Kmetijski inštitut Slovenije
Agricultural Institute of Slovenia

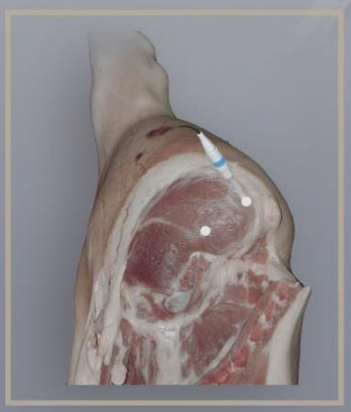
Univerza v Ljubljani
Veterinarska fakulteta

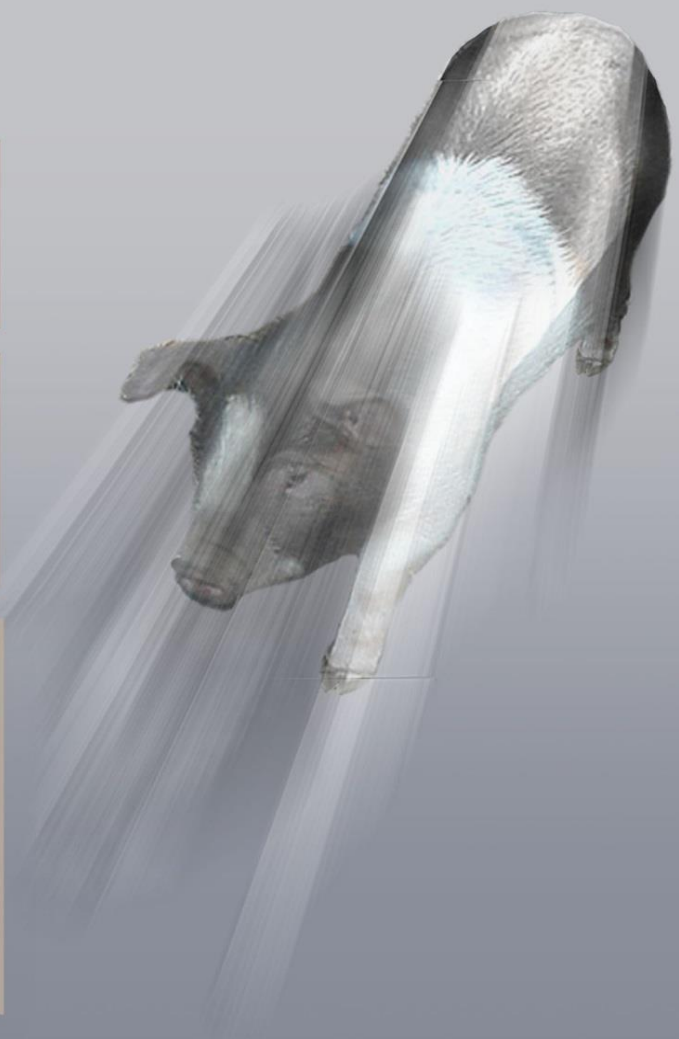
TRAINING SCHOOL

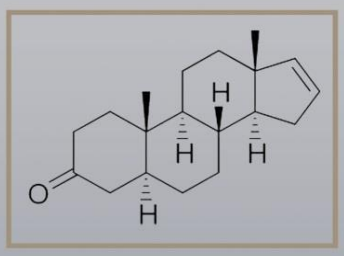
Harmonisation of methods in entire male and immunocastrate research

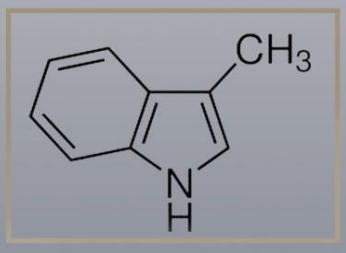
Ljubljana, November 20-22, 2017

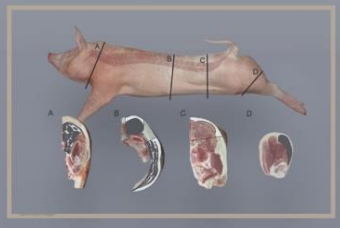
Lectures (handouts)











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EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

IPEMA

CA 15215: Innovative Approaches for Pork Production with Entire Males

COST is supported by the EU Framework Programme Horizon 2020

PROJECT: Sustainability in pork production with immunocastration (SuSI)
ERA-NET SUSAN

Kmetijski inštitut Slovenije
Agricultural Institute of Slovenia

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Veterinarska fakulteta

HARMONISATION OF METHODS IN ENTIRE MALE AND IMMUNOCASTRATE RESEARCH

Lectures of the Training school (Ljubljana, November 20-22, 2017)

Ljubljana 2017

Edited and published by:

KMETIJSKI INŠTITUT SLOVENIJE
Agricultural Institute of Slovenia
Hacquetova ulica 17, 1000 Ljubljana, SLOVENIA
Tel.: +386 1 / 28 05 262

Edited by: dr. Marjeta ČANDEK-POTOKAR

Series editor: Lili MARINČEK

Aquarelle on the cover painted by: mag. Blaž ŠEGULA

Publikacija bo izšla v elektronski obliki in bo objavljena na spletni strani Kmetijskega inštituta Slovenije http://www.kis.si

Kataložni zapis o publikaciji (CIP) pripravili v Narodni in univerzitetni knjižnici v Ljubljani
COBISS.SI-ID=293181696
ISBN 978-961-6998-17-8 (pdf)

FOREWORD

IPEMA – Innovative Approaches for Pork Production with Entire Males is a COST action (CA 15215) supported by the European Union within the framework programme Horizon 2020. Its main objective is to bring together the scientists and practitioners interested in the challenges that pork production sector is facing due to the ending of the practice of surgical castration of male piglets and who are conducting the research or evaluation of two most viable alternatives a) raising of entire males, and b) immunocastration.

Present publication results from the Training school on Harmonisation of methods in entire male and immunocastrate research organised in the frame of IPEMA, which had for the objective the presentation of wide spectre of research methods relevant for addressing the questions of interest or research hypotheses in studies of entire male and immunocastrate production. The emphasis was on the harmonisation of methodological approach in joint research projects. The goal of the training school was to help trainees understand which are the knowledge gaps in the respective research area and which methods to use (in harmonised way) to answer the research questions. The training school was conducted in collaboration with H2020 ERA-NET project SuSI (Sustainability of pig production with immunocastration) which represented or served as a case study of a joint research project. The methodology presented covered analytical procedures of boar taint substances, methods for on-line detection of boar taint, muscle and fat tissue analysis, welfare, behaviour, endocrine parameters to assess testicular function, body composition, carcass and meat quality, anatomy of reproductive tract, gastric ulcers, nutritional and environmental aspects, demonstrating multidisciplinary approach in searching of new knowledge and solutions for challenges in pork production with immunocastration.

The training school was held from 20th to 22nd November. It was attended by 27 trainees from 15 countries, 18 of them were from Target Inclusiveness Countries (Croatia, Czech Republic, Estonia, Portugal, Slovakia, Slovenia, FYR Macedonia, Serbia). The lectures given during the training school are compiled in the present handbook to serve as basic information and guidelines on methodological aspects.

Ulrike Weiler, IPEMA Chair

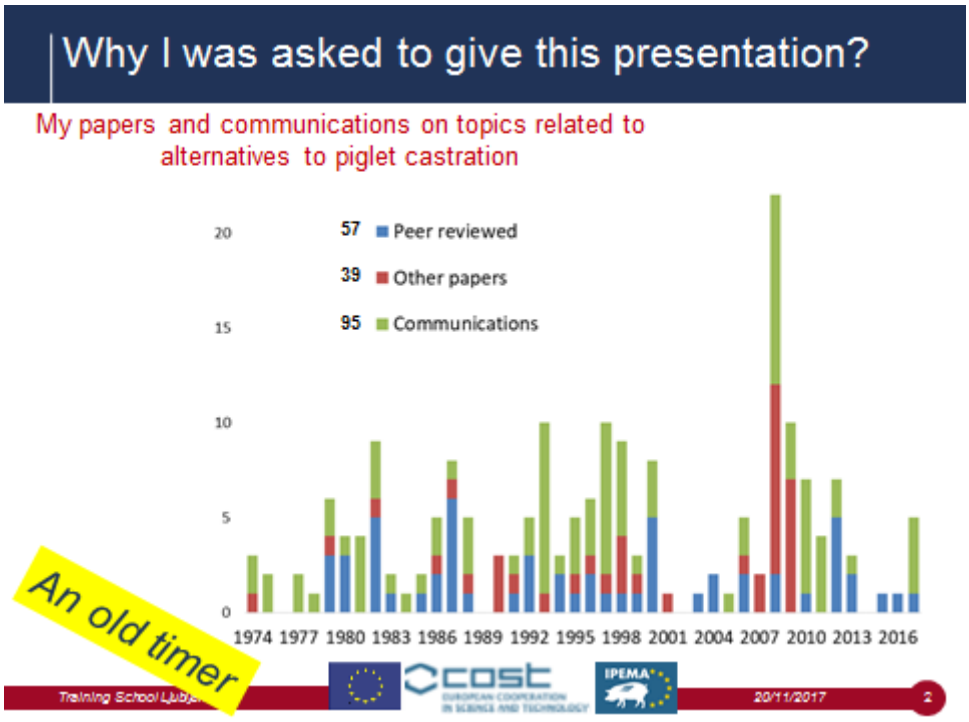
Marjeta Čandek-Potokar, editor

CONTENTS

Chapter 1

Pitfalls and problems in boar taint research

Michel BONNEAU[1]

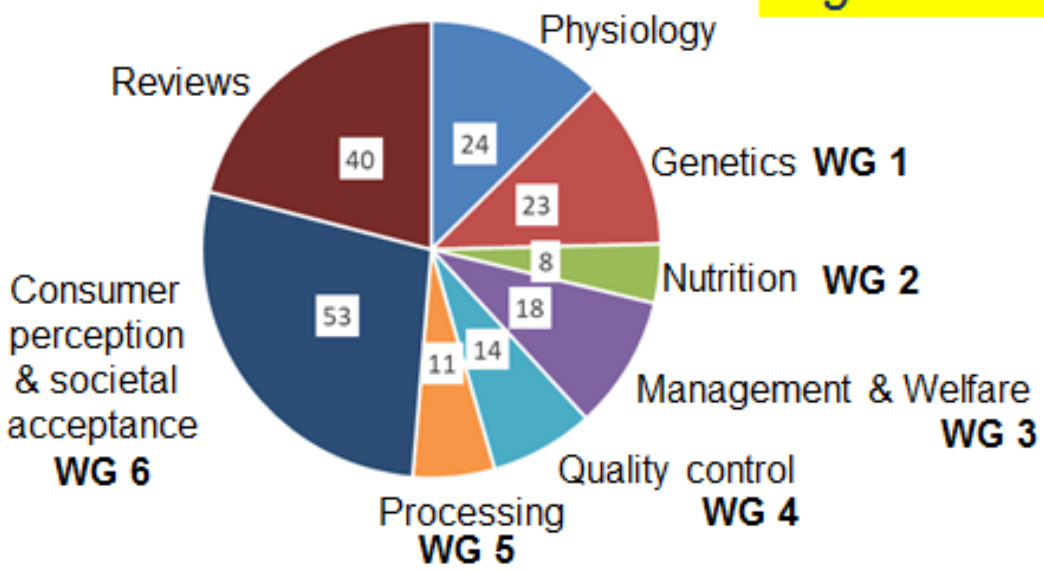


[1] The French Pork and Pig Institute (IFIP), La Motte au Vicomte, 35650 Le Rheu, France

Why I was asked to give this presentation?

My papers and communications on topics related to alternatives to piglet castration

A generalist



Problems and pitfalls in boar taint research

- What is boar taint?
  - Compounds responsible for boar taint
  - The gold reference for boar taint
  - Measurement of consumer dissatisfaction
    - The importance of sample preparation
  - The need for boar taint indicators
    - Test panel evaluation
    - Measurement of boar taint compounds
  - Thresholds for boar taint?
    - Boar Taint = f(skatole,androstenone)
- Genetic control of boar taint compounds
- Boar taint detection: How to measure accuracy?
- Chain approaches
  - To manage boar taint
  - To manage entire male production

What is boar taint ?

- Un unpleasant odour / flavour

  There are many unpleasant odours / flavours in pork meat (Fish, Rancid, Boar taint, ….)

- … that is specific of entire male pigs

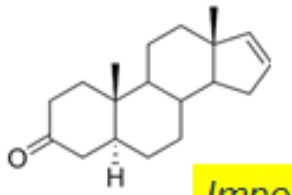
To be held as responsible for boar taint a compound must be

1. Perceived as unpleasant by at least a fraction of the consumers
2. Present at concentrations above perception level in pork from at least some entire males
3. Absent or below perception level in pork from castrates and gilts

All 3 conditions must be met

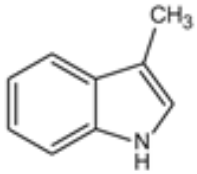
Compounds responsible for boar taint

As of today only 2 compounds were demonstrated to meet all 3 conditions



Patterson RLS 1968. 5α-androst-16-ene-3-one: Compound responsible for taint in boar fat. Journal of the Science of Food and Agriculture 19, 31–38.

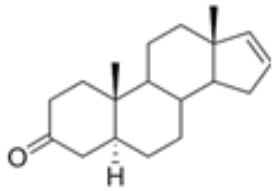
Important findings may be hidden in obscure journals



Vold E 1970. Fleischproduktionseigenschaften bei Ebern und Kastraten IV: Organoleptische und gaschromatographische Untersuchungen wasserdampfflüchtiger Stoffe des Rückenspeckes von Ebern. Meldinger fra Norges Landbrugshøgskole 49, 1–25.

Walstra P, Maarse G 1970. Onderzoek gestachlengen van mannelijke mestvarkens. IVO-rapport C-147, Rapport 2. Researchgroep voor Vlees en Vleeswaren , TNO, Zeist, The Netherlands.

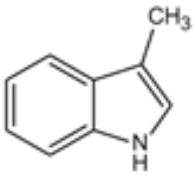
Compounds responsible for boar taint



- Testicular steroid
- Related compounds (androstenols) act as pheromones

There are obvious reasons for androstenone to be specific of entire males

For a long time I did not recognise the importance of skatole for boar taint



- Synthesized in the large intestine
- From the breakdown of tryptophan

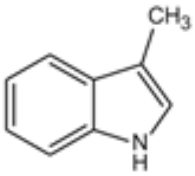
There was no obvious reason for skatole to be specific of entire males

Compounds responsible for boar taint

- *Rely on facts and evidence.*
- *Do not expect reality to fit one's own vision.*
- *One's vision must fit reality*

Results accumulated, demonstrating the importance of skatole as malodorous compound in pork fat and its specificity in entire males

Still I was not fully satisfied until two articles gave the explanation why skatole was specific of entire males



Claus R, Raab S and Röckle S 1996. Skatole concentrations in blood plasma of pigs as influenced by the effects of dietary factors on gut mucosa proliferation. Journal of Animal Physiology and Animal Nutrition 76, 170–179.

Doran E, Whittington FW, Wood JD, McGivan JD 2002b. Cytochrome P450IIE1 (*CYP2E1*) is induced by skatole and this induction is blocked by androstenone in isolated pig hepatocytes. Chemico-Biological Interactions 140, 81–92

Problems and pitfalls in boar taint research

- What is boar taint?
  - Compounds responsible for boar taint
  - The gold reference for boar taint
  - Measurement of consumer dissatisfaction
    - The importance of sample preparation
  - The need for boar taint indicators
    - Test panel evaluation
    - Measurement of boar taint compounds
  - Thresholds for boar taint?
    - Boar Taint = f(skatole,androstenone)
- Genetic control of boar taint compounds
- Boar taint detection: How to measure accuracy?
- Chain approaches
  - To manage boar taint
  - To manage entire male production

The gold reference for boar taint

- From an industry perspective, the gold reference for boar taint is consumer dissatisfaction caused by odour and or flavour
- Boar taint is the difference (if positive) in consumer dissatisfaction between pork from entire males and pork from control animals (females or castrates)

Boar taint = Differential Consumer Dissatisfaction with odour / flavour

Problems and pitfalls in boar taint research

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Measurement of consumer dissatisfaction

- What can be assessed by consumers?
  - Hedonic dimension (pleasant /unpleasant): dissatisfaction
  - ~~Presence/absence and intensity of an odour/flavour~~
- Many consumers are needed
  - >= 100 consumers for each product
  - No selection (*unless specific subpopulation is addressed), no training
- Monadic or paired comparison?
  - Paired comparison with a control because one wants to measure differential consumer dissatisfaction
- Sample preparation and serving conditions

Critically important

An illustration of the importance of sample preparation in consumer studies

Meat Science 54 (2000) 251–259

An international study on the importance of androstenone and skatole for boar taint: I. Presentation of the programme and measurement of boar taint compounds with different analytical procedures

M. Bonneau [a,*], A.J. Kempster [b], R. Claus [c], C. Claudi-Magnussen [d], A. Diestre [e], E. Tornberg [f], P. Walstra [g], P. Chevillon [h], U. Weiler [c], G.L. Cook [b]

An illustration of the importance of sample preparation in consumer studies

Abstract

An international study, involving 11 participants in 7 European countries, was conducted to provide scientific evidence for an objective measurement of boar taint in entire male pigs and its possible variation between countries. The specific objectives were to determine the respective contributions of androstenone and skatole to boar taint and their possible variations according to production systems and consumer populations. Over 4000 entire male pigs and 200 gilts were raised and slaughtered in 6 countries. Meat samples were taken from the loin and backfat samples were used for the rapid measurement of androstenone and skatole. A sub-population of 377 entire males and 42 gilts was then selected in such a way as to represent all combinations of skatole and androstenone levels. Androstenone and skatole levels in the selected samples were checked, using established reference methods. Meat samples from the selected animals were used for sensory evaluation by trained panels and for consumer surveys in 7 European countries.

An illustration of the importance of sample preparation in consumer studies

Meat Science 54 (2000) 271–283

An international study on the importance of androstenone and skatole for boar taint: III. Consumer survey in seven European countries

K.R. Matthews [a], D.B. Homer [a], P. Punter [b], M.-P. Béague [c], M. Gispert [d], A.J. Kempster [e], H. Agerhem [f], C. Claudi-Magnussen [g], K. Fischer [h], F. Siret [c], H. Leask [a], M. Font i Furnols [d], M. Bonneau [i,*]

An illustration of the importance of sample preparation in consumer studies

Materials and methods

Joints were roasted in an oven at 180°C to an internal temperature of 75°C. Fifteen millimetre slices of m. Longissimus thoracis et lumborum with 5 mm overlying subcutaneous fat were prepared. The ends of the m. Longissimus thoracis et lumborum were trimmed from each slice and the centre cut into 4 pieces which were placed in sealed aluminium foil containers. These were held chilled at 4°C prior to use […]. On the day of the test the samples for flavour evaluation were heated in their sealed containers for about 10 min in an oven at 180°C to achieve an internal temperature of 80°C. Samples for odour were heated to 95°C immediately prior to consumer testing.

An illustration of the importance of sample preparation in consumer studies

Table 2
Consumer scores for samples from entire male pigs and gilts

	Number of samples	Rating						
		Like very much	Like a lot	Like	Neither like nor dislike	Dislike	Dislike a lot	Dislike very much
(b) Distribution of odour scores (%)								
Gilts								
Overall	1529	5	15	28	24	18	7	3
Entire males								
Overall	13891	4	13	24	25	21	9	4

28

34

An illustration of the importance of sample preparation in consumer studies

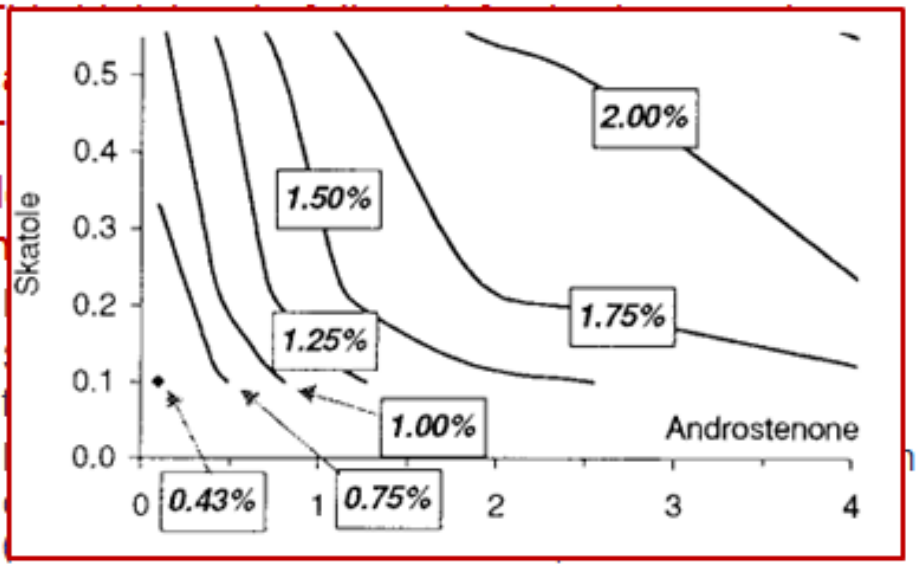
Results and discussion

It is, however, striking that such a high percentage of samples were disliked, particularly as consumers who said that they did not eat pork were excluded from the sample. It is possible that the samples were generally disliked due to their unusual presentation in comparison with the normal consumption situation. Additionally, the re-heating and chilled storage of samples may have resulted in the development of warmed over flavour which may have been exaggerated by the high serving temperature.

An illustration of the importance of sample preparation in consumer studies

- This high level of dissatisfaction in control meat casted doubt on the results of the study
- This was a pity because the study actually demonstrated very important knowledge at an international level
  - Dissatisfaction higher for Odour than for Flavour
  - Skatole contribution > Androstenone contribution for the whole population of consumers
  - High contribution of Androstenone in the subpopulation of consumers that are sensitive to androstenone
    (Weiler et al. Meat Science, 54, 297-304).

An illustration of the importance of sample preparation in consumer studies



- Better prediction of differential consumer dissatisfaction with an index = f(Androstenone, Skatole) than with thresholds

Measurement of consumer dissatisfaction

- What
  - Hedor
  - Prese
- Many
  - >= 10
  - No se
- Monad
  - Paire
    meas

- Many parameters to be considered
  - Which sample?
  - % fat in the sample
  - Presence / absence of other ingredients
  - Cooking method (oven / grill / frying pan / ...)
  - Cooking temperature
  - Cooking time
  - Container (open / closed)
  - Time delay between cooking and serving
  - .....

- **Sample preparation and serving conditions**
  - Depend on whether odour or flavour are assessed
  - Many possible mistakes

Measurement of consumer dissatisfaction

*Methods for the assessment of boar taint–related consumer dissatisfaction need to be **harmonized***

Meat Science 92 (2012) 319–329

Contents lists available at SciVerse ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Review

Consumer studies on sensory acceptability of boar taint: A review

Maria Font-i-Furnols

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  - To manage boar taint
  - To manage entire male production

The need for boar taint indicators

Boar taint = Differential Consumer Dissatisfaction with odour / flavour

- The measurement of differential consumer dissatisfaction
  - Is difficult, tedious and costly
  - Can be performed only on a limited number of samples

The need for boar taint indicators

- The measurement of differential consumer dissatisfaction cannot be used
  - For most of the research
    - Measuring the incidence of boar taint in a population
    - Genetic evaluation of and selection on boar taint
    - The effect of nutritional and environmental factors on boar taint
    - Measurement of the accuracy of a boar taint detection method
    - The effect of processing on boar taint
    - .....
  - For industry needs
- Intermediate indicators of boar taint are needed

Two families of indicators for boar taint

- Test panel evaluation
- Measurement of boar taint compounds

Test panel evaluations

- What can be assessed with a test panel?
  - ~~Hedonic dimension (pleasant /unpleasant)~~
  - Presence/absence and intensity of an odour/flavour
- Panel members
  - Selection (sensitivity to androstenone/ skatole)
  - Training
- Monadic or paired comparison?
  - Because boar taint is entire male specific, paired comparison with a control should be preferred but good panels can work monadically
- Sample preparation and serving conditions
  - As critical as for consumer surveys

Test panel evaluations

- What can be assessed with a test panel?
  - ~~Hedonic dimension (pleasant /unpleasant)~~
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- Sa nple preparation and serving conditions
  - As critical as for consumer surveys

Harmonization of methods for the assessment of boar taint by test panels would be most useful

Measurement of boar taint compounds

Meat Science 90 (2012) 9–19

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Review

Review of analytical methods to measure boar taint compounds in porcine adipose tissue: The need for harmonised methods

J.-E. Haugen [a,*], C. Brunius [b], G. Zamaratskaia [b]

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[b] Department of Food Science, BioCenter, Swedish University of Agricultural Sciences, 750 07, Uppsala, Sweden

ARTICLE INFO

Article history:
Received 23 April 2011
Received in revised form 6 July 2011
Accepted 8 July 2011

Keywords:
Androstenone
Skatole

ABSTRACT

This comprehensive review describes the analytical methods developed for quantification of the boar taint compounds skatole and androstenone in porcine adipose tissue. The following parts are considered: sampling, sample preparation, calibration and instrumentation. Additionally, method performance characteristics and level of validation of the existing methodology are discussed. It is concluded that there is a need for further validation of existing methods and need for standardisation of methodology to quantify boar taint compounds. Facing a possible near future ban of castration of male piglets would enforce further method harmonisation in this field.

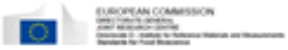
Measurement of boar taint compounds

- Until recently the measurement of androstenone and skatole was a real mess
  - Results differed widely between methods
  - Results were not expressed in the same way
    - / g fresh tissue
    - / g lipids

Haugen, J. E. (2009). The ALCASDE interlaboratory comparison study. In J. E. Haugen (Ed.), Report of the EAAP/ALCASDE boar taint detection workshop, Bologna, 28th October 2009 (pp. 38–40).

- Harmonisation of methods was badly needed
- Recent critical progress has been made
  - Consensus on expression of results / g lipids
  - Reference method now available

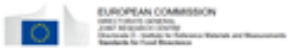
Reference method for the measurement of boar taint compounds



In house validation of a reference method for the determination of boar taint compounds by LC-MSMS

DOI: 10.2787/88600

http://publications.jrc.ec.europa.eu/repository/handle/JRC88197



Inter-laboratory validation of a reference method for the determination of boar taint compounds by GC-MS and LC-MSMS

DOI: 10.2787/96937

http://publications.jrc.ec.europa.eu/repository/handle/JRC91075

Methods used to measure androstenone and/or skatole levels should be compared to the reference method and the results of the comparison included in M&M section of the publications

Problems and pitfalls in boar taint research

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Thresholds for boar taint?

Can we relate differential consumer dissatisfaction to androstenone and skatole levels?

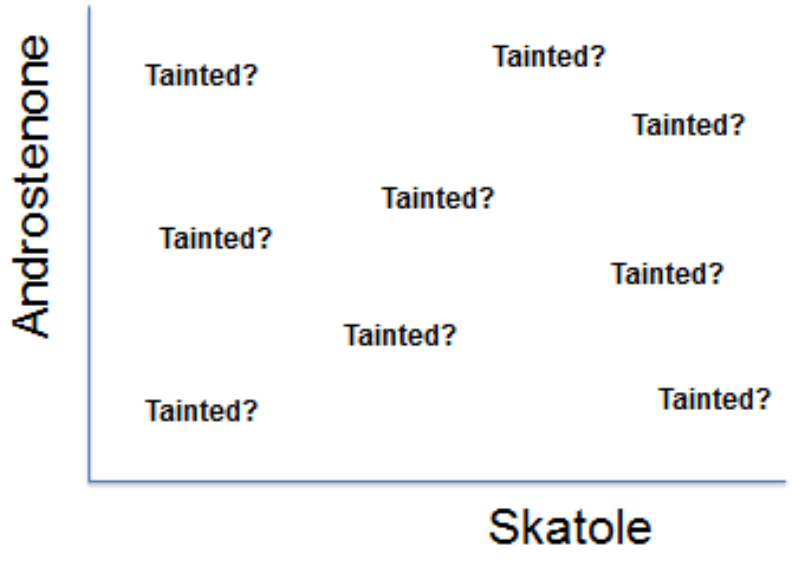
- Why is it important to do so?
  1. Test panel evaluations do not tell which compound is to blame for the presence of boar taint;
  2. Because the factors of variation of androstenone (mostly genetics) and skatole (nutrition, environment, genetics) are not the same, the actions to be taken to manage boar taint differ according to which compound is involved;
  3. Selection (particularly genomic selection), nutrition and environment control are much more efficient to address boar taint if directed on a given compound rather than on an olfactory assessment;

Thresholds for boar taint?

Can we relate differential consumer dissatisfaction to androstenone and skatole levels ?

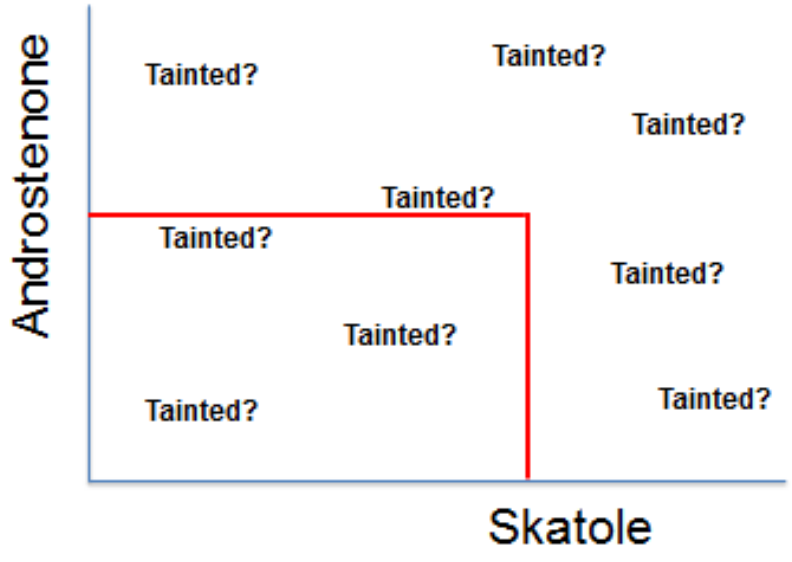
- Why is it important to do so? (continued)
  1.
  2.
  3.
  4. Instrumental methods for the detection of boar taint on the slaughter line are coming
     a. They are based on the measurement of androstenone and skatole levels
     b. Androstenone and skatole levels are useless for detection purpose unless we can relate them to differential consumer dissatisfaction

Thresholds for boar taint?



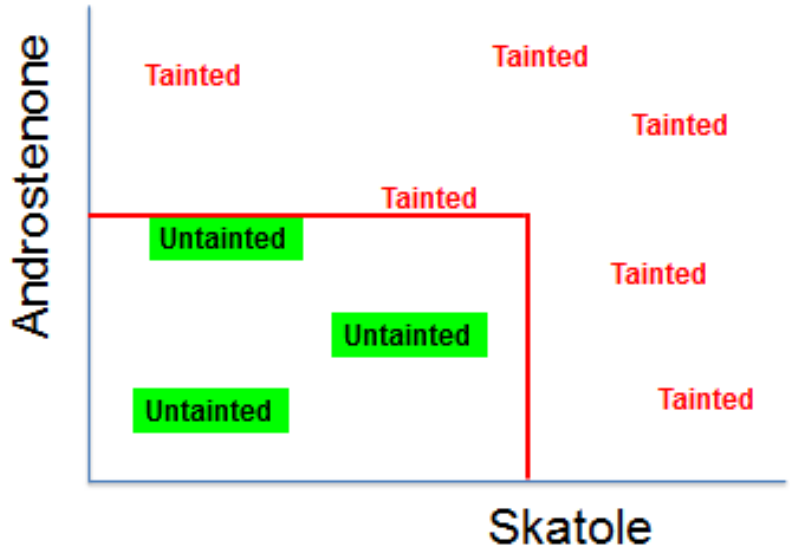
Thresholds for boar taint?

The classical approach with thresholds (cut-off) levels



Thresholds for boar taint?

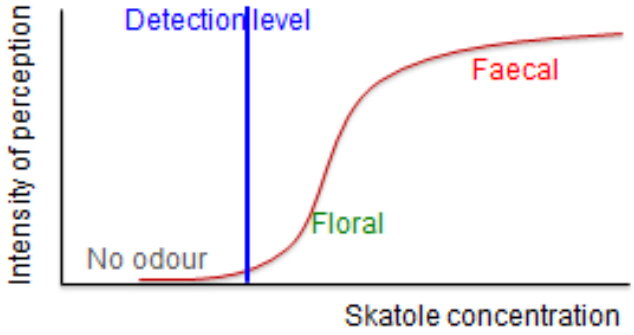
The classical approach with thresholds (cut-off) levels



Thresholds for boar taint?

- The threshold (cut-off) approach does not work because consumers differ from each other

1. Biological detection levels differ
2. Hedonic perception changes according to distance to detection level

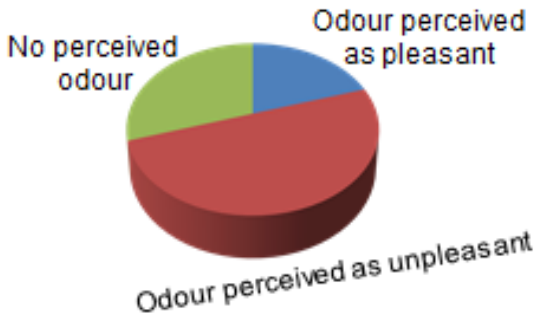


3. Hedonic perception is affected by culture and previous experience

Thresholds for boar taint?

- The threshold (cut-off) approach does not work because consumers differ from each other (continued)

1.
2.
3.
4. Some people are anosmic to androstenone
   Some perceive it as pleasant



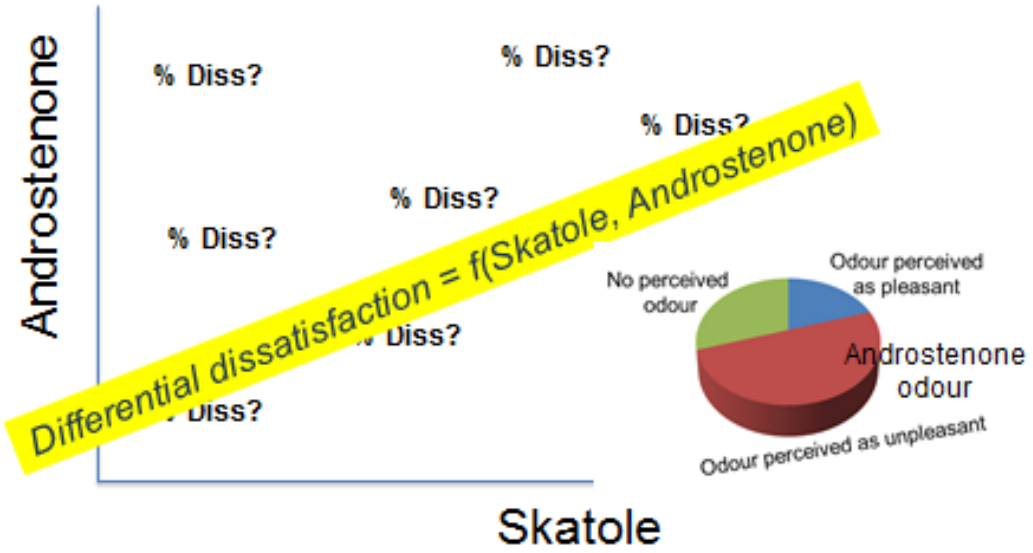
Thresholds for boar taint?

- The classical approach with threshold (cut-off) levels results in 2 populations of pork meat
  - The untainted ones
  - The tainted ones
- The reality is totally different
  - Some extreme consumers can detect very low levels
  - Other consumers cannot smell anything, whatever the levels are
  - Most consumers are somewhere in between

The probability of consumer dissatisfaction with odour / flavour increases with increasing levels of boar taint compounds

Boar Taint = f(Skatole,Androstenone)

The new approach proposed by CAMPIG



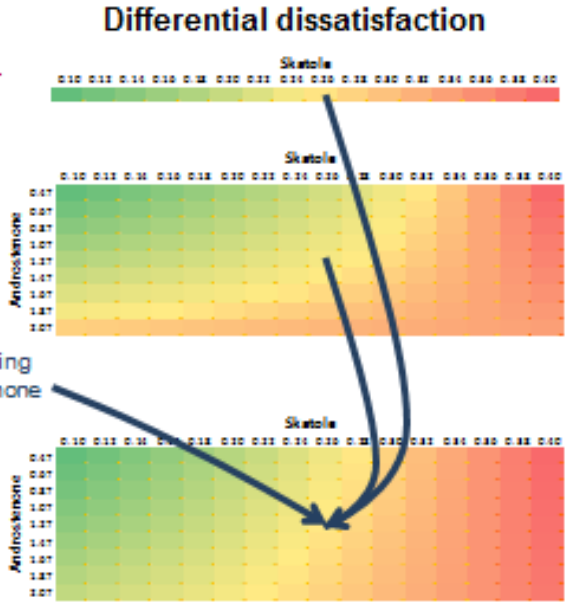
The approach proposed by CAMPIG

Consumers insensitive to androstenone or perceiving it as pleasant

Consumers sensitive to androstenone and perceiving it as unpleasant

Smell strip method
Meier-Dinkel et al., 2013. Meat Science, 94, 19-26

All consumers



The new approach proposed by CAMPIG

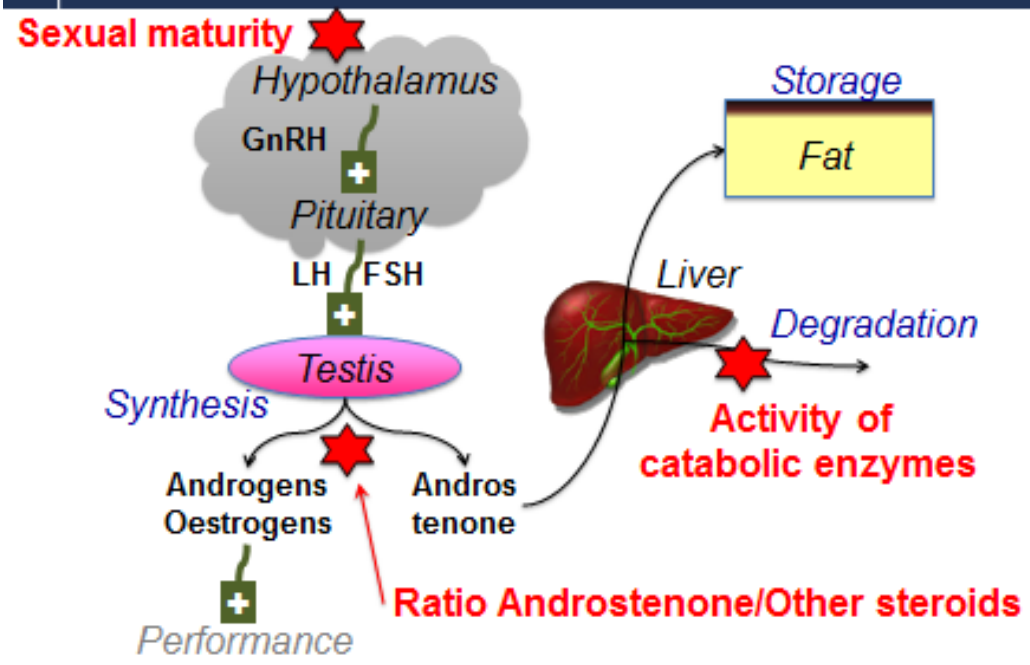
A remaining difficulty

- Boar taint perception depends on the product

Problems and pitfalls in boar taint research

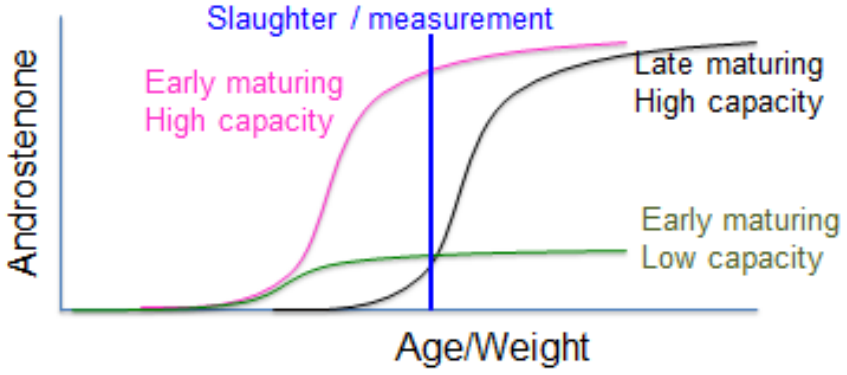
- What is boar taint?
  - Compounds responsible for boar taint
  - The gold reference for boar taint
  - Measurement of consumer dissatisfaction
    - The importance of sample preparation
  - The need for boar taint indicators
    - Test panel evaluation
    - Measurement of boar taint compounds
  - Thresholds for boar taint?
    - Boar Taint = f(skatole,androstenone)
- Genetic control of boar taint compounds
- Boar taint detection: How to measure accuracy?
- Chain approaches
  - To manage boar taint
  - To manage entire male production

Genetic control of Androstenone



Genetic control of Androstenone

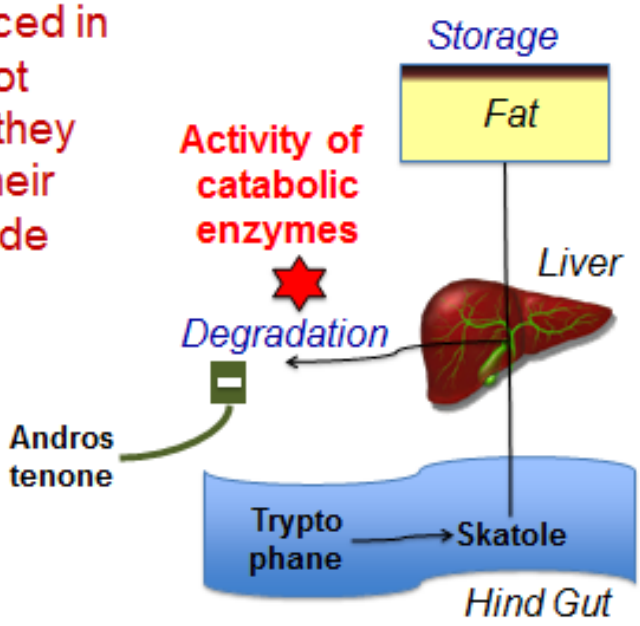
- Selection against androstenone results in negative side effects on reproductive performance



Sexual maturation must be taken into account when selecting against androstenone

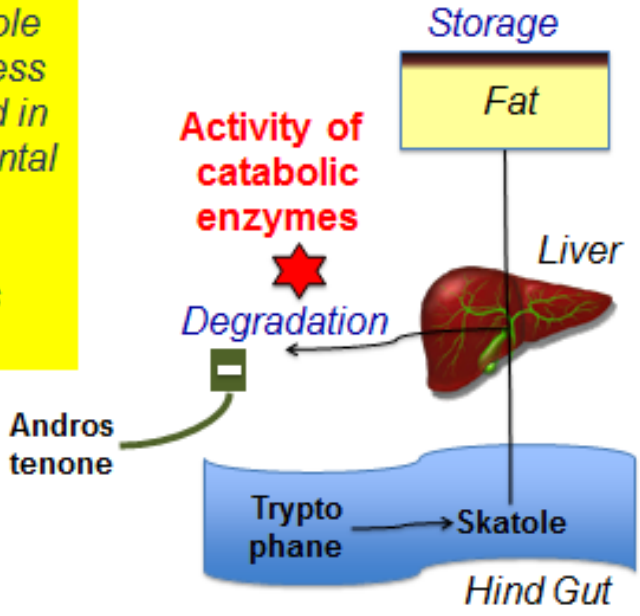
Genetic control of Skatole

- If the animals placed in test stations do not produce skatole, they cannot express their capacity to degrade skatole



Genetic control of Skatole

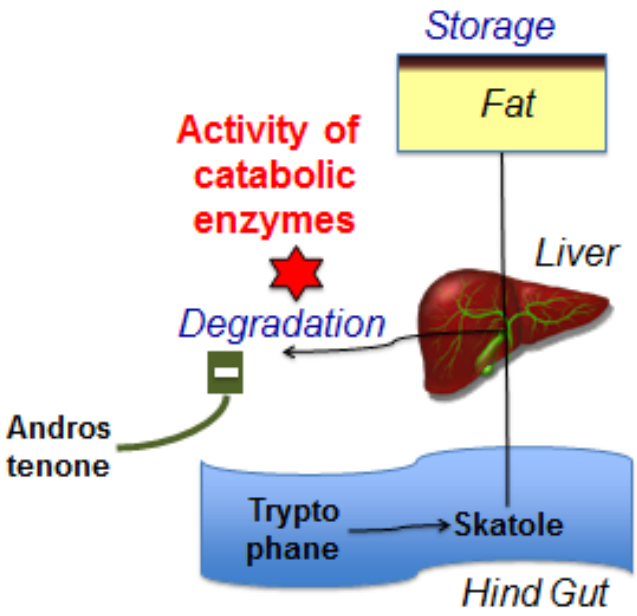
Selection against skatole cannot be efficient unless the animals are placed in nutritional / environmental conditions where they produce substantial amounts of skatole



Genetic control of Skatole

Selection against skatole cannot be efficient unless the animals are placed in nutritional / environmental conditions where they produce substantial amounts of skatole

The information on androstenone levels should be taken into account to evaluate the capacity of the animals to degrade skatole



Problems and pitfalls in boar taint research

- What is boar taint?
  - Compounds responsible for boar taint
  - The gold reference for boar taint
  - Measurement of consumer dissatisfaction
    - The importance of sample preparation
  - The need for boar taint indicators
    - Test panel evaluation
    - Measurement of boar taint compounds
  - Thresholds for boar taint?
    - Boar Taint = f(skatole,androstenone)
- Genetic control of boar taint compounds
- Boar taint detection: How to measure accuracy?
- Chain approaches
  - To manage boar taint
  - To manage entire male production

Boar taint detection: How to measure accuracy?


- Human nose methods
  - Currently in use in several countries
- Instrumental methods
  - After several decades of unsuccessful attempts, they are finally coming

- Are those methods accurate?
- None of the detection method has so far documented its accuracy in a satisfactory manner

Boar taint detection: How to measure accuracy?

- Accuracy is measured against a gold reference
- There is only one true gold reference
  - Differential consumer dissatisfaction with odour/flavour
- Problem:
  - Accuracy must be measured on a high number of samples
  - Consumer dissatisfaction can be measured, at a high cost, only on a very limited number of samples
- A way out of this dilemma?

Boar taint detection: How to measure accuracy?

- Yes, there is a way out of this dilemma
  - Use the detection method on a sufficient number of samples (typically 1 000)
  - Measure Androstenone and Skatole on the same samples, with a method that is sufficiently reliable compared to the reference method for measuring boar taint compounds
  - Calculate boar taint level in each sample, using the model: Differential dissatisfaction = f(Skatole,Androstenone)
  - Calculate accuracy in the classical way if thresholds are used

		Boar taint = f(skatole,androstenone)	
		Real untainted	Real tainted
Results of the detection method	Detected untainted	True negative	False negative
	Detected tainted	False positive	True positive

  - Calculate accuracy with correlations in the other cases

Boar taint detection: How to measure accuracy?

- A way out of this dilemma?
  - Use the detection metho… samples (typicall…
  - Measur… with a … referen… …ole on the same samples, …ciently reliable compared to the … for measuring boar taint compounds
  - Calculate boar taint level in each sample, using the model: Differential dissatisfaction = f(Skatole,Androstenone)
  - Calculate accuracy in the classical way if thresholds are used

Differential dissatisfaction = f(skatole,androstenone) The model is not available yet

		Boar taint = f(skatole,androstenone)	
		Real untainted	Real tainted
Results of the detection method	Detected untainted	True negative	False negative
	Detected tainted	False positive	True positive

  - Calculate accuracy with correlations in the other cases

Problems and pitfalls in boar taint research

- What is boar taint?
  - Compounds responsible for boar taint
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  - To manage boar taint
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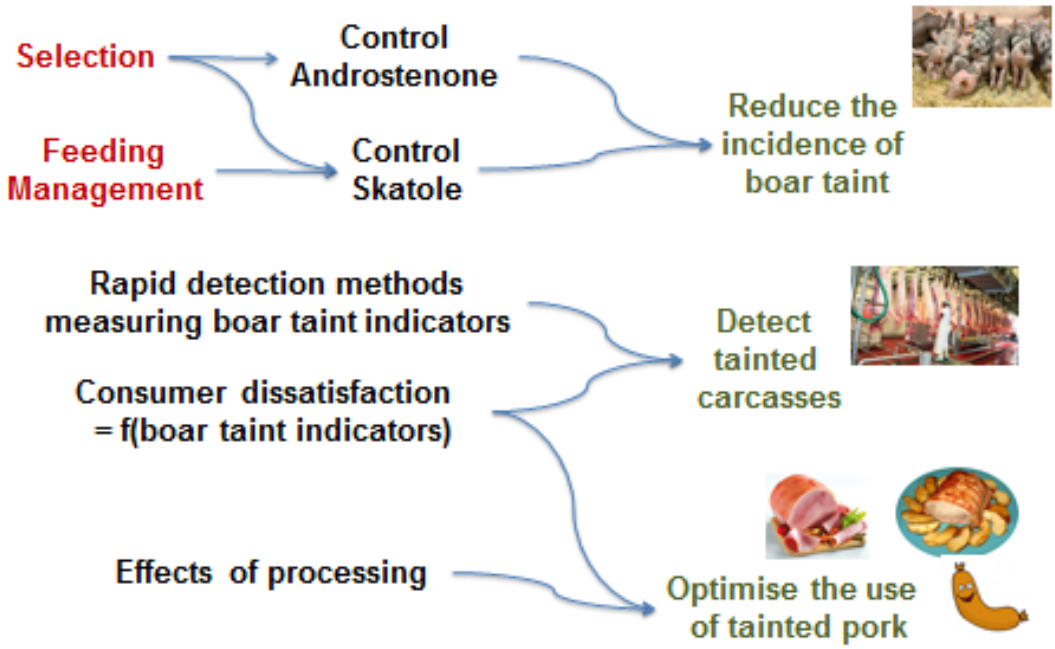
A chain approach to manage boar taint

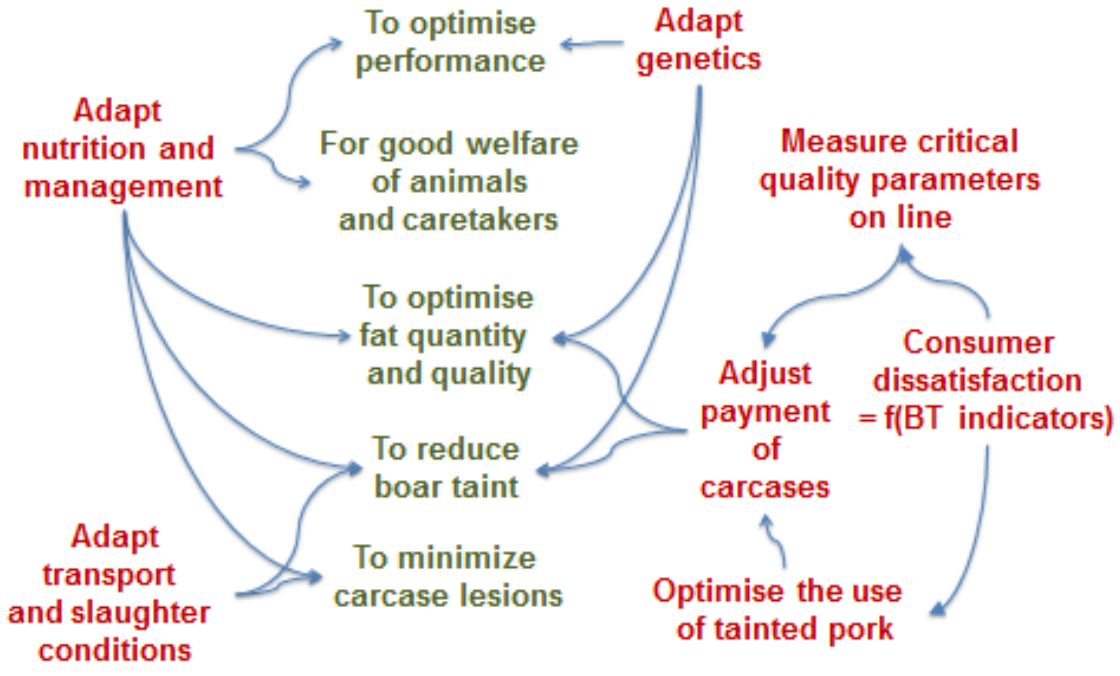


A chain approach to manage entire male production

Boar taint is not the only challenge facing entire male production

- Optimise performance
- Optimise the quantity and quality of fat
- Address the welfare concerns that are specific for entire males
- Measure all important quality parameters on/at the slaughter/processing lines
- Adapt processing to the different characteristics of entire male pork

A chain approach to manage entire male production



Thank you for your attention

The IPEMA consortium acknowledges the financial support of the EU, COST action CA15215.

Chapter 2

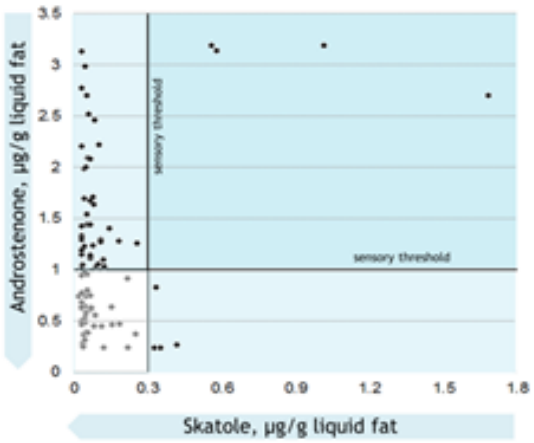
Boar taint compounds- analytical methods and sampling

Špela VELIKONJA BOLTA[1] and Nina BATOREK LUKAČ[1]

[1] Agricultural Institute of Slovenia (KIS), Hacquetova ulica 17, 1000 Ljubljana, Slovenia

Boar taint – sensory threshold levels (Walstra *et al.*, 1999)

- ANDROSTENONE : 0.5 – 1.0 µg/g liquid fat
- SKATOLE: 0.2 – 0.25 µg/g liquid fat

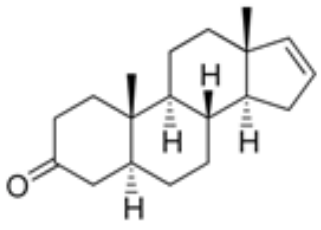


A and S concentration in backfat tissue of EM found in Slovenian studies (Batorek *et al.*, 2015)

Boar taint – analytical methods

Androstenone

Steroid compound



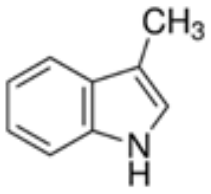
Non polar

M_r=272,214 (monoisotopic)

not soluble in water

Skatole

Indolic compound



More polar

M_r=131,18

Boar taint – analytical methods (Haugen et al, 2012, *Meat Science*)

- Sample preparation
- Extraction
  - steam distillation
  - liquid-liquid (methanol, hexane/2-propanol, dichloromethane, ethyl acetate, petroleum ether, Tris-acetone)
  - supercritical fluid extraction

  The use of internal standards to correct procedure errors!
- Clean-up

  SPE

  Bond-Elut 20H Diol

  Bond-Elut C18
- Saponification
- Derivatisation

Boar taint – analytical methods

Derivatisation

- Instrument dependent

Androstenone

- GC – ECD silylation or halogenation
- HPLC-FL derivatisation with dansyl hydrazine

Skatole

- Derivatisation with 4-dimethyl-aminobenzaldehyde (spectrophotometry)

Separation and detection

- GC-FID, NPD, ECD, MS LOD_a = 30 – 80 ng/g; LOD_s = 2 - 25 ng/g
- HPLC-UV, FL, MS (RP, isocratic elution, new columns with praticles <2 µm

  LOD_a = 125 – 200 ng/g; LOD_s = 4 - 50 ng/g

Boar taint – analytical methods

Mass spectrometry

- GC-MS SIM, androstenone
- LC-MS/MS both, APCI ion source!
- Headspace GC-MS
- Pyrolysis MS

Spectrophotometry

Immunological methods – ELISA - androstenone

Boar taint – JRC-IRMM ring test, 2014

Timing of the study

Initial stakeholder consultation	12.12.2012
Second stakeholder consultation	19.09.2013
Recruitment of participants	06.05.2013
Date of preparatory workshop	19.11.2013
Dispatch of samples	11.02.2014
Initial reporting dedline	17.03.2014
Reporting deadline extended on request of participants	15.04.2014

Provided by organiser
2 tissue samples
3 lard samples
Calibration check solution in toluene
Calibration check solution in methanol
SEC column
Deuterated internal standards

15 participants from 9 countries (DE, SI, BE, IT, DK, AT, SR, SP, FR)

Boar taint – analytical procedure JRC-IRMM- reference method (2014)

mill frozen sample

↓

liquefy in waterbath at 90 °C with the addition of Na_2SO_4 350 W

↓

centrifuge liquefied lipids for 5 min at 3220×g and 40°C

↓

transfer 1 ± 0.01 g water-free liquid fat in glass vial

↓

add 100 uL methanol of isotopically labelled internal standards and SEC eluent

(mix on vortex)

↓

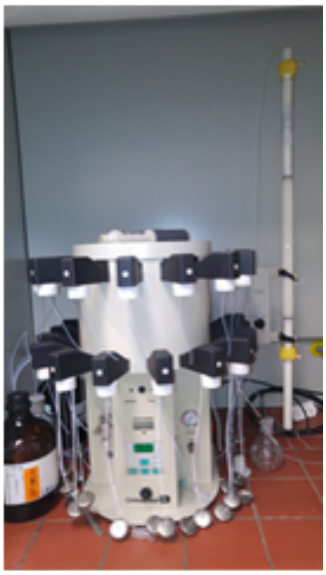
filter through 0,5 um PET syringe filter in a HPLC vial

↓

inject 750 uL, collect fraction between 25 and 37 min

↓

GC-MS LC-MS/MS



Boar taint – analytical procedure JRC-IRMM- continued (2013)

GC-MS

↓

add 100 uL of nonane

vacuum dry at 40 °C under nitrogen

dissolve in 300 uL toluenic IS

↓

GC conditions

HP 5 MS, 30 m * 0,25 mm, 0,25 µm film

Liner temperature 250 °C

Mobile phase: helium , 1 mL/min

LC-MS/MS

↓

add 100 uL of 1-octanol

vacuum dry at 40 °C under nitrogen

dissolve in 300 uL of methanolic IS

↓

HPLC conditions

Luna 150 x 4,6 mm, 5 um 100 A

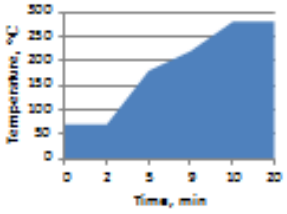
Flow 0,6 mL/min, V_{inj}=5 uL

Mobile phase: A: 0,1 % HCOOH

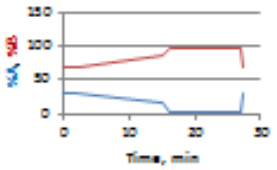
B 0,1% methanolic HCOOH

Compound	GC, SIM parameters	LC-MS/MS transitions	
indole	117, 90	118,0-65,1	118,0-91,0
indole-D7	123	124,0-95,9	
skatole	130, 103	132,1-117,0	132,1-89,1
Skatole-D3	132	135,1-117	
5-chloroindole	151	152,0-117	
Androstenone	272, 257	273,2,0-255,0	273,2-159,0
Androstenone- D4	276	277,2-259	

GC gradient



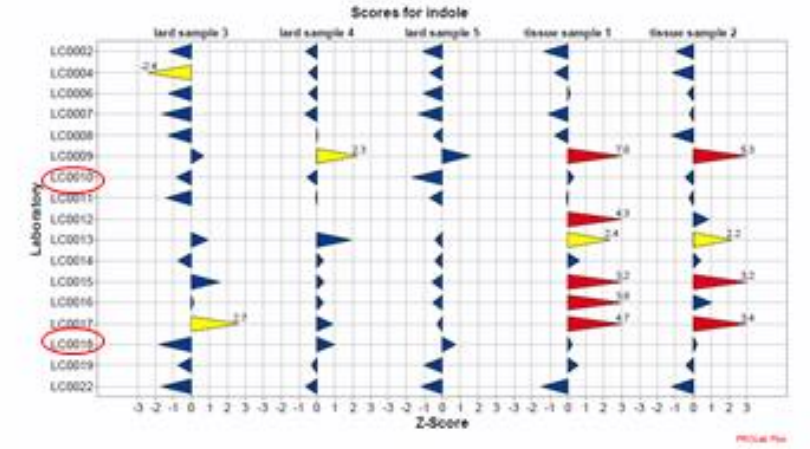
HPLC gradient



Boar taint – JRC-IRMM ring test - results

Figure 25: z-Scores for indole

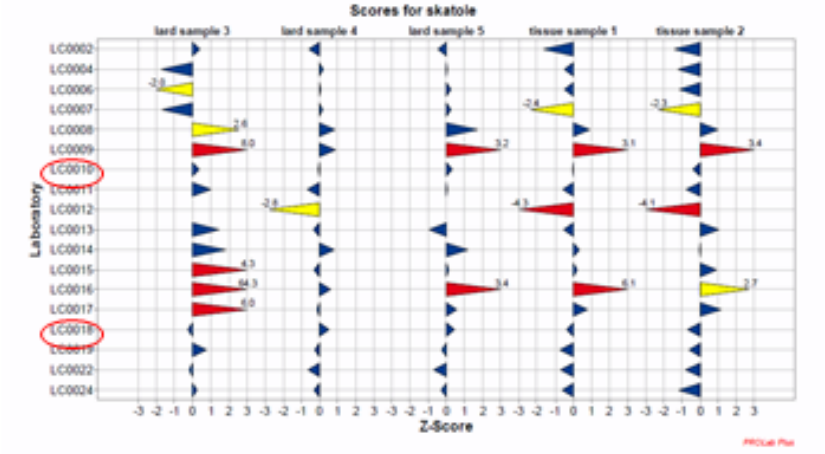
Blue triangles: z≤|2|; yellow triangles: |2|<z<|3|; red triangles: z≥|3|, score values presented next to the triangle



Boar taint – JRC-IRMM ring test - results

Figure 26: z-Scores for skatole

Blue triangles: z≤|2|; yellow triangles: |2|<z<|3|; red triangles: z≥|3|, score values presented next to the triangle



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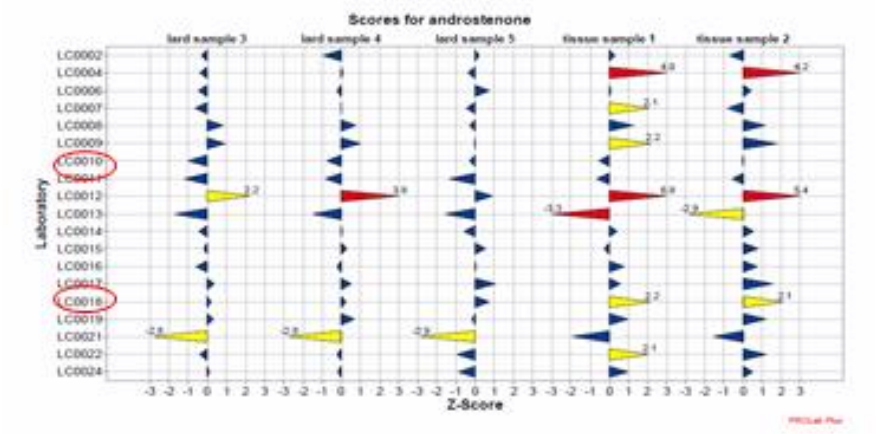
PROJECT: Sustainability in pork production with immunocastration (SuSI)
ERA-NET SUSAN

Boar taint – JRC-IRMM ring test - results

Figure 27: z-Scores for androstenone

Blue triangles: $z \leq |2|$; yellow triangles: $|2| < z < |3|$; red triangles: $z \geq |3|$, score values presented next to the triangle



Boar taint – IRMM ring test, conclusions

Conclusions

- RSD_r= 3 -10 %
- RSD_R= 10 - 30 %
- robust method, free from matrix interferences
- sensitive enough to determine the off-favour compounds at the sensory treshold values with acceptable analytical precision
- method performance characteristics are compliant with requirements for official control methods in the area of food contaminants

but

- expensive instrumentation
- time consuming

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Boar taint – analytical procedure in KIS lab - method according to Hansen-Møller (1994) and Pauly et al. (2008)

cut trimmed samples into 0.5 cm cubes

↓

liquefy in a microwave oven for 2×1 min at 350 W

↓

centrifuge liquefied lipids for 20 min at 11,200×g and 20°C

↓

transfer 0.5 ± 0.01 g water-free liquid fat in 2.5 mL tube

↓

add 1 mL methanol containing internal standards
(0.496 mg/L androstanone and 0.050 mg/L 2-methylindole)

↓

stirring for 30 s, incubate for 5 min at 30°C in an ultrasonic water bath

↓

on ice for 20 min

↓

centrifuge for 20 min at 11,200×g at 4°C→ transfer methanol phase into HPLC vials

Boar taint – analytical procedure in KIS lab - method according to Hansen-Møller (1994) and Pauly et al. (2008)

ANDROSTENONE

↓

submit 50 µL of the supernatant to derivatization with dansylhydrazine for exactly 2 min

↓

thereafter an aliquot of 10 µL of the derived mixture inject on HPLC column (THF – phospate buffer, isocratic)

↓

detect fluorescence
(excitation at 346 nm and emission at 521 nm)

↓

detection limit 0.24 µg/g liquid fat

SKATOLE and INDOLE

↓

inject 20 µL of the supernatant into the column
(methanol – phospate buffer, isocratic)

↓

detect fluorescence using HPLC system
(excitation at 285 nm and emission at 340 nm)

↓

detection limit 0.03 µg/g liquid fat

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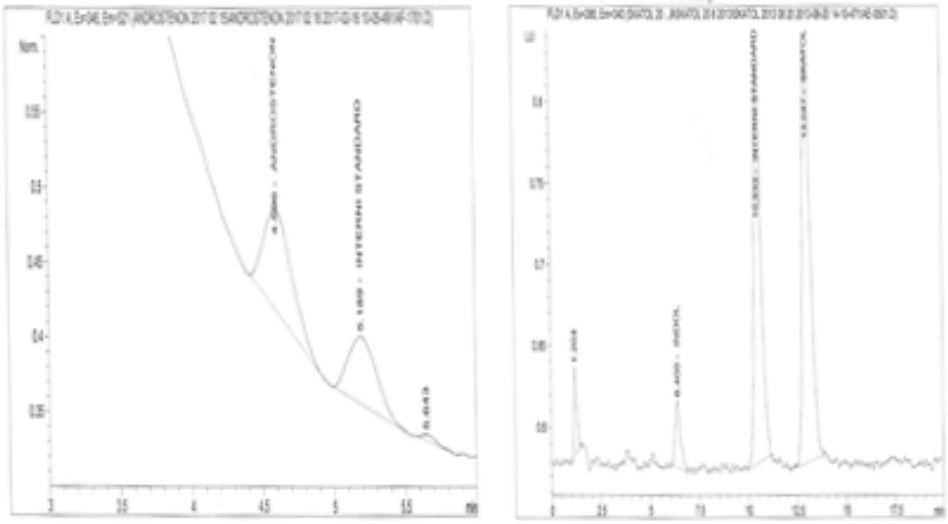
Boar taint – analytical procedure in KIS lab



Boar taint – analytical procedure in KIS lab

Conclusions

- robust method, free from matrix interferences
- sensitive enough to determine the off-favour compounds at the sensory treshold values with acceptable analytical precision
- fast
- very suitable for large number of samples

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Boar taint – sampling procedure

Sampling:

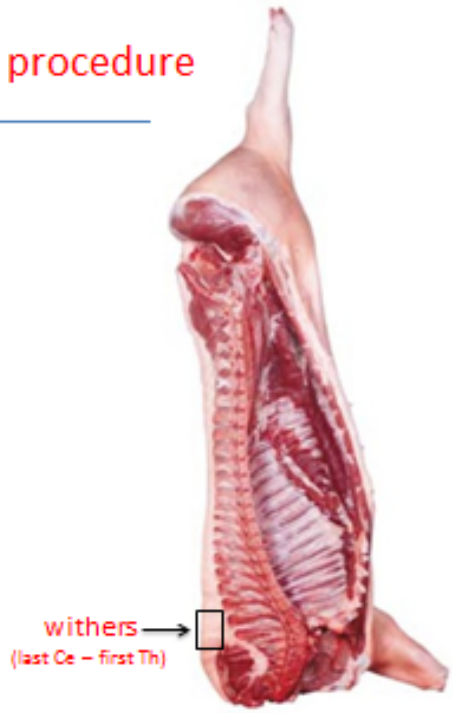
on cooled carcasses (approx. 24 h *post mortem*)

Location:

withers – position where subcutaneous backfat tissue is the thickest (important in EM)

Procedure:

excise a 10 x 10 cm piece of subcutaneous tissue using a sharp knife



Boar taint – sampling procedure

Sample preparation:

trim the excised tissue of the skin and muscle tissue

Sample storage:

store vacuum packed samples in freezer (- 20 ° C) until further analysis

TRAINING SCHOOL Harmonisation of methods in entire male and immunocastrate research, Ljubljana 20-22 November 2017
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Boar taint – harmonisation in joint research

- sampling location
- analytical method/same laboratory ?
- expression of results on the same basis – e.g. in liquid fat

Threshold levels:

ANDROSTENONE → 0.5 – 1.0 µg/g liquid fat

SKATOLE → 0.2 – 0.25 µg/g liquid fat

Example:

- sample of BF tissue (80 % fat)
  Androstenone 2.5 µg/g liquid fat → 2 µg/g sample/matrix
  Skatole 0.5 µg/g liquid fat → 0.4 µg/g sample/matrix
- sample of LD muscle tissue (1% IMF)
  Androstenone 2.5 µg/g liquid fat → 0.025 µg/g sample/matrix
  Skatole 0.5 µg/g liquid fat → 0.005 µg/g sample/matrix

Examples – expression of results:

- µg/g liquid fat
- µg/g fat
- µg/g sample
- µg/g

- µg/kg - fat
- µg/L - in serum
- ppm - sample ?
- mg/kg - sample

Fat content in certain tissue is important when interpreting results.

Thank you for your attention.

Need of harmonisation in:

- sampling location
- analytical method/same laboratory ?
- expression of results on the same basis – e.g. in liquid fat

TRAINING SCHOOL Harmonisation of methods in entire male and immunocastrate research, Ljubljana 20-22 November 2017

PROJECT: Sustainability in pork production with immunocastration (SuSI) ERA-NET SUSAN

Chapter 3

Welfare, behaviour (ethogram) and skin lesions recording

Volker STEFANSKI[1]

Welfare & Behavior Recording (Ethogram)

Volker Stefanski, University of Hohenheim, Germany

Behavioral Physiology of Livestock, Hohenheim

Part 1: Welfare

[1] University of Hohenheim, Garbenstr. 17, 70599 Stuttgart, Germany

Overview - Animal welfare

- Animal welfare legislation
- What is animal welfare?
- Animal welfare assessment
  - Ethological and physiological indicators
  - Motivation
- Excursus: Welfare Quality Report
- [Skin lesion recording]

Animal welfare is a state objective in Germany

Germany has one of the strictest animal welfare laws worldwide.

Animal welfare has been included as a state objective in the Basic Law since 2002 (Article 20a):

"Mindful also of its responsibility toward future generations, the state shall protect the natural foundations of life and animals by legislation and, in accordance with law and justice, by executive and judicial action, all within the framework of the constitutional order."

No other country in the EU so far has integrated animal welfare into its constitution.

Animal Welfare Act (Germany)

Tierschutzgesetz (TierSchG)

Principle (§1)

The aim of this Act is to protect the lives and well-being of animals, based on the responsibility of human beings for their fellow creatures. **No one may cause an animal pain, suffering or harm without good reason.**

Definitions: Pain, suffering, damage

Pain
Unpleasant sensory and emotional experience associated with acute or potential tissue damage or described as such damage.

Suffering
Significant impairment of well-being (except pain), especially anxiety, fear, stress.

Damage
Impairment of integrity (physically, mentally).

European Convention for the Protection of Animals kept for Farming

reflects the “Five Freedoms” (result of a 1965 UK government commission report led by Roger Brambell)

- Freedom from hunger and thirst
- Freedom from discomfort
- Freedom from pain, injury and disease
- Freedom to express normal behavior
- Freedom from fear and distress

https://ec.europa.eu/food/sites/food/files/animals/docs/aw_european_convention_protection_animals_en.pdf

Definition: What is Animal welfare?

Animal welfare in a scientific context adapted from Dawkins (2006)

There is no single definition as for humans, but it is certainly more than the absence of harm, physical suffering and illness.

In humans, poor welfare is not only associated with bad health, injury or illness (**physical symptoms**), but also with conditions such as stress, frustration, boredom, loneliness or grief (**mental symptoms**). That should be similar in animals.

Mental symptoms may or may not be correlated with **physical symptoms**: in humans, therefore, a distinction is made between physical and mental well-being. This distinction is basically also useful in animals.

Useful reading: Dawkins, M.S., 2006. A user's guide to animal welfare science. Trends in Ecology & Evolution 21, 77-82.

Welfare concepts

"If the animal's expectation copy (total set of expected values relate to good welfare) matches the perceptions of the environment, good welfare is achieved." (N. Sachser, 2000) Coping with Challenge - Welfare in animals including humans, Dahlem University Press.

"The welfare of an individual is its state as regards its attempt to cope with its environment." (D. M. Broom 1998) Applied Animal Behaviour Science, 20

Is "natural behavior" a requirement for welfare?

One of the "Five Freedoms" is: "... the ability to perform most natural patterns of behavior"

To what extent can "natural behavior" be equated with "welfare"?

Lessons from wildlife …

Useful reading: Bradley, A.J., McDonald, I.R., Lee, A.K., 1980. Stress and mortality in a small marsupial (Antechinus stuartii, Macleay). Gen Comp Endocrinol 40, 188-200.

How can good welfare be determined?

Two approaches

1) “Sum" of welfare indicators: behavior, physiology, health

2) Answer to the questions: “Are animals healthy and have they what they want?”

Clinical indicators of severely impaired welfare

Some clinical indicators of severely impaired welfare, which are usually very easy to recognize

- Strong reduction of body mass
- Illness
- Injury (lameness, wounds, etc.)
- Reduced life expectancy

Behavioral indicators for severely impaired welfare

- Impairment of food intake (feeding / drinking)
- Collapse of the species-specific diurnal activity pattern
- Frequent occurrence of conflict behaviors (e.g. stereotypes)
- Loss or severe reduction of comfort behavior
- Loss or severe reduction of exploratory and play behavior
- Apathy

Physiological indicators of impaired welfare

Indicators of poor welfare, often before clinical symptoms appear

- Increased stress hormone concentrations (but check for pitfalls: multifunctionality, diurnality, variability)
  - *Cortisol*
  - *ACTH*
  - *Endorphins*
  - *Catecholamines*
- Cardiovascular changes (heart rate, heart rate variability)
- Loss of normal day / night rhythm
- Reduced / modulated immune function
- Reduced reproductive capacity
- Gender changes (more female offspring)
- Reduced feed intake

Two crucial questions to decide if animal welfare is given

(after M.S. Dawkins, 2006)

Are the animals physically healthy?

Do the animals have what they want?

Which resources are important for the welfare of fur-farmed minks?

Mason, G.J., Cooper, J., Clarebrough, C., 2001. Frustrations of fur-farmed mink. Nature 410, 35-36.

Minks "work" for access to resources

Total expenditure

Seven additional rooms:

134 kg	• Water pool (1.5 x 0.5m)
115 kg	• Elevated platform
84 kg	• Novel objects
82 kg	• Alternative nesting site
34 kg	• Toy
26 kg	• Tunnel
9 kg	• Empty room

Foto: von Anna Wójtowicz - plWiki, uploaded by Arturek28, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=1610479

Mason et al. (2001) Frustrations of fur-farmed mink. Nature 410: 35-36

Loss of access to water pool causes frustation in minks

Von Anna Wójtowicz - plWiki, uploaded by Arturek28, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=1610479

Access blocked for 24 h

Deprived of	**Increase in cortisol (Urinary, to baseline)**
• Food	**50% increase**
• Water pool	**34 % increase**
• Raised platform	no increase
• Empty room	no increase

Mason et al. (2001)

Housing requirements of farm animals

Farm animals cannot adapt to all housing systems.

Certain elements must be present to achieve welfare.

→ Basic requirements that derive from social organization (e.g. interaction with social partners).

→ Essential elements of the habitat to which the wild ancestor was selected (e.g. structuring and nature of the habitat).

→ Basic patterns of behavior must be able to be performed.

Assessment of animal welfare on the basis of Welfare Quality®-Protocols

- Development of a European standard for the assessment of animal welfare of housing systems
- 44 institutions and universities in 13 EU countries and Latin America involved
- Pigs, cattle, poultry
- Currently no official use

Welfare Quality: Health-associated measures (pigs)

- Bursitis
- Body condition
- Manure on the body
- Wounds and scratches
- Tail biting
- Lameness
- Respiratory disorders
- Rectal prolapse
- Twisted snouts
- Hernias

Welfare Quality: Manure on the body

All surfaces contaminated with feces are put together imaginably (on side of the body)

Foto: N. Breßler

< 20% - 0
> 20-50% - 1
> 50% - 2

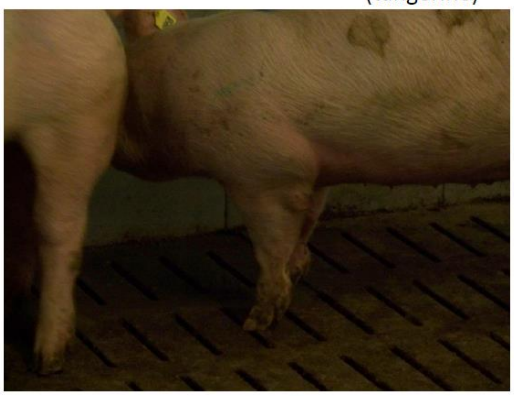
Welfare Quality: Bursitis

How many? What size?

Small (size of a grape) 1,5-2,0cm

Large 3,0-5,0cm

Extremely large (tangerine) 5,0-7,0cm

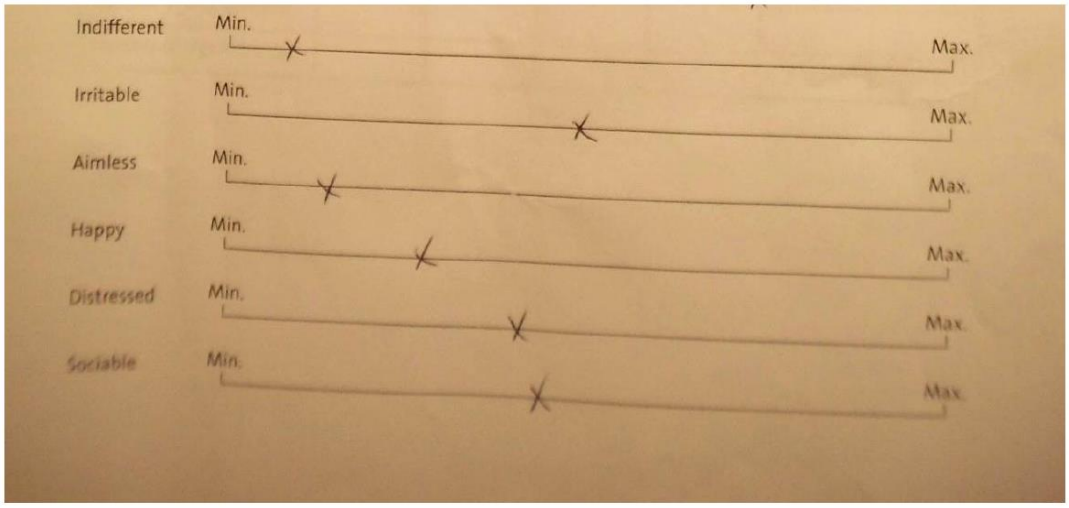


One or several small bursae on the same leg or one large bursa -1
Several large bursae at the same leg, or extremely large, or eroded -2

Photos: N. Breßler

Behavioral Physiology of Livestock, Hohenheim

Welfare Quality: Qualitative Behaviour Assessment (QBA)



N. Breßler, Master thesis, UHOH, 2013

Welfare quality

The „basic principles“ **good feeding, good housing** and **good health** are overall rather objective indicators

„Basic principle“ **appropriate behavior (as currently used) is highly questionable**:

- no clear definition of descriptors and parameters
- visual analogue scale (VAS)
- subjective and anthropomorphic
- not scientifically substantiated

→ not a suitable measure

Skin lesion recordings

Photo: Grün

Exact recording

“Lesion scores”

- Welfare Quality Assessment Protocols
- KTBL

Part 2: Behavior recording

Useful reading:

- Martin, P., Bateson, P., 2006. Measuring behaviour. Cambridge University Press, Cambridge, UK.
- (in German) Naguib, M., 2006. Methoden der Verhaltensbiologie. Springer-Verlag, Berlin Heidelberg, Germany

Overview – Behavior recording

- Observer and experimenter bias
- Recording medium
- Individual recognition of subjects
- Catalogue of behavior descriptions (ethogram)
- Broader categories of behavior (functional units)
- Quantitative assessment of behavior
- Selection of appropriate methods
- Inter- and intra-observer reliability
- How much behavior should be recorded?

Avoid observer and experimenter bias !

A) **Direct influence of the observer on the subjects (observer bias)**

B) **Experimenter bias**

Bias related to subtle cues given to the animals (Clever-Hans case)
Horse is responding to unconsciously given signals by the trainer

Bias related to recording or analyzing data
Unintentional influence of the experimenter on data. Strong expectations (hypothesis) about the outcome.

- Effect of castration on sexual behavior
- Determination of morphological parameters or body mass (e.g. male/ female differences, re-measurement of extreme values, interpretation as "miscalculation")

Gold standard is a **"blind" experimental design**, but sometimes this is not easy to achieve in behavioral studies

Recording medium

- Direct observation
- Video and audiotape
- Automatic recording devices

Direct observation

Photo: Sacha Dauphin, University of Hohenheim

Video and audiotape

Behavior analysis by professional software

Commerical software

e.g.:

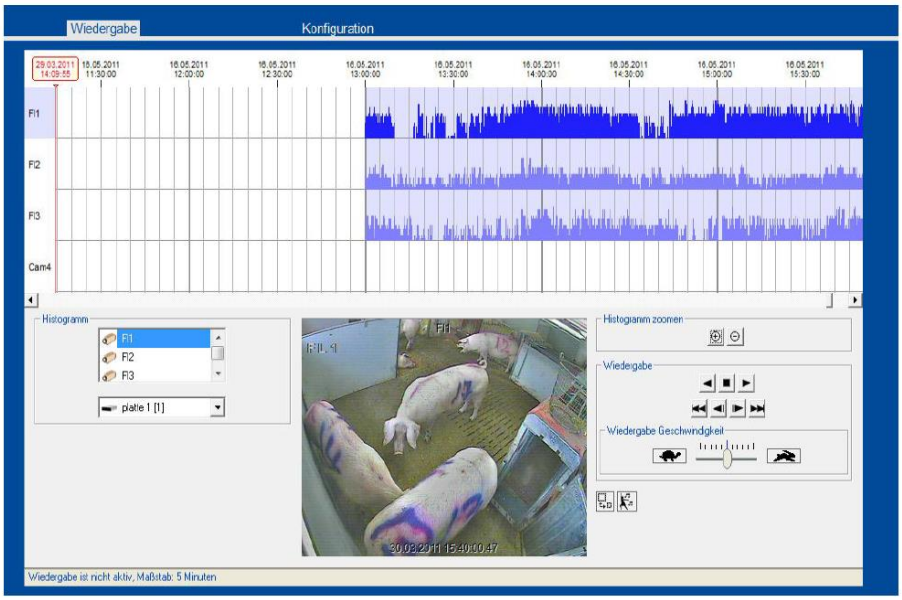
- The Observer XT (Noldus)
- Interact (Mangold)

Coding system

Video analysis

Inter-observer reliability

Playback of recorded behavior



Motion-/
Event
histogram

Videotaping versus direct observation

Advantages of video recordings

- if direct observation is not possible (disturbance of subjects, night)
- no observer effect
- archiving (later evaluation, relief during observation)
- playback in time-lapse or -lupe (time saving)
- "naive" observer (blind study design)

Disadvantages

- often time consuming
- incomplete recording of the whole group (sub-sections, possibly several cameras necessary)
- individual recognition may be difficult (small animals, diffused light conditions, shadow)

Automatic registration methods

Advantages

- Time savings (observer time)
- No subjective assessment
- Standardization (periods, experimenter)

Application

- Activity-related behavior
- Spatial distribution of individuals
- Additional recording of heart rate, temperature
- Hardly suitable for social interactions

Individual recognition of subjects

Individual recognition of the animals

Natural features (fur pattern etc.)

Markings (e.g. color marks, fur cuts, transponders, rings,

ear tags)

Designation of animals

Names (advantage: better to remember)

Number codes (advantage: no subjective properties, more scientific)

Catalogue of behavior descriptions (ethogram)

Accurate and detailed description of all behaviors (quality and quantity) occurring in the species concerned.

Avoid anthropomorphisms!

For convenience, a sub-ethogram is often made (e.g. sexual behavior).

Ethogram

interpretative	decriptive
- The pig threatens another	- The pig stands parallel or inverse parallel and pushes hard with the shoulders against another pig
- The pig is small	- The piglet weighs 10 kg
not verifiable	**verifiable**

Descriptive registration of behavior!

Pressing

The pigs stand parallel or inverse parallel and push hard with the shoulders against each other, throwing the head against the neck, head or flanks of the other pig.

Foto: V. Grün

Ethogram pigs

Ethogram Pigs (Hohenheim)
Part 1 – Behaviors & behavioral elements

Behavior (functional unit)	Behavioural elements (see part 2 for definitions)
Aggressive behaviour	Pressing Pressing-cum-bite Head knock Head knock-cum-bite Biting (attempt) Penis biting Levering Chasing
Defensive behaviour	Retreat Fleeing
Affiliative behaviour	Nosing, without anal-genital region
Sexual behaviour	Mounting (attempt) +/- pelvic thrusts +/- extruded penis Anal-genital nosing

Aggressive behaviour (Jensen 1980)	
Pressing	*The pigs stand parallel or inverse parallel and push hard with the shoulders against each other, throwing the head against the neck, head or flanks of the other pig.*
Pressing-cum-bite	*As above but with bites directed towards, head, ears and flanks of the other.*
Head knock	*A rapid thrust upwards or sideways with the head or snout against any part of the body of the other pig. Performer's mouth is shut.*
Head knock-cum-bite	*As above, but with bites. Performer's mouth is open.*

Ethogram Pigs (Hohenheim)

Part 1 - Behaviors & behavioral elements

Behavior (functional unit)	**Behavioral elements** (see part 2 for definitions)
Aggressive behavior	Pressing Pressing-cum-bite Head knock Head knock-cum-bite Biting (attempt) Penis biting Levering Chasing
Defensive behavior	Retreat Fleeing
Affiliative behavior	Nosing, without anal-genital region
Sexual behavior	Mounting (attempt) +/- pelvic thrusts +/- extruded penis Anal-genital nosing
Play behavior	Scamper Other play
Abnormal behavior	Body-nosing Chewing ear Chewing tail Chewing pen mate, without tail and ear
Active behavior Feeding/drinking Inactive behavior	Standing Locomotion Exploration Sitting Feeding/drinking Lying sternally Lying recumbently

Part 2 - Definitions

non-italic = own wording or modification
italic = wording of initial author

New behavior after 3 seconds of pause.

Aggressive behavior (Jensen 1980)	
Pressing	The pigs stand parallel or inverse parallel and *push hard with the shoulders against each other, throwing the head against the neck, head or flanks of the other pig.*
Pressing-cum-bite	*As above but with bites directed towards, head, ears and flanks of the other.*
Head knock	*A rapid thrust upwards or sideways with the head or snout against* any part of the body of the other pig. *Performer's mouth is shut.*
Head knock-cum-bite	*As above, but with bites. Performer's mouth is open.*
Biting (attempt) (Donaldson et al. 2002)	*Mouth opened and snapped shut against opponent.*
Penis biting	Biting (attempt) towards the extruded penis of another pig.
Levering	The pig *puts its snout under the body of another pig (in all observed cases from behind), and lifts it up in the air.*
Chasing	Following a fleeing animal at high speed.
Defensive behavior (modified after Jensen 1980)	
Retreat	The pig moves away from another pig in usual walking speed directly after a social interaction.
Fleeing	The pig *moves away from another pig rapidly with head high* directly after a social interaction. *Often accompanied by a shrill scream.*
Affiliative behavior (modified after Jensen 1980)	
Nosing	The nose of the pig approaches any part of the body except genital region of another pig up to at least 5 cm distance.

Sexual behavior (modified after Booth & Baldwin 1980)	
Mounting attempt	The pig lifts the front part of its torso to put it on top of the torso of another pig (usually from behind), but not successful.
Mounting…	The pig lifts the front part of its torso and puts it on top of the torso of another pig (usually from behind).
… with/without pelvic thrusts (+)	While holding the mounting position the pig moves its pelvis for- and backwards.
… and with/without emerging of penis (++)	While holding the mounting position the penis extrudes.
Mounting escape (attempt)	Occurs in response to mounting. The pig tries to or moves away from the mounting pig rapidly. The activity is often accompanied by a shrill scream.
Anal-genital-nosing (modified after Jensen 1980)	*The nose of the* pig *approaches the genital region of another pig* up to at least 5 cm.
Play behavior (Donaldson et al. 2002)	
Scamper	*A sequence of at least two forward hops in rapid succession, usually accompanied by ear flapping.*
Other play	***Pivot** (a jump on the spot in which the body is rotated rapidly at least 90° in the horizontal plane), **head toss** (exaggerated lateral displacements of the head and neck in the horizontal plane, involving at least one full movement to each side), **flop** (a rapid drop from an upright position to sternal or lateral recumbency in which the pig appeats to fall down by itself and not as a result of contact with another pig)*
Abnormal behavior (modified after Jensen et al. 2010)	
Chewing pen mate	Chewing movements towards a (mostly lying) pen mate, except ears and tail.
Chewing tail	*Making chewing movements while the tail of another pig in the mouth.*
Chewing ear	*Making chewing movements while the ear of another pig in the mouth.*

Body-Nosing (modified after Fraser 1978)	Body nosing is the rhythmic up-and-down movement of one pig rubbing the body, especially belly, of another with its snout.

Locomotor / activity behavior (modified after Ekkel et al. 2003)	
Standing	*Body supported by three or more legs* and head held high.
Locomotion	Walking or running, body supported by three or more legs, position change possible and head held high.
Feeding / drinking	Head at drinker or head at trough.
Exploration	Sniffing at the floor and feed trough, interaction with material (litter)
Lying sternally	The body not supported by any of the legs. The pig is lying on its sternum with head high or lowered down.
Lying recumbently	The pig *is lying half on the side and half on its belly or fully on the side with all four legs stretched out.*
Sitting	*Body supported by one or two front legs*, the rear part of the torso touches the floor.

Sources:

Booth, W.D., Baldwin, B.A. (1980). Lack of effect on sexual behaviour or development of testicular function after removal of olfactory bulbs in prepubertal boars. *Journal of Reproduction and Fertility 58: 173-182.*

Donaldson, T.M.; Newberry, R.C; Spinka, M.; Cloutier, S. (2002). Effects of early play experience on play behaviour of piglets after weaning. *Applied Animal Behaviour Science 79, 221-231.*

Ekkel, E. D..; Spoolder, H.A.M.; Hulsegge, I.; Hopster, H. (2003). Lying characteristics as determinants for space requirements in pigs. *Applied Animal Behaviour Science 80, 19-30.*

Fraser, D. (1978). Observations on the behavioural development of suckling and early-weaned piglets during the first six weeks after birth. *Anim. Behav. 26, 22-30.*

Jensen, P. (1980). An ethogram of social interaction patterns in group-housed sows. *Applied Animal Ethology 6, 341-350.*

Jensen, M.B.; Studnitz, M., Pedersen, L.J (2010). The effect of type of rooting material and space allowance on exploration and abnormal behaviour in growing pigs. *Applied Animal Behaviour Science 123: 87-92.*

Assignment of behavior to functional units

- Agonistic behavior
- Sexual behavior & reproduction
- Mother-infant behavior
- Feeding behavior
- Elimination behavior
- Resting and active behavior
- Comfort behavior
- Play and exploratory behavior
- Learning behavior

Sequence analysis

Sequential flow of behavior

- Classification in functional units
- Mechanisms and control of behavior
- Rules of decision-making

Quantitative assessment of behavior

Types of measure

Time parameters of behavior

Duration, occurrences & frequency

States versus events

Sampling rules

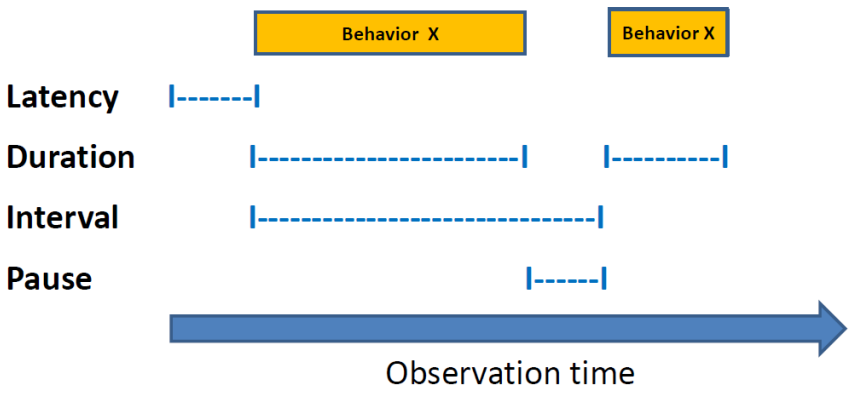
Recording methods

Continuous recording

Time sampling (instantaneous & one-zero)

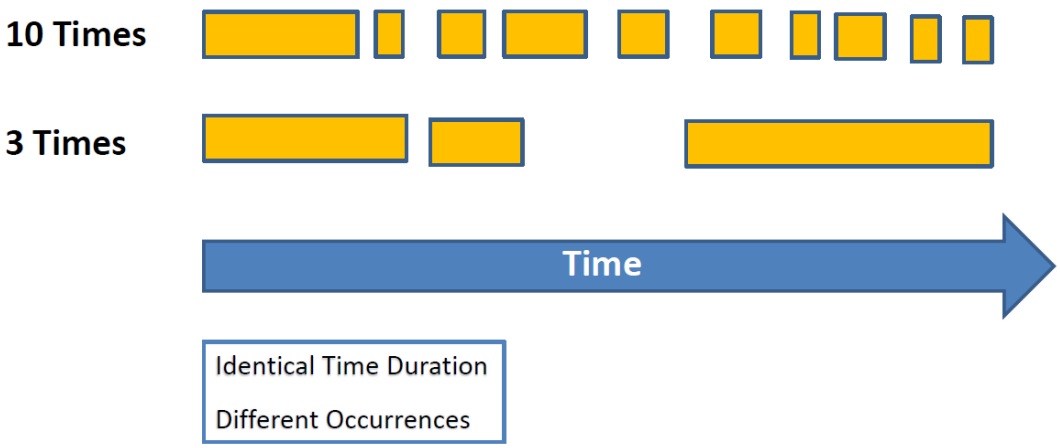
Types of Measures

Time parameters of behavior



Types of Measures

Duration and occurrences of behavior



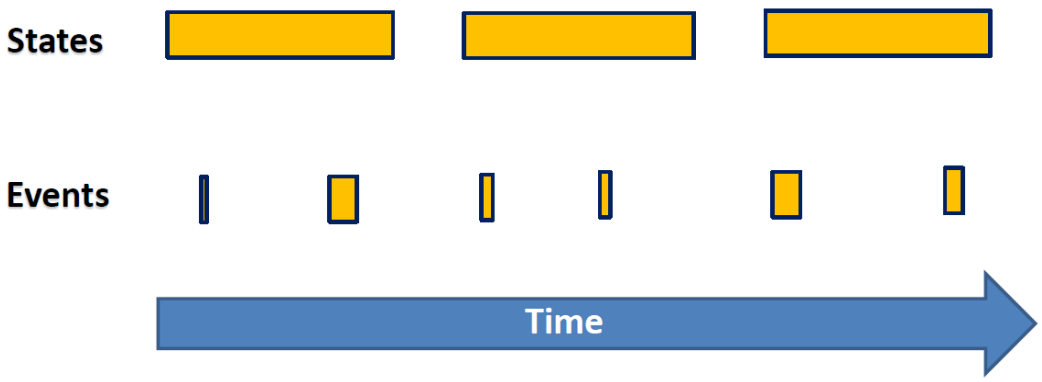
Frequency

(number of occurrences per unit time)

Total time: frequency of “A” twice as high as “B” or “C”
Total time: frequency “B” equals “C”

Day: frequency “A” equals “B”

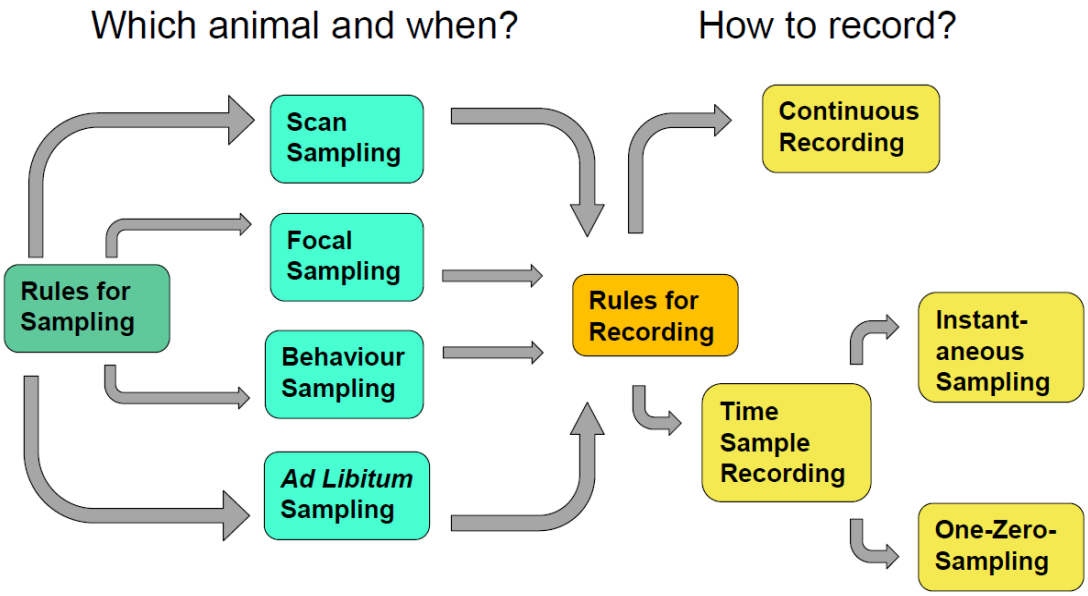
States versus events



States: e.g. resting behaviour

Events: e.g. social interaction, defecating behavior,...

Sampling and recording rules



Sampling rules

Which subjects to watch and what behavior when to record?

Behavioral Physiology of Livestock, Hohenheim

Sampling rules

- *Ad libitum* sampling (whole group, all occurrences of behaviors)
- Behavior sampling (whole group, all occutrences of a particular type of behavior (e.g. rare events, such as in an agonistic or sexual behavioral context)
- Focal animal sampling (one individual, all occurrences of its behavior)
- Scan sampling (whole group is scanned rapidly, behavior is recorded by instantaneous sampling → locomotor behavior, orientation, etc.)

Recording rules

How to record behavior?

Continuous recording

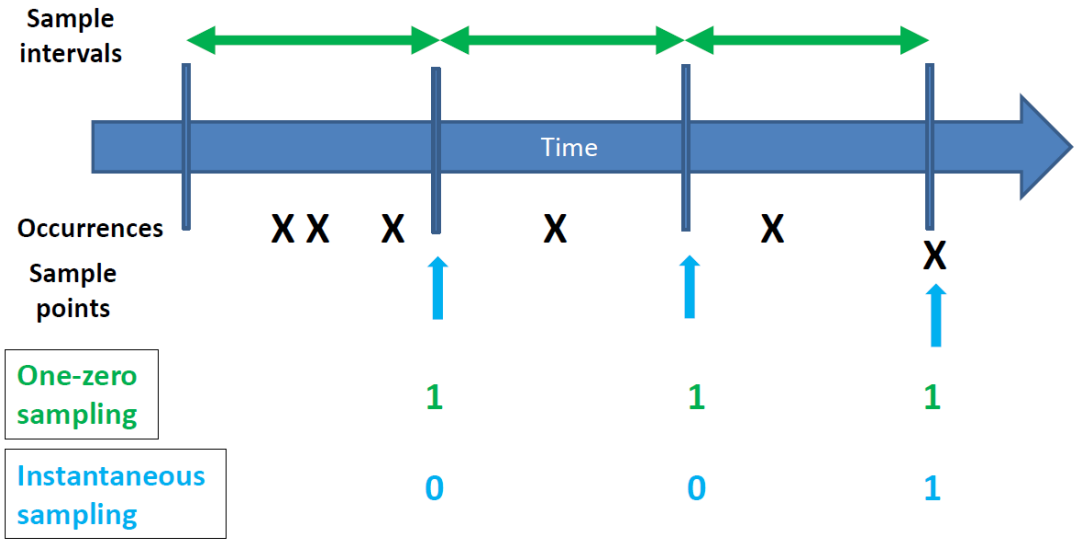
Recording: Duration & occurrences of behaviors

Assessment of continuous recording

Continuous recording

- measures true frequencies and durations accurately
- analysis sequences of behavior
- demanding for observer → time consuming
- less behavior can be recorded in a given period of time

Time sample recording



Assessment of time sampling methods

One-zero sampling

- does not provide true or unbiased estimates of duration or frequencies
- only practical method for intermittent behavior (e.g. play behavior)
- valid measurement for the "amount" of behavior (correlates with frequency and duration)
- may be more objective than continuous recording

Instantaneous sampling

- recording of behaviors that occur/do not occur at any instant in time (e.g. locomotor activity)
- not suitable for recording discrete events of short duration or rare events (many behaviors in social context)
- the accuracy depends on the length of the sample interval (the shorter the interval, the more accurately it reflects continuous recording)
- less time-consuming than continuous recording

adapted from Martin & Bateson (2007)

Intra- and inter-observer reliability

Variation exists between observations!

Intra-observer reliability

- Single observer obtains similar results in repeated counting of same sequences (e.g. videotape analysis)

Inter-observer reliability

- Two or more observers obtain similar results

Measuring reliability (Martin & Bateson 2007)

- Correlation coefficient (Pearson or Spearman rank)
- Index of concordance (total number of agreements/disagreements)
- Kappa coefficient (accounts for agreements that arise by chance)

How much behavior should be collected ?

Enough to get sufficient results!

Internal consistency (according to Martin & Bateson 2007)

- Divided data in subsets, analyzed separately and compared
- Split-half analysis (divided data for each behavioral category, plus correlation analysis, $r > 0,7$)

Necessary sample size

- Biometric analysis

Behavioral Physiology of Livestock, Hohenheim

How much behavior should be collected ?

Is information required on individual level?

- Individualized data collection
- More demanding (time)
- Allows intra-individual analysis
- Allows intra subject analysis behavior – physiology

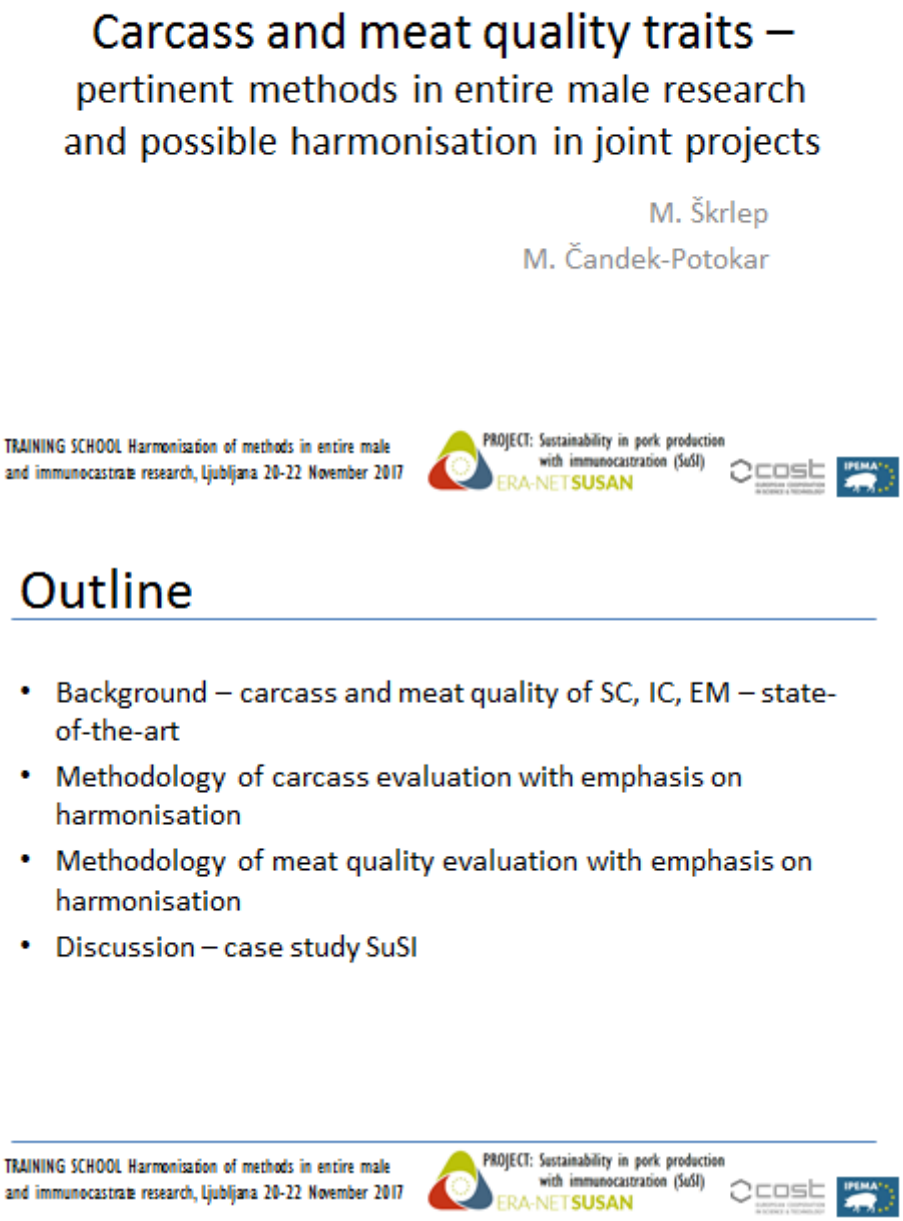
Information on group level sufficient?

- Less demanding
- Information on individual lost, okay when group comparison is sufficient
- Allows screening of large group sizes (scan sampling)

Chapter 4

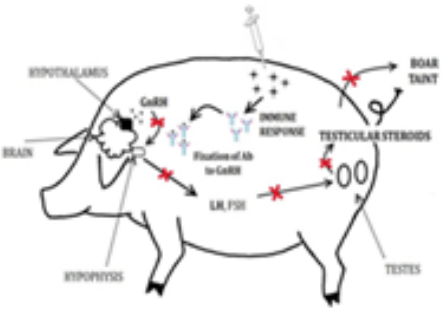
Carcass and meat quality traits – pertinent methods in boar taint research and possible harmonisation in joint projects

Martin ŠKRLEP[1] and Marjeta ČANDEK-POTOKAR[1,2]

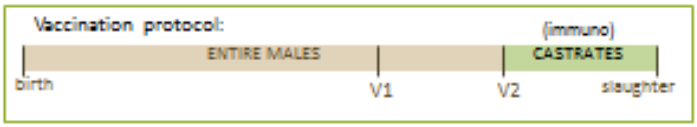


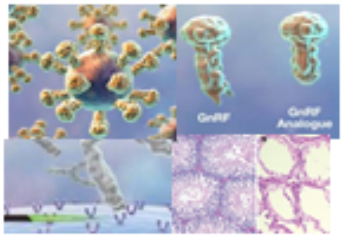
[1] Agricultural Institute of Slovenia (KIS), Hacquetova ulica 17, 1000 Ljubljana, Slovenia
[2] University of Maribor, Faculty of Agriculture and Life Sciences, Pivola 10, 2311 Hoče, Slovenia

IMMUNOCASTRATION



- vaccination against GnRH => disruption of hypothalamic–pituitary–gonadal axis
- 2 vaccinations needed; at least 4 wks apart
- regression of reproductive organs
- boar taint prevention
- no withdraw period => to eliminate boar taint, 4-6 weeks delay recommended
- affects performance and meat quality
- late IC - using boar-like growth potential





Formation of antibodies against GnRH, which binds to endogenous GnRF and blocks the release of LH and FSH hormones. This analogue of GnRH has no hormonal activity.

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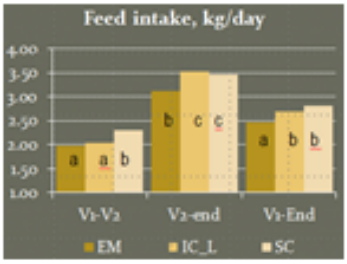
Why carcass traits/body composition?

- EM, SC and IC are metabolically different
- SC – early castration, loss of androgen potential
- IC – late castration (in case of standard vaccination protocol), short term androgen deprivation
- SC have higher feed intake than EM => effect on body composition
- After the effective vaccination, IC increase feed intake => effect on body composition
- Body composition => economic consequences

EM – entire males or boars
SC – surgically castrated male pigs
IC – immunocastrated male pigs



(Batorek et al., 2012)

	IC to SC			IC to EM		
Trait	*n*	θ_1 (CI)	*P*-value	*n*	θ_1 (CI)	*P*-value
DFI						
V1 to V2	12	−2.08 (−2.87, −1.89)	0.000	9	0.37 (−1.01, 0.75)	0.058
V2 to S	15	0.41 (−0.06, 0.87)	0.089	25	2.08 (1.50, 2.67)	0.000
V1 to S	13	−0.92 (−1.43, −0.40)	0.000	11	1.29 (0.63, 1.94)	0.000

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Differences in carcass traits/body composition?

- Lean meat content — SC < IC < EM
- Backfat thickness — SC > IC > EM
- Muscular dvlp (loin) — SC ≈ IC ≈ EM
- Muscular dvlp (ham, shoulder) — SC < IC ≈ EM

Table 1 *Summary of meta-analysis (effect size) for IC compared with the SC or EM* (Batorek et al., 2012)

	IC to SC			IC to EM		
Trait	*n*	θ_i (CI)	*P*-value	*n*	θ_i (CI)	*P*-value
Carcass traits						
Dressing	20	−0.86 (−1.14, −0.59)	0.000	16	−0.14 (−0.16, 0.44)	0.353
Lean meat	24	0.46 (0.31, 0.61)	0.000	24	−0.66 (−0.93, −0.39)	0.000
Muscle LD thickness	11	−0.08 (−0.21, 0.05)	0.248	6	0.30 (−0.06, 0.66)	0.105
Backfat thickness	28	−0.56 (−0.74, −0.36)	0.000	33	0.77 (0.47, 1.06)	0.000
Loin weight	5	−0.22 (−0.88, 0.45)	0.525	5	0.13 (−0.47, 0.75)	0.669
Ham weight	7	0.54 (0.22, 0.86)	0.001	6	0.04 (−0.17, 0.24)	0.723
Belly weight	4	−0.72 (−1.48, 0.04)	0.065	5	0.49 (0.27, 0.72)	0.000
Shoulder weight	4	0.84 (−0.02, 1.70)	0.057	5	−0.01 (−0.43, 0.43)	0.983

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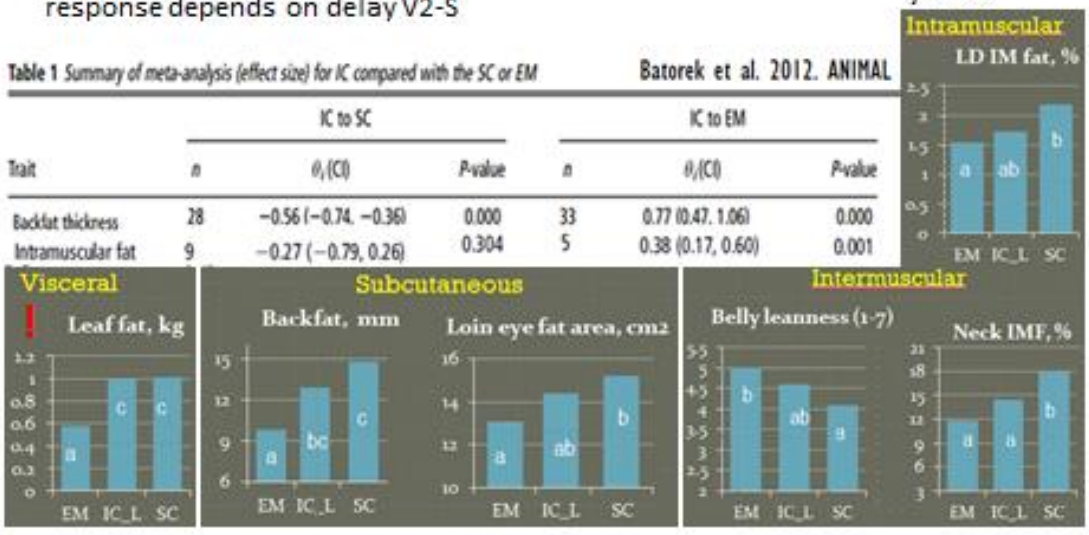
Differences in fat depots

Generally: SC > IC > EM;
response depends on delay V2-S

Batorek et al., 2012. J Anim Sci

Table 1 *Summary of meta-analysis (effect size) for IC compared with the SC or EM* Batorek et al. 2012. ANIMAL

	IC to SC			IC to EM		
Trait	*n*	θ_i (CI)	*P*-value	*n*	θ_i (CI)	*P*-value
Backfat thickness	28	−0.56 (−0.74, −0.36)	0.000	33	0.77 (0.47, 1.06)	0.000
Intramuscular fat	9	−0.27 (−0.79, 0.26)	0.304	5	0.38 (0.17, 0.60)	0.001



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What about meat quality*?

*other than boar taint problem typical for EM

	pH24	Color L*	drip	imf	tenderness
Trefan et al 2013	ns	EM < IC	ns	SC≈IC > EM	ns
Pauly et al 2012	EM < SC≈IC	EM < SC	IC > SC, EM	SC≈IC > EM	IC > SC > EM
Batorek et al 2012a	ns	ns	IC > EM	SC≈IC > EM	SC≈IC > EM
Aluwe et al 2013	EM < SC,IC	SC≈EM > IC	IC≈EM > SC	/	ns
Batorek et al 2012b	EM > SC	EM < SC	EM[a] > IC[ab] > SC[b]	SC[a] > IC[ab] > EM[b]	SC[a] > IC[ab] > EM[b]
Škrlep et al 2012	ns	ns	EM > SC=IC	SC≈IC > EM	ns
Škrlep et al 2010	ns	ns	ns	SC≈IC > EM	/
Van den Broeke et al 2016	EM > IC	ns	ns	EM≈IC	ns

Pauly et al 2012; increased PUFA in EM

What about meat quality*?

*other than boar taint problem typical for EM

Batorek et al. 2012. Animal 6(8):1330-38.

Table 1 *Summary of meta-analysis (effect size) for IC compared with the SC or EM*

	IC to SC			IC to EM		
Trait	*n*	θ_i (CI)	*P*-value	*n*	θ_i (CI)	*P*-value
Meat quality of LD						
Ultimate pH	12	−0.15 (−0.44, 0.15)	0.341	10	−0.16 (−0.35, 0.03)	0.093
L^*	6	0.47 (−0.56, 1.49)	0.376	8	0.28 (−0.03, 0.60)	0.076
a^*	5	−0.19 (−0.57, 0.33)	0.608	8	0.03 (−0.32, 0.39)	0.850
b^*	5	−0.06 (−0.44, 0.33)	0.774	8	0.07 (−0.24, 0.38)	0.648
Drip loss	7	0.10 (−0.05, 0.24)	0.190	7	0.30 (0.05, 0.55)	0.019
Shear force	2	−0.40 (−1.06, 0.26)	0.231	5	−0.56 (−1.03, −0.10)	0.017
Intramuscular fat	9	−0.27 (−0.79, 0.26)	0.304	5	0.38 (0.12, 0.60)	0.001

Pauly et al. 2012. Meat Sci 92:858-862

Trait	$D_{C\text{-}EM}$	$D_{IC\text{-}EM}$	$D_{IC\text{-}C}$
Lean meat (%)	−3.00 (16)	−1.99 (4)	0.79 (4)
IMF in the LD (%)	0.55 (13)	0.40 (1)	−0.20 (1)
L^*	0.57 (13)	1.09 (3)	0.13 (3)
Drip loss (%)	−0.01 (13)	0.63 (3)	0.56 (3)
Shear force (kg)	−0.17 (11)	−0.33 (1)	−0.25 (1)
SFA (%)	2.45 (4)	2.47 (2)	−0.37 (2)
MUFA (%)	0.89 (4)	0.70 (2)	−0.95 (2)
PUFA (%)	−3.38 (4)	−3.18 (2)	1.41 (2)
Sensory tenderness	0.00 (9)	0.63 (4)	0.03 (4)
Sensory juiciness	0.06 (8)	0.35 (4)	0.15 (4)

Batorek et al. 2012. J Anim Sci

Item	Treatment[1] EM	IC-L	SC	rmse[2]	*P*-value
Drip loss 24 h, %	3.1[b]	2.4[ab]	2.0[a]	1.1	0.011
Drip loss 48 h, %	5.5[b]	4.7[ab]	4.0[a]	1.6	0.036

Škrlep et al. 2012 CJAS

	EM	IC	SC	RMSE	*P*-value
Drip loss 24h (%)	5.3[b]	2.9[a]	3.6[a]	1.9	0.001
Drip loss 48h (%)	8.5[b]	5.5[a]	6.4[a]	2.1	< 0.001

Trefan et al. 2013. J Anim Sci 91:1480-1492

- IC vs EM: ↗IMF, ↗CIE L
- IC vs SC: most similar

What about meat quality*?

*other than boar taint problem typical for EM

• IMF	EM < IC < SC
• pHu	? study dependent
• Minolta L	? study dependent
• drip loss	?↗ in EM, IC …
• ~~Tenderness~~ toughness	↗ EM
• PUFA	↗EM

Literature is indicative of inferior meat quality of EM, while IC ≈ SC

➔ More studies needed

Questions of interest in EM/IC research where assessment of carcass and meat quality is pertinent

- Evaluating performances of EM/IC/SC in different production systems
  - ✓ Evaluating nutritional strategies for EM or IC
  - ✓ Evaluating vaccination protocols (early, late, adapted to special production systems)
  - ✓ Evaluating rearing, slaughter practices for EM, IC, SC
- Characterisation of meat quality (drawbacks) of EM, IC
- Evaluating aptitude of meat from EM/IC/SC for different further processing methods

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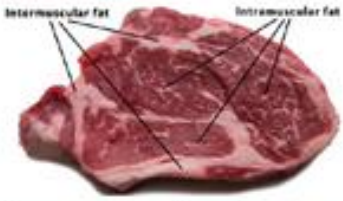
Carcass – fat depots

- **Subcutaneous**

- **Intermuscular**
  any fat between muscle(s) groups



- **Intramuscular**
  - the visible fat (marbling) as fat tissue within a muscle
  - located inside skeletal muscle fibers stored in lipid droplets

- **Intraperitoneal** (leaf fat…)

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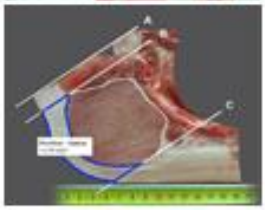
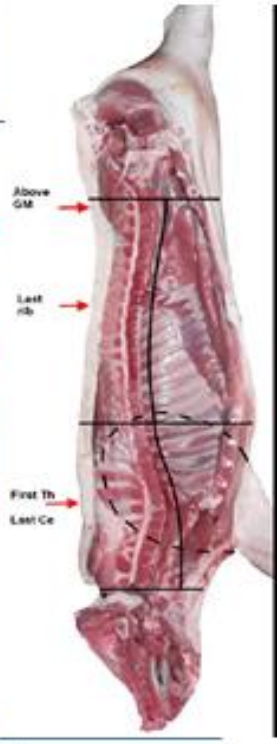
Subcutaneous fat

- measured at different anatomical locations
- as thickness or mass/weight
- with different tools
  - ➢ manual ruler, (digital) calliper
  - ➢ semi-automatic devices (HGP, CGM, FOM, Opti-grade)
  - ➢ weighing/scale

- Harmonisation
  - ➢ select common anatomical site(s)
  - ➢ define common tool





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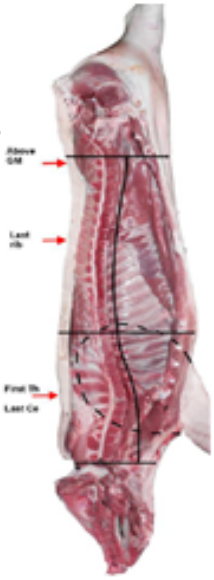
Harmonisation in multi-partner project example SuSI

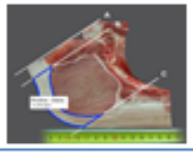
Common anatomical positions (measure fat + skin)

Split line

- above *gluteus medius* muscle (thinnest part)
- At last rib (above last thoracic/first lumbar vertebra)
- At withers (last cervical/first thoracic vertebra)

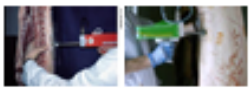


- Lateral – Fat thickness
- Last rib – lateral – Fat area



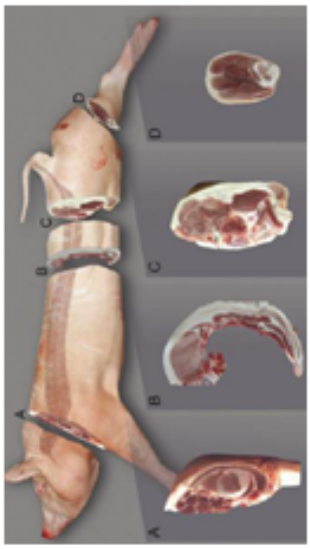
Probe or image of LD cross-section

- 3rd/4th last rib
- 6 cm laterally



Intermuscular fat

- **Different anatomical locations**
  - Carcass cross-sections
  - Digital images – surface measurement (cm^2; % of the cross-section)
  - Subjective evaluation/score
  - Chemical determination of fat or NIRS (defined anatomical slice)
  - pb. different cutting practice, carcass depreciation



- **Harmonisation**
  - select common anatomical position of cross-section
  - select way of measuring (subjective, objective)

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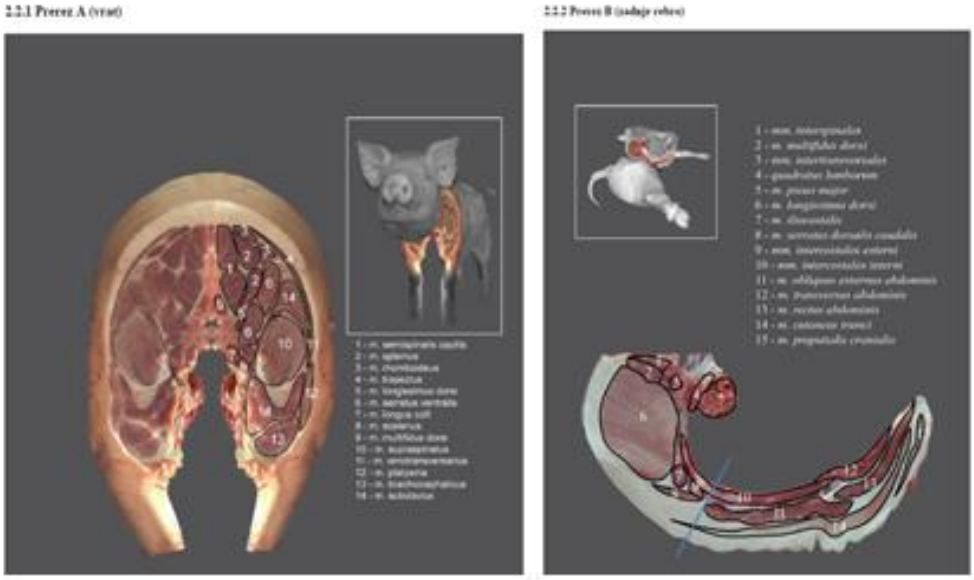
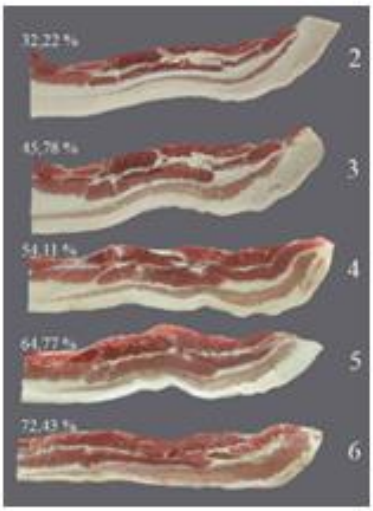
Cross-cut neck and last rib

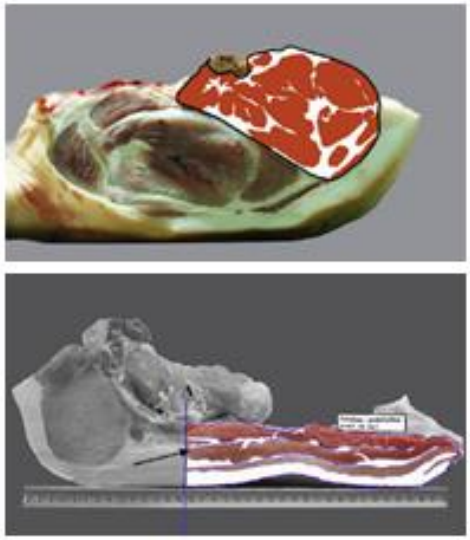


Intermuscular fat – measurement method

Visual evaluation score (1-7);



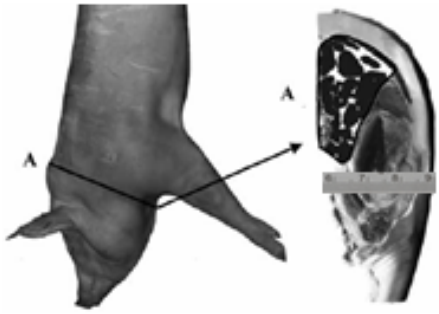
Area determination on digital images (fat:meat)



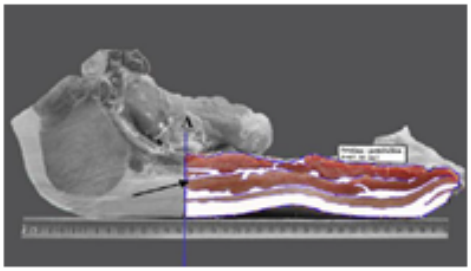
Harmonisation in multi-partner project example SuSI

Harmonisation

- Cross-section 3rd-4th cervical vertebrae
- Image (!! aspect/calibration scale)

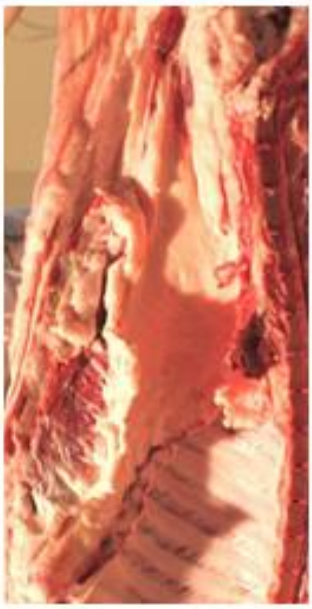


- Cross-section last rib
- Image (!! aspect/calibration scale)



Leaf (flare) fat

- Fat under the peritoneum (abdominal cavity)
- Recording weight at slaughterline
  - both halves if possible
  - removed thoroughly



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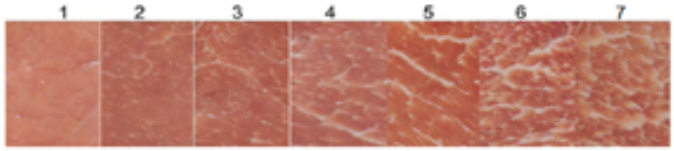
Intramuscular fat

- Deposited within fascia or muscle fiber bundles (visible=>marbling)
- located inside skeletal muscle fibers stored in lipid droplets

- Chemical determination (Folch, Soxhlet extraction) or with NIRS
- Visual determination (marbling score) <- clean cut, light conditions



Harmonisation in multi-partner project example SuSI

- Same muscle and site of sampling
- cross-section of LD at last rib, slice of lumbar LD (one vertebrae)
- Chemical determination or NIRS → ensure cleaning of adjacent connective and fat tissue
- Marbling on 1-7 scale using a reference common scale

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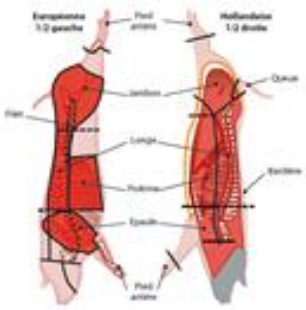
Carcass – muscular development

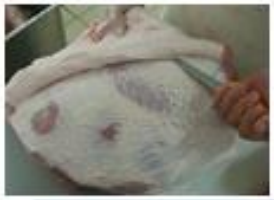
- Body composition - LMP
- Different dissection methods (anatomical sites of cross-cutting)
- neck, loin, ham/hindleg, belly/ribs, shoulder/foreleg
- recording weight, separating tissues (meat, bones, fat)

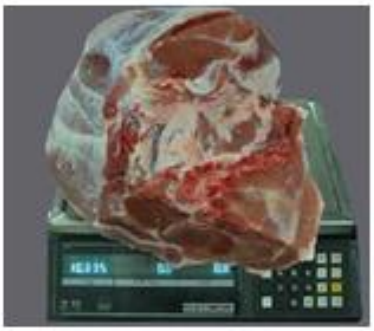


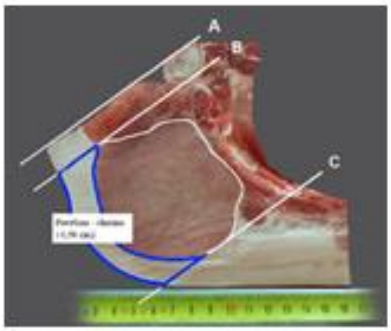
HARMONISATION
= common anatomical sites of cross-cuts

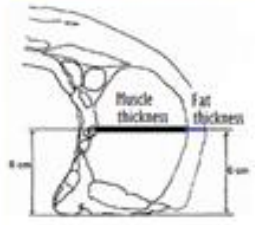
SEUROP - Walstra, Merkus dissection

Muscular development – prime cuts









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Carcass – lean meat assessment

- Manual dissection (weight)
- CT „dissection" (volume)
- On-line indirect methods (e.g. SEUROP) with different national methods to predict lean meat content (probes, AUTOFOM ...)
- Most common is measurements of fat and muscle thickness with probes
- EM, IC mainly not included in equations

Carcass – lean meat assessment

Harmonisation

- Same cutting positions for prime cuts
- Same dissection method

⇒ Laborious, expensive, time consuming, devalorisation of carcasses

⇩

Indirect method for LMP

- harmonisation of SEUROP equations is limited;
- Equations are developed for population and device;
- Non-negligible effect of country, dissection/CT and operator

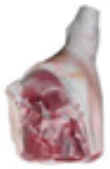
Harmonisation in multi-partner project example SuSI

Partner 3 => Dutch normalized procedure for cutting

Partner 2 => AUTOFOM printout

Partner 1 => Ham weight (cut off between last and last but one lumbar vertebrae and tarso-metatarsal joint) + trimmed ham weight

Partner 4 => Ham weight (cut off between last and last but one lumbar vertebrae and tarso-metatarsal joint) + trimmed ham weight

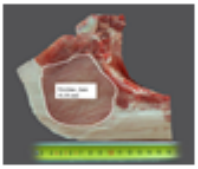


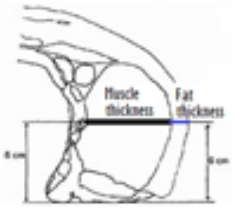
Commom measurements

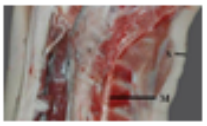
- Loin eye area (image - last rib)
- LD thickness (probe or image)
- M distance (ZP method)

- SEUROP LMP

? Common ZP equation according to Font i Furnols et al. 2016



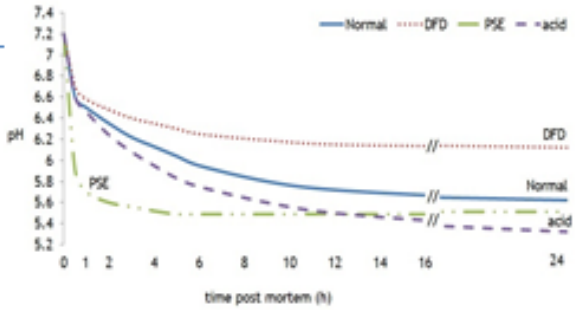




$$LMP = 54.43 - 0.670 \cdot ZP\_fat + 0.214 \cdot ZP\_muscle$$

Meat pH value

- In the moment of slaughter (in vivo) muscle pH is neutral (pH ≈7.2).
- Post-mortem glycolysis leads to lactate production =>lowering of pH value



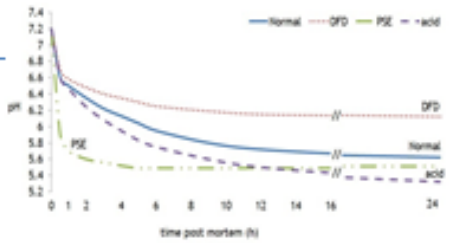
The rate of pH decline p.m.

- is proportional to the rate of the hydrolysis of ATP i.e. mATPase activity
- estimated with measurement of pH30/pH 45min/pH1h
- Fast rate coupled with high body T causes protein denaturation – loss of binding ability - PSE meat

Extent of pH decline.

- proportional to the quantity of produced lactate/available glycogen
- measured with pH24 or pHu
- limited amplitude (>6.0) DFD
- low pH – acid meat

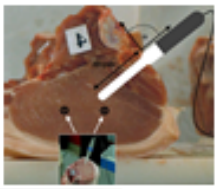
Meat pH value



- Dynamics of pH decline affects meat technological quality (WHC, colour, proteolysis …)

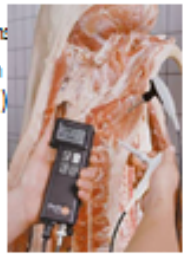
Measurement

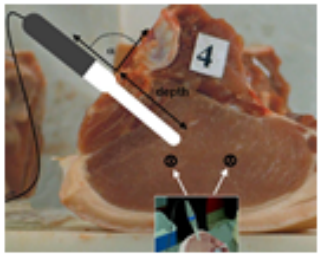
- Directly in meat (puncture electrode)
- In homogenate (distilled water; iodoacetate)
- Early p.m. pH (30 min, 45 min, 1h) – to control the rate of p.m. pH decline;
- Ultimate pH (24 h) – to control the extent of pH decline;
- ↗ accurracy if pH measured in homogenate (esp. 45 min p.m.)



Meat pH value - harmonisation

- Caution to measurement location (n=2-3)
  - LD cross section, defined locations/repetitions
  - Directly in carcass (electrode puncture depth and orientation to be sure of correct muscle)
- Measurement time p.m.
  - defined
- Measurement method
  - Defined i.e. directly or in homogenate
- Importance of correct electrode calibration
  - Maintenance of electrode
  - Fresh/clean buffers
  - At least 2-point calibration (buffer solutions 7.0 and 4.0)
  - **The most accurrate pH is obtained when T of calibration ≈ T of meat** (despite T correction)
  - Recording of mV (for control) esp. at calibration





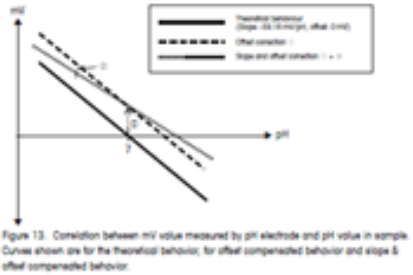
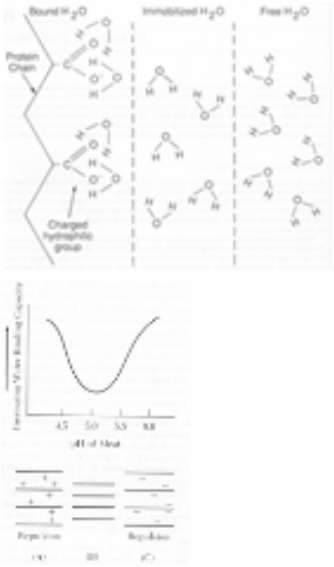


Figure 13. Correlation between mV value measured by pH electrode and pH value in sample. Curves shown are for the theoretical behavior, for offset compensated behavior and slope & offset compensated behavior.

Meat quality – Water holding capacity (WHC)

- ability to retain its own (or added water) when subject to external forces (gravity, cutting, heating, pressure etc.)
- water can be found in different states (chemically bound, immobilised, trapped within myofibrillar structure or as free water)
- WHC is the lowest in isoelectric point (pH≈5.1)

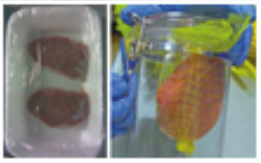


Meat quality – WHC measurement

- Amount of mobilised water depends on the method used

Methods

based on different principles, ±correlated, low repeatability



- No external force applied (gravity)
  - Drip loss - Bag method (Honikel, 1997)
  - Drip loss - EZ drip loss (Rasmunsen & Andersen, 1996)
  - Drip loss - Tray method (Allison et al., 2002)
- Methods with mechanical pressure
  - Filter paper press methods (Δ weight, surface)
  - Centrifugal force method

- Methods with thermal force (cooking loss)
- Other methods (vacuum loss, thawing loss, filter paper-soaking time)

Meat quality - WHC

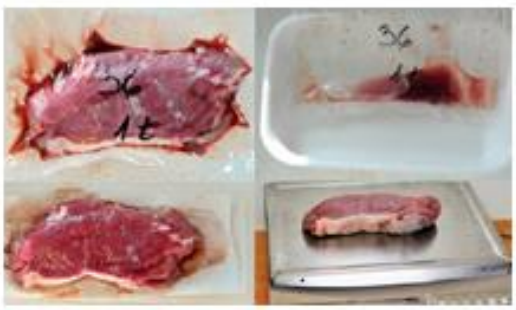
Harmonisation

- Uniform anatomical location, sample size and geometry, temperature
- EZ drip loss method (Christensen, 2003)
  - 2 cm transversal slice of LD at the level of last rib
  - 2 cylindrical samples of 1 inch (from the center of the muscle)
  - Storing in plastic containers at 4°C for 24h or more
  - Gently drain on paper towel before weighing
  - Use a scale with at least 0.01 g precision

Meat quality - WHC

Harmonisation

- Uniform anatomical location, sample size and geometry, temperature
- Thawing loss method
  - Preparing sample of defined geometry/weight e.g. cutting 8×5×4 cm (L×W×H) piece from the center of LD
  - Weighing, vacuum packing, freezing (! Equal conditions), thawing (24h at 4°C)
  - gently drain on paper towel before reweighing



Meat quality - WHC

Harmonisation

- Uniform anatomical location, sample size and geometry, temperature
- Cooking loss method
  - Preparing sample of defined geometry/weight e.g. cutting 8×5×4 cm (L×W×H) piece from the center of LD
  - Placing in plastic bag, cooking in water bath (80°C) until meat temperature reaches 71°C
  - Draining, cooling to 4°C (overnight, protect from desiccation)
  - Reweighing

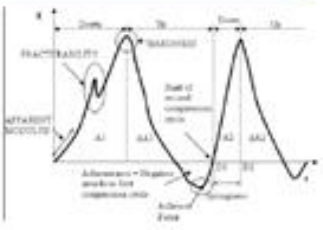


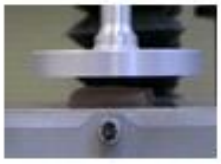
Meat quality – tenderness

➢ resistance to chewing or mechanical force applied

Depends on:

- structure (muscle fibre size, contractile protein, quantity and quality of connective tissue)
- composition (fat, moisture, collagen)
- rate/extent of p.m. conversion of muscle to meat

Methods

- sensory tenderness
- mechanical resistance (to shearing, compression, penetration; TPA, Volodkievich bite tenderometer, Kramer shear cell, Ottawa texture measuring system, SSF, WBSF, ...)

± well inversely correlated to sensory tenderness

Meat quality – tenderness (mechanical)- harmonisation

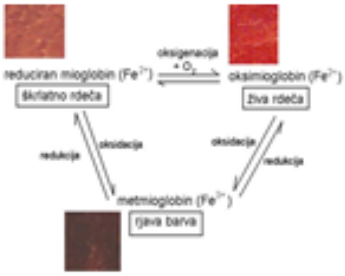
Warner-Bratzler shear force

- Blade (60° V-shaped)
- Sample preparation (1˝ thick, cooked to 71° C, chilled overnight)
- Equal core samples (sharp circular knife, ½'' diameter, minimum of 6 cores – covering entire cross-section, parallel to fibre direction, at room T)
- Shearing (blade speed 1mm/sec, through the center of the core, perpendicular to fibre direction
- Recording peak force - N

Meat quality – colour

Important for consumer purchase decision

- Typical for muscle type and species
- indicator of freshness/spoilage



Depending on

- muscle physiological function
- concentration of pigment-myoglobin
- chemical state of myoglobin (oxygenation/oxidation, denaturation)
- muscle micro structure (reflectance)

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Meat quality – colour

Methodology

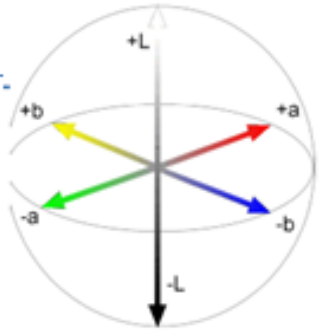
- Subjective (visual) evaluation, scale 1-6
  - Japanese colour scale (Nakai et al., 1975)

  - US National Pork Producers Council (NPPC, 2000)

Meat quality – colour

Methodology

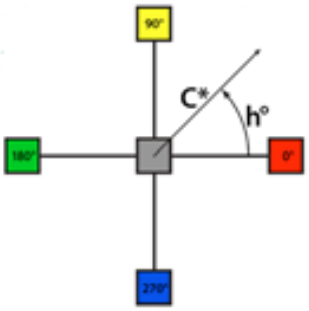
- **Objective (instrumental) evaluation**
- Any colour specified as a combination of red, green and blue
- 3-dimmensional "colour space" (Hunter Lab; CIE Lab):



L*a*b* colour space

- L* lightness (0=dark, 100=white)
- a* (-60 green to +60 red)
- b* (-60 blue to +60 yellow)

L*C*h° colour space



$\sqrt{a^2 + b^2}$ - C* (chroma, saturation), 0=unsaturated, grey to 60=max, high colour purity

$\tan^{-1}(a/b)$ - h° taint (hue angle), 0°=red(+a*), 90°=yellow(+b*), 180°=green(-a*), 270°=blue(-b*)

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Meat quality – colour

Harmonisation

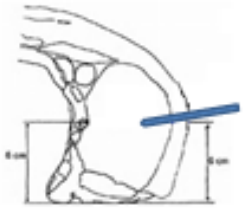
- CIE L*, a*, b* (C*, h°)
- Defining the device (Minolta Chromameter)
- Defined parameters (D65 illuminant, 11 mm diameter aperture, calibration against white tile)
- Time and location (>24h post mortem, cooled muscle, specified location – i.e. LD cross-section, >15 mm thick)
- Blooming (air exposure) defined (affects mainly b*)
  - recommended 1-2h for beef
  - not big influence on pork – stabilisation in <30 min (Škrlep and Čandek-Potokar, 2007)
- Measurement in triplicate (intramuscular variability in colour, marbling)

Harmonisation of meat quality assessment in multi-partner project: example SuSI

Commom measurements

Sampling – measurements 24h p.m.

➢ pH 45 min
- 45 min p.m. - direct measurement (with electrode puncture) in carcass



e.g. using hole made by probes FOM or HGP

➢ pH 24 h
- on LD sample

➢ colour
- (last rib) 24 h p.m.
- LD fresh cut, 3×, Minolta Lab

➢ EZ drip loss

last rib, LD, after 48 h storage at 4°C

➢ WBSF - LD sample 1

- Prepare 8×5×4 cm LD sample
- Weigh the sample, record weight
- Vacuum pack & send frozen for centralised measurement of WBSF (+ thawing, cooking loss)

➢ IMF - LD sample 2
- Slice of LD sample (2 cm)
- Clean off subcutaneous fat
- Vacuum pack & send frozen for centralised measurement with NIRS (imf, water, protein)

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Discussion – harmonisation case study SuSI

- Subcutaneous fat
- Intermuscular fat
- Intramuscular fat
- Muscularity
- Meat quality

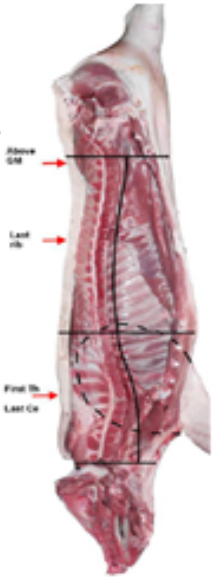
SUBCUTANEOUS FAT

Harmonisation in multi-partner project example SuSI

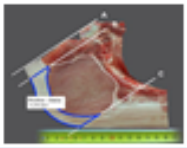
Common anatomical positions (measure fat + skin)

Split line

- above *gluteus medius* muscle (thinnest part)
- At last rib (above last thoracic/first lumbar vertebra)
- At withers (last cervical/first thoracic vertebra)



- Lateral – Fat thickness
- Last rib – lateral – Fat area



Probe or image of LD cross-section

- 3rd/4th last rib
- 6 cm laterally

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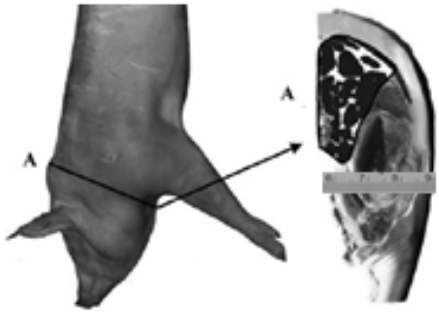
INTERMUSCULAR FAT

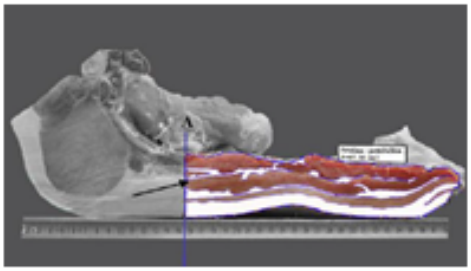
Harmonisation in multi-partner project example SuSI

Harmonisation

- Cross-section 3rd-4th cervical vertebrae
- Image (!! aspect/calibration scale)



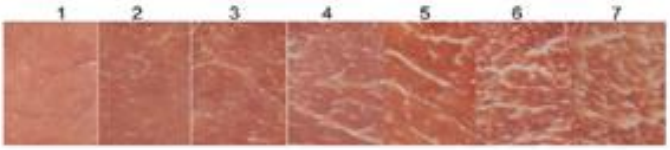
- Cross-section last rib
- Image (!! aspect/calibration scale)



INTRAMUSCULAR FAT

Harmonisation in multi-partner project example SuSI

- Same muscle and site of sampling
- cross-section of LD at last rib, slice of lumbar LD (one vertebrae)
- Chemical determination or NIRS → ensure cleaning of adjacent connective and fat tissue
- Marbling on 1-7 scale using a reference common scale



MUSCULARITY

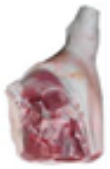
Harmonisation in multi-partner project example SuSI

Partner 3 => Dutch normalized procedure for cutting

Partner 2 => AUTOFOM printout

Partner 1 => Ham weight (cut off between last and last but one lumbar vertebrae and tarso-metatarsal joint) + trimmed ham weight

Partner 4 => Ham weight (cut off between last and last but one lumbar vertebrae and tarso-metatarsal joint) + trimmed ham weight

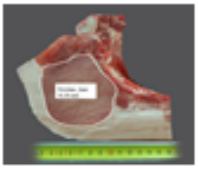


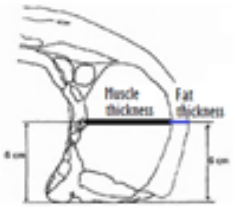
Commom measurements

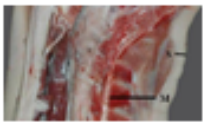
- Loin eye area (image - last rib)
- LD thickness (probe or image)
- M distance (ZP method)

- SEUROP LMP

? Common ZP equation according to Font i Furnols et al. 2016







$$LMP = 54.43 - 0.670 \cdot ZP\_fat + 0.214 \cdot ZP\_muscle$$

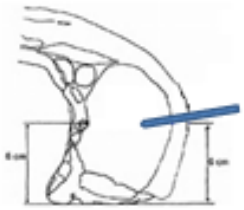
MEAT QUALITY

Harmonisation in multi-partner project example SuSI

Commom measurements

Sampling – measurements 24h p.m.

- pH 45 min
  - 45 min p.m. - direct measurement (with electrode puncture) in carcass



e.g. using hole made by probes FOM or HGP

- pH 24 h
  - on LD sample

- colour
  - (last rib) 24 h p.m.
  - LD fresh cut, 3×, Minolta Lab

- EZ drip loss

last rib, LD, after 48 h storage at 4°C

- WBSF - LD sample 1

- Prepare 8×5×4 cm LD sample
- Weigh the sample, record weight
- Vacuum pack & send frozen for centralised measurement of WBSF (+ thawing, cooking loss)

- IMF - LD sample 2
- Slice of LD sample (2 cm)
- Clean off subcutaneous fat
- Vacuum pack & send frozen for centralised measurement with NIRS (imf, water, protein, FA?)

Chapter 5

Anatomy of reproductive tract – measurements and sampling

Gregor FAZARINC[1]

Anatomy of reproductive tract – measurements and sampling

Prof. dr. Gregor Fazarinc
Veterinary faculty, University of Ljubljana
Institute of Preclinical Sciences

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cost

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RATIONALE

- Non-responders, not properly vaccinated pigs, pigs which escape vaccination exhibit boar taint similar to entire male
- Determination of boar substances (androstenone and skatole) is expensive and time consuming.
- Size of reproductive organs could serve as reliable indicator of successful immunocastration.
- INTENTION – to find a simple and applicable indicator of immunocastration efficiency on the basis of reproductive organs weights for the use in the slaughterhouse

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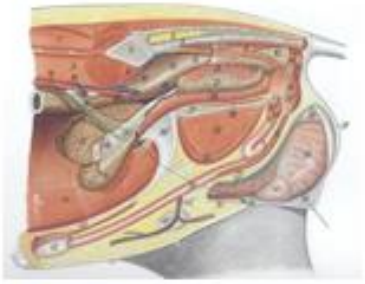
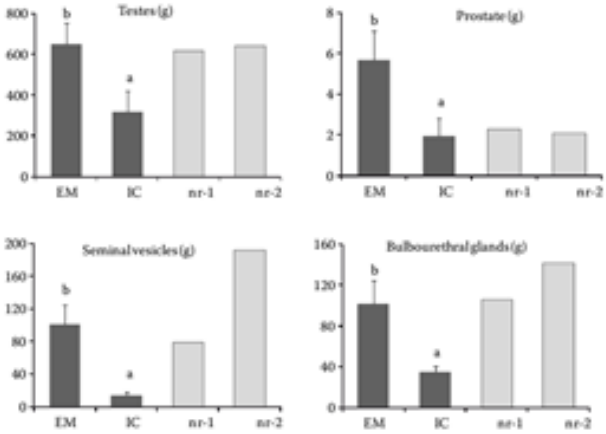
[1] University of Ljubljana, Veterinary Faculty, Gerbičeva 60, 1000 Ljubljana, Slovenia

Anatomy of reproductive organs in boar



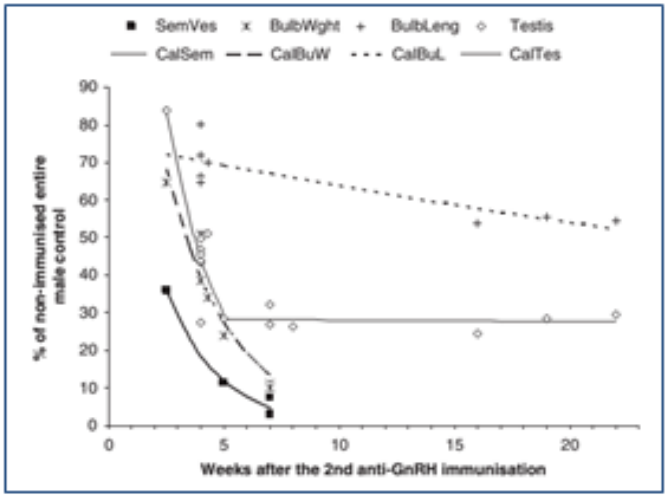
- **Large testis and epididymis**
  - Scrotum
  - Vaginal tunic
- **Perineal position**
- **Long spermatic cord**
- **Large accessory glands**
  - Vesicular gland
  - Prostate
  - Bulbourethral gland
- **Fibroelastic penis with the sigmoid flexure**

Size of reproductive organs - indicator of successful IC



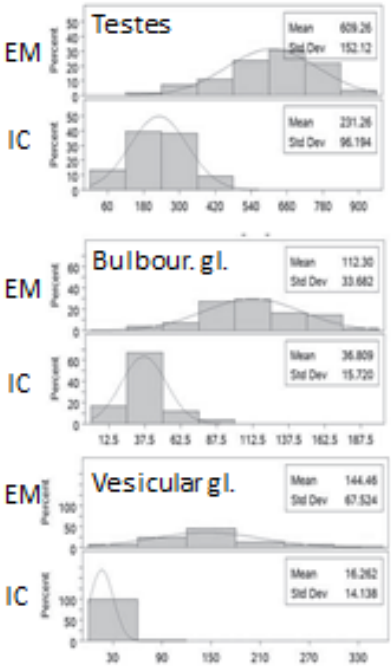
Weight of reproductive organs (mean ± SD) in the case of entire males (EM), immunocastrates (IC), and two non-responders (nr-1, nr-2). Pigs were slaughtered 5 weeks after second immunization. (*Škrlep et al., Czech J Anim. Sci. 2012*)

Vesicular gland and reproductive organs regression - the most reliable indicator of successful IC



Plot of genital tract development in immunocastrates (expressed as percentage of the corresponding development in entire male) against time after the second immunization. (*Bonneau, Animal 2010*)

Discriminant analysis of testes and accessory glands



Recognition rates of IC

- Testes: 94.7 %,
- Bulbourethral gland: 96.16 %
- Vesicular gland: 98.7 %
- All 3 criteria: 98.7 %

Candek-Potokar *et al.*, Proceedings of the International Symposium on Animal Science, 2014 Belgrade

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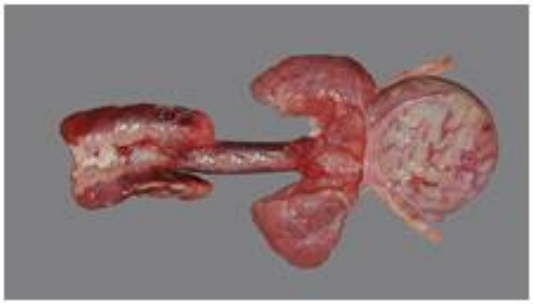
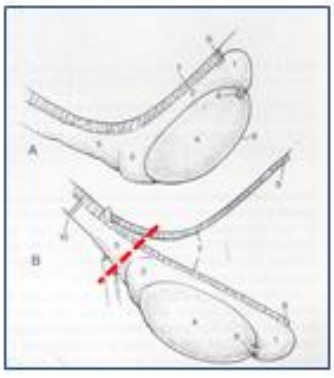
IPEMA

Procedure

- Separate rectum and anus from the pelvic urogenital tract
- Incise at the apex of the bladder and squeeze the urine out
- Clean the pelvic part of urogenital tract of excessive tissues
- Cut of the penis and penis muscles next to the caudal pole of the bulbourethral glands.
- **Weight the pelvic part of the urogenital tract together with the accessory glands and emptied bladder**
- Dissect and remove the vesicular and bulbourethral glands and weight them separately (use plastic tray of known weight)



- Remove both testis, epididymis and spermatic cords from the scrotum and vaginal tunic.
- Cut of the spermatic cord from the testis at the level of caput of the epididymis.
- Weight both testes together with epididymis



1 tail of epididymis
2 body of epididymis
3 head of epididymis
4 testis
5 spermatic cord
6 free border of testis
7 mesorchium
8 proper ligament of testis
9 ligament of tail of epididymis
10 section of spermatic cord to remove testis

Obtained weights

- Pelvic urogenital tract (including accessory glands, pelvic urethra and bladder)
- Seminal glands
- Bulbourethral glands
- Testes and epididymis

CALCULATE

- obtained weights/carcass weight (%)

Measurement option

Option 1

- Relative weights of the **pelvic urogenital tract including accessory glands**. This method is the fastest and easiest to perform.

Option 2

- Relative weights of **pelvic urogenital tract plus testis and epididymis**. This procedure demands some extra time because of testes and epididymis removal from the vaginal tunic sack .

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Chapter 6

Muscle tissue analysis - histochemistry

Milka VRECL FAZARINC[1]

[1] University of Ljubljana, Veterinary Faculty, Gerbičeva 60, 1000 Ljubljana, Slovenia

Muscle fiber characteristics – effect of immunocastration (IC)

- **immunocastrates have lower proportion of MyHC-IIb positive myofibers in the longissimus muscle than SC** *(Li et al., Meat Science 2015)*
- **immunocastrates have higher intramuscular fat content than EM** *(Batorek et al., Animal 2012)*

Histological methods

- Enzyme–/immuno–histochemistry
  - classification of myofiber types
- Histochemical staining
  - intramuscular fat (IMF)
  - connective tissue
- Morphometrical analysis
  - myofiber type percentage and cross sectional area
  - IMF and connective tissue quantification

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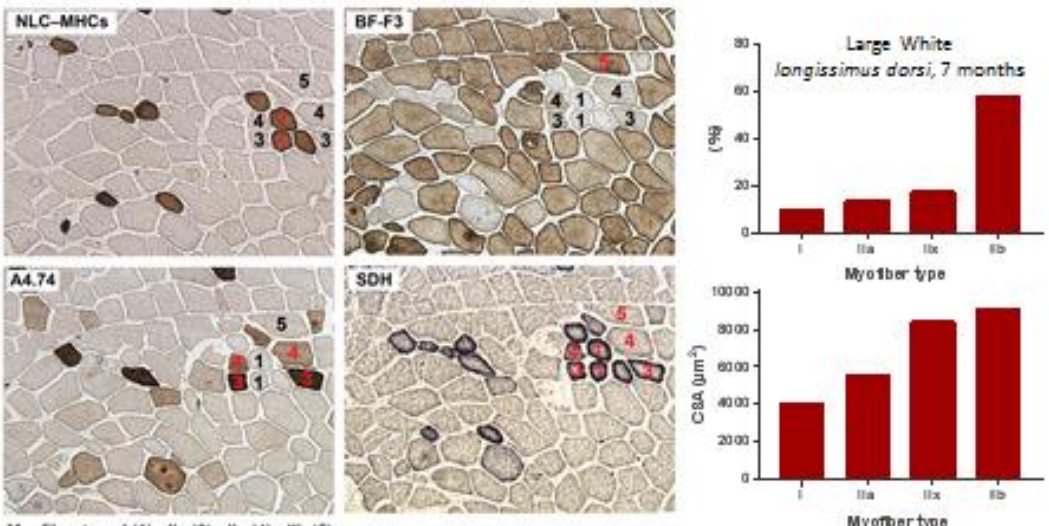
Characterization of pig myofiber types - immuno- and enzyme-histochemistry

		Myofiber type				
		I	I/IIa	IIa	IIx	IIb
Antibody	NLC-MHCs	++	++/+	–	–	–
	SC7.1/A4.74	–	++/+	++	+	–
	BF-F3	–	–	–	+/–	++
SDH		++	++	++	+	–

+ moderate positive reaction; ++ strong positive reaction; – negative reaction.

Fazarinc, Vrecl, Škorjanc *et al.* Animal 2017; 11(1):164-174.

Classification of myofiber types by immuno–/enzyme–histochemistry



Myofiber type I (1), -IIa (3), -IIx (4), -IIb (5).

Adopted from Fazarinc *et al.* Animal 2017; 11(1):164-174.

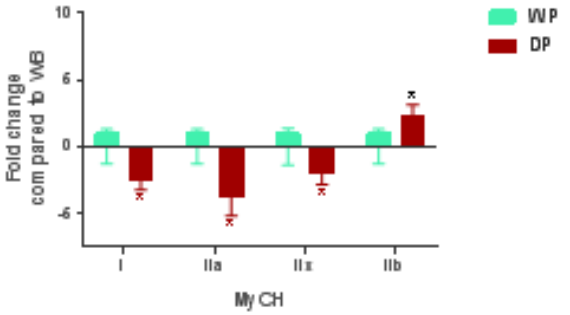
Quantitative polymerase chain reaction (qPCR)

MyHC isoform

- *MyHC–I*
- *MyHC–IIa*
- *MyHC–IIb*
- *MyHC–IIx*

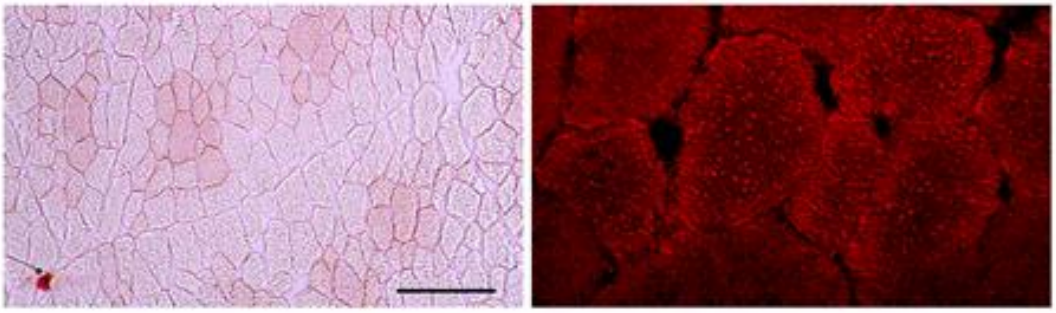
Lipid metabolism-related genes

- *PGC-1α*
- *PPARγ*
- *LPL*
- *CPT1B*



Intramyofiber lipids - Oil Red O staining

Wild pig, *longissimus dorsi*, 2 years



Oil red O staining protocol developed by Koopman et al., Histochem Cell Biol 2001; 116: 63-8.

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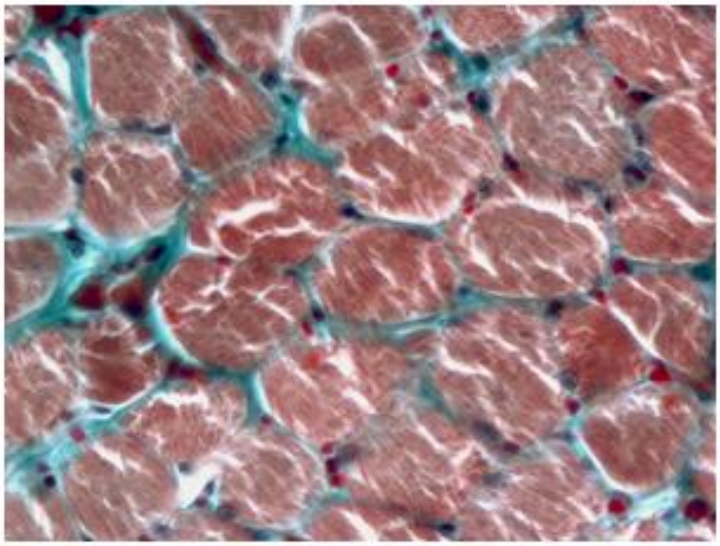
Connective tissue - Masson-Goldner trichrome staining



Muscle tissue samplling

M. longissimus dorsi

- approx. 1 cm^3 muscle sample from the central part of the muscle, at the level of the last rib

M. semispinalis capitis *(m. biventer cervicis and m. complexus major)*

- approx. 1 cm^3 muscle sample from the central part of the ***m. biventer cervicis,*** at the level of the 4^{th} cervical vertebra

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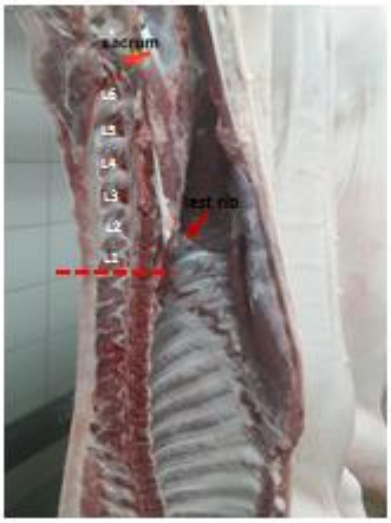
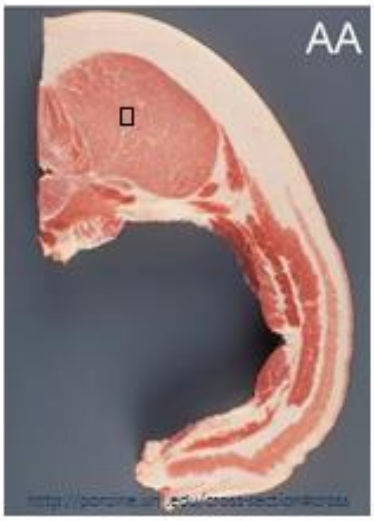
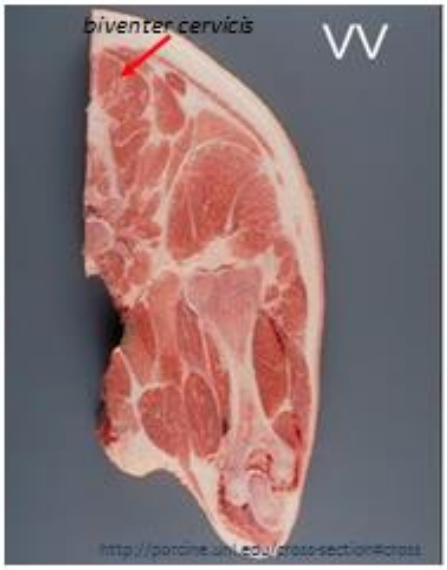
M. longissimus dorsi





M. semispinalis capitis (m. biventer cervicis)



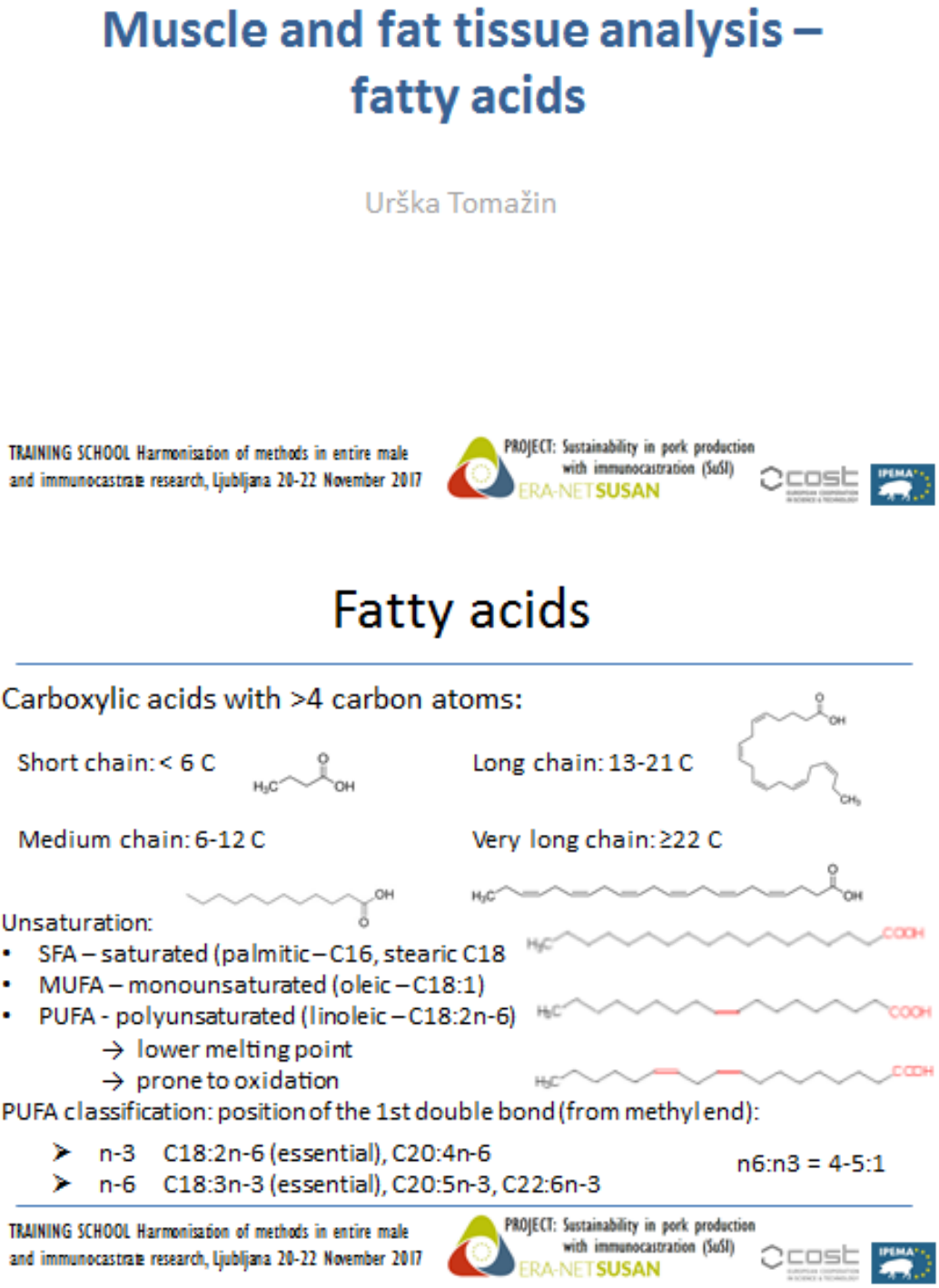
Sample handling/labeling

- froze sample in liquid nitrogen
- *wrap samples individually in aluminum foil* together with sample identifier
  - **Partner 5 – UL-VF -** number series of 5XXXX (partner, trial, set, animal number)
  - Please add LD (*longissimus dorsi)* and SC *semispinalis capitis*
- store samples at -80°C until shipped on dry ice.

Chapter 7

Muscle and fat tissue analysis – fatty acids

Urška TOMAŽIN[1]



[1] Agricultural Institute of Slovenia (KIS), Hacquetova ulica 17, 1000 Ljubljana, Slovenia

Fatty acids in pig meat/fat

Fat

Phospholipid fraction (polar)

- an essential component of cell membranes
- constant amount
- higher proportion of PUFA

Neutral fraction

- fat deposits (subcutaneous, intermuscular, intramuscular)
- increaseswith body fatness
- higher proportion of SFA

Table 4. Backfat and muscle lipid content of pigs fed crushed linseed for 20, 60, or 100 d

	Control diet			Linseed diet						
Lipid content	20 d	60 d	100 d	20 d	60 d	100 d	SE	D[a]	T[a]	D × T[a]
Total lipid content of backfat, g/100 g of tissue	69.4	85.3	86.0	75.7	82.4	86.7	0.74	NS[b]	**	NS
Phospholipid content of muscle, g/100 g of tissue	0.45	0.45	0.36	0.47	0.41	0.39	0.07	NS	*	NS
Neutral lipid content of muscle, g/100 of g tissue	0.53	0.80	0.92	0.50	0.59	0.84	0.14	*	***	NS
Total lipid content of muscle, g/100 g of tissue	0.98	1.25	1.28	0.97	1.00	1.23	0.16	*	***	NS

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
[a]D = diet effect; T = time on feed (20, 60, or 100 d); and D × T = diet × time on feed interaction.
[b]NS = no significant difference ($P > 0.05$).

Kouba et al., 2003

Effects on FA composition

NUTRITION: FA of feed >>> FA of pork

- Linseed → increased n-3 PUFA
- Pasture → increased PUFA, decreased SFA
- Traditional systems (acorn, chestnut feeding) → increased MUFA

BREED →
SEX → **ADIPOSITY**

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Effects on FA composition

BREED

Fatty acid composition according to genetic type (% of the total fatty acids).

Author	Breed	SFA	MUFA	PUFA
Comparison among pure breeds				
Labroue et al. (2000), on fresh subcutaneous fat	Basque	43.1a	45.2a	11.7a
	Gascon	46.6b	43.5b	9.9b
	Limousine	46.3b	43.9b	9.9b
	Blanc de l'Oueste	41.3b	46.8a	12.0a
	Large White	41.7c	42.4c	16.0c
Franci et al. (2005), on fresh subcutaneous fat	Cinta Senese	36.2a	50.3a*	10.4a
	Large White	37.6b	48.5b*	11.1b
Madonia et al. (2007), on salami	Nero Siciliano	33.39a	53.29a	13.33a
	Large White	37.71b	47.42b	14.87b

Local breeds: ⇧ MUFA
(differences in *de novo* synthesis)

Pugliese&Sirtori, 2012

Effects on FA composition

SEX: EM have lower content of all body fat tissues (subcutaneous fat, IMF, leaf fat)

	Entire males	IC	Castrates	Females	Reference
Backfat thickness, mm	13.2^{b}	15.1^{b}	18.3^{a}	/	Škrlep et al., 2010
Backfat thickness, mm	13.6^{a}	14.2^{a}	17.7^{b}	/	Aluwé et al. 2013
Backfat thickness, mm	17.8^{c}	19.3^{b}	24.9^{a}	/	Pauly et al. 2009
Backfat thickness, mm	17.6^{a}	18.3^{ab}	23.0^{c}	19.3^{b}	Grela et al., 2013
Backfat thickness, mm	15.9^{c}	21.0^{b}	24.6^{a}	18.6^{bc}	Gispert et al., 2010
IMF (SM muscle), %	1.84^{b}	2.07^{ab}	2.47^{a}	1.72^{b}	Gispert et al., 2010
IMF (LD muscle), %	1.56^{a}	1.58^{a}	1.98^{b}	/	Škrlep et al., 2010
Leaf fat, kg	1.23^{c}	1.68^{b}	2.12^{a}	1.61^{b}	Gispert et al., 2010
Leaf fat, kg	0.9^{c}	1.1^{b}	1.3^{a}	/	Škrlep et al., 2010

Effect on FA composition

Lower adiposity of EM→ lower SFA and higher PUFA concentrations

Table 4 *Fatty acid composition of the adipose tissue from barrows (C), immunocastrated (IC) and entire male pigs (EMG) raised in group pens (experiment 1)*[f]

	Experimental group			
	C	IC	EMG	s.e.
Total fatty acids	862.8[a]	838.1[b]	839.1[b]	5.10
Total SFA	43.79[a]	42.02[b]	39.49[c]	0.553
Total MUFA	42.59	42.87	42.78	0.407
Total PUFA	13.61[c]	15.10[b]	17.71[a]	0.439

FA content of subcutaneous adipose tissue of castrates, immunocastrates and entire males

	C	IC	EM
SFA	280.6[a]	250.9[a]	214.9[b]
MUFA	361.5[a]	312.9[b]	300.3[b]
n-3 PUFA	6.53	7.24	7.07
n-6 PUFA	91.5[a]	102.7[b]	115.8[b]

Pauly et al., 2009

Mackay et al., 2013

Effect of sex on FA composition

Table 4. TBARS* value and fatty acid composition (%) of the *longissimus* muscle fat.

Parameter	Experimental groups			
	EM	IM	CM	G
SFA	39.55[a]	40.53[b]	40.85[b]	39.06[a]
MUFA	48.99[a]	48.50[a]	50.06[b]	50.25[b]
PUFA	11.71[a]	10.49[b]	8.63[c]	10.42[b]
PUFA:SFA	0.30[a]	0.26[b]	0.21[c]	0.27[b]
n-6/n-3	18.85[a]	18.07[a]	21.13[b]	20.71[b]

Table 5. Fatty acid composition (%) of the backfat.

Fatty acids	Experimental groups			
	EM	IM	CM	G
SFA	37.24[a]	41.66[b]	41.99[b]	40.47[b]
MUFA	44.67[a]	42.98[b]	45.00[a]	44.86[a]
PUFA	13.20[a]	10.68[b]	8.69[c]	10.36[b]
PUFA:SFA	0.35[a]	0.26[b]	0.21[c]	0.25[b]
n-6/n-3	14.90[a]	15.18[a]	16.04[ab]	17.18[b]

Grela et al., 2013

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↑PUFA >>> ↑oxidation of lipids

Table 5
Total fatty acid composition (%) of intramuscular fat in longissimus muscle

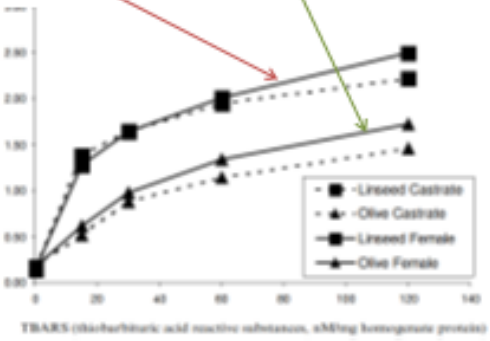
Fatty acids	Linseed group		Olive group		Significance ($p \leq 0.05$)
	Castrates, LSM_{SEM} ($N = 5$)	Females, LSM_{SEM} ($N = 7$)	Castrates, LSM_{SEM} ($N = 7$)	Females, LSM_{SEM} ($N = 6$)	
SFA (sum of saturated FA)	$35.1_{0.7}$	$32.6_{0.6}$	$35.6_{0.6}$	$32.2_{0.6}$	S
PUFA	$23.9_{1.0}$	$31.5_{1.1}$	$13.4_{1.1}$	$19.5_{1.0}$	S, F
n-3 fatty acids	$11.0_{0.6}$	$15.9_{0.7}$	$1.4_{0.1}$	$1.9_{0.1}$	S, F
n-6 fatty acids	$12.8_{1.1}$	$18.2_{1.3}$	$12.0_{1.1}$	$17.6_{1.0}$	S
n-6/n-3 ratio	$1.2_{0.1}$	$1.6_{0.1}$	$9.5_{0.6}$	$9.6_{0.6}$	F

S – significant influence of sex ($p \leq 0.05$), *F* – significant influence of feeding ($p \leq 0.05$), F×S – significant interaction between feeding and sex ($p \leq 0.05$).

Pigs fed linseed oil vs. olive oil suppl. diet

↑PUFA
↓oxidative stability
↓overall flavour

Neuernberg at al., 2005



TBARS (thiobarbituric acid reactive substances, nMol/mg homogenate protein)

Knowledge gap: lipid oxidation of EM compared to other sexes

FA of entire male pigs – boar taint

	LL	HH	s.e.	F-value	p-Value
Hot carcass weight, kg	86.6	84.7	2.00	0.47	0.4998
Age, d	177.7	182.2	5.41	0.35	0.5641
Back fat thickness, mm	17.7	18.5	0.13	0.19	0.6688
Lean meat yield*, %	62.9	59.2	0.62	17.99	0.0005
Intramuscular fat, %	1.04	1.19	0.13	0.69	0.4164
Androstenone, ng/g[b]	97.7	2983.7	305.53	44.61	<.0001
Skatole, ng/g[b]	37.5	464.2	38.41	61.71	<.0001
Indole, ng/g[b]	33.9	331.9	68.67	9.41	0.0066
Σ SFA	35.21	37.70	0.661	7.08	0.0159
Σ MUFA	41.43	42.57	0.703	1.32	0.2661
Σ PUFA	23.36	19.73	0.968	7.04	0.0162

High level of androstenone, skatole, indole (no diff. in backfat thickness or IMF)

↓

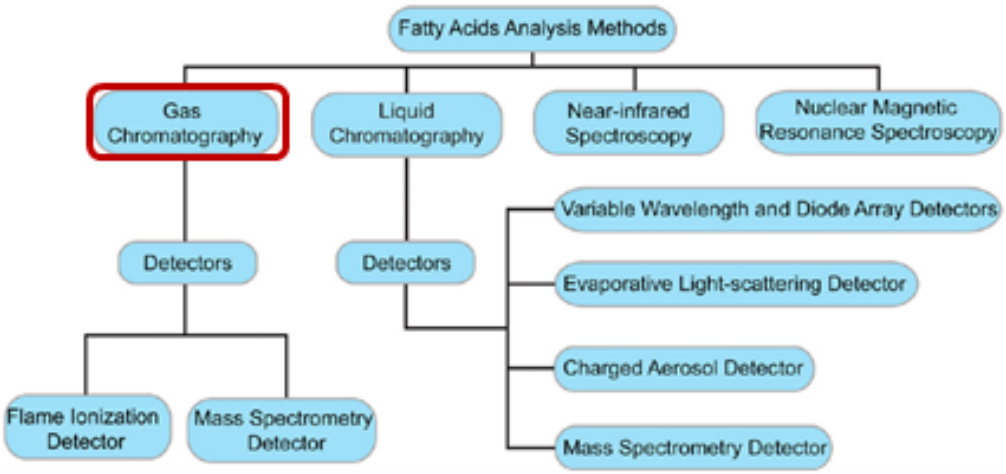
Higher proportion of SFA
Lower proportion of PUFA

???? Increased levels of A and S affect lipid synthesis and lipid metabolism related enzyme activity (androstenone inhibited by CYP activity in pigs' hepatocytes) ????

???? Decreased PUFA levels in animals with high levels of A and S could (partly) be due to oxidative processes because of free radical formation as induced by high S content ????

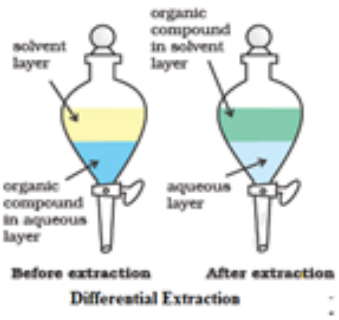
Mörlein&Tholen, 2015

FA analysis - methods



FA analysis

- **Storage:** at low temperature (-20°C or lower) to avoid oxidation of FA (PUFA!!!)
- **Extraction:**
  - Folch (1957): chloroform/methanol/water (8/4/3; v/v/v)
    (Blight&Dyer, 1959, Hara and Radin, 1978)
  - no extraction



- **Esterification (fatty acids are not volatile):**
  - acid-catalyzed (MetOH/HCl (5%), MetOH/H_2SO_4 (10%), MetOH/BF_3)
  - base-catalyzed
  - diazomethane

FA analysis – in situ (Park& Goins, 1994)

Sample
+CH_2Cl_2
+ 0.5 NaOH in MetOH
+internal standard (C:19)
Heating at 90°C
Esterification
+ BF_3
Heating at 90°C
+ water
+ hexane
Shaking, centrifuging
hexane phase is transfered to the vial

a direct transesterification:

+++ reducing the amount of toxic solvents needed for the extraction

+++ reducing the analysis time

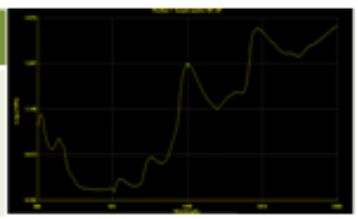
Detection by GC (non-polar column)

FA are expressed in wt% (g/100 g fat)

FA analysis - NIRS

- Allows the characterization of food and quality control throughout processing
- Based on physical principles of energy absorption of organic molecules at specific wavelength
- Applications in meat sector: fast, simple checks of quality of the raw material >>> amount of fat and fatty acid composition
- Control during processing, especially for products with long maturation time

Conventional methods	NIRS
- Destructive - Time consuming + High accuracy	+ Nondestructive + Rapid - Calibration



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FA analysis - NIRS

Subcutaneous fat of Iberian pigs; González-Martín et al., 2003

Table 2
Measurement with fibre optic. Intact samples. Calibration statistical descriptors for the NIR determination of the fatty acids

Components	Mathematical treatment	RSQ	SEC (%)	SECV (%)	No. of principal components	Probability explained (%)
C14:0	SNV-DT/1st derivative	0.6730	0.0947	0.1028	12	97.57
C16:0	SNV-DT/1st derivative	0.9377	0.5769	0.7352	12	98.28
C18:0	DT/2nd derivative	0.8649	0.6917	0.7968	12	99.20
C18:1	SNV-DT/2nd derivative	0.8917	0.9747	1.1868	12	99.20
C18:2	DT/2nd derivative	0.9528	0.4312	0.5224	12	99.21
C18:3	DT/2nd derivative	0.6115	0.1051	0.1278	12	99.21
C20:1	DT/1st derivative	0.5432	0.2102	0.2402	12	98.28
Σpolyunsaturated	DT/2nd derivative	0.9481	0.4746	0.6027	12	99.41
Σmonounsaturated	SNV/1st derivative	0.8967	0.9770	1.4966	12	98.28
Σsaturated	SNV-DT/1st derivative	0.9578	0.8633	1.1025	12	97.57

	Minimum	Maximum	Mean	SD
C14:0	0.78	1.77	1.28	0.17
C16:0	15.87	29.74	22.80	2.31
C18:0	4.61	15.90	10.25	1.88
C18:1	43.50	61.27	52.38	2.96
C18:2	2.03	13.94	7.98	1.99
C18:3	0.13	1.14	0.64	0.17
C20:1	0.45	2.32	1.38	0.31
Σpolyunsaturated	2.31	14.82	8.56	2.08
Σmonounsaturated	47.37	65.62	56.50	3.04
Σsaturated	22.09	47.31	34.70	4.20

Predictions are good for predominant FA:
- palmitic,
- stearic,
- oleic,
- linoleic acid,
- FA groups (SFA, MUFA, PUFA)

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FA analysis - NIRS

FA group (g/100 g fat)	Calibration R^2_c	Calibration se_c	Validation R^2_{cv}	Validation se_{cv}	RPD
	Fat tissue				
SFA	0.95	0.439	0.83	0.791	2.4
MUFA	0.98	0.350	0.91	0.696	3.2
PUFA	0.97	0.315	0.89	0.568	3.1
n-3 PUFA	0.96	0.035	0.83	0.076	2.6
n-6 PUFA	0.97	0.286	0.89	0.507	3.1
n-6/n-3 PUFA	0.80	0.480	0.30	0.894	1.3
	Muscle tissue				
SFA	0.98	0.255	0.58	1.332	1.5
MUFA	0.18	2.387	0.11	2.535	1.0
PUFA	0.78	1.508	0.53	2.209	1.4
n-3 PUFA	0.62	0.119	0.55	0.130	1.9
n-6 PUFA	0.77	1.428	0.52	2.075	1.4
n-6/n-3 PUFA	0.12	1.445	0.02	1.524	1.2

FAT samples:
results for the prediction of FA groups are very good

MUSCLE samples:
the accuracy is much worse (low fat content)

Prevolnik Povše et al., 2017

TRAINING SCHOOL Harmonisation of methods in entire male and immunocastrate research, Ljubljana 20-22 November 2017

PROJECT: Sustainability in pork production with immunocastration (SuSI)
ERA-NET SUSAN

SuSI: harmonisation of method

- Sampling: backfat tissue at withers (the same sample as for androstenone/skatole analysis)
- Storage: vacuum packed (-20°C or lower)
- Homogenisation into fine dust with liquid nitrogen
- In situ preparation (no extraction) → detection of FA methyl esters by GC
- Results are expressed in g FA/100 g fat
- NIRS → samples scanned intact: content of SFA, MUFA, PUFA

Thank you for your attention.

Chapter 8

Gastric ulcer scoring

Hanne MARIBO[1]

HOW TO EVALUATE ULCERS IN DENMARK

Svend Haugegaard & Hanne Maribo
Danish Pig Research Centre

Ljubljana 21/11-2017

SEGES

2

SEGES

[1] SEGES, Axeltorv 3, 1609 København V, Denmark

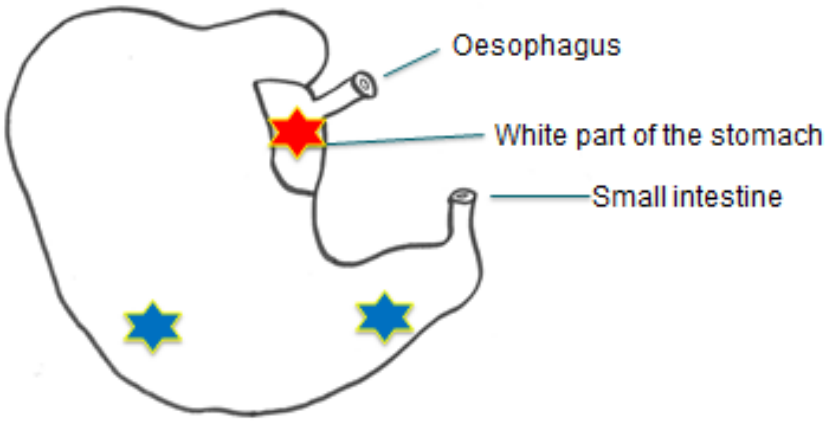
DO I HAVE A PROBLEM WITH ULCERS IN THE HERD

At least 20 pigs for USK

Finishers:

the middle if more than 1 delivery

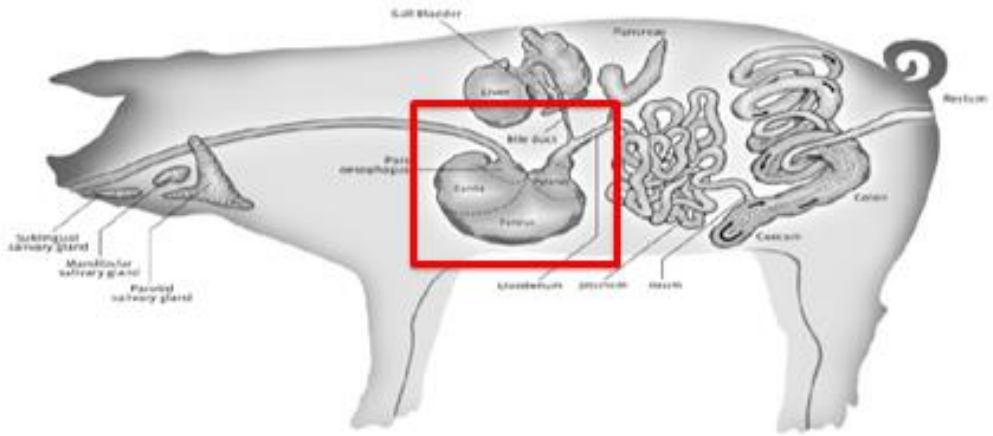
SEGES

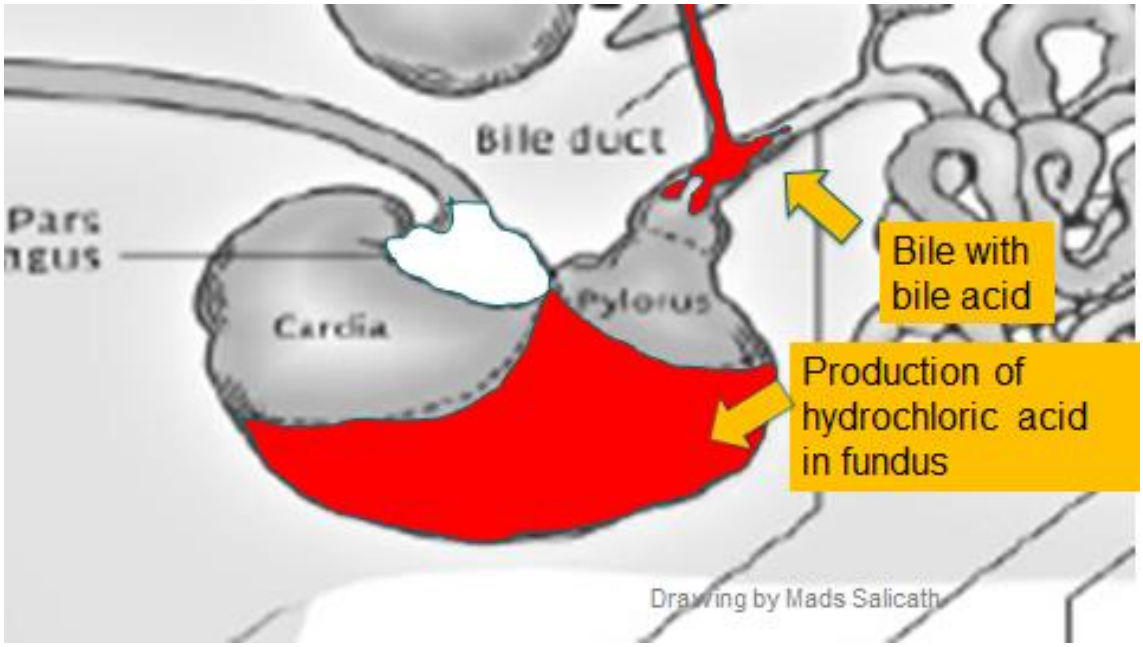


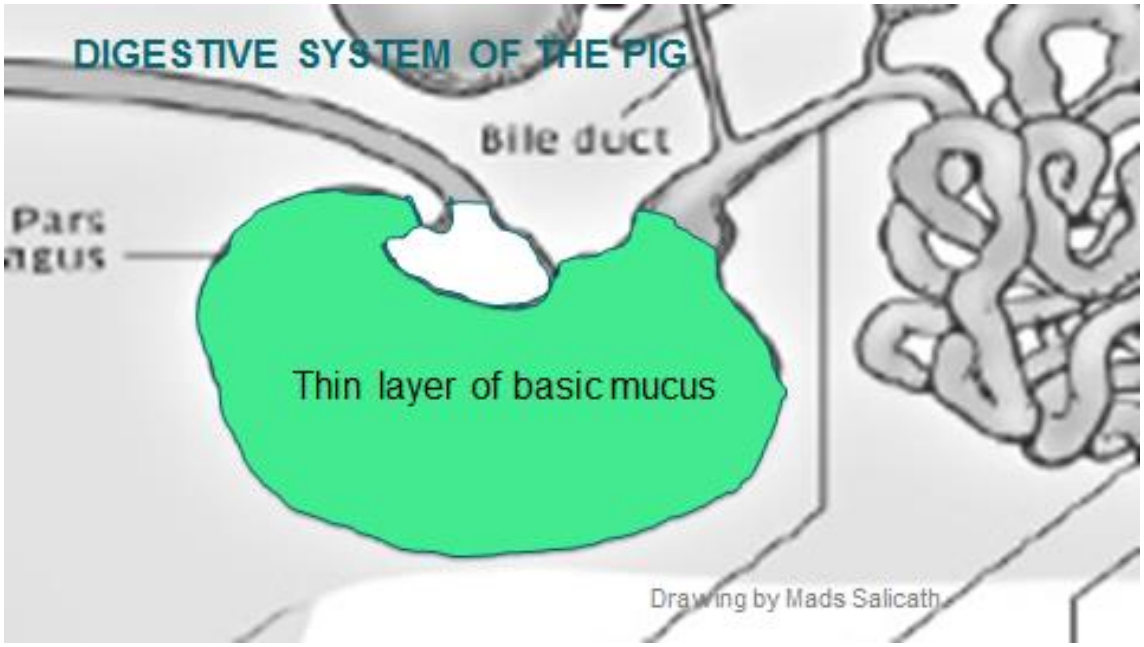
SEGES

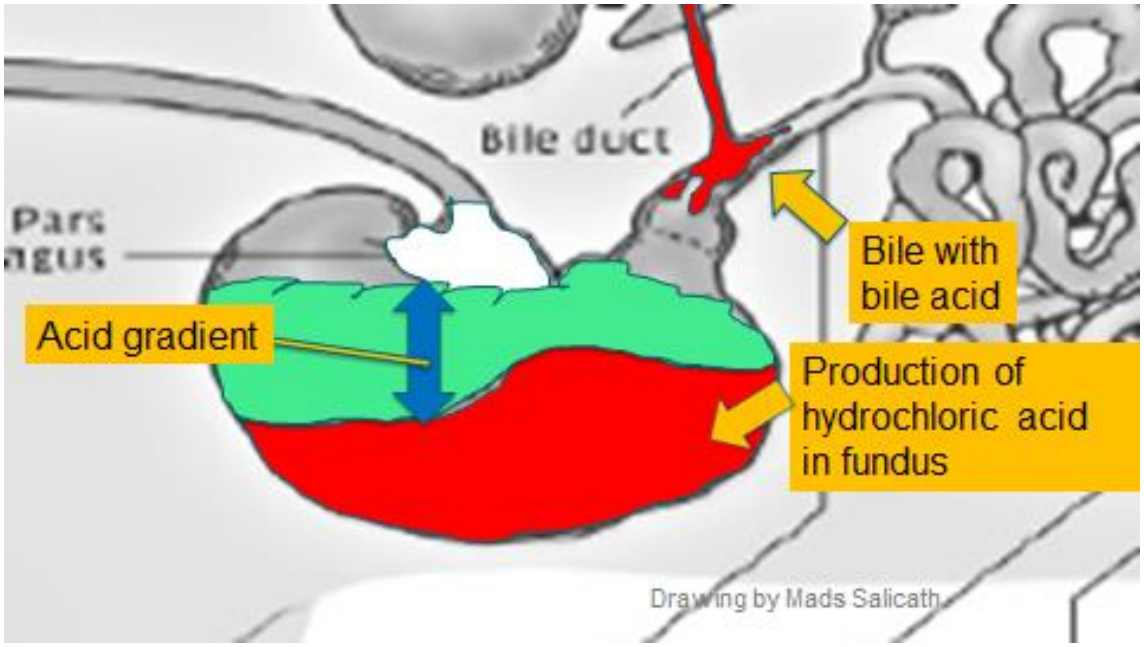
4

DIGESTIVE SYSTEM OF THE PIG



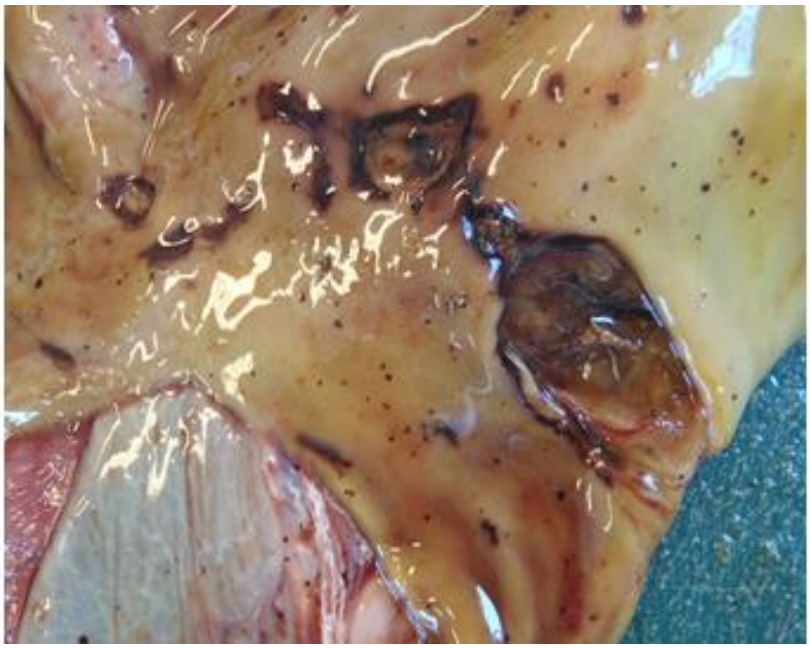



DIGESTIVE SYSTEM OF THE PIG
Bile duct
Pars
Thin layer of basic mucus
Drawing by Mads Salicath


Bile duct
Pars
Acid gradient
Bile with
bile acid
Production of
hydrochloric acid
in fundus
Drawing by Mads Salicath

9 Photos: Christian Fink Hansen

SEGES



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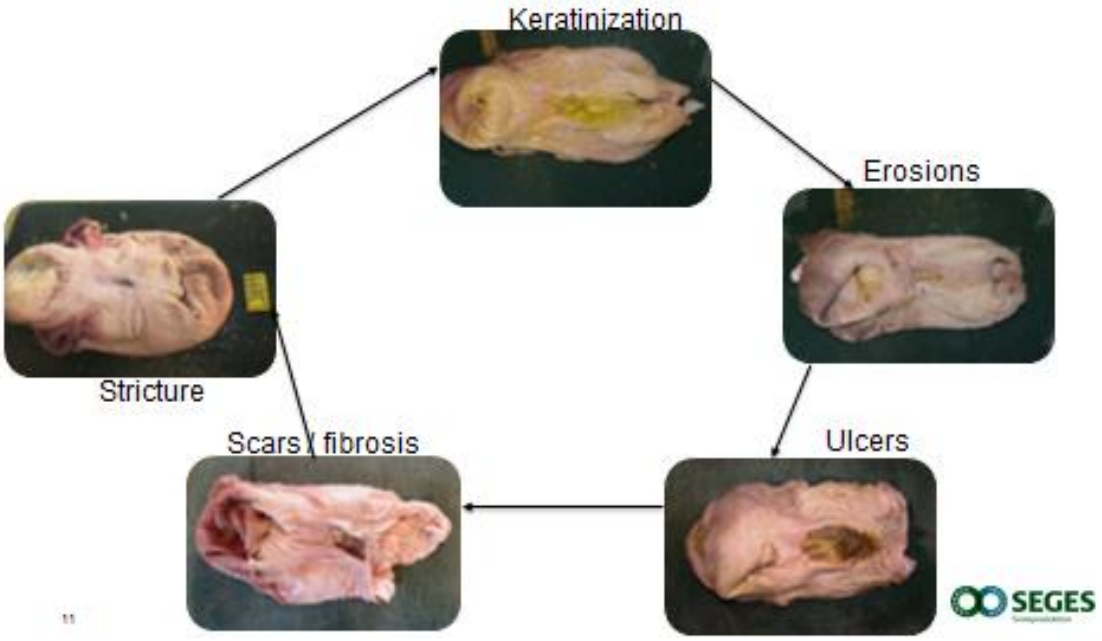
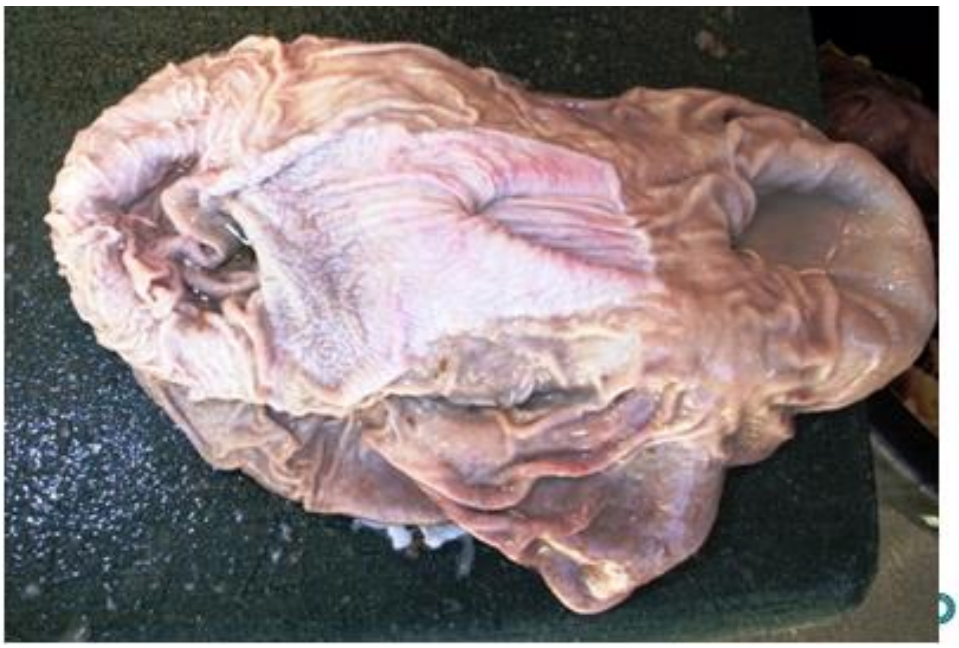


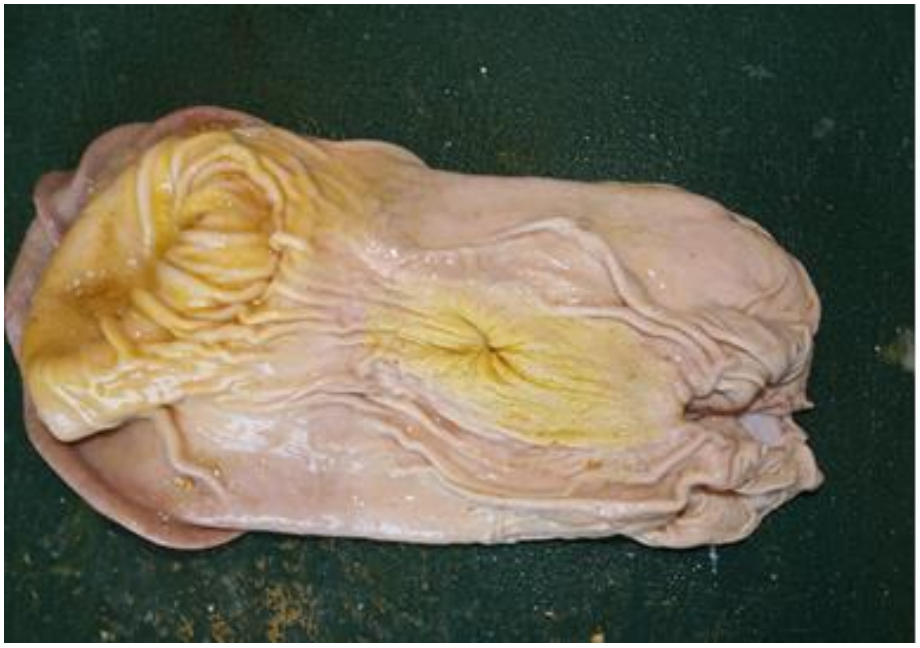
Keratinization

("Callus" on the white part of the stomach)

Score		Index
0	The white part is white, smooth and flexible	0
1	The white part is yellow and slightly rough, like medium coarse sand paper 1 mm.	1
2	The white part is yellow and rough, as in very coarse sand paper 2 mm.	2
3	The white part is yellow, rough and frayed.	3

SEGES

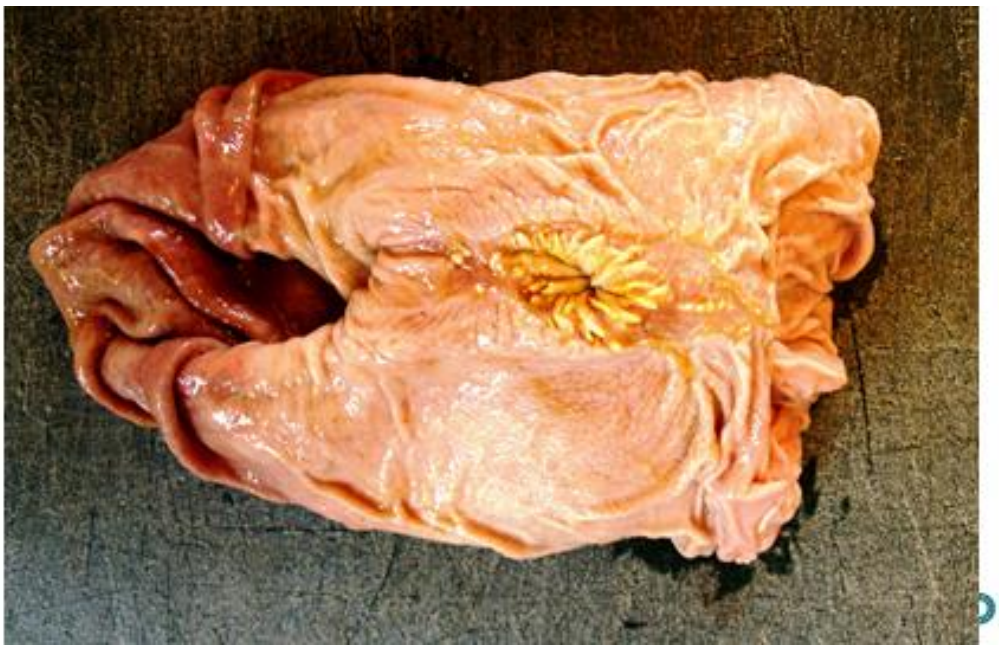




Erosions:

- The top layers of the mucous membrane are eroded.
- The tissue is below level, but it does not bleed; it can look red as blood vessels may shine through from profound structures. Erosions is a precursor to ulcers.

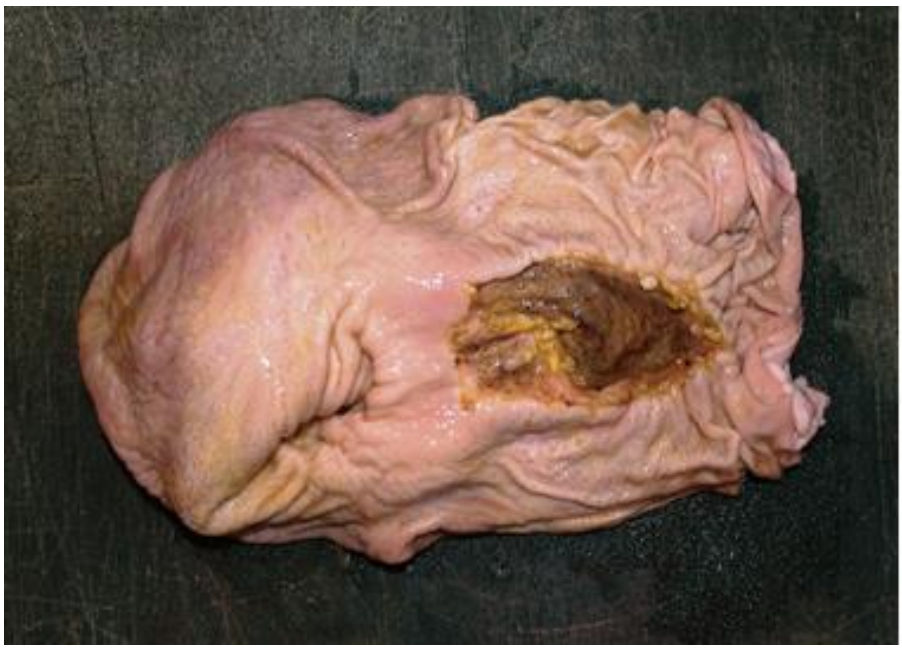
Score		Index
1	Erosions in < 10% of the white part	4
2	Erosions in 10-50% of the white part	5
3	Erosions in > 50% of the white	5



Ulcers:

- Bleed or evidence of bleeding, - brown-coloured tissue:
  - It can also be seen as loss of tissue in a depth of several mm.
- Ulcers may look highly different.
- They are scored by size in diameter and depth
  - i.e. deep wound is scored higher than a superficial wound.

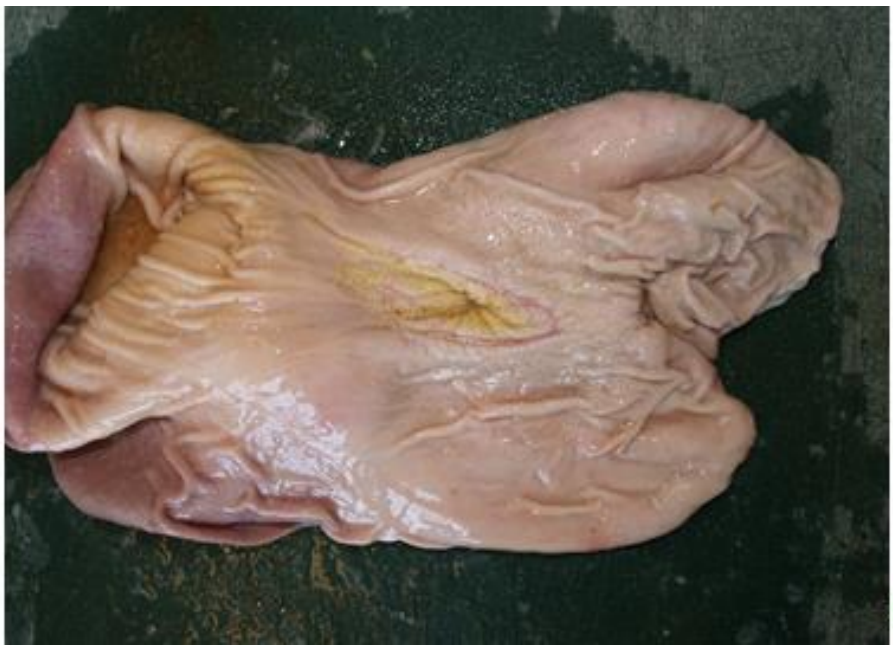
Score		index
1	Minor ulcers (up to about 0.5 cm in diameter)	6
2	Medium sized ulcers approx. 0.5 to 2 cm in diameter	7
3	Large ulcers more than 2 cm in diameter	8



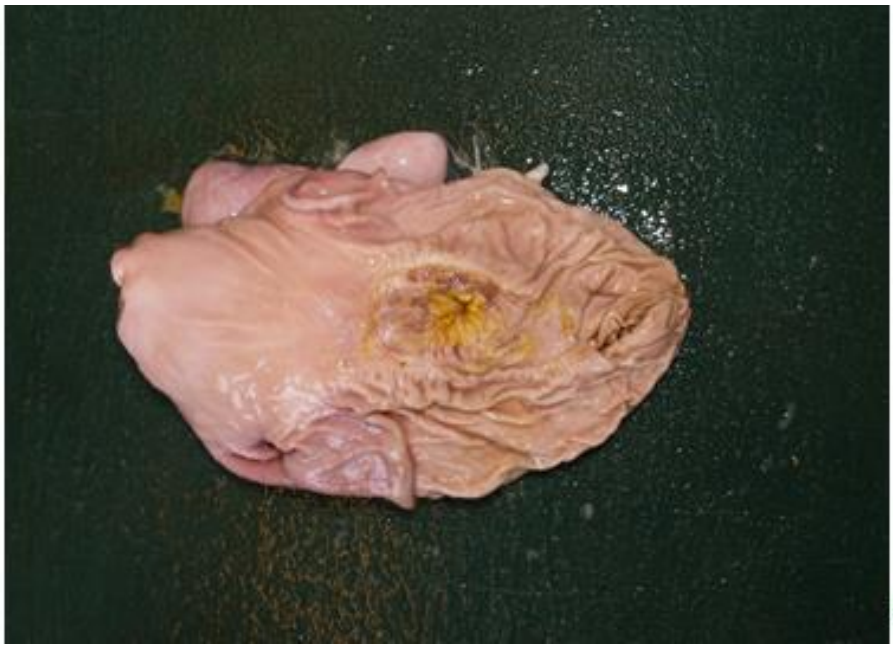
Scars / fibrosis:

- Scars are formed by healing of ulcers.
- Sometimes you see superficial scars in the mucosa
  - - are not evaluated, but rather the degree of fibrosis.
  - You have to feel it: insert two thumbs in the oesophageal opening and pull to the sides and feel the string formation

Score		Index
1	String formation in one or both sides of the white part	6
2	String is forming a ring, but soft or incomplete	7
3	String formation forms a solid ring	8



SEGES



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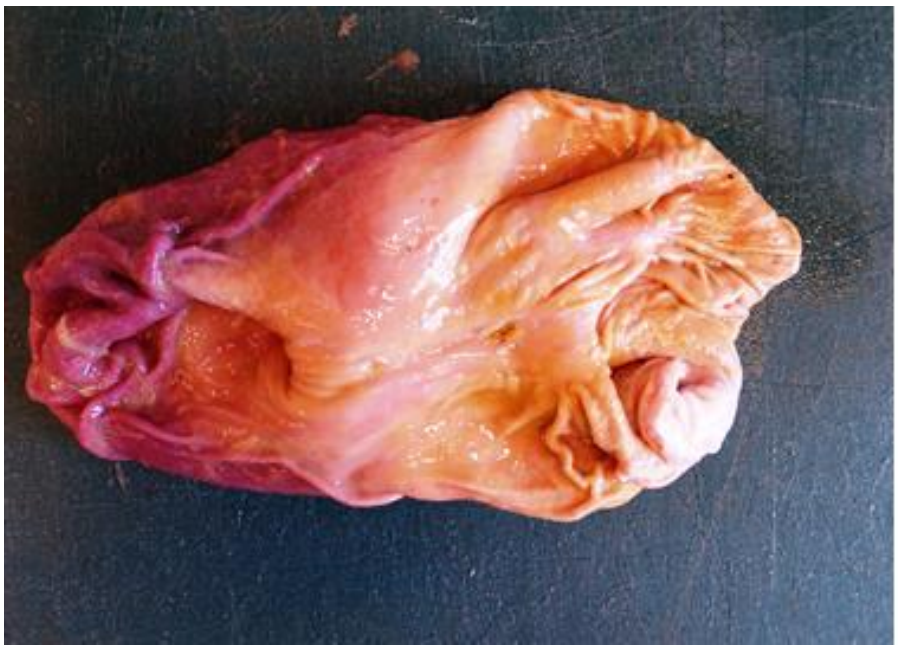

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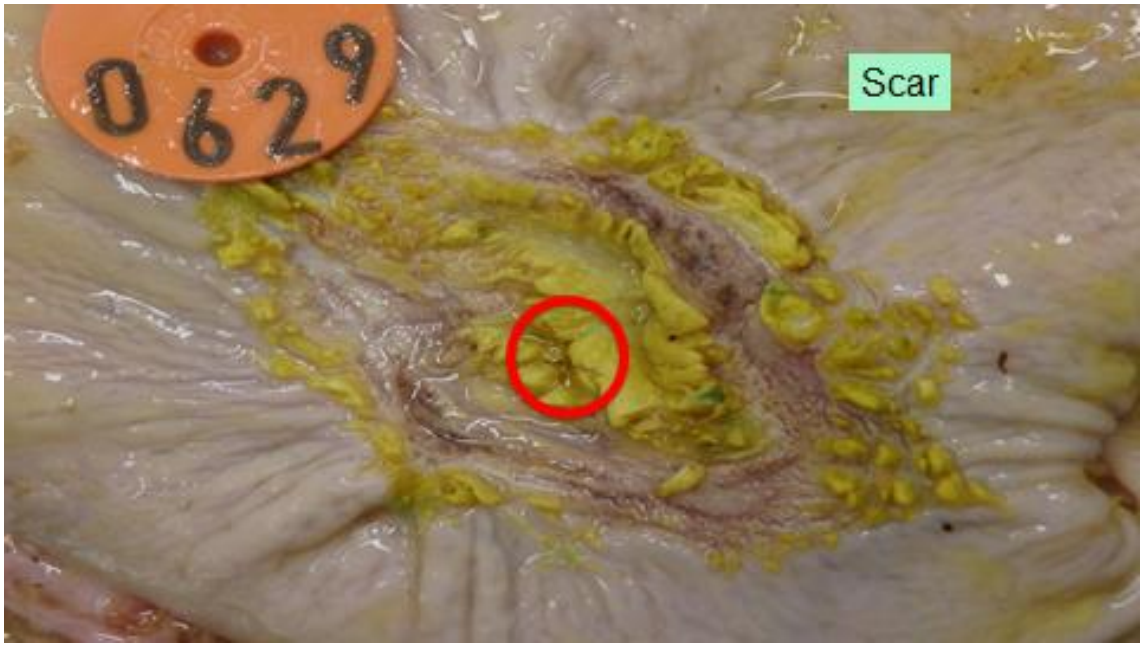
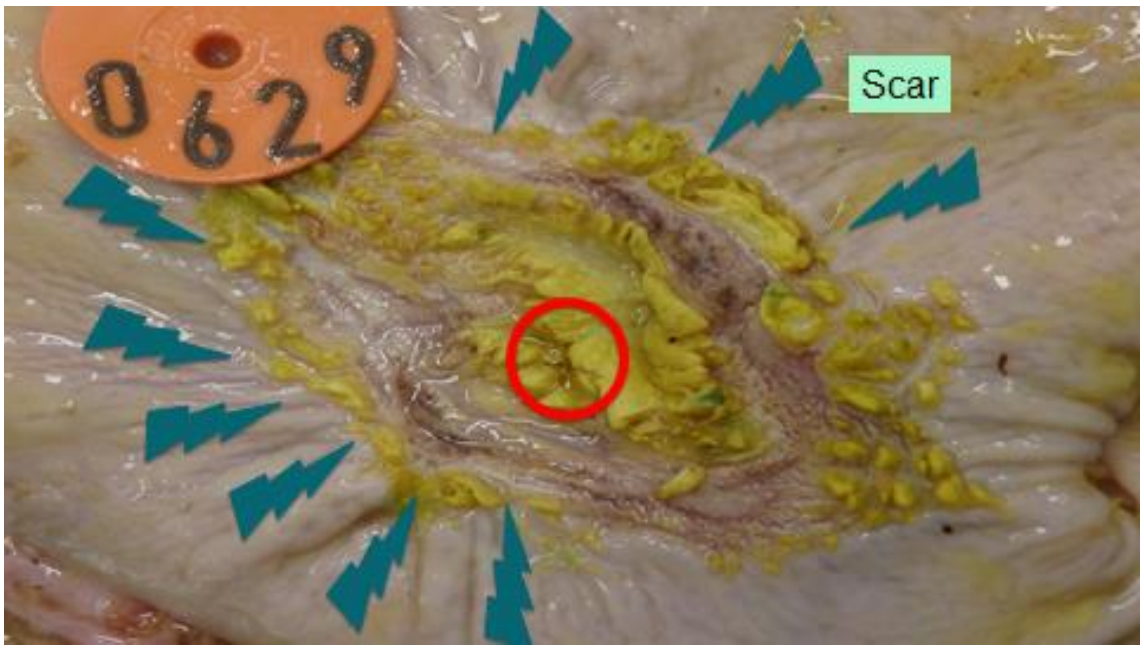
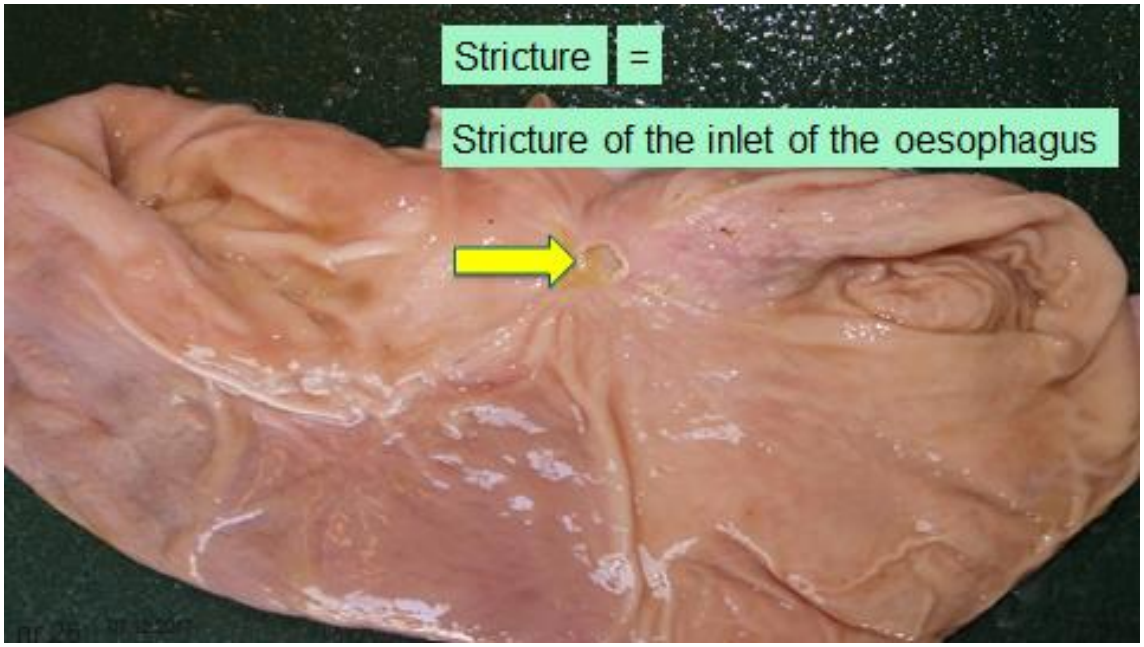
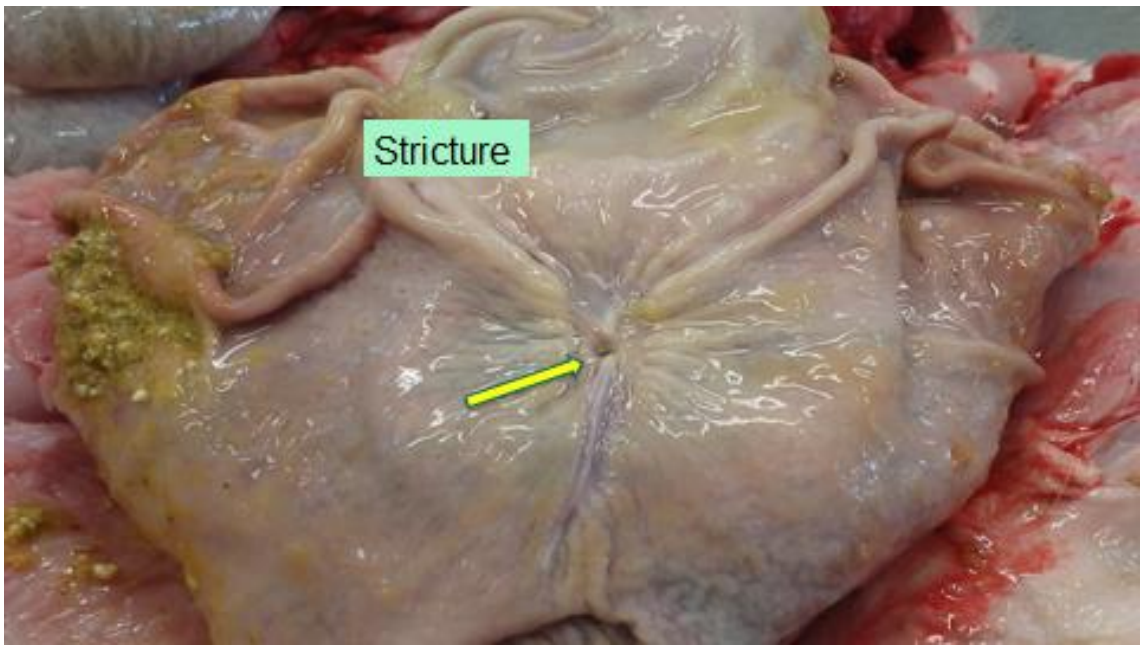
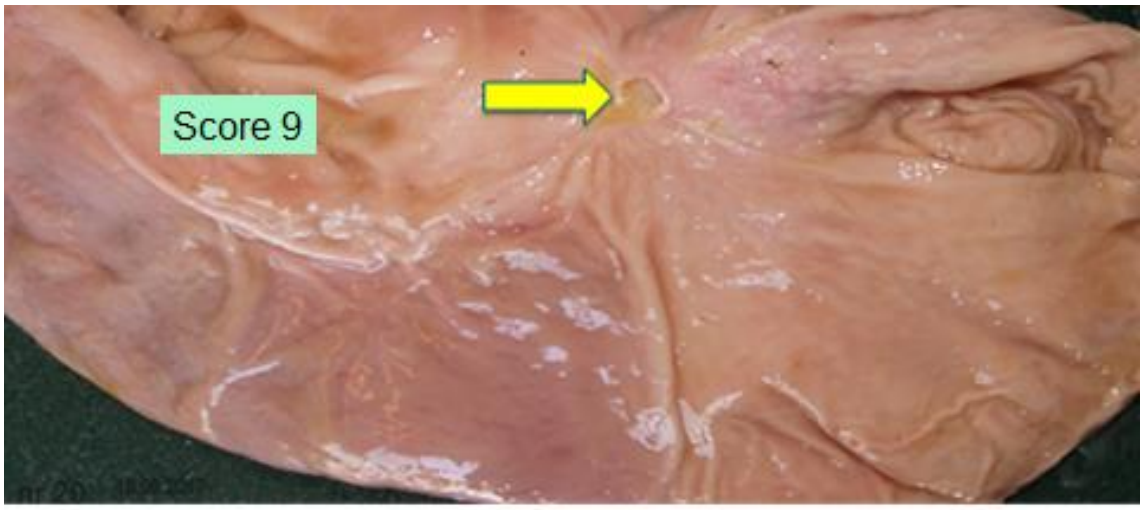



0629
Scar


Stricture =
Stricture of the inlet of the oesophagus





SEGES

29

Index, how to calculate

CURRENT INDEX

TOTAL INDEX

Index	0	1	2	3	4	5	6	7	8	9	10
Keratinization		1	2	3							
Erosions					1	2-3					
Ulcers							1	2	3		
Scars / fibrosis							1	2	3		
Stricture										~10 mm	~3 mm

SEGES

Current index

- Gastric index below 6 is considered insignificant
- **Current index** does not include string formation.
- Illustrates the actual level of gastric health (feed).
- Index ranges from 0 to 8.

SEGES

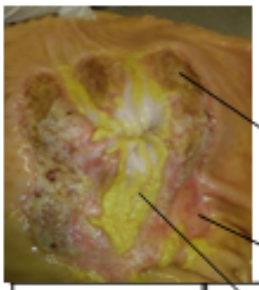
32

Total index

- **Total index** includes string formation.
- Illustrates the long term level of gastric health.
- Index ranges from 6 to 10.

33

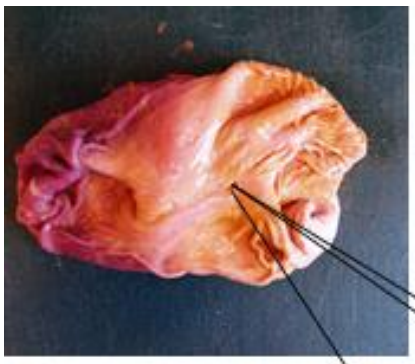
SEGES



CURRENT INDEX 7
TOTAL INDEX 7

Index	0	1	2	3	4	5	6	7	8	9	10
Keratinization		1	2	3							
Erosions					1	2-3					
Ulcers							1	2	3		
Scars / fibrosis							1	2	3		
Stricture										~10 mm	~3 mm

SEGES



CURRENT INDEX 2
TOTAL INDEX 10

Index	0	1	2	3	4	5	6	7	8	9	10
Keratinization		1	2	3							
Erosions					1	2-3					
Ulcers							1	2	3		
Scars / fibrosis							1	2	3		
Stricture										~10 mm	~3 mm

SEGES

Pig no.	Keratinization	Erosion	Ulcer, white part	Scar, white part	Diameter oesophageal opening, mm	Total index	Current index
11	2		1	1		6	6
12	2	2	1	1		6	6
13	1	2		1		6	5
14	2	1		1		6	4
15	2			1		6	2
16	1			1		6	1
17	1			1		6	1
18	1			1		6	1
19	1	2				5	5
20	2	1				4	4
21	2					2	2
22	2					2	2
23	2					2	2
24	1					1	1
Average gastric index						5.9	3.8

Conclusion:
- 18 in 24 stomachs with significant or highly significant changes.
- 7 stomachs with significant or highly significant current changes i.e. ulcers.
- 1 stomach with no or completely insignificant changes (index 0-1).

Pig no.	Keratinization	Erosion	Ulcer, white part	Scar, white part	Diameter oesophageal opening, mm	Total index	Current index
9	2					2	2
10	2					2	2
11	2					2	2
12	2					2	2
13	2					2	2
14	1					1	1
15	1					1	1
16	1					1	1
17	1					1	1
18	1					1	1
19	1					1	1
20	1					1	1
Average gastric index						2.8	2.1
Average gastric index, stomachs with significant changes						6.0	

Conclusion:
- 5 in 20 stomachs with significant or highly significant changes.
- 0 stomachs with significant or highly significant current changes i.e. ulcers
- 7 stomachs with no or completely insignificant changes (index 0-1).

SEGES

Pig no.	Keratinization	Erosion	Ulcer, white part	Scar, white part	Diameter oesophageal opening, mm	Total index	Current index
2201						0	0
1827						0	0
1805						0	0
Average gastric index						5.4	4.7
Average gastric index, stomachs with significant changes						7.4	6.4

Conclusion:
- 8 in 11 stomachs with significant or highly significant changes.
- 7 stomachs with significant or highly significant current changes i.e. ulcers.
- 3 stomachs with no or completely insignificant changes (index 0-1).

SEGES

Chapter 9

Significance and limitations of endocrine parameters to assess testicular function in EM and IC – matrix, sampling and analysis

Ulrike WEILER[1]

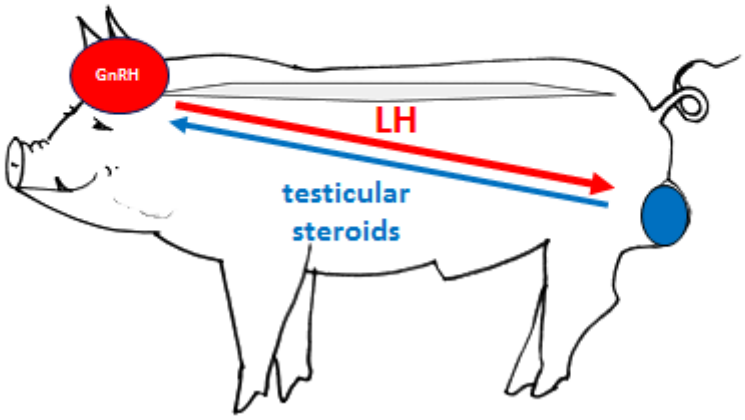
CA IPEMA Training School: „Harmonisation of methods in entire male and immunocastrate research"
Ljubljana, November 20-22, 2017

Significance and limitations of endocrine parameters to assess testicular function in EM and IC

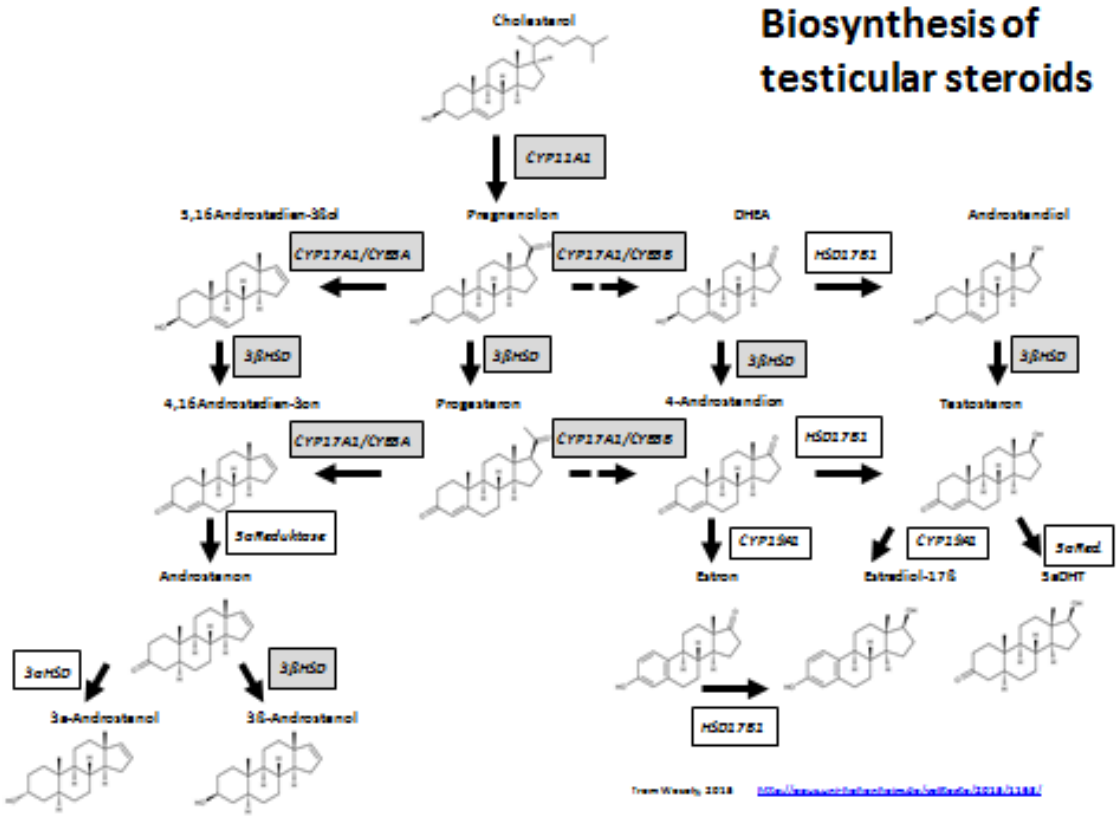
Ulrike Weiler
Universität Hohenheim

Endocrine regulation of testicular function

GnRH: Gonadtropin releasing hormone
LH: luteinizing hormone



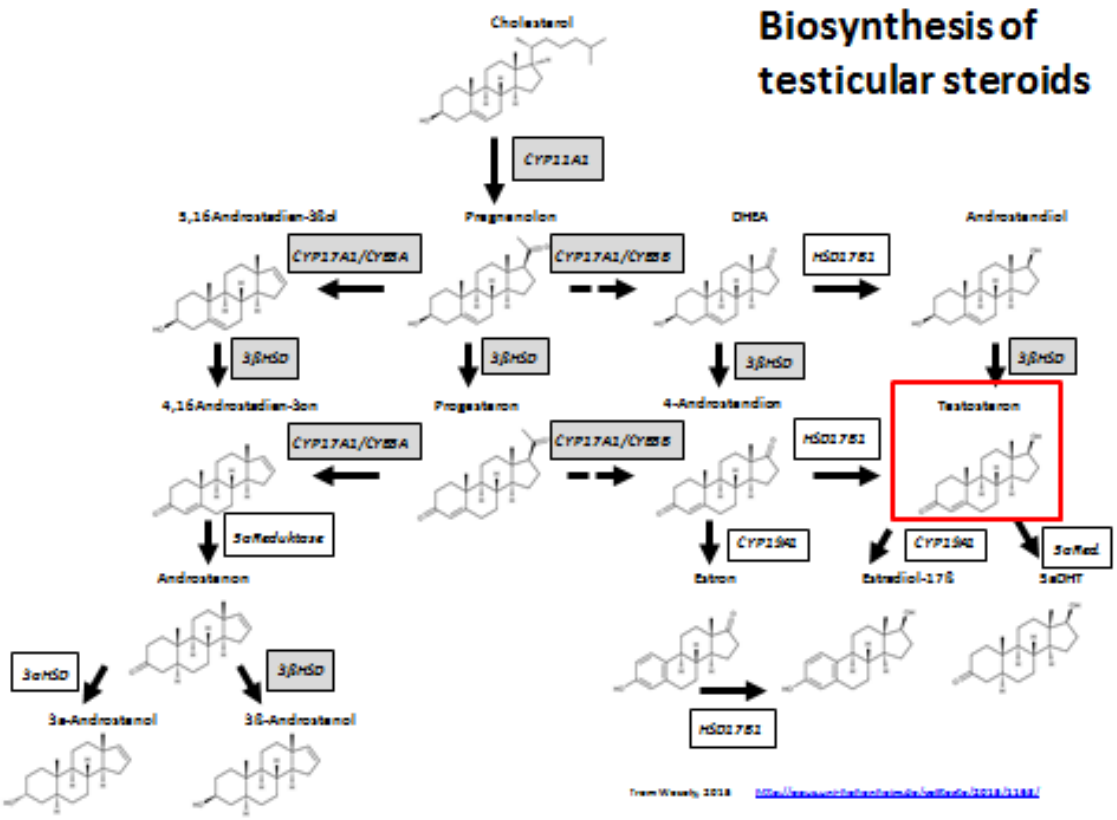
[1] University of Hohenheim, Garbenstr. 17, 70599 Stuttgart, Germany



Correlations between testicular steroids in blood plasma of an individual AI boar

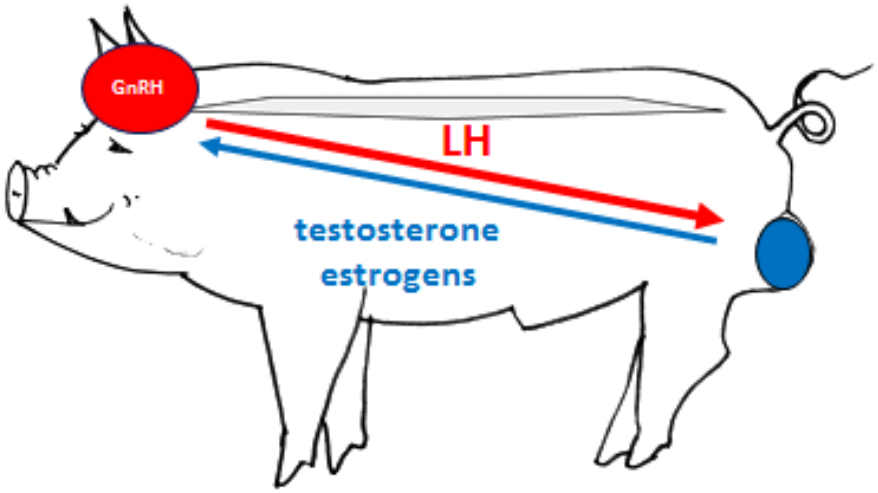
Samples (n=145)	Testosterone	5α DHT	Androst.	Unc. E
Conjugated Estrogens	0,78	0,78	0,66	0,66
Unconjugated Estrogens	0,88	0,67	0,78	
Androstenone	0,79	0,70		
5α DHT	0,72			

Claus et al., 1983; J Steroid Biochem. 19, 725-729



Endocrine regulation of testicular function

GnRH: Gonadtropin releasing hormone
LH: luteinizing hormone



Where to measure?

- **Blood** (substrate of choice, comparable date, established; easy at slaughter, but modified by stress; continuous profiles require catheter)
- **Saliva** (free testosterone, sampling?)
- **Urine** (86% of T excretion; sampling +/-, modified by water supply, species? What do the measurements represent? Pre-treatment of samples?)
- **Feces** (14% of T excretion; sampling easy, modified by feed, delay? What do the measurements represent; Pre-treatment of samples?)
- **Drip/Muscle** (after slaughter easy to sample, only one sample, reference values?)
- **Fat** (after slaughter easy to sample, only one sample, reference values?)

Principle of RIA and EIA determination of testosterone

Competitive antigen-antibody reaction

- **Antibody**
- **Tracer**
- **Bound-free separation**

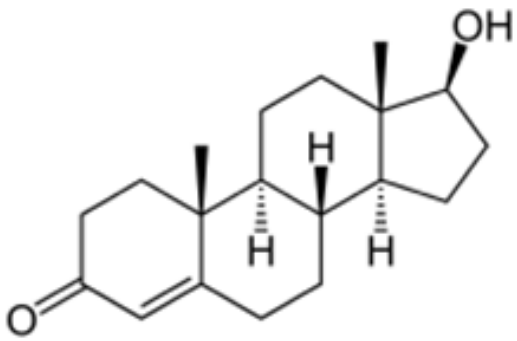
Principle of RIA and EIA determination of testosterone

Competitive antigen-antibody reaction

- **Antibody**
- **Tracer**
- **Bound-free separation**

How to get an antiserum....

- Size of the molecule: MW 288

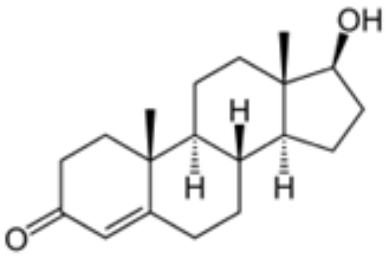


….just buy it!

….just buy it!

But what happens in the Black Box?

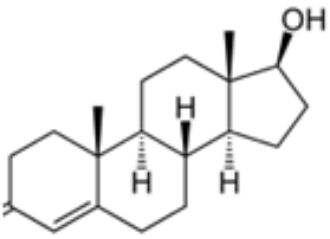
Hapten plus spacer…



$HOOC\text{-}CH_2\text{-}O\text{-}NH_2$ +

CMO: O-(Carboxymethyl)-hydroxylamine

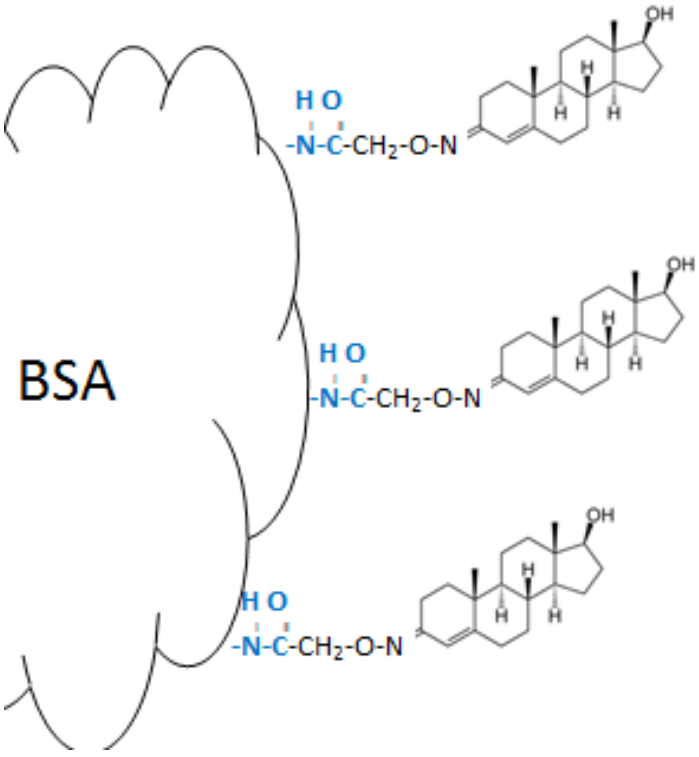
testosteone



$HOOC\text{-}CH_2\text{-}O\text{-}N$

T-3-CMO

Peptide coupling by Carbodiimid method



Molar ratio BSA/Δ16:

e.g. 1/200

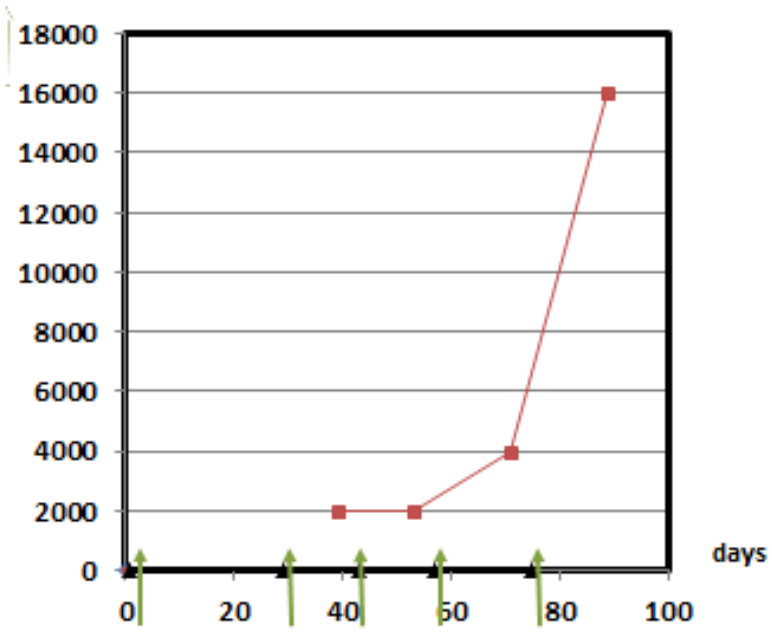
- dialysis
- lyophilization

Immunization

- **Animals:**
  rabbit (polyclonal) or mice (monoclonal)
- **First immunization (antigen amount:**
  rabbit: 1-2 mg, mice: 0.05-0.03 mg, e.g. complete Freunds adjuvans)
- **Booster injections in about 2 week intervals**
  (mice for monoclonal AB: 2,
  rabbits , polyclonals: 4 and more according titre control)

Development of the antiserum

= injection of antigen (1 mg)

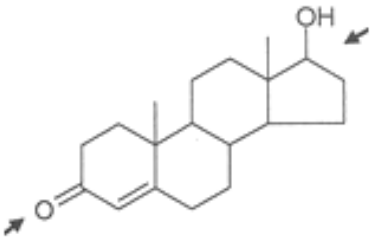


Characterization of the antiserum: Cross reactivity

Definition:

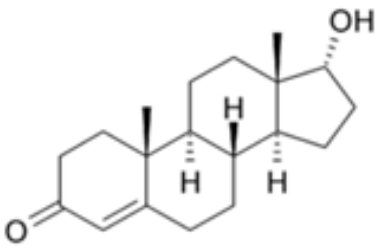
The ability of an antibody to bind an antigen, that did not stimulate its production.

Choice of test steroids ?

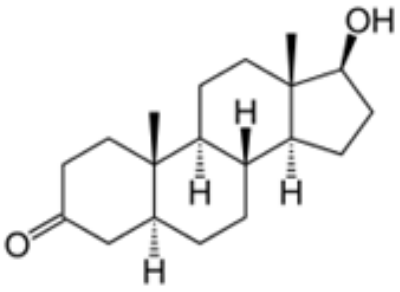


17-HS -Testosteron

3-CMO -Testosteron



17a-Testosteron



5a-DHT

Characterization of the antiserum: cross reactivity

Choice of test steroids:

- similarities in structure
- naturally occurring in pigs
- depending on the biological matrix

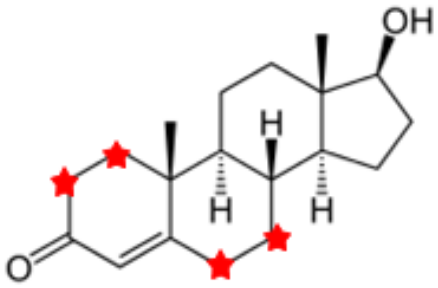
Principle of determination

Competitive antigen-antibody reaction

- Antibody
- Tracer
- Bound-free separation

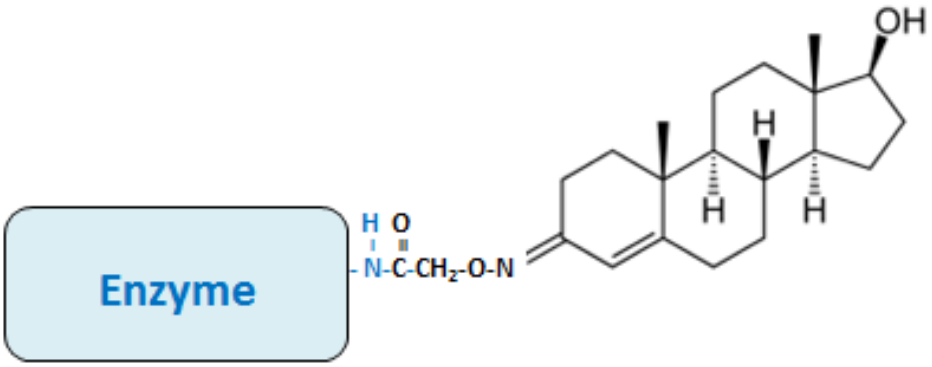
Tracer: ^{3}H-Testosterone

1,2,6,7-^{3}H-Testosteron (70 Ci/mmol; Perkin Elmer, NET 370);



radio immuno assay

Tracer: Enzyme - linked -Testosteone



Enzyme immuno assay

Detection: colorimetric, Substrate: TMB (Tetramethylbenzidinimine)

Testosterone determination

General RIA (radioimmunoassay) for Testosterone:

- Antiserum raised in rabbits against testosterone-3CMO-BSA.
  Cross reactivity: 67% with 5αDHT, and below 2% for other tested steroids.
  Working solution: 1: 100000
- Tracer: 1,2,6,7-^{3}H-Testosteron (70 Ci/mmol; Perkin Elmer, NET 370);
  Working solution: 12 000 cpm/100µl (corresponding to 150 pg/tube)
- Standard curve in Phosphate buffer or plasma: 0.005 ng/100µl up to 1 ng/100µl
- Mixture of sample diluted/suspended in Phosphate buffer (100µl), 100 µl Tracer, 500 µl Antiserum dilution; mixing, incubation at 37°C for 30 min, 1 h at ice.
- Bound free separation: Addition of Dextran coated charcoal (500 µl), centrifugation, transfer of supernatant into Szintillation fluid
- Counting: Beta counter (Liquid szintillation counter)

Where to measure?

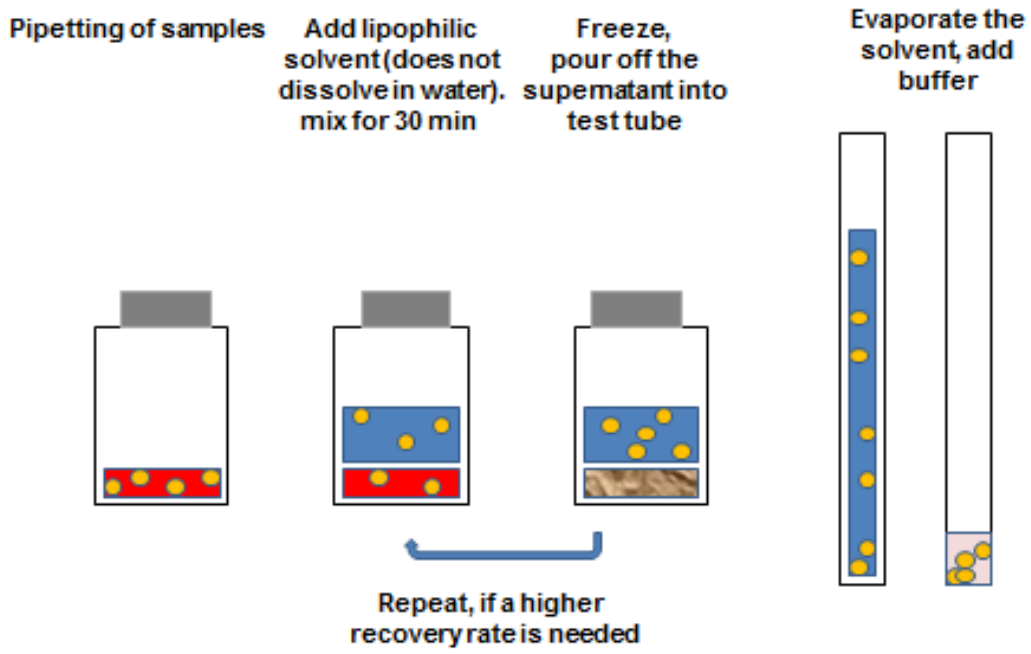
- **Blood** (substrate of choice, comparable … at slaughter, but modified by stress; con… catheter)
- **Saliva** (free testosterone, sa…)
- **Urine** (86% of excretion… by water supply, species? What do th… ent? Pre-treatment of samples?)
- **Feces** (14% … asy, modified by feed, delay? What do … esent; Pre-treatment of sampl…
- D… ghter easy to sample, only one sample,
- …ter easy to sample, only one sample, reference

For a radioimmunological analysis of testosterone concentrations in urine and fecal samples: extraction is prerequisite!

Determination of testosterone in plasma

- Testosterone concentrations in plasma are measured in duplicate without extraction with an in-house radioimmunoassay.
- 20 µl plasma are diluted with 100 µl of phosphate buffer and incubated with 3H- testosterone and antiserum. The antiserum was used at a final dilution of 1:144 000.
- A calibration curve in charcoal treated plasma (to remove endogenous testosterone) was used to compensate for substrate effects.
- Bound free separation was carried out by the addition of 0.5 ml ice cold solution of dextran coated charcoal (0.5%) in H20 and subsequent centrifugation.
- The supernatant was transferred into counting vials with scintillation fluid and counted in a beta-counter.
- Intra-assay and inter-assay variability was determined with pig plasma samples and were below 8% each. Precision was determined with samples of spiked pig plasma. The mean recovery rate of added concentrations was 110%.

Extraction of steroids



Testosterone determination in Urine: Exraction

- Per sample 10 µl urine are pipetted in duplicate and diluted with 100 µl aqua bidest.
- 3 ml of buthyl methyl ether are added and mixed for 30 min. Thereafter the sample is put into the freezer until the aeqous fraction is frozen an the supernatant is collectedinto a test tube and dried down.
- The residue is reconstituted with 100 µl phosphate buffer and the sample is ready for RIA.
- To compensate for procedural losses the recovery rate is determined with 3H-testosterone in each assay (about 90 to 95%)
- Precision is determined with spiked pig urine samples (low endogenous concentration = K0; plus 2.5 ng/ml, 5ng/ml and 10 ng/ml) and revealed a mean recovery rate of 82.9%. Intra-assay variation and inter-assay variation were determined with biological pig urine samples and was below 10% and below 15%, respectively. duplicate with

Testosterone determination in Fecal sampes: Extraction

- Testosterone is extracted from feces in a two-step solvent distribution.
- Fecal samples of about 0.5 g each are dissolved in 500 µl of water and 4 ml methanol is added, followed by mixing the sample for 30 min.
- Addition of 3 ml petroleum ether was added for solvent distribution. After mixing and centrifugation, the petroleum ether is discharged (to remove lipids)
- An aliquot of 100 µl of the remaining methanol/water mixture is further diluted with 600 µl water and extracted with 5 ml of 7:3 (v/v) petroleum ether/ethyl acetate.
- After incubation for 30 min and freezing the aequs fraction, the supernatant is collected and dried down.
- The residue is reconstituted with 100 µl phosphate buffer and the sample is ready for RIA.
- QA: To compensate for procedural losses, the recovery rate is determined with 3H-testosterone and is in an order 50%. Intra-assay variability and inter-assay variability are determined to characterize the repeatability. Precision is further determined with spiked fecal samples (recovery rate of 75 -80 %).

Why do we do that??????

Meat Science 99 (2015) 60–67

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Pre-slaughter conditions influence skatole and androstenone in adipose tissue of boars

Raffael Wesoly, Ina Jungbluth, Volker Stefanski, Ulrike Weiler *

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ARTICLE INFO

Article history:
Received 20 April 2014
Received in revised form 12 August 2014
Accepted 28 August 2014
Available online 6 September 2014

Keywords:
Androstenone
Skatole
Boar taint
Preslaughter conditions
Transport time

ABSTRACT

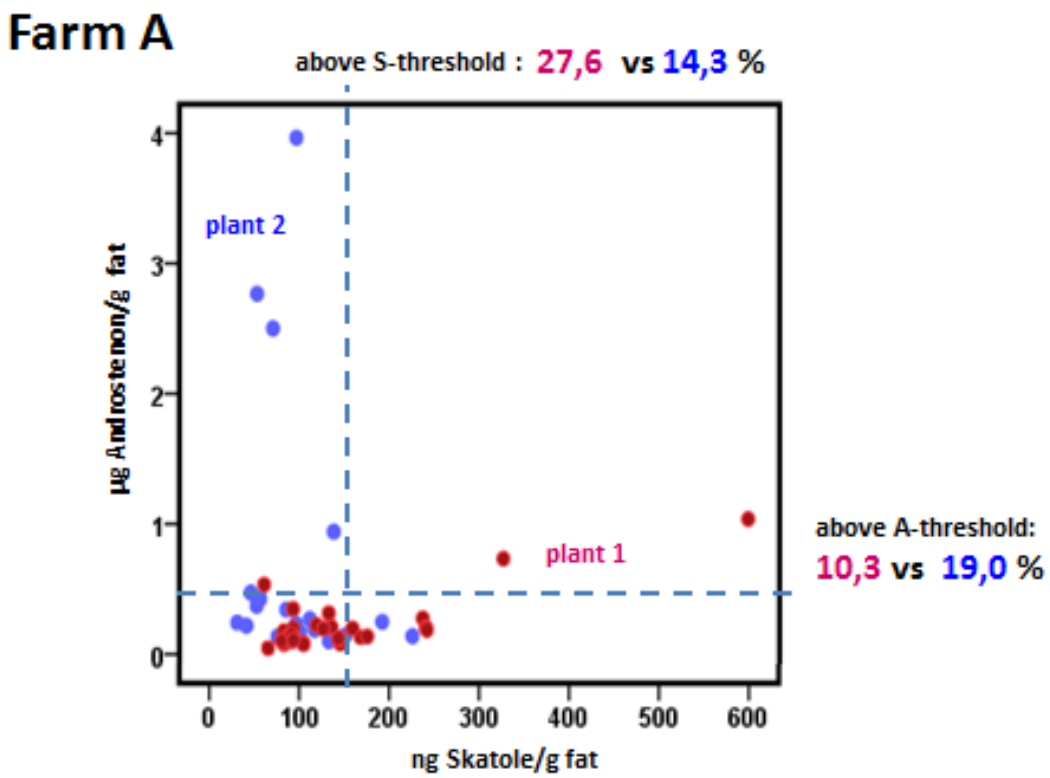
Boar taint in carcasses may vary between farms and abattoirs, although the underlying mechanisms are not yet fully understood. In the present study, 169 boars from three farms were split into two groups and slaughtered at two abattoirs. Duration of transport and the time between arrival at the abattoir and unloading (pre-unloading time) were recorded. During slaughter, blood, feces, and urine were collected to measure testosterone and cortisol levels. Carcasses were classified according to the number of skin lesions, and fat samples were taken to determine skatole, indole and androstenone levels. Androstenone in fat and testosterone in blood, feces, and urine were mainly influenced by the duration of transport. Skatole and indole concentrations were increased by both pre-unloading time and duration of transport, but were also related to skin lesions. Thus it is concluded that androstenone and skatole concentrations in fat are significantly modified by pre-slaughter conditions.

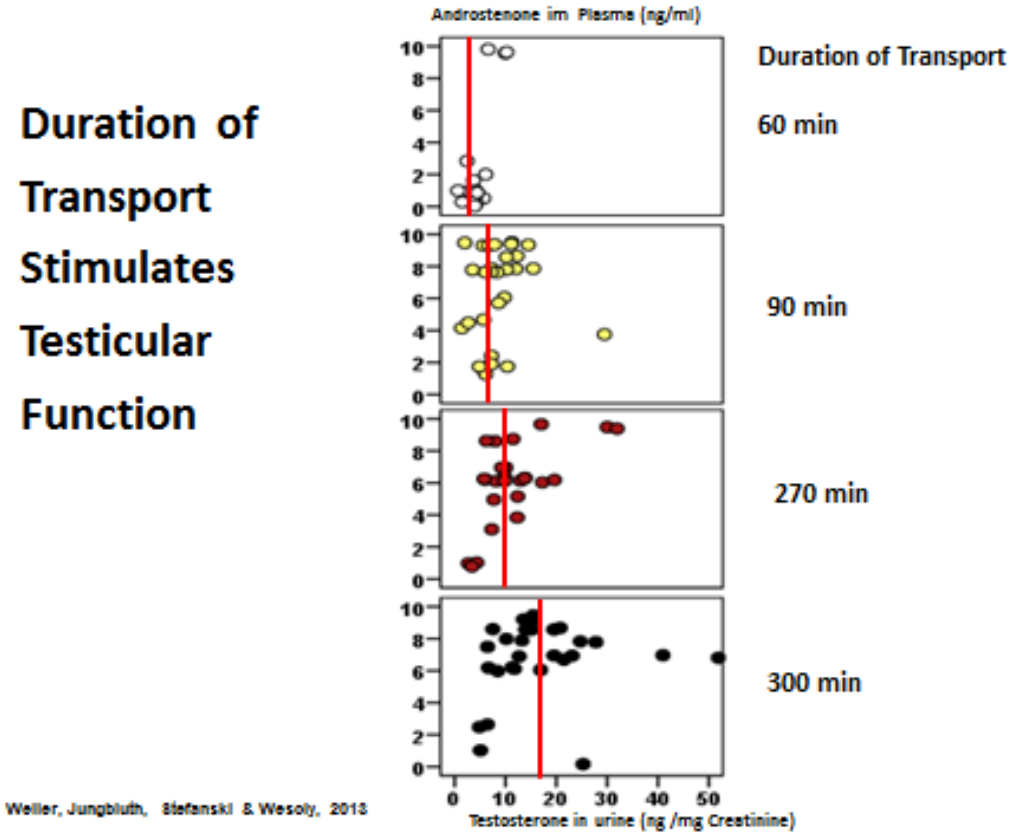
Table 2
LS-means ± SEM for the physiological parameters and estimated increase per hour of transport and pre-unloading times (ANOVA model 1).

Compound	Substrate	n	LS mean ± SEM	Increase/h transport	Increase/h pre-unloading
Androstenone	Fat (µg/g)	169	0.9 ± 0.1	+0.09	
Skatole	Fat (ng/g)	169	73.8 ± 5.0	+3.6	+21.5
Indole	Fat (ng/g)	169	34.1 ± 1.5	+6.8	+10.6
Testosterone	Plasma (ng/ml)	165	9.7 ± 0.8	+2.2	
	Urine (ng/mg creatinine)	153	10.1 ± 0.6	+1.6	
	Feces (ng/g)	124	22.6 ± 0.7	+1.4	
Cortisol	Urine (ng/mg creatinine)	153	71.7 ± 2.2		
	Feces (ng/g)	124	49.7 ± 1.65	+4.3	

Table 5
Pearson correlations between the analyzed parameters ((•): $p < 0.1$; •: $p < 0.05$; ••: $p < 0.01$; •••: $p < 0.001$).

	Indole in fat	Testosterone in urine	Testosterone in feces	Cortisol in urine
Androstenone in fat		0.49••	0.22••	0.17•
n		142	124	142
Skatole in fat	0.76••		0.26••	
n	169		124	
Indole in fat			0.23••	
n			124	
Testosterone in plasma	−0.22••	0.33•••		
n	165	138		
Testosterone in urine			0.41•••	
n			124	
Testosterone in feces				
n				
Cortisol in urine		0.16•	0.23••	
n		124	124	
Cortisol in feces	0.17(•)		0.28••	
n	124		124	


Farm A
above S-threshold : 27,6 vs 14,3 %
plant 2
plant 1
µg Androstenon/g fat
ng Skatole/g fat
above A-threshold:
10,3 vs 19,0 %


Duration of Transport Stimulates Testicular Function
Androstenone im Plasma (ng/ml)
Duration of Transport
60 min
90 min
270 min
300 min
Testosterone in urine (ng /mg Creatinine)
Weller, Jungbluth, Stefanski & Wesoly, 2013

Results: Duration of transport and pre-unloading time

Farm	Slaughter plant	Duration (min)	
		Transport	Pre - unloading
A	I	60	480
	II	240	202
B	I	150	165
	II	270	93
C	I	300	260
	II	90	17

Results Coefficients of Regression

Increasing transport time:

Androstenone (fat): 0.1 µg/h (LS-Mean: 0.89 µg/g)

Testosteron (urine): 1.58 ng/h (LS-Mean: 9.7 ng/mg)

Results: Duration of transport and pre-unloading time

Farm	Slaughter plant	Duration (min)	
		Transport	Pre - unloading
A	I	60	480
	II	240	202
B	I	150	165
	II	270	93
C	I	300	260
	II	90	17

Results Coefficients of Regression

Increasing transport time:

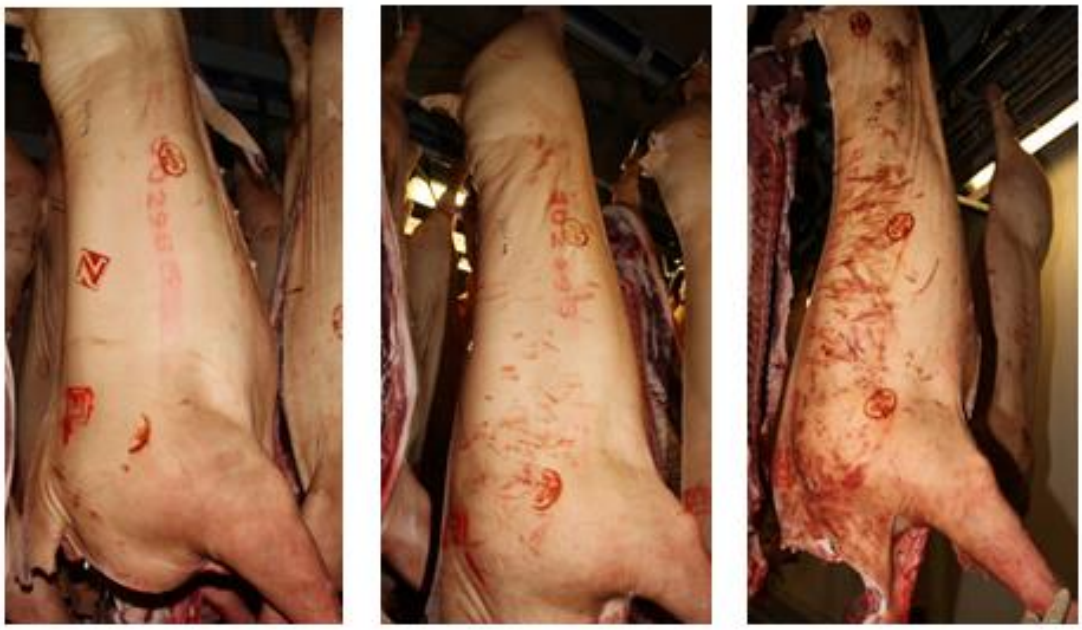
Androstenone (fat):	0.1 µg/h	(LS-Mean: 0.89 µg/g)
Testosteron (urine):	1.58 ng/h	(LS-Mean: 9.7 ng/mg)

Increasing pre - unloading time:

Skatole (fat):	21.5 ng/h	(LS-Mean: 74.2 ng/g)
Lesion score:	0.25 pts/h	(LS-Mean: 0.65 pts.)

Classification According Number of Lesions

Low (1-8 Lesions)	Medium (8-25 Lesions)	High (> 25 Lesions)

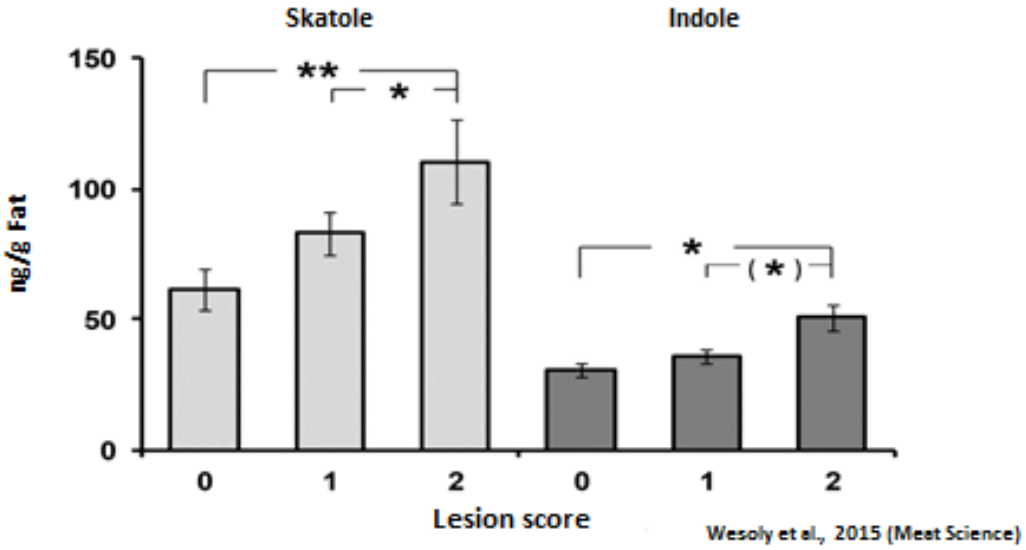


Classification of carcass lesions

- Less reliable in living animals (e.g. at arrival at the slaughter plant)
- Most reliable if evaluated at teh carcass
- Number and size (e.g. > 2 cm; classes?)

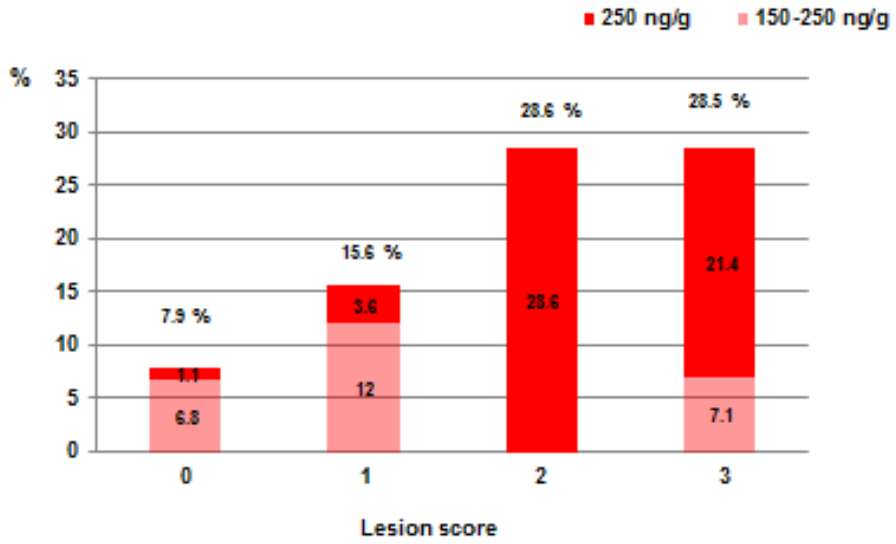
Relationship between carcass lesion score and skatole levels in fat

(Lesion scores: 0 = without any; 1 = low; 2 = medium and high; LS-means)



Wesoly et al., 2015 (Meat Science)

Carcasses with high skatole concentrations according to lesion score



Harmonization of methods:

- One lab measures samples of all groups: BEST
- Exchange of methods: recommended!
- Exchange of reference samples: crucial!
- In case of lesions: take pictures!

How to assess the success of vaccination against GnRH

Success of Improvac (field study)

	Time between 2nd vaccination and slaughter			Total
	3-5 weeks	>5-7 weeks	>7-10 weeks	
number of animals	50	71	54	175
„successful" (<1,5 ng T/ml)	92,0%	87,3%	77,8%	85,7%
„failed" (>1.5 ng T /ml)	8,0%	12,7%	22,2%	14,3%

Who did the bad job?

Gn-RH- Antibody quantification

AS-Sequence:

^{125}I

pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly

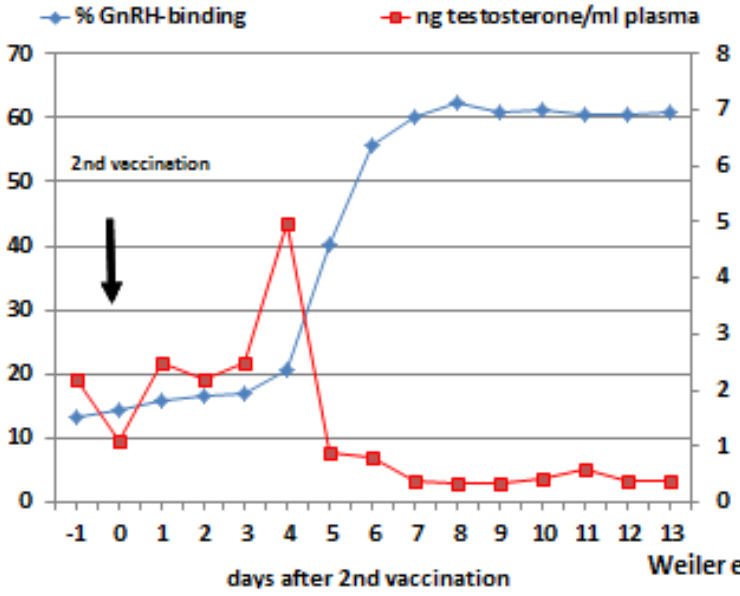
Dom, C. & Griesinger, G. Gynäkologische Endokrinologie (2009) 7: 161. doi:10.1007/s10304-009-0321-x

GnRH- binding Assay

GnRH-Iodination (solid phase Iodogen-method, 1µg/cup; resulting specific activity 200 µCi/µg)

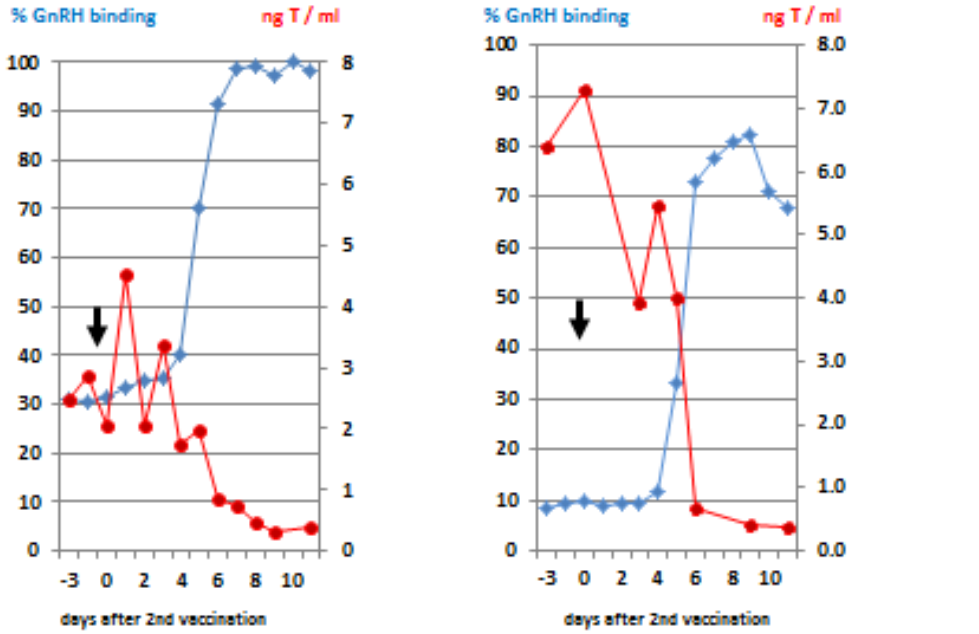
- **15 000 cpm ^{125}I-GnRH** (corresponding to 17,5 pg GnRH)
- **5 µl plasma** (in duplicate) **in 200µl PBSA** (BSA 0.1%)
- **Incubation at 4° for 24 h**
- **Bound-free separation** (1000 µl ice cold Dextran coated charcoal solution, centrifugation)
- **Counting of supernatant in a Gamma counter**
- **The Specific Binding of pool sample A** (vaccinated animals) **was 54,0; VK 10%,**
  the Non Specific Binding determined with pool sample B (unvaccinated animals) **was 4,5 %, VK 28% (range 5,81% to 2,36%).**

Changes after the 2nd vaccination (absolute binding)....



Weiler et al., 2016

Individual differences (relative binding)...



Weiler et al., unpubl.

Success of Improvac – assessment by determining testosterone and GnRH binding at slaughter

		Time between 2nd vaccination and slaughter		
		3-5 weeks	>5-7 weeks	>7-10 weeks
% GnRH binding (relative)	In „successful"	96,4	95,7	84,8
	In „failed"	91,4	70,9	61,0
ng testosterone /ml	In „successful"	0,90	0,50	0,20
	In „failed"	2,42	9,30	6,50

Weiler et al., in prep.

Harmonization of methods:

- AGAIN: One lab measures samples of all groups
- Exchange of methods: recommended!
- Critical point: specific activity of iodination! Half life of ^{125}Iodine: 59.5 days („old tracer“: decrease of specific binding, increase of NSB)
- Exchange of reference samples: crucial!

Why do we need continuous monitoring?

Frequency of penile injuries in EM and IC

Group	n	Number of scars per animal	Number of wounds per animal	% Animals with lesions	% Animals with severe injuries
IC	192	1.45 ± 2.35	0.14 ± 0.53	48.44	2.60
E	215	2.94 ± 3.05	0.40 ± 1.11	75.81	9.30

S. Reiter et al., 2017
doi:10.3390/ani7090071

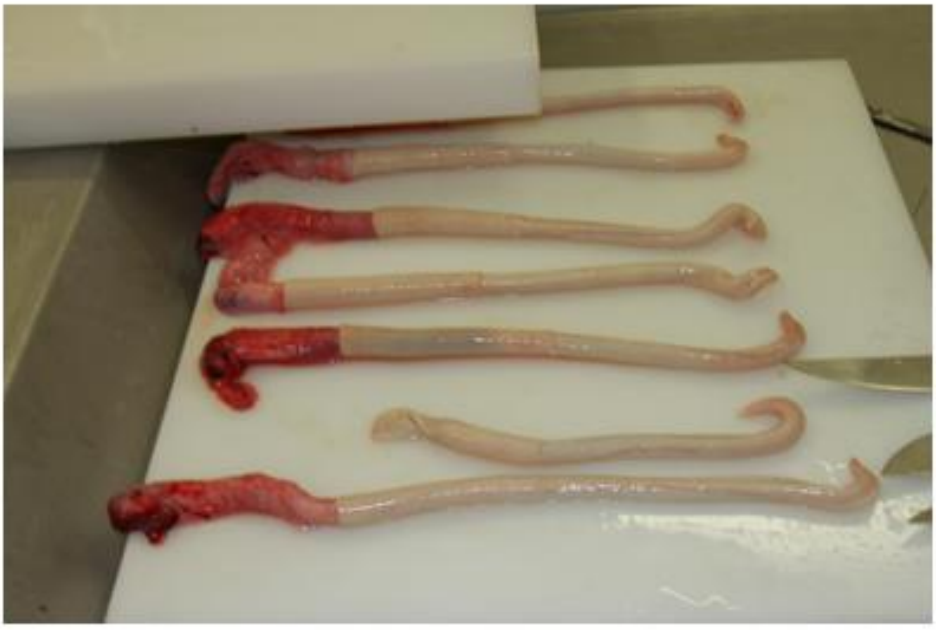
Sampling of specimen

The penis covered with the preputial sheet and some tissue is collected at the slaughter line during evisceration, where the genital tract is excised.

The specimen can be obtained by pushing the penis in a caudal direction within the preputial sheet and subsequently dissecting the preputial sheet without affecting the Pars libra penis or Glans penis, to get the Pars libra penis prepared for further evaluation.

Consecutively the Pars libra penis is evaluated for different types of lesions:
wounds, scars, hematomas (together: Total number of injuries)
Additionally changes of the ridge (slightly hypertrophic, slightly hypertrophic with abrasions).

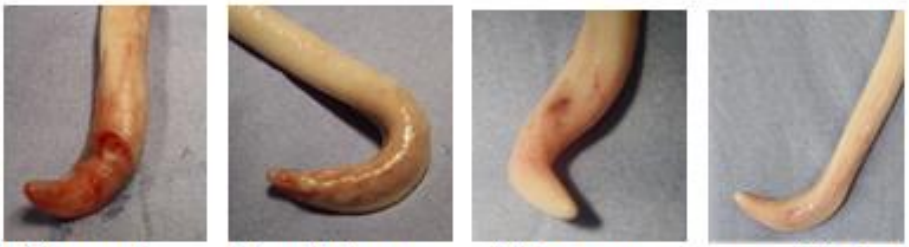
Also the size of the respective wounds and scars is recorded for each specimen according to a size-score 0.1-0.3cm, >0.3-0.6cm, >0.6-1cm, >1cm. Samples with injuries >1cm, with suppuration or losses of a part of penis are classified as "severe injuries" .



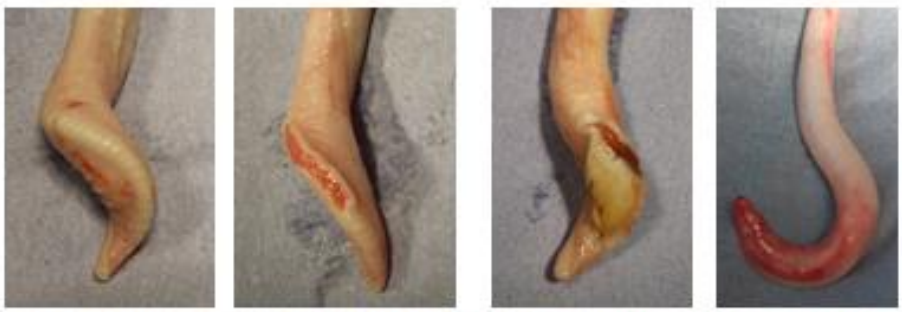
Comparison boar and barrow (same slaughter weight>)

Classifications

S. Reiter et al., 2017
doi:10.3390/ani7090071



(a) wounds (b) multiple scars; (c) hematoma; (d) no injuries



(e) slightly hypertrophic ridge (f) slightly hypertrophic ridge with abrasions; (g) ridge with hyperkeratosis (h) abrasion of the glans penis.

Exclude artefacts due to scalding!

Look really bad, but the animal did not feel anything….

cost
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IN SCIENCE AND TECHNOLOGY

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1818

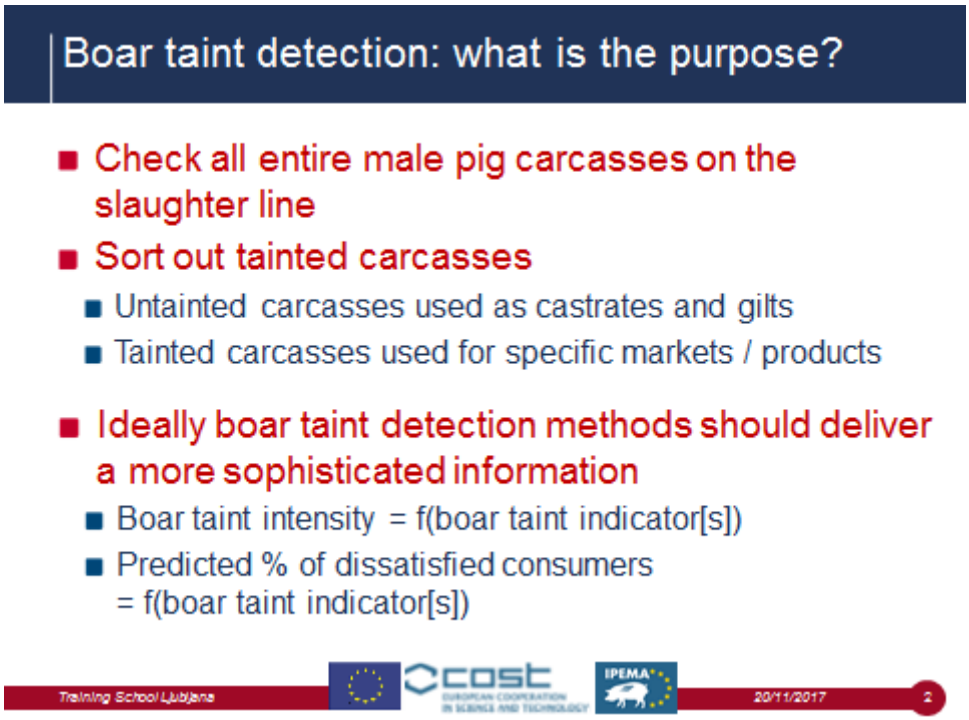
Thank you for your attention!

IPEMA

Chapter 10

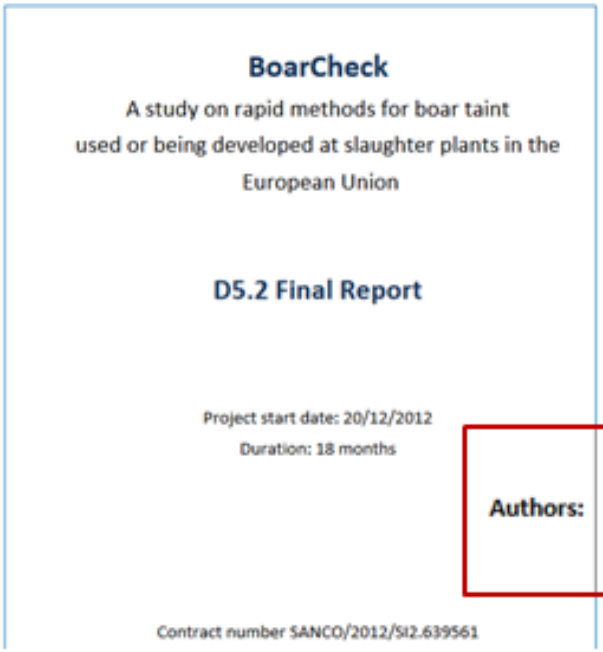
From human nose to instrumental methods for on-line detection of boar taint – Five decades of small steps forward

Michel BONNEAU[1]



[1] The French Pork and Pig Institute (IFIP), La Motte au Vicomte, 35650 Le Rheu, France

Boar taint detection: required specifications



BoarCheck

A study on rapid methods for boar taint used or being developed at slaughter plants in the European Union

D5.2 Final Report

Project start date: 20/12/2012
Duration: 18 months

Authors: **John-Erik Haugen (Nofima),** Coen van Wagenberg, Gé Backus (DLO), Bent Erling Nielsen, Claus Borgaard (DMRI), Michel Bonneau (IFIP), Nuria Panella-Riera (IRTA), Marijke Aluwé (ILVO).

Contract number SANCO/2012/SI2.639561

https://ec.europa.eu/food/sites/food/files/animals/docs/aw_prac_farm_pigs_cast-alt_research_boarcheck_20140901.pdf

Boar taint detection: required specifications

- Accurate
- Repeatable
- Rapid response
  - A few minutes to 1 hour
- High throughput
  - Up to 600 pigs per hour
- Usable in industrial conditions
  - Heat, humidity, off-odours
- Non specialised staff
- Low cost
  - Maxi 1-2 €

Boar taint detection: the available methods

- Human nose methods
  - Rapid, low cost, high throughput
  - Subjective
  - Operator dependent
  - Cannot be easily related to consumer dissatisfaction
- Instrumental methods
  - Objective
  - No operator effect
  - Can be eventually related to consumer dissatisfaction provided that they measure skatole and androstenone
  - Likely more expensive

Human nose methods

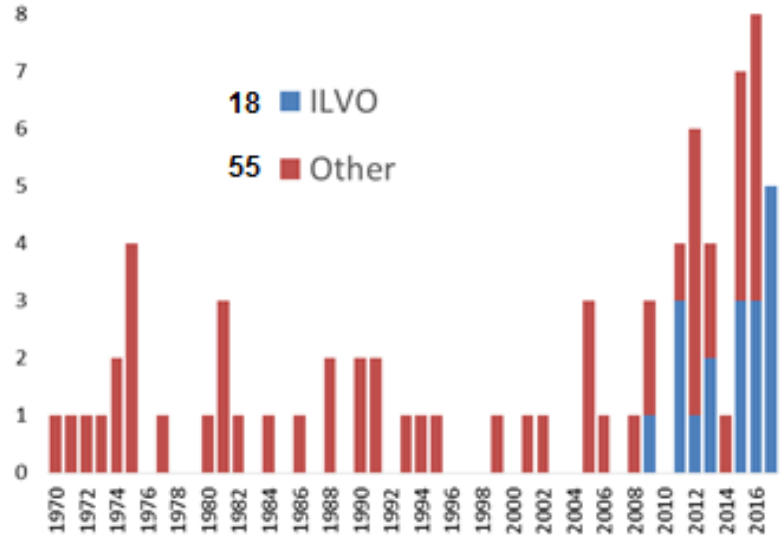
The first publication on a human nose method for rapid detection of boar taint

DETECTION OF TAINT (SEX ODOR) IN PORK
Leon Jarmoluk, A. H. Martin, H. T. Fredeen
Canadian Journal of Animal Science, 1970, 50(3): 750-752
https://doi.org/10.4141/cjas70-105

The instrument used was a 115-volt pistol-grip electric soldering gun with continuous heat build-up (i.e., no trigger switch). Application of the heated tip to a fat sample, specifically to the subcutaneous fat of the carcass or pork cut, was found to release the aromas observed in the cooking procedure. Further, the continuous heat burned all residue completely and rapidly, thus eliminating the need for cleaning or washing of the instrument between samples.

Human nose methods

Publications using, or referring to, soldering iron / hot iron



Human nose methods

- Were mostly used for research purpose
- Only recently (> 2012) did a handful of papers focus on rapid detection at industry level
- A lot of work was performed by the industry (Netherlands, Germany, France) that we know little about
  - Vion is an exception

Meat Science 91 (2012) 414–422

Contents lists available at SciVerse ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

A human nose scoring system for boar taint and its relationship with androstenone and skatole

P.K. Mathur [a,*], J. ten Napel [b], S. Bloemhof [a], L. Heres [c], E.F. Knol [a], H.A. Mulder [d]

Instrumental methods

BoarCheck

A study on rapid methods for boar taint used or being developed at slaughter plants in the European Union

D5.2 Final Report

Authors: **John-Erik Haugen (Nofima),** Coen van Wagenberg, Gé Backus (DLO), Bent Erling Nielsen, Claus Borgaard (DMRI), Michel Bonneau (IFIP), Nuria Panella-Riera (IRTA), Marijke Aluwé (ILVO).

Meat Science 90 (2012) 9–19

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/meatsci

Review

Review of analytical methods to measure boar taint compounds in porcine adipose tissue: The need for harmonised methods

J.-E. Haugen [a,*], C. Brunius [b], G. Zamaratskaia [b]

Instrumental methods

- The Danish colorimetric method (1982)
  - Skatole equivalents, androstenone not measured
  - Almost abandoned, no development outside of Denmark
- Numerous attempts in the last 20 years to have methods measuring both compounds
- No industrial method available so far

Instrumental methods

- Two promising methods have been recently announced in UK and Denmark
  - They both measure androstenone and skatole
  - They are not commercially available yet
- Performance
  - UK method unknown
  - Danish method: limited information
- Cost of the Danish method claimed to be < 1€
- Another method is in development in Belgium
  - Does not measure androstenone/Skatole
  - Still in lab/prototype phase

Instrumental methods: the UK method

Sensor and method for detecting androstenone or skatole in boar taint

EP 2966441 A1

https://google.com/patents/EP2966441A1?cl=en

ABSTRACT

The present application is concerned with a sensor system for and a method of detecting, and preferably quantifying, androstenone (CAS Reg.No. 18339-16-7) and/or skatole (CAS Reg. No. 83-34-1), the chemicals associated with boar taint. In a preferred embodiment, the sensor system comprises an array comprising (i) an enzyme electrode based on 3-hydroxysteroid dehydrogenase (3-HSD) which metabolises androstenone in the presence of cofactor NAD(P)H, together with a mediator, eg. Meldola's Blue (CAS Reg.No. 7057-57-0); and (ii) a sensor for the voltammetric detection of skatole, especially via direct oxidation at an electrode. The sensor system can be used to detect and quantify boar taint in pig carcasses or live pigs and so can be used to prevent the entrance of tainted carcasses into the food chain and to allow the grading of carcasses as "premium quality".

Instrumental methods: the Danish method

AIM

An increase in the number of slaughtered male pigs has led to demands for a rapid, reliable and inexpensive instrumental means of measuring androstenone and skatole. The aim was to develop an accurate method for measuring boar taint components in backfat from uncastrated male pigs, matching industrial demands for speed of operation and robustness.

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CONCLUSION

A rapid instrumental at-line method for simultaneous measurement of androstenone and skatole in back fat samples from entire male pigs has been developed. With an automated sample pre-treatment, it will be possible with a single LDTD-MS-MS system to keep up with a line speed of 360 male pig carcasses per hour and to run 16 hours per workday. Cost of operations is expected to be below 0.7€/carcass. Reproducibility on fully homogenized fat samples is better than 3% relative CV for androstenone and 5% relative CV for skatole.

cost
EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

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SCIENCE & IMPACT

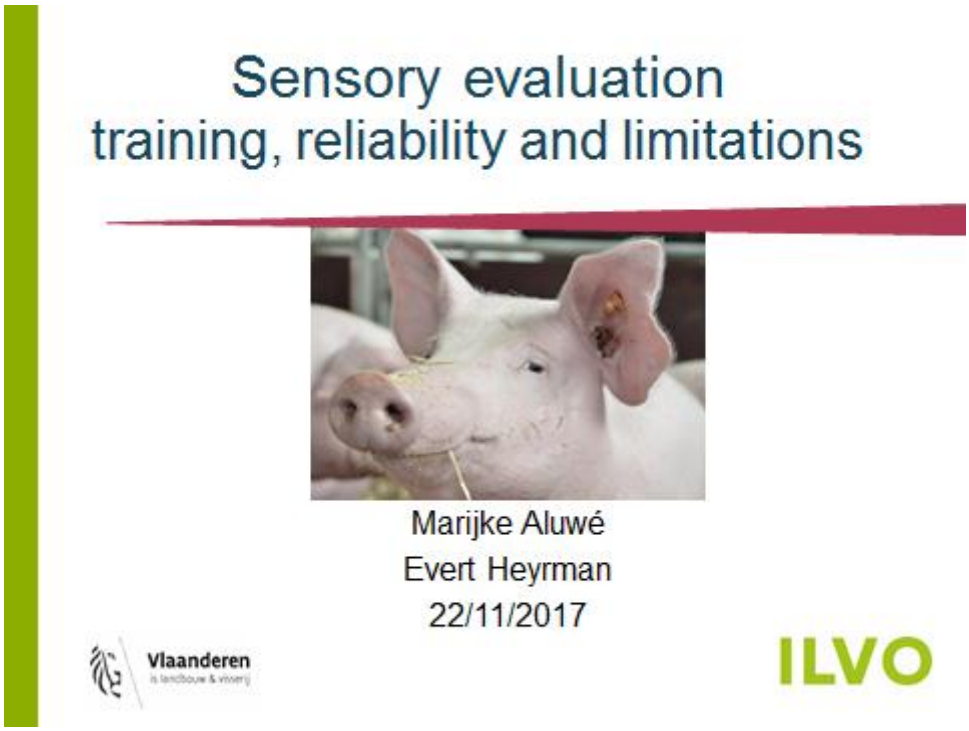
Thank you for your attention

The IPEMA consortium acknowledges the financial support of the EU, COST action CA15215.

Chapter 11

Human nose method – training, reliability and limitations

Marijke ALUWE[1]



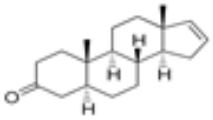
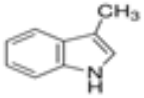
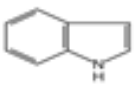
[1] Institute for Agricultural and Fisheries Research (ILVO), Scheldeweg 68, 9090 Melle, Belgium

Sensory evaluation

Soldering iron

ILVO

Sensory evaluation of boar taint

	Androstenone MP: 140°C	Skatole MP: 96°C	Indole MP: 53°C
			
[low]:	woody, floral	flowery	flowery
[high]:	sweat, urine	manure, napthalene	manure

± 50% sensitivity

± 99% sensitivity!

ILVO

Sensory evaluation

Step 1: Selection (AND sensitivity)

Step 2: Training with strips (BT and intensity)

Step 3: Use of HNS (soldering iron, BT and intensity)

Step 4: Training triangles

Step 5: Training series

ILVO

Sensory evaluation

Step 1: Selection (AND sensitivity)

Step 2: Training with strips (BT and intensity)

Step 3: Use of HNS (soldering iron, BT and intensity)

Step 4: Training triangles

Step 5: Training series

ILVO

Step 1: Selection of the experts

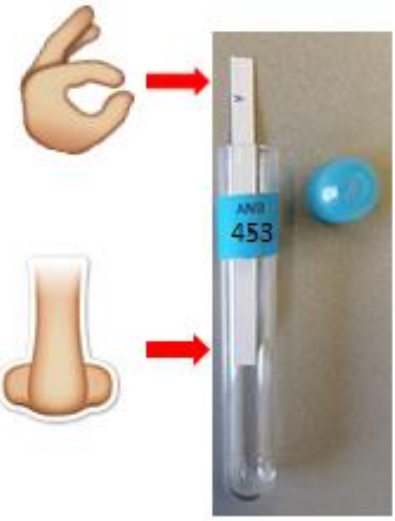
Androstenone sensitivity!

- Pure crystals
- Solutions => bottles/ strips
  - ✓ Crystals in water
  - ✓ Vaseline oil
  - ✓ Propyleneglycol
- Concentration: wide range:
  - ✓ SKA: 0.5 – 50 µg/g
  - ✓ AND: 0.2 – 50 µg/g
- ✓ Methodology

ILVO

Step 1: Selection of the experts

- Androstenone sensitivity!



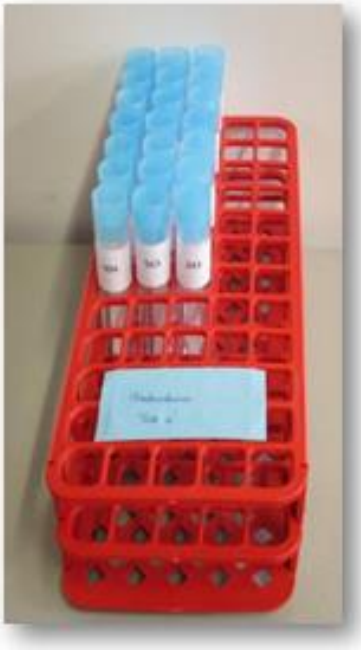
ILVO

Step 1: Selection of the experts

Triangle tests: identify the odd sample + liking/disliking

Tests	In random order		
Test 1	AND low	Blank	blank
Test 2	AND low	Blank	blank
Test 3	AND low	Blank	blank
Test 4	AND high	Blank	blank
Test 5	AND high	Blank	blank
Test 6	AND high	Blank	blank

Tests	In random order		
Test 1	SKA low	Blank	blank
Test 2	SKA low	Blank	blank
Test 3	SKA low	Blank	blank
Test 4	SKA high	Blank	blank
Test 5	SKA high	Blank	blank
Test 6	SKA high	Blank	blank



ILVO

Step 1: Selection of the experts

Preparation of the strips (based on Mörlein, 2013)

- Control strip: 20 µl propylene glycol
- Androstenone high: 20 µl of 5.0 µg/g AND solution
- Androstenone low: 20 µl of 0.5 µg/g AND solution
- Skatole high: 20 µl of 5.0 µg/g SKA solution
- Skatole low: 20 µl of 0.5 µg/g SKA solution

Odour tresholds trained experts

AND: 0.24 µg/g
SKA: 0.18 µg/g

Material

- Tubes: Carl Roth, Order no K938.1
- Lids: Carl Roth, white: E028.1; blue: E032.1, red: E030.1
- Sniffing strips: 240 g/m²

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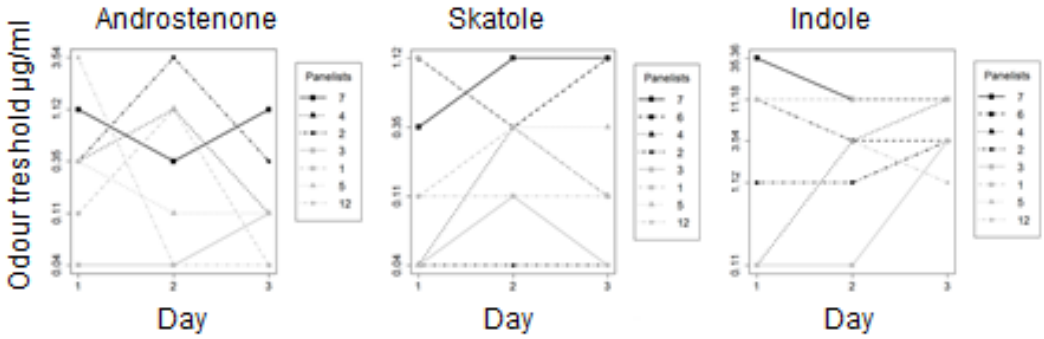
Odour thresholds



Based on staircase protocol (Heyrman)

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Androstenone sensitivity

Effect of concentration and repeated exposure

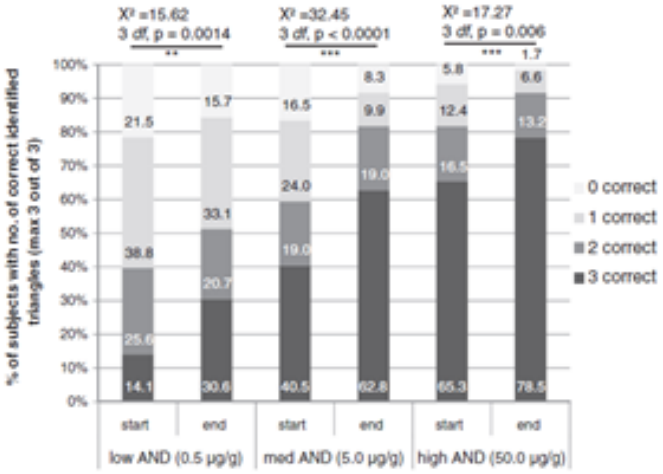


Fig. 1. Relative frequency of subjects (n = 121) with their number of correct discrimination of androstenone (AND) in various dilutions (0.5, 5.0, and 50.0 µg/g AND in propylene glycol on paper strips) at START and END of the six week experimental procedure. Distribution of subjects with respect to correct discrimination was tested for significant differences between START and END using Bhapkar's test in SAS PROC CATMOD.

(Mörlein, 2013)

ILVO

Sensory evaluation

Step 1: Selection (AND sensitivity)

Step 2: Training with strips (BT and intensity)

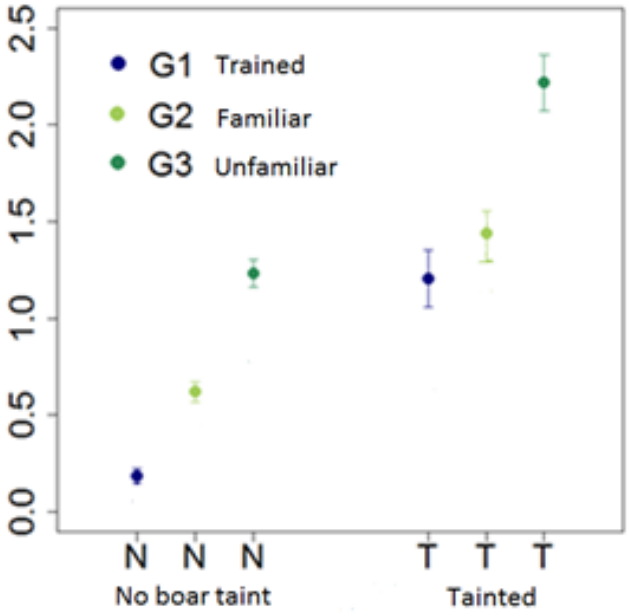
Step 3: Use of HNS (soldering iron, BT and intensity)

Step 4: Training triangles

Step 5: Training series

ILVO

Importance of training



(Heyrman, 2014)

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Importance of training

- Inter rater reliability: degree of agreement among raters
- Intra rater reliability: degree of agreement among repeated evaluations by a single rater

- Sensitivity: true positive rate
- Specificity: true negative rate

	G1 Trained	G2 Familiar	G3 unfamiliar
Inter rater reliability	0.45	0.29	0.16
Intra rater reliability	0.53	0.42	0.18
Sensitivity (HNS > 2)	0.34	0.44	0.62
Specificity (HNS > 2)	0.94	0.90	0.66

Heyrman, 2014

ILVO

Step 2: Training with odour strips

- Training with AND and SKA strips:
  - Recognise the boar taint compounds
  - Rank intensity of the boar taint compounds
  - Differentiate between AND and SKA and rank in intensity
    - Low: 0.5 µg/g
    - High: 5.0 µg/g
    - Very high: 50 µg/g

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Step 2: Training with odour strips

- Rank AND concentration: 3 out of 4 should be correct

Tests	In random order			
Test 1	AND low	AND high	AND very high	blank
Test 2	AND low	AND high	AND very high	blank
Test 3	AND low	AND high	AND very high	blank
Test 4	AND low	AND high	AND very high	blank

- Rank SKA concentration: 3 out of 4 should be correct

Tests	In random order			
Test 1	SKA low	SKA high	SKA very high	blank
Test 2	SKA low	SKA high	SKA very high	blank
Test 3	SKA low	SKA high	SKA very high	blank
Test 4	SKA low	SKA high	SKA very high	blank

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Step 2: Training with odour strips

- Differentiate between AND and SKA; and rank both

Tests	In random order						
Test 1	AND low	AND high	AND very high	blank	SKA low	SKA high	SKA very high
Test 2	AND low	AND high	AND very high	blank	SKA low	SKA high	SKA very high
Test 3	AND low	AND high	AND very high	blank	SKA low	SKA high	SKA very high
Test 4	AND low	AND high	AND very high	blank	SKA low	SKA high	SKA very high

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Sensory evaluation

Step 1: Selection (AND sensitivity)

Step 2: Training with strips (BT and intensity)

Step 3: Use of HNS (soldering iron, BT and intensity)

Step 4: Training triangles

Step 5: Training series

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Step 3: training the use of HNS

- scoring with the soldering iron
- boar taint compounds
- boar taint intensity

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Step 3: training the use of HNS

General guidelines

- Soldering iron: 350 °C (ERSA RDS80)

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Step 3: training the use of HNS

General guidelines

- Clean the soldering iron with ethanol in between each sample (lab settings)

Table 8
The mean value of sensory score given for the samples with and without boar taint and overall, Kendall's correlation coefficient (sens
with the concentration of the main boar taint compounds.

		Cleaning the soldering iron	
		Cleaning	Not-cleaning
Mean value of score	Samples with boar taint	52.9 ± 25.1^{a}	69.3 ± 24.3^{b}
	Samples without boar taint	2.3 ± 8.6^{a}	4.0 ± 10.4^{b}
	All samples	9.2 ± 21.2^{a}	13.0 ± 25.3^{b}
Correlation coefficient	Indole	0.48*	0.31*
	Skatole	0.45*	0.29*
	Androstenone	0.30*	0.27*

(Bekaert, 2013)

ILVO

Step 3: training the use of HNS

General guidelines

- Never singe on the same spot

Table 8
The mean value of sensory score given for the samples with and without boar taint and overall, Kendall's correlation coeffi with the concentration of the main boar taint compounds.

		Singeing on the same spot	
		1× singeing	2× singeing
Mean value of score	Samples with boar taint	76.1 ± 11.94	72.8 ± 10.5
	Samples without boar taint	3.7 ± 8.8[a]	1.8 ± 7.4[b]
	All samples	13.2 ± 25.9[a]	11.1 ± 25.5[b]
Correlation coefficient	Indole	0.49*	0.30*
	Skatole	0.43*	0.31*
	Androstenone	0.25*	0.26*

(Bekaert, 2013)

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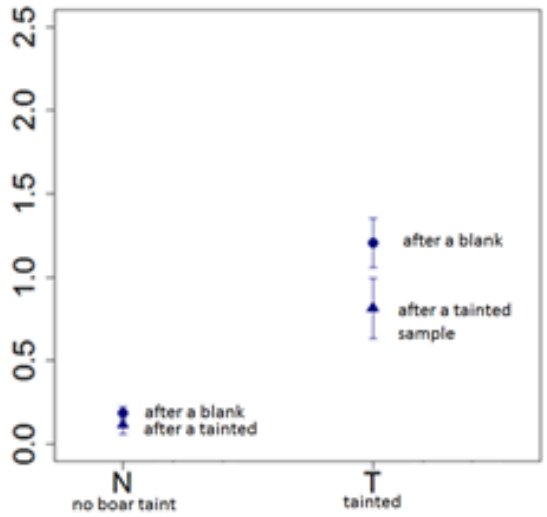
Step 3: training the use of HNS

General guidelines

- If you scored a sample as tainted, always score a blank sample before continuing



Heyrman, 2014
Bekaert, 2014

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Step 3: training the use of HNS

General guidelines

- Soldering iron: 350 °C (ERSA RDS80)
- Clean the soldering iron with ethanol in between each sample (lab settings)
- Never singe on the same spot
- If you scored a sample as tainted, always score a blank sample before continuing

- Well agreed scoring system

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Step 3: training the use of HNS

General guidelines

- Scoring system

Score	Description
0	No aberrant odour
1	Light boar taint
2	Moderate boar taint
3	Strong boar taint
4	Very strong boar taint
x (0-4)	X = Off-odour, but not boar taint Number = indicates the intensity

ILVO

y

Step 3: training the use of HNS

- Triangle tests with samples with known moderate and high boar taint levels and boar taint scores

- Identify tainted sample
- Score boar taint (0 to 4)
- Discuss the correctness and the intensity of the odour during scoring with trainer
- Can be repeated in case of doubt

Tests	In random order		
Test 1	AND	Blank	Blank
Test 2	AND	Blank	Blank
Test 3	AND	Blank	Blank
Test 4	SKA	Blank	Blank
Test 5	SKA	Blank	Blank
Test 6	SKA	Blank	Blank
Test 7	SKA + AND	Blank	Blank
Test 8	SKA + AND	Blank	Blank
Test 9	SKA + AND	Blank	Blank

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Sensory evaluation

Step 1: Selection (AND sensitivity)

Step 2: Training with strips (BT and intensity)

Step 3: Use of HNS (soldering iron, BT and intensity)

Step 4: Training triangles

Step 5: Training series

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Step 4: training triangles

Triangle tests (random order)	Triangle tests (random order)
Test 1	Test 1
Test 2	Test 2
Test 3	Test 3
Test 4	Test 4
Test 5	Test 5
Test 6	Test 6
Test 7	Test 7
Test 8	Test 8
Test 9	Test 9

Discuss results

Triangle tests (random order)	Triangle tests (random order)
Test 1	Test 1
Test 2	Test 2
Test 3	Test 3
Test 4	Test 4
Test 5	Test 5
Test 6	Test 6
Test 7	Test 7
Test 8	Test 8
Test 9	Test 9

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Step 5: training series

Series of 15 samples (random order)	
12 blank	3 tainted
Series of 15 samples (random order)	
12 blank	3 tainted
Series of 15 samples (random order)	
12 blank	3 tainted

Discuss results

Check correctness:

- Tainted samplesOK?
- Blank samplesOK?

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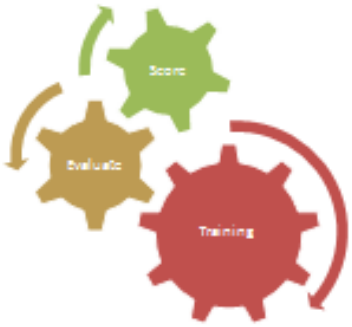
Sensory evaluation

Fig. 4. Graphical representation of the individual dichotomized ratings (hear, man) represented in black (deviant) and white (non-deviant). On the x-axes, the 72 samples are ordered by increasing androstenone and skatole levels (dual dichotomized ratings. The threshold for dichotomization was a score ≥2.

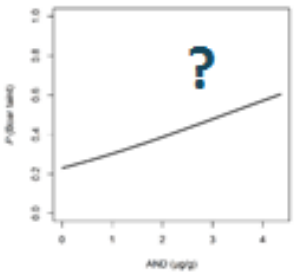
Trautmann, 2016

Evaluation of the experts

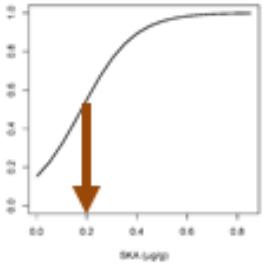


	G1 Trained
Inter rater reliability	0.45
Intra rater reliability	0.53
Sensitivity (HNS > 2)	0.34
Specificity (HNS > 2)	0.94

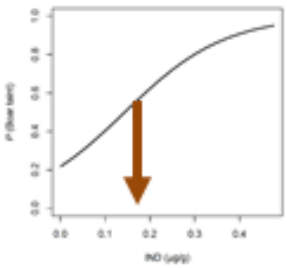
Androstenon



Skatol



Indol



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Sensory evaluation

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Sensory evaluation

- Maximum 100 - 120 samples per evaluation

- Lab scale
  - Minimum: 3 experts
  - Preferably: at least 3 experts on two consecutive days or 6 experts per sample

  - Depends on the aim of the experiment and the number of samples involved

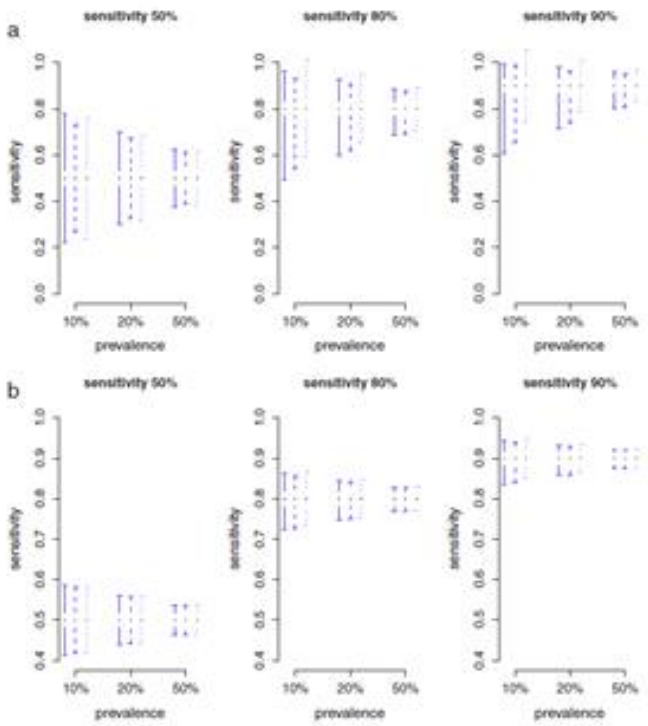

Fig. 3. Examples of 90% confidence intervals for sensitivity with respect to total sample size 100 (a), and 1000 (b), varying observed sensitivity (50, 80, 90%), and varying prevalence of truly tainted samples (10, 20, 50%). Several options were used to compute CIs, i.e., exact according to Clopper & Pearson (solid line), Wilson (dashed line), and asymptotic normal (dotted line).

Mörlein, 2015

Thank you for your attention

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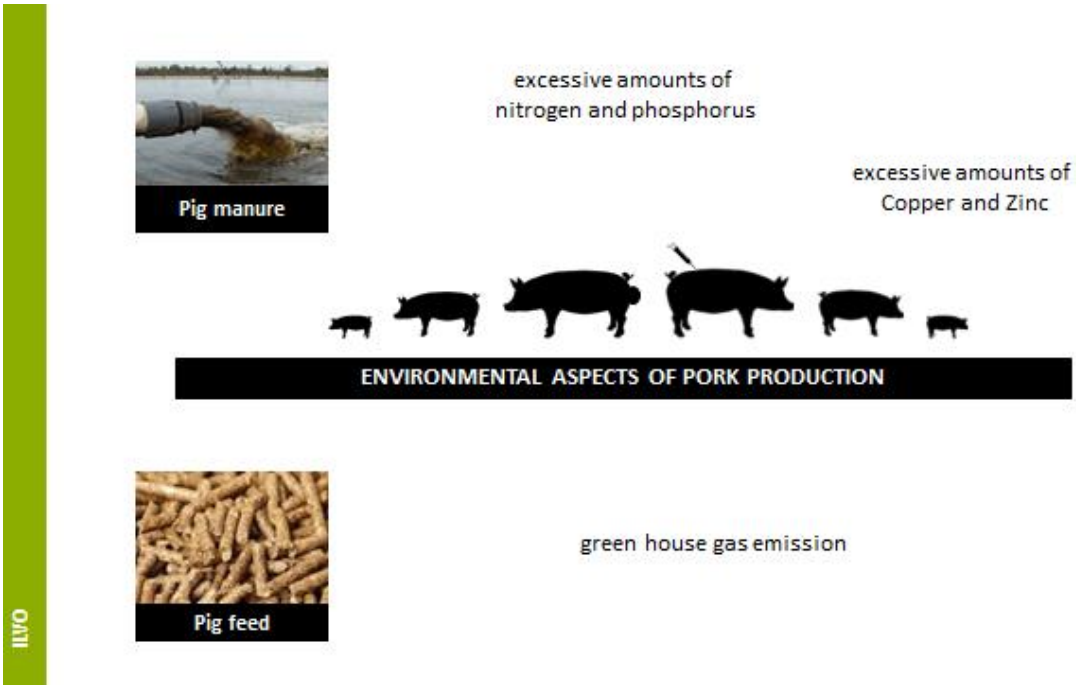
en Visserijonderzoek

Chapter 12

Nutritional and environmental aspects - indicators and recording

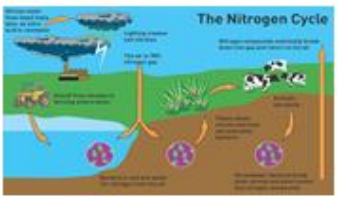
Alice van den BROEKE[1]

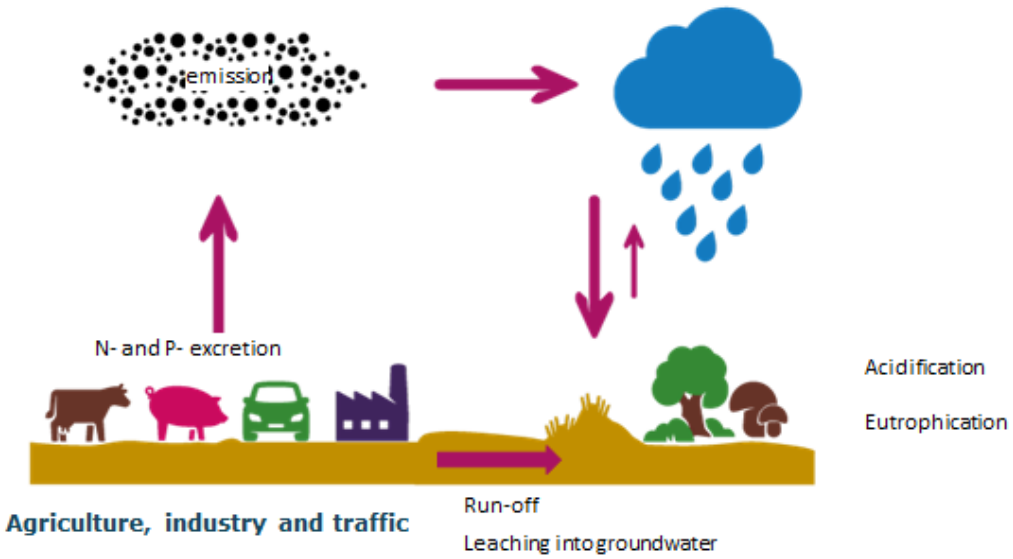


[1] Institute for Agricultural and Fisheries Research (ILVO), Scheldeweg 68, 9090 Melle, Belgium

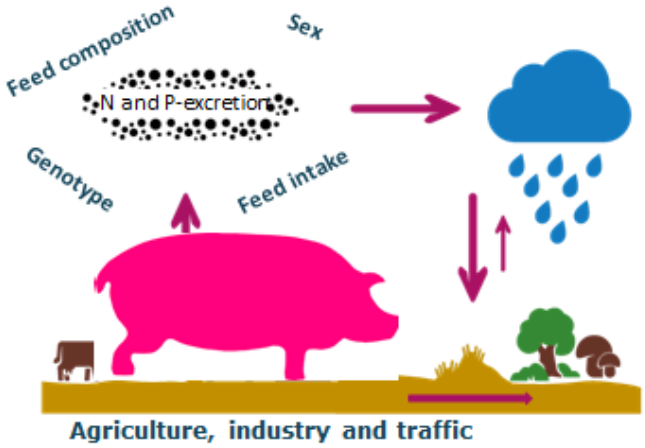
ENVIRONMENTAL ASPECTS OF PORK PRODUCTION

NUTRIENT EXCRETION: N AND P	CARBON FOOTPRINT FEED





Environmental sustainable pork production: aim at lowest possible N- and P-excretion



ILVO

Comparison nutrient excretion

SUSI

Sex: IC versus barrows and entire males

Feed intake: sex-dependent

Genotype: trials in different countries

Feed composition: for example high protein versus low protein diet

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Calculations nutrient balance growing-finishing pigs

Nutrient excretion = Nutrient intake – Nutrient retention

2 methods:

- Determine total nutrient excretion: digestibility cages

- Determine nutrient intake and retention and calculate excretion

Determine total nutrient excretion

Collection of all urine and manure on individual level

4-5 days collection per phase

Analysis of urine and manure in lab

Determination of total N- and P- excretion during growing-finishing period

Calculations nutrient balance growing-finishing pigs

Nutrient excretion = Nutrient intake – Nutrient retention

2 methods:

- Determine total nutrient excretion: digestibility cages

- Determine nutrient intake and retention and calculate excretion

Calculations nutrient balance growing-finishing pigs

Nutrient intake=

[mean **feed intake** pig per feeding phase × **Nutrient content** per feeding phase]

Nutrient retention=

[**Nutrient content pig** × weight of the pig)-(**Nutrient content piglet** × weight piglet)]

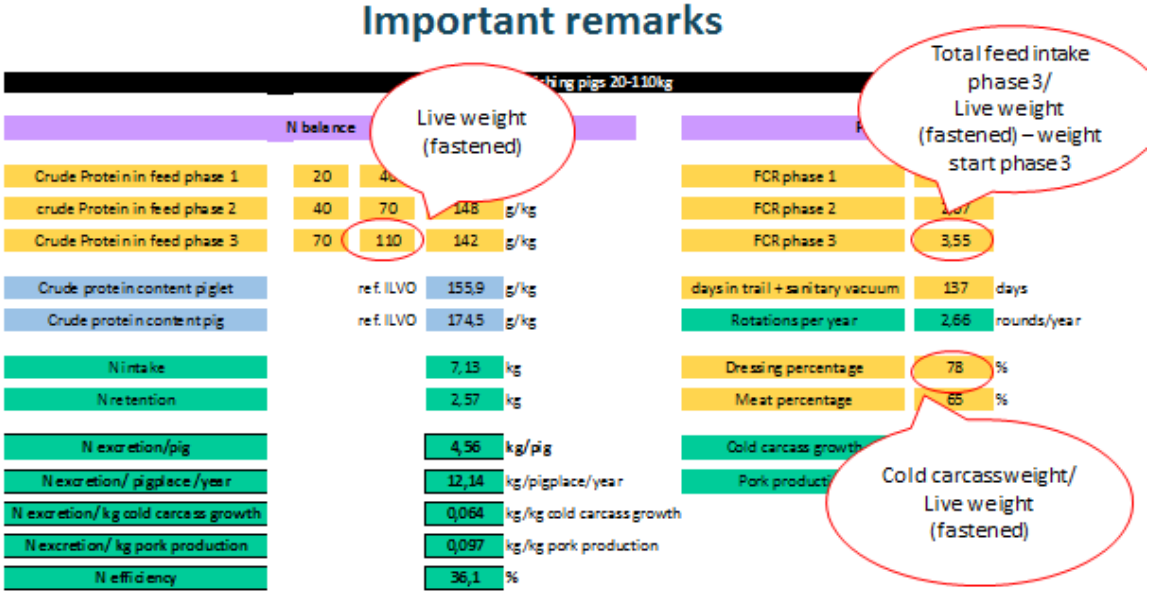
Calculation template

Growing-finishing pigs 20-110kg

N balance					Performance		
Crude Protein in feed phase 1	20	40	157	g/kg	FCR phase 1	4,00	
crude Protein in feed phase 2	40	70	148	g/kg	FCR phase 2	2,67	
Crude Protein in feed phase 3	70	110	142	g/kg	FCR phase 3	3,55	
Crude protein content piglet		ref. ILVO	155,9	g/kg	days in trail + sanitary vacuum	137	days
Crude protein content pig		ref. ILVO	174,5	g/kg	Rotations per year	2,66	rounds/year
N intake			7,13	kg	Dressing percentage	78	%
N retention			2,57	kg	Meat percentage	65	%
N excretion/pig			4,56	kg/pig	Cold carcass growth	70,8	kg
N excretion/ pigplace /year			12,14	kg/pigplace/year	Pork production	46,77	kg
N excretion/ kg cold carcass growth			0,064	kg/kg cold carcass growth			
N excretion/ kg pork production			0,097	kg/kg pork production			
N efficiency			36,1	%			

ILVO

Important remarks



ILVO

Important remarks

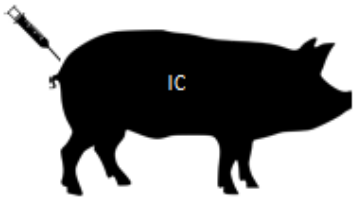
Growing-finishing pigs 20-110kg

N balance					Performance		
Crude Protein in feed phase 1	20	40	157	g/kg	FCR phase 1	4,00	
crude Protein in feed phase 2	40	70	148	g/kg	FCR phase 2	2,67	
Crude Protein in feed phase 3	70	110	142	g/kg	FCR phase 3	3,55	
Crude protein content piglet		ref. ILVO	155,9	g/kg	days in trail + sanitary vacuum	137	days
Crude protein content pig		ref. ILVO	174,5	g/kg	Rotations per year	2,66	rounds/year
N intake					Dressing percentage	78	%
N retention					Meat percentage	65	%
N excretion/pig					Cold carcass growth	70,8	kg
N excretion/ pigplace /year					Pork production	46,77	kg
N excretion/ kg cold carcass growth							
N excretion/ kg pork production			0,097	kg/kg pork production			
N efficiency			36,1	%			

Crude protein content based on trials ILVO

ILVO

Results ILVO trials



ILVO

Nutrient content pig and piglets

2 trials:
In total 178 pigs and 28 piglets euthanized

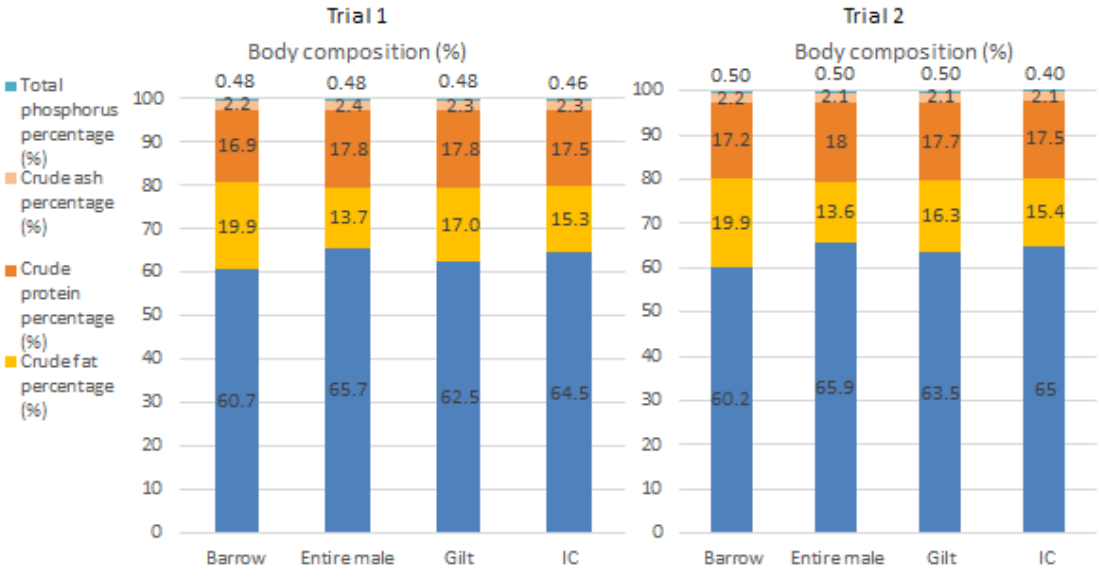
Carcass grinded
Representative subsample of 10 kg collected

Subsample autoclaved, mixed, lyophilized and analysed

Body composition:
Water, crude protein, crude fat, crude ash, total phosphorus concentration

ILVO

Nutrient content pig and piglets



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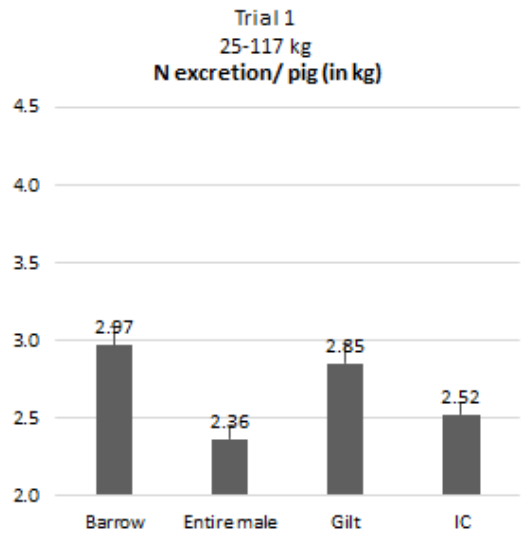
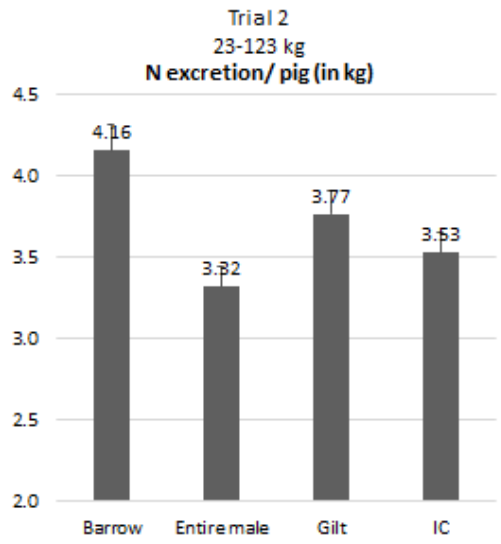
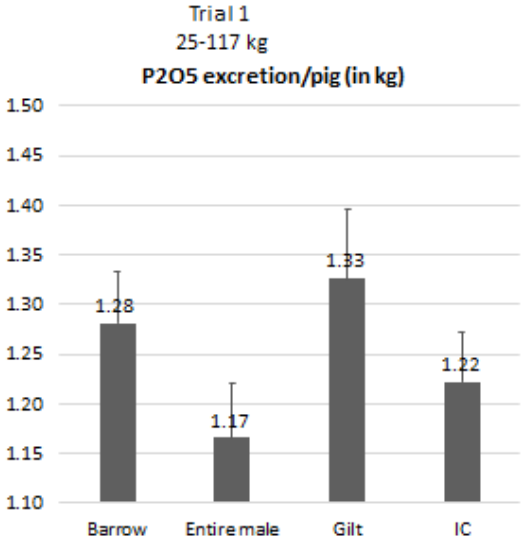
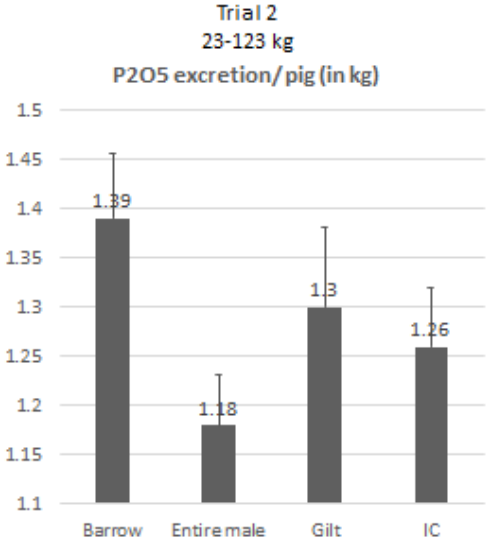
N excretion/pig





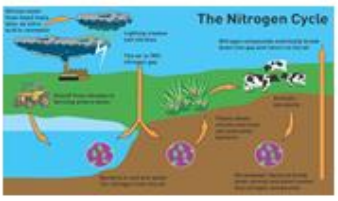
P_2O_5 excretion



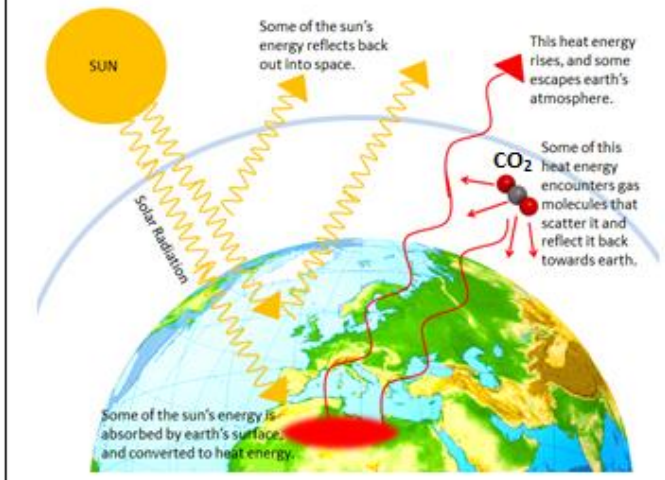


ECOLOGICAL ASPECTS OF PORK PRODUCTION

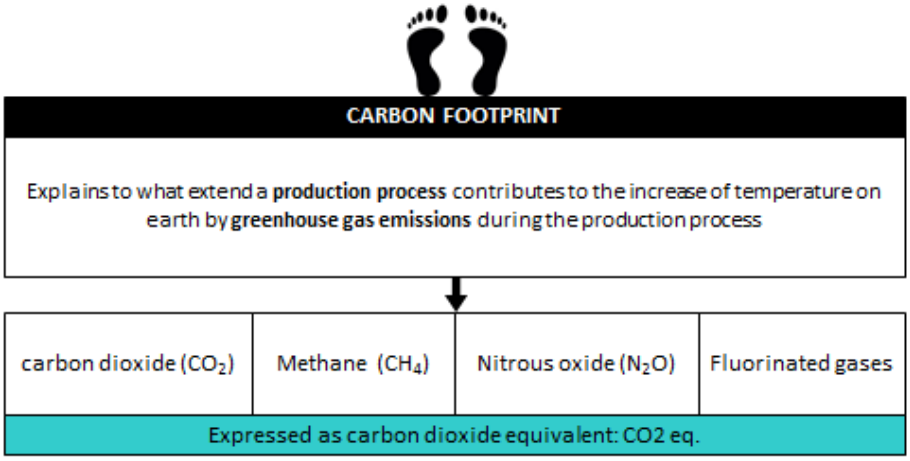
NUTRIENT EXCRETION N AND P	CARBON FOOTPRINT FEED

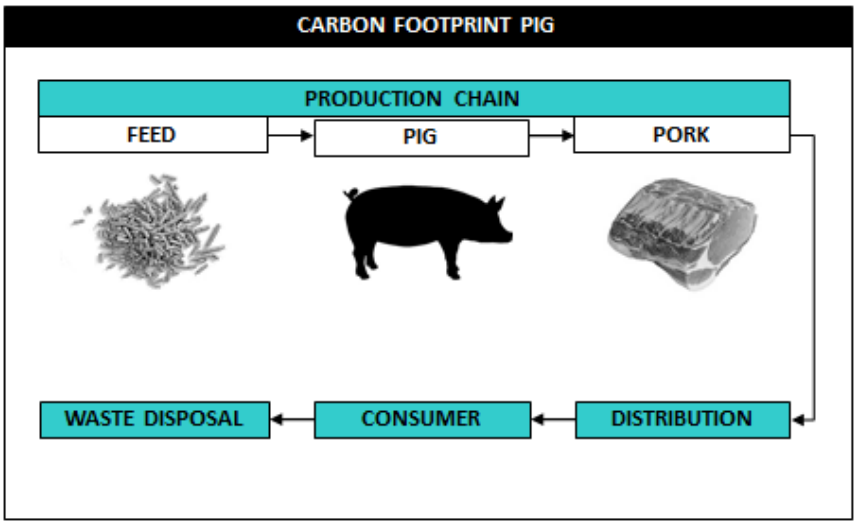


GREENHOUSE GAS EMMISION



MORE GREENHOUSE GAS EMMISION= HIGHER INCREASE OF GLOBAL WARMING


CARBON FOOTPRINT
Explains to what extend a production process contributes to the increase of temperature on earth by greenhouse gas emissions during the production process
carbon dioxide (CO_2)
Methane (CH_4)
Nitrous oxide (N_2O)
Fluorinated gases
Expressed as carbon dioxide equivalent: CO2 eq.


CARBON FOOTPRINT PIG
PRODUCTION CHAIN
FEED
PIG
PORK
WASTE DISPOSAL
CONSUMER
DISTRIBUTION

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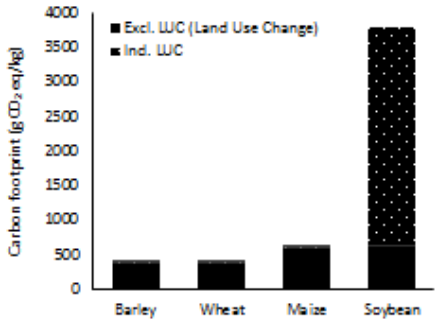
CARBON FOOTPRINT OF FEED PRODUCTION

FeedPrint databank: carbon footprint per ingredient



HIGH CARBON FOOTPRINT SOYBEANS

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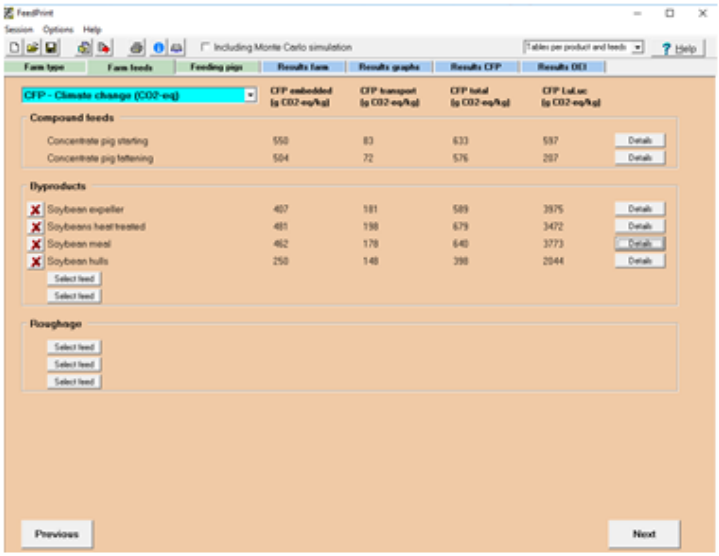
Calculation template

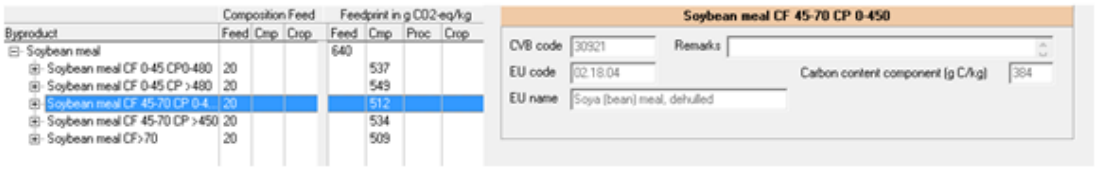
To calculate CO_2 eq/ kg feed, comprehensive description of ingredients needed

Carbon footprint				Performance		
Carbon footprint phase 1	20	40	627,08 g CO2 eq/kg	FCR phase 1	1,05	
Carbon footprint phase 2	40	70	634,47 g CO2 eq/kg	FCR phase 2	1,67	
Carbon footprint phase 3	70	110	599,37 g CO2 eq/kg	FCR phase 3	3,55	
				days in hall + sanitary vacuum	157	days
				Rotations per year	2,66	rounds/year
CO2eq produced for total intake			161,56 kg CO2 eq/ pig	Dressing percentage	78	%
				Meat percentage	65	%
CO2 eq / kg pig growth			1,80 kg CO2/kg pig growth	Cold carcass growth	70,5	kg
CO2 eq / kg cold carcass growth			2,28 kg CO2/kg cold carcass growth	Pork production	46,77	kg
CO2 eq / kg pork production			3,455 kg CO2/kg pork production			

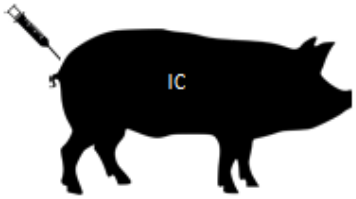
Feedprint database

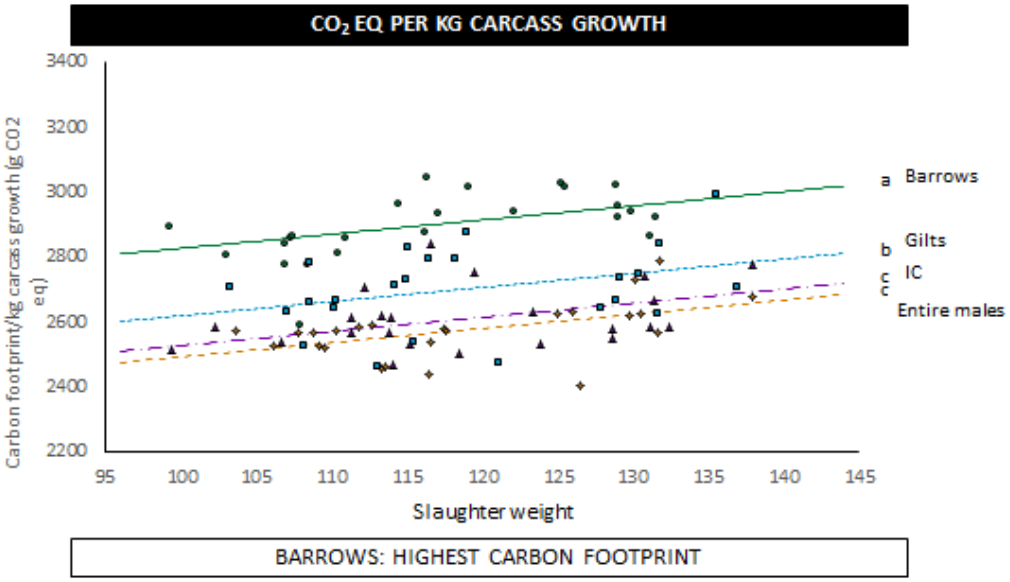


Feedprint database



Results ILVO trials





Thank you for your attention

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