DEVELOPMENT AND DETECTION OF BOAR TAINT

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Summary

The use of entire male pigs instead of castrates is an attractive method for improving the efficiency of pork production. Genetic selection programs would also benefit by being able to readily market their non-selected breeding stock. Concerns about animal welfare would also be addressed by eliminating the castration of boars. However, male pigs raised for meat are now castrated in many countries to prevent the possibility of taint in the meat. Boar taint is due to the presence of high levels of skatole and the 16-androstene steroids, particularly 5α-androstenone in the carcass. Skatole is produced by bacteria in the hindgut and skatole levels in carcasses are affected by dietary, environmental and genetic factors. Skatole production by the gut microflora can potentially be reduced by dietary means and the accumulation of skatole may be due to genetic differences between animals. The 16-androstenes can be reduced by interfering with the action of gonadotropins on the testis and by slaughter of the animal before sexual maturity. However, levels of both skatole and the 16-androstenes are low in the vast majority of market weight entire male pigs. Immunological, chromatographic and colorimetric methods for the analysis of skatole and the 16-androstenes have been described. Several of these methods may be suitable for use in packing plants when automated equipment is available. The pork industry in North America could now begin to put together procedures for raising and processing entire males and to develop markets for this product.

Introduction

Entire (uncastrated) male pigs are more efficient at converting feed into muscle than either females or castrated (reviewed in Squires *et al.*, 1993a) due to the anabolic effects of androgens produced by the testis. This result in lower feed consumption, increased lean meat yield, less backfat and, when feed is restricted, increased growth rate of entire males compared to females or castrates. All these factors can contribute to increased profitability of pork production from entire male pigs. In addition, selection pressure for lower backfat and increased lean could be reduced if entire male pigs were raised instead of castrates. The cost of genetic selection programs would also be lowered and the final selections could be sold as market hogs. Animal welfare concerns about the castration of farm animals also makes the use of entire male pigs desirable.

However, there are some potential problems in the use of entire male pigs for pork production. There may be increased aggression between entire males, causing injury and concern about the welfare of the animals and increasing the number of carcass demerits. However, this only appears to be a problem when different pigs are mixed at shipping and not when the pigs are raised in the same social groups. The carcasses of some entire males can be too lean to be handled by automated equipment. The fat can also be softer than that of castrates and gilts making it more difficult to cut and handle the meat and leading to separation of the fat from the meat (Kempster *et al.*, 1986). This softer fat is due to an increased content of unsaturated fatty acids in the fat (Barton-Gade, 1987), which may be more attractive to health conscious consumers. This problem may be controlled by decreasing the level of unsaturated fat in the diet (Warkup, 1992).

Several countries, including Denmark, Britain, Spain and Australia currently raise entire male pigs. However, in North America all male pigs that are raised for meat

are castrated shortly after birth in order to prevent the possibility of boar taint. Boar taint refers to the presence of off-odours and off-flavours found predominantly, but not exclusively, in the meat of some entire male pigs. The two main sources of boar taint are the 16-androstene steroids, primarily 5α-androstenone, and skatole.

The purpose of this paper is to summarize the factors responsible for the development of boar taint and methods used for measuring boar taint, particularly those methods which may be suitable for use in packing plants.

Boar taint due to 16-androstene steroids

The 16-androstene steroids, including 5α-androstenone, are produced by the testis of entire males and are released into the blood stream (reviewed in Bonneau, 1982). Due to their hydrophobic nature, they are concentrated in the fat and contribute to boar taint when the fat is heated. 5α-androstenone is also concentrated in the salivary glands by a specific binding protein called pheromaxein (Booth, 1984a) and is converted into 5α-androsten-3α-ol. This latter compound is released with the saliva and acts as a sex pheromone to induce the mating stance in female pigs in heat (Reed *et al.*, 1974) and to regulate the reproductive cycle of females (Booth, 1984b). 5α-androstenone was first identified as the most important 16-androstene steroid causing boar taint (Patterson, 1968) although 5α-androsten-3α-ol may also contribute a significant amount of taint (Brooks and Pearson, 1989).

The production of 16-androstene as well as other steroids in the testis is stimulated by increased levels of gonadotropins in the blood. The gonadotropins are produced by the pituitary gland in response to gonadotropin-releasing hormones from the hypothalamus. There are two peaks of steroid levels in the blood of entire male pigs: one shortly after birth up to about one month and another at about five months of age as the animal reaches sexual maturity (Schwarzenberge *et al.*, 1993). At these times, sufficient 16-androstene steroids may be produced to cause boar taint. The exact timing of the rise in 16-androstene steroid production in older animals varies among different animals and is a function of sexual maturity (Bonneau, 1982). The size of the bulbourethral gland and submaxillary salivary glands increases at sexual maturity and is correlated with the increased levels of 16-androstene steroids. These measurements may be useful to identify immature entire males that would not be tainted from high levels of 16-andrsotene steroids (Bonneau and Russeil, 1985; Squires and Deng, 1992).

However, we have observed low levels of 16-androstene steroids in some pigs that are sexually mature, so not all mature animals would necessarily be tainted. It may be possible to select for animals in breeding programs that are low in 16-androstene steroids but which produce normal levels of androgens. This is a moderately heritable trait, but selection programs so far have not been successful, resulting in either reproductive problems in females (Willeke *et al.*, 1987; Sellier and Bonneau, 1988) or failure to lower 16-androstene levels in entire males (Sellier *et al.*, 1993). Alternatively, levels of 16-androstene steroids in market weight pigs can be reduced by using fast growing and late maturing lines of pigs, feeding diets which optimize growth rate or by using growth promotants such as somatotropin (Bonneau *et al.*, 1992a). Some breeds of pigs, such as Duroc, have a tendency for high levels of 16-androstene steroids in the carcass (Squires *et al.*, 1992).

Production of the 16-androstene steroids can also be reduced by interfering with the effect of gonadotropins. This can be accomplished by generating antibodies against either gonadotropins or gonadotropin-releasing hormone by active immunization (Bonneau *et al.*, 1993) and possibly by passive immunization with antibodies (van der Lende *et al.*, 1993). Alternatively, use of agonists or antagonists to gonadotropins or gonadotropin-releasing hormone reduces the production of 16-androstenes by the testis (Xue *et al.*, 1993). The production of androgens is also reduced by these treatments, so their use must be timed to have an effect as late as possible to achieve maximal anabolic effects of the androgens. Antibodies could also be generated against 5α-androstenone (Williamson and Patterson, 1982), but methods to control boar tain in this manner have not been firmly established. The half life of 5α-androstenone in fat is in the range of a few days so that levels in a tainted animal should be reduced to acceptable levels by a few weeks after the inhibition of 16-androstene production (Bonneau *et al.*, 1982; 1993).

Boar tain due to skatole

Skatole (3-methyl indole) is not actually produced by the pig, but is absorbed from the hindgut where it is produced by bacterial degradation of tryptophan. Skatole is also found in carcasses from females and castrates, although levels can be higher in entire males (Hansson *et al.*, 1980; Bejerholm and Barton-Gade, 1993). High levels of skatole can also be found in some gilts and this may be the cause of so-called *gilt-tain*. Skatole may thus not be a cause of *sex* tain but does contribute to increased taint in entire males and is a component of boar taint. Levels of skatole in fat are correlated with levels of androstenone with correlation coefficients reported of about 0.4 (Andresen *et al.*, 1993; Lundstrom *et al.*, 1988) and 0.7 (Bonneau *et al.*, 1992b).

Lactobacillus sp. of bacteria in the hindgut are thought to be responsible for the production of skatole (Yokayama and Carlson, 1979) as well as indole, which does not contribute significantly to taint (Hansson *et al.*, 1980; Moss *et al.*, 1993). Studies *in vitro* have shown that the relative production of skatole to indole by the gut micoflora is affected by pH. The relative production of skatole to indole was increased at pH 5 but was dramatically reduced at pH 8 compared to pH 6.5 (Jensen and Jensen, 1993). This supports other results that feeding bicarbonate can reduce the production of skatole (Claus, 1992). Feeding inulin (Claus, 1992), coconut cakes or demolassed sugar beet pulp (Kjeldsen, 1993) to provide a source of energy for the bacteria reduced skatole production, presumably by reducing the metabolism of protein that was not fully digested and absorbed by the pig feeding tryptophan does not increase skatole production, since tryptophan is rapidly absorbed by the animal, but skatole is increased in *ad libitum* compared to restricted feeding (Deng *et al*, 1992). Restricted feeding for 48 hours and withholding feed for 12 hours before slaughter is recommended to lower levels of skatole in the carcass (Kjeldsen, 1993). Increasing the water consumption and feeding compounds such as avotan and virginiamycin which reduce the growth of *lactobacillus* bacteria also lower skatole levels (Kjeldsen, 1993). The half life for skatole in fat is about 10 hours (Friis, 1993), so it is removed from the fat much more rapidly than are the 16-androstenes. In practical terms, this suggest that any treatments used to reduce the levels of skatole in the carcass need only be done the last week before slaughter.

Wide variations in skatole levels among different herds have been reported (Kjeldsen, 1993). High levels of skatole in the carcass may be due to dietary, environmental and genetic factors. A sufficient population of *lactobacillus* in the gut of the pig is necessary along with diets which promote the degradation of tryptophan into skatole. Skatole can also be absorbed from the faeces, apparently through the lungs rather than through the skin (Hansen *et al.*, 1993). High skatole levels can therefore result from high stocking rates, particularly in the warmer months. Skatole is rapidly absorbed from the gut and metabolized in the liver (Abergaard and Laue, 1993). However, it has been suggested that the degradation of skatole in the liver is reduced in some pigs resulting in high skatole levels in the fat. This trait may be due to the presence of a recessive gene (Ska^1) which is only expressed under high environmental pressure for skatole (Lundstrom and Malmfors, 1993).

Importance of skatole versus the 16-androstenes to boar taint

There is considerable controversy over skatole or the 16-androstenes (particularly 5α-androstenone) is the most important component of taint.. Moreover, the maximum acceptable limits for these compounds have not been firmly established and range from 0.20 to 0.25 ppm for skatole (Berg *et al.*, 1993; Valhun, 1990) and from 0.5 to 1.0 ppm for 5α-androstenone (Bonneau, 1990). The ability to detect 5α-androstenone by humans is genetically determined and predominates in females. Only 56% of males but 92% of females are able to detect this substance and women find the substance more unpleasant than men (Wysocki and Beauchamp, 1984; Griffiths and Patterson, 1970). 5α-androstenone produces a urine-like odour while androstenol produces a musk odour. On the other hand, most people are able to smell skatole and find it unpleasant. It has a strong faecal smell and produces a bitter taste (Hansson *et al.*, 1980). The sensitivity to taint thus undoubtedly varies dramatically between different countries. It is therefore important to relate the results of analytical methods for skatole and androstenone to sensory evaluation of taint for different populations.

Consumer panels and trained sensory panels have been used to determine the relative contributions of skatole and 5α-androstenone to boar taint. Quite different results have been reported from different countries. Studies from Denmark have consistently found that skatole is the more important component of taint (Vahlun, 1990) and the Danish meat packing industry has spent a considerable amount of effort developing automated testing equipment for skatole and installing it in their slaughterhouses. An initial cutoff level of 0.20 ppm skatole in fat was used, but this was later raised to 0.25 ppm to reduce the number of rejected carcasses when few consumer complaints were found. Recent studies from Norway (Andresen *et al.*, 1993) and Denmark (Bejerholm and Barton-Gade, 1993) have shown that boar taint was more highly correlated with skatole than 5α-androstenone levels in fat. Androstenone was important only when skatole levels were low and particularly in products with a high content of fat (eg., belly) than for lean product. On the other hand, studies from France have shown that levels of 5α-androstenone were more highly correlated to boar taint than was skatole (Bonneau *et al.*, 1992b). However, meat from entire males with low levels of both skatole and 5α-androstenone was not as desirable as meat from castrates, suggesting that something else was also contributing to taint in these carcasses. Other workers have reported similar results but have been unable to identify the other factors that may contribute to taint (de Vries and Walstra, 1993). There is evidence of a

synergism between skatole and 5α-androstenone in causing taint (Hansson *et al.*, 1980; Berg *et al.*, 1993). The best way to express the acceptable limits for these compounds may be as a function of the amount of each that is present.

Measurement of boar taint in carcasses

Entire male pigs raised in Britain, Spain and Australia are not tested for boar taint. Consumer trials in Britain have indicated that boar taint is not a concern, but that other meat quality attributes such as the lack of tenderness and juiciness are more important. However, in other countries which raise entire males or are considering doing so, some form of test for taint is being used. Denmark has concentrated so far on the detection of skatole, but methods for the detection of androstenone are now being developed (Sandersen, 1993). Sweden and Norway, which are experimenting with the use of entire males, are only measuring levels of skatole, but result indicate that androstenone may also contribute to taint (Berg *et al.*, 1993).

Testing for boar taint is needed to decide if the levels are low enough for the carcass to be used for fresh pork products. Acceptable limits for 5α-androstenone are higher for meat that is used for cured and processed products (Bonneau *et al*, 1992c). This may be due to the loss of androstenone during processing or that the products are eaten cold. Carcasses with levels of taint too high for use as fresh product could therefore be used directly or mixed with untainted meat for use in processed products.

Ideally, carcasses should be tested on the slaughter line using precise, rapid and low cost methods that accurately predict the levels of boar taint. Individual carcasses would have to be identified so that the test results can be used to sort carcasses for fresh or processed products. The levels of the 16-androstene steroids in salivary glands are 10 to 100 times greater than in fat, making the salivary gland an ideal site for the estimation of the 16-androstene steroid levels in carcasses. In addition, the salivary gland is easily sampled and is sometimes removed along with the submaxillary lymph nodes for inspection by a veterinarian after slaughter. A good correlation between the levels of 16-androstene steroids in salivary glands and sensory scores for taint have been reported (Booth *et al.*, 1986; Squires *et al.*, 1991). Indirect measures of taint from 5α-androstenone can also be made using the length of the bulburethral gland and this may reduce the number of tests that need to be done. Danish slaughterhouses are equipped with sampling equipment which is used to remove backfat samples which are placed in plastic vials and sent to the lab for analysis of skatole. Individual samples and carcasses are identified using a bar code computerized system (Mortensen, 1993).

A number of different immunological, chromatographic and colorimetric methods have been developed for measuring the levels of skatole and 5α-androstenone in carcasses. Immunological methods utilize antibodies which should bind specifically to the compounds being tested and not to other compounds. The amount of antibody bound is then measured using radioisotopes in radioimmunoassay (RIA) or an enzyme which produces a coloured product in enzyme-linked immunospecific assay (ELISA). RIA's have been developed for androstenone (Andresen, 1979; Uzu and Bonneau, 1980) and ELISA's have been developed for skatole (Singh et al., 1988) and androstenone (Abouzied et al., 1990; Claus *et al.*, 1988). Immunological methods can be limited by the long time

required to develop equilibrium binding with the antibody and the necessity for careful extraction of the fat samples. These factors can also introduce a significant amount of variation in these assays, with coefficients of variation of more than 10% commonly found. A rapid extraction method for androstenone in fat has been developed for use in immunoassays (Dehnhard and Claus, 1992).

Chromatographic methods utilizing high performance liquid chromatography (HPLC) or gas chromatography (GC) have been reported for measuring skatole and androstenone. HPLC analysis of skatole has utilized reserve phase columns eluted with either isocratic or gradient solvent systems and using either ultraviolet or fluorescence detection (Garcia-Regeiro and Diaz, 1989; Lin *et al.*, 1991; Hansen-Moller, 1992). HPLC analysis of androstenone involves the conjugation to a chromophore for detection since it does not have a strong fluorescence or absorb strongly in the ultraviolet region. A combined method for the analysis of androstenone, skatole and indole is being developed (Hansen-Moller, personal communication). GC analysis of skatole and androstenone have been described with detection by flame ionization detector (Porter *et al.*, 1989), electron capture detector (de Brabander and Verbeke, 1986), thermionic specific detector (Peleran and Bories, 1985) and mass spectrometry (Garcia-Regueiro *et al.*, 1989; Edelhaeuser, 1989; Kwan *et al.*, 1992). Careful extraction of the samples is required for chromatographic analysis, both to insure good resolution of the samples and to maintain the useful life of the column. Rapid methods for the extraction of fat samples for HPLC analysis of skatole have been developed (Hansen-Moller, 1992; Dehnhard and Claus, 1992). Chromatographic methods can have the advantage of measuring a number of related compounds at one time and are usually quite specific and not affected by interfering compounds. However, they can be time consuming, technically difficult, expensive and prone to equipment failure. For these reasons, they are usually more suitable for use in experimental analysis rather than for the routine analysis of boar taint compounds on the slaughter line.

Colorimetric methods for the analysis of skatole and the 16-androstene steroids have been described. The test for skatole (Mortensen and Sorensen, 1984) involves extraction of the fat sample with tris-acetone and mixing the filtered extract with a colour reagent consisting of 4-dimethylaminobenzylaldehyde in sulfuric acid and ethanol. The intensity of the colour is then measured in a spectrophotometer at 580 nm. The limits of detection of the assay are about 0.02 ppm and the coefficient of variation for 0.25 ppm was 4%. However, other compounds such as indole, which do not contribute to taint, are detected by the assay and the colour reagents and reaction products are somewhat unstable. Automated equipment to perform this test is now being installed in all Danish slaughterhouses (Sandersen, 1993).

We have developed a method for the colourimetric analysis of the 16-androstene steroids in carcasses (Squires, 1990; Squires *et al.*, 1991; Squires *et al.*, 1993b). In this method, samples of fat or salivary glands are extracted with methanol and the 16-androstenes are concentrated on C18 cartridges. Cholesterol is removed from the fat extracts by a digitonin column placed before the C18 cartridges. The cartridges are then washed, dried and the steroids eluted from the columns and reacted with a colour reagent consisting of resorcylaldehyde and sulfuric acid in glacial acetic acid (Brooksbank and Haselwood, 1961). After heating, the colour intensity is measured in a spectrophotometer at 590 nm. Measurements are also

taken at 543 nm and used to correct for interference from cholesterol. All of the 16-androstenes produce a colour for this test, so the results are expressed as equivalents of 5α-androstene for fat samples and equivalents of 5α-androsten-3α-ol for salivary gland. The coefficient of variation in this method using salivary gland is less than 4%. Good correlations (r=0.8) have been found between off-aroma and off-flavour sensory scores from a trained sensory panel and total 16-androstene steroid levels in fat or salivary gland measured using this method. We are now working to automate this method for use in packing plants.

The detection of skatole, 16-androstenes and other compounds causing taint instantaneously on the slaughter line using some sort of probe would be the ideal situation. Suitable probes may be possible using immunosensors, electronic sensors, or chemical sensors. Immunosensors couple antibody-antigen reactions to an electronic signal generated by a transducer. In the surface plasmon resonance (SPR) immunosensor, the antibody-antigen reaction causes a change in the refractive index at the metal-liquid interface which is detected by a change in the intensity of a reflected laser beam. Electronic sensors are composed of organic semiconductors which characteristics change when a particular substance is absorbed onto the surface. Such a sensor has been built to detect the gases from truffles (Persaud, 1990), but the 16-androstenes absorb too strongly to the surface of currently available semiconductors to make a reusable probe feasible (K.Persaud, personal communication). It may be possible to build a combined sensor which can measure the levels of skatole and androstenone simultaneously. Chemical sensors could also be constructed in which chemical reactions specific to skatole or the 16-androstenes are converted into electronic signals. This might be achieved through the use of optical detectors to detect a colour reaction, but this reaction will have to be reversible for reuse of the probe.

Incidence of taint in entire males

Studies from Britain have shown that 10% of carcasses from entire males have levels of 5α-androstenone greater than 1.0 ppm and 7-10% have levels of skatole greater than 0.25 ppm (Kempster, 1993). The rejection rate of carcasses in Denmark for skatole levels greater than 0.25 ppm averages 5-10% (Kjeldsen, 1993). Preliminary results from Norway gave 14.4% of carcasses above 0.20 ppm skatole (Froystein *et al.*, 1993). We have measured the levels of 16-androstene steroids and skatole in a number of swine herds in Canada. The levels of skatole were quite variable among different herds. Levels of skatole in entire males from the Specific Pathogen Free herd at the University of Guelph were all below 0.25 ppm (Squire and Deng, 1992). Skatole levels in these animals were not increased by feeding a barley-soy based diet (Deng et al., 1992). In contrast, levels of skatole were above 0.25 ppm in 5-13% of purebred Yorkshire, Landrace, Hampshire and Duroc entire males from swine breeding herds in Ontario (Squire *et al.*, 1992). Levels of 16-androstene steroids were more consistent among different herds, with 6-8.5% of Yorkshire and Landrace entire males having levels of 16-androstene above acceptable limits (tentatively set at 65 ppm in salivary gland). However, 58.6% of Duroc pigs had levels of 16-androstenes above this limit. No data is available on the influence of Duroc in crossbred pigs on the levels of 16-androstenes in the carcass.

Future considerations

There are a few technical limitations at this time for using entire male pigs for pork production. The greatest limitation to the use of entire males in North America is not technology, but rather the lack of appropriate markets. The levels of taint in market weight (100 kg live weight) pigs are generally low, but likely can be further reduced by careful control of diet and environment and the use of appropriate genetic lines of pigs. These changes will also likely improve the quality of meat from gilts as well. Methods for the measurement of skatole and androstenone are available and much work is now proceeding on improving these methods. However, the existing technology could be used now to begin to put together the practical procedures for raising entire males and for testing and sorting carcasses for taint in packing plants. Once the use of entire males has been established, improved methods to control and measure taint will be necessary to improve efficiency.

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