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

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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 tryptophan. 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

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).
**Androstenone.** Androstenone is a steroid hormone. 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 20 -sec) 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 40 to pregnenolone. Thereafter, pregnenolone is hydroxylated to progesterone by 3β-hydroxysteroid dehydrogenase (Squires, 2003). Androstenone belongs specifically to the group of 16-androstan steroids, which are synthesized from progesterone in the testes (Melrose et al., 2009). 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 5α-reductase. Levels of this enzyme correlate with androstenone concentration (Cook 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-androstenedol and less frequently to 3α-androstenedol, by 3β-HSD and 3α-HSD enzymes in liver microsomes. In the following second phase of metabolism, androstenedols 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-hydroxy skatole, indole-3-carbinol, 3-hydroxy-3-methyloxindole (HMOI), 3-hydroxy-3-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-sulfooxy-skatol in sulphate or glucuronide bond with 5-hydroxy-3-methylindole or 3-hydroxy-3-methyloxindole (Diaz, Squires, 2006).
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

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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 (Claus, Raab, 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 (Losel et al., 2006; Pauly et al., 2008; Overland 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 (Shiotsubo, 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 (Overland 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
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 antibiotic 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 (Robertroid 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-1,3-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 (Pauly et al., 2011). It can be expected that less soluble β-glucans (ooligosaccharides) 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 Campulaceae. 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 (Jost et al., 2010; Overland et al., 2011; Zammulli et al., 2012). One of the first studies on this topic demonstrates a significant decrease of skatole concentration (by 50–70 %) in adipose tissue (Clauss 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 pigs.
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 (Vhile 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 (Güengerich, 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 (Güengerich, 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 (Urquhart 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 (Rasmussen 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 (Achour 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, Squires, 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 (Lemley 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 (Chang, 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 (Bais, Ravishankar, 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 (Rasmussen 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, esculentin, and esculin) would have on liver CYP mRNA in primary liver hepatocytes, compared to the total extract of chicory roots. It was
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 Ekstram 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.

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