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BOAR TAINT IN BELGIAN LANDRACE PIGS IN RELATION TO THE ANDROSTENONE CONTENT.

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INTRODUCTION

The impact of boar taint on the production economy of pigs is well known. The annual losses caused by the necessity of castrating male animals, raised for meat production, are considerable (1) (2). Since Patterson (3) laid the first link between a chemical substance : androstenone (AEON) and boar taint, considerable efforts have been made for working out analytical procedures, capable of on line selection of tainted carcasses in the slaughterhouse. A succesful selection may be realised if : 1) the number of tainters is small, 2) the relation between the chemical substance(s) and boar taint is unequivocal, (3) the analytical procedure has a high capacity at a low cost.

Most authors report off-flavour frequencies of 5 - 20% (4). The relation between boar taint and androstenone has been intensively studied : the best product-moment correlation coefficient obtained so far, r = 0.76 (5), accounts for 58% of the variation in boar taint explained by its androstenone content. However, in other studies lower correlation coefficients (magnitude 0.4 - 0.6) were found. Other malodorous compounds as skatole (1) (6) and volatile aldehydes (7) are suspected to contribute to boar taint.

A high capacity method for the on-line evaluation of boar taint on basis of its androstenone concentration is not yet realized. The ELISA method for androstenone, although announced in 1981 (2), is not yet commercially available. Mortenson and Sorenson (1) presented an automated spectrophotometric method for analysis of skatole and possibly related compounds, of which the results look very promising.

In this paper the boar taint of Belgian Landrace pigs, detected by a trained panel by ranking according to taint intensity, was compared with the androstenone concentration of the fat, determined by a new fused silica open tubular capillary gas chromatographic method with electron capture detection.

MATERIALS AND METHODS

- Sensory analysis

From 35 boars, 30 sows and 28 castrates backfat and cutlets were sampled after slaughtering at commercial ages (95 - 100 kg live weight). Panel assesment was performed with a trained panel (10 - 12 members) by ranking according to boar taint intensity of

1. Backfat samples (heated with a tip of a handsealer(from a boar, a sow and a castrate - 35 sessions using the different boars).
2. Roasted cutlets, which were kept warm on a hot plate and evaluated by sniffing. In each of the 23 sessions 4 samples were used : 2 sows, 1 castrate and 1 boar The evaluations intend to simulate the cooking process.

Statistical analysis was performed by addition of the ranks and comparing the rankssums (12 replications, 3 or 4 samples) with the totals required for significance at the 5% level (8). For correlations with the instrumental androstenone determinations a mean boar taint ranking score was calculated, ranging from 1 to 3 for handsealer-backfat procedure and from 1 to 4 for the cutlets-sniffing procedure. Androstenone was determined on 20 boars, 15 castrates and 16 sows.

- Gas chromatographic method

A fat sample (0.4 g) was placed into a 15 ml extraction tube with screw stopper and 1.4 ml toluene, 2 ml KOH solution (10% in methanol) and 20 µl internal standar solution (AAON, androstanone (5-α-androstan-3-one), Sigma (St-Louis, USA)) was added. The mixture was shaken and heated at 80°C during 1 hour. After cooling, 2.5 ml

methanol, 2 ml distilled water and 5 ml light petroleum was added. The mixture was shaken during 30 seconds. Phases were separated by centrifugation at 2000 rpm(500 g) for 10 min. The clear upper phase was transferred into a 8 ml silanized extraction tube. The volume was reduced to ca 1 ml under a jet of nitrogen. The content of the tube was transferred to a conical 5 ml extraction tube. The solvent was evaporated to dryness under a jet of nitrogen, 5 µl FLOROX reagent ((o-pentafluorobenzyl) hydroxylamine. HCl. 2,5 mg/ml in E.C. grade pyridine , Pierce (Rockford, IL. USA)) was added and the tube was heated at 100°C for 1.5 h. After cooling 40 µl cyclohexane was added and the tube was heated again for 5 min. at 100°C. 1 µl of the content was injected into the gas chromatograph.

The gas chromatograph used was a Varian 3700, equipped with a capillary injection system and an ECD. The column was a fused silica capillary (50 m x 0.23 mm I.D.) coated with CP Sil 5 (OV-1, SE-30 analogue) from Chrompack (Middelburg, The Netherlands). Hydrogen was used as a carrier gas at a rate of 1 ml/min. The splitting ratio was 1:25. Nitrogen was used as make-up gas for the ECD at 30 ml/min. The column, injector and detector temperatures were 270°C, 310°C and 330°C respectively. The retention times of the syn and the anti forms of the PFBHA derivatives of the steroids were : AEON, 8,4 and 8.8 min. ; AAON, 9.2 and 9.6 min.

RESULTS AND DISCUSSION

- Gas chromatographic determination of androstenone

In 1976 Kaufman et al. (9) described a procedure for the extraction of androstenone from adipose tissue, using a quaternary mixture of water-methanol-benzene-light petroleum (or hexane). In this procedure benzene was exchanged for the less toxic toluene. As the detection with electron capture is more sensitive than with FID the amount of sample and solvent can be scaled down (7.5 times) allowing the total procedure (saponification - extraction) to be carried out in one test tube.

Androstenone (resp. androstanone) was extracted for 93 ± 2,5% (resp. 98 ± 3,5%) in the upper phase of a single equilibration of 1.4 ml toluene (11%), 4,5 ml methanol (35%), 2 ml distilled water (15%) and 5 ml light petroleum (39%). After derivatisation of the extract with FLOROX reagent, the pentafluorobenzyl oxime derivatives (syn and anti) are baseline separated from each other by capillary gas chromatography. The androstenone - PFB oxime derivatives are also well separated from the internal standard derivatives and derivatives originating from the matrix.

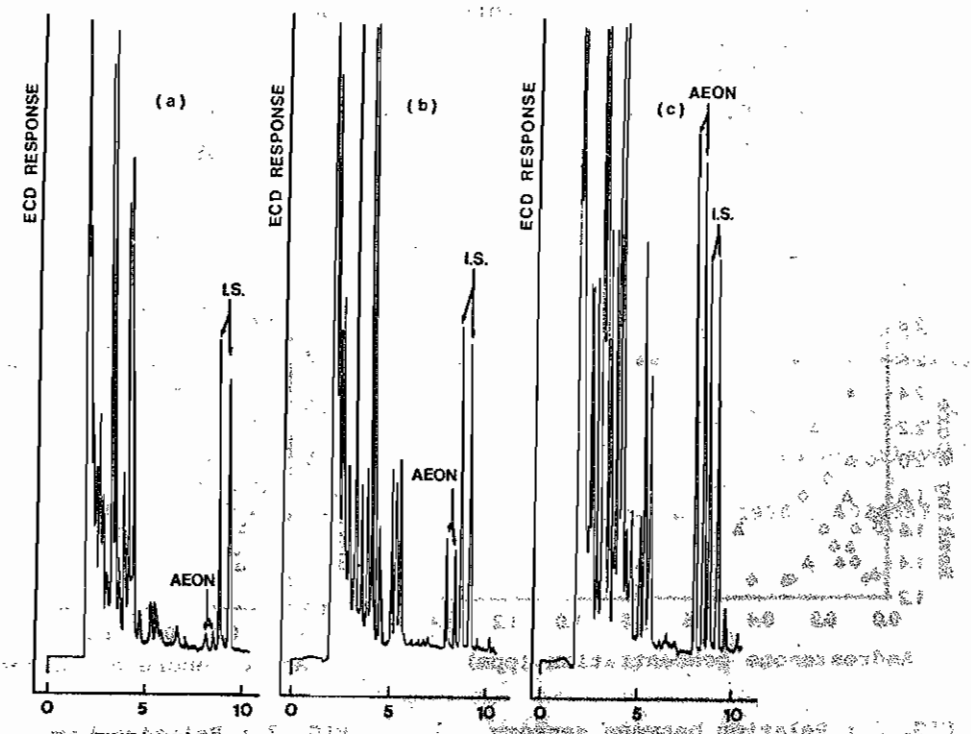

FIG. 1 : Gas chromatograms of adipose tissue from
a) female pig (0.08 ppm AEON) ; b) boar (0.66 ppm AEON)
c) boar (2.6 ppm AEON)
I.S. : internal standard equivalent with 2 ppm

. In fig. 1 chromatograms of three adipose tissues, extracted as described above are given. These figures illustrate that the clean-up, provided by the modified Kaufman procedure (9), meets the requirements of the cgc-EC determination of androstenone in the range of 0.08 - 2.6 ppm.

- Results of analysis

In table 1 the results of the sensory analysis and the androstenone determination are given. Boars show higher mean ranking scores and higher mean androstenone concentrations than sows or castrates ($p \leq 0.001$).

TABLE 1 : Results of sensory analysis and androstenone determinations

samples	ranking score (mean ± S.D.)		androstenone concentration		
	handsealer	sniffing	min.	max.	mean ± S.D.
sows (n=16)	1.6 ± 0.32	2.0 ± 0.39	0.04	0.77	0.3 ± 0.24
castrates (n=15)	1.6 ± 0.21	2.1 ± 0.25	0.02	0.53	0.3 ± 0.15
boars (n=20)	2.5 ± 0.38	3.6 ± 0.26	0.07	1.27	0.6 ± 0.26

The frequency of significant higher taint intensity ($p \leq 0.05$) in the ranking tests for boars, sows and castrates are given in table 2. In the backfat-handsealer procedure only 63% of boars were judged more tainted than sows or castrates. In the cutlets-sniffing procedure 83% of the boars were discriminated by the panel from the sows or castrates. The incidence of a significant higher taint intensity in sows or castrates was negligible. From the results it appears that cutlets-sniffing is a more sensitive procedure than backfat-handsealer in detecting low tainted carcasses (95 - 100 kg live weight).

TABLE 2 : Frequency of significant higher boar taint in the ranking test

Sensory procedure	boars	sows	castrates
Backfat-procedure	63%	0%	3%
Cutlets-sniffing	83%	0%	4%

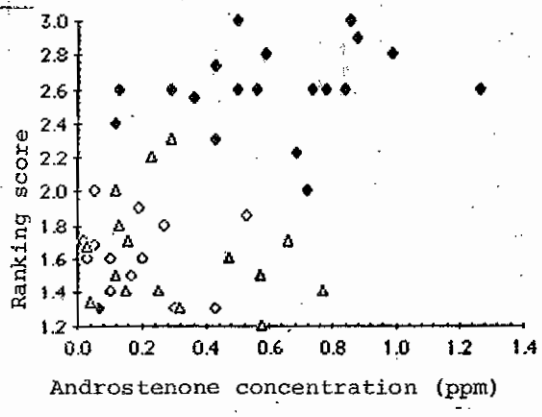

FIG. 2 : Relation between sensory analysis (backfat-handsealer) and androstenone concentration
(◆: boars ; ◇: castrates ; △ : sows)

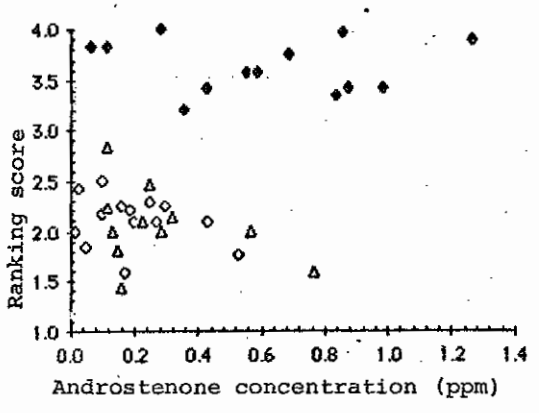

FIG. 3 : Relation between sensory analysis (cutlets-sniffing) and androstenone concentration
(◆: boars ; ◇: castrates ; △ : sows)

- Relation between sensory analysis and androstenone concentration

The relation between the sensory analysis and the androstenone concentration is shown in fig. 2 and fig. 3. In both figures the boars form a cluster, clearly separated from the cluster, formed by the sows and the castrates. However, the discrimination of boars from sows-castrates is mainly caused by differences in the ranking score. Although most boar fats have a higher androstenone concentration than sows or castrates no statistically significant correlation could be demonstrated between taint ranking and androstenone content of boar fats. This lack of correlation may be due to the low slaughter weight range of the pigs (95 - 100 kg live weight) and to panel assesment which was very sensitive in detecting boar taint but poor in ranking the different boar samples according to the intensity of taint.

CONCLUSIONS

The described fused silica open tubular-electron capture method, allows the accurate determination of androstenone in pig adipose tissue over the whole concentration range, needed for boar taint evaluation (0.1 - 6 ppm AEON). The method is designed for large-scale laboratory research on the still dubious relationship between boar taint and the androstenone content of fat. The method may also be useful in the evaluation of new and fast (immunological) methods.

Relative sensory procedures comparing, in one session, boars, sows and castrates, indicate too high a percentage of cooking odours in Belgian Landrace boars. From the boar samples 63% (backfat-handsealer), respectively 83% (cutlets-sniffing), had a significant ($p \leq 0.05$) higher taint intensity than the sows and castrates. The relative procedure used may be more discriminative than those using absolute scaling tests.

Between the boar taint ranking scores and androstenone content of fat no significant relationship was found for the populations (boars, castrates, sows) studied.

Taking into account the high percentage of tainters and the lack of correlation between boar taint and androstenone, it may be concluded that the conditions for a succesful selection of tainted carcasses on basis of its androstenone content, at least of Belgian Landrace pigs (live weight 90 -100 kg), are NOT fulfilled.

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