The role of P450IIE1 protein and mRNA expression in determining adipose tissue skatole level

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Introduction High concentrations of skatole in adipose tissue are a major factor in boar taint - the offensive odor and taste in cooked pork from some intact male pigs. Skatole is produced by bacterial fermentation of tryptophan in the large intestine, absorbed into the blood and transported into the liver, where it can be metabolised via the cytochrome P450 system. One reason for high skatole levels in pig adipose tissues could be low expression of hepatic cytochrome P450IIE1 in liver and hence a reduced rate of skatole clearance, but no correlation was found between the rate of skatole metabolism and the P450IIE1 content of isolated liver microsomes. The present study re-investigates the relationship between backfat skatole, liver P450IIE1 expression and the rate of microsomal skatole metabolism in two breeds of pigs.

Materials and Methods 85 intact male pigs of two breed types provided by the Cotswold Pig Development Company were used. These were Large White x Landrace (LW) and Meishan x Landrace (M). The pigs were fed a standard pelleted diet and slaughtered at the same age to provide carcasses of 63-74 kg. Liver samples were frozen in solid CO_2 and subsequently stored at –80°C. Skatole in backfat was measured by high resolution gas chromatography. Microsomes were isolated by differential centrifugation. Mitochondrial skatole metabolism was measured by separating skatole from its products by thin layer chromatography in hexane/ether. P450IIE1 levels in microsomes were measured by Western blotting with a commercial antibody. mRNA levels were measured by Northern blotting with a cDNA probe specific for P450IIE1.

Results The majority of the LW pigs had skatole levels in the range from 0 to 0.1 µg/g which is below the level characteristic of boar taint. Only about 5% of LW pigs had backfat skatole levels which were higher than this. In contrast, the M breed had very high backfat skatole levels (from 0.2 to 1 µg/g) most of which were above the boar taint threshold level (0.2µg/g). When microsomal metabolism was measured under appropriate conditions the rate of metabolism was always proportional to the microsomal P450IIE1 content. confirming that metabolism via P450IIE1 was the major route of skatole breakdown in liver. Fig 1 shows the correlation between backfat skatole and microsomal P450IIE1 protein. For the LW there was a good inverse correlation for all the pigs tested. All the M pigs expressed low levels of P450IIE1. Backfat skatole levels in M pigs were always high but varied over a ten-fold range. Fig.2 shows that there was an inverse correlation between backfat skatole and P450IIE1 mRNA expression in LW. In the M breed P450IIE1 mRNA levels were always low and backfat skatole levels were relatively high but there was no clear correlation between mRNA levels and backfat skatole.

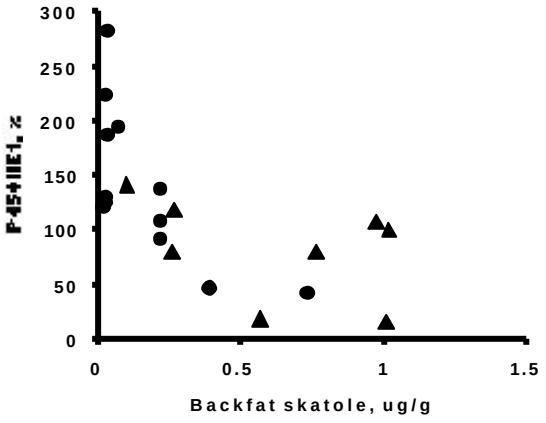

Fig 1. Microsomal P450IIE1 in LW (●) and M (▲) pigs

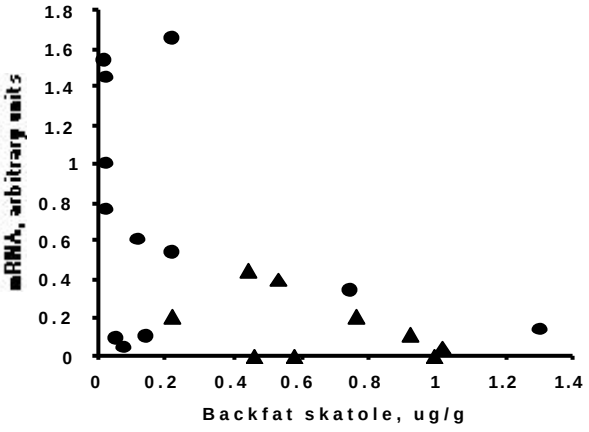

Fig 2. mRNA level in liver of LW (●) and M(▲)

Conclusions These results confirm the importanace of hepatic cytochrome P450IIE1 protein and mRNA expression in determining adipose tissue skatole levels. In the LW pigs high backfat skatole is clearly correlated with abnormally low cytochrome P450IIE1 expression. In M pigs some additional factor increases the skatole level above that determined by the low level of cytochrome P450IIE1 expression. An understanding of the molecular basis of boar taint will require elucidation of the factors controlling the expression of cytochrome P450IIE1 in pig liver.

Acknowledgements This work was supported by a grant from the Meat and Livestock Commission and by the Danish National Committee for pigs.