ČESKÁ ZEMĚDĚLSKÁ UNIVERZITA V PRAZE
Fakulta agrobiologie, potravinových a přírodních zdrojů

Katedra speciální zootechniky

Testace mutací vybraných kandidátních genů asociovaných s hladinami androstenonu a skatolu

Doktorská disertační práce

Doktorand: Ing. Kateřina Zadinová

Školitel: doc. Ing. Roman Stupka, CSc.

Konzultant: prof. Ing. Antonín Stratil, DrSc.

Praha 2017

Čestné prohlášení

Prohlašuji, že jsem předkládanou disertační práci na téma **„Testace mutací vybraných kandidátních genů asociovaných s hladinami androstenonu a skatolu“** vypracovala samostatně a použila jsem pramenů, které cituji a uvádím v seznamu použité literatury.

V Praze dne

Poděkování

Ráda bych touto cestou poděkovala především panu doc. Ing. Romanu Stupkovi, CSc. a prof. Ing. Antonínu Stratilovi, DrSc. za odborné vedení, trpělivost a cenné rady při zpracování disertační práce. Dále bych chtěla poděkovat doc. Ing. Jaroslavu Čítkovi, Ph.D. a kolegům z KSZ za pomoc a spolupráci.

V neposlední řadě chci poděkokat své rodině a přátelům za jejich podpou a trpělivost.

Obsah

1 Úvod

Vepřové maso patří v evropských státech k nejoblíbenějším a nejkonzumovanějším druhům masa. Jeho spotřeba v ČR činí cca 40 kg na osobu a rok, což přesahuje 50% veškeré spotřeby masa. Po vstupu do EU u nás došlo k prudkému poklesu stavů prasat a také soběstačnost v této komoditě výrazně klesala. Nyní se pohybuje okolo 60%. Jedním z důvodů je rovněž to, že hodně našich chovatelů má nepříznivé ekonomické výsledky a nejsou schopni vyprodukovat vepřové maso za ceny srovnatelné s chovatelsky vyspělými zeměmi.

V posledních letech je věnována velká pozornost životním podmínkám a prostředí, ve kterém hospodářská zvířata žijí. Upřednostňovány jsou produkty od zvířat chovaných v prostředí splňujícím požadavky na welfare zvířat. U kanečků na výkrm se běžně provádí kastrace krátce po narození (do 7. dne), aby se zabránilo výskytu kančího pachu ve vepřovém mase. Od roku 2018 doporučuje EU v členských státech ukončit chirurgickou kastraci kanečků bez použití anestezie. Toto opatření vzniklo pod tlakem ochranářských organizací s cílem zvýšit pohodu a welfare zvířat.

Výkrm kanečků se v porovnání s prasničkami a vepříky jeví jako výhodnější, především díky lepší růstové intenzitě, konverzi krmiva a dosažení vyšší zmasilosti. To je dáno působením androgenů (C-19 steroidů), syntetizovaných ve varlatech. Velkým problémem je ale výskyt tzv. kančího pachu ve vepřovém mase. Kančí pach se projevuje především u tepelně opracovaného masa a je způsoben hlavně androstenonem, skatolem a indolem. Androstenon je jedním ze samčích pohlavních hormonů (feromonů) a skatol a indol jsou látky vzniklé při trávení. Pokud nedojde k dostatečnému zmetabolizování v játrech, ukládají se tyto látky v tukové tkáni prasat. Takto zasažené maso není většinou spotřebiteli dobře přijímáno. Někdy lze u masa kanců detekovat kromě kančího pachu i nepříjemnou (abnormální) chuť masa. Ta je zapříčiněn stejnými látkami jako kančí pach tedy androstenonem, skatolem a indolem.

Nekastrovaní kanci jsou v některých zemích, jako je Velká Británie, Irsko nebo některé státy jižní Evropy, Španělsko, Portugalsko a Kypr, již využíváni na produkci vepřového masa. Kastrace kanečků do 7 dnů po narození je nejběžnějším způsobem jak předejít vzniku kančího pachu. Dojde-li k zákazu chirurgické kastrace bez použití anestezie, bude nutné hledat jiné alternativy, jak předejít nepříjemnému zápachu. Jednou z možností je využít poznatků molekulární genetiky a pokusit se výskyt kančího pachu a kančí chuti omezit pomocí genetické selekce, s využitím vhodných jednonukleotidových (SNP) mutací v kandidátních genech kódujících enzymy, které jsou zapojeny do syntézy a metabolismu androstenonu,

skatolu a indolu. Podle doposud známých zjištění je kančí pach ovlivněn větším počtem genů, které na sebe mohou vzájemně působit. Vzhledem k tomu, že některé geny se nacházejí ve stejných chromozomových oblastech, kde jsou lokalizovány geny pro syntézu pohlavních hormonů jako je testosteron, nebo pro znaky růstu a jatečné hodnoty, musíme brát v úvahu i vliv zmiňovaných genů na reprodukční a produkční ukazatele u prasat.

2 Literární přehled

2.1 Kančí pach

Kančí pach je potu a moči podobný zápach (Lee *et al.,* 2005; Strathe *et al.,*. 2013a), zapříčiněný substancemi, které vznikají především v souvislosti s dosažením pohlavní dospělosti kanců (Duijvesteijn *et al.,* 2010; Xue *et* Dial, 1997; Zamaratskaia, 2004; Zamaratskaia *et* Squires, 2009). Hlavní složky zodpovědné za vznik kančího pachu jsou androstenon (5α-androst-16-en-3-on), skatol (3-methylindol) (Zamaratskaia *et* Squires, 2009), dále také indol (Grindflek *et al.,* 2011b). Rovněž v malé míře 3α-androstenol (3α-OL) a 3β-androstenol (3β-OL) (Bonneau *et al.,* 2000a; Xue *et* Dial, 1997), které vznikají při metabolismu androstenonu (Zamaratskaia *et* Squires, 2009). Pokud nejsou tyto látky zmetabolizovány v játrech dochází k jejich akumulaci v tukové tkáni zvířat a vzniku kančího pachu. Toto maso není spotřebiteli dobře přijímáno (Engelsma *et al.,* 2007). Přijatelné hladiny pro spotřebitele u dvou nevýjznamnějších složek se pohybují mezi 0,5 – 1 μg/g tuku pro androstenon a 0,2 – 0,25 μg/g tuku pro skatol (Zamaratskaia *et al.,* 2004). Pro skatol mohou být uváděny hodnoty výrazně nižší kolem 150 ng/g (0,15 μg/g) (Mörlein *et al.,* 2012). Vnímavost kančího pachu a kančí chuti se však u konzumentů v jednotlivých státech Evropy liší (Bonneau *et al.,* 2000b).

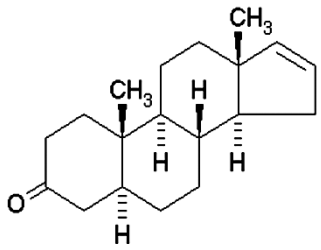
Aby se předešlo tomuto nepříjemnému zápachu, jsou kanečci v některých zemích kastrováni velmi brzy po narození (Moe *et al.,* 2008; Duijvesteijn *et al.,* 2010). Důvodem kastrace není jen zamenení nepříjemného pachu, ale také omezení agresivity a pohlavního chování mladých kanců. Způsob kastrace se stává problémem hlavně z hlediska etického a z hlediska welfare zvířat (Gray *et* Squires, 2013a), protože kastrace malých kanečků do 7. dne věku se provádí bez anestezie (Engelsma *et al.,* 2007). Jelikož takto prováděná kastrace způsobuje strach, bolest a může zhoršit zdravotní stav zvířat (Prunier *et al.,* 2006; Aleksić *et al.,* 2012; Lundström *et* Zamaratskaia, 2006), je snaha změnit tento způsob kastrace, na což reaguje i legislativa EU. Od roku 2018 začne platit doporučení neprovádět chirurgickou kastraci kanečků bez použití narkózy (Baes *et al.,* 2013; Mörlein *et al.,* 2012). Evropská deklarace počítá s ukončením chirurgické kastrace k 1.1. 2018 (European Declaration on Alternatives to Surgical Castration of Pigs, 2010) . Jedná se však pouze o doporučení, které je v jednotlivých státech EU postupně zaváděno do praxe s většími či menšími omezeními. Zákaz na úrovni legislativy je prozatím jen v některých státech jako jsou např. Dánsko (2011), Německo (2019), Švédsko (2016). Ve státech severní a západní Evropy se ve více jak 50% případů

používají analgetika bez legislativní úpravy. Země ve střední a východní Evropě kam patří i Česká republika většinou kastrují bez anestetik či analgetik. Některé státy - Španělsko, Velká Británie kastrují méně jak 20% procent zvířat, zde je kančí maso vnímáno jako součást národní kuchyně a většinou se praktikuje výkrm do nižší porážkové hmotnosti (okolo 80-90 kg).

Vzhledem k tomu je nutné hledat alternativy k odstranění kančího pachu, a tím k zabránění znehodnocení vepřového masa (Engelsma *et al.,* 2007; Grindflek *et al.,* 2011b).

2.1.1 Složky kančího pachu

2.1.1.1 Androstenon



Obrázek 1. - Chemická struktura androstenonu

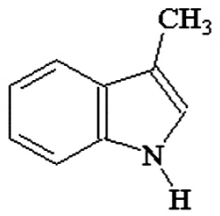
Androstenon (5α-androst-16-en-3-on) je steroidní hormon, produkovaný v Leydigových buňkách ve varlatech (Moe *et al.,* 2007a; Oskam *et al.,* 2008) společně s dalšími steroidními hormony, androgeny a estrogeny (Robic *et al*., 2011b). Ukládán je do tukové tkáně a slinných žláz odkud je vypouštěn do ovzduší jako feromon stimulující ochotu k páření u prasnic (Moe *et al*., 2008). Hromadění androstenonu v tukové tkáni způsobuje nepříjemný zápach (Zamaratskaia, 2004). Kromě toho byly zjištěny malé koncentrace androstenonu i v plazmě vepříků a prasnic, což naznačuje možnou produkci androstenonu také kůrou nadledvin (Claus *et al.,* 1971).

Prekurzorem pro biosyntézu androstenonu je pregnenolon. Od ostatních pohlavních hormonů se liší tím, že nemá hormonální funkce (Chen, 2007). Androstenon je syntetizován z pregnenolonu přes 5,16-androstadien-3β-ol pomocí andien-β syntázového systému. Při biosyntéze 5,16-androstadien-3β-olu hrají důležitou roli cytochrom P450C17 (CYP17A1) a cytochrom b5 (CYB5) (Davis *et* Squires, 1999). Produkce androstenonu a dalších testikulárních hormonů je řízena neuroendokrinním systémem, především prostřednictvím

luteinizačního hormonu (LH), který je řízen gonadotropin-releasing hormonem (GnRH) (Sinclair *et al.* 2001; Zamaratskaia *et* Squires, 2009).

Androstenon je metabolizován v játrech (Andresen, 2006; Doran *et al.,* 2004) a ve varlatech (Doran *et al.,* 2004). Velký podíl androstenonu syntetizovaného ve varlatech je okamžitě sulfokonjugován hydroxysteroid sulfotransferázami SULT2A1 (Sinclair *et al.,* 2005b) a SULT2B1 (Moe *et al.,* 2007a). Metabolismus probíhá ve dvou fázích. Ačkoliv játra jsou schopna produkovat metabolity obou fází, jejich hlavní úlohou je fáze II – sulfokonjugace (Sinclair *et al.,* 2005a). Ve fázi I je androstenon metabolizován 3β-hydroxysteroid dehydrogenázami za přítomnosti kofaktorů NADH a NADPH (Doran *et al.,* 2004). Míra exprese metabolismu androstenonu je dána mírou exprese jaterní 3β-hydroxysteroid dehydrogenázy (3βHSD) (Andresen, 2006; Doran *et al.,* 2004). Koncentrace androstenonu v tukové tkáni se zvyšuje s věkem kanců (Andersson *et al.,* 1999). Klíčovým enzymem fáze II je SULT2A1 (Sinclair *et al.,* 2006). Androstenon je metabolizován na 5α-androst-16-en-3a-ol a 5β-androst-16-en-3b-ol (Zamaratskaia *et* Squires, 2009), což představuje asi 68% všech metabolitů fáze II (Sinclair *et al.,* 2005a).

2.1.1.2 Skatol



Obrázek 2. - Chemická struktura skatolu

Druhou významnou složkou kančího pachu je 3-methyl indol neboli skatol. Skatol vzniká při rozkladu bílkovin z aminokyseliny L-tryptofanu, je produkován bakteriemi tlustého střeva u monogastrů (Lee *et al.,* 2005) a metabolizován jaterními enzymy (Zamaratskaia *et* Squires, 2009). Skatol je zodpovědný za zápach podobný moči a exkrementům. Z části je odváděn výkaly, z části metabolizován v játrech (Chen, 2007) a část je akumulována v tukové tkáni v důsledku jeho lipofilní povahy (Babol *et al.,* 1998b) a také v ledvinách.

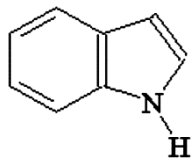
Na rozdíl od monogastrů, u kterých nezpůsobuje výrazné problémy, je skatol toxický pro přežvýkavce, u nichž způsobuje ABPE (acute bovine pulmonary edema and emphysema).

Prasata nejsou vysokými koncentracemi skatolu takto postižena, u nich způsobuje jen nepříjemný zápach a chuť masa (Deslandes *et al.*, 2001).

Syntéza skatolu probíhá ve dvou krocích za pomoci střevních bakterií. Nejprve z tryptofanu vzniká kyselina indol-3-octová působením bakterií *Escherichia coli* a *Clostridium spp. (Clostridium difficile, Clostridium sticklandii, Clostridium lituseburense, Clostridium subterminale* a *Clostridium putrefaciens)* (Yokoyama *et* Carlson, 1979) a následně z kyseliny indol-3-octové rody *Lactobacillus* a *Clostridium* vytvoří 3-methyl indol (skatol) (Jensen *et al*., 1995; Zamaratskaia et Squires, 2009).

Metabolismus skatolu zahrnuje dvě fáze – oxidační reakce a konjugaci. Během fáze I dojde k navázání hydroxylové skupiny, která je následně využita k připojení konjugátu ve fázi II (Zamaratskaia *et* Squires, 2009). Diaz *et al.,* (1999) identifikovali 7 meziproduktů fáze I (3-hydroxy-3-methylindolenine, 3-hydroxy-3-methyloxindole, 5-hydroxy-3-methylindole, 6-hydroxy-3-methylindole, 3-methyloxindole, indole-3-carbinol a 2-aminoacetophenone). Tyto metabolity pak slouží jako substrát pro další reakce ve druhé fázi metabolismu (Babol *et a*l., 1998a; Diaz *et* Squires, 2003). Enzymy fáze I zahrnují rodinu cytochromu P450 (CYP). Za hlavní enzymy jsou pokládány CYP2E1 (Babol *et al.*, 1998b), CYP2A6 (Diaz *et* Squires, 2000a) a rovněž aldehyd oxidáza (AO) (Diaz *et* Squires, 2000b). Fáze II pak zahrnuje sulfokonjugaci pomocí sulfotransferáz (SULT1A1), kyseliny glukuronové (Babol *et al*., 1998b) a UDP (urine-difosfát-glucuronosyltransferáza) (Diaz *et* Squires, 2003). Zde vznikají 5-sulphatoxyskatole, 6-sulphatoxyskatole, 5-hydroxyskatole glucuronide, 6-hydroxyskatole glucuronide a skatole-glutathione (Zamaratskaia *et* Squires, 2009). Metabolické produkty skatolu jsou následně vyloučeny močí z těla (Xue *et* Dial, 1997).

2.1.1.3 Indol



Obrázek 3. - Chemická struktura indolu

Indol (2,3-benzopyrol) (Verheyden *et al.,* 2007) vzniká stejně jako skatol při rozkladu aminokyseliny L-tryptofanu v trávicím traktu monogastrů. Je absorbován do krve a metabolizován v játrech (Yokoyama *et* Carlson, 1979). L-tryptofan je buď degradován na

indol, nebo z něho vzniká kyselina indol-3-octová a z ní následně skatol jak je popsáno výše (Chen, 2007). Na rozdíl od skatolu je do degradace na indol zapojeno široké spektrum bakterií (Deslandes *et al.,* 2001) *Escherichia coli, Proteus vulgaris, Paracolobactrum coliforme, Achromobacter lique-faciens, a Micrococcus aerogenes* (Yokoyama *et* Carlson, 1979).

Indol je podobně jako skatol ovlivňován expresí cytochromů CYP2E1 a CYP2A6. Skatol a indol mají rovněž stimulační účinky na expresi CYP2A6. V případě indolu je tento vliv výraznější než u skatolu (Chen *et al.,* 2008). Stejně jako androstenon a skatol, i indol se hromadí a byl detekován v tukové tkáni prasat. Jeho hladina může být ovlivněna i jinými než genetickými vlivy, například čistotou, respektive špínou v kotci nebo složením krmiva. Jeho vliv na kvalitu vepřového masa není tak výrazný jako v případě skatolu. Indol spíše jen zesiluje nepříjemný pach masa, ačkoliv indol a skatol mají velmi podobnou vůni. Indol je také spojován s intenzivnější abnormální chutí kančího masa (Chen, 2007).

V porovnání se skatolem není indol toxický pro přežvýkavce a nezpůsobuje u nich poškození plic. To naznačuje klíčovou roli 3-methylové skupiny (Deslandes *et al.,* 2001; Chen, 2007).

2.1.2 Faktory ovlivňující výskyt kančího pachu

Na výskyt kančího pachu má vliv řada důležitých faktorů, které můžeme rozdělit do dvou skupin - na faktory vnitřní a vnější (Zamaratskaia, 2004).

2.1.2.1 Vnitřní faktory

Geny: Vliv genů a jejich SNP na hladiny androstenonu a skatolu je popisován ve studiích Doran *et al*., 2004; Duijvesteijn *et al.,* 2010; Grindflek *et al.,* 2011b; Robic *et al.,* 2008; Strathe *et al*., 2013a. V případě androstenonu se jedná především o geny rodiny CYP, které působí na syntézu androstenonu (Davis *et* Squires, 1999;), dále pak o hydroxysteroid dehydrogenázy a sulfotransferázy, které ovlivňují degradaci androstenonu (Robic *et al*., 2008 Sinclair *et al.,* 2005).

Plemeno: Byly nalezeny rozdílné koncentrace androstenonu a skatolu mezi čistokrevnými plemeny i mezi hybridními kombinacemi (Chen, 2007; Oskam *et al.,* 2010). Primitivnější a ranější plemena jako je Meishan mají vyšší koncentrace androstenonu a skatolu v tukové tkáni a plasmě (Whittinghton *et al.,* 2004; Xue *et al*., 1996). U prošlechtěných plemen jsou nižší hladiny především androstenonu. I mezi těmito plemeny jsou patrny rozdíly – u kanců plemene Pietrain byly detekovány vyšší hladiny androstenonu a skatolu než u kanců plemene Belgická nebo Norská Landrace (Xue *et al.,* 1996).

Pohlaví: Androstenon, skatol a indol jsou produkovány jak kanci, tak prasnicemi a kastráty. Kanci mají ve srovnání s vepříky a prasničkami výrazně vyšší hladinu, především androstenonu, ale i skatolu a indolu (Claus *et al.,* 1971; Hansen *et al.,* 2008).

Věk: Koncentrace androstenonu a skatolu v tukové tkáni roste s věkem (Andersson *et al.,* 1999; Babol *et al.,* 2004; Zamaratskaia *et al.,* 2004). Nárůst hladiny androstenonu s věkem v tukové tkáni a plasmě byl pozorován u kanců a prasniček. U vepříků byl naopak zaznamenán pokles (Claus *et al.,* 1971).

Hmotnost: Hladiny androstenonu a skatolu jsou nižší u zvířat do 70 až 90 kg (Fàbrega *et al.* 2011; Zamaratskaia *et al.,* 2005a; Zamaratskaia *et al.,* 2006). Vyšší hmotnost souvisí rovněž s pohlavním dospíváním kanců a produkcí pohlavních hormonů (Andersson *et al.,* 1999; Zamaratskaia *et al*., 2004)

Funkce jater: Játra prasat jsou schopna zmetabolizovat mnohem více androstenonu a skatolu než kolik ho vyprodukuje tělo. Přesto dochází ke kumulaci části těcho látek do tukové tkáně (Zamaratskaia, 2004).

2.1.2.2 Vnější faktory

Výživa: Některé doplňky stravy mohou snižovat hladiny kančího pachu. Byl prokázán vliv čekanky resp. inulinu na pokles skatolu (Zammerini *et al.,* 2012; Rasmussen *et al.,* 2014) bramborového škrobu, cukrové řepy nebo lupiny (Hansen *et al.,* 2008; Wesoly *et* Weiler, 2012).

Systém ustájení: Čistota kotce a přítomnost výkalů ovlivňuje hladinu skatolu a indolu u kanečků (Chen, 2007; Walstra *et al.,* 1999). Hladina androstenonu a skatolu je rovněž ovlivněna přítomností prasniček v ustájovacích halách pro předvýkrm a výkrm (Fàbrega *et al.,* 2011).

Roční období a světelný režim: Hladina androstenonu klesá s narůstající délkou světelného dne (Andersson *et al.,* 1998; Walstra *et al.,* 1999; Zamaratskaia, 2004).

2.2 Eliminace kančího pachu za pomoci metod molekulární genetiky

V posledních letech je věnováno značné úsilí minimalizaci výskytu obou hlavních složek kančího pachu (androstenon a skatol) ve vepřovém mase. Jednou z cest je právě identifikace chromozómových oblastí, kandidátních genů a mutací (SNP – single nucleotide polymorphism) asociovaných s hladinami androstenonu a skatolu u komerčních populací

prasat (kanečků). Identifikace vhodných SNP způsobujících kančí zápach, by mohla urychlit jeho odstranění v rámci šlechtitelské práce v chovech, a tím odstranit potřebu kastrace kanečků (Duijvesteijn *et al.,* 2010).

Redukce kančího pachu pomocí genetické selekce se jeví jako slibné řešení, protože koncentrace složek kančího pachu jsou středně až vysoce dědivé (Robic *et al.,* 2008; Grindflek *et al.,* 2011b; Strathe *et al.,* 2013a). Heritabilita hladiny androstenonu se pohybuje v rozmezí 0,25 – 0,87 (Oskam *et al.,* 2010) a heritabilita hladiny skatolu 0,19 – 0,54 (Robic *et al.,* 2008). To znamená, že v populaci prasat se vyskytují jedinci s různě vysokým potenciálem pro jednotlivé úrovně androstenonu a skatolu (Oskam *et al.,* 2010). Tajet *et al.,* (2006) také uvádějí pozitivní genetickou korelaci mezi hladinami skatolu a androstenonu (0,36 - 0,62).

Lokusy pro kvantitativní znaky (QTL - Quantitative trait loci), které obsahují geny ovlivňující konkrétní vlastnost, lze identifikovat porovnáním genotypů neznámých markerů rozmístěných po celém chromozomu s fenotypem nebo s konkrétním zkoumaným znakem. Kandidátní geny mohou být identifikovány studiem genů, které se nacházejí v oblasti QTL dříve identifikované anonymními markery (Squires, 2006).

Quintanilla *et al.,* (2003) a Lee *et al.,* (2005) identifikovali několik QTL pro androstenon a skatol v experimentálních populacích kříženců (Meishan a Landrace). Lee *et al*., (2005) ve své práci popisují jako místo s nejvíce QTL, které mohou mít vliv na skatol a indol, chromozom (SSC – *Sus scrofa chromosome*) SSC14. Jako místa s QTL pro androstenon, uvádějí chromozomy SSC2, SSC4, SSC6, SSC7 a SSC9. Ovšem pouze na chromozomu SSC6 se nachází QTL i pro ovlivnění nepříjemné "kančí chuti" masa. Quintanilla *et al.,* (2003) v rámci projektu PORQTL identifikovali QTL ovlivňující hladiny androstenonu v tukové tkáni na chromozomech SSC3, SSC4, SSC7, SSC14 a také na koncích ramen chromozomů SSC6 a SSC9. To naznačuje, že na hladinách androstenonu, skatolu a indolu se podílí větší počet genů. Duijvesteijn *et al*., (2010) uvádějí rovněž SSC6, ale také chromozom SSC1 jako místo pro geny ovlivňující hladinu androstenonu.

Další možností při určování vhodných genů je studium polymorfismů v kandidátních genech. Nejčastěji se jedná o SNP. Kandidátní geny mohou kódovat důležité enzymy pro metabolismus jednotlivých složek kančího pachu a právě polymorfismy v kódující oblasti těchto genů, mohou působit na hladiny těchto látek (Squires, 2006). Studiem SNP se zabývaly například práce Lin *et al.,* (2004a); Lin *et al.,* (2006) u skatolu a Lin *et al*., (2005a); (2005b);

Kim *et al.*, (2013) u androstenonu. Ramos *et al.,* (2011) také nacházejí několik SNP markerů souvisejících s hladinou skatolu na distálním konci chromozomu 6p (SSC6p). Tyto SNP markery jsou seskupeny do 3 samostatných clusterů. Značné rozdíly jsou pozorovány především mezi homozygoty v jednotlivých skupinách. Jednotlivé SNP markery vysvětlují až 22% fenotypové variability. Tato oblast obsahuje několik kandidátních genů pro androstenon (Duijvesteijn *et al.*, 2010; Grindflek *et al*., 2011a).

Pro detekci QTL a SNP jsou často využívány genome-wide association studies (GWAS). Jako nejkomplexnější nástroj se používá Illumina Porcine SNP60 Genotyping BeadChip (de Campos *et al.*, 2015; Ernst *et* Steibel, 2013; Lukić *et al.,* 2015; Rowe *et al.,* 2014). Große-Brinkhaus *et al.,* (2015) touto metodou identifikovali 28 SNPs, které významně asociují alespoň s jednou ze složek kančího pachu. 5 popsaných SNPs nebylo dosud vůbec identifikováno v genomu prasat. Rovněž Lukić *et al.,* (2015) využili metodu Illumina Porcine SNP60 pro identifikaci téměř 62 153 SNP a přesnost odhadu fenotypu byla měřena 6 různými metodami – best linear unbiased model prediction (GBLUP) a pěti Bayesian regression methods (Bayes Lasso; Bayes A; Bayes B; Bayes C; Bayes SSVS). Metoda celogenomového hodnocení je přesnější než pouhá detekce QTL. Porovnáním metody Ridge regression Blup (RR-BLUP) a Bayesian LASSO (BL) se zabývá i práce de Campos *et al*., (2015).

Další metodou, kterou lze využít při studiu genů ovlivňující kančí pach i v rámci GWAS je next generation sequencing (NGS) (Grindflek *et al*., 2011). Jednou z metod NGS je pak RNA-seq, které umožňuje přesnější identifikaci jednotlivých transkriptů. Asociační analýza odhalila mutace v případě rodin monooxygenáz, hydroxysteroiddehydrogenáz, rodiny cytochromu P450 a nově v potenciálních kandidátních genech *IRG6, MX1, IFIT2, CYP7A1, FMO5* a *KRT18* (Gunawan *et al.,* 2013). Sahadevan *et al.,* (2015) se snažili identifikovat co-expression klastry v jaterní tkáni související s hladinou androstenonu v tuku prasat. Využili metodu RNA-seq a mikročipů u tří populací prasat (kanců) Duroc – otcovská linie, Duroc × F2 kanci a Norská Landrace. Geny nacházejícei se v klastru 2 se zdají být ve velmi úzkém vztahu s nízkou hladinou androstenonu.

2.3 Geny pro androstenon

Při syntéze androstenonu hrají klíčovou roli především geny řídící cytochrom P450C17 (*CYP17A*), cytochrom b5 (*CYB5A*) (Davis *et* Squires, 1999) a luteinizační hormon, beta polypeptid (*LHB*) (Duijvesteijn *et al.,* 2010). Lin *et al.,* (2005a) uvádějí, že mutace v genu *CYB5* významně ovlivňuje pokles hladiny androstenonu u prasat. Quintanilla *et al.,* (2003)

zmiňují ve své práci ještě možnost vlivu *CYP21* a *CYP11A* na chromozomu SSC7, ale v případě *CYP11A* nebyl vliv u jimy hodnocené populace prasat prokázán. Metabolismus androstenonu je v první fázi ovlivněn především *3βHSD* (Andresen, 2006; Doran *et al.* 2004; Duijvesteijn *et al.*, 2010; Chen, 2007*), CYP17A1* a *CYP21A2* (Davis et Squires, 1999; Wang *et al.*, 2015) a ve druhé fázi sulfotransferázami - *SULT2A1, SULT2B1* (Duijvesteijn *et al*., 2010).

Robic *et al.,* (2011a) zkoumali u populace kříženců Large White a Meishan ještě další potencionální geny, které mohou mít vliv na hladiny androstenonu (*C6ORF106, C6ORF81, CLPS, SLC26A8, SRPK1, MAPK14* a *TEAD3*). Ovšem ani u těchto genů se neprokázal žádný přímý vliv. Gregersen *et al.,* (2012) identifikovali ve své práci ještě geny *SRD5A2* (5α-reduktáza) a *LOC100518755* jako další kandidátní geny pro androstenon. Geny *CYP2C49, CYP2D6, NGFIB* a *CTNND1* také souvisí podle Moe *et al.,* (2009) s hladinou androstenonu v tukové tkáni prasat. Rowe *et al.,* (2014) nalezli asociace několika SNP s hladinou androstenonu na SSC5 a také na SSC13 a SSC17.

2.3.1 Geny ovlivňující syntézu androstenonu

2.3.1.1 Geny rodiny CYP (cytochrom P450)

2.3.1.1.1 CYP11A1

CYP11A1 (cytochrome P450 family 11 subfamily A member 1; ENSSSCG00000025273) (Ensembl; http://www.ensembl.org/) se u prasete nachází na chromozomu SSC7 (Quintanilla *et al.* 2003). Tento gen obsahuje 7 exonů a jeho velikost je 6 895 bp (Scaffold GL893363.2: 159 079-165 974 bp).

Enzym CYP11A1 katalyzuje první krok biosyntézy steroidních hormonů (Payne *et* Hales, 2004). Iniciuje přeměnu cholesterolu na pregnenolon (Robic *et al.*, 2008).

Kromě prasat byl tento gen zjištěn i u myší, potkanů (krys), lidí a mnoha dalších živočišných druhů. U lidí se tento gen nachází na chromozomu 15q23-q24 a jeho délka přesahuje 31 kb (Chung *et al*., 1986). Hlavní exprese *CYP11A1* je v kůře nadledvin, vaječnících, varlatech a placentě. Rovněž může být i v centrálním a periferním nervovém systému (Payne *et* Hayle, 2004).

O tomto genu se uvažovalo jako o možném kandidátním genu ovlivňujícím hladinu androstenonu, protože je na počátku jeho syntézy (Robic *et al.*, 2008; Robic *et al.*, 2011a). Moe *et al.,* (2007b) a Grindfleck *et al.,* (2010) zjistili rozdílnou expresi *CYP11A1* u kanců

s extrémně vysokými a nízkými hladinami androstenonu. Quintanillou *et al.*, (2003) byl *CYP11A1* však vyloučen jako potenciální kandidátní gen ovlivňující hladiny androstenonu u jím zkoumané populace prasat. Ani Robic *et al.*, (2011a) nepotvrdili ve své studii vliv *CYP11A1* nebo jeho mutace na hromadění androstenonu u prasat.

2.3.1.1.2 CYP17A1

Tento gen (steroid 17-alpha-hydroxylase/17,20 lyase; ENSSSCG00000010591) je u prasete lokalizován na chromozomu SSC7 (123 773 105-123 779 533 bp) (Davis *et* Squires, 1999; Ensembl).

CYP17A1 stojí na počátku syntézy steroidních hormonů z pregnenolonu. U člověka je gen *CYP17A1* na chromozomu 10q24.3. Jeho exprese je nejvíce patrná ve varlatech a vaječnících. Ovšem u některých druhů jako je člověk a opice byl prokázán také v placentě a nadledvinách (Payne *et* Hales, 2004; Pelletier *et al.*, 2001).

CYP17A1 je důležitý pro metabolismus androstenonu u prasat (Davis *et* Squires, 1999). Společně s CYB5A je na počátku syntézy androstenonu z pregnenolonu (Sinclair *et al.* 2006; Lin *et al.*, 2005a; Billen *et* Squires, 2009). Lin *et al*., (2005b) objevil substituci (T → A) (thymin → adenin) v nukleotidu 1317 (NM_214428.1:c.1220T>A), což způsobí změnu aminokyseliny leucin - Leu^{439} na histidin - His^{439}. Ačkoliv předpokládali vliv mutací v tomto genu na změnu enzymatické aktivity ovlivňující biosyntézu androstenonu nebo pohlavních hormonů, nic takového se nepotvrdilo.

U prasat nebyly pozorovány ani další polymorfismy, které jsou velice důležité pro správné interakce mezi *CYP17A1* a *CYB5A*. Jelikož existuje možnost, že projevy jednotlivých genů se mohou lišit podle plemene, provedli Moe *et al*., (2007b) studii na plemenech Landrace a Duroc. Ani u jednoho z testovaných plemen však nebyl prokázán významný rozdíl mezi projevem *CYP17A1* u zvířat s nízkou a vysokou hladinou androstenonu.

2.3.1.1.3 CYP21A2

Gen *CYP21A2* (cytochrome P450 family 21 subfamily A member 2; ENSSSCG00000001428) se nachází na chromozomu SSC7 (Grindflek *et al.* 2011a) v oblasti 27 722 776-27 725 977 bp (Ensembl).

CYP21A2 stojí na počátku syntézy androstenonu – katalyzuje hydroxylaci progesteronu. Progesteron je substrátem pro CYP21A2 a při této reakci není nutná exprese CYP17A1

(Payne *et* Hales, 2004). Exprese CYP21A2 se výhradně projevuje v kůře nadledvin (Wijesuriya *et al.*, 1999). Existují dvě varianty genu *CYP21*, známé jako *CYP21A2* a *CYP21B2*.

U lidí kóduje aktivní enzym pouze *CYP21B2* (Higashi *et al*. 1986). U lidí je gen lokalizován na 6p21.3, má 11 exonů a velikost přesahuje 6 kb (databáze NCBI; http://www.ncbi.nlm.nih.gov/). Je prokázáno, že velmi výrazně ovlivňuje plodnost u žen (Grindflek *et al*., 2010).

U prasat je kódujícím genem *CYP21A2* (Grindflek *et al*., 2010). Tento gen měl různou expresi u zvířat s nízkými a vysokými hladinami androstenonu (Grindflek *et al*., 2011a). V práci Grindflek *et al.*, (2010) je potvrzen jeho vliv na hladinu androstenonu pouze u hodnocené populace plemene Landrace a nikoliv u druhé populace plemena Duroc.

2.3.1.2 Geny rodiny CYB (cytochrom b)

2.3.1.2.1 CYB5A

CYB5A (cytochrome b5 type A (microsomal); ENSSSCG00000004875) se u prasat nachází na chromozomu SSC1 (165 902 018-165 937 614 bp) (Ensembl).

CYB5A je důležitý regulátor činnosti P450 17 (CYP17A1) (Billen *et* Squires 2009), nezbytného pro syntézu steroidů a především androstenonu (Nakamura *et al.*, 2011; Zamaratskaia *et al*., 2008a; Davis *et* Squires, 1999).

U lidí se nachází na chromozomu 18q23 a jeho velikost přesahuje 38 kb. U psů se zkoumají polymorfismy, které mohou souviset s hypersenzitivitou k sulfonamidům (Funk-Keenan *et al*., 2012).

Exprese proteinu CYB5A koreluje s úrovní hladiny androstenonu v tukové tkáni prasat (Davis *et* Squires, 1999; Lin *et al*., 2005a). To nabízí možnost snížení hladiny androstenonu výběrem zvířat s nízkou úrovní cytochromu B5 (Davis *et* Squires, 1999; Gray *et* Squires 2013b). Lin *et al*., (2005a) nalezli ve své studii polymorfismus (G → T) (guanin → thymin) v 5´ UTR oblasti (MN_001001770.1:c-8G>T), který je spojen s nižší hladinou androstenonu v tukové tkáni prasat. Zastoupení jednotlivých genotypů bylo *GG* 84,8%, *GT* 12,4% a *TT* 2,8%. Homozygotní jedinci s alelou *T* vykazují výrazně nižší aktivitu CYB5A. Tyto výsledky potvrzují i Peacock *et al*., (2008). Korelace *CYB5A* s vysokou hladinou androstenonu potvrzují i Leung *et al*., (2010). Zamaratskaia *et al.*, (2008a) v rozporu s předchozími

zjištěními uvádějí, že CYB5A má vliv na nízké hladiny androstenonu v plazmě a nízké hladiny skatolu v tukové tkáni prasat, ale už nenalézají žádný vliv na hladinu androstenonu v tuku. Dále také zmiňují i vliv živé hmotnosti prasat současně s působením CYB5A. Ale praktické využití těchto poznatků je zatím sporné z důvodu nízkého vlivu na androstenon a také z důvodu nízkého výskytu alely *T* v hodnocené populaci prasat.

2.3.1.3 LHB (gen kódující β řetězec luteinizačního hormonu LH)

LHB (ENSSSCG00000003151) je u prasat lokalizován na chromozomu SSC6 (Duijvesteijn *et al.*, 2010; Hidalgo *et al.*, 2014). Velikost tohoto genu je 1 047 bp (50 063 956-50 065 003 bp) (Ensembl). Tento gen navozuje syntézu steroidních hormonů v Leydigových buňkách varlat při nástupu puberty (Duijvesteijn *et al*., 2010) a rovněž ovlivňuje činnost vaječníků (Dasgupta *et al*., 2012).

U lidí se nachází na chromozomu 19q13.32, má 3 exony a celkovou velikost přesahující 1 kb (databáze NCBI). Mutace tohoto genu mohou u lidí souviset s rakovinou prsu (Giovangrandi *et al*., 2001) nebo s cystami na vaječnících (Dasgupta *et al*., 2012).

Duijvesteijn *et al*., (2010) uvažují o *LHB* jako o možném kandidátním genu pro androstenon, právě díky jeho lokalizaci na chromozomu 6. V této oblasti bylo doposud zjištěno několik SNP ovlivňujících hladinu androstenonu.

2.3.1.4 HSD – hydroxysteroid dehydrogenázy

Hydroxysteroid dehydrogenázy (HSD) jsou skupinou enzymů, které ovlivňují biosyntézu steroidních hormonů z cholesterolu u savců. Na rozdíl od enzymů rodiny CYP, které jsou řízeny jedním genem, existuje několik izoforem a izoenzymů dehydrogenáz, které jsou řízeny specifickými geny. Funkce těchto izoforem a izoenzymů se liší podle druhů živočichů a tkání (Payne *et* Hales, 2004).

2.3.1.4.1 17β – HSD (gen HSD17B7)

HSD17B7 (hydroxysteroid (17-beta) dehydrogenase 7; ENSSSCG000000063378) je u prasat lokalizován na chromozomu SSC6 (Duijvesteijn *et al*., 2010). Dosahuje velikosti 22 527 bp (95 571 648-95 594 175 bp) (Ensembl).

Tento enzym katalyzuje poslední krok syntézy androgenů a estrogenů (Payne *et* Hales, 2004). U člověka se nachází na chromozomu 17q11-q21, má 7 exonů a jeho velikost přesahuje 6,85

kb (databáze NCBI). U člověka se podílí na přeměně estrogenu na aktivní estradiol (Payne *et* Hales, 2004), ale u prasat toto zjištěno nebylo (Chen, 2007).

Chen *et al*., (2007) uvádějí i u jaterní 17β – HSD negativní vztah mezi expresí tohoto enzymu a hladinou androstenonu v tukové tkáni. Exprese 17β – HSD nalézající se ve varletní tkáni nepůsobí žádné rozdíly v hladinách androstenonu v tukové tkáni kanců. Rovněž byly zjištěny silné korelace mezi 3β – HSD a 17β – HSD u nekastrovaných kanců, což naznačuje podobné regulační mechanismy těchto dvou genů. Dále tato práce uvádí, že funkce 17β – HSD se může lišit podle druhu tkáně. Studie provedená Chen *et al*., (2007) odhalila silnou negativní korelaci mezi expresí jaterního genu *HSD17B7* a mezi expresí plazmové E1S (estrogenu); to naznačuje důležitou roli *HSD17B7* v metabolismu estrogenů u prasat.

2.3.1.5 RDH16

RDH16 (retinol dehydrogenase 16 (all-trans); ENSSSCG00000024919) je lokalizován na SSC5 v oblasti 24 133 018-24 139 024 (Ensembl). Tento gen je nově spojován s hladinou androstenonu u prasat, především proto že byly zjištěny výrazné exprese v játrech, varlatech a také placentě prasat (http://biogps.org; Rowe *et al.,* 2014).

U člověka je *RDH16* (ENSG00000139547) lokalizován na chromozomu 12. *RDH* geny rozpoznávají enzymy, které kódují deriváty androstenonu, 5α-androstan-3α,17β-diol a androsteron (Rowe *et al*., 2014) a dále se podílí na regulaci insulinu (Obrochta *et al*., 2015).

2.3.2 Geny ovlivňující metabolismus androstenonu

2.3.2.1 HSD – hydroxysteroid dehydrogenázy

2.3.2.1.1 3β – HSD (gen HSD3B1)

Prasečí *HSD3B* (3 beta-hydroxysteroid dehydrogenase; ENSSSCG00000006719) se nachází na chromozomu SSC4 (Doran *et al*., 2004) v oblasti 111 555 566 -111 564 192 bp (Ensembl).

Působí na začátku syntézy steroidů z pregnenolonu (Payne *et* Hales, 2004). Během metabolismu androstenonu působí v I. (oxidační) fázi (Doran *et al.,* 2004). U lidí se *HSD3B* nachází na chromozomu 1 (1p13.1), má 4 exony a délku 7,8 kb (databáze NCBI). Jeho exprese byla prokázána v placentě, kůži, prsní tkáni, vaječnících, nadledvinách, játrech a varlatech (Simard *et al*., 2005). Největší počet polymorfismů *HSD3B* byl detekován u myší (Payne *et* Hales, 2004).

Doran *et al.,* (2004) dospěli k závěru, že úroveň androstenonového metabolismu je určena mírou exprese jaterní 3β – HSD. Prasata s vysokou koncentrací androstenonu v tuku mají nízkou expresi genu *HSD3B* v játrech a ve varlatech (Chen *et al*., 2007). Exprese jaterní, ne testikulární, 3β – HSD vykazuje negativní vztah k úrovni androstenonu v tukové tkáni prasat.

Pomocí klonování a sekvenování kódující oblasti bylo zjištěno, že jak jaterní tak varletní 3β - HSD mají stejné sekvence. To vede k domněnce, že exprese jednoho genu je v játrech a ve varlatech prasat řízena různými mechanismy (Nicolau-Solano *et al*., 2006). Kim *et al*., (2013) identifikovali 8 polymorfismů. Polymosfismus SNP5 NM_003534677.2:g.165262G>A (guanin > adenin) souvisí podle této práce s hladinou androstenonu u plemene duroc. Prasata s genotypem *GG* mají výrazně nižší hladiny androstenonu v porovnání s ostatními genotypy. Různá exprese tohoto enzymu může být faktorem ovlivňujícím rychlost metabolismu androstenonu v játrech, která může ovlivnit hladinu jaterní CYP2E1, a tím i rychlost metabolismu skatolu v játrech. I v působení tohoto genu jsou patrny meziplemenné rozdíly. U plemene Meishan byla zjištěna menší míra exprese než u plemene Large White (Doran *et al*., 2004).

2.3.2.2 Sulfotransferázy

2.3.2.2.1 SULT2A1

SULT2A1 (sulfotransferase family 2A member 1; ENSSSCG00000003130) se u prasat nachází na chromozomu SSC6 (Hidalgo *et al.,* 2014; Lee *et al.,* 2005) v oblasti 49 108 567-49 119 941 bp (Ensembl).

Je klíčovým enzymem v testikulárním a jaterním metabolismu 5α-androstenonu (Moe *et al.,* 2007b; Sinclair *et al.,* 2006). U lidí se nachází na chromozomu 19q13.3, má 6 exonů a dosahuje délky 15,9 kb (databáze NCBI). U člověka ovlivňuje metabolismus xenobiotik a endogenních hormonů (Huang *et al.,* 2014).

Studie provedená Sinclair *et al*., (2006) ukazuje, že exprese *SULT1A2* může ovlivnit hromadění androstenonu (5α-androstenonu) v tukové tkáni prasat. Zvířata s vysokou koncentrací 5α-androstenonu a nízkou aktivitou *SULT2A1* mají rovněž nízké hladiny SULT2A1 proteinu v porovnání se zvířaty u kterých byly zjištěny nízké hladiny 5α-androstenonu. Vliv *SULT2A1* potvrzují také závěry Leung *et al.,* (2010), kteří zjistili negativní korelace s kančím pachem u kanců plemene Duroc, Norská Landrace a Yorkshire. Negativní korelace mezi hladinou androstenonu v tukové tkáni a aktivitou testikulární

SULT2A1 (r= −0,57; P<0,01) nalezl také Sinclair *et al.*, (2006). Nicméně Moe *et al*., (2007b) prokázali vysokou expresi mRNA genu *SULT2A1* ve varlatech kanců plemene Duroc a Ladrace, kteří mají vysoké hladiny androstenonu.

2.3.2.2.2 SULT2B1

SULT2B1 (sulfotransferase family, cytosolic, 2B, member 1; *ENSSSCG00000023280*) se nachází na chromozomu SSC6, (49 700 287-49 710 764 bp) (Ensembl).

U myší se vyskytuje ve dvou formách (*Sult2b1a* a *Sult2b1b*), které jsou řízeny jedním genem *Sult2b1* (Kugori *et al.,* 2010). *Sult2b1b* katalyzuje sulfonaci 3β-hydroxysteroidních hormonů a cholesterolu zatímco *Sult2b1a* přednostně katalyzuje sulfonaci pregnenolonu (Ji *et al*., 2007). Byly prokázány interakce mezi Sult2b1b a proteiny cytoskeletu a také podíl Sult2b1b na udržování epidermální bariéry (Kurogi *et al*., 2010).

V lidském genomu je gen lokalizován na 19q13.3, zahrnuje 8 exonů a jeho délka přesahuje 48 kb (databáze NCBI). U lidí byla identifikována SULT2B1b v cytosolu a buněčných jádrech (Falany *et al*., 2006). *SULT2B1b* je také spojován s výskytem rakoviny prsu. U populace žen v Africe je prokázána vyšší exprese *SULT2B1b* a nižší výskyt rakoviny prsu než u bělochů (Dumas *et al*., 2008).

Na projev tohoto genu u prasat může mít vliv plemenná příslušnost. V práci Moe *et al*. (2007a) nebyl rozdíl v projevu jaterní SULT2B1 v souvislosti s hladinou androstenonu u plemene Landrace. Nicméně u plemene Duroc byla exprese SULT2B1 u zvířat s vysokou hladinou androstenonu o 35% nižší ve srovnání s jedinci s nízkou hladinou androstenu. U varletní SULT2B1 byla nižší exprese pozorována u obou plemen, ale u Duroca to bylo výraznější. V případě SULT2B1 existují domněnky, že jeho exprese je ovlivněna testosteronem, jak uvádějí Panella-Riera *et al*. (2008). To ale vyvrací práce Zamaratskaia *et al*., (2012), která nenachází žádný vliv testosteronu ani estron sulfátu na expresi SULT2B1.

2.3.2.3 TEAD3 (TEF-5) - Transcriptional Enhancer Factor

TEAD3 neboli *TEF-5* (ENSSSCG00000001544) se u prasat nachází na SSC7 chromozomu (Robic *et al*., 2012) (36 085 701-36 100 686 bp) (Ensembl).

U člověka je lokalizován na chromozomu 6p21.2, má 13 exonů a velikost 23,4 kb (databáze NCBI). U lidí je známo, že *TEAD3* je schopen regulovat transkripci *HSD3B*, který významně reguluje degradaci androstenonu v játrech (Robic *et al.,* 2012). Projev transkripčních faktorů

je také zkoumán v kosterní svalovině, srdeční svalovině a placentě, kde byla prozatím zjištěna nejvýraznější exprese (Jacquemin *et al*., 1997). Ani zde ale nepůsobí přímo, ale prostřednictvím dalších genů, které ovlivňují embryonální vývoj (Jacquemin *et al*., 1999).

TEAD3 byl dle Robic *et al*., (2011a) považován za možný kandidátní gen pro androstenon právě díky jeho přítomnosti na SSC7, jeho přímý vliv ale nebyl zatím prokázán. U několika populací prasat bylo testováno 12 SNP, ale jen u jedné byl statisticky potvrzený vliv na hladinu androstenonu (NM_001037966: g.726C>T). U dvou populací Large White byl zjištěn statisticky průkazný vliv alely *C* na vysokou hladinu androstenonu ve srovnání s výskytem alely *T*. U populací kříženců byl naopak zjištěn vliv genotypu *TT* na vysokou hladinu androstenonu v porovnání s genotypy *CT* a *CC*. Byla nalezena vysoká korelace mezi *TEAD3* a *HSD3B* i u prasat, ale žádný rozdíl v expresi těchto genů v závislosti na genotypu ve sledovaném SNP (Robic *et al*., 2012).

2.4 Geny pro skatol

Výsledky dosavadních studií označují hlavně dva geny CYP rodiny, které mají velice významný vliv na I. fázi metabolismu skatolu v játrech. Jedná se především o *CYP2A6* a o *CYP2E1* (Diaz *et* Squires, 2000a). Wiercinska *et al*., (2012) uvádějí jako potenciálně významné regulátory metabolismu skatolu kromě *CYP2E1* také *CYP2A19* a *CYP2C49*. Skiner *et al*. (2006) testovali ještě *CYP2C18* na hybridní dánské populaci (Landrace – Yorkshire – Duroc) ale bez prokázání vlivu. Matal *et al*., (2009) potvrzují vliv *CYP2E1* a dále doporučují zkoumat vliv genů *CYP2A19* a *CYP1A2* na metabolismus skatolu a vznik jeho metabolitů. Významnou roli v II. fázi metabolismu skatolu hraje *SULT1A1* (Babol *et al*., 1998a). Lanthier *et al*., (2007) v rámci své studie na prepubertálních kanečcích studovali kromě vlivu výše zmíněných *CYP2E1*, *CYP2A6* a *SULT1A1* ještě vliv aldehyd oxidázy (*AO*) na metabolismus skatolu.

2.4.1 Geny ovlivňující I. fázi metabolismu skatolu

2.4.1.1 Geny rodiny CYP (cytochrom P450)

2.4.1.1.1 CYP2A6 (u prasat CYP2A19)

Podle Duijvesteijn *et al*., (2010) a Chen *et al*., (2008) je gen označovaný v lidském genomu jako *CYP2A6* shodný s prasečím genem *CYP2A19*. V mnohých studiích týkajících se kančího pachu je ovšem uváděn jako *CYP2A6*. *CYP2A6* (cytochrome P450, family 2, subfamily A,

polypeptide 2; ENSSSCG00000022808) je lokalizován na chromozomu SSC6 (Diaz *et* Squires, 2000a; Lin *et al*., 2004a) v oblasti 44 853 498-44 876 802 bp (Ensembl).

V lidském genomu se nachází na chromozomu 19q13.2, zahrnuje 9 exonů a dosahuje délky téměř 7 kb (databáze NCBI). Je spojován s metabolismem některých tabákových karcinogenů (Liu *et al*., 2013), především pak nikotinu (Yamazaki *et al*., 1999). Liu *et al*., (2013) zkoumali vliv polymorfismu CYP2A6*4 na výskyt rakoviny plic u kuřáků. Ačkoliv nebyla potvrzena významná souvislost mezi CYP2A6*4 a rakovinou plic v celé populaci, byl zjištěn významný vliv u asijské populace kuřáků.

U prasat je CYP2A6 považován za jeden z klíčových enzymů v metabolismu skatolu (Lin *et al.,* 2004a). Výsledky studie provedené Diaz *et* Squires, (2000a) rovněž potvrzují jeho významnou roli v metabolismu skatolu a předpokládají, že měření hodnot aktivity CYP2A6 může pomoci k regulaci hladin 3-methyl indolu (skatolu) u prasat. Prasata, u nichž byla zjištěna vysoká aktivita CYP2A6, mají nízké hladiny skatolu (Lin *et al*., 2004a). Nízká aktivita CYP2A6 je spojena s nadměrnou kumulací skatolu v tukové tkáni prasat (Chen *et al*., 2008).

Lin *et al*., (2004a) detekovali deleci guaninu v nukleotidu 421 (Accession number AY091516.1) což vede k posunu kódující oblasti a změně její délky z 1485 bp na 612 bp. Výsledky této studie naznačují, že tato delece je spojena s inaktivací *CYP2A6* a ztrátou enzymatické aktivity. To následně vede k vysokým hladinám skatolu u prasat.

2.4.1.1.2 CYP2E1

Dalším genem, který se velmi výrazně podílí na metabolismu skatolu v játrech je cytochrom P4502E1 (*CYP2E1*). *CYP2E1* (cytochrome P450, family 2, subfamily E, polypeptide 1; ENSSSCG00000010780) se u prasat nachází na chromozomu SSC14 (Skiner *et al*., 2005) a je lokalizován v poloze 153 477 961-153 490 404 bp) (Ensembl).

V lidském genomu je lokalizován na chromozomu 10q26.3, má 9 exonů a dosahuje délky 11,7 kb (databáze NCBI). Exprese *CYP2E1* je u lidí studována hlavně kvůli metabolismu xenobiotik a karcinogenů, na kterém se podílí (Lee *et al.,* 1996; Gonzalez, 2005). Dále je také studován kvůli metabolismu alkoholu, respektive ethanolu, v souvislosti s oxidačním stresem v hepatocytech a poškozením jater u alkoholiků (Cederbaum *et al*., 2001; Lu *et* Cederbaum, 2008). Mimo jiné se také podílí na metabolismu ketolátek, které se uvolňují při hladovění a tím ovlivňuje glukogenezi (Koop *et* Casazza, 1985). Jeho vliv na metabolismus karcinogenů

byl zkoumán nejen u lidí, ale také u myší (Konstandi *et al*., 2013), potkanů a králíků (Koop *et* Casazza; 1985). Konstandi *et al.,* (2013) se zabývali vlivem samičích hormonů na regulaci CYP2E1. Docházejí k závěru, že samičí pohlavní hormony ovlivňují CYP2E1, což může mít negativní dopad na metabolismus mnoha toxických a karcinogenních látek.

U prasat CYP2E1 spouští první krok degradace skatolu v jaterní tkáni (Robic *et al*., 2008). Aktivita CYP2E1 v játrech se velice významně podílí na celkové koncentraci skatolu a jeho metabolitů v tukové tkáni prasat (Babol *et al*., 1998b; Squires *et* Lundström, 1997). Exprese CYP2E1 je v negativní korelaci s akumulací skatolu u prasat. (Lin *et al*., 2006). Podle Whittington *et al*., (2004) právě zvýšená exprese CYP2E1 snižuje hladinu skatolu v tukové tkáni. Skinner *et al*., (2005) odhalili v oblasti promotoru genu *CYP2E1* SNP, který by mohl souviset s ukládáním skatolu u dánské produkční populace prasat. Nebyly však nalezeny žádné QTL pro skatol v této oblasti, proto se zdá být nepravděpodobné, že by tato mutace měla souvislost s tvorbou kančího pachu (Robic, 2008). Aktivita promotoru *CYP2E1* je ovlivňována dvěma faktory, COUP-TF1 a HNF-1α. Androstenon může snížit aktivitu promotoru prostřednictvím blokování vazby HNF-1α na promotor. To může vysvětlovat potlačení projevu CYP2E1 v izolovaných jaterních buňkách a rovněž pozorovanou nízkou expresi CYP2E1 u zvířat a vysokou hladinou androstenonu in vivo. Nicméně efektivní koncentrace androstenonu v játrech prasat není známa (Tambyrajah *et al*., 2004).

Lin *et al*., (2006) ve své studii identifikovali substituci G→A (guanin→adenin) v nukleotidu 1423 (NM_214421.1), jejímž výsledkem je záměna aminokyseliny alanin - Ala^{475} za aminokyselinu threonin - Thr^{475}. Tato mutace způsobuje významné snížení exprese CYP2E1. Hodnocená data ukazují, že tato substituce by mohla být alespoň částečně zodpovědná za vysoké hladiny skatolu u prasat. *CYP2E1* je řazen mezi geny ovlivňující nejen metabolismus skatolu ale také metabolismus indolu. Mörlein *et al.,* (2012) zkoumali vliv SNP AJ697882.1:g.2412 C>T (cytosin > thymin) v promotorové oblasti genu (–586 ATG) u dvou užitkových populací kříženců plemene Duroc. Frekvence jednotlivých genotypů byla *CC* (25%), *CT* (52%) a *TT* (23%). U genotypu *CC* byly zjištěny výrazně vyšší hladiny skatolu a indolu než u genotypů *CT* a *TT*.

2.4.1.2 CYB5A

Gen *CYB5A* byl již dříve popsán jako jeden z genů ovlivňujících syntézu androstenonu. Wiercinska *et al*., (2012) provedli studii vlivu CYB5A na metabolismus skatolu. Domnívají se, že CYB5A může působit i na jiné isoformy cytochromu P450, než je CYP17A1, buď

inhibičně nebo stimulačně, a tím ovlivňovat i průběh metabolismu skatolu. Dále zjistili, že vliv *CYB5A* je sice neprůkazný a navíc velmi specifický pro jednotlivé geny, nicméně vlivem především na *CYP2A19, CYP2E1* a *CYP2C49* by bylo možné regulovat hladiny skatolu.

2.4.2 Geny ovlivňující II. fázi metabolismu skatolu

2.4.2.1 Sulfotransferázy

2.4.2.1.1 SULT1A1

SULT1A1 (sulfotransferase family 1A member 1; ENSSSCG00000021557) byl u prasat detekován na chromozomu SSC3 (Lin *et al*., 2004b) v Scaffold GL892962.1: 15 128-17 110 (Ensembl).

Již dříve byly geny pro fenol sulfotransferázy a jejich funkce popsány u lidí (Babol *et al*., 1998a). *SUlT1A1* se v lidském genomu nalézá na chromozomu 16p12.1, zahrnuje 12 exonů a jeho velikost je 18 kb (databáze NCBI). U člověka jsou tyto geny předmětem výzkumu hlavně v oblasti výskytu rakoviny. Mutace především v *SULT1A1* jsou spojovány s několika druhy rakoviny (Lin *et al*., 2004b), jako je například rakovina plic (Wang *et al*., 2002), rakovina močového měchýře způsobená polymorfismem G → A (guanin → adenin), který mění arginin - Arg ^{213} → histidin - His^{213} (Zheng *et al*., 2003), rakovina jícnu u mužů (Wu *et al.*, 2003) nebo rakovina prsu u žen (Tengström *et al.*, 2012).

U prasat souvisí *SULT1A1* s II. fází metabolismu skatolu (Babol *et al*., 1998b). Babol *et al*. (1998a) popsal negativní korelaci mezi hladinami fenolsulfotransferázy a akumulací skatolu v tukové tkáni prasat. Lin *et al.*, (2004b) provedli studii, kdy izolovali *SULT1A1* u 69 kanců z evropských chovů (Yorkshire, Duroc, Landrace, Pietrain a kříženci Landrace x Duroc; Large White x Duroc a Large White x Pietrain) a identifikovali substituci (A → G) v nukleotidu 546 v kódující oblasti genu (AY193893.1:c439A>G). Tím dochází ke změně lysinu - Lys^{147} na glutamin - Glu^{147} a snížení aktivity SULT1A1. Tato genetická mutace může být alespoň částečně zodpovědná za snížení katalitické aktivity SULT1A1 a následně za zvýšení hladiny skatolu v tukové tkáni prasat.

Lin *et al*., (2004b) detekovali *SULT1A1* na chromozomu SSC3. Varona *et al.*, (2005) uvádějí, že se nepodařilo v této oblasti identifikovat QTL pro vysokou hladinu skatolu u jimi zkoumané populace prasat (Landrace). Je tedy nutné provést další studie a zohlednit i plemeno či hybridní kombinaci prasat. Jelikož se v prvním zkoumaném případě jednalo o plemena Yorkshire, Duroc, Pietrain, Landrace a také o kříženec Large White x Duroc, Large

White x Pietrain a Landrace x Duroc a ve druhém případě šlo o čistokrevné jedince plemene Landrace. Skinner *et al*., (2006) tato zjištění ověřovali u kříženců Large White a Meishan a také u dánské produkční populace prasat, ale výše uvedené mutace nenalezli.

SNP AJ885177:g.76G>A je dávána do souvislosti se zmasilostí prasat. Borowska *et al*., 2014 ve své práci hodnotili asociace 50 SNP u kandidátních genů ve vztahu ke zmasilosti prasat. Právě zmiňovaná mutace v genu *SULT1A1* vykazovala největší vliv na tyto znaky.

2.5 Vzájemný vztah mezi geny pro androstenon a pro skatol

Během puberty dochází ke zvyšování hladiny skatolu v tukové tkáni kanců a ta následně koreluje s hladinami androstenonu (Babol *et al*., 1999). Možný vliv některých genů na hladinu androstenonu a současně i na hladinu skatolu byl zaznamenán už ve výše zmiňovaných pracích. Wiercinska *et al*., (2012) zmiňují vliv CYB5 na geny rodiny P450 a jejich následné působení na koncentrace skatolu. Doran *et al*., (2004) zase uvádějí, že různá exprese HSD3B1 může ovlivnit rychlost metabolismu androstenonu. To sníží aktivitu CYP2E1 a zvýší hladinu skatolu v tuku prasat. Jak uvádějí Tambyrajah *et al*., (2004), vysoká hladina androstenonu, především u kanců v pubertě, může zablokovat vazbu faktorů (COUP-TF1 a HNF-1α) ovlivňujících aktivitu promotoru *CYP2E1*, a tím snížit jeho expresi v játrech a následně ovlivnit metabolismus skatolu.

2.6 Negativní vliv zkoumaných genů na užitkové vlastnosti prasat

2.6.1 Vliv na ukazatele jatečné hodnoty

Výkrm kanečků se jeví oproti ostatním kategoriím prasat (prasničky a vepříci) jako výhodnější, především díky lepší růstové intenzitě, lepší konverzi krmiva a dosažení vyšší zmasilosti díky produkci samčích pohlavních hormonů (Babol *et* Squires, 1995; Kim *et al*., 2013; Lundström *et* Zamaratskaia, 2006; Pauly *et al*., 2009; 2012; Regueiro *et* Ruis, 1998; Squires *et al*., 1993; 2006; Strathe *et al*., 2013a; Walstra *et al*., 1999; Xue *et al*., 1997;). Vyvstává tedy otázka, zda při snížené produkci tohoto hormonu nedochází následně ke zhoršení výše uvedených parametrů. Ať už je to vlivem nízké hladiny androstenonu nebo negativním působením genů. Je zde obava z možného snížení růstové schopnosti prasat v důsledku využití genetické selekce proti vysokým hladinám androstenonu (Lundström *et* Zamaratskaia, 2006).

Borowska *et al*., (2014) ve své práci uvádějí *SULT1A1* jako kandidátní gen pro zmasilost prasat a významný vliv jeho SNP na tento ukazatel. Rovněž zmiňují i možný vliv tohoto genu na hladinu skatolu v tukové tkáni prasat.

Grindflek *et al*., (2001) nacházejí QTL pro ukazatele kvality vepřového masa hlavně na chromozomech 4, 6 a 7. Z výše citovaných zdrojů vyplývá, že na těchto chromozomech jsou rovněž lokalizovány QTL obsahující geny kančího pachu. Neuhoff *et al.,* (2015) popisují ve své práci několik SNP mutací v genech *FMO1, FMO5, CYP21, ESR1, PLIN2 a SULT2A1*. Tyto geny jsou lokaizovány právě v oblastech kde se podle výše zmíněných autorů nachází QTL pro produkční a reprodukční užitkové znaky (např. SSC4 a SSC9). Tyto skutečnosti musí být brány v úvahu.

2.6.1.1 MC4R (melanocortin-4 receptor)

MC4R (ENSSSCG00000004904) se u prasat nachází na SSC1 v oblasti 178 553 488-178 555 219 bp. U lidí je MC4R lokalizován na chromozomu 18 (60 371 110-60 372 775 bp) (Ensembl). U člověka souvisí s rozvojem a ukládáním tukové tkáně a s obezitou (Wang *et al*., 2016; Kim *et al*., 2000).

U prasat je tento gen spojován především se znaky jatečné hodnoty a výkrmnosti (Kim *et al*. 2000; Maagdenberg *et al*., 2007; Dvořáková *et al*., 2011; Van den Broeke *et al*., 2015a, 2015b). SNP mutace Asp298Asn (AF087937:c.746G>A) má významný vliv na podíl hřbetního tuku u prasat. Alela *A* koreluje s vyšším podílem tuku a alela *G* s vyšším podílem

libové svaloviny (Dvořáková *et al.*, 2011). Maagdenberg *et al.*, (2007) popisují příznivý vliv této mutace na průměrný denní přírůstek. Ten je ovšem dán vyšším podílem tuku v jatečném těle prasat.

V souvislosti s kančím pachem je tato mutace uváděna jako marker pro složky kančího pachu. Van den Broeke *et al.*, (2015a) nalézají souvislost s touto mutací v genu *MC4R* a hladinou tří nejdůležitějších složek kančího pachu – androstenonu, skatolu a indolu. Výrazně vyšší hladinu všech tří látek popisují u kanců s genotypem *AA* ve srovnání s kanci *GG*.

2.6.2 Vliv na reprodukční ukazatele

Hladiny androstenonu a skatolu je možné snižovat, ale je třeba vyhnout se poklesu plodnosti kanců (Strathe *et al.*, 2013a). Podle výsledků (Moe *et al.*, 2009, Oskam *et al.*, 2008, 2010 či Strathe *et al.*, 2013b) se zdá být selekce proti kančímu pachu pomocí genů dobrým řešením, bez rizika současného negativního ovlivnění plodnosti. Většina QTL je ale specifická pro dané plemeno, z toho vyplývá, že výběr SNP musí být navržen samostatně pro každé plemeno (Grindflek *et al*., 2011a). Další prováděné studie nacházejí možné negativní dopady na plodnost zvířat.

Strathe *et al.*, (2013b) zmiňují možnost negativní korelace mezi složkami kančího pachu a ukazateli samčí plodnosti (kvalita spermatu a libido). Předpokládá, že selekce proti kančímu pachu nebude mít vliv na samotnou produkci spermií, ale spíše na morfologii reprodukčního systému. Další možné negativní dopady jsou změny v morfologii pohlavního ústrojí (Lervik *et al*., 2013), především velikost bulbouretrální žlázy jak uvádějí Tajet *et al.*, (2006) a Foilloux *et al.*, (1997). Velikost bulbouretrální žlázy je považována za významný ukazatel pohlavní dospělosti (Tajet *et al.*, 2006). Frieden *et al.*, (2011), Sellier *et al.*, (2000), Tajet *et al.*, (2006) a Zamaratskaia *et al.*, (2005) připouštějí možné negativní dopady na pohlavní dospívání jak u kanců, tak i u prasnic. Další negativní vlivy se mohou týkat pozdějšího nástupu puberty a pozdějšího dovršení pohlavní dospělosti jak uvádějí Engelsma *et al.*, (2007) a Frieden *et al.*, (2011). Výskyt korelace mezi androstenonem a ukazateli plodnosti u prasnic naznačují možný nárůst mortality vrhu a pozdější nástup říje (prodloužení intervalu odstav-říje) u prasnic po prvním odstavu (Engelsma *et al*., 2007; Frieden *et al*., 2011). U většiny dosud identifikovaných QTL pro hladinu androstenonu dochází zároveň k ovlivnění syntézy jiných steroidních a především pohlavních hormonů (Moe *et al.*, 2009, Grindflek *et al.*, 2011a). Dle Zamaratskaia *et al.*, (2004) hladina androstenonu pozitivně koreluje s hladinou testosteronu především v pozdějším věku kanců. Neuhoff *et al.*, (2015) uvádí jako jeden

z možných genů pro snížení hladiny androstenonu *ESR1*, který se v různé míře podílí na syntéze jak testosteronu tak androstenonu. Zmínění autoři se shodují v názoru, že je třeba provést další a podrobnější výzkumy v této oblasti, a tím přesněji potvrdit, které z genů určených k selekci proti kančímu pachu ovlivňují, a které neovlivňují ukazatele plodnosti.

3 Cíle práce a vědecké hypotézy hypotézy

3.1 Cíle práce

Na základě současných publikovaných zjištění o možnostech eliminace kančího pachu s využitím genetických metod byly stanoveny tyto cíle práce:

1. Identifikovat potenciální kandidátní geny ovlivňující syntézu steroidů a metabolismus androstenonu a skatolu.

2. Posoudit vliv polymorfismu vybraných kandidátních genů na hladinu androstenonu, skatolu a indolu.

3. Posoudit vliv různé hladiny androstenonu a skatolu na parametry růstu a jatečné hodnoty.

3.2 Vědecké hypotézy

Vzhledem ke stanoveným cílům byly v rámci disertační práce formulovány následující hypotézy:

H1. Syntéza a metabolismus androstenonu, skatolu a indolu jsou mimo jiné ovlivňovány geny. Geny asociované s hladinami androstenonu, skatolu a indolu se nacházejí na chromozomech, kde jsou lokalizoány QTL pro další užitkové vlastnosti prasat.

H2. Vybrané mutace genů, zejména z řad genů rodiny *CYP*, sulfotransferáz a monooxygenáz, průkazně ovlivňují hladinu androstenonu a skatolu v tukové tkáni prasat.

H3. Úroveň hladiny androstenonu a skatolu v těle prasat v závislosti na pohlaví ovlivňuje parametry růstu a jatečné hodnoty.

4 Materiál a metodika

V průběhu zpracování této práce byla u zvířat - kanců vykrmovaných na testační stanici v Ploskově u Lán Katedry speciální zootechniky ČZU v Praze - sledována hladina androstenonu, skatolu a indolu. Jednalo se o hybridní linie prasat Dx(BUxL) a ČBUx(ČBUxČL), běžně chované na výkrm a produkci vepřového masa v České republice.

Vzorky tukové tkáně pro chemické stanovení těchto látek byly odebrány při porážce, stejně jako krev, která byla použita pro izolaci DNA a stanovení genotypů u vybraných SNP metodou polymerázové řetězové reakce a polymorfismu restrikčních fragmentů DNA (PCR-RFLP).

Genetická analýza probíhala v laboratořích katedry speciální zootechniky ČZU v Praze a Ústavu živočišné fyziologie a genetiky AV ČR v Liběchově. Stanovení hladin androstenonu, skatolu a indolu, a rovněž hodnocení parametrů růstu a jatečné hodnoty probíhalo v laboratořích katedry speciální zootechniky a v testační stanici v Ploskově.

Stanovení hladiny androstenonu, skatolu a indolu bylo provedeno ze vzorku tukové tkáně odebrané do 24 hodin po porážce pomocí kapalinové chromatografie dle metodiky Hansena-Møllera, (1994) modifikované Okrouhlou *et al.*, (2016) – přístrojem HPLC Jasco řady 2000, Tokio, Japonsko. (Metodický okruh II a III)

Během výkrmu byly pravidelně sledovány parametry výkrmnosti a od průměrné hmotnosti 60 kg se zaznamenávala výška a plocha nejdelšího zádového svalu za účelem výpočtu procentuálního podílu masa v JUT, za použití ultrazvukového přístroje ALOKA SSD 500 se sondou UST 5011U – 3,5 MHz. (Metodický okruh III)

Statistické vyhodnocení získaných výsledků bylo provedeno v programu SAS 9.4 (Metodický okruh II a III).

Metodické postupy byly podrobně rozepsány v přiložených publikacích (Kapitola 5).

Na základě výše stanovených cílů a vědeckých hypotéz byla práce rozdělena do tří následujících okruhů.

4.1 Identifikace kandidátních genů a enzymů zapojených do syntézy a metabolismu složek kančího pachu

Na základě znalostí o metabolismu androstenonu a skatolu byly vytipovány enzymy, které by mohly ovlivňovat syntézu či metabolismus steroidních hormonů, především androstenonu. A dále enzymy podílející se na metobolismu skatolu a indolu, případně enzymy, které by mohly nějak ovlivnit činnost bakterií trávicího traktu a tím vznik těchto dvou látek. Jak bylo zmíněno výše, činnost těchto enzymů je kromě vnějších faktorů, například výživy, ovlivňována i geneticky. Dále byly vybrány kandidátní geny a jejich SNP mutace, které mohou působit na činnost těchto enzymů a tím ovlivňovat hladinu androstenonu, skatolu a indolu v tuku a svalovině prasat.

Výsledky plynoucí z prvního metodického okruhu jsou podrobněji popsány v publikacích 1 a 2 (Kapitola 5).

4.2 Testace vybraných SNP a jejich vlivu na hladinu androstenonu skatolu a indolu

Bylo testováno 6 kandidátních genů, zapojených do metabolismu androstenonu, skatolu a indolu, u kterých lze tedy očekávat vliv na hladinu těchto látek v tukové tkáni prasat: *CYP2E1, CYP17A1, SULT2B1, SULT1A1, CYB5A a MC4R.*

Pro tyto geny byly využity primery z již dříve publikovaných prací nebo byly navrženy vlastní primery pomocí programu OLIGO6. Primery a podmínky PCR reakce jsou uvedeny v tabulce 1. Výsledky PCR reakce byly kontrolovány na agarózovém gelu (1-2% - podle velikosti fragmentu). Získané PCR fragmenty byly štěpeny příslušným restrikčním enzymem (tabulka 2) a opět separovány v agarózovém gelu (1-3,5%). Na základě výsledků štěpení byly u testovaných zvířat stanoveny genotypy.

Výsledky plynoucí z druhého metodického okruhu a metodické postupy jsou podrobněji popsány v publikaci 3 (Kapitola 5).

V rámci řešení tohoto metodického okruhu bylo testováno celkem 13 SNP mutací u 6 výše popsaných prasečích genů. Publikace 3 v kapitole 5 zahrnuje jen výsledky týkající se SNP mutací v genu *CYP2E1*. U ostatních testovaných genů respektive SNP nebyla nalezena dostatečná frekvence jednotlivých genotypů v testované populaci prasat. Vzhledem k tomu

nelze očekávat signifikantní vliv na hladinu androstenonu a skatolu u testovaných populací prasat.

Dílčím výsledkem této části práce jsou sekvence genu *SULT1A1* v oblasti exonu 4-7 u plemene české bílé ušlechtilé a u plemene meishan, které byly vloženy do databáze GenBank pod čísly LN864417.1 a LN864418.1 (viz. příloha 10.1 a 10.2).

4.3 Vliv různé hladiny složek kančího pachu na užitkové vlastnosti prasat

U zvířat byly z pohledu výkrmnosti sledovány v týdenních intervalech následující ukazatele:

- průměrný denní přírůstek v testu (g/den),
- v průběhu růstu spotřeba krmiva a konverze krmiva (kg).

Pro posouzení jatečné hodnoty bylo sledováno:

- hmotnost jatečně upraveného těla (JUT) (kg),
- výška hřbetního tuku (mm) v místě 1, 2 a 3,
- podíl hlavních masitých částí (%),
- hmotnost hlavních masitých částí (kg),
- hmotnost jatečných partií (kg),
- podíl jatečných partií (%),
- partie tuk – hmotnost tukového krytí včetně kůže (kg),
- partie maso – hmotnost partie bez tukového krytí (kg),
- podíl svaloviny v %,
- měření plochy *musculus longissimus lumborum et thoracis* (MLLT).

Hodnoceny byly následující jatečné partie (plec, krkovice, pečeně, bok, kýta). Podíl svaloviny byl zjišťován pomocí přístroje Fat-o-Meater (FOM). Jatečná disekce byla provedena dle metodiky Walstry a Merkuse, (1995) 24 hodin po porážce.

Výsledky plynoucí ze třetího metodického okruhu a metodické postupy jsou podrobněji popsány v publikaci 4 (Kapitola 5)

Tabulka 1 Podmínky pro master mix a lokalizace SNP

Kandidátní gen	SNP (GenBank sekvence)	Lokalizace SNP	$MgCl_2$ (mM)	DNA polymeráza	DMSO (%)	Primery
CYP2E1	c.1423G>A (NM_214421.1)	exon 9	1,5	1U LA	2	A - GCT TAG GGT GAT GGT TTA CAC A
	c.1422C>T (NM_214421.1)	exon 9	1,5	1U LA	2	B - GGA ACC CAA CAC AGA CTC AA
	c.1502G>T (NM_214421.1)	3´UTR	1,5	1U LA	2	(tato práce)
	g.2412C>T (AJ697882.1)	promotor region	1,0	1U LA	2	L - GTCCCCAGAAACAACCTAACA N - AGCAACCCCAGTGGTACTGA (tato práce)
CYP17A1	c.1220T>A (NM_214428.1)	exon 7	1,5	1U LA	2	A - GCT GCC ACC CAC TGA CTA AT B - GCT GCA AAG AAT CTG GGT AAGT (tato práce)
SULT2B1	rs81218522	exon 4	1,0	1U LA	2	A - ATGTGCTGGTTTCGCTCTATCA B - AGCAATGCCACCGAGACAAG (tato práce)
	rs81218521	exon 5	1,0	1U LA	2	C - AAGAAGTGGCGGCCTATGT
	rs707488288	intron 4-5	1,0	1U LA	2	D - AAACAGGCCCAGAGAGGTTAAGT (tato práce)
SULT1A1	c.439A>G (AY193893.1)	exon 5	1,5	1U LA	2	C - GCC CAC ACT GCA GGT CGA CAG
	g.846T>C (LN864418.1)	exon 5	1,5	1U LA	2	D - CCC ACC CCT TCC TCC AAA GTC (tato práce)
CYB5A	c.8G>T (NM_001001770.1)	5´UTR	1,5	1U LA	2	A - ACA CGA GTT CTG GCC AAT CAT C
	rs8G>T (NM_001001770.1)	exon 1	1,5	1U LA	2	B - GTG CAG GTC TAT CCC GAC AT (tato práce)
MC4R	c.746G>A (AF087937)	exon 1	2,0	1U LA	2	A - TACCCTGACCATCTTGATTG B - ATAGCAACAGATGATCTCTTTG (Kim et al. 2000)

Tabulka 2 Podmínky PCR a délka fragmentů

Kandidátní gen	SNP (GenBank sekvence)	Anelační teplota (°C)	Počet cyklů	Délka fragmentu	Restrikční enzym	Alely (bp)
CYP2E1	c.1423G>A (NM_214421.1*	55	30	524 bp	*Bsp*68I	*A*: 524 / *B*: 301+223
	c.1422C>T (NM_214421.1)*	55	30	524 bp	*Hpy*188III	*A*: 465+59 / *B*: 302+163+59
					*Bsr*DI	*A*: 524 / *B*: 306+218
	c.1502G>T (NM_214421.1)	55	30	524 bp	*Taq*I	*T:* 318+206 / *G:*- 224+206+94
	g.2412C>T (AJ697882.1)	55	30	369 bp	*Tsc*AI	*C*: 271+92+6 / *T*: 363+6
CYP17A1	c.1220T>A (NM_214428.1)	57	30	659 bp	*Bsh*NI	*A*: 443+216 / *T*: 659
SULT2B1	rs81218522	55	30	602 bp	*Eco*24I	*T:* 236+223+132+10 / *G*: 223+163+132+73+10
	rs81218521	55	30	612 bp	*Btg*I	*G*: 389+113+110/*T:* 502+110
	rs707488288	55	30	612 bp	*Bse*GI	*A*: 297+256+59 / *G*: 297+162+94+59
SULT1A1	c.439A>G (AY193893.1)	55	30	271 bp	*Msc*I	*G:* 271 /*A:* 194+77
	g.846T>C (LN864418.1)	55	30	271 bp	*Sdu*I	*C:* 271 / *T:* 203+68
CYB5A	c.-8G>T (NM_001001770.1)	55	30	401 bp	*Bsh*1236I	*G:* 226+175 / *T*: 401
	rs80870484	55	30	401 bp	*Ava*II	*G:* 209+107+85/ *T:* 316+85
MC4R	c.746G>A (AF087937)	55	30	226 bp	*Taq*I	*A*: 226 / *G*: 70+156

*Pro zjištění genotypu c.1423G>A a c.1422C>T je nutné provést štěpení 3 restrikčními enzymy (*Bsp*68I; *Hpy*188III a *Bsr*DI) a poté určit genotyp. Tyto SNP se nacházejí vedle sebe a záměna jedné nebo druhé báze může ovlivnit vznik restrikčního místa pro použité enzymy.

5 Publikované práce

1. **Zadinová, K.,** Stupka, R., Stratil, A., Čítek, J., Vehovský, K., Urbanová, D. 2016. Boar Taint - The Effect of Selected Candidate Genes Associated with Androstenone and Skatole Levels - a Review. Animal Science Papers And Reports. 34 (2), 107-128.

2. Urbanová, D., Stupka, R., Okrouhlá, M., Čítek, J., Vehovský, K., **Zadinová, K.** 2016. Nutritional effects on boar taint in entire male pigs: a review. Scientia Agriculturae Bohemica. 47 (4), 154-163.

3. **Zadinová, K.,** Stupka, R., Stratil, A., Čítek, J., Vehovský, K., Lebedová, N., Šprysl, M., Okrouhlá, M. 2017. Association analysis of SNPs in the porcine *CYP2E1* gene with skatole, indole, and androstenone levels in backfat of a crossbred pig population. Meat Science. 131, 68-73.

4. Stupka, R., Čítek, J., Vehovský, K., **Zadinová, K**., Okrouhlá, M., Urbanová, D., Stádník, L. 2017. Effects of immunocastration on growth performance, body composition, meat quality and boar taint. Czech Journal of Animal Science. 62 (6), 249-258.

Animal Science Papers and Reports vol. 34 (2016) no. 2, 107-128
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Boar taint – the effects of selected candidate genes associated with androstenone and skatole levels – a review*

Kateřina Zadinová[1,] Roman Stupka[1], Antonín Stratil[2], Jaroslav Čítek[1], Karel Vehovský[1], Daniela Urbanová[1]**

[1] Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague Kamýcká 129, Prague - Suchdol 165 21, Czech Republic

[2] Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Rumburská 89, 277 21 Liběchov, Czech Republic

(Accepted Jauary 20, 2016)

Androstenone and skatole are the two main components causing the so-called boar taint as well as the unpleasant taste of pork meat from boars. These substances increasingly accumulate in the adipose tissue after reaching sexual maturity. For this reason, boars are less frequently used for pork production. The most commonly used method for boar taint elimination is the castration of male pigs shortly after birth. Another way how to reduce the boar taint is the identification of candidate genes and SNPs (single nucleotide polymorphisms) that affect the androstenone and skatole levels in the adipose tissue of pigs. The aim of this review is to present and systematize the current knowledge about the genetic influences on androstenone and skatole levels. In recent years several studies focused on the identification of potential candidate genes have been carried out and a study which purpose was to verify whether these genes or their mutations may have a significant effect on androstenone and skatole levels in pigs. Several genes and their SNPs that significantly influence the levels of androstenone (*CYP17A, CYB5, CYP21, SULT2A1, SULT2B1, HSD3B*) and skatole (*CYP2E1, CYP2A6, SULT1A1*) were identified. In addition, there are other candidate genes (*LH, TEAD3*) whose association with the components of boar taint has not been clearly confirmed yet. Several studies also mentioned the possibility of interactions between genes themselves as well as the possibility of negative effects of the tested genes on important pig production characteristics

**Corresponding author: zadinova@af.czu
This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. MSM 6046070901).

as fertility and carcass value. It is therefore clear that thorough studies on the effect of individual genes on different components of boar taint are desirable.

KEY WORDS: boar taint / androstenone / skatole / candidate genes / QTL / SNPs

Fattening of the entire male pigs, when compared with other pig categories (barrows and gilts), appears to be more profitable, mainly due to better growth intensity, feed conversion and improved carcass leanness. This is caused by the production of male sex hormone androstenone [Kim *et al*. 2013, Lundström and Zamaratskaia 2006, Moe *et al*. 2007b, Peacock *et al*. 2008, Strathe *et al*. 2013]. Non-castrated male pigs are already being used for the production of pork meat in some countries, however the occurrence of so called "boar taint" in this meat represents a significant problem [Babol *et al*. 1999]. Boar taint is a strong, faecal- and urine-like odour found in heat treated pork meat [Lee *et al*. 2005, Strathe *et al*. 2013]. In order to eliminate this odour, in many countries the male pigs are castrated very soon after being born [Moe *et al*. 2008, Duijvesteijn *et al*. 2010]. The methods of castration represent a problem from an ethical point of view and also endanger the welfare of animals in general [Gray and Squires 2013], because the castration of young male pigs less than 7 days old is performed without the use of any anesthetics [Engelsma *et al*. 2007]. Because this process is painful, frightening and can lead to a health status decline [Lundström and Zamaratskaia 2006], there are continuous efforts to change the way of pig castration, which is being supported by EU legislation as well. There is a new recommendation not to perform the surgical castration of male pigs without narcosis, which will come into effect in 2018 [Mörlein *et al*. 2012]. Considering all of these points, it is necessary to find alternatives to removing the boar taint and preventing the devaluation of pork meat [Engelsma *et al*. 2007, Grindflek *et al*. 2011b].

Boar taint

Boar taint is primarily caused by substances produced in pigs in relation with reaching sexual maturity [Duijvesteijn *et al*. 2010]. The main substances responsible for the unpleasant odour are androstenone (5α-androst-16-en-3-on), skatole (3-methylindole) and indole (2,3-benzopyrol) [Grindflek *et al*. 2011b]. It has also been shown that a minor part in taint production is played by 3α-androstenol (3α-OL) and 3β-androstenol (3β-OL) [Xue and Dial 1997] which are created during the metabolism of androstenone [Zamaratskaia and Squires 2009]. Acceptable levels of the two main substances differ between consumers and range between 0.5 - 1 μg/g for androstenone and 0.2 – 0.25 μg/g for skatole. However, acceptable skatole levels can be as low as 0.15 μg/g [Mörlein *et al*. 2012]. A study published by Bonneau *et al*. [2000] states that on average 6.5% of consumers can detect the unpleasant odour of boar meat, while about 3% of consumers can taste the unpleasant taste, although the published results varied for different countries.

Main substances causing boar taint

Androstenone

Androstenone is a steroid hormone produced by the Leydig cells located in the testis, which produce also other steroid hormones, androgens and estrogens. It is stored in the fat tissue and salivary glands, from which it is then released into the air as a pheromone stimulating the willingness toward mating in female pigs [Moe *et al*. 2008, Robic *et al*. 2011b]. Apart from this, lower concentrations of androstenone have also been detected in the blood serum of barrows and gilts, which suggests possible androstenone production in the cortex of suprarenal glands [Claus *et al*. 1971]. The organic precursor used for the androstenone biosynthesis is pregnenolone. It is synthesized from pregnenolone with the use of andiene-β synthase system (Fig. 1). An important role in the biosynthesis of 5,16-androstadien-3β-ol is played by cytochrome P450C17 (CYP17A1) and cytochrome b5 (CYB5) [Davis and Squires 1999]. The production of androstenone and other testicular hormones is controlled by the neuroendocrine system, mainly through the effects of luteinizing hormone (LH)

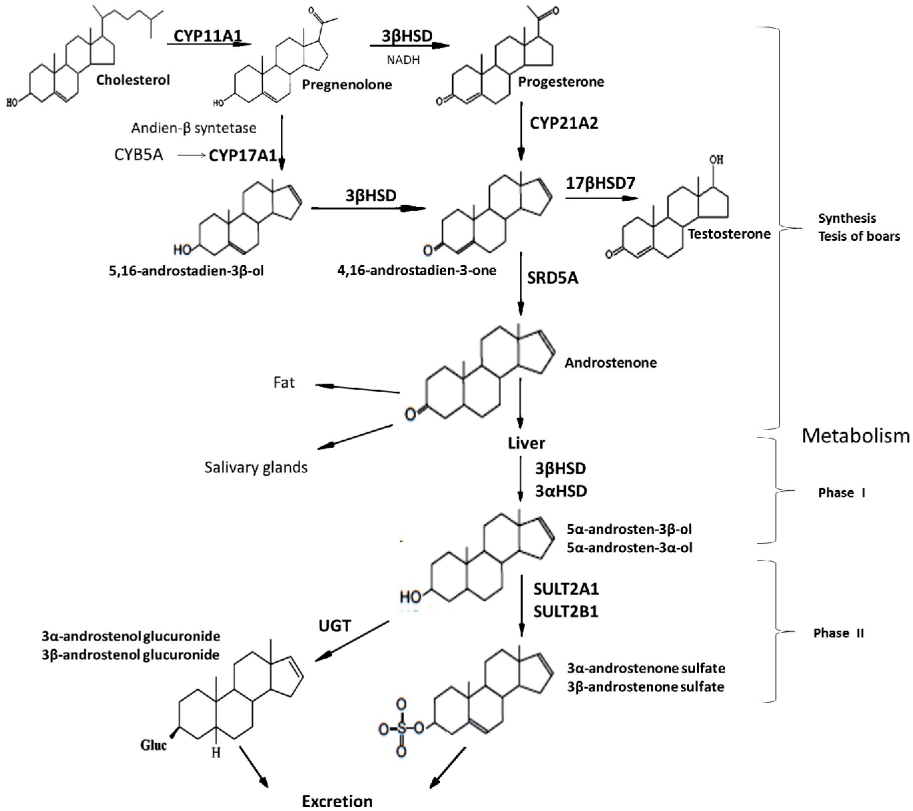


Fig. 1 Genes affecting androstenone synthesis and metabolism.

[Zamaratskaia and Squires 2009]. Androstenone is degraded in the liver and testis [Andresen 2006]. A large part of androstenone is immediately sulfoconjugated by the hydroxysteroid sulfotransferases SULT2A1 [Sinclair *et al*. 2006] and SULT2B1 [Moe *et al*. 2007a]. The metabolic degradation of androstenone is divided into two phases. During the phase I, androstenone is degraded by the 3β-hydroxysteroid dehydrogenases (3βHSD), using the NADH and NADPH cofactors [Doran *et al*. 2004]. The level of androstenone metabolism is directly related to the level of hepatic 3βHSD expression [Andresen 2006]. High levels of androstenone in the fat tissue correspond with low levels of 3βHSD [Dasgupta *et al*. 2007]. The concentration of androstenone in fat tissue also increases with the age of boars [Xue and Dial 1997]. Although the liver is capable of producing metabolites of both of these phases, its main role is in the phase II – sulfoconjugation. The key enzyme of the phase II is SULT2A1 [Sinclair *et al*. 2006]. Androstenone is cut into two main metabolites during its degradation: 5α-androst-16-en-3a-ol and 5β-androst-16-en-3b-ol [Zamaratskaia and Squires 2009], which represent about 68% of all the phase II metabolites [Sinclair *et al*. 2005] (Fig. 1).

Skatole

Skatole is formed from the amino acid L-tryptophan during the degradation of proteins. It is produced by the bacteria commonly found in the large intestine of monogastric animals [Lee *et al*. 2005]. It is responsible for giving the meat a faecal-like odour. A part of it is removed from the body by feces, another part is metabolised by the liver and a rest is stored in the kidneys as well as in the fat tissues due to its lipophilic character [Babol *et al*. 1998b]. Contrary to monogastric animals, ruminants are sensitive to skatole which is toxic for them and causes ABPE (acute bovine pulmonary edema and emphysema). Thus, high concentrations of skatole have no adverse effects on pigs [Deslandes *et al*. 2001]. Skatole synthesis is a two-step process which requires the assistance of intestinal bacteria. *Escherichia coli* and *Clostridium spp.* (*C. dýfficile, C. sticklandii, C. lituseburense, C. subterminale* and *C. putrefaciens*) transform tryptophan into indole-3-acetic acid [Yokoyama and Carlson 1979], which is then metabolised by *Lactobacillus* and *Clostridium* into skatole [Zamaratskaia and Squires 2009]. Skatole is metabolised within the liver tissues by enzymes P450 2E1 (CYP2E1), P450 2A6 (CYP2A6) [Diaz *et al*. 1999] and phenol sulfotransferase (SULT1A1) [Lin *et al*. 2004b]. The degradation of skatole consists of two phases – oxidative, followed by conjugation reactions. Phase I involves mainly the cytochrome P450 (CYP) family. The main enzymes are considered to be CYP2E1 [Babol *et al*. 1998b], CYP2A [Diaz and Squires 2000] as well as aldehyde oxidase (AO) [Lanthier *et al*. 2007]. Phase I results in an attachment of a hydroxyl group, which is then used to create a conjugate in phase II [Zamaratskaia and Squires 2009]. Diaz *et al*. [1999] describe the total of 7 intermediate products of phase I (see Fig. 2). These metabolites are then used as substrates for the reactions of phase II [Diaz and Squires 2003]. Phase II then includes sulfoconjugation with the use of sulfotransferases (SULT1A1),

glucuronic acid [Babol *et al*. 1998b] and uridine-diposphate-glucuronosyltransferase (UDP) [Diaz and Squires 2003]. For the final products of skatole degradation (Fig. 2) are then eliminated from the organism via urine [Xue and Dial 1997].

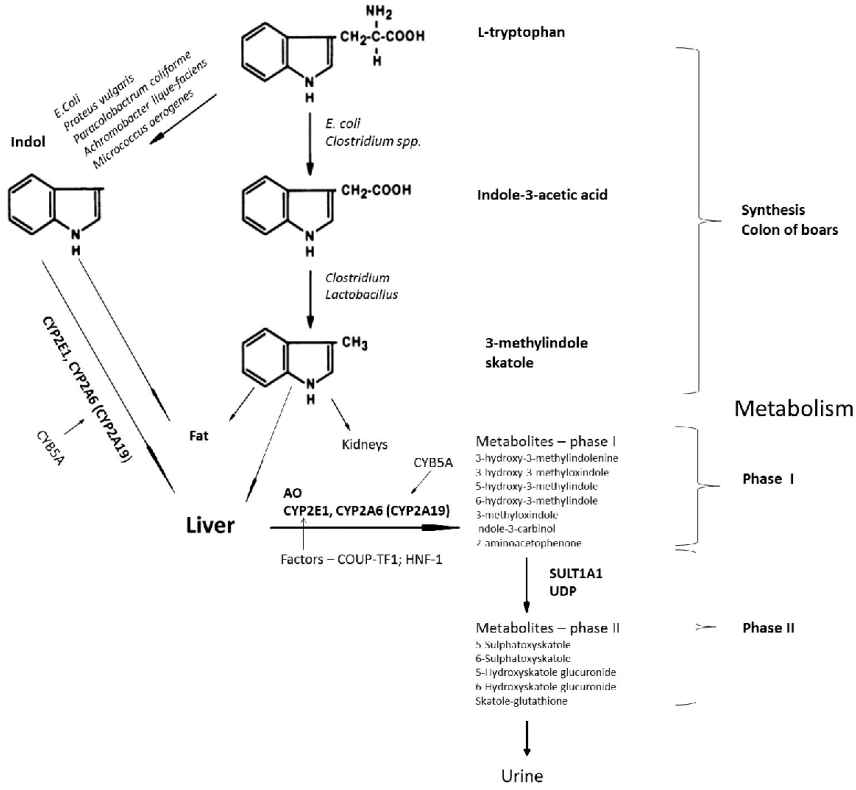


Fig. 2 Synthesis and metabolism of skatole; genes affecting skatole metabolism

Indole

Similarly to skatole, indole (2,3-benzopyrol) is produced by the degradation of L-tryptophan in the gastrointestinal tract (GIT) of monogastric animals. Indole is absorbed by blood and metabolised by the liver tissue. L-tryptophan can be either degraded in the GIT to indole or transformed to indole-3-acetic acid, which is the starting point in skatole synthesis as described above [Yokoyama and Carlson 1979]. As opposed to skatole, the degradation process resulting in indole uses a wide range of bacteria (figure 2) [Deslandes *et al*. 2001]. Similarly to skatole, indole production is influenced by the cytochrome CYP2A6 expression, on which both indole and skatole have the stimulating effect. It has also been found that in the case of indole this stimulating effect is a lot more significant than in skatole [Chen *et al*. 2008]. Indole too is accumulated in the fat tissue in pigs and its levels can be affected by other than

genetic influences, such as pen dirtiness or feed mixture composition. Indole appears to influence the quality of pork meat to a lower extent than skatole and it mostly just intensifies the unpleasant odour caused by skatole [Yokoyama and Carlson 1979].

Elimination of boar taint using genetic markers

During the recent years there has been a strong effort to minimalize the occurrence of the main substances causing boar taint in the pork meat. There are several approaches to study the genetic basis of boar taint: identification of QTL, genome-wide association study (GWAS), study of candidate genes and polymorphisms thereof (SNPs). The identification of suitable SNPs causing boar taint could help to accelerate its elimination through crossbreeding and thus remove the need for male pig castration [Duijvesteijn *et al*. 2010]. The reduction of boar taint through genetic selection seems to be a promising solution, because the levels of androstenone and skatole concentrations show medium heritability [Strathe *et al.* 2013]. Androstenone and skatole heritabilities range between 0.25-0.87 [Oskam *et al*. 2010] and 0.19-0.54 [Robic *et al*. 2008], respectively. Tajet *et al*. [2006] also report a positive gene correlation between the skatole and androstenone levels (0.36-0.62).

Quantitative trait loci (QTL) contain the genes affecting specific traits and can be identified by comparing the genotypes of unknown markers scattered along the chromosome with the phenotype or a specific trait under study. Quintanilla *et al.* [2003] and Lee *et al.* [2005] identified several QTL for androstenone and skatole in an experimental population of crossbred pigs (Meishan and Landrace). Lee *et al.* [2005] described the location with most skatole and indole affecting QTL to be the chromosome 14 (SSC14 – *Sus scrofa* chromosome 14). The locations with QTL for androstenone are SSC2, SSC4, SSC6, SSC7 and SSC9. However, only the SSC6 carries the QTL for causing the unpleasant ”boar taste“ of meat. Quintanilla *et al.* [2003] in their work concerning the PORQTL project identified QTL controlling the androstenone levels in the fat tissue on SSC3, SSC4, SSC7, SSC14 and also at the ends of short arms of SSC6 and SSC9. All these findings suggest that the androstenone and skatole levels are controlled by a large number of genes. Duijvesteijn *et al*. [2010] also mentioned SSC1 and SSC6 as the locations of genes affecting the androstenone levels.

The genome-wide association study (GWAS) is often used for detection of QTL and SNPs. The Illumina Porcine SNP60 Genotyping BeadChip is the most comprehensive genome-wide genotyping array for the porcine genome [de Campos *et al.* 2015, Ernst and Steibel 2013, Lukić *et al.* 2015, Rowe *et al.* 2014]. Große-Brinkhaus *et al.* [2015] identified 33 SNPs, of which 31 significantly associated with concentration in fat of one of boar taint compounds (androstenone or skatole). Only one SNP was significantly associated with concentration of both compounds. Lukić *et al.* [2015] used the Illumina SNP60 porcine BeadChip for detecting of 62 153 SNPs. The accuracy of predicting phenotypes was assessed based on the genotypes by cross-validation using six different evaluation methods: genomic best linear unbiased

prediction (GBLUP) and five Bayesian regression methods (Bayes Lasso, Bayes A, Bayes B, Bayes C, Bayes SSVS). In addition, this was compared with the accuracy of predictions using only QTL that showed genome-wide significance. The methods of GWAS gave greater accuracy than the QTL analysis. The aim of the de Campos *et al.* [2015] study was the comparison of two different Genome Wide Selection (GWS) methods (Ridge Regression BLUP - RR-BLUP and Bayesian LASSO - BL) to predict the genomic estimated breeding values of four phenotypes, including two boar taint compounds. Another method within the GWAS for study of candidate genes and SNPs associated with androstenone and skatole is next generation sequencing (NGS) [Grindflek *et al*. 2011a]. RNA-Seq is a recently developed NGS technology for transcriptome profiling which enables identification of new and less expressed transcripts. Association analysis identified boar taint candidate genes in flavin monooxygenease family, cytochrome P450 family and hydroxysteroid dehydrogenase family. Moreover, polymorphisms associated with boar taint were revealed in *IRG6*, *MX1*, *IFIT2*, *CYP7A1*, *FMO5* and *KRT18* genes. Sahadevan *et al.* [2015] studied gene co-expression clusters in liver tissue from three pig populatoins with low and high androstenone levels in backfat. The examined populations were Duroc sire line, Duroc × F_2 boars and Norwegian Landrace The RNA-seq and microarrays were used. Based on the results they assumed that low androstenone cluster 2 might be a signature co-expression cluster for androstenone metabolism in animals with low levels of androstenone in backfat.

Another possible method of identifying the suitable genes is to study candidate gene polymorphisms. These are most commonly found as SNPs. Several published studies have already focused on SNPs associated with skatole [Lin *et al.* 2004a, 2006] or androstenone [Lin *et al.* 2005a, Kim *et al.* 2013]. Ramos *et al.* [2011] also found several SNP markers affecting skatole levels at the distal end of SSC6p. These SNP markers are grouped into 3 individual clusters. Marked differences were observed mainly between the homozygotes in each group. Single SNP markers can account for up to 22% of the phenotypic variability [Grindflek *et al.* 2011a].

Genes for androstenone

The main genes involved in androstenone synthesis are *CYP17A1, CYB5A* [Davis and Squires 1999] and *LHB* – gene coding the β-chain of LH – luteinizing hormone [Duijvesteijn *et al.* 2010]. Lin *et al.* [2005a] stated that mutations in the *CYB5A* gene result in a decrease of androstenone levels in pigs. Quintanilla *et al.* [2003] also mentioned the possible effect of *CYP21A2* and *CYP11A1*, located on the chromosome SSC7. Phase I of androstenone metabolism is controlled mainly by 3βHSD, while phase II is under the control of sulfotransferases – SULT2A1, SULT2B1 [Duijvesteijn *et al.* 2010]. Robic *et al.* [2011a] studied crossbred pigs of Large White and Meishan genotypes and focused on several other potential genes which could have an effect on androstenone levels (*C6ORF106, C6ORF81, CLPS, SLC26A8, SRPK1, MAPK14* and

TEAD3). However, the study proved no significant direct effect of any of these genes. Gregersen *et al*. [2012] identified also gene *SRD5A2* (5α-reductase) as a candidate gene for androstenone. According to Moe *et al*. [2009], the *CYP2C49, CYP2D6, NGFIB* and *CTNND1* genes are connected with the androstenone levels in fat tissue in pigs.

Genes controlling androstenone synthesis

Genes of the CYP (cytochrome P450) family

CYP11A1 (cytochrome P450, family 11, subfamily A, polypeptide 1): In pigs, the CYP11A1 gene (ENSSSCG00000025273) (Ensembl; http://www.ensembl.org/index.html) is located on SSC7 [Quintanilla *et al*. 2003] (Scaffold GL893363.2: 159,079-165,974 bp) (Ensembl) CYP11A1 is involved in the first step of biosynthesis of steroid hormones. It initializes the transformation of cholesterol to pregnenolone [Robic *et al*. 2008]. Except for pigs, this gene has also been found in mice, rats, humans and many other species. In humans, *CYP11A1* gene is located on chromosome 15q23-q24 (Ensembl). CYP11A1 is expressed mainly in the cortex of suprarenal glands, ovaries, testis and placenta. Its transcript can also be found in the central and peripheral nervous system [Payne and Hayle 2004]. In pig this gene has been considered a possible candidate gene affecting the androstenone levels, because it is involved at the beginning of its synthesis [Robic *et al.* 2011b]. Grindfleck *et al.* [2010] found different expression levels in boars with extremely high and low androstenone levels. However, Quintanilla *et al.* [2003] in their work excluded *CYP11A1* as a candidate gene for their pig population. Similarly, Robic *et al.* [2011b] were also not able to confirm the effect of *CYP11A1* (or its mutations) on androstenone accumulation in pigs.

CYP17A1 (cytochrome P450, family 17, subfamily A, polypeptide 1): This gene (ENSSSCG00000010591) is located on SSC14 (123,773,105-123,779,533 bp) in pigs (Ensembl). *CYP17A1* is also involved at the beginning of steroid hormone synthesis from pregnenolone. In humans, *CYP17A1* is found on chromosome 10q24.3 (Ensembl). Its expression is most pronounced in the testes and ovaries. However in some species such as humans and monkeys, some expression was observed in the placenta and suprarenal glands as well [Payne and Hales 2004]. CYP17 is important for the metabolism of androstenone in pigs [Davis and Squires 1999, Nakamura *et al.* 2011]. Lin *et al.* [2005b] reported a substitution mutation (T>A) in the nucleotide 1317, (NM_214428.1:c.1220T>A) which causes the change of amino acid leucine - Leu^{439} to histidine - His^{439} However, despite their expectations, this mutation had no apparent effect on the enzymatic activity of the resulting proteins involved in adrostenone biosynthesis. Because there is a possibility that the effect of individual genes can vary between breeds, Moe *et al.* [2007b] performed a study on two individual breeds, Landrase and Duroc. Neither of the studied breeds, however, showed any significant differences in the expression of *CYP17A1* gene in pigs with low and high androstenone levels.

CYP21A2 (cytochrome P450, family 21, subfamily A, polypeptide 2): CYP21A2 (ENSSSCG00000001428) is located on the SSC7 (27,722,776-27,725,977 bp) (Ensembl). This gene is also involved at the beginning of androstenone synthesis and the encoded protein acts as a catalyst in the progesterone hydroxylation process. Progesterone is a direct substrate for CYP21A2 and this reaction does not require any expression of CYP17. The *CYP21A2* expression was found exclusively in the cortex of suprarenal glands [Payne and Hales 2004]. Human *CYP21A2* is located on chromosome 6p21.3. It has been proved that this gene has a very strong influence on sow fertility [Grindflek *et al.* 2010]. Grindflek *et al.* [2011a] reported different *CYP21A2* expressions in boars with high and low androstenone levels. However, in their study [Grindfleck *et al.* 2010], they were able to confirm the effect of *CYP21A2* on androstenone levels only in Landrace breed populations.

Gene of the CYB (cytochrome b) family

CYB5A (cytochrome P450, family 21, subfamily A, polypeptide 2): In pigs, *CYB5A* (ENSSSCG00000004875) is found on SSC1 (165,902,018-165,937,614 bp). *CYB5A* is a very important regulator of the *CYP17A1* function [Billen and Squires 2009]. In humans, it is located on chromosome 18q23 (Ensembl). The expression levels of *CYB5A* are closely correlated with androstenone levels in the fat tissue in pigs [Lin *et al.* 2005a]. This suggests that it could be possible to lower the androstenone levels by carefully selecting animals with low levels of cytochrome B5 [Gray and Squires 2013]. Lin *et al.* [2005a] identified a G>T polymorphism located at 8 bp upstream of the ATG codon (MN_001001770.1:c-8G>T) of the *CYB5A* gene associated with lower androstenone levels in fat. The frequencies of individual genotypes were *GG* 84.8%, *GT* 12.4% and *TT* 2.8%. The *TT* homozygotes have shown a significantly lower activity of *CYB5A*. These results were confirmed by Peacock *et al.* [2008] as well. On the other hand, the different effects of *CYB5A* gene have been reported by Zamaratskaia *et al.* [2008]. The *T* allele, particularly genotype *GT* was associated with low levels of androstenone in the serum and also with low levels of skatole in the fat tissue, but no relationship was observed between *CYB5A* and adrostenone levels in the fat tissue. Genotype *TT* was not included in the analysis, as only two homozygotes *TT* were detected. The effect of genotype was, however, live weight-dependent; androstenone levels were affected in lighter pigs and skatole levels in heavier pigs. However, any practical use of their data is arguable at this point, especially due to the small effect of *CYB5A* on adrostenone in fat and also due to a low frequency of the *T* allele in the observed population.

LHB gene (luteinizing hormone beta polypeptide)

LHB (ENSSSCG00000003151) in pigs is located on SSC6 (50,063,956-50,065,003 bp) (Ensembl). The gene is responsible for signalling the beginning of steroid hormone synthesis in Leydig cells at the onset of puberty [Duijvesteijn *et al.* 2010] and also affects the activity of ovaries [Dasgupta *et al.* 2012]. In humans, *LHB* is located on chromosome

19q13.32 (Ensembl). It has been reported that in humans mutations of this gene can be connected with breast cancer [Giovangrandi *et al.* 2001] or with the occurrence of ovarian cysts [Dasgupta *et al.* 2012]. Due to its location on SSC6, Duijvesteijn *et al.* [2010] considered this gene to be a possible candidate gene for androstenone levels.

Genes encoding HSD – hydroxysteroid dehydrogenases

HSD are a group of enzymes that take part in steroid hormone biosynthesis from cholesterol in mammals. Unlike the enzymes from CYP family, which are controlled by one gene, there are several isoforms for the *3HSD*s and several isozymes of the *17HSD*s, each a product of a distinct gene. The number of isoforms or isozymes varies in different species, in tissue distribution, catalytic activity (whether they function predominantly as dehydrogenases or reductases), substrate and cofactor specificity, and subcellular localization. [Payne and Hales 2004].

HSD17B7 (hydroxysteroid (17-beta) dehydrogenase 7): In pigs *HSD17B7* gene (ENSSSCG000000063378) is located on the SSC4 (95,571,648-95,594,175 bp) (Ensembl). The enzyme works as a catalyst of the last step in the androgen and estrogen synthesis [Payne and Hales 2004]. In humans, the *HSD17B7* gene is found on chromosome 1q23 (Ensembl). The study on human and mouse proved that 17βHSD7 catalyses the reduction of keto group in either 17- or 3- position of the substrate [Törn *et al.* 2003]. Chen *et al.* [2007] found a negative correlation between hepatic 17βHSD7 expression and androstenone levels in fat tissues. Testicular expression of 17βHSD7 does not seem to affect the androstenone levels in fat tissue in any way. There have also been detected strong correlations between 3βHSD and 17βHSD7 in non-castrated boars, which suggests that these two genes have similar regulatory mechanisms. This study discovered a strong negative correlation between the expression of hepatic 17βHSD7 and the expression of plasma E1S (estrogen), which suggests a very important role of 17βHSD7 in the estrogen metabolism of pigs.

Genes controlling androstenone metabolism

Genes encoding HSD – hydroxysteroid dehydrogenases

HSD3B (3 beta-hydroxysteroid dehydrogenase): In pigs, the *HSD3B* gene (ENSSSCG00000006719) is located on chromosome SSC4 (111,555,566-111,564,192 bp) (Ensembl). The enzyme coded by *HSD3B* is active during the beginning of steroid synthesis from pregnenolone [Payne and Hales 2004]. In humans, *HSD3B* is located on chromosome 1p13.1 (Ensembl). *HSD3B* expression in man has been confirmed in placenta, skin, breast tissue, ovaries, suprarenal glands, liver and testis [Simard *et al.* 2005]. Doran *et al.* [2004] reached a conclusion that the level of androstenone metabolism is determined by the level of hepatic 3βHSD expression. Pigs with high concentrations of androstenone in their fat have a low expression of 3βHSD in the liver and testis [Chen *et al.* 2007]. The expression of hepatic (non-testicular) 3βHSD shows a negative correlation with the fat tissue androstenone levels. With the help of

cloning and sequencing the gene-coding region it has been found that both hepatic and testicular 3βHSD have the same sequences, which leads to the assumption that the expression of one gene in the liver and testis is controlled by different mechanisms [Nicolau-Solano *et al.* 2006]. Kim *et al*. [2013] identified 8 polymorphisms, with the SNP5 polymorphism in the Duroc breed having a direct influence on androstenone levels. The study also found that pigs of the NM_003534677.2:g.165262G>A with genotype *GG* show significantly lower levels of androstenone, when compared with other genotypes. The different expression levels of this enzyme could be the defining factor for the rate of androstenone metabolism in the liver, which can affect the levels of hepatic CYP2E1 and thus the speed of skatole degradation in the liver. The effect of this gene is also different for different breeds, for example, the Meishan breed exhibited lower expression levels than the Large White breed [Doran *et al.* 2004].

Genes encoding sulfotransferases

SULT2A1 (sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA)-preferring, member 1): In pigs, *SULT2A1* (ENSSSCG00000003130) is located on SSC6 (49,108,567-49,119,941 bp) (Ensembl). SULT2A1 is a key enzyme in the testicular and hepatic metabolism of 5α-androstenone [Moe *et al.* 2007b]. In humans, this gene is found on chromosome 19q13.3 (Ensembl). Its function is in regulating the metabolism of xenobiotics and endogenous hormones [Huang *et al.* 2014]. A study performed by Sinclair *et al.* [2006] showed that the expression of SULT2A1 enzyme can affect the accumulation of 5α-androstenone in the fat tissue of pigs. Animals with high concentrations of 5α-androstenone and low activity of SULT2A1 also exhibit low levels of the SULT2A1 protein, when compared with animals showing low levels of 5α-androstenone. The effect of *SULT2A1* was also confirmed by the conclusions of Leung *et al*. [2010]. They found negative correlations between *SULT2A1* and boar taint in boars of the Duroc, Norwegian Landrace and Yorkshire breeds. Negative correlations between the fat tissue androstenone levels and activity of testicular SULT2A1 ($r = -0.57$; $P<0.01$) were also reported by Sinclair *et al.* [2006]. On the other hand, Moe *et al.* [2007b] found a high mRNA expression of the *SULT2A1* gene in the testis of Duroc and Landrace boars, which also showed high levels of androstenone.

SULT2B1 (sulfotransferase family, cytosolic, 2B, member 1): The *SULT2B1* gene (*ENSSSCG00000023280*) is found on SSC6 (49,700,287-49,710,764 bp) (Ensembl). In the mouse, the protein is found in two isoforms (Sult2b1a and Sult2b1b), which are controlled by the *Sult2b1* gene [Kurogi *et al.* 2010]. Human SULT2B1b catalyzes the sulfonation of 3-hydroxysteroid hormones and cholesterol, whereas SULT2B1a preferentially catalyzes pregnenolone sulfonation [Ji *et al.* 2007]. In mice, a number of interactions between the Sult2b1b and cytoskeletal proteins have been confirmed, as well as the role of Sult2b1b on keeping up the epidermal barrier [Kurogi *et al.* 2010]. In the human genome this gene is found on chromosome 19q13.3 (Ensembl). SULT2B1b has also been associated with breast cancer. Higher expression of

SULT2B1b was reported in the female populations in Africa, where there is a lower incidence of breast cancer when compared with Caucasian populations [Dumas *et al.* 2008]. The expression of this gene in pigs can depend on the breed. In the study published by Moe *et al.* [2007a] there was no difference in the expression of hepatic *SULT2B1* in connection with the androstenone levels in the Landrace breed. However, for the Duroc breed the expression of *SULT2B1* in animals with high levels of androstenone was 35% lower than in the animals with low levels of androstenone. Concerning the expression of testicular *SULT2B1*, both of the breeds showed lower expression levels, which was more pronounced in the Duroc breed. As suggested by Panella-Riera *et al.* [2008], there were speculations that the expression of *SULT2B1* is influenced by testosterone. Nevertheless, this was disproved by Zamaratskaia *et al.* [2012], who could not find any influence of testosterone nor estron sulfate on the *SULT2B1* expression.

TEAD3 (TEF-5) gene (*TEA domain family member 3*): *TEAD3* (ENSSSCG00000001544) is located on SSC7 (36,085,701-36,100,686 bp) in pigs. In humans, this gene is found on chromosome 6p21.2 (Ensembl). The human version of this gene is capable of regulating the *HSD3B* transcription, which significantly influences the degradation of androstenone in liver [Robic *et al.* 2012]. The manifestation of transcription factors was also studied in the skeletal muscles, cardiac muscle and placenta, which so far shows the highest levels of expression. Nonetheless, even here the effect of *TEAD3* is not direct and it works through the activation of other genes, which influence embryonal development [Jacquemin *et al.* 1999]. According to Robic *et al.* [2011a], *TEAD3* was considered to be one of the possible target genes for androstenone due to its location on chromosome SSC7; however, the study proved no direct effect of this gene. Several pig populations were tested for SNP at 726 bp in the 5´ UTR of the first exon (GenBank, Accession Number NM_001037966). The study found high correlations between *TEAD3* and *HSD3B* in pigs, but no difference was observed between expression levels measured in different genotypes of the SNP studied [Robic *et al.* 2012].

Genes for skatole

The results of current studies suggest that *CYP2A6* and *CYP2E1* are the two main genes of the CYP family which have the most significant effect on the phase I of skatole metabolism in the liver [Diaz and Squires 2000]. According to Duijvesteijn *et al.* [2010] and Chen *et al.* [2008], the gene known in the human genome as *CYP2A6* is identical with the pig *CYP2A19* gene, although in a number of studies concerning boar taint this gene is being referred to as *CYP2A6*. Wiercinska *et al*. [2012] stated that other potentially important metabolism regulators apart from *CYP2E1* are also *CYP2A19* and *CYP2C49*. Skinner *et al*. [2006] in their work also tested the effects of *CYP2C18* in a hybrid Danish population (Landrace - Yorkshire - Duroc), but the study proved no significant effect of this gene. Matal *et al.* [2009] also confirmed the influence

of *CYP2E1* and recommended further investigations of the effects of *CYP2A19* and *CYP1A2* genes on the skatole metabolism and the origin of its metabolites. A major part in phase II of skatole metabolism is played by *SULT1A1* [Babol *et al.* 1998a]. Lanthier *et al.* [2007] studied prepubescent boars and in addition to studies of the effects of *CYP2E1* and *SULT1A1* on the metabolism of skatole, their work was also focused on the influence of aldehyde oxidase (*AO*) on skatole metabolism.

Genes affecting phase I of skatole metabolism

Genes of the CYP (cytochrome P450) family

Gene CYP2A6 (CYP2A19) **(**cytochrome P450, family 2, subfamily A, polypeptide 6). This gene (ENSSSCG00000022808) is located on SSC6 [Diaz and Squires, 2000; Lin *et al.* 2004a] (44,853,498-44,876,802 bp)(Ensembl). In the human genome, the gene is found on chromosome 19q13.2 [Ensembl). The *CYP2A6* gene is associated with the metabolism of some of the tobacco carcinogens, mainly nicotine [Yamazaki *et al.* 1999]. Liu *et al.* [2013] studied the effect of gene polymorphism *CYP2A6*4* on the incidence of lung cancer in smokers. Although the study did not find any significant relationship in general population, it did confirm a significant effect on the Asian population of smokers. CYP2A6 is considered to be one of the most important enzymes regulating the skatole metabolism in pigs. Diaz and Squires [2000] and Lin *et al.* [2004a] also confirmed its significant role in skatole metabolism and assumed that a close monitoring of CYP2A6 levels and activity could help to regulate the levels of skatole in pigs. Pigs with a high activity of CYP2A6 also show very low levels of skatole [Lin *et al.* 2004a]. Conversely low CYP2A6 activity is connected with an excessive acccumulation of skatole in the fat tissue of pigs [Chen *et al.* 2008]. Lin *et al.* [2004a] detected a deletion mutation, in which a guanine base is deleted at the nucleotide position 421 (GenBank, Accession Number AY091516.1), which leads to the shift of reading frame and changes its length from 1485 to 612 bp. The results of this study suggest that this deletion leads to inactivation of *CYP2A6* and a loss of enzymatic activity, which results in excessively high levels of skatole in pigs.

CYP2E1 (cytochrome P450, family 2, subfamily E, polypeptide 1). Another gene with a significant role in the skatole hepatic metabolism is *CYP2E1* (ENSSSCG00000010780). It is found on chromosome SSC14 in location 153,477,961-153,490,404 bp. In the human genome this gene is located on chromosome 10q26.3 (Ensembl). The *CYP2E1* gene expression is a subject of intensive studies in humans as well, mainly due to its role in the metabolism of xenobiotics and carcinogens [Gonzalez 2005]. Its effect on carcinogens metabolism was studied not only in humans, but also in mice [Konstandi *et al.* 2013], rats and rabbits [Koop and Casazza 1985]. Konstandi *et al.* [2013] studied the effects of female hormones on regulation of CYP2E1 and reached the conclusion that CYP2E1 is indeed influenced by female hormones, which could have a negative effect on the metabolism of many toxic and carcinogenic substances. It is also being studied in connection with alcohol (ethanol) metabolism and oxidative stress in

hepatocytes, in relation to liver damage in alcoholics [Lu and Cederbaum 2008]. In addition, it also takes part in the metabolism of ketone bodies (which are released during periods of fasting) and thus influences gluconeogenesis [Koop and Casazza 1985]. The activity of CYP2E1 in the liver significantly determines the total concentration of skatole and its metabolites in the fat tissue of pigs [Babol *et al.* 1998b]. *CYP2E1* expression is in a negative correlation with skatole accumulation in pigs. *CYP2E1* expression and the level of skatole in fat tissue are in inverse proportion to each other [Lin *et al*. 2006]. Skinner *et al*. [2005] discovered a SNP in the promoter region of *CYP2E1*, which could be related to skatole fat deposition in Danish pig production populations. However, no QTL for skatole were found in this area and that is why it seems highly improbable that this mutation could be connected with the formation of boar taint [Robic *et al.* 2008]. Lin *et al.* [2006] identified a substitution mutation G>A in nucleotide 1423 in the coding region of *CYP2E1* gene (GenBank Accession Number NM_214421.1), which replaces the original amino acid alanine - Ala^{475} with Thr^{475}. This mutation causes a significant decrease in *CYP2E1* expression. The evaluated data suggest that the mutation could be at least partially responsible for high levels of skatole in pigs. *CYP2E1* is a gene which affects not only the concentrations of skatole, but also the metabolism of indole. Mörlein *et al.* [2012] studied the effect of SNP AJ697882.1:g.2412 C>T (in the promoter region) in two production populations of crossbred Duroc pigs. The frequencies of individual genotypes were *CC* (25%), *CT* (52%) and *TT* (23%), with the genotype *CC* showing significantly higher levels of skatole and indole than the other genotypes. The activity of *CYP2E1* promoter is affected by two main factors; COUP-TF1 and HNF-1α. Androstenone is capable of reducing the promoter's activity by binding with HNF-1α and blocking its binding with the promoter. This phenomenon could explain the inhibition of CYP2E1 activity in isolated hepatocytes and also the low CYP2E1 expression in animals with high levels of androstenone *in vivo*. [Tambyrajah *et al.* 2004].

CYB5A. The *CYB5A* gene has already been described above as one of the genes regulating androstenone synthesis. Wiercinska *et al.* [2012] studied the effect of CYB5A on skatole metabolism and believe, that CYB5A can affect not only CYP17A1, but also some other isoforms of cytochrome P450, in an inhibitory or stimulatory way and can thus influence the metabolism of skatole. The study found that the *CYB5A* effect is inconclusive and very specific for individual genes, however by influencing *CYP2A19, CYP2E1* and *CYP2C49* genes it could be possible to use it in order to regulate skatole levels.

Genes affecting phase II of skatole metabolism

Sulfotransferases

SULT1A1 (sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1): In pigs, the *SULT1A1* gene (ENSSSCG00000021557) is located on SSC3 [Lin *et al.* 2004b] in (Scaffold GL892962.1: 15,128-17,110) (Ensembl). Genes for phenol

sulfotransferases and their functions have been previously described for a human population [Babol *et al.* 1998a]. In human genome *SULT1A1* is located on chromosome 16p12.1 (Ensembl). In human medicine, this gene is intensively studied mainly due to its involvement in carcinogenesis. Several *SULT1A1* mutations have been connected with certain cancer types [Lin *et al.* 2004b], such as cancers of lung, colon, stomach, kidney, uterine cervix or urinary bladder, caused by a G>A polymorphism, which changes arginine (Arg^{213}) to histidine (His^{213}) [Zheng *et al.* 2003], oesophagus cancer in men [Wu *et al.* 2003] or breast cancer in women [Tengström *et al.* 2012]. The main role of *SULT1A1* in pigs lies in the phase II of skatole metabolism [Babol *et al.* 1998b]. Lin *et al* [2004b] studied *SULT1A1* polymorphism in 69 boars of European breeding populations (Yorkshire, Duroc, Landrace, Pietrain and crossbreds Landrace x Duroc; Large White x Duroc and Large White x Pietrain) and identified a substitution mutation (A>G) in the 546 nucleotide, located in the coding region of the gene (AY193893.1: c439A>G). This mutation causes the transformation of lysine - Lys^{147} to glutamine - Glu^{147} and results in reduced enzymatic activity of SULT1A1. This mutation could be at least partially responsible for the low catalytic activity of SULT1A1 and subsequently high concentrations of skatole in fat tissue. Whereas Lin *et al.* [2004b] detected *SULT1A1* on chromosome SSC3, Varona *et al.* [2005] were unable to detect the QTL in this area in a studied Landrace pig population. It is therefore necessary to conduct further studies and to take into account the breed or hybrid combination of the pigs studied. Skinner *et al.* [2006] attempted to confirm these conclusions in Large White and Meishan hybrids, as well as in the Danish production populations, but they were unable to find the previously mentioned mutation.

Relationship between the genes for androstenone and skatole

During the period of puberty there is an increase of skatole levels in the fat tissue of boars, which then correlates with the levels of androstenone [Babol *et al.* 1999]. However, no possible effect of certain genes on the androstenone and skatole level at the same time was discussed in the above mentioned publications of Wiercinska *et al.* [2012], Doran *et al.* [2004] and Tambyrajah *et al.* [2004].

Conclusion

Based on the information discussed in this review it is clear that careful breeding selection using selected candidate genes could present a suitable solution to problem of boar taint in the pork. However, this approach also has its risks, which need to be taken into consideration. Several potential candidate genes have been selected in order to regulate androstenone and skatole levels in fat tissue in pigs. In the case of some of these genes, their effect on androstenone and skatole levels has already been clearly demonstrated and researchers identified mutations causing either increase or decrease in the expression of these genes. Nonetheless, the effects of these mutations have not

been unequivocally confirmed. Several studies even describe the reciprocal effects of certain genes on each other – such as the effect of *CYB5A* on *CYP17A1* expression [Billen and Squires 2009]. Based on that, it is obvious that boar taint is the result of a larger number of genes, which mutually affect each other. Considering the fact that the genes affecting boar taint are located in the same chromosomal areas as genes coding enzymes necessary for sex hormones synthesis we also need to take into account any possible negative effects on fertility of the pigs. The above mentioned studies show possible negative effects of these genes on the fertility of boars and sows [Tajet *et al.* 2006; Strathe *et al.* 2013]. The potential negative effects of these genes could also be found in the area of meat quality traits. Due to low levels of androstenone there could be potential decrease in production parameters and carcass composition. Therefore it is appropriate to focus on such genes and SNPs which would be able to lower androstenone and skatole levels without affecting performance parameters [Moe *et al.* 2009].

REFERENCES

1. ANDRESEN O., 2006 – Boar taint related compounds: Androstenone/skatole/other substances. ***Acta Veterinaria Scandinavica*** 48 (Suppl 1), S5.
2. BABOL J., SQUIRES E.J., LUNDSTRÖM K., 1998a – Relationship between oxidation and conjugation metabolism of skatole in pig liver and concentrations of skatole in fat. ***Journal of Animal Science*** 76, 829-838.
3. BABOL J., SQUIRES J., LUNDSTRÖM K., 1998b – Hepatic metabolism of skatole in pigs by cytochrome P4502E1. ***Journal of Animal Science*** 76, 822-828.
4. BABOL J., SQUIRES E.J., LUNDSTRÖM K., 1999 – Relationship between metabolism of androstenone and skatole in intact male pigs. ***Journal of Animal Science*** 77, 84-92.
5. BILLEN M.J., SQUIRES E.J., 2009 – The role of porcine cytochrome b5A and cytochrome b5B in the regulation of cytochrome P45017A1 activities. ***Journal of Steroid Biochemistry and Molecular Biology*** 113, 98-104.
6. BONNEAU M., WALSTRA P., CLAUDI-MAGNUSSEN C., KEMPSTER A.J., TORNBERG E., FISCHER K., DIESTRE A., SIRET F., CHEVILLON P., CLAUS R., DIJKSTERHUIS G., PUNTER P., MATTHEWS K.R., AGERHEM H., BEAGUE M.P., OLIVER M.A., GISPERT M., WEILER U., VON SETH G., LEASK H., FURNOLS M.F.I., HOMER D.B., COOK G.L., 2000 – An international study on the importance of androstenone and skatole for boar taint: IV. Simulation studies on consumer dissatisfaction with entire male pork and the effect of sorting carcasses on the slaughter line, main conclusions and recommendations. ***Meat Science*** 54, 285-295.
7. DE CAMPOS C. F., LOPES M. S., E SILVA F. F., VERONEZE R., KNOL E. F., LOPES P. S., GUIMARÄES S. E. F., 2015 – Genomic selection for boar taint compounds and carcass traits in a commercial pig population. ***Livestock Science*** 174, 10-17.
8. CLAUS R., HOFFMANN B., KARG H. 1971 – Determination of 5α-androst-16-en-3-one, a boar taint steroid in pigs, with reference to relationships to testosterone. ***Journal of Animal Science*** 33, 1293-1297.
9. CHEN G., BOURNEUF E., MARKLUND S., ZAMARATSKAIA G., MADEJ A., LUNDSTRÖM K. 2007 – Gene expression of 3b-hydroxysteroid dehydrogenase and 17b-hydroxysteroid dehydrogenase in relation to androstenone, testosterone, and estrone sulphate in gonadally intact male and castrated pigs. ***Journal of Animal Science*** 85. 2457-2463.

10. CHEN G., CUE R.A., LUNDSTRÖM K., WOOD J.D., DORAN O., 2008 – Regulation of CYP2A6 protein expression by skatole, indole, and testicular steroids in primary cultured pig hepatocytes. ***Drug Metabolism and Disposition*** 36, 56-60.
11. DASGUPTA S., SIRISH P.V.S., NEELAVENI K., ANURADHA K., SUDHAKAR G., MOHAN REDDY B. 2012 – Role of luteinizing hormone β-subunit gene variants among South Indian women with polycystic ovary syndrome. ***GENE*** 494, 51-56.
12. DAVIS S.M., SQUIRES E.J., 1999 – Association of cytochrome b(5) with 16-androstene steroid synthesis and the testis and accumulation in the fat of male pigs. ***Journal of Animal Science*** 77, 1230-1235.
13. DESLANDES B., GARIEPY C., HOUDE A., 2001 – Review of microbiological and biochemical effects of skatole on animal production. ***Livestock Production Science*** 71, 193-200.
14. DIAZ G.J., SKORDOS K.W., YOST G.S., SQUIRES E.J., 1999 – Identification of phase I metabolites of 3-methylindole produced by pig liver microsomes. ***Drug Metabolism and Disposition*** 27, 1150-1156.
15. DIAZ G.J., SQUIRES E.J., 2000 – Metabolism of 3-methylindole by porcine liver microsomes: Responsible cytochrome P450 enzymes. ***Toxicological Sciences*** 55, 284-292.
16. DIAZ G.J., SQUIRES E.J., 2003 – Phase II *in vitro* metabolism of 3-methylindole metabolites in porcine liver. ***Xenobiotica*** 33, 485-498.
17. DORAN E., WHITTINGTON F.A., WOOD J.D., MCGIVAN J.D., 2004 – Characterisation of androstenone metabolism in pig liver microsomes. ***Chemico-Biological Interactions*** 147, 141-149.
18. DUIJVESTEIJN N., KNOL E.F., MERKS J.W.M., CROOIJMANS R., GROENEN M.A.M., BOVENHUIS H., HARLIZIUS B., 2010 – A genome-wide association study on androstenone levels in pigs reveals a cluster of candidate genes on chromosome 6. ***BMC Genetics*** 11, 42.
19. DUMAS N.A., HE D., FROST A.R., FALANY C.N., 2008 – Sulfotransferase 2B1b in human breast: Differences in subcellular localization in African American and Caucasian women. ***Journal of Steroid Biochemistry and Molecular Biology*** 111, 171-177.
20. ENGELSMA K. A., BERGSMA R., HARLIZIUS B., DUCRO-STEVERINK D., KNOL E., 2007 – Genetic parameters for components of boar taint and their relation with carcass quality and female fertility. EAAP 58th, Dublin, Ireland, August 26-29. Session 10, Poster 27.
21. ERNST C. W., STEIBEL J. P., 2013 – Molecular advances in QTL discovery and application in pig breeding. ***Trends in Genetics***, 29 (4), 215-224.
22. GIOVANGRANDI Y., PARFAIT B., ASHEUER M., OLIVI M., LIDEREAU R., VIDAUD M., BIECHE I., 2001 – Analysis of the human CGB/LHB gene cluster in breast tumors by real-time quantitative RT-PCR assays. ***CANCER Letters*** 168, 93-100.
23. GONZALEZ F.J., 2005 – Role of cytochromes P450 in chemical toxicity and oxidative stress: studies with CYP2E1. ***Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis*** 569, 101-110.
24. GRAY M.A., SQUIRES E.J., 2013 – Effects of nuclear receptor transactivation on steroid hormone synthesis and gene expression in porcine Leydig cells. ***Journal of Steroid Biochemistry and Molecular Biology*** 133, 93-100.
25. GREGERSEN V.R., CONLEY L.N., SORENSEN K.K., GULDBRANDTSEN B., VELANDER I.H., BENDIXEN C., 2012 – Genome-wide association scan and phased haplotype construction for quantitative trait loci affecting boar taint in three pig breeds. ***BMC Genomics*** 13, 22.
26. GRINDFLEK E., BERGET I., MOE M., OETH P., LIEN S., 2010 – Transcript profiling of candidate genes in testis of pigs exhibiting large differences in androstenone levels. ***BMC Genetics*** 11, 4.
27. GRINDFLEK E., LIEN S., HAMLAND H., HANSEN M.H.S., KENT M., VAN SON M., MEUWISSEN T.H.E., 2011a – Large scale genome-wide association and LDLA mapping study identifies QTLs for boar taint and related sex steroids. ***BMC Genomics*** 12, 362.

28. GRINDFLEK E., MEUWISSEN T.H.E., AASMUNDSTAD T., HAMLAND H., HANSEN M.H.S., NOME T., KENT M., TORJESEN P., LIEN S., 2011b – Revealing genetic relationships between compounds affecting boar taint and reproduction in pigs. ***Journal of Animal Science*** 89, 680-692.
29. GROßE-BRINKHAUS CH., STORCK L. C., FRIEDEN L., NEUHOFF CH., SCHELLANDER K., LOOFT CH., THOLEN E., 2015 – Genome-wide association analyses for boar taint components and testicular traits revealed regions having pleiotropic effects. ***BMC Genetics*** 16, 36.
30. GUNAWAN A., SAHADEVAN S., NEUHOFF CH., GROßE-BRINKHAUS CH., GAD A., FRIEDEN L., TESFAYE D., THOLEN E., LOOFT CH., UDDIN M. J., SCHELLANDER K., CINAR M. L., 2013 – RNA Deep sequencing reveals novel candidate genes and polymorphisms in boar testis and liver tissues with divergent androstenone levels. ***PLoS ONE*** 8, e63259**.**
31. HUANG C., ZHOU T., CHEN Y., SUN T., ZHANG S., CHEN G., 2014 – estrogen-related receptor err alpha regulation of human hydroxysteroid sulfotransferase (sult2a1) gene expression in human caco-2 cells. ***Journal of Biochemical and Molecular Toxicology*** 28, 32-38.
32. JACQUEMIN P., CHEN Z., MARTIAL J.A., DAVIDSON I., 1999 – Genomic structure and chromosomal mapping of the mouse transcription factor TEF-5 (*Tead3*) gene. ***Mammalian Genome*** 10, 632-634.
33. JI, Y., MOON I., ZLATKOVIC J., SALAVAGGIONE O.E., THOMAE B.A., ECKLOFF B.W., WIEBEN E.D., SCHAID D.J., WEINSHILBOUM R.M., 2007 – Human hydroxysteriod sulfotransferase SULT2B1 pharmacogenomics: Gene sequence variation and functional genomics. ***Journal of Pharmacology and Experimental Therapeutics*** 322, 529-540.
34. KIM J.M., AHN J.H., LIM K.S., LEE E.A., CHUN T., HONG K.C., 2013 – Effects of *hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1* polymorphisms on fat androstenone level and gene expression in Duroc pigs. ***Animal Genetics*** 44, 592-595.
35. KONSTANDI M., CHENG J., GONZALEZ F.J., 2013 – Sex steroid hormones regulate constitutive expression of *Cyp2e1* in female mouse liver. ***American Journal of Physiology-Endocrinology and Metabolism*** 304, E1118-E1128.
36. KOOP D.R., CASAZZA J.P., 1985 – Identification of ethanol – inducible P-450 isozyme 3A as the acetone and acetol monooxygenase of rabbit microsomes. ***Journal of Biological Chemistry*** 260, 3607-3612.
37. KUROGI K., SAKAKIBARA Y., KAMEMOTO Y., TAKAHASHI S., YASUDA S., LIU M.-C., SUIKO M., 2010 – Mouse cytosolic sulfotransferase SULT2B1b interacts with cytoskeletal proteins via a proline/serine-rich C-terminus. ***FEBS Journal*** 277, 3804-3811.
38. LANTHIER F., LOU Y., SQUIRES E.J., 2007 – Skatole metabolism in the intact pre-pubescent male pig: The relationship between hepatic enzyme activity and skatole concentrations in plasma and fat. ***Livestock Science*** 106, 145-153.
39. LEE, G.J., ARCHIBALD,A.L., LAW A.S., LLOYD S., WOOD J., HALEY C.S., 2005 – Detection of quantitative trait loci for androstenone, skatole and boar taint in a cross between Large White and Meishan pigs. ***Animal Genetics*** 36, 14-22.
40. LEUNG M.C.K., BOWLEY K.L., SQUIRES E.J., 2010 – Examination of testicular gene expression patterns in yorkshire pigs with high and low levels of boar taint. ***Animal Biotechnology*** 21, 77-87.
41. LIN Z., LOU Y., SQUIRES E.J., 2004a – Molecular cloning, expression and functional characterization of the *cytochrome P450 2A6* gene in pig liver. ***Animal Genetics*** 35, 314-316.
42. LIN Z.H., LOU Y.P., SQUIRES E.J., 2004b – Molecular cloning and functional analysis of porcine *SULT1A1* gene and its variant: a single mutation *SULT1A1* causes a significant decrease in sulfation activity. ***Mammalian Genome*** 15, 218-226.
43. LIN Z.H., LOU Y.P., PEACOCK J., SQUIRES E.J., 2005a – A novel polymorphism in the 5 ‘ untranslated region of the porcine cytochrome b5 (*CYB5*) gene is associated with decreased fat androstenone level. ***Mammalian Genome*** 16, 367-373.

44. LIN Z.H., LOU Y.P., SQUIRES E.J., 2005b – Identification of a single nucleotide polymorphism in porcine testis cytochrome P450-c17 (CYP17) and its effect on steroidogenesis. ***Biochemical Genetics*** 43, 531-542.
45. LIN Z.H., LOU Y.P., SQUIRES E.J., 2006 – Functional polymorphism in porcine CYP2E1 gene: Its association with skatole levels. ***Journal of Steroid Biochemistry and Molecular Biology*** 99, 231-237.
46. LIU Y., XU Y., LI F., CHEN H., GUO S., 2013 – CYP2A6 deletion polymorphism is associated with decreased susceptibility of lung cancer in Asian smokers: a meta-analysis. ***Tumor Biology*** 34, 2651-2657.
47. LU Y., CEDERBAUM A.I., 2008 – CYP2E1 and oxidative liver injury by alcohol. ***Free Radical Biology and Medicine*** 44, 723-738.
48. LUKIĆ B., PONG-WONG R., ROWE S. J., DE KONING D. J., VELANDER I., HALEY C. S., ARCHIBALD A. L., WOOLLIAMS J. A., 2015 – Efficiency of genomic prediction for boar taint reduction in Danish Landrace pigs. ***Animal Genetics*** 46, 607-616.
49. LUNDSTRÖM K., ZAMARATSKAIA G., 2006 – Moving towards taint-free pork – alternatives to surgical castration. ***Acta Veterinaria Scandinavica***. 48(Suppl 1). S1.
50. MATAL J., MATUSKOVA Z., TUNKOVA A., ANZENBACHEROVA E., ANZENBACHER P., 2009 – Porcine CYP2A19, CYP2E1 and CYP1A2 forms are responsible for skatole biotransformation in the reconstituted system. ***Neuroendocrinology Letters*** 30, 36-40.
51. MOE M., GRINDFLEK E., DORAN O., 2007a – Expression of 3 beta-hydroxysteroid dehydrogenase, cytochrome P450-c17, and sulfotransferase 2B1 proteins in liver and testis of pigs of two breeds: Relationship with adipose tissue androstenone concentration. ***Journal of Animal Science*** 85, 2924-2931.
52. MOE M., MEUWISSEN T., LIEN S., BENDIXEN C., WANG X.F., CONLEY L.N., BERGET I., TAJET H., GRINDFLEK E., 2007b – Gene expression profiles in testis of pigs with extreme high and low levels of androstenone. ***BMC Genomics*** 8, 405.
53. MOE M., LIEN S., BENDIXEN C., HEDEGAARD J., HORNSHOJ H., BERGET I., MEUWISSEN T.H.E., GRINDFLEK E., 2008 – Gene expression profiles in liver of pigs with extreme high and low levels of androstenone. ***BMC Veterinary Research*** 4,29.
54. MOE M., LIEN S., AASMUNDSTAD T., MEUWISSEN T.H.E., HANSEN M.H.S., BENDIXEN C., GRINDFLEK E., 2009 – Association between SNPs within candidate genes and compounds related to boar taint and reproduction. ***BMC Genetics*** 10, 32.
55. MÖRLEIN D., LUNGERSHAUSEN M., STEINKE K., SHARIFI A.R., KNORR C., 2012 – A single nucleotide polymorphism in the CYP2E1 gene promoter affects skatole content in backfat of boars of two commercial Duroc-sired crossbred populations. ***Meat Science*** 92, 739-744.
56. NAKAMURA Y., XING Y., HUI X.G., KUROTAKI Y., ONO K., COHEN T., SASANO H., RAINEY W.E., 2011 – Human adrenal cells that express both 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2) and cytochrome b5 (CYB5A) contribute to adrenal androstenedione production. ***Journal of Steroid Biochemistry and Molecular Biology*** 123, 122–126.
57. NICOLAU-SOLANO S.I., MCGIVAN J.D., WHITTINGTON F.M., NIEUWHOF G.J., WOOD J.D., DORAN O., 2006 – Relationship between the expression of hepatic but not testicular 3 beta-hydroxysteroid dehydrogenase with androstenone deposition in pig adipose tissued. ***Journal of Animal Science*** 84, 2809-2817.
58. OSKAM I.C., LERVIK S., TAJET H., DAHL E., ROPSTAD E., ANDRESEN O., 2010 – Differences in testosterone, androstenone, and skatole levels in plasma and fat between pubertal purebred Duroc and Landrace boars in response to human chorionic gonadotrophin stimulation. ***Theriogenology*** 74, 1088-1098.

59. PANELLA-RIERA N., MOE M., GRINDFLEK E., OLIVER M.A., WOOD J.D., DORAN O., 2008 – Effect of sex steroids on expression of sulfotransferase 2B1 immunoreactive protein in primary cultured porcine hepatocytes. ***Livestock Science*** 118, 223-230.
60. PAYNE A.H., HALES D.B., 2004 – Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. ***Endocrine Reviews*** 25, 947-970.
61. PEACOCK J., LOU Y., LUNDSTRÖM K., SQUIRES E.J., 2008 – The effect of a c.-8G > T polymorphism on the expression of *cytochrome b5A* and boar taint in pigs. ***Animal Genetics*** 39, 15-21.
62. QUINTANILLA R., DEMEURE O., BIDANEL J.P., MILAN D., IANNUCCELLI N., AMIGUES Y., GRUAND J., RENARD C., CHEVALET C., BONNEAU M., 2003 – Detection of quantitative trait loci for fat androstenone levels in pigs. ***Journal of Animal Science*** 81, 385-394.
63. RAMOS A.M., DUIJVESTEIJN N., KNOL E.F., MERKS J.W.M., BOVENHUIS H., CROOIJMANS R., GROENEN M.A.M., HARLIZIUS B., 2011 – The distal end of porcine chromosome 6p is involved in the regulation of skatole levels in boars. ***BMC Genetics*** 12, 35.
64. ROBIC A., LARZUL C., BONNEAU M., 2008 – Genetic and metabolic aspects of androstenone and skatole deposition in pig adipose tissue: A review. ***Genetics Selection Evolution*** 40, 129-143.
65. ROBIC A., FEVE K., LARZUL C., BILLON Y., VAN SON M., LIAUBET L., SARRY J., MILAN D., GRINDFLEK, E., BIDANEL, J.P., RIQUET J., 2011a – Expression levels of 25 genes in liver and testis located in a QTL region for androstenone on SSC7q1.2. ***Animal Genetics*** 42, 662-665.
66. ROBIC A., LE MIGNON G., FEVE K., LARZUL C., RIQUET J., 2011b – New investigations around *CYP11A1* and its possible involvement in an androstenone QTL characterised in Large White pigs. ***Genetics Selection Evolution*** 43,15.
67. ROBIC A., LARZUL C., GRINDFLEK E., CHEVILLON P., HOFER A., FEVE K., IANNUCCELLI N., MILAN D., PRUNIER A., RIQUET J., 2012 – Molecular characterization of the porcine *TEAD3 (TEF-5)* gene: examination of a promoter mutation as the causal mutation of a quantitative trait loci affecting the androstenone level in boar fat. ***Journal of Animal Breeding and Genetics*** 129, 325-335.
68. ROWE S. J., KARACAÖREN B., DE KONING D. J., LUKIĆ B., HASTINGS-CLARK N., VELANDER I., HALEY C. S., ARCHIBALD A. L., 2014 – Analysis of the genetics of boar taint reveals both single SNPs and regional effects. ***BMC Genomics***, 15, 424.
69. SAHADEVAN S., THOLEN E., GROßE-BRINKHAUS C., SCHELLANDER K., TESFAYE D., HOFMANN-APITIUS M., CINAR M. U., GUNAWAN A., HÖLKER M., NEUHOFF C., 2015 – Identification of gene co-expression clusters in liver tissues from multiple porcine populations with high and low backfat androstenone phenotype. ***BMC Genetics***, 16, 21.
70. SIMARD J., RICKETTS ML., GINGRAS S., SOUCY P., FELTUS F.A., MELNER M.H. 2005 – Molecular biology of the 3beta-hydroxysteroid dehydrogenase/delta5–4 isomerase gene family. ***Endocrine Reviews*** 26, 525–82.
71. SINCLAIR P.A., HANCOCK S., GILMORE W.J., SQUIRES E.J., 2005 – Metabolism of the 16-androstene steroids in primary cultured porcine hepatocytes. Journal of Steroid ***Biochemistry and Molecular Biology*** 96, 79-87.
72. SINCLAIR P.A., GILMORE W.J., LIN Z., LOU Y., SQUIRES E.J., 2006 – Molecular cloning and regulation of porcine *SULT2A1*: relationship between SULT2A1 expression and sulfoconjugation of androstenone. ***Journal of Molecular Endocrinology*** 36, 301-311.
73. SKINNER T.M., DORAN E., MCGIVAN J.D., HALE C.S., ARCHIBALD A.L., 2005 – Cloning and mapping of the porcine *cytochrome-p450 2E1* gene and its association with skatole levels in the domestic pig. ***Animal Genetics*** 36, 417-422.
74. SKINNER T.M., ANDERSON J.A., HALEY C.S., ARCHIBALD A.L., 2006 – Assessment of *SULT1A1*, *CYP2A6* and *CYP2C18* as candidate genes for elevated backfat skatole levels in

commercial and experimental pig populations. ***Animal Genetics*** 37, 521-522.

75. STRATHE A.B., VELANDER I.H., MARK T., KADARMIDEEN H.N., 2013 – Genetic parameters for androstenone and skatole as indicators of boar taint and their relationship to production and litter size traits in Danish Landrace. ***Journal of Animal Science*** 91, 2587-2595.
76. TAJET H., ANDRESEN, O., MEUWISSEN T., 2006 – Estimation of genetic parameters of boar taint; skatole and androstenone and their correlations with sexual maturation. ***Acta Veterinaria Scandinavica***. 48(Suppl 1). S9.
77. TAMBYRAJAH W.S., DORAN E., WOOD J.D., MCGIVAN J.D., 2004 – The pig CYP2E1 promoter is activated by COUP-TF1 and HNF-1 and is inhibited by androstenone. ***Archives of Biochemistry and Biophysics*** 431, 252-260.
78. TENGSTRÖM M., MANNERMAA A., KOSMA V.-M., HIRVONEN A., KATAJA V., 2012 – *SULT1A1* rs9282861 polymorphism-a potential modifier of efficacy of the systemic adjuvant therapy in breast cancer? ***BMC Cancer*** 12, 257.
79. TÖRN S., NOKELAINEN P., KURKELA R., PULKKA A., MENJIVAR M., GHOSH S., COCA-PRADOS M., PELTOKETO H., ISOMAA V., VIHKO P. 2003 – Production, purification, and functional analysis of recombinant human and mouse 17β-hydroxysteroid dehydrogenase type 7. ***Biochemical and Biophysical Research Communications*** 305, 37-45.
80. VARONA L., VIDAL O., QUINTANILLA R., GIL M., SANCHEZ A., FOLCH J.M., HORTOS M., RIUS M.A., AMILLS M., NOGUERA J.L., 2005 – Bayesian analysis of quantitative trait loci for boar taint in a Landrace outbred population. ***Journal of Animal Science*** 83, 301-307.
81. WIERCINSKA P., LOU Y., SQUIRES E.J., 2012 – The roles of different porcine cytochrome P450 enzymes and cytochrome b5A in skatole metabolism. ***Animal*** 6, 834-845.
82. WU M.T., WANG Y.T., HO C.K., WU D.C., LEE Y.C., HSU H.K., KAO E.L., LEE J.M., 2003 – *SULT1A1* polymorphism and esophageal cancer in males. ***International Journal of Cancer*** 103, 101-104.
83. XUE J.L., DIAL G.D., 1997 – Raising intact male pigs for meat: Detecting and preventing boar taint. ***Swine Health and Production*** 5, 151-158.
84. YAMAZAKI H., INOUE K., HASHIMOTO M., SHIMADA T., 1999 – Roles of CYP2A6 and CYP2B6 in nicotine C-oxidation by human liver microsomes. ***Archives of Toxicology*** 73, 65-70.
85. YOKOYAMA M. T., CARLSON J. R., 1979 – Microbial metabolites of tryptophan in the intestinal tract with special reference to skatole. ***American Journal of Clinical Nutrition*** 32, 173-178.
86. ZAMARATSKAIA G., LOU Y., PEACOCK J., RYDHMER L., ANDERSSON H.K., JUNEJA R.K., CHEN G., LUNDSTRÖM K., SQUIRES E.J., 2008 – Effect of polymorphism in the porcine cytochrome b5 (*CYB5A*) gene on androstenone and skatole concentrations and sexual development in Swedish pig populations. ***Animal*** 2, 190-196.
87. ZAMARATSKAIA G., SQUIRES E.J., 2009 – Biochemical, nutritional and genetic effects on boar taint in entire male pigs. ***Animal*** 3, 1508-1521.
88. ZAMARATSKAIA G., STEFANOVIC S., LUNDSTRÖM K., DORAN O., 2012 – Expression of the hepatic skatole- and androstenone-metabolising enzymes in entire male pigs of two live weights. ***Livestock Science*** 145, 124-130.
89. ZHENG L.Z., WANG Y.F., SCHABATH M.B., GROSSMAN H.B., WU X.F., 2003 – Sulfotransferase 1A1 (SULT1A1) polymorphism and bladder cancer risk: a case-control study. ***CANCER Letters*** 202, 61-69.

NUTRITIONAL EFFECTS ON BOAR TAINT IN ENTIRE MALE PIGS: A REVIEW*

D. Urbanová, R. Stupka, M. Okrouhlá, J. Čítek, K. Vehovský, K Zadinová

Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Prague, Czech Republic

Boar taint is one of topical problems in fattening pigs. It is caused by two main compounds — androstenone and skatole. Androstenone is a steroid feromone, which is synthesized and metabolized in liver and testes. Skatole is produced by intestinal bacteria by metabolization of trypthophan. Both these substances are metabolized by cytochrome P450 and the unmetabolized residues accumulate in adipose tissue. This review describes the possible nutritional effects on boar taint reduction. Skatole is the main component, which could be reduced by nutrition in entire male pigs. The presence in adipose tissue can be reduced by apoptosis of intestinal cells by raw potato starch. Another method is to influence the microbial population in the gastrointestinal tract by organic acids or fructooligosaccharides. Recently, attention has been directed towards the enzymatic system in the liver. There are a few possibilities of reducing skatole as well as androstenone by influencing the liver enzymatic system. They may be particularly affected by secondary plant metabolites and flavonoids. However, more research is required in this area to clarify physiological regularities and all the relationships in the metabolism detoxification from xenobiotic substances.

skatole, androstenone, cytochrome, diet, inulin, microbiota

DE GRUYTER OPEN

doi: 10.1515/sab-2016-0023
Received for publication on May 10, 2016
Accepted for publication on October 4, 2016

INTRODUCTION

Boar taint and its elimination is one of topical problems in fattening entire male pigs. Increasing publicity regarding food safety and quality and animal welfare brought about fundamental changes involving progressive limitations to prohibition of piglet castration without anaesthesia in fattening pigs. Castration leads to the elimination of characteristic boar taint. Surgical castration without anaesthesia, performed on piglets before instead of at 7 days of age in commercial farming, has become the subject of public attention in recent years. The European community has called for prohibition of these techniques. Based on these facts, the European Union has accepted recommendations on the prohibition of castration without anaesthesia from 2018 (http://ec.europa.eu/food/animal/welfare/farm/initiatives_en.htm). Therefore it is necessary to find possible and economically viable solutions for minimizing or eliminating boar taint in pork from entire male pigs. One of possible methods consists in boar nutrition.

Boar taint components

The distinctive boar taint in entire male pigs is caused by a high concentration of some compounds, specifically androstenone and skatole (Squires, 2003). However, there are other compounds which can influence the offensive odour of pork, such as 4-phenyl-3-buten-2-one, which causes higher sensitivity to androstenone and skatole in adipose tissue (Solé, García Requeiro, 2001) and also aldehydes, short chain fatty acids, alcohols, and ketones may influence the sensitivity of consumers (Rius et al., 2005).

* Supported by the Grant Agency of Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague (Project No. SV16-50-21320) and by the Ministry of Education, Youth and Sports of the Czech Republic ("S" Project).

Androstenone. Androstenone is a steroid feromone. It was found in adipose tissue of entire male pigs in 1968 as a substance with a typical urine odour (Patterson, 1968). Androstenone is synthesized in testes and it is released by specific binding protein from the lipocaline family to the salivary glands, where it acts as a pheromone (Marchese et al., 1998). Because of its hydrophobic properties, it is also stored in adipose tissue, where it causes urine-like odour when the fat is heated (Squires, 2005). Testicular hormones are synthesized in interstitial tissue by Leydig cells under the influence of the follicle-stimulating hormone (FSH), and luteinizing hormone (LH) under the influence of the gonadotropin-releasing hormone (GnRH).

The precursor of all steroid hormones is cholesterol, which originates from blood plasma. Many of the steps in steroid biosynthesis include the electron transport chain, with cytochrome P450 side-chain cleavage ($CYP450_{scc}$) enzyme at the end. After the stimulation by trophic hormones, esterase is activated. The newly formed free cholesterol moves to mitochondria, where it is converted by $CYP450_{scc}$ to pregnenolone. Thereafter, pregnenolone is hydroxylated to progesterone by 3β-hydroxysteroid dehydrogenase (Squires, 2003). Androstenone belongs specifically to the group of 16-androstene steroids, which are synthesized from progesterone in the testes (Melrose et al., 1971). The cytochrome, which catalyzes androstenone synthesizing reactions, is CYP17, along with CYB5. Levels of its protein and its total mRNA closely correlate with the rate of androstenone synthesis (Davis, Squires, 1999). The last step in androstenone synthesis is the reduction of double bond by 5-α-reductase. Levels of this enzyme correlate with androstenone concentration (Cooke et al., 1997). In the blood, steroids are binding into the proteins and transport to the final action place.

The degradation of androstenone takes place in testes and liver microsomes. In testes, androstenone is metabolized to 5α-androst-16-en-3α-ole and 5α-androst-16-en-3β-ole and these are further metabolized to more steroids with polar bond (Sinclair, Squires, 2005). Androstenone is reduced to 3β-androstenol and less frequently to 3α-androstenol, by 3β-HSD a 3α-HSD enzymes in liver microsomes. In the following second phase of metabolism, androstenols form glucuronide or sulphide bond. In this phase of metabolism, the sulphotransferase SULT2A1 is the most important enzyme (Sinclair et al., 2006). The synthesis and metabolism of androstenone are summarized in Fig. 1.

The residual metabolites are mainly transported to the salivary gland. If androstenone levels are high and the liver is not able to metabolize them, the residues are stored in adipose tissue (Doran et al., 2004) or can be excreted into bile (Devine, Dikeman, 2014). The concentration in plasma varies from several ng/ml up to 40–60 ng/ml (Andresen, 2006). It was found that a concentration of androstenone higher than 15 ng/ml usually leads to a very high concentration in adipose tissue (Andresen, 1976).

Overall, androstenone levels in tissues reflect the period of biosynthesis fluctuation in testes. The biological half-life of androstenone in tissues is relatively long and the reduction of its concentration in adipose tissue occurs after 3–6 weeks post-castration (Claus et al., 1994). It was also found that androstenone has a negative effect on CYP2E1 expression and thus on the metabolism of skatole in the liver, which may cause higher skatole levels in entire males (Doran et al., 2002).

Skatole. Skatole is a substance with a characteristic offensive faecal odour, which is formed by tryptophan degradation in anaerobic conditions. It is produced in the gastrointestinal tract by intestinal bacteria Escherichia coli, Clostridium ssp., and Lactobacillus ssp., which cleave to L-tryptophan. Most of these bacteria are able to metabolize tryptophan to indole and indole acetic acid, which is the main precursor of skatole. Indeed, only a small quantity of intestinal bacteria (less than 0.01 %) is able to catalyze the decarboxylation of indole acetic acid to skatole (Jensen, Jensen, 1993).

The main source of tryptophan, which is used for skatole synthesis by intestinal bacteria, is the cell debris from intestinal epithelium (Claus, Raab, 1999). Skatole production increases in the colon, with the highest concentration being in the distal part of the colon (Jensen, 2006). One part of produced skatole is excreted from the intestine by faeces and the second part is absorbed from the intestine by passive diffusion into the bloodstream, where it is transported to the liver by *vena cava caudalis* (Claus et al., 1994). There it is metabolized by the CYP450 enzymatic system. This system is considerably limited by androstenone in boars, because its levels are closely related to the levels of skatole (Babol et al., 1999).

Skatole metabolism is composed of two phases. The main metabolites in the first phase are: 6-hydroxyskatole, indole-3-carbinol, 3-hydroxy-3-methyloxindole (HMOI), 3-hydroxy3-methylindolenine (HMI), and 3-methyloxindole (3MOI) (Squires, 2003). The key enzymes of this phase are CYP2E1, CYP2A, and CYP1A2, which are located in the pig liver microsomes and their activity is influenced by androstenone physiological levels (Matala et al., 2009; Rasmussen et al., 2011b). It was also found that skatole induces the CYP2E1 protein expression but androstenone has the antagonistic effect on CYP2E1 expression when acting simultaneously (Doran et al., 2002). A high activity level of these enzymes is negatively correlated with skatole levels in adipose tissue (Diaz, Squires, 2000).

The main and final metabolites in the second phase of metabolism are: 6-sulfatoxy-skatol in sulphate or glucuronide bond with 5-hydroxy-3methylindole or 3-hydroxy-3-methyloxindole (Diaz, Squires,

2000). The main enzymes in this final phase are sulfotransferase (SULT1A1) and uridine-di-phosphate-glucoronsyltransferase (UGT) (Sinclair, Squires, 2005). The synthesis and metabolism of skatole are summarized in Fig. 2. The metabolites water solubility increases and leads to facilitation of urine excretion in the second phase of metabolism (Rasmussen et al., 2012b). In pigs, which have low levels of SULT1A1 and UGT enzymes, the levels of skatole in adipose tissue are higher due to inability to metabolize it (Squires, 2003).

The influence of nutrition on selected components of boar taint

In terms of the possibility of eliminating boar taint components through many food supplements, recent attention has been paid to boar nutrition. While androstenone is a steroid feromone produced by sexual glands in entire male pigs and the possibilities of its elimination by nutrition are very limited, skatole is the product of tryptophan degradation in the gastrointestinal tract and it can be better influenced by nutrition.

The influence of tryptophan availability in the gut on skatole formation. Skatole formation in the gastrointestinal tract can be influenced primarily by the availability of tryptophan in the gastrointestinal tract. As the main source of tryptophan is the cell debris of the intestinal epithelium, the objective is at most to reduce this cell debris through nutrition. One of the possibilities of cell debris reduction in the gut is to reduce apoptosis of intestinal cells, which can lead to a reduction of cell debris and, thereafter, the quantity of tryptophan required for skatole formation. The quantity of cell debris is related to the amount of cell mitosis. This phenomenon is influenced by the increasing factor IGF-I, whose expression increases with an increased quantity of feed purines. A higher

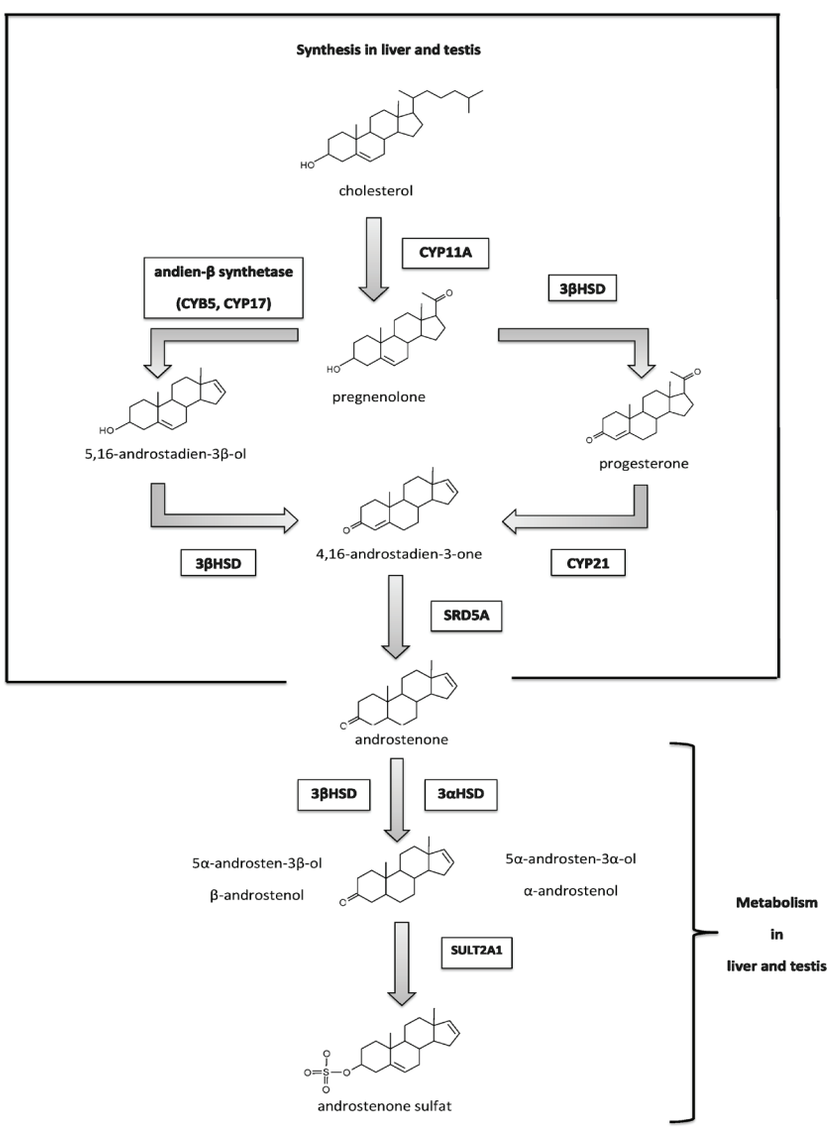


Fig. 1. Androstenone synthesis and metabolism

content of purines in feed can be achieved by the addition of dried brewer's yeast to the diet. These purines facilitate increased DNA and RNA synthesis, which leads to increased intestinal cell mitosis and subsequently a larger quantity of cell debris in the gut (C l a u s , R a a b , 1999).

Raw potato starch is a possible food supplement which can positively influence this phenomenon. It influences the formation of lactic acid in the gut and this acid subsequently inhibits the apoptosis of intestinal cells. In animals fed raw potato starch, a reduction of skatole concentration occurred in the colon, faeces, plasma, or adipose tissue (L o s e l et al., 2006; P a u l y et al., 2008; O v e r l a n d et al., 2011). Overland et al. (2011) stated that pelleting starch results in a loss of effect on skatole levels in adipose tissue. This could be explained by starch gelatinization, which occurs at temperatures higher than 52.5°C to which starch pellets are exposed (S h i o t s u b o , 1984). Feeding potato starch is therefore possible, but only in the raw form. By contrast, in animals which were separately fed lactic acid in the form of feed coated with Ca-butyrate, the skatole levels were not influenced (O v e r l a n d et al., 2008).

The influence of dietary composition on the microbial population in the gastrointestinal tract and skatole formation. During tryptophan metabolism, gastrointestinal bacteria produce two different volatile

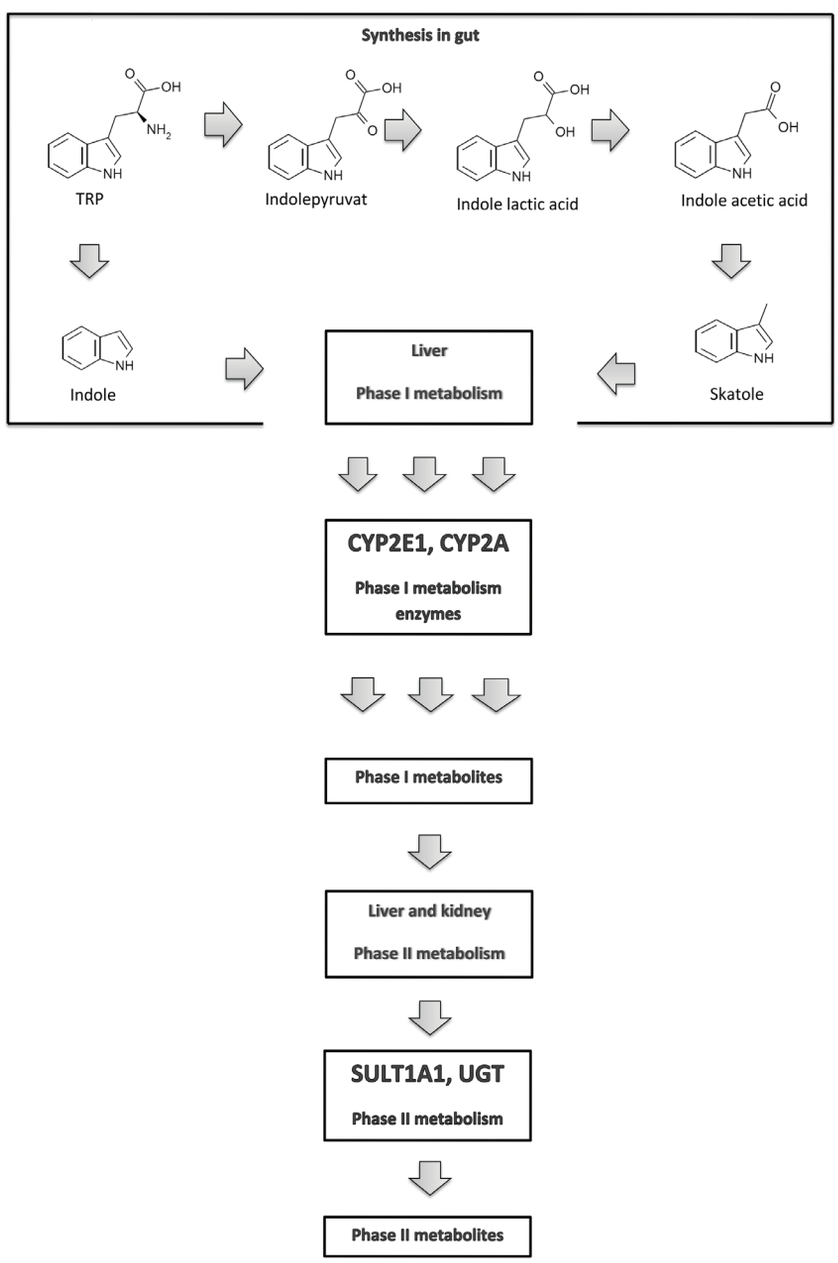


Fig. 2. Skatole synthesis and metabolism

lipophilic substances — indole and 3-methylindole. The activity of intestinal bacteria can be influenced by antibiotics, organic acids, selected plant extracts, or a diet rich in easily fermentable carbohydrates, which affect the pH of the gastrointestinal tract and hence decrease the formation and production of skatole (Wesloy, Weiler, 2012). The rate of skatole and indole production varies depending on pH. The microbial activity in the colon is also pH-dependent. Skatole formation increases with lower pH values. At pH values of approximately 6.5, the increased activity of skatole produces bacteria, while at pH values around 8.0 skatole production rapidly declines. On the other hand, indole production increases at pH 8.0, when indole producing bacteria activity increases, and decreases along with pH reduction (Jensen et al., 1995).

Feeding antibiotics is a logical consideration, but only on an experimental level (banned in the European Union since January 2006 due to increasing resistance across different strains of bacteria). However, treatment with Virginiamycin, Tylosin, and Bacitracin was tested, with Bacitracin being the only antibiotics statistically significantly affecting the decrease of skatole presence in the blood and adipose tissue (Hansen et al., 1997).

One of the possible alternatives could be the use of organic acids, which have a positive effect on growth across different pig categories (Partanen, Mroz, 1999). Moreover, it was found that organic acids have the ability to influence the bacterial population in the gastrointestinal tract of pigs because they have a bactericide effect. Overland et al. (2008) reported on the effect of a diet with organic acid supplement and its relation to skatole and indole production. Diet supplementation with formic, benzoic, and sorbic acids reduced the microbial content in the gastrointestinal tract, but no effect on skatole production in the colon was observed. Diet supplementation with formic and benzoic acids leads to decreasing skatole levels in plasma. The authors suggested that organic acids are able to influence the microbial population in the gastrointestinal tract, but not to the extent that boar taint could be reduced.

The type and quantity of polysaccharides entering the gastrointestinal tract could have a major impact on nitrogen metabolism and could influence skatole and indole synthesis (Hawe et al., 1992). Fructooligosaccharides (FOS) are a specific source of saccharides, which are not digested by digestive enzymes in the upper part of the digestive tract. They enter the colon in intact form and could be a nutrient source for some bacterial populations. Oligosaccharides support the activity and growth of bifidobacterium, and inhibit the growth of bacteria involved in skatole and indole formation, i.e. of the species *E. coli* and *Clostridium* (Roberfroid et al., 1998). A FOS supplement was tested under *in vitro* conditions. The results of the study support the hypothesis that lower skatole concentrations observed in the presence of FOS could be caused by reduced degradation of tryptophan due to a higher requirement of amino acids for bacterial cell protein. Further they could be caused by a shift of the microbial metabolism to indole production at the expense of skatole production, which could result from microbial ecosystem and pH changes (Xu et al., 2002).

Under *in vivo* conditions, the difference between oats and barley diets was tested. Oats and barley have high levels of β-D-glucans, which show a small difference between their structures. Oats β-glucans have a higher quantity of β-(1-4) than of β-(1-3) bonds as compared to β-glucans of barley and these make them harder to digest (Duss, Nyberg, 2004). Feeding an oats diet had a positive effect on the quantity of bacteria required (Paul y et al., 2011). It can be expected that less soluble β-glucans (oligosaccharides) have a prebiotic, thus positive, influence on the quantities of bifidobacterium and lactobacilli bacteria. This confirms the previous *in vitro* study.

One of the possibilities of utilizing the positive effect of polysaccharides is the feeding of inulin-rich diets. Inulin is a polysaccharide, which replaces starch as a storage substance in *Astraceae* and *Campalunaceae*. The animal organism cannot use it, because inulin is cleaved by gastrointestinal bacteria, not by amylase. The bacteria can cleave inulin, and use it as a source of energy, thus inulin is able to change the usual course of bacterial fermentation in the colon. In the hindgut it shows the same properties as soluble fibre and it primarily acts as prebiotic.

Examples of sources of inulin are the chicory root and Jerusalem artichoke. Numerous studies demonstrated that feeding chicory or pure inulin influenced the content of skatole in the excrement, blood, and adipose tissue (Hansen et al., 2006; Byrne et al., 2008). Further, it can be found in the literature that feeding chicory roots, dried chicory or pure inulin significantly decreased skatole levels in the adipose tissue of entire males (Kjos et al., 2010; Overland et al., 2011; Zammerini et al., 2012). One of the first studies on this topic demonstrates a significant decrease of skatole concentration (by 50–70 %) in adipose tissue (Claus et al., 1994). Hansen et al. (2006) reported that a higher amount of inulin in the diet, thus inclusion of chicory in the feeding diet, decreased skatole levels in plasma already after 3 days. They also recommend dried chicory as the best source of inulin, because of no negative influence on food intake at the beginning of feeding. It constantly decreases skatole levels without any impact on performance and, finally, it is easy to use all year round and is affordable.

Jerusalem artichoke has been confirmed to have the same impact as chicory. Feeding it one week before slaughter leads to a decrease of skatole levels in

the gut and adipose tissue. Decreased skatole levels can be associated with the decrease of *Clostridium perfrigens*, higher content of short chain fatty acids, and subsequent decrease of pH (V h i l e et al., 2012).

Based on these facts it could be concluded that chicory and artichoke are a good source of FOS for reducing boar taint, because of their affordability, and minimal influence on feed intake.

The influence of dietary composition on the enzymatic system influencing androstenone and skatole liver metabolism. There is relatively new publicity around the possible effect of diet on cytochrome activity in relation to cytochrome gene expression and the enzyme activity which participates in skatole and androstenone metabolism. This concerns cytochrome P450 (CYP450) and its group of proteins which, like enzymes, plays an essential role in bioactivation and metabolism detoxification from xenobiotic substances (G u e n g e r i c h , 2008). Metabolism of these substances consists of three phases. In Phase I, molecule polarization occurs, when the polar group is uncovered by oxidation, reduction or hydrolysis. Phase II is when the molecules from Phase I are conjugated by endogenous molecules, which leads to higher solubility in water and easier excretion of unfavourable substances in the urine. The transmembrane transfer from the cell to the exterior is referred to as Phase III (G u e n g e r i c h , 2007). In order for the organism to react correspondingly to the effect of xenobiotic substances, it has to regulate the chemical transition of these substances. In this case, the organism regulates it by changes in the expression of genes for biotransformation of enzymes by transcription. The receptors are involved in this, which function like transcription factors and also are activated by its ligands (U r q u h a r t et al., 2007). The mechanism of xenobiotic receptors activity is illustrated in Fig. 3.

There is a large quantity of these receptors. However, in relation to boar taint, only three of them were studied: the hydrocarbon receptor – AhR, the constitutive androstane receptor – CAR, and the pregnane X receptor – PXR (R a s m u s s e n et al., 2014). Each of them regulates different families (e.g. CYP1) and subfamilies (e.g. CYP1A) of CYP450. The prevalent isoforms of CYP450 in pig livers are CYP2A and CYP2D, which include 60 % of CYP450 proteins. The second largest groups are CYP2C and CYP3A (A c h o u r et al., 2011). CYP1A2, CYP2A, and CYP2E1 in particular participate in skatole metabolism, and are involved in the first phase of skatole metabolism. Their enzyme activity plays a major role in skatole accumulation in adipose tissue, because a lack of their activity leads to increased storage of skatole in adipose tissue (Diaz, S q u i r e s , 2000).

Chicory *Cichorium intybus* L. has been one of the most investigated plants in pig feeding in order to eliminate boar taint. Reduction of skatole formation in the gastrointestinal tract has been the prime expectation. However, studies on other animals showed that animals fed a fibre-rich diet showed higher CYP450 expression and activity (L e m l e y et al., 2010).

Growing public interest and the lack of scientific studies arouse scientists' interest in the chemical composition of these substances and their potential biological effect (C h a n g , 2009). *Cichorium intybus* L. was chosen as a potential plant to have, among other qualities, a hepatoprotective effect. It has beneficial inflammatory, antioxidant, and anticarcinogenic effects. Because of inulin contained in the roots, it has a prebiotic effect and, lastly, it produces secondary metabolites. For example, the most important are sesquiterpene lactones, essential oils with bioactive effects (B a i s , R a v i s h a n k a r , 2001).

A diet containing chicory root was fed to boars to determine the effect of chicory on pig liver microsomes activity. A diet containing 10 % of dried chicory root was fed 16 days before slaughter. A higher expression of CYP1A2 by ca.79 % and of CYP2A by ca. 20 % was detected in their liver. The mRNA expression in all cytochromes was increased (R a s m u s s e n et al., 2011a).

The next step in the studies focused on the effect of herbs and natural substances on cytochrome activity was studying their impact on xenobiotic receptors (AhR, CAR, PXR). One of these studies was focused on the potential induction of CYP mRNA, using the activation of xenobiotic receptors by secondary metabolites. An observation was made as to which effect each chicory secondary metabolite (artemisinin, scoparone, lactucin, esculetin, and esculin) would have on liver CYP mRNA in primary liver hepatocytes, compared to the total extract of chicory roots. It was

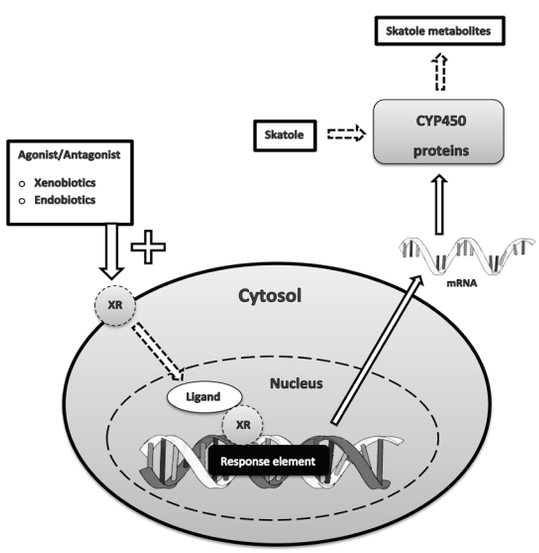


Fig. 3. Mechanism of xenobiotic receptors' activity

proved that artemisinin activates AhR, CAR, and PXR together, scoparone causes AhR and CAR activation, lactucin activates CAR, and esculetin can influence the activation of AhR. Only esculin, which is the esculetin metabolite, did not show any observable effect. The total chicory root extract decreased the expression in some cytochromes in high concentrations (Rasmussen et al., 2014). The conclusion is that purified secondary metabolites influence CYP expression and thereby detoxification in general, while the effects of the total extract differ from those of the single-component one.

Relative to previous findings, which prove that chicory influences CYP450 expression, scientists expected animals fed a chicory supplement diet to have lower steroid levels and higher expression of enzymes participating in their metabolism. It was investigated, with androstenone, how to influence 3β-hydroxysteroid dehydrogenase (3β-HSD) by diet. This is an enzyme which participates in steroid hormone metabolism. One of the studies proved that animals fed a diet with dried chicory roots had statistically significantly lower content of androstenone in adipose tissue, but showed increased mRNA expression of 3β-HSD protein (Rasmussen et al., 2012a).

Another study tested secondary metabolites from chicory root, specifically lactucin, esculetin and esculin. This study dealt with the single effect of metabolites on influence of xenobiotic receptors (AhR, CAR, PXR) on the expression of the enzymes which participate in androstenone metabolism, as in the previous study on skatole. In this study, it was found that lactucin increased mRNA expression of 3β-HSD and SULT2A1 by about 200 %. In contrast, the total chicory root extract decreased the expression of both these enzymes participating in androstenone metabolism. In conclusion, it could be stated that gene expression of these enzymes is complexly controlled by secondary metabolites (Rasmussen et al., 2014).

Other group of compounds to be investigated are dietary flavonoids as a potent inhibitor of various CYP450 isoforms and membrane transporters (Wahajuddin et al., 2013). An *in vitro* study of Ekstrand et al. (2015) revealed that the degree of inhibition of the major CYP450 isoforms by flavonoids is dependent on flavonoid structure, concentration, and on pigs'gender. It was found that some of the selected flavonoids (specifically myricetin, isorhamnetin, and quercetin) may affect the activities of porcine CYP1A, CYP3A, and CYP2E1. Nevertheless, this mechanism of action requires subsequent confirmation studies under *in vivo* conditions.

CONCLUSION

The elimination of boar taint, caused by a higher concentration of androstenone and skatole and their accumulation in adipose tissue of entire male pigs, is one of the topic issues in fattening pigs. Nutrition is one of the possible routes for the elimination of this adverse effect. Despite first studies showing that only skatole is possible to be reduced by nutrition, because androstenone as a steroid feromone cannot be influenced by diet, recent studies shed light on new possibilities to resolve this problem.

The accumulation of skatole and androstenone in adipose tissue of entire male pigs may not be so extensive if certain feeding strategies are followed. Intestinal cell apoptosis can first be limited, thus decreasing the quantity of tryptophan which is used in skatole formation. Thereafter, the bacterial ecosystem can be influenced by providing energy for bacteria feeding on FOS. The inclusion of FOS in the diet can shift the pH of the digestive tract to 8.0, if the intestinal tract is colonized by *Bifidobacterium spp.* not participating in skatole formation. In addition, the bacterial metabolism can be shifted from proteolytic to saccharolytic, because the quantity and type of proteins and saccharides in the diet have an important influence on nitrogen metabolism and subsequent skatole formation. Substances which contain these properties are soluble fibre and inulin from chicory or Jerusalem artichoke. These are not digestible in the small intestine and therefore can provide the energy for intestinal bacteria, when the skatole production decreases significantly.

The last step to successful androstenone and skatole elimination is increasing the expression of the proteins which participate in their metabolism. Fibre, inulin, and raw potato starch, as well as the newly tested chicory secondary metabolites (e.g. esculin, scoparone, lactucin) have a positive effect on protein expression.

Further research on this topic and on the precise mechanism of action and dietary doses of these substances promising for boar taint elimination through nutrition are needed.

REFERENCES

Achour B, Barber J, Rostami-Hodjegan A (2011): Cytochrome P450 pig liver pie: determination of individual cytochrome P450 isoform contents in microsomes from two pig livers using liquid chromatography in conjunction with mass spectroscopy. Drug Metabolism and Disposition, 39, 2130–2134. doi: 10.1124/dmd.111.040618.

Andresen O (1976): Concentrations of fat and plasma 5-alpha-androstenone and plasma testosterone in boars selected for rate of body-weight gain and thickness of back fat during growth, sexual-maturation and after mating. Journal of Reproduction and Fertility, 48, 51–59.

Andresen O (2006): Boar taint related compounds: androstenone/skatole/other substances. Acta Veterinaria Scandinavica, 48 (Suppl. I), S5. doi: 10.1186/1751-0147-48-S1-S5.

Babol J, Squires EJ, Lundström K (1999): Relationship between metabolism of androstenone and skatole in intact male pigs. Journal of Animal Science, 77, 84–92. doi: 10.2527/1999.77184x.

Bais HP, Ravishankar GA (2001): *Cichorium intybus* L. – cultivation, processing, utility, value addition and biotechnology, with an emphasis on current status and future prospects. Journal of the Science of Food and Agriculture, 81, 467–484. doi: 10.1002/jsfa.817.abs.

Byrne DV, Thamsborg SM, Hansen LL (2008): A sensory description of boar taint and the effects of crude and dried chicory roots (*Cichorium intybus* L.) and inulin feeding in male and female pork. Meat Science, 79, 252–269. doi: 10.1016/j.meatsci.2007.09.009.

Chang TKH (2009): Activation of pregnane X receptor (PXR) and constitutive androstane receptor (CAR) by herbal medicines. The AAPS Journal, 11, 590–601. doi: 10.1208/s12248-009-9135-y.

Claus R, Raab S (1999): Influences on skatole formation from tryptophan in the pig colon: tryptophan, serotonin and melatonin. Basic Aspects and Applications, 467, 679–684. doi: 10.1007/978-1-4615-4709-9_87.

Claus R, Weiler U, Herzog A (1994): Physiological aspects of androstenone and skatole formation in the boar – a review with experimental data. Meat Science, 38, 289–305. doi: 10.1016/0309-1740(94)90118-X.

Cooke GM, Pothier F, Murphy BD (1997): The effects of progesterone, 4,16-androstadien-3-one and MK-434 on the kinetics of pig testis microsomal testosterone-4-ene-5alpha-reductase activity. Journal of Steroid Biochemistry and Molecular Biology, 60, 353–359. doi: 10.1016/S0960-0760(96)00223-3.

Davis SM, Squires EJ (1999): Association of cytochrome b5 with 16-androstene steroid synthesis in the testis and accumulation in the fat of male pigs. Journal of Animal Science, 77, 1230–1235. doi:10.2527/1999.7751230x.

Devine C, Dikeman M (2014): Encyclopedia of meat sciences. Academic Press, London.

Diaz GJ, Squires EJ (2000): Metabolism of 3-methylindole by porcine liver microsomes: responsible cytochrome P450 enzymes. Toxicological Sciences, 55, 284–292. doi: 10.1093/toxsci/55.2.284.

Doran E, Whittington FW, Wood JD, McGivan JD (2002): Cytochrome P450IIE1 (CYP2E1) is induced by skatole and this induction is blocked by androstenone in isolated pig hepatocytes. Chemico-Biological Interactions, 140, 81–92. doi: 10.1016/S0009-2797(02)00015-7.

Doran E, Whittington FA, Wood JD, McGivan JD (2004): Characterisation of androstenone metabolism in pig liver microsomes. Chemico-Biological Interactions, 147, 141–149. doi: 10.1016/j.cbi.2003.12.002.

Duss R, Nyberg L (2004): Oat soluble fibers (beta-glucans) as a source for healthy snack and breakfast foods. Cereal Foods World, 49, 320–325.

Ekstrand B, Rasmussen MK, Woll F, Zlabek V, Zamaratskaia G (2015): *In vitro* gender-dependent inhibition of porcine cytochrome P450 activity by selected flavonoids and phenolic acids. BioMed Research International, 2015, 1–7. doi: 10.1155/2015/387918.

Guengerich FP (2007): Mechanisms of cytochrome P450 substrate oxidation: MiniReview. Journal of Biochemical and Molecular Toxicology, 21, 163–168. doi: 10.1002/jbt.20174.

Guengerich FP (2008): Cytochrome P450 and chemical toxicology. Chemical Research in Toxicology, 21, 70–83. doi: 10.1021/tx700079z.

Hansen LL, Larsen AE, Jensen BB, HansenMoller J (1997): Short time effect of zinc bacitracin and heavy fouling with faeces plus urine on boar taint. Animal Science, 64, 351–363. doi: 10.1017/S1357729800015927.

Hansen LL, Mejer H, Thamsborg SM, Byrne DV, Roepstorff A, Karlsson AH, Hansen-Moller J, Jensen MT, Tuomola M (2006): Influence of chicory roots (*Cichorium intybus* L.) on boar taint in entire male and female pigs. Animal Science, 82, 359–368. doi: 10.1079/ASC200648.

Hawe SM, Walker N, Moss BW (1992): The effects of dietary fiber, lactose and antibiotic on the levels of skatole and indole in feces and subcutaneous fat in growing pigs. Animal Production, 54, 413–419. doi: 10.1017/S0003356100020870.

Jensen BB (2006): Prevention of boar taint in pig production. Factors affecting the level of skatole. Acta Veterinaria Scandinavica, 48, S6. doi: 10.1186/1751-0147-48-S1-S6.

Jensen MT, Jensen BB (1993): Determination of indole and 3-methylindole (skatole) in bacterial cultures, intestinal content and faeces. Journal of Chromatography B, 665, 275–280.

Jensen MT, Cox RP, Jensen BB (1995): 3-Methylindole (skatole) and indole introduction by mixed population of pig fecal bacteria. Applied and Environmental Microbiology, 61, 3180–3184.

Kjos NP, Overland M, Fauske AK, Sorum H (2010): Feeding chicory inulin to entire male pigs during the last period before slaughter reduces skatole in digesta and backfat. Livestock Science, 134, 143–145. doi: 10.1016/j.livsci.2010.06.120.

Lemley CO, Wilmoth TA, Tager LR, Krause KM, Wilson ME (2010): Effect of a high cornstarch diet on hepatic cytochrome P450 2C and 3A activity and progesterone half-life in dairy cows. Journal of Dairy Science, 93, 1012–1021. doi: 10.3168/jds.2009-2539.

Losel D, Lacorn M, Buttner D, Claus R (2006): Flavor improvement in pork from barrows and gilts via inhibition of intestinal skatole formation with resistant potato starch. Journal of Agricultural and Food Chemistry, 54, 5990–5995. doi: 10.1021/jf0608017.

Marchese S, Pes D, Scaloni A, Carbone V, Pelosi P (1998): Lipocalins of the boar salivary glands binding odours and pheromones. European Journal of Biochemistry, 252, 563–568. doi:10.1046/j.1432-1327.1998.2520563.x.

Matala J, Matuskova Z, Tunkova A, Anzenbacherova E, Azenbacher P (2009): Porcine CYP2A19, CYP2E1 and CYP1A2

forms are responsible for skatole biotransformation in the reconstituted system. Neuroendocrinology Letters, 30, 36–40.

Melrose DR, Reed HC, Patterson RL (1971): Androgen steroids associated with boar odour as an aid to the detection of oestrus in pig artificial insemination. The British Veterinary Journal, 127, 497–502.

Overland M, Kjos NP, Borg M, Skjerve E, Sorum H (2008): Organic acids in diets for entire male pigs: effect on skatole level, microbiota in digesta, and growth performance. Livestock Science, 115, 169–178. doi: 10.1016/j.livsci.2007.07.007.

Overland M, Kjos NK, Fauske AK, Teige J, Sorum H (2011): Easily fermentable carbohydrates reduce skatole formation in the distal intestine of entire male pigs. Livestock Science, 140, 206–217. doi: 10.1016/j.livsci.2011.03.032.

Partanen KH, Mroz Z (1999): Organic acids for performance enhancement in pig diets. Nutrition Research Reviews, 12, 117–145. doi: 10.1079/095442299108728884.

Patterson RLS (1968): 5α-Androst-16-en-3-one: compound responsible for taint in boar fat. Journal of the Science of Food and Agriculture, 19, 31–38. doi: 10.1002/jsfa.2740190107.

Pauly C, Spring P, O'Doherty JV, Kragten SA, Bee G (2008): Performances, meat quality and boar taint of castrates and entire male pigs fed a standard and a raw potato starch-enriched diet. Animal, 2, 1707–1715. doi: 10.1017/S1751731108002826.

Pauly C, Spring P, Gahan D, O'Doherty JV (2011): The effect of cereal type and enzyme supplementation on carcass characteristics, volatile fatty acids and intestinal microflora and boar taint in entire male pigs. Animal, 5, 378–386. doi: 10.1017/S1751731110001849.

Rasmussen MK, Zamaratskaia G (2014): Regulation of porcine hepatic cytochrome P450 – implication for boar taint. Computational and Structural Biotechnology Journal, 11, 106–112. doi: 10.1016/j.csbj.2014.09.003.

Rasmussen MK, Zamaratskaia G, Ekstrand B (2011a): *In vivo* effect of dried chicory root (*Cichorium intybus* L.) on xenobiotica metabolising cytochrome P450 enzymes in porcine liver. Toxicology Letters, 200, 88–91. doi: 10.1016/j.toxlet.2010.10.018.

Rasmussen MK, Zamaratskaia G, Ekstrand B (2011b): *In vitro* cytochrome P450 2E1 and 2A activities in the presence of testicular steroids. Reproduction in Domestic Animals, 46, 149–154. doi: 10.1111/j.1439-0531.2010.01613.x.

Rasmussen MK, Brunius C, Zamaratskaia G, Ekstrand B (2012a): Feeding dried chicory root to pigs decrease androstenone accumulation in fat by increasing hepatic 3 beta hydroxysteroid dehydrogenase expression. Journal of Steroid Biochemistry and Molecular Biology, 130, 90–95. doi: 10.1016/j.jsbmb.2012.01.003.

Rasmussen MK, Brunius C, Ekstrand B, Zamaratskaia G (2012b): Expression of hepatic 3 beta-hydroxysteroid dehydrogenase and sulfotransferase 2A1 in entire and castrated male pigs. Molecular Biology Reports, 39, 7927–7932. doi: 10.1007/s11033-012-1637-5.

Rasmussen MK, Klausen CL, Ekstrand B (2014): Regulation of cytochrome P450 mRNA expression in primary porcine hepatocytes by selected secondary plant metabolites from chicory (*Cichorium intybus* L.). Food Chemistry, 146, 255–263. doi: 10.1016/j.foodchem.2013.09.068.

Rius MA, Hortós M, García-Regueiro JA (2005): Influence of volatile compounds on the development of off-flavours in pig back fat samples classified with boar taint by a test panel. Meat Science, 71, 595–602. doi: 10.1016/j.meatsci.2005.03.014.

Roberfroid MB, Van Loo JAE, Gibson GR (1998): The bifidogenic nature of chicory inulin and its hydrolysis products. Journal of Nutrition, 128, 11–19.

Shiotsubo T (1984): Gelatinization temperature of potato starch at the equilibrium state. Agricultural and Biological Chemistry, 48, 1–7. doi: 10.1080/00021369.1984.10866101.

Sinclair PA, Squires EJ (2005): Testicular sulfoconjugation of the 16-androstene steroids by hydroxysteroid sulfotransferase: its effect on the concentrations of 5 alpha-androstenone in plasma and fat of the mature domestic boar. Journal of Animal Science, 83, 358–365. doi: 10.2527/2005.832358x.

Sinclair PA, Gilmore WJ, Lin Z, Lou Y, Squires EJ (2006): Molecular cloning and regulation of porcine SULT2A1: relationship between SULT2A1 expression and sulfoconjugation of androstenone. Journal of Molecular Endocrinology, 36, 301–311. doi: 10.1677/jme.1.01847.

Solé MAR, García Regueiro JA (2001): Role of 4-phenyl-3-buten-2-one in boar taint: identification of new compounds related to sensorial descriptors in pig fat. Journal of Agricultural and Food Chemistry, 49, 5303–5309. doi: 10.1021/jf010482d.

Squires EJ (2003): Applied animal endocrinology. CABI Publishing, Wallingford.

Urquhart BL, Tirona RG, Kim RB (2007): Nuclear receptors and the regulation of drug-metabolizing enzymes and drug transporters: implications for interindividual variability in response to drugs. Journal of Clinical Pharmacology, 47, 566–578. doi: 10.1177/0091270007299930.

Vhile SG, Kjos NP, Sorum H, Overland M (2012): Feeding Jerusalem artichoke reduced skatole level and changed intestinal microbiota in the gut of entire male pigs. Animal, 6, 807–814. doi: 10.1017/S1751731111002138.

Wahajuddin IT, Arora S, Raju KSR, Siddiqui N (2013): Disposition of pharmacologically active dietary isoflavones in biological systems. Current Drug Metabolism, 14, 369–380. doi: 10.2174/1389200211314040002.

Wesloy R, Weiler U (2012): Nutritional influences on skatole formation and skatole metabolism in the pig. Animals, 2, 221–242. doi: 10.3390/ani2020221.

Xu ZR, Hu CH, Wang MQ (2002): Effects of fructooligosaccharide on conversion of L-tryptophan to skatole and indole

by mixed populations of pig fecal bacteria. Journal of General and Applied Microbiology, 48, 83–89. doi: 10.2323/jgam.48.83.

Zammerini D, Wood JD, Whittington FM, Nute GR, Hughes SI, Hazzledine M, Matthews K (2012): Effect of dietary chicory on boar taint. Meat Science, 91, 396–401. doi: 10.1016/j.meatsci.2012.01.020.

Corresponding Author:

Ing. Daniela U r b a n o v á , Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Animal Husbandry, Kamýcká 129, 165 21 Prague 6-Suchdol, Czech Republic, phone: +420 22438 3051, e-mail: urbanovad@af.czu.cz

Meat Science 131 (2017) 68–73

Contents lists available at ScienceDirect

Meat Science

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

Association analysis of SNPs in the porcine *CYP2E1* gene with skatole, indole, and androstenone levels in backfat of a crossbred pig population

Kateřina Zadinová[a,*], Roman Stupka[a], Antonín Stratil[b], Jaroslav Čítek[a], Karel Vehovský[a], Nicole Lebedová[a], Michal Šprysl[a], Monika Okrouhlá[a]

[a] Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic
[b] Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Liběchov, Czech Republic

ARTICLE INFO

Keywords:
Boar taint
PCR–RFLP
HPLC
Polymorphism
Genotyping
Allele frequencies

ABSTRACT

The occurrence of boar taint in meat from uncastrated males may significantly affect the economics of pork production. The aim of this study was to analyse associations of four single nucleotide polymorphisms (SNPs) in the porcine *CYP2E1* gene with skatole, indole, and androstenone levels in the Czech Large White–Czech Landrace commercial crossbreds. The SNPs were: g.2412C > T, c.1422C > T, c.1423G > A and c.*14G > T. Skatole, indole and androstenone levels were estimated by HPLC, and genotypes at the SNPs were determined by PCR–RFLP. SNPs c.1423G > A and c.*14G > T were in complete linkage disequilibrium. In boars, all SNPs were associated with the indole levels ($P < 0.05$; $P < 0.01$). There also were differences in the skatole levels in different genotypes, but these were not significant. No associations with androstenone levels were found. The associations of the SNPs with indole compounds should be studied in other commercial populations of boars to verify the favourable alleles and genotypes, with the prospect for their application in marker-assisted selection.

1. Introduction

Meat from uncastrated boars is usually characterized by an unpleasant odour known as boar taint. Boar taint can occur in 5–40% of uncastrated boars (Große-Brinkhaus et al., 2015). Differences in tissue concentrations of boar taint compounds (androstenone and skatole) were detected among different breeds (Xue et al., 1996). Comparing different breeds, Durocs and the early maturing Pietrains tend to have the least desirable taint scores (Frieden, Looft, & Tholen, 2011). In contrast to androstenone, the skatole levels are more likely to be influenced by nongenetic factors. To prevent boar taint, male piglets for meat production are usually surgically castrated without anaesthesia shortly after birth in some countries, which is unacceptable because of animal welfare and detrimental health effects (Aleksić et al., 2012; Lundström & Zamaratskaia, 2006; Prunier et al., 2006). It has been recommended that surgical castration of pigs should be abandoned by 2018 in the EU (European Declaration on Alternatives to Surgical Castration of Pigs, 2010). An alternative is to fatten intact male pigs (Bonneau et al., 2000a; Bidanel et al., 2006), and in some European countries the legislation has already been adopted that bans castration (Denmark, 2011; Sweden, 2016; Germany, 2019). In some Northern and Western European countries analgesics have been used in > 50%. In Great Britain and Spain, < 20% are castrated, or entire males are fattened to lower slaughter weight. For rearing of intact boars it is desirable to reduce boar taint through pig breeding and/or management and feeding.

Boar taint is a urine- and faecal-like odour of cooked pork from uncastrated males (Lee et al., 2005; Strathe, Velander, Mark, & Kadarmideen, 2013). The main components responsible for boar taint are androstenone (5α-androst-16-en-3-one), skatole (3-methylindole) (Zamaratskaia & Squires, 2009) and, to a lesser extent, indole (Claus, Weiler, & Herzog, 1994; Zamaratskaia, Chen, & Lundström, 2006). Androstenone is a testicular steroid which accumulates in adipose tissue. Skatole and indole are produced through degradation of L-tryptophan by bacteria in the colon. They are partly resorbed by the intestinal mucosa and partly metabolized in the liver. Non-metabolized skatole is accumulated mainly in adipose tissue and, to a lesser extent, also in muscle tissue (Claus et al., 1994; Zadinová et al., 2016; Zamaratskaia & Squires, 2009). Odour of indole is less pronounced as that of skatole. Indole rather increases the unpleasant smell of meat, although both skatole and indole have a very similar odour. Thus, indole contributes to an abnormal taste of the boar meat (Chen, Cue, Lundström, Wood, & Doran, 2008). The meat of these pigs is not well accepted by consumers (Engelsma, Bergsma, Harlizius, Ducro-Steverink, & Knol, 2007). Threshold average acceptance values are 0.5–1.0 μg/g fat for androstenone and 0.2–0.25 μg/g fat for skatole

* Corresponding author at: Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 00 Prague, Czech Republic.
E-mail address: zadinova@af.czu.cz (K. Zadinová).

http://dx.doi.org/10.1016/j.meatsci.2017.04.236
Received 18 November 2016; Received in revised form 8 April 2017; Accepted 28 April 2017
Available online 29 April 2017
0309-1740/

(Rowe et al., 2014). The values for skatole may be even lower, at 0.15 μg/g fat (Mörlein, Lungershausen, Steinke, Sharifi, & Knorr, 2012). The mean values of androstenone and skatole levels obtained on entire male pigs from different countries varied, in some cases exceeding the threshold values (Walstra et al., 1999).

The initial step of skatole metabolism in the liver involves mainly the enzymes CYP2E1 (cytochrome P450 family 2 subfamily E member 1; Babol, Squires, & Lundström, 1998; Doran, Whittington, Wood, & McGivan, 2002; Robic, Larzul, & Bonneau, 2008) and CYP2A6 (Zamaratskaia et al., 2006). Similarly, indole is metabolized in the liver by CYP2E1 and CYP2A6 as well (Zamaratskaia et al., 2006). The gene *CYP2E1* is found on chromosome SSC14 in location 153,477,961–153,490,404 bp (Ensembl, release 85, Sscrofa10.2; Groenen et al., 2012; http://www.ensembl.org/Sus_scrofa). CYP2E1 activity in the liver has a significant effect on the concentration of skatole in fat. Pigs which have high levels of the CYP2E1 protein in the liver and high catalytic activity of the enzyme have low levels of skatole in adipose tissue and *vice versa* (Lin, Lou, & Squires, 2006). The skatole levels in the fat of boars increase during puberty and are correlated with the fat androstenone levels (Robic et al., 2008). Potential simultaneous effects of several genes/enzymes on both the androstenone and skatole levels were reported. Wiercinska, Lou, and Squires (2012) showed the effect of CYB5A (involved in androstenone synthesis) on some CYP450s, which increased the production of skatole metabolites. Doran, Whittington, Wood, and McGivan (2004) have observed that different expressions of HSD3B may affect the rate of androstenone metabolism which in turn may influence the CYP2E1 expression and consequently the rate of skatole metabolism. According to Tambyrajah, Doran, Wood, and McGivan (2004), the high level of androstenone in boars at puberty can inhibit the binding of COUP-TF1 and HNF-1α factors that are acting on the promoter of CYP2E1. Consequently, the level of CYP2E1 in liver decreases, which has an effect on skatole metabolism.

The activities of enzymes or other proteins can be affected by mutations in coding regions of the genes and expressions of mRNA and protein can be affected by mutations in gene regulatory regions. Efforts have been devoted to finding mutations in porcine *CYP2E1* and studying their associations with the components of boar taint. Lin et al. (2006) identified a G → A substitution in nucleotide c.1423 (NM_214421.1) which causes the substitution of amino acid alanine (Ala^{475}) with threonine (Thr^{475}). This mutation was shown to be responsible for a significant decrease in expression of the CYP2E1 protein and lower catalytic activity. Skinner, Doran, McGivan, Haley, and Archibald (2005) found this SNP as well and searched for an association with skatole levels in Danish commercial pigs (Landrace/Yorkshire/Duroc cross). They found no evidence of any association. Moe et al. (2009), when studying associations of SNPs in candidate genes for boar taint, found several SNPs in *CYP2E1* which were associated with skatole and indole levels in Duroc and Norwegian Landrace boars. Another SNP, AJ697882:g.2412C > T (at −586 ATG) in the promoter region (Skinner et al., 2005), appeared to be associated with variation in skatole levels in the Danish population. Mörlein et al. (2012) studied associations of the promoter SNP with skatole and indole levels in two commercial Duroc-sired crossbred populations.

In a genome-wide association study (GWAS), Rowe et al. (2014) observed highly significant effects for skatole levels on SSC14, within the *CYP2E1* gene (SNP g.2412C > T in the promoter region) in a commercial population of Danish Landrace boars. Using GWAS approach, Gregersen et al. (2012) observed a QTL on SSC14 affecting indole in Danish Landrace and Yorkshire boars. Also Große-Brinkhaus et al. (2015), in GWAS, identified the largest QTL for indole on SSC14 in a Pietrain × crossbred population (B2). In both studies, the authors proposed *CYP2E1*, which was located in the SSC14 QTL intervals, as a candidate gene for indolic compounds.

Based on these earlier studies, including those of GWAS, we chose the porcine *CYP2E1* gene to search for polymorphisms and associations of these polymorphisms with skatole, indole, and androstenone levels in backfat of a commercial crossbred population (boars and gilts).

2. Materials and methods

2.1. Animals

Association analysis of SNPs in the *CYP2E1* gene with skatole, indole, and androstenone levels was performed in a commercial crossbred population of Czech Large White × (Czech Large White × Czech Landrace), denoted $CLW_S \times (CLW_D \times CL_D)$. There were 73 pigs in total, consisting of 49 boars and 24 gilts. All pigs were maintained in a testing station, in an air-conditioned barn, essentially under the conditions as described by Dvořáková et al. (2012) and housed in pens according to sex. They were fed *ad libitum* with complete feed mixtures the composition of which were continually adjusted with respect to the age and weight of the pigs (Šimeček, Zeman, & Heger, 2000). The pigs were slaughtered at 154 days of age and average live weight of 115.8 ± SE 8.59 kg. All pigs were slaughtered according to the protocols for certified Czech slaughterhouses under the supervision of an independent veterinarian.

Unrelated purebred pigs of eight breeds (Czech Large White, 30; Czech Landrace, 20; Czech Meat Pig, 15; Pietrain, 15; Prestice Black Pied, 16; Hampshire, 9; Duroc, 20; Meishan, 17), and Wild Boar (18) animals were genotyped to estimate the extent of genetic polymorphism in the studied SNPs.

2.2. Blood and tissue samples

Blood samples for DNA isolation from the experimental crossbred pigs were collected into K_2EDTA at slaughter and were stored at − 20 °C. Backfat samples in the neck region between cervical vertebrae 1 and 3 were taken 24 h after slaughter, vacuum packed, and frozen at − 80 °C. Blood samples from purebred pigs and Wild Boar animals were collected as well.

2.3. Isolation of DNA, PCR, sequencing, and genotyping

Genomic DNA was isolated from blood using a Genomic DNA Mini Kit (Blood/Cultured Cell) (Geneaid Biotech, New Taipei City, Taiwan) or QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) or was available as ready-to-use DNA. The DNA samples were stored at − 20 °C until use.

Fragments of the porcine *CYP2E1* gene were amplified by polymerase chain reaction (PCR) and used for sequencing and analysis of polymorphisms. PCR primers were designed on the basis of the *CYP2E1* genomic sequence available in the Pig Sscrofa10.2 Ensembl database (http://www.ensembl.org/Sus_scrofa/; gene ENSSSCG00000010780) using the OLIGO 6 program. The primers used for amplifying the fragments and sequencing are presented in Table 1.

Polymerase chain reaction was performed in 25 μl reaction volumes using 100 ng of genomic DNA, standard reaction buffer, 1.0 or 1.5 mM $MgCl_2$ (see Table 1), 200 μM of each dNTP, 10 pmol of each primer, 2% DMSO, and 1 U LA polymerase (Top-Bio, Prague, Czech Republic). The PCR profile was 2 min at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at 55 °C, and *x* seconds (see Table 1) at 68 °C. There was a final extension of 5 min at 68 °C. The PCR fragments were visualized on 1.5% agarose gel.

To confirm the identity of the amplicons and search for polymorphisms, the PCR fragments *CYP2E1* L-M and A–B were comparatively sequenced (SEQme, Dobříš, Czech Republic) by the Sanger dideoxy method using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The fragment L–M was sequenced in 1 Danbred, 1 Czech Large White, 1 Pietrain, and 1 Wild Boar pig. The fragment A–B was sequenced in 6 pigs of the hybrid combination Danbred, 1 $CLW_S \times (CLW_D \times L_D)$ cross, 1 Czech Large White, 1

Table 1
Primers and PCR conditions for amplification of *CYP2E1* fragments.

Primer name	Primer sequences (5′–3′)	Location	Amplicon length (bp)	$MgCl_2$ (mM)	T_a (°C)	Extension (sec)	Note
CYP2E1-L	GTCCCAGAAACAACCTAACA	5′ upstream	1055	1.5	57	70	Sequencing
CYP2E1-M	TCCTACCCCACAGAAACAATAT	Intron 1					
CYP2E1-L	GTCCCAGAAACAACCTAACA	5′ upstream	369	1.0	55	30	RFLP (*Tsp*RI)
CYP2E1-N	AGCAACCCCAGTGGTACTGA	5′ upstream					
CYP2E1-A	GCTTAGGGTGATGGTTTACACA	Intron 8	524	1.5	55	45	Sequencing; RFLP (*Bsp*68I, *Hpy*188III, *Bsr*DI; *Taq*I)
CYP2E1-B	GGAACCCAACACAGACTCAA	3′ downstream					

Pietrain, and 1 Meishan. The sequencing identified 6 SNPs in the studied *CYP2E1* fragments, of which 4 were genotyped in the pure breeds and in the commercial crossbred population.

Pigs were genotyped for SNP g.2412C > T in amplicon L-N (this amplicon is within fragment L-M) and for SNPs c.1422C > T, c.1423G > A, and c.*14T > G in amplicon A–B (see Table 1) using restriction fragment length polymorphism analysis of PCR-amplified fragments (PCR–RFLP). SNP g.2412C > T was genotyped with *Tsp*RI (New England Biolabs, Ipswich, MA, USA). SNPs c.1422C > T and c.1423G > A were genotyped with *Bsp*68I (Thermo Scientific®, Waltham, MA, USA), *Hpy*188III and *Bsr*DI (New England Biolabs). *Taq*I (Thermo Scientific®) was used for genotyping of SNP c.*14T > G. DNA fragments were separated on a 1.5% agarose gel (digestions with *Bsp*68I, *Hpy*188III, and *Bsr*DI) and 3.5% agarose gel (digestions with *Taq*I and *Tsp*RI).

2.4. Determination of skatole, indole, and androstenone levels

Skatole, indole, and androstenone levels were measured in backfat samples using high-performance liquid chromatography (LC-2000Plus HPLC system, Jasco, Tokyo, Japan) according to the method of Hansen-Møller (1994) as modified by Okrouhlá et al. (2016).

To measure skatole and indole, a Kinetex C18 100A column (5 μm, 50 × 4.60 mm ID) operated at 40 °C was used. The mobile phase buffers were: A – sodium phosphate buffer (10 mM) and B – methanol. The gradient profile was: 0–0.2 min, 90% A; 0.2–6.0 min, 90–55% A; 6.0–7.0 min, 55–0% A. The sample injection volume was 30 μl, and flow rate was 1.2 ml/min. Fluorescence detection was performed with excitation at 285 nm and emission at 340 nm. The standard calibration curves for indole and skatole were prepared and used to determine the content of the compounds.

To measure androstenone, an Agilent Eclipse XDB C18 column (5 μm, 150 × 4.60 mm ID) operated at 40 °C was used. The parameters of the mobile phase were: A – tetrahydrofuran: acetonitrile: sodium phosphate buffer (25 mM): acetic acid (34: 23.8: 41.4: 0.8) and B – methanol. Fluorescence detection was performed with excitation at 346 nm and emission at 521 nm. The standard calibration curve was used to determine the content of androstenone in the samples.

The records were evaluated using the programme ChromNAV (Jasco) and quantitation was performed on the basis of the retention times of the standards of androstanone and 2-methylindole.

2.5. Statistical analyses

The original data of androstenone, skatole and indole levels were log-transformed prior to statistical analysis. Levels of skatole, androstenone and indole are normally distributed for both groups - boars and gilts. Association analysis was performed using the general linear models (GLM) procedure in SAS (Statistical Analysis System, Inst. Version 9.4, 2012, SAS Institute, Cary, NC, USA). The data were analysed with two-way analysis of variance (ANOVA), and calculations were performed for each sex separately. The results are presented as the least squares means (LSM) ± standard error (SE). Differences between LSM were determined by Duncan's test ($P < 0.05$ and $P < 0.01$).

The statistical model was:

$$Y_{ij} = \mu + g_i + e_{ij},$$

where Y_{ij} = value of the trait, μ = overall mean, g_i = SNP (i = 1–3), and e_{ij} = random residual.

The Pearson correlation coefficients between levels of skatole, indole, and androstenone were calculated for boars.

3. Results

3.1. SNPs and genotyping

Two SNPs were found in amplicon L–M (which encompasses amplicon L–N): AJ697882.1:g.2412C > T (rs334863220; upstream variant) and AJ697882.1:g.2839C > T (rs332373188; upstream variant). In amplicon A–B, four SNPs were detected: NC_010456.4 (region 153,477,961–153,490,404):g.12007C > A (rs344385023; intron 8), NM_214421.1:c.1422C > T (rs328310183; exon 9, silent variant), NM_214421.1:c.1423A > G (rs809692284; exon 9, missense variant), and NM_214421.1:c.*14T > G (rs342692398; 3′ UTR variant).

Pigs were genotyped for the SNPs g.2412C > T in amplicon L–N (Fig. 1) and for the SNPs c.1422C > T, c.1423G > A, and c.*14T > G in amplicon A–B by PCR–RFLP analysis. Inasmuch as the SNPs c.1422C > T and c.1423G > A in amplicon A–B are next to each other, it is not possible to determine their genotypes by single enzymes, and therefore the amplicons were separately digested using three restriction enzymes: *Bsp*68I, *Hpy*188III, and *Bsr*DI. The genotypes of the two SNPs were determined on the basis of the three digestions (Table 2, Fig. 2). Genotypes at SNP c.*14T > G are shown in Fig. 3. Based on the sequencing results and genotyping of 82 pigs of pure breeds and crosses, it was found that polymorphisms in SNPs c.1423G > A and c.*14T > G are in complete linkage disequilibrium: Alleles *c.1423G* and *c.*14T* are in haplotype, and allele *A* with *G*. SNPs g.2839C > T (upstream variant) and g.12007C > A (intron 8 variant) were not genotyped, because they seemed to be less frequent in the

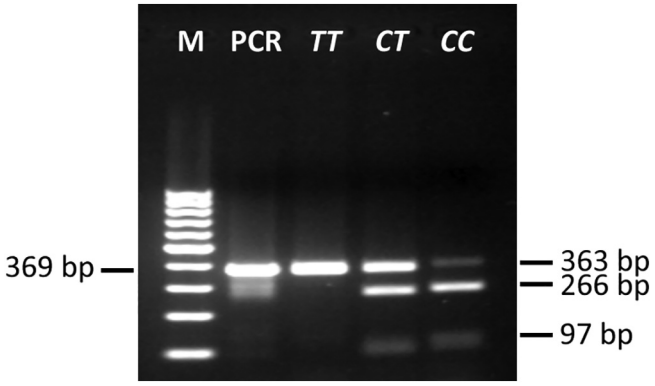


Fig. 1. Agarose gel electrophoresis (3.5%) showing genotypes (*TT*, *CT*, *CC*) at SNP g.2412C > T after digestion of amplicon L-N with restriction enzyme *Tsp*RI (allele *T* – 363 + 6 bp; allele *C* – 266 + 97 + 6 bp). M – marker 1000–100 bp; PCR – undigested PCR fragment.

Table 2
Determination of genotypes in SNPs c.1422C > T and c.1423A > G after digestion of amplicons A–B with restriction enzymes *Bsp*68I, *Hpy*188III, and *Bsr*DI.

SNPs/genotypes		Digestion with restriction enzymes[a]		
c.1422C > T	c.1423G > A	*Bsp*68I (*Nru*I) (tCG/cga)[b]	*Hpy*188III (tC/Nnga)[b]	*Bsr*DI (*Bse*MI) (nn/catTGc)[b]
CC	*GG*	B	B	A
TT	*GG*	A	A	B
CT	*GG*	AB	AB	AB
CC	*AA*	A	B	A
TT	*AA*	A	A	A
CT	*AA*	A	AB	A
CC	*AG*	AB	B	A
TT[c]	*AG*[c]	A	A	AB
CT	*AG*	A	AB	AB
TC	*AG*	AB	AB	A

[a] *Bsp*68I: A – 524 bp, B – 301 + 223 bp; *Hpy*188III: A – 465 + 59 bp, B – 302 + 163 + 59 bp; *Bsr*DI: A – 524 bp, B – 306 + 218 bp.
[b] The capital letters are nucleotides in positions c.1422 and c.1423.
[c] The genotype combination *TT/AG* was not found in the analysed samples.

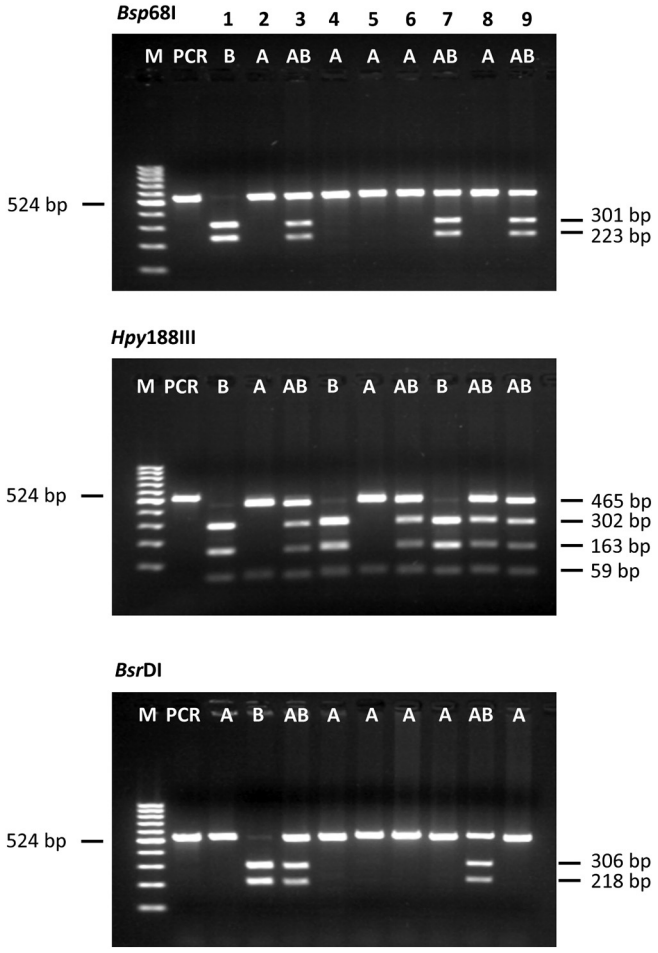


Fig. 2. Determination of genotypes at SNPs c.1422C > T and c.1423G > A following separate digestions of the A-B amplicons with restriction enzymes *Bsp*68I, *Hpy*188III, and *Bsr*DI. The amplicons (1–9) were the same in all three digestions. The designations (A, AB, B) define the results of the digestions, as shown in Table 2. Based on the combinations of all three digestions, the genotypes at SNPs c.1422C > T and c.1423G > A could be determined. The samples were electrophoresed in 1.5% agarose gel. M – marker 1000–100 bp; PCR – undigested PCR fragment.

studied pigs (as in our sequenced samples they appeared only once, and in a heterozygous genotype) and, based on their locations in the gene, no direct effect on the boar taint compounds was expected.

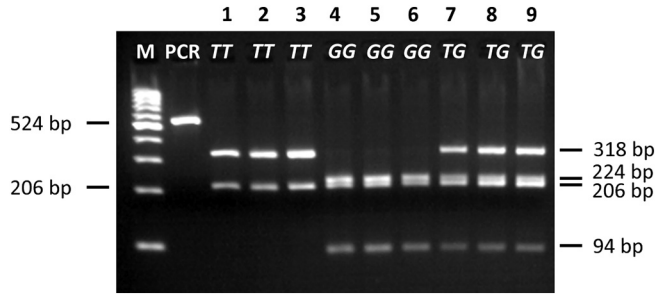


Fig. 3. Agarose gel electrophoresis (3.5%) showing genotypes (*TT*, *TG*, *GG*) at SNP c.*14G > T after digestion of amplicon A-B with restriction enzyme *Taq*I (allele *T* – 318 + 206; allele *G* – 224 + 206 + 94 bp). Samples 1–9 are identical with those in Fig. 2. M – marker 1000–100 bp; PCR – undigested PCR fragment.

Table 3
Allele frequencies at *CYP2E1* SNPs g.2412 C > T, c.1422C > T, and c.1423A > G in eight porcine breeds and Wild Boar pigs.

Breed	g.2412 C > T (L–N; upstream; *Tsp*RI)			c.1422C > T (A–B; exon 9; *Bsp*68I, *Hpy*188III, *Bsr*DI)			c.1423A > G[a] (A–B; exon 9; *Bsp*68I, *Hpy*188III, *Bsr*DI)	
	n	*C*	*T*	*n*	*C*	*T*	*A*	*G*
Czech Large White	30	0.42	0.58	20	0.60	0.40	0.53	0.47
Czech Landrace	20	0.27	0.73	15	0.90	0.10	0.40	0.60
Czech Meat Pig	12	0.50	0.50	15	0.97	0.03	0.33	0.67
Pietrain	15	0.11	0.89	15	0.93	0.07	0.93	0.07
Prestice Black Pied	16	0.41	0.59	16	0.75	0.25	0.50	0.50
Hampshire	9	0.50	0.50	9	0.83	0.17	0.22	0.78
Duroc	20	0.30	0.70	20	0.95	0.05	0.60	0.40
Meishan	17	0.65	0.35	16	0.97	0.03	0.09	0.91
Wild Boar	16	1.0	0	18	0.56	0.44	0.53	0.47

[a] The polymorphism in c.1423A > G was in complete linkage disequilibrium with SNP c.*14G > T. Allele *c.1423A* occurs with *c.*14G*, etc.

Allele frequencies at the three SNPs in various pig breeds are presented in Table 3. The fourth SNP (c.*14T > G) was in complete linkage disequilibrium with c.1423G > A.

3.2. Association analysis of the three SNPs with skatole, indole, and androstenone levels

The results of the association analysis of *CYP2E1* genotypes at SNPs g.2412C > T, c.1422C > T, and c.1423G > A are presented separately for boars and gilts in Table 4. Significant differences in indole levels are evident in the different genotypes of all three SNPs in boars. For g.2412C > T and c.1423G > A, the *P* value was < 0.05; for c.1422C > T, the *P* value was < 0.01. The most favourable genotypes (for low indole levels) were *g.2412 TT*, *c.1422 CC,* and *c.1423 AA*.

The indole levels were much lower in gilts, and the differences were not statistically significant. The indole levels (LSM) in boars were in the range of 0.027–0.097 μg/g fat, while in gilts the range was 0.009–0.020 μg/g fat.

For skatole, none of the tested SNPs in boars exhibited statistically significant differences (Table 4). The same genotypes as in the case of indole also had the lowest levels for skatole, however, indicating that there was a correlation between indole and skatole levels (correlation coefficient was 0.38). Skatole levels (LSM) were in the range of 0.037–0.094 μg/g fat for boars and 0.004–0.031 μg/g fat for gilts. For

Table 4
Association analysis of genotypes at SNPs g.2412C > T, c.1422C > T, and c.1423G > A with skatole and indole levels in backfat of boars and gilts of the $CLW_S \times (CLW_D \times CL_D)$ population.

SNP/genotype	Skatole (μg/g backfat)				Indole (μg/g backfat)			
	Boars		Gilts		Boars		Gilts	
	n	LSM ± SE[1]	*n*	LSM ± SE	*n*	LSM ± SE	*n*	LSM ± SE
g.2412C > T	49		24		49		24	
CC	15	0.094 ± 0.02	5	0.015 ± 0.01	15	0.081 ± 0.01[a,2]	5	0.009 ± 0.01
CT	26	0.067 ± 0.02	16	0.017 ± 0.01	26	0.045 ± 0.01[b]	16	0.017 ± 0.01
TT	8	0.047 ± 0.03	3	0.004 ± 0.01	8	0.029 ± 0.02[b]	3	0.012 ± 0.01
P value (GLM)		NS		NS		0.029		NS
c.1422C > T	49		24		49		24	
CC	10	0.037 ± 0.03	6	0.006 ± 0.01[a]	10	0.027 ± 0.02[A]	6	0.010 ± 0.01
CT	33	0.080 ± 0.02	11	0.009 ± 0.01[a]	33	0.053 ± 0.01[A]	11	0.013 ± 0.01
TT	6	0.075 ± 0.04	7	0.031 ± 0.01[b]	6	0.097 ± 0.02[B]	7	0.020 ± 0.01
P value (GLM)		NS		0.020		0.026		NS
c.1423G > A	49		24		49		24	
AA	11	0.055 ± 0.03	3	0.004 ± 0.01	11	0.033 ± 0.02[a]	3	0.012 ± 0.01
AG	23	0.074 ± 0.02	10	0.009 ± 0.01	23	0.046 ± 0.01[a]	10	0.015 ± 0.01
GG	15	0.075 ± 0.02	11	0.023 ± 0.01	15	0.079 ± 0.02[b]	11	0.015 ± 0.01
P value (GLM)		NS		NS		0.049		NS

CLW_S – Czech Large White sire line, CLW_D – Czech Large White dam line, CL_D – Czech Landrace dam line.
[1] LSM ± SE – least squares means (μg/g backfat) ± standard error.
[2] Values with different superscripts within columns are significantly different at: [a,b] $P < 0.05$, [A,B] $P < 0.01$.

c.1422C > T in gilts, the differences between genotypes were statistically significant ($P < 0.05$).

No statistically significant differences were observed in the levels of androstenone in different genotypes at all three SNPs (Supplementary Table 1). The levels (LSM) were in the range of 2.068–3.924 μg/g fat for boars and 0.047–0.112 μg/g fat for gilts. The correlation coefficients were 0.54 for androstenone–skatole and 0.25 for androstenone–indole.

4. Discussion

Porcine CYP2E1 is a key enzyme involved in phase I of skatole and indole metabolism (reviewed by Zadinová et al., 2016). We used PCR–RFLP analysis to genotype four polymorphisms in *CYP2E1* in different breeds and in a crossbred population. Because two of these SNPs are next to each other (c.1422C > T and c.1423G > A), it was not possible to determine the genotypes using single restriction enzymes. It is important to have a reliable method for genotyping of the two neighbouring SNPs. We therefore determined the genotypes following digestion of the amplicons A–B with three enzymes separately. Genotyping of the four SNPs in eight commercial breeds and Wild Boar revealed that all breeds are polymorphic and with different allele frequencies. It is therefore anticipated that these polymorphisms will also be present in crossbred populations and can be studied for their effects on the boar taint compounds.

In the association analysis, three SNPs (g.2412C > T, c.1422C > T, and c.1423G > A) were included. The fourth, c.*14T > G, was in complete linkage disequilibrium with c.1423G > A, and so there was no need to perform the analysis. All three SNPs were significantly associated with indole levels in boars of the $CLW_S \times (CLW_D \times CL_D)$ crossbred population. Favourable alleles were *T*, *C*, and *A*, respectively. The same alleles were associated with the lowest values for skatole, but those differences were not statistically significant.

In SNP g.2412C > T within the promoter region (Skinner et al., 2005), allele *C* appeared to be associated with high skatole levels in Danish pigs. Similarly, Mörlein et al. (2012) had recorded the highest skatole and indole values in homozygotes *CC* in boars of two commercial Duroc-sired crossbred populations. In our experiment, too, allele *C* was associated with a high level of indole ($P < 0.05$) as well as skatole (not significant). A reason for the non-significant results could be either a lower number of animals in the experiment, or a different crossbred population. In the earlier studies Duroc pigs were used to produce crossbreds, and this was not the case in our experiment. A highly significant association of this SNP with skatole levels was also observed by Rowe et al. (2014) in a genome-wide study within a population of 1000 commercial Danish Landrace boars.

The activity of CYP2E1 in the liver significantly influences skatole concentrations in fat. Pigs with high expression of the CYP2E1 protein in the liver have low levels of skatole in fat (Squires & Lundström, 1997). Lin et al. (2006) had found a missense mutation in c.1423 of the *CYP2E1* gene which affected the expression of the protein. Those authors had concluded that the G → A substitution, which significantly decreased CYP2E1 protein expression, would result in an increased skatole level in fat. This means that allele *A* is inferior. However, Moe et al. (2009) noted allele *A* to be favourable for SNP c.1423G > A in Duroc and Norwegian Landrace boars as regards levels of both skatole and indole. Yet another study (Skinner et al., 2005) found no association of any allele or genotype in c.1423G > A (AJ697885_1452) with high skatole in Danish commercial pigs. While the results of these three studies are discordant, our results are in agreement with those of Moe et al. (2009), which is to say that allele *A* was favourable. These differences can be due to the use of different pig populations in the studies, or by the presence of the other synonymous mutation in exon 9 (c.1422C > T), which was not taken into account in the analyses of Lin et al. (2006) and Skinner et al. (2005). This SNP, which is next to c.1423G > A, complicates its genotyping and it can lead to erroneous results.

Association of SNP c.1422C > T with the indole compounds was studied only by Moe et al. (2009). They observed significant association with indole only in Norwegian Landrace, and in the Duroc population this SNP was not recorded. Allele *C* was favourable. They found no association with skatole. In our study, genotype *CC* had highly significantly lower values for indole as compared to genotype *TT* ($P < 0.01$). Genotype *CC* was lower also for skatole, but those differences were not significant.

No associations of *CYP2E1* polymorphisms with androstenone levels were observed, and that is in agreement with Moe et al. (2009), Mörlein et al. (2012), and Rowe et al. (2014).

5. Conclusion

In conclusion, the results of this study show that SNPs g.2412C > T, c.1422C > T, c.1423G > A, and c.*14G > T are polymorphic in all

eight breeds studied. SNPs c.1423G > A and c.*14G > T were in complete linkage disequilibrium, and for others linkage disequilibrium was not observed. SNPs g.2412C > T, c.1422C > T, and c.1423G > A were associated with the levels of indole compounds in adipose tissue of boars of a Czech Large White × Czech Landrace commercial crossbred population. Favourable alleles were *T*, *C*, and *A*, respectively. None of these alleles can be considered causative at this stage of research. No association with androstenone levels was recorded. Associations between SNPs and indole compounds should be studied in other commercial populations of boars to verify the favourable alleles and genotypes.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.meatsci.2017.04.236.

Acknowledgements

We thank Prof. Alan L. Archibald (Roslin Institute, Midlothian, Scotland, UK) and Prof. Hermann Geldermann (University of Hohenheim, Stuttgart, Germany) for providing DNA samples, respectively, from Meishan and Pietrain. This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. MSM 6046070901), by the Internal Grant Agency of the Czech University of Life Sciences Prague (CIGA) (Project No. 20172005) and by the Institute of Animal Physiology and Genetics of the Academy of Sciences of the Czech Republic (RVO:67985904).

References

Aleksić, J., Dokmanović, M., Aleksić, Z., Teodorović, V., Stojić, V., Trbović, D., & Baltić, M.Ž. (2012). Investigation of the efficacy of immunocastration aimed at the prevention of sex odour in boar meat. *Acta Veterinaria (Beograd)*, *62*, 653–663.

Babol, J., Squires, J., & Lundström, K. (1998). Hepatic metabolism of skatole in pigs by cytochrome P4502E1. *Journal of Animal Science*, *76*, 822–828.

Bidanel, J. P., Riquet, J., Gruand, J., Squires, J., Bonneau, M., & Milan, D. (2006). Detection of quantitative trait loci for skatole and indole levels in Meishan × Large White F2 pigs. *Proceedings of the 8th world congress on genetics applied to livestock production. August 13–18, 2006, Belo Horizonte, MG, Brasil. Commun. No. 06-17*.

Bonneau, M., Kempster, A. J., Claus, R., Claudi-Magnussen, C., Diestre, A., Tornberg, E., ... Cook, G. L. (2000). An international study on the importance of androstenone and skatole for boar taint: I. Presentation of the programme and measurement of boar taint compounds with different analytical procedures. *Meat Science*, *54*, 251–259.

Chen, G., Cue, R. A., Lundström, K., Wood, J. D., & Doran, O. (2008). Regulation of CYP2A6 protein expression by skatole, indole, and testicular steroids in primary cultured pig hepatocytes. *Drug Metabolism and Disposition*, *36*, 56–60.

Claus, R., Weiler, U., & Herzog, A. (1994). Physiological aspects of androstenone and skatole formation in the boar — A review with experimental data. *Meat Science*, *38*, 289–305.

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

Doran, E., Whittington, F. M., Wood, J. D., & McGivan, J. D. (2004). Characterisation of androstenone metabolism in pig liver microsomes. *Chemico-Biological Interactions*, *147*, 141–149.

Dvořáková, V., Bartenschlager, H., Stratil, A., Horák, P., Stupka, R., Čítek, J., ... Geldermann, H. (2012). Association between polymorphism in the *FTO* gene and growth and carcass traits in pig crosses. *Genetics Selection Evolution*, *44*, 13.

Engelsma, K. A., Bergsma, R., Harlizius, B., Ducro-Steverink, D., & Knol, E. (2007). *Genetic parameters for components of boar taint and their relation with carcass quality and female fertility*. Dublin, Ireland: EAAP August 26–29.

European Declaration on Alternatives to Surgical Castration of Pigs (2010). http://ec.europa.eu/food/animals/welfare/practice/farm/pigs/castration_alternatives/index_en.htm.

Frieden, L., Looft, C., & Tholen, E. (2011). Breeding for reduced boar taint. *Lohman Information*, *46*, 21–27.

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

Groenen, M. A. M., Archibald, A. L., Uenishi, H., Tuggle, C. K., Takeuchi, Y., Rothschild, M. F., et al. (2012). Analyses of pig genomes provide insight into porcine demography and evolution. *Nature*, *491*, 393–398.

Große-Brinkhaus, C., Storck, L. C., Frieden, L., Neuhoff, C., Schellander, K., Looft, C., & Tholen, E. (2015). Genome-wide association analyses for boar taint components and testicular traits revealed regions having pleiotropic effects. *BMC Genetics*, *16*, 36.

Hansen-Møller, J. (1994). Rapid high-performance liquid-chromathography method for simultaneous determination of androstenone, skatole and indole in back fat from pigs. *Journal of Chromatography. B, Biomedical Applications*, *661*, 219–230.

Lee, G. J., Archibald, A. L., Law, A. S., Lloyd, S., Wood, J., & Haley, C. S. (2005). Detection of quantitative trait loci for androstenone, skatole and boar taint in a cross between Large White and Meishan pigs. *Animal Genetics*, *36*, 14–22.

Lin, Z. H., Lou, Y. P., & Squires, E. J. (2006). Functional polymorphism in porcine CYP2E1 gene: Its association with skatole levels. *The Journal of Steroid Biochemistry and Molecular Biology*, *99*, 231–237.

Lundström, K., & Zamaratskaia, G. (2006). Moving towards taint-free pork — Alternatives to surgical castration. *Acta Veterinaria Scandinavica*, *48*(Suppl. 1), S1.

Moe, M., Lien, S., Aasmundstad, T., Meuwissen, T. H. E., Hansen, M. H. S., Bendixen, C., & Grindflek, E. (2009). Association between SNPs within candidate genes and compounds related to boar taint and reproduction. *BMC Genetics*, *10*, 32.

Mörlein, D., Lungershausen, M., Steinke, K., Sharifi, A. R., & Knorr, C. (2012). A single nucleotide polymorphism in the CYP2E1 gene promoter affects skatole content in backfat of boars of two commercial Duroc-sired crossbred populations. *Meat Science*, *92*, 739–744.

Okrouhlá, M., Stupka, R., Čítek, J., Urbanová, D., Vehovský, K., & Kouřimská, L. (2016). Method for determination of androstenone, skatole and indole in dorsal fat of pigs (In Czech). *Chemicke Listy*, *110*, 593–597.

Prunier, A., Bonneau, M., von Borell, E. H., Cinotti, S., Gunn, M., Fredriksen, B., ... Velarde, A. (2006). A review of the welfare consequences of surgical castration in piglets and the evaluation of non-surgical methods. *Animal Welfare*, *15*, 277–289.

Robic, A., Larzul, C., & Bonneau, M. (2008). Genetic and metabolic aspects of androstenone and skatole deposition in pig adipose tissue: A review. *Genetics Selection Evolution*, *40*, 129–143.

Rowe, S. J., Karacaören, B., De Koning, D. J., Lukić, B., Hastings-Clark, N., Velander, I., ... Archibald, A. L. (2014). Analysis of the genetics of boar taint reveals both single SNPs and regional effects. *BMC Genomics*, *15*, 424.

Šimeček, K., Zeman, L., & Heger, J. (2000). *Nutrient requirements and tables of dietary requirements of feeds for pigs* (In Czech). . Brno: MZLU, 124.

Skinner, T. M., Doran, E., McGivan, J. D., Haley, C. S., & Archibald, A. L. (2005). Cloning and mapping of the porcine cytochrome-p450 2E1 gene and its association with skatole levels in the domestic pig. *Animal Genetics*, *36*, 417–422.

Squires, E. J., & Lundström, K. (1997). Relationship between cytochrome P450IIE1 in liver and levels of skatole and its metabolites in intact male pigs. *Journal of Animal Science*, *75*, 2506–2511.

Strathe, A. B., Velander, I. H., Mark, T., & Kadarmideen, H. N. (2013). Genetic parameters for androstenone and skatole as indicators of boar taint and their relationship to production and litter size traits in Danish Landrace. *Journal of Animal Science*, *91*, 2587–2595.

Tambyrajah, W. S., Doran, E., Wood, J. D., & McGivan, J. D. (2004). The pig CYP2E1 promoter is activated by COUP-TF1 and HNF-1 and is inhibited by androstenone. *Archives of Biochemistry and Biophysics*, *431*, 252–260.

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

Wiercinska, P., Lou, Y., & Squires, E. J. (2012). The roles of different porcine cytochrome P450 enzymes and cytochrome b5A in skatole metabolism. *Animal*, *6*, 834–845.

Xue, J. L., Dial, G. D., Holton, E. E., Vickers, Z., Squires, E. J., Lou, Y., ... Morel, N. (1996). Breed differences in boar taint: Relationship between tissue levels of boar taint compounds and sensory analysis of taint. *Journal of Animal Science*, *74*, 2170–2177.

Zadinová, K., Stupka, R., Stratil, A., Čítek, J., Vehovský, K., & Urbanová, D. (2016). Boar taint — The effect of selected candidate genes associated with androstenone and skatole levels — A review. *Animal Science Papers and Reports*, *34*, 107–128.

Zamaratskaia, G., Chen, G., & Lundström, K. (2006). Effects of sex, weight, diet and hCG administration on levels of skatole and indole in the liver and hepatic activities of cytochromes P4502E1 and P4502A6 in pigs. *Meat Science*, *72*, 331–338.

Zamaratskaia, G., & Squires, E. J. (2009). Biochemical, nutritional and genetic effects on boar taint in entire male pigs. *Animal*, *3*, 1508–1521.

doi: 10.17221/99/2016-CJAS

Effects of Immunocastration on Growth Performance, Body Composition, Meat Quality, and Boar Taint

Roman Stupka, Jaroslav Čítek, Karel Vehovský, Kateřina Zadinová, Monika Okrouhlá, Daniela Urbanová, Luděk Stádník*

Department of Animal Husbandry, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic

**Corresponding author: stupka@af.czu.cz*

ABSTRACT

Stupka R., Čítek J., Vehovský K., Zadinová K., Okrouhlá M., Urbanová D., Stádník L. (2017): **Effects of immunocastration on growth performance, body composition, meat quality, and boar taint**. Czech J. Anim. Sci., 62, 249–258.

The study objective was to evaluate the effect of immunocastration in the period between the first and second vaccinations and subsequently between the second vaccination and slaughter on growth performance, carcass composition, meat quality, and boar taint, and compare results in immunocastrated males (IC), uncastrated boars (UCM), surgically castrated barrows (CM), and gilts (FE). The study included 70 pigs of the Duroc × (Large White × Landrace) crossbreed. Upon the overall assessment of the selected fattening indicators (average daily gain, feed intake), significant differences between CM and the other groups were demonstrated. Meanwhile, no significant differences were found between the IC, UCM, and FE groups. In this test, immunocastrates showed no negative effect from the second vaccination in relation to those carcass value indicators evaluated in comparison with UCM and FE. CM showed adversely lower carcass value parameters compared the other groups. No significant differences in pH, meat colour, drip loss, shear force, and intramuscular fat were found. The values of these indicators obtained for IC converged with those measured in UCM and FE. It was demonstrated that immunocastration prevented the occurrence of undesired boar taint. Androstenone decreased by 77% and skatole by 71% in IC as compared to UCM.

Keywords: pig; castration; carcass value; androstenone; skatole

The requirement that good living conditions be ensured for farmed animals is gaining in importance across Europe. There is a discussion among the EU member states about the possibility to introduce a ban on surgical castration of pigs. Regulation No. 2008/120/EC determining the minimum standards for pig farming has been adopted on the basis of which the EU countries voluntarily undertake to stop the practice of boar surgical castration in 2018.

According to a number of experts, surgical castration, even if performed during the first week of a boar's life, is not only a stressful experience (Marsalek et al. 2015), but also constitutes a possible risk for infection. Surgical castration of males (boars) is routine and widely practiced on pig farms.

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. MSM 6046070901), by the Ministry of Agriculture of the Czech Republic (Project No. QJ1510191), by the EU Framework Programme for Research and Innovation Horizon 2020 (COST action IPEMA, CA15215), and by the Internal Grant Agency of the Czech University of Life Sciences Prague (CIGA) (Project No. 20172005).

doi: 10.17221/99/2016-CJAS

Conventional castration, which is performed several days after birth and has been used for many years, is intended to prevent the development of boar gonads, minimize an aggressive behaviour, and subsequently eliminate the occurrence of boar taint which damages the sensory quality of pork meat (Bonneau and Squires 2004). Conventional castration can currently be replaced by castration under anaesthesia, fattening boars without castration, or immunological castration.

Immunological castration consists in vaccinating the animals using gonadotropin-releasing hormone (GnRH) in a modified form conjugated to protein which induces the creation of anti-GnRH (Thun et al. 2006; Zamaratskaia et al. 2008). The dosage used is 2 ml of the vaccine in the muscle behind the ear twice within a period of 4 weeks. The first dose is administered 8 weeks and the second 4 weeks before the anticipated slaughter. This method prevents the occurrence of boar taint while preserving the positive effects of testicular steroids and anabolic hormones occurring in males. Boars have greater genetic potential for protein and feed utilization storage and better feed utilization than barrows (Pauly et al. 2009). Immunological castration allows the requirements for meat production to be fulfilled while avoiding the occurrence of boar taint (Cronin et al. 2003), although the meat characteristics performance can differ between boars and immunocastrates. Differences in growth performance, carcass value, and meat quality have been confirmed (Lundstrom et al. 2009). The results obtained differ very much in relation to the breed or hybrid combination (D'Souza and Mullan 2002), feeding strategy (*ad libitum* or restricted), time of the second vaccination (4 and more weeks before slaughter), or housing type (group or individual) (Skrlep et al. 2010).

Although studies have recently been published addressing the effect of immunocastration in pigs on the development and elimination of boar taint (Millet et al. 2011; Batorek et al. 2012), the qualitative and quantitative parameters of the carcass value are also important. It has been documented that the carcass value of immunologically castrated boars can be significantly affected by the time interval between the 1st vaccination (V1) and the 2nd vaccination (V2), and especially by the time interval between V2 and slaughter. Some studies have found that until administering V2 the results achieved are similar to those for fattened boars (Pauly et al. 2009) but that the meat performance subsequently worsens. The differences between the results achieved in boars and immunocastrates can diminish, however, due to physical and sexual activity and thus poorer food consumption by fattened boars (Cronin et al. 2003).

Despite the described favourable effect of immunocastration on the level of boar taint, some authors describe an adverse post vaccination effect on growth performance and carcass value. The aim of this study, therefore, was to evaluate the effect of immunocastration in the period between V1 and V2, and subsequently between V2 and slaughter on the growth performance, carcass composition, meat quality, and occurrence of boar taint.

MATERIAL AND METHODS

Animals and management. The study included 70 pigs of the Duroc × (Large White × Landrace) crossbreed. The experiment was approved by the Ethics Committee of the Central Commission for Animal Welfare of the Ministry of Agriculture of the Czech Republic. The pigs were 66 days old at the start of the experiment and their mean weight was 28.7 kg. The test continued for 74 days up to the age of 140 days. The pigs were identified by means of electronic ear chips. The following four groups were created: group 1: boars (UCM, uncastrated males, n = 18); group 2: immunocastrates (IC, n = 16); group 3: barrows (CM, castrated males, n = 18); and group 4: gilts (FE, females, n = 18). The male pigs in group 3 were surgically castrated on the 5th day after birth. The boars in group 2 were treated with Improvac® containing 200 µg of GnRH-protein conjugate/ml in water adjuvant solution. Using a syringe, a dosage of 2 ml was administered subcutaneously at the base of the ear in accordance with the technical manual in two dates: when the pigs were 94 and 115 days old. All groups were housed in a single testing barn. Two pigs of the same sex were housed in each pen. The micro-climate, temperature, gas concentration, and humidity were controlled automatically and monitored at hourly intervals so as to correspond to the animals' needs. The animals were fed according to standard nutrient requirements (Simecek et al. 2000) using completed feed mixes identified as P1, P2, and P3 (Table 1). All pigs were fed by the same commercial diets. Feeding was *ad libitum* by means of self-feeders for the two pigs in each

doi: 10.17221/99/2016-CJAS

pen. The animals also had *ad libitum* access to water during the test fattening period.

Growth performance. In order to obtain the data describing the growth characteristics of the tested animals, the following measurements were regularly (at the same hour in weekly intervals) monitored in each animal: live weight in kg (LW), daily feed intake in kg (FI), feed conversion ratio in kg per each kg gained (FCR), and average daily gain in g (ADG).

Carcass value. Since V2 on day 115 of age till slaughter, the *musculus longissimus lumborum et thoracis* (MLLT) depth and area as well as backfat depths were measured using an ALOKA SSD 500 – MICRUS ultrasound probe (Hitachi Aloka Medical Ltd., Japan). The measurements were taken along the central back line as described by Sprysl et al. (2010). The fat depth (FAT1) in the lumbar back region, the fat depth (FAT2) in the chest region, and the loin depth (MUSCLE2) were measured (all in mm). On the basis of repeated measurements in the same regions before slaughter, the development of the monitored dimensions of FAT1, FAT2, and MUSCLE2 (Difference: Final – V2) were calculated. After termination of the test at the age of 140 days and the average LW of 105.8 kg, all pigs were slaughtered at the same day in order to assess the quantitative and qualitative carcass value. The pigs were fasted 24 h before slaughtering. All carcasses were then subjected to analysis according to Walstra and Merkus (1995). Immediately after slaughtering, the following indicators were measured in the individual pigs: carcass weight CW (kg), carcass lean meat ZP (i.e. using a two-point method ZP) (%), muscle depth ZP (mm), fat thickness ZP (mm), and loin eye area of MLLT (mm^2) as described by Citek et al. (2015). To assess the quantitative carcass value, regular slaughter analysis was performed 24 h *post mortem* on all 70 pigs from the study groups (IC, UCM, CM, FE). The following indicators were determined in the carcasses: ham subcutaneous fat (kg), ham intramuscular fat IMF (%), ham meat + bone (kg and %), shoulder subcutaneous fat (kg), shoulder IMF (%), shoulder meat + bone (kg and %), loin subcutaneous fat (kg), loin IMF (%), loin meat + bone (kg and %), neck subcutaneous fat (kg), neck IMF (%), and neck meat + bone (kg and %), weights of testicles and bulbourethral glands (g and %).

Table 1. Ingredients and nutrient composition of diets according to pig live weight

Item	Diets		
	P1	P2	P3
Average live weight (kg)	28.7–34.9	35.0–64.9	65.0–106.0
Components (g/kg)			
Barley	353	432	500
Wheat	440	400	378
Soybean meal	177	140	95
Fattening premix[1]	30	28	27
Chemical composition			
Dry matter (%)	88.79	88.68	88.59
Crude protein (%)	18.00	16.51	14.74
Fat (%)	1.75	1.75	1.76
Crude fibre (%)	3.59	3.68	3.72
Metabolizable energy (MJ/kg)	12.92	12.84	12.75
Amino acids (g/kg)			
Lysine	10.7	9.6	8.3
Methionine	3.1	2.9	2.7
Threonine	6.7	6.1	5.4

[1]vitamin–mineral premix provided per kg diet: 400 000 IU retinol, 66 000 IU cholecalciferol, 3600 mg α-tocopherol, 100 mg menadione, 60 mg thiamine, 150 mg riboflavin, 800 mg niacin, 375 mg Ca pantothenate, 100 mg vitamin B_6, 1 mg vitamin B_{12}, 15 000 mg choline chloride, 15 mg folic acid, 3500 mg Fe as $FeSO_4 \cdot H_2O$, 3600 mg Zn as ZnO, 3100 mg Mn as MnO, 330 mg Cu as $CuSO_4 \cdot 5H_2O$, 175 mg I as $Ca(IO_3)_2$, 15 mg Co as $2CoCO_3 \cdot 3Co(OH)_2 \cdot H_2O$, 13 mg Se as Na_2SeO_3, 25 000 FTU 6-phytase (EC 3.1.3.26), 220 g Ca, 20 g P, 50 g Na, 10 g Mg, 85 g lysine, 15 g methionine, and 15 g threonine

Meat quality. The physical qualitative carcass value characteristics were evaluated at the cut between the 13th and 14th ribs in the loin (i.e. MLLT) and ham (i.e. *musculus semimembranosus,* MS). The pH_{45} was measured using a model 330i pH meter equipped with a SenTix Sp pH electrode (both WTW, Weilheim, Germany) 45 min *post mortem*. Electrical conductivity was determined 50 min *post mortem* (EC_{50}) using a conductometer/pigmeter (Czech Technical University in Prague, Czech Republic). Meat and fat colour values (L* = lightness, a* = redness, b* = yellowness) were measured by CM-2500d spectrophotometer (Minolta, Japan), shear force by Instron 3342 (Instron, USA), and drip loss 24 h *post mortem* according to the method of VanLaack and Smulders (1992). The samples were stored at 5°C for 24 h. Representative MLLT samples were taken from the

doi: 10.17221/99/2016-CJAS

right half-carcass, stored in plastic bags at –80°C for maximally 3 weeks, homogenized, and then analyzed chemically. The contents of water (difference of the sample weight before and after drying with sea sand) and IMF (gravimetric determination following extraction using petrol ether in a solvent extractor (SER 148; VELP Scientifica, Italy)) were measured. Androstenone and skatole levels in boars, immunocastrates, barrows, and gilts were analyzed. Fat samples from the neck region between the 1st and the 3rd cervical vertebrae were collected for the androstenone and skatole content analysis 24 h after slaughter and frozen without skin and muscles in a vacuum package at –80°C until the follow-up analysis. Contents of androstenone and skatole in the fatty tissue were determined according to the methodology of high-performance liquid chromatography modified by Hansen-Moller (1994). To determine the androstenone level, an Agilent Eclipse XDB C18 (5 µm, 150 × 4.60 mm ID) column tempered at 40°C was used. The mobile stage parameters were as follows: A – tetrahydrofuran : acetonitrile : sodium phosphate buffer (25 mM) : acetic acid (34 : 23.8 : 41.4 : 0.8), and B – methanol. The gradient profile program was as follows: 0–3.0 min, 90% A; 3.0–3.5 min, 90–45% A; 3.5–15.0 min, 45–5% A; 15.0–16.1 min, 5% A; 16.1–17.0 min, 5–90% A; 17.0–19.0 min, 90% A. The column flow rate was set at 1.2 ml/min with an injection volume of 40 µl. Fluorescence detection was performed with excitation at 346 nm and emission at 521 nm. We used the standard calibration curve to determine androstenone content in the actual sample. To determine the skatole level, a Kinetex C18 100A (5 µm, 50 × 4.60 mm ID) column tempered at 40°C was used. The mobile stage parameters were as follows: A – sodium phosphate buffer (10 mM) and B – methanol. The gradient profile program was as follows: 0–0.2 min, 90% A; 0.2–6.0 min, 90–55% A; 6.0–7.0 min, 55–0% A. The column flow rate was set at 1.2 ml/min with an injection volume of 30 µl. Fluorescence detection was performed with excitation at 285 nm and emission at 340 nm. We used the standard calibration curve to determine skatole content in the actual sample.

Statistical analysis. Data were analyzed using the GLM procedure of the SAS software (Statistical Analysis System, Version 9.4, 2012). All means presented herein are the Least Squares Means of each group along with standard errors of the mean (SEM), together with the significance levels for the main effects of sex. Treatment mean differences were tested using Tukey's test. Significance was declared at $P < 0.05$. Final body weight (BW) was used as a covariate in the carcass data analysis. Sex effect was included in the growth data analysis. Because no interaction was found between sex and dietary treatments, these interactions were removed from the final statistical growth model.

RESULTS AND DISCUSSION

Growth performance. Table 2 shows the values of growth performance from the beginning of the fattening test until V1 and V2 and subsequently until the test completion for all test groups of pigs. No significant differences in LW were found.

Regarding ADG, and with the exception of CM, there was no effect in relation to vaccination dates on change in growth intensity as compared to the other monitored groups. The CM group exhibited the highest ADG for the testing period as a whole (1193 g), followed by IC and FE (both 1181 g). The lowest ADG was measured in the UCM group (1169 g), and the difference between CM and UCM was 2.05%. Similar results had been obtained by D'Souza and Mullan (2002) and by Pauly et al. (2008). On the other hand, other authors (Skrlep et al. 2012) have recorded more favourable results in boars as compared to other groups. This inconsistency may be caused by a number of factors, such as (among others) a lower FI in boars due to sexual activity and their behaviour, which even can cause social stress (Pauly et al. 2008) and thus a reduced grow ability.

A comparison of the results obtained for FI and FCR between IC and UCM at each tested stage clearly indicates that higher values were reached in the IC group. Nevertheless, the recorded differences were not significant. The CM group reached the highest values. Lower FI in boars and higher FI in immunocastrates at the end of the finishing period were recorded by Weiler et al. (2013). Similar results of FI and FCR had been obtained by Fabrega et al. (2010). It is also clear that after V2 the levels of testosterone, aggressiveness, and sexual behaviour decrease in IC (Mackinnon and Pearce 2007). As a consequence of this fact, there is an increase in FI and simultaneously in FCR (Cronin et al. 2003). Based on overall assessment

doi: 10.17221/99/2016-CJAS

Table 2. Growth performance parameters of immunocastrated males (IC), castrated males (CM), uncastrated males (UCM), and females (FE)

Variable	IC	CM	UCM	FE	Significance
Body weight (kg)					
Initial BW	34.72 ± 3.62	33.28 ± 7.38	33.75 ± 3.67	36.39 ± 3.46	0.2387
V1 BW	56.28 ± 5.80	53.52 ± 10.57	55.12 ± 6.29	56.98 ± 4.93	0.9729
V2 BW	82.22 ± 9.87	80.75 ± 13.02	80.97 ± 7.32	81.22 ± 6.99	0.6411
Final BW	106.99 ± 10.29	105.95 ± 12.21	106.20 ± 8.49	104.17 ± 7.94	0.8588
ADG (g/day)					
Start to V1	1026 ± 129	964 ± 184	1017 ± 155	981 ± 137	0.5859
V1 to V2	1235 ± 223	1297 ± 161	1231 ± 171	1154 ± 158	0.1365
V2 to slaughter	1282 ± 197^{ab}	1319 ± 197^{a}	1260 ± 198^{ab}	1129 ± 120^{b}	0.0153
Overall	1181 ± 130^{ab}	1193 ± 115^{a}	1169 ± 88^{ab}	1181 ± 130^{b}	0.0243
Feed intake (kg/day)					
Start to V1	1.95 ± 0.21	1.84 ± 0.33	1.95 ± 0.29	1.99 ± 0.20	0.3619
V1 to V2	2.79 ± 0.24^{b}	3.04 ± 0.35^{a}	2.71 ± 0.22^{b}	2.77 ± 0.30^{b}	0.0045
V2 to slaughter	3.31 ± 0.30^{ab}	3.59 ± 0.26^{a}	3.19 ± 0.52^{cd}	2.98 ± 0.21^{bc}	< 0.0001
Overall	2.68 ± 0.19	2.83 ± 0.24^{a}	2.62 ± 0.29^{b}	2.58 ± 0.19^{b}	0.0127
Feed conversion ratio					
Start to V1	1.92 ± 0.28	1.94 ± 0.32	1.92 ± 0.12	2.06 ± 0.29	0.3693
V1 to V2	2.12 ± 0.27	2.18 ± 0.25	2.08 ± 0.15	2.25 ± 0.29	0.2995
V2 to slaughter	2.63 ± 0.37	2.78 ± 0.39	2.54 ± 0.25	2.67 ± 0.32	0.2196
Overall	2.29 ± 0.20	2.38 ± 0.24	2.23 ± 0.14	2.39 ± 0.28	0.1008

ADG = average daily gain, BW = body weight, V1 = first vaccination 1, V2 = second vaccination

$^{a–d}$means within the same row with different superscripts differ significantly at $P < 0.05$

of growth performance (ADG, FI, and FCR), it may be stated that no significant differences were found among the IC, UCM, and FE groups. On the other hand, significant differences between CM and other tested groups were observed. There was no significant effect of vaccination date on the monitored indicators (ADG, FI, and FCR) observed in comparing IC with UCM.

Table 3. Effect of the second vaccination (V2) on the body composition of immunocastrated males (IC), castrated males (CM), uncastrated males (UCM), and females (FE) (in mm)

Variable	IC	CM	UCM	FE	Significance
MLLT depth, MUSCLE2					
Up to V2	37.52 ± 3.63	38.43 ± 4.80	36.00 ± 3.56	38.91 ± 3.39	0.1315
Final	46.43 ± 3.89	46.93 ± 5.81	43.58 ± 3.71	46.57 ± 3.10	0.0795
Difference: Final – V2	8.91 ± 2.32	8.51 ± 2.80	7.58 ± 2.03	7.66 ± 2.21	0.2833
Backfat thickness, FAT2					
Up to V2	11.21 ± 1.01^{a}	11.21 ± 1.04^{a}	10.60 ± 0.60	10.30 ± 0.84^{b}	0.0057
Final	15.15 ± 1.61	15.70 ± 1.93	14.56 ± 1.55	15.16 ± 1.76	0.2770
Difference: Final – V2	3.94 ± 0.88	4.50 ± 1.41	3.97 ± 1.40	4.86 ± 1.34	0.1102
Backfat thickness, FAT1					
Up to V2	11.58 ± 1.02^{a}	11.30 ± 0.81^{a}	11.35 ± 1.09^{a}	10.22 ± 1.02^{b}	0.0006
Final	13.55 ± 1.26^{a}	13.75 ± 1.35^{a}	13.50 ± 1.26^{a}	12.43 ± 0.82^{b}	0.0064
Difference: Final – V2	1.98 ± 0.71	2.45 ± 0.85	2.15 ± 0.79	2.21 ± 0.71	0.3513

MLLT = *musculus longissimus lumborum et thoracis*

$^{a–b}$means within the same row with different superscripts differ significantly at $P < 0.05$

doi: 10.17221/99/2016-CJAS

Table 3 describes the effect of V2 on backfat (FAT1, FAT2) and MLLT muscle creation. No significant difference in MLLT depth after V2 was observed between IC and the other groups (CM, UCM and FE). As concerns backfat, an equal growth trend was found across all groups at FAT1. The highest FAT1 was reached by the CM group (13.75 mm) compared to the groups UCM (13.50 mm) and IC (13.55 mm). The lowest value was measured in the FE group (12.43 mm), which was significantly lower than in all the other groups. There were differences between groups as measured by change in FAT1 between V2 and slaughter. The smallest difference was recorded for the IC group (1.98 mm). The same trend had been found by Pauly et al. (2009) and Fabrega et al. (2010). At the end of the fattening test, FAT2 showed no significant differences between the pig groups. It can be therefore stated that no negative effect of V2 on the final indicators (MLLT depth, FAT1, and FAT2) was observed. However, there was detected an effect on the MLLT depth and FAT2 indicators in the growth of IC and CM groups compared to UCM, but without significant differences. This can be explained by the V2 date before slaughter. When the V2 date was extended to 7–9 weeks before slaughter, Kantas et al. (2014) found significant differences in these indicators.

Carcass composition. Table 4 presents the obtained carcass parameters. The highest CW was

Table 4. Carcass value parameters of immunocastrated males (IC), castrated males (CM), uncastrated males (UCM), and females (FE)

Variable	IC	CM	UCM	FE	Significance
Live weight (kg)	107.0 ± 10.3	106.0 ± 12.2	106.2 ± 8.5	104.2 ± 7.9	0.8588
Carcass weight (kg)	80.70 ± 8.1	81.8 ± 10.5	79.2 ± 7.2	79.5 ± 6.5	0.7733
Lean meat ZP[1] (%)	59.9 ± 1.3[b]	58.5 ± 1.5[a]	60.3 ± 1.1[b]	60.1 ± 1.2[b]	0.0003
Muscle depth ZP[1] (mm)	65.0 ± 5.8[b]	70.4 ± 6.8[a]	63.4 ± 5.4[b]	68.0 ± 4.7	0.0027
Backfat thickness ZP[1] (mm)	12.6 ± 2.6[b]	17.1 ± 3.8[a]	11.5 ± 2.4[b]	12.8 ± 2.7[b]	< 0.0001
MLLT area (mm^2)	3727 ± 485[b]	4361 ± 416[a]	3975 ± 412	4409 ± 395[a]	0.0026
Ham					
Subcutaneous fat (kg)	1.75 ± 0.33[b]	2.26 ± 0.37[a]	1.80 ± 0.24[b]	2.04 ± 0.30	0.0028
IMF (%)	3.08 ± 0.54[b]	3.69 ± 1.04	3.07 ± 0.86[b]	4.59 ± 1.41[a]	0.0054
Meat + bone (kg)	8.27 ± 1.04	8.53 ± 0.86	8.15 ± 0.52	9.04 ± 0.81	0.0945
Meat + bone (%)	21.72 ± 0.75[b]	21.89 ± 0.97	21.15 ± 0.87[b]	22.85 ± 1.03[a]	0.0019
Shoulder					
Subcutaneous fat (kg)	1.43 ± 0.40	1.35 ± 0.12	1.36 ± 0.29	1.21 ± 0.23	0.3691
IMF (%)	2.18 ± 0.42[bc]	2.82 ± 0.61[a]	2.43 ± 0.54[ac]	1.85 ± 0.25[b]	0.0006
Meat + bone (kg)	4.27 ± 0.50	4.26 ± 0.36	4.12 ± 0.37	4.36 ± 0.37	0.6073
Meat + bone (%)	11.23 ± 0.64	10.95 ± 0.73	10.69 ± 0.76	11.03 ± 0.41	0.3243
Loin					
Subcutaneous fat (kg)	1.63 ± 0.39	1.73 ± 0.25	1.42 ± 0.21	1.50 ± 0.23	0.0884
IMF (%)	2.13 ± 0.28	2.31 ± 0.43	2.18 ± 0.33	1.96 ± 0.35	0.1647
Meat + bone (kg)	4.51 ± 0.69	4.60 ± 0.45	4.58 ± 0.66	4.87 ± 0.39	0.5048
Meat + bone (%)	11.82 ± 0.80	11.82 ± 0.69	11.80 ± 0.75	12.32 ± 0.43	0.2662
Neck					
Subcutaneous fat (kg)	0.45 ± 0.11	0.51 ± 0.11	0.54 ± 0.11	0.42 ± 0.11	0.0851
IMF (%)	5.18 ± 1.10	6.37 ± 2.44	6.26 ± 2.79	4.51 ± 1.18	0.1385
Meat + bone (kg)	2.68 ± 0.35	2.59 ± 0.31	2.73 ± 0.15	2.68 ± 0.19	0.6850
Meat + bone (%)	7.05 ± 0.56	6.65 ± 0.55	7.09 ± 0.33	6.78 ± 0.36	0.1168

IMF = intramuscular fat, MLLT = *musculus longissimus lumborum et thoracis*

[1]measured by a two-point method ZP

[a–d]means within the same row with different superscripts differ significantly at $P < 0.05$

doi: 10.17221/99/2016-CJAS

reached by the CM group (81.8 kg) and the lowest by UCM (79.2 kg). The recorded differences were not significant. On the other hand, significant differences were found in carcass lean meat ZP ($P = 0.0027$), where the lowest values were reached by the CM group (58.5%), which also exhibited the highest backfat thickness ZP (17.1 mm) as well as the highest muscle depth (70.4 mm) and the second highest MLLT area (4361 mm^2). Although comparison of the IC and UCM groups indicated more favourable values for the UCM group, these differences were nevertheless not significant.

Evaluation of the selected carcass parts revealed a significantly higher proportion of IMF and subcutaneous fat in CM as compared to the other monitored groups. No significant difference was found between the groups IC and UCM. The lowest levels in all monitored parts were in the FE group. As regards the absolute and relative meat ratio (ham, shoulder, loin, and neck – in kg and %), no significant differences were found in the individual categories of the carcass parts. Some authors have pointed to differing muscle composition between the sexes. Fortin et al. (1987) documented that in comparison with gilts, boars have heavier neck and chest muscles and lighter pelvic and limb muscles. Similar results were found as regards carcass hind legs and shoulders, wherein boars showed the lowest proportion among all pig groups. Uttaro et al. (1994) reached just the opposite conclusion, but they did not evaluate hybrid populations.

Meat quality. Table 5 describes the physical meat quality parameters recorded. No significant differences were found between the groups in relation to pH, meat colour, drip loss, and shear force. The values recorded for IC converged with those recorded for UCM and FE. As concerns meat colour, Lundstrom et al. (1987) and Boler et al. (2014) came to the same findings as did we. Wood et al. (1986) found a slightly lighter colour of MLLT in boars than in gilts. On the other hand, Sather et al. (1991, 1993) recorded a slightly darker meat colour in boars in comparison to that from females. When assessing the shear force, our results corresponded to those of Sather et al. (1991) and Boler et al. (2014) showing no differences between

Table 5. Selected parameters of pork meat quality of immunocastrated males (IC), castrated males (CM), uncastrated males (UCM), and females (FE)

Meat quality	IC	CM	UCM	FE	Significance
Carcass (*n*)	16	18	18	18	
Carcass weight (kg)	80.7 ± 8.1	81.8 ± 10.5	79.2 ± 7.2	79.5 ± 6.5	0.7733
MLLT pH_{45}	6.4 ± 0.3	6.4 ± 0.3	6.4 ± 0.3	6.4 ± 0.3	0.8738
MS pH_{45}	6.5 ± 0.3	6.5 ± 0.3	6.4 ± 0.3	6.4 ± 0.3	0.8918
MLLT colour					
L*	52.3 ± 2.9	51.0 ± 3.0	52.2 ± 2.8	52.1 ± 3.3	0.7305
a*	–1.3 ± 0.5	–1.2 ± 0.7	–1.1 ± 0.6	–1.6 ± 0.6	0.4100
b*	8.3 ± 0.9	8.0 ± 1.1	8.7 ± 0.7	8.4 ± 1.1	0.3828
Backfat colour					
L*	80.7 ± 2.2	79.5 ± 2.6	81.3 ± 1.3	80.4 ± 1.3	0.2196
a*	–0.5 ± 0.6	–0.4 ± 0.7	–0.5 ± 0.6	–0.6 ± 0.6	0.9511
b*	7.4 ± 1.1	7.6 ± 0.9	7.6 ± 0.6	7.3 ± 0.9	0.7791
MLLT IMF (%)	2.13 ± 0.28	2.31 ± 0.43	2.18 ± 0.33	1.96 ± 0.35	0.1647
Drip loss (%)	4.7 ± 2.8	4.1 ± 1.7	4.8 ± 2.4	5.5 ± 3.0	0.6768
Shear force (N)					
MLLT raw	51.8 ± 7.7	45.4 ± 6.6	51.6 ± 8.2	50.9 ± 5.8	0.1553
MLLT boiled	27.9 ± 3.3	30.3 ± 4.4	28.6 ± 3.9	29.6 ± 4.6	0.5547
Raw fat	93.0 ± 24.8	71.7 ± 30.3	90.7 ± 18.0	84.8 ± 27.6	0.2594

IMF = intramuscular fat, MLLT = *musculus longissimus lumborum et thoracis*, MS = *musculus semimembranosus*, L* = lightness, a* = redness, b* = yellowness, pH_{45} = pH measured 45 min *post mortem*

[a–d]means within the same row with different superscripts differ significantly at $P < 0.05$

doi: 10.17221/99/2016-CJAS

Table 6. Least Squares Means of androstenone and skatole levels and the testes and bulbourethral glands variables of immunocastrated males (IC), castrated males (CM), uncastrated males (UCM), and females (FE)

Variable	IC	CM	UCM	FE	Significance
Androstenone (μg/g)	0.53 ± 0.70[a]	0.18 ± 0.14[a]	2.38 ± 0.67[b]	0.19 ± 0.17[a]	< 0.0001
Skatole (μg/g)	0.06 ± 0.05[a]	0.05 ± 0.02[a]	0.22 ± 0.06[b]	0.05 ± 0.03[a]	< 0.0001
Testes weight (g)	255.0 ± 160.3[a]		415.8 ± 100.3[b]		0.0012
Testes weight/live weight (%)	0.24 ± 0.14[a]		0.40 ± 0.11b		0.0008
Bulbourethral glands weight (g)	78.2 ± 25.1[a]		130.0 ± 35.6[b]		< 0.0001
Bulbourethral glands weight/live weight (%)	0.07 ± 0.02[a]		0.12 ± 0.04[b]		< 0.0001

[a–b]means within the same row with different superscripts differ significantly at $P < 0.05$

sexes. Lower shear force values in boar meat were found by Lundstrom et al. (1987), but the opposite finding was published by Sather et al. (1993). In assessing the drip loss in MLLT, Lundstrom et al. (1987) found no differences between sexes. Similar results were obtained in this work. To the contrary, Sather et al. (1991) suggested lower drip losses for boars as compared to gilts and castrates. IMF content is an important meat quality indicator, and no significant differences among groups were found. Considering that low IMF content can cause undesirable changes in meat taste and texture, it should be noted that the lowest values were achieved in FE (1.96%) and the highest in CM (2.31%). IC and UCM reached almost the same values (2.13 and 2.18%, respectively). Gispert et al. (2010) reported that meat from CM reached the greatest extent of IMF, boars showed the least, and gilts and CM were in between. Dubois et al. (2012) came to the conclusion that boars have lower proportion of fat and therefore also a lesser extent of IMF due to the anabolic effects of androgynous steroids, such as testosterone. This can cause problems, as even in CM the IMF in some muscles is less than the 2–3% which is recommended for optimal sensory quality. Nevertheless, in studies where such characteristic was monitored, no extremely low IMF levels were found in boars. Levels ranging from 1.5 to 3.5% were recorded, and the differences between sexes were relatively small (Barton-Gade 1987; Fortin et al. 1987).

As concerns the content of androstenone (Table 6), the significantly highest levels were recorded in the UCM group (2.38 μg/g). The IC group ranked second (0.53 μg/g) and was followed by the groups CM and FE with almost identical values (0.18 μg/g and 0.19 μg/g, respectively). A similar trend can be noted in the skatole level. Similar results were obtained by Zamaratskaia et al. (2008).

In assessing the weights of testicles and bulbourethral glands between the IC and UCM groups, highly significant differences ($P < 0.001$) were observed in all indicators. Mean testicular weight in boars reached 415.8 g compared to 255.0 g in immunocastrates. A similar trend was recorded for bulbourethral glands, where the weights in boars and immunocastrates, respectively, reached 130.0 g and 78.2 g. The effect of immunocastration on gonad development in boars was therefore fully demonstrated. This was manifested, too, in the low levels of androstenone and skatole. Forland et al. (1980) reported similar findings, as well as

Table 7. Proportion of animals with high concentrations of androstenone and skatole in backfat in the experimental groups

Variable	IC (n = 16)	CM (n = 18)	UCM (n = 18)	FE (n = 18)
High androstenone (≥ 0.50 μg/g)				
Animals (%)	31.2	11.1	100.0	11.1
Androstenone (μg/g)	1.44	0.52	2.38	0.64
High skatole (≥ 0.20 μg/g)				
Animals (%)	0	0	61.1	0
Skatole (μg/g)	–	–	0.26	–

IC = immunocastrated males, CM = castrated males, UCM = uncastrated males, FE = females

doi: 10.17221/99/2016-CJAS

a relatively high correlation between the size and weight of gonads and the androstenone level in fat.

Table 7 presents the proportion of animals with high concentrations of androstenone and skatole in the experimental groups. In UCM, 100% of animals exhibited above limit concentrations of androstenone (≥ 0.50 μg/g; on average 2.38 μg/g), followed by IC with 31% of animals (on average 1.44 μg/g). Only 11% of animals exceeded the limit in the other groups (CM and FE). Conversely, no animals with above limit concentrations of skatole (≥ 0.20 μg/g) were observed in IC, CM, and FE, whereas in UCM 61% of animals exceeded this limit (on average 0.26 μg/g). It is evident that the economics of pork production will be negatively affected especially in UCM but also in the IC group due to penalization of carcasses with excessive concentrations of androstenone and skatole.

CONCLUSION

The overall assessment of growth performance shows that there have been no differences between the groups of IC, UCM, and FE. On the other hand, significant differences between CM and the other groups were observed. No significant effect of vaccination date on the evaluated features of IC occurred as compared to UCM. It is nevertheless clear that under group boar housing mutual attacks will occur, and, as a consequence, fattening parameters will be adversely affected. It can also be stated that IC group pigs showed no negative effects from the second vaccination as measured by the final carcass value indicators, and their fat accumulation was not greater in comparison with UCM and FE. In this regard, the CM group exhibited the highest fat accumulation compared to the other groups.

Regarding the indicators characterizing pork meat quality, no significant differences in pH, meat colour, drip loss, shear force, or IMF proportion were found. The values recorded for IC converged very much with those for UCM and FE. As a consequence of immunocastration, the testicular weight decreased significantly, the occurrence of undesired boar taint was prevented.

REFERENCES

Barton-Gade P.A. (1987): Meat quality in boars, castrates and gilts. Livestock Production Science, 16, 187–196.

Batorek N., Candek-Potokar M., Bonneau M., Van Milgen J. (2012): Metaanalysis of the effect of immunocastration on the production performance, reproductive organs, and boar taint compounds in pigs. Animal, 6, 1330–1338.

Boler D.D., Puls C.L., Clark D.L., Ellis M., Schroeder A.L., Matzat P.D., Killefer J., McKeith F.K., Dilger A.C. (2014): Effects of immunological castration (Improvest) on changes in dressing percentage and carcass characteristics of finishing pigs. Journal of Animal Science, 92, 359–368.

Bonneau M., Squires E.J. (2004): Boar taint: causes and measurement. In: Devine C., Dikeman M., Jensen W.K. (eds): Encyclopedia of Meat Sciences. Elsevier, Oxford, UK, 91–97.

Citek J., Stupka R., Okrouhla M., Vehovsky K., Brzobohaty L., Sprysl M., Stadnik L. (2015): Effects of dietary linseed and corn supplement on the fatty acid content in the pork loin and backfat tissue. Czech Journal of Animal Science, 60, 319–326.

Cronin G.M., Dunshea F.R., Butler K.L., McCauley I., Barnett J.L., Hemsworth P.H. (2003): The effects of immuno- and surgical castration on the behaviour and consequently growth of group-housed, male finisher pigs. Applied Animal Behaviour Science, 81, 111–126.

D'Souza D.N., Mullan B.P. (2002): The effect of genotype, sex and management strategy on the eating quality of pork. Meat Science, 60, 95–101.

Dubois V., Laurent M., Boonen S., Vanderschueren D., Claessens F. (2012): Androgens and skeletal muscle: cellular and molecular action mechanisms underlying the anabolic actions. Cellular and Molecular Life Sciences, 69, 1651–1667.

Fabrega E., Velarde A., Cros J., Gispert M., Suarez P., Tibau J., Soler J. (2010): Effect of vaccination against gonadotropin-releasing hormone, using Improvac®, on growth performance, body composition, behaviour and acute phase proteins. Livestock Production Science, 132, 53–59.

Forland D., Lundstrom K., Andresen O. (1980): Relationship between androstenone content in fat, intensity of boar taint and size of accessory sex glands in boars. Nordisk Veterinaermedicin, 32, 201–206.

Fortin A., Wood J.D., Whelehan O.P. (1987): Breed and sex effects on the development, distribution of muscle, fat and bone, and the partition of fat in pigs. Journal of Agricultural Science, 108, 141–153.

Gispert M., Angels Oliver M., Velarde A., Suarez P., Perez J., Font Furnols M. (2010): Carcass and meat quality characteristics of immunocastrated male, surgically castrated male, entire male and female pigs. Meat Science, 85, 664–670.

Hansen-Moller J. (1994): Rapid high-performance liquid chromatographic method for simultaneous determination

doi: 10.17221/99/2016-CJAS

of androstenone, skatole and indole in back fat from pigs. Journal of Chromatography: B, Biomedical Applications, 661, 219–230.

Kantas D., Papatsiros V., Tassis P., Tzika E., Pearce M.C., Wilson S. (2014): Effects of early vaccination with a gonadotropin releasing factor analog-diphtheria toxoid conjugate on boar taint and growth performance of male pigs. Journal of Animal Science, 92, 2251–2258.

Lundstrom K., Malmfors B., Malmfors G., Stern S. (1987): Meat quality in boars and gilts after immediate slaughter or lairage for two hours. Swedish Journal of Agricultural Research, 17, 51–56.

Lundstrom K., Matthews K.R., Haugen J.E. (2009): Pig meat quality from entire males. Animal, 3, 1497–1507.

Mackinnon J.D., Pearce M.C. (2007): Improvac™ (Pfizer Animal Health): an immunological product for the control of boar taint in entire male pigs (II). Practical applications in pig production and potential production benefits. Pig Journal, 59, 68–90.

Marsalek P., Svoboda M., Bernardy J., Vecerek V. (2015): Concentrations of neopterin, biopterin, and cortisol associated with surgical castration of piglets with lidocaine. Czech Journal of Animal Science, 60, 473–478.

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

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

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

Sather A.P., Jones S.D.M., Joyal S. (1991): Feedlot performance, carcass composition and pork quality from entire male and female Landrace and Large White marketweight pigs. Canadian Journal of Animal Science, 71, 29–42.

Sather A.P., Tong A.K.V., Jeremiah L.E., Squires E.J., Jones S.D.M. (1993): Factors affecting the development of "sex taint" in entire male pigs. In: Proc. 39th Internat. Congress of Meat Science and Technology, Calgary, Canada, 200.

Simecek K., Zeman L., Heger J. (2000): Nutrient Requirements and Tables of Nutritive Value of Feeds for Pigs. Mendel University in Brno, Brno, Czech Republic. (in Czech)

Skrlep M., Segula B., Zajec M., Kastelic M., Kosorok S., Fazarinc G., Candek-Potokar M. (2010): Effect of immunocastration (Improvac®) in fattening pigs. II: Carcass traits and meat quality. Slovenian Veterinary Research, 47, 65–72.

Skrlep M., Batorek N., Bonneau M., Prevolnik M., Kubele V., Candek-Potokar M. (2012): Effect of immunocastration in group-housed commercial fattening pigs on reproductive organs, malodorous compounds, carcass and meat quality. Czech Journal of Animal Science, 57, 290–299.

Sprysl M., Citek J., Stupka R. (2010): Interaction of selected production indicators of the economy of pork production. Czech Journal of Animal Science, 55, 1–10.

Thun R., Gajewski Z., Janett F. (2006): Castration in male pigs: techniques and animal welfare issues. Journal of Physiology and Pharmacology, 57, 189–194.

Uttaro B., Aker C., Ball R.O. (1994): Growth characteristics and lean distribution in Ontario purebred swine. Ontario Swine Research Review, 1994, 53–55.

VanLaack R.L.J.M., Smulders F.J.M. (1992): On the assessment of water-holding capacity of hot- vs cold-boned pork. Meat Science, 32, 139–147.

Walstra A.P., Merkus G.S.M. (1995): Procedure for Assessment of the Lean Meat Percentage as a Consequence of the New EU Reference Dissection Method in Pig Carcass Classification. DLO Research Institute for Animal Science and Health (ID-DLO), Zeist, The Netherlands.

Weiler U., Gotz M., Schmidt A., Otto M., Muller S. (2013): Influence of sex and immunocastration on feed intake behavior, skatole and indole concentrations in adipose tissue of pigs. Animal, 7, 300–308.

Wood J.D., Jones R.C.D., Francombe M.A., Whelehan O.P. (1986): The effects of fat thickness and sex on pig meat quality with special reference to the problems associated with overleanness. 2. Laboratory and trained taste panel results. Animal Production, 43, 535–544.

Zamaratskaia G., Rydhmer L., Andersson H.K., Chen G., Lowagie S., Andersson K., Lundstrom K. (2008): Long-term effect of vaccination against gonadotropin-releasing hormone, using Improvac™, on hormonal profile and behaviour of male pigs. Animal Reproduction Science, 108, 37–48.

Received: 2016–11–10
Accepted after corrections: 2017–01–30

6 Výsledky a souhrnná diskuse

6.1 Identifikce kandidátních genů a enzymů zapojených do syntézy a metabolismu složek kančího pachu

H1. Syntéza a metabolismus androstenonu, skatolu a indolu jsou mimo jiné ovlivňovány geny. Geny asociované s hladinami androstenonu, skatolu a indolu se nacházejí na chromozomech, kde jsou lokalizoány QTL pro další užitkové vlastnosti prasat.

Publikace 1:

Zadinová, K., Stupka, R., Stratil, A., Čítek, J., Vehovský, K., Urbanová, D. 2016. Boar Taint - The Effect of Selected Candidate Genes Associated with Androstenone and Skatole Levels - a Review. Animal Science Papers and Reports. 34 (2), 107-128.

Cílem práce bylo podat ucelený přehled o možnostech snižování hladin kančího pachu pomocí genů a jejich SNP mutací. Dále pak, na základě poznatků o vlivu jednotlivých enzymů na metabolické dráhy těchto složek, identifikovat potenciální kandidátní geny zapojené do syntézy a metabolismu androstenonu a skatolu.

Z dosud publikovaných studií vyplývá, že genotypem prasat lze částečně ovlivnit tvorbu a ukládání androstenonu, skatolu a indolu v tukové a svalové tkáni prasat. Byly ovšem prokázány rozdíly mezi jednotlivými plemeny a dokonce mezi hybridními kombinacemi. Obecně se uvádí, že prasata prošlechtěných kulturních plemen ukládají tyto složky v menší míře než většina primitivních plemen (Squires *et al.,* 1997). U různých plemen a linií prasat existují geneticky podmíněné rozdíly v hladinách androstenonu a skatolu. Koeficienty dědivosti pro hladinu androstenonu se pohybují v rozmezí od 0,25 do 0,87. Hladiny skatolu mají hodnoty koeficientů dědivosti v rozmezí 0,19–0,54 (Oskam *et al.,* 2010; Robic *et al.,* 2008; Tajet *et al.,* 2006). Znamená to, že v populaci prasat se vyskytují jedinci s různě vysokým potenciálem pro jednotlivé úrovně těchto látek. A tedy, že ne každé zvíře v sobě nese dispozici pro vysokou produkci a hlavně akumulaci androstenonu, skatolu a indolu. Proto můžeme rovněž uvažovat o využití selekce na základě jednobodových mutací (SNP) ve vybraných genech prasat.

Z uvedené práce je zřejmé že, hladinu androstenonu a skatolu ovlivňuje větší počet genů, z nichž některé působí jen na hladinu androstenonu, některé jen na hladinu skatolu a indolu, a v případě některých genů se uvažuje o nepřímém vlivu na všechny výše uvedené substance

(Wiercinska *et al.,* 2012). V případě androstenonu patří mezi nejčastěji zmiňované geny *CYP17A1* (Lin *et al.,* 2005b), *CYB5A* (Lin *et al.,* 2005a), dále pak sulfotransferázy *SULT2A1* (Sinclair *et al.,* 2006) a *SULT2B1* (Zamaratskaia *et al.,* 2012) a hydroxysteroid dehydrogenázy zvláště *HSD3B1* a *HSD17B7* (Doran *et al.,* 2004). Na hladinu skatolu a zároveň indolu pak působí *CYP2E*1 (Lin *et al*., 2006; Morlein *et al.,* 2012) a *SULT1A1* (Lin *et al*., 2004b).

Na základě těchto poznatků lze říci, že selekce proti kančímu pachu na základě výskytu jednotlivých SNP je částečně možná. Je ale nutné brát v úvahu možná rizika pro reprodukční a užitkové vlastnosti prasat. Většina genů zapojených do syntézy a metabolismu androstenonu zároveň ovlivňuje i syntézu nebo metabolismu dalších pohlavních hormonů.

Publikace 2:

Urbanová, D., Stupka, R., Okrouhlá, M., Čítek, J., Vehovský, K., **Zadinová, K.** 2016. Nutritional effects on boar taint in entire male pigs: a review. Scientia Agriculturae Bohemica. 47 (4), 154-163.

Cílem této práce bylo popsat vliv výživy prasat, především specifický doplňků na metabolismus androstenou a skatolu u prasat a na výslednou hladinu těchto složek v tukové a svalové tkáni prasat.

Hlavní složkou, kterou lze tento nežádoucí jev u masa nekastrovaných jedinců pomocí výživy eliminovat je skatol. Jelikož skatol je produkt degradace L-tryptofanu v trávicím traktu prasat. Hlavním zdrojem tryptofanu je buněčný odpad výstelky tenkého střeva. Proto je cílem tento odpad co nejvíce omezit právě pomocí výživy. Jedním z krmných doplňků, o kterém se uvažuje, je bramborový škrob. Ten olivňuje tvorbu kys. mléčné, která následně snižuje odumírání střevních buňek, a tím i množství buněčného odpadu ve střevech. U zvířat, kterým byl podáván bramborový škrob, došlo k redukci skatolu v tlustém střevě, krevní plazmě i tuku (Claus *et al.,* 2003; Losel *et al.,* 2006).

Další možností jak snížit hladinu složek kančího pachu je prostřednictím vlivu diety na činnost cytochromů v souvislosti s expresí cytochromových genů a následnou aktivitou enzymů, které se podílejí na metabolismu skatolu a androstenonu. Konkrétně se jedná o cytochrom P450, a jeho skupinu proteinů, které hrají, jakožto enzymy, zásadní roli v bioaktivaci a detoxikaci organismu od látek xenobiotického původu (Guengerich, 2008).

Mezi hojně analyzované plodiny, zkrmované prasatům za účelem omezení výskytu androstenonu a skatolu je čekanka obecná - *Cichorium intybus L*. Původním předpokladem bylo, že její hlavní účinek je v jejím působení na formaci skatolu v trávicím traktu. Četné studie prokázaly, že se po zkrmování diety bohaté na vlákninu, zvyšuje exprese CYP450 (Nugon-Baudon, 1996; Lemley *et al.*, 2010). Rasmussen *et al.*, (2012) ve své studii potvrzuje, že u zvířat, kterým byly po dobu 16 dní před porážkou zkrmovány úsušky čekanky, byl statisticky průkazně nižší obsah androstenonu v podkožním tuku, přičemž tato zvířata měla zvýšenou expresi mRNA a proteinu 3β-HSD. Na druhou stranu nebyl zjištěn rozdíl v koncetraci skatolu ani v tuku ani v plazmě mezi kontrolní a pokusnou skupinou.

Mezi další uvažované plodiny s pozitivním vlivem na snížení hladiny kančího pachu patří rovněž slunečnice topinambur, bramborový škrob a cukrová řepa.

Z uvedevých poznatků vyplývá, že především tvorbu skatolu lze ovlivnit krmnými přídavky. A to jak na úrovni degradace L-tryptofanu v trávicím traktu, tak na úrovni metabolisnu v játrech.

6.2 Testace vybraných SNP a jejich vlivu na hladinu androstenonu skatolu a indolu

H2. Vybrané mutace genů, zejména z řad genů rodiny CYP, sulfotransferáz a monooxygenáz, průkazně ovlivňují hladinu androstenonu a skatolu v tukové tkáni prasat.

Publikace 3:

Zadinová, K., Stupka, R., Stratil, A., Čítek, J., Vehovský, K., Lebedová, N., Šprysl, M., Okrouhlá, M. 2017. Association analysis of SNPs in the porcine *CYP2E1* gene with skatole, indole, and androstenone levels in backfat of a crossbred pig population. Meat Science. 131, 68-73.

Cílem bylo prokázat vliv vybraných SNP mutací u prasečího genu *CYP2E1* na hladinu složek kančího pachu. U zkoumané populace prasat byly testovány známé mutace v genu *CYP2E1* na hladinu skatolu, indolu a také androstenonu. Ačkoliv *CYP2E1* působí především na hladinu skatolu a indolu, tedy metabolismus těchto složek v játrech, je nutné zabývat se i jeho vlivem na hladinu androstenonu. Už v dřívějších studiích byly popsány pozitivní korelace mezi hladinami androstenonu a skatolu.

U sledované populace prasat hybridní kombinace $ČBU_S$ x ($ČL_S$ x $ČBU_D$). Byl zjištěn statisticky průkazný vliv na hladinu indolu u všech tří sledovaných mutací g.2412C>T, c.1422C>T, c.1423G>A. Nebyl sice zaznamenán statisticky průkazný vliv na hladinu skatolu, nicméně u genotypů sledovaných mutací byl zaznamenán stejný trend jako v případě indolu. Stejná situace byla i v případě androstenonu.

V případě mutace g.2412C>T zvířata s genotypem *TT* měla nejnižší hodnoty skatolu oproti genotypu *CT* a *CC*, ale zde nebyl tento vztah statisticky významný. Hladina indolu u kříženců však významně ovlivněna byla. Zvířata s genotypem *TT* měla opět nejnižší koncentraci indolu v tuku. To je ve shodě se závěry Mörlein *et al.,* (2012), kteří rovněž potvrzují signifikantní vliv genotypu *CC* na zvýšení hladiny skatolu a indolu.

Lin *et al.,* (2006) a Moe *et al.,* (2009), popisují signifikantní vliv c.1423 G>A na hladinu skatolu. Lin *et al.,* (2006) nalezl asociaci alely *A* se zvýšenou hladinou skatolu v tuku prasat. Moe *et al.,* (2009) oproti tomu uvádějí pozitivní efekt alely *A* na hladinu skatolu a rovněž na hladinu indolu u populace prasat plemene duroc i u populace prasat plemene norské landrace. Avšak studie Skinner *et al.,* (2005), nenachází významné asociace mezi hladinou skatolu a

testovanou mutací u dánské komerční populace, která byla testována pro asociace s hladinou skatolu v tuku prasat. V naší testované populaci ($ČBU_S$ x ($ČL_S$ x $ČBU_D$)) byl rovněž pozorován významný vliv na hladinu indolu ($P<0,05$), kdy nejnižší koncentrace indolu byla zaznamenána u genotypu *AA* a nejvyšší u genotypu *GG*. Rozdílné výsledky mohou být zapříčiněny jednak rozdílnými populacemi prasat, jednak přítomností synonymní mutace (c.1422C>T) v exonu 9, která nebyla brána v úvahu ve zmiňovaných pracích Lin *et al.,* (2006) a Skinner *et al.,* (2005).

Výsledky této studie ukazují, že SNP: c.1423G>A; c.1422C>T; c.1502G>T a g.2412 C>T detekované v prasečím genu *CYP2E1* mají vliv na hladinu kančího pachu, především pak indolových sloučenin. Tyto SNP mohou napomoci při šlechtění proti vysoké koncentraci skatolu a indolu v tukové tkáni prasat. Je ale nutné vzít v úvahu rozdíly mezi hybridními kombinacemi, které mohou být způsobeny mimo jiné nestejnou frekvencí alel a genotypů. Ta byla rovněž studována na vzorku populace 8 kulturních plemen a prasete divokého. Rozdíly v zastoupení jednotlivých genotypů se mezi čistokrevnými zvířaty projevily, což může být komplikací při tvorbě vhodných hybridů pro užitkový chov.

6.3 Vliv různé hladiny složek kančího pachu na užitkové vlastnosti prasat

H3. Úroveň hladiny androstenonu a skatolu v těle prasat v závislosti na pohlaví ovlivňuje parametry růstu a jatečné hodnoty.

Publikace 4:

Stupka, R., Čítek, J., Vehovský, K., **Zadinová, K**., Okrouhlá, M., Urbanová, D., Stádník, L. 2017. Effects of immunocastration on growth performance, body composition, meat quality and boar taint. Czech Journal of Animal Science. 62 (6), 249-258.

Cílem práce bylo zhodnotit vliv imunokastrace na ukazatele výkrmnosti, utváření jatečných těl, kvalitu masa a eliminaci výskytu kančího pachu oproti vykrmovaným kanečkům, vepříkům a prasničkám v období mezi první a druhou vakcinací a následně mezi druhou vakcinací a porážkou.

Mezi sledovanými skupinami nebyly zjištěny statisticky významné rozdíly v živé hmotnosti. Přesto nejvyšší porážkové hmotnosti dosáhli imunokastráti (106,99 kg) a nejnižší prasničky (104,17 kg). Při hodnocení průměrného denního přírůstku lze konstatovat, že se neprojevil vliv doby vakcinace na změnu intenzity růstu u imunokastrátů oproti ostatním sledovaným skupinám s vyjímkou vepříků. Nejvyšší přírůstek za období testu byl zjištěn u vepříků (1193 g), dále u imunokastrátů a prasniček (1181 g), a nejnižší pak u skupiny kanečků (1169 g). Ke shodným výsledkům došli ve svých studiích rovněž Pauly *et al.,* (2008) a D´Souza *et* Mullan, (2002). Někteří autoři (Dostálová *et* Koucký, 2008, Škrlep *et al.,* 2012) naopak prokázali příznivější výsledky u kanečků oproti ostatním skupinám. Tyto rozdíly mohou ovšem souviset se sledovanou hyridní kombinací prasat či systémem ustájení. Je rovněž nutno počítat s výskytem agresivního pohlavního chování kanců, které má vliv na příjem krmiva. S poklesem pohlavních hormonů po 2. vakcinaci toto chování mizí a u zvířat stoupá spotřeba krmiva, a tím i průměrný denní přírůstek.

Lze také říci, že se u imunokastrátů neprojevil negativní vliv 2. vakcinace na sledované finální ukazatele jatečné hodnoty v porovnání s nekastrovanými kanečky a prasničkami. Oproti tomu vepříci dosahovali horších ukazatelů ve srovnání s ostatním sledovanými skupinami. U sledovaných ukazatelů charakterizujících kvalitu vepřového masa nebyly zjištěny žádné statisticky významné rozdíly, a to jak u pH, barvy masa, ztrát odkapem, síly střihu a podílu intramuskulárního tuku. Dosažené hodnoty u imunokastrátů se velmi přibližovaly zjištěným hodnotám u kanečků a prasniček.

Metoda kastrace měla statisticky průkazný vliv na obsah androstenonu a skatolu v tukové tkáni prasat, který se zjišťoval po porážce. Nejvyšších hodnot dosahovali nekastrovaní kanečci, potom imunokastráti a téměř shodných hodnot dosáhli prasničky a vepříci. Tyto výsledky potvrzují i Doran *et al.,* (2012) a Zamaratskaia *et al.,* (2008b).

Z výsledků této studie je zřejmé, že při hodnocení vybraných ukazatelů nebyly zjištěny průkazné rozdíly mezi imunokastráty, kanečky a prasničkami, ale byly zjištěny rozdíly mezi vepříky a ostatními skupinami.

7 Závěry a doporučení pro praxi

Vzhledem k blížícímu se zákazu chirurgické kastrace bez anestezie, která byla doposud jednou z hlavních metod, jak zabránit nepříjemnému kančímu pachu, je třeba hledat nové cesty jak řešit tento problém. Protože nepříjemný zápach kančího masa je způsoben několika různými substancemi - androstenonem, skatolem a indolem, není jeho odstranění jednoduché.

Cílem disertační práce bylo identifikovat geny, které se podílejí na syntéze a metabolismu androstenonu, skatolu a indolu, hlavních složek podílejících se na vzniku kančího pachu. Dále pak posoudit možný vliv těchto genů na hladinu androstenonu skatolu a indolu v tukové a svalové tkáni prasat. A také posoudit vliv různé úrovně hladiny těchto látek na parametry růstu, jatečné hodnoty a v neposlední řadě na kvalitu vepřového masa.

- Byly identifikovány geny, které řídí činnost enzymů, především CYP2E1, ale i sulfostransferázy SULT2A1, SULT2B1, SULT1A1, hydroxysteroid dehydrogenázy, zvláště HSD3B1 a HSD17B7. Jak vyplývá z předkládaných prací činnost těchto enzymů je možmo částečně ovlivnit genetickou selekcí.
- V případě genu *CYP2E1* byly identifikovány SNP především v promotorové oblasti (g.2412 C>T) a v exonu 9 (c.1423G>A; c.1422C>T), které průkazně ovlivňují hladinu indolu a částečně skatolu v tukové tkáni kanců.
- U polymorfismů g.2412 C>T a c.1422C>T byl statisticky průkazný vliv na hladinu indolu u testované hybridní populace prasat. Jedinci s genotypem *TT* (g.2412 C>T) a *CC* (c.1422C>T) mají průkazně nižší hladinu indolu oproti ostatním genotypům. Nebyl však prokázán vzájemný vztah mezi těmito SNP, alespoň ne u této testované populace.
- Další možností jak snížit intenzitu kančího pachu je prostřednictvím výživy, respektive krmných doplňků. Ty mohou jednak ovlivnit množství tzv. buněčného odpadu, který je zdrojem L-tryptofanu.
- V této souvislosti je vhodné sledovat vliv výživy na expresi výše zmíněných cytochromových genů především *CYP2E1*.
- Bylo prokázáno, že rozdílná hladina androstenonu a skatolu v případě porovnávání různých metod kastrace - chirurgická kastrace, imunokastrace nebo výkrm kanečků, nemusí výrazně ovlivňovat parametry růstu a jatečné hodnoty ani ukazatele kvality masa.
- V případě využití genetické selekce je ale třeba intenzivněji se zabývat vlivem genů na ukazatele výkrmnosti a jatečné hodnoty, jelikož některé kandidátní geny, které by mohly být

využity pro šlechtění proti kančímu pachu, se nacházejí na chromozomech, kde jsou i geny pro ukazatele jatečné hodnoty a kvalitu masa (SSC4, SSC6).

- Rovněž je třeba brát v úvahu vliv na reprodukci kanců. Některé geny (např. *HSD17B7*) zapojené do syntézy androstenonu se podílejí i na syntéze testosteronu a dalších pohlavních hormonů a jejich pokles by byl u kančí populace nežádoucí.
- Vzhledem k získaným poznatkům je třeba pečlivě vybírat geny a SNP pro případnou selekci zvířat.
- Předmětem dalších studií by měly být rovněž rozdíly mezi jednotlivými plemeny a hybridními populacemi prasat.
- Na základě zjištěných výsledků se jeví jako vhodná kombinace více metod pro odstranění kančího pachu nebo zabránění jeho vzniku. Například výběr populace zvířat s vhodným genotypem a využití přídavku krmného aditiva.

8 Seznam použité literatury

1. Aleksic, J., Dokmanovic, M., Aleksic, Z., Teodorovic, V., Stojic, V., Trbovic, D., Baltic, M. Z. 2012. Investigation of the efficacy of immunocastration aimed at the prevention of sex odour in boar meat. Acta Veterinaria-Beograd. 62, 653-663.

2. Andersson, H. K., Hullberg, A., Malmgren, L., Lundstrom, K., Rydhmer, L., Squires, J. 1999. Sexual maturity in entire male pigs - Environmental effects, relations to skatole level and female puberty. Acta Agriculturae Scandinavica Section and Animal Science. 49, 103-112.

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

4. Andresen, Ø. 2006. Boar taint related compounds: Androstenone/skatole/other substances. Acta Veterinaria Scandinavica. 48(Suppl 1):S5.

5. Babol, J., Squires E. J. 1995. Quality of meat from intact male pigs. Food Research International. 28, 201–212.

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

7. Babol, J., Squires, E. J., Lundstrom, K. 1999. Relationship between metabolism of androstenone and skatole in intact male pigs. Journal of Animal Science. 77, 84-92.

8. Babol, J., Squires, J., Lundstrom, K. 1998b. Hepatic metabolism of skatole in pigs by cytochrome P4502E1. Journal of Animal Science. 76, 822-828.

9. Billen, M. J., Squires, E. J. 2009. The role of porcine cytochrome b5A and cytochrome b5B in the regulation of cytochrome P45017A1 activities. Journal of Steroid Biochemistry and Molecular Biology. 113, 98-104.

10. Bidanel, J. P., Riquet, J., Gruand, J., Squires, J., Bonneau, M., Milan, D. 2006. Detection of quantitative trait loci for skatole and indole levels in meishan x large white F2 pigs. Proc. 8th World Congress on Genetics Applied to Livestock Production. Belo Horizonte. Minas Gerais. Brazil. WCGALP. Belo Horizonte. Minas Gerais.Brazil.

11. Bonneau, M., Kempster, A. J., Claus, R., Claudi-Magnussen, C., Diestre, A., Tornberg, E., Walstra, P., Chevillon, P., Weiler, U., Cook, G. L. 2000a. An international study on the importance of androstenone and skatole for boar taint: I. Presentation of the programme and measurement of boar taint compounds with different analytical procedures. Meat Science. 54, 251-259.

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

13. Borowska, A., Szwaczkowski, T., Koćwin-Podsiadła, M., Kamiński, S., Ruść, A., Krzęcio-Nieczyporuk, E. 2014. Associations of fifty single nucleotide polymorphisms within candidate genes with meatness in pigs. Czech Journal of Animal Science. 59 (5), 227-237.

14. Cederbaum, A. I., Wu, D. F., Mari, M., Bai, J. X. 2001. CYP2E1-dependent toxicity and oxidative stress in HEPG2 cells. Free Radical Biology and Medicine. 31, 1539-1543.

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

16. Claus, R., Hoffmann, B., Karg, H. 1971. Determination of 5-androst-16-en-3-one, a boar taint steroid in pigs, with reference to relationships to testosterone. Journal of Animal Science. 33, 1293–1297.

17. Chen, G. 2007. Physiological and Biochemical Factors Responsible for Boar Taint. Doctoral thesis, Swedish University of Agricultural Sciences. Uppsala. 53s.

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

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

20. Chung, B., Matteson, K. J., Voutilainen, R., Mohandas, T. K., Miller, W. L. 1986. Human cholesterol side-chain cleavage enzyme, P450SCC - cDNA cloning assignment of the gene to chromosome-15, and expression in the placenta. Proceedings of the National Academy of Sciences of the United States of America. 83, 8962-8966.

21. Dasgupta, S., Sirish,a P. V. S., Neelaveni, K., Anuradha, K., Sudhakar, G., Mohan Reddy, B. 2012. Role of luteinizing hormone β-subunit gene variants among South Indian women with polycystic ovary syndrome. Gene 494, 51–56.

22. Davis, S. M., Squires, E. J. 1999. Association of cytochrome b(5) with 16-androstene steroid synthesis and the testis and accumulation in the fat of male pigs. Journal of Animal Science. 77, 1230-1235.

23. Deslandes, B., Gariepy, C., Houde, A. 2001. Review of microbiological and biochemical effects of skatole on animal production. Livestock Production Science. 71, 193-200.

24. De Campos C. F., Lopes M. S., E Silva F. F., Veroneze R., Knol E. F., Lopes P. S., Guimarães S. E. F. 2015. Genomic selection for boar taint compounds and carcass traits in a commercial pig population. Livestock Science 174, 10-17.

25. Diaz, G. J., Skordos, K. W., Yost, G. S., Squires, E. J. 1999. Identification of phase I metabolites of 3-methylindole produced by pig liver microsomes. Drug Metabolism and Disposition. 27, 1150-1156.

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

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

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

29. Dostálová, A., Koucký, M. (2008). Výkrm kanečků v podmínkách ekologického zemědělství. Výzkumný ústav živočišné výroby, v.v.i., Praha-Uhříněves, Metodika, 46s.

30. D´Souza, D.N., Mullan, B.P. (2002). The effect of genotype, sex and management strategy on the eating quality of pork. Meat Science. 60, 95 –101.

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

32. Dumas, N. A., He, D., Frost, A. R., Falany, C. N. 2008. Sulfotransferase 2B1b in

human breast: Differences in subcellular localization in African American and Caucasian women. Journal of Steroid Biochemistry and Molecular Biology. 111, 171-177.

33. Dvořáková, V., Stupka, R., Šprysl, M., Čítek, J., Okrouhlá, M., Kluzáková, E., Kratochvílová, H. 2011. Effect of the missense mutation Asp298Asn in *MC4R* on growth and fatness traits in commercial pig crosses in the Czech Republic. Czech Journal of Animal Science. 56 (4), 176–180.

34. Engelsma, K. A., R. Bergsma, R., Harlizius, B., Ducro-Steverink, D., Knol, E. 2007. Genetic parameters for components of boar taint and their relation with carcass quality and female fertility. EAAP. Dublin, Ireland, August. 26–29.

35. Ernst C. W., Steibel J. P. 2013. Molecular advances in QTL discovery and application in pig breeding. Trends in Genetics. 29 (4), 215-224.

36. Fàbrega, E., Gispert, M., Tibau, J., Hortós, M., Oliver, M. A., Font i Furnols, M. 2011. Effect of housing system, slaughter weight and slaughter strategy on carcass and meat quality, sex organ development and androstenone and skatole levels in Duroc finished entire male pigs, Meat Science. 89, 434–439.

37. Falany, C. N., He, D., Dumas, N., Frost, A. R., Falany, J. L. 2006. Human cytosolic sulfotransferase 2B1: Isoform expression, tissue specificity and subcellular localization. Journal of Steroid Biochemistry and Molecular Biology. 102, 214-221.

38. Fouilloux, M. N., LeRoy, P., Gruand, J., Renard, C., Sellier, P., Bonneau, M. 1997. Support for single major genes influencing fat androstenone level and development of bulbo-urethral glands in young boars. Genetics Selection Evolution. 29, 357-366.

39. Frieden, L., Looft, C., Tholen, E. 2011. Breeding for reduce boar taint. Lohmann Information. 46 (1), 21-27.

40. Funk-Keenan, J., Sacco, J., Wong, Y. Y., Rasmussen, S., Motsinger-Reif, A.,

Trepanier, L. A. 2012. Evaluation of Polymorphisms in the Sulfonamide Detoxification Genes CYB5A and CYB5R3 in Dogs with Sulfonamide Hypersensitivity. Journal of Veterinary Internal Medicine. 26, 1126-1133.

41. Giovangrandi, Y., Parfait, B., Asheuer, M., Olivi, M., Lidereau, R., Vidaud, M., Bieche, I. 2001. Analysis of the human CGB/LHB gene cluster in breast tumors by real-time quantitative RT-PCR assays. Cancer Letters. 168, 93-100.

42. Guengerich, F. P., 2008. Cytochrome P450 and chemical toxicology. Chemical Research in Toxicology. 21, 70–83.

43. Gonzalez, F. J. 2005. Role of cytochromes P450 in chemical toxicity and oxidative stress: studies with CYP2E1. Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis. 569, 101-110.

44. Gray, M. A., Squires, E. J. 2013a. Effects of nuclear receptor transactivation on boar taint metabolism and gene expression in porcine hepatocytes. Journal of Steroid Biochemistry and Molecular Biology. 133, 110-119.

45. Gray, M. A., Squires, E. J. 2013b. Effects of nuclear receptor transactivation on steroid hormone synthesis and gene expression in porcine Leydig cells. Journal of Steroid Biochemistry and Molecular Biology. 133, 93-100.

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

47. Grindflek, E., Berget, I., Moe, M., Oeth, P., Lien, S. 2010. Transcript profiling of candidate genes in testis of pigs exhibiting large differences in androstenone levels. BMC Genetics. 11.

48. Grindflek, E., Lien, S., Hamland, H., Hansen, M. H. S., Kent, M., van Son, M., Meuwissen, T. H. E. 2011a. Large scale genome-wide association and LDLA

mapping study identifies QTLs for boar taint and related sex steroids. BMC Genomics. 12.

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

50. Grindflek, E., Szyda, J. Liu, Z., Lien, S. 2001. Detection of quantitative trait loci for meat quality in a commercial slaughter pig cross. Mammalian Genome. 12, 299–304.

51. Große-Brinkhaus, CH., Storck, L. C., Frieden, L., Neuhoff, Ch., Schellander, K., Looft, Ch., Tholen, E. 2015. Genome-wide association analyses for boar taint components and testicular traits revealed regions having pleiotropic effects. BMC Genetics. 16, 36.

52. Gunawan, A., Sahadevan, S., Neuhoff, Ch., Große-Brinkhaus, Ch., Gad, A., Frieden, L., Tesfaye D., Tholen, E., Looft, Ch., Uddin, M. J., Schellander, K., Cinar, M. L. 2013. RNA Deep sequencing reveals novel candidate genes and polymorphisms in boar testis and liver tissues with divergent androstenone levels. PLoS ONE 8, e63259.

53. Hansen-Møller, J. 1994. Rapid high-performance liquid-chromathography method for simultaneous determination of androstenone, skatole and indole in back fat from pigs. Journal of Chromatography B-Biomedical Applications. 661, 219-230.

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

55. Hidalgo, A. M., Bastiaansen, J. W. M., Harlizius, B., Megens, H. J., Madsen, O., Crooijmans, R. P. M. A., Groenen, M. A. M. 2014. On the relationship between an

Asian haplotype on chromosome 6 that reduces androstenone levels in boars and the differential expression of SULT2A1 in the testis. BMC Genetics. 15.

56. Huang, C., Zhou, T., Chen, Y., Sun, T., Zhang, S., Chen, G. 2014. Estrogen-Related Receptor ERR alpha Regulation of Human Hydroxysteroid Sulfotransferase (SULT2A1) Gene Expression in Human Caco-2 Cells. Journal of Biochemical and Molecular Toxicology. 28, 32-38.

57. Jacquemin, P., Chen, Z., Martial, J. A., Davidson, I. 1999. Genomic structure and chromosomal mapping of the mouse transcription factor TEF-5 (*Tead3*) gene. Mammalian Genome. 10, 632-634.

58. Jacquemin, P., Martial, J. A., Davidson, I. 1997. Human TEF-5 is preferentially expressed in placenta and binds to multiple functional elements of the human chorionic somatomammotropin-B gene enhancer. Journal of Biological Chemistry. 272, 12928-12937.

59. Ji, Y., Moon, I., Zlatkovic, J., Salavaggione, O. E., Thomae, B. A., Eckloff, B. W., Wieben, E. D., Schaid, D. J., Weinshilboum, R. M. 2007. Human hydroxysteriod sulfotransferase SULT2B1 pharmacogenomics: Gene sequence variation and functional genomics. Journal of Pharmacology and Experimental Therapeutics. 322, 529-540.

60. Kim, J. M., Ahn, J. H., Lim, K. S., Lee, E. A., Chun, T., Hong, K. C. 2013. Effects of hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1 polymorphisms on fat androstenone level and gene expression in Duroc pigs. Animal Genetics. 44, 592-595.

61. Kim, K. S., Larsen, N., Short, T., Plastow, G., Max F. Rothschild, M. F. 2000. A missense variant of the porcine melanocortin-4 receptor (*MC4R*) gene is associated with fatness, growth, and feed intake traits. Mammalian Genome. 11, 131–135.

62. Konstandi, M., Cheng, J., Gonzalez, F. J. 2013. Sex steroid hormones regulate constitutive expression of *Cyp2e1* in female mouse liver. American Journal of

Physiology-Endocrinology and Metabolism. 304, E1118-E1128.

63. Koop, D. R., Casazza, J. P. 1985. Identification of ethanol – inducible P-450 isozyme 3A as the acetone and acetol monooxygenase of rabbit microsomes. Journal of Biological Chemistry. 260, 3607-3612.

64. Kurogi, K., Sakakibara, Y., Kamemoto, Y., Takahashi, S., Yasuda, S., Liu, M.-C., Suiko, M. 2010. Mouse cytosolic sulfotransferase SULT2B1b interacts with cytoskeletal proteins via a proline/serine-rich C-terminus. Febs Journal. 277, 3804-3811.

65. Lanthier, F., Lou, Y., Squires, E. J. 2007. Skatole metabolism in the intact pre-pubescent male pig: The relationship between hepatic enzyme activity and skatole concentrations in plasma and fat. Livestock Science. 106, 145-153.

66. Lee, G. J., Archibald, A. L., Law, A. S., Lloyd, S., Wood, J., Haley, C. S. 2005. Detection of quantitative trait loci for androstenone, skatole and boar taint in a cross between Large White and Meishan pigs. Animal Genetics. 36, 14-22.

67. Lervik, S., Oskam, I., Krogenaes, A., Andresen, O., Dahl, E., Haga, H. A., Tajet, H., Olsaker, I., Ropstad, E. 2013. Androstenone and testosterone levels and testicular morphology of Duroc boars related to estimated breeding value for androstenone. Theriogenology. 79, 986-994.

68. Leung, M. C. K., Bowley, K. L., Squires, E. J. 2010. Examination of testicular gene expression patterns in yorkshire pig with high and low levels of boar taint. Animal Biotechnology. 21, 77-87.

69. Lin, Z., Lou, Y., Squires, E. J., 2004a. Molecular cloning, expression and functional characterization of the cytochrome P450 2A6 gene in pig liver. Animal Genetics. 35, 314-316.

70. Lin, Z. H., Lou, Y. P., Peacock, J., Squires, E. J., 2005a. A novel polymorphism in the 5 ' untranslated region of the porcine cytochrome b5 (*CYB5*) gene is associated

with decreased fat androstenone level. Mammalian Genome 16, 367-373.

71. Lin, Z.H., Lou, Y.P., Squires, E.J. 2004b. Molecular cloning and functional analysis of porcine *SULT1A1* gene and its variant: a single mutation *SULT1A1* causes a significant decrease in sulfation activity. Mammalian Genome. 15, 218-226.

72. Lin, Z. H., Lou, Y. P., Squires, E. J. 2005b. Identification of a single nucleotide polymorphism in porcine testis cytochrome P450-c17 (CYP17) and its effect on steroidogenesis. Biochemical Genetics. 43, 531-542.

73. Lin, Z. H., Lou, Y. P., Squires, E. J. 2006. Functional polymorphism in porcine CYP2E1 gene: Its association with skatole levels. Journal of Steroid Biochemistry and Molecular Biology. 99, 231-237.

74. Liu, Y., Xu, Y., Li, F., Chen, H., Guo, S. 2013. CYP2A6 deletion polymorphism is associated with decreased susceptibility of lung cancer in Asian smokers: a meta-analysis. Tumor Biology. 34, 2651-2657.

75. Lemley, C.O., Wilmoth, T.A., Tager, L.R., Krause, K.M., Wilson, M.E. (2010). Effect of a high cornstarch diet on hepatic cytochrome P450 2C and 3A activity and progesterone half-life in dairy cows. Journal of Dairy Science. 93, 1012–1021.

76. Losel, D., Lacorn, M., Buttner, D., Claus, R. 2006. Flavor improvement in pork from barrows and gilts via inhibition of intestinal skatole formation with resistant potato starch. Journal of Agricultural and Food Chemistry. 54, 5990–5995.

77. Lu, Y., Cederbaum, A. I. 2008. CYP2E1 and oxidative liver injury by alcohol. Free Radical Biology and Medicine. 44, 723-738.

78. Lukić B., Pong-Wong R., Rowe S. J., De Koning D. J., Velander I., Haley C. S., Archibald A. L., Woolliams J. A. 2015 Efficiency of genomic prediction for boar taint reduction in Danish Landrace pigs. Animal Genetics. 46, 607-616.

79. Lundström, K., Zamaratskaia, G. 2006. Moving towards taint-free pork – alternatives

to surgical castration. Acta Veterinaria Scandinavica. 48(Suppl 1), S1.

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

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

82. Moe, M., Lien, S., Aasmundstad, T., Meuwissen, T. H. E., Hansen, M. H. S., Bendixen, C., Grindflek, E. 2009. Association between SNPs within candidate genes and compounds related to boar taint and reproduction. BMC Genetics. 10.

83. Moe, M., Lien, S., Bendixen, C., Hedegaard, J., Hornshoj, H., Berget, I., Meuwissen, T. H. E., Grindflek, E. 2008. Gene expression profiles in liver of pigs with extreme high and low levels of androstenone. BMC Veterinary Research. 4.

84. Moe, M., Meuwissen, T., Lien, S., Bendixen, C., Wang, X. F., Conley, L. N., Berget, I., Tajet, H., Grindflek, E. 2007b. Gene expression profiles in testis of pigs with extreme high and low levels of androstenone. BMC Genomics. 8.

85. Morlein, D., Lungershausen, M., Steinke, K., Sharifi, A. R., Knorr, C. 2012. A single nucleotide polymorphism in the CYP2E1 gene promoter affects skatole content in backfat of boars of two commercial Duroc-sired crossbred populations. Meat Science. 92, 739-744.

86. Nakamura Y., Xing Y., Hui X. G., Kurotaki Y., Ono K., Cohen T., Sasano H., Rainey W. E. 2011. Human adrenal cells that express both 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2) and cytochrome b5 (CYB5A) contribute to adrenal androstenedione production. Journal of Steroid Biochemistry and Molecular Biology. 123, 122–126.

87. Neuhoff, C., Gunawan, A., Farooq, M. O., Cinar, M. U., Große-Brinkhaus C., Sahadevana, S., Frieden, L., Tesfaye, D., Tholen, E., Looft, C., Schellander, K., Uddin, M. U. 2015. Preliminary study of FMO1, FMO5, CYP21, ESR1, PLIN2 and SULT2A1 as candidate gene for compounds related to boar taint. Meat Science. 108, 67-73.

88. Nicolau-Solano, S. I., McGivan, J. D., Whittington, F. M., Nieuwhof, G. J., Wood, J. D., Doran, O. 2006. Relationship between the expression of hepatic but not testicular 3 beta-hydroxysteroid dehydrogenase with androstenone deposition in pig adipose tissued. Journal of Animal Science. 84, 2809-2817.

89. Nugon-Baudon, L., Roland, N., Flaoenoaoes, J-P., Beauhe, P. (1996). Hepatic cytochrome P450 and UDP-glucuronosyl transferase are affected by five sources of dietary fiber in germ-free rats. Journal of Nutrition. 126, 403–409.

90. Obrochta, K. M., Krois, CH, R., Campos, B., Napoli, J, L. 2015. Insulin Regulates Retinol Dehydrogenase Expression and All-trans-retinoic Acid Biosynthesis through FoxO1. The Journal of Biological Chemistry. 290 (11), 7259–7268.

91. Oskam, I. C., Lervik, S., Tajet, H., Dahl, E., Ropstad, E., Andresen, O. 2010. Differences in testosterone, androstenone, and skatole levels in plasma and fat between pubertal purebred Duroc and Landrace boars in response to human chorionic gonadotrophin stimulation. Theriogenology. 74, 1088-1098.

92. Oskam, I. C., Ropstad, E., Andersen Berg, K., Fredriksen, B., Larsen, S., Dahl, E., Andresen, Ø. 2008. Testicular germ cell development in relation to 5a-Androstenone levels in pubertal entire male pigs. Theriogenology. 69, 967–976.

93. Panella-Riera, N., Moe, M., Grindflek, E., Oliver, M. A., Wood, J. D., Doran, O. 2008. Effect of sex steroids on expression of sulfotransferase 2B1 immunoreactive protein in primary cultured porcine hepatocytes. Livestock Science. 118, 223-230.

94. Pauly, C., Spring,P., O´Doherty,J.V., Ampuero Kragten,S., Bee,G. (2008).

Performances, meat quality and boar taint of castrates and entire male pigs fed a standard and a raw potato starch-enriched diet. Anim,2 (11), 1707 –1715.

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

96. Payne, A. H., Hales, D. B. 2004. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. Endocrine Reviews. 25, 947-970.

97. Peacock, J., Lou, Y., Lundstrom, K., Squires, E. J. 2008. The effect of a c.-8G > T polymorphism on the expression of cytochrome b5A and boar taint in pigs. Animal Genetics. 39, 15-21.

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

99. Quintanilla, R., Demeure, O., Bidanel, J. P., Milan, D., Iannuccelli, N., Amigues, Y., Gruand, J., Renard, C., Chevalet, C., Bonneau, M. 2003. Detection of quantitative trait loci for fat androstenone levels in pigs. Journal of Animal Science. 81, 385-394.

100. Rasmussen, M.K., Brunius, C., Zamaratskaia, G., Ekstrand, B. (2012). Feeding dried chicory root to pigs decrease androstenone accumulation in fat by increasing hepatic 3 beta hydroxysteroid dehydrogenase expression. Journal of Steroid Biochemistry and Molecular Biology. 130, 90–95.

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

102. Ramos, A. M., Duijvesteijn, N., Knol, E. F., Merks, J. W. M., Bovenhuis, H.,

Crooijmans, R., Groenen, M. A. M., Harlizius, B. 2011. The distal end of porcine chromosome 6p is involved in the regulation of skatole levels in boars. BMC Genetics. 12.

103. Regueiro, J. A. G., Rius, M. A. 1998. Rapid determination of skatole and indole in pig back fat by normal-phase liquid chromatography. Journal of Chromatography A. 809, 246-251.

104. Robic, A., Feve, K., Larzul, C., Billon, Y., van Son, M., Liaubet, L., Sarry, J., Milan, D., Grindflek, E., Bidanel, J. P., Riquet, J. 2011a. Expression levels of 25 genes in liver and testis located in a QTL region for androstenone on SSC7q1.2. Animal Genetics. 42, 662-665.

105. Robic, A., Larzul, C., Bonneau, M. 2008. Genetic and metabolic aspects of androstenone and skatole deposition in pig adipose tissue: A review. Genetics Selection Evolution. 40, 129-143.

106. Robic, A., Larzul, C., Grindflek, E., Chevillon, P., Hofer, A., Feve, K., Iannuccelli, N., Milan, D., Prunier, A., Riquet, J. 2012. Molecular characterization of the porcine *TEAD3 (TEF-5)* gene: examination of a promoter mutation as the causal mutation of a quantitative trait loci affecting the androstenone level in boar fat. Journal of Animal Breeding and Genetics. 129, 325-335.

107. Robic, A., Le Mignon, G., Feve, K., Larzul, C., Riquet, J. 2011b. New investigations around *CYP11A1* and its possible involvement in an androstenone QTL characterised in Large White pigs. Genetics Selection Evolution. 43.

108. Rowe S. J., Karacaören B., De Koning D. J., Lukić B., Hastings-Clark N., Velander I., Haley C. S., Archibald A. L. 2014. Analysis of the genetics of boar taint reveals both single SNPs and regional effects. BMC Genomics. 15, 424.

109. Sahadevan S., Tholen E., Große-Brinkhaus C., Schellander K., Tesfaye D., Hofmann-Apitius M., Cinar M. U., Gunawan A., Hölker M., Neuhoff C. 2015. Identification of gene co-expression clusters in liver tissues from multiple porcine

populations with high and low backfat androstenone phenotype. BMC Genetics. 16, 21.

110. Sellier, P., Le Roy, P., Fouilloux, M. N., Gruand, J., Bonneau, M. 2000. Responses to restricted index selection and genetic parameters for fat androstenone level and sexual maturity status of young boars. Livestock Production Science. 63, 265-274.

111. Simard, J., Ricketts, M. L., Gingras, S., Soucy, P., Feltus, F. A., Melner, M. H. 2005. Molecular biology of the 3*β*-hydroxysteroid dehydrogenase/delta 5–4 isomerase gene family. Endocrine Reviews. 26, 525–82.

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

113. Skinner, T. M., Anderson, J. A., Haley, C. S., Archibald, A. L. 2006. Assessment of *SULT1A1, CYP2A6* and *CYP2C18* as candidate genes for elevated backfat skatole levels in commercial and experimental pig populations. Animal Genetics. 37, 521-522.

114. Skinner, T. M., Doran, E., McGivan, J. D., Haley, C. S., Archibald, A. L. 2005. Cloning and mapping of the porcine cytochrome-*p450 2E1* gene and its association with skatole levels in the domestic pig. Animal Genetics. 36, 417-422.

115. Squires, E. J. 2006. Possibilities for selection against boar taint. Acta Veterinaria Scandinavica. 48(Suppl 1), S8.

116. Squires E. J., Young L. G., Hacker R. R. & Adeola O. 1993 The role of growth hormones, ß-adrenergic agents and intact males in pork production: a review. Canadian Journal of Animal Science. 73, 1–23.

117. Squires, E. J., Lundstrom, K. 1997. Relationship between cytochrome P450IIE1 in liver and levels of skatole and its metabolites in intact male pigs. Journal of Animal

Science. 75, 2506-2511.

118. Strathe, A. B., Velander, I. H., Mark, T., Kadarmideen, H. N. 2013a. Genetic parameters for androstenone and skatole as indicators of boar taint and their relationship to production and litter size traits in Danish Landrace. Journal of Animal Science. 91, 2587-2595.

119. Strathe, A. B., Velander, I. H., Mark, T., Ostersen, T., Hansen, C., Kadarmideen, H. N. 2013b. Genetic parameters for male fertility and its relationship to skatole and androstenone in Danish Landrace boars. Journal of Animal Science. 91, 4659-4668.

120. Šimeček, K., Zeman, L., Heger, J. 1995. Potřeba živin a tabulky výživné hodnoty krmiv pro prasata. VÚZV Pohořelice, MZe ČR. Pohořelice. 103 s.

121. Škrlep, M., Batorek, N., Bonneau, M., Prevolnik, M., Kubele, V., ČandekPotokar, M. (2012). Effect of immunocastration in group-housed commercial fattening pigs on reproductive organs, malodorous compounds, carcass and meat quality. Czech Journal of Animal Science. 57 (6), 290–299.

122. Tajet, H., Andresen, O., Meuwissen, T. 2006. Estimation of genetic parameters of boar taint; skatole and androstenone and their correlations with sexual maturation. Acta Veterinaria Scandinavica. 48(Suppl 1) :S9.

123. Tambyrajah, W. S., Doran, E., Wood, J. D., McGivan, J. D., 2004. The pig CYP2E1 promoter is activated by COUP-TF1 and HNF-1 and is inhibited by androstenone. Archives of Biochemistry and Biophysics 431, 252-260.

124. Tengstrom, M., Mannermaa, A., Kosma, V. - M., Hirvonen, A., Kataja, V. 2012. *SULT1A1* rs9282861 polymorphism-a potential modifier of efficacy of the systemic adjuvant therapy in breast cancer?. Bmc. Cancer 12.

125. Van den Broeke, A., Aluwé, M., Tuyttens, F. A. M., Ampe, B., Vanhaecke, L., Wauters, J., Janssens, S., Coussé, A., Buys, N., Millet, S. 2015a. An intervention

study demonstrates effects of *MC4R* genotype on boar taint and performances of growing–finishing pigs. Journal of Animal Science. 93, 934–943.

126. Van den Broeke1, A., M. Aluwé1, M., Janssens, S., Wauters, J., L. Vanhaecke, L., Buys, N., Millet, S., Tuyttens, F. A. M. 2015b. The effect of the *MC4R* gene on boar taint compounds, sexual maturity and behaviour in growing-finishing boars and gilts. Animal, 1-10.

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

128. Verheyden, K., Noppe, H., Aluwe, M., Millet, S., Bussche, J. V., De Brabander, H. F. 2007. Development and validation of a method for simultaneous analysis of the boar taint compounds indole, skatole and androstenone in pig fat using liquid chromatography-multiple mass spectrometry. Journal of Chromatography A. 1174, 132-137.

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

130. Walstr, P., Merkus, G. S. M. 1995. Procedure for assessment of the lean meat percentage as a consequence of the new EU reference dissection metod in pig carcass classification. DLO- Research Institut for Animal Science and Healt Research Branch. Zeist. The Netherlands. 22.

131. Wang, Y. F., Spitz, M. R., Tsou, A. M. H., Zhang, K. R., Makan, N., Wu, X. F. 2002. Sulfotransferase (SULT) 1A1 polymorphism as a predisposition factor for lung cancer: a case-control analysis. Lung Cancer 35, 137-142.

132. Wesoly, R., Weiler, U. 2012. Nutritional influences on skatole formation and skatole metabolism in the pig. Animals. 2, 221-242.

133. Whittington, F. M., Nute, G. R., Hughes, S. I., McGivan, J. D., Lean, I. J., Wood, J. D., Doran, E. 2004. Relationships between skatole and androstenone accumulation, and cytochrome P4502E1 expression in Meishan x Large White pigs. Meat Science. 67, 569-576.

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

135. Wijesuriya, S. D., Zhang, G. R., Dardis, A., Miller, W. L. 1999. Transcriptional regulatory elements of the human gene for cytochrome P450c21 (steroid 21-hydroxylase) lie within intron 35 of the linked *C4B* gene. Journal of Biological Chemistry. 274, 38097-38106.

136. Wu, M. T., Wang, Y. T., Ho, C. K., Wu, D. C., Lee, Y. C., Hsu, H. K., Kao, E. L., Lee, J. M. 2003. *SULT1A1* polymorphism and esophageal cancer in males. International Journal of Cancer. 10, 101-104.

137. Xue, J. L., Dial, G. D. 1997. Raising intact male pigs for meat: Detecting and preventing boar taint. Swine Health and Production. 5, 151-158.

138. Xue J, Dial G. D., Pettigrew J. E. 1997. Performance, carcass, and meat quality advantages of boars over barrows: a literature review. Journal of Swine Health and Production. 5, 21–28.

139. Yamazaki, H., Inoue, K., Hashimoto, M., Shimada, T. 1999. Roles of CYP2A6 and CYP2B6 in nicotine C-oxidation by human liver microsomes. Archives of Toxicology. 73, 65-70.

140. Yokoyama, M. T., Carlson. J. R. 1979. Microbial metabolites of tryptophan in the intestinal tract with special reference to skatole. American Journal of Clinical

Nutrition 32, 173−178.

141. Zamaratskaia, G. 2004. Factors involved in the development of boar taint: Influence of breed, age, diet and raising conditions. Doctoral thesis, Swedish University of Agricultural Sciences. Uppsala. Sweden. 50s.

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

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

144. Zamaratskaia, G., Lou, Y., Peacock, J., Rydhmer, L., Andersson, H. K., Juneja, R. K., Chen, G., Lundstrom, K., Squires, E. J. 2008a. Effect of polymorphism in the porcine cytochrome b5 (*CYB5A*) gene on androstenone and skatole concentrations and sexual development in Swedish pig populations. Animal. 2, 190-196.

145. Zamaratskaia, G., Rydhmer, L., Andersson, H.K., Chen, G., Lowagie, S., Andersson, K., et al., 2008b. Long-term effect of vaccination against gonadotropin-releasing hormone, using Improvac™, on hormonal profile and behaviour in pigs. Animal Reproduction Science. 108, 37–48.

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

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

148. Zamaratskaia, G., Stefanovic, S., Lundstrom, K., Doran, O. 2012. Expression of the

hepatic skatole- and androstenone-metabolising enzymes in entire male pigs of two live weights. Livestock Science. 145, 124-130.

149. Zammerini, D., Wood, J. D., Whittington, F. M., Nute, G. R., Hughes, S. I., M. Hazzledine, M., Matthews, K. 2012. Effect of dietary chicory on boar taint. Meat Science. 91, 396–401.

150. Zheng, L. Z., Wang, Y. F., Schabath, M. B., Grossman, H. B., Wu, X. F. 2003. Sulfotransferase 1A1 (SULT1A1) polymorphism and bladder cancer risk: a case-control study. Cancer Letters. 202, 61-69.

8.1 Seznam databází webových odkazů

European Declaration on Alternatives to Surgical Castration of Pigs. (2010). 354 <http://ec.europa.eu/food/animals/welfare/practice/farm/pigs/castration_alternatives/index355 _en.htm>

Ensembl db – http://www.ensembl.org/Sus_scrofa/

NCBI db, SNP db – http://www.ncbi.nlm.nih.gov/

BioGPS db – http://biogps.org/

9 Seznam použitých zkratek

17β – HSD (HSD17B7) hydroxysteroid (17-beta) dehydrogenase 7

3´UTR – 3´untranslate reagion

3β – HSD (HSD3B) - hydroxysteroid (3-beta) dehydrogenase

5´UTR - 5´untranslate reagion

A – Adenin

ABPE – Acute Bovine Pulmonary Edema and Emphysema

Ala – Alanin

AO – Aldehyd oxidáza

Asn – Asparagin

Asp – Kyselina asparagová

ATG – Start kodón

BO – Bílé ušlechtilé otcovské

BU – Bílé ušlechtilé

C6ORF106 – chromosome 6 open reading frame 106

C6ORF81 – chromosome 6 open reading frame 81

CLPS – colipase

COUP–TF1 – Transcription Factor 1

CTNND1 – catenin delta 1

CYB5A – cytochrome b5 type A (microsomal)

CYP11A1 – cytochrome P450 family 11 subfamily A member 1

CYP17A1 – cytochrome P450 family 17 subfamily A member 1

CYP21A2 – cytochrome P450 family 21 subfamily A member 2

CYP2A19 – cytochrome P450 family 2 subfamily A member 19

CYP2A6 – cytochrome P450, family 2, subfamily A, polypeptide 6

CYP2C18 – cytochrome P450, family 2, subfamily A, polypeptide 18

CYP2C49 – cytochrome P450, family 2, subfamily C, polypeptide 49

CYP2D6 – cytochrome P450, family 2, subfamily D, polypeptide 6

CYP2E1 – cytochrome P450, family 2, subfamily E, polypeptide 1

CYP7A1 – cytochrome P450, family 7, subfamily A, polypeptide 1

DNA – deoxyribonucleotic acid (deoxyribonukleová kyselina)

ESR1 – estrogen receptor 1

FMO1 – flavin containing monooxygenase 1

FMO5 – flavin containing monooxygenase 5

FOM – Fat-o-Meater

G – Guanin

GBLUP – Genomic best linear unbiased prediction

Glu – Glutamin

GWAS – Genome-wide association study

H48 – hybridní linie BOxPN, PNxBO (PN-pietrain)

His – Histidin

HNF–1α – Hepatocyte nuclear factor 1 homeobox α

HSD – Hydroxysteroid dehydrogenase

IFIT2 – interferon induced protein with tetratricopeptide repeats 2

IRG6 – (RSAD2) radical S-adenosyl methionine domain containing 2

JUT – Jatečně upravený trup

KKS – Kompletní krmná směs

KRT18 – keratin 18, type I

L – Landrace

Leu – Leucin

LHB – gen kódující β řetězec luteinizačního hormonu LH

LOC100518755 – polypeptide N-acetylgalactosaminyltransferase-like 6

Lys – Lysin

MAPK14 - mitogen-activated protein kinase 14

MC4R - melanocortin-4 receptor

MLLT – musculus longissimus lumborum et thoracis

MX1 – MX dynamin-like GTPase 1

NCBI – National Center for Biotechnology information

NGFIB – (NR4A1) nuclear receptor subfamily 4 group A member 1

NGS – next generatio sequencing

PLIN2 – perilipin 2

PCR – Polymerase chain reaction – polymerázová řetězová reakce

PCR – RFLP – Restriction Fragment Lenght Polymorphism – polymerázová řetězová reakce a polymorfismu restrikčních fragmentů DNA

QTL – Quantitative trait loci

RDH16 – retinol dehydrogenase 16 (all-trans)

RNA – seq – RNA sequencing

RR-BLUP – Ride regession BLUP

SLC26A8 – solute carrier family 26 (anion exchanger), member 8

SNP – Single Nucleotide Polymorphismus (jednobodová mutace)

SRD5A2 – steroid-5-alpha-reductase, alpha polypeptide 2

SRPK1 – SRSF protein kinase 1

SSC – Sus Scrofa Chromosome – označení pro chromozom prasete

SULT1A1 – sulfotransferase family 1A member 1

SULT2A1 – sulfotransferase family 2A member 1

SULT2B1 – sulfotransferase family 2B member 1

Sult2b1a – sulfotransferase family 2B member 1a

Sult2b1b – sulfotransferase family 2B member 1b

T – Thymin

TEAD3 – TEA Domain Family Member 3

TEF-5 – Transcriptional Enhancer Factor

Thr – Threonin

UDP – urine-difosfát-glucuronosyltransferáza

10 Přílohy

10.1 Sus scrofa partial SULT1A1 gene for sulfotransferase family, cytosolic, 1A, phenol-preferring member 1, breed Czech Large White, exons 4-7

GenBank: LN864417.1

```
LOCUS       LN864417               1612 bp    DNA     linear   MAM 14-JUL-2015
DEFINITION  Sus scrofa partial SULT1A1 gene for sulfotransferase family,
            cytosolic, 1A, phenol-preferring member 1, breed Czech Large White,
            exons 4-7.
ACCESSION   LN864417
VERSION     LN864417.1  GI:886834884
KEYWORDS    .
SOURCE      Sus scrofa (pig)
  ORGANISM  Sus scrofa
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Suina; Suidae;
            Sus.
REFERENCE   1
  AUTHORS   Zadinova,K., Stratil,A., Stupka,R. and Citek,J.
  TITLE     Study on the associations of polymorphisms in the porcine CYP2E1,
            CYB5A and SULT1A1 genes with androstenone, skatole and indole
            levels
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1612)
  AUTHORS   Zadinova,K.
  TITLE     Direct Submission
  JOURNAL   Submitted (14-MAY-2015) Department of Animal Husbandry, Czech
            University of Life Sciences Prague, Kamycka 129, Prague, 160
            00,CZECH REPUBLIC
FEATURES             Location/Qualifiers
     source          1..1612
                     /organism="Sus scrofa"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9823"
                     /note="breed:Czech Large White"
     gene            <1..>1612
     CDS             join(<1..6,774..900,1010..1104,1527..>1612)
                     /gene="SULT1A1"
                     /EC_number="2.8.2.1"
                     /function="sulfotransferase activity"
                     /codon_start=1
                     /product="sulfotransferase family, cytosolic, 1A,
                     phenol-preferring member 1"
                     /protein_id="CRL58837.1"
                     /db_xref="GI:886834885"
                     /translation="VKVVYVARNAKDVAVSYYHFYRMAKVHPNPGTWDSFLEDFMAGE
                     VSYGSWYQHVQEWWELRHTHPVLYLFYEDMKENPKREIQKILEFVGRSLPEETVEDIV
                     QHT"
     exon            <1..6
                     /gene="SULT1A1"
                     /number=4
     intron          7..773
                     /gene="SULT1A1"
                     /number=4
     mobile_element  295..590
                     /gene="SULT1A1"
                     /mobile_element_type="SINE:PRE-1"
```

```
     variation       643
                     /gene="SULT1A1"
                     /experiment="heterozygous SNP identified by direct
                     sequencing"
                     /replace="c"
     exon            774..900
                     /gene="SULT1A1"
                     /number=5
     intron          901..1009
                     /gene="SULT1A1"
                     /number=5
     variation       949
                     /gene="SULT1A1"
                     /experiment="heterozygous SNP identified by direct
                     sequencing and confirmed by sequencing of a homozygous
                     animal"
                     /replace=""
     exon            1010..1104
                     /gene="SULT1A1"
                     /number=6
     intron          1105..1526
                     /gene="SULT1A1"
                     /number=6
     exon            1527..>1612
                     /gene="SULT1A1"
                     /number=7
ORIGIN
        1 gtcaaggtga atggcagagg aaggaggctg ctggggtcat cccagctgtg gccctgcctt
       61 ctggaaagat ctcctcactt cctccagcct ggtctcatgt taactggggg caccggcctg
      121 atgctttggt tggggtgggg gtggtcagat gacccacgaa gttgagactc ctgtgcgcag
      181 agtctgggtg ctcaggggcc tgtggaggag gggctgggag aatcccgcca gccttgttcc
      241 aaacctgggc agagcagcac tgaggggatt agattagtat aaaacacggt tccaggagtt
      301 cctgtcgtgg cgcagtggtt aacgaatccg actaggaacc atgaggttgc gggttcagtc
      361 cctgcccttg ctcagtgggt taaggatccg gcgttgccgt gagctatgtg gtgtaggttg
      421 cagacgcggc tcggatccgg cgttgctgtg gctctggcgt aggccggtgg ctacagctcc
      481 gattcgaccc ctagcctggg aacctccata tgccgcggga gcggcccaag aaataggaac
      541 aacaacaaca aaaaagacaa aagacaaaaa aaaaaaaaaa aaaaaaaaaa ccacagttcc
      601 agactgcagc atcagagcag ggttccagtc ccagctcagt ggtgcccaca ctgcaggtcg
      661 acagtaagac cgccttaggc ccccttctcc cttggaggga tgctgtgaag ggtgggacgc
      721 ctggcacgcc gcgtggttac agtgccgggg gcgggggtcc tctgcccctc caggtggtct
      781 acgtcgcccg caacgcaaag gacgtggccg tctcctatta ccacttctac cgtatggcca
      841 aagtgcaccc taaccctggc acgtgggaca gcttcctgga agacttcatg gccggagaag
      901 gtggggctga ctttggagga aggggtgggg gcggagccgg gggggggggt aaggcaccag
      961 ctgaaacaaa tgcccacccc tgtgtccacg tcccctggct tttccccagt gtcctacggg
     1021 tcctggtacc agcacgtgca ggagtggtgg gagctgcgtc acacccaccc tgtcctctat
     1081 ctcttctacg aggacatgaa ggaggtgagc agccctgctt ctgggtcctg ccatgacaca
     1141 cgtagggagg cagaaacctg gtggcgacgt gccaaggccc tcccagcgtg ccctccccca
     1201 gaccagtcct ggattccctg cgcaggactg agcgccccca gccccgcccc cccacttctc
     1261 tcccagcact gagtcagcaa cacgagcagg ccccagaagc tgtttaccgc ccttgcttca
     1321 gttacgaaag aggactgtgc ctcctctcac ttcaaaggaa ccagccatct gtactcaagg
     1381 gtcacgggtg gaaggcaaaa agagagccaa ggaacacagt gtgaggtctc caagttgaag
     1441 aaggggccag agccccacaa ggcgagagca gggcgtccct tctggtggct cagagagcaa
     1501 tacaagcctc ccctctgccg tcccagaacc ccaagaggga gattcagaag atcctggagt
     1561 ttgtggggcg ctcgctgcca gaggagacgg tggaggacat cgtccagcac ac
//
```

10.2 Sus scrofa partial SULT1A1 gene for sulfotransferase family, cytosolic, 1A, phenol-preferring member 1, breed Meishan, exons 4-7

GenBank: LN864418.1

```
LOCUS       LN864418               1616 bp    DNA     linear   MAM 14-JUL-2015
DEFINITION  Sus scrofa partial SULT1A1 gene for sulfotransferase family,
            cytosolic, 1A, phenol-preferring member 1, breed Meishan, exons
            4-7.
ACCESSION   LN864418
VERSION     LN864418.1  GI:886834902
KEYWORDS    .
SOURCE      Sus scrofa (pig)
  ORGANISM  Sus scrofa
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Suina; Suidae;
            Sus.
REFERENCE   1
  AUTHORS   Zadinova,K., Stratil,A., Stupka,R. and Citek,J.
  TITLE     Study on the associations of polymorphisms in the porcine CYP2E1,
            CYB5A and SULT1A1 genes with androstenone, skatole and indole
            levels
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1616)
  AUTHORS   Zadinova,K.
  TITLE     Direct Submission
  JOURNAL   Submitted (14-MAY-2015) Department of Animal Husbandry, Czech
            University of Life Sciences Prague, Kamycka 129, Prague, 160
            00,CZECH REPUBLIC
COMMENT     Sequencing Technology : Sanger dideoxy sequencing.
FEATURES             Location/Qualifiers
     source          1..1616
                     /organism="Sus scrofa"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9823"
                     /chromosome="3"
                     /note="breed:Meishan"
     gene            <1..>1616
                     /gene="SULT1A1"
     CDS             join(<1..6,762..888,997..1091,1514..>1616)
                     /gene="SULT1A1"
                     /EC_number="2.8.2.1"
                     /function="sulfotransferase activity"
                     /codon_start=1
                     /product="sulfotransferase family, cytosolic, 1A,
                     phenol-preferring member 1"
                     /protein_id="CRL58838.1"
                     /db_xref="GI:886834903"
                     /translation="VKVVYVARNAKDVAVSYYHFYRMAKVHPNPGTWDSFLEDFMAGE
                     VSYGSWYQHVQEWWELRHTHPVLYLFYEDMKENPKREIQKILEFVGRSLPEETVEDIV
                     QHTSFQEM"
     exon            <1..6
                     /gene="SULT1A1"
                     /number=4
     intron          7..761
                     /gene="SULT1A1"
                     /number=4
     variation       287
                     /gene="SULT1A1"
```

```
                     /experiment="heterozygous SNP identified by direct
                     sequencing"
                     /replace="t"
     mobile_element  295..578
                     /gene="SULT1A1"
                     /mobile_element_type="SINE:PRE-1"
     variation       485
                     /gene="SULT1A1"
                     /experiment="heterozygous SNP identified by direct
                     sequencing"
                     /replace="g"
     variation       545
                     /gene="SULT1A1"
                     /experiment="heterozygous SNP identified by direct
                     sequencing"
                     /replace="t"
     exon            762..888
                     /gene="SULT1A1"
                     /number=5
     intron          889..996
                     /gene="SULT1A1"
                     /number=5
     exon            997..1091
                     /gene="SULT1A1"
                     /number=6
     variation       1076
                     /gene="SULT1A1"
                     /experiment="heterozygous SNP identified by direct
                     sequencing"
                     /replace="c"
     intron          1092..1513
                     /gene="SULT1A1"
                     /number=6
     variation       1188
                     /gene="SULT1A1"
                     /experiment="heterozygous SNP identified by direct
                     sequencing"
                     /replace="g"
     variation       1373
                     /gene="SULT1A1"
                     /experiment="heterozygous SNP identified by direct
                     sequencing"
                     /replace="g"
     variation       1419
                     /gene="SULT1A1"
                     /experiment="heterozygous SNP identified by direct
                     sequencing"
                     /replace="g"
     exon            1514..>1616
                     /gene="SULT1A1"
                     /number=7
ORIGIN
        1 gtcaaggtga atggcagagg aaggaggctg ctggggtcat cccagctgtg gccctgcctt
       61 ctggaaagat ctcctcactt cctccagcct ggtctcatgt taactggggg caccggcctg
      121 atgctttggt tggggtgggg gtggtcagat gacccacgaa gttgagactc ctgtgcgcag
      181 agtctgggtg ctcaggggcc tgtggaggag gggctgggag aatcccgcca gccttgttcc
      241 aaacctgggc agagcagcac tgaggggatt agattagtat aaaacacggt tccaggagtt
      301 cccgtcgtgg cgcagtggtt aacgaatccg actaggaacc atgaggttgc gggtttggtc
      361 cctgcccttg ctcagtgggt taatgatccg gcgttgccgt gagctatgtg gtgtaggttg
      421 cagacgcggc tcggatccgg cgttgctgtg gctctggcat aggccggtgg ctacagctcc
      481 gattcgaccc ctagcctggg aacctccata tgccgcggga gcggcccaag aaataggaac
```

```
      541 aacaacaaaa gacaaaaaaa aaaaaaaaaa aaaaaaaacc acggttccag actgcagcat
      601 cagagcaggg ttccagtccc agctcagtgg tgcccacact gcaggtcgac agtaagactg
      661 ccttaggccc ccttctccct tggagggatg ctgtgaaggg tgggacgcct ggcacgccgc
      721 gtggttacag tgccgggggc gggggtcctc tgcccctcca ggtggtctac gtcgcccgca
      781 acgcaaagga cgtggccgtc tcctattacc acttctaccg tatggccaaa gtgcacccta
      841 accctggcac gtgggacagc ttcctggaag acttcatggc cggagaaggt ggggctgact
      901 ttggaggaag gggtgggggc ggagccgggg ggtggggaag gcaccagctg aaacaaatgc
      961 ccacccctgt gtccacgtcc cctggctttt ccccagtgtc ctacgggtcc tggtaccagc
     1021 acgtgcagga gtggtgggag ctgcgtcaca cccaccctgt cctctatctc ttctatgaag
     1081 acatgaagga ggtgagcagc cctgcttctg ggtcctgcca tgacacacgt agggaggcag
     1141 aaacctggtg gcgacgtgcc aaggccctcc cagcgtgccc tcccccaaac cagtcctgga
     1201 ttccctgcgc aggactgagc gcccccagcc ccgccccccc acttctctcc cagcactgag
     1261 tcagcaacac gagcaggccc cagaagctgt ttaccgccct tgcttcagtt acgaaagagg
     1321 actgtgcctc ctctcacttc aaaggaacca gccatctgta ctcaagggtc acaggtggaa
     1381 ggcaaaaaga gagccaagga acacagtgtg aggtctccaa gttgaagaag gggccagagc
     1441 cccacaaggc gagagcaggg cgtcccttct ggtggctcag agagcaatac aagcctcccc
     1501 tctgccgtcc cagaacccca agagggagat tcagaagatc ctggagtttg tggggcgctc
     1561 gctgccagag gagacggtgg aggacatcgt ccagcacacg tccttccagg agatga
//
```