Skatole levels in pigs selected on high and low protein diets

K. LUNDSTRÖM[1], B. MALMFORS[1], S. STERN[1], L. RYDHMER[1], A.B. MORTENSEN[2] AND H.P. MORTENSEN[2]

[1] Dept. of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden; [2] Danish Meat Research Institute, P.O. Box 57, DK-4000 Roskilde, Denmark

SUMMARY: Skatole levels were studied in two lines of pigs selected for lean tissue growth rate on either a low-protein diet containing yellow peas, or a high-protein diet based on conventional feedstuffs. Males from the low protein line had higher skatole levels than males fed the high protein diet or females fed either diet ($p \leq 0.001$). No difference was found between males and females from the high protein line. Only 2.7% of males in the high protein line exceeded the threshold value suggested for skatole (≥ 0.20 ppm), which should be compared with 23.1% of the males in the low protein line. Possible explanations for the divergence between lines are discussed.

INTRODUCTION: Boar taint, identifiable chiefly in heated fat of some entire male pig carcasses, is considered to be caused by two main compounds - androstenone and skatole. Androstenone (5α-androst-16-en-3-one), with a structure similar to the male sex hormone testosterone, acts as a pheromone but has no anabolic effect. Skatole (3-methylindole) is produced when the amino acid tryptophan is broken down by bacteria in the porcine gut. Skatole is produced in both sexes, but it is still not known why only entire male pigs store skatole in the body tissues in amounts that cause taint problems.

Several factors have been shown to influence the incidence of boar taint (for recent reviews, see e.g. BONNEAU, 1990; LUNDSTRÖM, 1990; MALMFORS et al., 1990). The level of androstenone in fat is highly dependent on genetic factors that affect both sexual maturity and potential for androstenone production. Other factors are important too, probably by virtue of their effect on sexual maturity, e.g. feeding level, age and weight, and presence of females. The skatole level in adipose tissue is highly influenced by environmental factors, such as diet, feeding level and health status, resulting in large differences between herds. Possible genetic influence on skatole storage in boar fat has so far been very little investigated.

The purpose of the present investigation was to study the effect on skatole storage in adipose tissue, when pigs were selected either on a low-protein diet including yellow peas, or on a high-protein diet based on conventional feedstuffs.

MATERIALS and METHODS: A selection experiment with purebred halothane-negative Yorkshire pigs was conducted at the Department of Animal Breeding and Genetics, Uppsala. Two lines were selected for lean tissue growth rate, on either a low (13.1% crude protein, 0.64% lysine) or a high protein diet (18.5% crude protein, 0.96% lysine). The energy level was 11.9 MJ/kg metabolizable energy (ME) in both diets, and the total ME offered was the same in both lines (see Table 1 for feed composition). When the selection lines were established, litters were split into both the high and the low protein lines with two replicates in each. The animals included in the present investigation were 164 entire males and 91 females from the 1st and 4th generations of the selection experiment. The two sexes were raised in separate buildings, with the replicates within line and sex in the same building. Males and females were mixed before being transported to the slaughterhouse (6 km), and kept in lairage for 2 hours before slaughter. All animals were slaughtered at a live weight of approximately 103 kg. Growth rate was calculated between 25 and 90 kg live weight.

Partial dissection and sample preparation: At jointing, one or two days after slaughter, the carcasses were partially dissected. Backfat was sampled from the lumbar region, vacuum packed and kept frozen until skatole analyses were performed. Before the selection experiment started, 200 carcasses were dissected completely. The estimation equation for lean meat percentage in the carcass was calculated by stepwise regression (STERN et al., 1991). The following equation ($R^2 = 0.87$) was used: lean meat percentage = 22.781 + 0.181 * carcass weight (kg) - 0.0103 * carcass length (mm) - 0.165 * sidefat thickness (mm) - 4.017 * fat in ham (kg) + 0.386 * %lean meat in ham + 0.666 * %ham of carcass without head.

Skatole analysis: Skatole was analysed with the spectrophotometric method developed in Denmark (MORTENSEN and SØRENSEN, 1984). The method is not specific for skatole, and compounds with

Table 1. Composition of the low and high protein diets

Ingredients, %	Low protein diet	High protein diet
Barley	62.2	53.9
Oats	10	10
Wheat	10	10
Soya meal	-	12
Yellow peas	10	-
Fish meal	1	3
Rapeseed	3	8
Monocalcium phosphate	1.3	0.8
Limestone	1.2	1.0
Sodium chloride	0.3	0.3
Vitamin	1.0	1.0
Crude protein	13.1	18.5
Crude fibre	4.3	5.0
Digestible energy	11.9	11.9
Lysine	0.64	0.96

chemical characteristics similar to skatole (e.g. indole) could interfere. The results should therefore be regarded as skatole equivalents, but are presented here as skatole (ppm in fat, wet weight).

Statistical analysis: All calculations were performed using the Statistical Analysis System (SAS INSTITUTE INC., 1985). The effects of generation, selection line, sire, dam, sex and the line by sex interaction were included in the model. In addition, a corresponding model but also including the effect of replicate within line as well as the replicate by generation interaction was tested within each sex.

RESULTS: The two selection lines differed widely at slaughter, both in skatole content and in growth rate and proportion of lean meat in the carcass. In pigs from the low protein line, growth was generally slower, resulting in a higher age at slaughter. Percentage lean meat was also lower (Table 2).

Males from the low protein line had significantly higher skatole levels ($p \leq 0.001$) than males fed the high protein diet or females fed either diet. No difference was found between males and females from the high protein line. Only 2.7% of males in the high protein line exceeded the threshold value suggested for skatole (≥ 0.20 ppm), which should be compared with 23.1% of the males in the low protein line. No females exceeded the threshold. The numbers of entire male pigs with skatole levels ≥ 0.20 ppm within line, generation and replicate are presented in Table 3, together with least-squares means for skatole concentration. In generation 4, males within one replicate of the low protein line had higher skatole levels than the other groups. In this replicate, all 10 pigs with high skatole concentrations were progenies of two sires and five dams. In those litters, most male full-sibs had increased levels.

Within diets, the skatole levels were not significantly correlated to either growth rate or lean meat percent. Across diets, correlations were higher and significant ($r=-0.3$ for both; $p \leq 0.001$). These can probably be regarded as autocorrelations reflecting the variation in skatole content between lines.

DISCUSSION: Our results clearly demonstrate that skatole levels were higher in pigs selected on a low than on a high protein diet. It is very unlikely, however, that the protein concentration **per se** is the reason for the difference. In a Danish study, three energy and three protein levels were fed to entire male pigs (MORTENSEN, 1989; cit. MALMFORS et al., 1990). Protein concentration in feed had no effect on the level of skatole in back fat, whereas a high energy content increased the skatole level. In our study, the energy level was the same for both diets.

The compositions of the diets given to the two lines differed widely. The low protein diet was based on protein sources produced entirely in Sweden, with yellow peas as the main protein

Table 2. Least-squares means for skatole, age and lean meat percent at slaughter for entire male and female pigs fed diets with low or high protein content

	Low protein diet		High protein diet	
Trait	Males	Females	Males	Females
Skatole, ppm	0.207[a]	0.093[b]	0.080[b]	0.079[b]
Age, days	196[a]	208[b]	183[c]	189[d]
Growth rate, g/day, 25-90kg	730[a]	669[b]	865[c]	782[d]
Lean, %	59.1[a]	59.7[a]	65.8[b]	63.2[c]

Means with same superscript are not significantly different ($p>0.05$).

Table 3. Number of entire male pigs with high skatole levels and least-squares means for skatole concentration within line, generation and replicate

	Low protein line				High protein line			
	Generation 1		Generation 4		Generation 1		Generation 4	
	Repl. 1	Repl. 2	Repl. 1	Repl. 2	Repl. 1	Repl. 2	Repl. 1	Repl. 2
Total number	22	28	20	21	25	26	9	13
Pigs with skatole ≥0.20 ppm	4	4	10	3	0	1	0	1
Skatole, ppm	0.14[a]	0.20[a b]	0.32[b]	0.14[a]	0.07[a]	0.08[a]	0.08[a b]	0.09[a]

source, while the high protein diet contained traditional protein sources, such as soya meal.

The starch in yellow peas is demonstrably less digestible than starch from barley or wheat (GRAHAM & ÅMAN, 1987). Since undigested starch is a good source for the microbial fermentation that takes place in the colon, it might increase the microbial activity and hence probably the production of skatole. Yellow peas are also rich in fibres, and a diet with a high fibre content has been shown to increase the skatole concentration in backfat (LUNDSTRÖM et al., 1988). These results may have been affected by the composition of the high-fibre diet as it contained yellow peas, though whether the increased skatole levels were due to the fibre content *per se* or to the yellow peas cannot be stated. However, dietary fibre did affect skatole output in the faeces when pigs were fed beet pulp (HAWE et al., 1989).

How exactly increased skatole concentrations in back-fat of entire male pigs and microbial activity in the colon are interdependent is not known. Brewery waste products have been shown to increase both skatole production in colon and skatole concentrations in adipose tissue, in females and in entire males (BORG JENSEN, 1988). Addition of Nebactin, a broad-spectrum antibiotic, reduced skatole production and storage to levels found in pigs fed a traditional diet.

Due to their limited protein supply, the pigs in the low protein line had a slower growth rate and were approximately two weeks older at slaughter. The content of the boar taint steroid androstenone is known to increase with increasing age and/or weight at slaughter (BONNEAU, 1990), but no direct evidence of a relationship between age/weight and skatole has yet been found. As male pigs age, the probability of their sexual maturity increases. This development is also connected with increasing length and weight of the bulbo-urethral glands (BONNEAU, 1990). The glandula weight is correlated not only to androstenone but also to skatole concentration in fat ($r=0.5$; BONNEAU, 1990). In analogy, oral administration of testosterone to entire male pigs has been shown to increase the level of skatole in back fat (MORTENSEN, 1991). Neither the size of the bulbo-urethral glands nor other aspects of sexual maturity in male pigs were investigated in the present selection experiment. Female sexual maturity was investigated, however, (ELIASSON, 1991), but no difference between lines was found in the proportions of

sexually mature females at slaughter (L. ELIASSON; pers. comm.). Differences in sexual maturity between lines are therefore unlikely to explain the variation in skatole levels.

The fact that a number of the male pigs with high skatole levels were closely related in our study, may signify a genetic influence on skatole, but evidence of a genetic explanation for skatole in pigs is so far rather scanty. In one Danish investigation, entire males of the Landrace breed had higher skatole concentrations than had Yorkshire, Hampshire and Duroc (MORTENSEN, 1989; cit. MALMFORS et al., 1990). In the progeny/performance testing scheme in Denmark, all entire males are now analysed for skatole at slaughter, which should make it possible to estimate genetic parameters in the future. It can not be excluded that genetic drift contributed to the difference between replicates in our experiment.

The question why only certain entire male pigs deposit large amounts of skatole in the adipose tissue still awaits answer. An increase in microbial activity in the colon due to fermentation of a suitable substrate and/or a fibre-rich diet is probably one prerequisite for an abundant skatole production. It should be noted, however, that despite the different feed compositions, no difference in skatole concentration in back fat was found between the female pigs from the two lines in our study. Skatole concentrations in colon (BORG JENSEN, 1988) and blood (SINGH et al., 1988) of pigs are probably independent of sex. To explain sex-related difference in skatole deposition, SINGH et al. (1988) suggested that skatole might be transported from blood to fat by a hydrophobic molecule such as androstenone. So far, no such transport mechanism has been identified.

CONCLUSIONS: It seems obvious that the diet given to entire male pigs has an influence on skatole levels in adipose tissue. The possible genetic background for skatole deposition, as well as any interdependence with male steroid concentrations need to be further investigated.

REFERENCES:

BONNEAU, M. (1990): Sexual odours in pork from entire males. Compounds responsible for boar taint and physiological basis of androstenone production and storage in young entire male pigs. Workshop on the Production of Pork from Entire Males, Ottawa, Canada, June 5-6.

BORG JENSEN, B. (1988): Mikrobiel omsætning i fordøjelsekanalen. Produktion af skatol (ornelugt) i grise. Annual Meeting of the National Institute of Animal Science, Denmark.

ELIASSON, L. (1991): Puberty and oestrous symptoms in gilts with special reference to the relationship with production traits. Diss., Swedish University of Agricultural Sciences, Uppsala.

GRAHAM, H. and ÅMAN, P. (1987): Whole-crop peas. II. Digestion of early- and late-harvested crops in the gastrointestinal tract of pigs. Anim. Feed Sci. Technol. 17: 33-43.

HAWE, M., MOSS, B.W., WALKER, N. and PORTER, M. (1989): Distribution of skatole and indole compounds in pigs: influence of dietary factors. Proc. 35th Int. Congr. Meat Sci. Techn., Copenhagen, pp. 1036-1038.

LUNDSTRÖM, K. (1990): Production of pork from entire males in Europe. Workshop on the Production of Pork from Entire Males, Ottawa, Canada, June 5-6.

LUNDSTRÖM, K., MALMFORS, B., MALMFORS, G., STERN, S., PETERSSON, H., MORTENSEN, A.B. and SÖRENSEN, S.E. (1988): Skatole, androstenone and taint in boars fed two different diets. Livest. Prod. Sci. 18:55-67.

MALMFORS, B., LUNDSTRÖM, K., ANDRESEN, Ö., BONNEAU, M., KEMPSTER, A.J. and PATTERSON, R.L.S. (1990): Boars for meat production - report from the EAAP working group, Spain 1989. Livest. Prod. Sci. 26:319-326.

MORTENSEN, A.B. and SØRENSEN, S.E. (1984): Relationship between boar taint and skatole determined with a new analysis method. Proc. 30th Europ. Meeting of Meat Research Workers, Bristol, pp 394-396.

MORTENSEN, H.P. (1991): Effect of testosterone and estradiol added in the feed on skatole and boar taint levels in entire male back fat. Proc. 37th Int. Congr. Meat Sci. Techn., Kulmbach.

SAS INSTITUTE INC. (1985): SAS User's guide: Statistics, Version 5 Ed. SAS Inst. Inc., Cary.

SINGH, P., SINHA, A., AFZAL, R. and BROCK, T.K. (1988): A practical enzyme-linked immunoassay for quantitation of skatole. Proc. 34th Int. Congr. Meat Sci. Techn., Brisbane, pp. 692-696.

STERN, S., RYDHMER, L., JOHANSSON, K. and ANDERSSON, K. (1991): A selection experiment in pig for lean tissue growth rate on high and low protein diets. I. Experimental design and selection response (in manuscript).