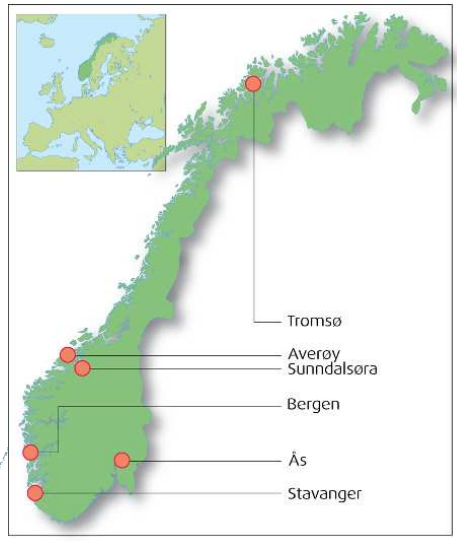
Ebergeruch und Nachweismethoden

John-Erik Haugen,
Nofima Mat

Workshop 9. März 2009, Kassel

Nofima – Food, Fisheries and Aquaculture Research, Norway

- Established 1 January, 2008
- Merger of former Akvaforsk, Fiskeriforskning, **Matforsk** and Norconserv
- Target groups: Food industry, aquaculture and fisheries
- Number of employees: 450
- Turnover 2007: NOK 430 mill.
- Main office in Tromsø
- Owner:
  - The Norwegian Government by the Department of Fisheries and Coastal Affairs 56,8%
  - Agricultural Food Research Foundation 33,2%
  - Akvainvest Møre og Romsdal county: 10 %



Management

Group CEO *Ørjan Olsvik*

Vice CEO:
Øyvind Fylling-Jensen

Head of communications:
Stein-Gunnar Bondevik

Business areas

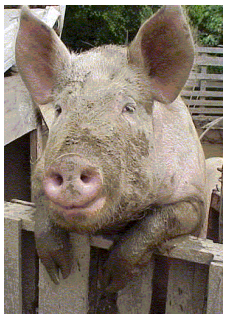
Nofima Marine
Breeding and genetics, fish health, sustainable and efficient production and catch, slaughtering, primary processing
Director Camilla Røsjø

Nofima Foods
Raw materials quality and processing, safe and lasting food, consumer understanding and sensory research, food and health, industrial gastronomy, innovation
Director Øyvind Fylling-Jensen

Nofima Ingredients
Analytical services, research and pilot production of raw materials and ingredients for the feed, food and pharmaceutical industries
Acting Director Bjørn Brekken

Nofima Market
Economic analyses, perspective and foresight analyses, market and consumer analyses, strategic consultancy
Director Bjørn Eirik Olsen

The boar taint case



- The issue boar taint
- Situation in Norway
- Stakeholder consequences
- Conventional analysis of boar substances
- Norwegian male pig research programme
- Rapid detection methodology
- Summary

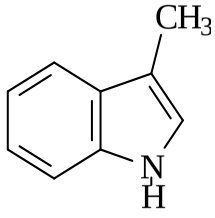
Boar taint - a food quality issue

- Sensory perceived off-odour/flavour in meat from entire/uncastrated male pigs
- Relates to sexual maturation of entire male pigs
- Caused by malodorous compounds, androstenone and skatole
- Androstenone is a pheromonal steroid hormone synthesised in the testes in parallel with anabolic hormones
- Skatole is produced in the large intestine by bacterial degradation of tryptophan
- The substances accumulate in fat tissue

Boar odour/flavour compounds

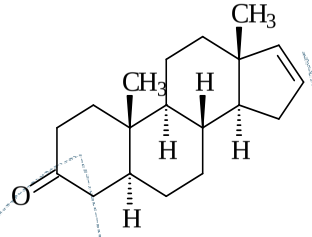
Skatole (3-methyl-indole)

MW: 131,2 M.pt: 96 ºC, B.pt: 265 ºC
Odour character: feacal, naphtalene, sweet, warm, fruity
Odour treshold (ortho-nasal): exogen: 0.15 ppm
fat phase: 0.5 ppm



Androstenone (5α-androst-16-en-3-one)

MW: 272,4 M.pt: 140 ºC, Bpt: 275 ºC
odour character: urine, sweat
odour treshold (ortho-onasal): exogen: 0.2-1.0 ppm
fat phase: 0.5-2.0 ppm



Strategie: Inhaltstoffe messen

Pig slaughter situation Norway

2007: 30 slaughterhouses receiving pigs

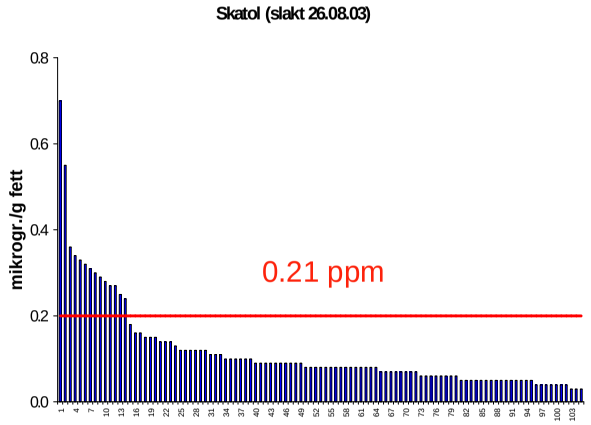
Distribution of pig slaughterhouses

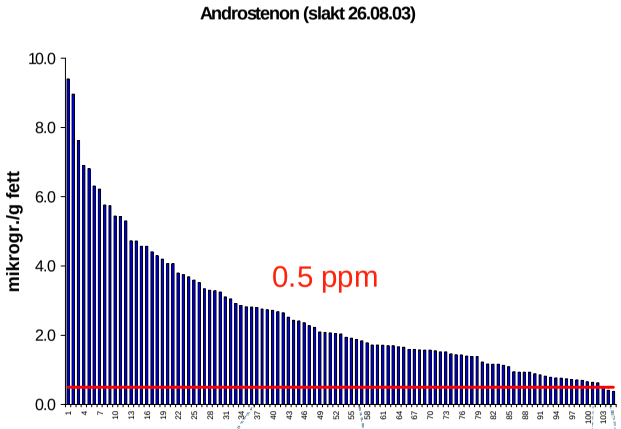
Number of pigs received for slaughter	Number of slaughterhouses
<100	11
100-1000	5
1000-10 000	8
10 000-50 000	8
50 000-100 000	3
100 000-200 000	3
>200 000	2

Situation at Norwegian slaughterhouses are very different. Small slaughterhouses would have to send their male pig samples to one of the laboratories established at the big slaughterhouses for S and A control.

Boar carcass sorting

Data from one herd with Noroc, Duroc and Norwegian landswine served dry feed, Sl. wt: 60-90 kg



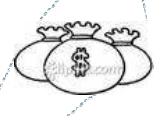


Data from Norwegian Meat Research Center

Ban on castration in Norway 2009

Economical consequences

- Expected percentage of tainted meat 20-80% ???
- Yearly loss for pig producers: 10 mill NOK (1.2 mill euro) for each percentage that have to be sorted out
- Need for detection assays
- Increase costs at the slaughterhouses
- Lost market shares because of consumer complains



Nofima

Ban on castration - stakeholder consequences

Strong international drive towards animal friendly production i.e. no castration of male piglets

- Swine producers
- Abbatoirs
- Retailers
- NGO's - animal welfare organisations
- Legislation/regulation

Nofima

NGO's are becoming more aggressive

"Let piglets keep their balls!" *(www.varkensinnood.nl.)*

From 2009, Dutch branches of supermarket giants Aldi and Lidl are only going to sell meat from pigs which have not been castrated

"Cut off your own balls!"
(Norwegian pig castration debate)

Regulation and legislation

- EU Regulation (854/2004) contains the general provision that **"meat is to be declared unfit [for human consumption] if it indicates…organoleptic anomalies, in particular a pronounced sexual odour".** Some German abattoirs are distinguishing between carcasses with sexual odour (androstenone) and faecal odour (skatole); the carcasses with sexual odour are condemned whereas the carcasses with faecal odour are not. Member States may establish their acceptability criteria and recognise a test method to ensure that carcasses with pronounced sexual odour will be detected.
- **At present, in the EU, there is no harmonised method for detecting boar taint**, but some Member States have established an appropriate test system; for example, in the UK occasionally a hot wire test may be used. An alternative is a soldering iron applied to the exposed backfat of the carcass. This also causes volatilisation of androstenone and skatole which can be detected by an operator. In Germany a cooking test and melting test are used to detect sexual odour in carcasses.This has proved successful in some situations involving small numbers of animals, but effective detection differs between operators and fatigue of the sensory response develops quickly.

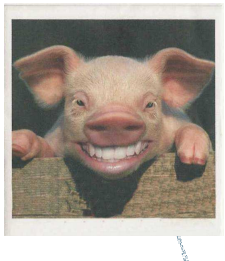
EFSA report 2004 " Welfare aspects of the castration of piglets"

Norwegian research programme 2004 - 2008

Total budget: 10 mill. Euro

Fundings are given to the following projects

- Genetics
- Testis physiology
- Feeding/managing
- Semen separation
- Analgesia effects oon piglets
- **Rapid detection methods**
- Consumer perception/Product development



Project financing

- The Norwegian Research Council
- The Research funds of the Ministry of Agriculture and Food
- The Producers Purchase Tax Fund

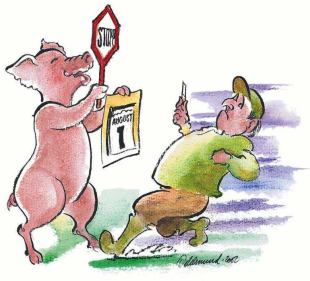
Nofima

Norway: castration ban postponed

Up until recently, Norway was facing a total ban on piglet castration as from January 1, 2009. However, these plans have been postponed in the end of 2007 as agriculture minister, Terje Riis-Johansen had started to wonder whether the 2009 deadline was actually achievable after a series of doubts were released around the castration issue. Questions had been around for a long time, as e.g. the issue of quality loss from non-castrated pigs was brought to light. In addition, it was publicly doubted whether Norwegian consumers would choose the stronger smelling pork from uncastrated pigs. To segregate the male pigs with stronger smells would cost pig processors around €25,000 annually. Eventually, the castration ban was postponed with no new date set as yet. According to researcher Bente Frederiksen, from the Norwegian meat & poultry research centre Animalia, "the aim of abandoning castration is still valid, and the research in this topic will go on, however, probably on a lower scale than the last four years."

Norwegian developments started back in 2002, when a new law stated that castration would only be allowed when using anaesthesia applied by veterinarians. With the 2009 total castration ban looming, the industry started looking for alternatives to make sure the public would continue to buy pork. Norwegian breeder Norsvin has been involved in a project promoting Duroc breeding; this breed's boars have three times more androsterone but half the skatole of other breeds of pigs. In addition, the Norwegian University of Life Sciences has focused on alternative feeding. Thirdly, a project on on-line detection is going on, lead by John-Erik Haugen from Nofima Food.

Extensive research on the current practice amongst veterinarians and pig producers showed that piglets were most often castrated using a combination of subcutaneous and intratesticular administration of lidocaine with adrenaline at an average age of ten days. **(Pig Porgress 24, No. 8, 2008)**



Nofima

Conventional analysis of boar substances

”Reference” analysis methods

Chromatographic methods (>30 methods published)

LC (UV, Fluor., MS),

GC (FID, TSD, MS, ECD)

Immunological methods (about 10 methods published)

EIA, RIA, TR-FIA

Different sampling and cleanup protocols

Carcass location, fat tissue, pure fate phase, solvents etc.

Different quantification protocols

Preparation of calibration/quantification standard

Use of ”external” quantification versus I.Std. method

Matrix based quantification

Results reported with and without recovery/loss correction

Comparison of methods

Ref: Harlizius et al., 2008

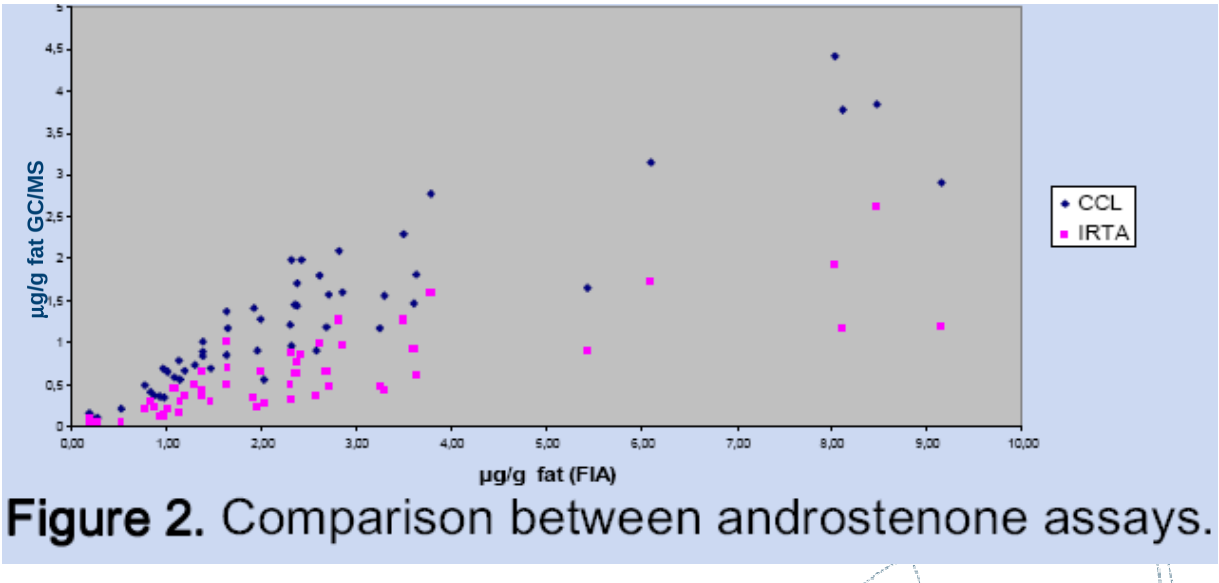


Figure 2. Comparison between androstenone assays.

Comparison of androstenone assays

53 fat samples from same slaughter day analysed by

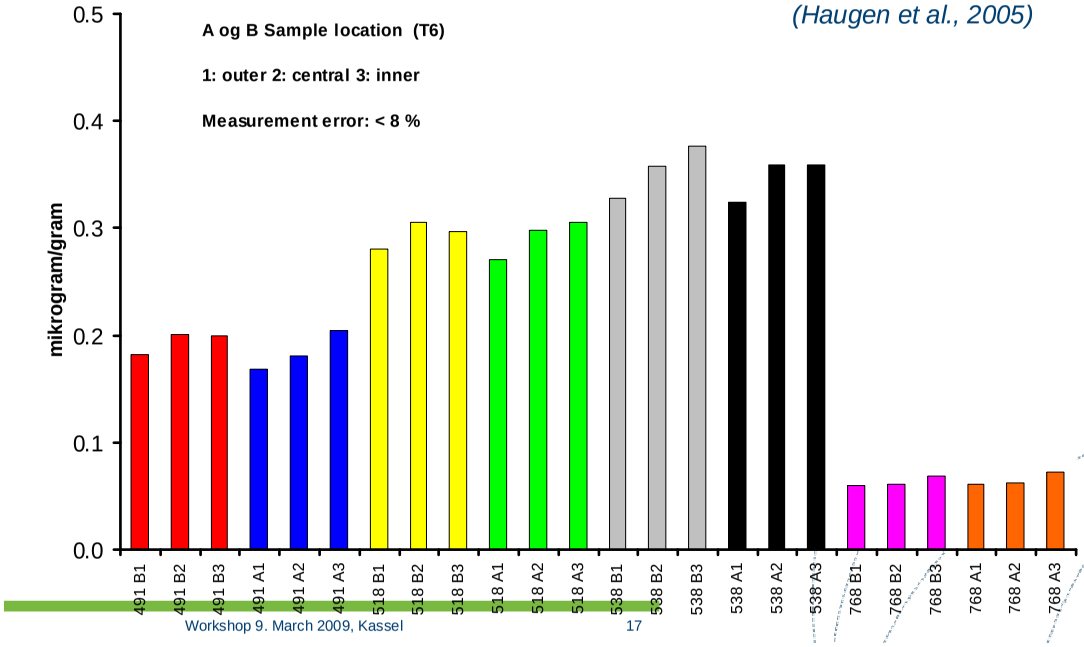
- FIA at NSVS (*Tuomola et al. 1997*)
- GC-MS at CCL (*Someya et al. 2006*) and IRTA (*Rius et al. 2005*)

Conclusions and outlook

Assay comparison is crucial to improve interpretation of results between studies and to determine consumer acceptance thresholds. Differences due to sample preparation, treatment of standards, and calibration are currently investigated.

Sampling – an issue (mostly fat samples)

Increasing concentrations from outer (skin side) to inner (muscle side) part of the back fat tissue



(Haugen et al., 2005)

Need for standardisation/harmonisation

- Many different method protocols i.e., variability in analysis protocols
- Mostly in-house validated methods, no completely externally validated
- Lack of info on performance characteristics and verification
- 2-3 inter-laboratory studies (not published)
- No collaborative validation studies according to internationally accepted guidelines
- Significant biases/deviations in results between labs

Great need for standardised and harmonised reference method(s)

Critical for definition of:

- sound sensory treshold levels
- sorting criteria for boar tainted carcasses
- calibration of non-specific rapid methods

Rapid detection methodology

Industrial requirements

- Simple (1 method both/all boar odour substances)
- Automated – sampling and detection
- High throughput (500-5000 /hr)
- Low cost (3-5 Euro pr analysis)
- No false negatives

- Rapid boar detection systems for sorting out the boar tainted carcasses at the slaughter line in combination with other actions like breeding and feeding will be essential to secure a future sustainable pig industry in European countries facing a possible ban on castration.

Rapid detection methods

Recent research: 2 methodological strategies:

Fingerprinting techniques *(gas and solid phase)*
Principle: indirect measurement of boar odour with non-specific methods

- *Chemical sensor arrays (e-noses)*
- *HS-Mass Spectrometry (MS)*
- *Spectroscopic*

Substance specific techniques *(gas and solid phase)*
Principle: single boar odour substances are measured specifically

- *Spectrophotometry*
- *Fast Gas Chromatography (GC)*
- *Gas phase spectrometry*
- *Biosensing*

Norwegian boar detection project (2004-2008)

3 methods under development

- Fat extraction combined with fast gas-chromatography
- FTIR Photoacustic (PA) gas-sensing (”electronic nose”)
- Biosensing using insects

Requirements (Norwegian sl.house conditions):

- 1 method - both skatole and androstenone
- Total analysis time <1hr
- Simple to operate
- Cheap i.e 2 - 4 Euro pr. sample

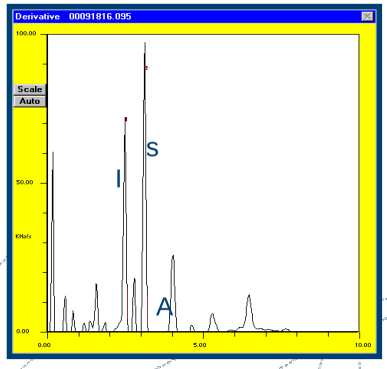
Fast gas chromatography

Fat extraction - SPE

Skatole, Indole and Androstenone can be isolated by one SPE step without sign. loss (85-100 % recovery)

SPE procedure can be performed automatically by using a commercial SPE robot

Sensitivity ~0.1-0.5 ppm

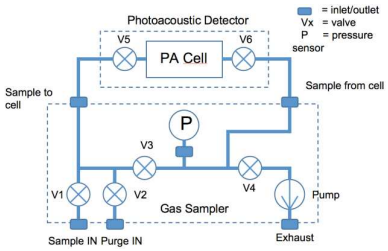


Fast GC 10 sec to analyse S,I and A (stds.)

FTIR-PA gas-sensing

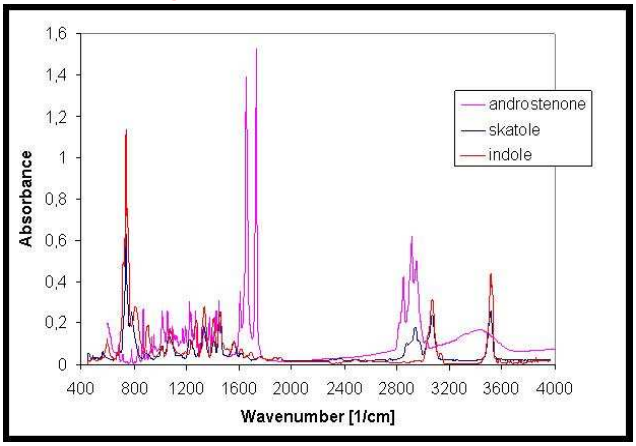
Measurement principle:

S,I og A in gas-phase absorbs IR light that causes an I ncrease in pressure in the photo acustic cell and a pressure interferogram is recorded. Pressure increase corresponds to concentration of boar substances in the vapour phase.



Skatole,Indole and Androstenone show distinguishable IR spectra in the gas-phase

FTIR- gas-phase spectra S, I, A



FTIR-PAS spectrum is highly linear, and therefore, water can be subtracted from the sample gas spectrum allowing detection of the Androstenone concentration

Nofima

Biosensing – using trained insects

Material

Bees: *Apis Melliferra*

Wasps: *Microplitis croceipes*

Test odours: Skatole, Indole and Androstenone

Training

Classical conditioning (associative learning). Solution of either androstenone, indole of skatole dissolved or mixed (1:1:1) in hexane at 0.001 – 20 mg/ml.

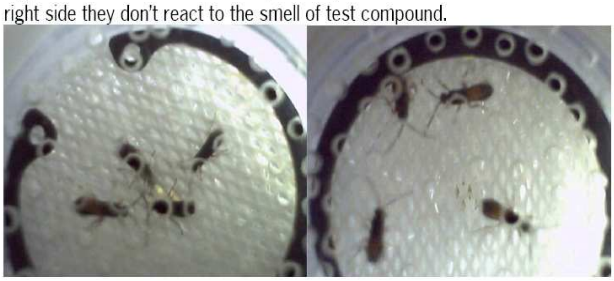
Reward/unconditioned stimulus: sucrose solution

Response

Bees: Proboscis (tongue) Extension Reflex (PER)

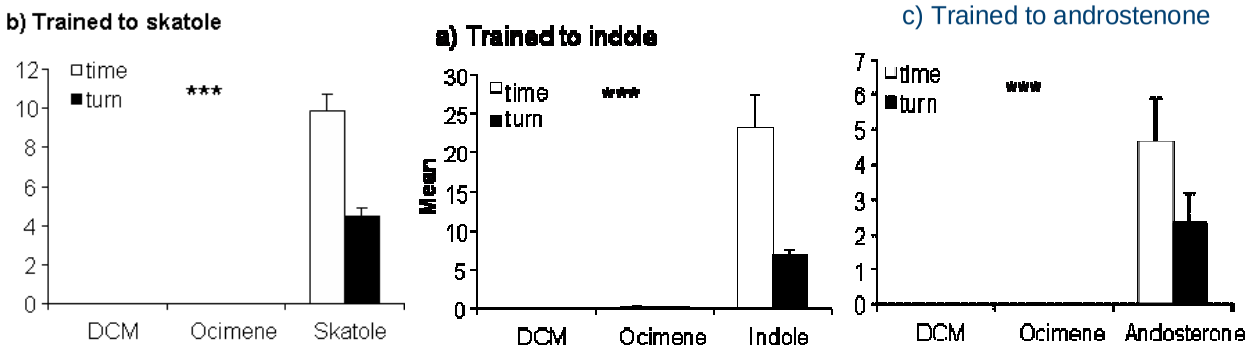
Wasps: "Head-banging"

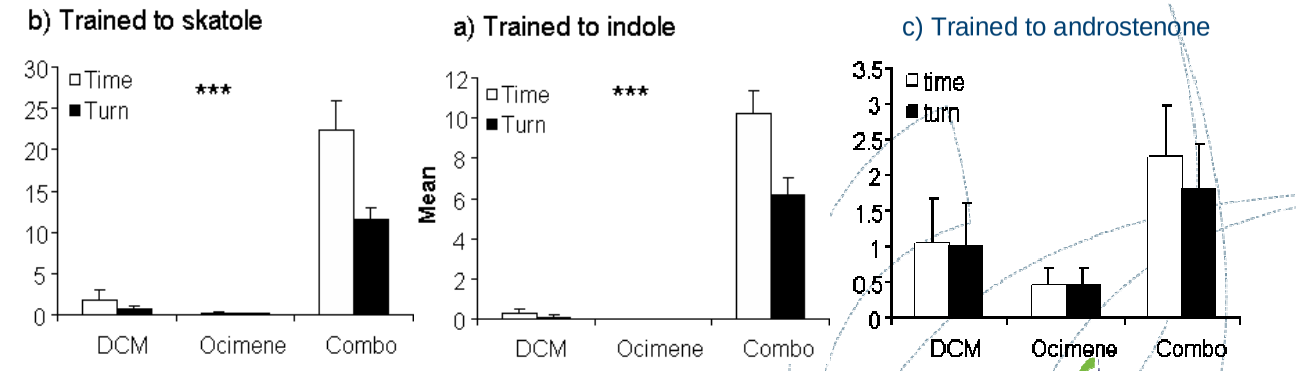
Figure 5: At the left side the wasps react positive to the smell of the test compo right side they don't react to the smell of test compound.



Nofima

Biosensing – results individual wasps





Wasp biosensing results

Classification rates of trained individual wasps (*mixtures)

Compound	Cl. Rate % (n)	FP %	FN %	Cl. Rate* % (n)	FP* %	FN* %
Indole	96 (25)	4(Oc)	4(I)	95 (20)	5	-
Skatole	95 (21)	-	5(S)	100 (20)	20	-
Androstenone	50 (20)	-	50(A)	40 (20)	30	60(A)

Results from wasp cohorts (6-8 ind) - higher class. rates > 80 %

EU project ALCASDE-SANCO/2008/D5/018

"Study on the improved methods for animal-friendly production, in particular on the **alternatives to the castration** of pigs and on alternatives to the dehorning of cattle"

WP1.2: Methods to detect boar taint at the slaughter line

- To develop sensors for simultaneous (or parallel) on-line skatole and androstenone detection in solid phase (fat) (J.Hart, O.Doran, UWE)
- **To develop methodology for on-line detection of boar taint compounds in gas phase (J.E.Haugen, Nofima)**
- To harmonise existing reference methodology for androstenone, skatole and indole analyses (S. Ampuero, AGROSCOPE)
- Interlaboratory comparison A/S reference methods
- To organise an international industry-orientated workshop (J.E.Haugen, S.Ampuero)

State of the art – rapid detection methods

Fingerprinting techniques

- Non-specific/indirect method
- Too high percentage of false negatives (5–20 %)
- Require calibration with sensory assessment or S, A ref. methods
- No standardised sampling system (solid/gas phase)
- No dedicated system for boar taint application

Substance specific techniques

- Specific – skatole and androstenone detection
- Skatole-equivalent method (colorimetric) lab method (rapid?)
- No androstenone method
- No dedicated system for boar taint application

Gaps and weaknesess in technology/knowledge

- Most of the potential rapid methods represent advanced and sophisticated technology that would require highly qualified staff to operate
- Few methods under development have short enough analysis times - sampling is the time consuming part of the analysis
- Methods are too costly - cost efficiency is the driver for industrial implementation of new measurement technology
- Fingerprinting based methods have too high percentage of false negatives (5–20 %)
- Still no dedicated measurement technology available for on/at-line detection of boar tainted carcasses that measures both androstenone and the indoles or boar taint
- Lab methods are available for both S and A (costs)

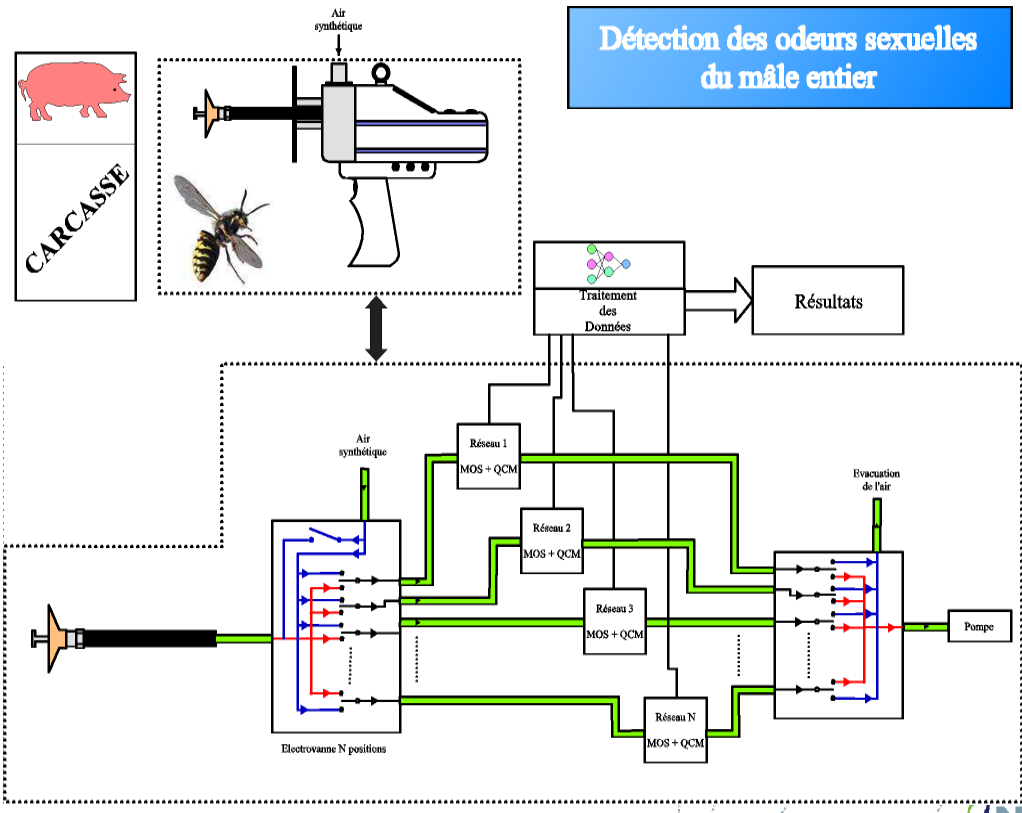
Needs and future research

- Need for standardised and harmonised S and A methodology
- Definition of sound sensory boar taint sorting criteria
- Need for rapid on/at-line methods for sorting boar carcasses
- Cost effective automated simple technological solutions in order to adapt a proper methodology to slaughter house conditions
- Sampling solutions that meet the industrial requirements for on-line/at-line use
- One detection method that measures both androstenone and indole or boar taint
- Method with sufficiently high analysis capacity (500-5000 carc./hr)

Collaboration partners

***Wäckers F.**, Lancaster University, UK*
***Olson, D.** , USDA AR, USA*
***Kauppinen, I,** Gasera Ltd, Finland*
***Ferber, A. ,** SINTEF ECY, Norway*
***de Wiel, D.,** WUR, The Netherlands*
***Lundström,** K., Univ. Agric. Sci., Sweden*
***Whittington, F.,** Univ. Bristol, UK*
***Briens, M.,** Inscentinel Ltd, UK*
***Tuyttens, F.**, ILVO, Belgium*
***Animalia,** Norway*
***Norsvin,** Norway*
***Lundby, F.** Nofima AS, Norway*

The sex-pistol-pistol (Concept by Alpha MOS, Toulouse)



Thank you for your attention!