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The sensitivity of Flemish citizens to androstenone: Influence of gender, age, location and smoking habits

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ABSTRACT

Skatole and androstenone are the main boar taint compounds. Whereas nearly everybody is sensitive to skatole, the sensitivity to androstenone is genetically determined and differs between countries. In this study the methodology for testing androstenone sensitivity was refined and applied to 1569 consumers that were approached at six shopping malls in Flanders. Participants were asked to smell the contents of four bottles (three were filled with water and one with androstenone solved in water) and to identify and describe the odour of the strongest smelling bottle. This test was performed twice. 45.3% of the respondents were classified as sensitive to androstenone (i.e. the percentage of participants that identified the correct bottle in both tests minus a guess correction). Sensitivity differed between sexes (men: 38.3%–women: 51.1%, $P<0.001$), according to age (older people were less sensitive, $P<0.001$), and between the test locations ($P<0.001$), but not between smokers versus non-smokers.

1. Introduction

Boar taint is an unpleasant odour that can occur when fat or meat from entire male pigs is heated. In most European countries pigs are surgically castrated to avoid boar taint (Fredriksen et al., 2009). Because of ongoing societal pressure, castration without anaesthesia has been forbidden in several countries over the last decade (e.g., the Netherlands and Norway). Alternative methods may be used instead such as castration with anaesthesia or anaelgesia, or immunocastration. Another alternative is the production of entire male pigs. It is known that boars have a better feed conversion ratio and leaner meat than barrows (Aluwe et al., 2009). However, in Belgium e.g. 4% of the meat of entire boars is classified as strongly tainted and 25% is classified as moderately tainted at slaughter (Aluwe et al., 2009). For this reason, all carcasses of entire male pigs are treated as inferior. This treatment will persist until a slaughter-line detection method (currently under development) has been implemented.

The main compounds known to be responsible for boar taint are skatole and androstenone (Patterson, 1968; Vold, 1970). Androstenone is a pheromone, which is synthesized in the boar testes and subsequently released into the bloodstream. Once androstenone is released it can be transported to the salivary gland or accumulate in adipose tissue (Bonneau, 1982). Skatole is produced from tryptophan by microbes in the hindgut and is partly absorbed into the blood. The majority is degraded in the liver, but unchanged skatole may also accumulate in adipose tissue (Jensen, Cox, & Jensen, 1995). Several studies have already shown that skatole levels can be reduced through management (e.g., diet composition and environmental factors (Hansen, Larsen, Jensen, Hansenmoller, & Bartongade, 1994; Hansen et al., 2006)), while the only way to reduce androstenone levels known so far is through genetic manipulation. Unfortunately, selection against androstenone is likely to result in negative effects on performance and sexual maturation of male pigs (Bonneau, 1998). Moreover, specific anosmia (insensitivity) to androstenone has been shown to differ between gender, age and country, among others, whereas nearly every individual tested so far has proved sensitive to skatole (Weiler, Fischer, Kemmer, Dobrowolski, & Claus, 1997). In order to test the sensitivity of a population to boar taint, it is therefore more relevant to focus on androstenone.

Sensitivity to androstenone is genetically determined (Keller, Zhuang, Chi, Vosshall, & Matsunami, 2007; Wysocki & Beauchamp, 1984) and a part of the consumers has been shown to be anosmic to adrostenone. This specific anosmia may result from defective or missing molecular receptors. Some anosmic individuals possess the receptors required but at sub-threshold density. However, an osmic condition may arise when individuals are exposed regularly to

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androstenone (Wysocki & Beauchamp, 1984; Wysocki, Dorries, & Beauchamp, 1989).

Gilbert and Wysocki (1987) estimated the percentage of anosmic women versus anosmic men to be 15.8 vs. 24.1% in Europe (excluding the UK), 29.5 vs. 37.2% in the USA and 17.2 vs. 25.5% in Asia. More recently, the proportion of anosmic people has been estimated to be much higher. Weiler et al. (2000) reported differences in androstenone insensitivity between countries of 65.9% for women and 69.7% for men in Germany, and corresponding percentages of 48.1% to 59.7% in Spain. In Norway 53.6% of the women and 73.7% of the men have been reported as insensitive to androstenone (Lunde, Skuterud, Nilsen, & Egelandsdal, 2009).

Comparison of the absolute percentages of androstenone sensitive people is problematic, as the methodology and operational definition used for categorizing a person as anosmic differs considerably between these studies. For example, in the study by Weiler et al. (2000) one bottle with pure crystals was offered to the consumers. A 7-point scale was used and people that marked a score of 3 or less were considered insensitive. Lunde et al. (2009) refined this methodology and provided the consumers with three bottles, one with androstenone dissolved in water and two with pure water. This test was done twice and people who correctly identified the bottle with androstenone in both tests and indicated on the label magnitude scale that the intensity of the smell was at least "strong" were considered sensitive.

In an international study with seven European countries (United Kingdom, Denmark, France, Sweden, Germany, Spain and the Netherlands) the odour of androstenone has been generally described as urine-like, but more refined terms like naphtalene/mothballs have been used as well (Dijksterhuis et al., 2000). Norwegian consumers described the odour of androstenone with terms such as ammonia and pungent rather than urine (Lunde et al., 2009). However, it cannot be verified whether the odour of androstenone is really perceived differently by different nationalities, as the list of odour descriptors differed between both studies.

The sensitivity to androstenone is also influenced by age. Matthews et al. (2000) reported from an international study that the oldest group of consumers had the lowest percentage of dislike scores. The effect of smoking to the sensitivity of androstenone has never been tested before, despite smoking being associated with a decreased ability to smell (Ishimaru & Fujii, 2007; Katotomichelakis et al., 2007; Vennemann, Hummel, & Berger, 2008).

The present study aimed to evaluate the sensitivity of Flemish citizens to androstenone. This has not been tested previously in Flanders (the northern part of Belgium), and cannot be derived from studies in other European countries, as sensitivity has been shown to differ between countries. In addition, it was also investigated whether androstenone-sensitivity among Flemish citizens is similarly affected by age and gender as has been described for other populations, and whether it is associated with other variables that have never been investigated before, such as smoking habits and test location.

2. Materials and methods

Androstenone sensitivity among 1569 consumers was tested in six shopping malls in different cities (Ostend, Kortrijk, Merelbeke, Oostakker, Antwerp and Hasselt) located across Flanders (Table 1). In five out of six locations, testing occurred outdoors.

The methodology to test sensitivity was based on Lunde et al. (2009). People were randomly approached and invited to participate. They were asked to smell the contents (10 ml) of four identical test bottles: three were filled with water, and one with androstenone dissolved in water at a concentration of 0.17 mg/ml (the undissolved crystals were not removed). The bottles were covered with aluminium foil, making it impossible to see their contents. In order to avoid contamination of the water-only bottles with the smell of androstenone, all water-only bottles were replaced with new bottles after approximately 20 consumer tests. The consumers were asked to smell the contents of each bottle once and to select the bottle with the strongest odour. This test was repeated with a new series of four bottles in order to reduce the likelihood of providing a correct answer by chance (from 1/4 to 1/16).

The consumers were asked to evaluate the odour intensity of the bottle which they perceived to smell the strongest on a labeled magnitude scale (LM scale) (Green et al., 1996). In making his/her judgment of intensity, the consumer was instructed to rate the odour of the solution relative to the strength of sensations he/she experiences in everyday life. Thus, "strongest imaginable" refers to the most intense sensation he/she had ever experienced. The intensity was scored after every test series with four bottles. The intensity scale was converted into a score from 0 to 100 (Green et al., 1996) and the mean value of both test series was calculated and used for further statistics. Subsequently, the volunteers were asked once to tick the term (only one term could be ticked) within the list of odour-descriptors ("no odour", "soap/perfume", "sweet", "spicy", "sweat", "urine", "pungent", "sour/bitter" and "other") they associated with the smell of the bottle. Between the two test series, consumers were asked to fill out information regarding their gender, age and smoking habits (smoker versus non-smoker) on the recording form.

The prevalence of consumers sensitive to androstenone was estimated as

$$\hat{\pi} = \frac{16}{15}\frac{X}{N} - \frac{1}{15}$$

where X is the number of persons that correctly identified the bottle with androstenone in both tests and N is the total number of persons tested. The effects of smoking, sex and age on sensitivity were

Table 1
Overview of the six test locations in Flanders: date of consumer testing, weather conditions, average age, number of females and males tested, number of smokers and non-smokers within the sample tested and number of consumers who had both tests, only one test and no tests correct.

	Merelbeke	Ostend	Kortrijk	Oostakker	Antwerp	Hasselt
Date	3-9-2009	8-9-2009	10-9-2009	15-9-2009	24-9-2009	29-9-2009
Temperature (°C)	16	18	17	15	21	14
Wind (m/s)	7	2	7	3		3
Cloudiness	65	20	60	80		80
Indoor/oudoor	Outdoor	Outdoor	Outdoor	Outdoor	Indoor	Outdoor
Average age (years)	45	38	47	41	35	34
Males	74	180	94	164	118	86
Females	130	139	163	110	116	195
Smokers		89	83	69	58	61
Non smokers		230	174	205	176	220
Both tests correct	122	138	107	142	117	140
One test correct	40	43	56	53	34	66
No test correct	42	138	94	79	83	75

evaluated by a generalized linear model with binomially distributed error term. The six test locations were also compared with each other by a generalized linear model with binomially distributed error term, but an adjustment was made for age and gender. Every odour term was evaluated by using the Fisher's exact test to detect odour-descriptors that were selected significantly more often by consumers sensitive to androstenone than by non-sensitive consumers (Moskowitz, 1988).

3. Results

In total 716 males and 853 females, aged between 7 and 88 years (mean = 39.97; SD = 17.8) were tested. Of those 1569 volunteers, 45.3% (95% CI: 42.6%–47.9%) were classified as sensitive to androstenone.

The percentage of the sample population sensitive to androstenone was smaller ($P<0.001$) for males (38.3%, with 95% CI: 34.5%–42.2%) than for females (51.1%, with 95% CI: 47.5%–54.7%), with an odds ratio of women compared to men for androstenone sensitivity equal to 1.6 (95% CI: 1.32–1.97). Androstenone sensitivity also depended on the age of the individual tested. The odds of a subject being sensitive amounted to 0.98 (95% CI: 0.97–0.98) of the odds of a subject being one year younger, meaning that sensitivity significantly decreases with age ($P<0.001$). The oldest group of persons also gave the lowest odour intensity score (Table 2). Interestingly, androstenone sensitivity was not significantly associated with smoking habit ($P=0.720$). Between the six test locations significant differences were found in sensitivity to androstenone ($P<0.001$) (Table 3). The following odour-description terms were used significantly more often by androstenone-sensitive consumers than by non-sensitive consumers: “spicy” ($P=0.002$), “sweat” ($P<0.001$), “urine” ($P<0.001$), “pungent” ($P<0.001$), “bitter” ($P<0.001$) and “sour” ($P<0.001$). Of these terms, “sweat” and “urine” were used most (21.5% and 20.9% of the correct answers, respectively); after this came “pungent” (17.4%), “bitter” (15.9%), “sour” (15.3%) and “spicy” (9%).

4. Discussion

In this sensory study conducted in Flanders, Belgium, 51.1% of the females and 38.3% of the males tested were considered to be sensitive to the boar taint component androstenone. These percentages can only superficially be compared with other studies, given significant differences in the methodology for testing androstenone-sensitivity and for defining someone as positive.

The methodology used in this study was based on the method developed by Lunde et al. (2009). Lunde et al. (2009) investigated which presentation form of androstenone (pure crystals, crystals dissolved in oil or water, or solutions with undissolved crystals removed or not) gave the most intense odour and the most negative response. They used androstenone crystals dissolved in distilled water, with undissolved crystals not removed, in foil-covered glass bottles. A questionnaire with a triangle approach combined with a LM scale was subsequently developed. In the present study, Lunde's methodology was slightly modified. Instead of two test series with two bottles filled with water alone and one with androstenone, we used two test series of three bottles with water and one with androstenone. This modification reduced the probability of correct answers by chance from 1/9 to 1/16. Lunde et al. (2009) considered consumers to be sensitive to androstenone when bottles were perceived correctly in both tests and when the mean value of intensity was indicated as ‘strong’ or higher. This definition rather separates the very sensitive consumers from the non-sensitive or the slightly sensitive consumers. However, some non-sensitive and slightly sensitive consumers can, after exposure to androstenone (for example, from exposure to boar meat contaminated with boar taint), become more sensitive (Wysocki et al., 1989). The definition used and proposed in the present study is therefore less conservative than the one used by Lunde et al. (2009) since people were considered sensitive when they scored both tests correctly independent of how strong they judged the intensity of the odour to be. The ability to distinguish androstenone from water, however, was tested more conservatively in the present study as compared to Lunde et al. (2009). Two tests with four (instead of three) bottles were used and a guess correction was used to correct for people who had both test-series correct by chance. When the criteria for categorizing an individual as androstenone-sensitive as used by Lunde et al. (2009) were applied to the dataset collected during the present study, the prevalence of Flemish consumers sensitive to androstenone was reduced considerably and became comparable to that of Norwegians (Table 4). Still, the Norwegians had been tested with 2×3 bottles whereas the Flemish were tested with 2×4 bottles.

Weiler et al. (2000) reported a sensitivity of 32.3% in Germany and 46.4% in Spain, but comparing this study with the present study is problematic for several reasons. First, the consumers in Germany and Spain were given a vial filled with crystals of pure androstenone while the consumers in Norway and Flanders received a bottle of androstenone dissolved in water (undissolved crystals not removed). We opted for the latter method as Lunde et al. (2009) established that pure crystals have a less intense odour than crystals solved in water. Second, Weiler et al. (2000) used a 7-point scale for recording the intensity of the odour and a different cut-off (score>3) procedure for categorizing individuals as sensitive to androstenone. The study of Lunde et al. (2009) and the present study, however, used a LM-scale. Third, Weiler et al. (2000) did not repeat the assay and the consumers were only allowed to smell a vial once, whereas during the study of Lunde et al. (2009) and the

Table 2
Average intensity of the androstenone odour perceived (scored on a scale from 0 to 100 on the LM) sorted by age group.

Age (years)	N	N^+(%)	Female (%)	Male (%)	Average intensity
0–20	220	39	66	34	32.9
20–40	585	42	59	41	33.5
40–60	494	59	63	37	31.1
60–80	261	53	56	44	26.4
80–100	9	66	50	50	24.0

N^+: Percentage of the population that is sensitive to androstenone.

Table 3
Odds ratios of androstenone sensitivity between test locations (differences in age and gender were accounted for) ($^{(*)}$: $0.05<p<0.1$; * $0,01<p<0.05$; ** $0.001<p<0.01$, *** $0.0001<p<0.001$; **** $p<0.0001$). The odds ratio refer to the odds of being sensitive to androstenone of a location in the first column versus a location in the first row.

	Kortrijk	Merelbeke	Hasselt	Oostende	Oostakker	Antwerpen
Kortrijk	1	–	–	–	–	–
Merelbeke	2.21^{****}	1	–	–	–	–
Hasselt	1.72^{*}	0.78	1	–	–	–
Oostende	$1.37^{(*)}$	0.62^{*}	0.8	1	–	–
Oostakker	1.85^{***}	0.84	1.08	$1.35^{(*)}$	1	–
Antwerpen	1.92^{***}	0.87	1.12	$1.40^{(*)}$	1.04	1

Table 4
Adaptation of the guess correction method employed for estimating the percentage of androstenone-sensitive males and females in Flanders to the method used by Lunde et al. (2009).

Method:	Population: Norway (Lunde et al., 2009)	Flanders (Lunde et al., 2009)	Flanders (guess correction)
Males	26 %	29 %	38%
Females	46 %	42 %	51 %

present study, consumers had to correctly identify the androstenone-positive bottle in two test series. Given that a consumer is sensitive to androstenone and that both tests are done directly after each other, the consumer is likely to smell in the first test the bottle with androstenone stronger than the bottle with androstenone in the second test. Therefore the intensity was scored after every test and the mean value of both test results calculated after the intensity scale was converted into numbers from 0 to 100 (Lunde et al., 2009).

As mentioned above, comparison between studies is problematic because of the use of different methodologies and operational definitions between the studies. Therefore, these types of studies should be harmonized. The most objective way to test the sensitivity to androstenone is to look for the specific receptor in each consumer since sensitivity is genetically determined (Keller et al., 2007). However, we believe that the cost to test consumers by this approach will probably be too high. An alternative is developing a screening test. Lunde et al. (2009) have tested different systems to present androstenone, including pure crystals, which have also been used in the studies of Weiler et al. (2000) and Furnols, Gispert, Diestre, and Oliver (2003). For the three reasons mentioned above we believe that the study of Lunde et al. (2009) better tests the sensitivity to androstenone than Weiler et al. (2000). However, it is advisable to apply some small experimental changes like we did in the present study e.g., use four bottles instead of three so the influence on guess correction is lower, use guess correction instead of the criterion that the mean value of intensity has to be “strong” or higher.

Although the aforementioned reasons explain to a large extent why the absolute percentages are not comparable between the different studies, the relative differences in androstenone sensitivity and in particular the impact of gender and age confirm previous findings. Indeed, the higher sensitivity of women compared to men has been reported previously (Weiler et al., 1997; Matthews et al., 2000). The negative association between age and sensitivity observed in the present study agrees with Matthews et al. (2000).

Intriguingly, we also found differences in sensitivity to androstenone between the various test locations within Flanders. The existence of significant differences in androstenone sensitivity between regions within a country has, to our knowledge, never been reported before. Differences between Kortrijk and the other locations are most pronounced (Table 3). It is unlikely that these differences were related to the weather conditions when the tests were conducted, as these conditions were not particularly different in Kortrijk compared to the other five locations (Table 1). Temperature and cloudiness were about average, while the wind velocity recorded was on average higher than in most cities but this was also true for Merelbeke, the location where the highest sensitivity for androstenone was measured. Antwerp was the only location where the test happened indoors, but the results are comparable to the other locations apart from Kortrijk. The composition of the sample of people tested at the different locations were not identical with regard to gender and age. The effect of both variables, however, were accounted for in the statistics. Possibly the difference between Kortrijk and the other locations may be attributed to other differences in the composition of the samples of people tested. Although such information was not collected, we have the impression that in Kortrijk a larger proportion of the people tested were foreigners as compared with the other locations. Unlike previous studies, and until there is more clarity about the underlying reason for these differences between locations, we recommend that people be tested under a range of conditions and locations in order to estimate the androstenone sensitivity of an entire nation or population.

Knowledge about the sensitivity of consumers to boar taint is important, particularly if castration of male pigs would be banned and the production of entire male pigs would be proposed as the alternative strategy. As at present it appears to be more feasible to reduce skatole levels among entire male pigs kept in commercial conditions as compared to androstenone levels, it is particularly relevant to investigate the sensitivity of consumers to androstenone. Better harmonization of such studies is warranted. Once the sensitivity of the population is known and a detection method is developed it will be easier to determine the impact of boar taint. Carcasses with a high amount of androstenone and a low amount of skatole will probably be a lesser problem than carcasses with a high amount of skatole. All consumers are sensitive to the presence of skatole, while only some consumers are sensitive to androstenone. However, the proportion of androstenone-sensitive consumers varies between countries/regions.

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