Phenotyping strategies for an efficient and holistic approach to reduced boar taint through genomic selection

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Abstract
Surgical castration without anaesthesia or analgesia of boars is about to be banned in Europe. This ancestral practice avoids the development of boar taint (BT), an unpleasant smell found in cooked meat of some uncastrated male pigs, mainly due to the accumulation of skatole and androstenone in adipose tissue. Animal breeding and especially genomic selection in the context of whole-male rearing has been recognised as a potentially promising strategy to avoid BT. However, challenges exist to define and to record BT associated traits. Required phenotypes must be cheap, available in routine as close as possible to the slaughterline and repeatable. This review will also cover new opportunities through different and innovative aspects of BT phenotype definitions allowing the potential identification of underlying genetic architecture and its use in the breeding against BT.

Introduction
Boar taint (BT), found in uncastrated male pigs, is a complex hormonal odour caused in majority by androstenone and skatole (Rius et al., 2005) that give respectively an urine and faecal odour to cooked meat (Font-i-furnols, 2012). Androstenone (i.e. *5α-androst-16-en-3-one*) is a testicular steroid that is synthesized in Leydig cells from pregnenolone (Squires et al., 2020) and in high concentrations since puberty (Bonneau, 1998). Skatole is an alkaloid derived from tryptophan (Claus et al., 1994; Jensen et al., 1995a). They are produced in the colon by the breakdown of indigestible fermentable food and dead intestinal cells by microbiota (Jensen et al., 1995b; Wesoly et al., 2012). Androstenone and skatole can be found in various tissues and fluids such as in fat and blood (Wesoly et al., 2012; Squires et al., 2020). Other compounds could contribute to BT (e.g., 2-aminoacetophenone) (Rius Solé et al., 2001; Fischer et al., 2014) or could be correlated (e.g., presence of high levels of saturated fatty acids (Mörlein et al., 2014)). Moreover, the odour intensity is affected by several factors such sexual maturity which particularly affects androstenone production, husbandry practices (e.g., nutrition particularly involves in skatole synthesis), the health and the raising environment, but also breed and genetics (Li et al., 2019; Squires et al., 2020), and lastly the interaction of all these. From one individual to another, the tainted characterisation is therefore highly variable. Despite extensive knowledge that was established about BT in the last years and related phenotypes based on biological and chemical facts, work is ongoing to advance on required phenotyping strategies for an efficient and holistic approach to do genomic selection for reduced BT while considering pig breeding as a whole.

Phenotypes
Direct classification of BT phenotypes. Phenotypes are characterised when boars are slaughtered as carcasses have to be checked for BT to determine what will be there use later on in the meat processing (e.g., consumption of pork chops for untainted boars or dilution of the meat in the case of tainted meat). Throughout Europe, the “human nose” method is generally used by trained operators online or at line except in Denmark where a colorimetric

method is used to detect and quantify indolic compounds (European Commission, 2019). From an animal breeding perspective this type of field data based on the current detection methods has the advantage of being fast, easily performed in routine and cheap, therefore on a large scale, but has the weakness of unreliability left by the subjective identification of an operator even if he is trained for "human nose method" and the lack of specificity for the colorimetric method (Burgeon et al., 2021a).

Detection of BT generating compounds in fat. An alternative to sensory analysis is accurate chemical analysis of odorous compounds. This implies normally that animal are dead but a biopsy tool has been developed by Baes et al. (2013) to test live boars and determine from their phenotype of BT compounds. Many chromatographic techniques have been developed that allow a high level of robustness, reliability, accuracy and sensitivity in the detection of BT compounds often found in trace amounts. Their disadvantages are in particular their rather high price, the difficulty of setting up such an instrument in terms of space and technical training in the slaughterhouse, and the lack of speed (including preparation). Portable instrumental methods such as biosensors, surface-enhanced Raman scattering, and Rapid Evaporative Ionisation Mass Spectrometry technologies are being studied but still required sample preparation. These research include the detection well-known BT compounds (e.g., androstenone and skatole) but can also be based on volatile organic compounds profile as Burgeon et al. (2021b) recently explored (Font-i-furnols et al., 2020; Burgeon et al., 2021a). Finally the Laser Diode Thermal Desorption–Tandem Mass Spectrometry is currently being tested in slaughterhouses in Denmark, probably to replace the colorimetric method, meeting the requirement of cost and reliability (Font-i-furnols et al., 2020; Burgeon et al., 2021a). Most of current detection methods are based on the detection of these compounds in fat matrix. However, other matrices could be studied such as blood or plasma and saliva since the presence of skatole and androstenone these matrices have been previously observed (Bone et al., 2019; Dubois et al., 2021).

Indirect traits used to detect risk of BT. Other traits related to sexual maturity were also studied although they were not always considered satisfactory for classification. Weight is used in the UK as an indicator of puberty implying slaughter below 110 kg (European Commission, 2019) but this method is at risk for pigs with early age maturity. Bernau et al. (2018) also correlated greater testicular volume and body fat with abundant androstenone content. If body fat is routinely assessed on slaughterlines measuring testis volume may present a greater challenge. Fatty acids have also been identified as potential indicators of BT. It would appear that tainted fats are significantly richer in saturated fatty acids and poorer in unsaturated fatty acids than an untainted subcutaneous adipose tissue (Mörlein et al., 2014; Liu et al., 2017). Measuring fatty acids using appropriate techniques may be reliable in routine using appropriate tools (e.g. near-infrared reflectance spectrometry) (Font-i-furnols et al., 2020).

Classical breeding against boar taint

Classical genetic tools. Breeding programmes need to use different precited phenotypes to identify boars with a low risk of transmitting odour development to their offspring (Drag et al., 2017). In Europe, there are various BT selection programmes (e.g., Topigs Norsvin, Nucleus) (Squires et al., 2020) often nowadays exploiting genomics (Drag et al., 2017).

Holistic approach towards pig breeding. If the previously mentioned indirect traits were relatively, but not totally, i.e., body fat, in neutral relationships to breeding objectives, BT is suspected to have negative genetic correlations with meat quality, including intramuscular fat and fat thickness, and fertility and that selecting for reduced BT incidence would deteriorate the selection response in these traits. Genetic correlations of reproductive and aggressiveness traits, aspects concerning farmers for breeding, with BT compounds have been reported as related by Duarte et al. (2021). However, these correlations depend on the breed and the gender and their capacity to develop associated traits to BT compounds development.

Future opportunities and challenges

Innovative phenotypes. BT is complex. Its compounds, some of which are well known, are widely studied. Currently, genetic and genomic studies for selection against BT generally focus on the main compounds responsible for BT (skatole, androstenone and indole at a smaller extent) as well as on sexual maturity related traits. To characterise BT and optimize selection to make it fast and cheap for use in routine, research should open up to other ways of working or go down again paths that are sometimes abandoned due to lack of funds or technological resources. For example, the study of volatile organic compounds related to BT (e.g., Burgeon et al., 2021b) is a path that is again being opened in particular through the development of more refined and potentially faster chemical techniques. Technological phenotyping has to explore the BT characterization in fat and other matrices which contain these compounds. BT compounds could be related in particular to the physiology of boars and sows and indicator traits (traditional and novel ones). Finally, this innovative phenotyping is a gateway to genetics, genomics and gene expression studies for known and currently undetermined BT molecules. Where fundamental science brings technologies to phenotyping, these could be linked to establish the most accurate genetic selection possible to respond to producers and consumers requirements.

Gene pathways. Skatole and androstenone have been extensively studied, including at genomic level to determine which genes are involved in the physiological pathways (e.g., Squires et al., 2020). As genome wide association studies (GWAS) on skatole, androstenone, indole and other usual BT traits have already identified candidate genes, research could also find new gene pathways from exploiting these novel phenotypes. These new identified biomarkers could be then used.

Gene expressions. The study of these genes and their expression in different tissues could bring new opportunities to define expression quantitative trait loci (eQTL) of interest. Drag et al. (2018) have already characterised eQTLs function according to regulation systems of traditional BT compounds. However, since BT reduction is desired, it is necessary to monitor possible overlaps for other traits as highlighted by Drag et al. (2018).

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