevalence of carcasses with boar taint is on average 3 to 4 % in Holland. However, it varies across individual farms from 0 % to 8 % (110). In the study of Mathur et al. (2012), the proportion of samples with boar taint measured by the HNS method was 9 % (377). However, if commonly used thresholds for androstenone (1 μ g/g) and skatole (0.25 μ g/g) are considered, the estimated proportion of samples with boar taint was 44 %. However, changing the threshold values to 0.30 μ g/g for skatole and 3.0 μ g/g for androstenone resulted in levels comparable to those found by the human nose method (377). As also pointed out by the authors this questions at which levels the threshold should be set to reflect the consumers ability to perceive tainted meat.

The hot wire method, which is analogous with the HNS method, is occasionally used at slaughter houses in UK (378), Belgium (119, 157, 166) and Germany (379).

In Denmark, an accredited human nose test based on heating 5 g fat in 75 ml boiling water for 2 min following assessment (380) is used together with the colorimetric method for measuring skatole equivalents (Chapter 4.1.2.1) to test all organic entire male pigs produced (currently only pigs involved in research projects are left as entire males in organic pig production in Denmark). Conventional raised entire male pigs (4,000 to 5,000 per week) are only tested by the colorimetric method.

4.1.1.2 Biosensors

Biosensors based on utilizing the chemical learning abilities of insects have been suggested for detection of boar taint (381). It has been shown that the wasp, *Microplitis croceipes*, could be trained to report either a low, medium or high concentration of indole, skatole and androstenone in boar fat at room temperature. However, the learning was based on a combined odour of the three boar taint components, and not the individual compounds. It remains to be shown whether the wasp can be trained to report the individual boar taint compounds. However, use of wasp biosensors for the commercial on-line detection of boar taint may have potential.

4.1.2 Chemical tests

The development in rapid chemical detection methods for boar taint has been characterized by two methodological strategies. One is based on vapour fingerprinting methods, which aims at developing indirect measurements of boar odour by applying methods based on non-specific gas sensor arrays (e-nose) or direct mass spectrometry. The other approach is to measure the boar taint compounds directly using specific techniques, such as fast gas chromatography and spectroscopy/colorimetry, to separate and quantify the single compounds.

4.1.2.1 Colorimetric methods

A colorimetric method for measuring skatole equivalents (0. 442 * indole + 0.795* skatole + 0.027; 382) in adipose tissue was developed in Denmark (383). Basically, it is an at-line method, as back fat samples are physically removed from the carcass and taken to an automated analyser in a lab at the abattoir, and the results are used for sorting carcasses later down the production line. The benefits with this method are that it is rapid and simple (180 samples/hour). However, it does not provide information on the levels of the other important boar taint component androstenone and from a technical point of view it is not up to date. All entire male pigs slaughtered in Denmark (currently 4,000 to 5,000 per week) are tested by this method, and pigs with a skatole equivalent above 0.25 μ g/g are sorted out and only used for processed products.

A colorimetric method for androstenone determination in fat was developed by Squires (1990) (384). The analysis is group specific for 16 androstene steroids and thus reflects the total amount of 16 androstene steroids, not only androstenone. This means that the method is also sensitive to cholesterol, which might be present in significant amounts in adipose tissue. Elimination of cholesterol requires a solid phase extraction, which is time consuming. Thus, the method has, to our knowledge, never been used at slaughterhouses.

4.1.2.2 Electronic nose methods (gas sensor arrays)

Recently, gas phase detection by use of gas sensor array technology combined with multivariate data processing methods, the so called "electronic nose", has been investigated (7, 385). However, these methods remain to be properly validated. As concluded by Lundström et al. (2009), gas sensor array-based methods replace neither complex analytical equipment nor odour panels but may be a valuable supplementing tool. It is essential for further work in this field that these techniques are being validated properly to prove their suitability (7). Unless selectivity and sensitivity are improved with regard to boar taint substances, these methods will not be applicable to rapid boar taint detection.

4.1.2.3 Direct mass spectrometry

Direct mass spectrometry (MS) has also been applied to measure boar odour. This technique is based on direct transfer of vapour samples into the iron-source of the MS. Using cut-off values of 1.0 μ g/g for androstenone and 0.16 μ g/g for skatole, high classification rates have been obtained with the technique (386).

4.1.2.4 Fast gas chromatography

It has been demonstrated that androstenone, skatole and indole, in principle, can be separated and detected within 10 s using ultra-fast gas chromatography (GC) (378). However, sampling is critical, as a proper clean-up step to isolate the boar taint compound is necessary. Using a single solid-phase extraction (SPE) step, Haugen et al. (2012) demonstrated detection limits of 0.2 μ g/g for skatole and 0.5 μ g/g for androstenone. The method enables quantification of all three boar taint compounds

(androstenone, skatole and indole) in one analysis and can be automated using commercial GCinterfaced SPE systems (378). However, it will still be an at-line method, since samples have to be taken from the carcasses and transferred to the analysis system.

4.1.2.5 Gas-phase spectrometry

Another technique under development for boar taint detection is gas phase Fourier transformed infrared spectroscopy (IR) combined with photo acoustic spectroscopy (PAS) (7). It has been shown that the boar taint compounds have distinguishable gas-phase IR spectra that would enable direct detection of each compound. As it is a very fast technique, it has potential for on-line use (7). However, gas sampling is a critical point that needs to be adapted to the slaughter line.

4.2 Methods used to quantify boar taint compounds

Numerous approaches have been developed to quantify boar taint compounds in porcine adipose tissue. Gas chromatography (GC), normal and reversed phase high performance liquid chromatography (HPLC) and spectrophotometric methods are generally used to measure indolic compounds, while GC, HPLC and different immunological methods are used to measure androstenone.

4.2.1 Gas chromatography

Gas chromatography (GC) was the first technique used to detect androstenone concentrations in adipose tissue (387, 388). Currently, several GC-based methods for measurement of androstenone exist (378). The limitation of GC-based methods for quantification of skatole and androstenone includes high cost of equipment and long duration of the analysis for androstenone.

4.2.2 Liquid chromatography

Various HPLC methods to quantify indole, skatole and androstenone have been published. Indoles show an intense auto-fluorescence emission, which makes them easy to detect at very low concentrations. Because androstenone does not have native fluorescence derivation is needed to make use of the high sensitivity of fluorescence detection (382). A rapid HPLC method for simultaneous determination of androstenone, skatole and indole has been developed (382), and with minor modifications this method is the most used method to analyse indole, skatole and androstenone in adipose tissue today. The drawback of HPLC methods for detection of boar taint compound is the high cost and the complexity of the equipment.

4.2.3 Mass spectrometry

The detection power of mass spectrometry (MS) has lately made this the choice as detector for boar taint compound, and several methods have been published where MS in combination with separation

techniques such as GC or HPLC are used to quantify androstenone, skatole and indole in adipose tissue (389, 390). Use of MS as detectors also enables analyses of plasma androstenone and skatole. However, use of MS makes the equipment even more expensive and complex to run.

4.2.4 Immunological methods

As reviewed by Haugen et al. (2012), various immunological techniques have been developed for the analysis of androstenone in carcasses, e.g. radioimmunoassay (RIA), enzyme immunoassay (EIA) and fluoroimmunoassay (FIA) (378). RIA assays were shown to be sensitive and reasonably accurate (108, 391); however, they suffer by the use of radioisotopes, which restricts the use to specific facilities and is problematic in relation to the working environment. In EIA, an enzyme labelled antigen/antibody complex is detected by adding a substrate that reacts with the enzyme and thereby produces a colour (392). FIA uses europium chelaters as flourophoric labels. Background fluorescence is an issue but is overcome by measuring fluorescence sometime after excitation when the background fluorescence has disappeared (time-resolved fluoroimmunoassay; TR-FIA) (393). The common disadvantage of the antibody against androstenone, which results in a high cross-reaction with other steroids in fat (47, 108, 394). To our knowledge, no immunological based commercial kit (or antibodies) is available for measurement of androstenone in adipose tissue, which means that each lab has to prepare its own antibodies.

Application of immunological methods for skatole analyses is rare as it is even more difficult to make specific antibodies against a smaller molecule, such as skatole. However, Tuomola et al. (2000) developed a monoclonal antibody against skatole (395), and a competitive FIA method has been described (396), but information on the application of the method for skatole analyses on larger number of samples is not available.

4.3 Concluding remarks

Overall, there is so far no international accepted and validated on-line method available for the measurements of boar taint in carcasses that throughout fulfils the requirement for a highly streamlined industry at the slaughterhouses.

There is a need for a method that 1) measures both skatole and other boar taint compounds including androstenone 2) is simple and does not require highly qualified personal to operate it 3) is rapid enough to handle up to several hundred carcasses per hour, 4) is accurate and gives a high sensitivity as well as specificity of the classification and 5) has low costs.

A number of chemical methods exist for quantification of androstenone and skatole in adipose tissue. However, these methods are not applicable on the slaughter line since they involve complicated sample preparation steps and are usually labour intensive, but they permit quantification of androstenone and skatole which is essential for research purposes. However, only few inter-laboratory comparison studies have been conducted on the various methods, and significant differences between laboratories may exist, which emphases the need for further harmonisation and standardisation of methods (378).

Several rapid chemical boar taint detection methods have been tested. Apart from the spectrophotometric method that has been used with success for skatole equivalent analysis in Danish slaughterhouses, none of these methods has been properly evaluated. The limitation with the Danish skatole equivalent method is that it is not up to date, androstenone is not measured, and only 180 samples/ hour can be tested.

The presence of boar taint in pork products might be evaluated by sensory analysis, but the use of sensory test has been postulated not to be efficient due to high cost, high variability and low accuracy. In addition, there is to date still no established internationally approved definition of the sensory perception of boar taint. Further investigations are required to implement a generally accepted, rapid and sensitive method for systematic analyses of boar carcasses, which includes sound, sensory perception thresholds with regard to consumer acceptance as well as boar taint rejection criteria for sorting tainted carcasses at the slaughter line. Of the human nose methods tested, the hot iron method used in the Netherlands seems to be the best validated method and it seems to be promising.

In spite of the variety of analytical protocols available for measurements of boar taint, a harmonized, approved method for detecting boar taint at the slaughter line in the EU is still lacking. Until a reliable on-line method that both measure skatole and androstenone is developed a reasonable approach may be to use an up to date modification of the Danish colorimetric method for measuring skatole equivalents, which has been shown to be valid as an at-line method, combined with the hot iron method used in the Netherlands, which seems to be valid as an on-line method. The combination of these two methods will address the problem with skatole measured by the colorimetric method, which seems to be the most important compound for boar taint, as well as the fact that some samples will have an odour even though skatole level is low, either due to high concentrations of androstenone or other compounds.

Chapter 5 - Time horizon, stakeholder attitudes and economic feasibility of alternatives to surgical castration

Within the alternatives to surgical castration described in chapter 3, a number of strategies have the potential to reduce boar taint and improve animal welfare. Some of the alternatives are, however, not applicable within 2 to 5 years, or evaluation of their feasibility relies on analyses by the pig industry followed by a focused effort, if the initial analysis gives reasons for it. In this chapter we first shortly address the alternatives only found relevant in a longer time perspective or requiring specific focus by the pig industry. Next, the alternatives that are almost immediately implementable are discussed and the economic feasibility is analysed.

As described in chapter 3.3, sperm sexing may be relevant in a longer time perspective, but the technique needs more development and is not expected to be practically useful within the next 2 to 5 years. In addition, genetic selection (Chapter 3.4) is considered highly relevant. However, by conventional genetic selection it is estimated to take between 8 and 12 years to reduce the number of male pigs with androstenone levels above the acceptable limit from 20 % to 5 %. By applying molecular genetic selection approaches, it would be realistic to obtain these results within 5 years if the pig industry prioritizes this alternative (Chapter 3.4). However, as most of the other alternatives, a genetic approach needs to be combined with improved methods for detection of boar taint at the slaughterhouses. An evaluation on whether molecular genetic selection traits as well as the costs of a breeding program and has to be based on data and analyses by the pig industry (Pig Research Centre). Thus, the economic calculation of this alternative is not included in our evaluation of economic consequences.

Production of entire males (EM, Chapter 3.1) and immunocastrated male pigs (IC, Chapter 3.2) may, theoretically, be implementable within a short time, although these alternatives require fast and reliable methods for on-line detection of boar taint, at least until experience gathering has occurred, and the alternatives at present require approval according to the rules from the Danish classification authority. However, the control may be redundant for immunocastrated pigs in the long term if the level of tainted pigs is lower than 1 % also in large-scale. Reliable methods for on-line detection are still lacking (Chapter 4), but provided that these are available within the next couple of years, strategies of EM production and IC are considered applicable within a timeframe of 2 to 5 years. However, a major barrier in implementation of these alternatives is the uncertainty on whether the consumers will accept the methods and resulting pork quality, hence continuing to purchase pork, as this is the key factor for maintaining the profitability in the whole production chain. In addition, any alternative method has to be profitable at herd level to maintain the meat production. Thus, in this chapter, we

summarize the attitudes and interests by the stakeholders and evaluate the economic feasibility at herd level of production of EM and IC pigs in Denmark through cost-benefit analyses.

5.1 Stakeholders - attitudes and interests

The success of any alternative to surgical castration will depend of its acceptance by the stakeholders throughout the production chain. The main stakeholders are the consumers, the farmers, as well as the slaughterhouses and the food manufacturing industry.

5.1.1 Consumers

Consumer perceptions and attitudes are key determinants for the applicability and economic feasibility of an alternative strategy to SC. In general, the consumer attitudes depend on factors such as national traditions and androstenone sensitivity (Chapter 2), experience with tainted meat (397) and knowledge about boar taint and the methods used to avoid it (398). The latter is relatively limited according to most studies (311, 398-402). This means that the information given, including choice of words and the information condition, may be important for attitude (310, 397, 400). Furthermore, besides meat quality priorities of the consumers may also include high animal welfare, naturalness and responsibility in relation to use of medicals (400, 401, 403). Other important issues in terms of the consumers can be associations to loaded words or items (e.g. hormones, medical treatment or vaccination) and feelings such as fear of changes, unknown side effects and confidence with authorities (400).

The majority of the available studies focused on IC. Although unjustified by facts, common concerns about IC are fear of residues (397, 399-401, 403) and unknown long-term effects due to associations to hormones (397, 400, 401, 403). Surveys of consumer attitudes to immunocastration that are independent of the vaccine manufacturer only inform on a small part of the total market (Table 13) including Australia (404), Flandern in Belgium (399, 402), Switzerland (397), Norway (400), Sweden (405) and organic (401, 403) as well as general consumers in Germany (398).

Differences between countries in the attitudes of consumers would be expected but the result also varies between studies and the alternatives included in the surveys (Table 13). Overall, if surgical castration with anaesthesia was presented as one of the alternatives to SC, this method was rated more acceptable than IC and EM, which may reflect that SC is a known and reliable method, whereas IC and EM are less known leaving some uncertainty. However, as completely effective methods of sedation and pain relief in pigs that are cost-effective and possible to use at farm level are still lacking (1-6), surgical castration with anaesthesia may be questionable as a general alternative from a welfare point of view. Excluding surgical castration with anaesthesia as an alternative, IC seems generally acceptable by most consumers in Australia, Belgium and Sweden (Table 13). In addition, Sattler & Schmoll (2012) concluded that IC would be an acceptable method in Germany if the consumers were correctly informed (398), whereas the results from Switzerland are ambiguous (Table 13). In Norway, where the

common practice during the recent years has been SC with local anaesthesia performed by a veterinarian, the consumers were sceptical to IC but due to a high confidence to the control authorities, introduction of IC is believed not to change consumer purchasing habits (400). Organic consumers may be more critical to IC as evident in the surveys of German organic consumers (401, 403).

Table 13. Consumer attitudes with respect to the use of surgical castration (SC), surgical castration with anaesthesia (SCA), immunocastration (SC), and rearing of entire males (EM) in surveys independent of the vaccine manufacturer.

Method	Belgium ^a	Belgium ^b	Switzerland ^c	Norway ^d	Sweden ^e	Germany ^f organic	Germany ^g organic	Germany ^h	Australia ⁱ
Year of survey	2008	2009	2006	2008	2005	2009	2009	2010	2004 ⁱ
SC	21	base	base	5 + 10	base	+	59.1	19	NA
SCA	NA	40	(17.6)	67 + 22	NA	++++	86.4	NA	NA
IC	60	21	(47.2)	51 + 23	more	++	52.3	41	100
EM	NA	1	(40.2-42.4)	17 + 15	less	+++	79.3	NA	NA
Neutral*	19	NA	53-82%	NA	NA	NA	NA	NA	NA

NA: not assessed or not informed; * no preferences

^a percentages that evaluate SC without anaesthesia better than IC, or IC better than SC without anaesthesia (399). ^b percentages willing to pay an extra price for pork from the alternatives to SC (402).

^c the percentages represent 'disagree or disagree strongly' that the method is an acceptable alternative to SC as 'agree or agree strongly with the acceptability of the alternative' did not occur (397).

^d percentage that answered 'totally acceptable' + 'possibly acceptable' for each method (400).

^e Willing to pay more or less for pork from IC pigs and EM compared to pork from SC (405).

 $^{\rm f}$ qualitative study – the authors conclusion with respect to preferences between methods is illustrated by the number of plus signs, where higher number of plus signs express higher preference (401).

^g percentage that are willing to buy the meat (calculated by subtracting the 'percentage that are not willing to buy the meat' from 100 %)(403).

^h preference between use of SC or IC. Fourty percent were not able to make an opinion (398).

¹qualitative study – percentage that preferred IC over SC (404).

^jYear of publishing as year of survey is not informed.

In addition to these studies, the vaccine manufacturer has performed interviews comparing the preferences for IC and for SC with anaesthesia, respectively, as alternatives to SC without anaesthesia in Switzerland, Germany, France, Belgium and the Netherlands. According to these sources, approximately 61 to 74 % of the German, French, Belgian and Dutch consumers prefer IC over SC with anaesthesia as an alternative with the lowest preference for IC found in Germany (311, 406). Moreover, 82 to 90 % of the consumers were willing to eat pork from IC pigs (311). Among the Swiss consumers, 77 % preferred IC over SC with or without anaesthesia, and 95 % were willing to eat meat from IC (407). These results contradict the above mentioned preferences found in other studies treating SC with anaesthesia as an alternative to SC without anaesthesia.

Consumer attitudes at other markets were not surveyed. However, immunocastration is considered unacceptable at the Chinese, Japanese, Canadian and the US markets (116, 408).

In the few studies including both IC and EM, the attitude of consumers towards EM is in general less acceptable than towards IC except in studies that include organic consumers (Table 13). This

scepticism is linked to the risk of boar taint and reduced meat quality (400). The result may, however, be biased by less profound information provided on this method compared to IC in the studies (400).

In summary, the risk of tainted meat in production of EM and the consumers' uncertainty of whether there are risks to food safety by IC are issues that should be taken into account in the decisions on which alternatives to surgical castration that should be implemented. The consumers' knowledge about castration methods is limited and information about the methods, especially on whether they imply risks for the consumers, is important for consumer acceptance of IC, whereas acceptance of production of EM demands a focused effort on avoiding tainted meat.

5.1.2 Pig producers

In addition to consumers, the pig producers are also important stakeholders as they have to implement the alternative strategy in practice. Nevertheless, studies focusing on pig producers' attitude towards alternatives to SC are rare. Opposite to the attitude of consumers, the attitude of pig producers is likely to be influenced by the expected production costs and labour implications of the alternative strategies (400). In a Belgian study, participants with farmer experience in general had a less positive attitude towards alternatives to SC compared to participants with no farmer experience, especially to SC with anaesthesia and EM (409). In accordance, Tuyttens et al. (2012) revealed that Belgian pig producers preferred the current practice with SC whereas the production of EM was strongly rejected by a large proportion of the producers, mainly due to expectations of reduced pork quality, elevated level of aggression between animals and decreased farm profitability (409). The negative perception of EM production seems, however, to be less pronounced in producers with experience in rearing of EM as on-farm implementation of both SC with anaesthesia, IC and EM on 19 farms changed the perception of the alternatives in favour of EM. The latter was mainly due to lower problems with boar taint and behaviour than expected (410). The organic producers in Sweden have, however, expressed a desire to use IC, but at present this does not seem compatible with the EU regulation on organic production (264). The Danish organic producers do not regard IC to be an option in organic pig production (411-413).

In summary, previous studies indicate that the producers seem to have a less positive attitude towards implementation of alternatives to SC than the consumers and are mainly concerned on labour implications, the risk of boar taint and the expected reduction of profit of the alternatives. However, experience with these alternatives can change their attitude. The attitude of Danish producers has not been surveyed.

5.1.3 Pig Industry

In 2012, Danish pig products were exported to 78 countries all over the world, amounting to DKK 32.3 bn., where 66 % arose from export to EU27 countries, whereas 34 % arose from exported to countries outside EU (414). The major markets outside EU that mainly buy by-products or special cuts are

China, Japan and Russia (414). This means that every pig is split into many pieces and exported to several countries which all have to accept the kind of meat this pig delivers. A change in the production like replacing surgical castration with an alternative method thus requires that consumers in a lot of different cultures will accept the new product. If not, the export of parts of the pig might be at risk. Therefore, the pig industry has put a lot of effort into avoiding this situation.

A recent Danish report concerning the expected acceptance of alternatives to SC on important Danish export markets states that the German, Japanese, Chinese, Polish and Dutch markets at present are not expected to accept meat from IC pigs, based on interviews with either stakeholders in the pig industry, independent researchers or both groups (415). In addition, meat from production of EM is assumed not to be accepted at the Chinese, Japanese and the US markets, whereas Russia, UK, Italy, Belgium and Spain are expected to accept both alternatives (415). The lack of acceptance of meat from entire male pigs in Japan and China are due to lack of confidence on the methods for detection of boar taint at the abattoir and may therefore change as reliable methods are developed. According to the report (415), the lack of acceptance of meat from IC pigs is suggested to be more permanent, especially at the Asian markets, as animal welfare is not important for the consumer attitudes in these countries. However, the information from these countries was only based on assumptions about consumer attitude by the pig industries.

The Danish pig industry is positive to the ban on surgical castration and they regard production of entire males to be the best alternative to surgical castration (408). Therefore, one of the main issues for the slaughterhouses is the development of fast and reliable on-line detection of boar taint, which at present is expected to be refined in the future by including analysis of androstenone (408). However, full-scale production of entire male pigs implies an increase in the total amount of meat sorted out as tainted and has to be used for cold, smoked processed products. The market for and handling of the boar tainted meat are thus two other main issues concerning production of EM, because this market will easily be saturated and an excess of tainted meat will constitute a large loss to the meat industry. Therefore, methods for reducing the amount of boar tainted meat also have high priority in the pig industry. Furthermore, the change to production of EM presents a challenge in relation to the current changes in the market towards bigger cuts favouring a higher slaughter weight (408) as this increases the risk of higher levels of fat androstenone and more tainted meat (Chapter 3.1.2.1.1).

With respect to immunocastration, the Danish pig industry is, at the moment, reluctant to implementing the method, both due to the uncertainty of the consumers' acceptance, especially on export markets in Asia, and because they worry about the risk of self-injection by the farmers (408). Until now less than 0.5 self-injection per million doses administered have been reported, and none have resulted in lasting or severed adverse effects (337). The pig industry has the possibility to inform the consumers at the domestic market about the safety of the meat, while it will be much more difficult to inform and affect consumers at the export markets who buy the majority of the Danish pig products.

5.2 Economic aspects at farm level and cost-benefit analyses of non-breeding alternatives

5.2.1 Introduction

The economic aspects of alternatives to surgical castration have only been the focus of a few studies. Economic consequences of slaughtering at a low slaughter weight have been modelled as a scenario with ban on castration in the Netherlands concluding economic losses to the pig production sector of 10 % (416). In addition, economic implications of a) SC with local anesthesia, b) SC with general anaesthesia, c) IC and d) EM were evaluated for nine of the most important pig-producing member states of the EU (417). The authors concluded that using local anaesthesia implied the lowest costs for the farmers, but might not be practically feasible for smaller farms due to the cost of the veterinarian; IC has many pros, is economically neutral compared to SC, but has a low consumer acceptance in many countries, while raising EM can be economically profitable in many countries depending on the percentage of males with boar taint at slaughter. A report investigating the cost effectiveness of EM production in different scenarios and including two countries, the Netherlands and France, concluded that raising boars and gilts in separate groups is more cost effective than mixed-sex rearing, and breeding programs combining selection on boar taint and economics were more cost-effective than programs focusing on boar taint only (418). A Danish study evaluated the effect on boar taint in EM of skatole-reducing feeding just before slaughter (419) and concluded that addition of 15 % chicory for 14 days before slaughter had the highest feed costs (DKK 50 /pig presupposing a cost of DKK 7/kg), while feeding pure grain for 4 days before slaughter reduced revenue due to lower slaughter weight (DKK 13). Both methods reduced the amount of skatole. Similarly, a Danish study on IC showed an increased gross margin per pig space per year on DKK 114 to 154 compared to SC if costs for vaccination (around DKK 82 per pig space per year) and a potential EM-deduction (DKK 100 per pig space per year) were not included (420).

To supply previous evaluations of the economic consequences of implementing EM production or IC with focus on Danish pig production, the present cost-benefit analyses include the alternatives that, based on the review of the alternatives in chapter 3, are regarded to be efficient in reducing boar taint and are technically applicable in Denmark within a timeframe of 5 years. These alternatives are:

- 1. Entire males
 - Entire males, traditional production conditions
 - Entire males, chicory feeding before slaughter with or without reduced slaughter weight
 - Entire males, pure grain feeding before slaughter with or without reduced slaughter weight
- 2. Immunocastration
 - Standard vaccination, ad libitum feeding
 - Standard vaccination, restricted feeding after second vaccination
 - Early second vaccination, restricted feeding after second vaccination
 - Early second vaccination, ad libitum feeding

A number of factors are difficult to apply a monetary value because market mechanisms (supply/demand/competition) and political decisions related to alternatives will be able to cause changing prices. As a considerable number of stakeholders, including the pig farmers, consumers (both national and international market), slaughter houses, feed producers, veterinarians, pharmaceutical companies and politicians are being influenced by and influence the costs and benefits of the different alternatives, the price levels may be affected both in the short and long term perspectives.

5.2.2 Method and assumptions behind cost-benefit analysis

The cost-benefit analysis compares the production of each alternative to the production of surgically castrated males, and it includes all factors that affect the gross margin differently between the scenarios. Factors normally included in the gross margin (variable revenues and costs directly linked to the production) but which do not differ between SC and the alternatives are not included. In addition, fixed costs and costs for potential new investments are not included. Price levels for October 2013 are used in the calculations, but they will probably be affected by market mechanisms which will be discussed afterwards. Three factors are in common of all alternative scenarios: a) reduced labour and costs by omitting castration (DKK 6/pig according to Maribo (2013) (116)), b) a deduction for controlling the carcasses for boar taint at the slaughterhouse (DKK 25/pig, and additionally DKK 7/pig if the Dutch human nose test is used) and c) the risk of getting sorted out during slaughter due to boar taint which in 2013 implies a reduction in slaughter price of DKK 2 per kg. For each alternative, the effect of each relevant factor will be based on conclusions from summaries in the earlier chapters, literature and personal communication with relevant persons. The calculation of the effect on gross margin per pig space per year is based on a spreadsheet based on the model published by the Danish pig research Centre: <u>http://vsp.lf.dk/Aktuelt/Noteringer/Beregn%20DB%20slagtesvin.aspx</u>. This model was visited in week 45 2013, and calculations are based on prices from that week. The model from the Danish pig research Centre calculates gross margin of conventionally produced pigs based on national means from the pig production, and from that model we have set the following parameters for SC: Feed efficiency rate: 2.85; lean meat percentage 60.0 %, daily weight gain: 905 g; feed price: DKK 1.73/ Feed unit (7.72 MJ net energy), price of 30 kg piglet: DKK 425. All other prices are described in appendix 1 to 10. For all alternative scenarios it is relevant to evaluate the economic latitude for improving animal welfare by reducing stocking density to reduce risk of aggressive behaviour, which is probably increased in all scenarios compared to SC, and all results are thus recalculated where area per pig is increased by 10 % in accordance with the German guidelines for space allowance in EM production (Chapter 3.1.1). An increased aggression level will probably also result in meat and skin damage (243). However, damage to the meat and skin occurring at the farm normally heals before slaughter, and only damage resulting from fighting just before leaving the farm and at the slaughter house will be registered at the slaughterhouse and give deductions (421). Valid estimation on the incidence of deduction is, however, not available, and therefore not included. Until now, the production of EM is so small that the amount of discarded skin is no problem according to the meat industry. In case of a full scale EM production it might, however, turn into a problem if the aggression level is increased and is not reduced by other causes.

5.2.2.1 Production of entire male pigs

Production of entire males implies the risk that some of the pigs produced will be sorted out due to boar taint, but androstenone may be reduced by reducing the slaughter weight and skatole may be reduced by specialized feeding the last days before slaughter (Chapter 3.1).

5.2.2.1.1 Production of entire male pigs with a carcass weight of 83 kg and managed like surgically castrates

In this scenario, we assume conditions that resemble conventional production with ad libitum dry feeding, except that castration of male pigs is omitted. The input parameters are based on information from chapter 3.1 and the change in performance in *ad libitum* dry feeding of EM compared with SC pigs in a recent Danish study (239): Improved feed conversion rate (kg feed per kg gain: -0.24), higher lean meat percentage (+1.3 %). Although considerable variation exists between studies daily weight gain is oftenly reduced in Danish EM production (Chapter 3.1.3.2, Table 12). This parameter was therefore reduced by 20 g compared to SC according to a Danish study (237). Incidence of meat and skin damage due to increased aggression will probably, in general, be higher in EM production but as mentioned above, no valid estimates on the incidence and deduction are available and this is not included in the analysis.

As previously described, the risk of an entire male of being out-sorted at slaughter due to boar taint varies according to the criteria for sorting (Chapter 2 and 4). If alternatives to SC imply more tainted meat the sorting criteria at present are not fully satisfactory and are likely to be revised in the future. Therefore, the consequences of different sorting criteria have been calculated. As the sorting criteria at present are not fully satisfactory and are likely to be revised in the future. Therefore, the consequences of different sorting criteria have been calculated. As the sorting criteria at present are not fully satisfactory and are likely to be revised in the future if alternatives to SC imply more tainted meat calculation of the consequences of different sorting criteria has been made. According to the present procedure used in conventional pig production approved for production of EM in Denmark, the out-sorting in herds not using specific strategies for prevention of boar taint may

be expected to be at least 8 % based on fat skatole above 0.25 μ g/g (Chapter 3.1.2). Sensory tests may detect more pigs as tainted (Chapters 2.1 and 4). The combined sorting criteria used in Danish organic production (a sensory test and fat skatole above 0.25 μ g/g) may detect 50 % more pigs as tainted pigs than if fat skatole above 0.25 μ g/g is used as the only sorting criteria (117). Assuming that this also would be the case in conventional pig production, the combined criteria would result in sorting out of 12 %. If the critical limit for sorting is changed to 1.0 μ g/g for androstenone and 0.25 μ g/g for skatole, the share of sorted out pigs could be expected to be 30 % (Chapter 3.1.2). However, it has recently been indicated that androstenone might be of less importance if skatole is low (Chapters 2.1 and 4.1.1.1). Assuming that the critical threshold for androstenone may be set to 2.0 μ g/g if skatole is below 0.2 μ g/g, the risk of out-sorting on 10 % seems reasonable (80).

Handling of EM on the farm does not seem to be different from that of castrates and thus implies no extra labour or risks (Chapters 3.1.1 and 3.1.3.4). The male pigs could be raised separately from the females to avoid pregnancy, which may also reduce aggression (Chapter 3.1.3.1.1). We assume no extra costs because the pigs are usually sorted anyway in Danish pig production.

5.2.2.1.2 Production of entire male pigs with a carcass weight of 83 kg and using skatolereducing feeding strategies

At present, the sorting out of meat in Danish pig production may be considerably reduced if skatolereducing feeding strategies are added for the last 4 to 7 days before slaughter as the sorting procedures based on skatole are used. In the model it is assumed that females and males are fattened separately, which means that skatole-reducing feed before slaughter can be added to males only.

Chicory as skatole-reducing strategy before slaughter

Our assumptions related to chicory feeding are that adding 15 % dried chicory root to the feed the last 7 days before slaughter reduces the amount of skatole by two thirds but has no effect on androstenone in plasma and fat (Chapter 3.1.2.2.2). This reduction implies a risk of being sorted out due to skatole above 0.25 μ g/g of 2.7 %. Sorting based on skatole above 0.25 μ g/g and a sensory test is set to 4.1 % using the same assumption as in chapter 5.2.2.1.1. Besides that, health, daily weight gain and feed efficiency are not affected (116, 160). The extra cost for feeding with chicory is set to DKK 27 per pig assuming a cost of chicory of DKK 7/kg, a feed intake of 3.1 kg/pig from one week before slaughter of the first pig in a pen, a symmetrical distribution of the individual weights within pens and delivery to the slaughterhouse of pigs from the same pen within 4 weeks with a reduction in intake of the standard feed of 15 % (116, 422, 423). The scenario includes no extra labour. In 2013 there is no commercial production of chicory roots in Denmark, all chicory is imported. But in case of an increasing demand there is a good basis for restarting the production - especially in the southern and warmer parts of Denmark there is a basis for good production results and maybe establishment of more chicory-manufacturing plants and thereby creating jobs. Thereby, the price of chicory may be reduced a little compared to imported chicory (422). We have, however, used the current price in the scenario.

Pure grain as skatole-reducing strategy before slaughter

In a Danish study, feeding a mixture of 50 % barley and 50 % wheat the last 4 days before slaughter reduced the skatole level by 25 %, but it also caused a reduction in daily weight gain and feeding efficiency (419). At present, the potential of this strategy is further studied reducing the duration of the feeding period and it may be higher than 25 %. Of this reason, two scenarios are made; one suggesting a reduction in skatole of 25 % and one suggesting a reduction comparable with the other feeding strategies, i.e. of 66 %. The risk of getting sorted out on basis of skatole above 0.25 μ g/g is set to 6 % and 2.7 %, respectively, whereas the risk of tainted meat measured by a combination of skatole measurements and a sensory test is set to 9 % and 4.1 %, respectively. The costs for feed were not affected, but the reduction of revenue foregone (reduced slaughter value) was estimated to be DKK 13 per pig (419).

5.2.2.1.3 Production of entire male pigs with a carcass weight of 75 kg and use of skatolereducing feeding strategies

With a live weight of 98 kg at slaughter (carcass weight 75 kg), androstenone level is reduced compared to normal slaughter weight (Chapter 3.1.2.1.1), which is important if sorting based on levels of fat androstenone is implemented, although it is not expected to affect skatole (Chapter 3.1.2.1.1). With a live weight of 98 kg at slaughter, the level of androstenone may be reduced by 20 % according to a Danish field study (121). Based on the data from Walstra et al. (1999) (80), this means that 25 % of the pigs are expected to exceed 1.0 μ g/g for androstenone and 0.25 μ g/g for skatole, whereas 7.4 % are expected to affect skatole, which is the present criterion for sorting. Therefore, the strategy is combined with the same skatole-reducing feeding strategies and sorting rates for skatole-based outsorting rates as described for the scenarios with a carcass weight of 83 kg (Chapter 5.2.) for the last 4 to 7 days before slaughter. The scenario is like the others based on price levels for October 2013.

In the model it is assumed that females and males are fattened separately, which means that skatolereducing feed before slaughter can be added to males only. For the male groups, the usual four fattening cycles per year can be replaced by 4.45 fattening cycles per year (in producing 11 % more male pigs) due to lower weight at slaughter and the fact that actual surplus of produced piglets in Denmark can fill this gap. The assumption in this calculation was that EM has a reduced daily weight gain of 20 g compared to SC (237) which were further 10 g reduced due to the lower weight (423).

Lower feed costs are expected both because the males are slaughtered at a lower weight and because of better feed efficiency of EM at lower weight. The feed conversion rate, kg feed per kg gain, was assumed to be 2.5 kg/kg (423). Lean meat percentage will be higher (62.3 %), because it increases 1 % for every 10 kg the live weight at slaughter goes down (424). All in all, the feed costs per male will be reduced by DKK 93/pig.

5.2.2.2 Immunocastrated males

Immunocastrating male pigs by vaccinating twice with Improvac® reduces the risk of boar taint to a level close to that of SC pigs, but the effect varies a little between studies referred to in chapter 3.2. In the cost-benefit analysis we assume a risk of being sorted out of 1 %. This sorting ratio is comparable with the sorting rate when using SC, and costs for controlling the carcasses for boar taint at the slaughterhouse are therefore not included. Initially, during implementation of IC, the sorting rate may be higher than 1 % and controlling cost should be included as well as a deduction in slaughter price per pig sorted. This will also be the case if large-scale use of IC at a long term shows a higher sorting rate than 1 %. As no estimates are available on potential sorting rates at large-scale use, it has been set to 2 % in the present analyses. In addition, from chapter 3.2 we have the information that in the majority of studies, IC neither compromises meat quality measures nor sensory evaluation of meat quality compared to SC. It is also described that IC has the potential to improve feed conversion and increase lean meat percentage, while back fat thickness is reduced compared to SC. The cost of using Improvac® per pig (two vaccinations) is estimated to DKK 19.50 (337). That includes safety vaccinator to avoid the risk of self-injection. The two injections take approximately 2 minutes per pig. If simultaneous feeding is practised it can be performed by one person, and there is no practical problem related to applying the injection (116). With ad libitum feeding with less than one feeding space per pig the vaccination may require two persons. Therefore, in the cases with simultaneous restrictive feeding the total price of DKK 24 is entered the model presuming a labour cost on DKK 135 per hour, whereas a total price of DKK 28.50 is used in cases with ad libitum feeding which usually implies a restricted number of feeding spaces. An earlier second vaccination would improve welfare because the period where the aggression level is comparable to EM is reduced. In addition, restricted feeding is suggested to improve feed conversion rate and lean meat percentage. Presumed that all pigs in a pen are fed simultaneously as is usual with liquid feeding in Denmark, the feeding method is considered adequate from a welfare point of view. By combining these possibilities, four different scenarios are suggested.

5.2.2.2.1 IC scenario 1: standard vaccination, ad libitum feeding

Improvac[®] is applied as recommended by the manufacturer: First dose of Improvac is given when moved to finishing pens at 30 kg BW and again 4 to 6 weeks before the expected date of slaughter when the pigs are 19 to 21 weeks old. Based on results from a Danish study in which the pigs from one of the included farms were fed semi ad libitum (328, 425), we assume a feed conversion rate (kg feed per kg gain) of 0.14 better than SC, a daily weight gain like SC and a lean meat percentage of 1.1 % point higher than SC fed ad libitum.

5.2.2.2.2 IC scenario 2: standard vaccination, restricted feeding after second vaccination

Vaccination is applied as in scenario 1. By feeding IC restrictively after the second vaccination, 50 % further improvement of the feed conversion ratio and 84 % further increase in lean meat percentage have been found compared to the improvement by IC in ad libitum fed pigs (291). Therefore, the improvement of the feed conversion ratio and lean meat percentage compared to ad libitum fed SC is

set to 0.21 and 2 % based on values used in scenario 1, and daily weight gain is assumed unaffected. Furthermore, restrictive feeding requires one feeding space per pig, which means that the number of pigs produced per space per year is reduced from 3.99 to 3.78.

5.2.2.3 IC scenario 3: Early second vaccination, restricted feeding after second vaccination

First vaccination is applied at an age of 10 to 11 weeks as in scenario 1 and 2. Second vaccination is, however, performed at an age of 14 to 15 weeks to improve animal welfare (Chapter 3.2.4.1). Anderson et al. (2012) have compared early vaccination (week 10 and 14) with standard vaccination (week 16 and 20) in restrictively fed pigs and found that the only difference in performance was the lean meat percentage, which was reduced by 0.4 % compared to scenario 2 (315). Feed conversion ratio, daily weight gain and the number of pigs produced per space per year are set as in scenario 2.

5.2.2.2.4 IC scenario 4: Early second vaccination, ad libitum feeding

Vaccination is applied like in scenario 3. Early second vaccination combined with ad libitum feeding has never been investigated, but based on Batorek et al. (2012) (291) the feed conversion ratio is weighted according to the number of days between and after vaccinations assuming that an improved feed conversion will only last for 4 weeks after the second vaccination (Chapter 3.2.4.2). In addition, the feed conversion is adjusted to the size of the effect of IC found in the Danish study (328, 425), which gives an assumption of an improved ratio of 0.06 kg feed/kg growth compared to SC. Lean meat percentage is assumed to be reduced by 0.4 % points compared to scenario 1.

5.2.3 Results and discussion of the cost-benefit analyses

As explained above, we have made cost-benefit analyses for eleven different management strategies: Four strategies with production of entire males with or without feed additives at a slaughter weight at 83 kg, three with production of entire males at a slaughter weight at 75 kg and four strategies implying immunocastration. To show the main elements of the cost-benefit analysis, Table 14 shows SC and two random scenarios. In appendix 1 to 10, the model is demonstrated showing all scenarios. Results for production of entire males at a live weight of 108.7 kg (comparable to a carcass weight of 83 kg) are shown in Table 15, and they are presented as both gross margin per pig and gross margin per pig space per year as well as gross margin per pig space per year in case of 10 % decreased stocking density to improve animal welfare and reduce aggression level. The difference between the results of scenarios and the results from production of SC at the space allowance required according to the legislation is listed in brackets behind every value. Furthermore, these three figures are repeated with four different criteria for sorting out due to boar taint: Current practice in Denmark (skatole $< 0.25 \,\mu g/g$), the current method combined with a sensory test, such as the Dutch human nose method (skatole $<0.25 \ \mu g/g+$ human nose method), selection based on the common thresholds of boar taint compounds (and rostenone < 1.00 μ g/g, skatole < 0.25 μ g/g) and a less restrictive thresholds of boar taint compounds recently indicated to be acceptable (androstenone <2.00 µg/g, skatole <0.20 µg/g;

Chapters 2.1, 4.1.1.1 and 5.2.2.1.1). As grain-end-feeding seems very promising, but not very well documented, we have included two scenarios representing two different skatole reducing effects, i.e. a reduction on 25 % as previously found (419) and 66 % as the expected effect of feeding with chicory. In Table 16, scenarios applying skatole-reducing feed are repeated with a reduced slaughter weight with the aim of reducing the androstenone content and reduce the overall risk of getting sorted out. Also, these scenarios are repeated for all four sorting criteria. In Table 17, results of the four IC scenarios are presented both as results expected to be obtained if control for boar taint at the abattoir is required either permanently or in an implementation period (normally around half a year) and in a routine scenario based on the assumption that less than one percentage of the meat is tainted and control for boar taint is unnecessary.

The calculations are only made for conventional production because we lack several parameter estimates to make the equivalent calculations for organic production. The organic farmers get an additional price of DKK 4/kg meat but this is partly counterbalanced by feed prices that are the double of the prices for conventional feed. In the discussion we address whether the different scenarios are likely to be implementable in organic production and which effects and complications that might be involved.

	SC		EM + 15% chicory root 75 kg carcass weight		IC - standard vaccination fed ad libitum	
Revenues (Dkr/pig)	Assumption	Economic	Assumption	Economic	Assumption	Economic
		effect		effect		effect
Slaughter weight live/carcass (kg)	108.7/83		98.3/75		108.7/83	
Feed conversion ratio (FEsv/kg)	2.85		2.5		2.75	
Lean meat %	60		62.3		61	
Pigs produced per space/year	3.99		4.45		3.99	
Risk of boar taint	1 %		4.1 %		1 %	
Revenue per pig (DKK)*		1011		932 (-79)		1021(+11)
Costs (DKK / pig)						
Transferred 30 kg pig		425		425 (0)		425 (0)
Feed		388		295 (-93)		375 (-13)
Price reduction boar taint (DKK 2/kg)		1.7		25 (+25)		1,7 (0)
Deduction for control boar		0		32 (+32)		0 (0)
taint at slaughterhouse						
Labor saved by not castrating		0		-6 (-6)		-6 (-6)
Costs for feed additives (DKK/pig)		0		27 (+27)		0 (0)
Other costs (eg.vet, transport,DAKA,)		45		45 (0)		45 (0)
Costs for vaccinating		0		0 (0)		28.50
						(+28.50)
Gross Margin (DDK)						
Per pig (DKK)		153		105 (-48)		159 (+7)
Per pig space/year (DDK)		609		468 (-141)		636 (+26)
Per space/year, decreased stocking rate*		554 (-60)		426 (-184)		578 (-32)

Table 14. Assumptions, revenues and costs in surgically castrated pigs and 2 out of 36 scenarios illustrating parameters included in the cost-benefit analysis. The difference compared to the same parameter/calculation in surgically castrated pigs is shown in brackets.

* The values in the brackets are the loss in gross margin compared to production of surgically castrated pigs at the space allowance required according to the legislation.

EM: Entire male pigs; IC: immunocastrated pigs.

5.2.3.1 Profitability

5.2.3.1.1 Profitability of entire male production

Production of entire male pigs with a carcass weight on 83 kg (live-weight 108.7 kg)

The cost-benefit analysis (Table 15) indicates that under current prices and sorting procedures (only based on skatole detection), production of EM without using skatole-reducing feeding strategies and slaughtering the pigs at the common slaughter weight are very profitable compared to SC. In case of a full scale production of entire males we expect, however, that regulations are needed to reduce the amount of tainted meat only usable for processed products. If the price reduction for boar taint is increased from DKK 2 to > DKK 3.8 per kg EM, the production will not be profitable anymore compared to SC.

Table 15. Economic results of different scenarios within production of entire male pigs (EM) and in brackets the difference compared to surgically castrated males. Four different strategies for producing entire males are included: 1) entire males fed traditionally and slaughtered at a carcass weight of 83 kg (live-weight 108.7 kg); 2) entire males fed 15 % chicory root 7 days before slaughter, carcass weight of 83 kg (live-weight 108.7 kg) and 3) and entire males fed with grains only the last 4 days before slaughter, carcass weight of 83 kg (live-weight 108.7 kg).

			EM, § 83 kg carc	grain, ass weight
	EM - traditional EM		Skatole	Skatole
	Production	chicory	reduction	reduction
Out sorting criterion	83 kg carcass weight	83 kg carcass weight	25 %	66 %
			/.	
Skatole > 0.25 μ g/g, current practice in Denmark	8 %	2.7 %	6 %	2.7 %
Gross margin per pig (DDK)	168 (+15)	150 (-3)	158 (+6)	164 (+11)
GM (GM) per pig space/year (DDK)	656 (+46)	585 (-25)	618 (+8)	639 (+30)
GM per space/year, decreased stocking rate (DDK)	596 (-14)	531 (-78)	562 (-48)	581 (-29)
Skatole > 0.25 μg/g + human nose	12 %	4.1 %	9 %	4.1 %
Gross margin per pig (DDK)	154 (+2)	141 (-12)	146 (-6)	155 (+2)
GM (GM) per pig space/year(DDK)	602 (-7)	548 (-61)	571 (-38)	603 (-7)
GM per/space/year, decreased stocking rate (DDK)	548 (-62)	498 (-111)	519 (-90)	548 (-61)
Androstenone > 1.00 μg/g or skatole > 0.25 μg/g	30 %	30 %	30 %	30 %
Gross margin per pig (DDK)	132 (-21)	105 (-48)	119 (-34)	119 (-34)
GM (GM) per pig space/year (DDK)	513 (-96)	408 (-202)	462 (-147)	462 (-147)
GM per space/year, decreased stocking rate (DDK)	466 (-143)	371 (-239)	420 (-189)	420 (-189)
			4.0.0/	
Androstenone > 2.00 μg/g if skatole < 0.20 μg/g	10 %	10 %	10 %	10 %
Gross margin per pig (DDK)	165 (+12)	138 (-15)	152 (-1)	152 (-1)
GM (GM) per pig space/year (DDK)	643 (+33)	537 (-72)	592 (-18)	592 (-18)
GM per space/year, decreased stocking rate (DDK)*	584 (-25)	488 (-121)	538 (-71)	538 (-71)

Feed conversion rate: 2.61; lean meat percentage: 61.3 %; produced pigs per pigs space per year: 3.9, daily weight gain: 885 g.

Four different criteria for sorting out are shown.

* The values in the brackets are the loss in gross margin compared to production of surgically castrated pigs at the space allowance required according to the legislation.

In case of full scale production of EM, the introduction of more sensitive sorting methods could be expected to ensure the market for pig products. Table 15 lists the expected economic effects in current prices if other sorting procedures get available and are introduced in the future. In this case, the economy of EM production only equals the production of SC as long as a maximum of 10 to 12 % of the

pigs are sorted out. The amounts of sorted out meat resulting from a full production of EM will, however, still comprise a problem and loss to the pig industry due to the high amounts of tainted meat that have to be processed. Especially sorting based on androstenone results in a high frequency of sorted out meat. However, with decreasing levels of skatole, higher levels of androstenone may be acceptable (Chapters 2.1 and 4.1.1.1). Therefore, the effects of the following taint-reducing scenarios are considered.

Use of skatole-reducing feeding strategies combined with a carcass weight on 83 kg

When only skatole is measured as in current practice we expect that around 8 % will be sorted out in case of full-scale production. Currently, 4 % are sorted out, but they arise from farms specialized in, and certified for, entire male production. However, we assume that in case of a full-scale production, it is possible to obtain a sorting rate of 2.7 to 6 % by using skatole-reducing feeding methods if detection methods are not changed. In this case, the profitability for grain-end-feeding is better than for SC (DKK 8 to 37 per pig space/year) as the increased feeding efficiency and higher lean meat percentage of EM pay for the reduction of revenue and the boar taint control. The profitability of using chicory is, however, lower than the current profitability of SC (DKK -25 per pigs space/year) due to the high price of this feed additive.

Use of skatole-reducing feeding strategies combined with a reduced carcass weight

With the aim of reducing the androstenone level, which increases the amount of out-sorted meat considerably if included in the criterion of out-sorting, we suggest a reduced slaughter weight, and to obtain an effective reduction, the slaughter weight should be reduced to around 80 kg live weight (61.5 kg carcass weight). With the current sorting methods and combined with skatole-reducing feeding strategies this scenario, however, resulted in a gross margin of DKK 455 to 514 less per pig space/year compared to SC production, and the scenario was therefore considered irrelevant. Therefore, we suggest scenarios with a carcass weight of 75 kg (live weight 98.3 kg) combined with the skatole-reducing feeding strategies. None of these were, however, profitable compared to SC irrespectively of the sorting criteria (Table 16). If sorting methods in the future will include androstenone, the reduction in androstenone at a carcass weight of 75 kg seems to result in a too low decrease in androstenone to be profitable.

Table 16. Economic results of different scenarios within production of entire males (EM), four of them with reduced slaughter weight and in brackets the difference compared to surgically castrated males. Four different strategies for producing EM are included: 1) EM fed traditionally and slaughtered at a carcass weight of 83 kg (live-weight 108.7 kg), 2) EM fed 15 % chicory root 7 days before slaughter, carcass weight 75 kg (live-weight 98.3 kg) and 3) EM fed with grains only the last 4 days before slaughter, carcass weight 75 kg (live-weight 98.3 kg).

			EM, grain,	
			75 kg carca	iss weight
	EM - traditional		Skatole	Skatole
	production	EM, chicory	reduction	reduction
Out sorting criterion	83 kg carcass weight⁺	75 kg carcass weight ²	25 %	66 %
Skatole > 0.25 µg/g, current practice in Denmark	8 %	2.7 %	6 %	2.7 %
Gross margin per pig (DDK)	168 (+15)	121 (-32)	130 (-23)	135 (-18)
GM (GM) per pig space/year (DDK)	656 (+46)	537 (-72)	578 (-32)	600 (-10)
GM per space/year, decreased stock. Rate (DDK)	596 (-14)	488 (-121)	525 (-84)	545 (-64)
Skatala > 0.25 ug/g + human nasa	13.9/	4 1 9/	0.9/	4 1 0/
Skatole > 0.25 µg/g + human hose	12 %	4.1 %	9%	4.1 %
Gross margin per pig (DDK)	154 (+2)	105 (-48)	112 (-41)	119 (-34)
GM (GM) per pig space/year(DDK)	602 (-7)	468 (-141)	498 (-112)	530 (-79)
GM per space/year, decreased stocking rate (DDK)	548 (-62)	426 (-184)	452 (-157)	482 (-127)
Androstenone > 1.00 μg/g or skatole > 0.25 μg/g	30 %	25 %	25 %	25 %
Gross margin per pig (DDK)	132 (-21)	87 (-65)	101 (-51)	101 (-51)
GM (GM) per pig space/year (DDK)	513 (-96)	389 (-221)	451 (-159)	451 (-159)
GM per space/year, decreased stocking rate (DDK)	466 (-143)	353 (-256)	410 (-200)	410 (-200)
Androstonono > 2.00 μ_{g}/g if skatolo < 0.20 μ_{g}/g	10.9/	7 / 9/	7 4 9/	7 4 9/
Androstenone > 2.00 µg/g II skatole < 0.20 µg/g		7.4 %	7.4 %	7.4 %
	105 (+12)	114 (-39)	128 (-25)	128 (-25)
Givi (Givi) per pig space/year (DDK)	643 (+33)	506 (-103)	568 (-41)	568 (-41)
GM per space/year, decreased stocking rate (DDK)*	584 (-25)	460 (-150)	517 (-93)	517 (-93)

¹ Feed conversion rate: 2.61; lean meat percentage: 61.3 %; produced pigs per pigs pace per year: 3.9; daily weight gain: 885 g.

² Feed conversion rate: 2.5; lean meat percentage: 62.3 %; produced pigs per pigs pace per year: 4.45; daily weight gain: 875 g.

* The values in the brackets are the loss in gross margin compared to production of SC at the space allowance required according to the legislation

Although the assumptions are not directly applicable to our conditions as we suggest a higher live weight these results are consistent with the only other study (417) that refers to costs and benefits of raising entire males with reduced live weight at slaughter of 80 kg nationwide in the Netherlands (416) resulting in economic losses to the pig production sector of 10%.

However, only a small increase in the meat prices is necessary to pay these extra costs for the farmer and obtain the same profit per pig space as with SC. With the current sorting methods the meat price paid to the farmer has to increase with DKK 0.10 per kg to compensate in case of grain-end-feeding and DKK 0.27 per kg to compensate in case of chicory-end-feeding. In the worst case (most sensitive sorting procedure), the meat price has to be increased by DKK 0.48 to 0.67 per kg to pay the extra costs for the farmer. It may, however, be difficult to get additional payment on the export market, especially at markets where the causative factor determining an increased price, i.e. animal welfare, is not important for the consumer attitudes. In addition, production of EM at reduced slaughter weight may entail some logistic problems in relation to optimal pen utilization if FE pigs are slaughtered at the normal slaughter weight. Pen utilization may be optimized by allocating more pens for female pigs. All-in-all-out operations may, however, be impeded and health management thereby compromised depending on the housing dimension. Alternatively, the FE pigs may be slaughtered at reduced weight as well. Assuming that FE pigs have 0.14 lower feed conversion ratio (kg feed per kg gain) and about 20 g lower daily gain than SC and a meat percent comparable with EM pigs as found in previous Danish studies (237, 239) production of FE at a carcass weight of 75 kg results in a reduced profit per pig space per year on DDK 21 with the current price level of meat compared with production of FE at a carcass weight of 83 kg.

Furthermore, a potential negative aspect of slaughtering at a lower slaughter weight is that the market is changing towards bigger cuts these years favouring a higher slaughter weight (408). Danish Crown pays a base list price for pigs within the range of 70 to 88 kg (upper limit changing a little from week to week). Within near future this range will be increased by 4 kg signalling the smaller value of small pigs. By choosing an average weight of 75 kg in our scenario, this change in list price should have only a negligible effect on our calculations. However, if an increasing amount of the pigs is slaughtered at a reduced weight the lower limit of the range may be further increased making production at reduced slaughter weight uneconomic. Concerning routines in the slaughterhouse, a large-scale production of smaller pigs (live-weight 98 kg) has no practical implications as the equipment (robots etc.) is already able to handle a large variation in carcass sizes (426).

Use of chicory or pure grain as skatole-reducing feeding strategies

The cost-benefit analysis indicates that feeding only grain (50 % wheat, 50 % barley) the last 4 days before slaughter at a carcass weight of 98 kg seems to be most profitable way to reduce the skatole level, also if the effect is only a 25 % reduction. The feeding efficiency and daily weight gain were, however, reduced resulting in a loss of DKK 13 per pig (419) but include no additional costs for feed. Although this method seems promising and easy to implement, it has only been tested in few studies and further ongoing studies on the effect on skatole, duration of optimal feeding period and welfare issues should take place before implementation in large-scale. Although well-studied and effective in reducing skatole, feeding by chicory for the last week before slaughter is not profitable at the current prices as a minor increase in payment of DKK 0.08 per kg carcass weight to the farmer is necessary. This extra payment is, however, low compared to the usual variation in payment over a year. In addition, more inulin-rich crops exist and an increased demand for these products may in the future improve production methods and lower the prices.

Chicory has been produced in Denmark since the end of the 19th century for coffee-surrogate (Rich's) and again few years ago as a feed additive, but the demand decreased and production was stopped again. The production machinery used for washing, cutting, drying and pelleting can be started up again, but for a large-scale production, one more chicory-processing plant in the country will probably

be needed giving new jobs. Chicory shows the best outcome in the southern parts of Denmark and requires relatively fertile soils. Besides chicory, other inulin-rich alternatives are relevant to consider – amongst these especially Jerusalem artichokes could be relevant in Denmark. The inulin content in Jerusalem artichoke is around 650 g/kg dry matter, and to ensure a sufficient amount we suggest that 15 % of the feed is substituted by Jerusalem artichokes the last week. This strategy is assumed to have the same skatole-reducing effect as the above mentioned chicory-strategy. There are indications that free-range pigs foraging on Jerusalem artichokes and fed very restrictedly with concentrated feed may consume up to 9 kg tubers per day per pig (427). Free-range foraging on Jerusalem artichokes is possible from August to April if heavy frost is avoided. For use in housed pig production the Jerusalem artichokes have to be pelleted. No practical or health related problems have been reported related to feeding with pelleted chicory or Jerusalem artichoke. Jerusalem artichokes have only been produced and sold as a raw vegetable in Denmark. They can be sown and harvested with machinery already commonly available, and a research project has shown that they can be turned into pelleted feed just like chicory (428). A commercial production can be started up quite easily as they can be grown all over Denmark with reasonable yields, also on sandy soil, and no new machinery is needed as machinery for potato production can be used (428). The top of the plant might be used for biogas production. For in-housed pig production Jerusalem artichokes have to be washed, dried and pelleted just like chicory roots. Nobody was able to give an estimate on the price, but it is assumed to be more expensive than feeding with chicory root, amongst others because the inulin content is lower, and larger amounts have to be supplied to the pigs.

In summary, end-gain-feeding may have the potential of making EM production profitable at the same time as it to some extent limits too large amounts of tainted meat only usable for processed products. However, further limitation of tainted meat may be obtained by feeding inulin-rich crops which only requires small price-changes that are well below the usual variation in payment over a year in the payment per kg carcass to the farmer. In addition, an increased demand for inulin-rich crops may improve production methods and alternative use of waste products which will reduce the prices and may result in profitability of the use as skatole-reducing feed for pigs. Furthermore, production of inulin-rich crops may create new jobs.

Organic production

In a Danish research project with six herds raising organic entire males, an average of 68 % of the pigs, varying from 21 to 95 %, would be sorted out if using the criterion of exceeding 1 μ g/g of androstenone and 0.25 μ g/g of skatole (117). Under similar conditions in conventional production, we expect 30 % to be sorted out due to this criterion. In the study of Maribo (2012), the production of organic EM was found profitable if less than 4 % were sorted out due to boar taint (117). The study also demonstrated that there can be a large variation between farms, and it indicates that organic farms might face larger problems with boar taint than conventional production. Therefore, methods to reduce the risk of boar taint may be even more necessary in organic production.

The grain feeding method is easiest to implement in organic pig production, as organic wheat and barley is already available. The use of chicory or other inulin-rich crop, such as Jerusalem artichokes, requires the establishment of either an industrialized organic production of these products or they can be produced locally and fed directly. This will, however, imply storage problems (quite problematic) and extra labour. Nobody has been able to put an estimate on these challenges.

Reduced slaughter weight is expected to have the same effects in organic production and will probably be more relevant in organic than in conventional EM production, as some organic farms tend to have very high risks of boar taint and reducing methods are thereby required. The organic pig production might have better possibilities to market meat from smaller pigs as this consumer group is supposed to be more open to alternative products and willing to pay a little more for specialized products.

5.2.3.1.2 Profitability of immunocastration

We have assumed that the DKK 25 deduction for boar taint control is unnecessary at the long term because of an apparently low risk of boar taint related to this method (Chapter 3.2). However, there is some doubt that this will be the case as some sensory evaluations indicate that more than 1 % of the meat may be experienced as tainted although the thresholds of the boar taint compounds are not exceeded. Some strategies seem profitable but the income depends on vaccination strategy, feeding regimes, duration of the period of implementation and the successfulness of use in large-scale.

Vaccination strategy

Presuming that less than 1 % of the IC pigs are tainted and boar taint control is unnecessary, the costbenefit analysis on scenario 1 indicates that production of IC males, reared under comparable conditions as the majority of SC pigs and vaccinated according to the standard procedure, is economically attractive at the long term if there is a market for products from these pigs. However, if IC pigs have to be controlled for boar taint it is not profitable compared to SC. If second vaccination is done earlier to improve animal welfare and is combined with ad libitum feeding during the whole growth period, it also results in loss of revenue compared with SC irrespective of whether control is needed. **Table 17**. Economic results of different scenarios within production of immunocastrated pigs (IC) and in brackets the difference compared to surgically castrated males. In the implementation period, a deduction of DKK 25 to control of boar taint is included and 2 % are supposed to be sorted out due to boar taint. In the scenarios beneath, the risk of sorting out is 1 % and there is no deduction for boar taint control. Four different strategies for producing IC are included: 1) IC standard vaccinated, fed ad libitum, 2) IC standard vaccinated, fed restrictively with one feeding space per pig, 3) IC early second vaccination, fed restrictively with one feeding space per pig and 4) IC early second vaccination, fed ad libitum.

IC1 ¹	IC2 ²	IC3 ³	IC4 ⁴
133 (-20)	155 (+2)	151 (-2)	118 (-35)
529 (-80)	586 (-24)	570 (-39)	469 (-140)
481 (-128)	532 (-77)	518 (-91)	426 (-183)
159 (+7)	182 (+29)	177 (+25)	144 (-9)
636 (+26)	687 (+77)	671 (+61)	576 (-34)
578 (-32)	624 (+15)	610 (0)	523 (-86)
	IC1 ¹ 133 (-20) 529 (-80) 481 (-128) 159 (+7) 636 (+26) 578 (-32)	IC1 ¹ IC2 ² 133 (-20) 155 (+2) 529 (-80) 586 (-24) 481 (-128) 532 (-77) 159 (+7) 182 (+29) 636 (+26) 687 (+77) 578 (-32) 624 (+15)	IC1 ¹ IC2 ² IC3 ³ 133 (-20) 155 (+2) 151 (-2) 529 (-80) 586 (-24) 570 (-39) 481 (-128) 532 (-77) 518 (-91) 159 (+7) 182 (+29) 177 (+25) 636 (+26) 687 (+77) 671 (+61) 578 (-32) 624 (+15) 610 (0)

¹ Feed conversion rate: 2.75; lean meat percentage: 61.0 %; produced pigs per pigs pace per year: 3.99.

² Feed conversion rate: 2.70; lean meat percentage: 61.8 %; produced pigs per pigs pace per year: 3.78 (corrected for 1 pig less per pen due to liquid feeding).

³ Feed conversion rate: 2.70; lean meat percentage 61.4 %; produced pigs per pigs pace per year: 3.78 (corrected for 1 pig less per pen due to liquid feeding).

⁴ Feed conversion rate: 2.81; lean meat percentage: 60.6 %; produced pigs per pigs pace per year: 3.99.

* The values in the brackets are the loss in gross margin compared to production of surgically castrated pigs at the space allowance required according to the legislation.

Feeding regime

Irrespective of strategies of vaccination, the profit seems in general to be considerably enhanced by restrictive feeding during the last 4 weeks before slaughtering compared to ad libitum fed SC and IC, as restrictive feeding prevents the consequences of increased appetite in IC pigs after the second vaccination (Chapter 3.2.4.1). Under these circumstances, early vaccination is also accompanied with a considerably improved production economy although still less than if the standard vaccination is used. Although restrictive feeding also has the potential of improving the feed conversion and lean meat percentage of SC (429), the combination of this feeding strategy with IC accounts for a large part of the improved income as feed conversion and lean meat percentage, based on the literature, were assumed to be improved in restrictively fed IC compared to SC. However, at present, only about half of the Danish pig producers have feeding facilities that enable synchronous feeding of restrictive feed pigs (430) and are acceptable from a welfare point of view.

Period of implementation

An issue of the income of IC at herd level is, however, the general need for experience with new management procedures to obtain successful results. With respect to IC, successful results with low occurrence of boar taint depend on adequate management with respect to handling and storage of the vaccine as well as surveillance and re-vaccination of animals that escape vaccination (Chapter 3.2). Although IC is expected to result in less than 1 % of tainted meat, the percentage of rejected meat may be higher during implementation of the method at herd level. To secure a market for meat from IC pigs
this may imply a temporary additional cost for controlling of the carcasses for boar taint at the slaughterhouse during the implementation at herd level as well as a deduction in slaughter price per kg tainted meat. The duration of the implementation period has not been investigated, but in Danish pig production the duration of implementation with respect to changed production procedures is in general expected to be about half a year. The expected additional costs during an implementation period, presuming a rejection of 2 % of the pigs, are presented in Table 17. As evident from the table, the economic consequences of this handling of the implementation of IC result in a considerable, although probably temporary, loss of income for all IC strategies ranging from DDK 24 to DDK 140 per pig space per year.

Successfulness of use in large scale

Furthermore, as mentioned in chapter 3.2, large-scale studies on the effect of IC are very limited. This means that rejection of tainted meat may turn out to be higher than 1 %. If this happens, costs comparable with those mentioned for the implementation period in Table 17 will persist, and in that case none of the IC scenarios becomes economically profitable.

Organic production

Immunocastration is not regarded to be a realistic alternative within organic pig production, mainly because of disagreement with organic ideals for animal husbandry and the risk of self-injection. It is not mentioned in the rules for organic farming, but it has been debated in their organization (411-413). The Swedish organic farmer organisation interprets the EU regulation as a ban.

5.2.3.2 Economic latitude for improving animal welfare

Entire male production and IC may compromise welfare due to the increased aggression and mounting compared to SC pigs that are shown for more or less of the growth period as well as during transport of EM to the slaughterhouse, although the negative consequences for welfare of surgical castration are avoided. At present it is not known whether the risk of aggression and mounting in EM and IC in some cases are of a magnitude that exceeds the welfare benefits of omitting surgical castration. To represent a real improved alternative to SC from a welfare point of view, aggression and mounting in production of EM or IC should, therefore, be minimised as much as possible. Aggression levels at the farm may be reduced by a general enrichment of the environment (e.g. providing straw and roughage as rooting material) as well as by reducing stocking density (Chapters 3.1.3.1 and 3.2.4.1). In addition, earlier second vaccination in IC may reduce the duration of the period with increased aggression and mounting compared with SC (Chapter 3.2.4.1).

No specified advises for the environmental improvements are available but according to the German guidelines for space allocation in EM production (Chapter 3.1.1), a 10 % increase in the available space per pig is advised. Therefore, we re-calculated the gross margin per pig space for all scenarios with an area/pig increased by 10 % to evaluate the economic latitude for improving welfare. As evident from

Table 15 to 17, the only scenarios that have economical latitude for improving animal welfare through an increase in space allowance by 10 % are those with production of IC with restrictive feeding after the second vaccination if permanent control for boar taint is redundant. In this case welfare of restrictively fed IC may even be further improved by earlier vaccination and still be profitable. However, as mentioned above, only about half of the Danish pig producers at present have feeding facilities that enable restrictive feeding with synchronous feeding which is important premise to ensure animal welfare by restrictive feeding. In addition, restrictive feeding implies the risk that a minor part of the pigs, which may have a relatively large appetite or eat slowly, are too restrictively fed and are hungry for part of the time.

Overall, in comparable environment, IC pigs are likely to have better welfare than EM due to the reduction of the duration of the period with increased levels of mounting and aggression with at least 25 to 40 % (Chapter 3.2.4.1) as well as the reduced aggressiveness at the time of delivery. Furthermore, in the condition of restrictive feeding IC seems to possess the economic latitude to further improve animal welfare compared with production of EM by increasing the space allowance and earlier application of the second vaccination. However, only a small increase in the meat price has to occur (DKK 0.05 per kg), which is very low compared to the usual variation in payment over a year, to pay the costs for the farmer to increase space allowance by 10 %. Thus, there is considerable economic latitude for less expensive initiatives to improve animal welfare in EM slaughtered at a carcass weight of 83 kg if no skatole-reducing feed is added. This, in combination with the fact that the economic latitude for reduction of aggression and mounting in IC only is achievable in the 50 % of the Danish farms with facilities for synchronous restrictive feeding, entails that animal welfare in practise may not generally be better in IC than in EM. Thus, from a welfare point of view IC would only have the potential of being the definite best alternative to SC if facilities for simultaneous restrictive feeding are available.

5.3 Concluding remarks

Within the alternatives described in chapter 3, a number of strategies have the potential of reducing boar taint and improving animal welfare. However, sperm sexing is not applicable within 2 to 5 years, and evaluation of the feasibility of molecular genetic selection approaches rely on an impact analysis by the pig industry followed by a focused effort if the initial analysis gives reasons for it.

Production of entire male and immunocastrated pigs may, theoretically, be implementable within a short time, but the methods require reliable methods for on-line detection of boar taint, which may be available within the next couple of years. However, another major barrier in implementation of these alternatives is the uncertainty on whether the consumers will accept the methods and resulting pork quality, hence continuing to purchase pork, as this is the key factor for maintaining the profitability in

the whole production chain. In addition, to ensure a future production of meat an alternative to surgical castration has to be profitable for the farmer.

The risk of tainted meat in production of entire male pigs and the consumers' uncertainty of whether there are risks to food safety by immunocastration are issues that should be taken into account in the decisions on which alternatives to surgical castration that should be implemented. The consumers' knowledge about castration methods is limited and the provided information about alternative methods, especially on whether they imply risks for the consumers, is important for consumer acceptance of immunocastration, whereas acceptance of production of meat from entire male pigs demands a focused effort on avoiding tainted meat.

Based on the literature, the producers seem in general to have a less positive attitude than the consumers towards implementation of alternatives to surgical castration and mainly concern on labour implications, the risk of boar taint and the expected profit of the alternatives, but experience with the alternatives can change their attitude. The attitude of Danish producers has not been surveyed.

The Danish pig industry is positive to the ban on surgical castration and they regard production of entire males to be the best alternative to surgical castration. Therefore, one of the main issues for the slaughterhouses is the development of fast and reliable on-line detection of boar taint. In addition, development of methods for reducing the amount of tainted meat has high priority as full-scale production of entire male pigs implies an increase in the total amount of meat sorted out, which has to be used for processed products. The market for processed products will, however, easily be saturated.

The cost-benefit analyses indicate that production of entire males with the current sorting methods is very profitable if using the usual carcass weight of 83 kg. In case of full-scale production, however, the amount of boar tainted meat will constitute a big problem to the pig industry due to the large amount of tainted meat that has to be processed and a reduction in the payment per kg carcass may occur. This problem will be further enlarged if sorting is also based on androstenone, which is suggested to be implemented in order to protect market shares. However, with decreasing levels of skatole, higher levels of androstenone may be acceptable. Therefore, there will be an urgent need to reduce skatole, among others by special end-feeding. By feeding pure grain the last days before slaughter, profitability is still better with EM at a carcass weight of 83 kg than by producing SC pigs, while it is a little lower than with surgical castration when adding chicory to end-feed instead as long as current sorting methods are used.

In case new sorting methods including the level of fat androstenone are implemented, a reduction in slaughter weight may also contribute to a reduction in sorting out if combined with skatole-reducing feeding. But with the current prices and sorting methods, the profitability of slaughtering at a reduced

weight is only at the same level as SC production if the farmers are paid DKK 0.1 - 0.27 more per kg carcass when combined with feeding pure grain or chicory, respectively. However, in general, EM production is not profitable compared to SC if more restrictive sorting methods are introduced but again the farmers only have to get a little bit more for the meat to be able to pay the extra costs. In the worst case (most sensitive sorting procedure: Androstenone <1.00 μ g/g, skatole <0.25 μ g/g), the meat price has to be increased by DKK 0.48 to 0.67 per kg to pay these extra costs, which is still less than the variation in the listed meat price during the year.

Supplying the feeding of male pigs with chicory or pure grain for the last 4 to 7 days before slaughter is able to reduce the amount of skatole significantly, but with the current price-level only end-feed with pure grain is profitable, and only in case of normal slaughter weight. Feeding with pure grain will be easy to implement, but the effect is less documented than the effect of inulin-containing feeds like chicory and Jerusalem artichoke. If the profitability of using inuline-based feed is improved, e.g. through a minor increase in meat prices or reduced price of the crops, both crops can be grown and processed in Denmark with good outcomes, but at present, the industry is non-existing. Already existing agricultural machinery from beet and potato production can be used, but the washing, drying and pelleting industrial facilities have to be built up.

At the current prices, immunocastration will only be profitable provided that less than 1 % of the meat is tainted, and the control for boar taint at the abattoir therefore is redundant. The highest profit is obtained by vaccination in accordance with the manufacturer's recommendation and restrictive feeding after the second vaccination.

In comparable environment, IC pigs are likely to have better welfare than EM due to the reduction of the duration of the period with increased levels of mounting and aggression as well as the reduced aggressiveness at the time of delivery. Furthermore, in the condition of restrictive feeding IC seems to possess the economic latitude to further improve animal welfare compared with production of EM by increasing the space allowance and earlier application of the second vaccination. However, only a small increase in the meat price has to occur (DKK 0.05 per kg), which is very low compared to the usual variation in payment over a year, to pay the costs for the farmer to increase space allowance by 10 %. Thus, there is considerable economic latitude for less expensive initiatives to improve animal welfare in EM slaughtered at a carcass weight of 83 kg if no skatole-reducing feed is added. This, in combination with the fact that the economic latitude for reduction of aggression and mounting in IC only is achievable in 50 % of the Danish farms with facilities for synchronous restrictive feeding, entails that animal welfare in practise may not generally be better in IC than in EM. Thus, from a welfare point of view, IC would only have the potential of being the definite best alternative to SC if facilities for simultaneous restrictive feeding are available.

The cost-benefit analyses did not include organic farming due to lack of several parameter estimates. However, based on previous Danish research projects, organic farms might face larger problems with boar taint than conventional production, and methods to reduce the risk of boar taint may, therefore, be even more necessary in organic production. The grain feeding method is easy to implement in organic pig production as organic wheat and barley are already available. Reduced slaughter weight is expected to have the same effects in organic production and will probably be more relevant in organic than in conventional EM production. The organic pig production might have better possibilities to market meat from smaller pigs as this consumer group is supposed to be more open to alternative products and willing to pay a little more for specialized products. Immunocastration is not regarded to be a realistic alternative within organic pig production.

Chapter 6 – Current relevance of alternatives to surgical castration in Danish pig production

At present, surgical castration of male piglets is a common practice in the pig production in many countries to prevent boar taint mainly caused by skatole and androstenone. However, due to animal welfare concerns there is an increased desire to stop surgical castration at least in the European countries. The European Commission and representatives of European pig farmers, meat industry, retailers, scientists, veterinarians and animal welfare NGOs have recently committed themselves to voluntarily end surgical castration of pigs in the European Union by January 1, 2018. Therefore, realistic alternatives to surgical castration are needed.

The most promising alternatives to surgical castration are production of entire male pigs, immunocastration, sperm sexing and breeding. However, sperm sexing is not applicable within the next 2 to 5 years. Selective breeding to reducing the level of boar taint by conventional selection might have a time frame of 8 to 12 years depending upon the intensity of selection. Combined with molecular genetic approaches selection will likely have a time frame shorter than 5 years. However, evaluation of the feasibility of the molecular genetic approaches of selective breeding relies on an impact analysis of the expected effects on other production and reproduction traits, which need to be performed by the pig industry before decision making and the first steps of action can be taken. Production of entire male pigs and immunocastrated pigs may, theoretically, be implementable within a short time. However, except sperm sexing all alternatives require access to international accepted and reliable online methods for detection of boar taint that throughout fulfils the requirement for a highly streamlined industry at the slaughterhouses. Such methods may be available within the next 2 to 5 years.

In addition, the major barrier for implementation of these alternatives is mainly the uncertainty regarding sufficient consumer acceptance and pork quality, to ensure continuing purchase of pork, as this is the key factor for maintaining profitability in the whole production chain. The consumers' knowledge about castration methods is limited and information about alternatives to surgical castration is important for consumer acceptance. Regarding acceptance of IC, the concern is especially whether IC implies risks to the consumer. The Danish pig industry has the possibility to inform about the lack of risk of immunocastration for the consumers of the domestic market, while it will be much more difficult to inform and affect consumers at the export markets who buy a large part of the Danish pig products. Due to the risk of loss of market share and the consequences for a highly refined industry, the Danish pig industry is reluctant to accept immunocastration. The consumers' acceptance of production of entire male pigs mainly relies on the risk of tainted meat, which by the pig industry is expected to be resolved by future implementation of reliable on-line methods for detection of boar taint, including measurement of androstenone. However, high sorting out of tainted meat will affect

the marketing as tainted meat is inappropriate for fresh meat products and therefore has to be used in processed products.

Irrespective of the exact nature of the alternative it has to be accepted by the farmer and to be profitable at herd level. The attitude of Danish producers is not known but the present review and costbenefit analyses indicate that in Denmark, production of entire males with the current sorting methods is very profitable at the common slaughter weight of 83 kg. In case of full-scale production, however, the expected amount of boar tainted meat will increase and as such the amount of meat that has to be processed. Skatole-reducing feeding strategies during the last 4 to 7 days before slaughter have the potential to reduce this problem considerably but are only profitable at farm level if the cost for the feeding strategy is about DKK 13 or less as is the case with grain feeding. At present, the well documented and probably more effective feeding strategies to lower skatole, based on crops containing inulin such as chicory and Jerusalem artichoke, are unprofitable for the farmers compared to the production of surgical castrates, but the costs can be out-weighted by a minor increase in meat price to the farmer of DDK 0.08 to 0.23 per kg depending on sorting method. Both crops can be grown and processed in Denmark with good outcomes, but at present, the industry is non-existing. Already existing agricultural machinery from beet and potato production can be used, but the washing, drying and pelleting industrial facilities have to be built up. Thus, the costs of the feeding strategies may be reduced if a market for the crops arises. At present, no well-documented feeding solution to the androstenone problem is known.

The industrial problem of high amounts of out-sorted meat, only usable for processed products, will be further enlarged if future sorting-out methods are also based on androstenone, as suggested by the meat industry, to protect market share. This is also the case when taking into account that decreasing levels of skatole allow higher levels of androstenone before meat quality is compromised. The level of fat androstenone may, to some extent, be reduced by a reduction in weight at slaughter, but with the current prices and even less restricted sorting methods, reduced weight at slaughter is not profitable for the farmer. However, with the current sorting methods the meat price paid to the farmer only has to increase with DKK 0.10 per kg to compensate for the lower slaughter weight in case of grain-end-feeding and DKK 0.27 per kg to compensate in case of chicory-end-feeding, which is less than the normal variation in list prices during a year.

At the current meat and vaccine prices, immunocastration will only be profitable provided that less than 1 % of the meat is sorted out and the control for boar taint at the abattoir therefore is redundant. The highest profit is obtained by vaccination in accordance with the manufacturer's recommendation and restrictive feeding after the second vaccination.

The cost-benefit analyses did not include organic farming due to lack of several parameter estimates. However, based on previous Danish research projects, organic farms might face larger problems with boar taint than conventional production, and methods to reduce the risk of boar taint may therefore be even more necessary in organic production. The organic pig production might have better possibilities to market meat from smaller pigs as their consumer group is supposed to be more open to alternative products and willing to pay a little more for specialized high quality products. Immunocastration is not regarded to be a realistic alternative within the concept of organic pig production. However, selective breeding, excluding boars known to produce offspring with high level of boar taint might be an alternative.

From a welfare point of view, both production of entire males and immunocastration may improve the animal welfare in early life due to the omission of the painful surgical castration procedure. However, the level of aggression and sexual male behaviour such as mounting are increased during the growing period compared to surgically castrated pigs and may compromise animal welfare as these types of behaviour have the risk of injuries on pen mates. Whether this in practice will lead to an impaired animal welfare is likely to depend on the social environment and the housing conditions. At present it is not known whether the risk of higher aggression and mounting in entire male pigs and immunocastrated pigs in some cases are of a magnitude that exceeds the welfare benefits of omitting surgical castration.

In comparable environments, immunocastrated pigs are likely to have better welfare than entire male pigs due to the reduction of the duration of the period with increased levels of mounting and aggression as well as the reduced aggressiveness at the time of delivery. However, the economic latitude for improving animal welfare by increased space allowance and early vaccination is only significant if the immunocastrated pigs are restrictively fed, which from a welfare point of view requires that the pigs are synchronously fed. At present, the facility for synchronous, restrictive feeding exists, however, only in about half of the Danish farms. In production of entire males at a carcass weight of 83 kg and no skatole reducing feed only a very small increase in the meat price has to occur (DKK 0.05 per kg) to pay the costs of increasing welfare by increasing space allowance by 10 %. This, in combination with the fact that the economic latitude for reduction of aggression and mounting in immunocastrated pigs only are achievable in 50 % of the Danish farms with facilities for synchronous, restrictive feeding, entails that animal welfare in practise may not generally be better by immunocastration than in production of entire males.

Future research and activities should primary focus on acceptable thresholds of skatole, androstenone and potential other relevant compounds that take the interdependence between individual boar taint compounds into consideration as well as an elaboration of an international accepted, reliable on-line method for sorting that fulfils the requirement for a highly streamlined industry at the slaughterhouses, as this is a precondition for implementation of all alternatives to surgical castration that may be relevant within 2 to 5 years.

If entire male production is intended to be implemented in traditional as well as organic pig production further research in strategies for reducing boar taint may improve the profitability considerable and should preferential include refinement of the most reliable strategies influencing boar taint (e.g. the optimal duration of adding grain or chicory before slaughter or guidelines with respect to hygiene) as well as identification of new, potential reliable strategies (e.g. availability of feed; dietary supplementation of organic acids, bioactive components or adsorbent materials; changing in the dietary ratio of different grain types or the fat ratio and its composition; optimal slaughter weight; choice of breeds and the social and physical environment).

In case immunocastration is preferred it should be taken into account that the experience in practise within EU is limited and large scale studies informing on the amount of tainted meat, performance, animal welfare and working environment is restricted.

As breeding seems to be a sustainable approach reducing the level of boar taint, an evaluation of the feasibility of molecular genetic selection approaches should preferentially be done by the pig industry followed by a focused breeding effort if the initial analysis gives reasons for it.

Furthermore, although all alternatives are based on omission of surgical castration and as such improve animal welfare in the early rearing period, the welfare consequences of the alternatives in relation to surgical castration taking the whole growing period into consideration are poorly elucidated. Presumed that decisions on alternatives to surgical castration should be based on knowledge further studies on animal welfare and strategies for improving it have to be performed.

Finally, the effect of a change towards production of entire male pigs or immunocastrated pigs on overall resource-use and environmental impact may be relevant but was not within the scope of this report. Due to the improved feed conversion ratio in entire male, and partly in immunocastrated pigs, compared to surgical castrated pigs a reduction in environmental effects is expected compared to the present pig production.

In conclusion, sperm sexing is not applicable within the next 2 to 5 years, but selective breeding is a sustainable approach to reduce the level of boar taint and may be relative fast if a combination of genomic selection and candidate SNP test is used and provides the opportunity to restrict selection pressure to haplotypes showing little or no adverse effects on other traits. Selective selection relies, however, on an impact analysis of the expected effects on other production and reproduction traits, which has to be performed by the pig industry before decision making and the first steps of action can be taken. Production of entire male pigs as well as immunocastration may be implemented within 2 to 5 years and improve animal welfare in early life compared to surgical castration, but both alternatives may compromise welfare due to increased aggression and mounting behaviour in the growing period, if no initiatives to improve animal welfare is taken. At present, it is not possible to evaluate whether the alternatives overall improve welfare compared to surgical castration. In addition, ranking of the

possibilities of animal welfare in the respective alternatives is not possible. Both alternatives may be economical profitable at farm level depending on the specific conditions but imply new challenges for the pig industry, especially concerning the potential increased amounts of tainted meat requiring improved sorting methods and only usable for processing as well as consumers' attitudes on export markets. The national economic consequences of this have to be evaluated. Implementation of sperm sexing or selection against boar taint are promising alternatives as well but need further development or a focused decision based on analyses by the pig industry.

For all alternatives an evaluation of the environmental impact will be highly relevant. In addition, future research on entire male pig production should focus on identification of acceptable thresholds for boar taint compounds; elaboration of reliable and international accepted on-line method for sorting; refinement of the most reliable strategies influencing boar taint and identification of new, potential reliable strategies. In case immunocastration is preferred large scale studies informing on the amount of tainted meat, performance, animal welfare and working environment is desirable. Furthermore, the welfare consequences of the alternatives in relation to surgical castration taking the whole growing period into consideration is essential for decision making and elaboration of guidelines for optimizing animal welfare in the specific productions are needed.

References – according to the order of citation

1. Prunier, A., Bonneau, M., von Borell, E.H., Cinotti, S., Gunn, M., Fredriksen, B., Giersing, M., Morton, D.B., Tuyttens, F.A.M., Velarde, A. 2006. A review of the welfare consequences of surgical castration in piglets and the evaluation of non-surgical methods. Animal Welfare 15: 277-289

2. von Borell, E., Baumgartner, J., Giersing, M., Jäggin, N., Prunier, A., Tuyttens, F.A.M., Edwards, S.A. 2009. Animal welfare implications of surgical castration and its alternatives in pigs. Animal 3:1488-1496

3. Kluivers-Poodt, M., Houx, B. B., Robben, S. R. M., Koop, G., Lambooij, E., Hellebrekers, L. J. 2012. Effects of a local anaesthetic and NSAID in castration of piglets, on the acute pain responses, growth and mortality. Animal 6:1469–1475

4. Kluivers-Poodt, M., Zonderland, J. J., Verbraak, J., Lambooij, E., Hellebrekers, L. J. 2013. Pain behaviour after castration of piglets; effect of pain relief with lidocaine and/or meloxicam. Animal 7:1158–1162

5. Schmidt, T., König, A., von Borell, E., 2012. Impact of general injection anaesthesia and analgesia on post-castration behaviour and teat order of piglets. Animal 6:1998–2002

6. Sutherland, M. A., Davis, B. L., Brooks, T. A., Coetzee, J. F. 2012. The physiological and behavioral response of pigs castrated with and without anesthesia or analgesia. Journal of Animal Science 90:2211-2221

7. Lundström, K., Matthews, K.R., Haugen, J.E. 2009. Pig meat quality from entire males. Animal 3:1497-1507

8. Bonneau, M., Walstra, P., Claudi-Magnussen, C., Kempster, A.J., Tornberg, E., Fischer, K., Diestre, A., Siret, F., Chevillon, P., Claus, R., Dijksterhuis, G., Punter, P., Matthews, K. R., Agerhem, H., Béague, M.P., Oliver, M.A., Gispert, M., Weiler, U., von Seth, G. Leask, H., Furnols, M., Homer, D.B., Cook, G.L. 2000. An international study on the importance of androstenone and skatole for boar taint: IV. Simulation studies on consumer dissatisfaction with entire male pork and the effect of sorting carcasses on the slaughter line, main conclusions and recommendations. Meat Science 54:285-295

9. Zamaratskaia, G., Squires, E.J. 2009. Biochemical, nutritional and genetic effects on boar taint in entire male pigs. Animal 3:1508-1521

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

11. Gower, D.B. 1972. 16-unsaturated C₁₉ steroids - a review of their chemistry, biochemistry and possible physiological role. Journal of Steroid Biochemistry 3:45-103

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

13. Meier-Dinkel, L., Trautmann, J., Frieden, L., Tholen, E., Knorr, C., Sharifi, A.R., Bücking, M., Wicke, M., Mörlein, D. 2013. Consumer perception of boar meat as affected by labelling information, malodorous compounds and sensitivity to androstenone. Meat Science 93: 248-256

14. Weiler, U., Font i Furnols, M., Fischer, K., Kemmer, H., Oliver, M.A., Gispert, M., Dobrowolski, A., Claus, R. 2000. Influence of differences in sensitivity of Spanish and German consumers to perceive

androstenone on the acceptance of boar meat differing in skatole and androstenone concentrations. Meat Science 54:297-304

15. Font i Furnols, M., Gispert, M., Diestre, A., Oliver, M.A. 2003. Acceptability of boar meat by consumers depending on their age, gender, culinary habits, and sensitivity and appreciation of androstenone odour. Meat Science 64:433-440

16. Lunde, K., Egelandsdal, B., Skuterud, E., Mainland, J.D., Lea, T., Hersleth, M., Matsunami, H. 2012. Genetic Variation of an Odorant Receptor OR7D4 and Sensory Perception of Cooked Meat Containing Androstenone. PLoS ONE 7:e35259

17. Font i Furnols, M. 2012. Consumer studies on sensory acceptability of boar taint: A review. Meat Science 92:319-329

18. Bekaert, K.M., Tuyttensm F.A.M., Duchateaum L., De Brabanderm H.F., Aluwém M., Milletm S., Vandendriesschem F., Vanhaeckem L. 2011. The sensitivity of Flemish citizens to androstenone: Influence of gender, age, location and smoking habits. Meat Science 88:548-552

19. Bonneau, M., Chevillon, P. 2012. Acceptability of entire male pork with various levels of androstenone and skatole by consumers according to their sensitivity to androstenone. Meat Science 90:330-337

20. Aaslyng M.D., Christensen R., Brockhoff P., Broge E.H.de L. 2013. Age related changes in consumers ability to detect the boar taint compounds androstenone and skatole. Danish consumers' liking of loin chops from entire males and castrates. Presentation at EAAP Working Group 2013, Production and Utilization of Meat from Entire Male Pigs, Monells, Girona, Spain, 2nd to 3rd December, 2013. 21. Patterson, R.L.S. 1967. A possible contribution of phenolic components to boar odour. Journal of the Science of Food and Agriculture 18:8-10

22. Annor-Frempong, I.E., Nute, G.R., Whittington, F.W., Wood, J.D. 1997. The problem of taint in pork-II. The influence of skatole, androstenone and indole, Presented individually and in combination in a model lipid base, on odour perception. Meat Science 47: 49-61

23. Rius Solé, M.A., García Regueiro, J.A. 2001. Role of 4-Phenyl-3-buten-2-one in Boar Taint: Identification of New Compounds Related to Sensorial Descriptors in Pig Fat. Journal of.Agricultural and Food Chemistry 49:5303-5309

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

25. Jensen, M. T., Cox, R.P., Jensen, B.B. 1995b. Microbial-production of skatole in the hind gut of pigs given different diets and its relation to skatole deposition in backfat. Animal Science 61:293-304

26. Jensen, B.B., Jensen, M. T. 1998. Microbial production of skatole in the digestive tract of entire male pigs. In: Skatole and boar taint, (ed. W.K.Jensen), pp. 41-76. Danish Meat Research Institute. Roskilde, Denmark.

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

28. Jensen, B.B. 2006. Prevention of boar taint in pig production. Factors affecting the level of skatole. Acta Veterinaria Scandinavica 48:S6

29. Wesoly, R., Weiler, U. 2012. Nutritional Influences on Skatole Formation and Skatole Metabolism in the Pig. Animals 2:221-242

30. Agergaard, N., Laue, A. 1998. Absorption of skatole to portal vein blood and liver turnover in entire male pigs using an *in vivo* animal model. In: Jensen, W.K. (ed.), Skatole and Boar Taint, results from

an integrated national research project investigating causes of boar taint in Danish pigs, pp. 77-96, Danish Meat Research Institute, Roskilde, Denmark.

31. Hansen, L.L., Larsen, A.E., Jensen, B.B., Hansen-Møller, J., Barton-Gade, P. 1994. Influence of stocking rate and faeces deposition in the pen at different temperatures on skatole concentration (boar taint) in subcutaneous fat. Animal Science 59:99-110

32. Jensen, R.L. 2012. Feed interventions and skatole deposition. Characterisation of a skatole producing bacterium isolated from the gastrointestinal tract of pigs. Master thesis, Department of Large Animal Scenic, Copenhagen University, 50 pp.

33. Xiaoqiong, L., Jensen, R.L., Canibe, N., Højberg, O., Jensen, B.B. 2013. Characterisation of a skatole producing bacterium isolated from the gastrointestinal tract of pigs. In preparation.

34. Hawe, S., Walker, N., Moss, B. 1992. The effects of dietary fiber, lactose and antibiotic on the levels of skatole and indole in feces and subcutaneous fat in growing pigs. Animal Production 54: 413-419

35. Jensen B.B. 1990. [Skatole (boar taint), microbial production of skatole in the gastro-intestinal tract of pigs]. Skatol (ornelugt). Mikrobiel produktion af skatol i mave-tarmkanalen hos grise. Meddelelse, Statens Husdyrbrugsforsøg [NIAS], Landbrugsministeriet, Meddelelse no. 772, 4 pp.

36. Leong, J., Morel, P.C.H, Purchas, R.W., Wilkinson, B.H.P. 2011. Effect of dietary components including garlic on concentrations of skatole and indole in subcutaneous fat of female pigs. Meat Science 88:45-50

37. Raab, S., R. Leiser, H. Kemmer, aR. Claus. 1998. Effects of energy and purines in the diet on proliferation, differentiation, and apoptosis in the small intestine of the pig. Metabolism, Clinical and Experimental 47:1105-1111

38. Claus, R, Weiler, U., Herzog, A. 1994. Physiological aspects of androstenone and skatole formation in the boar – a review with experimental data. Meat Science 38: 289-305

39. Škrlep, M., Batorek, N., Bonneau, M., Fazarinc, G., Šegula, B., Candek-Potokar, M. 2012a. Short Communication. Elevated fat skatole levels in immunocastrated, surgically castrated and entire male pigs with acute dysentery. The Veterinary Journal 194:417–419

40. Puccinelli, E., Gervasi, P.G., Longo, Xenobiotic, V.2011. Metabolizing Cytochrome P450 in Pig, a Promising Animal Model. Current Drug Metabolism 12:507-525

41. Parkinson, A. 2001. Biotransformation of xenobiotics. In: Toxicology: The basic science of poisons, sixth edition, edited by Klaassen C.C., New York, McGraw-Hill

42. Matal, J., Matuskova, Z., Tunkova, A., Anzenbacherova, E., Anzenbacher, P. 2009. Porcine CYP2A19, CYP2E1 and CYP1A2 forms are responsible for skatole biotransformation in the reconstituted system. Neuroendocrinology Letters 30:36-40

43. Terner, M.A., Gilmore, W.J., Lou, Y.P., Squires, E.J. 2006. The role of CYP2A and CYP2E1 in the metabolism of 3-methylindole in primary cultured porcine hepatocytes. Drug Metabolism and Disposition 34:848-854

44. Wiercinska, P., Lou, Y., Squires, E.J. 2012. The roles of different porcine cytochrome P450 enzymes and cytochrome b5A in skatole metabolism. Animal 6:834-845

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

46. Babol, J., Squires, E.J., Lundström, K. 1998. Hepatic metabolism of skatole in pigs by cytochrome P4502E1. Journal of Animal Science 76:822-828

47. Squieres, E.J., Lundström, K. 1997. Relationship between cytochrome P450IIE1 in liver and levels of skatole and its metabolites in intact male pigs. Journal of Animal Science 75:2506-2511

48. Zamaratskaia, G., Squires, E.J., Babol, J., Andersson, H.K., Andersson, K., Lundstrøm, K. 2005. Relationship between the activities of cytochromes P4502E1 and P4502A6 and skatole content in fat in entire male pigs fed with and without raw potato starch. Livestock Production Science 95:83-88

49. Zamaratskaia, G., Chen, G., Lundstrom, K. 2006. Effects of sex, weight, diet and hCG administration on levels of skatole and indole in the liver and hepatic activities of cytochromes P4502E1 and P4502A6 in pigs. Meat Science 72:331-338

50. Zamaratskaia, G., Stefanovic, S., Lundstrom, K., Doran, O.2012. Expression of the hepatic skatoleand androstenone-metabolising enzymes in entire male pigs of two live weights. Livestock Science 145:124-130

51. Diaz, G.J., Squires, E.J. 2000. Role of Aldehyde Oxidase in the Hepatic in Vitro Metabolism of 3-Methylindole in Pigs. Journal of Agricultural and Food Chemistry 48:833-837

52. Diaz, G.J., Skordos, K.W., Yost, G.S., Squires, E.J. 1999. Identification of Phase I Metabolites of 3-Methylindole Produced by Pig Liver Microsomes. Drug Metabolism and Disposition 27:1150-1156

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

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

55. Pascussi, J.M., Gerbal-Chaloin, S., Drocourt, L., Assénat, E., Larrey, D., Pichard-Garcia, L., Vilarem, M.J., Maurel, P. 2004. Cross-talk between xenobiotic detoxication and other signalling pathways: clinical and toxicological consequences. Xenobiotica 34:633-664

56. Tompkins, L.M., Wallace, A.D. 2007. Mechanisms of cytochrome P450 induction. Journal of Biochemical and Molecular Toxicology 21:176-181

57. Brunius, C., Rasmussen, M. K., Lacoutière, H., Andersson, K., Ekstrand, B., Zamaratskaia, G. 2012. Expression and activities of hepatic cytochrome P450 (CYP1A, CYP2A and CYP2E1) in entire and castrated male pigs. Animal 6:271-277

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

59. Kojima, M., Sekimoto, M., Degawa, M. 2008. A novel gender-related difference in the constitutive expression of hepatic cytochrome P4501A subfamily enzymes in Meishan pigs. Biochemical Pharmacology 75:1076-1082

60. Gillberg, M., Skaanild, M.T., Friis, C.2006. Regulation of Gender-Dependent CYP2A Expression in Pigs: Involvement of Androgens and CAR. Basic & Clinical Pharmacology & Toxicology 98:480-487

61. Zamaratskaia, G., Zlabek, V., Chen, G., Madej, A. 2009. Modulation of porcine cytochrome P450 enzyme activities by surgical castration and immunocastration. Animal 3:1124-1132

62. Whittington, F., Nute, G., Hughes, S., McGivan, J., Lean, I., Wood, J. et al. 2004. Relationships between skatole and androstenone accumulation, and cytochrome P4502E1 expression in meishan x large white pigs. Meat Science 67:569-576

63. Niemelä, O., Parkkila, S., Pasanen, M., Viitala, K., Villanueva, J.A., Halsted, C.H. 1999. Induction of cytochrome P450 enzymes and generation of protein-aldehyde adducts are associated with sexdependent sensitivity to alcohol-induced liver disease in micropigs. Hepatology 30:1011-1017 64. Zamaratskaia, G., Oskam, I. C., Ropstad, E., Tajet, H., Dahl, E., Andresen, Ø. 2008. Effects of hCG Stimulation on Hepatic Activities of Cytochromes P4502E1 and P4502A in Pubertal Male Pigs. Reproduction in Domestic Animals 43:147-152

65. Zamaratskaia, G., Zlabek, V., Ropstad, E., Tajet, H., Andresen, Ø. 2010. Hepatic Ethoxy-, Methoxyand Pentoxyresorufin O-Dealkylase Activities in Landrace and Duroc Pigs Stimulated with hCG. Reproduction in Domestic Animals 45:e269-e274

66. Zamaratskaia, G., Zlabek, V., Ropstad, E., Andresen, Ø. 2012. In vitro and In vivo Association of Porcine Hepatic Cytochrome P450 3A and 2C Activities with Testicular Steroids. Reproduction in Domestic Animals, 47: 891-898

67. Kojima, M., Sekimoto, M., Degawa, M. 2010. Androgen-mediated down-regulation of CYP1A subfamily genes in the pig liver. Journal of Endocrinology 207:203-211

68. Chen, G., Cue, R.A., Lundstrom, K., Wood, J.D., Doran, O. 2008. Regulation of CYP2A6 protein expression by skatole, indole, and testicular steroids in primary cultured pig hepatocytes. Drug Metabolism and Disposition 36:56-60

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

70. Tolson, A.H., Wang, H. 2010. Regulation of drug-metabolizing enzymes by xenobiotic receptors: PXR and CAR. Advanced Drug Delivery Reviews 62:1238-1249

71. Rasmussen, M.K., Zamaratskaia, G. and Ekstrand, B. 2011. In vitro cytochrome P450 2E1 and 2A activities in the presence of testicular steroids. Reproduction in Domestic Animals 46:149-154.

72. Zamaratskaia, G., Gilmore, W.J., Lundstrom, K., Squires, E.J. 2007. Effect of testicular steroids on catalytic activities of cytochrome P450 enzymes in porcine liver microsomes. Food and Chemical Toxicology 45: 676-681.

73. Zamaratskaia, G., Rasmussen, M., Herbin, I., Ekstrand, B., Zlabek, V. 2011. In vitro inhibition of porcine cytochrome P450 by 17b-estradiol and 17a-estradiol. Interdisciplinary Toxicology 4:78-84

74. Zamaratskaia, G., Babol, J., Andersson, H., Lundström, K. 2004. Plasma skatole and androstenone levels in entire male pigs and relationship between boar taint compounds, sex steroids and thyroxine at various ages. Livestock Production Science 87:91-98

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

76. Brooks, R.I., Pearson, A.M. 1986. Steroid Hormone Pathways in the Pig, with Special Emphasis on Boar Odor: A Review. Journal of Animal Science 62:632-645

77. Gundlach, H. 1968. Brutfürsorge, Brutpflege, Verhaltensontogenese und Tagesperiodik beim Europäischen Wildschwein (*Sus Scrofa* L.). Zeitschreift für Tierpsychologie 25:955-995

78. Andersson, H., Rydhmer, L., Lundstrom, K., Wallgren, M., Andersson, K., Forsberg, M. 1998a. Influence of artificial light regimens on sexual maturation and boar taint in entire male pigs. Animal Reproduction Science 51:31-43

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

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

81. Fredriksen, B., Lium, B.M., Marka, C.H., Heier, B.T., Dahl, E., Choinski, J.U., Nafstad, O. 2006. Entire male pigs in a farrow-to-finish system. Effects on androstenone and skatole. Livestock Science 102:146-154

82. Pedersen, L.J. Jensen, K.H., Jørgensen, E. 1996. Pre- and postpubertal LH and estradiol pattern in gilts subjected to intermittent inescapable electroshock. Acta Veterinaria Scandinavica 37:153-161

83. Moberg, G.P., Mench, J.A. 2000. The Biology of Animal Stress, CABI Publishing, Wallingford, UK, 377p.

84. Pedersen, L.J. 2007. Sexuel behavior in female pigs. Hormones and Behavior, 53:64-69

85. Denhard, M., Rohrmann, H., Kauffold J. 2013. Measurement of 16-Androstenes (5α -Androst-16-en-3-one, 5α -Androst-16-en- 3α -ol, 5α -Androst-16-en- 3β -ol) in salvia of German landrace and Göttingen minipig boars. Chapter 30 in: M.L. East and M. Denhard (eds.) Chemical Signals in Vertebrates 12.

86. Doran, E., Whittington, F.M., Wood, J.D., McGivan, J.D. 2004. Characterisation of androstenone metabolism in pig liver microsomes. Chemico-Biological Interactions 147:141-149

87. Sinclair, P.A., Hancock, S., Gilmore, W.J., Squires, E. J. 2005. Metabolism of the 16-androstene steroids in primary cultured porcine hepatocytes. The Journal of Steroid Biochemistry and Molecular Biology 96:79-87

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

89. Moe, M., Grindflek, E., Doran, O. 2007. Expression of 3b-hydroxysteroid dehydrogenase, cytochrome P450-c17, and sulfotransferase 2B1 proteins in liver and testis of pigs of two breeds: Relationship with adipose tissue androstenone concentration. Journal of Animal Science 85:2924-2931

90. Nicolau-Solano, S.I., Whittington, F.M., Wood, J.D., Doran, O. 2007. Relationship between carcass weight, adipose tissue androstenone level and expression of the hepatic 3b-hydroxysteroid dehydrogenase in entire commercial pigs. Animal 1:1053-1059

91. Chen, G., Li, S., Dong, X., Bai, Y., Chen, A., Yang, S., Fang, M., Zamaratskaia, G., Doran, O. 2012. Investigation of Testosterone, Androstenone, and Estradiol Metabolism in HepG2 Cells and Primary Culture Pig Hepatocytes and Their Effects on 17bHSD7. Gene Expression. PLoS ONE 7:e52255

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

93. Rasmussen, M.K., Brunius, C., Ekstrand, B., Zamaratskaia, G. 2012. Expression of hepatic 3bhydroxysteroid dehydrogenase and sulfotransferase 2A1 in entire and castrated male pigs. Molecular Biology Reports 39:7927–7932

94. Nicolau-Solano S.I. and Doran O. 2008. Effect of testosterone, estrone sulphate and androstenone on 3β -hydroxysteroid dehydrogenase protein expression in primary cultured hepatocytes. Livestock Science 114:202–210

95. Rasmussen, M.K., Ekstrand, B. 2013. Regulation of 3beta-hydroxysteroid dehydrogenase and sulphotransferase 2A1 gene expression in primary porcine hepatocytes by selected sex-steroids and plant secondary metabolites from chicory (Cichorium intybus L.) and wormwood (Artemisia sp.). Submitted to GENE.

96. Dong, X., Bai, Y., Xin, Y., Xu, Q., Chen, G., Fang, M. 2012. Investigation on the transcription factors of porcine 3b-hydroxysteroid dehydrogenase and 17b-hydroxysteroid dehydrogenase genes. Gene 499:186-190

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

98. Labrie, F., Simard, J., Van, L., Bélanger, A., Pelletier, G.1992. Structure, function and tissuespecific gene expression of 3b-hydroxysteroid dehydrogenase/5-ene-4-ene isomerase enzymes in classical and peripheral intracrine steroidogenic tissues. The Journal of Steroid Biochemistry and Molecular Biology 43:805-826

99. Roberts, M.S., Magnusson, B.M., Burczynski, F.J., Weiss, M. 2002. Enterohepatic Circulation: Physiological, Pharmacokinetic and Clinical Implications. Clinical Pharmacokinetics 41:751-790

100. Jen, K., Squires, E.J. 2011a. Efficacy of nonnutritive sorbent materials as intestinal binding agents for the control of boar taint. Animal 5:1814-1820

101. Adlercreutz H., Martin, F. 1980. Biliary excretion and intestinal metabolism of progesterone and estrogens in man. Journal of Steroid Biochemistry 13:231-44

102. Ruoof ,W.L., Dziuk, P.J. 1994. Absorption and metabolism of estrogens from the stomach and duodenum of pigs. Domestic Animal Endocrinology 11:197-208

103. Andresen, Ø. 1975. 5a-androstenone in peripheral plasma of pigs, diurnal variation in boars, effects of intravenous hcg administration and castration. Acta Endocrinologica 78:385-391

104. Bonneau M., Meusy-Dessolle N., Léglise P.C., Claus, R. 1982. Relationships between fat and plasma androstenone and plasma testosterone in fatty and lean young boars following castration. Acta Endocrinologica 101:129-133

105. Claus R. 1976. Messung des Ebergeruchsstoffes im Fett von Schweinen mittels eines Radioimmunotests. 2. Mitteilung. Zeitlicher Verlauf des Gerruchsdepotabbaues nach der Kastration. Zeitschrift für Tierzüchtung und Züchtungsbiologie 93:38-47

106. Klassificeringsudvalget for svin, kvæg og får, 2012. [Classification Committee for pigs, cattle and sheep. Statistics about slaughter data regarding weighing and classification of pigs in Denmark in 2012]. Statistik over slagtedata vedrørende vejning og klassificering af svin i Danmark i 2012. Axeltorv, København. Available online:

http://www.klassificeringskontrollen.dk/Svin/Statistiske_klassificeringsdata_for_svin.aspx [downloaded May 2013].

107. Bonde, M. 2013. [Organic entire male pig production in Denmark 2012]. Økologisk hangriseproduktion i Danmark 2012, frasortering pga. ornelugt. Udviklingscenter for Husdyr på Friland, 29. Maj, 2013. 5pp.

108. Andersen, R. 1997. Production of entire male pigs; The Danish perspective. Slagteriernes forskningsinstitut, DMRI, report 01.705. 11 pp.

109. Fredriksen, B., Font-i-Furnols, M., Lundström, K., Migdal, W., Prunier, A., Tuyttens, F.A.M., Bonneau. 2009. Practice on castration of piglets in Europe. Animal 3:1480-1487.

110. van Wagenberg, C.P.A., Snoek, H.M., van der Fels, J.B., van der Peet-Schwering, C.M.C., Vermeer, H.M., Heres, L. 2013. Farm and management characteristics associated with boar taint. Animal 7:1841-1843

111. Thomsen, M. 2011. [German abattoir banks on entire male pigs]. Tysk slagteri satser på hangrise. Landbrugsavisen. Available online: <u>www.landbrugsavisen.dk</u>. Cited May 2013.

112. Hansen, G. 2013. Pig adviser, Personal communication., GEFION, Denmark.

113. Vermeer, H.M. 2013. Scientist at Wageningen UR Livestock Resarch, Personal Communication. May, 2013

114. Kompass Jungebermast, 2013. [Entire male production. Recommendations from practicians to practicians]. Empfehlungen von Praktikerne für Praktiker. QS Qualität und sicherheit GmbH, Dr. Hermans-Josef Nienhoff (V.i.S.d.P.) Schedestrasse 1-3, 53113 Bonn. 31pp.

115. AHDB, 2013. Pig Pocketbook 2013. Agriculture & Horticulture Development board 35 pp.

116. Maribo, H. 2013. Personal communication. Pig Research Centre, Denmark.

117. Maribo, H. 2012. [Screening of organic entire males]. Screening af økologiske hangrise. Message No. 955. Cited 1 November 2012 Available online: <u>http://vsp.lf.dk/Publikationer/Kilder/lu_medd/2012/955.aspx</u>

118. Babol, J., Squires, E.J., Gullett, E.A. 2002. Factors affecting the level of boar taint in entire male pigs as assessed by consumer sensory panel. Meat Science 61:33-40

119. Aluwe, M., Bekaert, K.M., Tuyttens, F.A.M., Vanhaecke, L., De Smet, S., De Brabander, H.F., De Brabander, D.L., Millet, S. 2011a. Influence of soiling on boar taint in boars. Meat Science 87:175-179

120. Fabrega, E., Gispert, M., Tibau, J., Hortos, M., Oliver, M.A., Furnols, M.F. 2011. Effect of housing system, slaughter weight and slaughter strategy on carcass and meat quality, sex organ development and androstenone and skatole levels in Duroc finished entire male pigs. Meat Science 89:434-439

121. Maribo, H. 2013. NO CAST. Reduction of boar taint – the practical way. Presentation at Workshop on organic pig production. June 13th. Hovborg, Denmark. Available online: <u>http://www.udviklingscenter.com/projekter/igangvaerende/scientific-workshop.html</u>

122. Jensen, B.B. 2013. Unpublished results.

123. Thomsen, R. 2013. Unpublished results.

124. Andersson, H.K., Andersson, K., Zamaratskaia, G., Rydhmer, L., Chen, G., Lundström, K. 2005. Effect of single-sex or mixed rearing and live weight on performance, technological meat quality and sexual maturity in entire male and female pigs fed raw potato starch. Acta Agriculturae Scandinavica, Section A 55:80-90

125. Jensen, H.F., Andersen, B.H. 2005. [Rearing of uncastrated male pigs together with siblings: Early slaughter increases meat quality and can solve behaviour problems]. Opdræt af ukastrerede hangrise sammen med kuldsøskende: Tidlig slagtning giver højere kødkvalitet og kan løse adfærdsproblemer. Sveriges lantbruksuniversitet (SLU), Forskningsnytt om økologisk landbruk i Norden, No. 4.

126. Aldal, I., Andresen, Ø., Egeli, A.K., Haugen, J.E., Grødum, A., Fjetland, O., Eikaas, J.L. 2005. Levels of androstenone and skatole and the occurrence of boar taint in fat from young boars. Livestock Production Science 95:121-129

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

128. Giersing, M., Lundstrom, K., Andersson, A. 2000. Social effects and boar taint: Significance for production of slaughter boars (Sus scrofa). Journal of Animal Science 78:296-305

129. Zamaratskaia, G., Babol, J., Andersson, H.K., Andersson, K., Lundström, K. 2005a. Effect of live weight and dietary supplement of raw potato starch on the levels of skatole, androstenone, testosterone and oestrone sulphate in entire male pigs. Livestock Production Science 93:235-243

130. Patterson, R.L.S., Lightfoot, A.L. 1984. Effect of sex grouping during growth on 5α [|]-androstenone development in boars at three commercial slaughter weights. Meat Science 10:253-263

131. Salmon, L. 2007. Factors influencing the performance, behaviour and meat quality of heavy weight pigs. Pre-publication PhD thesis. School of agriculture, food and rural development, University of Newcastle upon Tyne. pp 93-130

132. Courboulay, V., Leroy, C., Quiniou, N., Chevillon, P. 2013. Effect of mixing entire males with females and slaughter strategy on behaviour, growth and boar taint. Book of abstracts of the 64th Annual Meeting of the European Federation of Animal Science. Nantes, France, 26-30 August. Pp. 562.

133. Allen, P., Joseph, R.L., Lunch, P.B. 1997. Effect of nutrition and management on the incidence of boar taint. EAAP European association for Animal Production Publication, Issue 92:88-91

134. Rydhmer, L., Hansson, M., Lundström, K., Brunius, C., Andersson, K. 2013. Welfare of entire male pigs is improved by socialising piglets and keeping intact groups until slaughter. Animal 7:1532-1541

135. Jungbluth, I., Wesoly, V., Stefanski, V., Weiler, U. 2013. Pre slaughter conditions influence skatole and androstenone in adipose tissue and blood of boars. (Abstract). Book of abstracts of the 64th annual meeting of the European Federation of animal science. Nantes, France

136. Hansen, L.L., Larsen, A.E., Hansen-Møller, J. 1995. Influence of keeping pigs heavily fouled with faeces plus urine on skatole and indole concentration (Boar taint) in subcutaneous fat. Acta Agriculturae Scandinavica, Section A 45: 178-185

137. Aluwé, M., Millet, S., Bekaert, K.M., Tuyttens, F.A.M., Vanhaecke, L., De Smet, S., De Brabander, D.L. 2011b. Influence of breed and slaughter weight on boar taint prevalence in entire male pigs. Animal 5:1283-1289

138. Bekaert, K.M., Aluwé, M., Millet, S., Goethals, K., Nijs, G., Isebaert, S., De Brabander, D.L., Verheyden, K., De Brabander, H.F., Vanhaecke, L., Tuyttens, F.A.M. 2012. Predicting the likelihood of developing boar taint: Early physical indicators in entire male pigs. Meat Science 92:382-385

139. Andersson K., Schaub, A., Andersson, K., Lundström, K., Thomke, S., Hansson, I. 1997. The effect of feeding systems, lysine level and gilt contact on performance, skatole levels and economy of entire male pigs. Livstock Production Science 51:131-140

140. Kjeldsen, N., Udesen F. 1998. Practical experience from field studies with entire male pigs. In: Skatole and boar taint. Ed: Jensen W.K., Danish Meat Research Institute 1998:129-136.

141. Hansen, L., Mikkelsen, L., Agerhem, H., Laue, A., Jensen, M., Jensen, B. 2000. Effect of fermented liquid food and zinc bacitracin on microbial metabolism in the gut and sensoric profile of m. longissimus dorsi from entire male and female pigs. Animal Science 71:65-80

142. Jensen, M. T., Cox, R.P., Jensen, B.B. 1995a. 3-methylindole (skatole) and indole production by mixed populations of pig fecal bacteria. Applied and Environmental Microbiology 61:3180-3184

143. Claus R., Raab S. 2000. Influence on skatole formation from tryptophan in the pig colon Advances in Experimental Medicine and Biology 467:679-684

144. Petersen J.K., Mortensen, A.B., Madsen, A., Mortensen, H.P., Hyldegaard-Jensen, J. 1986. [Effect of feed composition on boar taint in pig meat]. Foderets indflydelse på ornelugt i svinekød. Statens Husdyrbrugsforsøg, Meddelelse 638:1-7

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

146. Madsen, A., Østerballe, R., Mortensen, H.P., Bejerholm, C., Barton, P. 1990. [Effect of fed composition on entire male pig meat quality]. Foderets indflydelse på råvarekvaliteten hos slagtesvin. 673 Beretning Fra statens Husdyrbrugsforsøg, København

147. Madsen, A., Petersen, J., Mortensen, H.P., Bjerholm, C., Barton, P. 1991. [Entire male pigs fed restrictively or ad libitum with increasing amounts of soy beans]. Hangrise fodret efter norm eller ædelyst med stigende mængder sojaskrå. Meddelelse 810. Statens Husdyrbrugsforsøg. Foulum. Danmark.

148. Xu, Z. R., Hu, C.H., Wang M.Q. 2002. Effects of fructooligosaccharide on conversion of Ltryptophan to skatole and indole by mixed populations of pig fecal bacteria. Journal of General and Applied Microbiology 48:83-90

149. Sun, T., Lærke, H.N., Jørgensen, H., Knudsen, K.E.B. 2006. The effect of extrution cooking of different starch sources on the in vitro and in vivo digestibility in rowing pigs. Animal Feed Science and Technology 131:67-86

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

151. Lösel, D. and Claus, R. 2005. Dose-dependet effects of resistant potato starch in the diet on intestinal skatole formation and adipose tissue accumulation in pig. Journal of Veterinary Medicine Series A 52:209-212

152. Lösel, D., Lacorn, M., Büttner, D., Claus, R. 2006. Flavor improvement in pork barrows and gilts via inhibition of intestinal skatol formation with resistant potato starch. Journal of Agricultural and Food Chemistry 54:5990-5995

153. Chen, G., Zamaratskaia, G., Anderson, H.K., Lundström, K. 2007. Effect of raw potato starch and live weight on fat and plasma skatole, indole and androstenone levels measured by different methods in entire male pigs. Food Chemistry 101:439-448

154. Pauly, C, Spring, P., O'Doherty, J.V., Ampero Kragten, S., Bee, G. 2008. Performances, meat quality and boar taint of castrates and entire male pigs fed a standard and a raw potato starch-enriched diet. Animal 2:1707-1715

155. Pauly, C., Spring-Staehli, P., O'Doherty, J.V., Kragten, S.A., Dubois, S., Messadene, J., Giuseppe, B. 2010. The effects of method of castration, rearing condition and diet on sensory quality of pork assessed by a trained panel. Meat Science 86:498-504

156. Øverland, M., Kjos, N.P., Fauske, A.K., Teiger, J., Sørum, H. 2011. Easily fermentable carbohydrates reduce skatole formation in the distal intestine of entire male pigs. Livstock Science 40:206-217

157. Aluwé, M., Millet, S., Nijs, G., Tuyttens, F.A.M., Verheyden, K., De Brabander, H.F., De Brabander, D.L., Oeckel, M.J. 2009. Absence of an effect of dietary fibre or clinoptilolite on boar taint in entire male pigs fed practical diets. Meat Science 82:346-352

158. Knudsen, K. E. B., Hedemann, M.S., Laerke, H.N. 2012. The role of carbohydrates in intestinal health of pigs. Animal Feed Science and Technology 173:41-53

159. Rideout, T. C., Fan, M.Z., Cant, J.P., Wagner-Riddle, C., Stonehouse, P. 2004. Excretion of major odor-causing and acidifying compounds in response to dietary supplementation of chicory inulin in growing pigs. Journal of Animal Science 82:1678-1684

160. Hansen, L.L., Mejer, H., Thamsborg S.M., Byrne D.V., Roepstorff, A., Karlsson, A.H. 2006. Influence of chicory roots (cichorium intybus L) on boar taint in entire male and female pigs. Animal Science 82:359-368

161. Vhile, S. G., Kjos, N.P. Sorum, H., Overland, M. 2012. Feeding jerusalem artichoke reduced skatole level and changed intestinal microbiota in the gut of entire male pigs. Animal 6:807-814

162. Hansen, L. L., Stolzenbach, S., Jensen, J.A., Henckel, P., Hansen-Moller, J., Syriopoulos K., Byrne, D.V. 2008. Effect of feeding fermentable fibre-rich feedstuffs on meat quality with emphasis on chemical and sensory boar taint in entire male and female pigs. Meat Science 80:1165-1173

163. Maribo, H., Magnussen, C., Jensen, B.B. 2010. [Entire male pigs fed 15 % chicory]. Hangrise fodret med 15 % cikorie. Pig Research Center. Message No. 876. Cited 25th September 2012 Available online: <u>http://vsp.lf.dk/Publikationer/Kilder/lu_medd/2010/876.aspx</u>

164. Rasmussen, M.K., Brunius, C., Zamaratskaia, G., Ekstrand, B. 2012a. Feeding dried chicory root to pigs decrease androstenone accumulationin fat by increasing hepatic 3β hydroxysteroid dehydrogenase expression. The Journal of Steroid Biochemistry and Molecular Biology 130:90-95

165. Zammerini, D., Wood, J.D., Whittington, F.M., Nute, G.R., Hughes, S.I., Hazzledine, M. et al. 2012. Effect of dietary chicory on boar taint. Meat Science 91:396-401

166. Aluwe, M., Langendries, K.C.M., Bekaert, K.M., Tuyttens, F.A.M., De Brabander, D.l. 2013. Effect of surgical castration, immunocastration and chicory-diet on the meat quality and palatability of boars. Meat Science 94:402-407

167. Gill, B.P., Hardy, B., Perrott, J.G., Wood, J.D., Hamilton, M. 1993. The effect of dietary fibre on the meat eating and fat quality of finishing pigs fed ad libitum. Animal Production 56:421-422

168. Van Oeckel, M.J., Warnats, M. De Paepe, Casteels, M., Boucqué, Ch.V. 1998. Effect of fiber-rich diets on the backfat skatole content or entire male pigs. Livstock Production Science 65:173-180

169. Hansen, L.L., Claudi-Magnussen, C. 2004. Feeding with lupines reduces the amount of skatole in organic pigs. DARCOFenews. Newsletterfrom Danish Research Center for Organic Farming, December 2004, No 4.

170. Wood, D.J., Nute, G.R., Whittington, F.M., Kay, R.M., Perrott, J.G. 1994. Effect of molassed sugar beet feed on pig meat quality. Animal Production 58:471-472

171. Pauly, C., Spring, P., Gahan, D., O'Doherty, J.V.O. 2011. The effect of ceral type and enzyme supplementation on carcass characteristics, volatile fatty acids and intestinal microflora and boar taint in entire male pigs. Animal 5: 378-386.

172. Kouba, M., Enser, M., Whittington, F.M., Nute, G.R., Wood, J.D. 2003. Effect of a high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pig. Journal of Animal Science 81:1967-1979

173. Hansen, L.L., Larsen, A.E., Jensen, B.B., Hansen-Møller, J. 1997. Short time effect of zinc bacitracin and heavy fouling with faeces plus urine on boar taint. Animal Science 64:351-363

174. Staun, H., Mortensen. H.P. 1992. [Feed additives to entire male pigs]. Tilsætningsstoffer til hangrise. Meddelelse 838. Statens Husdyrbrugsforsøg, 8830 Tjele, Danmark.

175. Hansen, L.L., Larsen, A.E. 1994. Effect of antibiotic feed additives on the level of skatole in fat of male pigs. Livstock Production Science 39:269-274.

176. Øverland, M., Kjos, N.P., Borg, M., Skjerve, E., H. Sørum, H. 2008. Organic acids in diets for entire male pigs: Effect on skatole level, microbiota in digesta, and growth performance. Livestock Science 115:169-178

177. Burk, O. Arnold, K.A., Nussler, A.K., Schaeffeler, E., Efimova, E. Avery, B.A., Avery, M.A., Fromm, M.F., Eichelbaum, M. 2005. Antimalarial Artemisinin Drugs Induce Cytochrome P450 and MDR1 Expression by Activation of Xenosensors Pregnane X Receptor and Constitutive Androstane Receptor. Molecular Pharmacology 67:1954-1965

178. Simonsson, U.S.H., Lindell, M., Raffalli-Mathieu, F., Lannerbro, A., Honkakoski, P., Lang, M.A. 2006. In vivo and mechanistic evidence of nuclear receptor CAR induction by artemisinin. European Journal of Clinical Investigation 36:647-653

179. Greger, D.L., Morel, C., Bee, G., Ampuero, S., Baumrucker, C.R., Barbato, G., Stahl, .C.A., Blum, J.W.2008. Transcriptional profiling of hepatic constitutive androstane receptor (CAR) and CAR target genes: New opportunities for reducing boar taintin uncastrated pigs. EAAP workgroup meeting, Girona 2008.

180. Cavin, C., Delannoy, M., Malnoe, A., Debefve, E., Touché, A., Courtois, D., Schilter, B. 2005. Inhibition of the expression and activity of cyclooxygenase-2 by chicory extract. Biochemical and Biophysical Research Communications 327:742-749

181. Kisiel,W., Zielinska., K. 2001. Guaianolides from Cichorium intybus and structure revision of Cichorium sesquiterpene lactones. Phytochemistry 57:523-527

182. de Kraker, W., Franssen, M.C.R., Joerink, M., de Groot, A., Bouwmeester, H.J. 2002. Biosynthesis of Costunolide, Dihydrocostunolide, and Leucodin. Demonstration of Cytochrome P450-Catalyzed Formation of the Lactone Ring Present in Sesquiterpene Lactones of Chicory. Plant Physiology 129:257-268

183. Rasmussen, M.K., Zamaratskaia, G., Ekstrand, B. 2011. In vivo effect of dried chicory root (Cichorium intybus L.) on xenobiotica metabolising cytochrome P450 enzymes in porcine liver. Toxicology Letters 200:88-91

184. Rasmussen, M.K, Zamaratskaia, G., Andersen, B., Ekstrand, B. 2012c. Dried chicory root modifies the activity and expression of porcine hepatic CYP3A but not 2C - Effect of in vitro and in vivo exposure. Food and Chemical Toxicology 50:4175-4179

185. Rasmussen, M.K., Klausen, C.L., Ekstrand, B. 2014. Regulation of cytochrome P450 mRNA expression in primary porcine hepatocytes by selected secondary plant metabolites from chicory (Cichorium intybus L.). Food Chemistry 146:255-263

186. Jeong H.G., Yun, C.H. 1995 Induction of Rat Hepatic Cytochrome P450 Enzymes by Myristicin. Biochemical and Biophysical Research Communications 217: 966-971

187. Haw-Wen, C., Jaw-Ji, Y., Chia-Wen, T., Wu, J.J. 2001. Dietary fat and garlic oil independently regulate hepatic cytochrome P(450)2B1 and the placental form of glutathione S-transferase expression in rats. The Journal of Nutrition 131:1438-1443

188. Reicks, M.M. Crankshaw, D.L. 1996. Modulation of rat hepatic cytochrome PGÇÉ450 activity by garlic organosulfur compounds. Nutrition and Cancer 25:241-248

189. Zhang, C.L., Zeng, T., Zhao, X.L., Xie, K.Q. 2013. Garlic oil attenuated nitrosodiethylamineinduced hepatocarcinogenesis by modulating the metabolic activation and detoxification enzymes. International journal of biological sciences 9:237-245

190. Jen, K., Squires, E.J. 2011b. In vitro assessment of the effectiveness of non-nutritive sorbent materials as binding agents for boar taint compounds. Animal 5:1821-1828

191. Heimer, G.M., Englund, D.E. 1986. Enterohepatic recirculation of oestriol: inhibition by activated charcoal. Acta Endocrinologica 113:93-95

192. Jørgensen L. 1993. [Activated charcoal in feed to entire male pigs]. Aktivt kul i foder til hangrise. Videncenter for Svineproduktion, Erfaring nr. 9317, 3 pp. Available online: <u>http://vsp.lf.dk/Publikationer/Kilder/lu_erfa/erfa/9317.aspx</u>

193. Gregersen, V.R., Conley, L.N., Sorensen, K.K., Guldbrandtsen, B., Velander, I.H., Bendixen, C. 2012. Genome-wide association scan and phased haplotype construction for quantitative trait loci affecting boar taint in three pig breeds. BMC Genomics 13:1-22

194. Grindflek, E., Meuwissen, T.H.E., Aasmundstad, T., Hamland, H., Hansen, M.H.S., Nome, T., Kent, M., Torjesen, P., Lien, S. 2011. Revealing genetic relationships between compounds affecting boar taint and reproduction in pigs. Journal of Animal Science 89:680-692

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

196. Duijvesteijn, N., Knol, E.F., Merks, J.W., Crooijmans, R.P., Groenen, M.A., Bovenhuis, H., Harlizius, B. 2010. A genome-wide association study on androstenone levels in pigs reveals a cluster of candidate genes on chromosome 6. BMC Genetics 11:42

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

198. Signoret, J.P. 1976. Influence of anabolic agents on behaviour. Environmental quality and safety, Supplement volume 5:143-150 (Abstract)

199. EFSA (European Food Safety Authority) 2004. 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. pp. 1-100

200. Rydhmer, L., Zamaratskaia, G., Andersson, H.K., Algers, B., Guillemet, R., Lundström, K. 2006. Aggressive and sexual behaviour of growing and finishing pigs reared in groups, without castration. Acta Agriculturae Scandinavica, Section A 56: 109-119

201. Boyle, L.A., Björklund, L. 2007. Effects of Fattening Boars in Mixed or Single Sex Groups and Split Marketing on Pig Welfare. Animal Welfare 16:259-262

202. Fredriksen, B., Hexeberg, C. 2009. The effect of removing animals for slaughter on the behaviour of the remaining male and female pigs in the pen. Research in Veterinary Science 86:368-370

203. Fàbrega, E., Velarde, A., Cros, J., Gispert, M., Suàrez, P., Tibau, J., Soler, J. 2010. Effect of vaccination against gonadotrophin-releasing hormone, using Improvac, on growth performance, body composition, behaviour and acute phase proteins. Livestock Science 132:53-59

204. Vanheukelom, V., Van Beirendonck, S., Van Thielen, J., Driessen, B. 2012. Behavior, production results and meat quality of intact boars and gilts housed in unmixed groups: A comparative study. Applied Animal Behaviour Science 142:154-159

205. Cronin, G.M., Dunshea, F.R., Butler, K.L., McCauley, I., Barnett, J.L., Hemsworth, P.H. 2003. The Effects of Immuno- and Surgical-castration on the Behaviour and Consequently growth of Grouphoused, Male Finisher Pigs. Applied Animal behaviour Science 81:111-126

206. Tuyttens, F.A.M., De Groot, J, Van Reenen, K., De Bourdeaud'huy, A., Struelens, E. 2008. Differences in Aggressive and Sexual Behaviour in Entire Male Pigs versus Barrows. In Proceedings of the EAAP Working Group on Production and Uttilisation of Meat from Entire male Pigs, 26-27 March 2008, Monells, Spain, pp. 34-35

207. Fredriksen, B., Lium, B.M., Marka, C.H., Mosveen, B., Nafstad, O. 2008. Entire Male Pigs in Farrow-to-finish Pens – Effects on Animal Welfare. Applied Animal Behaviour Science 110:258-268

208. Thomsen, R., Bonde, M., Kongsted, A.G., Rousing, T. 2012. Welfare of entire males and females in organic pig production when reared in single-sex groups. Livestock Science 149:118-127

209. Rydhmer, L., Lundström, K., Andersson, K. 2010. Immunocastration reduces aggressive and sexual behaviour in male pigs. Animal 4:956-972

210. Hintze, S., Scott, D., Turner, S., Meddle, S.L., D'Eath, R.B. 2013. Mounting behaviour in finishing pigs: Stable individual differences are not due to dominance or stage of sexual development. Applied Animal Behaviour Science 147:69-80

211. Hemsworth, P.H., Tilbrook, A.J. 2007. Sexual behaviour of male pigs. Hormones and behaviour 52:39-44

212. McGlone, J.J. 1985. Quantitative ethogram of aggressive and Submissive behaviors in recently regrouped pigs. Journal of Animal Science 61:556-566

213. Puppe, B., Tuchscherer, M., Tuchscherer, A. 1997. The effect of housing conditions and social environment immediately after weaning on the agonistic behaviour, neutrophil/lymphocyte ratio, and plasma glucose level in pigs. Livestock Production Science 48:157-164.

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

215. Andersson, H. K., Hullberg, A., Malmgren, L., Lundström, K., Rydhmer, L. Squires, J. 1999. Sexual Maturity in Entire Male Pigs, Environmental Effects, Relations to Skatole Level and Female Puberty. Acta Agriculture Scandinavica, Section A, Animal Science 49:103–112

216. Giersing, M., Andersson, A. 1998. How does former acquaintance affect aggressive behaviour in repeatedly mixed male and female pigs? Applied Animal Behaviour Science 59:297-306

217. Turner, S.P., Edwards, S.A. 2004. Housing immature domestic pigs in large social groups: implications for social organisation in a hierarchical society. Applied Animal Behaviour Science 87:239-253

218. Fabrega, E., Puigvert, X., Soler, J., Tibau, J., Dalmau, A. 2013. Effect of on farm mixing and slaughter strategy on behaviour, welfare and productivity in Duroc finished entire male pigs. Applied Animal Behaviour Science 143:31-39

219. Conte, S., Lawlor, P., O'Connell, N., Boyle, L. 2012. Effect of split marketing on the welfare, performance, and carcass traits of finishing pigs. Journal of Animal Science 90: 373-380

220. Sather, A.P., Jones, S.D.M., Squires, E.J., Schaefer, A.L., Robertson, W.M., Tong, A.K.W., Zawadski, S. 1995. Ante mortem handling effects on the behaviour, carcass yield and meat quality of market weight entire male pigs. Canadian Journal of Animal Science 75:45-56

221. Hagelsø Giersing, M., Studnitz, M. 1996. Characterization and Investigation of Aggressive Behaviour in the Pig. Acta Agriculturae Scandinavica, Section A, Animal Science. Suppl. 27:56-60

222. Simonsen, H.B. 1990. Behaviour and distribution of fattening pigs in the multi-activity pen. Applied Animal Behaviour Science 27:311-324

223. O'Connell, N.E., Beattie, V.E. 1999. Influence of environmental enrichment on aggressive behaviour and dominance relationships in growing pigs. Animal Welfare 8:269-279

224. Olsen, A.W. 2001. Behaviour of growing pigs kept in pens with outdoor runs I. Effect of access to roughage and shelter on oral activities. Livestock Production Science 69:255-264

225. Tuyttens, F.A.M. 2005. The importance of straw for pig and cattle welfare: A review. Applied Animal Behaviour Science 92:261-282

226. Scott, K., Chennells, D.J., Campell, F.M., Hunt, B., Armstrong, D., Taylor, L., Gill, B.P., Edwards, S.A. 2006. The welfare of finishing pigs in two contrasting housing systems: Fully-slatted versus strawbedded accommodation. Livestock Science 103:104-115

227. Olsen, A.W., Simonsen, H.B., Dybkjær, L. 2002. Effect of Access to Roughage and Shelter on Selected Behavioural Indicators of Welfare in Pigs Housed in a Complex Environment. Animal Welfare 11:75-87

228. Høøk Presto, M., Algers, B., Persson, E., Andersson, H.K. 2008. Different Roughages to Organic Growing/Finishing Pigs – Influence on Activity Behaviour and Social Interaction. Livestock Science 123:55-62

229. Hansen, L.L., Hagelsø, A.M., Madsen, A. 1982. Behavioural results and performance of bacon pigs fed 'ad libitum' from one or several self-feeders. Applied Animal Ethology 8:307-333

230. Prunier, A., Brillouët, A., Merlot, E., Meunier-Salaün, M.C., Tallet, C. 2013. Influence of housing and season on pubertal development and skin lesions of male pigs. Animal 7:2035-2043.

231. Salmon, L., Edwards, S.A. 2008. Influence of housing system on behaviour and carcass taint when rearing entire males. University of Newcastle, Unpublished.

232. Pauly, C., Spring P., O'Doherty, J. V., Ampuero Kragten, S., Bee, G. 2009. Growth performance, carcass characteristics and meat quality of group-penned surgically castrated, immunocastrated (Improvac®) and entire male pigs and individually penned entire male pigs. Animal 3:1057-1066.

233. Millet, S., Gielkens, K., De Brabander, D., Janssens, G.P.J. 2011. Considerations on the performance of immunocastrated male pigs. Animal 5:1119-1123

234. McCauley, I., Cronin, G.M., Barnett, J.L., Butler, K.L., Hennessy, D.P., Campbell, R.G., Luxford, B., Smits, R.J., Tilbrook, A.J., Dunshea, F.R. 2000 An immunocastration vaccine (Improvac®) increases growth in individually and group-housed boars. Journal of Animal Science 78 (supl 1):138

235. Zeng, X.Y., Turkstra, J.A., Jongbloed, A.W., van Diepen, J.Th.M., Meloen, R.H., Oonk, H.B., Guoa, D.Z., van de Wiel, D.F.M. 2002. Performance and hormone levels of immunocastrated, surgically castrated and intact male pigs fed ad libitum high- and low-energy diets. Livestock Production Science 77:1-11

236. Zamaratskaia, G, Andersson, H.K., Chen, G., Andersson, K., Madej, A., Lundström, K. 2008a. Effect of a Gonadotropin-releasing Hormone Vaccine (Improvac[™]) on Steroid Hormones, Boar Taint Compounds and Performance in Entire Male Pigs. Reproduction in Domestic Animals 43:351–359

237. Udesen, F. 1988. [Entire male pig production]. Ornegriseproduktion. Meddelelse 139, Den rullende afprøvning, landsudvalget for svin og videncenter for svineproduktion. 7 pp.

238. Andersen, S, Pedersen, B. 1993. [Performance differences between entire male pigs and female pigs]. Forskel i produktionsegenskaber mellem hangrise og galte. Notat 9328, Landudvalget for svin og Danske slagterier. 5 pp.

239. Maribo, H., Christiansen, M.C. 2013. [Economy in entire male pig production in two herds]. Økonomi i hangriseproduktion i 2 besætninger. Meddelelse nr 984. Videncenter for svineproduktion, den rullende afprøvning. 15 pp. Available online: http://vsp.lf.dk/Publikationer/Kilder/lu_medd/2013/984.aspx

240. Pauly C, Luginbühl, W., Ampuero, S., Bee, G. 2012. Expected effects on carcass and pork quality when surgical castration is omitted – results from a meta-analysis study. Meat Science 92:858-862

241. Gispert M, Ángels, O.M., Velarde, A., Suarez, P., Pérez, J., Font-i-Furnols, M. 2010. Carcass and meat quality characteristics of immunocastrated male, surgically castrated male, entire male and female pigs. Meat Science 85:664-670

242. Banon, S, Andreu, C., Laencina, J., Garrido, M.D. 2004. Fresh and eating pork quality from entire versus castrate heavy males. Food quality and preference 15:293-300

243. Babol, J., Squires, E.J. 1995. Quality of Meat from Entire Male Pigs. Food Research International 28:201-212

244. Van Oeckel M.J, Casteels, M., Warnants, N., De Boever, J.L., vand Renterghem, R., Boucqué, Ch.V. 1996. Production of entire males with Belgian Landrace and hybrid pigs: the incidence of sensory aberrations. Journal of Animal Physiology and Animal Nutrition 76:111-121

245. Ellis, M, Smith, W.C., Clark, J.B.K., Innes, N. 1983. A comparison of boars, gilts and castrates for bacon manufacture. 1. On farm performance, carcass and meat quality characteristics and weight-loss in the preparation of sides for curing. Animal Production 37:1-9

246. Petersen, J.S., Busk, H. 1994. [Carcass and meat quality of castrates and entire male pigs]. Slagteog kødkvalitet hos galte, so- og hangrise. Husdyrforskning nov/dec nr 6: 15-16.

247. Kjaersgaard, N.C., Hviid, M. 2012. Comparison of product yield for entire males and castrate pigs based on CT-scanning. 58th International congress of Meat Science and Technology, 12-17th August 2012, Montreal, Canada. 3 pp.

248. Kempster, A.J., Lowe, D.B. 1993. Meat production with entire males. Growth performance and carcass characteristics as influenced by genotype and environment. In: Proceedings of the 44th Annual Meeting of the European Association for Animal Production, Aarhus

249. Alonso, V, M del Mar Campo, L Provincial, P Roncalés, JA Beltrán, 2010: Effect of protein level in commercial diets on pork meat quality. Meat Science 85:7-14

250. Teye, G.A, Sheard, P.R., Whittington, F.M., Nute, G.R., Stewart, A., Wood, J.D. 2006. Influence of dietary oils and protein level on pork quality. 1. Effects on muscle fatty acid composition, carcass, meat and eating quality. Meat Science 73:157-165

251. Claudi-Magnussen, C., Hansen, L.L. 2005. [Pig feeding – feed utilization, productivity and health (OrganicPigFeed)]. Svinefodring - næringsstofudnyttelse, produktkvalitet og sundhed (OrganicPigFeed), WP 3: Produktkvalitet. Slutrapport. Slagteriernes Forskningsinstitut. 33 pp. Available online: <u>http://orgprints.org/5310</u> [15-09-2013].

252. Sundrum, A., Acosta Aragon, Y., Schulza-Langenhorst, C., Bütfering, L., Henning, M., Stalljohann, G. 2006. Implications of genotype and amino acid supply on pork quality. Paper presented at Joint Organic Congress, Odense, Denmark, May 30-31

253. Xue, J, Dial, G.D., Pettigrew, J.E., 1997. Performance, carcass and meat quality advantages of boars over barrows: A litterature review. Swine health Production 1:21-28

254. Bonneau, M. 1998. Use of Entire Males for Pig Meat in the European Union. Meat Science 49: Suppl.1: 257-272

255. Moss, B.W., Robb, J.D. 1978: The effect of preslaughter lairage on serum thyroxine and cortisol levels at slaughter, and meat quality of boars, hogs and gilts. Journal of the Science of Food and Agriculture 29:689-696

256. Hansen, L.L., Claudi-Magnussen, C., Andersen, H.J. 2001. Meat and eating quality from organic produced fattening pigs. In: Jakobsen, K and JH Hermansen (Ed) Intern rapport nr 145, 2001 fra Danmarks Jordbrugsforskning. Temamøde d. 21. Aug 2001, Økologisk og udendørs svineproduktion, hvor star vi?: 39-47

257. Hadorn, R., Eberhard, P., Guggisberg, D., Piccinali, P., Schlichtherle-Cerny, H. 2008. Effect of fat score on the quality of various meat products. Meat Science 80:765-770

258. Courboulay, V., Temple, D., Velarde, A., Dalmau, A., Fàbrega, E. 2010. Welfare assessment of entire males and females on pig farms. EAAP 2010. Poster

259. EMA, 2013. Improvac Summary of Product Characteristics (online) [last updated 04 Jun 2013]. Available from: <u>http://www.ema.europa.eu/</u> [20 Jun 2013].

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

261. Oonk, H.B., Turkstra, J.A., Schaaper, W.M.M., Erkens, J.H.F., Schuitemaker-de Weerdt, M.H., van Nes, A., Verheijden, J.H.M., Meloen, R.H. 1998. New GnRH-like peptide construct to optimize efficient immunocastration of male pigs by immunoneutralization of GnRH. Vaccine 16:1074-1082

262. Turkstra, J.A., Oonk, H.B., Schaaper, W.M.M., Meloen, R.H. 2002a. The role of the individual amino acids of a GnRH-tandem-dimer peptide used as an antigen for immunocastration of male piglets determined with systematic alanine replacements. Vaccine 20:406–412

263. Zeng, X.Y., Turkstra, J.A., Meloen, R.H., Liu, X.Y., Chen, F.Q., Schaaper, W.M.M., Oonkb, H.B., Guo, D.Z., van de Wiel, D.F.M. 2002b. Active immunization against gonadotrophin-releasing hormone in Chinese male pigs: effects of dose on antibody titer, hormone levels and sexual development. Animal Reproduction Science 70:223-233

264. Brunius, C. 2011. Early Immunocastration of Male Pigs - Effects on Physiology, Performance and Behaviour. Doctoral Thesis, Swedish University of Agricultural Sciences, Uppsala, Acta Universitatis Agriculturae Sueciae 84: 77 pp.

265. Bonneau, M., Dufour, R., Chouvet, C., Roulet, C., Meadus, W., Squires, E. J. 1994. The effects of immunization against luteinizing hormone-releasing hormone on performance, sexual development, and levels of boar taint-related compounds in intact male pigs. Journal of Animal Science 72:14-20

266. Oonk, H.B., Turkstra, J.A., Lankhof, H., Schaaper, W.M.M., Verheijden, J.H.M., Meloen, R.H. 1995. Testis size after immunocastration as parameter for the absence of boar taint. Livestock Production Science 42:63-71

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

268. Zeng. X.Y., Turkstra, J.A., van de Wiel, D.F.M., Guo, D.Z., Liu, X.Y., Meloen, R.H., Schaaper, W.M.M., Chen, F.Q., Oonk, H.B., Zhang, X. 2001. Active Immunization against Gonadotrophinreleasing Hormone in Chinese Male Pigs. Reproduction in Domestic Animals 36:101-105

269. Metz, C., Hohl, K., Waidelich, S., Drochner, W., Claus, R. 2002. Active immunization of boars against GnRH at an early age: consequences for testicular function, boar taint accumulation and N-retention. Livestock Production Science 74:147-157

270. Turkstra, J.A., van der Staay, F.J., Stockhofe-Zurwieden, N., Woelders, H., Meloen, R.H., Schuurman, T. 2011. Pharmacological and toxicological assessment of a potential GnRH vaccine in young-adult male pigs. Vaccine 29:3791-3801

271. Metz, C., Claus, R. 2003. Active immunization of boars against GnRH does not affect growth hormone but lowers IGF-I in plasma. Livestock Production Science 81:129-137

272. Wagner, A., Claus, R. 2004. Involvement of glucocorticoids in testicular involution after active immunization of boars against GnRH. Reproduction 127:275–283

273. Jaros, P., Bürgi, E., Stärk, K.D.C., Claus, R., Hennessy, D., Thun, R. 2005. Effect of active immunization against GnRH on androstenone concentration, growth performance and carcass quality in intact male pigs. Livestock Production Science 92:31-38

274. Hilbe, M., Jaros, P., Ehrensperger, F., Zlinszky, K., Janett, F., Hässing, M., Thun, R. 2006. Histomorphological and immunohistochemical findings in testes, bulbourethral glands and brain of immunologically castrated piglets. Schweiz. Arch. Tierheilkd. 148:599-608

275. Claus R., Lacorn M., Danowski K., Pearce M.C., BauerA. 2007. Short-term endocrine and metabolic reactions before and after second immunization against GnRH in boars. Vaccine 25: 4689-4696.

276. Claus, R., Rottner, S., Rueckert, C. 2008. Individual return to Leydig cell function after GnRHimmunization of boars. Vaccine 26: 4571-4578

277. Zamaratskaia, G., Rydhmer, L., Andersson, H.K., Chen, G., Andersson, S.L.K., Lundström, K. 2008b. Long-term effect of vaccination against gonadotropin-releasing hormone, using Improvac[™], on hormonal profile and behaviour of male pigs. Animal Reproduction Science 108:37–48

278. Bauer, A., Lacorn, M., Danowski, K., Claus, R. 2008. Effects of immunization against GnRH on gonadotropins, the GH-IGF-I-axis and metabolic parameters in barrows. Animal 2:1215–1222

279. Bauer, A., Lacorn, M., Claus, R. 2009. Effects of two levels of feed allocation on IGF-I concentrations and metabolic parameters in GnRH-immunized boars. Journal of Animal Physiology and Animal Nutrition 93:744–753

280. Einarsson, S., Andersson, K., Wallgren, M., Lundström, K., Rodriguez-Martinez, H. 2009. Shortand long-term effects of immunization against gonadotropin-releasing hormone, using Improvac[™], on sexual maturity, reproductive organs and sperm morphology in male pigs. Theriogenology 71:302-310

281. Einarsson, S., Brunius, C., Wallgren, M., Lundström, K., Andersson, K., Zamaratskaia, G., Rodriguez-Martinez, H. 2011. Effects of early vaccination with Improvac® on the development and function of reproductive organs of male pigs. Animal Reproduction Science 127:50-55

282. Fuchs, T., Thun, R., Parvizi, N., Nathues, H., Koehrmann, A., Andrews, S., Brock, F., Klein, G., Sudhaus, N., grosse Beilage, E. 2009. Effect of a gonadotropin-releasing factor vaccine on folliclestimulating hormone and luteinizing hormone concentrations and on the development of testicles and the expression of boar taint in male pigs. Theriogenology 72:672-680

283. Pearce, M., Andrews, S., Brock, F., Allison, J. 2009. Effects of vaccination with Improvac® on boar taint and carcase quality of male pigs reared under commercial conditions in Europe. Proceedings: The 55th International Congress of Meat Science and Technology (ICoMST), Copenhagen, Denmark, 16-21 August, PE1.08: 59-63

284. Schmoll, F., Kauffold, J., Pfützner, A., Baumgartner, J., Brock, F., Grodzycki, M., Andrews, S. 2009. Growth performance and carcass traits of boars raised in Germany and either surgically castrated or vaccinated against gonadotropin-releasing hormone. Journal of Swine Health Production 17:250–255

285. Morales. J., Gispert, M., Hortos, M., Pérez, J., Suárez, P., Piñeiro, C. 2010. Evaluation of production performance and carcass quality characteristics of boars immunised against gonadotropin-releasing hormone (GnRH) compared with physically castrated male, entire male and female pigs. Spanish Journal of Agricultural Research 8:599-606

286. Fang, F., Li, H., Liu, Y., Pu, Y., Wang, L., Wang, S., Zhang, X. 2010a. Immunogenicity of Recombinant Maltose-binding Protein (MBP)-Gonadotropin Releasing Hormone I (GnRH-I). Systems Biology in Reproductive Medicine 56:478-486

287. Fang, F., Li, H., Liu, Y., Zhang, Y., Tao, Y., Li, Y., Cao, H., Wang, S., Wang, L., Zhang, X. 2010b. Active immunization with recombinant GnRH fusion protein in boars reduces both testicular

development and mRNA expression levels of GnRH receptor in pituitary. Animal Reproduction Science 119:275-281

288. Brunius, C., Zamaratskaia, G., Andersson, K., Chen, G., Norrby, M., Madej, A., Lundström, K. 2011. Early immunocastration of male pigs with Improvac[®] – Effect on boar taint, hormones and reproductive organs. Vaccine 29:9514-9520

289. Škrlep, M., Šegula, B., Zajec, M., Kastelic, M., Košorok, S., Fazarinc, G., Čandek-Potokar, M. 2010a. Effect of immunocastration (Improvac ®) in fattening pigs I: growth performance, reproductive organs and malodorous compounds. Slovenian Veterinary Research 47:57-64

290. Škrlep, M., Batorek, N., Bonneau, M., Prevolnik, M., Kubale, V., Čandek-Potokar, M. 2012b. Effect of immunocastration in group-housed commercial fattening pigs on reproductive organs, malodorous compounds, carcass and meat quality. Czech Journal of Animal Science 57:290–299

291. Batorek, N., Škrlep, M., Prunier, A., Louveau, I., Noblet, J., Bonneau, M., Čandek-Potokar, M., 2012b. Effect of feed restriction on hormones, performance, carcass traits, and meat quality in immunocastrated pigs. Journal of Animal Science 90:4593–4603

292. Kubale, V., Batorek, N., Škrlep, M., Prunier, A., Bonneau, M., Fazarinc, G., Čandek-Potokar, M. 2013. Steroid hormones, boar taint compounds, and reproductive organs in pigs according to the delay between immunocastration and slaughter. Theriogenology 79:69–80

293. Turkstra, J.A., Zeng, X.Y., van Diepen, J.Th.M., Jongbloed, A.W., Oonk, H.B., van de Wiel D.F.M., Meloen, R.H. 2002b. Performance of male pigs immunized against GnRH is related to the time of onset of biological response. Journal of Animal Science 80:2953-2959

294. Lealiifano, A.K., Pluske, J.R., Nicholls, R.R., Dunshea, F.R., Campbell, R.G., Hennessy, D.P., Miller, D.W., Hansen, C.F., Mullan, B.P. 2011. Reducing the length of time between slaughter and the secondary gonadotropin-releasing factor immunization improves growth performance and clears boar taint compounds in male finishing pigs. Journal of Animal Science 89:2782-2792

295. Allison J., Tolasi, G., Donna, R., Solari Basano, F., Nazzari, R., Minelli, G., Pearce, M. 2009. Efficacy of Improvac® for controlling boar taint in heavy male pigs under commercial field conditions in Italy. Proceedings: The 55th International Congress of Meat Science and Technology (ICoMST), Copenhagen, Denmark, August 2009, PE1.09, p. 64-67

296. Rottner, S., Claus, R. 2009. Return of testicular function after vaccination of boars against GnRH: consequences on testes histology. Animal 3:1279-1286

297. Font i Furnols, M., Gispert, M., Soler, J., Diaz ,M., Garcia-Regueiro, J.A., Diaz, I., Pearce, M.C. 2012. Effect of vaccination against gonadotrophin-releasing factor on growth performance, carcass, meat and fat quality of male Duroc pigs for dry-cured ham production. Meat Science 91:148-154

298. Bonneau, M. 2010. Accessory sex glands as a tool to measure the efficacy of immunocastration in male pigs. Animal 4:930–932

299. Trujillo, J.H.A., Pineda, J.E., González, P.A.G. 2011. Immunocastration: a humane and effective alternative to surgical castration of adult boars. Revista Colombiana de Ciencas Pecuarias, 24:254-262

300. Bilskis, R., Sutkeviciene, N., Riskeviciene, V., Januskauskas, A., Zilins, H. 2012. Effect of active immunization against GnRH on testosterone concentration, libido and sperm quality in mature AI boars. Acta Veterinaria Scandinavica 54:33-39

301. Font i Furnols, M., Gonzalez, J., Gispert, M., Oliver, M.A., Hortos, M., Perez, J., Suarez, P., Guerrero, L. 2009. Sensory characterization of meat from pigs vaccinated against gonadotropin releasing factor compared to meat from surgically castrated, entire male and female pigs. Meat Science 83:438-442

302. Batorek, N., Čandek-Potokar, M., Bonneau, M., Van Milgen, J. 2012a. Meta-analysis of the effect of immunocastration on production performance, reproductive organs and boar taint compounds in pigs. Animal 6:1330–1338

303. Andreasen, M., Maribo, H. 2012. Comparison of the productivity of Improvac vaccinated entire male pigs with surgically castrated male pigs and gilts. In: International Pig Veterinary Society, 22nd IPVS Congress, Jeju, Korea, p. 310

304. Aleksic, J., Dokmanovic, M., Aleksic, Z., Teodorovic, V., Stojic, V., Trbovic, D., Baltic, M. 2012. Investigation of the efficacy of immunocastration aimed at the prevention of sex odour in boar meat. Acta Veterinaria (Beograd), 62:653-663

305. Weiler, U., Götz, M., Schmidt, A., Otto, M., Müller, S. 2013. Influence of sex and immunocastration on feed intake behavior, skatole and indole concentrations in adipose tissue of pigs. Animal 7:300–308

306. Font i Furnols, M., Gispert, M., Guerrero, L., Velarde, A., Tibau, J., Soler, J., Hortós, M., Garcia-Regueiro, J.A., Perez, J., Suarez, P., Oliver, M.A. 2008. Consumers' sensory acceptability of pork from immunocastrated male pigs. Meat Science 80:1013-1018

307. Campbell, R. 2007. An Australian view on immunocastration. Pig Progress 23:24-25

308. Jeong, J.-Y., Choi, J.-H., Choi, Y.-S., Han, D.-J., Kim, H.-Y., Lee, M.-A., Lee, D.-H., Kim, C.-J. 2011. The Effects of Immunocastration on Meat Quality and Sensory Properties of Pork Bellies. Korean Journal for Food Science of Animal Resources 31:372-380

309. D'Souza, D. 2013. Boar taint vaccine and the Australian pork industry position. Danske Svineproducenter 27.02.2013. Available online: <u>http://www.danskesvineproducenter.dk/index.php?action=read_article&article_id=180</u>

310. Tuyttens, F.A.M., Vanhonacker, F., Langendries, K., Aluwé, M., Millet, S., Bekaert, K., Verbeke, W. 2011. Effect of information provisioning on attitude toward surgical castration of male piglets and alternative strategies for avoiding boar taint. Research in Veterinary Science 91:327–332

311. Vanhonacker, F., Verbeke, W. 2011. Consumer response to the possible use of a vaccine method to control boar taint v. physical piglet castration with anaesthesia: a quantitative study in four European countries. Animal 5:1107–1118

312. Molenaar, G.J., Lugard-Kok, C., Meloen, R.H., Oonk, R.B., de Koning, J., Wensing, C.J.G. 1993. Lesions in the hypothalamus after active immunisation against GnRH in the pig. Journal of Neuroimmunology 48:1-12

313. Albrecht, A.-K., grosse Beilage, E., Kanitz, E., Puppe, B., Traulsen, I., Krieter, J. 2012. Influence of immunisation against GnRF on agonistic and mounting behaviour, serum testosterone concentration and body weight in male pigs compared with boars and barrows. Applied Animal Behaviour Science 138:28–35

314. Brewster, V., Nevel, A. 2013. Immunocastration with Improvac[™] reduces aggressive and sexual behaviours in male pigs. Applied Animal Behaviour Science 145:32-36

315. Andersson, K., Brunius, C., Zamaratskaia, G., Lundström, K. 2012. Early vaccination with Improvac®: effects on performance and behaviour of male pigs. Animal 6:87-95

316. Baumgartner, J., Laister, S., Koller, M., Pfützner, A, Grodzycki, M., Andrews, S., Schmoll, F. 2010. The behaviour of male fattening pigs following either surgical castration or vaccination with a GnRF vaccine. Applied Animal Behaviour Science 124:28–34

317. Schmidt, T., Calabrese, J.M., Grodzycki, M., Paulick, M., Pearce, M.C., Rau, F., von Borell, E. 2011. Impact of single-sex and mixed-sex group housing of boars vaccinated against GnRF or physically castrated on body lesions, feeding behaviour and weight gain. Applied Animal Behaviour Science 130:42-52

318. Dunshea, F.R., Cronin, G.M., Barnett, J.L., Hemsworth, P.H., Hennessy, D.P., Campbell, R.G., Luxford, B., Smits, R.J., Tilbrook, A.J., King, R.H., McCauley, I. 2011. Immunisation against gonadotrophin-releasing hormone (GnRH) increases growth and reduces variability in group-housed boars. Animal Production Science 51:695-701

319. Quiniou, N., Monziols, M., Colin, F., Goues, T., Courboulay, V. 2012. Effect of feed restriction on the performance and behaviour of pigs immunologically castrated with Improvac®. Animal 6:1420-1426

320. Randolph, J.H., Cromwell, G.L., Stahly, T.S., Kratzer, D.D. 1981. Effects of Group Size and Space Allowance on Performance and Behavior of Swine. Journal of Animal Science 53:922-927

321. Meunier-Salaun, M.C., Vantrimponte, M.N., Raab, A., Dantzer, R. 1987. Effect of floor area restriction upon performance, behavior and physiology of growing-finishing pigs. Journal of Animal Science 64:1371-1377

322. Morales, J. I., Serrano, M. P., Cámara, L., Berrocoso, J. D., López, J. P., Mateos, G. G. 2013. Growth performance and carcass quality of immunocastrated and surgically castrated pigs from crossbreds from Duroc and Pietrain sires. Journal of Animal Science 91:3955-3964

323. Morales, J. I., Camara, L., Berrocoso, J. D., Lopez, J. P., Mateos, G. G., Serrano, M. P. 2011. Influence of sex and castration on growth performance and carcass quality of crossbred pigs from 2 Large White sire lines. Journal of Animal Science 89:3481-3489

324. dos Santos, A.P., KieferII, C., Martins, L.P., Fantini, C.C. 2012. Feeding restriction to finishing barrows and immunocastrated swine. Ciência Rural, Santa Maria 42:147-153

325. Lanferdini, E., Lovatto, P.A., Melchior, R., Orlando, U.A.D., Ceccantini, M., Poleze, E. 2013. Feeding surgically castrated, entire male and immunocastrated pigs with different levels of amino acids and energy at constant protein to energy ratio with or without ractopamine. Livestock Science 151:246-251

326. Rikard-Bell, C., Curtis, M. A., van Barneveld, R. J., Mullan, B. P., Edwards, A. C., Gannon, N. J., Henman, D. J., Hughes, P. E., Dunshea, F. R. 2009. Ractopamine hydrochloride improves growth performance and carcass composition in immunocastrated boars, intact boars, and gilts. Journal of Animal Science 87:3536-3543

327. Dunshea, F. R., Allison, J. R. D., Bertram, M., Boler, D. D., Brossard, L., Campbell, R., Crane, J. P., Hennessy, D. P., Huber, L., de Lange, C., Ferguson, N., Matzat, P., McKeith, F., Moraes, P. J. U., Mullan, B. P., Noblet, J., Quiniou, N., Tokach, M. 2013. The effect of immunization against GnRF on nutrient requirements of male pigs: a review. Animal 7:1769 -1778

328. Andreasen, M., Maribo, H. 2011. [Improvac vaccinated male pigs performes as female pigs]. Improvac-vaccinerede hangrise ligner sogrise i produktivitet. Svin, December 11, p. 36

329. Boler, D. D., Killefer, J., Meeuwse, D. M., King, V. L., McKeith, F. K., Dilger, A. C. 2012. Effects of slaughter time post-second injection on carcass cutting yields and bacon characteristics of immunologically castrated male pigs. Journal of Animal Science 90:334–344

330. Škrlep, M., Šegula, B., Prevolnik, M., Kirbiš, A., Fazarinc G., Čandek-Potokar, M. 2010b. Effect of immunocastration (Improvac ®) in fattening pigs II: Carcass traits and meat quality. Slovenian Veterinary Research 47:65-72

331. D'Souza D.N., Mullan B.P. 2003. The effect of genotype and castration method on the eating quality characteristics of pork from male pigs. Animal Science 77:67-72.

332. Boler, D.D., Kutzler, L.W., Meeuwse, D.M., King, V.L., Campion, D.R., Mceith, F.K., Killefer J. 2011. Effects of increasing lysine on caarcass composition and cutting ields of immunologicallycastrated male pigs. Animal Science 89:2189-2199

333. Mackay, J., Pearce, M.C., Thevasagayam, S., Doran, O. 2012. Fatty acid composition and lipogenic enzyme protein expression in subcutaneous adipose tissue of male pigs vaccinated against boar taint, barrows, and entire boars. Journal of Animal Science 91:395-404

334. Clarke, I., Walker, J. Hennessy, D., Kreeger, J., Nappier, J., Crane, J. 2008. Inherent food safety of a synthetic gonadotrtopin-releasing factor (GnRF) vaccine for the control of boar taint in entire male pigs. International Journal of Applied Veterinary Medicine 6:7-14

335. Tomankova, J., Rasmussen, M.K., Andersson, K., Ekstrand, B., Zamaratskaia, G. 2012. Improvac does not modify the expression and activities of the major drug metabolizing enzymes cytochrome P450 3A and 2C in pigs. Vaccine 30:3515-3518

336. Meeusen, E.N.T., Walker, J., Peters, A., Pastoret, P.P., Jungersen, G. 2007. Current Status of Veterinary Vaccines. Clinical Microbiology Reviews 20:489–510

337. Jensen, Jens Christian, 2013. Personal communication Orion Pharma, Denmark.

338. Manger, M., Bostedt, H., Schill, W.B., Mileham A.J. 1997 Effect of sperm motility on separation of bovine X- and Y-bearing spermatozoa by means of free-flow electrophoresis. Andrologia 29:9-15

339. Garner, D.L., Evans, M., Seidel. Jr G. 2013. Sex-sorting sperm using flow cytometry/cell sorting. Chapter 26 in: Carrell, D.T., Aston, K.I. (eds.), Spermatogenesis: Methods and Protocols, Methods in Molecular Biology: 927, Springer Verlag: 279-295

340. Spinaci, M., Chlapanidas, T., Bucci, D., Vallorani, C., Perteghella, S., Lucconi, G., Communod, R., Vigo, R., Galeati, G., Faustini, M., Torre, M.L. 2013. Encapsulation of sex sorted boar sperm: Sperm membrane status and oocyte penetration parameters. Theriogenology 79:575-581

341. OvaSort (low cost sperm sorting system): www.bristol.ac.uk/red/research-commercial/uobcompanies/ovasort.html

342. Videncenter for SvineProduktion. 2012. Kønssorteret ornesæd ændrer ikke kønsfordelingen. Meddelelse af 27. januar

343. Cytomation (sperm sexing flow cytometer): www.cyto.purdue.edu/cdroms/cyto5/sponsors/cytomate/sx.htm

344. XY LLC (sperm sexing flow cytometer): <u>www.xyinc.com/index.php</u>

345. Roca, J., Parrilla, I., Rodriguez-Martinez, H., Gil, M.A., Cuello, C., Vazquez, J.M., Martinez, E.A. 2011. Approaches towards efficient use of boar semen in the pig industry. Reproduction in Domestic Animals 46: Suppl 2:79-83

346. Vazquez, J.M., Martinez, E.A., Parrilla, I., Roca, J., Gil, M.A., Vazquez, J.L. 2003. Birth of piglets after deep intrauterine insemination with flow cytometrically sorted boar spermatozoa. Theriogenology 59:1605-1614

347. Sellier, P., Le Roy, P., Foilloux, M.N., Gruand, J., Bonneau, M. 2000. Responses to restricted index selection and genetic parameters for fat androstenone level and sexual maturity status of young boars. Livestock Production Science 63:265-274

348. Tajet, H., Andersen, Ø., Meuwissen, T.H.E. 2006. Estimation of genetic parameters of boar taint; skatole and androsterone and their correlations with sexual maturation. Acta Veterinary Scandinavica 48:1-4

349. Bergsma, R., Knol, E., Feitsma, H. 2007. Parameters of Al boars and predicted correlated responses of selection against boar taint. Proc. EAAP Meeting, Dublin

350. Windig, J., Mulder, H., Ten Napel, J., Knol, E., Mathur, P., Crump, R. 2012. Genetic parameters for androstenone, skatole indole and human nose scores as measures of boar taint and their relationship with finishing traits. Journal of Animal Science 90: 2120-2129

351. Strathe, A. B., Velander, I.H., Mark, T., Kadarmideen, H.N. 2013a. Genetic parameters for androstenone and skatole as indicators of boar taint and their relationship to production and litter size traits in Danish Landrace. Journal of Animal Science 91:2587-2595

352. Grindflek, E., Lien, S., Hamland, H., Hansen, M.H.S., Kent, M., van Son, M., Meuwissen, T.H. 2011b. Large scale genome-wide association and LDLA mapping study identifies QTLs for boar taint and related sex steroids. BMC Genomics 12:362

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

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

355. Robic, A., Larzul, C., Bonneau, M. 2008. Genetic and metabolic aspects of androstenone and skatole deposition in pig adipose tissue: A review. Genetics, Selection, Evolution 40:129–143

356. Gunawan, A., Sahadevan, S., Neuhoff, C., Große-Brinkhaus, C., Gad, A., Frieden, L., Tesfaye, D., Tholen, E., Looft, C., Uddin, M.J., Schellander, K., Cinar, M.U. 2013. RNA Deep Sequencing Reveals Novel Candidate Genes and Polymorphisms in Boar Testis and Liver Tissues with Divergent Androstenone Levels. PLOS one 8:e63259

357. Leung, M.C., Bowley, K.L., Squires, E.J. 2010. Examination of testicular gene expression patterns in Yorkshire pigs with high and low levels of boar taint. Animal Biotechnology 21:77-87

358. Moe, M., Lien, S., Bendixen, C., Hedegaard, J., Hornshoj, H., Berget, I., Meuwissen, T.H., Grindflek, E. 2008. Gene expression profiles in liver of pigs with extreme high and low levels of androstenone. BMC Veterinary Research 4:29

359. Moe, M., Meuwissen, T., Lien, S., Bendixen, C., Wang, X., Conley, L.N., Berget, I-, Tajet, H., Grindflek, E. 2007. Gene expression profiles in testis of pigs with extreme high and low levels of androstenone. BMC Genomics 8:405

360. Moe, M., Lien, S., Aasmundstad, T., Meuwissen, T.H.E., Hansen, M.H.S., Bendixen, C., Grindflek, E. 2009. Association between SNPs within candidate genes and compounds related to boar taint and reproduction. BMC Genetics 10:32

361. Strathe, A. B., Velander, I.H., Mark, T., Ostersen, T., Hansen, C., Kadermideen, H.N. 2013b. Genetic parameters for male fertility and its relationship to Skatole and Androstenone in Danish Landrace Boars. Journal of Animal Science 91:4659-4668

362. Ren, D., Xing, Y., Lin, M., Wu, Y., Li, K., Li, W., Yang, S., Gao, T., Ren, J., Ma, J., Lan, L., Huang, L. 2009. Evaluation of boar gonad development, spermatogenesis with regard to semen characteristics, libido and serum testosterone levels based on large White Duroc x Chinese Erhualian crossbred boars. Reproduction in Domestic Animals 44:913-919

363. Walker, S., Robinson, O.W., Whisnant, C.S., Cassady, J.P. 2004. Effects of divergent selection for testosterone production on testicular morphology and daily sperm production in boars. Journal of Animal Science 82:2259-2263

364. Pedersen, B. 1998. Heritability of skatole in back fat. In: W. K. Jensen, editor, Skatole and boar taint. Danish Meat Research Institute, Roskilde, Denmark, p. 129-136.

365. Baes, C., Mattei, S., Luther, H., Ampuero, S., Sidler, X., Bee, G., Spring, P., Hofer, A. 2012. A performance test for boar taint compounds in live boars. Animal 7:714–720

366. Frieden L., Looft, C., Tholen, E. 2011. Breeding for reduced boar taint. Lohmann Information 46:21-27

367. Topigs <u>http://www.sapork.biz/genetic-opportunities-exist-to-stop-castration/</u>

368. Jacobsen, M., Cirera, S., Joller, D., Esteso, G., Kracht, S.S., Edfors, I., Bendixen, C., Archibald, A.L., Vogeli, P., Neuenschwander, S., Bertschinger, H.U., Rampoldi, A., Andersson, L., Fredholm, M., Jørgensen, C.B. 2011. Characterisation of five candidate genes within the ETEC F4ab/ac candidate region in pigs. BMC Research Notes 4:225

369. Gibbons, R.A., Sellwood, R., Burrows, M., Hunter, P.A. 1977. Inheritance of resistance to neonatal E. coli diarrhoea in the pig: Examination of the genetic system. Theoretical and Applied Genetics 51:65-70

370. Danish Pig Research Centre: Annual Report 2010: Breeding & Genetics. Available online: <u>http://vsp.lf.dk/~/media/Files/PDF%20-</u>%20Aarsberetning%20VSP%20English/ANNUAL%20REPORT%202010.ashx

371. Goddard, M.E., Hayes, B.J. 2007. Genomic selection. Journal of animal breeding and genetics (Zeitschrift fur Tierzuchtung und Zuchtungsbiologie) 124:323–330

372. Su, G., Christensen, O.F., Ostersen, T., Henryon, M., Lund, M.S. 2012. Estimating Additive and Non-Additive Genetic Variances and Predicting Genetic Merits Using Genome-Wide Dense Single Nucleotide Polymorphism Markers. PLOS ONE 7: e45293

373. Hayes, B.J., Bowman, P.J., Chamberlain, A.J., Goddard, M.E. 2009a. Invited review: Genomic selection in dairy cattle: Progress and challenges. Journal of Dairy Science 92:433–443

374. Hayes, B.J., Bowman, P.J., Chamberlain, A.C., Verbyla, K., Goddard, M.E. 2009b. Accuracy of genomic breeding values in multi-breed dairy cattle populations. Genetics Selection Evolution 41:51

375. Muir, W.M. 2007. Comparison of genomic and traditional BLUP-estimated breeding value accuracy and selection response under alternative trait and genomic parameters. Journal of Animal Breeding and Genetics 124:342-355

376. Whittington, F.M., Zammerini, D., Nute, C.R., Baker, A., Wood, J.D. 2011. Comparison of heating methods and the use of different tissues for sensory assessment of abnormal odours (boar taint) in pig meat. Meat Science 88:249-255

377. Mathur, P.K., ten Napel, J., Bloemhof, S., Heres, L., Knol., E.F., Mulder, H.A. 2012. A human nose scoring system for boar taint and its relationship with androstenone and skatole. Meat Science 91:414-422

378. Haugen, J.E., Brunius, C., Zamaratskaia, G. 2012. Review of analytical methods to measure boar taint compounds in porcine adipose tissue: The need for harmonised methods. Meat Science 90:9-19

379. Meinert, L., Claudi-Magnussen, C., Støier, S. 2013. Limits for the detection of boar taint. Fleischwischaft International 2:100-103

380. Klassificeringsudvalget for svin, kvæg og får, 2012. [Description of an approved method for odour test of small boars, half boars, feral boars and hermaphrodite pigs.] Beskrivelse af en godkendt metode til lugttest af små orner, halvorner, urorner og tvekønnet svin (Uddybning af pkt. 40 i "Regler for registrering, afregning og afdisponering af slagtede hangrise, små orner, halvorner, urorner og tvekønnet svin samt orner brugt til avlsbrug"). Axeltorv, København. Available online:

<u>http://www.klassificeringskontrollen.dk/Brancheregler_for_svin/Han-_og_ornegrise.aspx</u> [downloaded November 2013].

381. Olson, D., Wäckers, F., Haugen, J.E. 2012. Thersold detection of boar taint chemicals using parasitic wasps. Journal of Food Science 77:S356-S361

382. Hansen-Møller, J., Andersen, J.R. 1994. Boar-taint analytical alternatives. Fleshwirtch 74:963-966

383. Mortensen, A.B., Sørensen, S.E. 1984. Relationship between boar taint and skatole determination with a new analysis method. In Proceeding at the 30^{th} European Meeting of Meat Research Workers, Bristol, UK

384. Squiers, J. 1990. Studies on the suitability or a colometric test for androst-16-ene steroids in the submaxillary gland and fat of pigs as a simple chemical test for boar taint. Canadian Journal of Animal Science 70:1029-1040

385. Haugen, J.E. 2006. The use of chemical sensor array technology, the electronic nose, for detection of boar taint. Acta Veterinaria Scandinavica 48:S15

386. Ampuero, S., Bee, C. 2006. The potential to detect boar tainted carcasses by using an electronic nose based on mass spectrometry. Acta Veterinaria Scandinavica 48:S15

387. Claus, R. 1970. Bestimmung von testesteron und 5α -androst-16-en-3-one, einem Ebergerusstoff, bei Sweinen. Disseration, Universität Weihenstephan

388. Vold., E. 1970. Fleischproductionseigsigenschaften bei Ebern und Kastraten IV: Organoleptische und gaschromatgraphische Untersuchungen wasserdampfflüchtige Stoffe des Rückenspeckes von Ebben. Meldung fra Norges Landbrugshøgskole 49, 1-25.

389. Verheyden, M., Valva, M., Kallio, H. 1996. High performance chomatographydetermination of skatole and indole levels in pig serum, subcutanrous fat, and submaxillary salvia glands. Journal of Agricultural and Food Chemistry 44:1265-1270

390. Zamaratskaia, G., Wiklund, T., Jastrebova, J. 2006. Application of LC-MS for determination of indole and 3-methylindole in porcine adipose tissue. Chromatographia 64:435-439

391. Claus, R. 1974. Dosage rasioimmunologique du 5 α -androst-16-en-3-one steroideresponsable de l'odeur de verrat dans le tissue adipeux des porcs. Comptes Rendus des séamces de l'Académie des Sciences Série D 278:299-302

392. Booth, W.D., Willamson, E.D., Patterson, R.L.S. 1986. 16-androstene steroids in the submaxillary gland of the boar in relation to measures of boar taint in carcases. Animal Production 42:145-152

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

394. Claus, R., Mahker, C., Münster, E. 1988. Determination of the boar taint steroid 5α -androst-16en-3-one in adipose tissue of pigs with a rapid microtitre plate enzyme-immunoassay (MET). Arch Lebesmittelhygiene 39:87-90

395. Tuomola, M., Harpio, R., Mikol., H., Knuuttila, P., Lindström, M., Mukkala, V.M. 2000. Production and characterisation of monoclonal antibodies against a small hepten, 3-methylindole. Journal of Immunological Methods 240:111-124

396. Aguilar-Caballos, M.P., Hämä, H., Tuomola, M., Lövgren, T., Gómez-Hens, A. 2002. Homogeneous stopped-flow fluoroimmunuassay using europium as label. Analytica Chimica Acta 460:271-277

397. Huber-Eicher, B., Spring, P. 2008. Attitudes of Swiss consumers towards meat from entire or immunocastrated boars: A representative survey. Research in Veterinary Science 85:625-627.

398. Sattler, T., Schmoll, F. 2012. Impfung oder Kastration zur Vermeidung von Ebergeruch – Ergebnisse einer repräsentativen Verbraucherumfrage in Deutschland. Journal für Verbraucherschutz und Lebensmittelsicherheit 7:117-123.

399. Vanhonacker, F., Verbeke, W., Tuyttens, F.A.M. 2009. Belgian consumers' attitude towards surgical castration and immunocastration of piglets. Animal Welfare 18:371-380.

400. Fredriksen, B., Johnsen, A.M.S., Skuterud, E. 2011. Consumer attitudes towards castration of piglets and alternatives to surgical castration. Research in Veterinary Science 90:352-357.

401. Heid, A., Hamm, U. 2012. Consumer Attitudes Towards Alternatives to Piglet Castration Without Pain Relief in Organic Farming: Qualitative Results from Germany. Journal of Agricultural and Environmental Ethics 25:687-706.

402. Van Beirendonck, S., Driessen, B., Geers, R. 2013. Belgian Consumers' Opinion on Pork Consumption Concerning Alternatives for Unanesthetized Piglet Castration. Journal of Agricultural and Environmental Ethics 26:259-272.

403. Heid A., Hamm. U. 2013. Animal welfare versus food quality: Factors influencing organic consumers' preferences for alternatives to piglet castration without anaesthesia. Meat Science 95:203–211.

404. Hennessy D., Newbold, R. 2004. Consumer attitudes to a boar taint vaccine, IMPROVAC – A qualitative study. In: International Pig Veterinary Society, 18th IPVS Congress, Hamburg, Germany, 2: 612.

405. Lagerkvist, C.J., Carlsson, F., Viske, D. 2006. Swedish consumer preferences for animal welfare and biotechnology: A choice experiment. Agrobiotechnology management & economics 9:51-58.

406. Allison, J., Wright, N., Martin, S., Wilde, N., Izumi, E. 2008. Consumer Acceptance of the Use of Vaccination to Control Boar Taint. In: Book of Abstracts of the 59th Annual Meeting of the European Associaton of Annimal Production, Vilnius, Lithuania, p. 97.

407. Giffin, B.J., Allison, J.R.D., Martin, S., Ward, P., Tschopp, A. 2008. Consumer acceptance of the use of vaccination to control boar taint. In: International Pig Veterinary Society, 20th IPVS Congress, Durban, South Africa, 1:267.

408. Christoffersen, N., 2013. Personal communication. Danish Agriculture and Food Council, Denmark.

409. Tuyttens, F.A.M., Vanhonacker, F., Verhille, B., De Brabander, D., Verbeke, W. 2012. Pig producer attitude towards surgical castration of piglets without anaesthesia versus alternative strategies. Research in Veterinary Science 92:524–530.

410. Aluwé, M., Vanhonacker, F., Verbeke, W., Millet, S., Tuyttens, F.A.M. 2013. Farmers expectations and experiences with alternatives for surgical castration. In: Book of Abstracts of the 64th Annual Meeting of the European Federation of Animal Science, 26-30 August no. 19. P. 563.

411. Bonde, M.K., 2013. Personal communication. Center of Development for Outdoor Livestock Production, Denmark.

412. Serup, T. 2013. Personal communication. Knowledge Centre for Agriculture, Denmark.

413. Ingvorsen, B. 2013. Personal communication. Knowledge Centre for Agriculture, Denmark.

414. Landbrug & Fødevarer, 2013. Statistics 2012, Pigmeat. Landbrug & Fødevarer. Availabel online: <u>http://www.lf.dk/Tal_og_Analyser/Aarstatistikker/Statistik_svin/Tidligeres_statistikker.aspx#</u>
415. Grunert, K.G. 2013. [Evaluation of alternatives to surgical castration of male pigs in relation to relevant Danish export markets]. Vurdering af alternativer til kastration af hangrise på forskellige markeder af relevans for den danske svinekødssektor. Report elaborated for Danish Agriculture & Food Council by MAPP Centre for Research on Customer Relations in the Food Sector, Aarhus University, 32 pp.

416. Baltussen, W.H.M., Hennen, W.H.G.J., Backus, G.B.C. 2010. Ban on castration of boars in the Netherlands: Modelling economic concequences of options. Proceedings from the 59th Conference of the European Association for Animal Production (EAAP), Vilnius, Lithuania, 24 - 27 August, 2008.

417. de Roest, K., Montanari, C., Fowler, T., Baltussen, W. 2009. Resource efficiency and economic implications of alternatives to surgical castration without anaesthesia. Animal 3:11:1522–1531.

418. Valeeva, N.I., De Smet, A., Hoste, R., Backus, G.B.C. 2010. Economics of boar taint prevention without surgical castration in the pork chain. Proceedings of the 9th Wageningen International Conference on Chain and network Management, Wageningen, 26-28 May 2010.

419. Maribo, H., Møller, S., 2013. [Grain and chicory reduces skatole levels of entire males]. Korn og Cikorie reducerer skatoltal hos hangrise. SVIN SPECIAL Nr. 1, 2013. Available online: http://vsp.lf.dk/~/media/Files/PDF%20-%20Viden/Viden%20-%20Artikler%20fra%20SVIN/2013/Specialudgave%20nr%201/Svinspecial4HMASEMKornogcikorier educererskatoltalhoshangrise.ashx

420. Støier, S., Maribo, H. 2012. [Production of entire males – what happens now?] Hangriseproduktion - hvad sker der?. Presentation from the Danish swinecongress 2012.

421. Blaabjerg, L.O. 2013. Personal communication. Danish Meat research Institute, Denmark.

422. Nygaard, B., 2013. Personal communication. Dan-Cikorie, Denmark.

423. Udesen, F. 2002. [Daily weight gain and feeding efficiency in different weight intervals]. Daglig tilvækst og foderudnyttelse I forskellige vægtintervaller. VSP erfaring nr 0212. Available online:

http://vsp.lf.dk/Publikationer/Kilder/lu_erfa/erfa/0212.aspx

424. Vitek M., Pulkrabek J., Valis L., David L. 2012. The prediction of lean meat content in pig carcasses berfore evisceration using the UFOM-300 apparatus. Research in pig breeding, 6, 1012.

425. Andreasen, M., Maribo, H. 2010. [Entire male pigs and castration update 2010]. Hangrise og kastration update 2010. Præsentation ved Kongres for Svineproducenter, 2010, Herning. Foredrag 24. Available online:

http://vsp.lf.dk/~/media/Files/Kongres%202010/Foredrag%20Kongres%202010/24Hangrise_og_k_astration_update_2010.ashx

426. Nedergaard, K. 2013. Personal communication. Danish Crown, Denmark.

427. Kongsted, AG, Hermansen, J, Horsted, K. 2013. Free-range pigs foraging on Jerusalem artichokes (*Helianthus tuberosus* L.) – effect of feeding strategy on growth, feed conversion and animal behaviour. Acta Agriculturae Scandinavica, Section A, Animal Science 63: 76-83.

428. Grevsen, K., 2013. Personal communication. Aarhus University, Institute of Food Science, Denmark.

429. Pedersen A.Ø., 2013. Personal communication. Pig Research Centre, Denmark.

430. Jørgensen L., 2013. Personal communication. Pig Research Centre, Denmark.

References – alphabetic ordered

Please find the references at

http://www.dca.au.dk/fileadmin/DJF/DCA/dcarapport42-referencer.pdf

Glossary

Adipose tissue: fat tissue.

Adjuvant: chemicals, macromolecules or entire cells of certain killed bacteria, which enhance the immune response to an antigen, and may be included in a vaccine.

AI station: see artificial insemination station.

Allele: one of a number of alternative versions of the same gene at a particular genetic locus.

Androstenone: a pheromone synthesized in the testis of male pigs along with the steroid sex hormones at onset of puberty. It can accumulate in fat where it may contribute to boar taint.

Anaesthesia: the condition of having sensation, including the feeling of pain, temporarily taken away. The state is pharmacologically induced and reversible.

Anosmia: inability to perceive odour.

Artificial insemination station: a herd where sperm from pure breed boars are collected for use by other farmers.

At-line method: a method, where samples are physically removed from the carcass and taken to an automated analyser in the abattoir, and the results are used for sorting carcasses later down the production line.

Atrophy: partial or complete wasting away of a part of the body.

Autosomal dominant Mendelian trait: a trait that is controlled by a dominant allele at a single locus on a chromosome that is found in both sexes.

Best linear unbiased prediction: the statistical method used for calculating the selection index of boars.

Bioactive components: nonessential biomolecules that are present in foods and exhibit the capacity to modulate one or more metabolic processes.

Booster vaccination: a vaccination given after a previous vaccination with the same antigen to enhance the immunity.

Colorimetric method/colorimetry: a chemical method to determine the concentration of colored compounds in a solution by measuring its absorbance at a specific wavelength of light.

Candidate genes: any gene thought likely to cause a particular trait.

Catheter: a thin tube.

Colon: the large intestine.

Coprophagy: consumption of faeces.

Cortisol: the main glucocorticoid produced in the adrenal cortex of humans and pigs and released at exposure to stressors. See also glucocorticoids and HPA axis.

CYP: see cytochrome P450 monooxygenases.

Cytochrome P450 monooxygenases: heme-containing enzymes oxidizing a wide range of substrates in collaboration with NADPH oxidoreductase and/or cytochrome b_5 . The different isoforms of CYPs are classified according to their amino acid sequence with isoforms sharing more than 40 % being members of the same family (CYP1, CYP2, CYP3 etc.) and isoforms sharing more than 55 % being members of the same subfamily (e.g. CYP1A and CYP1B, or CYP2A and CYP2B). The individual isoforms are named by an Arabic number following the designation of the subfamily (CYP1A1, CYP1A2, CYP1B1 etc.).

Direct mass spectrometry (MS): an analytical technique that produces spectra of the masses of the atoms or molecules comprising a sample of material. The spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules and to elucidate the chemical structures of molecules. Mass spectrometry works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios. The atoms or molecules can be identified by correlating known masses to the identified masses or through a characteristic fragmentation pattern.

Endogenous: a substance that originate from within an organism, tissue or cell.

Enterocytes: intestinal absorptive cells that are simple columnar epithelial cells found in the small and large intestines and in appendix.

Enzyme: a protein that speeds up chemical reactions in the body, thereby promting the conversion of a substrate to a product (metabolite) without being altered itself.

Enzyme immunoassay (EIA): measures the concentration of specific molecule in a solution by use of antibodies of the molecule and enzyme mediated color reactions.

Extrinsic: not contained in or belonging to a body.

Fast gas chromatography (GC): speed and cost optimized routine gas chromatographic analysis that may be used at-line or online.

Fluoroimmunoassay (FIA): a very sensitive form of immunoassay that uses fluorescence spectrometry.

Gas chromatography: a method used in analytical chemistry for separating out volatile compounds of a chemical mixture onto an absorbent material. The volatile compounds can be individually analyzed because different compounds are caught on the material at different rates.

Gas sensor array technology: an array of gas sensors with a learning system (ElectronicNose).

Gas phase Fourier transformed infrared spectroscopy (IR): a technique used to analyse the chemical composition of many biological and chemical samples. A beam of infrared light is focused on the sample using all-reflective optics. The light absorbed at different wavelengths is unique for almost every organic compound. FTIR analysis is not only qualitative (identification), but can with relevant standards also be used for quantitative analysis.

Genes: a hereditary unit, ordinarily at a fixed position on a chromosome that influences a particular trait and consists of sequences of deoxyribonucleic acid (DNA). Genes are the part of the DNA that can be translated into protein.

Genetic correlations: the proportion of variance that two traits share due to genetic causes.

Genomic selection: a novel approach to traditional marker-assisted selection where selection is made based on few markers, in most cases SNPs. Rather than seeking to identify individual loci significantly associated with a trait, genomic selection uses a set of marker data as predictors of performance. In an attempt to increase the accuracy of the prediction of breeding and genotypic values, genomic prediction uses a combination of marker data with phenotypic traits as well as pedigree data when available. This is done by using the associations between markers and traits in a background reference population.

Glucocorticoids: a class of steroid hormones produced in the adrenal cortex and involved in responses to stressors. They regulate or support a variety of important cardiovascular, metabolic, immunologic and homeostatic functions. Cortisol (or hydrocortisone) is the most important glucocorticoid in humans and pigs. See also HPA-axis.

GnRH: gonadotropin-releasing hormone. See HPG-axis.

Haplotype: either a collection of markers linked together by few recombination events back in time or in this case the inferred phased pattern for a collection of SNPs highly associated to a given trait located within the same locus.

Hepatic: states that the specified compound or process is related to the liver.

Hepatocytes: main cell type found in the liver, having the ability to perform detoxifying processes.

Heritability: the proportion of the variance that can be explained solely by the genetic background of a population.

High performance liquid chromatography (HPLC): High performance liquid chromatography (formerly referred to as high-pressure liquid chromatography), HPLC, is a chromatographic technique used to separate the components in a mixture, to identify each component and to quantify each component. It relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with a sorbent, leading to the separation of the sample components.

Histomorphological: the use of histology to study the morphology of cells. Histology is the study of the microscopic anatomy of cells and tissues. It is performed by examining a thin slice (section) of tissue under a light microscope or electron microscope.

HPA-axis: the hypothalamic-pituitary-adrenal axis that is a major part of the neuroendocrine system that controls reactions to stress and influences many body processes, including digestion, the immune system, mood and emotions, sexuality and energy storage and expenditure. The basic hormones of the HPA-axis are corticotropin-releasing hormone (CRH) from hypothalamus, adrenocorticotropic hormone (ACTH) from the pituitary gland and glucocorticoids, including cortisol, from the adrenal cortex.

HPG-axis: hypothalamic-pituitary-gonadal axis. This axis controls development, reproduction and aging in animals. The hypothalamus produces gonadotropin-releasing hormone (GnRH). The anterior portion of the pituitary gland produces luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and the gonads (testis) produce estrogen and testosterone.

Hydrophilic: a hydrophilic molecule or portion of a molecule is one that has a tendency to interact with or be dissolved by water and other polar substances.

Ileal digestion or digestibility: means that digestion of feed takes place in the small intestine.

Immunogenicity: the ability of a particular substance, such as an antigen or epitope, to provoke an immune response in the body of a human or animal.

Indolic compound: indole is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring. Compounds that contain an indole ring are called indoles or indolic componds.

Insemination: when sperm from males is collected with the aim to bring it to females and put it into their vagina to make them pregnant without sexual intercourse.

Intrinsic: situated within or belonging solely to the organ or body part on which it acts.

Libido: reproduction instinct.

Ligand: a molecule that binds specifically and reversibly to a receptor and initiates or modifies a biological process.

Lipids: a group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins monoglycerides, diglycerides, triglycerides, phospholipids and others.

Lipophilic: states that molecules are attracted to lipids.

Marker Assisted Selection (MAS): an indirect selection process selecting for a trait of interest by only considering markers closely linked to the trait, typically in the form of SNPs or haplotypes.

Metabolize, metabolism: the physical and chemical processes in an organism by which its material substance is produced, maintained and destroyed, and by which energy is made available. Metabolism includes processes for cell growth, reproduction, response to environment, survival mechanisms, sustenance and maintenance of cell structure and integrity.

Monoclonal antibody: monospecific antibodies that are the same because they are made by identical immune cells that are all clones of a unique parent cell, in contrast to polyclonal antibodies which are made from several different immune cells. Monoclonal antibodies have monovalent affinity, in that they bind to the same epitope.

Monomers: a molecule that may bind chemically to other molecules to form a polymer. The most common natural monomer is glucose, which is linked by glycosidic bonds into polymers such as cellulose and starch.

Monosaccharaides: the most basic units of carbohydrates. They are the simplest form of sugar and are usually colorless, water-soluble, crystalline solids. Some monosaccharides have a sweet taste. Examples of monosaccharides include glucose (dextrose), fructose and galactose. Monosaccharides are the building blocks of disaccharides (such as sucrose) and polysaccharides (such as cellulose and starch).

Morphological: the form and structure of organisms without consideration of function.

Mucosa: a mucous tissue lining various tubular structures, such as the intestine, consisting of epithelium, lamina propria, and, in the digestive tract, a layer of smooth muscle.

Mutations: a change on a single position within the DNA.

Non-specific gas sensor arrays: combine the responses of several low cost, nonspecific sensors (sensor arrays) to achieve an ensemble performance known as "electronic nose" which tries to imitate the human olfactory system.

Oligosaccharides: a saccharide polymer containing a small number (typically three to nine) of simple sugars (monosaccharides).

On-line methods: direct measurements of boar taint compounds at the carcass on the slaughter line.

Oocyte: unfertilized egg cell; the female germ cell for the reproductive process.

Phenotype: a measurable or observable characteristic caused by differences in the genetic background between individuals.

Pheromone: a secreted or excreted chemical factor (e.g. androstenone) that triggers a social response in members of the same species.

Photo acoustic spectroscopy (PAS): Photo acoustic spectroscopy (PAS) is recognized as a sensitive, zero background method for trace gas analysis. When an infrared (IR) beam penetrates a sample, the absorption of the radiation heats up the gas increasing both the temperature and the pressure. Thus, the IR radiation modulated by a chopper with a certain frequency will create temperature and pressure variations in the sample gas with the same frequency. These variations form a sound wave, which is usually measured with a capacitive microphone. The selectivity is achieved by using an optical filter or a laser with a certain wavelength.

Polymer: a polymer is a large molecule composed of many repeated subunits, known as monomers. Because of their broad range of properties, both synthetic and natural polymers play an essential and ubiquitous role in everyday life. Polymers range from familiar synthetic plastics such as polystyrene (or styrofoam) to natural biopolymers such as carbohydrates, DNA and proteins that are fundamental to biological structure and function.

Polymerisation: polymerization is a process of reacting monomer molecules together in a chemical reaction to form polymer chains or three-dimensional networks.

Polysaccharides: polysaccharides are polymeric carbohydrate molecules composed of long chains of monosaccharide units bound together by glycosidic bonds. They range in structure from linear to highly branched. Examples include storage polysaccharides such as starch and glycogen, and structural polysaccharides such as cellulose and chitin.

QTL, Quantitative trait loci: chromosomal regions affecting a phenotypic trait.

Radioimmunoassay (RIA): a very sensitive in vitro assay technique used to measure concentrations of antigens (for example, hormone levels in the blood) by use of antibodies and labelling of antibody-antigen complexes with radioactive isotopes.

Sedation: a state of calm or reduced nervous activity.

Selection index: an estimate of a boar's breeding value based on phenotypic measures.

Skatole: 3-methylindole, a microbial degradation product originating from microbial fermentation of the feed in the large intestine that is absorbed into the blood and may accumulate in fat and contributes to boar taint.

SNP, Single Nucleotide Polymorphism: changes, caused by mutations of the base pairs at a single position within the double helix DNA strand that leads to different variations within and/or among individuals.

Solid phase extraction (SPE): a separation process by which compounds that are dissolved or suspended in a liquid mixture are separated from other compounds in the mixture according to their physical and chemical properties. Analytical laboratories use solid phase extraction to concentrate and purify samples for analysis.

Spectroscopy/ Spectrophotometric methods: spectroscopy is the spectroscopic technique used to assess the concentration or amount of a given chemical (atomic, molecular or ionic) species. In this case, the instrument that performs such measurements is a spectrometer, spectrophotometer or spectrograph. Spectroscopy/spectrometry is often used in analytical chemistry for the identification and quantification of substances through the spectrum emitted from or absorbed by them.

Spermatogenesis: production of sperm cells.

Spermatozoon: a motile sperm cell; the male germ cell for the reproductive process.

Steroidogenesis: synthesis of steroids.

Subcutaneous: located or placed just beneath the skin.

Sugar: the generalized name for a class of chemically-related sweet-flavoured substances, most of which are used as food. They are mono- or disaccharide, composed of carbon, hydrogen and oxygen. There are various types of sugar derived from different sources. Simple sugars are called monosaccharides and include glucose (also known as dextrose), fructose and galactose. The most customarily used sugar in food is sucrose, a disaccharide (in the body, sucrose hydrolyses into fructose and glucose).

Sus scrofa chromosome (SSC): refers to the different pig chromosomes.

Testicular steroids: steroids produced in testis, e.g. testosterone, androstenone and oestrogens.

Time-resolved fluoroimmunoassay (TR-FIA): a fluoroimmunoassay in which the fluorescence intensity is measured after a selected delay time which almost completely eliminates background fluorescence, which has a fast decay time.

Tryptophan: the amino acid that is precursor for skatole.

Xenobiotics: a foreign chemical substance found within an organism that is not normally naturally produced by or expected to be present within that organism, e.g. pesticides and antibiotics.

Appendix

Appendix 1: Cost-benefit model where surgical castration (SC) is compared to variations of **entire male pig production** (EM): No feed additives, chicory added the last 7 days and pure grain feeding the last 4 days (reducing skatole 25 % or 66 %).

Boar taint out-sorting criteria: Current method in Denmark, skatole > $0.25 \mu g/g$.

Costs and benfits of alternativs to surgical castration of male pig						
Scenarios:	SC	EM	EM+Chicory	EM+grain25	EM+grain66	
Assumptions						
Danish Crow listed meat price week 45 (DKK)	11.7	11.7	11.7	11.7	11.7	
Price correction at the end of the year (DKK)	0.85	0.85	0.85	0.85	0.85	
Risk of out sorting (DKK)	0.01	0.08	0.027	0.06	0.027	
Average price deduction per pig due to boar taint (DKK)	-0.02	-0.16	-0.054	-0.12	-0.054	
Price correction (meat %, transport etc.) (DKK)	-0.35	-0.19	-0.19	-0.19	-0.19	
Final price per kg meat (DKK)	12.18	12.2	12.306	12.24	12.306	
Carcass weight (kg)	83	83	83	83	83	
Live weight (kg)	108.73	108.73	108.73	108.73	108.73	
Average revenue per slaghtered pig (DKK)	1,010.94	1,012.6	1,021.398	1,015.92	1,021.398	
Weight of purchased piglet (DDK)	30	30	30	30	30	
List price purchased piglet (DDK)	425	425	425	425	425	
Price of purchased piglet (DDK)	425	425	425	425	425	
Feed price per Feed Unit (7.72 MJ net energy) (DKK)	1.73	1.73	1.73	1.73	1.73	
No. of produced pigs per space per year	3.99	3.9	3.9	3.9	3.9	
Feed conversion ratio (kg gain per kg feed)	2.85	2.61	2.61	2.61	2.61	
Daily weight gain	905	885	885	885	885	
Lean meat %	60	61.3	61.3	61.3	61.3	
Dead and discarded (%)	3.6	3.6	3.6	3.6	3.6	
Gross profit per pig (DKK)	585.94	587.6	596.398	590.92	596.398	
Costs due to death (DKK)	15.588	15.588	15.588	15.588	15.588	
Costs for feed (DKK)	388.178	355.489	355.489	355.489	355.489	
Veterinarian costs (DKK)	5.71	5.71	5.71	5.71	5.71	
Various costs (Straw, pick-up of dead animals etc.) (DKK)	10.79	10.79	10.79	10.79	10.79	
Deduction for control of boar taint (DKK)	0	25	25	25	25	
Costs for transportation slaughterhouse (DKK)	12.92	12.92	12.92	12.92	12.92	
IC vaccination incl. labour (DKK)	0	0	0	0	0	
Labour saved when not castrating (DKK)	0	-6	-6	-6	-6	
Cost for feed additives before slaughter (DKK)	0	0	27	13	13	
Skin lesions (DKK)		0	0	0	0	
Results						
Gross margin per pig per year (DKK)	152.753	168.102	149.9	158.422	163.9	
Gross margin per pig space per year (DKK)	609.487	655.599	584.611	617.847	639.211	
Gross margin/pig space, space allowance 10% higher (DKK)	554024	595.939	531.412	561.623	581.043	

Appendix 2: Cost-benefit model where surgical castration (SC) is compared to variations of **entire male pig production** (EM): No feed additives, chicory added the last 7 days and pure grain feeding the last 4 days (reducing skatole 25 % or 66 %).

Boar taint out-sorting criteria: Skatole > 0.25 $\mu g/g$ + human nose.

Costs and benfits of alternativs to surgical castration of male pig						
Scenarios:	SC	EM	EM+Chicory	EM+grain25	EM+grain66	
Assumptions					-	
Danish Crow listed meat price week 45 (DKK)	11.7	11.7	11.7	11.7	11.7	
Price correction at the end of the year (DKK)	0.85	0.85	0.85	0.85	0.85	
Risk of out sorting (DKK)	0.01	0.12	0.041	0.09	0.041	
Average price deduction per pig due to boar taint (DKK)	-0.02	-0.24	-0.082	-0.18	-0.082	
Price correction (meat %, transport etc.) (DKK)	-0.35	-0.19	-0.19	-0.19	-0.19	
Final price per kg meat (DKK)	12.18	12.12	12.278	12.18	12.278	
Carcass weight (kg)	83	83	83	83	83	
Live weight (kg)	108.73	108.73	108.73	108.73	108.73	
Average revenue per slaghtered pig (DKK)	1,010.94	1,005.96	1,019.074	1,010.94	1,019.074	
Weight of purchased piglet (DDK)	30	30	30	30	30	
List price purchased piglet (DDK)	425	425	425	425	425	
Price of purchased piglet (DDK)	425	425	425	425	425	
Feed price per Feed Unit (7.72 MJ net energy) (DKK)	1.73	1.73	1.73	1.73	1.73	
No. of produced pigs per space per year	3.99	3.9	3.9	3.9	3.9	
Feed conversion ratio (kg gain per kg feed)	2.85	2.61	2.61	2.61	2.61	
Daily weight gain	905	885	885	885	885	
Lean meat %	60	61.3	61.3	61.3	61.3	
Dead and discarded (%)	3.6	3.6	3.6	3.6	3.6	
Gross profit per pig (DKK)	585.94	580.96	594.074	585.94	594.074	
Costs due to death (DKK)	15.588	15.588	15.588	15.588	15.588	
Costs for feed (DKK)	388.178	355.489	355.489	355.489	355.489	
Veterinarian costs (DKK)	5.71	5.71	5.71	5.71	5.71	
Various costs (Straw, pick-up of dead animals etc.) (DKK)	10.79	10.79	10.79	10.79	10.79	
Deduction for control of boar taint (DKK)	0	32	32	32	32	
Costs for transportation slaughterhouse (DKK)	12.92	12.92	12.92	12.92	12.92	
IC vaccination incl. labour (DKK)	0	0	0	0	0	
Labour saved when not castrating (DKK)	0	-6	-6	-6	-6	
Cost for feed additives before slaughter (DKK)	0	0	27	13	13	
Skin lesions (DKK)		0	0	0	0	
Results						
Gross margin per pig per year (DKK)	152.753	154.462	140.576	146.442	154.576	
Gross margin per pig space per year (DKK)	609.487	602.403	548.248	571.125	602.848	
Gross margin/pig space, space allowance 10 % higher (DKK)	Not relevant	547.584	498.357	519.153	547.988	

Appendix 3: Cost-benefit model where surgical castration (SC) is compared to variations of **entire male pig production** (EM): No feed additives, chicory added the last 7 days and pure grain feeding the last 4 days (reducing skatole 25 % or 66 %).

Most restrictive boar taint out-sorting criteria: Androstenone > 1.00 $\mu g/g,$ skatole > 0.25 $\mu g/g.$

Costs and benfits of alternativs to surgical castration of male pig						
Scenarios:	SC	EM	EM+Chicory	EM+grain25	EM+grain66	
Assumptions						
Danish Crow listed meat price week 45 (DKK)	11.7	11.7	11.7	11.7	11.7	
Price correction at the end of the year (DKK)	0.85	0.85	0.85	0.85	0.85	
Risk of out sorting (DKK)	0.01	0.3	0.3	0.3	0.3	
Average price deduction per pig due to boar taint (DKK)	-0.02	-0.6	-0.6	-0.6	-0.6	
Price correction (meat %, transport etc.) (DKK)	-0.35	-0.19	-0.19	-0.19	-0.19	
Final price per kg meat (DKK)	12.18	11.76	11.76	11.76	11.76	
Carcass weight (kg)	83	83	83	83	83	
Live weight (kg)	108.73	108.73	108.73	108.73	108.73	
Average revenue per slaghtered pig (DKK)	1,010.94	976.08	976.08	976.08	976.08	
Weight of purchased piglet (DDK)	30	30	30	30	30	
List price purchased piglet (DDK)	425	425	425	425	425	
Price of purchased piglet (DDK)	425	425	425	425	425	
Feed price per Feed Unit (7.72 MJ net energy) (DKK)	1.73	1.73	1.73	1.73	1.73	
No. of produced pigs per space per year	3.99	3.9	3.9	3.9	3.9	
Feed conversion ratio (kg gain per kg feed)	2.85	2.61	2.61	2.61	2.61	
Daily weight gain	905	885	885	885	885	
Lean meat %	60	61.3	61.3	61.3	61.3	
Dead and discarded (%)	3.6	3.6	3.6	3.6	3.6	
Gross profit per pig (DKK)	585.94	551.08	551.08	551.08	551.08	
Costs due to death (DKK)	15.588	15.588	15.588	15.588	15.588	
Costs for feed (DKK)	388.178	355.489	355.489	355.489	355.489	
Veterinarian costs (DKK)	5.71	5.71	5.71	5.71	5.71	
Various costs (Straw, pick-up of dead animals etc.) (DKK)	10.79	10.79	10.79	10.79	10.79	
Deduction for control of boar taint (DKK)	0	25	25	25	25	
Costs for transportation slaughterhouse (DKK)	12.92	12.92	12.92	12.92	12.92	
IC vaccination incl. labour (DKK)	0	0	0	0	0	
Labour saved when not castrating (DKK)	0	-6	-6	-6	-6	
Cost for feed additives before slaughter (DKK)	0	0	27	13	13	
Skin lesions (DKK)		0	0	0	0	
Results						
Gross margin per pig per year (DKK)	152.753	131.582	104.582	118.582	118.582	
Gross margin per pig space per year (DKK)	609.487	513.171	407.871	462.471	462.471	
Gross margin/pig space, space allowance 10% higher (DKK)	Not relevant	466.472	370.755	420.386	420.386	

Appendix 4: Cost-benefit model where surgical castration (SC) is compared to variations of **entire male pig production** (EM): No feed additives, chicory added the last 7 days and pure grain feeding the last 4 days (reducing skatole 25 % or 66 %).

Boar taint out-sorting criteria: Androstenone >2.00 μ g/g, skatole > 0.20 μ g/g.

Costs and benfits of alternativs to surgical castration of male pig						
Scenarios:	SC	EM	EM+Chicory	EM+grain25	EM+grain66	
Assumptions						
Danish Crow listed meat price week 45 (DKK)	11.7	11.7	11.7	11.7	11.7	
Price correction at the end of the year (DKK)	0.85	0.85	0.85	0.85	0.85	
Risk of out sorting (DKK)	0.01	0.1	0.1	0.1	0.1	
Average price deduction per pig due to boar taint (DKK)	-0.02	-0.2	-0.2	-0.2	-0.2	
Price correction (meat %, transport etc.) (DKK)	-0.35	-0.19	-0.19	-0.19	-0.19	
Final price per kg meat (DKK)	12.18	12.16	12.16	12.16	12.16	
Carcass weight (kg)	83	83	83	83	83	
Live weight (kg)	108.73	108.73	108.73	108.73	108.73	
Average revenue per slaghtered pig (DKK)	1,010.94	1,009.28	1,009.28	1,009.28	1009.28	
Weight of purchased piglet (DDK)	30	30	30	30	30	
List price purchased piglet (DDK)	425	425	425	425	425	
Price of purchased piglet (DDK)	425	425	425	425	425	
Feed price per Feed Unit (7.72 MJ net energy) (DKK)	1.73	1.73	1.73	1.73	1.73	
No. of produced pigs per space per year	3.99	3.9	3.9	3.9	3.9	
Feed conversion ratio (kg gain per kg feed)	2.85	2.61	2.61	2.61	2.61	
Daily weight gain	905	885	885	885	885	
Lean meat %	60	61.3	61.3	61.3	61.3	
Dead and discarded (%)	3.6	3.6	3.6	3.6	3.6	
Gross profit per pig (DKK)	585.94	584.28	584.28	584.28	584.28	
Costs due to death (DKK)	15.588	15.588	15.588	15.588	15.588	
Costs for feed (DKK)	388.178	355.489	355.489	355.489	355.489	
Veterinarian costs (DKK)	5.71	5.71	5.71	5.71	5.71	
Various costs (Straw, pick-up of dead animals etc.) (DKK)	10.79	10.79	10.79	10.79	10.79	
Deduction for control of boar taint (DKK)	0	25	25	25	25	
Costs for transportation slaughterhouse (DKK)	12.92	12.92	12.92	12.92	12.92	
IC vaccination incl. labour (DKK)	0	0	0	0	0	
Labour saved when not castrating (DKK)	0	-6	-6	-6	-6	
Cost for feed additives before slaughter (DKK)	0	0	27	13	13	
Skin lesions (DKK)		0	0	0	0	
Results						
Gross margin per pig per year (DKK)	152.753	164.782	137.782	151.782	151.782	
Gross margin per pig space per year (DKK)	609.487	642.651	537.351	591.951	591.951	
Gross margin/pig space, space allowance 10% higher (DKK)	Not relevant	584.17	488.452	538.083	538.083	

Appendix 5: Cost-benefit model where surgical castration (SC) is compared to variations of **entire male pig production** (EM) with **slaughtered weight reduced to 75 kg:** No feed additives, chicory added the last 7 days and pure grain feeding the last 4 days (reducing skatole 35 % or 66 %).

Boar taint out-sorting criteria: Current method in Denmark, skatole > $0.25 \ \mu g/g$.

Costs and benfits of alternativs to surgical castration of male pig						
Scenarios:	SC	EM	EM+Chicory	EM+grain25	EM+grain66	
Assumptions						
Danish Crow listed meat price week 45 (DKK)	11.7	11.7	11.7	11.7	11.7	
Price correction at the end of the year (DKK)	0.85	0.85	0.85	0.85	0.85	
Risk of out sorting (DKK)	0.01	0.08	0.027	0.06	0.027	
Average price deduction per pig due to boar taint (DKK)	-0.02	-0.16	-0.054	-0.12	-0.054	
Price correction (meat %, transport etc.) (DKK)	-0.35	-0.19	-0.07	-0.07	-0.07	
Final price per kg meat (DKK)	12.18	12.2	12.426	12.36	12.426	
Carcass weight (kg)	83	83	75	75	75	
Live weight (kg)	108.73	108.73	98.25	98.25	98.25	
Average revenue per slaghtered pig (DKK)	1,010.94	1,012.6	931.95	927	931.95	
Weight of purchased piglet (DDK)	30	30	30	30	30	
List price purchased piglet (DDK)	425	425	425	425	425	
Price of purchased piglet (DDK)	425	425	425	425	425	
Feed price per Feed Unit (7.72 MJ net energy) (DKK)	1.73	1.73	1.73	1.73	1.73	
No. of produced pigs per space per year	3.99	3.9	4.45	4.45	4.45	
Feed conversion ratio (kg gain per kg feed)	2.85	2.61	2.5	2.5	2.5	
Daily weight gain	905	885	875	875	875	
Lean meat %	60	61.3	62.3	62.3	62.3	
Dead and discarded (%)	3.6	3.6	3.6	3.6	3.6	
Gross profit per pig (DKK)	585.94	587.6	506.95	502	506.95	
Costs due to death (DKK)	15.588	155.88	15.588	15.588	15.588	
Costs for feed (DKK)	388.178	355.489	295.181	295.181	295.181	
Veterinarian costs (DKK)	5.71	5.71	5.71	5.71	5.71	
Various costs (Straw, pick-up of dead animals etc.) (DKK)	10.79	10.79	10.79	10.79	10.79	
Deduction for control of boar taint (DKK)	0	25	25	25	25	
Costs for transportation slaughterhouse (DKK)	12.92	12.92	12.92	12.92	12.92	
IC vaccination incl. labour (DKK)	0	0	0	0	0	
Labour saved when not castrating (DKK)	0	-6	-6	-6	-6	
Cost for feed additives before slaughter (DKK)	0	0	27	13	13	
Skin lesions (DKK)		0	0	0	0	
Results						
Gross margin per pig per year (DKK)	152.753	168.102	120.76	129.81	134.76	
Gross margin per pig space per year (DKK)	609.487	655.599	537.385	577.657	599.685	
Gross margin/pig space, space allowance 10 % higher (DKK)	554024	595.939	488.483	525.09	545.113	

Appendix 6: Cost-benefit model where surgical castration (SC) is compared to variations of **entire male pig production** (EM) with **slaughtered weight reduced to 75 kg:** No feed additives, chicory added the last 7 days and pure grain feeding the last 4 days (reducing skatole 35 % or 66 %).

Boar taint out-sorting criteria: Skatole > 0.25 μ g/g + human nose.

Costs and benfits of alternativs to surgical castration of male pig						
Scenarios:	SC	EM	EM+Chicory	EM+grain25	EM+grain66	
Assumptions						
Danish Crow listed meat price week 45 (DKK)	11.7	11.7	11.7	11.7	11.7	
Price correction at the end of the year (DKK)	0.85	0.85	0.85	0.85	0.85	
Risk of out sorting (DKK)	0.01	0.12	0.041	0.09	0.041	
Average price deduction per pig due to boar taint (DKK)	-0.02	-0.24	-0.082	-0.18	-0.082	
Price correction (meat %, transport etc.) (DKK)	-0.35	-0.19	-0.07	-0.07	-0.07	
Final price per kg meat (DKK)	12.18	12.12	12.398	12.3	12.398	
Carcass weight (kg)	83	83	75	75	75	
Live weight (kg)	108.73	108.73	98.25	98.25	98.25	
Average revenue per slaghtered pig (DKK)	1,010.94	1,005.96	929.85	922.5	929.85	
Weight of purchased piglet (DDK)	30	30	30	30	30	
List price purchased piglet (DDK)	425	425	425	425	425	
Price of purchased piglet (DDK)	425	425	425	425	425	
Feed price per Feed Unit (7.72 MJ net energy) (DKK)	1.73	1.73	1.73	1.73	1.73	
No. of produced pigs per space per year	3.99	3.9	4.45	4.45	4.45	
Feed conversion ratio (kg gain per kg feed)	2.85	2.61	2.5	2.5	2.5	
Daily weight gain	905	885	875	875	875	
Lean meat %	60	61.3	62.3	62.3	62.3	
Dead and discarded (%)	3.6	3.6	3.6	3.6	3.6	
Gross profit per pig (DKK)	585.94	580.96	504.85	497.5	504.85	
Costs due to death (DKK)	15.588	15.588	15.588	15.588	15.588	
Costs for feed (DKK)	388.178	355.489	295.181	295.181	295.181	
Veterinarian costs (DKK)	5.71	5.71	5.71	5.71	5.71	
Various costs (Straw, pick-up of dead animals etc.) (DKK)	10.79	10.79	10.79	10.79	10.79	
Deduction for control of boar taint (DKK)	0	32	32	32	32	
Costs for transportation slaughterhouse (DKK)	12.92	12.92	19.38	19.38	19.38	
IC vaccination incl. labour (DKK)	0	0	0	0	0	
Labour saved when not castrating (DKK)	0	-6	-6	-6	-6	
Cost for feed additives before slaughter (DKK)	0	0	27	13	13	
Skin lesions (DKK)		0	0	0	0	
Results						
Gross margin per pig per year (DKK)	152.753	154.462	105.2	111.85	119.2	
Gross margin per pig space per year (DKK)	609.487	602.403	468.143	497.735	530.443	
Gross margin/pig space, space allowance 10 % higher (DKK)	Not relevant	547.584	425.542	452.441	482.172	

Appendix 7: Cost-benefit model where surgical castration (SC) is compared to variations of **entire male pig production** (EM) with **slaughtered weight reduced to 75** kg: No feed additives, chicory added the last 7 days and pure grain feeding the last 4 days (reducing skatole 35 % or 66 %).

Most restrictive boar taint out-sorting criteria: Androstenone > 1.00 $\mu g/g,$ skatole > 0.25 $\mu g/g.$

Costs and benfits of alternativs to surgical castration of male pig						
Scenarios:	SC	EM	EM+Chicory	EM+grain25	EM+grain66	
Assumptions						
Danish Crow listed meat price week 45 (DKK)	11.7	11.7	11.7	11.7	11.7	
Price correction at the end of the year (DKK)	0.85	0.85	0.85	0.85	0.85	
Risk of out sorting (DKK)	0.01	0.3	0.25	0.25	0.25	
Average price deduction per pig due to boar taint (DKK)	-0.02	-0.6	-0.5	-0.5	-0.5	
Price correction (meat %, transport etc.) (DKK)	-0.35	-0.19	-0.07	-0.07	-0.07	
Final price per kg meat (DKK)	12.18	11.76	11.98	11.98	11.98	
Carcass weight (kg)	83	83	75	75	75	
Live weight (kg)	108.73	108.73	98.25	98.25	98.25	
Average revenue per slaghtered pig (DKK)	1,010.94	976.08	898.5	898.5	898.5	
Weight of purchased piglet (DDK)	30	30	30	30	30	
List price purchased piglet (DDK)	425	425	425	425	425	
Price of purchased piglet (DDK)	425	425	425	425	425	
Feed price per Feed Unit (7.72 MJ net energy) (DKK)	1.73	1.73	1.73	1.73	1.73	
No. of produced pigs per space per year	3.99	3.9	4.45	4.45	4.45	
Feed conversion ratio (kg gain per kg feed)	2.85	2.61	2.5	2.5	2.5	
Daily weight gain	905	885	875	875	875	
Lean meat %	60	61.3	62.3	62.3	62.3	
Dead and discarded (%)	3.6	3.6	3.6	3.6	3.6	
Gross profit per pig (DKK)	585.94	551.08	473.5	473.5	473.5	
Costs due to death (DKK)	15.588	15.588	15.588	15.588	15.588	
Costs for feed (DKK)	388.178	355.489	295.181	295.181	295.181	
Veterinarian costs (DKK)	5.71	5.71	5.71	5.71	5.71	
Various costs (Straw, pick-up of dead animals etc.) (DKK)	10.79	10.79	10.79	10.79	10.79	
Deduction for control of boar taint (DKK)	0	25	25	25	25	
Costs for transportation slaughterhouse (DKK)	12.92	12.92	12.92	12.92	12.92	
IC vaccination incl. labour (DKK)	0	0	0	0	0	
Labour saved when not castrating (DKK)	0	-6	-6	-6	-6	
Cost for feed additives before slaughter (DKK)	0	0	27	13	13	
Skin lesions (DKK)		0	0	0	0	
Results						
Gross margin per pig per year (DKK)	152.753	131.582	87.31	101.31	101.31	
Gross margin per pig space per year (DKK)	609.487	513.171	388.532	450.832	450.832	
Gross margin/pig space, space allowance 10 % higher (DKK)	Not relevant	466.472	353.176	409.807	409.807	

Appendix 8: Cost-benefit model where surgical castration (SC) is compared to variations of **entire male pig production** (EM) with **slaughtered weight reduced to 75 kg**: No feed additives, chicory added the last 7 days and pure grain feeding the last 4 days (reducing skatole 35 % or 66 %).

Boar taint out-sorting criteria: Androstenone >2.00 μ g/g, skatole > 0.20 μ g/g.

Costs and benfits of alternativs to surgical castration of male pig						
Scenarios:	SC	EM	EM+Chicory	EM+grain25	EM+grain66	
Assumptions					-	
Danish Crow listed meat price week 45 (DKK)	11.7	11.7	11.7	11.7	11.7	
Price correction at the end of the year (DKK)	0.85	0.85	0.85	0.85	0.85	
Risk of out sorting (DKK)	0.01	0.1	0.074	0.074	0.074	
Average price deduction per pig due to boar taint (DKK)	-0.02	-0.2	-0.148	-0.148	-0.148	
Price correction (meat %, transport etc.) (DKK)	-0.35	-0.19	-0.07	-0.07	-0.07	
Final price per kg meat (DKK)	12.18	12.16	12.332	12332	12.332	
Carcass weight (kg)	83	83	75	75	75	
Live weight (kg)	108.73	108.73	98.25	98.25	98.25	
Average revenue per slaghtered pig (DKK)	1,010.94	1,009.28	924.9	924.9	924.9	
Weight of purchased piglet (DDK)	30	30	30	30	30	
List price purchased piglet (DDK)	425	425	425	425	425	
Price of purchased piglet (DDK)	425	425	425	425	425	
Feed price per Feed Unit (7.72 MJ net energy) (DKK)	1.73	1.73	1.73	1.73	1.73	
No. of produced pigs per space per year	3.99	3.9	4.45	4.45	4.45	
Feed conversion ratio (kg gain per kg feed)	2.85	2.61	2.5	2.5	2.5	
Daily weight gain	905	885	875	875	875	
Lean meat %	60	61.3	62.3	62.3	62.3	
Dead and discarded (%)	3.6	3.6	3.6	3.6	3.6	
Gross profit per pig (DKK)	585.94	584.28	499.9	499.9	499.9	
Costs due to death (DKK)	15.588	15.588	15.588	15.588	15.588	
Costs for feed (DKK)	388.178	355.489	295.181	295.181	295.181	
Veterinarian costs (DKK)	5.71	5.71	5.71	5.71	5.71	
Various costs (Straw, pick-up of dead animals etc.) (DKK)	10.79	10.79	10.79	10.79	10.79	
Deduction for control of boar taint (DKK)	0	25	25	25	25	
Costs for transportation slaughterhouse (DKK)	12.92	12.92	12.92	12.92	12.92	
IC vaccination incl. labour (DKK)	0	0	0	0	0	
Labour saved when not castrating (DKK)	0	-6	-6	-6	-6	
Cost for feed additives before slaughter (DKK)	0	0	27	13	13	
Skin lesions (DKK)		0	0	0	0	
Results						
Gross margin per pig per year (DKK)	152.753	164.782	113.71	127.71	127.71	
Gross margin per pig space per year (DKK)	609.487	642.651	506.012	568.312	568.312	
Gross margin/pig space, space allowance 10% higher (DKK)	Not relevant	584.17	459.965	516.596	516.596	

Appendix 9: Cost-benefit model where surgical castration (SC) is compared to four different strategies for producing **immunocastrated pigs** (IC): 1) IC standard vaccinated, fed ad libitum, 2) IC standard vaccinated, fed restrictively, 3) IC early second vaccination, fed restrictively and 4) IC early second vaccination, fed ad libitum.

In the **implementation period or a situation with permanent control a deduction of DKK 25 per pig to control of boar taint is included and 2 % are supposed to be sorted out due to boar taint.**

Costs and benfits of alternativs to surgical castration of male pig						
Scenarios:	SC	IC1	IC2	IC3	IC4	
Assumptions						
Danish Crow listed meat price week 45 (DKK)	11.7	11.7	11.7	11.7	11.7	
Price correction at the end of the year (DKK)	0.85	0.85	0.85	0.85	0.85	
Risk of out sorting (DKK)	0.01	0.02	0.02	0.02	0.02	
Average price deduction per pig due to boar taint (DKK)	-0.02	-0.04	-0.04	-0.04	-0.04	
Price correction (meat %, transport etc.) (DKK)	-0.35	-0.23	-0.13	-0.18	-0.28	
Final price per kg meat (DKK)	12.18	12.28	12.38	12.33	12.23	
Carcass weight (kg)	83	83	83	83	83	
Live weight (kg)	108.73	108.73	108.73	108.73	108.73	
Average revenue per slaghtered pig (DKK)	1,010.94	1,019.24	1,027.54	1,023.39	1,015.09	
Weight of purchased piglet (DDK)	30	30	30	30	30	
List price purchased piglet (DDK)	425	425	425	425	425	
Price of purchased piglet (DDK)	425	425	425	425	425	
Feed price per Feed Unit (7.72 MJ net energy) (DKK)	1.73	1.73	1.73	1.73	1.73	
No. of produced pigs per space per year	3.99	3.99	3.78	3.78	3.99	
Feed conversion ratio (kg gain per kg feed)	2.85	2.71	2.64	2.64	2.79	
Daily weight gain	905	905	905	905	905	
Lean meat %	60	61.1	62	61.6	60.7	
Dead and discarded (%)	3.6	3.6	3.6	3.6	3.6	
Gross profit per pig (DKK)	585.94	594.24	602.54	598.39	590.09	
Costs due to death (DKK)	15.588	15.588	15.588	15.588	15.588	
Costs for feed (DKK)	388.178	369.109	359.575	359.575	380.006	
Veterinarian costs (DKK)	5.71	5.71	5.71	5.71	5.71	
Various costs (Straw, pick-up of dead animals etc.) (DKK)	10.79	10.79	10.79	10.79	10.79	
Deduction for control of boar taint (DKK)	0	25	25	25	25	
Costs for transportation slaughterhouse (DKK)	12.92	12.92	12.92	12.92	12.92	
IC vaccination incl. labour (DKK)	0	28.5	24	24	28.5	
Labour saved when not castrating (DKK)	0	-6	-6	-6	-6	
Cost for feed additives before slaughter (DKK)	0	0	0	0	0	
Skin lesions (DKK)						
Results						
Gross margin per pig per year (DKK)	152.753	132.622	154.956	150.806	117.575	
Gross margin per pig space per year (DKK)	609.487	529.162	585.734	570.047	469.127	
Gross margin/pig space, space allowance 10% higher (DKK)	554024	481.008	532.433	518.173	426.437	

Appendix 10: Cost-benefit model where surgical castration (SC) is compared to four different strategies for producing **immunocastrated pigs** (IC): 1) IC standard vaccinated, fed ad libitum, 2) IC standard vaccinated, fed restrictively, 3) IC early second vaccination, fed restrictively and 4) IC early second vaccination, fed ad libitum.

In the implementation period or with permanent control a deduction of DKK 25 to control of boar taint is included and the manufacturer indicates a **risk of being sorted out due to boar taint of 1** %. In this case we assume no deduction for boar taint control.

Costs and benfits of alternativs to surgical castration of male pig						
Scenarios:	SC	IC1	IC2	IC3	IC4	
Assumptions						
Danish Crow listed meat price week 45 (DKK)	11.7	11,7	11,7	11,7	11,7	
Price correction at the end of the year (DKK)	0.85	0,85	0,85	0,85	0,85	
Risk of out sorting (DKK)	0.01	0,01	0,01	0,01	0,01	
Average price deduction per pig due to boar taint (DKK)	-0.02	-0.02	-0.02	-0.02	-0.02	
Price correction (meat %, transport etc.) (DKK)	-0.35	-0.23	-0.13	-0.18	-0.28	
Final price per kg meat (DKK)	12.18	12.3	12.4	12.35	12.25	
Carcass weight (kg)	83	83	83	83	83	
Live weight (kg)	108.73	108.73	108.73	108.73	108.73	
Average revenue per slaghtered pig (DKK)	1,010.94	1,020.9	1,029.2	1,025.05	1,016.75	
Weight of purchased piglet (DDK)	30	30	30	30	30	
List price purchased piglet (DDK)	425	425	425	425	425	
Price of purchased piglet (DDK)	425	425	425	425	425	
Feed price per Feed Unit (7.72 MJ net energy) (DKK)	1.73	1.73	1.73	1.73	1.73	
No. of produced pigs per space per year	3.99	3.99	3.78	3.78	3.99	
Feed conversion ratio (kg gain per kg feed)	2.85	2.71	2.64	2.64	2.79	
Daily weight gain	905	905	905	905	905	
Lean meat %	60	61.1	62	61.6	60.7	
Dead and discarded (%)	3.6	3.6	3.6	3.6	3.6	
Gross profit per pig (DKK)	585.94	595.9	604.2	600.05	591.75	
Costs due to death (DKK)	15.588	15.588	15.588	15.588	15.588	
Costs for feed (DKK)	388.178	369.109	359.575	359.575	380.006	
Veterinarian costs (DKK)	5.71	5.71	5.71	5.71	5.71	
Various costs (Straw, pick-up of dead animals etc.) (DKK)	10.79	10.79	10.79	10.79	10.79	
Deduction for control of boar taint (DKK)	0	0	0	0	0	
Costs for transportation slaughterhouse (DKK)	12.92	12.92	12.92	12.92	12.92	
IC vaccination incl. labour (DKK)	0	28.5	24	24	28.5	
Labour saved when not castrating (DKK)	0	-6	-6	-6	-6	
Cost for feed additives before slaughter (DKK)	0	0	0	0	0	
Skin lesions (DKK)						
Results						
Gross margin per pig per year (DKK)	152.753	159.282	181.616	177.466	144.235	
Gross margin per pig space per year (DKK)	609.487	635.535	686.509	670.822	575.501	
Gross margin/pig space, space allowance 10% higher (DKK)	554024	577.701	624.037	609.777	523.131	

DCA - National Centre for Food and Agriculture is the entrance to research in food and agriculture at Aarhus University (AU). The main tasks of the centre are knowledge exchange, advisory service and interaction with authorities, organisations and businesses.

The centre coordinates knowledge exchange and advice with regard to the departments that are heavily involved in food and agricultural science. They are:

Department of Animal Science Department of Food Science Department of Agroecology Department of Engineering Department of Molecular Biology and Genetics

DCA can also involve other units at AU that carry out research in the relevant areas.