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THE USE OF ELECTRONIC NOSE TO DIFFERENTIATE ENTIRE MALES FROM IMMUNOCASTRATES (ASSOCIATION WITH LEVELS OF ANDROSTENONE AND SKATOLE)

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ABSTRACT

The aim of this work was to test the ability of the electronic nose (designed and fabricated by CNR-Institute for Microelectronics and Microsystems Rome, Italy) for the differentiation of subcutaneous fat samples from entire or immunocastrated pigs. Seven pigs immunocastrated with Improvac® (IC) and eight entire males (EM) from genotype Pietrain × (Large White × Landrace) were used. After the slaughter, samples of backfat were taken for the determination of androstenone and skatole concentrations. Thighs from experimental pigs (n = 30) were processed to dry-cured ham *Kraški pršut*. After the "*riposo*" phase, subcutaneous fat was sampled from thighs for the analysis with electronic nose. The device is based on eight gravimetric sensors (quartz crystal microbalances-QCMs) oscillating at 20 MHz of fundamental frequency and coated with a proper set of organic polymers and macromolecules. Principal Component Analysis (PCA) was used to discriminate IC and EM, based on the signal acquired with electronic nose. The first two components explained 93.5% of variance. The discrimination of EM from IC was good but not flawless, because four EM samples were false negative, but only one pig was false negative for both samples/repetitions. It can be concluded that the results are promising and that the tested electronic nose is able to discriminate fat samples of EM, however, due to the methodological uncertainties (sampling fat after "*riposo*") results should be considered preliminary and further work is needed.

Key words: boar taint / pig / immunocastration / electronic nose / principal component analysis

1 INTRODUCTION

Surgical castration of piglets is a routine practice in pig production. It is used as a preventive measure to avoid unpleasant smell, the so-called boar taint, which occurs in non-castrated males at the onset of puberty. The two main compounds are held responsible for boar taint, androstenone, related mostly to "urine", and skatole, related mostly to "manure" (Gunn *et al*., 2004, Dijksterhuis *et al*. 2000). Castration as practiced nowadays (without analgesia/anaesthesia) is presently being questioned in the EU and there is a strong initiative to end it until 2018. One of the alternatives of surgical castration is the so-called immunocastration, i.e. the vaccination against gonadotropin-releasing-hormone (GnRH), which effectively disrupts the reproductive hormonal axis and thus reduces the levels of compounds (androstenone and skatole) responsible for boar taint (Batorek *et al*., 2012). Boar taint is perceived by human nose when the meat or fat is heated. Because of the differences in human sensibility and due to non-harmonized methods, the perception of boar taint does not perfectly match the levels of androstenone and skatole concentrations in pork (Bonneau *et al*., 1992). To detect boar taint, sensory analysis or chemical determination of responsible compounds can be used; however, chemical analysis is time-consuming,

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expensive and not adapted for use on the slaughter line. In this respect, the use of the electronic nose for odour detection is of interest. The objective of the study was to test the electronic nose developed by CNR-Institute for Microelectronics and Microsystems Rome, Italy for its ability to differentiate back fat samples of entire from immunocastrated male pigs.

2 MATERIAL AND METHODS

Immunocastrated (IC, N = 7), and entire males (EM, N = 8) of the genetic line Pietrain × (Large White × Landrace) were used. Experimental pigs come from the trial conducted at INRA UMR 1348 PEGASE (one IC pig died in the course of the experiment). Pigs of IC group received two vaccinations with Improvac®, the first one (V1) at 11 weeks of age and the second one (V2) at 16 weeks of age. Pigs were slaughtered at 24 weeks of age. Backfat was sampled to assess the concentrations of androstenone and skatole (Pauly *et al.*, 2008). One day after slaughter, 30 thighs (16 EM + 14 IC) were harvested and sent to Slovenia to be processed to dry-cured ham "*Kraški pršut*". After "*riposo*" phase, the samples of subcutaneous fat were taken from left and right thighs (according to two methods differing in the content of salt) and analysed with an electronic nose designed and fabricated by CNR-Institute for Microelectronics and Microsystems Rome, Italy. Measurements were performed on headspace of sealed bottles (100 mL) that contained 4 g of fat (12 hours after thawing) which were placed on a disk of silicone treated filter paper (Whatman). The electronic nose consisted in eight gravimetric sensors – quartz crystal microbalances-QCMs – oscillating at 20 MHz of fundamental frequency and coated with a proper set of organic polymers and macromolecules. The sensors recorded a frequency shift related to the mass of volatile compounds absorbed on their surfaces according to Sauerbrey's equation ($\Delta f = -Cf \times \Delta m$, Δf –the observed frequency change, Δm the change in mass per unit area and Cf the constant factor depending on the crystal used). Sample measurements were carried out at room temperature until a dynamic equilibrium was reached between the sensors and the volatile compounds absorbed and no further frequency change was recorded (steady state). Thirty minutes were adopted to restore the sensors by flowing filtered and dry air throughout the measurement chamber (10 cc) before initiating a new measurement. Cluster analysis based on androstenone and skatole (with SAS 9.2, SAS Institute Inc., Cary, NC, USA) was made to confirm a discrimination between EM and IC. The signals/responses (from eight microbalances) of the electronic nose were analysed by Principal Component Analysis (PCA) using statistical software (PLS Toolbox by Eigenvector Research, Inc., Wenatchee, WA, USA). The responses were calculated as the difference between frequencies during the measurement and cleaning phase. Autoscaling of the data was performed prior to PCA.

3 RESULTS AND DISCUSSION

Cluster analysis (Fig. 1) was used to validate "group status" (IC or EM) based on androstenone and skatole concentration. It can be noted that two groups were well separated, however some subgroups within the main ones could be detected, in particular within EM. One IC

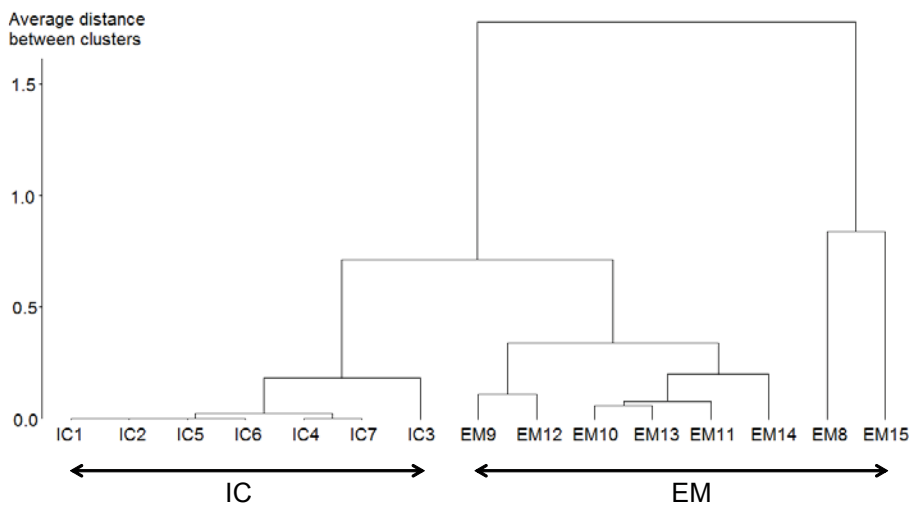

Figure 1: *Cluster analysis based on androstenone and skatole concentration*

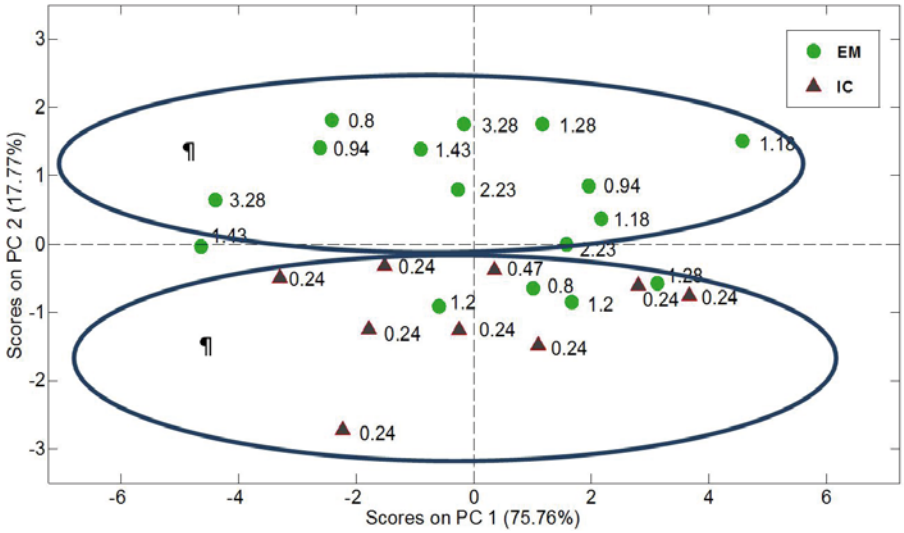

Figure 2: *Scoreplot of PCA from electronic nose measures. Sample labels indicate the level of androstenone (µg/g fat).*

pig was also slightly apart from the others with androstenone level (0.47 µg/g fat) being close to the sensory perception threshold in fat phase (0.5–1.0 µg/g fat; Haugen, 2012) and is suspicious for being a non-responder.

Discrimination of EM and IC based on the signal acquired by the electronic nose was good (Fig. 2, 3). Figures 2 and 3 show the PCA plots as related to the level of androstenone or skatole, respectively. The first two components (PC1, PC2) explained a very high part of the variance (93.5%). The component PC2 was able to distinguish EM (positive values) from IC (negative values). However, the distinction was not faultless; there were some EM samples that were false negatives, i.e. positioned in the group of IC. However, only one pig was false negative for both samples/repetitions. It is also worth noting that two EM samples with very high androstenone and skatole concentrations were found on the border of the cloud. On the other hand, there were no false positive cases, i.e. no IC samples positioned in the group of EM. The same observations can be made when considering the levels of skatole (Fig. 3). All the samples, which were above the threshold limit (0.2 µg/g fat) were classified in the EM group.

It is worth noting that in the present study the samples of fat were taken after salting and "*riposo*" phase, from the experiment where two salting levels were applied, which could influence the results. Indeed, salt is known to have prooxidant effect on lipids (Min and Ahn,

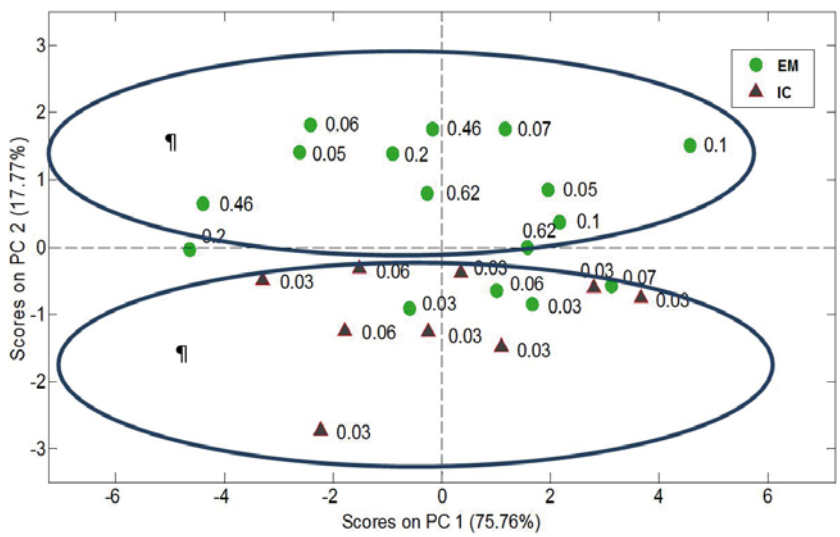

Figure 3: *Scoreplot of PCA from electronic nose measurements. Sample labels indicate the level of skatole (µg/g fat).*

2005) and because the samples were taken app. three months later than the samples for androstenone and skatole determination this could explain why in some cases, two samples from the same animal were ambiguously classified, once as EM and once as IC. Another explanation could be that other aromatic compounds are involved in boar taint. As suggested by Vestergaard *et al.* (2006), there are other major compounds present in the head-space of fat samples which can be involved or can interfere with compounds responsible for boar taint. Various electronic devices to detect either boar taint or responsible compounds (in particular androstenone) have been tried in and validated in the past (Bourrounet *et al.*, 1995; Di Natale *et al.*, 2003; Vestergaard *et al.*, 2006; Kirsching *et al.*, 2012). As concluded by Haugen (2006) in his review, the potential of electronic noses is promising, but there is still a need for further research and development.

4 CONCLUSION

Results obtained in the present study show that the electronic nose which was tested has the potential to discriminate EM. However, there were some false negative samples that were classified as IC despite relatively high (0.8–1.2 μg/g) androstenone levels. The reason might be due to the methodological circumstances, and thus the present results should be considered merely preliminary. Further studies are needed to test the ability of electronic nose to detect boar taint, in particular in view of other (presently unknown) compounds potentially involved or interfering.

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