CONTRIBUTION OF THE HALOTHANE GENE TO PSE LEVELS IN AUSTRALIAN PORK.

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SUMMARY

Although many factors interact to produce PSE, the presence of the Hal (Halothane) or PSS gene in the slaughtered pigs is believed to be one of the major contributing factors. In spite of this, it is not known to what extent the gene contributes to PSE in Australian production and processing systems. Therefore this study was carried out to quantify the contribution of the gene to PSE. Approximately 150 pigs were selected that had some level of PSE from three different slaughter plants. The carcases were selected based on FOP readings at four different sites on each carcase and were classified on a scale of 0.5 to 4.0 based on the extent and intensity of PSE. At the same time, tissue samples were taken for PSS genotype testing using a DNA test. The results showed that in carcases with low PSE scores (i.e., < 2.0) there was very little relationship between the frequency of the Halothane gene and PSE - only 13% of these carcases were homozygous (nn) or heterozygous (Nn) for the gene. In contrast, of the pigs with high PSE scores (i.e. >2.0) more than 40% of the pigs were either nn or Nn. Hence, it appears that the Halothane gene is one of the main contributing factors in severe cases of PSE but not so for less severe cases. The less severe cases of PSE are presumably caused by poor handling, processing or other similar factors.

INTRODUCTION

PSE (Pale Soft Exudative) pork results in major economic losses to the Australian pig processing industry. It has been estimated that the Australian pig industry loses approximately $24M per year due to PSE (Whan, 1993). Although many factors interact to produce PSE, the presence of the Hal gene in the slaughtered pigs is believed to be one of the major contributing factors (Murray et al., 1989; Lundstrom et al., 1989; Webb and Simpson 1986; McPhee et al., 1994.). In spite of this, it is not known the extent to which the Hal gene contributes to PSE in commercial pigs produced and slaughtered under Australian conditions.

From the studies carried out to date, it is not possible to ascertain the effect of the Hal gene on the incidence and severity of PSE in commercially slaughtered pigs. The reason for this is that the studies carried out to determine the relationship between the Hal gene and PSE have been carried out with pigs breed specifically to contain the Hal gene and hence the pigs are quite different to those from commercial herds. Moreover, in most of these studies the animals are not slaughtered under commercial conditions. In such a study in Canada, Murray et al., (1989) determined that depending on preslaughter conditions, 30-60% of pigs heterozygous for the Hal gene would produce PSE. In another study in Canada of commercial pigs, Pommier and Houde (1992) found that only 32% of the pigs heterozygous for the Hal gene produced PSE. Neither of the two studies just cited are conclusive since in both studies the researchers evaluated PSE only in the loin (which is not representative of the PSE level in the carcase) and they did not rate the severity of the PSE. In determining the relationship between the Hal gene and PSE, it is difficult to extrapolate results obtained from different countries and from different slaughter plants since both production and slaughtering practices will influence the extent and severity of PSE.

Until recently, it was difficult to determine the relationship between the Hal gene and PSE in commercial pigs since it was not possible to determine the Hal genotype of pigs once they had been slaughtered. However, this situation has changed with the recent development of the DNA test for the Hal gene (Hughes et al., 1992). Using this test, it is now possible to determine the Hal genotype of pigs which have been slaughtered and have produced PSE. This information can then be used to identify and eliminate the causes of PSE in commercial slaughter plants.

Hence, the objective of this study was to relate the frequency of the Hal gene to the incidence and severity of PSE in pigs slaughtered in Australian slaughter plants.

MATERIALS AND METHODS.

Selection of PSE carcases. On five separate occasions over a one month period 150-250 pig carcases at a local boning room were surveyed for the extent and intensity of PSE. All carcases were from the previous day's kill and had been chilled for 18-24 hours. The deep muscle temperature of each carcase was in the range 5-10°C. The extent and intensity of PSE in each carcase was evaluated using a Fibre Optic Probe (FOP) (Pro Electro Optics, Barnsley, Yorkshire, UK). Each carcase was probed in four sites: 1) the anterior end of the *m. longissimus dorsi* between the 8/9 thoracic vertebrae, 2) the lumbar portion of the *m. longissimus dorsi* between the 2/3 lumbar vertebrae, 3) the centre of *m. biceps femoris*, and 4) the centre of *m. semimembranosus*. Three measurements were made at each site at depths of approximately 2.0, 4.0, and 6.0 cm. Carcases were selected for further investigation if, according to the FOP readings, at least one site had some level of PSE. At each visit, approximately 24 carcases were selected that had a range of PSE levels that covered the range normally found in commercial slaughter plants in Australia (the criteria for selecting and classifying PSE carcasses is outlined below). A fat sample from each selected carcase was taken for subsequent DNA analysis to determine the Hal genotype.

FOP calibration and PSE classification. The FOP was calibrated at 25°C using the following two point calibration procedure: The instrument was adjusted to read 1) zero in the dark [away from direct light] and 2) 98 in a 0.25% PVC suspension of particle size 399 $\pm$6nm (Cat. No. 44430, Serva Chemicals, Heidelberg, Germany). The intensity of PSE was categorised using the following procedure: Normal - FOP < 45; PSE - FOP > 45 < 58; Extreme PSE - FOP > 58 (Trout, 1992). An overall PSE index which takes into consideration the intensity and extent of PSE was calculated as follows: 1) Each of the four sites was allocated a PSE score of 0.0, 0.5 or 1.0 for each of the three pork quality classifications of Normal, PSE and Extreme PSE, respectively and 2) the PSE index was calculated by summing the PSE scores at the four sites measured. For example, a carcase that had a PSE classification of normal, normal, normal, normal for the four sites measured would have a PSE index of zero. Similarly, a carcase with a PSE classification of Extreme PSE, Extreme PSE, Extreme PSE, Extreme PSE would have a PSE index of 4.0.

DNA extraction and Halothane genotyping. The DNA was extracted from the fat samples by incubating approximately 50 mg of fat with 200 microlitres of 20mM pH 8.0 Tris buffer and 16 microlitres of 20mg/ml Proteinase K for 3 hours at 56 °C, inactivated at 99°C for 5 minutes and centrifuged. The procedure used for determining Hal genotype was that described by Hughes (1992) except the primers were changed to produce a larger 314 base pair DNA fragment and the PCR (polymerise chain reaction) conditions were optimised for maximum PCR product from the crude DNA extract obtained from the fat sample.

RESULTS AND DISCUSSION:

The results of the DNA genotyping showed that 142 pig carcases tested had the following distribution of genotypes: NN=70.4%; Nn=28.8% and nn=0.7%. The pigs carcases tested were from approximately 20 different producers in the region. Hence, this distribution of genotypes would expected to be reasonably representative of the commercial pig population in this region. Information from the Australian National Hal-Gene Testing Centre indicates that of the 3019 pigs tested to date, 28% were Nn and 4% were nn.

The results of the PSE evaluation of the 142 carcases indicated that carcases had the following distribution of PSE Index values: 3.5-4.0 - 15.5%; 2.5-3.0 - 22.5%; 1.5-2.0 - 40.1%; and 0.5-1.0 - 21.1%. These figures indicate that there was a fairly uniform range of carcasses of different PSE Index values.

The results in Fig. 1 show the relationship between the extent and severity of PSE, as indicated by the PSE index value and the percentage of carcases with the Hal gene (either nn or Nn). It is quite apparent from this figure that carcases with high PSE index values have a high frequency of the Hal gene. For example, 45.5% of the carcases with a PSE index value of 3.5-4.0 contained the Hal gene. In contrast, only 13.3% of the carcases with a PSE index value of 0.5-1.0 contained the Hal gene. These results are consistent with those of Pommier and Houde (1992) who found that approximately 40% of carcases with extreme PSE in the loin (i.e., an L* value > 55) contained the Hal gene (it has been previously found that an L* value > 53.5 is equivalent to the extreme PSE as defined in this study [Trout, 1992]). The results are in contrast, however, to earlier studies (Webb and Simpson, 1986) that indicated that pigs heterozygous for the Hal gene produced meat of similar quality to that from normal (NN) animals.

CONCLUSION:

This study has shown that the Hal gene is one of the major contributing factors in severe cases of PSE. The results also indicate that the major cause of less severe cases of PSE is not due to the Hal gene. This then indicates that the less severe cases of PSE can only be reduced by improving pre-slaughter management so as to reduce stress and by improving slaughtering practices.

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