

Effect of in-feed indigestible protein content on fat skatole deposition in entire male pigs

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The aim of this study was to determine whether reducing the indigestible protein content in the finishing diet is a viable way to decrease skatole content in the fat of entire male pigs. Skatole and androstenone are the two major compounds that cause pork from entire male pigs to sometimes give off the unpleasant sex odour called 'boar taint'. Skatole is formed by the microbial degradation of proteins in the large intestine where, once absorbed by the intestinal mucosa, it can then deposit and accumulate in fat tissue.

This study was designed to test whether increasing in-feed ileal protein digestibility to reduce the amount of undigested proteins reaching the large intestine also reduces skatole production. Effect comparison is based on skatole deposition in carcass fat tissue. Increasing the ileal protein digestibility of the diet given to pigs in the last four weeks of finishing was not found to reduce fat skatole content. In fact, fat skatole content was increased significantly, as 30.2% of carcasses from pigs fed the more-protein-digestible diet showed a skatole content greater than the 0.2 µg/g cut-off versus just 15.2% in the less-protein-digestible diet group. This effect was probably connected to the fibre content decrease of the feed that results from the better protein digestibility. Indeed, fibre is widely thought to promote lower intestinal skatole production, as it shifts the balance of fermentation processes in the large intestine away from proteins and towards sugars.

Effet de la teneur en protéines non digestibles de l'aliment sur le dépôt de scatol dans le gras de porcs mâles entiers

Cette étude a pour objectif de déterminer s'il existe un intérêt de réduire la teneur en protéines non digestibles de l'aliment distribué en fin d'engraissement, afin de diminuer la teneur en scatol des gras de porcs mâles entiers. Le scatol fait partie avec l'androstenone des deux composants majeurs à l'origine des odeurs sexuelles dégagées parfois par les viandes issues de porcs mâles entiers. Il provient de la dégradation des protéines dans le gros intestin et peut se déposer dans le gras des animaux après avoir été absorbé par la muqueuse intestinale.

L'objet de l'étude consiste à analyser l'incidence de l'augmentation de la digestibilité iléale des protéines de l'aliment permettant une réduction de la quantité de protéines non digérées parvenant dans le gros intestin et donc potentiellement une réduction de la production de scatol. Le critère de comparaison se base sur le dépôt tissulaire de scatol dans le gras des carcasses.

L'augmentation de la digestibilité iléale des protéines de l'aliment distribué au cours du dernier mois d'engraissement ne conduit pas à la réduction de la teneur en scatol des gras. Au contraire, celle-ci est significativement accrue, puisque 30,2% des carcasses issues du régime le plus digestible sur le plan protéique, présentent une teneur en scatol supérieure à 0,20 µg/g, pour 15,2 % au régime le moins digestible. Cet effet est vraisemblablement à relier à la réduction de la teneur en fibres de l'aliment qui est la conséquence de l'amélioration de la digestibilité des protéines. Or les fibres sont généralement considérées comme favorables à la réduction de la production intestinale de scatol, car elles limitent l'importance des fermentations protéiques siégeant dans le gros intestin au profit des fermentations de nature glucidique.

Keywords: entire male pig, fat, skatole, androstenone, digestibility, fattening, odour, porc

Mots clés : mâles non castrés, gras, scatol, androstenone, digestibilité, engraissement, odeurs, porc

Background

The upcoming ban on current castration practices will likely bring about a rise in the proportion of entire male pigs on French pig farms. However, pork from entire male pigs can sometimes give off offensive sex odour called 'boar taint'. Androstenone (urine-like odour) and skatole (faecal-like odour) deposited in fat tissues have been pinpointed as the main causes of this boar taint defect (Bonneau *et al.* 1991, Chevillon *et al.* 2011). Androstenone is a steroid compound produced in the testes, whereas skatole is a bacterial metabolite of tryptophan degradation in the hindgut (Bonneau, 1988).

Even though the 'boar taint risk' in entire male pig fat appears relatively low in France (pig farms count from 0 to 10% of entire male pigs testing positive at a cut-off of 0.2 µg skatole per g fat) compared to other EU countries (2009 EU AlCasDe [Alternatives to Castration and Dehorning] Programme), the data available point to a sizeable but as-yet unexplainable variation in boar taint between pig farms. If the production of entire male pigs gains ground in France, it will bring a need for farm system and/or feed system management guidelines to minimize the boar taint risk.

On the diet front, giving low-digestibility proteins is thought to promote intestinal skatole production and thus increase skatole deposition in fat tissue (Jensen, 2006). As undigested proteins reaching the large intestine tend to increase bacterial proteolytic activity in the colon, it follows that they ultimately increase the amount of substrate potentially available for skatole formation.

Objectives and protocol

Objectives

The focus of this research is the effect of feed indigestible protein content on fat skatole deposition in entire male pigs. Undigested proteins in the small intestine can effectively serve as substrate for bacteria in the caecum and colon, therefore promoting intestinal skatole production and consequently skatole deposition in the fat of primal cuts used for fresh meat or processed products.

The main objective of this study was **to gain deeper insight into the effect of ileal protein digestibility parameters on skatole deposition in fat tissue**. It is therefore expected to inform on whether reducing the in-feed indigestible protein fraction can effectively reduce on-farm boar taint risk.

Protocol

This trial used 48 intact crossbred male pigs [(Large White × Landrace) sows mated with (Landrace × Pietrain) boars]. The pigs were taken from post-weaning and housed individually in two identically-configured finishing stalls right through to transfer to the slaughterhouse. They were given a growth-type feed ration (total protein: 16%, NE: 9.70 MJ, digestible lysine per MJNE: 0.89 g). After an 8 week finishing period, the pigs were individually weighed fasted. They were then batched into age-matched and sex-matched pairs and each member was randomized into one of two diet groups. The pigs were then fed for 41 days on the experimental diets which were given ad libitum up to an intake capped at 24 MJNE per day. Any refusals were weighed and measured for dry matter content. All 48 same-group pigs were slaughtered in a single all-in-one batch. Carcass characteristics (hot carcass weight, fat thickness and lean thickness) were measured immediately on the slaughter line. Backfat samples were taken to be assayed for skatole and androstenone content by the INRA's Saint-Gilles (northwest France) research lab.

The experimental diets were established based on nutritional values given in the INRA-AFZ feed tables (2004), chiefly the standardized ileal digestibility coefficients of the feed material amino acids. The control diet offers high ileal amino acid digestibility as it uses soybean meal as main protein source with animal requirements completed by synthetic amino acids. This feed has a particularly straightforward composition, as it only counts two cereals blended with soybean meal. It has a relatively low protein content (13%). The test diet is a more complex ration mix as it incorporates several different protein sources: peas, wheat bran and rapeseed meal. These feed materials were chosen on the basis that they feature lower-digestibility amino acids. The test diet has a 15% protein content, which is closer to the protein content of a regular finisher-ration feed. The net energy concentrations of the two diets (control diet: 10.2 MJNE; test diet: 9.7 MJNE) are different due to the diet characteristics targeted by design. The feed materials used in the test-diet feed bring added fibre compared to the control diet to help reduce the energy content of the feed. Given the technological limits to the amount of oil that manufacturers can viably incorporate in feed pellets, we elected to limit their inclusion in the test diet and to adopt an iso-energetic feed distribution plan. Note too that digestible lysine per MJNE was identical between diets (0.76 g/MJNE) and that digestible methionine, methionine-plus-cystine, threonine and tryptophan contents fitted with industry-standard ratios for porker feed (at 30%, 60%, 65% and 19% of digestible lysine content, respectively).

Certain feed materials were deliberately excluded from the experimental diets by design on the basis that they would have delivered an over-high proportion of fermentative substrate to intestinal bacteria (β -glucans from barley, pectins from beet pulp) and consequently interfere with the indigestible protein fraction under study.

Feeds samples were collected every week over the course of the trial then pooled to form a representative sample of the feeds given to the animals in the experiment. Analyses led on these samples served to verify conformity of feed manufacture.

Results

Table 2 reports the mean per-diet data obtained for each measured parameter. Batch-1 pigs received the experimental diets from 75 kg BW and were slaughtered at an average weight of 114 kg, Batch-2 pigs started at 81 kg BW and were slaughtered at 118 kg liveweight. Control-diet pigs showed significantly improved animal performances ($p < 0.05$): growth rate improved by 5%, feed conversion ratio by 9% and feed energy conversion ratio by 4%. In number terms, weight at slaughter was not significantly different, although carcass weight tended to be higher (+2.1 kg; $p < 0.10$) in control-diet pigs. Control-diet pigs showed significantly higher carcass yield (+0.9%), but they also showed a significant increase in carcass backfat thickness (+1.1 mm) that penalized their mean LMP score (-0.8%) as the M2 lean thicknesses remained identical.

Table 1: Composition and nutritional characteristics of the experimental diets

Diet	Control	Test
Composition, kg/T		
Wheat	583	227
Maize	300	300
Wheat bran		80
Field peas		222
Soybean meal	86	
Rapeseed meal		99
Sunflower meal		30
Vegetable oil		18
Estimated nutritional characteristics¹		
Dry matter (%)	87.1	87.3
Total nitrogen (%)	13.0	15.0
Digestible nitrogen (%)	85.0	79.5
Lipids (%)	2.1	4.0
Crude cellulose (%)	2.5	5.0
Total ash (%)	4.1	4.6
Lysine (g/kg)	8.4	8.9
Digestible lysine (g/kg)	7.70	7.35
DE (MJ/kg)	13.7	13.4
NE (MJ/kg)	10.2	9.7
Dig. lys per MJNE (g/MJ)	0.75	0.76

¹: estimated based on composition and nutritional value tables for feed materials (INRA, 2004)

The fat skatole and androstenone contents in entire male pigs measured here are on average higher than in Chevillon *et al.* (2010). Proportion of entire male pigs with high fat skatole (i.e. over the 0.2 $\mu\text{g/g}$ cut-off) was

Table 2: Animal performances, carcass characteristics and skatole and androstenone contents compared by diet

Diet	Control	Test	Statistics ¹	
			Effects	RSD
Initial weight (kg)	77.9	78.2	B**	6.1
ADG ² (g/d)	942	896	D**, B**	78
ADI ² (kg/d)	2.39	2.49	D**	0.03
FCR ² (kg/kg)	2.56	2.81	D**, B**	0.23
eFCR ² (MJNE/kg)	26.10	27.26	D*, B**	2.29
Slaughter weight (kg)	116.5	114.9	B**	6.2
Carcass weight (kg)	91.1	89.0	D ¹ , B**	5.1
Carcass yield ² (%)	78.3	77.4	D**	0.0
Fat thickness—G2 (mm)	13.1	12.0	D*	2.3
Lean thickness—M2 (mm)	56.4	56.4	B**	5.5
LMP ² (%)	60.7	61.5	D*	1.9
Skatole ($\mu\text{g/g}$)	0.22	0.13	D*	0.17
Androstenone ($\mu\text{g/g}$)	1.15	1.15	B*	0.88

¹: based on ANOVA factorizing the effects of diet, batch and diet \times batch interaction; RSD = residual standard deviation, D = diet effect, B = batch effect. Levels of significance = t: $p < 0.10$, *: $p < 0.05$, **: $p < 0.01$. Data are reported as adjusted means.

²: ADG: average daily gain, ADI: average daily intake, FCR: feed conversion ratio, eFCR: feed energy conversion ratio indexed as net energy intake ratioed to weight gain, LMP: lean meat percentage calculated from M2 and G2 measurements.

22% here, whereas Chevillon *et al.* (2010) reported a 3% rate based on measurement in 347 pigs from 6 different pig farms. Recalculating to a 0.1 µg/g cut-off, the proportion found here would reach 64% compared to just 12% in Chevillon *et al.*'s 2010 dataset. Likewise, proportion of entire male pigs with high fat androstenone (i.e. over a 1.0 µg/g cut-off) was 40% here, whereas Chevillon *et al.*'s 2010 dataset gives a 20% rate. Recalculating to a 0.5 µg/g cut-off, the proportion would reach 80% here vs 50% in Chevillon *et al.*'s 2010 dataset.

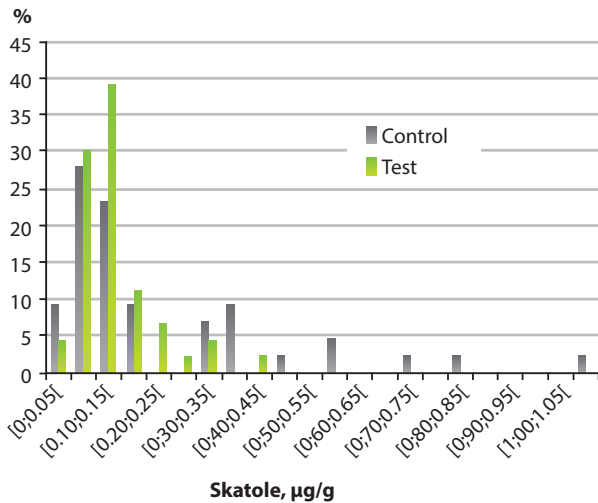


Figure 1: Carcass fat skatole contents plotted per diet

Diet effect was significant on skatole content: control-diet pig carcasses showed a higher mean skatole content than test-diet pig carcasses. Proportion of carcasses with high-skatole content (i.e. over a 0.2 µg/g cut-off) was 30.2% with the control diet vs 15.2% with the test diet. Diet effect was not significant on androstenone content, but a significant batch effect was observed: average fat androstenone content was 0.94 µg/g in batch-1 pigs vs 1.38 µg/g in batch-2 pigs.

Table 3: Correlation coefficients between fat skatole and androstenone contents and animal performance and carcass characteristic parameters

	Skatole	Androstenone
Initial weight (kg)	0.12	0.17
ADG (g/d)	-0.15	-0.21 ^t
FCR (kg/kg)	0.07	0.21*
eFCR (MJNE/kg)	0.15	0.23*
Slaughter weight (kg)	0.06	0.07
Carcass weight (kg)	0.12	0.06
Fat thickness—G2 (mm)	0.13	0.12
Lean thickness—M2 (mm)	0.06	0.10

Table 3 reports the observed correlation coefficients between fat skatole and androstenone contents and animal performance and carcass characteristic parameters. Skatole content did not correlate with any of the parameters studied. Androstenone content was significantly correlated with feed conversion ratios, whether when calculated in kg feed intake or MJNE intake, and tended to correlate with growth rate: androstenone content increases with increasing feed conversion ratio but decreases with increasing growth rate. However, the correlation coefficients calculated remain relatively low.

Likewise, the correlation coefficient computed between fat skatole and androstenone contents was significant but still relatively low (+0.23). The data obtained is reported in two sets stratified as over and under a cut-off, i.e. with androstenone contents < 1.0 µg/g (Figure 2) and

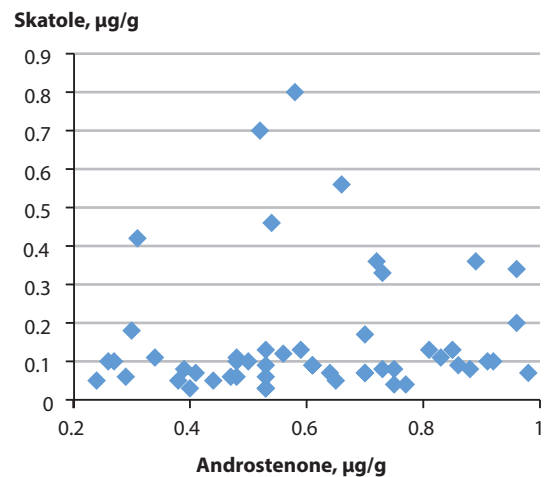


Figure 2: Skatole contents per carcass fat androstenone content with androstenone contents < 1.0 µg/g

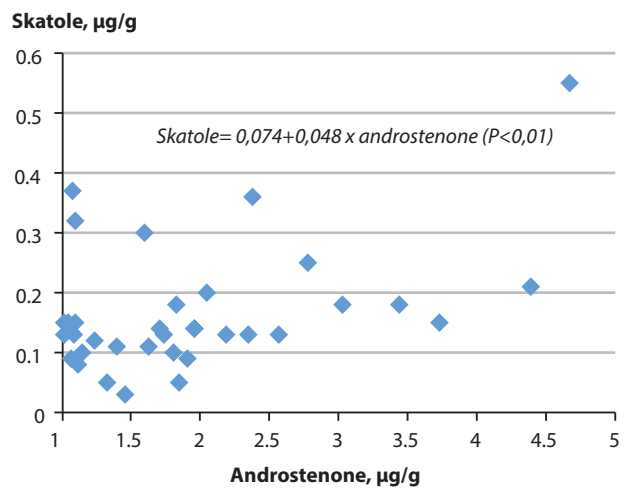


Figure 3: Skatole contents per carcass fat androstenone content with androstenone contents > 1.0 µg/g

with androstenone contents $> 1.0 \mu\text{g/g}$ (Figure 3). This stratification reveals that when androstenone content is $< 1.0 \mu\text{g}$, carcass fat skatole and androstenone contents are unrelated, whereas when androstenone content is $> 1.0 \mu\text{g}$, skatole content increases linearly with increasing androstenone content (at around $0.1 \mu\text{g/g}$ extra skatole per $2 \mu\text{g/g}$ androstenone).

Discussion

Skatole and androstenone contents as measured here were higher than values routinely reported, but are known to vary widely farm-to-farm (Chevillon *et al.* 2010). Our data confirm that animal performance factors (growth rate and feed conversion ratio) have little effect on carcass fat skatole and androstenone contents. The observed trend between growth rate and androstenone content may even appear to run counter to the supposedly beneficial effect of this diet restriction (EFSA, 2004), although the correlation remains weak. Likewise, we found no slaughter weight effect on fat skatole and androstenone contents, although note that the trial was deliberately designed to minimize slaughter weight variability.

This study found that the test diet had a clear effect on carcass fat skatole content—but the effect was the reverse of what we initially expected to find. Increasing the ileal protein digestibility of the feed, which equates to decreasing the fraction of undigested proteins leaving the small intestine and reaching the large intestine, not only turned out ineffective against boar taint risk but actually proved counter-productive. Proportion of carcasses with a skatole content greater than the $0.2 \mu\text{g/g}$ cut-off was doubled in the more-protein-digestible diet compared to the less-protein-digestible diet. Jensen (2006) posits that giving a low-ileal-digestibility feed would risk stimulating skatole production. Our findings thus run counter to this hypothesis. Jensen *et al.* (1995) built their conclusion on their previous work comparing casein and brewer's dried grain.

However, the negative effect on boar taint risk of the better-protein-digestibility diet could be explained by the lower proportion of fibre in the test feed, as its crude cellulose content was minimal (2.5%) and limited to just half that found in the 15% protein diet. However, Jensen (2006) claimed that good sources of dietary fibre can help reduce skatole levels, although the mechanisms of action were not clearly identified. Jensen (2006) cited fructooligosaccharides and native potato starch as skatole-reducing compounds. Other potentially skatole-reducing feed materials include lupins, and possibly also beet pulp although the results so far are conflicting.

Lastly, comparison of the combined skatole and androstenone contents in carcass fat suggests that high androstenone levels amplify the risk of finding high skatole levels. There is a logical interaction between androstenone production and skatole deposition, since androstenone decreases skatole catabolism in the liver, which consequently increases the risk of skatole deposition in fat tissues (Babol *et al.*, 1999), and our results concur with this pattern.

Conclusion

The fat skatole and androstenone contents in entire male pigs measured in this study led at the Romillé experimental pig farm in northwest France were inexplicably high compared to values routinely reported. This result, although confirming the strong farm-factor effect on boar taint, is also important in that this research piggery is run in conditions that make it a good model for investigating ways to reduce the carcass content of these unpleasant odour compounds.

Our study showed that skatole deposition in carcass fat was increased with a higher ileal protein digestibility diet. This finding appears to run counter to the literature data, but may be explained by the reduction in added dietary fibre that comes with reducing the indigestible protein nitrogen fraction, as fibre is widely thought to promote lower skatole production.

Our findings also highlight a potential linkage between androstenone production in the testes and skatole deposition in fat, as high androstenone tends to increase the risk of high skatole.



Acknowledgements

This study was financed under the French national programme for rural and agricultural development. Inaporc provided financial support.

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How to cite

- Gaudré D., Chevillon P., Gault E., Lhommeau T., Le Roux A., 2016. Effect of in-feed indigestible protein content on fat skatole deposition in entire male pigs. *Les Cahiers de l'IFIP*, 3(1), 19-24.