Incidence of boar taint in entire male pigs in Europe, assessed by chemical assay of androstenone and skatole

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Abstract— Boar taint is associated with the accumulation of androstenone and/or skatole in the fat of entire male pigs. To minimize the risk of boar taint most male pigs in Europe today are physically castrated early in life, but alternative strategies are being investigated, including raising boars to current market weights. The incidence of high androstenone and skatole concentrations in such animals is therefore of interest and the objective of this study was to review the information available from a multi-study database. During the development and market introduction of Improvac[®] (Pfizer Animal Health), a vaccine alternative to physical castration for the reduction of boar taint, multiple European studies were conducted; many including untreated entire males as positive controls. Fifteen studies from 8 countries were identified in which fat samples were taken from entire males at slaughter and assayed for androstenone and skatole by validated HPLC methods. From a total of 1036 animals, 39.4% were considered likely to pose a significant risk of boar taint on the basis of androstenone and/or skatole concentrations above those considered detectable by sensitive consumers (>1.0 μ g/g or >0.2 μ g/g respectively). Of the 1036 pigs tested 18.5% were considered to have a particularly high risk by having either both compounds above the threshold values, or at least the second at a moderately elevated concentration (>0.5 μ g/g or >0.1 μ g/g respectively). The results suggest that entire male production to current weights could reduce consumer satisfaction and be detrimental to the pork industry.

Index Terms - Boar taint, androstenone, skatole, entire boars.

I. INTRODUCTION

Boar taint is an unpleasant odour and taste predominantly associated with the cooking and eating of pork from some sexually mature or maturing male pigs. An unpleasant experience with boar taint can result in consumer rejection of pork. The main compounds responsible for boar taint are androstenone and skatole [1, 3, and 13]. Androstenone is a steroid hormone produced directly by the testicles of the post-pubertal male pig. It serves as a pheromone and accumulates in the salivary gland. Being highly lipophilic, it also accumulates in fat where it can contribute to boar taint [1, 3, and 13].

Skatole is produced, in boars, castrates and female pigs, by bacterial digestion of the amino acid tryptophan in the pig's hind gut from where it is absorbed and, if not cleared by the liver, accumulates in fatty tissues. In intact male pigs the liver is less efficient at metabolizing skatole than in females or castrated males, leading to an accumulation of this component of boar taint [1, 3, and 13].

Skatole has been demonstrated to enhance the sensory perception of the unpleasant odours associated with androstenone [3]. The strongest objections associated with boar taint are found with pork from pigs that have elevated concentrations of both compounds [3]. These pigs in particular should not enter the food chain if consumer confidence in pork is to be maintained.

Boar taint is traditionally controlled by physical castration early in life. However, problems with castration make it undesirable. Compared to intact boars, castrates are less efficient at converting feed into weight gain and are fatter with less lean meat in the carcass [2]. There are also significant animal health and welfare concerns with physical castration [3] and largely driven by these it has become controversial, particularly in Europe where there is an intention to end the practice by 2018 and earlier in some countries. Potential alternatives include the use of vaccination against GnRF (immunological castration) in the late fattening phase and the rearing of entire boars to slaughter. The viability of the latter approach, however, is dependent on the level of boar taint likely to be found in pigs taken to commercial slaughter weights, and the practicality of screening out and disposing of those carcasses found to be unacceptable.

This paper reports on the background concentrations of the boar taint chemicals, androstenone and skatole, found in boars raised under commercial production conditions in Europe. The data were drawn from a multi-study database.

II. MATERIALS AND METHODS

A. Data base

During the development and market introduction of Improvac, a vaccine alternative to physical castration for the reduction of boar taint, controlled clinical studies were conducted throughout Europe. In many of these studies noncastrated entire boars were included as positive controls. The entire boars were slaughtered at weights representative of current commercial slaughter weights for the respective countries.

In total taint data were available from 1036 entire boars from 15 studies conducted in 8 European countries (Czech Republic, Denmark, France, Germany, Hungary, Netherlands, Spain, and UK). In all studies the boars were housed in the same buildings, but in separate pens, from the other treatments (Improvac vaccinated pigs, physical castrates and occasionally females, depending on site). In all studies the entire boars were fed the same commercial diets and subjected to the same husbandry procedures as the other treatments. Most studies were conducted on commercial farms with the remainder being performed in research facilities, but under conditions similar to normal production practice.

B. Samples

Following slaughter, a sample of at least 10 g of subcutaneous belly fat was collected from around the second and third nipple, from each pig. Samples were stored below -15° C prior to analysis.

C. Sample analysis

 5α -androstenone (5- α -androst-16-en-3-one) was extracted from approximately 0.5 g of pork fat by heating and sonicating in a water bath for approximately one hour in the presence of methanol [4] and 100 µL of a 10.0 µg/mL androstanone solution was added as an internal standard. The methanol extract was then filtered through a packed bed on the SPE plate. A 400 µL volume of hexane was added for washing using a vortex mixer, then centrifuging the plate to separate the layers. After evaporation of the bottom layer, the sample was reconstituted in 250 µL of methanol to remove interfering lipids. A 20 µL sample of the methanol extract was analyzed for 5 α -androstenone concentration by a highperformance liquid chromatographic/mass spectrometry/mass spectrometry (LC/MS/MS) method. Separation was achieved with a Phenomenex Synergi Hydro-RP, 150 mm x 2.0 mm, 4 μ m column and a water:acetonitrile:formic acid mobile phase. Ionization of 5 α -androstenone was improved by derivitization prior to injection in the spectrometer. The calibration curve for 5 α -androstenone ranged from 0.2 μ g/g to 8.0 μ g/g.

Skatole was extracted from an approximately 0.5 g sample of pork fat by heating and sonicating in a water bath for approximately one hour in the presence of hexane:isopropanol diluent. Before the extraction, 100 μ L of a 7.12 mg/mL solution of 7-ethylindole was added as an internal standard. Interfering lipids were partially removed by precipitation during cooled (5°C) centrifugation. A 50 μ L sample of the hexane:isopropanol extract was analyzed using a normal phase high-performance liquid chromatography separation with fluorescence detection. The separation was accomplished using three Hypersil APS-2, 250 mm x 4.0 mm, 5 μ m columns in series. The mobile phase was isocratic using the hexane:isopropanol extraction solvent. The calibration curve for skatole ranged from 0.0187 μ g/g to 0.719 μ g/g.

III. RESULTS AND DISCUSSION

At commercial slaughter weights, the reported incidence of boar taint is quite variable ranging from around 10% up to 75% according to different studies [e.g. 2, 5, 6, 8, 9, 10 and 12]. There are many reasons for these differences, but one particular difficulty is the lack of a common, objective definition of boar taint. Ultimately it is a human sensory experience, but the susceptibility of individuals varies greatly so surveys based on subjective sensory evaluation, using different panels and methods, have an inherent variation. Also, although results are frequently reported in a way that suggests that boar taint is a yes or no phenomenon, it is in reality more of a continuous variable, with increasing concentrations of the boar taint compounds leading to an increasing proportion of the population noticing their presence and those individuals finding the experience increasingly objectionable. The penalty of failing to adequately control boar taint, therefore, is not only the risk of specific consumer rejection, which is likely to require a high level of taint or a particularly sensitive person, but also a more insidious, longterm decline in the reputation of pork as consumers suffer disappointing eating experiences.

In the analysis of the current data set each animal was objectively classed as having a high, medium or low risk of being detected as tainted by consumers, based on the concentrations of androstenone and skatole. The concentrations used and the risk categories are shown in Table 1. A "high risk" animal was defined as one that had a high concentration of one primary taint compound (>1.00 and >0.20 μ g/g for androstenone and skatole respectively) and a moderate (0.50-1.00 and 0.10-0.20 μ g/g for androstenone and skatole respectively) or high concentration of the other

primary taint compound. A "medium risk" animal was defined as having a high concentration of one major taint compound but a low level of the other major taint compound. "Low risk" animals were those with low concentrations of both compounds, or a low level of one compound and only a moderate level of the other compound. Animals with moderate levels of both taint compounds (0.50-1.00 and 0.10-0.20 μ g/g for androstenone and skatole respectively) have also been classified as low, although whether such animals should be classified as low or medium is debatable.

These categories were chosen because concentrations above $1.0 \ \mu g/g$ and rostenone in fat are generally considered to result in tainted meat [3]. The skatole threshold is generally considered to be $0.20 \ \mu g/g$ [3].

The pooled results are summarised in Table 2 and the detailed results by study are shown in Table 3. The shaded cells in Table 2 represent a high risk of boar taint and overall 18.5% of the 1036 boars tested fell into this category. An additional 22.4% fell into the medium risk area, giving a total of 40.9% for undesirable animals. The percentage of boars with both major taint compounds above the sensory thresholds was also high at 9.1%. These animals represent a particularly high risk. In comparison only just over half (59.0%) of the pigs tested would be considered as low risk.

In terms of the individual thresholds for androstenone and skatole 37.6% of these 1036 entire boars had androstenone above 1.0 μ g/g; while 12.5% had a skatole concentration greater than 0.20 μ g/g.

This European data set is in general agreement with previous published reports from Europe and other regions [2, 5, 7, 11, 12].

IV. CONCLUSION

If the incidence of boar tainted carcasses reported in this study is representative of the wider European pig population, the use of entire males presents significant challenges if pork quality is to be maintained. Even if the 18.5% of high risk carcasses can be identified and diverted, perhaps for utilization in low value processed products where their meat can be diluted with that from non-tainted animals, a large number of medium risk carcasses will still enter the pork supply chain. While rarely generating direct consumer complaints, the marketing of sub-optimal products may have a negative effect on consumer perceptions and lead to a longterm decline in the reputation of pork.

References

 Bonneau, M., Le Denmat, M., Mortensen, A. B. and Mortensen, H. P. (1993). Relationships between fat androstenone and skatole levels and the organoleptic assessment of pork and cooked ham. In Measurement and Prevention of Boar Taint in Entire Male Pigs, ed. M. Bonneau. INRA Editions, Paris, pp. 81-86.

- [2] Dunshea, F. R., Colantoni, C., Howard, K., McCauley, I., Jackson, P., Long, K.A., Lopaticki, S., Nugent, E.A., Simons, J.A., Walker, J. and Hennessy, D.P. (2001). Vaccination of boars with a GnRH vaccine Improvac eliminates boar taint and increases growth performance. Journal of Animal Science. 79:2524–2535.
- [3] EFSA report. (2004). Welfare Aspects of the Castration of Piglets. EFSA Journal. 91:1-18.
- [4] Hansen- Moller, J. (1994). Rapid high-performance liquid chromatographic method for simultaneous determination of androstenone, skatole and indole in back fat from pigs. Journal of Chromatography B, 661 (1994) 219-230.
- [5] Hennessy, D., Changwei, Ma, Zhinan, Liu, Qilin Wu and Hanchun, Yang. (2009). Growth performance of male pigs vaccinated with the boar taint vaccine, Improvac® and the effects on boar taint assessment. Pages 122-125 in Proceedings 55th International Congress of Meat Science and Technology. Copenhagen, Denmark (ICoMST), Copenhagen, Denmark, 16-21 August 2009.
- [6] Malmfors, B. and Hansson, I. (1974). Incidence of boar taint in Swedish Landrace and Yorkshire boars. Livestock Production Science. 1: 411-420.
- [7] Pauly, C., Spring, P., O'Doherty, J.V., Ampuero Kragten, S. and Bee, G. (2008). Performances, meat quality anboar taint of castrates and entire male pigs fed a standard and a raw potato starch-enriched diet. Animal. 2:1707–1715.
- [8] Rhodes, D. N. and Patterson, R. L. S. (1971). Effects of partial castration on growth and the incidence of boar taint in the pig. Journal of the Science of Food and Agriculture. 22: 320-324
- [9] Williams, L. D., Pearson, A. M. and Webb, N.B. (1963). Incidence of sex odor in boars, sows, barrows and gilts. Journal of Animal Science. 22: 166-168.
- [10] Xue, J., Dial, G.D., Holton, E.E., Vickers, Z., Squires, E.J., Lou, Y., Godbout, D. and Morel, N. (1996). Breed differences in boar taint: relationship between tissue levels of boar taint compounds and sensory analysis of taint. Journal of Animal Science. 74: 2170-2177
- [11] Zamaratskaia, G., Rydhmer, L., Andersson, H.K., Chen, G., Lowagie, S., Andersson, K. and Lundstrom, K. (2007). Long-term effect of vaccination against gonadotropin- releasing hormone, using ImprovacTM, on hormonal profile and behaviour of male pigs. Animal Reproduction Science 108, 37–48.
- [12] Zamaratskaia, G., Andersson, H.K., Chen, G., Andersson, K., Madej, A. and Lundstrom, K. (2008). Effect of a gonadotropin-releasing hormone vaccine (Improvac[™]) on steroid hormones, boar taint compounds and performance in entire male pigs. Reproduction in Domestic Animals 43, 351–359.
- [13] Zamaratskaia, G. and Squires, E.J. (2009) Biochemical, nutritional and genetic effects on boar taint in entire male pigs. Animal 3:11, 1508-1521

 and skatole in fat tissue.									
Skatole concentration	Androstenone concentration $(\mu g/g)$								
$(\mu g/g)$	< 0.50	0.50 - 1.00	>1.00						
<0.100	Low risk	Low risk	Medium risk						
0.100 - 0.200	Low risk	Low risk	High risk						
>0.200	Medium risk	High risk	High risk						

 Table 1. Risk of detection boar taint relative to the concentration of androstenone and skatole in fat tissue.

Table 2. Distribution of androstenone and skatole in fat tissue of 1036 entire

 boars sampled during 15 clinical studies in 8 European countries. Data presented

 as the percentage of the total.

Skatole concentration	Androstenone concentration (µg/g)						
(µg/g)	< 0.50	0.50 - 1.00	>1.00				
<0.100	32.8	18.2	20.9				
0.100 - 0.200	4.6	3.3	7.6				
>0.200	1.5	1.8	9.1				

Table 3. Distribution of androstenone and skatole by study. Data presented as number of pigs and (for total only) as percentage

	Age at	No. pigs	Androstenone <0.5 μg/g Skatole μg/g			Androstenone 0.5 -1.0 µg/g Skatole µg/g			Androstenone >1.0 μg/g Skatole μg/g		
Country	slaughter (weeks)										
			-0.1	0.1-		-0.1	0.1-		-0.1	0.1-	
			<0.1	0.2	>0.2	<0.1	0.2	>0.2	<0.1	0.2	>0.2
Czech Republic	27-29	17	15	0	0	2		0	0	0	0
Denmark	21-22	28	6	0	0	9	2	0	2	4	5
France	23	23	16	1	0	3	1	0	1	0	1
Germany	27-29	17	15	1	0	0	0	1	0	0	0
Germany	22-29	127	55	1	0	17	2	1	39	5	7
Hungary	28-31	71	22	11	7	4	7	2	4	6	8
Netherlands	26-28	27	8	5	6	2	1	4	0	0	1
Spain	24-26	35	12	0	0	14	2	0	6	1	0
Spain	22-24	36	18	5	1	1	5	2	1	2	1
Spain	24-26	9	3	2	0	2	0	0	1	1	0
Spain	25-28	15	9	3	1	1	0	0	0	0	1
UK	21-23	99	29	0	0	36	0	0	32	1	1
UK	22	46	9	1	0	9	2	1	15	7	2
UK	22-26	333	92	9	1	65	8	2	92	31	33
UK	19-22	153	31	9	0	24	4	6	24	21	34

Total (number)	1036	340	48	16	189	34	19	217	79	94
Total (%)		32.8	4.6	1.5	18.2	3.3	1.8	20.9	7.6	9.1