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# Energy metabolism, growth performance, body composition, meat quality and boar taint compounds in immunocastrated pigs

Nina Batorek

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UNIVERZA V MARIBORU  
FAKULTETA ZA KMETIJSTVO IN BIOSISTEMSKO VEDE

AGROCAMPUS-OUEST  
ECOLE DOCTORALE DE RENNES (VIE, AGRO, SANTE)

ENERGY METABOLISM, GROWTH PERFORMANCE, BODY  
COMPOSITION, MEAT QUALITY AND BOAR TAIN COMPOUNDS IN  
IMMUNOCASTRATED PIGS  
PH. D. THESIS

METABOLIZEM ENERGIJE, PITOVNE LASTNOSTI, TELESNA  
SESTAVA, KVALITETA MESA IN SUBSTANCE VONJA PO MERJASCU  
PRI IMUNOKASTRIRANIH PRAŠIČIH  
DOKTORSKA DISERTACIJA

METABOLISME ENERGETIQUE, PERFORMANCES DE CROISSANCE,  
COMPOSITION CORPORELLE, QUALITE DE VIANDE ET LES  
COMPOSES DE L'ODEUR DE VERRAT CHEZ LES PORC  
IMMUNOCASTRES  
THESE DE DOCTORAT

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Nina BATOREK LUKAČ





Faculty of Agriculture  
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## THESE / AGROCAMPUS OUEST

Sous le label de l'Université Européenne de Bretagne  
pour obtenir le diplôme de :

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Ecole Doctorale : « VIE, AGRO, SANTE »

présentée par :

« **NINA BATOREK LUKAČ** »

**METABOLISME ENERGETIQUE, PERFORMANCES DE CROISSANCE,  
COMPOSITION CORPORELLE, QUALITE DE VIANDE ET LES COMPOSES DE  
L'ODEUR DE VERRAT CHEZ LES PORC IMMUNOCASTRES**

soutenue le « 8. Juillet 2015 » devant la commission d'Examen

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**Errata:**

Present doctoral dissertation was carried out in co-supervision between the University of Maribor (UM), the Faculty of Agriculture and Life Sciences (FKBV), Maribor, Slovenia and Agrocampus Ouest, Rennes, France. It is based on a young researcher's project entitled "*Immunocastration as a way to prevent boar taint: effects on growth, body composition and meat quality*" financed by the Slovenian Research Agency. The experimental part of the thesis was performed at the Agricultural Institute of Slovenia (KIS) and at INRA PEGASE, Saint Gilles, France in accordance with current legislation on animal welfare and animal breeding.

The Senate of UM has approved the disposition of doctoral dissertation at the 26<sup>th</sup> regular session on 17<sup>th</sup> December 2013. Assoc. prof. dr. Marjeta Čandek-Potokar and Jacob van Milgen, PhD, HDR have been appointed as mentor and co-mentor. The Senate of FKBV has named prof. dr. Dejan Škorjanc, prof. dr. Janez Salobir and Giuseppe Bee, PhD to the commission for the evaluation of doctoral dissertation on 22<sup>nd</sup> of April 2015. The report of the commission for evaluation of doctoral dissertation has been addressed on 27<sup>th</sup> of May at the correspondence session of the Senate of FKBV, where the commission for the defense of the dissertation was appointed by the Senate.

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## **Energy metabolism, growth performance, body composition, meat quality and boar taint compounds in immunocastrated pigs**

UDK: 636.4.082.2:636.4.084.52:637.5.04/.07(043.3)=111

In recent years, immunocastration, a vaccination against gonadotropin releasing hormone, has been proposed as an alternative to surgical castration of intact male pigs without anaesthesia. However, the effects of immunocastration on metabolic changes, nutritional requirements and growth potential have not been quantified. Thus in the present thesis, the meta-analytical approach was used to quantitatively synthesize the effects of immunocastration and four studies were conducted to evaluate the effects of quantitative and qualitative feed restriction on growth performance, heat production and energy metabolism, carcass and meat quality. Results show that boar taint compounds are dramatically reduced in immunocastrates, still they remain slightly higher than in surgical castrates. Immunocastrates are less feed efficient, fatter but grow more rapidly and may have better meat quality than entire males. Compared to surgical castrates, immunocastrates have superior performance with no difference in meat quality. It is more economical to fatten immunocastrates than surgical castrates, but production costs and carcass quality are less favourable than in entire males because of the huge increase in voluntary feed intake during the weeks preceding slaughter. Applying quantitative feed restriction after active immunization did not significantly affect growth performance or carcass composition; however, restrictively fed immunocastrates were closer to entire males, whereas ad libitum fed immunocastrates were closer to surgical castrates. Meat quality is also unchanged by the restriction. However, higher incidence of carcass lesions in restricted immunocastrates together with higher plasma cortisol level indicates more aggression prior to slaughter and can be considered as a negative aspect of the quantitative restriction. Total heat production, assumed to be the major factor contributing to the differences in feed efficiency of pigs, is changed after the immunocastration. Additional energy retained due to higher feed intake is directed towards lipid deposition. Increased fatness of immunocastrates is a result of increased daily lipid deposition caused by higher energy intake, lower fasting heat production and improved energy retention. Hence, dietary energy intake should be limited after immunocastration. Moreover, according to the results of the nitrogen balance study, dietary crude protein content could also be decreased. By reducing net energy concentration of the diet up to 10 % using higher fibre content, similar energy efficiency (gain to net energy intake ratio) may be achieved, with an advantage in terms of reduced lipid deposition, and no effect on growth rate after effective immunization. This is particularly important when immunocastrated pigs are fattened to higher body weights, i.e. when longer delays between full immunization and slaughter are practiced.

Keywords: pig / immunocastration / growth performance / carcass characteristics / meat quality / heat production / lipid metabolism / fat deposition / feed restriction

NO: xiv, 222 p., 2 tab., 11 ph., 282 ref.

## **Metabolizem energije, pitovne lastnosti, telesna sestava, kvaliteta mesa in substance vonja po merjascu pri imunokastriranih prašičih**

UDK: 636.4.082.2:636.4.084.52:637.5.04/07(043.3)=111

V preteklih letih je bila kot alternativa kirurški kastraciji merjaščkov brez uporabe anestezije predlagana imunokastracija, pri kateri gre za vakcinacijo proti gonadotropin sproščujočemu hormonu. Vendar pa učinki imunokastracije na presnovo energije, prehranske potrebe in rastni potencial imunokastratov še niso bili ovrednoteni. V sklopu doktorske disertacije smo tako s pomočjo meta-analize kvantitativno sintetizirali učinek imunokastracije in izvedli štiri poskuse, v katerih smo proučevali količinsko ter energijsko omejitev krme ter produkcijo toplote in presnovo energije pri imunokastriranih prašičih. Rezultati kažejo, da se po imunokastraciji vonj po merjascu drastično zmanjša, še vedno pa ostaja nekoliko višji kot pri kirurških kastratih. Imunokastrati manj učinkovito izkoriščajo krmo, so bolj zamaščeni, vendar hitreje priraščajo in imajo boljše kakovost mesa od merjascev. V primerjavi s kirurškimi kastrati odlikujejo imunokastrate boljše pitovne lastnosti brez razlik v kakovosti mesa. Tako je reja imunokastratov bolj ekonomična kot reja kirurških kastratov, vendar pa so proizvodni stroški in klavna kakovost manj ugodni kot pri reji merjascev. Količinska omejitev krme po polni imunizaciji ne vpliva značilno na prirast ali klavne lastnosti, vendar so restriktivno krmljeni imunokastrati v omenjenih lastnostih bližje merjascem, imunokastrati krmljeni po volji pa kirurškim kastratom. Kakovost mesa je kljub restrikciji krme, v primerjavi s kirurškimi kastrati, nespremenjena. Višja incidenca lezij na klavnih trupih restriktivno krmljenih imunokastratov (podobna kot pri merjascih) skupaj z višjo plazemsko koncentracijo kortizola kaže na večjo agresijo pred zakolom in se lahko smatra kot negativni vidik količinskega omejevanja krme. Skupna produkcija toplote, glavni dejavnik, ki prispeva k razlikam v izkoriščanju krme med prašiči, se je po učinkoviti imunokastraciji spremenila. Dodatna naložena energija, ki je posledica večjega zauživanja krme po imunokastraciji, se usmeri v nalaganje maščob. Povečana zamaščenost imunokastratov je posledica povečanega dnevnega nalaganja maščob zaradi večjega vnosa energije, nižje proizvodnje toplote na tešče in boljšega nalaganja energije. Zato priporočamo, da se vnos energije po drugi vakcinaciji imunokastratom omeji, prav tako je glede na izsledke ocene bilance dušika, potrebno po drugi vakcinaciji omejiti vsebnost surovih beljakovin v krmi. S povečanjem količine vlaknin v obroku smo zmanjšali koncentracijo neto energije krmne mešanice do 10 % in s tem dosegli podobno energijsko učinkovitost (prirast preračunan na količino zaužite neto energije v obroku) po učinkoviti imunizaciji. S tem smo omejili nalaganje maščob in zagotovili boljše učinkovitost prireje, kar je še posebej pomembno, kadar so imunokastrati namenjeni za pitanje na višje teže, ali ko je pričakovati daljši razmik med polno imunizacijo in zakolom.

**Ključne besede:** prašič / imunokastracija / pitovne lastnosti / klavna kakovost / kakovost mesa / produkcija toplote / nalaganje maščob / restriktivno krmljenje

OP: xiv, 222 s., 2 pregl., 10 sl., 282 ref.

## **Metabolisme energetique, performances de croissance, composition corporelle, qualite de viande et les composes de l'odeur de verrat chez les porc immunocastres**

UDK: 636.4.082.2:636.4.084.52:637.5.04/.07(043.3)=111

Au cours des dernières années, l'immunocastration, caractérisée par la double injection d'un vaccin anti gonadotropique, a été proposée comme alternative à la castration chirurgicale sans anesthésie des porcs mâles entiers. Cependant, les effets de l'immunocastration sur les changements métaboliques, les besoins nutritionnels et le potentiel de croissance des porcs n'ont pas été quantifiés. Ainsi, dans cette thèse, une approche par méta-analyse a été utilisée pour synthétiser quantitativement les effets de l'immunocastration sur les performances de croissance et la qualité de la carcasse et quatre études ont été menées pour évaluer l'effet d'une restriction alimentaire quantitative et qualitative sur les performances de croissance, la production de chaleur et le métabolisme énergétique et la qualité de la carcasse et de la viande chez les porcs immunocastres. Les résultats montrent que les composés responsables des odeurs sexuelles sont considérablement réduits dans les carcasses des porcs immunocastres, même s'ils restent encore légèrement supérieurs à ceux rencontrés chez les porcs castrés chirurgicalement. Les porcs immunocastres sont moins efficaces et plus lourds que les porcs mâles entiers mais ont des performances de croissance plus élevées et ont une meilleure qualité de viande que les mâles entiers. Les porcs immunocastres ont des performances de croissance supérieures aux porcs castrés chirurgicalement mais sans différence de qualité de la viande. Il est également plus économique d'engraisser des porcs immunocastres que des porcs mâles castrés chirurgicalement, mais les coûts de production et la qualité de carcasse est moins intéressante que lors de l'engraissement de mâles entiers. La restriction alimentaire quantitative après vaccination effective ne modifie pas les performances de croissance mais améliore l'efficacité alimentaire et la composition des carcasses des porcs immunocastres. La qualité de la viande n'est également pas influencée par la restriction quantitative. Cependant, l'incidence plus élevée de lésions de la carcasse chez les porcs immunocastres et alimentés avec restrictions (similaire à celle de mâles entiers) et un niveau plus élevé de cortisol plasmatique indiquent une agressivité supérieure avant l'abattage et peut être considéré comme un aspect négatif de la restriction quantitative. La production de chaleur, principal facteur contribuant aux différences dans l'efficacité alimentaire chez les porcs est modifiée après l'immunocastration. L'énergie supplémentaire retenue en raison de la prise alimentaire plus élevée est dirigée vers le dépôt de lipides. Le niveau d'engraissement supérieur des carcasses des porcs immunocastres est le résultat d'une augmentation des dépôts de lipides causée par une plus grande consommation d'énergie, un métabolisme basal inférieur et une meilleure efficacité de rétention de l'énergie. Par conséquent, les apports énergétiques doivent être limités après l'immunocastration. En outre, les résultats de l'étude du bilan azoté des porcs immunocastres indiquent que la teneur en protéine brute l'aliment peut également être diminuée. En réduisant la concentration en énergie nette de l'alimentat de 10% par l'ajout de fibres alimentaires, une efficacité énergétique similaire (rapport entre le gain pondéral et l'ingéré d'énergie nette) peut être obtenue, en limitant le dépôt de lipides mais sans affecter les performances de croissance chez les porcs immunocastres. Ceci est particulièrement important lorsque les porcs immunocastres sont engraisés jusqu'à un poids vif plus élevé que la pratique courante, c'est-à-dire lorsque des délais plus longs entre la vaccination effective et l'abattage sont pratiqués.

Les mots clés: porc / immunocastration / performances de croissance/ carcasse / qualité de la viande / production de chaleur / métabolisme des lipides / dépôt de lipides / restriction alimentaire

NO: xiv, 222 p., 2 tab., 10 ph., 282 ref.

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### Abbreviations and symbols

ADF	acid detergent fibre
ADFI	average daily feed intake
ADG	average daily gain
ADL	acid detergent lignin
AHP	heat production due to physical activity
BFT	backfat thickness
BW	body weight
CIE a*	redness
CIE b*	yellowness
CIE L	lightness
CIE	Commission Internationale de l'Elclairage
CV	coefficient variation
DM	dry matter
DMI	dry matter intake
ELISA	enzyme-linked immunosorbent assay
EM	entire males
EU	European Union
FA	fatty acids
FCR	feed conversion ratio
FHP	fasting heat production
FSH	follicle-stimulating hormone

G:F	gain to feed ratio
GC	gas chromatography
GE	gross energy
GH	growth hormone
GnRH	gonadotropin releasing hormone
HFD	high fat diet
HI	heat increment
HP	heat production
HPG axis	hypothalamic-pituitary-gonadal axis
HPLC	high-performance liquid chromatography
i.m.	intra-muscular
IC	immunocastrated pigs
IC-R	immunocastrated pigs fed restrictively
IC-L	immunocastrated pigs fed <i>ad libitum</i>
IGF-I	Insulin-like growth factor 1
IMF	intramuscular fat
LD	lipid deposition
LFD	low fat diet
LH	luteinizing hormone
ME	metabolizable energy
NDF	neutral detergent fibre
NE	net energy
NIMF	neck intermuscular fatness

NIRS	near-infrared spectroscopy
NSAID	non-steroidal anti-inflammatory drug
PD	protein deposition
pH24	pH measured 24 h post mortem
pH45	pH measured 45 minutes post mortem
RE	retained energy
RIA	radioimmunoassay
RQ	respiratory quotient
S	slaughter
s.c.	subcutaneous
S1	stage 1
S2	stage 2
SC	surgical castrates
SD	standard deviation
SID	standardized ileal digestible
TEF <sub>lt</sub>	long-term thermic effect of feeding
TEF <sub>st</sub>	short-term thermic effect of feeding
TEF <sub>t</sub>	thermic effect of feeding
V1	first vaccination
V1-S	overall experiment period
V2	revaccination or second vaccination
WHC	water holding capacity
Θ	standardized effect size

## 1 INTRODUCTION

### 1.1 Definition of the problem

Castration or gonadoextomy is a surgical procedure performed on male piglets for centuries in which testis and epididymis are physically removed from scrotal sack. It can be done without anaesthesia or analgesia within the first 7 days of life (Council Directive 2008). It is practiced to avoid presence of boar taint in meat and meat products, which makes pork undesirable for sensitive consumers, and also to prevent sexual behaviour in group housing. The substances that are considered the primary cause of boar taint are androstenone, a male pheromone (Patterson 1968) and skatole, a by-product of tryptophan breakdown in large intestine (Vold 1970, Walstra and Maarse 1970). Skatole has a faecal like odour, detected by vast majority of people (Weiler et al. 2000), whereas androstenone has a urine-like odour and flavour that has large variation in consumers' sensitivity. Some people can detect androstenone in very low concentration and others are anosmic to it (Wysocki and Beauchamp 1984). Androstenone (5 $\alpha$ -androst-16-ene-3-one) is an anabolic steroid without androgenic effect (Claus et al. 1971). It is produced by the Leydig cells in the testis of sexually mature male pigs (Gower 1972). Due to its lipophilic character, it accumulates in adipose tissue in much higher amounts than other sex hormones (Pearce et al. 1988). Part of androstenone is stored in salivary glands and serves as a pheromone to promote sexual behaviour in female pigs (Pearce et al. 1988). It has also been detected in the plasma of females and castrates in low levels, indicating its possible production in adrenal cortex and ovary (Claus et al. 1971). Unlike androstenone, skatole (3-methyl-indole) does not seem to have a biological function in pig. It is a by-product of amino acid tryptophan, broken down by bacterial microflora in cecum and colon of pig. Therefore the amount of skatole produced is primarily regulated by the availability of tryptophan, which mainly originates from gut-mucosa cells (Claus et al. 1994; Claus and Raab 1999) and the activity of intestinal bacteria, especially *Escherichia coli*, *Lactobacillus* sp. and *Clostridium* sp. (Jensen et al. 1995a). Skatole that is being produced in the colon is partly absorbed through



intestinal wall, released into bloodstream and is thereafter partially metabolised in the liver. Similarly like androstenone, skatole is lipophilic, so it accumulates in adipose tissue, but only in app. 10% of entire male pigs (Bonneau et al. 1992). Because the European Union (EU) regulations aim at avoiding physical castration without analgesia and/or anaesthesia, alternatives have been proposed to limit boar taint. Among them, a method called immunocastration was developed and has been commercially available in the EU since 2009. Immunocastration consists of vaccination against gonadotropin releasing hormone (GnRH) and uses natural immune system of pig for the achievement of castration-like effect. It triggers the production of anti-GnRH antibodies blocking the hypothalamic-pituitary-gonadal (HPG) axis which results in suppression of testis growth and function (Caraty and Bonneau 1986; Falvo et al. 1986; Awoniyi et al. 1988). It seems an acceptable alternative to physical castration, performed without analgesia or anaesthesia, because it offers a solution for boar taint problem avoiding pain, as no surgical procedure is needed (Prunier et al. 2006). Also, it reduces aggressive behaviour that is seen when rearing entire males (EM) (Cronin et al. 2003; Velarde et al. 2007; Rydhmer et al. 2010). Moreover, physiological effects of immunocastration are not expressed until seven days after revaccination (V2) (Claus et al. 2007), performed 4-6 weeks preceding slaughter, which allows benefiting of EM feed efficiency and lean tissue growth through most of the growth-finishing period (Millet et al. 2011). However, after V2 a substantial increase in average daily feed intake (ADFI) is observed in immunocastrated pigs (IC) resulting in a progressive decrease in feed efficiency and an increase in body fatness of IC in comparison to EM (Batorek et al. 2012a). The latter could theoretically be limited by either restricting IC after V2 or shortening the period from V2 until slaughter, giving the pigs less time to accumulate additional fat. Reducing the delay between V2 and slaughter has been tested by Lealiifano et al. (2011) and shows that in the period following V2 (P2) fat depth levels were reduced with V2 closer to the slaughter and that concentrations of boar taint compounds in adipose tissue were suppressed in all animals. Results of Kubale et al. (2013) also confirmed that immunocastration is effective in fattening pigs already two weeks after V2. However, due to market situation, the delay between V2 and slaughter could also be prolonged. Hence, restricted feed intake of IC after V2 would be

favourable to avoid pigs getting too fat. Quiniou et al. (2012) reported that reducing daily feed intake for 15 or 22% in IC in group-housing conditions reduced average daily gain (ADG) in P2 but had no effect on feed conversion ratio (FCR) or carcass leanness in comparison with *ad libitum* feed control. They observed more aggression among restricted IC resulting in high level of lesion scores, which raises questions concerning welfare because of the feed intake restriction. As an alternative, energy restriction of the diet (decreasing the energy content with no limitation of voluntary feed intake) may be proposed. The decrease in dietary energy content may be achieved by decreasing fat and starch content or by increasing the fibre content. This could increase skatole production in the gut (Jensen et al. 1995b) which would merit to be verified.

During the past years, effectiveness of immunocastration, performance and carcass composition of IC have been drawn (Millet et al. 2011; Batorek et al. 2012a, Dunshea et al. 2013), but numerous questions concerning metabolic changes following the effective immunisation, nutritional requirements and growth potential of IC remain unanswered.

## 1.2 Objectives of doctoral dissertation

### **Effect of immunocastration on performance, body composition, reproductive organs, boar taint compounds and meat quality traits (Study 1)**

The objective of the first study was to assess the magnitude and heterogeneity of the effects of immunocastration in pigs through a meta-analysis of currently available research results, focussing on performance, carcass characteristics, meat quality, reproductive organs and boar taint substances.

### **Effect of feed restriction on hormones, performance, carcass traits, and meat quality in immunocastrated pigs (Study 2)**

The aim of the second study was to evaluate the effect of restricted feed allowance applied to IC after the effective immunization on performance, carcass and meat quality traits, in comparison with *ad libitum* fed EM, surgical castrates (SC) and IC. In addition, reproductive

organs and concentrations of boar taint compounds in fat were assessed. Serum Insulin-like growth factor 1 (IGF-I) and leptin concentrations were measured to better understand the physiological mechanisms leading to the increased feed consumption following immunocastration and the potential benefits of feed restriction.

**Effect of immunocastration in combination with different dietary fat content on energy metabolism, protein and lipid deposition and growth composition in male pigs (*Study 3*)**

The objective of the third study was to determine the partitioning of retained energy (RE) between protein deposition (PD) and lipid deposition (LD) before and after effective immunocastration in male pigs receiving diets differing in their lipid content, using nitrogen and energy balance techniques. Additionally, relationships between digested nutrients and their final outputs for catabolic and metabolic processes were determined to better understand what happens at the level of energy metabolism after immunocastration.

**Effect of dietary net energy content on growth performance, carcass characteristics and meat quality in immunocastrated male pigs (*Study 4*)**

The objective of the last study was to evaluate the effects of reduced dietary net energy (NE) in IC slaughtered eight weeks after V2 on growth performance, carcass composition (with emphasis on fat deposition), meat quality, involution of testes and concentrations of boar taint compounds.

### **1.3 Theses of the doctoral dissertation**

We have set the following working hypothesis:

1. We assume that immunocastration successfully limits boar taint i.e. deposition of androstenone and skatole in adipose tissue.
2. We assume that IC exhibit improved performance and leanness in comparison to SC, and better meat quality in comparison to EM.
3. We assume that restricting feed intake of IC after the V2 limits fat deposition and improves their performance, however it may negatively affect the behaviour.
4. We assume that after immunocastration the energy metabolism changes, LD is enhanced, but there is no effect on PD.
5. We assume that dietary energy enrichment (addition of fat) of IC after V2, emphasizes LD.
6. We assume that dietary energy dilution (NE reduction) of IC after V2 limits fat deposition.







## 2 LITERATURE REVIEW

### 2.1 Challenges in modern pig production

Castration or gonadoextomy is a surgical procedure performed on male pigs for centuries in which testis and epididymis are physically removed from scrotal sack, without anaesthesia or analgesia, in the first days of life (Council Directive 2008), mainly to get calmer and fatter pigs that do not exhibit boar taint and to prevent sexual behaviour in group housing (EFSA 2004). For conventional production in the EU, castration is performed on 80 to 100% of male pigs (with the exception of Ireland 0%, United Kingdom 2.1%, Portugal 11.2%, Spain 33.3%, Cyprus 39% and Greece 75.6%), 97% of which are castrated without the use of anaesthesia (EFSA 2004). Although generally used, this form of castration has been recently criticized as being painful for the piglets (McGlone et al. 1993, Prunier et al. 2006) and may be considered even unnecessary in modern pig production with the emergence of new, alternative techniques, especially if pigs are raised for meat consumption where raising EM would be more efficient (Bonneau 1998, Trefan et al. 2013). Improving feed efficiency is one of the major objectives in modern pig production and has become also one of the breeding goals (Barea et al. 2010), because feed represents the major cost in pork production (Noblet 2007). Moreover, the payment system of pig carcasses in most European countries rewards leanness, which gives EM additional advantage over SC, provided that the carcasses are not being devalored due to the presence of boar taint. Thus, taking into account the welfare as well as the economic aspects it has been decided at the EU level that pig producers will voluntary stop castrating until 2018 ([http://ec.europa.eu/food/animal/welfare/farm/initiatives\\_en.htm](http://ec.europa.eu/food/animal/welfare/farm/initiatives_en.htm); <http://boars2018.com>) and at the same time, intensive research of alternative methods to surgical castration, diagnostic methods for boar taint analysis and harmonization of analytical methods is being supported.



## 2.2 Alternatives to surgical castration of pigs

Possible alternatives to surgical castration with explanations of their way of action and the positive and negative aspects of their use/application are summarized in Table 1. Among them the alternatives that are the most promising are genetic selection for reduced boar taint, semen sorting and rearing only females, immunocastration, surgical castration with pain relief (the use of analgesia/anaesthesia) and rearing of EM. Although one of the easiest ways to neutralize male fertility would be using substances that lead to local destruction of testicular tissue (e.g. formaldehyde, zinc or silver salt, acetic acid), because they are easy to administer, not expensive and cause no hemorrhages, the use of such substances causes inflammatory reaction, leading to swelling, necrosis and subsequently pain (Fahim 1994, EFSA 2004) and is therefore unacceptable from a welfare point of view. Also the use of endogenous hormones (GnRH agonists or antagonists) in order to neutralize GnRH provides no alternative to surgical castration in the EU, because their use is strictly limited to medical purposes, mostly for the treatment of reproductive disorders (Council Directive 1996, Stephany 2001). Thus, from a practical point of view only immunocastration, rearing of EM and castration with pain relief are acceptable alternatives in the EU, which can be applied immediately, whereas semen sorting and genetic selection for low androstenone level are long term, but sustainable and welfare friendly solutions (EFSA 2004).

Norway and Switzerland have already prohibited surgical castration without pain relief and have introduced alternative methods – the castration is performed by veterinarians and anaesthesia is mandatory (EFSA 2004). Castration under anaesthesia or/and with the use of analgesia is basically the same surgical procedure as is the one being performed nowadays, except that with the application of pharmacological agents to the piglets prior or/and during the operation the pain is omitted or reduced. Hence, the use of analgesia/anaesthesia raises animal welfare of piglets significantly, but it generates extra costs on pig farms which depend on the substances being used and the form of their application. The costs namely increase significantly if the anaesthesia cannot be administered by farmers and when the veterinarian has to be called (de Roest et al. 2009). There are numerous possibilities of reducing or

avoiding pain caused by castration that can be in general divided in local or general anaesthesia and analgesia alone, however they all require additional handling of the piglets inducing additional stress and greatly extending the time required for performing the castration (EFSA 2004). The method of choice should result in a significant reduction or elimination of pain, not only sedation, discomfort and stress for the piglets; moreover the substance has to be approved for the use in pigs reared for meat consumption (under Council Regulation No 2377/90), the dose of the substance has to be consistently adhered, because the toxic or even lethal dose can easily be exceeded in young piglets, and it would be preferable that it can be administered by the farmer alone, which significantly narrows down the available options. The remaining options, giving satisfactory results in term of pain relief, registered for use in animals raised for meat consumption, which might be used without additional equipment on the farm are:

- use of pre-operative analgesia alone – intra-muscular (i.m.) administration of non-steroidal anti-inflammatory drug (NSAID; e.g. Ketoprofen, Meloxicam);
- use of combination of pre-operative analgesia and local anaesthesia – i.m. administration of NSAID and intratesticular or intrafunicular administration of 1% lidocaine with adrenalin.

Other options all have some drawbacks; the application of anesthetic mixture via nasal spray is insufficient to induce adequate depth of anaesthesia (Axiak et al. 2007), general anaesthesia by injection was insufficient in significant number of piglets and has to be administered by veterinarian (Leeb et al. 2008), general anaesthesia by inhalation (isoflurane or sevoflurane – none is currently registered for the use in pigs) requires special equipment, veterinarians' assistance plus application of analgesic (NSAID), because it does not provide sufficient pain relief when used alone (Kupper et al. 2008, Schulz 2007, Hodgson 2007, EFSA 2004). Moreover there is a thin line between lethal and anesthetic dose, plus inhalation anaesthesia and CO<sub>2</sub> are aversive prior to the loss of consciousness (EFSA 2004).

Raising EM (boars) is a common practice in some EU countries (United Kingdom, Ireland, Spain, Portugal) and is gradually being introduced by some pig chains in the Netherlands, Germany, Belgium and France. Compared with SC rearing of EM it is more cost effective (no

cost and wound infections due to castration, better FCR and better carcass conformation-higher meat percentage; Bonneau 1998) . However, this type of farming is more demanding for the farmer and more stressful for the animals (more aggression and sexual behavior as they mature; Prunier et al. 2006); moreover there is a possibility for occurrence of boar taint in meat and meat products from EM (rejected by majority of consumers; Malmfors and Lundström 1983, Weiler et al. 2000), lower meat quality (decreased tenderness and lower water holding capacity, reduced intramuscular fatness; Babol and Squires 1995, Trefan et al. 2013) and subsequently decreased suitability for processing into high quality meat products (Diestre et al.1990, Banon et al. 2003). Because the occurrence of boar taint at commercial slaughter weights is very variable, ranging from 10 to 75 % (EFSA 2004), depending on breed, slaughter weight, rearing and management conditions, the production of EM requires screening and sorting of carcasses on the slaughter line. Currently there are only subjective methods that can be applied (cooking test and use of soldering iron applied on exposed backfat of the carcass; Bundesanzeiger 2007, Jarmoluk et al. 1970) that require trained panellist, as the objective methods that would give immediate or quick result are either still experimental (electronic nose and mass spectrometry; Annor-Frempong et al. 1998, Ampuero and Bee 2006) or detect only one component of boar taint (spectrophotometric method for skatole analysis and enzyme-linked immunosorbent assay (ELISA) kit for androstenone analysis; Mortensen and Sornsen 1984, Walstra et al. 1999). In recent years, several strategies to prevent or reduce occurrence of boar taint meat have been developed. They include the use of special feeding strategies (different polysaccharides and additives; Albrecht 2011), slaughter at a lower weight (prior sexual maturity; Bonneau 1987) and improving rearing conditions (farrow-to-finish system, clean environment, maximal ventilation rate and temperature in termoneutral zone; Salmon and Edwards 2006, Hansen et al. 1994, Andersson et al. 2005). Thus, if detection of the tainted meat on the slaughter line and improved processing of tainted meat is provided, the rearing of EM can easily be applied. The only currently available accurate and potentially cost effective approaches for achieving sex preselection is separating X- from Y- chromosome-bearing sperm with high volume sperm sexing technology (sperm sexing kit; Ter Beek 2007) or on the basis of the difference in electric charge by using

modified flow-cytometer/cell sorter system (Johnson et al. 1989). Cell sorting system can produce about 18 million X-sperm per h of 85-90% of purity (Johnson 2000) whereas semen sorting kit results in 70% purity, which gives the possibility of breeding exclusively female offspring. However, both methods are technologically under developed and currently unsuitable for commercial use in the pig (Hofmo 2006), as the boar semen is rather sensitive to manipulation compared to other animal species and consequently relatively large insemination dose is required (3 billion spermatozoa in 80-100 mL of fresh diluted semen; Dominiek et al. 2011). However, using post-cervical or deep intrauterine insemination allows 3 to 20 fold reduction in the number of spermatozoa in the insemination dose compared to the standard transcervical artificial insemination (Vazquez et al. 2008), but special skills are needed for catheter handling and there is a possibility of damaging cervical or uterine tissue (Dominiek et al. 2011). Controlled-release capsules containing 2.5 billion boar spermatozoa per dose were developed to extend the preservation time of spermatozoa and maximize the efficiency of a single artificial insemination (Vigo et al. 2009) in swine. Thus, sperm sexing and insemination with X-sperm have a great potential in pig production provided that the reproductive performance is optimized by the use of accurate and precise insemination technique and that semen sorting results in a larger quantity of high quality semen, that would meet the market requirements.

Genetic selection for low androstenone level is another long-term and welfare friendly solution that is still in development, although selection of breeds that have a natural low incidence of boar taint could provide short-term solution (EFSA 2004). Sexually mature boars that have low fat androstenone but normal plasma testosterone and luteinizing hormone (LH) values have been identified almost 30 years ago (Bonneau 1987). However, single nucleotide polymorphisms associated with boar taint compounds that can be applied in practical breeding to reduce boar taint in intact boars without aversive effect on sexual maturity have been identified only recently (Moe et al. 2009) and require further research before being implemented in practice.

In conclusion, if surgical castration is associated with pain and risk to the health of animals, non-castration is associated with social stress and fighting occurring when boars reach

puberty, resulting in skin lesions and ultimate carcass damage. It is more profitable to raise EM pigs due to their enhanced FCR and greater carcass leanness; however the quality of meat from boars may be deteriorated by its odour or taste, lower intramuscular fat (IMF) content and reduced tenderness. Thus, raising EM and applying castration when they approach puberty would mean combining benefits of the two alternatives; benefiting from boar performance during most of the growth-finish period, while avoiding the risk of boar taint and aggression among pigs. This becomes possible with the use of late immunocastration, a method that will be in detail described in the following sections and is the subject of this PhD.

Table 1: Summary of alternative methods to surgical castration with explanations of their way of action and its pro et contra (for and against)

ALTERNATIVE			WAY OF ACTION	PRO	CONTRA	REFERENCE
<b>Raising entire male pigs</b>	Detection of boar taint	Trained panellists on slaughter line	Cooking test	Detecting tainted carcasses regardless to their source	Useful only on small number of animals, subjective method	Bundesanzeiger 2007
			Soldering iron applied to the exposed backfat of the carcass	On line method for boar taint detection	Possibility of false positive or false negative results, subjective	Jarmoluk et al.1970
		Skatole analysis on slaughter line	Spectrophotometric method	Successful and relatively fast (180 samples/hour)	Androstenone is not detected	Mortensen and Sorensen 1984
		Electronic nose	Chemical electronic sensors which measure androstenone and skatole	Measures both substances at one occasion, all tainted carcasses are detected	Only experimental, 16% false positive results	Annor-Frempong et al. 1998 Ampuero and Bee 2006
		Mass spectrometry	Pyrolysis-mass spectrometry	High classification rates	Experimental	Ampuero and Bee 2006 Ampuero et al. 2008
	Slaughter at lower weight		Slaughter of pigs below 90 kg	Reduces the incidence of tainted carcasses, higher % lean meat, better feed efficiency	Requires detection of boar taint on slaughterline, lighter carcasses are less profitable, meat is unsuitable for dry-meat products	Bonneau 1987 Zamaratskaia et al. 2005a Aldal et al. 2005 Chen et al. 2007
	Controlling boar taint through nutrition and rearing conditions		Farrow-to-finish system, feeding diets rich in indigestible carbohydrates, clean environment, ideal temperature and max. ventilation rate	Reduced skatole level, no adverse effect on growth	No effect on androstenone level	Hansen et al. 1994 Jensen et al. 1995b Rideaut et al. 2004 Zamaratskaia et al. 2005a Andersson et al. 2005
<b>Blocking reproductive axis</b>	Targeting GnRH	GnRH agonists	Negative feedback of GnRH agonist on GnRH synthesis		Short-term effect, hormonal treatment is expensive and not permitted in EU	Reid et al. 1996 Sinclair et al. 2001
		GnRH antagonists	Permanent occupation of GnRH receptors by antagonist			Bruessow et al. 2011 Ziecik et al. 1989
		Passive immunization	Application of antiGnRH serum	Immunization effect in 24h	Short-term effect, large amounts of	van der Lende et al. 1993

ALTERNATIVE		WAY OF ACTION		PRO	CONTRA	REFERENCE
					antisera and frequent administration is required	
		GnRH targeted toxins	Destruction of GnRH receptors bearing cells by GnRH coupled to cytotoxins	Single treatment reduces testosterone to zero for approximately 20 weeks	Experimental, not used on pigs	Sabeur et al. 2003 Harrison et al. 2004
	Targeting androstenone	Immunization against 5 $\alpha$ -androst-16-en-3-one	Ig that are produced neutralize androstenone in circulation	Reduction of androstenone conc.in adipose tissue, advantages of male type performance and carcass composition is preserved	Experimental	Williamson et al. 1985
<b>Surgical castration</b>	With local anesthesia		Intrafunicular and/or intratesticular application of local anesthetic (commonly lidocaine with adrenalin)	Reduces the acute pain and stress induced by castration	No economic benefits, additional cost, requires veterinary assistance, pain is not eliminated	EFSA 2004 Prunier et al. 2006 Fredricksen and Nafstad 2006
	With general anesthesia	By injection	Intramuscular application of anesthetic mixture prior castration	Reduction of acute pain	Long recovery period, losses are 3-5 %, insufficient depth of anesthesia, additional cost and veterinary assistance	Lahrmann et al. 2006 Leeb et al. 2008 Zankl 2007
		By inhalation	Anesthesia with isouflurane by mask	Reduction of pain	Expensive, time consuming, requires veterinarian assistance, high mortality of piglets	Shulz 2007 Kupper et al. 2008
			CO <sub>2</sub> inhalation	Fast introduction and recovery	Exposure to CO <sub>2</sub> is aversive until loss of consciousness, thin line between anesthetic and lethal dose	Svendsen et al. 2005 Kluivers-Poodt et al. 2007
		By nasal spray	Administration of anesthetic mixture (Ketamin, Azeperone	Induction in 10 min and fast recovery,	Insufficient depth of anesthesia,	Axiak et al. 2007

ALTERNATIVE		WAY OF ACTION	PRO	CONTRA	REFERENCE	
			and Climazolame) via nasal spray	reduction of pain	possibility of overdose	
	With analgesia	Meloxicam/ Metamizol / Ketoprofen	Application of analgesic prior castration	Effective pain relief	Additional cost and veterinary assistance is required	Heinritz et al. 2006 Zols et al. 2006
<b>Chemical castration</b>		Formaldehyde, zinc or silver salt, acetic acid	Local destruction of testicular tissue	Easy to administer, not expensive and no hemorrhages	Painful inflammatory reaction	Fahim 1994 Giri et al.2002
<b>Sperm sexing</b>	Sperm Sexing Technology		Sex preselection (producing female litters) by flow-cytometry cell sorting	Females do not exhibit boar taint	To slow for commercial use, requires intrauterine insemination, 85-95% purity for female production, less efficient production and fatter carcasses than boars	Johnson et al. 1989 Johnson et al.2000
	Semen sexing kit (high volume sperm sexing technology)		Binding x-bearing cells by Ig, filtration deagglutination of X-bearing cells	Faster than BSST, produces high-volume of x-bearing sperm (good potential for commercial use)	Experimental, litters of 70% females	ter Beek 2007
<b>Genetic selection</b>	For low androstenone		Identifying genes responsible for low androstenone production or high androstenone and skatole metabolism and selection of such animals	It would be the most welfare friendly method, which would not require castration or boar taint detection on line	It is a long term solution, still in development	Willeke et al. 1987 Bonneau et al. 1987 Willeke and Pirchner 1989 Sellier et al. 2000 Moe et al. 2007





### 2.3 Immunocastration

Two major compounds responsible for boar taint: androstenone (Patterson 1968) and skatole (Vold 1970) have been identified 45 years ago, but the idea of immunocastration, as a method to limit boar taint accumulation, originates from the late eighties (Falvo et al. 1986) and is has been intensively studied in recent years, since the registration of the first commercially available product for immunocastration of pigs (Improvac<sup>®</sup>, Zoetis Florham Park, NJ, USA) and because of the increasing questioning on the welfare of pigs surgically castrated without anesthesia in the EU. Immunocastration is an active vaccination against GnRH, it causes the formation of specific antibodies that bind and neutralise the GnRH and by that, block the HPG axis, thereby effectively inhibit testis growth and sexual steroids synthesis (see Figure 1). Therefore, it can be used as an alternative to physical castration and fertility control in farm animals, companion animals and wildlife species (Thompson 2000, Ferro et al. 2004, Delves and Roitt 2005). Although passive immunization against GnRH has been developed and tested, it is less effective, because it has short term effects and requires frequent administration of relatively large amount of antisera (van der Lende et al. 1993). Furthermore, castration via vaccination cannot only be directed towards GnRH, but also towards LH or androstenone, however both methods have been proven to be less effective (Falvo et al. 1986, Williamson and Patterson 1982) than immunocastration and remained at experimental level. Although the first studies using immunocastration in pigs were performed more than twenty years ago (Caraty and Bonneau 1986, Falvo et al. 1986, Awoniyi et al. 1988), due to the presence of strong adjuvants, they were unsuitable for practical use. The only currently available commercial vaccine - Improvac<sup>®</sup> (Zoetis Florham Park, NJ, USA) was introduced on the market in Australia and New Zealand in the nineties, and is now registered for use in more than 50 countries around the world, including the EU (since 2009). According to the manufacturers' instructions, the vaccine should be administered subcutaneously (s.c.) as two doses of 2 mL at least four weeks apart. Although no withdrawal period is needed, it is recommended that the V2 is performed four to six weeks before slaughter, in order to ensure

clearance of boar taint substances from adipose tissue  
<http://www.improvac.co.nz/sites/improvac/en-NZ/Pages/technicalinformation.aspx>).

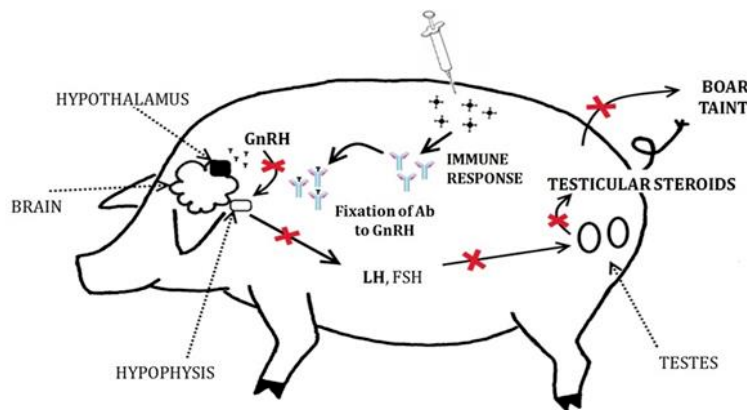


Figure 1: Physiological response to immunocastration in male pig: after revaccination a large quantity of antibodies against GnRH are produced that neutralise GnRH. As GnRH is no longer available, there is no stimulus for follicle-stimulating hormone (FSH) and LH release from hypophysis and spermatogenesis and production of androgen hormones in testis is on hold, meaning that there is no semen production nor male behaviour.

### 2.3.1 Effect of immunocastration on steroid and metabolic hormones

Reproduction in male is controlled by a synchronised action of three hormonal glands forming an HPG axis (see Figure 2). The preoptic area of hypothalamus secretes GnRH that travels via hypophyseal portal system to adeno-hypophysis where it binds to GnRH receptors on secretory cells (Schally et al. 1971). The binding of GnRH stimulates the synthesis of LH and FSH and their secretion into the blood stream (Pawson and McNeilly 2005, Schneider et al. 2006). The release of these two hormones is controlled by the size and the frequency of GnRH pulses, as well as by the feedback from androgens and oestrogens (Fink 1988). Low-frequency pulses lead to FSH release, whereas high-frequency pulses stimulate LH release. LH controls production of steroids (androgens, oestrogens and androstenes) by Leydig cells and FSH is responsible for spermatogenesis in the seminiferous tubules of the testes (Gorbman and Sower 2003). While androgens and oestrogens are necessary for spermatogenesis, sexual behaviour (Babol et al. 1998a, Babol et al. 1998b) and influence metabolism in favour to lean tissue

production, androstenes, especially androstenone, serve as pheromones (Pearce et al. 1988). Immunocastration provokes rapid development of anti-GnRH antibodies that bind to endogenous GnRH, preventing the stimulus for pituitary LH secretion, reliably reducing the production of testicular steroids (Bonneau et al. 1994, Zamaratskaia and Squires 2009) within the week following V2 (Claus et al. 2007). Interestingly, only LH drops to basal level after immunocastration (Falvo et al. 1986, Zeng et al. 2002a, Turkstra et al. 2002, Metz and Claus 2003, Wagner and Claus 2004), whereas FSH remains unaffected (Wagner and Claus 2004). Moreover, immunocastrates have been shown to preserve high growth hormone (GH) concentrations (up to 31 % higher than SC, Metz and Claus 2003, Wagner and Claus 2004, Bauer et al. 2008), a result which remains unexplained (Bauer 2010) and could account for the slow decrease of IGF-I levels after V2 (Claus et al. 2007).

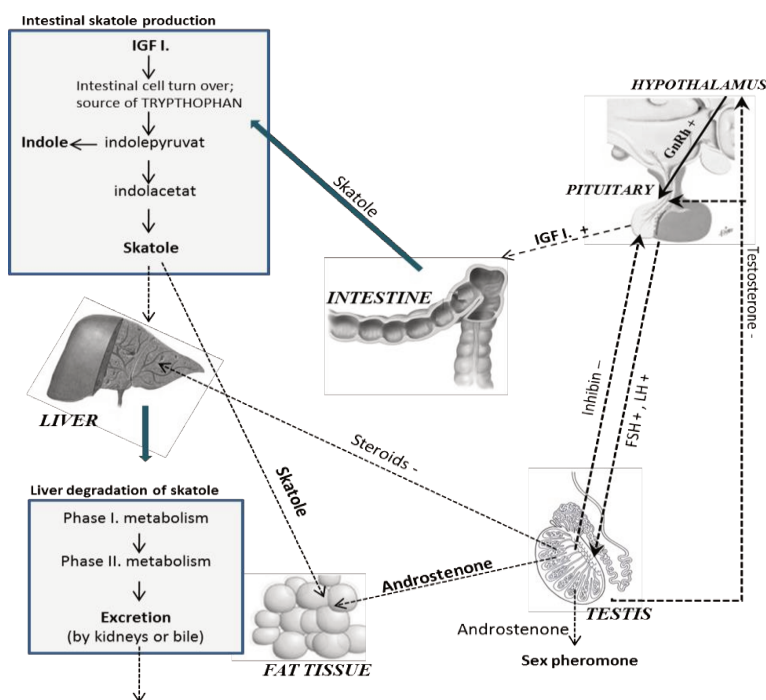


Figure 2: Relationships between the HPG axis, androstenone production in testes and skatole formation from tryptophan in intestine and their metabolism in liver. In boar, the production of testicular steroids, including androstenone, primarily a male pheromone, inhibits hepatic metabolism of skatole. Androstenone and skatole are therefore, due to their lipophilic character, deposited in adipose tissue.

### 2.3.2 Effect of immunocastration on boar taint compounds and reproductive organs

Boar taint refers to the offensive odour or taste, evident during cooking or eating of meat or meat products from some, mostly EM pigs (Bonneau et al. 1992). It makes pork undesirable for sensitive consumers (Weiler et al. 2000). The substances that are considered the primary cause of boar taint are androstenone, a male pheromone, (Patterson 1968) and skatole, a by-product of tryptophan breakdown in large intestine (Vold 1970, Walstra and Maarse 1970). Androstenone ( $5\alpha$ -androst-16-ene-3-one) is an anabolic steroid, without androgenic effect (Claus et al. 1970), but with pronounced urine-like odour and flavour. It is produced by the Leydig cells in the testis of sexually mature male pigs (Gower 1972). Due to its lipophilic character, it accumulates in adipose tissue in much higher amounts than other sex hormones (Pearce et al. 1988). Part of androstenone is stored in salivary glands, bound to specific binding protein pheromaxein (Booth and White 1988), and after being realised in the saliva, it serves as a pheromone to promote sexual behaviour in female pigs (Pearce et al. 1988). It has also been detected in plasma from females and castrates in low levels, indicating possible production in adrenal cortex and ovary (Claus et al. 1971). Unlike androstenone, skatole (3-methyl-indole) does not seem to have a biological function in pig. It is a by-product of amino acid L-tryptophan breakdown by bacterial microflora in large intestine of pig. Thus the amount of skatole produced is primarily regulated by the availability of L-tryptophan which mainly originates from gut-mucosa cell debris (Claus et al. 1994, Claus and Raab 1999) and the activity of intestinal bacteria, especially *Escherichia coli*, *Lactobacillus* sp., *Clostridium* sp. (Jensen et al. 1995a) and *Olsenella* sp. (Jensen 2012). Skatole that is produced in the colon is then partly excreted with faeces and partly absorbed through intestinal wall and released into the bloodstream. The metabolism and degradation of skatole in liver is inhibited by steroid hormones (Doran et al. 2002) or pharmacological substances (Škrlep et al. 2012a), and due to its lipophilic nature, it also accumulates in adipose tissue. The odour of skatole is described as faecal like or sometimes naphthalene like and can be detected by the vast majority of people (Weiler et al. 2000). In contrary, androstenone has a large variation in consumer's sensitivity, as some people can detect it in very low concentrations, whereas approximately 25% are

anosmic to it (Wysocki and Beauchamp 1984, Claus et al. 1994). Even though studies using immunocastration have been conducted on different types of animals in various conditions and using numerous different vaccination schedules, they are consistent in effectiveness of Improvac to control boar taint and diminish the size of testes and sexual glands in IC compared to EM (Dunshea et al. 2001, Jaros et al. 2005, Zamaratskaia et al. 2008). Androstenone in blood declines rapidly after V2 (Claus et al. 2007), but for the clearance of the boar taint compounds from adipose tissue more time is needed (3-4 weeks; Bonneau et al. 2000, Dunshea et al. 2001). Although the vaccine producer recommends that a 4-week interval between V2 and slaughter is respected, recent study (Lealiifano et al. 2011) indicated that the concentration of androstenone and skatole in fat tissue drop below the limit of sensory detection already two weeks after V2 in pigs of commercial slaughter weight. Simultaneously with the decrease of steroid hormones and boar taint compounds concentrations, the effect of immunocastration is visible on the reproductive organs through the reduced size of the testes and accessory sex glands (Bonneau 2010), with the strongest impact on the size of vesicular gland, followed by the size of the bulbourethral gland. Regression of the reproductive tract is consistent with the loss of functional activity as shown by histological changes of testicular tissue (i.e. atrophy of Leydig cells and germinal epithelium, the cessation of spermatogenesis, the reduction of the size of glandular acini and the disappearance of secretory function of accessory sex glands; Hilbe et al. 2006, Einarsson et al. 2009, Fang et al. 2010, Einarsson et al. 2011). Although studies suggest that the effect of immunocastration persisted up to 22 weeks (Zamaratskaia et al. 2008), it should not be permanent, because a progressive restoration of testicular function has been demonstrated together with an increase of the androstenone blood concentration 12 weeks following V2 (Claus et al. 2008). However, the study using different time schedules of vaccination (Einarsson et al. 2009) indicated an irreversible loss of reproductive capacity, probably related to the earlier vaccination. Regarding the effectiveness of immunization, it should be noted that some animals do not react to it (so-called "non-responders") due to poor immunological response or technically improper vaccination (Zeng et al. 2002a, Jaros et al. 2005, Škrlep et al. 2012a). The number of non-responders is relatively low (1-3%) and is expected to be similar to the number of cryptorchids obtained after surgical castration. Thus,

there is a need of a fast, simple but effective method for boar taint detection on the slaughter line also when rearing IC. Due to the large variability in size of testes among animals, the evaluation of the effectiveness of immunocastration on slaughter line based on the size of testes is not reliable and the size of the accessory sex glands should be take into account as well (Bonneau 2010, Čandek-Potokar et al. 2014), which would be rather unpractical.

### 2.3.3 Effect of immunocastration on feed intake, growth performance and nutrient requirements

Because immunocastration from the physiological perspective becomes effective in weeks following V2 (Claus et al. 2007), the growth characteristics of IC are similar to those of EM prior to that point (Škrlep et al. 2010a, Millet et al. 2011, Dunshea et al. 2013), which encounters for the most of the growing-finishing period if recommended vaccination schedule is respected (V2 applied 4-6 weeks prior to slaughter). As a result, similar like boars, IC have higher ADG and improved FCR when compared to barrows over the entire production phase (Dunshea et al. 2001, Turkstra et al. 2002, Pauly et al. 2009, Škrlep et al. 2010a, Morales et al. 2011). However, due to higher ADFI following V2, IC have higher ADG but lower FCR than boars over the entire production phase (Dunshea et al. 2001, Turkstra et al. 2002, Pauly et al. 2009, Škrlep et al. 2010a, Morales et al. 2011). While the differences in growth rate between boars and barrows can be directly linked to the feed intake (if fed *ad libitum*) and efficiency of feed utilization (Millet et al. 2011), the growth rate of IC is influenced by the biological response to immunization - to changes in hormonal status (i.e. low concentration of steroid hormones and relatively high residual levels of IGF-I and somatotropin; Bauer et al. 2009) as well as a more barrow-like behaviour (increased time spent feeding and decreased time spent on social, especially aggressive and sexual behaviour; Dunshea et al. 2001, Cronin et al. 2003). Because the existence of two metabolical phases, prior to and after immunocastration, implementation of two phase feeding might be beneficial in IC. According to the results of Zeng et al. (2002b), a high energy diet is preferable (9.7 MJ NE/kg) in the first growth period, whereas in the second growth period a low energy diet (8.3

MJ/kg) should be fed in order to improve growth rate and lean meat yield. However, feed restriction (to 2.75 or 2.50 kg feed/day) in the late finishing stage did not improve the feed efficiency of IC, whereas their growth rate was reduced (Quiniou et al. 2012). Dunshea et al. (2013) suggests that dietary standardized ileal digestible (SID) lysine concentration might be increased to 110% of gilt requirements in boar phase, whereas it should be decreased to 94% of gilt requirement in immunocastrate phase. Thus available results suggest the use of a high energy and protein diet in boar-like phase and low energy and protein diet in immunocastrate phase, but the exact energy and amino acid requirements still need to be determined.

#### 2.3.4 Effect of immunocastration on carcass composition and meat quality

Regarding carcass characteristics, boars are known to have lower dressing yield and carcass weight than barrows, whereas in most studies IC take intermediate position (Bonneau et al. 1994, Dunshea et al. 2001, Pauley et al. 2009, Gispert et al. 2010, Škrlep et al. 2010b, Morales et al. 2011, Boler et al. 2012). Lower dressing yield in IC has been contributed to the higher gut fill (Dunshea et al. 2001, Gispert et al. 2010, Boler et al. 2014), the presence and removal of reproductive tract (Boler et al. 2012, Boler et al. 2014) along with heavier internal organs - liver and kidneys (Pauly et al. 2008, Pauly et al. 2009, Boler et al. 2014). Nevertheless, IC take advantage of boar-like growth performance and thus produce leaner carcasses due to increased muscling and decreased fatness in comparison to SC, but become fatter than EM (Dunshea et al. 2001, Jaros et al. 2005, Pauly et al. 2009, Škrlep et al. 2010b, Morales et al. 2011) due to high feed intake following V2 that enhances fat deposition. In general, fat deposition in IC largely depends on the timing of immunization - the length of period between immunization and slaughter (Turkstra et al. 2002, Lealiifano et al. 2011, Škrlep et al. 2012b). The longer the time between V2 and slaughter the greater the fat content in the carcass. Although the greater fatness (especially IMF content) is desirable due to greater sensory quality, the payment system of pig carcasses in most EU countries rewards leanness, so extra fat deposition after V2 is not desirable when pigs are being reared for meat consumption, although it improves meat quality extensively. The weight of the main carcass cuts of IC and EM seems similar,



whereas greater weight of hams, shoulders and lower weight of loin in comparison to barrows have been measured (Pauly et al. 2009, Gispert et al. 2010, Boler et al. 2012). Regarding meat quality, IC and SC have an advantage over EM. Their meat has a higher IMF content and increased tenderness, which is favourable especially in terms of sensory quality. On the other hand, the meat from IC was associated with a slightly higher drip loss, lower pH and lighter colour (Batorek et al. 2012b) compared to EM. In comparison to SC, IC did not differ in any of investigated meat quality traits (*Longissimus* muscle pH, colour, drip loss, shear force, IMF content; Pauly et al. 2009, Morales et al. 2011, Boler et al. 2012, Font i Furnols et al. 2012, Trefan et al. 2013), except that the pork from IC has been shown to have a lighter colour (Trefan et al. 2013). Moreover, immunocastration does not have any negative effects on fat quality (Font i Furnols et al. 2012) and further processing characteristics of pork (Boller et al. 2012, Škrlep et al. 2014).

### **3 MATERIAL AND METHODS**

In order to confirm or reject research hypothesis, four separate studies have been planned and executed between 2010 and 2012.

#### **3.1 General experimental design, diets and feeding**

##### **3.1.1 Meta-analysis of the effect of immunocastration on production performance, reproductive organs and boar taint compounds in pigs (Study 1)**

The search for articles was performed using the bibliographic databases Web of Science (<http://www.isiwebofknowledge.com/>), CAB Direct (<http://www.cabdirect.org/>) and the internet. Only studies published before July 2011 were considered. To be included in the meta-analysis, studies had to be original research work reporting the mean and variability (per treatment group or pooled) of the response traits. Only the studies that provided the results for the treatment group (IC) and at least one of the control groups (EM and/or SC) were considered. When several independent experiments were reported within an article, they were considered as separate studies. When other treatments were applied in addition to immunocastration within a study (e.g. ractopamine, somatotropine, different stages of immunization, different adjuvants, different types of the vaccine, different doses, different energy levels of the diet, different vaccination protocols, different periods between revaccination and slaughter), these treatments and their relevant controls were also considered as separate studies. The complete database consisted of 68 “studies” from 41 articles, involving 7,288 pigs (n=3,483 IC, n=1,162 EM and n=2,643 SC). The meta-analysis was performed only for those traits for which a sufficient number of studies (n $\geq$ 5) was available, with the exception of the weights of the belly, shoulder, bulbourethral glands (n=4), seminal vesicle weight (n=3) and shear force (n=2) in the comparison between IC and SC. Growth rate, feed intake and feed efficiency were considered for three periods: from first vaccination

(V1) to the V2, from V2 to slaughter (S) and for the overall fattening (experimental) period (V1-S). Carcass traits included dressing percentage, *longissimus dorsi* muscle thickness, subcutaneous backfat thickness (BFT), lean meat percentage and weights of the carcass prime cuts (i.e. loin, ham, belly and shoulder). The value of pH measured 24 h post mortem (pH24), Commission Internationale de l'Elclairage (CIE) colour measurements ( $L^*$ ,  $a^*$ ,  $b^*$ ), water holding capacity (WHC) assessed as drip loss, shear force and IMF content were the meat quality traits considered in the meta-analysis. Boar taint compounds included skatole and androstenone levels in fat. Regarding reproductive organs, the weights and lengths of testes and bulbourethral glands and seminal vesicle weight were compared between IC and EM, whereas IC and SC were compared only for bulbourethral gland and seminal vesicle weight.

### 3.1.2 Effect of feed restriction on hormones, performance, carcass traits, and meat quality in IC (Study 2)

The effect of quantitative feed restriction on performance, carcass and meat quality, metabolic hormones, reproductive organs and boar taint compounds was evaluated on 88 crossbred pigs (Pietrain x (Large White x Landrace)) from 37 litters on a commercial pig farm in Slovenia. One piglet per litter was surgically castrated within seven days after birth. At an average age of 24 days, piglets were weaned and allocated within litter to one of four treatment groups as presented in Figure 3: SC (n=22), EM (n=24), IC fed *ad libitum* (IC-L; n=21) and IC restrictively fed (IC-R; n=21). In the growing phase, the piglets were group-housed according to their treatment group. At the average age of 83 days, the V1 against GnRH (Improvac<sup>®</sup>, Zoetis Florham Park, NJ, USA) was applied to IC-L and IC-R pigs according to the manufacturers' instructions. From the time of V1 onwards, pigs were individually housed in pens (1.2 m × 2.1 m) with concrete slatted floors and fed a commercial diet containing 13.0 MJ/kg of ME, 16% crude protein, 2.0% crude fat, 5% crude fiber, 6% crude ash and 0.88% lysine. *Ad libitum* feeding was applied to EM, IC-L and SC pigs, whereas IC-R pigs were submitted to the restricted feeding one week after the V2, performed at the average age of 130 days. Feed allowance given to IC-R pigs corresponded to approximately 80% of the average

*ad libitum* feed intake of the SC group (calculated for the previous week). Pigs were sent to slaughter in two batches (six days apart, in average 165 day of age) with all treatment groups equally represented and slaughtered according to standard procedures (approximately half an hour transport, two hours lairage, CO<sub>2</sub> stunning). During transport and lairage, pigs of the different treatment groups were not mixed.

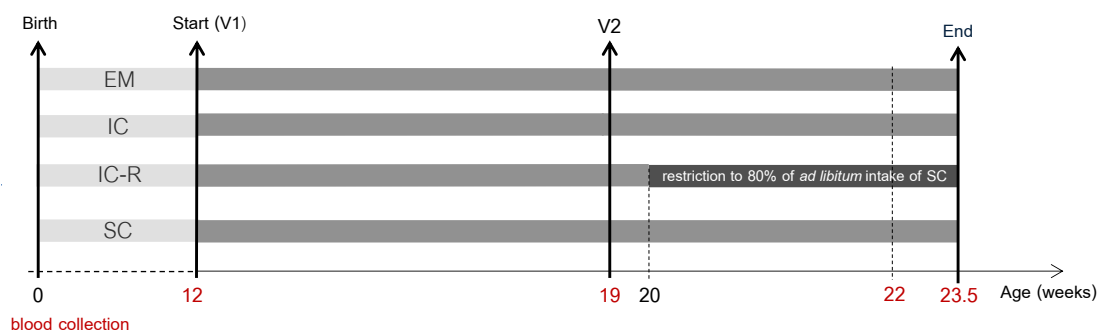


Figure 3: Experimental design of study 2 showing time schedule of the experiment with four experimental groups (entire males-EM, immunocastrates-IC, immunocastrates on feed restriction-IC-R and surgical castrates-SC): the first (V1) and second (V2) vaccination with Improvac<sup>®</sup>, time of restriction feeding in IM-R group and the four blood collections (marked with red).

### 3.1.3 Effect of immunocastration in combination with different dietary fat content on energy metabolism, PD and LD and growth composition in male pigs (Study 3)

The study was designed to evaluate the effect of immunocastration in combination with two dietary lipid contents on PD, LD and heat production (HP) in eight crossbred IC (Pietrain x (Large White x Landrace)) from the INRA experimental herd (Saint-Gilles, France). Four repetitions, each composed of two littermates divided to Group 1 (G1) and Group 2 (G2), were carried out simultaneously (see Figure 4). The measurements were performed in two subsequent stages: before (stage 1, S1) and after (stage 2, S2) revaccination. During S1, all pigs received a low fat diet (LFD) and at the end of the S1, feed transition to high fat diet (HFD) was applied in G2, whereas pigs in G1 continued consuming the LFD during S2. Each

stage was composed of an adaptation period (3 - 5 weeks) in metabolic cages followed by 8-9 days of nitrogen and energy balance measurements, plus 1 day of fasting in order to measure the fasting heat production (FHP).

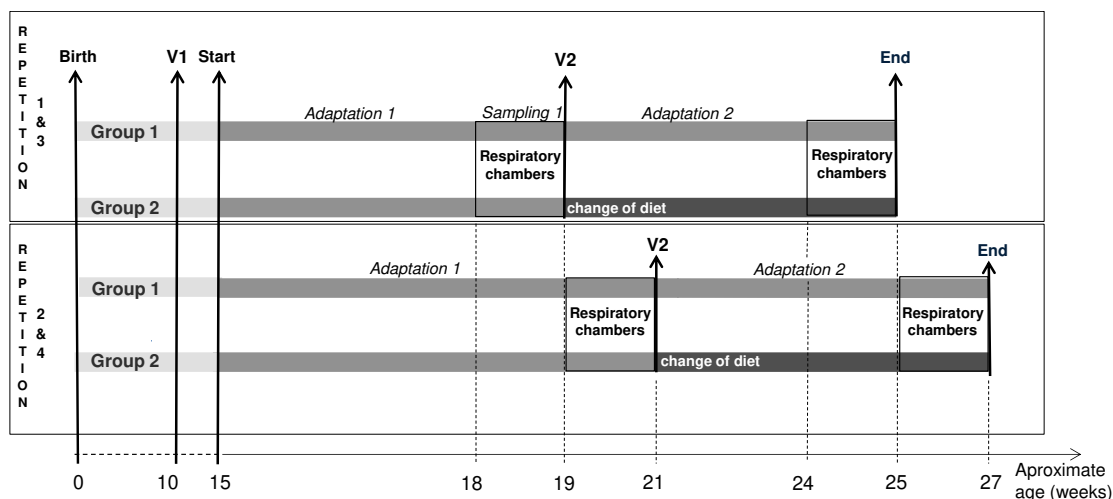


Figure 4: Experimental design of study 3 showing start and end of experiment performed in four repetitions, divided in two adaptation periods (Adaptation 1 and Adaptation 2) each followed by measurement period in respiration chambers (Sampling 1 and Sampling 2), first (V1) and second (V2) vaccination with Improvac<sup>®</sup>, two experimental groups (Group 1 and Group 2) and change of diet in Group 2 after V2.

Individual measurements of littermates occurred simultaneously in two 12 m<sup>3</sup> open-circuit respiration chambers, designed as those described by Vermorel et al. (1973) and presented in Photo 1. The respiration chambers were additionally equipped with force sensors to record physical activity of the pig (Quiniou et al. 2001) and two infrared barriers that detected standing, lying or sitting of the animal (van Milgen et al. 1997). The temperature (24°C) and relative humidity (70%) were kept constant and light was switched on between 8.00 and 20.00.



Photo 1: Two 12 m<sup>3</sup> open-circuit respiration chambers used in experiment 3 for nitrogen and energy balance measurements.

The second dose of Improvac<sup>®</sup> (Zoetis Florham Park, NJ, USA) was administered to all pigs at the end of the S1 (ie. 4-5 wks before starting measurements of the S2). LFD was a standard fattening diet with the addition of 0.5% of rapeseed oil, resulting in a dietary fat content of 2.8%, whereas HFD was made of the same basic ingredients as LFD but with lipid content up to 8.9% achieved by addition of corn and linseed oils to balance the ratio between C18:3 and C18:2 fatty acids (FA). The pigs were restricted to 90% of predicted *ad libitum* feed intake calculated according to the INRAPorc<sup>®</sup> software (van Milgen et al. 2008). Daily feed allowances were prepared for each pig and feed was offered twice a day in adaptation periods and four times a day (at 9, 12, 15 and 18.00) during the measurements. Water was provided *ad libitum*. Calculations included nitrogen and energy balances and oxidation of dietary nutrients.

#### 3.1.4 Effect of NE concentration of the diet on growth performance, carcass characteristics and meat quality in IC (Study 4)

The experiment was performed in the facilities of INRA Saint Gilles, France between June and September 2012 using 45 male crossbreed pigs (Pietrain x (Large White x Landrace) in growth trial and five in digestibility trial (performed according to Noblet et al. 1993) for

measuring digestive and metabolic utilization of three experimental diets in order to accurately calculate their NE content (Noblet et al. 1994). At the age of 70 days, pigs in growth trial were transferred to experimental stables with individual housing and concrete floor (as shown in Photo 2) and divided according to litter in three dietary treatment groups (High NE, Medium NE and Low NE) as demonstrated by experimental design in Figure 5.



Photo 2: Stable with pigs in individual cages (experiment 4).

The pigs were fed: High NE, Medium NE and Low NE diet, formulated using wheat, corn, barley and soybean meal and they contained (% of dry matter (DM)), respectively as follows: crude protein 18.5, 18.0, 17.7; crude fibre 3.4, 6.0, 8.0; fat 4.2, 3.4, 3.3; ash 5.4, 5.6, 6.0; neutral detergent fibre (NDF) 14.7, 18.8, 22.8; acid detergent fiber (ADF) 4.4, 7.4, 10.0; starch 51.8, 47.0, 41.6; NE (MJ/kg of DM) 11.6, 11.1, 10.5. In Medium NE and Low NE diet wheat, corn and barley were partly replaced with soybean hulls, dried beet pulp and rapeseed meal in order to reduce their NE content. Feeding with the three experimental diets began at the age of 84 days with five days of feed transition. Feed (distribution three times per day at 9.00, 12.00 and 15.00 h) and water were provided *ad libitum*. Two vaccinations with Improvac<sup>®</sup> (2 mL, s.c. application, Zoetis Florham Park, NJ, USA) were performed at the age of 77 and 112 days. Slaughter was performed in two batches (at an average age of 172 days) in experimental

abattoir of INRA Saint Gilles according to standard procedure (app. 20 h feed deprivation, electrical stunning and immediate bleeding).

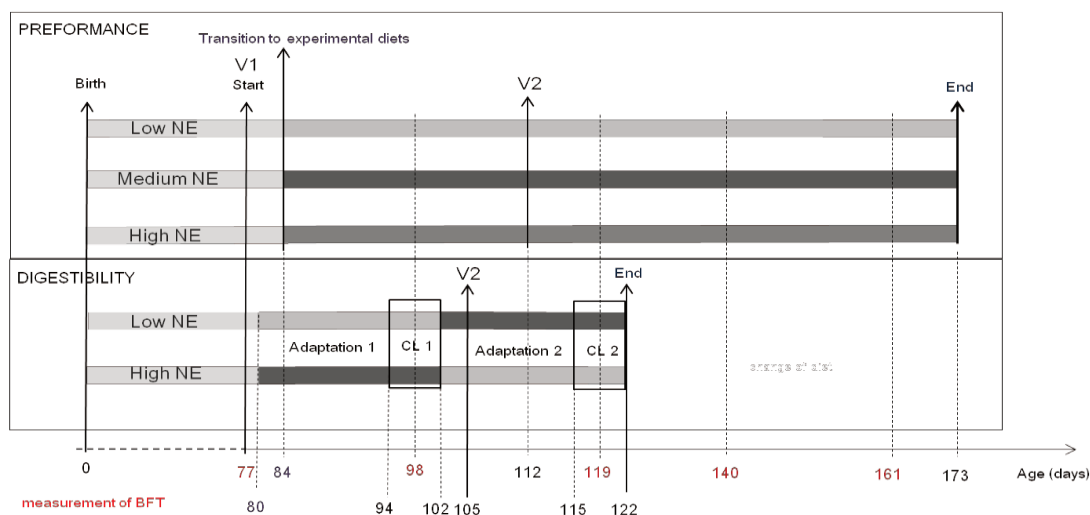


Figure 5: Experimental design of study 4 showing the main experiment (PERFORMANCE) with three experimental groups on Low NE, Medium NE and High NE diets and digestibility trail (DIGESTIBILITY) showing start (0) and end (End) of experiment, first (V1) and second (V2) vaccination with Improvac<sup>®</sup>, feed transition to experimental diets, the measurements of backfat thickness in main experiment and the two adaptation periods (Adapt 1 and Adapt 2) and two collection (CL 1 and CL 2) periods in digestibility trail.

## 3.2 Sample collection, measurements and laboratory analyses

### 3.2.1 Measurements of feed intake, body weight and BFT

The voluntary feed intake was individually recorded on a daily (study 3) or weekly basis (studies 2 and 4) and pigs were weighed at V1, at V2 and at the end of the experiment (in study 2), weekly (in study 4 and during adaptation periods in study 3). In study 3, pigs were additionally weighed before entering the respiration chambers and on the morning of the last day (ie before fasting). In study 4, ultra-sound (Noveko, VetkoPlus, 3.5 MHz) measurements of BFT on the left and right side of the back, 6 cm lateral to the spine in the middle of the back



(level of the last rib) were performed every three weeks from V1 onwards to obtain five subsequent measurements on each animal. The difference between the final and initial BFT divided with the number of days between the two measurements was expressed as BFT gain per day.

### 3.2.2 Blood sample collection

In study 2, blood samples were collected into chilled glass tubes immediately before V1, V2, and 24 days after V2 from *vena cava cranialis* (9 mL) and during exsanguination at slaughter from all experimental animals. Collected blood samples were immediately placed on ice and delivered within one hour to the laboratory where blood was left to clot at 4°C overnight. Blood serum was then centrifuged at 1,800 rpm (355×g) for 10 min and supernatant was stored at -20°C until analyzed.

### 3.2.3 Measurements at slaughter

#### 3.2.3.1 Carcass characteristics

At the slaughter line, carcass weight was recorded and dressing percentage was calculated as the percentage of carcass in relation to live weight measured on the day before slaughter. Carcass lean meat content (%), based on measurements of muscle and fat thickness) were determined, using a method approved for Slovenia in study 2 and method approved for France in study 4. Leaf fat was excised and weighed. Carcasses were cooled overnight until the internal carcass temperature dropped below 7°C. On the day following slaughter, additional carcass traits were assessed. The carcass was cut at two sites, between 3<sup>rd</sup> and 4<sup>th</sup> cervical vertebra (cross-section A, Figure 6) and after the last rib (cross-section B, Figure 6) and images of the cross-sections were taken using a digital photo camera (Canon PowerShot G3, Canon Inc., Tokyo, Japan). LUCIA.NET 1.16.5 image analysis software (Laboratory Imaging

s.r.o., Prague, Czech Republic) was used to determine the % of neck intermuscular fat (NIMF) on cross-section A (the evaluated area is marked with circle in Photo 3), loin eye area (marked with a circle in Photo 4) and corresponding fat area (loin eye fat area, between two lines in Photo 4) on cross-section B.

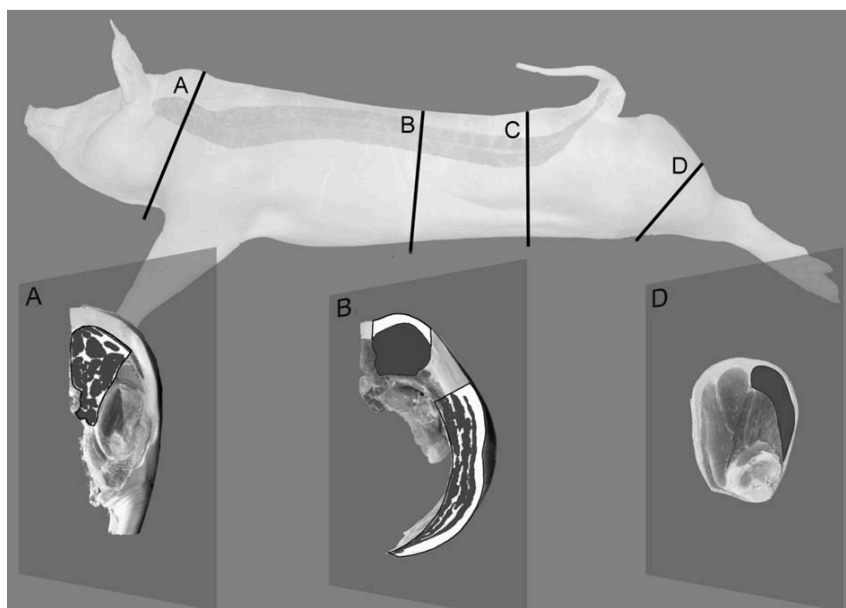


Figure 6: Locations of cross-sections in study 2 and 4 for the evaluation of carcass properties. A = cross section between 3<sup>rd</sup> and 4<sup>th</sup> cervical vertebra, B = cross section cut after last rib, C = cross section cut between 6<sup>th</sup> and 7<sup>th</sup> lumbar vertebra, D = cross section cut through the knee joint.



Photo 3: The picture shows a photo of the carcass cut made between 3<sup>rd</sup> and 4<sup>th</sup> cervical vertebra for the evaluation of neck intermuscular fatness expressed in %.



Photo 4: The picture shows the measurement of loin eye area (marked with a circle) and loin eye fat area (between the two lines), which were evaluated in the photo of carcass cut made at the level of the last rib and expressed in  $\text{cm}^2$ .

Additionally, in study 2, belly leanness on cross-section B was visually assessed using a 1 to 7 point scale (1 denoting the lowest and 7 the highest leanness). The hind leg was weighed and calculated as % of carcass weight (Ham %). Skin and subcutaneous fat were then removed and % of lean with bones in ham was calculated.

In study 4, the left half carcasses were additionally divided in primary cuts according to the Dutch normalized procedure (Institut Technique du Porc 1990) and weights of head, tail and feet, neck and shoulder, side, total loin, backfat and ham were recorded.

### 3.2.3.2 Carcass lesions evaluation

Carcass lesions were assessed on the skin of the cooled carcasses (disregarding regions distally from the tarsal and carpal joints). Only the fresh lesions inflicted during the pre-slaughter period were recorded according to etiology, i.e. as lesions caused either by teeth or by hoofs (Škrlep et al. 2011).

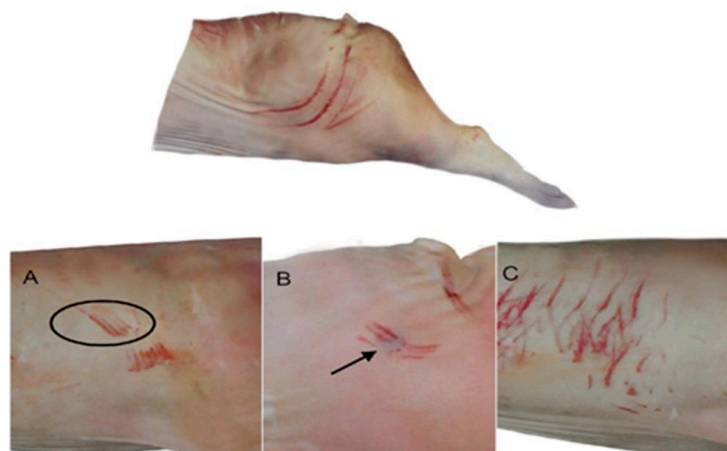


Photo 5: An injury made by hooves (picture on the top). Injuries made by teeth (pictures on the bottom): A – an injury made with incisors, B - bite (in the middle a bruising is visible), C - injuries made by canines (Škrlep et al. 2011).

### 3.2.3.3 Meat quality measurements

The pH value in *longissimus dorsi muscle* was determined 45 min *post mortem* (pH<sub>45</sub>) using a combined glass electrode (MP120 Mettler-Toledo, GmbH, 8603 Schwarzenbach, Switzerland with Metter-Toledo InLab427 electrode) directly on the carcass (study 2) or in iodoacetate homogenate (study 4). One day after slaughter the colour and pH<sub>24</sub> (using pH meter) were measured on a freshly cut surface of *longissimus dorsi muscle*. The colour of *longissimus dorsi muscle* was assessed subjectively using a 1 - 6 point Japanese colour scale (Nakai et al. 1975) and objectively using CIE (1976) L\* (lightness), a\* (redness) and b\* (yellowness) colour space. Measurements were taken in triplicates using a Minolta Chroma Meter CR-300 (Minolta Co. Ltd, Osaka, Japan) with an 11 mm aperture, D65 illuminant, calibrated against a white tile. Muscle pH<sub>24</sub> was determined in two replicates in the central area of *longissimus dorsi muscle*. Caudally from the level of the last rib, two 2.5 cm thick slices of *longissimus dorsi muscle* were removed from the loin for the determination of drip loss and IMF in both studies and cooking loss and shear force in study 2. Drip loss was determined according to the EZ method (Christensen 2003). Shortly, two cylindrical samples were excised from the central

part of *longissimus dorsi* muscle, weighed and placed in plastic containers. The samples were reweighed after 24 and 48 h of storage at 4°C. Drip loss was expressed as the difference (%) from the initial sample weight. Samples of *longissimus dorsi* muscle were minced and IMF content estimated using near-infrared spectroscopy (NIRS; NIR System model 6500 Spectrometer, Silver Spring, MD, USA; Prevornik et al. 2005). The second *longissimus dorsi* muscle slice was trimmed from fat and connective tissue, vacuum packed and frozen at -20°C until analyzed. For the determination of cooking loss and shear force in study 2, the *longissimus dorsi* muscle samples were thawed (overnight at 4°C), weighed and cooked in a thermostatic water bath (ONE 7-45, Memmert GmbH, Schwabach, Germany) until the internal temperature reached 72°C. The samples were reweighed for cooking loss evaluation and cooled at 4°C overnight. The next day, two 2.5 cm wide cylindrical cores were excised and shear force (maximum cutting force) was measured using TA Plus texture analyzer (Ametek Lloyd Instruments Ltd., Fareham, UK) equipped with 60° V-shaped rectangular edged blade and a crosshead speed set at 3.3 mm/sec.

#### 3.2.3.4 Weight of reproductive organs

At the slaughter line, testes (in study 2 and 4) and accessory sex glands (in study 2) were removed and taken to the laboratory for dissection and weighing. This was done for all pigs in study 4 and for EM (n = 21) and half of IC-R (n = 10) and IC-L pigs (n = 10), by random selection in study 2.

#### 3.2.4 Sample collection and measurements in respiration chambers

In the measurement periods, a sample of each diet was collected during the preparation of daily feed allowances for DM measurement, subsequently pooled for each stage and stored at 4°C until further analyses. During the first 8-9 days of the measurement periods, feed refusals were collected daily and analyzed for their DM content; faeces were collected daily and stored at 4°C; urine was collected in plastic tanks of 10 L containing 120 mL of 10% sulfuric acid,

weighed and a representative sample (2%) was taken and cumulated in plastic bottles containing samples from entire balance period and placed at 4°C until further laboratory analyses. On the last day, total collected faeces were weighed, mixed and 3 representative samples were taken, two of them were used for DM determination and the third sample was freeze-dried, grinded and stored at 4°C until further laboratory analyses. To collect ammonia evaporated in the respiration chambers, condensed water from the air conditioning system of the respiration chambers was collected in plastic tanks containing 120 mL of a 10% sulfuric acid solution and a quantified aliquot of the outgoing air was bubbled in a sulfuric acid solution during the entire balance period. Condensed water and bubbling solution were weighed and sampled as described by Noblet et al. (1987). Feed consumption (using weighing scale placed under the trough), physical activity (force sensors and infrared barriers), gas concentrations (CO<sub>2</sub>, O<sub>2</sub> and CH<sub>4</sub>) of outgoing air, ventilation rate and physical characteristics of the air in the respiration chambers were measured continuously (60 times/s), averaged over 10 s intervals and recorded for further calculations (van Milgen et al. 1997).

### 3.2.5 Laboratory analysis

#### 3.2.5.1 Hormone analysis in blood samples

For hormone assays, all samples were analyzed within a single assay. The serum level of cortisol at slaughter was determined using a commercially available ELISA kit (IBL International GmbH, Hamburg, Germany) according to the manufacturers' instructions. The intensity of the colour was read at 450 nm using spectrophotometer Varioscans Flash and SkanIt Software Version 2.4.3. RE (Thermo Fisher Scientific Inc. Waltham, MA, USA). The sensitivity of the method was 2.5 ng/mL, and the intra assay coefficient variation (CV) 8.1% at 43.5 ng/mL and 3.2 % at 226.5 ng/mL. Serum leptin concentrations were determined using the multi-species radioimmunoassay (RIA) kit (Millipore, St. Charles, MO, USA) previously validated for use in pigs (Qian et al. 1999). The sensitivity of the method was 1 ng/mL and the intra-assay CV was 5 % at 1.8 ng/mL. Serum IGF-I concentrations were determined using a

double antibody radioimmunoassay (Louveau and Bonneau 1996) after an acid-ethanol extraction (Daughaday et al. 1980). Detection limit was 0.31 ng/mL and the intra-assay CV was 7.3% at 302 ng/mL.

### 3.2.5.2 Boar taint compounds analysis

For determination of boar taint compounds in studies 1 and 4, samples of subcutaneous fat were taken at the level of the last rib, vacuum packed and stored at -20°C, until further laboratory analyses. Androstenone, skatole and indole concentrations were measured by high-performance liquid chromatography (HPLC) according to the procedures of Hansen-Møller (1994) adapted by Pauly et al. (2008). In brief, adipose tissue samples were liquefied in a microwave oven for 2×1 min at 350 W. The liquefied lipids were centrifuged for 20 min at 11,200×g and 20°C. After centrifugation, fat was heated to 50°C and 0.5 ± 0.01 g water-free liquid fat was transferred in 2 mL eppendorf tubes where 1 mL of internal standard containing 0.496 mg/L androstanone and 0.050 mg/L 2-methylindole in methanol was added. After stirring for 30 s, the tubes were incubated for 5 min at 30°C in an ultrasonic water bath, kept on ice for 20 min and centrifuged for 20 min at 11,200×g at 4°C. For androstenone determination, 50 µL of the supernatant was submitted to derivatization with dansylhydrazine and BF<sub>3</sub> for exactly 2 min. Thereafter, an aliquot of 10 µL of the derived mixture was injected into a HPLC column and fluorescence was detected (excitation at 346 nm and emission at 521 nm) using HP1200 (Agilent Technologies, Waldbronn, Germany). For skatole and indole determination, 20 µL of the supernatant was injected into the column and fluorescence was detected (excitation at 285 nm and emission at 340 nm) using the same HPLC system. Concentrations were expressed per g of the lipid fraction from adipose tissue. The detection limits were 0.24 µg/g for androstenone and 0.03 µg/g for skatole and indole.

### 3.2.5.3 Chemical analysis of feed, faeces, urine samples and condensated air

Pooled diet samples were analyzed for DM, ash, nitrogen, starch, crude fiber, crude fat and gross energy (GE) according to AOAC (1990). The NDF, acid detergent fibre (ADF) and ADL contents of pooled samples of diets were analyzed according to Van Soest et al. (1991). Samples of faeces were analyzed for DM, ash, nitrogen, crude fibre, crude fat and GE contents. Fresh samples of urine were analyzed for nitrogen content, whereas GE content was evaluated after freeze-drying approximately 30 mL of urine in polyethylene bags. In study 3, the ammonia content in bubbling solution and condensed water were determined using enzymatic method (Enzytec™ fluid Ammonia, Thermo Fisher Scientific Oy, Vantaa, Finland).

Assuming a converting factor of 6.25, crude proteins were calculated from nitrogen content.

## 3.3 Calculations and statistical analysis

### 3.3.1 Calculations in meta-analysis

Pooled within-study standard deviations (SD) were calculated (according to Saville and Rowarth 2008) from the reported variabilities that were expressed in different ways (e.g. standard error, standard error of mean, standard error of difference, least significant difference). The effect of the immunocastration on the studied traits was evaluated using the effect size method (Borenstein et al. 2007) which allows the comparison of two populations. For each variable, the standardized effect size ( $\Theta$ ) was calculated as the difference between the treatment (IC) and control groups (EM or SC) divided by its pooled SD. The effect size method requires information on intra-study variability for the computation of a weighted mean, with more weight given to studies with lower variation and vice versa. The effect size was calculated according to a random model, which assumes that the studies were drawn from populations that differ from each other in ways that could have an impact on the treatment effect (e.g. early or late immunisation, ractopamine or somatotropine treatment, energy level



of the diet). The heterogeneity or between-studies variability was assessed using Cochran's Q chi-square test. However, since the Q statistic does not provide information on the extent of true heterogeneity (only on its significance), the  $I^2$  statistic was calculated, which denotes the percentage of the total variability that is due to between-studies variability (Higgins and Thompson 2002). All calculations were made according to Borenstein et al. (2007).

### 3.3.2 Calculations of growth performance

In study 3, ADG for the balance period was calculated using body weight (BW) on the morning of the first day in respiration chambers and on the morning of the fasting day. In studies 2 and 4, ADG and gain to feed ratio (G:F) were calculated on a weekly basis and subsequently pooled per periods V1-V2, V2-slaughter and V1-slaughter. Additionally, in study 4, NE intake was calculated from DM intake and NE concentration of experimental diets was used in calculation of gain to NE intake ratio.

### 3.3.3 Calculations of digestibility, heat production, partitioning of energy and lipogenesis

In studies 3 and 4, the dry matter intake (DMI) and apparent digestibility coefficients of organic matter, nitrogen, GE and fat were calculated according to standard procedures.

In study 3, nitrogen retention was calculated as the difference between nitrogen intake and nitrogen losses in faeces, urine, condensed water and outgoing air. Respiratory quotient (RQ) was calculated as the ratio between  $\text{CO}_2$  production and  $\text{O}_2$  consumption. Total HP was calculated according to Brouwer (1965) from  $\text{O}_2$  consumption,  $\text{CO}_2$  and  $\text{CH}_4$  production, and urinary nitrogen losses calculated as the sum of nitrogen recovered in the urine and evaporated as ammonia. The first day in the respiration chambers was considered as an adaptation day and was not included in the calculation of HP. Total RE during balance period was calculated as the difference between GE intake and energy losses in faeces and urine, and as methane and HP. Retained energy as protein was obtained from nitrogen balance considering that the

energy content of protein gain equalled 23.6 kJ/g. Assuming that the energy content of fat gain equalled 39.7 kJ/g, fat deposition was calculated from the difference between RE and RE as protein. The model of van Milgen et al. (1997) was used to calculate the components of HP using data from gas exchange, signals of force sensors and feed intake (ingested quantity and time of feed distribution) in respiration chambers. The HP due to physical activity (AHP), feed intake (short-term thermic effect of feeding,  $TEF_{st}$ ), resting metabolism and FHP were calculated according to the formula of Brouwer (1965) from the respective volumes of  $O_2$  and  $CO_2$  which were determined by the model. The difference between HP due to resting metabolism and FHP was considered as a long-term thermic effect of feeding ( $TEF_{lt}$ ) and combined with  $TEF_{st}$ , it represented total thermic effect of feeding ( $TEF_t$ ). Additionally, HP and its components were corrected for the difference in physical activity (assuming a constant AHP:ME ratio) as described by Labussière et al. (2011). Components of HP were used to calculate heat increment (HI) as the sum of HP due to physical activity and thermic effect of feeding. Lipogenesis, oxidation of nutrients and their contribution to fat retention were calculated according to Chwalibog et al. (1992). All energy measurements were expressed relative to  $BW^{0.60}$  (Noblet et al. 1999).

#### 3.3.4 Statistical analysis

The effect of the treatment group in study 2 was analyzed using the general linear model procedure of SAS (SAS Inc., Cary, NC, USA). Carcass weight was used as a covariate in the model for carcass traits and IMF. For meat quality traits, slaughter batch was added to the model. For the analysis of leptin and IGF-I, a repeated measures analysis was performed as a split plot in time. Due to the significant interaction between the treatment group and sampling time, a slice option was used and multiple comparisons of lsmeans performed within the treatment group or sampling time using MULTTEST procedure (Holm adjustment). In study 3, analysis of variance was performed using mixed model procedure of SAS (PROC MIXED, SAS Inst., Cary NC, USA) on repeated measurements of nitrogen and energy balances, RQ, nutrient deposition and BW gain composition data (n=16) with nested design including fixed

effects of stage and group within stage and random effect of pig (included in the repeated statement). To evaluate the IC/growth effect (IC confounded with age and weight i.e. growth), the pigs on LF diet were compared in stage 1 and 2 (group1 in stage 1 was contrasted to group 1 in stage 2). A contrasts analysis was also used for evaluating the effect of HF diet by comparing group 1 and group 2 in stage 2. It is worth noting that, due to the absence of any notable difference between groups 1 and 2 in stage 1, the results (least squares means) for this stage were presented as pooled values. In study 4, analysis of variance was also performed using mixed model procedure of SAS (PROC MIXED, SAS Inst., Cary NC, USA). Repeated measurements of digestibility and nitrogen balances data (N=8) in digestibility trial (trial1) were evaluated by including the fixed effects of dietary group and stage and their interaction and the random effect of pig (included in the repeated statement). The effect of NE concentration of the diet in growth trial (trial 2) on growth performance was evaluated considering the fixed effects of dietary group and litter for the phases before (d 84 to d 114) and after V2 (d 115 to d 170) whereas, for the separate two 4-week periods within the phase after V2, the model included dietary group, period, their interaction and litter, with pig as repeated variable. Because the effect of immunocastration on performance is well established, and this was not the interest of the present study, the comparisons of phases before and after V2 are not presented. Estimated parameters for the generalized relationships between dietary energy intakes and BW in P1 and P2 were evaluated for the effect of diet using nonlinear procedure of SAS (PROC NLIN, SAS Inst., Cary NC, USA). Data on carcass and meat quality traits were analyzed using a model with fixed effects of dietary treatment group, litter within slaughter batch and slaughter batch (PROC MIXED, SAS Inst., Cary NC, USA). The individual pig was the experimental unit for the analysis of all data. Least squares means were compared using Tukey test with statistical significance based on  $P < 0.05$ .

## 4 RESULTS

### 4.1 Meta-analysis of the effect of immunocastration on production performance, reproductive organs and boar taint compounds in pigs

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## Meta-analysis of the effect of immunocastration on production performance, reproductive organs and boar taint compounds in pigs

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*Meta-analytical approach was used to quantitatively synthesize the effect of immunocastration on growth, carcass, meat quality, reproductive organs and boar taint compounds. Altogether, 41 papers were collected for effect size ( $\theta$ ) calculation and the comparisons were made with entire males (EM) and surgical castrates (SC). The data for reproductive organs and growth performance are numerous enough to draw firm conclusions. In contrast, data for carcass and meat quality are more limited. Results of meta-analysis show efficient immunocastration with the magnitude of the response being by far the largest for reproductive organs ( $\theta = -2.8$  to  $-5.0$ ) and boar taint substances ( $\theta = -2.8$  and  $-0.8$  for androstenone and skatole, respectively). However, compared with SC, the immunocastrates exhibit larger bulbourethral glands ( $\theta = 1.3$ ) and slightly higher concentrations of androstenone and skatole ( $\theta = 0.1$  and  $\theta = 0.2$ , respectively). The impact of immunocastration is also remarkable on performance, where the main advantage of the immunocastrates is their boar-like performance until revaccination. In the period following the second vaccination, they eat much more than EM ( $\theta = 2.1$ ), resulting in large effect size for growth rate compared with both EM and SC ( $\theta = 1.1$  and  $\theta = 1.4$ , respectively). Considering the whole fattening period, their feed conversion ratio is higher compared with EM ( $\theta = 0.6$ ) and much lower than that of SC ( $\theta = -1.3$ ), although exhibiting moderately faster growth compared with both ( $\theta = 0.6$  and  $\theta = 0.2$ , respectively). With regard to carcass quality, the immunocastrates take intermediate position between EM and SC. Besides, our analysis suggests no difference in meat quality with SC and some meat quality advantages of immunocastrates over EM because of higher intramuscular fat content ( $\theta = 0.4$ ) and lower shear force ( $\theta = -0.6$ ).*

**Keywords:** meta-analysis, immunocastration, performance, boar taint, pigs

### Implications

A meta-analysis of the literature data obtained from different experimental conditions was performed to obtain a quantitative synthesis of the responses to immunocastration. Boar taint compounds are significantly reduced in immunocastrates, and yet remain slightly higher than that in surgical castrates (SC). Immunocastrates are less efficient, fatter but grow more rapidly and may have better meat quality than entire males (EM). Compared with SC, they have superior performance with no difference in meat quality. It is more economical to fatten immunocastrates than SC; yet, production costs and carcass quality are less favourable than that of EM.

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### Introduction

Surgical castration of male piglets is a traditional practice used to avoid boar taint, an unpleasant odour and flavour of meat from entire male (EM) pigs that has been ascribed to the presence of androstenone (Patterson, 1968) and skatole (Vold, 1970; Walstra and Maarse, 1970). Androstenone is a testicular steroid producing a urine-like smell, whereas skatole has a faecal-like odour and is produced by bacteria in the large intestine. Recently, the citizen concern on animal welfare has been exerting increasing pressure on pig producers to stop surgical castration without anaesthesia because it is painful for the animal. A ban on surgical castration without pain relief is already enforced in Norway, Switzerland and the Netherlands and is also under consideration by the European Union. The alternatives currently



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under consideration include surgical castration with anaesthesia and/or analgesia, raising EM, sperm sexing and immunocastration (attitude, practices and state of the art regarding piglet castration in Europe; PIGCAS, 2009). The latter method is a vaccination against the gonadotrophin-releasing hormone (GnRH), which induces the formation of specific antibodies that bind and neutralize GnRH, and thus disrupt the hypothalamic–pituitary–gonadal axis. Early studies, using experimental vaccines, demonstrated the effectiveness of immunocastration to prevent sexual development and occurrence of boar taint (Caraty and Bonneau, 1986; Falvo *et al.*, 1986; Awoniyi *et al.*, 1988). The first commercial product for immunocastration of male pigs (Improvac<sup>®</sup>) has been released in Australia and New Zealand in 1998 and since then has been registered in 53 countries, including the European Union in 2009. Although it is widely used in Australia, New Zealand and Brazil, its use in Europe is still under intensive testing in view of local conditions and consumer acceptability. In addition to the effectiveness of immunocastration to prevent sexual development and boar taint, the published studies indicate better production performance (i.e. growth rate, feed intake, feed efficiency and leanness) of immunocastrated pigs compared with surgical castrates (SC; for review see Millet *et al.*, 2011). However, the results are not always consistent and factors associated with the different studies can interfere with the effect of immunocastration. Meta-analysis is a research methodology to integrate and to quantify experimental results of different origins. The aim of this study was to assess the magnitude and heterogeneity of the effects of immunocastration in pigs through a meta-analysis of currently available research results, focussing on performance, carcass characteristics, meat quality, reproductive organs and boar taint substances.

## Material and methods

### Data collection

The search for articles was carried out using the bibliographic databases Web of Science (<http://www.isiwebofknowledge.com/>), CAB Direct (<http://www.cabdirect.org/>) and the internet. Only studies published before July 2011 were considered. To be included in the meta-analysis, studies had to be original research work reporting the mean and variability (per treatment group or pooled) of the response traits. Because of these conditions, many studies published only as abstracts at congresses were not included. Pooled within-study standard deviations were calculated from the reported variabilities that were expressed in different ways (e.g. standard error, standard error of mean, standard error of difference, least significant difference). Calculations were made according to Saville and Rowarth (2008). Only the studies that provided the results for the treatment group (immunocastrated males, IM) and at least one of the control groups (EM and/or SC) were considered. When several independent experiments were reported within an article, they were considered as separate studies. When other treatments were applied in addition to immunocastration

within a study (e.g. ractopamine, somatotrophin, different stages of immunization, different adjuvants, different types of the vaccine, different doses, different energy levels of the diet, different vaccination protocols, different periods between revaccination and slaughter; see Supplementary Table S1), these treatments and their relevant controls were also considered as separate studies. The complete database thus consisted of 68 'experiments' from 41 articles, involving 7288 pigs ( $n = 3483$  IM,  $n = 1162$  EM and  $n = 2643$  SC). The meta-analysis was performed only for those traits for which a sufficient number of studies ( $n \geq 5$ ) was available, with the exception of the weights of the belly, shoulder, bulbourethral glands ( $n = 4$ ), seminal vesicle weight ( $n = 3$ ) and shear force ( $n = 2$ ) in the comparison between immunocastration treatment (IC) and SC. Growth rate, feed intake and feed efficiency were considered for three periods: from the first vaccination (V1) to the second vaccination (V2), from V2 to slaughter (S) and for the overall fattening (experimental) period (V1 to S). Carcass traits included dressing percentage, *longissimus dorsi* (LD) muscle thickness, subcutaneous backfat thickness, lean meat percentage and weights of the carcass prime cuts (i.e. loin, ham, belly and shoulder). The value of pH measured 24 h *post mortem* (pH24), CIE colour measurements ( $L^*$ ,  $a^*$ ,  $b^*$ ), water holding capacity assessed as drip loss, shear force and intramuscular fat content were the meat quality traits considered in the meta-analysis. Boar taint compounds included skatole and androstenone levels in fat. Regarding reproductive organs, the weights and lengths of testes and bulbourethral glands and seminal vesicle weight were compared between IC and EM, whereas IC and SC were compared only for bulbourethral gland and seminal vesicle weight.

### Meta-analysis

The effect of the immunocastration on the studied traits was evaluated using the effect size method (Borenstein *et al.*, 2007), which allows the comparison of two populations. For each variable, the standardized effect size ( $d$ ) was calculated as the difference between the treatment (IC) and control groups (EM or SC) divided by its pooled standard deviation (s.d.). The effect size method requires information on intra-study variability for the computation of a weighted mean, with more weight given to studies with lower variation and vice versa. The effect size was calculated according to a random model, which assumes that the studies were drawn from populations that differ from each other in ways that could have an impact on the treatment effect (e.g. early or late immunization, ractopamine or somatotrophin treatment, energy level of the diet). The forest plot of effect sizes for average daily gain (ADG) in the period V1 to S (comparison between IC and EM) is given in Figure 1 to illustrate the variation within a study and the weight given to each study. The heterogeneity or between-studies variability was assessed using Cochran's  $Q$   $\chi^2$  test. However, as the  $Q$  statistic does not provide information on the extent of true heterogeneity (only on its significance), the  $I^2$  statistic was calculated, which denotes the percentage of the total variability, that is, due to



### Meta-analysis of immunocastration in male pigs

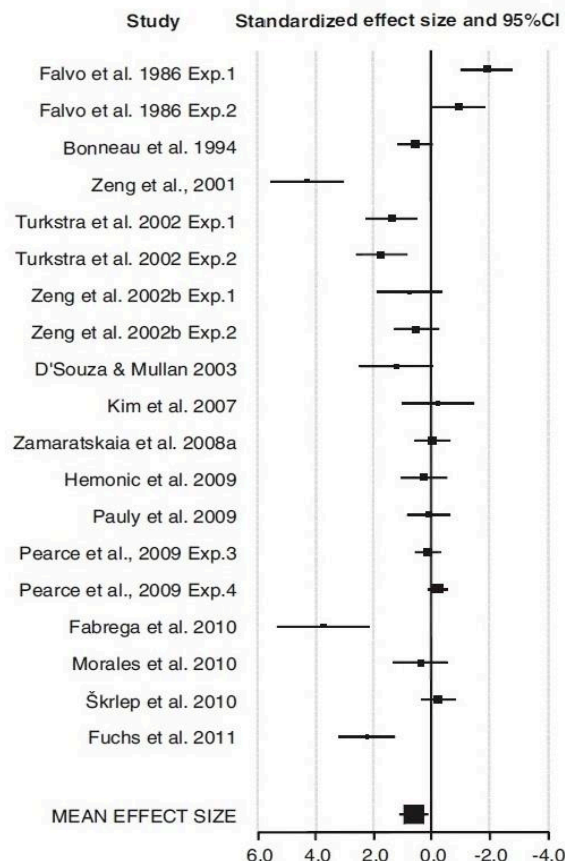


Figure 1 Forest plot of immunocastration effect (comparison with entire males) on average daily gain from the first vaccination to slaughter.

between-studies variability (Higgins and Thompson, 2002). All calculations were made according to Borenstein *et al.* (2007). It should be mentioned that in some cases (e.g. shear force for comparison IC to SC) the number of studies was very small, which may represent a problem for estimating between-studies variance and consequently draw any firm conclusions. However, in such cases, the effect size was insignificant and therefore not considered and discussed.

## Results

### *The importance of immunocastration effect with regard to the studied traits*

The magnitude of the effect of immunocastration is given in Table 1 and this magnitude was different for different traits of interest. The strongest impact (effect size ranging from  $-2.8$  to  $-5.0$ ) was observed for reproductive organs and androstenone concentration (comparison IC v. EM). High values for effect size (absolute value  $>0.8$ ) were also observed for certain traits of performance, whereas low (absolute value  $<0.4$ ) to moderate effect sizes (absolute

value in the range of 0.4 to 0.8) were observed for carcass and meat quality traits.

### *Performance*

Effect sizes for ADG, daily feed intake (DFI) and feed conversion ratio (FCR; Table 1) were derived from 29 references (Supplementary Table S1) that dealt with the effect of the immunocastration in comparison with SC or EM.

*The comparison of IC and EM.* There was no significant difference in performance between IC and EM in the period between the two vaccinations (V1 and V2). However, growth rate tended to be greater in IC than in EM ( $P = 0.058$ ). After the second vaccination (V2 to S), IC consumed more and grew faster than EM. Considering the overall period (V1 to S), consumed more and grew faster than EM but had a higher FCR. The comparison of effect sizes between traits demonstrates that the largest difference between IC and EM pertains to the higher feed intake of IC after the second vaccination. For illustration, the non-standardized (raw) differences in performance traits between IC and EM are given in Figure 2. In general, the heterogeneity of the results as estimated by the  $Q$  statistic was significant ( $P < 0.10$  for DFI and for ADG in the period V1 to V2;  $P < 0.05$  for the other traits; data not shown). The  $I^2$ , denoting the degree of variability between studies, ranged from 40% to 90% (data not shown). The heterogeneity of the results is also illustrated in the forest plot for ADG in the period V1 to S (Figure 1).

*The comparison of IC and SC.* In the period (V1 to V2) before the second vaccination, IC consumed and grew less, and had a lower FCR than SC. After the second vaccination (V2 to S), IC tended to have a higher feed intake and had a higher growth rate and lower FCR than SC. Considering the overall fattening period (V1 to S), IC consumed much less, grew faster and had a lower FCR than SC. The comparison of effect sizes between the traits shows that the biggest difference between IC and SC pertains to the lower feed intake in the period before immunization. The comparison of IC and SC is further illustrated by the non-standardized differences shown in Figure 2. The heterogeneity of the results of different studies was significant and high ( $I^2 > 75\%$ , data not shown), except for daily gain for the overall fattening period ( $I^2 = 16\%$ ).

### *Carcass traits*

Effect sizes for carcass traits (Table 1) were calculated on data gathered from 30 studies (Supplementary Table S1). The majority of studies dealt with dressing percentage, backfat thickness and lean meat percentage. Studies reporting more detailed data on carcass body composition or prime cuts were scarce.

*The comparison of IC and EM.* Compared with EM, IC pigs had a greater backfat thickness, resulting in lower carcass lean meat percentage. Concerning the carcass prime cuts, IC pigs were similar to EM, except for belly weight, which was



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

Trait	IC to SC			IC to EM		
	<i>n</i>	$\theta_i$ (CI)	<i>P</i> -value	<i>n</i>	$\theta_i$ (CI)	<i>P</i> -value
Daily gain						
V1 to V2	19	-0.58 (-1.05, -0.10)	0.017	16	0.15 (-0.16, 0.45)	0.343
V2 to S	21	1.38 (0.89, 1.86)	0.000	30	1.09 (0.53, 1.65)	0.000
V1 to S	26	0.22 (0.12, 0.33)	0.000	19	0.62 (0.14, 1.09)	0.011
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
FCR						
V1 to V2	10	-1.12 (-1.73, -0.51)	0.000	9	-0.36 (-1.18, 0.46)	0.391
V2 to S	13	-1.18 (-1.93, -0.43)	0.002	25	0.24 (-0.20, 0.67)	0.283
V1 to S	13	-1.33 (-1.89, -0.76)	0.000	12	0.65 (0.26, 1.03)	0.001
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
Meat quality of LD						
Ultimate pH	12	-0.15 (-0.44, 0.15)	0.341	10	-0.16 (-0.35, 0.03)	0.093
<i>L</i> *	6	0.47 (-0.56, 1.49)	0.376	8	0.28 (-0.03, 0.60)	0.076
<i>a</i> *	5	-0.19 (-0.57, 0.33)	0.608	8	0.03 (-0.32, 0.39)	0.850
<i>b</i> *	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 (0.17, 0.60)	0.001
Reproductive organs						
Testis weight				39	-4.21 (-4.88, -3.55)	0.000
Testis length				8	-2.84 (-3.72, -1.96)	0.000
Bulbourethral gland weight	4	1.29 (0.56, 2.02)	0.001	10	-3.55 (-4.60, -2.51)	0.000
Bulbourethral gland length				12	-3.59 (-4.50, -2.68)	0.000
Seminal vesicle weight	3	0.26 (-0.22, 0.74)	0.284	5	-4.99 (-7.51, -2.47)	0.000
Boar taint compounds						
Androstenone	7	0.12 (-0.00, 0.24)	0.053	23	-2.80 (-3.44, -2.15)	0.000
Skatole	6	0.18 (0.01, 0.35)	0.039	14	-0.77 (-0.98, -0.56)	0.000

IC = immunocastration treatment; SC = surgical castrates; EM = entire males.

$\theta_i$  = mean effect size calculated according to random effect model; CI = 95% confidence interval; V1 = first vaccination; V2 = second vaccination; S = slaughter; LD = *longissimus dorsi* muscle; *L*\**a*\**b*\* are colorimetric parameters.

higher in IC. The comparison of effect sizes between carcass traits shows that the most important difference between IC and EM concerns body fatness. Non-standardized trait differences given in Figure 2 also illustrate this difference. In general, the heterogeneity of the results of different studies was significant, except for ham and belly weight, and the  $I^2$  ranged from 0% to 93% (data not shown).

**The comparison of IC and SC.** Compared with SC, dressing percentage was lower for IC. In contrast, IC pigs were leaner (lower backfat thickness and higher lean meat percentage). Concerning the prime cuts, IC pigs had heavier ham and shoulder weights ( $P = 0.06$ ) but lower belly weight

( $P = 0.07$ ). The comparison of the effect size shows the greatest impact on dressing percentage and shoulder weight. The differences between IC and SC are further illustrated in Figure 2 for non-standardized traits. The heterogeneity among studies was significant and high (58%  $< I^2 < 93\%$ ), except for LD muscle thickness ( $I^2 = 31\%$ ; data not shown).

#### Meat quality

Data for the calculation of effect sizes for pH24, CIE colour measurements (i.e. *L*\*, *a*\*, *b*\*), drip loss, shear force and intramuscular fat content were obtained from 12 studies (Supplementary Table S1) and results are presented in Table 1.

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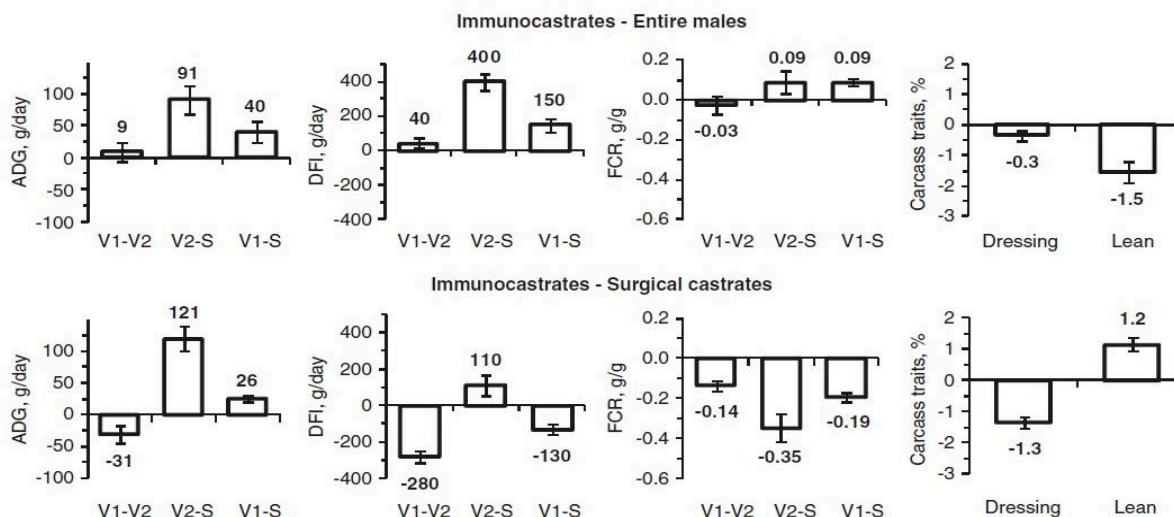


Figure 2 Unstandardized (raw) differences between immunocastrates and entire males or surgical castrates (SC) for average daily gain (ADG), average daily feed intake (DFI), feed conversion ratio (FCR) and carcass traits (dressing %, lean meat %).

*The comparison of IC and EM.* The higher drip loss observed for IC compared with EM is consistent with the slightly lower pH24 and higher  $L^*$  values ( $P < 0.10$ ). In contrast, intramuscular fat content was higher and shear force was lower in IC than in EM. Regarding meat quality traits, the largest effect size was observed for shear force. The heterogeneity of studies was mostly insignificant and moderate ( $I^2 < 55\%$ ; data not shown).

*The comparison of IC and SC.* Differences between IC and SC were not significant for any of the meat quality traits. The heterogeneity of studies was significant for pH24, colour values  $L^*$ ,  $a^*$  and intramuscular fat, with  $I^2$  varying from 12% to 96% (data not shown).

#### Reproductive organs and boar taint compounds

Studies addressing the development of reproductive organs were the most abundant (Supplementary Table S1). Among 41 investigated studies, 26 reported reproductive organ size or weight, mostly testes weight, whereas 15 studies presented data for the concentration of boar taint compounds (Table 1).

*The comparison of IC and EM.* Effect size for the weights of testes and accessory sex glands indicated drastic regression of reproductive organs in IC pigs. The most marked effect was noted for the seminal vesicle, followed by testis and the bulbourethral gland, as also confirmed by the non-standardized differences that indicated a 87%, 65% and 66% weight reduction for the respective organs. Because of immunocastration, levels of androstenone and skatole were also strongly reduced. However, the impact was more important for androstenone than for skatole. The heterogeneity of the results from different studies was significant

and high for reproductive organs and androstenone concentration ( $I^2 > 90\%$ ) and low for skatole concentration ( $I^2 < 40\%$ ; data not shown).

*The comparison of IC and SC.* With regard to the accessory sex glands, the comparison of IC and SC indicated a strong effect size for the bulbourethral gland, but not for the seminal vesicles. Although the effect sizes for androstenone and skatole concentrations were small (0.12 and 0.18, respectively), they were significant ( $P = 0.05$  and  $P = 0.04$ , respectively). The heterogeneity of data was low ( $I^2 < 40\%$ ) for both substances (data not shown).

### Discussion

Immunocastration is a new, alternative technique to control boar taint. As indicated by the review of Millet *et al.* (2011), immunocastration alters animal performance, but the results are not always consistent. This is why we carried out a quantitative analysis of available results, using all relevant published information to date, in order to compare and quantify the importance of the effects for different traits.

#### Performance

Before the second vaccination, IC appeared to be (physiologically) similar to EM, with mostly no difference in performance. However, feed intake tended to be higher in IC than in EM. This could be explained with an early response to the vaccination. Indeed, Turkstra *et al.* (2002) observed that in some pigs (qualified as early responders), LH and testosterone levels were reduced after V1. After the second vaccination, IC produce a large quantity of antibodies, sufficient for the neutralization of all secreted GnRH. Within 5 days, LH



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and steroid secretions are suppressed and the metabolism adapts in 7 days (Claus *et al.*, 2007). As a result, major performance changes occur in IC after V2. The largest effect is a drastic increase in feed intake compared with EM, in relation with the sharp reduction in the production of androgens and oestrogens, which are known to negatively affect feed intake (Claus and Weiler, 1987). Cronin *et al.* (2003) observed that IC had spent more time eating after V2, possibly in relation with their reduced sociosexual behaviour.

Our analysis shows that feed intake after V2 is also slightly higher in IC compared with SC. A possible explanation for this effect pertains with leptin. Because IC pigs are leaner than SC at the time of V2, they may have lower circulating levels of leptin (Ramsay *et al.*, 1998), which is known to be an inhibitor of feed intake (Houseknecht *et al.*, 1998; Ramsay, 1999). The faster growth of IC compared with SC results to a small extent not only from the increased intake, but also, and mostly, from a much better feed efficiency demonstrated by the lower FCR. It could be hypothesized that the better feed efficiency of IC relative to SC results from the fact that immunocastration does not impair growth hormone (GH) secretion compared with EM, whereas it is much lower in SC (Metz and Claus, 2003). However, the secretion of IGF1, which is the real active hormone mediating the effect of GH on growth and feed efficiency, is impaired in IC relative to EM (Metz and Claus, 2003; Brunius *et al.*, 2011). Compared with SC, IGF1 levels of IC were found similar (Metz and Claus, 2003) or higher (Brunius *et al.*, 2011). It can be argued, however, that because circulating IGF1 has a long half-life (Claus *et al.*, 2007) and because high feed intake stimulates IGF1 secretion (Bauer *et al.*, 2008), IGF1 decreases only slowly after the second vaccination is fully effective, which occurs only 1 week after the second injection (Claus *et al.*, 2007). Therefore, there is a transient period after the second vaccination when the IC still benefits from higher IGF1 levels, resulting in better feed efficiency and higher feed intake, and thus resulting in faster growth.

Overall for the whole fattening period (V1 to S), IC are much more efficient than SC (because they behave like EM before the second vaccination and also some time after) but less efficient than EM because they are slaughtered much after the transient period is finished, when IC behave more or less like EM.

#### *Carcass traits*

Dressing percentage of EM is usually slightly lower than that of barrows or gilts (Babol and Squires, 1995), mostly because of the weight of the genital tract. In that respect, IC seem to differ from SC, but not from EM. Although the total weight of the reproductive organs is lower in IC compared with EM, it is still higher than that in SC because the testes are still present and the accessory sex glands have not regressed to the same extent as in SC. Moreover, there are other factors that can explain impaired dressing percentage in IC, such as increased abdominal fat (Škrlep *et al.*, 2010a and 2010b) and higher weights of intestinal tract, kidneys and liver (Pauly *et al.*, 2009; Gispert *et al.*, 2010).

As discussed above, the hormonal status of the IC is similar to that of the EM for most of the fattening period, except for the last few weeks before slaughter, when the transient period after the second vaccination is finished. This is why lean content in IC is intermediate between those in EM and SC. As could be expected, backfat thickness in IC increases with the time between V2 and slaughter (Lealiifano *et al.*, 2011). IC submitted to an early vaccination schedule (Falvo *et al.*, 1986) or exhibiting a response after the first vaccination (early responders described by Turkstra *et al.*, 2002) may actually be fatter than SC.

The heavier belly weight in IC than in EM is consistent with the higher fat content of the carcass. The higher ham and shoulder weights and the lower belly weight in IC compared with SC can be ascribed to the fact that muscles of the fore and hind limbs develop earlier in life than muscles of posture (e.g. loin, belly muscles) and may be less affected by the reduced anabolic potential after immunocastration (Pauly *et al.*, 2009).

#### *Meat quality*

The synthesis of published data on meat quality as affected by the immunocastration shows that IC do not differ from SC, whereas IC present some advantages over EM, namely higher intramuscular fat content and lower shear force. Higher intramuscular fat content has been a consistent finding in all five studies and could partly explain the lower shear force (van Laack *et al.*, 2001). Another possible explanation pertains with the much increased growth rate in the weeks before slaughter, resulting in enhanced protein turnover *in vivo*, and hence increased proteolysis *post mortem* (Therkildsen *et al.*, 2004; Lametsch *et al.*, 2006). Compensatory growth has been proposed to have a positive effect on pork tenderness (Kristensen *et al.*, 2002).

However, IC also have some disadvantages compared with EM, including a higher drip loss, which is consistent with a tendency for lower ultimate pH and higher *L* value. The lower ultimate pH is consistent with the reduced aggressive behaviour and physical activity of IC (Cronin *et al.*, 2003). Increased activity in EM is indeed known to result in higher ultimate pH (Sather *et al.*, 1995) because muscle glycogen is depleted to a greater extent before slaughter.

Because of the limited number of available data, more studies are needed to confirm the positive influence of immunocastration on meat quality.

#### *Reproductive organs and boar taint compounds*

Immunocastration results in a dramatic regression of the genital tract. This has been shown in all studies, despite the high heterogeneity between studies because of variation in vaccination protocols. The effects on reproductive organs are indeed larger for early vaccination than for late vaccination. The meta-analysis shows that the largest reduction is observed for seminal vesicle weight, which supports the suggestion of Bonneau (2010) to use this as a diagnostic tool to assess the success of immunization at slaughter. This outcome can be related to their anatomical structure of



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liquid-containing vesicles, which can be quickly resorbed. The other accessory glands and testes have a more firm structure and thicker walls and they take more time to regress (Bonneau, 2010). This can also explain why bulbourethral glands are larger in IC than in SC, whereas no difference is observed for seminal vesicles. Regression of the reproductive tract is consistent with loss of functional activity as shown by histological observations (Falvo *et al.*, 1986; Grizzle *et al.*, 1987; Awoniyi *et al.*, 1988). Immunocastration reduces the mean diameter of the seminiferous tubules, the number of spermatogonia and spermatocytes, induces atrophy of the Leydig and reduces the weight of gland tissues and secretory products of accessory sexual glands. These changes were shown to be dependent on the time of immunization (Einarsson *et al.*, 2011; Kubale *et al.*, 2011).

The impairment of testis functionality is also apparent from the reduction of boar taint compounds. These are reduced substantially in IC as compared with EM, although the elimination is not fully complete, as shown by the small but significant differences between IC and SC for both skatole and androstenone. A first possible explanation could be that in some studies, the relatively short time between V2 and slaughter was insufficient for complete elimination of androstenone and skatole from the fat depots. This is, however, unlikely because the half-life of these compounds in fat is short (Claus, 1976; Bonneau *et al.*, 1982; Friis, 1993) compared with the time interval between full effectiveness of the second vaccination and slaughter. Another explanation is that some animals do not react to the vaccination, or have not been correctly vaccinated. The so-called non-responders have been identified by Zeng *et al.* (2002a), Jaros *et al.* (2005) and Hilbe *et al.* (2006).

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### Supplementary materials

For supplementary material referred to in this article, please visit <http://dx.doi.org/doi:10.1017/S1751731112000146>

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Table 2: Summary of the 41 references used to construct the final database of meta-analysis.

Study	Experimental treatment				Vaccination			Reported traits					Comments
	IM	EM	SC	Other treatments	Type	Protocol (wks) V1-V2-S	Housing	Performance	Carcass	MQ	Reprod. organs	Boar taint compounds	
Falvo <i>et al.</i> , 1986	x	x	x	different adjuvant	Other	12-(16,18)-24	individual	x	x		x		V3 at 18 wk, Boar taint compounds not considered, only data for anti-GnRH vaccination were used
Grizzle <i>et al.</i> , 1987	x	x			Other	48-(58,62,66,70)-72 <sup>1</sup>	individual				x		V3 at 58 wk, V4 at 62 wk, V5 at 66 wk, V6 at 70 wk, sexually mature boars
Awoniyi <i>et al.</i> , 1988	x	x			Other	12-(18,20)-24	individual				x		V3 at 20 wk
Bonneau <i>et al.</i> , 1994	x	x	x		Other	10-20-22 <sup>2</sup>	individual	x	x		x	X	
Meloen <i>et al.</i> , 1994	x	x		monomer and tandem vaccine	Other	9.5-17.5-25.5 <sup>3</sup>	individual				x	X	group mean and SD calculated from individual data
Dunshea <i>et al.</i> , 2001	x	x	x		Improvac	15-19-23 18-22-26	group	x	x		x	X	2 vaccination protocols
Zeng <i>et al.</i> , 2001	x	x	x		Other	13-21-31	group	x			x	X	traditional Chinese breed
Metz <i>et al.</i> , 2002	x	x	x		Improvac	10-(16,23)-26	individual	x	x		x		V3 at 23 wk, 2 replicates
Turkstra <i>et al.</i> , 2002	x	x	x	early, late responders	Other	9-17-22.5	group	x	x				
Zeng <i>et al.</i> , 2002a	x	x		different dose (10, 62.5, 125 and 250µg)	Other	13-21-31	group				x	X	traditional Chinese breed
Zeng <i>et al.</i> , 2002b	x	x	x	high and low energy diet	Other	10-17-27 <sup>4</sup>	group	x	x			X	
Cronin <i>et al.</i> , 2003	x	x	x		Improvac	14-18-23	group	x					behavioural study
D'Souza and Mullan, 2003	x	x	x	2 different genotypes	Improvac	10-18-22 <sup>4</sup>	individual	x	x	x			genotype not considered
McCaughey <i>et al.</i> , 2003	x	x		somatotropine treatment	Improvac	14-18-22	individual	x	x		x	X	gilts were not considered



Study	Experimental treatment				Vaccination		Housing	Reported traits					Comments
	IM	EM	SC	Other treatments	Type	Protocol (wks) V1-V2-S		Performance	Carcass	MQ	Reprod. organs	Boar taint compounds	
Oliver <i>et al.</i> , 2003	x	x		somatotropine treatment	Improvac	13-17-21	group	x	x		x		gilts were not considered
Jaros <i>et al.</i> , 2005	x	x	x		Improvac	16-21-25 <sup>4</sup> 14-20-25 <sup>4</sup>	group	x	x		x	X	2 experiments (different farms)
Hilbe <i>et al.</i> , 2006	x	x		different times of S	Improvac	13-18- (19,20,21,22,23,24,25,26,29,33,78)	group				x		group mean and SD calculated from individual data
Kim <i>et al.</i> , 2007	x	x	x		Improvac	16-20-25 <sup>4</sup>	group	x	x	x	x		
Zamaratskaia <i>et al.</i> , 2008a	x	x	x		Improvac	(13,16)-20-24 <sup>2</sup>	group	x	x	x	x	X	2 trials
Zamaratskaia <i>et al.</i> , 2008b	x	x		two times of S	Improvac	15-21-37 14-18-40	group				x	X	two vaccination protocols
Medina <i>et al.</i> , 2008	x		x		Improvac	14-18-23	group	x					congress proceeding
Andrews <i>et al.</i> , 2009	x		x		Improvac	11.5-20.5-25.5	group			x	x		congress proceeding
Fuchs <i>et al.</i> , 2009	x		x		Improvac	13.5-20.5-25 <sup>3</sup>	group		x				
Hemonic <i>et al.</i> , 2009	x	x	x		Improvac	8.5-18.5-23.5 <sup>3</sup>	group	x	x	x	x	X	EM housed separately, gilts were not considered
Moore <i>et al.</i> , 2009	x	x		ractopamine treatment	Improvac	13-17-22 <sup>4</sup>	individual	x	x	x			
Pauly <i>et al.</i> , 2009	x	x	x		Improvac	9.5-18.5-23.5	group	x	x	x	x	X	only experiment 1 was considered
Pearce <i>et al.</i> , 2009	x	x	x	different studies	Improvac	13-18-22.5 <sup>2,4</sup>	group	x	x				experiments in 5 countries, congress proceeding
Rikard-Bell <i>et al.</i> , 2009	x	x		ractopamine treatment	Improvac	11-17-21.5	group	x	x				gilts were not considered
Schmoll <i>et al.</i> , 2009	x	x	x		Improvac	10-21-25.5	group	x	x		x		
Januskauskas <i>et al.</i> , 2009	x		x		Improvac	13.5-20-26	group			x			congress proceeding
Einarsson <i>et al.</i> , 2009	x	x		different times of S (4, 16 or 22)	Improvac	16-20-24 15-21-37 13-18-40	group				x		2 experiments

Study	Experimental treatment				Vaccination		Housing	Reported traits					Comments
	IM	EM	SC	Other treatments wk after V2)	Type	Protocol (wks) V1-V2-S		Performance	Carcass	MQ	Reprod. organs	Boar taint compounds	
Fabrega <i>et al.</i> , 2010	x	x	x		Improvac	11-21-25	group	x	x				gilts were not considered
Fang <i>et al.</i> , 2010	x	x	x		other	9-17-25	group				x		traditional Chinese bread
Gispert <i>et al.</i> , 2010	x	x	x		Improvac	11-21-26	group		x	x	x		gilts were not considered, SD obtained by personal communication
Morales <i>et al.</i> , 2010	x	x	x		Improvac	10.5-20.5-24.5 <sup>3</sup>	group	x	x		x	X	gilts were not considered
Škrlep <i>et al.</i> , 2010a,b	x	x	x		Improvac	12-19-24	individual	x	x	x	x	X	
Fuchs <i>et al.</i> , 2011	x	x	x		Improvac	13.5-20.5-24.5 <sup>3</sup>	group	x					
Font i Furnols <i>et al.</i> , 2011	x		x		Improvac	12.5-24.5-31.5	group	x	x	x			gilts were not considered
Lealiifano <i>et al.</i> , 2011	x	x		different times of V2 (2,3,4,6 wk before S)	Improvac	10-14-20 10-16-20 10-17-20 10-18-20	group	x	x		x	X	EM received V1
Morales <i>et al.</i> , 2011	x		x	2 different genotypes	Improvac	11-18-25 <sup>3</sup>	group	x	x	x			gilts were not considered in meta-analysis, growth performance results of 2 genotypes were pooled
Zankl <i>et al.</i> , 2011	x	x	x		Improvac	10.5-20-25 <sup>2</sup>	group	x	x				EM not included in the study results

IM- immunocastrated males, EM- entire males, SC- surgical castrates, V1-1<sup>st</sup> vaccination, V2-2<sup>nd</sup> vaccination, S- slaughter, MQ- meat quality, wk- week, <sup>1</sup> pigs were castrated not slaughtered, <sup>2</sup> estimated vaccination time, <sup>3</sup> average vaccination time, <sup>4</sup> estimated slaughter time



#### **4.2 Effect of feed restriction on hormones, performance, carcass traits, and meat quality in immunocastrated pigs**

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## Effect of feed restriction on hormones, performance, carcass traits, and meat quality in immunocastrated pigs<sup>1</sup>

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**ABSTRACT:** The aim of this study was to assess the effect of feed restriction applied to immunocastrated pigs in the period after the second vaccination (V2) against GnRH on hormonal status, performance, carcass traits, and meat quality. Immunocastrated pigs (IC) were compared with entire males (EM) and surgical castrates fed ad libitum. Pigs (Large White × Landrace) × Pietrain were either left entire or surgically castrated within 1 wk after birth (SC, n = 22). At 83 d of age, the entire males were further allotted to treatment groups (individual housing) of ad libitum fed EM (n = 24), ad libitum fed IC (IC-L, n = 21), or restrictively fed IC (IC-R, n = 21). At that time, the first vaccination (V1) was applied to IC-L and IC-R pigs. One week after V2 (age 130 d), feed restriction (~80% of the ad libitum feed intake of SC pigs) was applied to IC-R pigs. The experiment ended 5 wk after V2, when pigs were 165 d old. Immunocastration successfully reduced boar taint compounds and size of reproductive organs. At 130 d, serum leptin concentrations were similar in all groups, whereas IGF-I concentration was less in SC ( $P \leq 0.002$ )

than in the other groups. Three weeks after V2, leptin concentrations of both IC groups were in between EM (least) and SC (greatest). The reverse was observed for IGF-I. Feed restriction had no effect on leptin or IGF-I concentrations in IC pigs. In the period V1 to V2, performance differed mainly between EM and SC, whereas both IC groups had feed intake and feed conversion ratio similar to EM and intermediate daily BW gain, not differing from either EM or SC. After V2, IC-L pigs increased their feed intake to the concentrations of SC, with faster growth compared with the other 3 groups ( $P < 0.05$ ) and fatter carcasses compared with EM pigs ( $P = 0.007$ ). Similar performance and carcass leanness were observed for IC-R and EM pigs. During preslaughter handling more carcass lesions were noted in EM and IC-R than in IC-L or SC pigs ( $P < 0.002$ ). Neither immunocastration nor feed restriction had any effect on meat quality, but EM had greater drip loss, less intramuscular fat, and decreased tenderness than SC pigs. In conclusion, restricting feed intake can increase production efficiency but also aggressiveness of IC pigs.

**Key words:** carcass characteristics, feed restriction, immunocastration, meat quality, performance, pig

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### INTRODUCTION

Immunocastration refers to the vaccination against GnRH, causing the formation of specific antibodies that

bind and neutralize GnRH and hence suppress the hypothalamic-pituitary-gonadal axis. It has been demonstrated that vaccination against GnRH stops testes growth and testicular steroid synthesis in male pigs (Caraty and Bonneau, 1986; Falvo et al., 1986; Awoniyi et al., 1988). IMPROVAC (known as IMPROVEST in the United States) is the only commercially available product for immunocastration of pigs. It was authorized for commercial use in pigs in 1998 in Australia and New Zealand and in May 2009 in the European Union. Immunocastration offers a solution for the boar taint problem and avoids pain inflicted by surgical castration and aggressive behavior observed in entire males (EM; Prunier et al., 2006).

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Moreover, until the immunization is effective, the immunocastrates (**IC**) are actually EM and thus behave like them in terms of feed efficiency and lean tissue growth (Millet et al., 2011), which justifies the advantage of late immunization just a few weeks before slaughter. A recently published meta-analysis of the effects of immunocastration (Batorek et al., 2012) has demonstrated the strongest effect on feed intake (increased) after immunization, reflected in better growth rate of IC, but with a disadvantage of increasing fat deposition when compared with EM. Hence, the objective of the present study was to evaluate the effect of restricted feed allowance applied to IC after the immunization on performance and carcass and meat quality traits compared with ad libitum fed EM, surgically castrated pigs (**SC**), and IC. In addition, reproductive organs and concentrations of boar taint compounds in fat were assessed. Serum IGF-I and leptin concentrations were measured to better understand the physiological mechanisms behind the increased feed consumption after immunocastration as well as the potential benefits of feed restriction.

## MATERIALS AND METHODS

The experiment was carried out in accordance with the Slovenian law on animal protection (Zakon o zaščiti živali, 2007) and took place on a commercial pig farm between December 2010 and February 2011.

### *Animals and Fattening Trial*

Crossbred pigs (progeny of Large White × Landrace dams sired by Pietrain boars) from 37 litters born within 3 d of each other were used for the study. One piglet/litter was surgically castrated within 7 d after birth. At an average age of 24 d piglets were weaned and allocated within litter to 4 treatment groups: SC, EM, and **IC-L** pigs were given ad libitum access to feed throughout the experiment, whereas the **IC-R** group was designated to be restricted after immunization. In the growing phase, the piglets were group housed according to their treatment group. At the average age of 83 d, pigs were selected for the experiment on the basis of their BW (i.e., to obtain a similar average BW and exclude the extremes) and litter, giving a total of 88 pigs (n = 24, 21, 21, and 22 for EM, IC-R, IC-L, and SC, respectively). At that time, the first vaccination (**V1**) against GnRH (IMPROVAC, Pfizer Animal Health, Berlin, Germany) was applied to IC-L and IC-R pigs according to the manufacturer's instructions. During the experiment, from the time of V1 onward, pigs were individually housed in pens (1.2 m × 2.1 m) with concrete slatted floors and were fed a commercial diet containing 13.0 MJ/kg of ME, 16% CP, 2.0% crude fat, 5% crude fiber, 6% crude ash, and 0.88% lysine. Ad libitum feeding was applied to EM,

IC-L, and SC pigs, whereas IC-R pigs were subjected to the restricted feeding 1 wk after the second vaccination (**V2**), at an average age of 130 d. Feed allowance given to IC-R pigs corresponded to approximately 80% of the average ad libitum feed intake of the SC group (calculated for the previous week). Daily feed intake was individually recorded, and pigs were weighed at V1, at V2, and at the end of the experiment (i.e., 165 d of age). During the experiment, 2 SC pigs died, another 1 (IC-R) was an outlier (low growth rate) and was hence removed from the data set, thus leaving 24 EM, 20 IC-R, 21 IC-L, and 20 SC pigs for the statistical evaluation. Pigs were sent to slaughter in 2 batches (6 d apart), with all treatment groups equally represented in the 2 batches. One day before slaughter, pigs were provided feed in the morning, but in the afternoon the feeders were emptied to assure fasting before slaughter. They were transported (20 km) to a commercial abattoir and slaughtered according to standard procedures (approximately 30 min transport, 2 h lairage, and CO<sub>2</sub> stunning). During transport and lairage, pigs from the different treatment groups were not mixed. At the slaughter line, testes and accessory sex glands were removed and taken to the laboratory for dissection and weighing. This was done for EM (n = 21) and one-half of IC-R (n = 10) and IC-L pigs (n = 10, random selection).

### *Blood Sampling and Hormone Analyses*

Blood samples were collected immediately before V1, V2, and 24 d after V2 from vena cava cranialis (9 mL) and during exsanguination at slaughter. Blood samples were collected into chilled glass tubes, immediately placed on ice, and delivered within 1 h to the laboratory, where blood was left to clot at 4°C overnight. Blood serum was then centrifuged at 355×g for 10 min at 4°C, and supernatant was stored at -20°C until analyzed. For hormone assays all samples were analyzed within a single assay.

The serum concentration of cortisol at slaughter was determined using a commercially available ELISA kit (IBL International GmBH, Hamburg, Germany) according to the manufacturer's instructions. The intensity of the color was read at 450 nm using spectrophotometer Varioscans Flash and SkanIt Software version 2.4.3. RE (Thermo Fisher Scientific Inc., Waltham, MA). The sensitivity of the method was 2.5 ng/mL, and the intra-assay CV was 8.1% at 43.5 ng/mL and 3.2% at 226.5 ng/mL. Serum leptin concentrations were determined using the multi-species RIA kit (Millipore, St. Charles, MO) previously validated for use in pigs (Qian et al., 1999). The sensitivity of the method was 1 ng/mL, and the intra-assay CV was 5% at 1.8 ng/mL. Serum IGF-I concentrations were determined using a double-antibody RIA (Louveau and



Bonneau, 1996) after an acid-ethanol extraction (Daughaday et al., 1980). The detection limit was 0.31 ng/mL, and the intra-assay CV was 7.3% at 302 ng/mL.

### Carcass Lesion Evaluation

Carcass lesions were assessed on the skin of the cooled carcasses (disregarding regions distally from the tarsal and carpal joints). Only the fresh lesions inflicted during the preslaughter period were recorded according to etiology (i.e., as lesions caused either by teeth or by hoofs; Škrlep et al., 2011).

### Carcass Traits

At the slaughter line, carcass lean meat content (%) was determined using a method approved for Slovenia (European Commission, 2008), which consisted in taking 2 measurements at the carcass split line: back fat (minimum thickness over the gluteus medius muscle) and loin depth (shortest distance between cranial end of gluteus medius muscle and dorsal edge of vertebral canal); lean meat content (%) was then calculated as

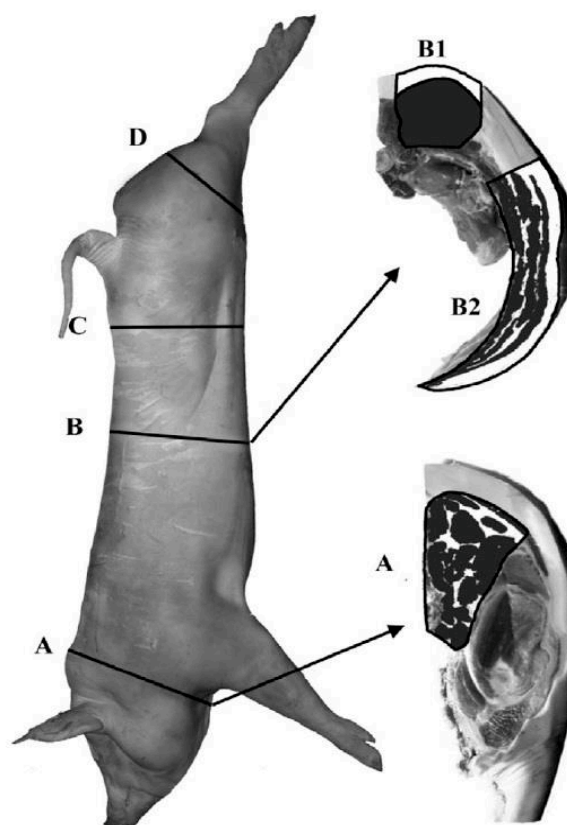
$$\text{lean meat content (\%)} = 60.81879 - 0.72992 \times \text{back fat (mm)} + 0.12127 \times \text{loin depth (mm)}.$$

Leaf fat was excised and weighed. Carcasses were cooled overnight until the internal carcass temperature declined below 7°C. On the day after slaughter, additional carcass traits were assessed. The carcass was cut at 4 sites, as shown in Figure 1, and images of the cross sections were taken using a digital photo camera (Canon PowerShot G3, Canon Inc., Tokyo, Japan). LUCIA.NET 1.16.5 image analysis software (Laboratory Imaging s.r.o., Prague, Czech Republic) was used for determining the percentage of neck intermuscular fat on cross section A, loin eye area, and corresponding fat area (loin eye fat area) on cross section B. Additionally, belly leanness on cross section B was visually assessed using a 1 to 7 point scale (1 denoting the lowest and 7 denoting the greatest leanness). The hind leg (between cross sections C and D) was weighed and calculated as percentage of carcass weight (ham %). Skin and subcutaneous fat were then removed, and percentage of lean with bones in ham was calculated.

### Meat Quality Traits

Measurement of pH ( $\text{pH}_{45}$ ) in the LM was taken 45 min postmortem using a MP120 Mettler Toledo pH meter (Mettler-Toledo, GmbH, Schwarzenbach, Switzerland) fitted with a combined glass electrode (InLab427) and previously calibrated at pH 4.0 and 7.0. One day

after slaughter the color and pH ( $\text{pH}_{24}$ ) were measured on a freshly cut surface of LM (Figure 1; cross section B). Color of LM was assessed subjectively using a 1 to 6 point Japanese color scale (Nakai et al., 1975). Color of LM was also assessed objectively using International Commission on Illumination (1976)  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (yellowness) color space. Measurements were taken in triplicate using a Minolta Chroma Meter CR-300 (Minolta Co. Ltd, Osaka, Japan) with an 11-mm aperture and  $D_{65}$  illuminant, calibrated against a white tile. Muscle  $\text{pH}_{24}$  was determined in 2 replicates in the central area of LD. Caudally from the level of last rib, two 2.5-cm-thick slices of LD were removed from the loin to determine drip loss, intramuscular fat, cooking loss, and shear force. Drip loss was determined according to the EZ method (Christensen, 2003). Soon after, 2 cylindrical samples were excised from the central part of the LM, weighed, and placed in plastic containers. The samples were reweighed after 24 and 48 h of storage at



**Figure 1.** Locations of cross sections for the evaluation of carcass traits: A = between 3rd and 4th cervical vertebra, B = after last rib, C = between 6th and 7th lumbar vertebra, D = knee joint. Highlighted areas on cross sections A and B represent loin eye and corresponding fat area (B1), belly leanness (B2), and neck intermuscular fat (A).



4°C. Drip loss was expressed as the difference (%) from the initial sample weight. Samples of LM were minced, and intramuscular fat content (**IMF**) was estimated using near-infrared spectral analysis (**NIRS**: NIR System model 6500 Spectrometer, Silver Spring, MD; Prevotnik et al., 2005). The second LM slice was trimmed from fat and connective tissue, vacuum packed, and frozen at -20°C until analyzed. To determine cooking loss and shear force, the LM samples were thawed (overnight at 4°C), weighed, and cooked in a thermostatic water bath (ONE 7-45, Memmert GmbH, Schwabach, Germany) until the internal temperature reached 72°C. The samples were reweighed for cooking loss evaluation and cooled at 4°C overnight. The next day, two 2.5-cm-wide cylindrical cores were excised, and shear force (maximum cutting force) was measured using a TA Plus texture analyzer (Ametek Lloyd Instruments Ltd., Fareham, UK) equipped with a 60° V-shaped rectangular-edged blade and a crosshead speed set at 3.3 mm/s.

#### *Analysis of Boar Taint Compounds*

For determination of boar taint compounds, samples of subcutaneous fat were taken at the level of the last rib. Androstene, skatole, and indole concentrations were measured by HPLC according to the procedures of Hansen-Møller (1994) and Pauly et al. (2008). Briefly, adipose tissue samples were liquefied in a microwave oven for 2 × 1 min at 350 W. The liquefied lipids were centrifuged for 20 min at 11,200 ×g at 20°C. After centrifugation, fat was heated to 50°C and 0.5 ± 0.01 g water-free liquid fat was transferred in 2.5 mL Eppendorf tubes with 1 mL methanol containing 0.496 mg/L androstane and 0.050 mg/L 2-methylindole (internal standards). After stirring for 30 s, the tubes were incubated for 5 min at 30°C in an ultrasonic water bath, put on ice for 20 min and centrifuged for 20 min at 11,200 ×g at 4°C. For androstene determination, 50 µL of the supernatant was submitted to derivatization with dansyl hydrazine for exactly 2 min. Thereafter, an aliquot of 10 µL of the derived mixture was injected into an HPLC column, and fluorescence was detected (excitation at 346 nm and emission at 521 nm) using HP1200 (Agilent Technologies, Waldbronn, Germany). For skatole and indole determination, 20 µL of the supernatant was injected into the column, and fluorescence was detected (excitation at 285 nm and emission at 340 nm) using the same HPLC system. Concentrations were expressed per gram of the lipid fraction from adipose tissue. The detection limits were 0.24 mg/g for androstene and 0.03 mg/g for skatole and indole, and these values were assigned to pigs with concentrations below those limits.

#### *Statistical Analyses*

The effect of treatment group was analyzed using the GLM procedure (SAS Inst., Inc., Cary, NC). Carcass weight was used as a covariate in the model for carcass traits and IMF. For meat quality traits, slaughter batch was added to the model. Significant differences ( $P < 0.05$ ) in least squares (**ls**) means between treatment groups were compared with the Tukey test. For the analysis of leptin and IGF-I, a repeated measures analysis was performed as a split plot in time. Because of the significant interaction between treatment group and sampling time, a slice option was used, and multiple comparisons of **ls** means were performed within treatment group or sampling time using the MULTTEST procedure (Holm adjustment).

## **RESULTS AND DISCUSSION**

#### *Reproductive Organs and Boar Taint Compounds*

The effectiveness of the immunocastration was judged on the basis of the size of the reproductive organs and concentrations of boar taint compounds in fat tissue. In the present study, we can consider all pigs to be successfully immunocastrated, and no nonresponders were identified. The results presented in Table 1 show that immunocastration caused a significant weight ( $P < 0.001$ ) reduction of testes (approximately 71% reduction) and accessory sex glands (78%, 93%, and 67% for bulbourethral glands, seminal vesicles, and prostate, respectively) in IC pigs compared with EM, and no difference ( $P > 0.10$ ) was observed between the IC-R and IC-L groups. The concentrations of boar taint compounds were also strongly reduced ( $P < 0.001$  and  $P < 0.05$  for androstene and skatole, respectively) in IC pigs, with the exception of indole, for which the values were minimal for all treatment groups (Table 1).

These results are consistent with the literature on immunocastration effect, as recently summarized in a meta-analysis (Batorek et al., 2012). Even though examined studies were conducted on different types of animals, in various conditions, and using numerous different vaccination schedules, they all report successful elimination of boar taint compounds and regression of testes and sexual glands in IC pigs. Regression of the reproductive tract is consistent with the loss of functional activity, as shown by histological changes of testicular tissue (Hilbe et al., 2006; Einarsson et al., 2009; Fang et al., 2010; Einarsson et al., 2011). The more extensive decrease of seminal vesicles than of the other reproductive organs is also consistent with the literature, as reviewed by Bonneau (2010). To our knowledge, the present results are the first report on the effect of feed restriction in IC on the size of reproductive organs and boar taint com-



pounds; the absence of effect could be related to the fact that development of reproductive organs was stopped by immunocastration when feed restriction started.

### Performance

In the period between V1 (start of experiment) and V2, similar feed intakes were recorded for EM, IC-L, and IC-R, with these values being less ( $P < 0.005$ ) than in SC (Table 2). After V2, feed intake of IC-L was similar ( $P > 0.10$ ) to SC and greater than EM or IC-R ( $P < 0.001$ ). In agreement with the experimental design, the IC-R had less feed intake than IC-L pigs; it was also less than EM and SC pigs ( $P < 0.001$ ).

At the time of V1, there was no difference ( $P > 0.10$ ) in BW between groups. Over the period between V1 and V2, growth rate tended to differ ( $P = 0.06$ ) only between EM (least) and SC pigs (greatest), whereas after V2, IC-L pigs grew faster than EM, IC-R, or SC pigs ( $P < 0.05$ ). Overall daily BW gain, and consequently BW at the time of slaughter, was greater for IC-L than EM pigs ( $P = 0.005$ ), whereas SC and IC-R were positioned in between, not differing ( $P > 0.10$ ) from either IC-L or EM.

With regard to feed conversion ratio (F:G), SC pigs were less efficient than others groups in the first phase (V1 to V2), before the immunization of the IC pigs was effective. Restricted feed allowance of IC-R pigs after V2 resulted in the smallest feed conversion ratio, which differed from SC and EM ( $P = 0.045$ ) but not IC-L pigs. The

latter pigs (IC-L) did not differ ( $P > 0.10$ ) from either EM or IC-R, but they were closer to EM and had a better feed efficiency than SC ( $P = 0.003$ ). Similar findings were observed for the overall feed efficiency (V1 to the end of experiment). Concerning the overall performance, it should be noted that IC-R pigs had similar daily feed intake and BW gain but a better feed conversion ratio than EM ( $P = 0.011$ ) and that IC-L had a greater daily BW gain than EM ( $P = 0.005$ ) and a better feed conversion ratio than SC ( $P < 0.001$ ). The difference in overall feed efficiency between IC-R and IC-L pigs was, however, not significant ( $P > 0.10$ ).

It is well known that boars exhibit better feed efficiency than SC pigs (Walstra, 1974), which was reconfirmed in the present study. When IC pigs are compared with SC or EM, data from the literature are inconsistent and depend upon the experimental conditions (in particular, the timing of vaccination). Even though recently published reviews (Millet et al., 2011; Batorek et al., 2012) suggest that ad libitum IC grow faster than SC and EM (because of their greater feed intake after V2), they are usually in between SC and EM for their feed efficiency. It is worth noting that feed conversion ratio was greater in ad libitum fed EM than in restricted IC, indicating that the latter had decreased energy expenditure for maintenance. The reasons could be less physi-

**Table 1.** Effect of immunocastration and feed restriction on reproductive organs and boar taint compounds at slaughter in pigs

Item	Treatment <sup>1</sup>				rmse <sup>2</sup>	P-value
	EM	IC-R	IC-L	SC		
<b>Reproductive organs</b>						
No.	21	10	10			
Testes, g	540 <sup>b</sup>	167 <sup>a</sup>	147 <sup>a</sup>		112	<0.001
Bulbourethral glands, g	120 <sup>b</sup>	33 <sup>a</sup>	27 <sup>a</sup>		26	<0.001
Seminal vesicles, g	149 <sup>b</sup>	12 <sup>a</sup>	9 <sup>a</sup>		45	<0.001
Prostate, g	4.3 <sup>b</sup>	1.4 <sup>a</sup>	1.4 <sup>a</sup>		1.1	<0.001
<b>Boar taint compounds</b>						
No.	24	21	20	20		
Androstenone, mg/g	1.29 <sup>b</sup>	0.24 <sup>a</sup>	0.24 <sup>a</sup>	0.24 <sup>a</sup>	0.36	<0.001
Skatole, mg/g	0.06 <sup>b</sup>	0.03 <sup>a</sup>	0.04 <sup>ab</sup>	0.04 <sup>ab</sup>	0.03	0.019
Indole, mg/g	0.03	0.03	0.03	0.03	0.01	0.490

<sup>a-c</sup>Means within a row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>EM = entire males fed ad libitum; IC-R = immunocastrates with restricted feeding; IC-L = immunocastrates fed ad libitum; SC = surgical castrates fed ad libitum.

<sup>2</sup>rmse = residual mean square error.

**Table 2.** Effect of immunocastration and feed restriction on performance in pigs

Item <sup>1</sup>	Treatment <sup>2</sup>				rmse <sup>3</sup>	P-value
	EM	IC-R	IC-L	SC		
No.	24	21	20	20		
<b>BW, kg</b>						
V1	27.3	28.4	28.1	29.8	3.8	0.220
V2	69.9 <sup>a</sup>	72.3 <sup>ab</sup>	71.7 <sup>ab</sup>	75.0 <sup>b</sup>	7.1	0.048
End	102.4 <sup>a</sup>	106.9 <sup>ab</sup>	111.3 <sup>b</sup>	108.0 <sup>ab</sup>	10.0	0.032
<b>Daily BW gain, g/d</b>						
V1 to V2	883	935	928	963	96	0.057
V2 to End	956 <sup>a</sup>	989 <sup>a</sup>	1131 <sup>b</sup>	941 <sup>a</sup>	166	0.001
V1 to End	914 <sup>a</sup>	958 <sup>ab</sup>	1015 <sup>b</sup>	954 <sup>ab</sup>	98	0.011
<b>Feed intake, kg/d</b>						
V1 to V2	1.98 <sup>a</sup>	2.08 <sup>a</sup>	2.04 <sup>a</sup>	2.30 <sup>b</sup>	0.20	<0.001
V2 to End	3.11 <sup>b</sup>	2.76 <sup>a</sup>	3.52 <sup>c</sup>	3.46 <sup>c</sup>	0.28	<0.001
V1 to End	2.46 <sup>a</sup>	2.37 <sup>a</sup>	2.68 <sup>b</sup>	2.80 <sup>b</sup>	0.19	<0.001
<b>F:G, kg feed/kg BW gain</b>						
V1 to V2	2.25 <sup>ab</sup>	2.23 <sup>a</sup>	2.21 <sup>a</sup>	2.39 <sup>b</sup>	0.18	0.007
V2 to End	3.35 <sup>b</sup>	2.83 <sup>a</sup>	3.15 <sup>ab</sup>	3.84 <sup>c</sup>	0.62	<0.001
V1 to End	2.71 <sup>b</sup>	2.48 <sup>a</sup>	2.64 <sup>ab</sup>	2.96 <sup>c</sup>	0.24	<0.001

<sup>a-c</sup>Means within a row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>First vaccination (V1), second vaccination (V2), end of experiment (End); V1 to V2 = interval between V1 and V2 (47 d); V2 to End = interval between V2 and End (35 d); V1 to End = interval between V1 and End (82 d).

<sup>2</sup>EM = entire males fed ad libitum; IC-R = immunocastrates with restricted feeding; IC-L = immunocastrates fed ad libitum; SC = surgical castrates fed ad libitum.

<sup>3</sup>rmse = residual mean square error.



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cal activity (unlikely, because pigs of both groups were housed individually) or reduced maintenance requirements as reported in pigs selected for reduced residual feed intake (Barea et al., 2010; Boddicker et al., 2011). The present results associated with IGF-I and leptin data (see below) further support these observations. The idea that restricted feed allowance of IC pigs after V2 would improve feed efficiency and carcass leanness of IC pigs was recently investigated by Quiniou et al. (2012), who found slower growth with no improvement of feed efficiency in restrictively fed group-housed IC pigs, suggesting that feed restriction of IC pigs after V2 has no practical interest. Our results, on the contrary, show that despite a slower growth of feed-restricted IC pigs, feed efficiency was improved, and performance traits were similar to those in ad libitum EM. Again, the discrepancy in the results can be ascribed to the experimental conditions, in particular the housing system (individual vs. group), indicating that further studies are needed.

#### Indicators of Preslaughter Aggression and Stress

The number of lesions inflicted by teeth (Table 3) was similar in IC-R and EM and greater than in IM-L or SC pigs ( $P < 0.002$ ). This result suggests a greater level of aggression in EM and IC-R pigs during the preslaughter period (pigs were raised individually). A similar pattern ( $P = 0.05$ ) was observed for carcass skin lesions caused by hoofs. It is well known that EM show more aggressive behavior than their castrated counterparts (von Borell et al., 2009), which was also the case in the present study for EM compared with IC-L and SC pigs. Fewer agonistic and sexual behaviors were reported for vaccinated males once they were effectively immunized (Cronin et al., 2003; Zamaratskaia et al., 2008; Rydhmer et al., 2010), along with fewer lesions on carcasses after slaughter (Fàbrega et al., 2010; Rydhmer et al., 2010). The greater incidence of lesions in IC-R pigs indicates more aggression (comparable with EM) during preslaughter handling and may be linked to feed restriction, which is known to induce chronic stress and greater excitability in pigs and, for that reason, has been criticized from a welfare point of view (D'Eath et al., 2009). Greater incidence of lesions during fattening has also been reported for restrictively fed IC pigs by Quiniou et al. (2012). With the demonstrated successful immunization of all IC pigs, the possible presence of unsuccessfully castrated male pigs, behaving like EM, which could have explained more aggressive behavior, can be ruled out.

The greatest serum cortisol concentration at slaughter was observed for IC-R and the least for EM pigs ( $P = 0.008$ ), whereas IC-L and SC pigs (exhibiting very similar concentrations) were intermediate, not differing

from either IC-R or EM pigs (Table 3). Greater cortisol concentrations in IC-R pigs are probably related to the greater level of aggression before slaughter in this group, given that fighting of pigs is known to increase their serum cortisol (Fernandez et al., 1994; Otten et al., 1997). Because we did not measure the concentrations of cortisol before slaughter, it is difficult to make any conclusion in regard to preslaughter stress. Regarding the least concentration of cortisol in EM, it could be related to the negative association between the anabolic (e.g., testosterone, GH, IGF-I) and catabolic hormones (e.g., cortisol; Weiler et al., 1998; Elsaesser et al., 2002).

#### Carcass Characteristics

Carcass traits (Table 4) were affected by the treatment, with the exception of 2 muscle development indicators (i.e., loin depth and loin eye area). The IC-L and SC pigs had heavier ( $P \leq 0.002$ ) carcasses than EM, and IC-R pigs were intermediate. The yield of ham, as percentage of carcass weight, was similar in both IC groups, which were closer to SC (greatest percentage) than EM (least percentage), not differing significantly ( $P > 0.10$ ) from either of them. All carcass traits associated with fat deposition (back fat thickness, lean meat content, loin eye fat area, belly leanness, neck IMF, ham leanness, and leaf fat) demonstrated an intermediate position of both IC groups between the SC (fattest) and EM (leanest) pigs. The 2 groups of IC pigs did not differ ( $P > 0.10$ ) from each other (except for leaf fat, which was significantly less in IC-R pigs); however, IC-L pigs were closer to SC, and IC-R pigs were more similar to EM pigs. Whereas IC-R differed significantly from EM pigs only in the case of leaf fat (more in IC-R,  $P = 0.019$ ), the IC-L differed from SC pigs only in the case of neck IMF (less in IC-L pigs,  $P = 0.004$ ) and were generally fatter

**Table 3.** Effect of immunocastration and feed restriction on cortisol concentration at slaughter and indicators of preslaughter aggression (skin lesions) in pigs

Item	Treatment <sup>1</sup>				mse <sup>2</sup>	P-value
	EM	IC-R	IC-L	SC		
No. of pigs	24	21	20	20		
Cortisol at slaughter, ng/mL	64.6 <sup>a</sup>	96.2 <sup>b</sup>	84.8 <sup>ab</sup>	82.2 <sup>ab</sup>	30.9	0.015
Lesions caused by teeth, No.	8.4 <sup>b</sup>	9.8 <sup>b</sup>	3.1 <sup>a</sup>	2.7 <sup>a</sup>	4.6	<0.001
Lesions caused by hoofs, No.	3.3	3.9	1.1	1.0	3.5	0.050

<sup>a-c</sup>Means within a row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>EM = entire males fed ad libitum; IC-R = immunocastrated with restricted feeding; IC-L = immunocastrated fed ad libitum; SC = surgical castrates fed ad libitum.

<sup>2</sup>mse = residual mean square error.



than EM pigs.

The results of the present study confirm the generally reported intermediate position of IC pigs compared with EM and SC pigs in regard to fat deposition (Millet et al., 2011; Batorek et al., 2012). This could be related to the hormonal status of IC pigs (being actually entire males until V2), which is similar to that of the EM pigs until the immunization is effective. However, most of the literature on the immunocastration effect deals with back fat thickness or lean meat content, whereas a more detailed carcass evaluation, such as reported in the present study, has not been commonly presented. Our findings associated with recent studies (Pauly et al., 2009; Gispert et al., 2010; Morales et al., 2010; Škrlep et al., 2010, 2012) indicate that immunocastration has no major effect on lean meat content but increases fat deposition. Different responses of the various fat depots to immunocastration were observed in the present study, where IC-L did not differ from SC pigs for leaf fat, were closer to SC in the case of subcutaneous fat, and were closer to EM in the case of intermuscular fat, whereas they were intermediate between SC and EM for other depots. This is consistent with the relative growth rate of the various fat depots at the end of the fattening period, which is known to be the greatest in leaf fat, followed by subcutaneous and intermuscular fat (Jones, 2004; Kouba and Bonneau, 2009; Kouba and Sellier, 2011).

**Table 4.** Effect of immunocastration and feed restriction on carcass characteristics in pigs

Item	Treatment <sup>1</sup>				rmse <sup>2</sup>	P-value
	EM	IC-R	IC-L	SC		
No.	24	21	20	20		
Carcass weight, kg	80.3 <sup>a</sup>	81.9 <sup>ab</sup>	86.0 <sup>b</sup>	86.0 <sup>b</sup>	6.4	0.006
Back fat, mm	9.8 <sup>a</sup>	11.9 <sup>ab</sup>	12.9 <sup>bc</sup>	14.8 <sup>c</sup>	2.9	<0.001
Loin depth, mm	68.7	70.6	67.4	68.4	4.4	0.210
Lean meat content, %	62.0 <sup>c</sup>	60.7 <sup>bc</sup>	59.6 <sup>ab</sup>	58.3 <sup>a</sup>	2.2	<0.001
Loin eye area, cm <sup>2</sup>	46.9	47.4	45.6	45.9	3.6	0.450
Loin eye fat area, cm <sup>2</sup>	13.1 <sup>a</sup>	13.9 <sup>ab</sup>	14.4 <sup>ab</sup>	15.2 <sup>b</sup>	1.8	0.005
Belly leanness <sup>3</sup>	5.0 <sup>b</sup>	4.6 <sup>ab</sup>	4.6 <sup>ab</sup>	4.1 <sup>a</sup>	0.5	<0.001
Intermuscular fat, % <sup>4</sup>	11.8 <sup>a</sup>	13.6 <sup>a</sup>	14.4 <sup>a</sup>	17.9 <sup>b</sup>	3.2	<0.001
Ham, % carcass weight	24.7 <sup>a</sup>	25.3 <sup>ab</sup>	25.3 <sup>ab</sup>	25.5 <sup>b</sup>	0.9	0.040
Lean with bones in ham, %	86.3 <sup>c</sup>	84.5 <sup>bc</sup>	83.4 <sup>ab</sup>	81.5 <sup>a</sup>	2.6	<0.001
Leaf fat, kg	0.58 <sup>a</sup>	0.78 <sup>b</sup>	0.99 <sup>c</sup>	1.01 <sup>c</sup>	0.21	<0.001

<sup>a-c</sup>Means within a row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>EM = entire males fed ad libitum; IC-R = immunocastrates with restricted feeding; IC-L = immunocastrates fed ad libitum; SC = surgical castrates fed ad libitum.

<sup>2</sup>rmse = residual mean square error; carcass weight covariate in the GLM model.

<sup>3</sup>Subjective score using a 1 to 7 scale.

<sup>4</sup>Intermuscular fat in neck, assessed by image analysis.

With regard to the effect of restriction in IC pigs, there was a general trend for IC-R pigs to be leaner than IC-L pigs, but the difference was significant only for leaf fat. The fact that leaf fat was the only significantly affected fat depot in the short period of restriction can be explained by its fast relative growth, as already discussed. To our knowledge, there are only 2 publications dealing with restricted feeding of IC pigs (dos Santos et al., 2012; Quiniou et al., 2012), which also observed no significant effect on carcass leanness. Anyhow, there may still be a potential benefit of restricted feeding because IC-R had 2.4 percentage points greater carcass leanness than SC, whereas IC-L had only 1.3 percentage points.

### Metabolic Hormones

Until V2, leptin serum concentrations were similar in all treatment groups (Table 5). Thereafter, the concentrations of serum leptin increased significantly in SC and both IC groups and did not change in EM pigs. Three weeks after V2, the concentration of serum leptin in both IC groups was intermediate between SC (greatest) and EM pigs (least), in agreement with their intermediate position for body fatness indicators. This is consistent with the well-known positive correlation between serum leptin concentration and the amount of fat in the body (for a review, see Barb et al., 2001; Steiner and Romanovsky, 2007).

At the time of V1 (start of experiment), concentrations of serum IGF-I did not differ significantly between treatment groups (Table 5). These concentrations increased sharply from V1 (83 d) to V2 (130 d) in all treatment groups, the rise being more pronounced for EM (2.5 times,  $P < 0.001$ ) and IC (3.9 times,  $P < 0.001$ ) than for SC pigs (1.4 times,  $P = 0.013$ ). By the time of V2, IGF-I concentrations were 1.6 to 1.9 times greater ( $P \leq 0.002$ ) in EM and IC pigs than in SC pigs. From V2 (age 130 d) until last blood sampling (154 d), serum IGF-I concentrations remained steady for SC and IC pigs, whereas they continued to increase in EM pigs. These data corroborate a previous work reporting similar IGF-I concentrations in EM and SC at 45 d but greater concentrations in EM than in SC at 140 d of age (Louveau et al., 1991). Three weeks after V2, at a time when testicular steroids already declined significantly (Claus et al., 2007), IC pigs still exhibited a greater IGF-I concentrations than SC pigs ( $P \leq 0.001$ ), which is in agreement with the slow decrease of IGF-I concentration after the second immunization reported by Claus et al (2007). Increased concentrations of GH have been observed in immunized boars (Metz and Claus, 2003; Bauer et al., 2009). Even if this phenomenon remains unexplained (Bauer, 2010), it could account for the greater IGF-I con-



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**Table 5.** Effect of immunocastration and feed restriction on serum leptin and IGF-I concentrations (ng/mL) in pigs

Item	Treatment <sup>1</sup>				P-value
	EM	IC-R	IC-L	SC	
No.	24	21	20	20	
Leptin <sup>2</sup>					
Age					
83 d (V1)	2.38	2.10 <sup>A</sup>	2.29 <sup>A</sup>	2.23 <sup>A</sup>	0.351
130 d (V2)	2.51	2.34 <sup>AB</sup>	2.22 <sup>A</sup>	2.31 <sup>A</sup>	0.323
154 d	2.41 <sup>A</sup>	2.68 <sup>B,ab</sup>	2.70 <sup>B,ab</sup>	2.97 <sup>B,b</sup>	0.008
P-value	0.685	0.003	0.008	<0.001	
IGF-I <sup>2</sup>					
Age					
83 d (V1)	93 <sup>A</sup>	66 <sup>A</sup>	72 <sup>A</sup>	102 <sup>A</sup>	0.101
130 d (V2)	236 <sup>B,b</sup>	259 <sup>B,b</sup>	276 <sup>B,b</sup>	144 <sup>B,a</sup>	<0.001
154 d	358 <sup>C,c</sup>	234 <sup>B,b</sup>	250 <sup>B,b</sup>	151 <sup>B,a</sup>	<0.001
P-value	<0.001	<0.001	<0.001	0.011	

<sup>a-c</sup>Within a column, means assigned different uppercase letters differ significantly between ages ( $P < 0.05$ ).

<sup>a-c</sup>Within a row, means assigned different lowercase letters differ significantly between treatment groups ( $P < 0.05$ ).

<sup>1</sup>EM = entire males fed ad libitum; IC-R = immunocastrates with restricted feeding; IC-L = immunocastrates fed ad libitum; SC = surgical castrates fed ad libitum.

<sup>2</sup>Probability concentrations obtained from the repeated measures analysis as split plot in time: for leptin, treatment ( $P = 0.601$ ), age ( $P < 0.0001$ ), treatment  $\times$  age interaction ( $P = 0.011$ ), root-mean-square error = 0.51 ng/mL; for IGF-I, treatment ( $P < 0.0001$ ), age ( $P < 0.0001$ ), treatment  $\times$  age interaction ( $P < 0.0001$ ), root-mean-square error = 54 ng/mL.

centrations in immunized pigs after V2 than in SC pigs. Contrary to the present study, Metz and Claus (2003) observed no difference between immunized boars and barrows. The difference in the findings must be related to the experimental conditions. Namely, in the study of Metz and Claus (2003), the reported IGF-I concentration corresponds to a mean value of daily blood samples taken 2 to 7 wk after V2, indicating that the measurement was taken (on average) later than in the present study. In addition, in their study, feed allowance in castrated pigs (IC and SC) was restricted to the level of EM, which could influence the results. It has been shown that greater energy availability increases IGF-I concentrations (Bauer et al., 2009).

Altogether, these data demonstrate that effective castration (SC or IC) is associated with decreased IGF-I concentrations, the reduction being less marked in IC than in SC pigs. When relating results on hormones to growth, it could be speculated that the absence of testicular steroids and the presence of relatively small amounts of leptin in IC after V2 compared with SC pigs result in greater feed intake in IC pigs (both estrogens and leptin are known to reduce appetite; Claus and Weiler, 1994; Barb et al., 1998). Together with the presence of greater IGF-I, which contributes to improved feed efficiency, the increased feed intake enables stimulated growth (as observed for IC-L pigs; Table 2).

## Meat Quality

The comparison of treatment groups revealed significant differences for the majority of meat quality traits (Table 6). Feed restriction in IC was not a source of differences in meat quality, and both IC groups were mainly intermediate between EM and SC. In the case of IMF, both IC groups were closer to EM (least) than to SC (greatest) but not different from either of them. As indicated by pH<sub>45</sub>, pH declined less rapidly ( $P < 0.04$ ) postmortem in IC-R and EM than in SC, whereas IC-L pigs were intermediate. Greater ultimate pH (pH<sub>24</sub>) was observed for EM than for the other 3 groups ( $P < 0.05$ ). This was reflected in the darker meat color (greater color score and decreased Minolta L\*) of EM, which differed from IC-R ( $P = 0.004$ ) and IC-L ( $P = 0.005$ ). Despite greater pH<sub>45</sub> and pH<sub>24</sub>, EM exhibited reduced water-holding capacity (greater drip and cooking loss) than SC ( $P < 0.05$ ), whereas IC pigs were intermediate (drip loss) or closer to EM (cooking loss). Regarding meat tenderness, as evaluated by shear force, IC pigs did not differ ( $P > 0.10$ ) from either EM (the toughest) or SC (the most tender).

In regard to IMF, a recently published meta-analysis (Batorek et al., 2012) has shown IC to be positioned closer to SC, which was not the case in the present study, where both IC-L and IC-R were intermediate but closer to EM. The absence of an effect of restricted feed allow-

**Table 6.** The effect of immunocastration and feed restriction on meat quality (LM) in pigs

Item	Treatment <sup>1</sup>				rmse <sup>2</sup>	P-value
	EM	IC-R	IC-L	SC		
No.	24	21	20	20		
Intramuscular fat, %	1.54 <sup>a</sup>	1.71 <sup>ab</sup>	1.73 <sup>ab</sup>	2.18 <sup>b</sup>	0.56	0.010
pH <sub>45</sub>	6.43 <sup>b</sup>	6.46 <sup>b</sup>	6.38 <sup>ab</sup>	6.34 <sup>a</sup>	0.16	0.007
pH <sub>24</sub>	5.58 <sup>b</sup>	5.51 <sup>a</sup>	5.51 <sup>a</sup>	5.51 <sup>a</sup>	0.07	0.003
Subjective color <sup>3</sup>	4.3 <sup>b</sup>	3.9 <sup>a</sup>	3.9 <sup>a</sup>	4.0 <sup>ab</sup>	0.3	0.001
Objective color <sup>4</sup>						
L*	46.8 <sup>a</sup>	49.9 <sup>b</sup>	48.9 <sup>b</sup>	48.7 <sup>ab</sup>	2.1	<0.001
a*	6.8	6.6	6.9	6.9	0.8	0.570
b*	2.5	2.6	2.4	2.3	0.5	0.250
Drip loss 24 h, %	3.1 <sup>b</sup>	2.5 <sup>ab</sup>	2.4 <sup>ab</sup>	2.0 <sup>a</sup>	1.1	0.011
Drip loss 48 h, %	5.5 <sup>b</sup>	4.9 <sup>ab</sup>	4.7 <sup>ab</sup>	4.0 <sup>a</sup>	1.6	0.036
Cooking loss, %	28.8 <sup>b</sup>	26.7 <sup>ab</sup>	27.3 <sup>b</sup>	22.5 <sup>a</sup>	4.0	0.004
Shear force, N	211 <sup>b</sup>	186 <sup>ab</sup>	186 <sup>ab</sup>	172 <sup>a</sup>	35	0.022

<sup>a-c</sup>Means within a row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>EM = entire males fed ad libitum; IC-R = immunocastrates with restricted feeding; IC-L = immunocastrates fed ad libitum; SC = surgical castrates fed ad libitum.

<sup>2</sup>rmse = residual mean-square error; slaughter batch effect was added to the GLM model except for intramuscular fat, where carcass weight was added as a covariate.

<sup>3</sup>Japanese color scale (1 to 6), greater values denoting darker color.

<sup>4</sup>L\*, greater value indicates a lighter color; a\*, greater value indicates a redder color; b\*, greater value indicates a more yellow color.



ance on IMF could be related to the fact that the applied feed restriction was moderate (–20%) and short (4 wk). The greater pH<sub>45</sub> observed in IC-R and EM is difficult to explain because the increased aggression (Table 3) observed in these 2 groups before slaughter rather suggests the opposite. Namely, the postmortem rate of pH decline depends on the rate of ATP hydrolysis (i.e., ATPase activity; Bendall, 1973), which is greater in more stressful conditions, in fast and glycolytic muscles. To our knowledge, there is no evidence in the literature to show an effect of sex or restricted feed intake on the proportion of different muscle fibers or enzymes associated with muscle metabolism that could explain our result. On the other hand, stress and high activity before slaughter (transportation and manipulation) can deplete muscle glycogen and consequently increase the ultimate pH (Karlsson et al., 1999; Przybylski et al., 2006), which could explain greater pH<sub>24</sub> (associated with darker color) in EM. Although the meta-analysis of Batorek et al. (2012) indicated that IC could present greater drip loss than EM, IC-L and IC-R were intermediate between EM (greatest) and SC (least) in the present study. An intermediate position of both IC groups was also found for tenderness: EM pigs presented reduced tenderness compared with SC, which is consistent with their reduced IMF and water-holding capacity. Overall, in regard to the effect of feed restriction, no consequence for meat quality was observed in the case of IC pigs, which corroborates the literature reports for other categories of pigs (see the review of Lebreton, 2008). In view of the alternatives to SC, which is presently being questioned in the EU, more data on the immunocastration effect are needed regarding meat quality and possible negative consequences for meat quality (besides boar taint) when raising EM because the published results are rare and inconsistent (Batorek et al., 2012).

### Conclusion

The interest in immunocastration resides in the more boarlike performance of pigs compared with SC. However, after immunization, feed intake is considerably increased, which diminishes the advantages in performance. The restricted feed allowance tested in the present study did not significantly affect performance and carcass composition; however, restrictively fed IC were closer to EM, whereas ad libitum fed IC were closer to SC. Meat quality was also not affected by the restriction; some negative aspects (IMF, drip loss, and tenderness) were only observed for EM compared with SC. Greater incidence of carcass lesions in restricted IC (similar to EM) indicates more aggression before slaughter and can be considered a negative aspect of the restriction. In commercial piggeries where pigs are raised in groups,

it is likely that such problems would be exacerbated because of the social competition during the whole fattening period. For future research, it would be worthwhile to test if energetic dilution of the feed provided ad libitum could help in achieving a positive outcome for the producers without compromising animal welfare.

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### **4.3 Effect of immunocastration in combination with high fat diet on heat production and protein and lipid deposition in male pigs**

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## **Effect of immunocastration in combination with high dietary fat content on heat production and protein and lipid deposition in male pigs<sup>‡</sup>**

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<sup>‡</sup> *Preliminary results have been partly presented at the 21<sup>st</sup> International Scientific Symposium on Nutrition of Farm Animals Zadravčevi-Erjavčevi dnevi 2012 in Radenci, Slovenia and at 4<sup>th</sup> EAAP International Symposium on Energy and Protein Metabolism and Nutrition in Sacramento, California, USA.*

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### **Running head**

Energy metabolism in immunocastrated pigs

### **Abstract**

In immunocastrated (IC) pigs, revaccination (V2) increases lipid deposition (LD) because of increased voluntary feed intake; but little is known on associated effect of

diet composition on partitioning of nutrients in IC pigs. Digestibility measurements and nitrogen and energy balances in respiration chambers were performed in two subsequent stages, prior (stage 1) and after (stage 2) V2 with Improvac<sup>®</sup>, in four replicates of two male littermates to determine the effect of IC/growth and increased dietary fat content on energy utilization between 85 (stage 1) and 135 (stage 2) kg live weight. During stage 1, pigs received a standard low fat diet (LF; 2.5% DM of fat fed at 2.27 MJ ME/kg BW<sup>0.60</sup> per day) whereas, during stage 2, feed intake was increased to 2.47 MJ ME/kg BW<sup>0.60</sup> per day and one littermate was fed the LF diet whereas the second received a fat enriched diet (HF; 8.9% DM of fat). Results from nitrogen balance and measurements of gas exchanges were used to calculate respiratory quotient (RQ), heat production (HP), nutrients contribution to fat retention, components of HP, and protein (PD) and lipid deposition (LD). Nutrients and energy apparent digestibility coefficients, methane losses and nitrogen retention ( $P < 0.05$ ) increased with stage. Despite higher ME intake, total HP remained similar (1365 kJ/kg of BW<sup>0.60</sup> per day;  $P = 0.26$ ) and fasting HP tended to decrease (823 to 697 kJ/kg of BW<sup>0.60</sup> per day;  $P = 0.07$ ) between stages. Consequently, total retained energy (RE) increased with stage (from 916 to 1078 kJ/kg of BW<sup>0.60</sup> per day;  $P < 0.01$ ) with a higher fat retention (625 to 807 kJ/kg BW<sup>0.60</sup> per day,  $P < 0.01$ ) originating mainly from carbohydrates associated with a higher lipogenesis (536 to 746 kJ/kg BW<sup>0.60</sup> per day;  $P < 0.01$ ) and RQ (1.095 to 1.145;  $P < 0.01$ ). Both PD (from 178 to 217 g/day;  $P = 0.04$ ) and LD (from 227 to 384 g/day;  $P < 0.01$ ) increased due to growth/IC. Feeding HF diet after IC was associated with increased crude fat digestibility ( $P < 0.01$ ) and increased RE as fat (807 to 914 kJ/kg BW<sup>0.60</sup> per day,  $P = 0.05$ ), originating mainly from dietary fat ( $P < 0.01$ ) and resulting in increased LD (384 to 435 g/day,  $P = 0.02$ ) and lower RQ (from 1.145 to 1.073,  $P < 0.01$ ). Altogether, present results indicate that increased fatness of IC pigs is a result of increased daily LD caused by higher energy intake, lower basal metabolic rate and improved energy retention. Additionally, LD is further enhanced by dietary energy enrichment with fat after V2.

**Key words:** pig, immunocastration, heat production, dietary fat, lipid metabolism

### **Implications**

Higher lipid deposition rather than decreased protein deposition seems to be the origin of increased fatness and reduced feed efficiency of immunocastrated pigs as compared to entire males. After immunocastration, dietary energy content should be reduced in order to conserve the advantage of entire males for lean meat percentage. Additionally, dietary crude protein content could be decreased after immunocastration to reduce N emissions.

### **Introduction**

Surgical castration without pain relief, traditionally used in fattening male pigs to prevent development of boar taint, is being questioned from an ethical, welfare and also economical point of view (Bonneau, 1998). In order to replace current practice, immunocastration (**IC**) uses the natural immune system of the pig, stimulated by active vaccination, to produce antibodies against gonadotropin releasing hormone (**GnRH**) that blocks hypothalamo-pituitary-gonadal axis and further sexual development (Caraty and Bonneau, 1986). After administering two doses of vaccine, containing an incomplete analogue of GnRH conjugated to a carrier protein, at least four weeks apart, testicular steroids drop sharply and physiological effects of IC are expressed in the week following revaccination (**V2**) (Claus *et al.*, 2007). This transition from entire male (**EM**) to castrate-like status after IC, leads to major changes in the performances of the pigs. As demonstrated in recently published meta-analyses (Batorek *et al.*, 2012; Dunshea *at al.*, 2013), the most pronounced effect is an increase in feed intake associated with a faster growth, increased lipid deposition (**LD**) and reduced feed efficiency. This would indicate that IC does not affect daily protein deposition (**PD**), but only increases daily LD. Therefore, the objective of the present study was to modify lipid metabolism of male pigs from two perspectives i) to evaluate the changes following IC (due to combined effects of IC, age and increased

feed intake of immunocastrates), and ii) to test if a high lipid diet further stimulates lipid deposition in immunocastrates. For that purpose, nitrogen and energy balance techniques were used to determine the partitioning of retained energy (**RE**) between PD and LD, on the same pigs before and after revaccination with Improvac<sup>®</sup> (Zoetis Florham Park, NJ, USA).

## Materials and methods

The experiment was supervised by E. Labussière (authorization no. 35-110) and performed in accordance with French laws on animal experimentations.

### *Experimental design*

Eight crossbred male pigs (Piétrain x (Large White x Landrace)) from INRA experimental herd (Saint-Gilles, France) were used in the study. The experiment was designed to evaluate the effect of IC in combination with two dietary lipid contents on PD, LD and heat production (**HP**) in male pigs. The study was carried out in four replicates, each composed of two littermates which were divided in Groups 1 and 2 to be fed low fat (**LF**) or high fat diet (**HF**) after IC, respectively (Figure 1). The measurements were performed in two successive stages: before (stage 1) and after V2 (i.e. effective IC; stage 2) with Improvac<sup>®</sup> (Zoetis Florham Park, NJ, USA). Each stage consisted of an adaptation period (3-5 weeks) followed by 9 days of nitrogen and energy balance measurements. At the end of each measurement period, pigs were fasted for one day in order to measure their fasting HP. Individual measurements of littermates were recorded simultaneously in two 12 m<sup>3</sup> open-circuit respiration chambers described by van Milgen *et al.* (1997). In the respiration chamber, each pig was kept in an individual metabolic cage; the trough was placed on a load cell in order to measure the time, size and duration of each meal and water was freely available from low-pressure water nipple drinkers. The cage was additionally equipped with force sensors to record physical activity of the pig (Quiniou *et al.*, 2001). The temperature (24°C) and relative humidity (70%) were kept constant and light was switched on between 0800h and 2000h.

Vaccination with Improvac<sup>®</sup> (Zoetis Florham Park, NJ, USA) was performed according to the producer's instructions (2 mL, subcutaneous administration), the first dose being administered at the age of 10 weeks (before the start of experiment) and the second dose at the age of 19 (replicates 1 and 3) or 21 (replicates 2 and 4) weeks. (*i.e.* 4-5 weeks before starting stage 2 measurements).

## INSERT FIGURE 1

### *Experimental diets and feed allowance*

The LF diet was prepared with 0.5% rapeseed oil, resulting in a dietary fat content of 2.8% of dry matter (**DM**), whereas the HF diet was composed of the same basic ingredients but with 8.9% lipid content per DM achieved by addition of corn oil and linseed oil in order to have a C18:3 to C18:2 ratio equal to 0.16 in both diets. During stage 1, all pigs received the LF diet. Thereafter, a seven-day feed transition to the HF diet was applied in group 2. In both stages, pigs were restricted to 90% of predicted *ad libitum* net energy (**NE**) intake for growing pigs (adapted to BW and sex) which was estimated using InraPorc<sup>®</sup> model (van Milgen *et al.*, 2008). Individual daily feed allowances were prepared and feed was offered twice during the adaptation period or four times per day (at 0900, 1200, 1500 and 1800h) during the measurements period. Water was provided *ad libitum*.

## INSERT TABLE 1

### *Measurements and sampling procedures*

Pigs were weighed before entering the respiration chambers and on the morning of the fasting day. During each measurement period, a sample of each diet was collected during the preparation of daily feed allowances for determination of DM content. The samples of each feed were subsequently pooled for each stage and stored at 4°C until further analyses. During the measurement period, feed refusals of



each pig were collected daily, cumulated over the measurement period, and analysed for their DM content. Faeces were collected daily, pooled per pig for the entire measurement period and stored at 4°C. Urine was collected daily in 10 L plastic tanks containing 120 mL of 10% sulphuric acid, weighed each morning and representative samples (2%) were taken, accumulated in plastic bottles and stored at 4°C until further laboratory analyses. At the end of each measurement period, total collected faeces were weighed, homogenised and three representative samples were taken; two of them were used for DM determination and the third sample was freeze-dried, ground and stored at 4°C until further laboratory analyses. To collect ammonia evaporated in the respiration chambers from urine, condensed water from the air conditioning system of the respiration chambers was collected in plastic tanks containing 120 mL of 10% sulphuric acid; a quantified aliquot of the outgoing air was also bubbled in a sulphuric acid solution during the entire balance period. Condensed water and bubbling solution were weighed, analysed for ammonia content and ammonia evaporation was calculated as described by Noblet *et al.* (1987). Feed consumption (using load cell placed under the trough), physical activity (using force sensors and infrared barriers), gas concentrations (CO<sub>2</sub>, O<sub>2</sub> and CH<sub>4</sub>) of outgoing air, ventilation rate and physical characteristics of the air in the respiration chambers (atmospheric pressure, temperature, relative humidity) were measured continuously (60 times/s), averaged over 10 s intervals and recorded for further calculations (van Milgen *et al.*, 1997).

#### *Chemical analyses*

Pooled diet samples of each stage were analysed for DM, ash, nitrogen, starch, crude fibre, crude fat with prior acid hydrolysis and gross energy (**GE**) according to AOAC (1990). The NDF, ADF and ADL contents of pooled diet samples were analysed according to van Soest *et al.* (1991). Samples of faeces were analysed for DM, ash, nitrogen, crude fat with prior acid hydrolysis and GE contents according to the same methods as for diets. Fresh samples of urine were analysed for nitrogen content, whereas GE content was measured after freeze-drying 30 mL of urine in polyethylene

bags. The ammonia contents in bubbling solution and condensed water were determined using an enzymatic method (Enzytec™ fluid Ammonia, Thermo Fisher Scientific Oy, Vantaa, Finland). Assuming a converting factor of 6.25, crude protein content was calculated from nitrogen content.

### *Calculations*

Average daily gain for the balance period was calculated using BW on the morning of the first day in respiration chambers and BW on the morning of the fasting day. Dry matter intake and apparent digestibility coefficients of organic matter, nitrogen, GE and fat were calculated according to standard procedures. Nitrogen retention was calculated as the difference between nitrogen intake and nitrogen losses in faeces and urine (including nitrogen losses as ammonia in condensed water and outgoing air of the respiration chambers). Respiratory quotient was the ratio between CO<sub>2</sub> production and O<sub>2</sub> consumption. Total HP was calculated according to Brouwer (1965) from O<sub>2</sub> consumption, CO<sub>2</sub> and CH<sub>4</sub> production, and urinary nitrogen losses calculated as the sum of nitrogen recovered in the urine and evaporated as ammonia. The first day in the respiration chambers was considered as an adaptation day and was not included in the calculation of HP and nutrient balance. Total RE during the balance period was calculated as the difference between GE intake and energy losses in faeces, urine, methane and HP. With regard to RE as protein, it was calculated from nitrogen balance taking into account that energy in PD (nitrogen retention × 6.25) equals 23.6 kJ/g. Energy retained as LD was calculated as the difference between total RE and RE as protein; LD (g) calculation assumed that the energy content of LD equals 39.7 kJ/g. Oxidation of nutrients and their contribution to LD were calculated according to Chwalibog *et al.* (1992) from nitrogen excreted in urine (oxidation of proteins) or from gas exchange measurements (oxidation of carbohydrate and fat). The values obtained for the oxidation of carbohydrates and fat were interpreted as “apparent values”, corrected for apparent oxidation of lipids, because the respiratory quotient unrelated to protein oxidation was above 1.0, which caused oxidation of fat to be negative. The retained energy as fat originating from

dietary carbohydrates, proteins and fat was expressed in % of total LD and subsequently recalculated in kJ/kg of  $BW^{0.60}$  per day. The model of van Milgen *et al.* (1997) was used to calculate the components of HP using data from gas exchange, signals of force sensors and feed intake (time and ingested quantity of each meal) in respiration chambers. The HP due to physical activity, feed intake (short-term thermic effect of feeding), resting metabolism and fasting HP were calculated according to the formula of Brouwer (1965) from the respective volumes of  $O_2$  and  $CO_2$  which were determined by mathematical modelling of gas exchanges. The difference between HP due to resting and fasting was considered as long-term thermic effect of feeding. Total thermic effect of feeding was calculated as the sum of short- and long-term thermic effect of feeding. Additionally, HP and its components were corrected for differences in physical activity among animals in each replicate (assuming constant ratio between activity HP and ME throughout the experiment) as described by Labussière *et al.* (2011). Net energy was calculated as the sum of total RE and fasting HP. All energy measurements were expressed relative to  $BW^{0.60}$  (Noblet *et al.*, 1999).

#### *Statistical analyses*

Analysis of variance was performed using mixed model procedure of SAS (PROC MIXED, SAS Inst., Cary NC, USA) on repeated measurements of nitrogen and energy balances, respiratory quotient, nutrient deposition and BW gain composition data (n=16) with nested design including fixed effects of stage and group within stage and random effect of pig (included in the repeated statement). To evaluate the IC/growth effect (IC confounded with age and weight i.e. growth), the pigs on LF diet were compared in stage 1 and 2 (group1 in stage 1 was contrasted to group 1 in stage 2). A contrasts analysis was also used for evaluating the effect of HF diet by comparing group 1 and group 2 in stage 2. It is worth noting that, due to the absence of any notable difference between groups 1 and 2 in stage 1, the results (least squares means) for this stage were presented as their averaged value.

## Results

There were no major difficulties during the experiment. The chemical composition of the diets corroborated with the predicted one (Table 1). Experimental pigs increased their BW ( $P < 0.01$ ) over the experiment (i.e. with stage), but their ADG during the balance periods did not differ between stages and diets and it averaged 1.40 kg/day (Table 2). According to the protocol, DM intake increased with stage ( $P < 0.01$ ), from 2196 g/day in stage 1 to 3060 and 2848 g/day for group 1 (LF diet) and group 2 (HF diet) in stage 2, respectively (Table 2).

### *Digestive utilization of nutrients and energy*

Apparent digestibility coefficients of nutrients and GE were higher in stage 2 ( $P < 0.01$ ; Table 2). As demonstrated by contrasts analysis, in the case of DM, organic matter, GE and crude protein, this increase due to IC/growth was in the range of 1.4 to 2.7 points ( $P \leq 0.02$ ). On the other hand, the difference in apparent digestibility of crude fat was not significant for IC/growth effect (4.4 points;  $P = 0.15$ ). Although methane emissions and subsequently energy loss as methane increased markedly with stage due to IC/growth (5.2 vs 10.3 L/day and 0.60 vs 0.84 % of DE in stage 1 and stage 2, respectively;  $P < 0.01$ ), ME/DE ratio averaged 95.6 % and only tended to be lower due to IC/growth effect (-0.7 point;  $P = 0.08$ ).

Within stage 2, diet affected only crude fat digestibility (increase of 18 points;  $P < 0.01$ ; Table 2), whereas no effect of diet ( $P > 0.10$ ) was noted, neither on the digestibility coefficients of DM, organic matter, GE or crude protein, nor on methane emissions or ME/DE ratio.

## INSERT TABLE 2

### *Nitrogen and energy balance*

An effect of IC/growth was noted on nitrogen balance data, resulting in higher nitrogen retention (28.5 vs 34.8 g/day in stages 1 and 2;  $P = 0.04$ ); however, the

relative nitrogen retention in stage 2 was lower (52.7 vs 43.0 % of digested nitrogen in stages 1 and 2;  $P=0.10$ ).

In agreement with the design of the study, the ME intake increased from 2274 to 2450 kJ/kg of  $BW^{0.60}$  per day between stages 1 and 2 in order to mimic IC/growth effect ( $P<0.01$ ). On the other hand, NE intake did not differ between stage and group and it averaged 1757 kJ/kg of  $BW^{0.60}$  per day in stage 1 and 2 ( $P>0.05$ ). Despite the difference in ME intake, total HP remained similar in both stages (1365 kJ/kg of  $BW^{0.60}$  per day;  $P=0.26$  for IC/growth effect) in connection with a lower fasting HP that counterbalanced the higher thermic effect of feeding. As shown by contrasts analysis, fasting HP tended to decrease (from 823 to 697 kJ/kg  $BW^{0.60}$  per day between stages 1 and 2;  $P=0.07$ ), whereas total thermic effect of feeding increased (from 311 to 423 kJ/kg  $BW^{0.60}$  per day between stages 1 and 2;  $P=0.05$ ). The total thermic effect of feeding expressed as % of ME was similar regardless of IC/growth effect (15.5 % of ME;  $P=0.12$ ) and the HP related to physical activity was also unchanged between stages (8-9% of ME intake). As a consequence, total RE increased with stage due to IC/growth (from 916 to 1078 kJ/kg of  $BW^{0.60}$  per day, respectively;  $P<0.01$ ) that benefited exclusively to a higher RE as fat (from 625 to 807 kJ/kg  $BW^{0.60}$  per day, respectively;  $P<0.01$ ); RE as protein averaged 281 kJ/kg  $BW^{0.60}$  per day in stages 1 and 2 ( $P>0.10$ ). The origin of RE as fat increase with IC/growth was mainly from carbohydrates (from 489 to 671 kJ/kg  $BW^{0.60}$  per day;  $P<0.01$ ) and to a much lower extent from proteins (from 50 to 68 kJ/kg  $BW^{0.60}$  per day;  $P<0.01$ ). Accordingly, an increase with stage due to IC/growth was noted for lipogenesis (from 536 to 746 kJ/kg  $BW^{0.60}$  per day;  $P<0.01$ ) and the associated respiratory quotient (from 1.095 to 1.145 L  $CO_2$ / L  $O_2$ ;  $P<0.01$ ) as shown by contrasts.

Neither ME intake nor NE intake were influenced by dietary treatment within stage 2 (average of 2472 and 1826 kJ/kg  $BW^{0.60}$  per day, respectively;  $P>0.10$ ), whereas the HF diet tended to reduce total HP (from 1372 to 1327 kJ/kg  $BW^{0.60}$  per day;  $P=0.08$ ) and then to increase total RE (from 1078 to 1165 kJ/kg  $BW^{0.60}$  per day;  $P=0.06$ ) and RE as fat (from 807 to 914 kJ/kg  $BW^{0.60}$  per day;  $P=0.05$ ). The higher RE as fat

originated mainly from deposition of dietary fat (67 vs 224 for LF and HF diet;  $P < 0.01$ ). Consequently, lipogenesis was not altered by dietary treatment within stage 2 (average of 714 kJ/kg BW<sup>0.60</sup> per day;  $P = 0.24$ ), whereas respiratory quotient decreased in the HF diet (from 1.145 to 1.073 L CO<sub>2</sub>/ L O<sub>2</sub>;  $P < 0.01$ ). Finally, the components of HP were not affected by diet composition in stage 2, except a numerically lower thermic effect of feeding with the HF diet.

### **INSERT TABLE 3**

#### *Components of BW gain*

Both protein gain (from 178 to 217 g/day;  $P = 0.04$ ) and fat gain (from 227 to 384 g/day;  $P < 0.01$ ) increased due to IC/growth, but the difference in nutrients deposition resulted in a slightly increased fat/protein ratio in BW gain (1.29 vs 1.80 in stages 1 and 2, respectively;  $P = 0.10$ ). Protein gain relative to BW was not affected by IC/growth ( $P = 0.17$ ), whereas fat and energy gain increased with IC/growth (from 164 to 281 g and from 9.5 to 14.9 MJ/kg, respectively;  $P < 0.05$ ). Feeding HF diet within stage 2 increased fat gain (from 384 to 435 g/day;  $P = 0.02$ ) and tended to increase fat/protein ratio (from 1.80 to 2.19 g/g;  $P < 0.13$ ), whereas BW gain composition (in kg of gain) was not altered by dietary treatment ( $P > 0.10$ ).

### **INSERT TABLE 4**

#### **Discussion**

In the course of the experiment (with BW gain i.e. growth), digestive utilization of nutrients and GE increased. This agrees with the literature data showing continuous and linear increase of digestibility coefficients with BW (Le Goff and Noblet, 2001; Noblet *et al.*, 2013) as a consequence of better degradation and absorption of nutrients, especially dietary fibre fractions, in the hindgut of heavier or more mature pigs (Noblet and Shi, 1993). Higher apparent digestibility coefficient of crude fat

observed on fat enriched diet in the present study also fully agrees with the literature data (Le Goff and Noblet, 2001) and, according to Shi and Noblet (1994), can be attributed to the dilution of the inevitable endogenous fat faecal losses by higher amounts of undigested dietary fat. According to previous results (Noblet and Shi, 1993), the higher methane losses (% DE) in the present study associated with IC/growth effect are caused by a lower rate of passage due to a larger volume of hindgut (Noblet and Le Goff, 2001). Nevertheless, this only contributes to a low extent of the decreased ME/DE ratio with stage (i.e. IC/growth) in the present study (only for 20% of the difference). At a given stage of growth (or production), the ratio ME/DE is linearly related to dietary protein content (Noblet and Perez, 1993) and reflects the variation of nitrogen excretion in the urines with variation of nitrogen intake. Similarly, the decreased ME/DE ratio with IC/growth is related to increase feed intake and apparent digestibility coefficients of crude protein that resulted in higher nitrogen absorption whereas PD remained constant. The exceeding nitrogen was then oxidized, increasing urinary nitrogen excretion thus urinary energy (Noblet, 2007). Moreover, higher nitrogen losses with urine observed in the present study due to IC/growth effect as a consequence of higher feed (and nitrogen) intake at similar daily nitrogen gain indicate that daily nitrogen or protein content in the diet should be reduced with growth and IC. In agreement, plasma urea nitrogen concentrations was shown to increase in IC pigs within the first week after V2 (Bauer *et al.*, 2009; Claus *et al.*, 2007) or shortly after (Huber *et al.*, 2013) indicating increased amino acid catabolism. These results confirm that crude protein content of the diet for IC could be decreased after V2 in order to reduce unnecessary nitrogen emissions. Similar recommendations were given by Dunshea *et al.* (2013) based on a meta-analysis.

In the present experiment, ME intake (per kg metabolic BW) was increased in stage 2 to mimic IC effect, but it was not accompanied by any difference in total HP. Likewise, higher ME intake at stage 2 was not associated with an increased fasting HP as observed by de Lange *et al.* (2006) in pigs of a given BW. A decrease in fasting HP was even noted, which illustrates the important changes in the physiology and

metabolism due to IC. Higher body fatness (van Milgen *et al.*, 1998) and changed hormonal status after IC (castrate vs. male, Labussière *et al.*, 2013) have both been associated with decreased basal metabolic rate and might explain the lower fasting HP after IC. In the present study, there was also no effect of IC/growth on total thermic effect of feeding (as % ME). Consequently, energy gain after V2 was more pronounced than expected from ME intake change only. Nevertheless, Labussière *et al.* (2013) have shown that IC pigs have higher total thermic effect of feeding than EM, due to higher feed intake and because more dietary protein is used as a source of energy with a low energetic efficiency for lipogenesis (van Milgen *et al.*, 2001). The latter may have been counterbalanced by the age related effect on thermic effect of feeding (Barea *et al.*, 2010)

In addition to the stimulation of fat metabolism by IC, the effect of a dietary stimulation of fat metabolism was investigated in the present study. The increased dietary fat after V2 additionally stimulated retention of energy in the form of fat. However, the contribution of lipogenesis to retained energy as fat was reduced, because additional dietary fat was deposited in carcass as such without oxidation of dietary fat and *de novo* synthesis (Chwalibog *et al.*, 1992; Bruininx *et al.*, 2011). Indeed, a lower intensity of lipogenesis from carbohydrate in pigs consuming HF diet was confirmed by a reduced respiratory quotient in the present study. Moreover, additional fat ingested in pigs on HF diet was associated with a numerical decrease of thermic effect of feeding (% ME). However, this reduction is lower than the one observed by van Milgen *et al.* (2001) using barrows (0.017% vs 0.024% per g of digested fat, respectively). In view of the presented results, the feeding strategy of IC pigs should be based on energy limitation after V2.

In the present study, PD and LD were increased in IC pigs after V2. However, it is difficult to discriminate between IC and growth effect. Comparing to the literature, it can be hypothesized that PD was likely increased due to growth, but not affected by IC, as Labussière *et al.* (2013) showed similar PD in IC and EM pigs five weeks after V2 (and superior to barrows). On the other hand, LD seems to be increased not only



with growth, but also due to IC. Moreover, decrease in fasting HP following IC, shown in the present study, emphasizes the effects of increased feed intake on fat gain in IC pigs. The increase in lipid content of BW gain after IC is consistent with reported differences in carcass composition between IC and EM pigs (thicker backfat resulting in lower carcass lean meat %; Batorek *et al.*, 2012). Moreover, the increased dietary fat after V2 was associated with a considerable increase in daily LD, which fully agrees with previous studies in barrows (van Milgen *et al.*, 2001, Bruininx *et al.*, 2011). However, it is worth noting that our results point out that the increased fatness of IC pigs is a result of increased daily LD caused by higher energy intake, lower basal metabolic rate and improved energy retention and not by decreased daily PD. Consequently, due to increased LD stimulated by higher feed intake and an increased protein catabolism with low efficiency, IC pigs are less feed efficient than EM. However, if dietary composition after IC would be optimized in energy and protein content, feed efficiency might be improved substantially.

In conclusion, the energy balance results indicate that the dynamics of HP, assumed to be the major factor contributing to the differences in feed efficiency among pigs, is changed after IC. Additional energy retained because of higher feed intake is directed towards LD. Increased fatness of IC pigs is a result of increased daily LD caused by higher energy intake, lower basal metabolic rate and improved energy retention. Hence, dietary energy intake should be limited after IC. Moreover, according to the results of the nitrogen balance study, dietary crude protein content could be decreased after IC. However, the patterns of tissue deposition rates following changes in energy metabolism after IC should be further explored in order to establish exact nutrient requirements of IC pigs and develop a feeding strategy which would allow maximizing their growth potential and minimizing the nitrogen losses.

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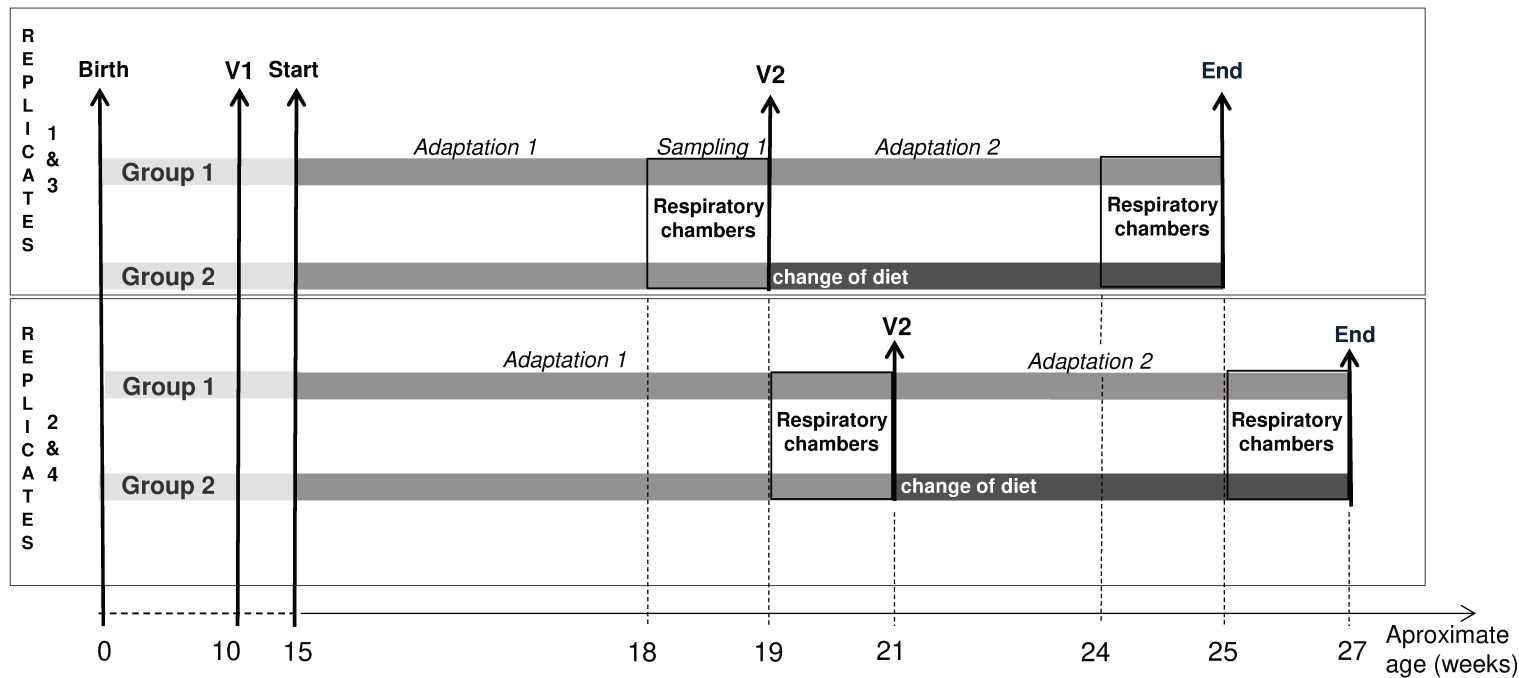
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**Figure 1** Experimental design showing start and end of experiment performed in four replicates, divided in two adaptation periods (Adaptation 1 and Adaptation 2) each followed by measurement period in respiratory chambers (Sampling 1 and Sampling 2), first (V1) and second (V2) vaccination with Improvac<sup>®</sup>, two experimental groups (Group 1 and Group 2) and change of diet in Group 2 after V2.



**Table 1** *Composition of experimental diets fed to male pigs before and after the second vaccination for immunocastration*<sup>1</sup>.

	Low fat diet	High fat diet
Ingredients (%)		
Wheat	35.72	29.86
Barley	35.72	29.86
Wheat bran	3.50	3.50
Soybean meal	19.40	25.19
Cane molasses	2.50	2.50
Rapeseed oil	0.50	0.50
Corn oil	-	5.20
Linseed oil	-	0.80
L-Lysine-HCL	0.12	0.07
L-Threonine	0.02	0.01
DL-Methionine	0.02	0.04
Sodium chloride	0.45	0.45
Calcium carbonate	0.62	0.58
Bicalcium phosphate	0.94	0.94
Minerals and vitamins <sup>2</sup>	0.50	0.50
Chemical composition (% DM) <sup>3</sup>		
Crude protein (N x 6.25)	19.7	21.7
Fat	2.8	8.9
Ash	5.7	6.0
Starch	47.0	39.0
Crude fiber	4.1	4.1
Neutral detergent fiber	15.6	13.7
Acid detergent fiber	5.4	4.8
Acid detergent lignin	0.7	0.3
Gross energy (MJ/kg of DM)	18.06	19.50



Calculated composition<sup>4</sup>

Digestible energy (MJ/kg of DM)	13.53	14.70
Metabolizable energy (MJ/kg of DM)	12.943	14.09
Net energy (MJ/kg of DM)	9.55	10.56
SID Lys (g/MJ NE)	0.85	0.83

DM = dry matter; SID Lys= standardized ileal digestible lysine.

<sup>1</sup> See text for details.

<sup>2</sup> The minerals and vitamins mixture provided the following (/kg of diet): Vitamin A 5000 UI; vitamin D3 1000 UI; vitamin E 20 UI; vitamin B1 2 mg; vitamin B2 4 mg; pantothenic acid 10 mg; vitamin B6 1 mg; vitamin B12 0.02 mg; niacin 15 mg; vitamin K3 2 mg; folic acid 1 mg; biotin 0.2 mg; choline chloride 500 mg; iron 80 mg; copper 10 mg; zinc 100 mg; magnesium 40 mg; cobalt 0.1 mg; iodine 0.2 mg; selenium 0.15 mg.

<sup>3</sup> As measured.

<sup>4</sup> Values were calculated according to Sauvant *et al.* (2004) and subsequently adjusted for 89% DM content of the diet.



Feces	8.5	10.0	9.0	0.8	0.05	0.41	0.05	0.22
Urine <sup>6</sup>	25.5	46.1	49.9	3.5	<0.01	0.26	<0.01	0.20
N retention	28.5	34.8	32.3	3.9	0.05	0.44	0.04	0.42
N retention (% of digested N)	52.7	43.0	39.3	4.9	<0.01	0.26	0.10	0.33

r.s.d. = residual standard deviation; DM = dry matter; DE = digestible energy; ME = metabolizable energy.

<sup>1</sup> LF diet=low fat diet; HF diet=high fat diet; for diet composition, see Table 1.

<sup>2</sup> Values were tested for effects of stage and group within stage (group(stage)); results are least squares means, the measurement was considered repeated per stage. Due to the absence of significant difference between groups 1 and 2 in stage 1, the LS means for this stage are presented as their averaged value.

<sup>3</sup> IC+growth= effect evaluated as the difference between group 1 in stage 1 and group 1 in stage 2; Diet= effect evaluated as the difference between group 1 and group 2 in stage 2.

<sup>4</sup> Average of the BW taken in the morning of the first day and the morning of the fasting day.

<sup>5</sup> According to experimental design, the DM intake increased between stage 1 and 2 to mimic the increase in voluntary feed intake after second vaccination reported for immunocastrated pigs.

<sup>6</sup> Including ammonia evaporation.

**Table 3:** Effects of stage and experimental group of pigs on energy metabolism in male pigs prior (stage 1; 7 days) and after (stage 2; 7 days) second vaccination with *Improvac*<sup>®</sup> according to dietary fat level.

	Stage 1 <sup>2</sup>		Stage 2		r.s.d.	Significance <sup>2</sup>		Contrast <sup>3</sup>	
	Groups 1 & 2		Group 1	Group 2		Stage	Group (Stage)	IC+growth	Diet
	LF diet		LF diet	HF diet					
Number of pigs used	8		4	4		8	8	4	4
Mean BW <sup>4</sup>	85.9		135.7	134.4	2.4	<0.01	0.79	<0.01	0.81
Energy balance (kJ/kg of BW <sup>0.60</sup> per day)									
ME intake	2274		2450	2493	19	<0.01	0.18	<0.01	0.14
NE intake <sup>5</sup>	1739		1775	1877	80	0.08	0.37	0.58	0.21
Heat production									
Fasting HP	823		697	712	73	0.02	0.98	0.07	0.86
Activity HP	212		197	201	20	0.25	0.95	0.31	0.86
Corrected activity HP <sup>6</sup>	198		213	217	—	—	—	—	—
Thermic effect of feeding									
Short-term	206		249	217	28	0.11	0.14	0.14	0.12
Long-term	105		174	153	79	0.20	0.82	0.20	0.74
Total	311		423	371	65	0.05	0.60	0.05	0.41
Total	1358		1372	1327	17	0.34	0.02	0.26	0.08
Retained energy									
As protein	291		271	251	32	0.12	0.35	0.84	0.37
As fat <sup>7</sup>									

from dietary carbohydrates	489	671	615	33	<0.01	0.50	<0.01	0.26
from dietary proteins	50	68	76	5	<0.01	0.13	<0.01	0.12
from dietary fat	86	67	224	16	<0.01	<0.01	0.14	<0.01
Total	625	807	914	30	<0.01	<0.01	<0.01	0.05
Total	916	1078	1165	20	<0.01	<0.01	<0.01	0.06
Lipogenesis	536	746	681	39	<0.01	0.47	<0.01	0.24
Respiratory quotient (L CO <sub>2</sub> /L O <sub>2</sub> )	1.095	1.145	1.073	0.002	0.15	0.01	<0.01	<0.01
Dietary energy content (MJ/kg DM)								
Metabolizable energy	14.9	15.1	16.5	0.1	<0.01	<0.01	0.08	<0.01
Net energy	11.4	11.0	12.5	0.5	0.32	0.03	0.24	0.01
Total thermic effect of feeding (% ME)	13.7	17.3	14.9	2.9	0.16	0.58	0.12	0.39

r.s.d.= residual standard deviation; ME = metabolizable energy; NE = net energy.

<sup>1</sup> LF diet=low fat diet; HF diet=high fat diet; for diet composition see Table 1.

<sup>2</sup> Values were tested for effects of stage and group within stage (group(stage)); results are least squares means, the measurement was considered repeated per stage. Due to the absence of significant difference between groups 1 and 2 in stage 1, the LS means for this stage are presented as their averaged value.

<sup>3</sup> IC+growth= effect evaluated as the difference between group 1 in stage 2 and group 1 in stage 1; Diet= effect evaluated as the difference between group 2 and group 1 in stage 2.

<sup>4</sup> Mean BW during the entire nitrogen and energy balance period.

<sup>5</sup> NE was calculated as the sum of total retained energy and fasting HP.

<sup>6</sup> Corrected activity HP = activity HP corrected for the difference in physical activity among animals (assuming a constant 8.7 % AHP:ME through experiment) according to Labussière *et al.* (2011).

<sup>7</sup> Oxidation of nutrients and their contribution to lipid deposition were calculated according to Chwalibog *et al.* 1992.

**Table 4** Effects of stage and experimental group of pigs on BW, protein, fat and energy gain in male pigs prior (stage 1; 7 days) and after (stage 2; 7 days) second vaccination with *Improvac*<sup>®</sup> according to dietary fat level.

	Stage 1 <sup>2</sup>		Stage 2		r.s.d.	Significance <sup>2</sup>		Contrast <sup>3</sup>	
	Groups 1 & 2		Group 1	Group 2		Stage	Group (Stage)	IC+group	Diet
	LF diet		LF diet	HF diet					
Number of pigs used	8		4	4		8	8	4	4
Components of BW gain (g/day)									
Protein	178		217	202	24	0.05	0.44	0.04	0.42
Fat	227		384	435	14	<0.01	0.01	<0.01	0.02
Fat/Protein (g/g)	1.29		1.80	2.19	0.28	<0.01	0.18	0.10	0.13
BW gain composition (/kg gain)									
Protein (g)	128		158	150	29	0.14	0.87	0.17	0.68
Fat (g)	164		281	323	46	<0.01	0.42	0.02	0.28
Energy (MJ)	9.5		14.9	16.4	2.4	<0.01	0.64	0.03	0.41

r.s.d. = residual standard deviation.

<sup>1</sup> LF diet=low fat diet; HF diet=high fat diet; for diet composition see Table 1.

<sup>2</sup> Values were tested for effects of stage and group within stage (group(stage)); results are least squares means, the measurement was considered repeated per stage. Due to the absence of significant difference between groups 1 and 2 in stage 1, the LS means for this stage are presented as their averaged value.

<sup>3</sup> IC+growth= effect evaluated as the difference between group 1 in stage 2 and group 1 in stage 1; Diet= effect evaluated as the difference between group 2 and group 1 in stage 2.



#### **4.4 Effect of net energy concentration of feed on feed intake, performance, carcass and meat quality traits in growing-finishing immunocastrated pigs housed individually**

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## Energy restriction in immunocastrated pigs

### **Effect of net energy concentration of feed on growth performance and carcass and meat quality in growing-finishing immunocastrated pigs housed individually<sup>1,2</sup>**

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**ABSTRACT:** Immunocastration offers the opportunity to benefit from long term boar-like performance of pigs, with reduced boar taint at slaughter. However, feed intake is considerably increased after the full immunization (V2), which diminishes the advantages in performance. An experiment was performed to evaluate the effects of decreasing NE concentration of feed in 42 immunocastrated pigs (IM) slaughtered 8 wk after V2 on growth performance, carcass and meat quality traits with emphasis on fat deposition before (P1) and after V2 at 112 days of age (P2), and in first and second 4-wk following V2. Pigs were divided in 3 groups and were fed experimental diets with low, medium or high NE content (LNE, MNE and HNE diets; 8.5, 9.3 and 10.0 MJ NE/kg, respectively) from 84 d of age onwards. Additionally, a digestibility trial was performed in order to accurately calculate the NE content of 3 diets using 4 pigs that received successively LNE and HNE extreme diets. Diet composition had no effect on ADFI in any period of the experiment ( $P>0.10$ ), whereas higher NE intake in mega joule per day ( $P=0.08$ ) was noted in the second 4-wk phase after V2 (d 143 to d 170) for pigs receiving HNE diet. There was no effect ( $P>0.10$ ) of the reduction of NE concentration of the diet on ADG or gain to NE intake ratio (G:NE) in any stage of experiment. A difference in backfat gain was observed only in the second phase after V2 (d 143 to d 170) as higher ( $P<0.01$ ) backfat gain in HNE group. In agreement with the absence of diet effect on BW, no effect ( $P>0.10$ ) was noted on HCW, dressing percentage or prime carcass cuts. The differences among treatment groups were noted on the carcass as lower backfat (as the percentage of HCW;  $P=0.01$ ) and as area of fat layer over LD ( $P=0.05$ ) in LNE and MNE pigs. Additionally higher lean content ( $P=0.04$ ) of LNE pigs was observed. Intermuscular and LD i.m. fat deposition were not affected ( $P>0.05$ ) by reduction of NE concentration of feed. No effect of dietary NE concentration was noted on meat quality, with the exception of lighter color score ( $P=0.04$ ) of LD muscle in the HNE group. Overall, present results demonstrate that reducing NE concentration of the diet up to 10 % with higher fiber content ingredients did not affect growth performance and energy efficiency (G:NE) but reduced lipid deposition in IM pigs after full immunization. This is particularly important when IM pigs are fattened to higher weights i.e. when longer delays between V2 and slaughter are practiced.

**Key words:** pig, immunocastration, net energy, lipid deposition, carcass traits, meat quality

## INTRODUCTION

In most countries, entire males (EM) are surgically castrated shortly after birth to prevent meat boar taint. As an alternative, vaccination against GnRH (i.e. immunocastration) has been proposed. Using standard vaccination protocol, physiological effects of immunocastration are expressed in week after revaccination (V2; Claus et al., 2007), allowing benefiting from EM advantages for lean tissue growth and feed efficiency (Millet et al., 2011) for most of the fattening period. However, after V2, a substantial increase in ADFI is observed in immunocastrated pigs (IM), resulting in a decrease in G:F ratio and an increase in body fatness in comparison to EM (Batorek et al., 2012a). Both negative consequences can be limited either by restricting feed allowance to IM after V2 (Batorek et al., 2012b) or shortening the delay between V2 and slaughter, giving the pigs less time to deposit fat (Lealiifno et al., 2011). Quantitative feed restriction in IM after V2 has been shown to improve performance (Batorek et al., 2012b), but can increase aggression in restricted animals (Quiniou et al., 2012), raising questions of welfare degradation. As an alternative, reduced dietary energy content in feed without limitation of allowance may be applied. Because energy component of feed represents the major cost in pork production (Noblet et al., 1994), replacing energy rich starch with relatively inexpensive fibrous feedstuffs, such as cereal by-products might be interesting in finishing diets for IM pigs. As a longer delay between V2 and slaughter can emerge in some market situations, limiting fat deposition may also become economically important. Hence, the objective of this study was to evaluate the effects of reduction of NE concentration of feed in IM on growth performance, carcass and meat quality traits with emphasis on fat deposition in 3 successive phases of growth (i.e. before V2 and in first and second 4-weeks following V2).

## MATERIALS AND METHODS

The study was performed in facilities of INRA at Saint Gilles, France from June to September 2012 in accordance with French laws on animal experimentation (permission of E. Labussière for animal experimentation number 35-110). The study included a digestibility and N balance trial (Trial 1) and a growth trial (Trial 2) to compare the utilization of 3 diets differing in their NE content (low, medium or high NE content; LNE, MNE and HNE diets, respectively). Trial 1 was performed in 2 successive periods on each of 4 pigs on the LNE and HNE extreme diets in order to accurately calculate the NE content of the 3 diets. In Trial 2, 45 IM pigs (immunocastrated at the age of 77 and 112 d and slaughtered at the age of 172 d) were divided in 3 experimental groups, each fed an experimental diet with low, medium or high NE content (8.5, 9.3 and 10.0 MJ NE/kg; Table 1).

### *Diets*

For the purpose of the study, 3 wheat, barley and corn based diets differing in NE content were prepared (Table 1). Concentration of NE in feed was reduced from 10.0 MJ/kg in the HNE diet to 9.3 and 8.5 MJ/kg in the MNE and LNE diets, respectively (Table 1).

## **INSERT TABLE 1**

### *Trial 1*

In order to accurately calculate NE content (Noblet et al., 1994), a digestibility and N balance study was performed. Four pigs were immunocastrated using Improvac<sup>®</sup> (2 mL, s.c. application at age of 77 and 105 d, Zoetis Florham Park, NJ, USA) and they were fed the 2 extreme experimental diets during 2 successive periods so that 2 pigs were fed each diet in each period. Each period consisted of an adaptation sub period (14 d, allowing pigs to adapt to feed, digestibility cage and environmental conditions) and a subsequent collection sub period (7 d), when feed intake was recorded and feces and urine were totally collected. Pigs were weighed at the beginning (age of 77 d) of the trial and at the beginning and the end of each collection period. During the collection period, feed allowance was restricted to the level measured individually during the last week of the adaptation period (in gram per kilogram of

BW<sup>0.60</sup>). Water was freely available. Feed was prepared daily and distributed 3-times per d (at 0900, 1200 and 1500 h), whereas feed refusals were collected each morning and stored at 4°C until analysis of DM (24 h in ventilated oven at 103°C). A representative sample of each diet was taken during the collection period and stored at 4°C until further laboratory analyses. During the collection period, feces and urine (collection in a 10 L plastic container containing 120 mL of 10% sulfuric acid) were collected each morning. Feces were stored at 4°C and accumulated during the collection period. On the last day of collection, total collected feces were weighed and homogenized. Three representative samples were taken and 2 of them were dried in oven at 100°C during 48 h for DM determination whereas the third sample was freeze-dried, ground (1 mm-grind) and stored at 4°C until further laboratory analyses. The containers for urine collection were replaced each morning, the pH was measured and, if necessary, adjusted by adding 10% sulfuric acid so that it was inferior to 2.0. Afterwards, urine was weighed, homogenized and a representative sample (1%) was taken, cumulated over the entire collection period and stored at 4°C.

### ***Trial 2***

At the age of 70 d, 45 pigs were transferred to the experimental stables (individual housing, concrete floor, water freely available) and fed a commercial fattening diet. Two vaccinations with Improvac<sup>®</sup> (2 mL, s.c. application, Zoetis Florham Park, NJ, USA) were performed at the age of 77 and 112 d. At the age of 84 d, pigs were allocated within litter origin to 3 treatment groups, HNE (N=15), MNE (N=15) and LNE (N=15) and they received experimental diets with a progressive transition that lasted 5 d.

During the course of experiment, pigs were fed individually 3-times per d at 0900, 1200 and 1500 h. Daily meals were prepared in buckets containing the expected *ad libitum* feed intake for 3 or 4 d plus 10% (calculated according to the INRAPorc software; van Milgen et al., 2008). At the end of each 3 or 4-d period, non-offered feed which remained in the buckets and accumulated feed refusals were weighed. Feed refusals were not contaminated by urine or saliva and therefore, they were not dried and their DM content was considered as for the offered feed, which was measured at each meal preparation.



Pigs were weighed weekly in the morning without any previous limitation of feed allowance. Ultra-sound (Noveko, VetkoPlus, 3.5 MHz) measurements of backfat thickness (BFT) were performed at the level of the last rib (measured on the left and right side of the back, 6 cm lateral to the spine and subsequently averaged per pig) every 3-wk from first vaccination (V1) onwards in order to obtain 5 subsequent measurements on each animal during the course of the experiment. The difference between final (on the day before slaughter) and initial BFT (at V1) divided by the number of d between the 2 measurements was calculated to assess daily BFT gain.

Pigs were slaughtered in 2 batches (balanced according to experimental groups) within 1-wk interval (at average age of 172 d) in the experimental slaughterhouse of INRA Saint-Gilles according to standard slaughter procedure (app. 20 h feed deprivation, electrical stunning and immediate vertical bleeding). At the slaughter line, hot carcass weight (HCW) was recorded. Testes were removed (stored at 4 °C during 5 h), dissected and weighed. Weight of right and left testis (epididymis included) was recorded for each pig. Leaf fat was excised from both half-carcasses and weighed. Carcasses were cooled overnight until the internal carcass temperature dropped below 7°C. On the day following slaughter, additional carcass traits were assessed. Neck i.m. fat (in percentage), loin eye area (in square centimeters) and loin eye fat area (in square centimeters) were assessed at a cross-section between third and fourth cervical vertebra and last rib, respectively, as described by Batorek et al. (2012b). The left half carcasses were additionally divided into prime cuts according to the Dutch normalized procedure (Institut Technique du Porc, 1990) and weights of head, tail and feet, neck and shoulder, side, total loin, backfat and ham were recorded.

The pH (pH<sub>45</sub>) was determined in a LD muscle sample taken 45 min *post mortem*, immediately frozen with liquid N and stored at - 80°C. This sample was then homogenized in iodoacetate and pH was measured in homogenate (Čandek, 1991) using a combined glass electrode (MP120 Mettler-Toledo, GmbH, 8603 Schwarzenbach, Switzerland with Mettler-Toledo InLab427 electrode). The day after slaughter, color and pH (pH<sub>24</sub>) were measured on a freshly cut surface of LD. Color of LD was visually assessed, using a 1 to 6-point Japanese color scale (Nakai et al., 1975), and objectively, using CIE (1976) L\* (lightness), a\* (redness)

and b\* (yellowness) color space, measured in triplicates using a Minolta Chroma Meter CR-300 (Minolta Co. Ltd, Osaka, Japan) with an 11 mm aperture, D65 illuminant, calibrated against a white tile. Caudally from the level of the last rib, one 2.5 cm thick slice of LD was removed from the loin for the determination of i.m. fat and drip loss (EZ drip according to the method of Christensen, 2003). EZ drip loss was expressed as the difference (in percentage) from the initial sample weight after 24 and 48 h storage at 4°C. Intramuscular fat was estimated on minced samples of LD using NIRS (NIR System model 6500 Spectrometer, Silver Spring, MD, USA; Prevolnik et al., 2005). For determination of boar taint compounds, samples of subcutaneous fat were taken at the level of last rib, to be vacuum packed and stored at - 20°C until further laboratory analysis.

### ***Chemical analyses***

Pooled feed samples were analyzed for DM, ash, N, starch, crude fiber and ether extract contents according to AOAC (1990). Gross energy content was measured using an adiabatic bomb calorimeter (IKA, C5000, Staufen, Germany). The NDF and ADF contents of pooled samples of diets were analyzed according to Van Soest et al. (1991). Samples of feces were analyzed for DM, ash, N, crude fiber, ether extract with prior acid hydrolysis, NDF, ADF and GE contents according to the same methods as for feed samples. Fresh samples of urine were analyzed for N content, whereas the GE content was evaluated after freeze-drying approximately 30 mL of urine in polyethylene bags.

Androstenone and skatole concentrations were measured in subcutaneous fat samples by HPLC according to the procedures of Hansen-Møller (1994), as described in detail by Batorek et al. (2012b). Concentrations were expressed per gram of liquid fat. The detection limits were 0.24 µg/g for androstenone and 0.03 µg/g for skatole.

### ***Calculations***

Dry matter feed intake and apparent digestibility coefficients of OM, CP, GE, ether extract, crude fiber, NDF and ADF were calculated from values obtained in Trial 1 according to standard procedures. Dietary NE concentration was calculated for HNE and LNE according

to Noblet et al. (1994) using average of values obtained by equations 3, 4 and 5 (with DE as predictor) and NE content of MNE diet was assumed to be the mean of values calculated for HNE and LNE diets. In Trial 2, ADG and G:F were calculated for each of 4-wk experimental periods: d 84 to d 114, d 115 to d 142, d 143 to d 170. Additionally, NE intake was calculated from DM feed intake and NE concentration of experimental diets as estimated from Trial 1 and it was used in the calculation of BW gain to NE intake ratio (**G:NE**). The energy values of experimental diets, ADFI, NE intake, G:F and G:NE were calculated on 100% DM and subsequently adjusted to 89% DM content.

Dressing percentage was calculated as the ratio between HCW and final BW, measured 24 h before slaughter without prior fasting.

### *Statistical analyses*

Analysis of variance was performed using mixed model procedure of SAS (PROC MIXED, SAS Inst., Cary NC, USA). Repeated measurements of digestibility and N balances data (N=8) in Trial 1 were evaluated by including the fixed effects of dietary group and stage and their interaction and the random effect of pig (included in the repeated statement). The effect of reduced NE concentration of feed in Trial 2 on growth performance was evaluated considering the fixed effects of dietary group and litter for the phases before (d 84 to d 114) and after V2 (d 115 to d 170) whereas, for the separate two 4-week periods within the phase after V2, the model included dietary group, period, their interaction and litter, with pig as repeated variable. Because the effect of immunocastration on performance is well established, and this was not the interest of the present study, the comparisons of phases before and after V2 are not presented.

Data of feed intake measurements and BW were used for calculation of relationship between cumulative energy intakes ( $y$ ; GE, DE, ME or NE) and BW according to the following model:

If age < 112:

$$y = a_{\text{diet before V2}} \times BW^{c_{\text{diet}}} + b_{\text{pig}}$$

If  $\text{age} \geq 112$ :

$$y = a_{\text{diet after } V2} \times BW^{c_{\text{diet}}} + (a_{\text{diet before } V2} - a_{\text{diet after } V2}) \times BW_{\text{age}=112} + b_{\text{pig}}$$

with  $a_{\text{diet before } V2}$  and  $a_{\text{diet after } V2}$  the slopes of the relationship for each diet, before and after V2, respectively,  $c_{\text{diet}}$  the exponent applied to BW for each diet,  $BW_{\text{age}=112}$  the BW at 112 days of age, and  $b_{\text{pig}}$  the intercept of the relationship for each pig. Parameters of the model were evaluated using NLIN procedure of SAS (PROC NLIN, SAS Inst., Cary NC, USA). Hypothesis for a single  $c_{\text{diet}}$  exponent applied to BW for all diets was tested for GE, DE, ME and NE intake using the extra sum of squares reduction test (Ratkowski, 1993). As this test was never significant ( $P > 0.10$ ), a single exponent (1.43) was considered for further statistical analyses to test the effect of diet and stage on slopes of the relationship.

Data on carcass and meat quality traits were analyzed using a model with fixed effects of dietary treatment group, litter within slaughter batch and slaughter batch. The individual pig was the experimental unit for analysis of all data. Least squares means were compared using Tukey test with statistical significance based on  $P < 0.05$ .

## RESULTS

Chemically determined values for dietary CP, crude fiber, ether extract and starch concentrations in the representative samples of LNE, MNE and HNE diets were in line with expected values (Table 1). No specific problem occurred in Trial 1, whereas in Trial 2, 1 pig died (from HNE group), 2 were excluded due to umbilical hernia (1 in LNE and MNE groups) and 1 due to broken forelimb (from MNE group) providing 41 pigs weighting on average 122.5 kg at the end of trial 2 (Table 3).

The effectiveness of immunocastration was assessed taking into consideration androstenone and skatole concentration in backfat and testes weight at slaughter. Androstenone and skatole concentrations averaged  $0.27 \pm 0.08$  and  $0.05 \pm 0.03$   $\mu\text{g/g}$  of liquid fat, respectively, regardless of treatment group in trail 2 ( $P > 0.05$ ). Weight of testes with epididymis averaged  $247 \pm 147$  g and no differences were observed between treatment groups in trail 2 ( $P > 0.05$ ).

### ***Nutrient digestibility of experimental diets (Trial 1)***

Results of Trial 1 (Table 2) show a lower ( $P < 0.05$ ) digestibility of OM, CP, ether extract, and energy in the LNE diet in connection with its higher crude fiber content. Higher crude fiber digestibility was noted on LNE diet ( $P < 0.01$ ), whereas NDF and ADF digestibility were not affected ( $P > 0.05$ ) by diet composition. Likewise, N intake and N retention were similar with LNE and HNE dietary treatment ( $P > 0.10$ ) whereas higher N fecal losses ( $P < 0.01$ ) and lower N urinary losses ( $P = 0.04$ ) occurred with LNE diet. In line with the objective of the study, the experimental diets differed in their energy contents; DE ( $P = 0.01$ ), ME ( $P = 0.02$ ) and calculated NE ( $P < 0.01$ ) contents of HNE and LNE diets were 13.9 vs 12.7, 13.4 vs 12.3 and 10.4 vs 9.3 MJ/kg (Table 2).

### **INSERT TABLE 2**

### ***Feed intake and performance (Trial 2)***

The effect of reduced NE concentration of feed on feed intake and performance was evaluated in P1- period before V2 (d 84 to d 114), P2- period after V2 (d 115 to d 170) and separately in two 4-wk phases (d 115 to d 142 and d 143 to d 170) within P2 where the effect of phase and the interaction between phase post V2 and dietary treatment was taken into account. No effect of the diet ( $P > 0.10$ ; Table 3) was noted on BW at any stage of the experiment. The effect of phase after V2 was significant for all parameters of feed intake and growth performance ( $P < 0.01$ ), whereas the phase and dietary treatment interaction after V2 was significant only for backfat gain ( $P < 0.01$ ). Diet composition had no effect on ADFI in any period of the experiment ( $P > 0.10$ ), whereas reduction of NE concentration of feed influenced NE intake in P2. To be more precise, higher NE intake in mega joule per day ( $P = 0.08$ ) was noted in the second 4-wk phase after V2 (d 143 to d 170) for HNE dietary group, whereas the difference in NE intake expressed as mega joule per kilogram of  $BW^{0.60}$  per day was noted only between HNE and MNE dietary groups in P2 ( $P = 0.03$ ). There was no effect ( $P > 0.10$ ) of reduction of NE concentration of feed on ADG in any stage of experiment, whereas reduction

of NE concentration of feed decreased G:F ( $P<0.01$ ) in P1 (d 84 to d 114) and P2 (d 115 to d 170) in LNE dietary group only. However, within P2, the difference in G:F between dietary groups ( $P<0.01$ ) was noted only in the first 4-wk phase (d 115 to d 142). On the other hand, no effect ( $P>0.10$ ) of reduction of NE concentration of feed was noted for G:NE in any period of experiment. A difference in backfat gain was observed only in the second phase after V2 (d 143 to d 170) where higher ( $P<0.01$ ) backfat gain was noted for the HNE group which was also reflected as a tendency ( $P=0.13$ ) towards higher backfat gain in P2 (d 115 to d 170) in HNE dietary group.

The slopes of the relationships between cumulative energy intake and BW were tested for the effects of stage and diet on slopes of the relationships. For each diet, the slopes never differed between stages for cumulative GE, DE, ME and NE intake ( $P>0.10$ ; Table 4). Before V2, the slopes of the relationship between cumulative GE intake and BW differed between HNE and LNE diets (4.18 and 5.48 MJ/kg BW<sup>1.43</sup>, respectively;  $P<0.01$ ) and tended to differ between MNE and LNE diets. After V2, the slopes of the relationship between cumulative GE intake and BW differed between dietary treatments; they varied from 4.48 to 4.97 MJ/kg BW<sup>1.43</sup> for HNE and LNE diets, respectively. At each stage, the slopes of the relationships between cumulative DE or ME intake and BW tended to differ between the 2 extreme LNE and HNE diets ( $P<0.10$ ). The slopes of the relationship between cumulative NE intake and BW did not differ between stages and diets and averaged 2.86 MJ/kg BW<sup>1.43</sup>.

#### **INSERT TABLE 3 and 4**

#### ***Carcass characteristics and meat quality (Trial 2)***

In agreement with the absence of diet effect on BW, no effect ( $P>0.10$ ) was noted on HCW, dressing percentage or prime carcass cuts. However, it should be noted that the numerical variation in dressing percentage between dietary treatment groups was rather high; a 1.5 and 0.9 points decrease with LNE and MNE diet relative to HNE diet was noted ( $P=0.11$ ). In agreement with the effect of dietary treatment on *in vivo* backfat gain measured with

ultrasound, the differences among treatment groups were noted on the carcass as lower backfat expressed as the percentage of HCW ( $P=0.01$ ) and as area of fat layer over LD ( $P=0.05$ ) in LNE and MNE pigs than in pigs fed HNE diet. However, this was not confirmed by backfat thickness at the level of the last rib ( $P=0.88$ ), despite higher final BFT in HNE group measured with ultra-sonography at corresponding position at 168 d of age ( $P=0.02$ ). Likewise, intermuscular (evaluated as NIMF) and LD i.m. fat deposition were not affected ( $P>0.05$ ) by reduction of NE concentration of feed. Nevertheless, decreased subcutaneous fatness was reflected in higher lean content ( $P=0.04$ ) of LNE pigs. No effect of dietary NE concentration was noted on meat quality, with the exception of a tendency ( $P=0.10$ ) towards higher yellowness (CIA b\*) and lighter color score ( $P=0.04$ ) of LD muscle in the HNE group.

## **INSERT TABLE 5**

## **DISCUSSION**

Rapid changes in hormonal status of IM pigs approximately 10 days after full immunization (Claus et al., 2007) result in increased voluntary feed intake, higher ADG (Batorek et al., 2012a; Dunshea et al., 2013) and altered energy metabolism followed by increased lipid deposition (Labussière et al., 2013) leading to higher backfat thickness (Dunshea et al., 2013) and lower lean content (Batorek et al., 2012a). However, the majority of studies deals with pigs slaughtered 4 to 6 wk after V2, whereas little is known about what happens when the delay between V2 and slaughter is prolonged beyond recommendation. In the present study this delay increased up to 8 wk with no adverse effects on meat boar taint: taking into account androstenone and skatole levels in backfat together with the testis weight, all pigs can be considered fully immunized. In agreement, Kubale et al. (2013) observed no sign of functional or morphological restoration of testicular function within 8 wk after V2. Likewise, neither quantitative feed restriction in IM (Batorek et al., 2012b) nor feeding fiber-rich diets to EM pigs (Van Oeckel et al., 1998) increased the risk for skatole formation and boar taint accumulation in fat. Also, high digestibility of energy in low fiber diet (i.e. high



starch diet) observed in Trial 1 is consistent with previous results (Noblet and Shi, 1993; Le Goff and Noblet, 2001; Wilfart et al., 2007) and confirms that dietary fiber can be successfully used as an energy diluting factor in growing-finishing diets (Le Goff et al., 2002). Indeed, by increasing crude fiber content (plus 26 and 46 g/kg DM in MNE and LNE diet, respectively), the calculated NE content of the diets in the present study was reduced by approximately 5 and 10% in MNE and LNE diet.

Pigs are generally capable of adjusting voluntary feed intake to dietary characteristics, and energy density is usually the first determinant of ADFI as long as the maximum capacity of digestible tract is not reached (Henry, 1985; Quiniou and Noblet, 2012). However, in the present study, in agreement with the results of Zeng et al (2002), reduction of NE concentration of feed did not significantly affect ADFI of IM, which might indicate that pigs already reached their maximal feed intake capacity or that the dietary source used had a marked effect on the satiety of pigs due to higher water-holding capacity of fiber as discussed by Quiniou and Noblet (2012) and Len et al. (2008). However, since ADFI of IM in the first weeks after full immunization is higher than the one of barrows (for approximately 105 g/d; Millet et al., 2011; Batorek et al., 2012a; Dunshea et al., 2013) it could be, that physical limits for ADFI are reached whatever the diet energy content. Thus, it can be expected that NE intake (MJ/kg) would be lower if NE concentration of feed was reduced. Yet in the present study only a tendency for a lower NE intake was noted for LNE and MNE diet in the second 4-wk period after V2, despite that ADFI was not altered by dietary NE concentration. However, expressed on metabolic BW (MJ/kg BW<sup>0.60</sup> per day) only pigs consuming MNE diet reached significantly lower NE intake after V2. This might indicate that LNE pigs compensated for the lower NE dietary content with numerically higher ADFI, whereas this was not the case in MNE pigs. It should however be noted that the NE content of the MNE diet was calculated as the average of the one measured for LNE and HNE and not estimated from a digestibility trial. Additionally, the variability in NE intake per metabolic BW (MJ/kg BW<sup>0.60</sup> per day) is much smaller, meaning that variability in NE intake and also in ADFI is partly explained by variability in BW which was numerically lower for LNE and MNE groups. In agreement, the slope of the relationship between cumulative GE intake and BW

varied between diets and small differences can also be reported in the slopes for cumulative DE and ME intake (Table 4) whereas no difference was observed in the slope of the relationship between cumulative NE intake and BW between stages and diets. Thus, it can be considered that maximal NE intake was achieved regardless of the dietary energy content and can be described as  $2.86 \text{ MJ/kg} \times \text{BW}^{1.43} - 463 \text{ MJ}$ .

In order to eliminate the influence of differential gut content and visceral tissue because of differences in ADFI (Quiniou and Noblet 2012), ADG was adjusted for dressing percentage, and was unaffected by reduction of NE concentration of feed in the present study. In the case of more severe quantitative feed restriction in IM, a decrease of ADG was reported (by 20% and 12% at 78% and 85% of *ad libitum* feed intake, respectively), with no improvement in G:F (Quiniou et al., 2012). Due to energy dilution in the present study, growth efficiency was evaluated on NE basis as gain to NE ratio (G:NE), in order to express energy requirements and dietary energy values on the same scale (Noblet and van Milgen, 2004). In accordance with results of Quiniou and Noblet (2012) in barrows, G:NE ratio was similar in all 3 dietary groups, indicating that reduction of NE concentration of feed did not influence the performance of IM and that the utilization of energy was similar in all dietary groups. In agreement with the aim of the study, reduction of NE concentration of feed (10% relative to standard diet containing 10.4 MJ NE/kg of feed) resulted in reduced backfat deposition; however the difference between 3 dietary treatments in the rate of backfat thickness gain was significant only during the second 4-wk following V2, resulting in a 28 % lower rate of backfat deposition. In general, lipid deposition increases gradually with BW (Cisneros et al., 1996), while IM are characterized by an intense post V2 increase in lipid deposition and subsequent increase in LD:PD ratio (Labussiere et al., 2013); therefore it was expected, and proven in the present study, that the difference in backfat gain due to reduced dietary NE concentration would become notable only a prolonged time after V2.

The results of carcass traits confirm the results of performance. In agreement with a similar study in barrows (Quiniou and Noblet, 2012), carcass weight of IM in the present study was not affected by reduction of NE concentration of feed, whereas lean content was improved. Although not significant, the numerical variation in dressing yield was rather high

and consistent with literature that shows a lower dressing percentage with higher dietary fiber diets due to the increased weight of visceral organs, gastrointestinal tract and digesta (Jørgensen et al., 1996). Weights of prime cuts were similar in all 3 dietary treatment groups, whereas response of fat depots varied. Only s.c. fat depot was significantly reduced with reduction of dietary energy content, whereas leaf fat and i.m. fat deposition were only numerically reduced. Intramuscular fat content was below 2 to 3 % required to confer flavor and succulence to pork meat (Wariss, 1995) in all dietary groups, due to the crossbred used (Pietrain × (Large White × (Landrace))). In contrary, the lack of influence of dietary NE concentration on leaf fat deposition is unexpected in view of relative growth rate of various fat depots in barrows, known to be the highest for leaf fat, followed by subcutaneous and intermuscular (and i.m.) fat deposition (Jones, 2004; Kouba and Bonneau, 2009; Kouba and Sellier, 2011). With regard to the effect of feed restriction in IM pigs, to our knowledge, there are only 3 publications, which either observed no significant effect on carcass leanness and fat deposition (dos Santos et al., 2012; Quiniou et al., 2012) or only an effect on leaf fat weight (Batorek et al., 2012b). The lack of effect of restricted feed allowance on carcass leanness in the above mentioned studies might be related to a shorter duration of the applied restriction. As shown in the present study, backfat deposition significantly increases only after a substantial time post V2 (4 to 8 wk after V2).

Reduction of dietary energy content in the present study was not a source of differences in meat quality, in agreement with results in heavy barrows (Fraga et al., 2009) and quantitatively restricted IM (Batorek et al., 2012b). However, visually assessed lighter color and a tendency towards greater yellowness of pork from HNE group were noted. While the first might be the consequence of higher carotenoid content (not measured), for the second we have no explanation. To dilute energy content, corn was partly replaced by fiber in MNE and LNE diets, thus dietary carotenoid content might have been reduced, influencing LD meat yellowness, which corroborates the results of Akiba et al. (2001) in poultry and Fraga et al. (2009) in pigs.

Taken together, the results of the present experiment suggest that energy dilution in growing and finishing diet offered *ad libitum* to IM limits subcutaneous fat deposition without

adverse effect on meat quality. From a practical point of view, this indicates that it would be reasonable to apply feed restriction (quantitative or qualitative) especially when prolonged time between V2 and slaughter is expected.

## CONCLUSION

The interest of immunocastration resides in a long term boar-like performance of pigs; however, after the immunization, feed intake is considerably increased, which diminishes the advantages in performance. Overall, our results demonstrate that by reducing NE concentration of the diet up to 10 % with higher fiber content ingredients, similar energy efficiency (gain to NE intake ratio) may be achieved, and this technique provides an advantage in terms of reduced lipid deposition, without affecting growth rate in IM after full immunization. This is particularly important when IM are fattened to higher weights i.e. when longer delays between V2 and slaughter are practiced.

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**Table 1:** Ingredients and chemical composition of experimental diets differing in their NE content, fed to male pigs between 84 and 172 days of age

Item	Diet		
	Low NE	Medium NE	High NE
Ingredient composition (%)			
Wheat	17.77	21.35	24.93
Corn	17.77	21.35	24.93
Barley	17.77	21.35	24.93
Wheat bran	15.00	8.75	2.50
Soybean hulls	10.00	5.00	-
Dried beet pulp	5.00	2.50	-
Soybean meal	9.18	12.46	15.74
Rapeseed meal	1.97	0.99	-
Cane molasses	3.00	3.00	3.00
Sunflower oil	-	0.50	1.00
L-Lysine HCL	0.24	0.27	0.29
L-Threonine	0.07	0.08	0.09
L-Tryptophan	0.01	0.01	0.02
DL-Methionine	0.01	0.02	0.03
L-Valine	0.00	0.01	0.02
NaCl	0.45	0.45	0.45
Limestone	0.29	0.41	0.54
Dicalcium phosphate	0.97	1.00	1.03
Vitamin-mineral premix <sup>1</sup>	0.50	0.50	0.50
Measured chemical composition, g/kg of DM			
Ash	60	56	54
CP	180	175	185
Starch	416	470	518
Ether extract	33	34	42
Crude fiber	80	60	34
NDF	228	188	147
ADF	100	74	44
ADL	21	20	14
GE, MJ/kg of DM	18.17	18.17	18.31
Nutritional value <sup>2</sup>			

DE, MJ/kg of feed	12.24	13.00	13.89
NE, MJ/kg of feed	8.50	9.25	9.99
SID lysine <sup>3</sup> , g/kg of feed	7.1	7.8	8.4
SID threonine, g/kg of feed	4.6	5.0	5.4
SID tryptophan, g/kg of feed	1.4	1.5	1.8
SID methionine, g/kg of feed	2.0	2.3	2.6
SID valine, g/kg of feed	5.4	5.9	6.5

<sup>1</sup> Supplied per kilogram of final diet (as-fed basis): vitamin A, 5,000 UI; vitamin D3, 1,000 UI; vitamin E, 20 UI; vitamin B1, 2 mg; vitamin B2, 4 mg; pantothenic acid, 10 mg; vitamin B6, 1 mg; vitamin B12, 0.02 mg; niacin, 15 mg; vitamin K3, 2 mg; folic acid, 1 mg; biotin, 0.2 mg; choline chloride, 500 mg; iron, 80 mg; copper, 10 mg; zinc, 100 mg; magnesium, 40 mg; cobalt, 0.1 mg; iodine, 0.2 mg; selenium, 0.15 mg.

<sup>2</sup> Calculated from the feed ingredients according to Sauvant *et al.*, 2004, adjusted to 89 % DM content of the diet.

<sup>3</sup> The SID lysine/NE ratio was constant among diets at a level of 0.84 g/ MJ.

**Table 2:** Digestibility of nutrients and energy values of diets.<sup>1</sup>

Item	Diet		RSD <sup>2</sup>	P-value <sup>3</sup>
	LNE	HNE		
BW at first stage, kg	47.1	44.7	6.4	0.71
BW at second stage, kg	67.7	70.5	7.2	0.70
Digestibility coefficients, %				
DM	80.0	85.6	1.1	0.02
Organic matter	81.6	87.2	1.0	0.02
CP	73.2	82.2	2.1	0.02
Ether extract	58.3	69.1	2.5	0.02
Crude fiber	44.7	29.7	3.7	0.02
NDF	52.1	52.8	4.2	0.83
ADF	45.0	38.9	3.6	0.12
Energy	78.9	85.1	1.2	0.02
Energy values <sup>4</sup> (MJ/kg of feed)				
DE	12.70	13.87	0.20	0.01
ME <sup>5</sup>	12.31	13.42	0.25	0.02
NE <sup>6</sup>	9.29	10.38	0.15	<0.01
N balance (g/day)				
N intake	60.6	58.4	1.7	0.18
N excretion				
N feces	16.2	10.3	0.8	<0.01
N urine	13.6	20.5	2.2	0.04
N retained	30.7	27.6	3.9	0.34
N retention (% of digested N)	69.4	59.2	6.5	0.14

LNE= Low NE diet; HNE= High NE diet.

<sup>1</sup> Trial 1 consisted of digestibility trail and N balance study and was performed using 4 pigs (immunocastrated with Improvac<sup>®</sup> at age of 77 and 105 d) fed the 2 extreme experimental diets during 2 successive periods (first prior and second after revaccination with Improvac<sup>®</sup>) consisted of one adaptation sub period (14 d) and a subsequent collection sub period (7 d), so that 2 pigs were fed each diet at each stage.

<sup>2</sup> RSD= Residual SD.

<sup>3</sup> Values were tested for the effect of diet; results are least squares means, the measurement was considered repeated per period.

<sup>4</sup> Adjusted for 89% DM content.

<sup>5</sup> It should be noted that ME was not accounted for the loss of E as CH<sub>4</sub>.

<sup>6</sup> Calculated according to Noblet *et al.*, 1994, average of equations 3, 4 and 5.

**Table 3:** Effect of reduction of NE concentration of feed on feed intake and growth performance in growing-finishing immunocastrated<sup>1</sup> pigs during three successive 4-wk phases of growth.<sup>2</sup>

Item	Treatment group			RSD <sup>3</sup>	P-value <sup>4</sup>	
	LNE	MNE	HNE		Diet	Diet×phase
No. of pigs	14	14	14			
BW, kg						
d 84	33.6	32.8	32.8	3.5	0.80	-
d 114	55.1	54.5	55.9	7.0	0.88	-
d 142	85.8	86.0	89.4	10.4	0.61	-
d 170	120.8	120.0	126.8	11.0	0.24	-
ADFI <sup>5</sup> , kg/d						
before V2: d 84 to 114	1.85	1.69	1.70	0.22	0.14	-
after V2: d 115 to 142	2.97	2.74	2.75			
d 143 to 170	4.07	3.68	3.95	0.30	0.14	0.16
d 115 to 170	3.51	3.21	3.37	0.72	0.33	-
NE intake <sup>5</sup> , MJ/d						
before V2: d 84 to 114	19.2	18.6	19.8	2.5	0.47	-
after V2: d 115 to 142	30.7	30.6	32.1			
d 143 to 170	42.4	41.0	46.3	3.2	0.08	0.09
d 115 to 170	36.6	35.6	39.2	8.0	0.24	-
NE intake <sup>6</sup> , MJ/ kg BW <sup>0.60</sup> per d						
before V2: d 84 to 114	2.16	2.13	2.25	0.12	0.08	-
after V2: d 115 to 142	2.49 <sup>A</sup>	2.46 <sup>A</sup>	2.58 <sup>A</sup>			
d 143 to 170	2.67 <sup>AB</sup>	2.58 <sup>AB</sup>	2.84 <sup>B</sup>	0.20	0.04	0.40
d 115 to 170	2.58 <sup>ab</sup>	2.52 <sup>a</sup>	2.71 <sup>b</sup>	0.25	0.03	-
ADG <sup>7</sup> , kg/d						
before V2: d 84 to 114	0.75	0.76	0.76	0.11	0.94	-
after V2: d 115 to 142	1.09	1.11	1.15			
d 143 to 170	1.27	1.21	1.31	0.14	0.17	0.61
d 115 to 170	1.18	1.16	1.23	0.16	0.27	-
G:F <sup>5</sup> , g BW /g of feed						
before V2: d 84 to 114	0.40 <sup>a</sup>	0.45 <sup>b</sup>	0.45 <sup>b</sup>	0.03	<0.01	-
after V2: d 115 to 142	0.37 <sup>B</sup>	0.40 <sup>C</sup>	0.42 <sup>C</sup>			
d 143 to 170	0.31 <sup>A</sup>	0.33 <sup>A</sup>	0.34 <sup>A</sup>	0.03	<0.01	0.15

	d 115 to 170	0.34 <sup>a</sup>	0.37 <sup>b</sup>	0.38 <sup>b</sup>	0.05	<0.01	-
G:NE <sup>6</sup> , g BW/MJ NE							
before V2:	d 84 to 114	38.6	40.6	38.9	3.1	0.21	-
after V2:	d 115 to 142	35.2	36.7	36.5			
	d 143 to 170	29.8	30.1	29.0	10.6	0.48	0.35
	d 115 to 170	32.5	33.4	32.7	4.4	0.76	-
Initial backfat thickness, mm							
		5.0	4.9	5.0	0.4	0.64	-
Backfat gain, mm/d							
before V2:	d 84 to 114	0.07	0.07	0.07	0.02	0.79	-
after V2:	d 115 to 142	0.10 <sup>A</sup>	0.11 <sup>A</sup>	0.12 <sup>A</sup>			
	d 143 to 170	0.21 <sup>B</sup>	0.22 <sup>B</sup>	0.29 <sup>C</sup>	0.04	<0.01	0.01
	d 115 to 170	0.16	0.17	0.20	0.09	0.13	-

<sup>a-c</sup> Least square means within a row with different superscripts differ (P<0.05).

<sup>A-C</sup> Least square means within a row and column with different superscripts differ (P<0.05).

<sup>1</sup> Vaccination with Improvac<sup>®</sup> (2 mL, s.c. application, Zoetis) was performed at the age of 77 and 112 days.

<sup>2</sup> Growth performance data were obtained over 3 successive 4-wk phases of growth (d 84 to 114, d 115 to 142, d 143 to 170 of age) shown as the phase before second vaccination with Improvac<sup>®</sup> (before V2), the phase after second vaccination with Improvac<sup>®</sup> (after V2) and separated first (d 115 to d 142) and second (d 143 to d 170) 4-week phases following V2.

<sup>3</sup> RSD = residual SD, which is the root-mean-square of the error that applies to the whole model.

<sup>4</sup> In periods before and after V2, the effect of dietary treatment (Diet) and litter were included in the model, whereas for the 2 separate 4-wk phases in the period after V2, the effect of phase and its interaction with dietary treatment (Diet×Phase) were considered. Effect of phase was always significant (P<0.01) and is therefore not reported in the table.

<sup>5</sup> Adjusted for 89% DM content of the diet.

<sup>6</sup> Calculated using the dietary NE concentration calculated from trial 1.

<sup>7</sup> For ADG dressing percentage was added to the model, in order to eliminate the influence of energy accumulated in the gut content and gut tissue (Quiniou and Noblet, 2012).





**Table 4.** Estimated parameters for the generalized relationship between cumulative dietary energy intake before and after effective immunocastration and BW by diet<sup>1</sup> in growing-finishing immunocastrated<sup>2</sup> pigs (n=41).

	Cumulative GE intake, MJ			Cumulative DE intake, MJ			Cumulative ME intake, MJ			Cumulative NE intake, MJ		
	Before V2	After V2	P-value <sup>3</sup>	Before V2	After V2	P-value <sup>3</sup>	Before V2	After V2	P-value <sup>3</sup>	Before V2	After V2	P-value <sup>3</sup>
Exponent applied to BW (c)	1.43	0.48		1.43	0.47		1.43	0.47		1.43	0.47	
Slope, MJ/ kg BW <sup>c</sup>												
HNE	4.18	4.48	0.46	3.58	3.84	0.43	3.47	3.72	0.44	2.69	2.88	0.43
MNE	4.51	4.68	0.62	3.73	3.87	0.61	3.61	3.75	0.61	2.76	2.87	0.61
LNE	5.48	4.97	0.26	4.33	3.94	0.29	4.20	3.81	0.28	3.17	2.89	0.29
P-values for test difference between <sup>4</sup>												
HNE and LNE	<0.01	<0.01		0.06	0.07		0.06	0.05		0.11	0.68	
HNE and MNE	0.53	<0.01		0.63	0.56		0.63	0.53		0.65	0.66	
MNE and LNE	0.06	<0.01		0.17	0.29		0.17	0.26		0.22	0.65	
Calculated intercept, MJ <sup>5</sup>												
HNE	-652	-733		-555	-626		-537	-605		-416	-469	
MNE	-705	-751		-580	-619		-562	-600		-430	-459	
LNE	-946	-808		-744	-637		-721	-617		-544	-466	

Residual standard deviation, MJ <sup>6</sup>	94	77	75	57
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<sup>1</sup> Parameters are estimated for the generalized relationship between cumulative GE/DE/ME/NE intake (MJ) and body weight (kg) before and after second vaccination for immunocastration and different diets (HNE=high NE diet, MNE=medium NE diet and LNE=low NE diet) in form:

If age<112:

$$y = a_{diet\ before\ v2} \times BW^{c_{diet}} + b_{pig}$$

If age≥112:

$$y = a_{diet\ after\ v2} \times BW^{c_{diet}} + (a_{diet\ before\ v2} - a_{diet\ after\ v2}) \times BW_{age=112} + b_{pig}$$

with  $a_{diet\ before\ v2}$  and  $a_{diet\ after\ v2}$  the slopes of the relationship for each diet, before and after V2, respectively,  $c_{diet}$  the exponent applied to BW for each diet,  $BW_{age=112}$  the BW at 112 days of age, and  $b_{pig}$  the intercept of the relationship for each pig.

<sup>2</sup> Vaccination with Improvac<sup>®</sup> (2 mL, s.c. application, Zoetis) was performed at the age of 77 and 112 days.

<sup>3</sup> *P-values* that the residual sum of squares of the reduced model (single exponent c for all diets, or single exponent c for all diets plus single slope for both stages for each diet) is equal to that of the full model. Hypothesis for which P>0.05 is indicative that the reduced model is adequate to describe the data.

<sup>4</sup> *P-values* that the residual sum of squares of the reduced model (single exponent c for all diets plus single slope for 2 diets at each stage) is equal to that of the full model. Hypothesis for which P>0.05 is indicative that the reduced model is adequate to describe the data.

<sup>5</sup> Intercept calculated as the average per diet of individual  $b_{pig}$  values for stage 1, and as the average per diet of individual  $[(a_{diet\ before\ v2} - a_{diet\ after\ v2}) \times BW_{age=112} + b_{pig}]$  for stage 2.

<sup>6</sup> Residual standard deviation for the full model calculated as the square root of residual sum of squares divided by residual degrees of freedom.

**Table 5:** Effect of reduction of NE concentration of feed on carcass and meat quality traits in growing-finishing immunocastrated<sup>1</sup> pigs.

Item	Treatment			RSD <sup>2</sup>	P-value
	LNE	MNE	HNE		
No. of pigs	14	14	14		
Measurements before and at slaughter					
Live weight, kg	125.0	123.2	129.9	11.1	0.29
Final backfat thickness, mm	15.7 <sup>a</sup>	16.1 <sup>a</sup>	18.5 <sup>b</sup>	2.5	0.02
HCW <sup>3</sup> , kg	96.9	96.3	102.6	9.6	0.20
Dressing percentage, %	77.5	78.1	79.0	1.8	0.11
Measurements on carcass <sup>4</sup>					
Lean content <sup>5</sup> , %	56.8 <sup>b</sup>	56.3 <sup>ab</sup>	54.8 <sup>a</sup>	1.8	0.04
Head, %	5.4	5.4	5.3	0.3	0.66
Tail and feet, %	0.6	0.6	0.6	0.1	0.57
Backfat, %	5.4 <sup>a</sup>	5.4 <sup>a</sup>	6.2 <sup>b</sup>	0.7	0.01
Ribs, %	11.0	11.1	11.0	0.5	0.79
Neck and shoulder, %	23.4	23.1	23.1	0.9	0.56
Ham, %	31.8	31.8	31.3	1.1	0.34
Loin, %	22.7	22.7	22.5	0.8	0.81
Leaf fat, %	1.4	1.4	1.5	0.3	0.57
Backfat thickness <sup>6</sup> , mm	18.2	18.1	18.9	4.4	0.88
NIMF <sup>7</sup> , %	22.7	21.2	24.7	4.1	0.30
LD area <sup>8</sup> , cm <sup>2</sup>	51.1	51.3	52.5	5.4	0.77
Fat over LD area <sup>8</sup> , cm <sup>2</sup>	17.4 <sup>a</sup>	18.2 <sup>ab</sup>	20.5 <sup>b</sup>	3.2	0.05
Meat quality					
LD intramuscular fat, %	1.54	1.45	1.68	0.62	0.66
pH <sub>45</sub>	6.07	6.00	6.01	0.15	0.40
pH <sub>24</sub>	5.49	5.48	5.48	0.05	0.72
CIA L*	57.0	57.3	58.8	2.8	0.21
CIA a*	7.8	7.9	8.1	1.2	0.74
CIA b*	5.0	4.9	5.7	1.0	0.10
Color (1-6) <sup>9</sup>	2.8 <sup>ab</sup>	3.0 <sup>b</sup>	2.4 <sup>a</sup>	0.5	0.04
EZ drip loss <sup>10</sup> 24h, % of initial weight	5.85	5.78	6.91	3.15	0.55
EZ drip loss <sup>10</sup> 48h, % of initial weight	7.49	7.93	9.36	3.24	0.31

<sup>a-c</sup> Least squared means within a row with different capitalized superscripts differ (P<0.05).

<sup>1</sup> Vaccinations with Improvac<sup>®</sup> (2 mL, s.c. application, Zoetis) was performed at age of 77 and 112 d, slaughter was performed in two batches within 5 days, at an average age of 172 d.

<sup>2</sup> RSD = residual SD, which is the root-mean-square of the error that applies to the whole model.

<sup>3</sup> HCW= hot carcass weight with head.

<sup>4</sup> Carcass with head. Primary carcass cuts are expressed as % of cold carcass weight with head.

<sup>5</sup> Calculated from backfat (F34) and muscle (M34) thicknesses:  $62.19 - 0.729 \times F34 + 0.144 \times M34$  (<http://uniporc-ouest.com>).

<sup>6</sup> Backfat thicknesses measured at the level of the last rib.

<sup>7</sup> NIMF= neck intermuscular fatness, assessed by image analysis (Batorek et al. 2012b).

<sup>8</sup> LD area and fat over LD area were assessed by image analysis (Batorek et al. 2012b)

<sup>9</sup> Japanese color scale (1-6), higher values denoting darker color.

<sup>10</sup> EZ drip loss was determined according to the method of Christensen et al. (2003).

## 5 ADDITIONAL RESULTS

### 5.1 Effect of immunocastration in group-housed commercial fattening pigs on reproductive organs, malodorous compounds, carcass and meat quality

**Article:** Škrlep M, Batorek N, Bonneau M, Prevolnik M, Kubale V, Čandek-Potokar M. 2012. Effect of immunocastration in group-housed commercial fattening pigs on reproductive organs, malodorous compounds, carcass and meat quality (Czech J. Anim. Sci. 57: 290–299.)

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## Effect of immunocastration in group-housed commercial fattening pigs on reproductive organs, malodorous compounds, carcass and meat quality

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**ABSTRACT:** In the present study, the effect of immunocastration on carcass traits, meat quality, reproductive organs development, and boar taint compounds was investigated. Male piglets (50% Duroc crosses) were randomly assigned to three treatment groups: entire males (EM;  $n = 19$ ), surgical castrates (SC;  $n = 20$ ) and immunocastrates (IC, vaccinated with Improvac<sup>®</sup> at the age of 79 and 142 days;  $n = 21$ ). Pigs were fed *ad libitum* and weighed at the time of first and second vaccination and before slaughter (176 days of age). No differences between treatment groups were detected for carcass weight. In the case of backfat thickness, carcass lean meat content, and belly leanness score, IC were intermediate between EM (the leanest) and SC (the fattest), differing ( $P < 0.05$ ) from both control groups. Regarding loin eye fat area, neck intermuscular fatness, ham leanness, and leaf fat weight, IC were similar to EM and were less fat than SC ( $P < 0.01$ ). IC had lower intramuscular fat than SC ( $P < 0.01$ ) and higher average pH 24 than both EM and SC ( $P < 0.01$ ), resulting in darker colour. IC also demonstrated lower drip loss than EM ( $P < 0.05$ ). Immunocastration caused a significant reduction of reproductive organs and concentrations of boar taint compounds ( $P < 0.01$ ) which were comparable with the levels observed for SC.

**Keywords:** pigs; immunocastration; carcass; meat quality; reproductive organs; boar taint

Surgical castration of male animals is a routine and widely used practice in the pig breeding industry. It is aimed at preventing boar taint, an unpleasant smell of the meat attributed to the accumulation of skatole and androstenone in the fatty tissue of sexually mature male pigs (boars), causing negative perception and rejection of such meat by the consumers (Font i Furnols et al., 2003; Bonneau and Squires, 2004). Besides the absence of boar taint, castrates exhibit less aggressive behaviour

(Cronin et al., 2003) than boars. There is, however, one important drawback. Due to lower feed efficiency and higher carcass fat deposition, the fattening of castrates is less cost-effective (Xue et al., 1997; de Roest et al., 2009). Moreover, surgical castration without anaesthesia has been severely criticised because of the pain inflicted to the animals during the procedure (Giershing et al., 2006). Several European countries have already prohibited surgical castration without anaesthesia and a gen-

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eral ban on castration is envisaged in the EU by the year 2018 (EC declaration, 2010). In view of these changes, immunocastration is one of the possible alternatives. The method is based on vaccination against GnRH (gonadotropin releasing hormone) and may improve animal welfare, since no surgical procedure is needed (Thun et al., 2006). Two vaccinations are needed for a full response, causing reduction of testes and accessory reproductive organs and elimination of boar taint (Dunshea et al., 2001; Cronin et al, 2003; McCauley et al., 2003; Jaros et al., 2005; Zamaratskaia et al., 2008a, b). Notable changes in metabolism and behaviour of animals have also been reported (Cronin et al, 2003; Claus et al., 2007). Immunocastration enables exploiting boar-like growth potential until the second vaccination, when the castration is effective. In the last few years, several studies have investigated the effect of immunocastration in pigs (reviewed by Millet et al., 2011 and Batorek et al., 2012), however, they were mostly focused on growth performance and boar taint elimination, whereas there is much less information on carcass and meat quality. Moreover, because the results of the studies are not always consistent due to numerous factors interfering with the effect of immunocastration, there is a need for testing this alternative in the local conditions. The present paper presents the results of a study conducted in commercial rearing conditions, evaluating the effects of immunocastration on performance, carcass and meat quality traits, development of reproductive organs and boar taint elimination.

## MATERIAL AND METHODS

### Animals and fattening trial

The experiment took place in a commercial pig farm. Initially, seventy two male crossbred (progeny of Large White Landrace dams sired by Duroc boars) pigs were selected from 34 litters farrowed within two weeks. Within each litter, male pigs were either left entire or surgically castrated (SC,  $n = 24$ ) during the first week of life. At the average age of  $79.2 \pm 3.3$  days, when pigs entered the fattening unit, half of the entire male pigs (IC,  $n = 24$ ) received the first vaccination against GnRH (2 ml of Improvac<sup>®</sup> vaccine per animal, Pfizer Animal Health), whereas the remaining half (EM,  $n = 24$ ) was untreated. Pigs of each treatment group were

Table 1. Performance traits (mean  $\pm$  standard deviation) in entire males, immunocastrates, and surgical castrates according to the phase of experiment

	EM $n = 19$	IC $n = 21$	SC $n = 20$
<b>Weight (kg)</b>			
V1	$29.5 \pm 1.7$	$29.8 \pm 2.5$	$30.7 \pm 3.2$
V2	$79.2 \pm 7.6$	$77.3 \pm 8.9$	$79.9 \pm 8.7$
Slaughter	$111.2 \pm 7.2$	$109.0 \pm 7.8$	$110.5 \pm 8.2$
<b>Daily gain (g/day)</b>			
V1–V2	$790 \pm 118$	$753 \pm 118$	$780 \pm 128$
V2-slaughter	$966 \pm 103$	$970 \pm 186$	$925 \pm 136$
V1-slaughter	$852 \pm 95$	$828 \pm 94$	$830 \pm 95$

EM = entire males, IC = immunocastrates, SC = surgical castrates, V1 = at the time of the first vaccination, V2 = at the time of the second vaccination

group-housed in two pens of 12 pigs. The second vaccination of IC pigs was performed 9 weeks later (average age was  $142.2 \pm 3.3$  days), i.e. 5 weeks prior to slaughter, as recommended by the vaccine producer. Pigs were fed *ad libitum* a commercial diet containing 13.0 MJ/kg of metabolisable energy, 17% crude protein, 2.6% crude fat, 4% crude fibre, 6% crude ash, and 1% lysine. Feed intake was not recorded. Pigs were weighed three times, at the first and second vaccination, and at the end of the experiment (three days prior to slaughter). Information on body weight and growth rate according to the phase of experiment is provided in Table 1. Pigs were slaughtered within the same week (at an average age of  $175.5 \pm 4.5$  days) in two batches, with all pens equally represented in both batches. The slaughter took place in a commercial abattoir according to standard procedure including approximately 1 h transport, 2 h lairage, CO<sub>2</sub> stunning (86 vol.% in the air), vertical exsanguination, vapour scalding and evisceration. During transport and lairage the pigs were not mixed. At the slaughter line, testes and accessory sex glands were removed and taken to the laboratory for dissection and weighing. This was done for half of the EM and IC pigs, taken at random.

### Blood sampling

At slaughter, blood samples from all experimental animals (approximately 9 ml) were taken into glass



tubes (containing no anticoagulant) and immediately placed on ice. Within 1 h the blood samples were taken to the laboratory and left to coagulate overnight at 4°C. After 24 h, coagulum was removed, while serum was centrifuged at 1800 rpm for 10 min and the supernatant collected and stored at –20°C until analysed.

### Carcass and meat quality measurements

At the slaughter line, after the evisceration, leaf fat (i.e. subperitoneal fat) was removed and weighed. The pig carcasses were weighed and classified according to SEUROP by official classification body, using a method approved for Slovenia (OJ EU L56/28, 2008) which consists of taking two measurements at the carcass split line: DM fat (minimal fat thickness over the *gluteus medius* muscle – GM) and DM muscle (shortest distance between cranial end of GM and dorsal edge of vertebral canal). Measurement of pH ( $\text{pH}_{45}$ ) in *longissimus dorsi* muscle (LD) was taken 45 min *post mortem* using a MP120 Mettler Toledo pH meter (Mettler-Toledo, GmbH, Schwarzenbach, Switzerland) fitted with a combined glass electrode (InLab427) and previously calibrated at pH 4.0 and 7.0. The carcasses were cooled overnight at 0–2°C until the internal carcass temperature dropped below 7°C.

The next day following slaughter, additional carcass traits were assessed. The hind leg was cut off the carcass between the 6<sup>th</sup> and 7<sup>th</sup> lumbar vertebra and the shank removed. The weight of the leg (ham) was recorded before and after the removal of the skin and subcutaneous fat. Ratio between ham weight and carcass weight (ham/CW, %) and ratio between ham muscle with bones and entire ham weight (ham leanness, %) were calculated. Two further cross-sections of carcass were made, one at the level of the last rib (cross-section A) and one between the 3<sup>rd</sup> and 4<sup>th</sup> cervical vertebra (cross-section B). A digital image of each cross-section was taken using a digital photo camera (Canon PowerShot G3, Canon Inc., Tokyo, Japan). Image analysis using LUCIA.NET 1.16.5 software (Laboratory Imaging s.r.o., Prague, Czech Republic) was used for determination of intermuscular fat of the neck (NIME, in %) on cross-section B and LD surface (LEA) and the surface of the corresponding fat (LEA fat, in %) on cross section A. Additionally, belly leanness was visually assessed on cross-section

A using a 1–7 point scale (from 1 for extremely fat to 7 for extremely lean).

Colour and pH ( $\text{pH}_{24}$ ) were measured on a freshly cut surface of LD (cross-section A). Colour of LD was assessed using a 1–6 point Japanese colour scale (Nakai et al., 1975). Colour parameter measurements (CIE  $L^*$ ,  $a^*$ ,  $b^*$ ) were taken in triplicate using a Minolta Chroma Meter CR-300 (Minolta Co. Ltd, Osaka, Japan) with an 11 mm aperture,  $D_{65}$  illuminant, calibrated against a white tile. Muscle ultimate pH ( $\text{pH}_{24}$ ) was determined in two replicates in the central area of LD. Caudally from the level of the last rib two 2.5 cm thick slices of LD were removed from the loin for the determination of drip loss and intramuscular fat. Drip loss was determined according to the EZ drip loss method (Christensen et al., 2003). Shortly, two cylindrical samples were excised from the central part of LD, weighed, and placed in special plastic containers. The samples were reweighed after 24 h and after 48 h of storage at 4°C. Drip loss (drip loss 24 h and drip loss 48 h) was expressed as a difference (%) to initial sample weight. Samples of LD muscle were minced and intramuscular fat content (IMF, %) estimated using NIRS (NIR System model 6500 Spectrometer, Silver Spring, USA) (Prevolnik et al., 2005). On the next day (48 h after slaughter) the second LD slice was cleaned of fat and connective tissue, vacuum packed, and frozen at –20°C until further analysis. For determination of cooking loss and tenderness the LD samples were thawed overnight at 4°C, weighed, and cooked in a thermostatic water bath (ONE 7-45, Memmert, GmbH, Schwabach, Germany) until the internal temperature reached 72°C. Then the drained LD samples were weighed again (difference in weights was used for cooking loss % calculation) and cooled at 4°C overnight. The next day, two 2.5 cm wide cylindrical cores were excised and shear force (maximum cutting force) was measured using TA Plus texture analyser (Ametek Lloyd Instruments Ltd., Fareham, UK) equipped with 60° V-shaped rectangular-edged blade and a crosshead speed set at 3.3 mm/s.

### Carcass lesions evaluation

The evaluation took place on the skin of the cooled carcasses, with the exception of the regions distally from the tarsal and carpal joints. The lesions were counted (only fresh lesions, inflicted

during pre-slaughter period were included) and sorted according to aetiology i.e. as lesions caused either by teeth, hoofs, or hits (strokes).

### Cortisol determination

The levels of cortisol were determined in collected serum samples using a commercially available ELISA kit (IBL International, GmBH, Hamburg, Germany). The intensity of the colour was read at 450 nm using a Varioscan Flash spectrophotometer and SkanIt Software Version 2.4.3. RE (Thermo Fisher Scientific Inc., Waltham, USA). Plasma concentrations of cortisol were expressed in ng/ml.

### Androstenone, skatole, and indole determination

Samples of subcutaneous fat were taken at the level of the last rib for the determination of boar taint compounds. Androstenone, skatole, and indole concentrations were measured by HPLC as described by Pauly et al. (2008), and the levels were expressed as µg/g of liquid fat. The detection limits were 0.24 µg/g for androstenone and 0.03 µg/g for skatole and indole.

### Statistical analysis

Analysis of variance was performed using the MIXED procedure of statistical package SAS (SAS Institute Inc., Cary, USA). For reproductive organs and boar taint compounds, the model included the fixed effects of treatment group and pen nested within treatment group. For carcass traits, the carcass weight was additionally included as a covariate. For meat quality traits, cortisol concentration, and carcass lesions, the model comprised also the slaughter batch as a random effect. When a significant effect of the treatment group ( $P < 0.05$ ) was detected, the least square means (LSM) were compared using Tukey's test.

## RESULTS AND DISCUSSION

During the experiment, six animals (four EM and two SC) were lost due to illness, death or due to balancing the number of pigs per pens. After slaughter and following preliminary data analysis, further six pigs were removed from the data set: one SC pig was actually a cryptorchid, two IC pigs did not respond to the vaccination (as shown by genital tract development), and three pigs (one from each treatment group) exhibited extremely slow growth.

Table 2. Carcass traits (LSM) in entire males, immunocastrates, and surgical castrates

	EM <i>n</i> = 19	IC <i>n</i> = 21	SC <i>n</i> = 20	RMSE	<i>P</i> -value
Carcass weight (kg)	79.4	76.6	79.0	6.0	0.29
DM fat (mm)	6.7 <sup>a</sup>	8.4 <sup>b</sup>	11.8 <sup>c</sup>	2.0	< 0.001
DM muscle (mm)	68.9	68.1	69.3	4.2	0.65
DM meat (%)	64.3 <sup>c</sup>	63.0 <sup>b</sup>	60.6 <sup>a</sup>	1.5	< 0.001
LEA (cm <sup>2</sup> )	42.9	41.9	43.6	4.7	0.54
LEA fat (cm <sup>2</sup> )	10.6 <sup>a</sup>	11.6 <sup>a</sup>	13.6 <sup>b</sup>	2.0	< 0.001
Belly leanness (1–7)	5.9 <sup>c</sup>	5.5 <sup>b</sup>	4.6 <sup>a</sup>	0.4	< 0.001
NIMF (%)	13.0 <sup>a</sup>	15.5 <sup>a</sup>	19.1 <sup>b</sup>	3.3	< 0.001
Ham/CW (%)	23.6	23.6	23.7	1.0	0.94
Ham leanness (%)	86.5 <sup>b</sup>	85.3 <sup>b</sup>	81.1 <sup>a</sup>	1.9	< 0.001
Leaf fat (kg)	0.48 <sup>a</sup>	0.65 <sup>a</sup>	0.91 <sup>b</sup>	0.3	< 0.001

EM = entire males, IC = immunocastrates, SC = surgical castrates, RMSE = root mean square error, DM fat = minimal fat thickness over the *gluteus medius* muscle, DM muscle = shortest distance between cranial end of GM and dorsal edge of vertebral canal, DM meat = carcass lean meat content according to the DM5 method, LEA = loin eye area, LEA fat = fat corresponding to loin eye area, NIMF = neck intermuscular fat (%) surface relative to the entire surface of neck cross-section area, Ham/CW = ratio between ham weight and carcass weight

<sup>a,b,c</sup>LSM values within a row with different superscript letters are significantly different ( $P < 0.05$ )



### Carcass traits

No difference was observed between treatment groups on carcass weight and most muscle development indicators including DM muscle, LEA and ham/CW weight ratio (Table 2). The differences between treatment groups were, however, significant for all traits associated with fat deposition. IC were intermediate and differed from both EM and SC in backfat thickness (DM fat), lean meat content (DM meat), and belly leanness score. The 2.4% point difference in carcass lean meat content (DM meat) between SC and IC shows an economically important advantage of the latter. In case of backfat corresponding to LEA, neck intermuscular fat, ham leanness, and leaf fat, the IC were closer to EM and both groups exhibited significantly lesser fat depots than SC.

In general, the available literature provides information about the immunocastration effect on backfat thickness or lean meat content, whereas more detailed carcass quality evaluation is rarely presented. In the present study, we evaluated the effect of immunocastration on various fat depots (subcutaneous, intermuscular, intramuscular, and subperitoneal fat) which are known to vary in deposition rate according to anatomical position, and age (Kouba et al., 1999; Stupka et al., 2008) and also to have different value for meat industry and consumers. In agreement with the results of the present

research, less subcutaneous fat depots than SC and more than EM were reported for IC by several studies (Pauly et al., 2009; Gispert et al., 2010; Morales et al., 2010). Others observed IC to be either similar to EM (Bonneau et al., 1994; Dunshea et al., 2001; Škrlep et al., 2010), closer to SC (Zeng et al., 2002; D'Souza and Mullan, 2003; Fabrega et al., 2010) or even not different from both EM and SC (Metz et al., 2002; Kim et al., 2007). The variability of the responses can be explained by the differences in experimental designs, slaughter weight and, most important, timing of the second vaccination. The longer the time that elapses between the second vaccination and slaughter, the higher is the fat deposition, as demonstrated by Turkstra et al. (2002) and Lealiifano et al. (2011). In agreement with the present study, recently performed meta-analysis showed that in terms of carcass leanness IC are intermediate between SC and EM (Batorek et al., 2012).

In case of belly leanness, the present study agrees with Fuchs et al. (2009) reporting IC to have leaner bellies than SC, but not with Škrlep et al. (2010) positioning IC closer to SC than EM. In the case of NIMF, IC pigs were similar to EM and lower than SC, in agreement with our previous observations (Škrlep et al., 2010). For leaf fat, Gispert et al. (2010) and Škrlep et al. (2010) showed the intermediate position of IC, differing from both EM and SC, whereas in the present study IC were similar to EM.

Table 3. Meat quality traits measured in *Longissimus dorsi* muscle (LSM) in entire males, immunocastrates, and surgical castrates

	EM <i>n</i> = 19	IC <i>n</i> = 21	SC <i>n</i> = 20	RMSE	<i>P</i> -value
IMF (%)	1.4 <sup>a</sup>	1.5 <sup>a</sup>	1.9 <sup>b</sup>	0.4	< 0.001
pH <sub>45</sub>	6.27 <sup>a</sup>	6.31 <sup>ab</sup>	6.40 <sup>b</sup>	0.17	0.04
pH <sub>24</sub>	5.42 <sup>a</sup>	5.52 <sup>b</sup>	5.44 <sup>a</sup>	0.08	< 0.001
Colour (1–6)	3.3 <sup>a</sup>	3.6 <sup>b</sup>	3.5 <sup>ab</sup>	0.4	0.04
<i>L</i> <sup>*</sup>	54.2 <sup>b</sup>	50.6 <sup>a</sup>	53.5 <sup>b</sup>	2.8	< 0.001
<i>a</i> <sup>*</sup>	7.8 <sup>b</sup>	6.8 <sup>a</sup>	7.1 <sup>ab</sup>	1.0	0.01
<i>b</i> <sup>*</sup>	3.6 <sup>b</sup>	2.9 <sup>a</sup>	3.4 <sup>ab</sup>	0.7	0.01
Drip loss 24h (%)	5.3 <sup>b</sup>	2.9 <sup>a</sup>	3.6 <sup>a</sup>	1.9	0.001
Drip loss 48h (%)	8.5 <sup>b</sup>	5.5 <sup>a</sup>	6.4 <sup>a</sup>	2.1	< 0.001
Cooking loss (%)	28.4	28.6	26.6	4.4	0.29
Shear force (N)	139	144	136	23	0.54

EM = entire males, IC = immunocastrates, SC = surgical castrates, RMSE = root mean square error, IMF = intramuscular fat content, pH<sub>45</sub> = muscle pH value 45 min after slaughter, pH<sub>24</sub> = muscle pH value 24 h after slaughter, Colour = subjective LD muscle colour evaluated according to a 6-point Japanese colour scale

<sup>ab</sup>LSM values within a row with different superscript letters are significantly different ( $P < 0.05$ )

### Meat quality traits

IC had lower LD intramuscular fat content than SC but similar to EM (Table 3). This is in agreement with our previous study (Škrlep et al., 2010), whereas Gispert et al. (2010) and Morales et al. (2010) reported intermediate position of IC compared to SC and EM for IMF in *semimembranosus* and *gluteus medius* muscle, respectively. Studies comparing only IC and SC reported either no difference (Morales et al., 2011) or lower LD intramuscular fat in IC (Boler et al., 2011). If the deposition of IMF is related to other fat depots, such as leaf fat, LEA fat, and neck intermuscular fat, a similar pattern is observed, i.e. IC similar to EM and lower than SC.

The rate of post-mortem pH decline was not affected by immunocastration, but IC had higher  $\text{pH}_{24}$  than EM and SC, resulting in darker (higher subjective colour score and lower Minolta  $L^*$ ), less red (lower Minolta  $a^*$ ), and less yellow (lower Minolta  $b^*$ ) meat. IC had also lower drip loss than EM (making up a difference of 3.0% points for drip loss 48 h). The higher  $\text{pH}_{24}$  in IC is not consistent with the usually observed higher  $\text{pH}_{24}$  in entire males, due to more aggressive behaviour and higher level of physical activity (Fernandez et al., 1994; Sather et al., 1995; D'Souza et al., 1998, 1999;

Cronin et al., 2003). There are not many studies dealing with the immunocastration effect on meat quality. They show for the most part the absence of any effect on  $\text{pH}_{24}$  (Pauly et al., 2009; Gispert et al., 2010; Škrlep et al., 2010; Boler et al., 2011; Morales et al., 2011;), drip loss or colour (Pauly et al., 2009; Škrlep et al., 2010; Boler et al., 2011). On the other hand, some reports indicate IC to have darker meat than SC (Silveira et al., 2008), lighter than EM (Gispert et al., 2010) or lower drip loss than SC (Miclait-Sonaco et al., 2008).

### Reproductive organs and boar taint compounds

The vaccination was not successful for all the immunocastrates. Two of the vaccinated pigs had boar-like weights of reproductive organs (Figure 1). These pigs were considered non-responders and therefore excluded from the dataset used for the statistical analysis. Non-responders have also been identified in previous studies (Zeng et al., 2002; Jaros et al., 2005; Hilbe et al., 2006). The non-response to vaccination has been ascribed either to the lack of immunological response or to acute illness.

Disregarding the non-responders, the immunocastration caused notable reduction of the size of

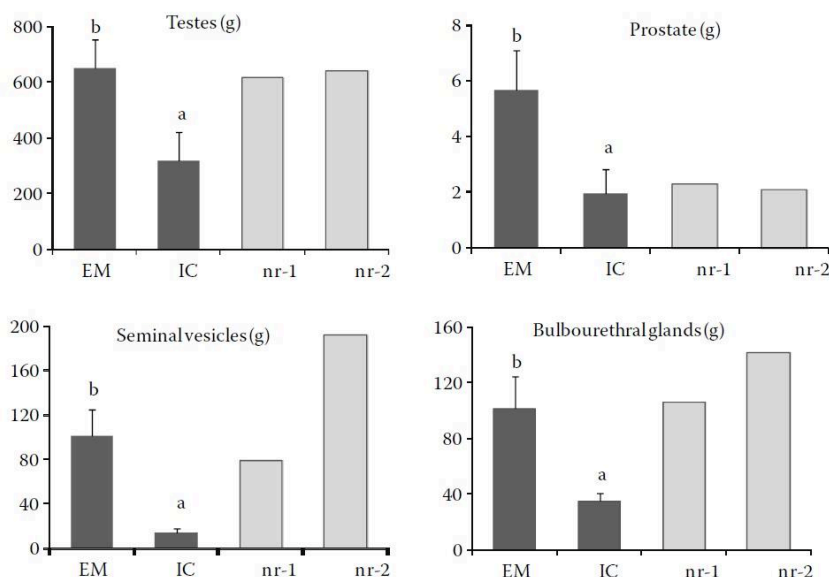


Figure 1. Weight of reproductive organs (mean  $\pm$  standard deviation) in the case of entire males (EM), immunocastrates (IC), and two non-responders (nr-1, nr-2)



testes and accessory reproductive organs (Figure 1). In IC the weight of testes was reduced to 50%, bulbourethral gland to 34%, seminal vesicles to 14%, and prostate to 35% of that measured in EM. In consistence with the reduction of reproductive organs, immunocastration significantly reduced the amount of boar taint compounds (Figure 2). The concentration of androstenone in IC dropped to around one third of the level found in EM and was similar to that found in SC. The situation was similar for skatole, while concentrations of indole were below detection limit (0.03 µg/ml liquid fat) regardless of the treatment group (data not shown). Because none of the IC or SC pigs had androstenone concentrations higher than the proposed sensory threshold (0.5 to 1.0 µg/g fat tissue, i.e. 0.9 to 1.7 µg/g liquid fat; Walstra et al., 1999), it can be concluded that vaccination was efficient in preventing androstenone-related boar taint. None of the pigs had skatole concentration above the proposed sensory threshold (0.20 to 0.25 µg/g fat tissue, i.e. 0.32 to 0.35 µg/g liquid fat).

In accordance with the present study, numerous studies have proven the effectiveness of anti-GnRH vaccine in elimination of boar taint compounds and reduction of reproductive organ size (Bonneau et al., 1994; Dunshea et al., 2001; Metz et al., 2002; Turkstra et al., 2002; Jaros et al., 2005; Zamaratskaia et al., 2008a, b; Pauly et al., 2009; Gispert et al., 2010). In the mentioned studies a reduction from 16% to 60% for the testes, 50% to 90% for the bulbourethral glands, and 36% to 90% for the seminal vesicles is reported. The comparison with the absolute values reported in the literature data is, however, difficult due to the differences in the experimental design (timing of the second vaccination, type of animals used) and

differences in analytical procedures. In the present study the recommendations of the vaccine producer were respected concerning the delay between the immunisation and slaughter. The second vaccination gives a rise of anti-GnRH antibody titres, causing then a rapid decrease of plasma testosterone levels (Bonneau et al., 1994; Dunshea et al., 2001; Zamaratskaia et al., 2008a). Consequently, a relatively sharp drop of fat androstenone concentration occurs due to the cessation of testes function. According to Claus et al (1994) at least three weeks are needed for complete clearance of androstenone from fat tissue. However, a recently published study (Lealiifano et al., 2011) suggests that only two weeks after the second vaccination might be enough for sufficient clearance (below sensory thresholds) of skatole and androstenone.

#### Carcass lesions and cortisol

There were no differences between treatment groups in the level of cortisol and incidence of carcass skin lesions (data not shown). Incidence of skin lesions was proposed as indicator of aggressiveness between pigs prior to slaughter (Turner et al., 2006). According to the literature, entire males show more aggressive behaviour in comparison to their castrated counterparts (either surgical or immunocastrates) (Cronin et al., 2003; von Borell et al., 2009; Rydhmer et al., 2010) which was not observed in the present study. The reason for the absence of any difference may be due to the fact that the pigs of different groups were not mixed even during pre-slaughter handling. Namely mixing of unfamiliar animals is one of the reasons triggering the aggression (Bolhuis et al., 2005).

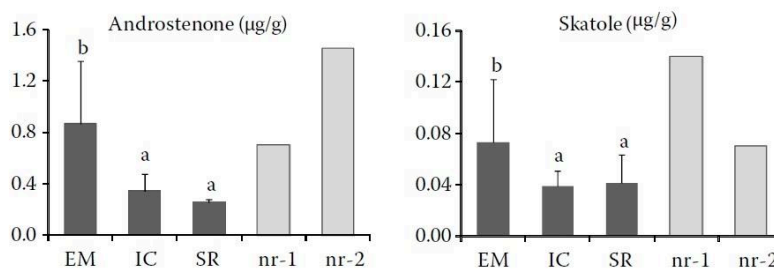


Figure 2. Fat androstenone and skatole concentrations (mean ± standard deviation) in the case of entire males (EM), immunocastrates (IC), surgical castrates (SC), and two non-responders (nr-1, nr-2)

## CONCLUSIONS

The elimination of boar taint using the immunocastration in group-housed Slovenian commercial fatteners was efficient for the majority of vaccinated pigs. Improved leanness in comparison to surgical castrates and lower meat drip loss in comparison to entire males was found proving the benefits of this new alternative to surgical castration.

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## **5.2 Steroid hormones, boar taint compounds, and reproductive organs in pigs according to the delay between immunocastration and slaughter**

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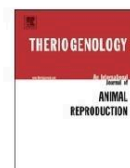
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## Steroid hormones, boar taint compounds, and reproductive organs in pigs according to the delay between immunocastration and slaughter

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Histology

### ABSTRACT

The producer of vaccine against GnRH recommends that immunocastrated pigs are to be slaughtered within 4 to 6 weeks after the second vaccination (V2). The objective of the study was to examine the effect of shorter or longer delay on steroid hormones, boar taint compounds, and morphologic and histologic traits of reproductive organs. Forty male pigs (individually housed and fed a commercial diet) were assigned within litter to four treatment groups, 10 pigs were left entire (EM27) and the others were vaccinated against GnRH (Improvac, Pfizer Animal Health) at the age of 12 and 19 weeks. Pigs were slaughtered at 21 (IC21), 24 (IC24), and 27 (IC27 and EM27) weeks of age. Two EM27 pigs died during the experiment, one IC21 pig was excluded because of illness, one IC27 pig was a nonresponder, and two pigs (IC24 and IC27) were hermaphrodites. To assess the effect on steroid hormones, blood was taken at 12, 15, 19, 21, and 24 weeks of age. Subcutaneous fat and reproductive organs were sampled after slaughter for determination of androstenone, skatole, morphologic, and histologic measurements. Immunocastration interrupted the rise of estrogen and caused a substantial fall of testosterone in IC21, IC24, and IC27 pigs. As a result, androstenone and skatole levels were successfully reduced regardless of the time elapsed from V2. The weight of the reproductive organs was also drastically reduced, the shrinkage being proportional to the length of the interval between V2 and slaughter and was the most evident for vesicular glands, followed by bulbourethral glands, and testes. Corresponding changes were observed also on a histologic level with a progressive decrease in the size and number of Leydig cells, a diminishing immunoreactivity of 3 $\beta$ -hydroxysteroid dehydrogenase/ $\Delta$ -5-4 isomerase, and luteinizing hormone receptor, along with a shrinkage of tubuli seminiferi, atrophy of seminiferous epithelium, and a loss of germ cells, indicating a disruption in testicular spermatogenetic function. Regression of the glandular tissue with a decreasing amount of secretory cells was also observed for bulbourethral and vesicular glands. The investigated physiologic, morphologic, and histologic traits were progressive with the increasing delay to slaughter (clearly seen already 2 weeks after V2), though no signs of functional or morphological restoration was observed within 8 weeks after V2.

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### 1. Introduction

Immunocastration, i.e., active immunization against gonadotrophin-releasing hormone (GnRH), represents an

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alternative to the raising of surgical castrates because it offers a solution for boar taint problems while avoiding the pain inflicted by surgical castration [1], and also for aggressive behavior observed in entire male pigs [2]. Presently, surgical castration is being questioned in the European Union and major European pork chains committed themselves to voluntarily end this practice by 2018. Since the registration of the vaccine Improvac (Pfizer Animal Health) in the European Union, this alternative has been extensively studied. As recently shown in a meta-analysis [3], immunocastration effectively eliminates boar taint compounds, is beneficial to animal welfare because of less aggression and mounting [2,4]. It is also beneficial to the performance [3,5], because the anabolic potential of boar is preserved during most of the productive life of the animals [6,7]. For the effective immunization, two doses of the vaccine need to be administered at least four weeks apart, resulting in disruption of the hypothalamic-pituitary-gonadal axis, and hence an arrest of testicular steroid synthesis by Leydig cells. Levels of testicular steroids drop sharply within a week after the effective immunization [7], but for the clearance of boar taint compounds a 4- to 6-week delay is recommended by the vaccine producer. However, castration experiments demonstrated that a shorter delay could also be considered [8] and recently Lealiifano et al. [9] demonstrated that levels of androstenone and skatole are below sensory threshold already 2 weeks after the second vaccination. This timing is important from the perspective of pig production. The shorter the delay, the more benefit from the boar-like performance can be expected. On the other hand, fluctuations in the demand or other specific market needs could also lead to a prolonged delay and pig producers need to be sure that there is no risk of restoration of testicular function and consequently boar taint. The little available literature suggests that the immunocastration effects can persist for 22 weeks after the second vaccination, as observed for androstenone and skatole levels, size of reproductive organs [10], and histomorphologic traits [11]. However, Claus et al. [12] reported a progressive restoration of testicular function starting approximately 10 weeks after second vaccination for testosterone and estradiol and, approximately 12 weeks for androstenone. Although the physiologic response to the immunization has been relatively well described, there is still a need for more information on the events in the period shortly after the effective immunization, in particular at the histologic level. Such information could improve the understanding of changes associated with the loss or eventual restoration of the function of reproductive organs. Therefore, the present study was designed to follow the dynamics of the changes induced by immunocastration on morphologic and histologic traits of reproductive organs along with the levels of steroid hormones and boar taint compounds in the period from 2 to 8 weeks after the second immunization.

## 2. Materials and methods

### 2.1. Animals and fattening trial

The experiment was carried out in accordance with the Slovenian law on animal protection [13] and took place in

a commercial pig farm between December 2010 and February 2011.

Forty crossbred pigs (progeny of Large White × Landrace breeds) were selected from 10 litters farrowed within 5 days. At the age of 12 weeks, they were transferred to the experimental stable and individually housed. Within each litter, one pig was left uncastrated, and the remaining animals were vaccinated at 12 (V1) and 19 (V2) weeks of age by means of anti-GnRH vaccine application (Improvac, Pfizer Animal Health). Pigs were fed *ad libitum* a commercial diet containing 13.0 MJ/kg of metabolizable energy, 17% crude protein, 2.6% crude fat, 4% crude fiber, 6% crude ash, and 1% lysine. Feed intake was individually monitored and pigs were weighed at the time of first and second vaccination and at slaughter. Vaccinated pigs were slaughtered at 21 weeks (IC21; N = 10), 24 weeks (IC24; N = 10), and 27 weeks (IC27; N = 10) of age at a live weight of  $84.8 \pm 2.4$ ,  $100.9 \pm 3.8$ , and  $112.3 \pm 6.6$  kg, respectively. The group of entire male pigs (EM27; N = 8) was slaughtered at the same time as IC27 pigs at a live weight of  $118.3 \pm 8.7$  kg. Slaughter took place in a commercial abattoir according to the standard procedure (approximately 1 hour transport, 2 hours of lairage, CO<sub>2</sub> stunning). At the slaughter line, testes and accessory reproductive glands were removed and taken to the laboratory for morphometric and histochemical analysis. Six pigs were removed from the dataset, one pig IC21 was removed because of illness and consequently slow growth, one IC27 was a nonresponder, two were hermaphrodites (one IC24 and one IC27), and two EM27 pigs died in the course of the experiment.

### 2.2. Blood sampling

Blood samples (approximately 9 mL) were collected from the animals (by puncturing vena cava cranialis) at 12 (V1), 15, 19 (V2), 21, and 24 weeks of age. After blood collection, the tubes were immediately placed on ice and delivered to the laboratory within 1 hour, where the blood was left to clot at 4 °C overnight. After the removal of the clot, the remaining blood serum was centrifuged at 1800 rpm for 10 minutes and supernatant stored at –20 °C until further analyses.

### 2.3. Estradiol and testosterone determination

The levels of estradiol and testosterone were determined in serum samples using commercially available ELISA kits (IBL International GmbH, Hamburg, Germany). The intensity of the color was read at 450 nm using spectrophotometer Varioscan Flash and SkanIt Software Version 2.4.3. RE (Thermo Fisher Scientific Inc., Waltham, MA, USA). For estradiol, the sensitivity of the method was 9.7 pg/mL, and the intra-assay coefficient of variation 6.8% at 91 pg/mL, and in the case of testosterone the sensitivity of the method was 0.07 ng/mL and the intra-assay coefficients of variation 5.4% at 0.68 and 7.8 ng/mL.

### 2.4. Skatole and androstenone determination

For determination of boar taint compounds, samples of subcutaneous fat were taken at the level of last rib. Androstenone, skatole, and indole concentrations were measured



by high performance liquid chromatography according to the procedure of Hansen-Møller [14] as described by Batorek et al. [15]. Concentrations were expressed as  $\mu\text{g/g}$  of liquid fat. The detection limits were 0.24  $\mu\text{g/g}$  for androstenedione and 0.03  $\mu\text{g/g}$  for skatole and indole.

### 2.5. Histologic examination

Microscopic evaluation was performed on the organs of five randomly selected pigs from each experimental group. Tissue samples from the lateral part of the vesicular gland and from the central part of the testis (above mediastinum) and bulbourethral gland, as well as the whole prostate were collected and fixed in 5% buffered formalin. After fixation, the samples were paraffin-embedded, cut to 5  $\mu\text{m}$  thick slices, stained with hematoxylin-eosin, and examined microscopically with a Nikon FXA microscope (Nikon Instruments Europe B.V., Badhoevedorp, The Netherlands). Regarding the morphometry of testes the following measurements were taken on 10 microscopic fields: (1) the cross-sectional area of tubuli seminiferi, (2) the height of seminiferous epithelium on eight tubuli seminiferi, (3) the area of the cytoplasm, as well as the nuclear area in Leydig cells, and (4) number of Leydig cells. The presence of mature spermatids was also assessed in tubuli seminiferi. The cross-sectional area of glandular acini (average of 25 acini) was measured in the bulbourethral gland, and in the vesicular gland and prostate the height of the epithelium (of 50 gland acini) was determined. Additionally the quantity (as surface) of glandular and connective tissue of the prostate was measured and the proportion of the two tissues calculated (10 microscopic fields). All morphometric traits were assessed using Lucia-G image analysis software (Laboratory Imaging Ltd., Prague, Czech Republic) and observed under a Nikon FXA microscope.

### 2.6. Immunohistologic examination

To demonstrate cell proliferation in the tubuli seminiferi of the testes, antibodies against human proliferating cell nuclear antigen (PCNA), which is expressed in the S-phase of cell division, were used (clone PC10; Dako, Glostrup, Denmark). The number of PCNA-positive spermatogonia lying next to the basal membrane of tubuli seminiferi was counted. 3- $\beta$ -Hydroxysteroid dehydrogenase/ $\Delta$ -5-4 isomerase (3 $\beta$ -HSD) was used as a marker of androgen biosynthesis in Leydig cells. To test the activity of the enzyme, antibodies against 3 $\beta$ -HSD (Novus Biologicals, Cambridge, UK) were used. The luteinizing hormone receptor (LHR) is a seven-transmembrane receptor found on the surface of Leydig cells and stimulates the production of testosterone [16]. To evaluate the quantity of LHR on Leydig cells, antibodies against LHR (Sigma Aldrich, St. Louis, MO, USA) were used. For the immunohistochemical staining, paraffin sections were mounted on the slides and covered with poly-L-lysine (Sigma Aldrich). After deparaffinization and rehydration, sections were pretreated by the antigen retrieval technique (microwave oven for 20 minutes at 1000 W in the sodium citrate buffer [pH 6.0], treated with endogenous peroxidase [20 minutes at ambient temperature] and washed with 0.05 Tris-HCl, pH 7.2–7.4 with addition of 0.15 M NaCl).

Sections were then incubated with different primary antibodies or without them (negative controls) in dilution 1:200 overnight at 4 °C. The reaction was visualized with peroxidase DAB (Envision Detection System, Dako). At the end, contrasting of sections with hematoxylin was performed.

### 2.7. Statistical analysis

The effect of the experimental group on hormones, reproductive organs, boar taint compounds, and histologic traits were analyzed, using the GLM procedure of SAS (SAS Institute, Cary, NC, USA) with treatment group as fixed effect. If a significant effect ( $P < 0.05$ ) was observed, means were compared with the Tukey test. Statistical significance was based on  $P < 0.05$ .

## 3. Results

The immunization was not successful for all vaccinated pigs. Based on the weight of testes, accessory reproductive glands and testosterone level in week 24 (data shown as a star in Figs. 1 and 2) one animal from the IC27 group was identified as a nonresponder and subsequently removed from further analyses.

### 3.1. Steroid hormones

Before V2, all the experimental pigs were physiologically entire male pigs, as confirmed by statistically insignificant differences in the levels of testosterone and estradiol between immunocastrated and entire male pigs (Fig. 1). After week 19 (V2), the levels of steroid hormones decreased in immunocastrated pigs. On the other hand, estradiol levels continued to rise in the EM27 group (1.5- and 3.1-fold increase for the periods 19–21 and 19–24 weeks of age, respectively), but an unexpected drop of testosterone was observed in week 21 for EM27 pigs. Despite that drop the level of testosterone was still six times higher in EM27 than in immunocastrated pigs ( $P < 0.0001$ ). In the subsequent period, i.e., week 24, testosterone levels increased markedly in EM27 pigs, and levels remained very low in immunocastrated pigs. At the end of the observations (age 24 weeks) considerably higher testosterone (48 times) and estradiol (2.9 times) levels were found in EM27 than in immunocastrated pigs.

### 3.2. Boar taint compounds

As shown in Figure 2, the expected effect of the immunocastration, i.e., a decrease in androstenedione (and skatole) levels, was evident in all immunocastrated groups, regardless of the interval between V2 and slaughter. All immunocastrated pigs had androstenedione concentration below the detection limit of the assay. No significant difference in skatole level was detected among groups of immunocastrated pigs.

### 3.3. Reproductive organs

The most remarkable difference in weight (in comparison with EM27 pigs, Fig. 3) was observed for vesicular

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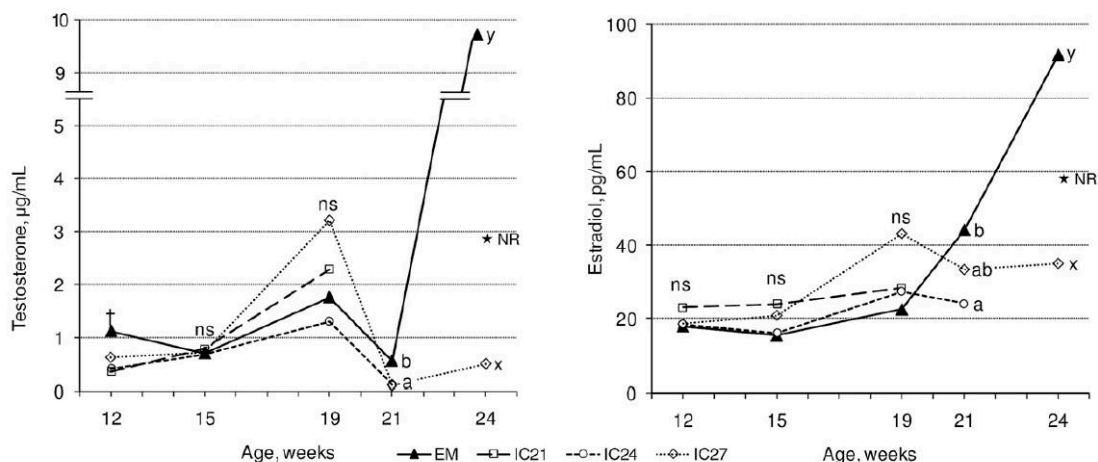


Fig. 1. Testosterone and estradiol concentrations (means) of entire male pigs (EM) from group EM27 and immunocastrated pigs slaughtered 2 (IC21), 5 (IC24), and 8 weeks (IC27) after the second vaccination (V2 at 19 weeks of age). Within age (sampling), the absence of statistical significance is denoted by 'ns', a tendency ( $P < 0.10$ ) is denoted with †; in case of significant effect ( $P < 0.05$ ) differences between groups are indicated with different lowercase letters. The stars denote the values (not included in the statistical analyses) observed for a nonrespondent (NR) pig in the IC27 group.

glands (8.3, 14.3, and 16.7 times lower for IC21, IC24, and IC27, respectively) followed by bulbourethral glands (2.9, 3.6, and 4.8 times lower for IC21, IC24, and IC27, respectively), testes (2.6, 3.1, and 3.1 times lower for IC21, IC24, and IC27, respectively), or prostate (2.1, 4.3, and 2.2 times lower for IC21, IC24, and IC27, respectively). Although the weight of reproductive organs was statistically similar in vaccinated pigs slaughtered 2, 5, and 8 weeks after V2, the difference with EM27 pigs was, except for prostate, numerically larger in pigs with a longer interval between V2 and slaughter.

#### 3.4. Histologic parameters

Histomorphologic properties of testes were dramatically affected by immunocastration, with a dynamic that is related to the interval between V2 and slaughter (Fig. 4). Leydig cell

area was bigger in EM27 than in any of the immunocastrated groups (Fig. 4A) and decreased progressively with a longer delay to slaughter (being 1.9, 2.9, and 3.1 times lower in IC21, IC24, and IC27, respectively, than in EM27). The nuclear area of Leydig cells (Fig. 4B) was also bigger in EM27 than in IC24 or IC27 pigs but did not differ from that observed in IC21 pigs. Again, a progressive regression was observed with increased interval between V2 and slaughter (1.2 and 2.2 times for IC24 and IC27, respectively). The number of Leydig cells (Fig. 4C) was significantly lower only in IC27 as compared with EM27 pigs. Morphologic changes of Leydig cells were also observed as an outcome of the immunocastration. Compared with EM27 pigs, Leydig cells were not only progressively smaller in IC21, IC24, and IC27 pigs, but were also losing their polygonal shape. Already 2 weeks after V2 (in IC21), a smaller volume of interstitial tissue was observed, and in the most affected clusters of IC24 and even more IC27 pigs, a disruption of the

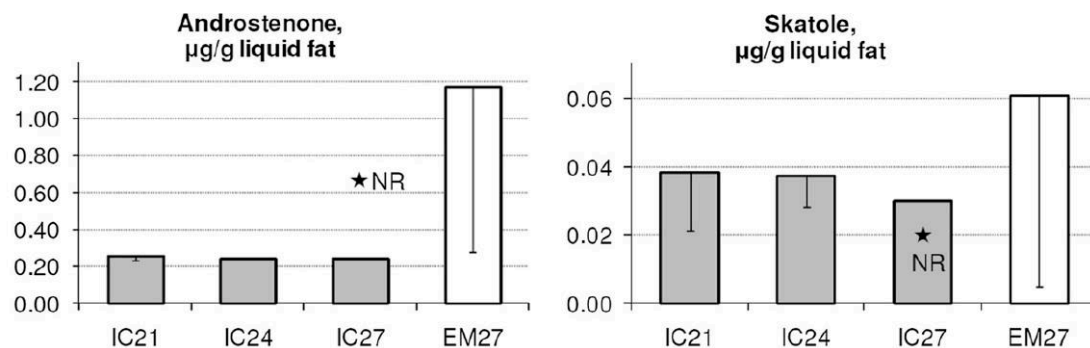


Fig. 2. Androstenone and skatole levels (mean  $\pm$  SD) in entire male pigs (EM27) and immunocastrated pigs slaughtered 2 (IC21), 5 (IC24), and 8 (IC27) after the second vaccination. Statistically significant ( $P < 0.05$ ) differences between treatment groups are denoted by different lowercase letters. The stars denote the values (not included in the statistical analyses) observed for a nonrespondent (NR) pig in the IC27 group.



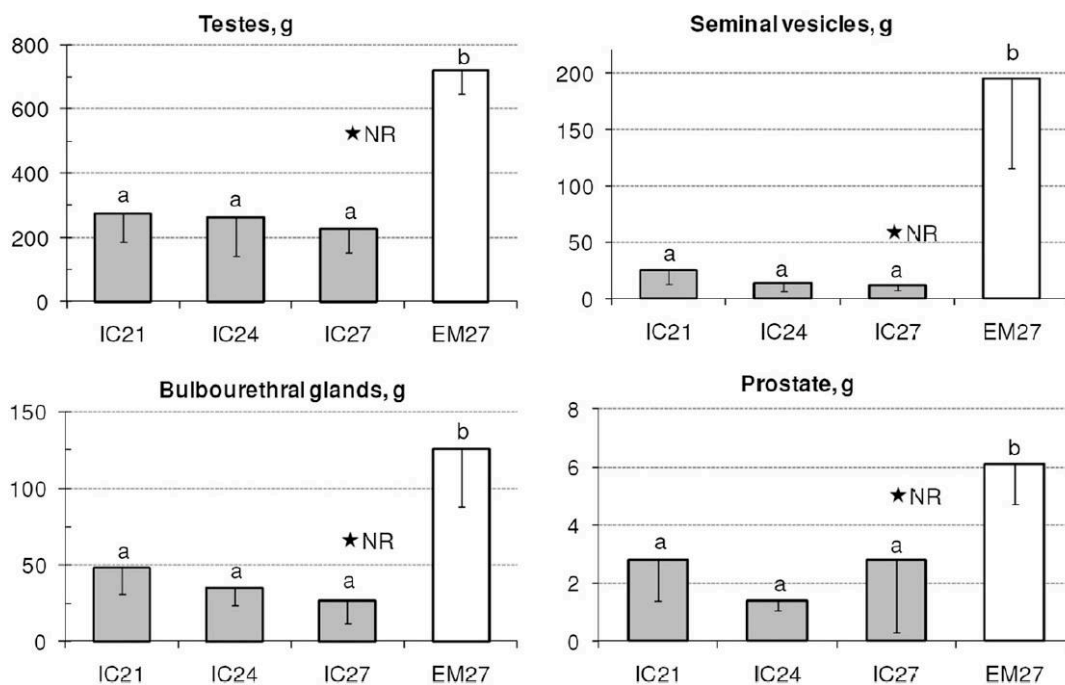


Fig. 3. Weight of reproductive organs (mean  $\pm$  SD) in entire male pigs (EM27) and immunocastrated pigs slaughtered 2 (IC21), 5 (IC24), and 8 weeks (IC27) after the second vaccination. Statistically significant ( $P < 0.05$ ) differences between treatment groups are denoted by different lowercase letters. The stars denote the values (not included in the statistical analyses) observed for a nonrespondent (NR) pig in the IC27 group.

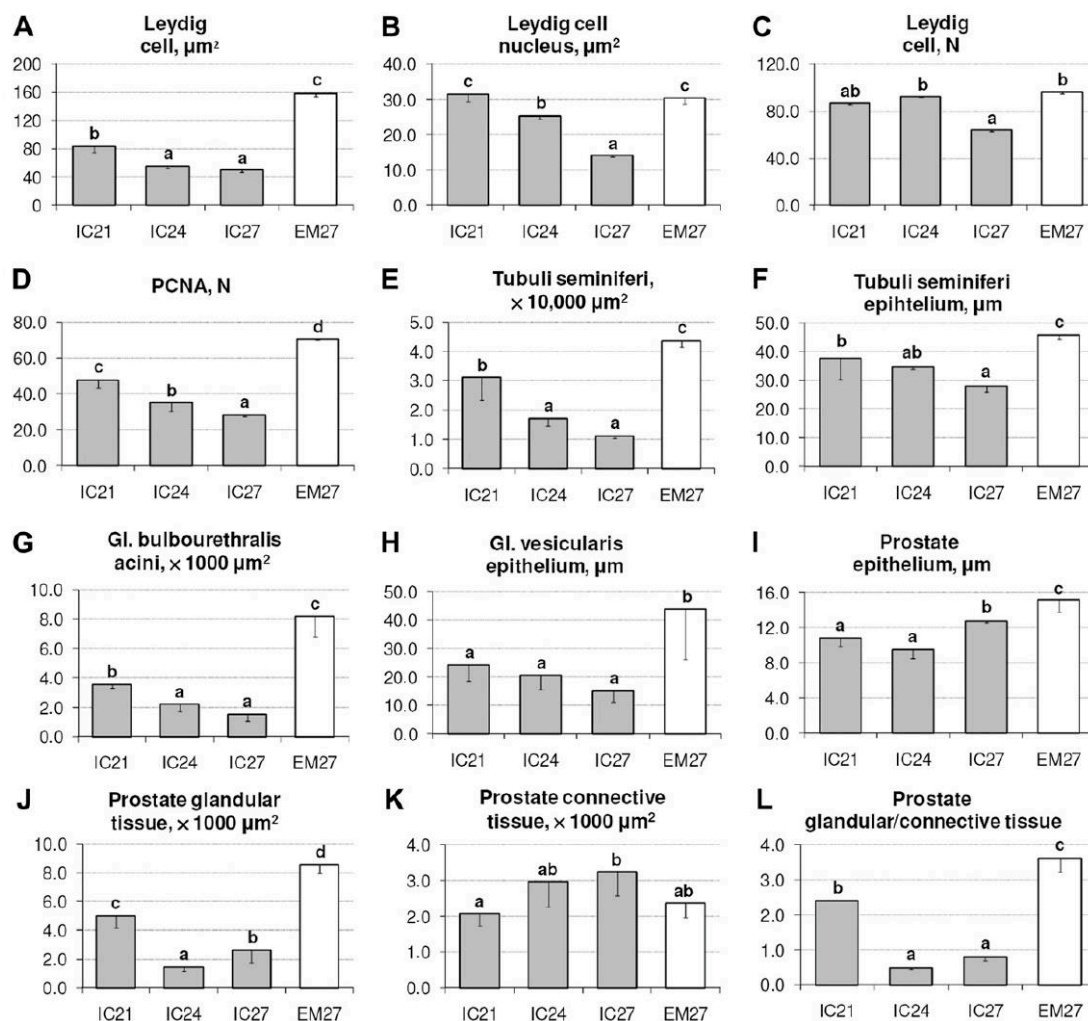
interstitial tissue could be noted (Fig. 5). Prominent nucleus and nucleolus were not displayed in Leydig cells of immunocastrated pigs and appeared shrunken, with borders between cells difficult to determine (Figs. 6 and 7). With the reduction of cell volume a more abundant collagen layer in the interstitial space was observed.

Localization of steroidogenic enzymes essential for the synthesis of sex steroid hormones in the Leydig cells ( $3\beta$ -HSD immunostaining), indicated strong immunoreactivity, mostly in the testicular interstitium (Fig. 6). Leydig cells stained with  $3\beta$ -HSD were clearly visible, large and prominent in EM27, however they were progressively diminishing in size and staining intensity with a longer interval between V2 and slaughter. Immunoreactivity for  $3\beta$ -HSD was observed in the whole cytoplasm of the Leydig cells and was the most prominent in EM27 pigs; some activity was also observed in IC21 pigs, whereas it was diminishing in IC24 and IC27 pigs.

The immunostaining of LHR, important for the regulation of Leydig cells function, showed similar pattern as observed in the case of  $3\beta$ -HSD. In testicular tissue of EM27 pigs, the LHR reaction was moderate to strong and confined to Leydig cells. Individual Leydig cells showed heterogeneous response (from weak to very strong staining) of cytoplasmic antigens. Some immunoreactivity was still observed in IC21 pigs, but much less in IC24 pigs, whereas it was completely absent in the shrunken Leydig cells of IC27 pigs (Fig. 7).

A fully developed testicular tissue was revealed in EM27 pigs as observed by microscopic assessment of morphologic features (Fig. 5), additionally visualized with PCNA staining for a detection of spermatogonia proliferation. All signs of active spermatogenesis were present, including all the phases of spermatocyte development (from spermatogonia up to spermatozoa), spermatids with well distinguished heads, and dense and long nucleus and tail in the lumen of tubuli seminiferi. The spermatogenesis process was on the other hand clearly disrupted in immunocastrated pigs. Only in IC21 pigs mild atrophy signs of tubuli seminiferi were observed, seen as spermatocyte loss, lower number of germ cells, and impaired spermatogenesis. The disruption was more advanced in IC24 pigs and progressed further in IC27 pigs as demonstrated by a severe loss of germ cells (visible as a decrease in the number of germ cell layers, including almost complete disappearance of germ cells, undifferentiated germ cells, whereas in some tubules only a single layer of germ cells or the 'Sertoli-cell-only' phenotype was observed). Tubuli seminiferi were shrunken, many empty spaces were visible inside tubules with almost no spermatocytes or round spermatids, pointing out the impaired spermatide maturation. Observed spermatogonia were mostly without mitotic features, mitosis was reduced, and only a few or no mature spermatids were noticed.

In all examined pigs, tubuli seminiferi were lined with the spermatogonia and Sertoli cells (Fig. 5). Immunohistochemical staining with antibodies against PCNA (detecting



**Fig. 4.** Histologic measurements (mean  $\pm$  SD) for entire male pigs (EM27) and immunocastrated pigs slaughtered 2 (IC21), 5 (IC24), and 8 weeks (IC27) after the second vaccination (V2 at 19 weeks of age). (A) Leydig cell area, (B) Leydig cell nuclear area, (C) number of Leydig cells, (D) number of PCNA-positive spermatogonia at basal lamina of tubuli seminiferi, (E) area of tubuli seminiferi, (F) epithelium height of tubuli seminiferi, (G) acini area of bulbourethral glands, (H) epithelium height of vesicular glands, (I) epithelium height of prostate gland, (J) prostate glandular tissue area, (K) prostate connective tissue area, and (L) ratio between prostate glandular and connective tissue. Statistically significant ( $P < 0.05$ ) differences between treatment groups are denoted by different lowercase letters. Gl, glandula; PCNA, proliferating cell nuclear antigen.

the proliferation of spermatogonia) indicated a progressive decrease of proliferation in immunocastrated pigs, as demonstrated by lower PCNA-positive cell counts (1.5, 2, and 2.5 times lower than in EM27, for IC21, IC24, and IC27, respectively; Fig. 4D). In addition, a progressively reduced tubuli seminiferi area (1.4, 2.6, and 4 times lower than in EM27, for IC21, IC24, and IC27, respectively; Fig. 4E) and a reduction of the height of seminiferous epithelium (1.2, 1.3, and 1.6 times lower than in EM27 for IC21, IC24, and IC27 pigs, respectively; Fig. 4F) were also observed.

As regards to stereological measurements of accessory sexual glands, i.e., bulbourethral gland, vesicular gland, and

prostate, their histologic characteristics were also clearly affected by immunocastration. The analysis showed significant differences between immunocastrated pigs and EM27 and also among the various groups of immunocastrated pigs in agreement with progressive weight loss of the glands. The most dramatic effect in relation to EM27 was observed for the area of glandular tissue of bulbourethral gland (2.3, 3.7, and 5.3 times smaller than EM27 in IC21, IC24, and IC27 pigs, respectively; Fig. 4G). Furthermore, a progressive reduction was observed also for the epithelium height of the vesicular gland (2.2, 2.1, and 2.9 times smaller than EM27 for IC21, IC24, and IC27 pigs,



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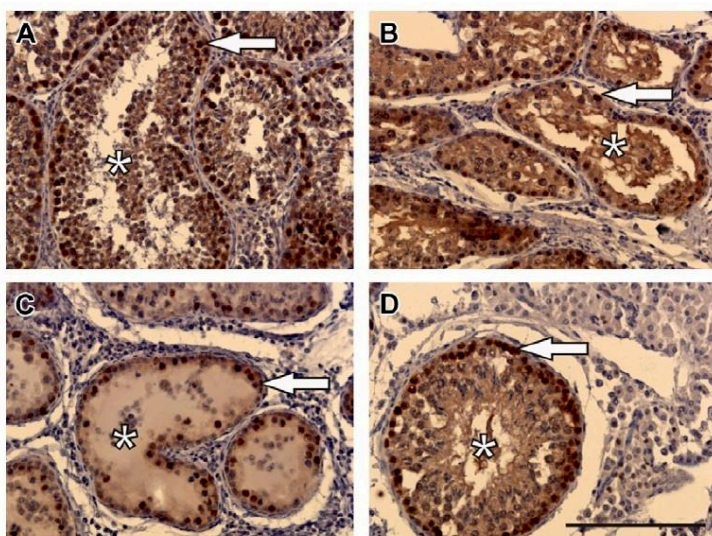


Fig. 5. Light microscopy photographs of testicular tissue in immunocastrated pigs slaughtered 2 (IC21) (A), 5 (IC24) (B), and 8 (IC27) (C) weeks after the second vaccination in comparison with EM27 pigs (D), i.e., entire male pigs slaughtered at the same time as IC27 pigs. Immunohistochemical staining for PCNA demonstrating proliferation in spermatogonia in the layer adjacent to the basal membrane of tubuli seminiferi (arrows). Note: A diminishing diameter of tubuli seminiferi (asterisk) and number of proliferating spermatogonia, along with a decreasing epithelium height is observed from IC21 to IC27 as compared with EM27 pigs (fully developed and functional). Bar = 500  $\mu$ m. PCNA, proliferating cell nuclear antigen.

respectively; Fig. 4H). It is worth noting, that in the case of the prostate, an increase of the epithelium height was observed in IC27, after the initial atrophy found in IC21 and IC24 pigs (1.4, 1.6, and 1.2 times lower than in EM27 for IC21, IC24, and IC27 pigs, respectively; Fig. 4I). Nevertheless, it remained significantly lower than in EM27 pigs. Similarly, prostate glandular tissue area (although significantly decreased by the immunocastration) was increased in IC27 pigs after an initial reduction in IC21 and IC24 pigs (Fig. 4J). On the other hand, prostate connective tissue progressively increased with the time elapsed from V2 to slaughter (Fig. 4K). As a result (Fig. 4L), a ratio between prostatic glandular and connective tissue was similar for IC21 and EM27 pigs (3.6 and 2.9 respectively), however this ratio was considerably reduced in favor of connective tissue in IC24 and IC27 pigs (0.5 and 0.8, respectively).

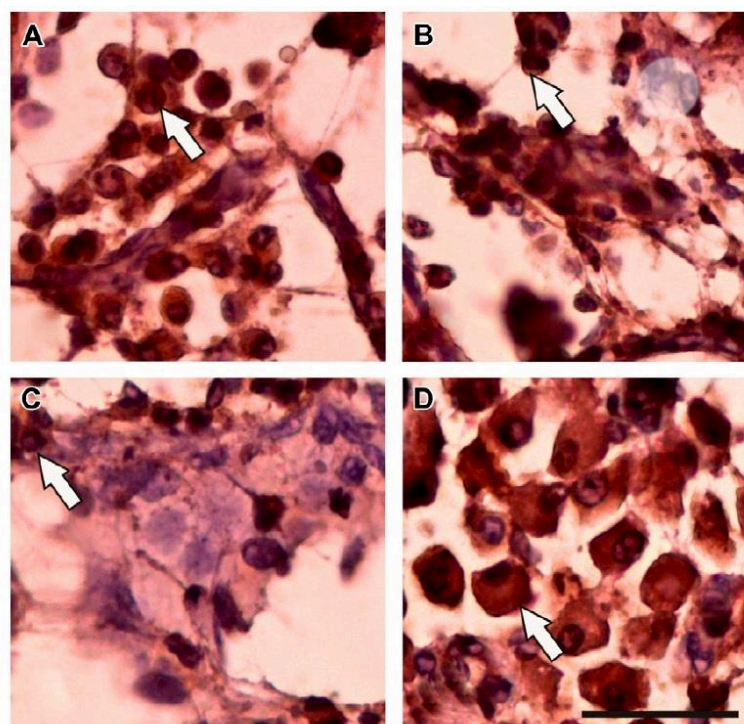
In agreement with stereological measurements, microscopic examination of bulbourethral glands revealed a regression of glandular tissue having less secretory and more connective tissue in immunocastrated than in EM27, the changes being more intensive with increased time elapsed from V2 (Fig. 8). In vesicular glands, a distinctive progressive regression of glandular epithelium was observed for immunocastrated; only a few glandular tubes and connective tissue skeleton of the glands remained in IC21 and IC24, and the amount of secretory diminished progressively in IC27 (Fig. 9). A similar trend (i.e., decreasing amount of glandular and increasing amount of connective tissue) could be observed in the case of prostate (Fig. 10), however for IC27 an increase of epithelium and glandular tissue was found in comparison with IC24.

#### 4. Discussion

The main focus of our experiment was to study the effect of immunocastration in the period (2–8 weeks after V2) after the second vaccination, on the changes of steroidogenesis (level of steroid hormones and boar taint compounds), spermatogenesis (morphologic, histologic, and immunohistochemical changes of reproductive organs), along with the changes in accessory reproductive glands: bulbourethral glands, vesicular glands, and prostate. As far as we know this is the first study presenting a deeper insight into the morphologic and histologic changes occurring in accessory glands and testes because of immunocastration using different immunohistochemical markers in parallel with the effect on the levels of testosterone, estradiol, and boar taint compounds. Overall, our data indicate that the regression of reproductive glands was more pronounced when the delay between the second vaccination and slaughter increased. It could be that the influence of the delay between V2 and slaughter was biased by an effect of age because vaccinated pigs were not compared with entire pigs at the same ages except those from the IC27 group. However, reproductive organs are still developing in entire pigs between 21 and 27 weeks of age and hence the decrease with age in the ratios between vaccinated and entire pigs of the same age would have been even more pronounced for IC21 and IC24 groups.

In the present experiment, GnRH was effectively 'immunoneutralized' in 29 out of 30 vaccinated pigs, as confirmed by the levels of sexual hormones, boar taint compounds, histomorphologic, and immunohistochemical measurements. Several reports [17–19] demonstrated that





**Fig. 6.** Light microscopy photographs of Leydig cells in immunocastrated pigs slaughtered 2 (IC21) (A), 5 (IC24) (B), and 8 (IC27) (C) weeks after the second vaccination in comparison with EM27 pigs (D), i.e., entire male pigs slaughtered at the same time as IC27 pigs. Immunohistochemical staining for 3 $\beta$ -HSD demonstrating steroidogenic activity in Leydig cells (arrows). Note: progressive diminishing in size and steroidogenic activity of Leydig cells with the time elapsed from the second immunization from (A) to (C) and in comparison with entire male pigs (fully active). Bar = 100  $\mu$ m. 3 $\beta$ -HSD, 3- $\beta$ -hydroxysteroid dehydrogenase/ $\Delta$ -5-4 isomerase.

anti-GnRH vaccination is not always successful. In the present study, only one vaccinated pig was denoted as a nonresponder (not included in the analysis) based on higher weight of not only testis but also other accessory sexual glands as well as plasma testosterone.

#### 4.1. Steroid hormones

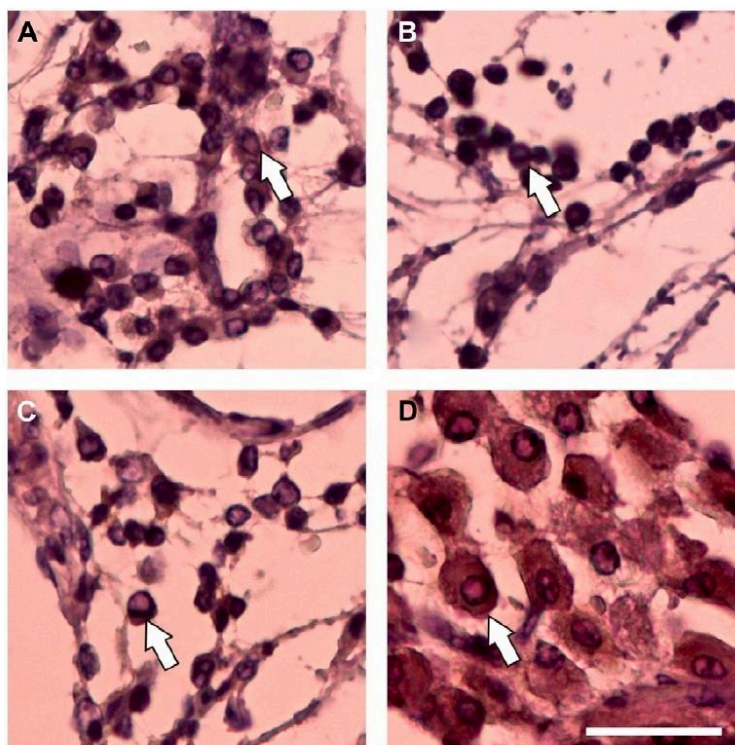
An initial rise of estradiol and testosterone was observed between weeks 15 and 19 as a consequence of early pubertal development. In pigs, this process starts at the age of 15 to 17 weeks and leads to the peak of steroids at the age of approximately 6 months [20,21]. Thereafter the levels of sexual hormones slightly decrease and stabilize [20,22,23]. Because estradiol and testosterone levels in entire pigs demonstrated a continual increase in the period from week 19 to 24, it can be assumed, that V2 was conducted at an early stage of the pubertal development. The second vaccination (week 19) interrupted the rise of estrogen levels and provoked a considerable fall of testosterone in immunocastrated pigs (recorded at week 21 and week 24), in agreement with the results of Claus et al. [7,12] reporting a rapid increase in GnRH antibody titers after V2, followed by a marked decrease of gonadal steroids within 5 to 10 days after V2. The drop of testosterone observed in entire male

pigs in week 21 was not expected, and could be because of 'uncontrolled' stress or to health problems, that are known to reduce testosterone levels [24,25]. Despite this drop, six times higher testosterone concentration in EM27 than immunocastrated pigs together with the results for estradiol (no drop in EM27 pigs in week 21) confirm the effective disruption of steroidogenesis because of the immunocastration already 2 weeks after V2. A prolonged delay until slaughter (8 weeks after V2 in the present experiment) was not accompanied by a restoration of steroidogenesis. This result agrees with the studies showing that immunocastrated pigs present low levels of testosterone [11,26] and estradiol [27] for approximately 4 to 10 weeks after V2.

#### 4.2. Boar taint compounds

As a result of the interrupted steroidogenesis, the levels of androstenone and skatole deposited in fat were sufficiently reduced in immunocastrated pigs to fall well below the established sensory detection thresholds (0.5–1.0  $\mu$ g/g for androstenone; 0.20–0.25  $\mu$ g/g for skatole), even in those slaughtered 2 weeks after the second immunization. Because of the experimental design (steroids still rising when pigs were effectively immunized), we cannot conclude whether 2 weeks were sufficient for the clearance of the compounds





**Fig. 7.** Light microscopy photographs of Leydig cells in immunocastrated pigs slaughtered 2 (IC21) (A), 5 (IC24) (B), and 8 (IC27) (C) weeks after the second vaccination in comparison with EM27 pigs (D), i.e., entire male pigs slaughtered at the same time as IC27 pigs. Immunohistochemical staining for LHR demonstrating Leydig cells function. Immunohistochemistry was performed using polyclonal antibodies against the extracellular domain of the LHR. Note: LHR protein was detectable in the Leydig cells (arrows), mostly in EM27, but less in IC21 or was almost totally absent in IC24 and IC27 pigs. Bar = 100  $\mu$ m. LHR, luteinizing hormone receptor.

or if their levels were still relatively low at the time of the second immunization. A successful elimination of boar taint was demonstrated at least 4 weeks after V2 [18,26], whereas Claus [28], based on the experiment with surgical castration of adult boars, proposed a delay of 3 to 6 weeks, depending on the age of boars. The latest study of Lealiifano et al. [9], focusing on the onset of the slaughter relative to V2, however, concluded that 2 weeks should be enough. Their study, similarly as in the present one, dealt with relatively young and light pigs (slaughter age and weight were 22 weeks and 106 kg with control boars exhibiting relatively low levels of skatole and androstenone), thus making the results applicable only for standard commercial fatteners.

#### 4.3. Reproductive organs

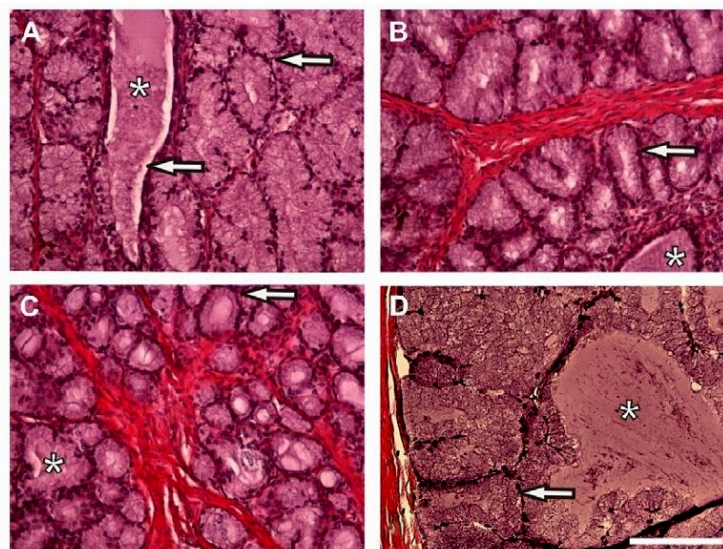
It has been shown that immunocastration drastically reduces the size of reproductive organs [3]. Moreover, the literature review by Bonneau [29] demonstrated that this shrinking is proportional to the length of the interval between V2 and slaughter, which was also observed in the present study. Size reduction was the most important for vesicular glands, followed by bulbourethral glands and testes, as previously stated by Bonneau [29]. Our results indicate no recovery within 8 weeks after V2, in accordance

with the study of Einarsson et al. [30] observing no restitution of testes and bulbourethral gland for 22 weeks post V2, or with Hilbe et al. [17] who reported a single case of testes regeneration and fertility only 60 weeks after the last (fifth) immunization.

#### 4.4. Histologic parameters

Entire male pigs at 27 weeks of age exhibit fully developed testicular structures (i.e., tubuli seminiferi composed of seminiferous epithelium with wide intratubular lumen, large interstitium with peritubular myoid cells, collagen fibers, intracellular spaces, and Leydig cells that were largely dispersed in clusters surrounding blood vessels) in accordance with previous observations [31,32]. Compared with EM27, testicular histology was clearly affected by immunocastration. Interstitial Leydig cells, the principal steroid-producing cells, were notably affected by immunocastration in the present study. The changes in the morphology of Leydig cells were consistent with the loss of the function (i.e., decrease in testosterone, androstenone). In agreement with other studies [11,17,30], immunocastration decreased size and number of Leydig cells along with a shrinkage of cytoplasm and nucleus and loss of the distinctive shape. The extent of changes increased with the

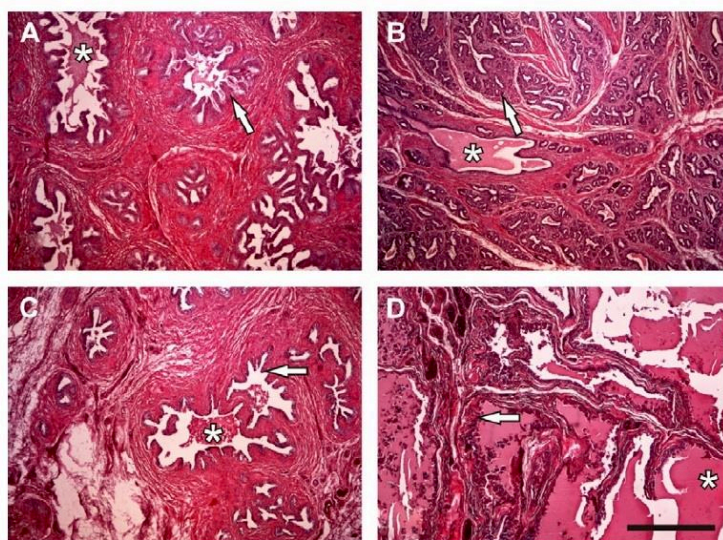




**Fig. 8.** Light microscopy photographs of bulbourethral gland tissue in immunocastrated pigs slaughtered 2 (IC21) (A), 5 (IC24) (B), and 8 (IC27) (C) weeks after the second vaccination in comparison with EM27 pigs (D), i.e., entire male pigs slaughtered at the same time as IC27. Note: a diminishing size of glandular acini (arrows) and secretory products (asterisks) in bulbourethral gland of vaccinated pigs as compared with EM27 pigs. Bar = 500  $\mu$ m.

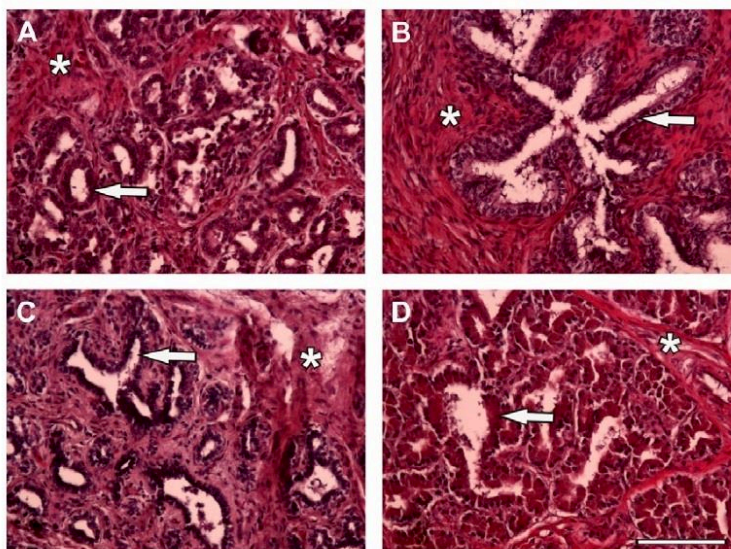
duration from V2 to slaughter, however changes were notable already 2 weeks after the second vaccination. It is difficult to conclude that smaller Leydig cell area in IC21 (than in EM27) is only because of immunocastration, because Leydig cells in pigs were reported to enlarge until the age of 22 to 25 weeks [22,33]. Nevertheless, 2 weeks after V2 (in IC21) Leydig cell nuclear area was not reduced

as it was in IC24 and IC27. Diminishing immunoreactivity for  $3\beta$ -HSD (key steroidogenic enzyme) in immunocastrated pigs further substantiates the loss of Leydig cells ability to produce gonadal steroids. The observed progressive diminution of  $3\beta$ -HSD immunoreactivity in immunocastrated pigs is in agreement with Leydig cell hypoplasia, denoted for IC27, in which Leydig cells were practically



**Fig. 9.** Light microscopy photographs of vesicular gland tissue in immunocastrated pigs slaughtered 2 (IC21) (A), 5 (IC24) (B), and 8 (IC27) (C) weeks after the second vaccination in comparison with EM27 pigs (D), i.e., entire male pigs slaughtered at the same time as IC27. Note: a diminishing size of glandular acini (arrows) and secretory products (asterisks) in vesicular gland of vaccinated pigs as compared with EM27 pigs. Bar = 1000  $\mu$ m.





**Fig. 10.** Light microscopy photographs of prostate gland tissue in immunocastrated pigs slaughtered 2 (IC21) (A), 5 (IC24) (B), and 8 (IC27) (C) weeks after the second vaccination in comparison with EM27 pigs (D), i.e., entire male pigs slaughtered at the same time as IC27 pigs. Note: the diminishing in the size of glandular acini (arrows) and increase in the amount of connective tissue (asterisks) in vaccinated pigs in comparison with EM27 pigs. Bar = 1000  $\mu$ m.

devoided of 3 $\beta$ -HSD immunoreactivity. Lack of 3 $\beta$ -HSD staining, reflecting a deficiency of the key enzyme in testosterone biosynthesis, can explain a suppression of testicular steroidogenesis in Leydig cells of immunocastrated pigs. In addition, staining against LHR showed a marked decrease in intensity from IC21 to IC27 (completely absent). The reduction of testicular LHR after anti-GnRH immunization was also shown by Awoniyi et al. [34] and is probably because of the lack of LH resulting from the hypothalamic-pituitary-gonadal axis disruption [35].

The changes occurring in the Leydig cells coincide with alterations in the morphology and functionality of the tubuli seminiferi (i.e., spermatogenesis). Regression of the Leydig cells notably affected histomorphometric and functional properties of seminal tubules. The changes included time-dependant shrinking of tubuli seminiferi along with progressive diminution of germ cell layers. Whereas the entire boars fully developed spermatogenesis at the time of the slaughter, milder signs of tubuli seminiferi atrophy were observed in IC21 pigs (seen as spermatocyte loss, lower number of layers of germ cells, impaired spermatogenesis). In the case of IC24 and IC27 pigs, even more drastic atrophy (shrinkage of tubuli seminiferi), and loss or even absence of germ cells with severely impaired spermatid maturation, were noted, indicating a clear disruption of spermatogenesis at that stage. The presence of tubuli with only Sertoli cells is indicative of the irreversibility of the process, as previously suggested by Einarsson et al. [11] for early vaccinated pigs. Focusing on spermatogenesis Einarsson et al. [30] pointed out a persisting effect of immunocastration (up to 22 weeks after V2) and the importance of the vaccination timing, with the severity of the changes being far more prominent (or even irreversible) in early vaccinated pigs [11].

Regression of the glandular tissue accompanied by a decreasing amount of secreta was observed in the case of bulbourethral and vesicular glands, indicating the loss of steroids. Similarly as for testes, the extent of the changes increased with the time elapsed from V2. Interestingly, prostate did not show the same trend and in IC27 glandular tissue was increased indicating a possible recovery, which had been proved on a hormonal level after approximately 10 [12] or 22 weeks post V2 [10]. In general, our results agree with the previously published literature regarding the regression of accessory reproductive glands and their morphology [11,17,30]. However, the mentioned studies mainly focused on the morphology, and the present study provides additional information about the time-related changes on a histophysiological level.

## 5. Conclusions

The results of the present study, addressing time-related effect of the immunocastration on physiologic parameters related to boar taint, along with morphometric and histologic parameters related to reproductive function, demonstrate a disruption of the steroidogenesis and deposition of boar taint compounds, suppressed growth of reproductive organs, and disturbance of spermatogenic function already 2 weeks after the effective immunocastration. The changes observed were progressive with an increasing delay until slaughter, with nearly no signs of functional restoration observed within 8 weeks after the second vaccination. It should however be stressed that, regarding the restoration of the reproductive function, the present results are valid in the case of relatively young and light-weight pigs, such as used in conventional pig fattening, and additional experiments would be needed for mature boars.



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## 6 GENERAL DISCUSSION

Major compounds responsible for boar taint, androstenone (Patterson 1968) and skatole (Vold 1970), have been identified 45 years ago, but the idea of immunocastration, as a method to limit boar taint accumulation, originates from the late eighties (Falvo et al. 1986) and is being intensively studied in recent years, since the registration of the first commercially available product for immunocastration of pigs (Improvac<sup>®</sup>, Zoetis Florham Park, NJ, USA) and because of the increasing questioning on the welfare of pigs surgically castrated without anaesthesia in EU. Immunocastration uses natural immune system of the pig for production of specific antibodies that binds and neutralises the GnRH and by that, blocks the HPG axis. It effectively inhibits testis growth and sexual steroids synthesis (Hilbe et al. 2006, Einarsson et al. 2009, Fang et al. 2010, Einarsson et al. 2011) and can be successfully used as alternative to physical castration of pigs (Dunshea et al. 2001). According to the manufacturer's instructions, the vaccine should be administered in two doses at least four weeks apart. Although no withdrawal period is needed, it is recommended that the V2 is performed four to six weeks before slaughter, in order to ensure clearance of boar taint substances from adipose tissue. However, our results (see 4.4 and 5.2-Kubale et al. 2013) in agreement with literature demonstrated that a shorter (Lealiifano et al. 2011, 5.2-Kubale et al. 2013) or longer delay could be considered (4.4, Claus et al. 2008, Einarsson et al. 2009). Levels of androstenone and skatole are below sensory threshold already two weeks after V2 at commercial slaughter weights (Lealiifano et al. 2011, 5.2-Kubale et al. 2013) and the effect of immunocastration can persist for at least 10 weeks after V2 (Claus et al. 2008, Zamaratskaia et al. 2008b, Einarsson et al. 2009). This timing is important from the perspective of pig production. The shorter the delay, the more benefit from the boar-like performance can be expected, whereas fluctuations in the demand or other specific market needs could lead to a prolonged delay and pig producers need to be sure that there is no risk of boar taint.

## 6.1 Effect of immunocastration on boar taint compounds and reproductive organs

The effectiveness of immunocastration has been assessed in the first part of present thesis with meta-analytical approach, combining the results from studies conducted with experimental vaccines as well as with the commercially available anti-GnRH vaccine Improvac<sup>®</sup>. The results of meta-analysis (see 4.1-Batorek et al. 2012a) show efficient immunocastration with the magnitude of the response being by far the largest for reproductive organs and boar taint substances. This has been shown in all studies, despite the high heterogeneity between studies due to different vaccination protocols used. In agreement with Brunius (2011), the effects on reproductive organs are larger for early vaccination than for late vaccination. The meta-analysis shows that the largest reduction is observed for seminal vesicle weight. This is related to their anatomical structure of liquid-containing vesicles, which can be quickly resorbed, whereas the other accessory glands and testes take more time to regress due to firmer structure (Bonneau 2010). This result supports the suggestion of Bonneau (2010) to use seminal vesicles as a diagnostic tool to assess the success of immunocastration at slaughter. Consistently with the regression of the reproductive tract, a loss of functional activity is observed at a histological level (Falvo et al. 1986; Grizzle et al. 1987, Awoniyi et al. 1988). Our results (see 5.2-Kubale et al. 2013) confirm that immunocastration reduces the mean diameter of the seminiferous tubules, the number of spermatogonia and spermatocytes, induces atrophy of the Leydig cells and reduces the weight of glandular tissues and secretory products of accessory sexual glands. However, the intensity of changes occurring at a histological level is dependent on the time of immunization (5.2-Kubale et al. 2011 and Einarsson et al. 2011). The impairment of testis functionality is also results in the reduction of boar taint compounds in adipose tissue (see 4.1-Batorek et al. 2012a), in IC compared to EM. However the elimination of androstenone and skatole is not fully complete, as shown by the small but significant differences between IC and SC for both substances in present meta-analysis. Furthermore, our results indicate that neither quantitative feed restriction (see 4.2-Batorek et al. 2012b) nor feeding fibre-rich diets (see 4.4) to IC increased the risk for skatole

formation in hindgut and boar taint accumulation in fat. In agreement Van Oeckel et al. (1998) have found no effect of fiber-rich diets fed to EM pigs on skatole production in hindgut.

## 6.2 Effect of immunocastration and different feeding strategies on growth performance

Physiologically IC appears to be similar to EM before V2, with mostly no difference in growth performance (see 4.1-Batorek et al. 2012a). However feed intake tended to be higher in IC than in EM, which might be explained by an early response to the vaccination in some pigs, shown to have reduced LH and testosterone levels after V1 (Turkstra et al. 2002). The majority of IC produces a sufficient quantity of antibodies to neutralize all secreted GnRH only shortly after V2. Consequently LH and steroid secretions are suppressed and the metabolism adapts itself in approximately seven days after V2 (Claus et al. 2007). The largest effect observed is a drastic increase in feed intake comparatively to EM (for approximately 450 g/day; 4.1-Batorek et al. 2012a, Millet et al. 2011, Dunshea et al. 2013). This effect was related firstly to the sharp reduction in the production of androgens and oestrogens, known to negatively affect feed intake (Claus and Weiler 1987), and secondly to reduced socio-sexual behaviour in IC compared to EM (Cronin et al. 2003). Likewise, feed intake of IC after V2 is slightly higher compared with SC (for approximately 105 g/day, 4.1-Batorek et al. 2012a, Millet et al. 2011, Dunshea et al. 2013) and our results (see 4.4) also indicate, that daily maximal NE intake is achieved by IC following V2 regardless of the dietary energy content and can be described as  $2.86 \text{ MJ/kg} \times \text{BW}^{1.43} - 463 \text{ MJ}$ . A possible explanation for this effect pertains in hormonal changes following V2 as well. The absence of testicular steroids and the presence of relatively low amounts of leptin in IC after V2 compared with SC (see 4.2-Batorek et al. 2012b) might cause high feed intake in IC, because both oestrogens and leptin are known to reduce appetite (Claus and Weiler 1994, Barb et al. 1998). Together with the presence of higher IGF-I in *ad libitum* feed IC compared to SC (see 4.2-Batorek et al. 2012b and Claus et al. 2007), which contributes to improved feed efficiency, the increased feed intake enables stimulated growth. Indeed, it was shown that the ADG of IC in the period following V2 is

approximately 145 and 117 g/day higher compared to SC and EM, respectively (4.1- Batorek et al. 2012a, Millet et al. 2011, Dunshea et al. 2013). Eventually feed efficiency in IC following V2 is improved compared to SC (for approximately 0.10 points, Millet et al. 2011, Dunshea et al. 2013) but reduced compared to EM (for approximately 0.35 points, Millet et al. 2011, Dunshea et al. 2013). Considering the whole fattening period (V1-S) IC are much more efficient than SC because physiologically they are EM before V2 and also some time after, however they are less efficient than EM because they are slaughtered much after the transient period is finished, but they exhibit moderately faster growth compared with both, SC and EM (see 4.1-Batorek et al. 2012a). The idea that restricted feed allowance of IC after V2 would improve feed efficiency and carcass leanness of IC was further investigated. Our results (see 4.2-Batorek et al. 2012b) show that despite a slower growth of feed-restricted IC, feed efficiency is improved and performance traits are similar to those in *ad libitum* fed EM. On the contrary, Quiniou et al. (2012) suggest that feed restriction of IC pigs after V2 has no practical interest, because restrictive feeding of group-housed IC results in slower growth with no improvement of feed efficiency. The discrepancy in the results can perhaps be ascribed to different experimental conditions, namely the housing system (individual vs. group) and different genetics of pigs used. However, both studies agree that restrictive feeding of IC is associated with higher incidence of lesions during fattening (Quiniou et al. 2012) or pre-slaughter (4.2-Batorek et al. 2012b) and thus indicate more aggression among pigs (comparable to EM) perhaps due to chronic stress and higher excitability caused by feed restriction (D'Eath et al. 2009) and, for that reason, might be questioned from a welfare point of view. Reduced dietary energy content in feed by inclusion of fibre-rich ingredients without limitation of feed allowance was applied as an alternative (see 4.4) and growth efficiency was evaluated on NE basis as G:NE ratio in order to express energy requirements and dietary energy values on the same scale (Noblet and van Milgen 2004). In accordance with results of Quiniou and Noblet (2012) in barrows, reduction of NE concentration of feed did not influence G:NE, indicating that the performance and utilization of energy was similar regardless of dietary NE concentration or the composition of nutrients to provide energy in *ad libitum* fed IC. As anticipated, reduction of NE concentration of feed (10% relative to standard

diet containing 10.4 MJ NE/kg of feed) resulted in reduced backfat deposition. However the difference in backfat gain due to reduced dietary NE concentration become notable only during the second 4-wk following V2. From a practical point of view, this indicates that it would be reasonable to apply feed restriction especially when prolonged time between V2 and slaughter is expected.

### **6.3 Effect of immunocastration and dietary energy concentration on energy metabolism**

In order to evaluate the effect of immunocastration combined with growth on energy metabolism (see 4.4) ME intake (per kg metabolic BW) was increased after V2 in IC, but it was not accompanied by any difference in total HP. Likewise, higher ME intake after immunocastration was not associated with an increased fasting HP as observed by de Lange et al. (2006) in pigs of a given BW. A decrease in fasting HP was even noted, which illustrates the important changes in the physiology and metabolism due to immunocastration. Higher body fatness (van Milgen et al. 1998) and changed hormonal status after immunocastration (castrate vs. male, Labussière et al. 2013) have both been associated with decreased basal metabolic rate and might explain the lower fasting HP after immunocastration. Moreover, there was no effect of immunocastration combined with growth on total thermic effect of feeding (as % ME). Consequently, energy gain after V2 was more pronounced than expected from ME intake change only. Nevertheless, Labussière et al. (2013) have shown that IC have higher total thermic effect of feeding than EM at similar age and BW, due to higher feed intake and because more dietary protein is used as a source of energy with a low energetic efficiency for lipogenesis (van Milgen et al. 2001). Although PD and LD were increased in IC after V2 (see 4.4) it is difficult to discriminate between immunocastration and growth effect. It can be assumed that PD more likely increased due to growth than by immunocastration, as Labussière et al. (2013) showed similar PD in IC and EM five weeks after V2 (and superior to barrows). On the other hand, LD seems to be increased not only with growth, but also due to

immunocastration. Moreover, decrease in fasting HP following immunocastration (see 4.4), emphasizes the effects of increased feed intake on fat gain in IC. The increase in lipid content of BW gain after immunocastration is consistent with reported differences in carcass composition between IC and EM discussed above, namely higher backfat thickness and consequently lower carcass lean meat percentage (4.1-Batorek et al. 2012a). In addition to the stimulation of fat metabolism by immunocastration, the effect of a dietary stimulation of fat metabolism was investigated (see 4.4). The increased dietary fat after V2 additionally stimulated retention of energy in the form of fat. However, the contribution of lipogenesis to retained energy as fat was reduced, because additional dietary fat was deposited in carcass as such without oxidation of dietary fat and de novo synthesis (Chwalibog et al. 1992, Bruininx et al. 2011). Indeed, a lower intensity of lipogenesis from carbohydrate in pigs consuming high fat diet was confirmed by a reduced respiratory quotient (see 4.4). The increased dietary fat after V2 was associated with a considerable increase in daily LD, which fully agrees with previous studies in barrows (van Milgen et al. 2001, Bruininx et al. 2011). However, it is worth noting that our results point out that the increased fatness of IC is a result of increased daily LD caused by higher energy intake, lower basal metabolic rate and improved energy retention and not by decreased daily PD. Consequently, due to increased LD stimulated by higher ADFI, and an increased protein catabolism with low efficiency, IC are less feed efficient than EM. Yet, if dietary composition after immunocastration would be optimized in energy and protein content, feed efficiency might be improved substantially.

#### **6.4 Effect of immunocastration and different feeding strategies on carcass composition and meat quality**

The results of two meta-analysis show that IC have lower dressing percentage than SC (for approximately 2.0 percentages, 4.1-Batorek et al. 2012a, Dunshea et al. 2013) and, although not always significantly, also than EM (for approximately 0.3 percentage, 4.1-Batorek et al. 2012a, Dunshea et al. 2013). Lower dressing percentage in IC has been contributed to the



presence of reproductive tract (Boler et al. 2012, Boler et al. 2014), higher gut fill due to increased ADFI (Dunshea et al. 2001, Gispert et al. 2010, Boler et al. 2014), increased weight of abdominal fat (Škrlep et al. 2010a, Škrlep et al. 2010b) and also to the heavier internal organs, namely liver and kidneys (Pauly et al. 2008, Pauly et al. 2009, Boler et al. 2014). Because physiologically immunocastration becomes effective in the last weeks before slaughter, when the transient period after V2 is finished, IC take advantage of boar like growth performance for most of growing-fattening period. Hence they produce leaner carcasses compared to SC (lean content is increased for approximately 1.0 percentage, Millet et al. 2011, 4.1-Batorek et al. 2012a, Pauly et al. 2009) due to increased muscling and decreased fatness. According to Dunshea et al. (2013) backfat thickness is approximately 2.64 mm lower in IC compared to SC, but approximately 1.53 mm higher than in EM at slaughter. Latter can be attributed to high ADFI following V2 that enhances fat deposition and consequently lowers lean content of the carcass in IC compared to EM (for approximately 1.5 percentages, Millet et al. 2011, 4.1-Batorek et al. 2012a, Pauly et al. 2009). However, fat deposition in IC largely depends on the timing of immunization - the length of period between immunization and slaughter (Leailifano et al. 2011) and possibly feeding strategy applied. However, feed restriction in IC had either no significant effect on fat deposition and carcass leanness (dos Santos et al. 2012, Quiniou et al. 2012) or only reduced leaf fat weight (4.2-Batorek et al. 2012b). The lack of effect of restricted feed allowance on carcass leanness in the above mentioned studies might be related to a shorter duration of the applied restriction, because backfat deposition significantly increases only after a substantial time post V2 (see 4.4). In contrary, reduction of NE concentration of feed in IC improved lean content, whereas carcass weight was not affected, in agreement with a similar study in barrows (Quiniou and Noblet 2012). However, only backfat depot was significantly reduced with reduction of dietary energy content, whereas leaf fat and IMF deposition were not affected. In general, the weight of the main carcass cuts of IC and EM seems similar, with exception of heavier belly weight (4.1-Batorek et al. 2012a), which is consistent with the higher fat content of the carcass. Whereas the higher ham and shoulder weights and the lower belly weight in IC compared with SC reported in meta-analysis (4.1-Batorek et al. 2012a) can be ascribed to the fact that

muscles of the fore and hind limbs develop earlier in life than muscles of posture (e.g. loin, belly muscles) and may be less affected by the reduced anabolic potential after immunocastration as discussed by Pauly et al. (2009). The synthesis of published data on meat quality shows that IC do not differ from SC (4.1-Batorek et al. 2012a, Pauly et al. 2012), whereas IC present some advantages over EM, namely higher IMF content and lower shear force (4.1-Batorek et al. 2012a, Pauly et al. 2012). Later might be contributed to increased growth rate in the weeks prior to slaughter, resulting in enhanced protein turn-over *in vivo* and increased proteolysis *post-mortem* (Therkildsen et al. 2004, Lametsch et al. 2006). Although compensatory growth has been proposed to have a positive effect on pork tenderness (Kristensen et al. 2002), neither sensory tenderness nor juiciness were affected by castration method or sex (Pauly et al. 2012, Trefan et al. 2013). Our meta-analysis also indicated, that IC might have some disadvantages in meat quality compared with EM (4.1-Batorek et al. 2012a), including a higher drip loss, which is consistent with a tendency for lower ultimate pH and higher L value. However this was not confirmed by Pauly et al. (2009) and Trefan et al. (2013), with exception of lighter meet colour (Trefan et al. 2013). Moreover, neither quantitative feed restriction, nor a reduction of dietary energy content was a source of differences in meat quality in our studies (see 4.2-Batorek et al. 2012b, 4.4).

## 7 CONCLUSIONS

During the past years, the effectiveness of immunocastration and its effect on performance and carcass composition of pigs have been studied intensively. However, the available information has not been analysed in a systematic way. In addition, numerous questions concerning the metabolic changes following immunisation, nutritional requirements and growth potential of IC remain unanswered. Thus in the first part of the present thesis a meta-analytical approach was used to quantitatively analyse the effects of immunocastration, whereas in the second part four experimental studies were conducted to evaluate the effect of immunocastration and quantitative and qualitative restriction on heat production and energy metabolism in pigs. Additional studies were performed to evaluate the effect of immunocastration on performance, carcass and meat quality in group-housed conditions and to assess if the delay between slaughter and V2 at standard slaughter weight can be shorter than recommended by the vaccine producer. The following working hypotheses have been set:

1. Immunocastration successfully limits boar taint i.e. deposition of androstenone and skatole in adipose tissue.
2. IC exhibit improved performance and leanness in comparison with SC, and better meat quality in comparison to EM.
3. Restricting feed intake of IC after the V2 limits fat deposition and improves their performance in comparison to *ad libitum* feed IC, however it may negatively affect behaviour.
4. After immunocastration the energy metabolism changes, LD is enhanced but there is no effect on PD.
5. Dietary energy enrichment (addition of fat) of IC after V2 emphasizes LD.
6. Dietary energy dilution (NE reduction) of IC after V2 limits fat deposition.

The results of meta-analysis show that the concentrations of boar taint compounds in adipose tissue and the size of reproductive organs are dramatically reduced in IC compared to EM,

confirming the first hypothesis of the present doctoral thesis. IC are less efficient and fatter but grow more rapidly and may have better meat quality than EM. Compared to SC, IC have superior performance with no difference in meat quality. Taken together, this results confirm the second hypothesis of the present doctoral thesis and indicate that it is more economical to fatten IC than SC, still production costs and carcass quality are less favourable than with EM. However, due to the limited number of available data it should be noted, that more studies are needed to confirm the positive influence of immunocastration on meat quality.

The quantitative feed restriction after full immunization tested in the present thesis has been shown to improve feed efficiency and carcass composition of IC in comparison to *ad libitum* feed control, whereas meat quality was not influenced by the restriction. However, higher incidence of carcass lesions in restricted IC (similar as in EM) together with higher plasma cortisol level indicates more aggression prior to slaughter and can be considered a negative aspect of quantitative feed restriction in IC. The results fully confirm the third hypothesis tested in the present thesis.

The dynamics of heat production prior and after immunocastration were studied in the present thesis. The results indicate that additional energy retained because of higher feed intake is directed towards LD. Increased fatness of IC is a result of increased daily LD caused by higher energy intake, lower basal metabolic rate and improved energy retention. Additionally, LD is further enhanced by dietary energy enrichment with fat after V2. This results confirm the fourth and fifth hypothesis of the present doctoral thesis, also they suggest that dietary energy intake should be limited after immunocastration. Moreover, the results of the nitrogen balance study indicate that the dietary crude protein content could be decreased after immunocastration without affecting performance. However, the patterns of tissue deposition rates following changes in energy metabolism after immunocastration should be further explored in order to establish exact nutrient requirements of IC and develop a feeding strategy which would allow maximizing their growth potential and minimizing the nitrogen losses.

By reducing net energy concentration of the diet up to 10% with higher fibre content ingredients, similar energy efficiency (gain to net energy intake ratio) was achieved in the present thesis, providing an advantage in terms of reduced LD, without affecting growth rate

in IC after full immunization. These results confirm the sixth hypothesis of the present doctoral thesis. Such feeding strategy could be particularly beneficial when IC would be fattened to higher weights i.e. when longer delays between full immunization and slaughter would be practiced, however the results should be further confirmed in group housed conditions.

Additional results, provided in the present thesis, have shown that the elimination of boar taint using the immunocastration in group-housed conditions is efficient for the majority of vaccinated pigs. Improved leanness in comparison to SC, and lower meat drip loss in comparison to EM provide the benefits of this new alternative to surgical castration.

The disruption of the steroidogenesis and deposition of boar taint compounds, suppressed growth of reproductive organs and disturbance of spermatogenetic function exists already two weeks after the effective immunocastration. The changes observed are progressive with an increasing delay until slaughter, with nearly no signs of functional restoration within eight weeks after the V2. However, it should be stressed that as regards the restoration of the reproductive function, the present results are valid in the case of relatively young and light-weight pigs, such as used in conventional pig fattening, whereas further experiments would be needed for mature boars.









## 8 SUMMARY

In most countries male pigs are surgically castrated shortly after birth to prevent meat boar taint. In spite of its wide use, this form of castration has been criticized as being painful for the piglets (McGlone et al. 1993, Prunier et al. 2006) and may be considered even unnecessary in modern pig production especially if pigs are raised for fresh meat consumption. In such a case raising EM would be much more efficient (Bonneau 1998, Trefan et al. 2013). As an alternative, an active vaccination against gonadotropin releasing hormone (GnRH; i.e. immunocastration) has been proposed, which uses natural immune system of the pig for the formation of specific antibodies that bind and neutralize GnRH. It consists of two vaccinations, as a result hypothalamic-pituitary-gonadal axis is blocked and testes growth and sexual steroids synthesis are effectively inhibited. Physiologically the immunocastration becomes effective in a week following second vaccination (V2; Claus et al. 2007), therefore in case of late revaccination during the month preceding slaughter, growth characteristics of immunocastrated pigs (IC) are similar to those of entire males (EM; Škrlep et al. 2010a, Millet et al. 2011, Dunshea et al. 2013) and better production performance (i.e. growth rate, feed intake, feed efficiency and carcass leanness) of IC compared with surgical castrates (SC; for review see Millet et al. 2011) is observed. However, the results are not always consistent and effects of immunocastration on metabolic changes, nutritional requirements and growth potential have not yet been quantified. Thus, the aims of the present thesis were i) to assess the effects of immunocastration in pigs through a meta-analysis of available research results, focusing on growth performance, carcass characteristics, meat quality, reproductive organs and boar taint substances; ii) to evaluate the effects of quantitative and qualitative feed restriction on growth performance, carcass and meat quality and iii) to assess lipid metabolism of IC from two perspectives; firstly to evaluate the changes following immunocastration (due to combined effects of immunocastration, age and increased feed intake of IC), and secondly to test if a high lipid diet stimulates lipid deposition in IC. In meta-analysis, altogether 41

papers were collected, effect size ( $\theta$ ) was calculated for comparison of IC with EM and SC. Results of meta-analysis confirmed the efficiency of immunocastration with the magnitude of the response being by far the largest for reproductive organs ( $\theta=-2.8$  to  $-5.0$ ) and boar taint substances ( $\theta=-2.8$  and  $-0.8$  for androstenone and skatole, respectively). However, compared with SC, IC exhibited bigger bulbourethral glands ( $\theta =1.3$ ) and slightly higher concentrations of androstenone and skatole ( $\theta =0.1$  and  $\theta =0.2$ , respectively). The impact of immunocastration was also remarkable on growth performance, where the main advantage of the IC is their boar-like performance until revaccination. In the period following V2 they consume much more feed than EM ( $\theta =2.1$ ) resulting in large effect size for growth rate compared with both EM and SC ( $\theta =1.1$  and  $\theta =1.4$ , respectively). Considering the whole fattening period, IC exhibited higher FCR than EM ( $\theta =0.6$ ) but much lower than SC ( $\theta =-1.3$ ) with moderately faster growth compared to both EM and SC ( $\theta =0.6$  and  $\theta =0.2$ , respectively). Regarding carcass quality (carcass weight, dressing yield, leanness, backfat thickness), IC take intermediate position between EM and SC. Furthermore, meta-analysis suggests no difference in meat quality when IC are compared with SC and some meat quality advantages of IC over EM due to higher intramuscular fat (IMF) content ( $\theta =0.4$ ) and lower shear force ( $\theta =-0.6$ ). To conclude, the key impacts after V2 are a substantial increase of ADFI in IC, resulting in a decrease of G:F ratio and an increase of body fatness in comparison to EM. The second aim of the present thesis was thus to assess the effect of feed restriction applied to IC in the period following V2 on hormonal status, performance, carcass traits and meat quality. In this study IC (fed either restrictively or *ad libitum*) were compared with EM and SC fed *ad libitum*. One pig per litter was surgically castrated within a week after birth (SC,  $n = 22$ ), the other male pigs were left entire. At 83 days of age, the EM were further allotted to treatment groups of *ad libitum* fed EM ( $n = 24$ ), *ad libitum* fed IC (IC-L,  $n = 21$ ) and restrictively fed IC (IC-R,  $n = 21$ ). At that time, the first vaccination was also applied to IC-L and IC-R pigs. One week after V2 (age 130 days), feed restriction ( $\approx 80\%$  of the *ad libitum* feed intake of the SC) was applied to IC-R pigs. During the experiment, pigs were housed individually, and the experiment ended 5 week after V2, when pigs were 165 days old. Immunocastration successfully reduced boar taint compounds and size of reproductive organs. At 130 days of

age (V2), serum leptin levels were similar in all groups, while IGF-I level was lower in SC ( $P \leq 0.002$ ) than in the other groups. Three weeks after V2, leptin levels of both IC groups were in-between EM (lowest) and SC (highest). The reverse was observed for IGF-I. Feed restriction had no effect neither on leptin nor on IGF-I levels in IC pigs. In the period from first to second vaccination (V1-V2), growth performance differed mainly between EM and SC, whereas both IC groups had similar feed intake and feed conversion ratio as EM and intermediate daily gain that did not differ from either EM or SC. After V2, IC-L pigs increased their feed intake to the levels of SC, with faster growth than other three groups ( $P < 0.05$ ), resulting in fatter carcasses compared to EM pigs ( $P = 0.007$ ). Similar performance and carcass leanness were observed for IC-R and EM pigs. During pre-slaughter handling, more carcass lesions were noted in EM and IC-R than in IC-L or SC pigs ( $P < 0.002$ ). Neither immunocastration nor feed restriction had any effect on meat quality. It was concluded that restricting feed intake can increase production efficiency, but also aggressiveness of IC pigs. Because of increased voluntary feed intake after full immunization, increased lipid deposition is observed, but little is known on associated effect of diet composition on partitioning of nutrients in IC pigs. Thus, the next study aimed at evaluating nutrient digestibility and nitrogen and energy balances of IC. This study was conducted in open-circuit respiration chambers in two subsequent stages, prior (stage 1) and after (stage 2) V2 for immunocastration, and four replicates of two male littermates, to determine the effect of immunocastration (effect of immunocastration was associated with the effect of growth) and increased dietary fat content on energy utilization between 85 (stage 1) and 135 (stage 2) kg live weight. During stage 1, pigs received a standard low fat diet (LF; 2.5% DM of fat fed at 2.27 MJ ME/kg BW<sup>0.60</sup> per day) whereas, during stage 2, feed intake was increased to 2.47 MJ ME/kg BW<sup>0.60</sup> per day and one littermate was fed LF diet whereas the second one received fat enriched diet (HF; 8.9% DM of fat). Results from nitrogen balance and measurements of gas exchanges were used to calculate respiratory quotient (RQ), heat production (HP), nutrients contribution to fat retention, components of HP, and protein deposition (PD) and lipid deposition (LD). Nutrients and energy apparent digestibility coefficients, methane losses and nitrogen retention increased with stage ( $P < 0.05$ ). Despite higher ME intake, total HP remained

similar (1365 kJ/kg of  $BW^{0.60}$  per day;  $P=0.26$ ) and fasting HP tended to decrease (823 to 697 kJ/kg of  $BW^{0.60}$  per day;  $P=0.07$ ) between stages. Consequently, total retained energy (RE) increased with stage (from 916 to 1078 kJ/kg of  $BW^{0.60}$  per day;  $P<0.01$ ) with a higher fat retention (625 to 807 kJ/kg  $BW^{0.60}$  per day,  $P<0.01$ ) originating mainly from carbohydrates associated with a higher lipogenesis (536 to 746 kJ/kg  $BW^{0.60}$  per day;  $P<0.01$ ) and RQ (1.095 to 1.145;  $P<0.01$ ). Both PD (from 178 to 217 g/day;  $P=0.04$ ) and LD (from 227 to 384 g/day;  $P<0.01$ ) increased due to growth/immunocastration. Both diets differed in crude fat digestibility ( $P<0.01$ ). Feeding HF diet after immunocastration was associated with increased RE as fat (807 to 914 kJ/kg  $BW^{0.60}$  per day,  $P=0.05$ ), originating mainly from dietary fat ( $P<0.01$ ) and resulting in increased LD (384 to 435 g/day,  $P=0.02$ ) and lower RQ (from 1.145 to 1.073,  $P<0.01$ ). Altogether, these results indicate that increased fatness of IC is a result of increased daily LD caused by higher energy intake, lower basal metabolic rate and improved energy retention. Additionally, LD is further enhanced after V2 by dietary energy enrichment with fat. Thus the last aim of the present thesis was to evaluate the effects of reduction of NE concentration of feed in IC slaughtered eight weeks after V2 on growth performance, carcass and meat quality traits with an emphasis on fat deposition before (P1) and after V2 (P2) and in first and second 4-week following V2. Pigs ( $n=41$ ) divided in three groups, were fed experimental diets with low ( $n=14$ ), medium ( $n=13$ ) or high ( $n=14$ ) NE content (LNE, MNE and HNE diets; 8.5, 9.3 and 10.0 MJ NE/kg, respectively) from 84 days of age onward. Additionally a digestibility trial was performed in two phases using four pigs on the LNE and HNE extreme diets in order to accurately calculate the dietary NE concentration. Diet composition had no effect on ADFI in any period of the experiment ( $P>0.10$ ), whereas higher NE intake in MJ per day ( $P=0.08$ ) was noted in the second 4-week phase after V2 (day 143 to day 170) for HNE dietary group. There was no effect ( $P>0.10$ ) of reduction of NE concentration of feed on average daily gain (ADG) or gain to net energy ratio (G:NE) in any stage of experiment. A difference in backfat gain was observed only in the second phase after V2 (day 143 to day 170) and it was higher ( $P<0.01$ ) in HNE group. In agreement with the absence of diet effect on BW, no effect ( $P>0.10$ ) was noted on hot carcass weight (HCW), dressing percentage or prime carcass cuts. The differences among treatment



groups were noted for carcass traits i.e. lower backfat expressed as the percentage of HCW (P=0.01) and smaller area of fat layer over *longissimus dorsi* muscle (P=0.05) in LNE and MNE pigs. Consequently higher lean meat content (P=0.04) of LNE pigs was observed. Intermuscular and *longissimus dorsi* intramuscular fat deposition were not affected (P>0.05) by reduction of NE concentration of feed. No effect of dietary NE concentration was noted on meat quality, with the exception of lighter color score (P=0.04) and a tendency (P=0.10) towards higher yellowness (CIA b\*) of *longissimus dorsi* muscle in the HNE group. Overall, present results demonstrate that by reducing NE concentration of the diet up to 10 % using high fiber content ingredients, similar energy efficiency (G:NE) may be achieved, providing an advantage in terms of reduced LD, without affecting growth rate in IC after full immunization. This is particularly important when IC are fattened to higher weights than usually practiced, i.e. when longer delays between V2 and slaughter are to be expected.







## 9 POVZETEK

V večini držav se prakticira kirurška kastracija samcev prašičev kmalu po rojstvu, s čimer se prepreči neprijeten vonj mesa po merjascu. Kljub široki uporabi je ta oblika kastracije v zadnjem času vse bolj kritizirana, saj je boleča za pujske (McGlone in sod. 1993, Prunier in sod. 2006) in v komercialni reji morda tudi nepotrebna, še posebej kadar so prašiči namenjeni za konzum svežega mesa, saj bi bila v tem primeru reja merjascev (EM) veliko bolj ekonomična (Bonneau 1998, Trefan in sod. 2013). Ena izmed alternativ kirurški kastraciji je aktivna vakcinacija proti gonadotropin sproščujočemu hormonu (GnRH, t.i. imunokastracija), ki spodbudi naravni imunski sistem prašiča, da tvori specifična protitelesa, ki se vežejo in nevtralizirajo GnRH. Sestoji iz dveh vakcinacij; slednja povzroči blokado hipotalamo-hipofizno-gonadne osi, njena posledica pa je regresija spolnih organov in prekinitev sinteze spolnih hormonov v modih. Fiziološko postane imunokastracija učinkovita v tednu po drugi vakcinaciji (V2; Claus in sod. 2007), zato dosegajo imunokastrirani prašiči (IC) v primeru izvedbe pozne imunizacije (v mesecu pred zakolom) podobne proizvodne rezultate (prirast, konverzijo krme in delež mesa v klavnem trupu) kot EM (Škrlep in sod. 2010a, Millet in sod. 2011, Dunshea in sod. 2013) in boljše v primerjavi s kirurškimi kastrati (SC; glej Millet in sod. 2011). Rezultati raziskav niso vedno enotni, poleg tega učinki imunokastracije na energetsko presnovo, prehranske potrebe in rastni potencial IC še niso bili ovrednoteni. Iz tega razloga so bili cilji doktorske disertacije sledeči: i) oceniti učinke imunokastracije prašičev z meta-analizo rezultatov dostopnih raziskav, s poudarkom na rastnih parametrih, sestavi trupa, kakovosti mesa, reprodukcijskih organih in substancah vonja po merjascu; ii) oceniti učinke kvantitativne in kvalitativne restrikcije krme na rastne parametre, klavno kakovost in kakovost mesa in iii) oceniti spremembe v metabolizmu energije pri IC, ki nastanejo po imunokastraciji (zaradi kombiniranih učinkov imunokastracije, starosti in povečane konzumacije), ter dodatno stimulirati nalaganje maščob po imunokastraciji s povečanim vnosom maščob. V meta-analizo smo vključili 41 znanstvenih člankov, jakost učinka ( $\theta$ ) pa smo izračunali za primerjavo IC z

EM in SC. Rezultati meta-analize potrjujejo učinkovitost imunokastracije z največjo magnitudo odziva na velikost reprodukcijskih organov ( $\theta = -2,8$  do  $-5,0$ ) in koncentracijo substanc vonja po merjascu ( $\theta = -2,8$  in  $-0,8$  za androstenonon in skatol). V primerjavi z SC imajo IC nekoliko večje bulbouretralne žleze ( $\theta = 1,3$ ) in nekoliko višje koncentracije androstenona in skatola ( $\theta = 0,1$  in  $\theta = 0,2$ ). Vpliv imunokastracije je močan tudi na rastne parametre, kjer je glavna prednost IC izkoriščanje ravnega potenciala EM do V2. V obdobju po V2 se v primerjavi z EM močno poveča konzumacija krme ( $\theta = 2,1$ ), posledično pa tudi dnevni prirast tako v primerjavi z EM kot SC ( $\theta = 1,1$  in  $\theta = 1,4$ ). V celotnem obdobju pitanja imajo IC višjo konverzijo krme kot EM ( $\theta = 0,6$ ), vendar precej nižjo kot SC ( $\theta = -1,3$ ) in zmerno hitrejšo rast tako v primerjavi z EM kot SC ( $\theta = 0,6$  in  $\theta = 0,2$ ). Glede kakovosti klavnega trupa (klavna masa, klavni izplen, delež mesa, debelina hrbtna slanina) se IC nahajajo med EM in SC. Meta-analiza nadalje kaže, da med IC in SC ni razlike v kakovosti mesa, medtem ko se v primerjavi z EM pri IC nakazujejo določene prednosti v kakovosti mesa, kot so višja vsebnost intramuskularne maščobe (IMF;  $\theta = 0,4$ ) in nižja trdota mesa ( $\theta = -0,6$ ). Če povzamemo, so ključne spremembe, ki nastopijo pri IC po V2, precejšnje povečanje konzumacije, kar ima za posledico višjo konverzijo krme in povečano nalaganje maščob v primerjavi z EM. Na osnovi tega smo kot drugi cilj doktorske naloge želeli oceniti učinek restrikcije krme IC v obdobju po V2 na pitovne lastnosti, klavno lastnosti, kakovost mesa in koncentracijo presnovnih hormonov. V tej raziskavi smo primerjali IC, krmljene bodisi restriktivno ali *ad libitum*, z EM in SC, krmljenimi *ad libitum*. V tednu po rojstvu smo enega prašička na gnezdo kirurško kastrirali (SC,  $n = 22$ ). Pri starosti 83 dni smo EM razdelili v poskusne skupine: EM ( $n = 24$ ), krmljeni *ad libitum*, IC (IC-L,  $n = 21$ ), krmljeni *ad libitum* in IC (IC-R,  $n = 21$ ), krmljeni restriktivno. Istočasno smo izvedli prvo vakcinacijo IC-L in IC-R prašičev. En teden po V2 (v starost 130 dni) je bila konzumacija krme IC-R prašičev omejena na 80% *ad libitum* konzumacije SC prašičev. Tekom celotnega poskusa so bili prašiči nastanjeni individualno. Poskus se je zaključil pet tednov po V2, ko so bili prašiči v povprečju stari 165 dni. Imunokastracija je uspešno zmanjšala koncentracijo spojin vonja po merjascu in velikost reprodukcijskih organov. Pri starosti 130 dni (V2), so bile serumske koncentracije leptina podobne pri vseh poskusnih skupinah, medtem ko je bila raven IGF-I nižja pri SC ( $P \leq$



0,002) v primerjavi z ostalimi skupinami. Tri tedne po V2 je bila raven leptina obeh skupin IC med EM (najnižja) in SC (najvišja). Pri koncentraciji IGF-I smo ugotovili obraten učinek. Omejitev krme ni imela vpliva na serumske koncentracije leptina ali IGF-I pri IC. V obdobju med prvo in drugo vakcino (V1-V2) so se pitovne lastnosti razlikovale predvsem med EM in SC, konzumacija in konverzija v obeh skupinah IC pa je bila podobna kot pri EM, medtem ko se dnevni prirast ni razlikoval niti od EM niti od SC. Po V2 so IC-L povečali konzumacijo na isto količino kot SC, vendar so priraščali intenzivneje kot ostale tri poskusne skupine ( $P < 0,05$ ), kar je rezultiralo v večji zamaščenosti trupov v primerjavi z EM ( $P = 0,007$ ). Pitovne lastnosti in delež mesa sta bila podobna pri IC-R in EM. V obdobju pred zakolom je bilo zaznati več lezij trupov pri EM in IC-R v primerjavi z IC-L ali SC prašiči ( $P < 0,002$ ). Imunokastracija in restrikcija krme nista imeli vpliva na kakovost mesa. Ugotovljeno je bilo, da lahko restriktivno krmljenje poveča učinkovitost proizvodnje, vendar tudi agresivnost IC. Zaradi povečane konzumacije po V2 se poveča nalaganje maščob, vendar je malo znanega o učinku sestave krme na nalaganje hranil pri IC. Tako je bil cilj naslednje raziskave oceniti prebavljivost hranil in bilanco dušika ter energije pri IC. Ta raziskava je bila izvedena v respiratornih kletkah v dveh fazah, pred (faza 1) in po (faza 2) V2 ter štirih ponovitvah z dvema sorojencema. Namen je bil oceniti učinek imunokastracije (v povezavi z rastjo) in učinek povečane konzumacije maščob na presnovo energije med 85 (faza 1) in 135 (2 fazi) kg žive teže. V fazi 1 so prašiči prejeli standardno krmo (LF; 2,27 MJ metabolne energije na kg  $TM^{0,60}$  na dan z 2,5% vsebnostjo maščobe na suho snov). V fazi 2 je bil vnos krme povečan na 2,47 MJ metabolne energije na kg  $TM^{0,60}$  na dan in en sorojenec je dobival krmo LF, medtem ko je drugi sorojenec prejemal krmo, obogateno z maščobami (HF; 8,9% maščobe na suho snov). Rezultate bilance dušika in meritve izmenjave plinov v respiratorni kletki smo uporabili za izračun respiratornega količnika (RQ), proizvodnje toplote (HP), količine hranil naloženih v obliki maščobe, komponente HP in nalaganje proteinov (PD) ter maščob (LD). Koeficienti prebavljivosti hranil in energije, izgube metana in zadrževanje dušika so se povečali s fazo poskusa ( $P < 0,05$ ). Kljub povečanemu vnosu metabolne energije je ostala HP podobna (1365 kJ na kg  $TM^{0,60}$  na dan;  $P = 0,26$ ), medtem ko se je HP na tešče zmanjševala (iz 823 na 697 kJ na kg  $TM^{0,60}$  na dan;  $P = 0,07$ ) med fazama. Iz tega razloga se je s fazo poskusa povečala

količina skupne zadržane energije (iz 916 na 1078 kJ na kg  $TM^{0.60}$  na dan;  $P < 0,01$ ), predvsem v obliki maščob (iz 625 na 807 kJ na kg  $TM^{0.60}$  na dan,  $P < 0,01$ ), ki so večinoma izvirale iz ogljikovih hidratov v povezavi z intenzivnejšo lipogenezo (iz 536 na 746 kJ na kg  $TM^{0.60}$  na dan;  $P < 0,01$ ) in višjim RQ (iz 1,095 na 1,145;  $P < 0,01$ ). PD (iz 178 na 217 g na dan;  $P = 0,04$ ) in LD (iz 227 na 384 g na dan;  $P < 0,01$ ) sta je povečala z rastjo oziroma imunokastracijo. Poskusni krmi sta se razlikovali v prebavljivosti surovih maščob ( $P < 0,01$ ). Krmljenje HF krme po V2 je povzročilo povečanje količine zadržane energije v obliki maščob (iz 807 na 914 kJ na kg  $TM^{0.60}$  na dan,  $P = 0,05$ ), ki je izvirala predvsem iz zaužite maščobe ( $P < 0,01$ ) in rezultirala v povečanju LD (iz 384 na 435 g na dan,  $P = 0,02$ ) in nižjem RQ (iz 1,145 na 1,073;  $P < 0,01$ ). Ti rezultati kažejo, da je povečana zamaščenost IC posledica povečanega dnevnega LD zaradi višjega vnosa energije, nižjega bazalnega metabolizma in izboljšane izkoriščenosti energije. Ker z dodatkom maščobe v krmo po V2 še dodatno stimuliramo LD, je bil zadnji cilj doktorske disertacije oceniti učinke nižje koncentracije neto energije (NE) krmne mešanice v obroku IC, ki so bili imunokastrirani osem tednov pred zakolom, na pitovne lastnosti, kakovost klavnega trupa in mesa, s poudarkom na nalaganju maščob pred (P1) in po V2 (P2) ter v prvem in drugem 4-tedenskem obdobju po V2. Prašiči ( $n = 41$ ), razdeljeni v tri skupine, so bili od 84. dneva starosti krmljeni s poskusno dieto z nizko ( $n = 14$ ), srednjo ( $n = 13$ ) ali visoko ( $n = 14$ ) koncentracijo NE (LNE, MNE in HNE krma, 8,5, 9,3 in 10,0 MJ NE na kg). Z namenom natančnega izračuna vsebnosti NE v poskusni krmi smo izvedli prebavljivostni poskus v dveh fazah, s štirimi prašiči na LNE in HNE krmi. Sestava krme v nobenem izmed preučevanih obdobj ni imela vpliva na konzumacijo ( $P > 0,10$ ), medtem ko je višja konzumacija NE ( $P = 0,08$ ) opazna v drugem 4-tedenskem obdobju po V2 (od 143. do 170. dneva) pri HNE. Zmanjšana koncentracija NE krmne mešanice ( $P > 0,10$ ) v nobenem obdobju ni vplivala na dnevni prirast (ADG), ali konverzijo na NE bazi (G:NE). Razlika v intenzivnosti nalaganja hrbtna slanina je postala značilna šele v drugem 4-tedenskem obdobju po V2 (od 143. do 170. dneva), in sicer višja ( $P < 0,01$ ) pri HNE skupini. V skladu z odsotnostjo vpliva zmanjšanja koncentracije NE krmne mešanice na telesno maso med poskusnimi skupinami nismo opazili razlik ( $P > 0,10$ ) v masi toplih klavnih trupov (HCW), klavnem izplnu ali masi klavnih kosov. Razlike med poskusnimi skupinami so bile

ugotovljene za nekatere pokazatelje zamaščenosti, t.j. nižja masa hrbtne slanine, izražena v odstotku HCW ( $P = 0,01$ ) in manjše območje maščobe nad *longissimus dorsi* mišico ( $P = 0,05$ ) pri LNE in MNE. Posledično je bil delež mesa v trupu višji ( $P = 0,04$ ) pri LNE. Količina medmišične in intramuskularne maščobe v mišici *longissimus dorsi* sta ostali nespremenjeni ( $P > 0,05$ ) ne glede na količino NE v krmi. Zmanjšanje NE v obroku prav tako ni vplivalo na kakovost mesa, z izjemo svetlejše barve ( $P = 0,04$ ) in težnje ( $P = 0,10$ ) v smeri bolj rumenega odtenka (CIA b \*) mišice *longissimus dorsi* v skupini HNE. Rezultati so tako pokazali, da lahko z zmanjšanjem koncentracije NE v krmni mešanici do 10% z uporabo sestavin z visoko vsebnostjo vlaknine pri IC po V2 dosežemo podobno učinkovitost prireje na NE bazi (G:NE), in kljub temu omejimo nalaganje maščob brez negativnega vpliva na prirast. Slednje je še posebej pomembno, kadar IC pitamo do višjih klavnih tež oziroma kadar pričakujemo daljši razmik med V2 in zakolom.









## 10 RÉSUMÉ

Les porcs mâles sont généralement castrés chirurgicalement juste après la naissance dans la plupart de pays afin d'éviter les odeurs sexuelles dans la viande. Bien que largement utilisée, la castration chirurgicale telle que pratiquée aujourd'hui (sans anesthésie et analgesie) est douloureuse pour les porcelets et elle est critiquée par le public (McGlone et al. 1993, Prunier et al. 2006). De plus, elle peut être considérée comme inutile dans l'élevage contemporain des porcs destinés à la consommation de viande fraîche. Dans ce cas-là, l'élevage d'mâles entiers (ME) serait beaucoup plus efficace (Bonneau 1998, Trefan et al. 2013). En alternative, une vaccination contre l'hormone gonadotrope (GnRH, appelée immunocastration) a été proposée; elle utilise le système immunitaire naturel du porc pour la formation d'anticorps spécifiques qui se fixent et neutralisent la GnRH. La procédure d'immunocastration repose sur deux vaccinations, ce qui interrompt l'axe hypothalamo-hypophyso-gonadique avec des répercussions sur la taille des testicules (réduite) et l'inhibition de synthèse des stéroïdes sexuels. Physiologiquement, l'immunocastration devient efficace au cours de la semaine suivant la deuxième vaccination (V2; Claus et al. 2007). Par conséquent (en cas de revaccination tardive i.e. dans le mois précédent l'abattage) les caractéristiques de croissance de porcs immunocastre (IC) sont similaires à ceux d'ME (Škrlep et al. 2010a, Millet et al. 2011, Dunshea et al. 2013) et les porcs IC présentent de meilleures performances que les porcs castrés chirurgicalement (SC ; vitesse de croissance, efficacité alimentaire et taux de viande maigre ; Millet et al. 2011). Cependant, les résultats ne sont pas toujours cohérents et les effets de l'immunocastration sur le métabolisme, les besoins nutritionnels et le potentiel de croissance n'ont pas encore été quantifiés. Ainsi, les objectifs de cette thèse étaient i) d'évaluer les effets de l'immunocastration chez les porcs par une méta-analyse des résultats publiés, en se concentrant sur les performances de croissance, la composition corporelle (caractéristiques de la carcasse), la qualité de viande, l'impact sur les organes génitaux et les substances

responsables des odeurs sexuelles; ii) d'évaluer les effets de la restriction alimentaire quantitative et qualitative sur les performances de croissance, la qualité de carcasse et de viande et iii) d'évaluer le métabolisme lipidique chez les porcs IC pour quantifier les modifications après l'immunocastration (dues aux effets combinés de l'immunocastration, de l'âge et de l'ingestion plus élevée des porcs IC), et pour vérifier si un apport élevé de lipides stimule le dépôt des lipides chez les porcs IC. Dans la méta-analyse, au total 41 articles ont été collectés, l'ampleur de l'effet ( $\theta$ ) a été calculé pour la comparaison entre les porcs IC contre ME ou SC. Les résultats de la méta-analyse ont confirmé l'efficacité de l'immunocastration avec l'ampleur de l'effet la plus grande pour les organes génitaux ( $\theta = -2,8$  à  $-5,0$ ) et les substances responsables des odeurs sexuelles ( $\theta = -2,8$  et  $-0,8$  pour l'androsténone et skatole, respectivement). Par rapport aux porcs SC, les porcs IC ont une taille de glandes bulbourethrales supérieure ( $\theta = 1,3$ ) et des concentrations en androsténone et skatole légèrement plus élevées ( $\theta = 0,1$  et  $\theta = 0,2$ , respectivement). L'effet de l'immunocastration était aussi important sur les performances de croissance, où le principal avantage des porcs IC provient de leurs performances de croissance similaires à celles des ME jusqu'à la revaccination. Après V2, les porcs IC augmentent leur niveau d'ingestion, qui surpasse celle d'EM ( $\theta = 2,1$ ), ce qui se traduit par une ampleur de l'effet sur la vitesse de croissance plus importante par rapport aux ME ou SC ( $\theta = 1,1$  et  $\theta = 1,4$ , respectivement). Pour toute la période d'engraissement, les porcs IC présentent une plus faible efficacité alimentaire i.e. un indice de consommation plus élevé que les porcs ME ( $\theta = 0,6$ ), mais beaucoup plus faible que les porcs SC ( $\theta = -1,3$ ) avec une vitesse de croissance légèrement plus élevée que les porcs ME ou SC ( $\theta = 0,6$  et  $\theta = 0,2$ , respectivement). Concernant la qualité de carcasse (poids de la carcasse, rendement de carcasse, épaisseur de lard dorsal), les porcs IC présentent des caractéristiques intermédiaires entre les porcs ME et SC. De plus, la méta-analyse n'indique aucune différence de qualité de viande entre les porcs IC et SC, mais indique quelques avantages des porcs IC par rapport aux porcs ME avec notamment plus de gras intramusculaire ( $\theta = 0,4$ ) et une viande moins dure ( $\theta = -0,6$ ). En conclusion, les principaux effets après V2 chez les porcs IC sont une augmentation importante de la consommation alimentaire, qui résulte en une diminution de l'efficacité alimentaire et une augmentation de

l'adiposité par rapport aux porcs ME. Le second objectif de la thèse était d'évaluer les effets de la restriction alimentaire appliquée chez les porcs IC au cours de la période suivant V2 sur le statut hormonal, les performances de croissance, les caractéristiques de la carcasse et de la qualité de viande. Dans cette étude, les porcs IC (alimentés soit restrictivement soit *ad libitum*) ont été comparés aux porcs ME et SC alimentés *ad libitum*. Un porc par portée a été castré chirurgicalement au cours de la semaine suivant la naissance (SC, n = 22), les autres mâles de la portée ont été gardés à l'état de mâles entiers. A l'âge de 83 jours, les porcs ME ont été distribués (intra- portée) aux traitements suivants : ME alimentés *ad libitum* (n = 24), IC alimentés *ad libitum* (IC-L, n = 21) et IC alimentés restrictivement (IC-R, n = 21). Le même jour, la première vaccination a été appliquée aux porcs IC-L et IC-R. Une semaine après V2 (à l'âge de 130 jours), une restriction alimentaire ( $\approx 80\%$  de l'apport alimentaire des porcs SC alimentés *ad libitum*) a été appliquée aux porcs IC-R. Les porcs expérimentaux étaient logés individuellement jusqu'à la fin de l'expérimentation 5 semaines après V2, quand les porcs avaient 165 jours de l'âge. L'immunocastration a réduit le taux des substances responsables des odeurs sexuelles et la taille des organes génitaux. À l'âge de 130 jours (V2), les concentrations de leptine sanguine étaient similaires dans tous les groupes, alors que le niveau d'IGF-I était plus faible chez les porcs SC ( $P \leq 0,002$ ) que chez les porcs d'autres groupes. Trois semaines après la V2, le niveau de leptine chez les porcs IC était intermédiaire entre celui des porcs ME (plus bas) et SC (plus élevé). L'inverse a été observé pour le niveau d'IGF-I. La restriction alimentaire chez les porcs IC n'a pas eu d'effet sur les taux de leptine et d'IGF-I. Pour la période entre V1 et V2, la vitesse de croissance étaient différentes entre les porcs ME et SC, alors que les deux groupes IC avaient un niveau d'ingestion et une efficacité alimentaire similaire aux porcs ME, mais avec une vitesse de croissance intermédiaire entre les porcs ME et SC. Après la V2, les porcs IC-L ont augmenté leur consommation alimentaire au niveau de celles des porcs SC, avec une croissance plus rapide par rapport aux trois autres groupes ( $P < 0,05$ ). Par conséquent, leurs carcasses étaient plus grasses que celles des porcs ME ( $P = 0,007$ ). Les performances de croissance et le taux de viande maigre des carcasses étaient similaires pour les porcs IC-R et ME. Au cours de la manipulation pré-abattage, une incidence plus importante de lésions de la carcasse a été notée chez les porcs ME et IC-R que

chez les porcs IC-L ou SC ( $P < 0,002$ ). Ni l'immunocastration ni la restriction alimentaire n'ont eu d'effet sur la qualité de viande. Il a été conclu que la restriction alimentaire peut augmenter l'efficacité alimentaire en engraissement, mais aussi l'agressivité des porcs IC. En raison d'un niveau d'ingestion volontaire plus important après la V2, le dépôt de lipides est plus important mais les effets du niveau alimentaire et de la composition de l'aliment sur l'utilisation des nutriments chez les porcs IC ne sont pas connus. Ainsi, l'étude suivante visait à évaluer la digestibilité des nutriments et les bilans d'azote et d'énergie chez les porcs IC. Cette étude a été réalisée en utilisant les chambres respiratoires lors de deux stades consécutifs, avant (stade 1) et après (stade 2) l'immunocastration (V2). Quatre répétitions de deux porcs mâles de la même portée ont été utilisées, afin de déterminer l'effet de l'immunocastration (étant associé avec l'effet de la croissance) et l'effet du taux de lipides de la ration sur le métabolisme énergétique entre 85 (phase 1) et 135 (stade 2) kg de poids vif. Pendant le stade 1, les porcs ont reçu un régime standard i.e. à basse teneur en matières grasses (LF; 2,5% de lipides par rapport à la MS, distribué à 2,27 MJ EM / kg PV<sup>0,60</sup> par jour) alors que, durant le stade 2, le niveau d'ingestion énergétique a été augmenté à 2,47 MJ EM / kg PV<sup>0,60</sup> par jour et un porc de chaque répétition a reçu le régime LF alors que l'autre porc a reçu un aliment enrichi en matières grasses (HF; 8,9% de matières grasses par rapport à la MS). La méthode des bilans azotés et énergétiques avec mesure des échanges respiratoires a été utilisée pour calculer le quotient respiratoire (RQ), la production de chaleur (PC), et la contribution des nutriments à la rétention de matières grasses, les composants de PC, et le dépôt de protéines (DP) et de lipides (DL). Les coefficients de digestibilité des nutriments et de l'énergie, les pertes de méthane et la rétention d'azote ont augmenté avec le stade ( $P < 0,05$ ). Malgré une augmentation de la consommation de EM, PC au total est restée similaire (1365 kJ / kg de PV<sup>0,60</sup> par jour;  $P = 0,26$ ) et la PC au jeûne a présenté une tendance à diminuer entre les stades (de 823 à 697 kJ / kg de PV<sup>0,60</sup> par jour;  $P = 0,07$ ). Par conséquent, l'énergie retenue au total (ER) a augmenté entre les stades (916 à 1078 kJ / kg par jour de PV<sup>0,60</sup>;  $P < 0,01$ ) et avec une rétention de la matière grasse plus élevée (625 à 807 kJ / kg par jour PV<sup>0,60</sup>,  $P < 0,01$ ) provenant principalement de glucides et associée à une lipogenèse (536 à 746 kJ / kg PV<sup>0,60</sup> par jour;  $P < 0,01$ ) et RQ (1,095 à 1,145;  $P < 0,01$ ) plus élevés. Ainsi, le DP (178-217 g / jour;  $P$

= 0,04) et le DL (227-384 g / jour,  $p < 0,01$ ) sont augmentés par les effets combinés de la croissance et de l'immunocastration. Les deux régimes expérimentaux avaient différente digestibilité des matières grasses brutes ( $P < 0,01$ ). Pour l'aliment HF, après l'immunocastration, une augmentation de ER sous forme de lipides (de 807 à 914 kJ / kg PV<sup>0,60</sup> par jour,  $P = 0,05$ ) ont été observées, provenant principalement de lipides alimentaires ( $P < 0,01$ ), et se traduisant par un DL plus élevé (384-435 g / jour,  $P = 0,02$ ) et un RQ réduit (1,145 à 1,073,  $P < 0,01$ ). Ces résultats indiquent que l'augmentation de l'adiposité de porcs IC est le résultat de l'augmentation du DL par jour entraîné par l'ingestion plus élevée d'énergie, un plus faible métabolisme basal et meilleure rétention de l'énergie. De plus, le DL est encore renforcé après V2 par l'apport supplémentaire d'énergie provenant de l'ajout de matières grasses. Par la suite, le dernier objectif de thèse était d'évaluer les effets de la diminution de EN dans la ration des porcs IC abattus 8 semaines après la V2 sur la croissance, la qualité de carcasse et de viande et en particulier sur les dépôts de gras avant (P1) et après V2 (P2) ainsi que pendant les quatre premières et les quatre secondes semaines après la V2. Les porcs ( $n = 41$ ) divisés en trois groupes expérimentaux, ont reçu à partir de 84 jours d'âge les aliments expérimentaux se distinguant par un niveau bas ( $n = 14$ ), moyen ( $n = 13$ ) ou haut ( $n = 14$ ) de concentration en EN (LEN, MEN et HEN avec 8.5, 9.3 et 10,0 MJ EN / kg, respectivement). En plus, un essai de digestibilité a été réalisé avec quatre porcs nourris avec les régimes extrêmes (LEN et HEN) en deux phases afin de déterminer précisément la concentration en EN des aliments. La composition de l'aliment n'a pas eu d'effet sur la consommation quotidienne quelle que soit la période de l'expérience ( $P > 0,10$ ), alors qu'une consommation de EN en MJ par jour plus élevée ( $P = 0,08$ ) a été notée lors de la dernière phase (4 à 8 semaines après V2 i.e. jour 143 à jour 170 d'âge) pour le groupe HEN. Une absence d'effet ( $P > 0,10$ ) de la réduction de EN alimentaire a été notée sur le gain moyen quotidien (GMQ) et le rapport entre le gain quotidien et la consommation d'énergie nette (G: EN) dans tous les stades de l'expérimentation. Un effet sur le gain de l'épaisseur de lard dorsal a été observé uniquement dans la seconde phase après la V2 (jour 143 à jour 170) qui était plus élevé ( $P < 0,01$ ) dans le groupe HEN. En accord avec l'absence d'effet de régime sur PV, aucun effet ( $P > 0,10$ ) a été noté sur le poids de carcasse chaude (HCW), le rendement de carcasse ou sur le

poids des pièces principales. Des différences entre les groupes expérimentaux ont été observées pour le poids de gras dorsal (en pourcentage des HCW plus faible ( $P = 0,01$ ) et plus petite surface du gras recouvrant le muscle *longissimus dorsi* ( $P = 0,05$ ) chez les porcs recevant les régimes LEN et MEN. Par conséquent, un taux de viande maigre plus élevé ( $P = 0,04$ ) a été observé chez les porcs LEN, alors que les dépôts de gras intermusculaire et intramusculaire (dans le *longissimus dorsi*) n'étaient pas affectés ( $P > 0,05$ ) par la réduction de l'EN alimentaire. Aucun effet de la teneur en EN alimentaire a été noté sur la qualité de viande, à l'exception d'une couleur plus claire ( $P = 0,04$ ) et une tendance ( $P = 0,10$ ) de teinte plus jaune (CIA b \*) du muscle *longissimus dorsi* notées pour le groupe HEN. Globalement, les résultats actuels prouvent qu'en diminuant la concentration de EN du régime alimentaire jusqu'à 10% par le biais d'une teneur élevée en fibres, une efficacité énergétique similaire (G: EN) peut être obtenue, fournissant un avantage en termes de plus faible LD, sans affecter la croissance de porcs IC après la vaccination efficace. Ceci est d'autant plus important si les porcs IC sont engraisés à des poids vifs plus élevés que la pratique habituelle ou quand un délai plus long entre V2 et l'abattage est prévu.



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## ANNEX 1

COBISS Co-operative Online Bibliographic system & services COBISS

Nina Batorek Lukač [33232]

Personal bibliography for the period 2001-2015

### ARTICLES AND OTHER COMPONENT PARTS

#### 1.01 Original scientific article

1. ČANDEK-POTOKAR M, ŠKRLEP M, BATOREK LUKAČ, N., ZAMARATSKAIA, G., PREVOLNIK POVŠE, M., VELIKONJA BOLTA, Š., KUBALE, V., BEE, G. Hydrolysable tannin fed to entire male pigs affects intestinal production, tissue deposition and hepatic clearance of skatole. The veterinary journal, ISSN 1090-0233, 2015, doi: 10.1016/j.tvjl.2015.02.012. [COBISS.SI-ID 4650600]
2. BATOREK LUKAČ, N., ŠKRLEP, M., PRUNIER, A., LOUVEAU, I., NOBLET, J., BONNEAU, M., ČANDEK-POTOKAR, M. Effect of feed restriction on hormones, performance, carcass traits, and meat quality in immunocastrated pigs. Journal of animal science, ISSN 0021-8812, 2012, vol. 90, str. 4593-4603, doi: 10.2527/jas.2012-5330. [COBISS.SI-ID 3891560]
3. KUBALE, V., BATOREK LUKAČ, Nina, ŠKRLEP, Martin, PRUNIER, A., BONNEAU, Michel, FAZARINC, Gregor, ČANDEK-POTOKAR, Marjeta. Steroid hormones, boar taint compounds, and reproductive organs in pigs according to the delay between immunocastration and slaughter. Theriogenology, ISSN 0093-691X. [Print ed.], 2013, vol. 79, iss. 1, str. 69-80, ilustr., doi: 10.1016/j.theriogenology.2012.09.010. [COBISS.SI-ID 3945064]
4. BATOREK LUKAČ, Nina, ČANDEK-POTOKAR, Marjeta, BONNEAU, Michel, VAN MILGEN, J. Meta-analysis of the effect of immunocastration on production performance, reproductive organs and boar taint compounds in pigs. Animal, ISSN 1751-7311, 2012, vol. 6, iss. 8, str. 1330-1338, ilustr., doi: 10.1017/S1751731112000146. [COBISS.SI-ID 3768168]
5. ŠKRLEP, Martin, BATOREK LUKAČ, Nina, BONNEAU, Michel, PREVOLNIK, Maja, KUBALE, Valentina, ČANDEK-POTOKAR, Marjeta. Effect of immunocastration in group-housed commercial fattening pigs on reproductive organs, malodorous compounds, carcass and meat quality. Czech Journal of Animal Science, ISSN 1212-1819, 2012, vol. 57, no. 6, str. 290-299. [COBISS.SI-ID 3862120]
6. BATOREK LUKAČ, Nina, VOGRIN BRAČIČ, Marija, ŠKORJANC, Dejan. Serum concentrations of leptin, ghrelin and cortisol are not direct related to the loss of backfat thickness, body weight, protein and fat at

weaning of second parity of crossbreed sows (Landrace x Large White). Journal of animal and veterinary advances, ISSN 1680-5593, 2013, vol. 12, št. 6, str. 766-774. [COBISS.SI-ID 3610924]

## 1.02 Review article

7. ŠKRLEP, Martin, BATOREK LUKAČ, Nina, PREVOLNIK POVŠE, Maja, ČANDEK-POTOKAR, Marjeta. Theoretical and practical aspects of immunocastration. Stočarstvo, ISSN 0351-0832, 2014, vol. 68, no. 2, str. 39-49. [COBISS.SI-ID 4679784]

## 1.03 Short scientific article

8. ŠKRLEP, Martin, BATOREK LUKAČ, Nina, BONNEAU, Michel, FAZARINC, Gregor, ŠEGULA, Blaž, ČANDEK-POTOKAR, Marjeta. Elevated fat skatole levels in immunocastrated, surgically castrated and entire male pigs with acute dysentery. The veterinary journal, ISSN 1090-0233, 2012, vol. 194, str. 417-419, doi: 10.1016/j.tvjl.2012.04.013. [COBISS.SI-ID 3836520]

## 1.04 Professional article

9. ŠKORJANC, Dejan, BATOREK LUKAČ, Nina, VOGRIN BRAČIČ, Marija. Hormonska dogajanja pri odstavljenih svinjah. Kmetovalec, ISSN 1318-4245, avg. 2013, letn. 81, št. 8, ilustr., graf. prikazi. [COBISS.SI-ID 4251240]
10. BATOREK LUKAČ, Nina, ŠKRLEP, Martin, ŠEGULA, Blaž, ČANDEK-POTOKAR, Marjeta. Kastracija pujskov. Kmečki glas, ISSN 0350-4093, 9. mar. 2011, letn. 68, št. 10, str. 8, ilustr. [COBISS.SI-ID 3533416]
11. ČANDEK-POTOKAR, Marjeta, BATOREK LUKAČ, Nina, ŠKRLEP, Martin. Če mi pismo ni všeč, je kriv poštar - odgovor na odmev v zvezi s prispevkom o kastraciji pujskov. Kmečki glas, ISSN 0350-4093, 6. apr. 2011, letn. 68, št. 14, str. 12. [COBISS.SI-ID 3563624]
12. ŠKRLEP, Martin (author, photographer), BATOREK LUKAČ, Nina, ČANDEK-POTOKAR, Marjeta. Vsebnosti skatola in androstenona pri mladih merjascih in s tem povezan problem vonja mesa v slovenskih rejskih razmerah. Kmetovalec, ISSN 1318-4245, dec. 2011, letn. 79, št. 12, str. 14-16, ilustr., graf. prikazi. [COBISS.SI-ID 3713640]
13. BATOREK LUKAČ, Nina, ŠKRLEP, Martin, ŠEGULA, Blaž, ČANDEK-POTOKAR, Marjeta. Kastrirani ali ne kastrirani (pujske), to je zdaj vprašanje za prihodnost. Reja prašičev, ISSN 1408-600X, 2011, letn. 14, št. 1, str. 13-17. [COBISS.SI-ID 3597416]
14. BATOREK LUKAČ, Nina, ŠKORJANC, Dejan. Uravnavanje apetita pri svinjah v laktaciji. Reja prašičev, ISSN 1408-600X, 2010, letn. 13, št. 2, str. 8-11, ilustr. [COBISS.SI-ID 2977068]

## 1.08 Published scientific conference contribution

15. ŠKRLEP, Martin, BATOREK LUKAČ, Nina, ŠEGULA, Blaž, ZAJEC, Marta, KOŠOROK, Stane, GLAVAČ-VNUK, Marija, KUBALE, Valentina, FAZARINC, Gregor, ČANDEK-POTOKAR, Marjeta. Effect of immunocastration on performance of Slovenian pig fatteners. V: KAPŠ, Miroslav (ur.). Animal science days, (Agriculturae Conspectus Scientificus, ISSN 1331-7768, vol. 76, Number 3, 4). [Zagreb]: Universities of the quadrilateral agreement, 2011, str. 205-208. [COBISS.SI-ID 3659368]
16. KUBALE, Valentina, FAZARINC, Gregor, BATOREK LUKAČ, Nina, ŠKRLEP, Martin, ŠEGULA, Blaž, BONNEAU, Michel, ČANDEK-POTOKAR, Marjeta. Dinamika vpliva imunizacije s cepivom ImprovacR na morfologijo mod in pomožnih spolnih žlez pri prašičih. V: 4. slovenski veterinarski kongres 2011, Portorož, 18. do 19. november 2011. MAJDIČ, Gregor (ur.). Program in zbornik referatov, (Slovenian veterinary research, ISSN 1580-4003, Supplement, 13). Ljubljana: Veterinarska fakulteta, 2011, vol. 48, suppl. 13, str. 198-202. [COBISS.SI-ID 3444602]
17. ŠKRLEP, Martin, BATOREK LUKAČ, Nina, ŠEGULA, Blaž, KUBALE, Valentina, FAZARINC, Gregor, ČANDEK-POTOKAR, Marjeta. Incidenca kožnih poškodb na klavnih trupih prašičev : primerjava merjascev, imunokastratov in kirurških kastratov. V: 4. slovenski veterinarski kongres 2011, Portorož, 18. do 19. november 2011. MAJDIČ, Gregor (ur.). Program in zbornik referatov, (Slovenian veterinary research, ISSN 1580-4003, Supplement, 13). Ljubljana: Veterinarska fakulteta, 2011, vol. 48, suppl. 13, str. 203-206. [COBISS.SI-ID 3444858]
18. ŠKRLEP, Martin, ČANDEK-POTOKAR, Marjeta, BATOREK LUKAČ, Nina, PREVOLNIK, Maja, VELIKONJA BOLTA, Špela, LISJAK, Klemen. Effect of salting duration on salt content and proteolysis in Kraški pršut dry hams after the resting phase. V: ČANDEK-POTOKAR, Marjeta (ur.). 8th International Symposium on the Mediterranean Pig, Slovenia, Ljubljana, October 10th-12th, 2013, (Acta agriculturae slovenica, ISSN 1854-4800, Supplement, 2013, 4). Ljubljana: Biotechnical Faculty, 2013, suppl. 4, str. 193-196, tabeli. [COBISS.SI-ID 4310120]
19. LABUSSIÈRE, Etienne, BATOREK LUKAČ, Nina, BESNARD, Jean-Charles, ČANDEK-POTOKAR, Marjeta, NOBLET, Jean. Effet de la teneur en énergie nette du régime sur la consommation volontaire et les performances de croissance des porcs mâles immunocastrés = Effect of dietary net energy content on voluntary feed intake and growing performances of immunologically castrated male pigs. V: 46e Journées de la recherche porcine, Paris 4 et 5 février 2014, INRA, (Journées de la Recherche Porcine, ISSN 0767-9874). Paris: Institut technique du porc, 2014, ann. 46, str. A14. [COBISS.SI-ID 4491368]
20. BATOREK LUKAČ, Nina, ŠKRLEP, Martin, PREVOLNIK POVŠE, Maja, ČANDEK-POTOKAR, Marjeta. Teoretični in praktični vidiki imunokastracije = Theoretical and practical aspects of immunocastration. V: ČEH, Tatjana (ur.), KAPUN, Stanko (ur.). Zbornik predavanj = Proceedings of the 23rd International

Scientific Symposium on Nutrition of Farm Animals, 13th and 14th November 2014. Murska Sobota: Kmetijsko gozdarska zbornica Slovenije, Kmetijsko gozdarski zavod, 2014, str. 11-17. [COBISS.SI-ID 4588904]

21. ČANDEK-POTOKAR, Marjeta, ŠKRLEP, Martin, BATOREK LUKAČ, Nina, KUBALE, Valentina, FAZARINC, Gregor, SALOBIR, Janez, REZAR, Vida, LEVART, Alenka, FANEDL, Lijana, AVGUŠTIN, Gorazd, PREVOLNIK POVŠE, Maja, ŠKORJANC, Dejan. Reja merjašchkov s stališča problematike spolnega vonja in kakovosti proizvodov - predstavitev projekta in prvih rezultatov = Raising entire males with an outlook on boar taint and product quality - preliminary project results. V: ČEH, Tatjana (ur.), KAPUN, Stanko (ur.). Zbornik predavanj = Proceedings of the 23rd International Scientific Symposium on Nutrition of Farm Animals, 13th and 14th November 2014. Murska Sobota: Kmetijsko gozdarska zbornica Slovenije, Kmetijsko gozdarski zavod, 2014, str. 25-30. [COBISS.SI-ID 3469704]
22. ŠKRLEP, Martin, ČANDEK-POTOKAR, Marjeta, POTOČNIK, Boris, PREVOLNIK POVŠE, Maja, BATOREK LUKAČ, Nina, ŽEMVA, Marjeta, LISJAK, Klemen. Zmanjšanje soli v kraškem pršutu - predstavitev rezultatov projekta Agrotur = Reducing salt content in "Kraški pršut" dry ham - results from Agrotur project. V: ČEH, Tatjana (ur.), KAPUN, Stanko (ur.). Zbornik predavanj = Proceedings of the 23rd International Scientific Symposium on Nutrition of Farm Animals, 13th and 14th November 2014. Murska Sobota: Kmetijsko gozdarska zbornica Slovenije, Kmetijsko gozdarski zavod, 2014, str. 167-172. [COBISS.SI-ID 4590696]
23. PUGLIESE, Carolina, ŠKRLEP, Martin, MACAGNANO, Antonella, ZAMPETTI, Emiliano, BATOREK LUKAČ, Nina, ČANDEK-POTOKAR, Marjeta, LABUSSIÈRE, Etienne. The use of electronic nose to differentiate entire males from immunocastrates (association with levels of androstenone and skatole). V: ČANDEK-POTOKAR, Marjeta (ur.). 8th International Symposium on the Mediterranean Pig, Slovenia, Ljubljana, October 10th-12th, 2013, (Acta agriculturae slovenica, ISSN 1854-4800, Supplement, 2013, 4). Ljubljana: Biotechnical Faculty, 2013, suppl. 4, str. 167-170, ilustr. [COBISS.SI-ID 4309864]
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25. ŠKRLEP, Martin, ČANDEK-POTOKAR, Marjeta, BATOREK LUKAČ, Nina, ŠEGULA, Blaž, PREVOLNIK, Maja, PUGLIESE, Carolina, BONNEAU, Michel. Length of the interval between immunocastration and slaughter in relation to boar taint and carcass traits. V: PETRIČ, Nežika (ur.).

Livestock production as a technological and social challenge, (Acta agriculturae slovenica, ISSN 1854-4800, Supplement, 2012, 3). Ljubljana: Biotechnical Faculty, 2012, suppl. 3, str. 247-251. [COBISS.SI-ID 3923816]

26. BATOREK LUKAČ, Nina, DUBOIS, Serge, NOBLET, Jean, BONNEAU, Michel, ČANDEK-POTOKAR, Marjeta, LABUSSIÈRE, Etienne. Vpliv količine zaužitih maščob na presnovo energije ter nalaganje proteinov in maščob pri imunokastriranih merjaških = Effects of dietary fat intake on energy metabolism and protein and lipid deposition in immunocastrated male pig. V: 21. mednarodno znanstveno posvetovanje o prehrani domačih živali, Zdravčevi-Erjavčevi dnevi 2012. ČEH, Tatjana (ur.). Zbornik predavanj = Proceedings of the 21st International Scientific Symposium on Nutrition of Farm Animals : [being] Zdravec-Erjavc Days 2012, Radenci, November 8th and 9th, 2012. Murska Sobota: Kmetijsko gozdarska zbornica Slovenije, Kmetijsko gozdarski zavod Murska Sobota, 2012, str. 131-137. [COBISS.SI-ID 3954536]

### **1.10 Published scientific conference contribution abstract (invited lecture)**

27. KUBALE, Valentina, BATOREK LUKAČ, Nina, ŠKRLEP, Martin, TOČAJ, Vesna, FAZARINC, Gregor, ČANDEK-POTOKAR, Marjeta, LABUSSIÈRE, Etienne. Histological adaptations of the gastrointestinal tract of immunocastrated pigs affected by varied dietary net energy content. V: 2nd Congress of Slovenian Society for Laboratory Animals, Ljubljana, 19-20 June 2014. MAJDIČ, Gregor (ur.). Proceedings = Zbornik prispevkov, (Slovenian veterinary research, ISSN 1580-4003, 2014, suppl. 15). Ljubljana: Veterinarska fakulteta, 2014, str. 29. [COBISS.SI-ID 3843450]
28. KUBALE, Valentina, FAZARINC, Gregor, BATOREK LUKAČ, Nina, ŠKRLEP, Martin, BONNEAU, Michel, ČANDEK-POTOKAR, Marjeta. Dynamic effects of the Improvac vaccination on reproductive organs in pigs according to the delay between immunocastration and slaughter. V: FIETZ, Simone (ur.). Program and abstracts : July 17-20, 2013 Leipzig, (Leipziger Blaue Hefte). Berlin: Lehmanns Media-Verlag, 2013, str. 25. [COBISS.SI-ID 3705978]

### **1.12 Published scientific conference contribution abstract**

29. KUBALE, Valentina, BATOREK LUKAČ, Nina, ŠKRLEP, Martin, TOČAJ, Vesna, FAZARINC, Gregor, ČANDEK-POTOKAR, Marjeta, LABUSSIÈRE, Etienne. Effects of dietary net energy content on intestinal morphology and cell proliferation in immunocastrated pigs in comparison on entire males. Anatomia, Histologia, Embryologia, ISSN 0340-2096. [Print ed.], 2014, vol. 43, suppl. 1, str. 58. [COBISS.SI-ID 3858554]
30. KUBALE, Valentina, BATOREK LUKAČ, Nina, ŠKRLEP, Martin, TOČAJ, Vesna, FAZARINC, Gregor, ČANDEK-POTOKAR, Marjeta, LABUSSIÈRE, Etienne. Morfološke spremembe tankega in debelega

- črevesja pri imunokastriranih prašičih krmljenih z različno neto energetsko vrednostjo krmila. V: MAJDIČ, Gregor (ur.). 5. slovenski veterinarski kongres 2014 = 5th Slovenian Veterinary Congress 2014, Portorož, 14.-15. November 2014, (Slovenian veterinary research, ISSN 1580-4003, 2014, suppl. 16). Ljubljana: Veterinarska fakulteta, 2014, vol. 51, suppl. 16, str. 100-102. [COBISS.SI-ID 4584296]
31. ŠKRLEP, Martin, ČANDEK-POTOKAR, Marjeta, BATOREK LUKAČ, Nina, PREVOLNIK POVŠE, Maja, PUGLIESE, Carolina, FLORES, Mónica, LABOUSSIERE, Etienne. Use of entire males and salt reduction in dry-cured ham production. 1, Green ham properties, processing yields, chemical composition. V: TOLDRÁ, Fidel (ur.), FLORES, Mónica (ur.). Book of abstracts : 2nd International Symposium on Fermented Meat, 20-23 October, Valencia, Spain, (Cost Action FA 1102: Farm Animal Imaging (FAIM)). [S. l.]: [S. n.], 2014, str. 45. [COBISS.SI-ID 4567912]
32. ŠKRLEP, Martin, ČANDEK-POTOKAR, Marjeta, BATOREK LUKAČ, Nina, PREVOLNIK POVŠE, Maja, PUGLIESE, Carolina, FLORES, Mónica, LABOUSSIERE, Etienne. Use of entire males and salt reduction in dry-cured ham production. 2, Rheological traits and sensory profile. V: TOLDRÁ, Fidel (ur.), FLORES, Mónica (ur.). Book of abstracts : 2nd International Symposium on Fermented Meat, 20-23 October, Valencia, Spain, (Cost Action FA 1102: Farm Animal Imaging (FAIM)). [S. l.]: [S. n.], 2014, str. 74. [COBISS.SI-ID 4568168]
33. FANEDL, Lijana, ČANDEK-POTOKAR, Marjeta, ŠKRLEP, Martin, BATOREK LUKAČ, Nina, LABOUSSIERE, Etienne, SLAVEC, Andreja, LIPOGLAVŠEK, Luka, STRES, Blaž, ŠKET, Robert, AVGUŠTIN, Gorazd. Molekularno biološka analiza vpliva imunokastracije in dodatka taninov v krmi na intestinalno mikrobioto pri merjaščkih. V: 6. kongres Slovenskega mikrobiološkega društva, 24.-26. september 2014, Bled, Slovenija. KUŠAR, Darja (ur.), OCEPEK, Matjaž (ur.). Knjiga povzetkov : kongres SMD 2014. Ljubljana: Veterinarska fakulteta, 2014, str. 132. [COBISS.SI-ID 3448200]
34. BATOREK LUKAČ, Nina, NOBLET, Jean, BONNEAU, Michel, ČANDEK-POTOKAR, Marjeta, LABOUSSIERE, Etienne. Effect of dietary net energy content on performance and lipid deposition in immunocastrated pigs. V: Book of Abstracts of the 64th Annual Meeting of the European Federation of Animal Science, Nantes, France, 26-30 August 2013, (EAAP book of abstract series, ISSN 1382-6077, 19). Wageningen: Wageningen Academic Publishers, 2013, str. 560. [COBISS.SI-ID 4295016]
35. ŠKRLEP, Martin, BATOREK LUKAČ, Nina, ČANDEK-POTOKAR, Marjeta. Supplementing pig diet with hydrolysable tannins: effect on performance and level of androstenone and skatole in boars. V: Production and utilization of meat from entire male pigs : EAAP Working Group 2013, Monells, Girona, 2nd - 3rd December : [abstracts]. Monells: IRTA, 2013, str. 20. [COBISS.SI-ID 4370792]
36. BATOREK LUKAČ, Nina, DUBOIS, Serge, NOBLET, Jean, BONNEAU, Michel, ČANDEK-POTOKAR, Marjeta, LABOUSSIERE, Etienne. Vpliv količine zaužitih maščob na presnovo energije ter nalaganje proteinov in maščob pri imunokastriranih merjaščkih = Effects of dietary fat intake on energy metabolism and

- protein and lipid deposition in immunocastrated male pig. V: ČEH, Tatjana (ur.), KAPUN, Stanko (ur.). Zbornik izvlečkov predavanj = Abstracts of the proceedings of the 21st International Scientific Symposium on Nutrition of Farm Animals : [being] Zdravec-Erjavec Days 2012, Radenci, November 8th and 9th, 2012. Murska Sobota: Kmetijsko gozdarska zbornica Slovenije, Kmetijsko gozdarski zavod, 2012, str. 31. [COBISS.SI-ID 3956328]
37. BATOREK LUKAČ, Nina, ČANDEK-POTOKAR, Marjeta, ŠKRLEP, Martin, BONNEAU, Michel. Effect of feed restriction in immunocastrates on performance, carcass and meat quality - comparison with ad libitum fed boars and surgical castrates. V: Boars headig for 2018 : [abstracts satellite program]. Amsterdam: LEI - Wageningen University and Research Centre, 2011, 1 str. [COBISS.SI-ID 3743592]
38. BATOREK LUKAČ, Nina, VOGRIN BRAČIČ, Marija, ŠKORJANC, Dejan. Effect of application time of PG600 on sows` weaning-to-oestrus interval. V: VÁCLAVKOVÁ, Eva (ur.). Research in pig breeding : International workshop, Kostelec nad Orlicí, 2010 : [proceedings book. Kostelec nad Orlicí: Výzkumný ústav živočišné výroby, 2010, str. 5. [COBISS.SI-ID 3425384]

## **MONOGRAPHS AND OTHER COMPLETED WORKS**

### **2.05 Other educational material**

39. ŠKRLEP, Martin (author, photographer), ČANDEK-POTOKAR, Marjeta, ŠEGULA, Blaž (author, photographer), ŽABJEK, Andreja, HORVAT, Anja, BATOREK LUKAČ, Nina, PREVOLNIK POVŠE, Maja, REPIČ, Milan, JANŽEKOVIČ, Marjan. Merjenje lastnosti kakovosti mesa pri govedu. Ljubljana: Kmetijski inštitut Slovenije, 2013. 20 str., ilustr. [COBISS.SI-ID 4083816]
40. ČANDEK-POTOKAR, Marjeta (author, editor), BATOREK LUKAČ, Nina (author, editor), ŠKRLEP, Martin (author, editor). Pitanje prašičev glede na uporabnost in kakovost klavnih trupov in mesa : priročnik za prašičerejce v sklopu usposabljanja "Konverzija krme pri prašičih", dne 12. decembra 2012 na Kmetijsko gozdarskem zavodu Murska Sobota. Ljubljana: Kmetijski inštitut Slovenije, 2012. 1 zv. (loč. pag.), ilustr. [COBISS.SI-ID 3991400]

## **PERFORMED WORKS (EVENTS)**

### **3.15 Unpublished conference contribution**

41. BATOREK LUKAČ, Nina. Predstavitev uspešnih zgodb sodelovanja : primer sodelovanja raziskovalcev s podjetji : predavanja na posvetu Prenos znanja iz akademske sfere v gospodarstvo, 24. apr. 2013. 2013. [COBISS.SI-ID 4307304]





## ANNEX 2

### BIOGRAPHY (Europass Curriculum Vitae)


#### PERSONAL INFORMATION



Nina Batorek

 Zgomje Hoče 26D, SI-2311 Hoče, Slovenia

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 ++386 41 928 457

 [nina.batorek@kis.si](mailto:nina.batorek@kis.si)

Date of birth 14/05/1985 | Nationality Slovenian

FOR

Doctoral thesis

#### WORK EXPERIENCE

from November 2010

assistant researcher

**Agricultural institute of Slovenia**, Hacquetova 17, SI-100 Ljubljana, Slovenia

Animal Science Department

 [www.kis.si](http://www.kis.si)

- research work

from April 2013

assistant

University of Maribor, **Faculty of agriculture and life sciences**, Pivola 10, SI-2311 Hoče, Slovenia

Animal Science Department

 [www.fk.uni-mb.si](http://www.fk.uni-mb.si)

- Physiology and anatomy of domestic animals

#### EDUCATION AND TRAINING

1/2015 VLAG course Advanced Food Analysis 2015

The Graduate School VLAG, Wageningen, Nederland

10/2013 training for experiment leader in animal experimentation

University of Ljubljana, Veterinary faculty, Slovenia

04/2013 training from entrepreneurship for young researchers

LUI- Ljubljana university incubator, Ljubljana, Slovenia



- from June 2011 to October 2012 **research work**  
 INRA, UMR 1348 Pegase, F-35590 Saint Gilles, France  
 Physiology, Genetics and Environment for Animal and Livestock Systems
- HPLC method of boar taint analysis in pork fat tissue under supervision of A. Prunier
  - training in the field of animal nutrition (study of energy metabolism in respiration cages, the technique of nitrogen and energy balance, comparative slaughter technique) under supervision of E. Labussiere
  - determination of fatty acid composition in biological samples under supervision of E. Labussiere and J. Mouro
- from 2010 **Ph. D. Student**  
 University of Maribor, Faculty of agriculture and life sciences, Pivola 10, SI-2311 Hoče, Slovenia  
 and  
 Agrocampus-Ouest, UMR 1348 Pegase, F-35000 Rennes, France
- 2003-2009 **doctor of veterinary medicine (dr.vet.med.)**  
 University of Ljubljana, Veterinary faculty, Gerbičeva ulica 60, SI-1000 Ljubljana, Slovenia
- Knowledge in the field of veterinary medicine (physiology, anatomy, histology, pathology, microbiology, pharmacology, animal health, food safety,...)
- 1999-2003 **high school graduate**  
 II. Gimnazija Maribor, Trg Miloša Zidanška 1, SI-2000 Maribor, Slovenia

**PERSONAL SKILLS**

Mother tongue Slovenian

Other languages	UNDERSTANDING		SPEAKING		WRITING
	Listening	Reading	Spoken interaction	Spoken production	
English	C2	C2	C2	C2	C2
French	B2	B1	B2	B2	B1
Croatian	C2	C2	C2	C2	C2

Levels: A1/2: Basic user - B1/2: Independent user - C1/2 Proficient user  
 Common European Framework of Reference for Languages

- Job-related skills
- biochemical analysis of biological material
  - measurements of carcass and meat quality
  - analysis of boar taint compounds
- Computer skills
- Microsoft Office, SAS, R

Driving licence

- B category



## ANNEX 3

UNIVERZA V MARIBORU  
Fakulteta za kmetijstvo in biosistemske vede

### IZJAVA DOKTORSKEGA KANDIDATA

Podpisana Nina Batorek-Lukač, vpisna številka 51060975 izjavljam, da je doktorska disertacija z naslovom: *Energy metabolism, growth performance, body composition, meat quality and boar taint compounds in immunocastrated pigs / Metabolizem energije, pitovne lastnosti, telesna sestava, kvaliteta mesa in substance vonja po mesjascu pri imunokastriranih prašičih;*

- rezultat lastnega raziskovalnega dela. *N. B. Lukač*
- da predložena disertacija v celoti ali v delih ni bila predložena za pridobitev izobrazbe po študijskem programu druge fakultete ali univerze,
- da so rezultati korektno navedeni in
- da nisem kršil-a avtorskih pravic in intelektualne lastnine drugih.

*N.B. V skladu s konvencijo o sodelovanju med VRT in Agriscampus Ovest 14. dne 1. 3. 2011, je bila doktorska naloga kategorizirana kot ta FFBV predložitveni kodi in doktorski deli Agriscampus Ovest, Reunis, Franceja.*

*N. B. Lukač*

Datum in kraj:

Podpis doklerandke:

Ljubljana, 15. 4. 2015

Nina Batorek-Lukač

*N. B. Lukač*

ANNEX 4

UNIVERZA V MARIBORU

Fakulteta za kmetijstvo in biosistemske vede

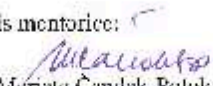
IZJAVA KANDIDATOVEGA MENTORJA O USTREZNOSTI DOKTORSKE  
DISERTACIJE

Podpisana **Marjeta Čandek-Potokar** mentorica doktorski kandidark, izjavljam, da je doktorska disertacija z naslovom: *Energy metabolism, growth performance, body composition, meat quality and boar taint compounds in immunocastrated pigs / Metabolizem energije, pitovne lastnosti, telesna sestava, kvaliteta mesa in substance vonja po mešascu pri imunokastriranih prašičih*, ki jo je izdelala doktorska kandidatka **Nina Batorek-Lukač**, v skladu z odobreno temo, Pravilnikom o pripravi in zagovoru doktorske disertacije ter mojimi navodili in predstavlja izvirni prispevek k razvoju znanstvene discipline.

Datum in kraj:

Ljubljana, 15.4.2015

Podpis mentorice:

  
izr. prof. dr. Marjeta Čandek-Potokar



ANNEX 5

UNIVERZA V MARIBORU

Fakulteta za kmetijstvo in biosistemске vede

IZJAVA O OBJAVI ELEKTRONSKE VERZIJE DOKTORSKE DISERTACIJE IN  
OSEBNIH PODATKOV, VEZANIH NA ZAKLJUČEK ŠTUDIJA

Ime in priimek doktorandke: **Nina Batorck-Lukač**

Vpisna številka: **51060975**

Študijski program: **FKBV doktorski študij kmetijstvo**

Naslov doktorskega dela: *Energy metabolism, growth performance, body composition, meat quality and boar taint compounds in immunocastrated pigs / Metabolizem energije, pitovne lastnosti, telesna sestava, kvaliteta mesa in substance vonja po merjascu pri imunokastriranih prašičih*

Mentorica: **izr. prof. dr. Marjeta Čandek-Potokar**

Somentor: **Jacob van Milgen, PhD, HDR**

Podpisana Nina Batorck-Lukač, soglašam z objavo doktorske disertacije v Digitalni knjižnici Univerze v Mariboru.

Tiskana verzija doktorske disertacije je istovetna elektronski verziji, ki sem jo oddala v Digitalno knjižnico Univerze v Mariboru.

Podpisana hkrati izjavljam, da dovoljujem objavo osebnih podatkov, vezanih na zaključek študija (ime, priimek, leto in kraj rojstva, datum diplomiranja, naslov diplomskega dela) na spletnih straneh in v publikacijah Univerze v Mariboru.

Datum in kraj:

Ljubljana, 15.4.2015

Podpis doktorandke:

Nina Batorck-Lukač

