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Review

Nuclear Receptor Pathways Mediating the Development of Boar Taint

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Abstract: The nuclear receptors PXR, CAR, and FXR are activated by various ligands and function as transcription factors to control the expression of genes that regulate the synthesis and metabolism of androstenone and skatole. These compounds are produced in entire male pigs and accumulate in the fat to cause the development of a meat quality issue known as boar taint. The extent of this accumulation is influenced by the synthesis and hepatic clearance of androstenone and skatole. For this reason, PXR, CAR, and FXR-mediated signaling pathways have garnered interest as potential targets for specialized treatments designed to reduce the development of boar taint. Recent research has also identified several metabolites produced by gut microbes that act as ligands for these nuclear receptors (e.g., tryptophan metabolites, short-chain fatty acids, bile acids); however, the connection between the gut microbiome and boar taint development is not clear. In this review, we describe the nuclear receptor signaling pathways that regulate the synthesis and metabolism of boar taint compounds and outline the genes involved. We also discuss several microbial-derived metabolites and dietary additives that are known or suspected nuclear receptor ligands and suggest how these compounds could be used to develop novel treatments for boar taint.

Keywords: boar taint; metabolism; nuclear receptor; signaling pathways; cytochrome P450; PXR; CAR; FXR

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1. Introduction

Nuclear receptors are a family of transcription factors that regulate the expression of genes controlling numerous physiological processes [1,2]. All nuclear receptors share a common structure, which includes a DNA binding domain (DBD) and a C-terminal ligand-binding domain (LBD) [2]. The LBD contains a binding pocket that recognizes specific endogenous and exogenous ligands that activate the nuclear receptor upon binding [3]. The DBD contains zinc fingers, which recognize and bind to specific DNA sequences called response elements [4]. Nuclear receptors also contain a ligand-dependent activation function (AF-2) within the LBD and an N-terminal ligand-independent activation function (AF-1), which recruit coactivating proteins to synergistically regulate gene transcription [3,5–9].

The nuclear receptors pregnane X receptor (PXR), constitutive androstane receptor (CAR), and farnesoid X receptor (FXR) are class II nuclear receptors that form heterodimers with the retinoid X receptor (RXR) and bind to response elements primarily organized as direct repeats [1,10].

Initial studies characterized PXR as a ligand-dependent transcription factor for several genes belonging to the cytochrome P4503A (CYP3A) subfamily, activated by naturally occurring pregnane steroids such as pregnenolone and progesterone [11]. PXR is now known to regulate the expression of nearly 500 genes in human hepatocytes alone, and numerous ligands, including drugs, natural products, and endogenous ligands, such as secondary bile acids or metabolites produced by gut microbes, have been identified [12–15].

CAR was first described as a constitutive receptor and was found to be inactivated by 3α-hydroxy, 5α-reduced androstane steroids [16]. Several activators of CAR have since been

identified, including phenobarbital, steroids such as 5β-pregnane-3,20-dione, several drugs, and natural products; however, they do not necessarily interact directly with the receptor and instead can regulate CAR activity by promoting nuclear translocation [12,17–22]. PXR and CAR are highly expressed in the liver and intestinal tract and are key regulators of xenobiotic metabolism [23,24].

The activation of FXR was first demonstrated in response to farnesol derivatives, and the receptor was named accordingly [25]. However, bile acids such as chenodeoxycholic acid (CDCA), cholic acid (CA), deoxycholic acid (DCA), lithocholic acid (LCA), and their conjugates were later identified as natural ligands for FXR under physiological conditions, with CDCA being the most effective agonist [10,26–29]. FXR is highly expressed in the liver, kidney, and intestinal villi and controls a wide spectrum of metabolic pathways; most notably, FXR mediates the autoregulatory effects of bile acids on bile acid homeostasis [25,30]. FXR can also function as a transcription factor for *PXR* and can modulate the production of bile acid metabolites that activate or repress PXR and CAR signaling pathways [31–33].

Porcine orthologues of PXR, CAR, and FXR share a high degree of sequence homology with human receptors and respond to similar ligands [34–36]. These nuclear receptors, as well as several splice variants, have been isolated from the liver and testis, where they have been shown to modulate the synthesis and metabolism of compounds produced in entire male pigs that are responsible for a meat quality issue known as boar taint [37,38]. Boar taint describes an off-odour or off-flavour that results from the accumulation of 5α-androst-16-ene-3-one (androstenone) and 3-methylindole (skatole) in the fat, which has traditionally been prevented by surgically castrating male pigs shortly after birth. However, surgical castration has come under increasing public scrutiny due to animal welfare concerns, and the negative environmental impact associated with raising surgical castrates, which consume more feed and grow less efficiently than entire male pigs [39]. For this reason, PXR, CAR, and FXR are potentially attractive targets for treatments designed to prevent boar taint without castration.

2. Nuclear Receptor Signaling and Boar Taint

2.1. Nuclear Receptor-Mediated Regulation of Testicular Steroid Synthesis

The testis of the boar is a dynamic organ that is the primary site of synthesis for various steroid hormones, including the 16-androstene steroid androstenone. Androstenone is a sex pheromone that is produced by the boar at the onset of puberty, which can regulate female reproductive behaviour. Like all steroids, androstenone is derived from the stepwise conversion of cholesterol. A key step in this pathway involves the conversion of pregnenolone to the 16-androstene steroid 5,16-androstadien-3β-ol (androstadienol), a precursor of androstenone [40]. This is catalyzed by the andien-β synthase activity of cytochrome P45017A1 (CYP17A1) and is regulated by several accessory proteins such as cytochrome b5A (CYB5A) and cytochrome b5 reductase (CYB5R3) [41,42].

Nuclear receptor activation in porcine Leydig cells (Figure 1A) has been shown to alter the synthesis of the 16-androstene steroids, which is presumably mediated by nuclear receptor-induced changes in the expression of *CYB5A* and *CYB5R3* [37]. Activation of PXR and CAR increased *CYB5A* and *CYB5R3* expression and decreased the production of sex steroids while increasing 16-androstene steroid synthesis. FXR activation also decreased the production of sex steroids but did not affect the synthesis of 16-androstene steroids [37]. The 17,20-lyase reaction that is required for the conversion of pregnenolone to sex steroids is also catalyzed by CYP17A1 and is prioritized over the andien-β synthase reaction when levels of CYB5A are limiting [43]. This suggests that the activation of PXR and CAR promotes the synthesis of 16-androstene steroids by upregulating *CYB5A* and *CYB5R3* to favour the andien-β synthase activity of CYP17A1.

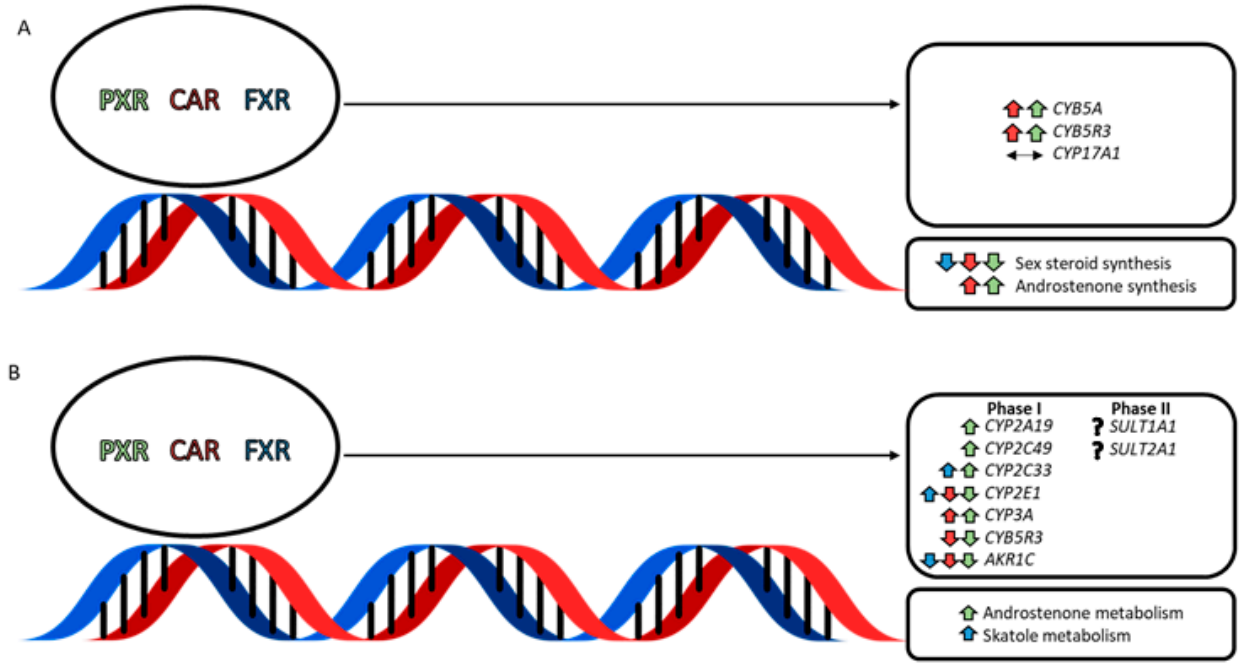

Figure 1. The effects of nuclear receptor activation reported by Gray and Squires [37,38] in porcine (**A**) Leydig cells [37] and (**B**) hepatocytes [38]. PXR, CAR, and FXR, shown in green, red, and blue, respectively, increase (↑) or decrease (↓) the expression of genes in the testis that regulate steroidogenesis and Phase I and II metabolism in the liver. Symbols in black represent no effect (↔) or no characterized effect (?) of PXR, CAR, or FXR on the genes of interest. The overall effect on the synthesis or metabolism of androstenone and skatole is shown.

2.2. Nuclear Receptor-Mediated Regulation of Boar Taint Metabolism

The liver is the primary site for the Phase I and Phase II metabolic reactions that promote the hepatic clearance of androstenone and skatole (Figure 1B). During Phase I metabolism, oxidation, reduction, or hydrolysis reactions result in an addition of a hydroxyl group to the compound, which can serve as a site for conjugation during Phase II metabolic reactions. Phase I oxidation reactions are modulated by the cytochrome P450 (CYP450) enzyme superfamily and the accessory proteins NADPH-cytochrome P450 oxidoreductase (POR) or CYB5A [44,45]. The total expression of hepatic CYP450s is similar between humans and pigs; however, differences in the relative quantity of CYP450 subfamilies have been reported [46]. In particular, the expression of the CYP2A, CYP2D, and CYP2E subfamilies are more abundant in pigs, while CYP1A2 and the CYP3A and CYP2C subfamilies are more abundant in humans [46].

2.2.1. Nuclear Receptor-Mediated Effects on Skatole Metabolism

The porcine CYP450 isoforms CYP1A1, CYP2A19, CYP2C33v4, CYP2C49, CYP2E1, and CYP3A regulate the Phase I metabolism of skatole [45,47–49]. The Phase I metabolites of skatole with the most notable implications for the development of boar taint include 3-hydroxy-3-methyloxindole (HMOI), indole-3-carbinol (I3C), and 6-hydroxy-3-methylindole (6-OH-3MI), which is sulfoconjugated by the sulfotransferase enzyme SULT1A1 during Phase II metabolism to form 6-OH-3MI sulfate [50,51]. However, four additional metabolites have been identified and include 3-methyloxindole (3MOI), 5-hydroxy-3-methylindole (5-OH-3MI), 2-aminoacetophenone (2AAP), and 3-hydroxy-3-methylindolenine (3-OH-MI), which is the most abundant skatole metabolite produced during Phase I metabolism [52]. Higher plasma and urine concentrations of HMOI and urine concentrations of I3C have been noted in animals with high fat concentrations of skatole, while animals with low concentrations of skatole in the fat tend to produce greater concentrations of the metabolite

6-OH-3MI sulfate [51,53]. Therefore, HMOI, I3C, and 6-OH-3MI are important biomarkers for boar taint from skatole.

Schelstraete et al. [46] reported that the protein expression of CYP2A19 and CYP2E1 in porcine hepatocytes accounted for nearly half of the total CYP450 protein quantified (31% and 13%, respectively). Achour et al. [54] observed similar relative protein expression profiles indicating that CYP2A19 and CYP2E1 are highly expressed in the liver of pigs. Both CYP2A19 and CYP2E1 produce large quantities of the key Phase I metabolite 6-OH-3MI relative to the other CYP450s regulating skatole metabolism and are downregulated in animals with high levels of androstenone and skatole the fat [45,48,51,55]. Androstenone has been shown to have an inhibitory effect on the protein expression and activity of CYP2E1 and CYP2A6, the human orthologue of CYP2A19 [48,56,57]. Therefore, the hepatic expression of *CYP2A19* and *CYP2E1* has important implications for the development of boar taint. CYP2C49 has also been identified as an important regulator of skatole metabolism as it produces significant quantities of 6-OH-3MI; however, *CYP2C49* is upregulated in animals with high fat androstenone concentrations along with *CYP2C33* [45,55].

Activation of PXR, but not CAR, has been found to upregulate the expression of *CYP2A19* in porcine hepatocytes [38], despite *CYP2A6* being a gene target for both nuclear receptors in humans [46,58]. PXR activation also increased the expression of *CYP2C49* and *CYP2C33*, while decreased expression of *CYP2E1* and *CYB5R3* resulted from the activation of both PXR and CAR. Puccinelli et al. [58] reported similar effects on gene expression following phenobarbital administration in vivo. Phenobarbital, a known activator of PXR and CAR, increased the expression and activity of CYP2C33, CYP2C49, and CYP3A in the liver of pigs. These changes in gene expression were associated with an increase production of I3C by activation of PXR and HMOI by activation of CAR in porcine hepatocyte culture [38]. The production of 6-OH-3MI was not altered despite the upregulation of *CYP2C49* and *CYP2A19* by PXR; however, the activity of these enzymes may have been reduced by the downregulation of *CYB5R3*, which works alongside CYB5A to increase the production of 6-OH-3MI by CYP2C49, CY2A19, and CYP2E1 [45]. In contrast, FXR activation in porcine hepatocytes upregulated the expression of *CYP2E1* without altering *CYB5R3* expression and significantly increased the production of 6-OH-3MI [38].

2.2.2. Nuclear Receptor-Mediated Effects on Androstenone Metabolism

Phase I metabolites of androstenone include 3α-androstenol and 3β-androstenol, which are produced from the reduction in androstenone by aldo-keto reductase (AKR1C) or 3β-hydroxysteroid dehydrogenase (HSD3B), respectively [40]. These 16-androstene steroids are substrates for the sulfotransferase enzyme SULT2A1, which promotes Phase II sulfoconjugation reactions [59]. The metabolism of androstenone also occurs in the testis, and a negative correlation between testicular SULT2A1 activity and fat androstenone concentrations has been reported [60]. This suggests that the sulfoconjugation of androstenone and its metabolites during Phase II metabolism is essential for reducing the development of boar taint. However, we have recently shown that androstenone sulfate can be transported into the adipose tissue and deconjugated by the enzyme steroid sulfatase (STS) [61]. The deconjugation of androstenone sulfate was positively correlated with fat androstenone concentrations in early but not late-maturing boars, which suggests that the sulfoconjugation of the 16-androstene steroids may not reduce boar taint development in all animals. Thus, androstenone metabolism in the testis may primarily function to convert excess concentrations of androstenone to an inactive reservoir, which can be deconjugated during low periods of steroid synthesis to return free androstenone. Such is the case for sulfoconjugated estrogens in the boar [62]. In contrast, androstenone metabolism in the liver may instead favour hepatic clearance and excretion.

The activation of PXR has been found to significantly increase androstenone metabolism by porcine hepatocytes, but the mechanism behind this is not well understood. Gray and Squires [38] reported a significant decrease in the initial percentage of androstenone following treatment with rifampicin, a potent agonist of PXR in pigs and humans. The activation

of PXR also decreased the expression of *AKR1C* but did not alter the production of the Phase I metabolite 3α-androstenol; however, 3β-androstenol synthesis was decreased. Interestingly, the expression of *AKR1C* was also decreased following CAR and FXR activation, but no effect on the metabolism of androstenone was observed. These results suggest that PXR activation may increase the metabolism of androstenone by promoting the synthesis of Phase II conjugated metabolites.

In humans, the activation of PXR and CAR results in the upregulation of several genes that control Phase II metabolic reactions, including *SULT2A1*, and FXR activation induces the expression of *SULT1A1* [33,63]. However, the effect of nuclear receptor activation on the Phase II metabolism of androstenone and skatole has not been reported. Therefore, future research is needed to evaluate the effect of PXR, CAR, and FXR activation on the expression of genes regulating Phase II metabolic reactions and the production of conjugated metabolites of androstenone and skatole.

2.3. Coregulatory Proteins and Nuclear Receptor Crosstalk

The effect of nuclear receptor activation on the hepatic metabolism of boar taint compounds may also be influenced by the availability of different nuclear receptor coactivators (NCOAs) and nuclear receptor corepressors (NCORs) that regulate the transcriptional activity of PXR, CAR, and FXR.

NCOA1, NCOA2, and NCOA3 are well-characterized members of the p160 steroid receptor coactivator (SRC) protein family that are recruited to the enhancer region of target genes by ligand-activated nuclear receptors to promote gene transcription [5,64]. NCOAs share a common structure: an N-terminal basic helix–loop–helix-PER-ARNT-SIM (bHLH/PAS) domain, a serine/threonine rich domain, an "NR" box containing LXXLL motifs, which are recognized by the AF-2 region within the nuclear receptor LBD, and two C-terminal transactivation domains (AD1/AD2) [65–67]. Within each domain, there are sites for various post-translational modifications, including acetylation, phosphorylation, methylation, ubiquitination, and sumoylation [68–70]. NCOAs form multi-subunit coactivator complexes by recruiting secondary coactivator proteins (e.g., protein arginine methyltransferase 1 (PRMT1), coactivator-associated arginine methyltransferase 1 (CARM1), and CBP/p300), which remodel chromatin and modify histones to promote gene transcription [64,65,71].

In contrast, nuclear receptors recruit NCORs to the repressor region of target genes in the absence of ligand binding to inhibit gene transcription. The NCORs include NCOR1 and NCOR2, which is better known as silencing mediator for retinoid and thyroid hormone receptor (SMRT) [65]. NCOR1 contains a deacetylase activating domain (DAD), three N-terminal repression domains (RD), and three C-terminal "CoRNR" box motifs (LXX I/H I XXX I/L), which serve as sites for nuclear receptor interaction. SMRT is similar in structure but contains an additional RD and one less nuclear receptor interaction site [72–74]. NCORs exert their inhibitory effect on gene transcription through interactions with histone deacetylase 3 (HDAC3) and transducin β-like 1 X-linked/transducin β-like 1 X-liked receptor 1 (TBL1X/TBL1XR1), which bind within the DAD and RDs, respectively, as well as the recruitment of additional corepressor complexes [70,74].

Nuclear receptor crosstalk can also regulate the transcriptional activity of PXR, CAR, and FXR and this may be important to consider for future studies investigating the effect of nuclear receptor activation on the development of boar taint. Recent studies have demonstrated that activation of FXR in the testis can induce the transcription of dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene-1 (DAX-1) or small heterodimer partner (SHP) to repress steroidogenesis in pubertal mice [31,75]. In the liver, SHP represses the transcriptional activity of PXR, CAR, and FXR by inhibiting interactions with coactivator proteins; however, PXR crosstalk can regulate SHP expression [71]. PXR inhibits the recruitment of peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1α by hepatocyte nuclear factor 4 alpha (HNF4α) to repress *SHP* transcription, which restores interactions between nuclear receptors and coactivator proteins to promote the

transcription of various target genes [71,76,77]. Competition between nuclear receptors or transcription factors for common coregulatory proteins is another source of nuclear receptor crosstalk, and this occurs between PXR and CAR, which compete for the common coactivator NCOA1 [78]. Specific ligands can also differentially regulate nuclear receptor signaling pathways and promote nuclear receptor crosstalk. For example, PXR and FXR have several common agonists (e.g., bile acids such as CA, 12-ketolithocholic acid, and some bile acid derivatives), which have an inhibitory effect on CAR activity [79]. Additionally, nuclear receptors can interact with different coactivator proteins in a ligand-dependent manner [80]. Therefore, further work is required to characterize the impact of coregulatory proteins and crosstalk events on the development of boar taint in pigs.

3. The Gut–Liver Axis

The gut microbiome is a complex organ that is typically comprised of a couple hundred bacterial species expressing nearly 2 million different genes, which promote the biotransformation of xenobiotics and endogenous compounds and regulate the production of microbial metabolites in response to dietary, genetic, and environmental factors [81,82]. Microbiota-derived compounds function as signaling molecules between different bacterial species to synchronize bacterial behaviours by altering the microbial population or the gene expression within the gut microbiome, which is known as quorum sensing [83]. Gut-derived compounds also modulate metabolic pathways in the liver and intestines and act as ligands for nuclear receptors and other xenobiotic sensing transcription factors [82,84]. In response, the liver produces bile to provide feedback to the gut microbiota and regulate further metabolite production [85]. This bidirectional communication between the liver and the gut is referred to as the gut–liver axis and represents an important link between the gut microbiome and nuclear receptor signaling pathways. Despite this, research examining the relationship between the gut–liver axis and the development of boar taint is surprisingly limited.

Several studies have suggested that the metabolism of boar taint compounds may occur in extra-hepatic tissues. PXR, CAR, and FXR are constitutively expressed in the porcine intestinal tract along with CYP1A1, CYP3A29, CYP3A22, and CYP3A46 [34,35,86]. Moreover, dietary supplementation with phenobarbital was reported to induce additional CYP450s in porcine enterocytes such as CYP2C49 and CYP2C33, and it has been suggested that ligands for other nuclear receptors or transcription factors may induce different skatole metabolizing CYP450s such as CYP2E1 [58,86]. This suggests that the activation of nuclear receptors by microbial-derived compounds may regulate the metabolism of boar taint compounds in the intestinal tract and liver; however, the combined effect of extra-hepatic and hepatic metabolism on the development of boar taint is not well established.

3.1. Gut-Derived Tryptophan Metabolites

Indole-3-propionic acid (IPA) is an indole derivative synthesized from the reductive metabolism of tryptophan in the gut [87]. In this reductive pathway, tryptophan is converted to indole-3-lactic acid (ILA), indole-3-acrylic acid (IA), and IPA by several species of *Clostridium* and *Peptostreptococcus* expressing the phenyllactate dehydratase gene cluster (*fldAIBC*) [87–89]. IPA is an endogenous ligand and activator of PXR, which primarily regulates PXR signaling pathways within the gut to improve gastrointestinal barrier function [14]. IPA is also a ligand for the aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor that shares 88% of its known activators with PXR [90]. AhR is also expressed in the liver and intestinal tract of pigs, and crosstalk between AhR and PXR was previously found to mediate the cytostatic properties of IPA in breast cancer cells [86,91]. However, PXR signaling is also regulated by negative crosstalk with AhR, which may explain why IPA induces AhR, but not PXR, target genes in the liver of mice [92].

AhR is activated by several other microbial-derived metabolites of tryptophan, including indole and indole-3-acetamide (IAD), which were recently identified as low- and medium- affinity ligands of human PXR, respectively [93]. Indole is produced from the hy-

drolysis of tryptophan by over 85 different bacterial species expressing tryptophanase [94], and the conversion of tryptophan to IAD is catalyzed by tryptophan-2-monooxygenase [95]. Illés et al. [93] reported that indole and IAD bind directly to the LBD of PXR and induce the PXR target genes, CYP3A4 and multidrug resistance 1 (MDR1), in human intestinal LS180 cells as well as CYP3A4 in primary human hepatocytes. Moreover, the intestinal anti-inflammatory properties of IPA via PXR are significantly enhanced in the presence of indole [14].

Skatole is also a microbial-derived metabolite of tryptophan that is produced in response to the transient accumulation of indole acetic acid (IAA) in the hindgut of pigs [96]. Numerous bacterial species modulate the conversion of tryptophan to IAA, and several tryptophan metabolites have been identified as precursors of IAA in mice [97]. However, the production of skatole from IAA is limited to four bacterial species in pigs that belong to the *Clostridium* and *Olsenella* genera [98,99]. Skatole is a low affinity ligand and partial agonist of human PXR, but a strong inverse agonist of PXR, CAR, and FXR in pigs [34,100]. Based on this, skatole may indirectly regulate boar taint development by suppressing nuclear receptor signaling pathways that promote the metabolism of boar taint compounds, in addition to accumulating in the fat directly. However, Gray and Squires reported contradictory effects of skatole in primary porcine Leydig cells [37] and hepatocytes [38]. Skatole decreased the expression of *CYP2B22*, the porcine orthologue of *CYP2B6*, and *CYB5R1* in Leydig cells and altered the ratio of 3α/3β-androstenol production by hepatocytes. However, skatole did not affect the total production of 16-androstene or sex steroids, nor the metabolism of androstenone in the testis and liver, respectively. Moreover, skatole did not alter the expression of several genes induced by activators of PXR, CAR, and FXR in both Leydig cells and hepatocytes, suggesting that crosstalk with other transcription factors may influence the suppressive effect of skatole on nuclear receptor signaling pathways.

Skatole is a weak activator of AhR in humans and was found to decrease the mRNA expression of PXR in HepaRG cells along with several nuclear receptor target genes, including *CYP3A4*, *CYP2B6*, and *CYP2A6*, and to inhibit the induction of *CYP3A4* by rifampicin. However, the activation of AhR was proposed to de-regulate an unidentified factor mediating crosstalk between AhR, PXR, and basal CYP expression as skatole decreased the expression of CYP2E1, which is not a known target gene of PXR [90]. It is unclear if skatole is a ligand for AhR in pigs; however, the induction of CYP1A by a standard activator of AhR (β-napthoflavone) was demonstrated in primary porcine hepatocyte culture and was presumed to result from the activation of AhR [101,102]. Thus, future research should investigate the skatole-mediated activation of AhR in pigs, and potential crosstalk that is established with other nuclear receptor signaling pathways, to better understand the impact of skatole on the metabolism of boar taint compounds.

3.2. Short-Chain Fatty Acids

Acetate, propionate, and butyrate are the primary short-chain fatty acids (SCFAs) produced from the microbial fermentation of dietary fibre (e.g., pectin, hemicellulose, lignin, inulin, resistant starch) by anaerobic bacteria in the hindgut [103,104]. As extracellular signaling molecules, SCFAs target G-protein coupled receptors (GPR41, GPR43, GPR109a) to regulate protein kinase-dependent intracellular signaling pathways in the liver and the gut [105,106]. Moreover, propionate and butyrate inhibit histone deacetylases (HDACs) to regulate gene transcription [107]. This suggests that there are several opportunities for crosstalk between SCFAs and nuclear receptor signaling pathways. Interestingly, methoxyacetic acid and valproic acid, which are xenobiotics derived from the SCFAs acetate and valerate, respectively, have been found to enhance the activity of several steroid-activated nuclear receptors (e.g., estrogen receptor, progesterone receptor) via crosstalk involving mitogen-activated protein kinase signaling and inhibition of histone deacetylase [108]. Acetate, propionate, and butyrate induced histone acetylation and CYP1A1 in both Caco-2 and YAMC cells and enhanced the recruitment of AhR to the promoter [109]. Moreover, the effects of 1,4-dihydroxy-2-naphthoic acid (DHNA), a known activator of AhR, were

enhanced in mice cotreated with butyrate and resulted in a 50-fold induction of CYP1A1 in the liver [109]. Butyrate was also reported to regulate CYP1A1 expression directly as a ligand and activator of AhR in human intestinal cells and was shown to induce PXR expression in Caco-2 cells [110,111]. This suggests that SCFAs such as butyrate may regulate nuclear receptor signaling pathways to control the metabolism of boar taint compounds.

3.3. Bile Acids

The primary bile acids CDCA and CA are cholesterol metabolites produced in the liver. Following synthesis, the primary bile acids are conjugated with glycine or taurine and incorporated into the bile. CDCA and CA are deconjugated by bile salt hydrolase enzymes, which are expressed by many bacterial species, including *Lactobacillus* [112–114], *Enterococcus* [115], *Bifidobacterium* [116,117], *Clostridium* [118], and *Bacteroides* [119]. Following deconjugation, approximately 95% of the bile acids released into the gut are re-absorbed and transported back to the liver via the hepatic portal vein bound to albumin or lipoproteins in what is known as the enterohepatic circulation [120–122]. Bile acids that escape re-absorption are metabolized in the colon by bacterial flora with 7α-dehydroxylation activity, resulting in the production of the secondary bile acids DCA and LCA from CA and CDCA, respectively [123,124].

As endogenous ligands and activators of FXR, bile acids induce the expression of several enzymes in the liver and gastrointestinal tract to autoregulate subsequent bile acid synthesis, transport, and metabolism and mitigate their potential cytotoxic effects. Upon activation, FXR increases the expression of SHP, which interacts with HNF4α and liver receptor homolog-1 (LRH-1) to inhibit CYP7A1 expression and bile acid synthesis [125]. Bile acids also work through FXR to directly increase the transcription of PXR, which functions as a target receptor for LCA and DCA [32,126]. The bile acid-mediated activation of FXR and PXR upregulates the expression of SULT2A1, UGT2B4, and CYP3A4 to promote bile acid metabolism/detoxification [127–129]. Some bile acids, bile acid conjugates, and bile acid metabolites also have inhibitory effects on CAR activity in humans and mice [130]. The metabolism of androstenone and skatole is dependent on many of the same hydroxylation and conjugation reactions that promote bile acid detoxification. Therefore, circulating levels of bile acids may indirectly affect the development of boar taint.

Like bile acids, androstenone is also thought to be recycled in the gut through the enterohepatic circulation. The inclusion of non-nutritive sorbent materials, most notably activated charcoal, in finishing diets was previously reported to significantly decrease fat androstenone concentrations [131]. While the mechanism behind this is unclear, it was proposed that dietary sorbent materials may disrupt the enterohepatic circulation of androstenone to promote excretion. This suggests that dietary sorbent materials may also indirectly alter nuclear receptor signaling pathways by reducing circulating levels of bile acids and bile acid derivatives. Therefore, future research aimed at characterizing the disruption of the enterohepatic circulation by dietary sorbent materials should also consider the potential effect of these binding agents on the activation or inhibition of PXR, CAR, and FXR, and the downstream consequences on the metabolism of androstenone and skatole.

3.4. Diet

The production of microbiota-derived compounds in the gut is highly dependent on the composition of the diet, and several dietary compounds have been investigated as a treatment strategy for boar taint. Most notably, raw potato starch, sugar beet pulp, chicory inulin, and other fermentable fibre sources can significantly reduce the synthesis of skatole in the hindgut; however, the exact mechanism behind this is not well understood.

Claus et al. [132] attributed the effects of fermentable carbohydrates to the production of butyrate, which was shown to act in the gastrointestinal tract to inhibit apoptosis of colon crypt cells and reduce the production of cell debris that would otherwise provide a source of tryptophan for skatole synthesis. However, opposite effects on skatole synthesis and

apoptosis have been reported following butyrate treatment via intracecal infusion [133]. Interestingly, butyrate can promote either growth stimulatory or apoptotic effects in human colorectal tumour cell lines in the absence and presence of glucose, respectively [134]. Therefore, this may explain the controversial effects of butyrate on skatole synthesis.

Diets containing high levels of sugar beet pulp or chicory root effectively decrease fecal skatole concentrations and simultaneously increase the synthesis of IPA [133,135]. Although it has been suggested that fermentable carbohydrates may alter the microbial metabolism of tryptophan to favour the synthesis of IPA over skatole, IPA may alternatively act through nuclear receptor signaling pathways to promote skatole metabolism and clearance. Although the exact mechanism is unclear, the hepatic expression of CYP2E1 was increased in pigs fed sugar beet pulp [136]. Moreover, dietary supplementation with dried chicory root was reported to significantly increase the hepatic expression of CYP1A2 and CYP2A19 at the mRNA and protein level and CYP2E1 at the mRNA level relative to boars fed a standard control diet [137]. This suggests a possible link between IPA synthesis from the fermentation of dietary fibre, nuclear receptor activation, and boar taint metabolism. However, chicory root contains several sesquiterpene lactones, and some of these compounds (e.g., artemisinin) are established nuclear receptor agonists [138], which may alternatively explain these results.

In addition to chicory root, several plant species and herbal medicines contain active compounds capable of selectively regulating nuclear receptor signaling pathways [139,140]. For example, oleanolic acid is a selective modulator of FXR found in many plant species and is used in Chinese herbal medicine for its hepatoprotective and anti-inflammatory effects [141]. Diallyl sulfide is an active ingredient found in garlic and an agonist of CAR, which has been reported to inhibit the activity of CYP2E1 in vivo and induce the expression of several CYP450s, including CYP1A, CYP2B, and CYP3A [20,141–143]. Moreover, hyperforin, from *Hypericum perforatum* or St. John's Wort, is a high-affinity agonist of PXR [144], and the phytoestrogen coumestrol is a PXR antagonist [145]. Several compounds are also targets for multiple nuclear receptors, including ginkgolide A, a terpenoid found in *Ginkgo biloba*, which is an agonist of both PXR and CAR [146–148] and (Z)-guggulsterone, a plant sterol found in guggul plant (*Commiphora mukul*) resin, which is an agonist of PXR, antagonist of FXR, and inverse agonist of CAR [149,150]. Many natural products also contain fermentable carbohydrates that can increase the production of SCFAs, and several have been reported to modulate the composition of the gut microbiome (reviewed in [151]). Therefore, natural products may be a promising dietary treatment strategy for preventing the development of boar taint.

4. Recommendations for Future Research

Nuclear receptor signaling pathways have been extensively studied and well characterized in humans and mice, but studies investigating these effects in pigs are limited. Few studies have examined the impact of nuclear receptor transactivation on the synthesis and metabolism of boar taint compounds. The studies aimed at understanding these effects in pigs have predominantly been performed in vitro using primary cell culture systems, and only a limited number of agonists for PXR, CAR, and FXR have been investigated. These compounds have typically included rifampicin and 6-(4-Chlorophenyl)imidazo[2,1-b] [1,3] thiazole-5-carbaldehyde O-3,4-dichlorobenzyl) oxime (CITCO), which are pharmaceutical agents and the primary bile acid CDCA. Future research is needed to establish the effect of selective nuclear receptor activation on the synthesis and metabolism of androstenone and skatole in vivo to determine the efficacy of this method as a treatment for boar taint. However, the potential cytotoxic effects of dietary supplementation with rifampicin, CITCO, or CDCA have not been well characterized in a whole animal system.

In a clinical trial, 10 mg/kg of rifampicin was provided as a treatment for patients with primary biliary cirrhosis daily over 14 months; however, 37.5% of the patients withdrew from the study after six months of treatment due to rifampicin-induced side effects [152]. CITCO (20 mg/kg) has been safely provided to hCAR-transgenic mice over six days as

an adjuvant to enhance the tumour suppressing effects of the chemotherapy combination "CHOP", which contains cyclophosphamide, doxorubicin, vincristine, and prednisone [153]. Moreover, a dietary inclusion level of 200 mg/kg for CDCA was previously found to increase body weight and average daily gain and improve intestinal health in piglets over 30 days [154]. However, it is not clear if these treatment durations would be sufficient for the prevention of boar taint or if these inclusion levels could be safely used in different species or older animals, as the hepatic expression of nuclear receptors and CYP450 enzymes increases significantly at the onset of puberty [155]. The use of these compounds may also create concerns regarding food safety due to the potential presence of drug residues in pork products from treated animals. Therefore, natural products may be a more suitable treatment for the prevention of boar taint, but in vitro studies are first needed to investigate the ability of these natural product compounds to selectively activate or inhibit porcine PXR, CAR, or FXR-mediated signaling pathways, which control the synthesis and metabolism of androstenone and skatole. This will allow for the identification of compounds that can maximize the metabolism of androstenone and skatole in the liver, without compromising the synthesis of sex steroids or increasing androstenone production in the testis. These studies should also consider the effect of coregulatory proteins and crosstalk events that may alter the response to treatment in a whole animal model in the presence of other endogenous ligands, which regulate the same or similar signaling pathways.

Numerous factors and physiological systems differentially regulate the development of boar taint in individual animals. Consequently, the response to most existing treatments for boar taint varies significantly between individual animals. We have summarized these factors and their impact in a recent review [156]. This suggests that nuclear receptor transactivation may only regulate the synthesis and metabolism of boar taint compounds in a subset of animals, and this aspect should be carefully considered for future studies. The use of a defined whole animal model would allow for the identification of biomarkers that are common amongst animals that respond favourably to treatment. This could be accompanied with 16S rRNA sequencing to identify microbial communities in the gut that may act through the gut–liver axis to promote favourable treatment outcomes. The production of microbial metabolites in response to treatment should also be quantified using appropriate analytical methods including mass spectrometry or chromatography. Moreover, an interaction between the gut microbiome and the genome of the host should be considered due to the complex nature of boar taint. Transcriptome analysis using RNA sequencing has recently led to the identification of novel biomarkers and expression quantitative trait loci (eQTLs), which correspond to concentrations of androstenone and skatole in the fat [157–159]. These genetic markers complement the various candidate genes associated with high and low levels of boar taint that have previously been identified in the testis and liver (reviewed in [160]) and may also be useful for developing a gene profile associated with a favourable treatment outcome to reduce boar taint.

5. Conclusions

Nuclear receptor signaling pathways can regulate various physiological processes, from bile acid homeostasis to detoxifying drugs and other exogenous compounds. There is also evidence to suggest that the nuclear receptors PXR, CAR, and FXR regulate the expression of genes controlling the synthesis and metabolism of compounds that are responsible for the development of boar taint. However, these effects need to be evaluated using whole animal studies. Several endogenous compounds such as tryptophan metabolites, SCFAs, or bile acids, which are produced or metabolized by microbes in the gut, have been suggested to alter nuclear receptor signaling pathways. Based on this, it may be possible to prevent the development of boar taint by providing dietary sources of fermentable carbohydrates and related compounds to promote the activation or inhibition of PXR, CAR, or FXR-mediated signaling pathways by altering the basal production of these endogenous compounds. Several natural products commonly found in plants have been identified as nuclear receptor ligands in humans and mice. These compounds could potentially be included as a dietary

supplement to selectively activate or inhibit nuclear receptor-mediated signaling events for the prevention of boar taint.

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