Distribution of skatole and indole compounds in pigs: influence of dietary factors.

M. Hawe[1], B.W. Moss[2], N. Walker[1] and M. Porter[1]

[1] Agricultural Research Institute of Northern Ireland, Hillsborough, Co Down.

[2] Agricultural and Food Science Centre, Newforge Lane, Belfast BT9 5PX.

INTRODUCTION

The use of a rapid method of skatole measurement has been suggested as a means of identifying boar taint samples on a production line (Mortensen & Sorensen 1984). Mortensen & Sorensen (1984) point out that substances other than skatole may participate in the colour reaction. Although correlations between skatole equivalents and taint and androsterone level have been published (Lundstrom et al 1984) there is no information on the relationship between skatole equivalents and skatole and indole measured by GC.

In vivo studies in ruminants have identified a number of possible metabolites of skatole (Bradley & Carlson 1982) but there is little information on skatole metabolism in pigs. Dietary fibre content has been reported to alter adipose tissue skatole levels (Lundstrom et al 1984) but whether this is due to a change in skatole production within the gut or absorption was not investigated.

The aim of the experiments reported here was to investigate the effect of some other indoles, reported to be metabolites of skatole within ruminants, on the colorimetric method and the relationship of skatole measured by colorimetric method and by a chromatographic method. In addition the effect of skatole infusion and fibre content of the diet was also investigated.

MATERIALS AND METHODS

Experiment 1: Stock solutions of Skatole, Indole, Indole-3-carbinol and 3 indoxyl sulphate were prepared in acetone/tris buffer pH 7.5 (100 mg l^{-1}). Working standards covering the range 0 to 10 g ml^{-1}, were prepared and assayed using the method described by Mortensen and Sorensen (1984).

Experiment 2: Samples of adipose tissue were collected froms the neck, belly, back and kidney regions of 29 pigs. Skatole and indole were measured by the gas chromatographic (GC) method of Porter, Hawe and Walker (1989). In addition skatole was measured as skatole equivalents by the colorimetric method (Mortensen & Sorensen 1984) on the samples from the neck region.

Experiment 3: Two entire male pigs were fitted with a T piece cannula in the terminal ileum. Faeces and urine were collected for 2 d, then the pigs were given infusions of skatole into the terminal ileum for a period of 3 days (infusions of 1 g skatole in 2.5 ml ethanol were given at 4 h intervals). Faeces and urine were collected during the 3 d infusion period and 3 d post infusion period. After a period of 2 weeks the infusion was repeated as before except that doses of 2 g in 5 ml ethanol were infused at 8 h intervals. In both cases the pigs received 18 g skatole over the 3 d period. The faeces collected were analysed for skatole and indole content by GC.

Experiment 4: Sixteen pigs (8 boars, 8 gilts) were allocated according to littermate pairs to either a control diet or a diet high in fibre from 35 to 85 kg liveweight. The control diet contained (in g kg^{-1}) 715 ground wheat, 260 extracted soya bean meal, and 25 mineral plus vitamins supplement. The high fibre diet was prepared by partially replacing some of the constituents of the control diet with sugar beet pulp, maintaining similar energy density.

Faeces were collected over two 4 d periods one at 60 kg liveweight and the other at 80 kg liveweight.

RESULTS

Experiment 1: The other indoles tested did not give as great absorbence as skatole. The ratio of slopes relative to skatole was 0.082 for indole, 0.022 for indole-3-carbinol, 0.009 for 3 indoxyl sulphate. Oxindole showed no detectable absorbence change over the range 0 to 10 g ml^{-1}. These results indicate that interference from other indoles which have previously been reported as metabolites of skatole, have little influence on skatole determination by the colorimetric method.

Experiment 2: There was a significant difference in the skatole and indole concentration in the adipose tissue from the four regions studied (Table 1). Skatole was significantly lower in adipose tissue from the back and kidney regions than in that from the neck and belly regions. Indole concentration was significantly lower in adipose tissue taken from the back than from other regions. There was no statistically significant correlation between skatole equivalents determined by the colorimetric method and the level of skatole and indole determined by GC (r = 0.128, 0.24 and 0.11 for skatole, indole and total indoles respectively). The skatole measured by GC ranged from 0.02 to 0.10 g g^{-1}, whilst skatole equivalents determined colorimetrically were at least ten fold greater. In these initial studies a 1 cm light path length was used and absorbence readings were low (<0.03 abs units at the proposed cut off point of 0.2 ppm. Further studies are being undertaken using longer light path length cuvettes. Errors in the colorimetric method due to low absorbence readings may explain the poor correlation but not the magnitude of difference between the GC method and colorimetric method.

These results suggest that both the site for sampling and method of analysis should be specified if skatole determinations are to be used in commercial practice for taint identification.

Table 1. Skatole and indole in adipose tissue.

Region	Skatole g g^{-1}	Indole g g^{-1}	Ratio[a]
Neck	0.048	0.020	14.2
Belly	0.049	0.011	8.2
Back	0.036	0.002	19.4
Kidney	0.035	0.011	4.2
Significance of difference	***	***	-
sem.	0.0027	0.0024	-

sem - standard error of mean

[a] mean ratio of skatole/indole calculated from individual samples.

Experiment 3: In both pigs (A and B) faecal skatole and indole output during infusion peaked at about 3 to 4 times that of the pre and post infusion levels. The faecal skatole:indole ratio increased during the infusion period. Skatole and indole output in the faeces over the 3 day infusion and post infusion accounted for less than 5% of the skatole infused. This low percentage of excretion of skatole may be explained by (1) absorption of skatole and indole through the gut wall or (2) metabolism of these compounds by gut microflora. Analysis of urine from these animals may give some indication of body absorption and metabolism.

Experiment 4: There was no statistically significant sex difference in the faecal output of

skatole or indole (Table 2). This suggests that the sex difference in skatole in adipose tissue (Lundstrom et al) is most likely to be related to sex differences in body metabolism. Increasing the fibre content of the diet significantly increased faecal output of skatole ($p<0.05$) and indole ($p<0.01$). The faecal skate:indole ratio was decreased by increasing the fibre content of the diet.

Table 2. Effect of sex and dietary fibre on faecal output of skatole and indole

	Boars	Gilts
Skatole mg d^{-1}		
Control	23.4	15.1
Fibre	28.2	31.3
Indole mg d^{-1}		
Control	7.25	3.16
Fibre	12.17	16.13
Skatole/Indole		
Control	6.99	7.61
Fibre	2.73	2.75

CONCLUSION

The results of these experiments indicate that the colorimetric method overestimates adipose tissue skatole content. The interfering substances would not appear to be those indoles previously identified as metabolites of skatole. Increasing the fibre content of the diet increased faecal output of skatole and indole. Further work on the metabolism of skatole within the gut and its absorption should be undertaken.

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