Mini Review

Regulation of Porcine Hepatic Cytochrome P450 — Implication for Boar Taint

Martin Krøyer Rasmussen a,b,⁎, Galia Zamaratskaia c

a Department of Food Science, Aarhus University, Denmark
b INSERM U1040, University of Montpellier, France
c Department of Food Science, Swedish University of Agricultural Science, Uppsala, Sweden

Abstract

Cytochrome P450 (CYP450) is the major family of enzymes involved in the metabolism of several xenobiotic and endogenous compounds. Among substrates for CYP450 is the tryptophan metabolite skatole (3-methylindole), one of the major contributors to the off-odour associated with boar-tainted meat. The accumulation of skatole in pigs is highly dependent on the hepatic clearance by CYP450s. In recent years, the porcine CYP450 has attracted attention both in relation to meat quality and as a potential model for human CYP450. The molecular regulation of CYP450 mRNA expression is controlled by several nuclear receptors and transcription factors that are targets for numerous endogenously and exogenously produced agonists and antagonists. Moreover, CYP450 expression and activity are affected by factors such as age, gender and feeding. The regulation of porcine CYP450 has been suggested to have more similarities with human CYP450 than other animal models, including rodents. This article reviews the available data on porcine hepatic CYP450s and its implications for boar taint.

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1. Introduction

Regulation of cytochrome P450 (CYP450) and its importance for xenobiotic clearance in the body has been the focus of numerous studies over the last two decades. Moreover, the involvement of CYP450 enzymes in the metabolism of several endogenously produced compounds...
is well documented. The superfamily of enzymes belonging to the group of CYP450s are hemoproteins with a spectrophotometric peak at 450 nm in their reduced state in complex with CO. CYP450s are often situated in the membranes of the endoplasmic reticulum or mitochondria, oxidising a wide range of substrates in collaboration with NADPH oxidoreductase and/or cytochrome b5. These reactions are an important part of the general detoxification process usually conducted in two phases, where CYP450 enzymes are responsible for Phase I metabolism [1].

The CYP450 family consists of at least 57 genes in the human body [1]. They are arranged into families based on their amino acid sequence, with isoforms sharing more than 40% being members of the same family (e.g., CYP1, CYP2) and isoforms sharing more than 55% being members of the same subfamily (e.g., CYP1A1, CYP1B1). Individual isoforms are identified by an additional Arabic number (e.g., CYP1A1, CYP1A2). CYP450s are widely expressed in all living species, with more or less conserved isoforms. Studies have determined high homology between the human and porcine versions of the CYP450, ranging from ~90% for human CYP2A6 and porcine CYP2A19 to ~60% for human CYP2C8 and porcine CYP2C33 [2].

Mammalian CYP450s are expressed in a variety of tissues, including the liver, intestine, kidney, gonads and brain. For most of the CYP450s the highest expression is detected in the liver. The current knowledge on porcine CYP450 identification and tissue-distribution has been summarised by Puccinelli et al. [2].

Similar to general detoxification, the tryptophan metabolite skatole (3-methylindole) is metabolised in two phases, with CYP450 enzymes being involved in Phase I metabolism [3]. Skatole accumulation in pigs has been associated with negative sensory perception of the meat upon heating and consumption, which is a phenomenon known as boar taint [3]. The current practice in several countries to overcome the accumulation of skatole is surgical castration of male piglets before the age of 7 days. However, this practice is highly questioned due to increasing focus on animal welfare and negative production impacts. In this context, alternative methods are needed. In this review, we summarise the current knowledge on the regulation of porcine CYP450 isoforms involved in skatole metabolism (particularly CYP1A1, 2A and 2E1), and we suggest how this knowledge might be used to enhance the activity of hepatic CYP450 and thereby potentially minimise the accumulation of skatole in pig meat.

2. Xenobiotic receptors and regulation of mRNA expression

The expression of individual CYP450s is regulated by ligand binding receptors constitutively expressed in hepatocytes and other cell types (e.g., enterocytes), often collectively referred to as xenobiotic receptors (XR) (Fig. 1). Several receptors are known to be involved in the initiation of gene expression, either by direct binding to promoter regions of the gene or by crosstalk with other receptors [4,5]. With respect to the control of skatole metabolising CYP450, the major XRs controlling them are the aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR) and pregnane X receptor (PXR). All of these receptors control a battery of genes, including different CYP450s, Phase II enzymes and drug transporters. Other receptors (e.g., farnesoid X receptor and liver X receptor) and co-factors are also likely involved in tuning the activity of the XRs as co-activator and co-repressors or via crosstalk; however, it is beyond the scope of this review to cover this topic. Readers interested in more detailed information about these regulatory events are directed to other reviews [4,5].

2.1. Aryl hydrocarbon receptor

The AhR is known to control the expression of genes such as CYP1A1, 1A2 and 1B1. AhR is located in the cytosol where it is kept in complex
with other proteins, including heat shock protein 90 (HSP90). Upon binding of its ligands, AhR dissociates from HSP90 and translocates into the nucleus, where it binds with AhR nuclear translocator (Arnt). The AhR–Arnt complex then binds to the response element of the gene, initiating transcription. The class of ligands able to activate AhR is diverse and includes both endogenous and exogenous compounds [6]. A prototypical AhR ligand is TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), which strongly increases mRNA expression of several genes including CYP1A1 and 1A2 [7]. Treatment of porcine primary hepatocytes with β-naphthoflavone (β-NF), another commonly used AhR activator, increased the mRNA and protein expression as well as the activity of the CYP1A family [8,9]. In agreement with this observation, in vivo results from studies treating pigs with β-NF showed increased expression and activity of CYP1A in several tissues, including the liver [10–12]. AhR activation and increased CYP1A mRNA and protein expression are also observed in the presence of several naturally occurring compounds, among them metabolites of tryptophan [13–15]. In human bronchial epithelia, skatole was shown to increase the expression of CYP1A1 by interacting with AhR [16]. It is unknown if skatole is an agonist of porcine hepatic AhR, but this is a reasonable suggestion due to a number of common activators of human and porcine AhR. Moreover, a known metabolite of skatole is indole-3-carbinol, which is known to be a strong activator of human AhR, increasing CYP1A expression [17].

2.2. Constitutive androstane receptor

CAR (NR1I3) belongs to the class of orphan nuclear receptors and is named for its constitutively active properties. However, it is still debated if the receptor is truly constitutively active in vivo [7]. Similar to AhR, CAR is situated in the cytoplasm, where it translocates to the nucleus upon ligand binding, and initiates gene transcription when in complex with retinoic acid receptor (RXR). CAR is mainly expressed in the mammalian liver and intestine, where it regulates the transcription of several genes, including the CYP2A and 2B family. Several agonists/activators of CAR have been identified, including phenobarbital, TCPOBOP (4-bis-[2-(3,5 dichloropyridyloxy)]benzene) and CITCO (6-(4-chlorophenyl)imidazo[2,1-beta] [1.3]thiazole-5-carbaldehyde-O-[3,5-dichlorobenzoyl] oxime). Species-specific differences in CAR have been demonstrated, as CITCO is an activator of human and porcine CAR, while TCPOBOP has no effect [18–20]. Accordingly, TCPOBOP activates mouse CAR but not human and porcine CAR [19,21]. However, other studies did not report increases in CYP2A19 mRNA expression after CITCO treatment using primary porcine hepatocytes [9,20]. A study by Gray et al. [22] showed that a similar response for human and porcine CARs in 10 out of 12 treatments compared to only 4 out of 12 for human and mouse CARs. Using a reporter gene assay, skatole has been determined to decrease the activity of CAR in a dose-dependent manner [23].

2.3. Pregnane X receptor

Similarly to CAR, PXR (NR112) belongs to the class of orphan receptors and is found in several tissues, including liver and intestine [12,23]. PXR has previously been named SRX (steroid and xenobiotic receptor) and PAR (pregnane-activated receptor), indicating the broad range of ligands for this receptor. Once activated, PXR translocates from the cytosol into the nucleus, where it forms a complex with e.g., RXR, initiating transcription of numerous genes, including the CYP3A and 2C family [8,9,20,24]. PXR and CAR share the control of several genes. An ever-evolving list of both natural and synthetic compounds has been identified as agonists for PXR, while reports of antagonists are scarce. The list includes the commonly used CYP3A inducer rifampicin, as well as several natural compounds such as artemisinin and hyperforin [8,9,25–27]. Furthermore, porcine PXR has been suggested to be a good model for human PXR [23,28,29]. As for CAR, skatole has been shown to reduce porcine PXR activity in a reporter gene assay in a dose-dependent manner [23].

2.4. Other receptors involved in CYP450 regulation

Several other receptors are also involved in the regulation of CYP450 transcription, either as direct receptors, co-factors or via crosstalk.

The peroxisome proliferator activated receptor (PPAR) is another important receptor found in hepatocytes and other tissues, expressed in three isoforms, which has been shown to regulate CYP4A, an isoform involved in the metabolism of fatty acids. Fatty acids as well as several other compounds are known agonists for PPARs. No studies have shown involvement of CYP4A in the metabolism of skatole, but PPAR activation has been shown to inhibit expression of AhR regulated genes, including CYP1A [30]. Moreover, PPAR agonists have been shown to regulate other CYP450 families [31].

Another known example is the chick ovalbumin upstream promoter transcription factor 1 (COUP-TF1), which has been shown to bind to the promoter region of porcine CYP2E1 [32]. The same study showed that binding of COUP-TF1 to this promoter region was inhibited by androstenone, a compound often found in high amounts in boar-tainted meat.

3. Protein expression

As described in the central dogma of biology, protein expression is dependent on translation of mRNA, meaning that protein expression is to some degree positively correlated to mRNA expression. However, this is a simplified assumption as other events like, mRNA turnover and protein stabilisation are also important in determining the given protein amount. In fact, for CYP2E1, it has been suggested that events such as protein stabilisation are more important for protein expression than mRNA expression [33]. This suggestion is consistent with results on the effects of castration and specific feeding components, as both castration and bioactive dietary compounds increased porcine CYP2E1 mRNA expression without affecting protein expression and activity [34,35]. Using primary porcine hepatocytes treated with activators of the XRs (rifampicin, dexamethasone, phenobarbital, 3-methylcholanthrene, dimethyl sulfoxide) for 3 days, Baldini et al. [36] found no changes in CYP2E1 protein expression. The protein expression of CYP2E1 in primary porcine hepatocytes has been determined to increase after treatment with skatole in a dose- and time-dependent manner [37]. The skatole-induced increase in protein expression was eliminated by co-treatment with androstenone. Likewise, it has been shown that skatole and indole induce CYP2A19 expression in primary porcine hepatocytes, while androstenone down-regulates protein expression [38]. There were no observed changes in CYP2A19 protein expression by treatment with other steroids such as testosterone and oestrone sulphate. It has also been suggested that androgens (testosterone) can decrease the expression of CYP1A proteins [39,40]. However, this effect is breed-dependent.

4. CYP450 activity

The catalytic activities of individual CYP450 isoforms are generally estimated from the rate of metabolism of specific probe substrate(s). To date, only limited data on the specificities of probe substrates for porcine CYP450 are available. Thus, to estimate activities of individual porcine CYP450 isoforms, typical probe substrates for human CYP450 are often used, showing both similarities and differences between the catalytic activity of human and porcine CYP450 [41,42]. This makes the interpretation of results on porcine CYP450 substrate metabolism challenging.

The catalytic activity of CYP1A is usually estimated as the rate of ethoxyresorufin O-deethylation (EROD). EROD activity was detected in microsomes from minipigs, although the obtained values were lower than in microsomes from humans [43] as well as from human.
recombinant CYP1A [44]. In conventional pigs, EROD activity has also been detected; however, it was suggested that EROD might not truly reflect specific CYP1A1 activity, as it is most likely metabolised by two different CYP450 isoforms in pigs [45].

Chlorozoxazone 6-hydroxylation is the most used probe reaction to assess human CYP2E1 activity [46], while in pigs, the kinetics of chlorozoxazone 6-hydroxylation differed from that found in human microsomes [47,48]. It has been suggested that chlorozoxazone metabolism in pigs is not entirely due to CYP2E1 activity because other isoforms, such as CYP1A1, CYP2A19, and CYP2C33v4, are also involved [48,49]. P-nitrophenol is currently used as CYP2E1 specific substrates, although its specificity towards porcine CYP2E1 has also been questioned [49].

Catalytic activities of CYP2A are commonly measured by the hydroxylation of coumarin, showing large correlation with the expression of the protein [50]. Moreover, studies showed that coumarin 7-hydroxylation could be inhibited by anti-human CYP2A6 antibodies [42,50].

For estimation of CYP3A activity several different probe substrates have been used, including testosterone [27,42] and nifedipine, both showing strong correlation with expression of CYP3A5 [51]. Additionally, the metabolism of 7-benzoxoxyresorufin and 7-benzooxyquinoline has been shown to be inhibited by ketoconazole, which is a known inhibitor of CYP3A activity [52].

Attempts to use cocktails of substrates for the simultaneous determination of several porcine CYP450 activities have not been successful [53].

4.1. Skatole metabolism

As stated in the Introduction, porcine CYP450 is of special interest because it mediates the metabolic transformation of skatole, one of the main contributors to boar taint, an unpleasant odor in meat from intact (un-castrated) male pigs. Skatole is a hydrophobic compound, which makes it difficult to eliminate from the body. The biological significance of skatole metabolism is to produce more hydrophilic metabolites to facilitate its excretion. Bark et al. [54] identified several skatole metabolites in porcine blood and urine, with the major ones being 3-hydroxy-3-methylxindole and 6-sulfatoxyskate. Moreover, in vitro study using porcine liver microsomes identified seven Phase I metabolites, with the major metabolite being 3-hydroxy-3-methylindolenine [64]. 3-hydroxy-3-methylindolene and indole-3-carbinol and 2-aminoacetophenone, are produced in the liver through Phase Ioxidation reactions by CYP450 [55,56]. Some of these metabolites undergo Phase II reactions, sulphation and glucuronidation [55,57]. Porcine CYP1A, 2A19, 2C33v4, 2C49, 2E1 and 3A were identified as the major skatole-metabolising isoforms (Table 1) [56,58,59]. Similarly, CYP1A2, 2E1, 2A6 and 3A have been shown to metabolise skatole in humans [59–61]. Originally, CYP2E1 was suggested to be the main skatole-metabolising isomorph [62]. Later, Diaz and Squires [56] demonstrated the involvement of CYP2A. Moreover, in vivo skatole levels in fat were more strongly related to CYP2A than to CYP2E1 activity [63] and unpublished observations). Recently, involvement of CYP1A in skatole metabolism was also suggested [61]. Interestingly, the co-factor cytochrome B5A (CYB5A) was shown to be of importance for CYP450-dependent skatole metabolism [59]. The exact mechanism of CYB5A involvement is not yet understood; however, a role as an electron donor has been suggested [59]. Disagreements between the relative importance of different isoforms for skatole metabolism exist, which might be partly due to breed-related variations in the formation of skatole metabolites, as well as in differences in experimental conditions (e.g., studies have used different in vitro systems to identify skatole metabolites, including hepatic microsomes [62,64], primary cultured pig hepatocytes [58] and individual purified porcine enzymes [61]). Wiercinska et al. [59] studied the contribution of porcine CYP450s in hepatic skatole metabolism by cloning and expressing them individually in the human embryonic kidney HEK293-FT cell line. In porcine liver microsomes and hepatocytes, the involvement of CYP450s was studied using probe reactions and specific inhibitors for human CYP450s. However, as discussed above, their specificity towards porcine CYP450 might differ from that of human CYP450s, causing diverse results. Even various solvents to solubilise inhibitors may have different effects on CYP450 probe reactions. Moreover, due to genetic variation, environmental and physiological factors, as well as in intrinsic limitations of in vitro systems, the quantitative prediction of in vivo skatole metabolism in pigs remains a challenge.

4.2. Hormonal status has an impact on CYP450 expression and activity

The hormonal status of the pig is one of the crucial factors regulating CYP450 catalytic activities and expression. Several in vivo studies demonstrated the involvement of testicular steroids in CYP450 regulation. It has repeatedly been shown that mature pigs with high levels of testicular steroids possess low CYP450 activities [19,34,39,40,65,66]. Accordingly, both castration and immunocastration (subcutaneous injection with a GnRH analogue to promote the intrinsic production of GnRH antibodies) increased gene expression and activities of most hepatic CYP450s [65,67]. Treating pigs with human chorionic gonadotropin (hCG), thus inducing a temporary increase in the level of testicular steroids, reduced CYP450 activities [68–70]. The role of testicular steroids

Table 1 Overview of known porcine CYP450 isoforms and their importance for skatole Phase I metabolism.

| Sub-family | Isomorph | Importance for boar taint |
|-----------|----------|--------------------------|
| CYP1      | CYP1A    | Both human and porcine versions metabolise skatole in vitro [59,92] |
|           | CYP1A2   | Both human and porcine versions metabolise skatole; most likely more than CYP1A1. Has also been suggested to be the most active human CYP450 in the metabolism of skatole [60,61,92] |
| CYP2      | CYP2B    | Human version metabolises skatole to a small extent in vitro; porcine version not known [92] |
|           | CYP2C    | Has been shown to metabolise skatole to a low degree [59] |
|           | CYP2E    | Both human and porcine versions metabolise skatole; has been suggested to be the most important in the metabolism of skatole, together with CYP2A19 [56,58–62,92]. |


in the regulation of porcine CYP450 is however breed-dependent [40,66]. Further studies on breed-related differences in hepatic CYP450 are relevant not only for meat science but also for veterinary medicine, as CYP450s are involved in the metabolism of veterinary drugs. In vitro studies have been used to investigate the mechanism of testicular steroid–CYP450 interactions and determine gender-related differences of these interactions. Apart from the previously discussed down-regulating effect of androstenedione on the protein expression of CYP2E1 [32,37] and CYP2A [38], androstenedone also directly inhibits CYP2E1 activity [71–73]. Special interest is focused on 17β-oestradiol (E2), as it was shown to affect CYP450 activities only in male pigs [71,73]. The results, however, differed in terms of the inhibition mode of E2. Zamaratskaia et al. [73] reported that E2 is a mixed-mode inhibitor of CYP2E1 in microsomes from prepubertal male pigs, although E2 was later reported to act more as an irreversible inhibitor [72]. While a major step forward, the in vitro studies fail to replicate the precise conditions of an organism and are hampered by the lack of correlation to in vivo studies. Thus, physiological consequences of the inhibition of CYP450 activities by oestradiol remain uncertain, especially in the light of a recent in vivo study which did not show any differences in CYP450 activities between pigs with physiological and artificially reduced oestradiol levels [74].

4.3. Dietary factors and CYP450

Dietary compounds are another key factor regulating CYP450 metabolic activity in humans and pigs. Between-individual variations in CYP450 expression/activity and in the magnitude of response to drug-treatment [75–78] are due to genetic background, previous drug intake or hormonal status as well as differences in exposure to dietary ingredients. Thus, bioactive components in the diet modify CYP450 activity and thus interact with the metabolism of xenobiotic substances. In humans, there have been several cases of food–drug interaction, situations where the consumption of specific dietary compounds affects the outcome of a simultaneous drug treatment. Most of these cases have been traced back to compounds originating from food or herbal medicine capable of modifying the expression or activity of the CYP450 enzyme system. Examples include the phytoestrogen coumestrol, which has been shown to be an antagonist of human PXR [79], and grapefruit juice, which has been shown to down-regulate CYP3A4 expression and thereby augment the bioavailability of several drugs [80]. Moreover, the inclusion of herbal medicines containing ginkgo biloba or St. John’s-wort in diets interferes with drug clearance by CYP3A4 [81]. In pigs, increased hepatic CYP2E1 activity or protein expression has been observed following exposure to high-fat/high-cholesterol diets [82], feeding with sugar beet [67] or after administration of ethanol with a folate-deficient diet [83]. Additionally, in pigs, the administration of the plant secondary metabolite quercetin has been shown to alter the bioavailability of co-administered drugs [84,85]. Following administration of chicory root, increased expression and activity have been observed for several porcine hepatic CYP450s, including CYP1A, 2A and 3A [35,86]. Accordingly, purified secondary metabolites found in chicory induce mRNA expression of CYP1A, 2A and 2E1, together with 3A, in porcine primary hepatocytes [9]. A number of secondary plant metabolites have also been shown to directly interact with the CYP450 enzymes, affecting their activity [87]. These different levels of interactions make the study of the effects of specific dietary compounds challenging and make the outcome of a given treatment a function of numerous factors, including the time of exposure.

Remarkably, our unpublished results suggested that regulation of CYP450 activities might differ between genders within the same species [Borrisser-Pairó F., Rasmussen M.K., Ekstrand B., Zamaratskaia G. Accepted for publication in Animal]. We demonstrated that CYP3A activity was inhibited by myricetin and CYP2E1 by quercetin in microsomes from male but not from female pigs. In support of this finding, we have shown that an extract of chicory root inhibits CYP3A activity in microsomes from male pigs, while increasing the activity in microsomes from female pigs [86]. Further investigations are needed to determine the physiological significance of these gender-related differences and to determine the mechanisms behind this difference.

5. Future research focus

Skatole metabolism has mainly been studied in the liver because it is considered the major site of skatole metabolism; however, biotransformation of skatole might also occur in extra-hepatic tissues, such as intestines and blood. As the first boundary, skatole has to cross the intestinal wall before entering the hepatic portal vein and ultimately reaching the liver. Several CYP450 isoforms have been found in the enterocytes (e.g., CYP1A, 2A, 2E and 3A). As for their hepatic counterparts, it is likely that they also metabolise skatole. The importance of this “first-pass-metabolism” for the occurrence of boar taint has gained surprisingly little attention. As an example, it has been determined that the intestinal metabolism of the drug midazolam is of the same magnitude as the hepatic clearance in humans [88]. Another example of extra-hepatic CYP450 location is the presence of CYP2E1, the major skatole-metabolising enzyme, in human peripheral blood lymphocytes [89]. The role of this enzyme in blood remains highly speculative, but a possibility of its involvement in skatole metabolism could be of interest, and has never been studied in pigs. Additionally, the search for polymorphism of CYP2E1 and other skatole-metabolising enzymes using blood samples would be useful in the identification of genetic markers for the selection of pigs with low fat skatole levels. Pigs with high CYP2E1 activity can then be selected to produce pigs with a lower incidence of boar taint. Moreover, biomarkers for intestinal and/or hepatic CYP450 activity can be identified in the faeces or urine of the pig and used for non-invasive detection of pigs with high risk of boar taint.

An important research area is the modulation of CYP450 activities by specific dietary compounds. Targeting regulation of the skatole-metabolising enzymes to enhance skatole metabolism and reduce the risk of boar taint would be an attractive alternative to surgical castration and immunocastration. However, this research is challenging because little is known about the effectiveness of bioactive compounds in the regulation of porcine CYP450s. Several cases of food–drug interactions are reported in humans, as stated above. Thus, with the close similarities between human and porcine XRs, it is likely that the same events will occur in pigs. This opens the possibility of targeting specific XRs in the liver, up-regulating skatole-metabolising CYP450s, and thereby increasing the clearance of skatole from the pig (Fig. 1), which may be a consumer-acceptable and easily implementable method.

Another important point of view to the regulation of porcine CYP450, apart from the importance for boar taint, is the usefulness of porcine CYP450 as a model for human CYP450. Due to limited availability and high costs, human primary hepatocytes for basic research are not common. However, the substitution of human hepatocytes with porcine hepatocytes for basic trials can be the future; porcine livers for isolation of hepatocytes are available on request, giving the possibility of using the exact age and gender needed. However, the high variation between humans and the fact that the gender of the pigs is a factor for CYP450 activity also need to be addressed to fully evaluate the potential of pigs as a model. Moreover, there seems to be differences between the isoform distribution when comparing human and porcine livers (e.g. in humans CYP3A4 is the most predominant isoform, while CYP2A19 is the most predominant isoform in pigs [90,91]).

Finally, many questions related to genetic variations in the mechanism of CYP450 regulation remain to be addressed.

6. Conclusion

Knowledge about the regulation of porcine CYP450 and the factors/mechanisms behind it is very important in the context of meat quality in pigs. We currently know to a large extent how the expression of specific
CYP450 is controlled and how the activity is affected under different experimental conditions. This knowledge will potentially enable us to use tools such as dietary compounds to modulate the CYP450 expression and activity, and thereby controlling the metabolism of skatole in pigs. However, research is still needed to cover the level from cell models to whole animal studies.

It should be emphasised that CYP450s only controls the first stage of skatole metabolism. Skatole deposition also depends on the second stage of skatole metabolism as well as on the rate of its production. Thus, in the studies on the control of boar taint, complex interactions between production, metabolism and clearance of boar taint components should be considered.

Conflict of interest

The authors declare no conflict of interests.

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It should be emphasised that CYP450s only controls the first stage of skatole metabolism. Skatole deposition also depends on the second stage of skatole metabolism as well as on the rate of its production. Thus, in the studies on the control of boar taint, complex interactions between production, metabolism and clearance of boar taint components should be considered.
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