

Not only due to their generally lower odour thresholds, but also because of their more disagreeable odours, as they are nitrogen and sulphur compounds as e.g. skatole and dimethyldisulphide, which means they have a relatively higher negative impact on the air quality. The produced compounds can also be combined, e.g. volatile fatty acids can be combined with alcohols to form esters, which have other odour characteristics usually with less offensive odour notes e.g. fruity notes. It should also be kept in mind that the odour quality of a compound can change with varying concentrations, e.g. the odour of skatole is pleasantly floral at very low concentrations, but faecal at high concentrations.

The strategy for changing the composition of the odour active compounds (and thereby increasing the air quality) would then be to increase the amount of less odour offensive compounds (from carbohydrate degradation) on the expense of the more odour active compounds (from protein degradation). If the odour active compounds also include synthesis of esters, the odour quality would be improved further. Previous results in connection with studies of boar taint have shown that this strategy is feasible as the concentration of skatole in colon and rectal contents of pigs was significantly reduced by adding fermentable carbohydrates to the food (Jensen *et al.*, 1995, Claus, 1992). In humans a comparable observation has been made with haemodialysis patients, where the group given fermentable carbohydrates (fructooligosaccharides) showed a significant decrease in the amount of putrefactive products such as indole, skatole and p-cresol compared with control group patients (Takahashi *et al.*, 1996).

A convenient method to reduce the amount of malodorous compounds in manure would therefore be to feed the pigs with components reducing the presence of these compounds. The food compound should be a convenient crop fitting naturally into the crop rotation, containing a good feeding value and fermentable carbohydrates, which reduce the degradation of non-digested protein in the colon and rectum of pigs. The inulin-rich chicory roots of the cultivar Orchies (*Cichorium intybus* L) fulfil these conditions. In addition to its nutritive value, this plant also contains bioactive secondary metabolites, which could positively affect the health of the pigs, e.g. by reducing the presence of parasites and pathogenic micro-organisms (Bais and Ravishankar, 2001). Inulin is a mixture of fructopolysaccharides and fructooligosaccharides consisting of a chain of variable length of fructose units (2 to 60) and a single glucose unit in the end of the molecule (Roberfroid, 1993). Inulin is found in many plants, but most

prominently in chicory (*Cichorium intybus* L) and Jerusalem artichoke (Farnworth *et al.*, 1995). Chicory roots have a better crop yield of inulin than Jerusalem artichoke and are easy to manufacture. Chicory roots are consequently used in the production of inulin on an industrial scale in Europe, North and South America and Australia and are thereby feasible to implement in the present swine production and feeding systems as a freshly chopped or dried food component.

The aim of this study was to show that the composition of malodorous compounds in the colon and rectum of pigs could be positively modified by feeding diets with a high amount of fermentable carbohydrates such as inulin, which is present in significant amounts in chicory roots. This reduction of malodorous compounds is expected to have a positive effect on the air quality of pig houses and the surrounding environment, especially in the warm summertime. Although the perception of odour quality is highly individual, we believe that a general reduction in the amount of malodorous compounds from animal production units would be of significant interest.

Material and methods

Animals and diets

The 16 pigs (eight entire males and eight females) used were Danish standard DLY crossbred pigs from Duroc sires and dams which were zigzag crossbred sows between Danish Landrace × Large White at Research Centre Foulum. At an average live weight of 55 kg, the 16 pigs were distributed to two treatments (no. = 8) in individual pens according to live weight, replicate (= litter) and sex (female and entire male pigs) (see Table 1). Treatment 1 was a conventional control group given 100 energy % organic concentrate according to scale (Madsen *et al.*, 1990) and no roughage. The animals in treatment 2 were given 25% chopped, organic chicory roots on energy basis plus 70% organic concentrate until slaughter (totally 95 energy %), and a 5% energy decrease was estimated compared with the control treatment to ensure that all the 25% bitter, chopped chicory roots were eaten. The chicory amount of 25% was chosen to secure a significant response on the composition of odour active compounds. The pigs were given food twice a day. The eight male and eight female pigs were slaughtered on the days 63 and 65, respectively, of the study. The pigs were given food for the last time on the afternoon of the day before slaughter. The pigs from the two treatments were equally distributed to the slaughtering procedure and slaughter hours (time), both as regards male and female pigs. The live weights at slaughter and the daily gain during

Table 1 Experimental design for the finishing feeding period of the two treatments feeding with or without the chicory roots from 55 kg live weight until slaughter (9 weeks)

| Treatment | No. of pigs | Food composition and energy level compared with 100% energy according to scale [†] | Roughage |
|-----------|---------------------|---|---|
| 1 | 8 4 female + 4 male | 100% organic concentrate from 55 to 123 kg live weight | None |
| 2 | 8 4 female + 4 male | 70% organic concentrate + 25% chicory roots from 55 to 115 kg live weight | Chicory roots (2.1 to 3.0 kg per day) from 55 kg to slaughter |

[†] Madsen *et al.* (1990).

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the experimental period of treatments 1 and 2 were 123 kg and 1070 g/day and 115 kg and 934 g/day, respectively.

The composition of the diet during the experiment is shown in Table 2. The concentrate diet contained 8.57 MJ net energy (1.16 feed units (FUp)) and 149.7 g digestible protein per kg food. The inulin content (fructan) in the chicory roots of the cultivar Orchies (*Cichorium intybus* L.) for the experiment was 15% and the chicory roots contained 2.11 MJ net energy (0.28 Scandinavian Feed Units (FUp)) and 23.4 g digestible protein per kg chicory roots.

Collection of samples and sample preparation

Immediately after slaughter, the gastro-intestinal tract (GIT) was removed, and the colon and rectum were separated from the rest of the GIT. The contents from colon and rectum were quantitatively transferred to a basket and mixed so that a representative sample could be obtained. The samples were stored at -20°C before analysis. In order to prepare the samples for analysis, 3 g were transferred to 10 ml vials with addition of 3 ml saturated NaCl; the samples were mixed and stored at -80°C before analysis. The saturated NaCl was added to increase the transfer of volatiles to the gas phase and to stop further microbial activity in the samples. On the day of analysis, the samples were transferred to an oven held at 40°C (approx. the body temperature of pigs), thawed and equilibrated at this temperature for 25 min with occasional shaking to increase the transfer of volatiles from the medium to the headspace. For extraction a solid-phase micro-extraction (SPME) fibre (75 μm polydimethylsiloxane/-carboxen, Supelco) was exposed to the headspace for 1 min and immediately transferred to the injection port of the gas chromatograph for desorption. As the contents from colon and rectum were lost from one of the experimental pigs on treatment 2, the results are based on eight control pigs and seven chicory-fed pigs.

GC-MS measurement of volatiles

The gas chromatograph was a Varian model STAR 3400 CX. The column was an HP5-MS (Agilent) 30 m long, 0.25 mm i.d. and with a 0.25 μm film thickness. Injection temperature was set to 250°C and the column temperature programme was as follows: hold at initial temperature 35°C for 10 min, then increase to 130°C with $3^{\circ}\text{C}/\text{min}$, finally

increase to 250°C with a rate of $40^{\circ}\text{C}/\text{min}$ and hold at this temperature for 5.34 min. The carrier gas was high purity helium with a linear flow rate of 29 cm/s at 35°C , the samples were run one at a time to ensure that the samples were treated in exactly the same way. The temperature of the transfer line between the gas chromatograph and the mass spectrometer was set to 275°C . The mass spectrometer was a Varian model Saturn 2000 operated in electron impact mode, with the following settings: detection mass range: 35 to 300 m/z; multiplier voltage: 1800; trap temperature: 200°C ; and manifold temperature: 52°C .

Identification and correction for differences in odour thresholds

The compounds were identified by comparison with standard spectra from NIST/EPA/NIH or by comparison with spectra from original standards. Table 3 shows odour threshold values and odour descriptors of the selected compounds found in colon and rectal contents. The relative odour activity of the individual compounds was calculated by dividing the area of the compound by the odour threshold. Thereby a compound, which is present in low amounts, may result in a high odour impact, if the odour threshold is low. The relative 'odour activity' of the two experimental treatments can therefore be compared. In order to do so, the raw data were divided by the odour threshold values found in the literature (Gemert and Nettenbreijer, 1977; Zahn *et al.*, 2001). The odour threshold values vary widely; both the lowest and the highest values were therefore applied to give an impression of the effect on the potential odour. Only one threshold value for indole was found in the literature and it was not possible to find literature values of odour threshold for ethyl-2-methylbutyrate and propylbutyrate, so they were omitted from the calculations.

Statistical analysis

The statistical analyses were carried out using the program package from the Statistical Analysis Systems Institute (2002). Analysis of variance was performed using the GLM procedure. Least-square means and standard error of the means for the odour impact compounds from colon and rectum were calculated (Y). The models included the fixed effects of diet, replicate (= litter of origin) and sex as well as the interaction between diet and sex (model 1) although no significant interactions were noticed.

$$Y = \mu + a_{\text{diet}} + b_{\text{replicate}} + c_{\text{sex}} + ac_{\text{diet} \times \text{sex}} + e_{\text{error}} \quad (\text{model 1})$$

Y = odour impact compounds: dimethylsulphide, 2-butanone, acetic acid, 2-pentanone, dimethyldisulphide, 1-pentanol, 2-methylpropanoic acid, ethylbutyrate, propylpropionate, butyric acid, 3-methylbutanoic acid, propylbutyrate, ethyl-2-methyl butanoate ethylester, dimethyltrisulphide, p-cresol, indole and skatole; μ = mean; treatment diets = control, chicory, $a_{\text{diet}} = 2$ (d.f. = 1); replicates = litters of origin selected for the experiment, $b_{\text{replicate}} = 8$ (d.f. = 7); sex = female and entire male pigs, $c_{\text{sex}} = 2$ (d.f. = 1). The raw uncorrected data (Y) of the GC-MS areas of the odour compounds were analysed by the GLM- model 1 to investigate the effect of the two diets.

Table 2 Composition of control (treatment 1) and experimental (treatment 2) diets

| | Treatment 1 | Treatment 2 |
|----------------------------|------------------|------------------------------------|
| Dry matter (g/kg) | 880 [†] | 880 [†] /250 [‡] |
| Contents (g/kg wet matter) | | |
| Crude chicory | 0 | 564 |
| Rapeseed cake | 145 | 63 |
| Peas | 240 | 103 |
| Wheat | 223 | 97 |
| Barley | 220 | 95 |
| Oat | 50 | 22 |
| Soya bean | 100 | 43 |
| Vitamins and minerals | 22 | 13 |

[†]Organic concentrate.

[‡]Crude chicory roots.

Table 3 Odour descriptors and odour thresholds in air of odorous compounds found in colon and rectal contents (mg/m³)

| Compound | Odour descriptor [†] | Low threshold (4) | High threshold (4) |
|--|---|-------------------|--------------------|
| Dimethylsulphide | Cooked vegetable, garlic, hydrogen sulphide (1) | 0.002 | 0.65 |
| 2-Butanone | Acetone, varnish (1) | 0.75 | 250 |
| Acetic acid | Vinegar (1) | 0.025 | 76 |
| 2-Pentanone | Jasmine, geranium, varnish (1) | 11 | 48 |
| Dimethylsulphide | Decayed vegetables (3) | 0.003 | 0.029 |
| 1-Pentanol | Alcohol, medicinal (1) | 0.1 | 1100 |
| 2-Methylpropanoic acid | Sweaty, bitter, sour (1) | 0.00 072 (3) | 0.0072 (3) |
| Ethylbutyrate | Butter, sweetish, apple, perfumed (1) | 0.13 | 0.28 |
| Propylpropionate | Complex fruity odour (apple banana) (2) | 0.23 | 0.26 |
| Butyric acid | Buttery, cheesy, sweaty (1) | 0.0004 | 9 |
| 2-Methylbutanoic acid ethyl ester | Odour descriptor not found in literature | ‡ | ‡ |
| Propylbutyrate | Pineapple, apricot (2) | ‡ | ‡ |
| 3-Methylbutanoic acid (isovaleric acid) | Cheese, sweaty (1) | 0.005 | 3 |
| Dimethyltrisulphide (methyltrithiomethane) | Fresh onion (2) | 0.0073 | 0.0073 |
| p-Cresol (4-methyl-phenol) | Phenol-like (2) | 0.00 005 | 0.04 |
| Indole | Floral (highly pure) otherwise faecal (2) | 0.0006 | 0.0006 |
| 3-Methylindole (skatole) | Faecal (high conc.) floral (low conc.) (2) | 0.00 035 | 0.1 |

[†] (1) Meilgaard (1975). (2) Burdock (1995). (3) Zahn *et al.* (2001). (4) Gemert and Nettenbreijer (1977).

[‡] It has not been possible to find odour threshold values in the literature for 2-methylbutanoic acid ethyl ester or propylbutyrate.

Multivariate data analysis using principal component analysis (PCA) was also carried out on the raw uncorrected data of GC-MS area of the odour compounds, as well as data corrected for low and high odour thresholds. Full cross validation (leave one out) was applied. PCA was carried out using the software The Unscrambler version 7.8 (Camo AS, Oslo, Norway). The three different PCA analyses show which odour compounds have the greatest effect using either the raw uncorrected data of GC-MS area of the odour compounds or data corrected for low and high odour thresholds.

PCA is a multivariate data analysis method used for data mining of large data sets. By using this method it is possible to identify in a data set the components, which were most responsible for the variations, found in the data. The results are presented in 'spider webs' where the most influential data components are given the highest weight.

Results

Table 4 shows the peak mean area of GC-MS analyses of selected odour impact compounds found in headspace over the colon and rectum samples. The compounds 2-pentanone, ethylbutyrate, propylpropionate, butanoic acid, ethyl-2-methylbutyrate, p-cresol, indole and skatole showed significant difference between the two treatments. The esters, which have relatively pleasant, and often fruity, odours, increased in treatment 2 (factorial difference below 1), whereas the malodorous compounds, p-cresol, indole and skatole decreased in treatment 2 (factorial difference above 1).

Figure 1 shows the PCA analysis of the data set from the raw data. Treatment 1 (control) and treatment 2 (chicory addition) were clearly separated with no overlap between the treatments. The first principal component (*x* axis) was controlled by p-cresol (protein degradation product), whereas the second (*y* axis) was controlled by butyric acid and propyl propionate, which are both degradation products of carbohydrate.

Figure 2 shows the PCA analysis of the raw data divided by the low odour threshold values to give odour activity-corrected values. The two sets of treatment results were clearly separated, and the clusters of points were tightly grouped, especially for the pigs given the diet containing chicory. The first principal component (92%) was controlled mostly by p-cresol, whereas the second was controlled by butyric acid (7%).

Figure 3 shows the PCA-analysis of the raw data divided by the high odour threshold values. The results for the pigs on the control diet were more dispersed and overlapped the chicory-fed pigs. In contrast, the results for the pigs on the chicory diet were very tightly grouped. The first principal component was in this case controlled by indole (protein degradation product), whereas the second was controlled by dimethyl disulphide, 2-methyl propanoic acid and to a lesser degree dimethyl trisulphide (all protein degradation products).

Discussion

In this study the samples analysed were a mixture of colon and rectum contents instead of faeces collected in the pens. It is our experience that faeces collected in the pens is highly irregular in composition due to difference in time of excretion and difference in time from excretion to sampling. By using samples collected directly from a mixture of colon and rectum contents this source of error was eliminated. Although the negative influence on ambient air quality originates from odorous compounds released from manure, it has been shown that feeding a diet containing fermentable dietary fibre as fructans, the smell of manure can be improved (Farnworth *et al.*, 1995). By using a food component as chicory, which acts as a prebiotic, the problem with adaptation to the diet as seen with antibiotics is avoided.

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Table 4 Raw data of GC-MS areas of selected compounds from the colon and rectum in chicory roots and control-fed fattening pigs (least-square means and s.e.)

| Compound | Treatment† | | | | | |
|--------------------------------------|------------|--------|---------|--------|---|---|
| | 1 | | 2 | | Significant difference between treatments | 1/3 Factorial difference between treatments |
| | LS mean | s.e. | LS mean | s.e. | | |
| Dimethylsulphide | 83736 | 6827 | 48145 | 8078 | | 1.74 |
| 2-Butanone | 54274 | 7681 | 59512 | 9088 | | 0.91 |
| Acetic acid | 252338 | 42504 | 286741 | 50292 | | 0.88 |
| 2-Pentanone | 22742 | 7513 | 48500 | 8889 | * | 0.47 |
| Dimethyldisulphide | 132354 | 52309 | 128911 | 61893 | | 1.03 |
| 1-Pentanol | 29277 | 6201 | 47543 | 7337 | | 0.62 |
| 2-Methylpropanoic acid | 43571 | 9416 | 29886 | 11141 | | 1.46 |
| Ethylbutyrate (ester) | 5026 | 28026 | 48440 | 33161 | | 0.10 |
| Propylpropionate (ester) | 23718 | 40419 | 174429 | 47824 | ‡ | 0.14 |
| Butyric acid | 935596 | 118921 | 878861 | 140710 | | 1.06 |
| Butanoic acid, 2-methyl-,ethyl ester | 2663 | 1599 | 8679 | 1892 | * | 0.31 |
| Propylbutyrate (ester) | 3208 | 1145 | 7760 | 1355 | * | 0.41 |
| 3-Methylbutanoic acid | 96309 | 12822 | 64413 | 15171 | | 1.50 |
| Dimethyltrisulphide | 7196 | 2755 | 6252 | 3260 | | 1.15 |
| p-Cresol | 347725 | 27566 | 72516 | 32616 | ** | 4.8 |
| Indole | 19943 | 2487 | 6690 | 2942 | ‡ | 3.0 |
| 3-methylindole (skatole) | 25322 | 4954 | 3740 | 5862 | ** | 6.8 |

† See Table 1 for description of treatments. Data were lost from one of the pigs on treatment 2 so the results are based on only seven animals.

‡ Approaching significance ($P < 0.1$).

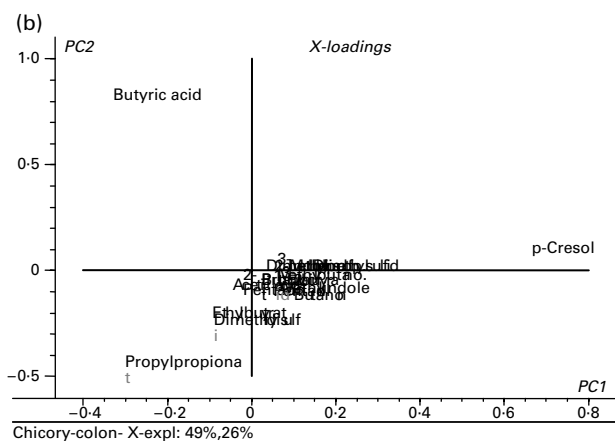
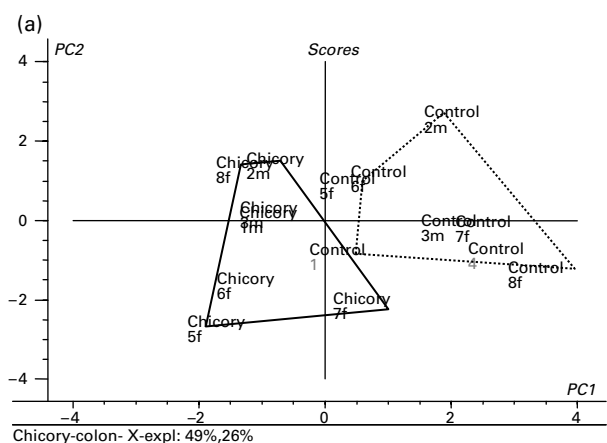


Figure 1 (a) The scores of odorous compounds of raw data from colon and rectal contents and (b) the loadings of the odorous compounds of control-fed and chicory-fed pigs.

We used headspace analysis of the odours instead of liquid extraction due to the wide differences in vapour pressure between the individual odour compounds; e.g. 3-methylindole is a solid compound whereas dimethylsulphide is a gas at room temperature. Liquid extraction of the intestinal contents would therefore not give the correct impression of the odour composition in the air above the samples and consequently a total chemical analysis of the compounds in the intestinal contents would be pointless.

The pigs ate the high amount of raw, bitter and chopped chicory roots without problems after one week of adaptation during which increasing amounts of chicory roots were provided. No adverse behaviour or reactions of the pigs were observed. The health status and daily growth rate were good in both treatments, and the daily gain corresponded to the energy intake of the two treatments. Percentage of meat in carcass was 0.8% higher in the chicory-fed pigs.

The raw GC-MS areas in Table 4 and Figure 1 show that when the pigs were given the inulin-containing chicory roots, the fermentation pattern in the colon and rectum shifted from protein fermentation to carbohydrate fermentation. The result was a change in composition of odorous compounds from the protein fermentation products, such as p-cresol and skatole, to the less offensive smelling esters. The latter are produced when alcohols and carboxylic acids, compounds with relatively negative odour impressions, react. Farnworth *et al.* (1993 and 1995) observed this improvement in odour in chicken and weaner piglet houses. The PCA plot also confirmed that the fermentation product pattern is well separated and mostly controlled by p-cresol and butyric acid.

Although the sensory impression of a mixture of odorous compounds is a combination of all compounds in the mixture, some of the compounds may have a higher impact

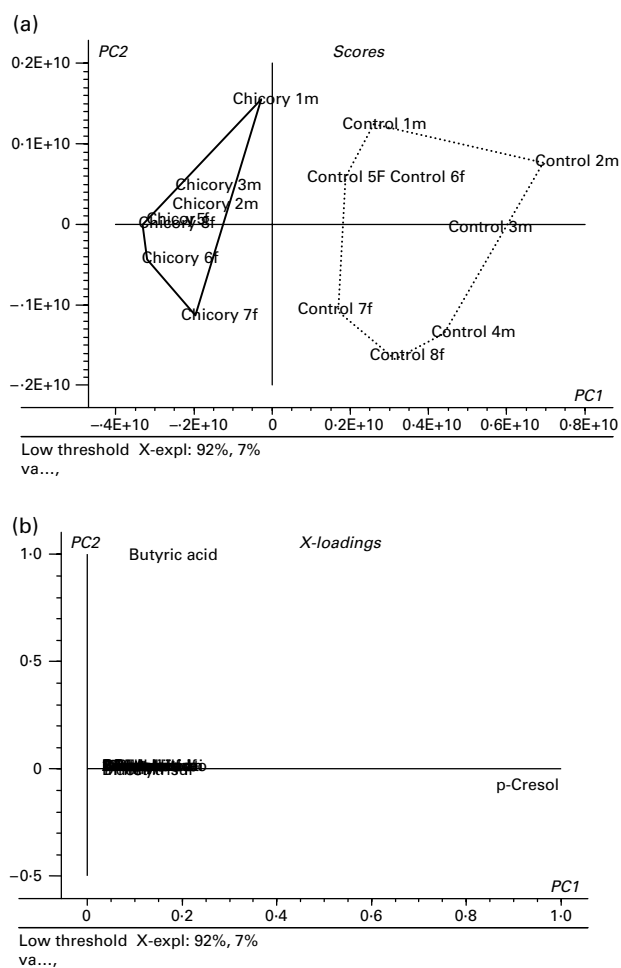


Figure 2 (a) The scores of raw GC-MS data corrected by dividing with low threshold values of odorous compounds from colon and rectal contents and (b) the loadings of low threshold values of the odorous compounds of control-fed and chicory-fed pigs.

on the odour impression due to their low threshold values. In addition to the threshold values of the odorous compounds, the odour quality of the compounds should be taken into consideration. The odour quality of a compound may change according to concentration, e.g. skatole has a pleasant flower-like odour at very low concentrations, whereas the same compound is nauseating at higher concentrations. In contrast some groups of compounds have a relatively pleasant odour description even at higher concentrations, e.g. esters, which usually have fruity and sweet odour notes. By dividing the raw GC-MS data (Table 4) by the odour thresholds (Table 3) of selected compounds, we illustrated the impact of odours with widely different odour thresholds (Figures 2 and 3). As the reported values in the literature of odour thresholds can vary widely, Figures 2 and 3 illustrate the extremes. By incorporating the odour thresholds in the raw data, a picture of the impact of sensoric impression of the mixture was created, in contrast to those of the individual compounds (Figure 1). As it appears from both Figures 2 and 3, the plot of points from the chicory-fed pigs were more tightly grouped when corrected for high or low threshold values compared with the plot of points from the control pigs. Chicory was therefore

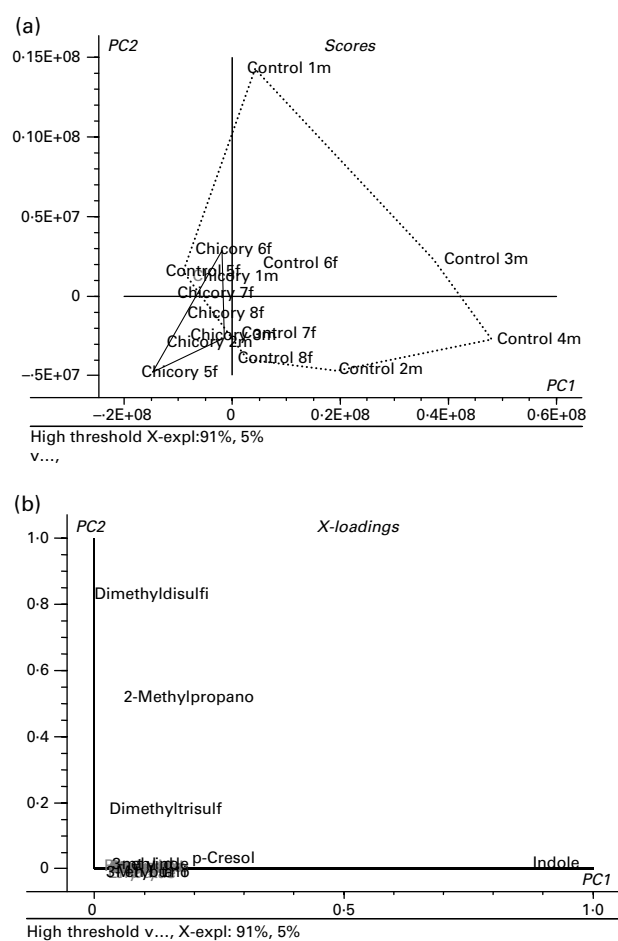


Figure 3 (a) The scores of raw GC-MS data corrected by dividing with high threshold values of odorous compounds from colon and rectal contents and (b) the loadings of high threshold values of the odorous compounds of control-fed and chicory-fed pigs.

able to control the production of odorous compounds in the colon and rectum, and effectively change the fermentation from protein fermentation to carbohydrate fermentation.

Sensory analysis of the meat from the experimental pigs showed that addition of chicory to the diets resulted in an overall improved eating quality (D.V. Byrne and L.L. Hansen, unpublished). Furthermore, reduction of the boar taint compound skatole was very significant (Hansen *et al.*, 2006). Feeding chicory therefore had a positive influence on the meat quality.

In addition to the reduction of the odorous compounds mentioned above, feeding with chicory roots might reduce the production of ammonia. The fermentation of inulin in the caecum, colon and rectum of pigs results in production of short chain fatty acids. Higher amounts of short-chain fatty acids reduce the pH, which has a positive influence on the retention of ammonia in the faeces and manure. This results in an improved environment in the stable and in the surroundings (Lenis and Jongbloed, 1999; Sutton *et al.*, 1999). The ammonia emission can be further reduced as the bacteria switch from protein fermentation to carbohydrate

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fermentation when feeding with inulin. Furthermore, as the bacteria grow, the nitrogen will be used for production of proteins in the bacterial biomass and is therefore not available for production of ammonia or odorous compounds.

It is not necessary to completely eliminate the presence of odorous compounds in the colon and rectum of pigs in order to reduce the odor impact on ambient air quality. A reduction should be sufficient to improve the ambient air quality to an acceptable level. The amount of chicory roots necessary for a sufficient reduction of odorous compounds in the colon and rectal contents of pigs remains therefore to be determined. If this amount can be decreased, the method will be more cost effective. As the content of inulin in chicory roots probably vary with season and growth conditions, it must be fed in a sufficient amount to secure an adequate concentration of inulin in the caecum and colon. In addition to the odour-reducing effects, the chicory root has the following benefits: Easy to grow in the present agricultural systems; can be handled by equipment used for other crops such as sugar beets; is in itself a valuable food component; contains bioactive secondary metabolites (Amaducci and Pritoni, 1998; Baert *et al.*, 1992; Candilo *et al.*, 1997). The product can be dried for all-year use. As the offensive odours from pig and poultry houses are produced in much higher amounts during the warm summer time periods (Spoelstra, 1977), the use of dried chicory roots in such periods may be enough to bring down the number of complaints from neighbours to pig and poultry production units.

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