J. agric. Engng Res. (1997) **67,** 267–275

Preliminary Investigation of Breath Sampling as a Monitor of Health in Dairy Cattle

R. J. Elliott-Martin*; T. T. Mottram†; J. W. Gardner‡; P. J. Hobbs§; P. N. Bartlett*

* Department of Chemistry, University of Southampton, Southampton SO17 1BJ, ‡ Department of Engineering, University of Warwick, Coventry CV4 7AL, § Institute of Grassland and Environmental Research, North Wyke, Okehampton, EX20 2SB, † Siloe Research Institute, Wrest Park, Silsoe, Bedford, MK45 4HS, U.K.

(Received 18 December 1995; accepted in revised form 25 March 1997)

A nasal breath sampling device was developed for use with dairy cattle. This device was tested as a means of supplying breath for analysis by a gas sensor array, gas chromatograph-mass spectrometer, and Fourier transform infrared spectrophotometer. Concentrations of methane, dimethyl sulphide, butan-2-one, and propanone (acetone) in cow nasal breath, under field conditions, are reported for the first time, and were detected at 70–1000 p.p.m., 80–100 p.p.m., 70–80 p.p.m., and 1–10 p.p.m., respectively. The output from a six-element gas sensor array was analysed using chemometric techniques. Breath samples were successfully classified in terms of known input odours by referencing to serum 3-hydroxybutanoate (β-HB) concentrations. These preliminary breath odour sensing studies suggest that it may be possible to discriminate between healthy cows and cows with ketosis by developing an application-specific electronic nose (ASEN) and sampler.

1. Introduction

Every year the dairy industry uses feeds with a value of £1000 M to produce milk.[1] In early lactation, ketosis is associated with an inefficient use of feed, causes economic loss and compromises cow health.[2] Marked improvements in this situation may be attainable if a simple, cheap, automatic diagnosis of bovine ketosis was readily available. Ketosis or acetonaemia is traditionally identified at the clinical stage by symptoms such as inappetance and the smell of acetone on the breath.[2] The detection of acetone on the breath by the human nose is prone to variation in sensitivity both between and within individuals depending upon, for example their state of health.[3] Subclinical ketosis can be diagnosed by analysis of blood and milk samples in the laboratory. However, laboratory analyses of body fluids are time consuming, costly and intrusive to the animal as well as being inherently difficult to automate. We propose a rapid and non-invasive method of sampling breath in dairy cattle. Chemical analysis of the sample can be performed using laboratory techniques, Fourier transform infrared spectroscopy (FTIR) and gas chromatography-mass spectrometry (GC-MS), and in addition, artificial olfactometry[4] (or odour sensing) using an ''electronic nose''. The latter technique may have potential application in the automatic detection of subclinical ketosis.

The quality of industrially important odours is traditionally monitored by a combination of analytical techniques such as GC-MS and human sensory panels, both of which are expensive to implement in practice. Discrete devices consisting of a single gas sensor (e.g. Odor Monitor by Sensidyne Inc.) can be used to quantify odour intensity, but they cannot be used to discriminate between different types of complex odours. Instruments employing an array of non-specific electronic gas sensors (e.g. chemoresistors, field effect transistors or surface acoustic wave devices) termed ''electronic noses'' have recently been developed in attempts to mimic the human olfactory system by utilization of artificial intelligence.[4–6] Odour classification using an electronic nose (*Fig. 1*) can be achieved in the following way. The active material of each sensor i (where $i = 1$ to n and n is the total number of sensors in the array) converts the chemical input of odour j into a time-dependent electrical signal, $V_{ij}(t)$. The response pattern from the array is then a vector, X_j which can be analysed using a suitable data processing technique.[7] This process is known as pattern recognition (PARC) in odour sensing and in practice usually involves the selection of a

0021-8634/97/080267 + 09 $25.00/0/ag970168

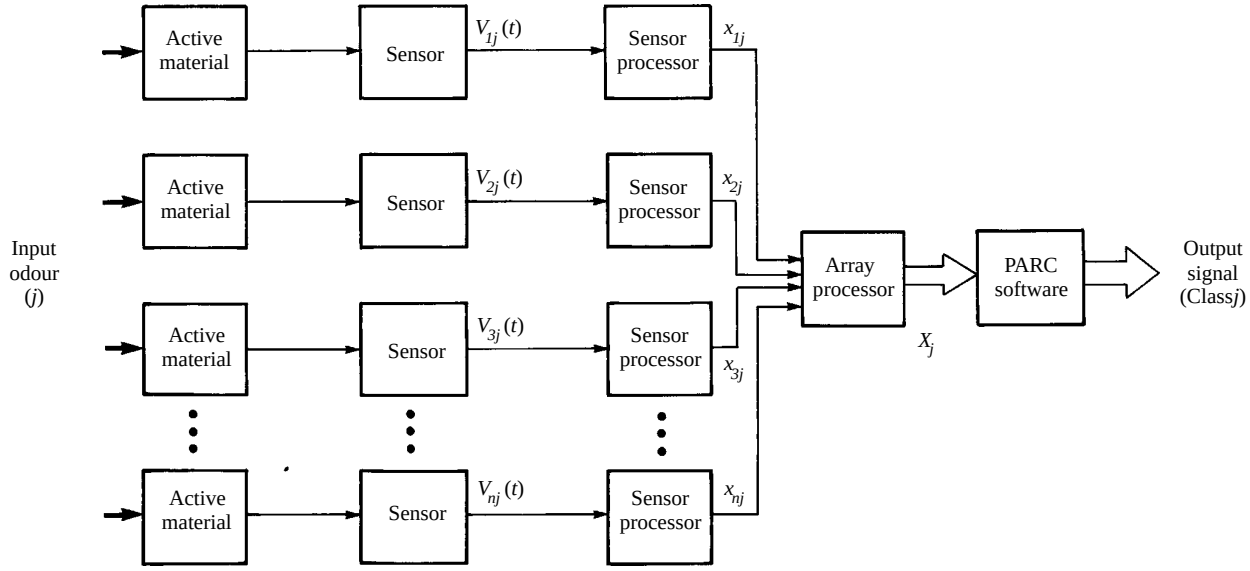

Fig. 1. Schematic diagram of the processing of signals with an electronic nose

preprocessing algorithm, x_{ij}, e.g. the normalisation of the array output to reduce experimental variation. Electronic nose technology is, at present, application specific, that is to say each application requires a specific experimental protocol and PARC technique. To date, interest has been concerned primarily with the measurement of odours from food and beverage. Instruments have been shown to discriminate, for example, between standard and artificially tainted beers[5] and different coffee aromas,[6] amongst other consumer products. More recently, in the field of agricultural engineering, an electronic nose was shown to successfully distinguish between odours from pig and poultry slurries[8] at concentrations above 6×10^4 odour units/m^3 in air.

Mottram[9] proposed that electronic sensors could be used to detect ketosis at an early stage by sampling the breath of the cow. A sensor system could either be deployed to sample periodically or as a hand-held device used by a herdsperson or veterinarian. A programme of work was planned in three stages, first to develop a repeatable method of gaining samples for analysis, secondly to evaluate suitable sensor systems, and thirdly to correlate the results of breath sensors with blood profiles in a controlled trial.

The object of the work reported in this paper is to report the first part of the programme, to devise a repeatable method of sampling the breath of a cow and to develop a reference method of calibrating sensors which does not require the presence of a cow. Three problems have been addressed, namely, variability of sampling, lack of prior knowledge of chemical constituents in the breath, and sources of error.

2. Materials and methods

2.1. *Nasal sampling system*

A prototype hand-held device[9] for sampling breath directly from the nostril (*Fig. 2*) was designed as it was assumed that this would avoid cross-contamination from odours emitted from the mouth.

A rigid tube contains a flexible inner tube made from poly(terephthalic ester) film (Nalophan NA, Hoechst) which serves as a disposable gas sampling bag (capacity of 0.55 to 0.60 1). Nalophan was selected because it is impermeable to aromas and water vapour, neutral in odour, and contains no plasticizer. At one end, the inner tube is sheathed around the lip of an all poly(tetrafluoroethene) sampling head which in turn has a trim fit to the outer Perspex case. The opposite end of the film is enfolded around the rear rim of the cylinder with the latter sealed by a bung, also housing a duct to enable the application of a partial vacuum or positive pressure between the inner and outer tube. A breath sample can be drawn into and discharged from the disposable, inert material bag.

Breath is drawn into the sample bag by evacuation of the annular volume between the rigid Perspex cylinder and the inflatable tube, using a positive displacement pump. The pump is manually controlled with a switch positioned in close proximity to the poly(tetrafluoroethene) head. The operator is able to move the device in order to retain the inlet port in the cow's nostril even whilst the cow is moving. As the

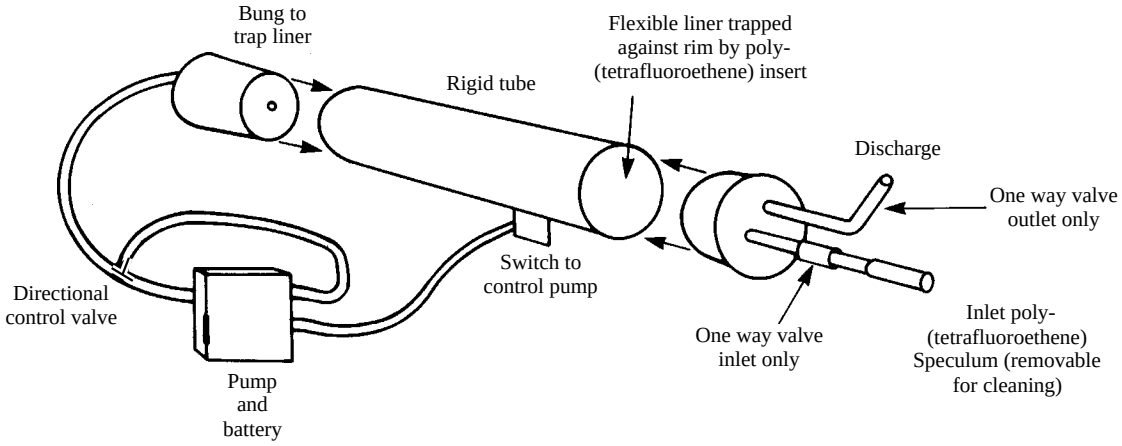

Fig. 2. Exploded diagram of breath sampling device

cow exhales (indicated by the animal's breath mist or whisker movement), the pump is activated to fill the sample bag with breath. The bag is discharged by pumping air into the annular space between the Perspex chamber and the flexible sample tube. Breath sampling times varied although generally an extraction of about 0.5 l breath could be accomplished in less than 30 s. Gas discharge times were of the order of 10 to 30 s.

The inner Nalophan NA film was replaced with a new piece of tube prior to each breath sample collection, thus avoiding any contamination from previous specimens. Likewise the speculi were removed, wiped with a paper towel in order to remove any mucus or particulate matter, and rinsed in deionized water before drying and remounting on the poly(tetrafluoroethene) head.

The device was used for sampling from several animals belonging to different herds (assigned as herds A to E). Sampling from different herds was necessary as different analytical instruments were located in different parts of the country. Not all techniques were applied to all five herds.

2.2. *Odour sensing*

For the purposes of odour monitoring, a Fox 2000 Intelligent Nose (Alpha MOS, France) based on an array of six commercially available metal oxide gas sensors was employed. Each sensor (Table 1) was interchangeable and capable of running at one of two operating temperatures, controlled by the applied voltage. Further details of the operation, sensing mechanism and selectivity of these gas sensors have been reported by Bartlett and Gardner.[10] Data acquisition and handling was carried out using an Intel 486-based microcomputer running National Instruments "LabVIEW" software. Instrumental interface and signal conditioning were obtained through utilization of a National Instruments LPM-16 analogue-to-digital converter (ADC) card.

A small internal pump ensured a continuous flow of gaseous sample (0.01 to 0.7 l/min) across the sensor array, and out via an exhaust port. Even when not testing, a continuous flow of background air was passed over the sensors thus defining the baseline signal and the conditions for which sensor resistance

Table 1
Designation of metal oxide (MOS) gas sensors used in electronic nose (sensor 6 was not used in the analysis due to missing data)

Sensor		*Specification*
1	NFI N43	High sensitivity to ammonia, amines
2	STAQ 1A	Air quality
3	NFI 1813	For detection of various combustible gases
4	TGS 825	High sensitivity to hydrogen sulphide, 5 to 100 p.p.m.
5	TGS 880	Food odours and tobacco smoke
6	TGS 822	High sensivity to alcohols and other organic solvents >50 p.p.m.

was calibrated. A ''burning-in'' period of at least 1 h was necessary before sampling to allow the sensors to reach an equilibrium state under the conditions of air flow rate and sensor voltage employed. After this, a routine calibration of sensor resistance was carried out. Sensor responses to breath samples were recorded over a period of 120 s. Complete sensor recovery was established within 15–20 min before subsequent measurements were made.

Samples presented to the electronic nose were collected over five experimental sessions (21, 43, 64, 85, and 120 ds after calving) in herd E only. Only at this site was it possible to set up the instrument in a room which was sufficiently near to the cows to enable rapid transport of the sample to the apparatus. At least three replicate samples were taken for each cow in any given test day. Blood samples, analysed by the MAFF State Veterinary Service, were taken simultaneously with the first breath sample of the day for each cow.

2.3. *GC-MS analysis*

Silica and carbon based adsorbents were used to concentrate 0.5 l of cow breath (from herds A to D) prior to analysis by GC-MS.

A GC-mass spectrometer [Hewlett Packard 5890 II Series gas chromatograph and a 5972A mass selective detector (MSD II)] was used to analyse samples collected on the adsorbents. Samples were introduced into the GC-MS system with an optic thermal desorption unit (Ai Cambridge Ltd.), at an initial temperature of 30°C increasing at a rate of 16°C/s and held at 280°C for 1 min. Chromatographic separation was performed using a split mode on a 25 m fused silica (cross linked with methyl silicone) column (i.d. of 0.2 mm and a 0.34 μm film) with a 1 m deactivated fused silica guard column (i.d. 0.25 mm). The flow-rate was 0.40 ml/min. Electronic pressure control was used to offset the effect of increasing pressure resistance with increasing GC column temperature to improve the chromatography of later eluting peaks. The GC-mass spectrometer interface was at a temperature of 280°C. The GC oven conditions were; initial temperature of 30°C, then increased to 150°C at 25°C/min. The mass spectrometer scanned from 32 to 150 mass units every 0.18 s to give a sensitivity down to 30 pg. Trapped volatile compounds were identified using an eight peak probability based matching algorithm and the NIST mass spectral library. Compounds were declared unknown if their matching probability was less than 0.80 (1.0 being a perfect match).

2.4. *FTIR spectroscopy*

Approximately 30–40 l of breath was collected from the nostril by discharging 0.5 l samples into a poly(tetrafluoroethene) gas sampling bag. FTIR spectra were obtained using a variable path length, Biorad FTS165 spectrophotometer fitted with BaF_2 endwalls. The path length was set to 10 m giving a cell volume of 1.75 l, which was consequently maintained at 40°C. Initially the apparatus was purged with cow breath. Spectra were recorded over the range, 4000 to 740 cm^{-1} at ambient pressures. A total of 64 scans to an instrumental resolution of 2 cm^{-1} was carried out for each sample. Due to the need to transport large volumes of fresh breath, only samples from herds D and E were studied by FTIR.

3. Results

3.1. *GC-MS analysis*

The breath metabolites detected from late lactation cows sampled from four different herds are presented in Table 2. Various analyte concentrations, where measured, have been included. The breadth constituents varied depending upon herd although dimethyl sulphide was commonly found in cows at all locations. Dimethyl sulphide and butan-2-one may be inferred to be protein and fatty acid breakdown products, respectively. The cellulose microbiological breakdown product ethanoic acid appeared among cows from herds B and C. Methyl benzene, previously observed in pig slurry odours,[8] was detected in cows sampled from herd C only. These cows (4–7) were suspected by the farmer as being ketotic. An acetone concentration of 1 to 10 p.p.m. in the breath was indicative of ketosis which was also confirmed by blood analysis. The absence of volatile organic compounds (VOCs) in cow 8 could be due to the occurrence of condensation in the sampling bag after storage prior to analysis. Water soluble VOCs would likewise condense out of the gaseous phase.

3.2. *FTIR spectroscopy*

The FTIR spectra of three discrete breath samples originating from cows in herd D exhibit the characteristic methane absorption peaks centred around 3000 cm^{-1} and 1310 cm^{-1} (*Fig. 3*). The characteristic P

Table 2
GC-MS analytes detected in late lactation cows: the number of experiments are given in parenthesis

Cow	*Analyte*	*Concentration/p.p.m.*	*Herd*
1(1)	dimethyl sulphide	80–100	A
	butan-2-one	70–80	
2(3)	dimethyl sulphide	80–100	A
	butan-2-one	70–80	
3(2)	dimethyl sulphide	—	B
	ethanoic acid	—	
	di(ethyleneglycol)	—	
3(2)†	acetone	saturated	C
4(1)	ethanoic acid	—	C
5(1)	ethanoic acid	—	C
	methyl benzene	—	
6(1)	acetone	<1	C
	ethanoic acid	—	
	methyl benzene	—	
7(2)	acetone	1–10	C
	dimethyl sulphide and other sulphur compounds, methyl benzene	—	
8(4)	No compounds detected		D

† Denotes gaseous sample spiked with acetone

and R branches on either side of the main peak correspond to the molecular rotations associated with the molecular vibration. Two broad absorptions, observed above 3500 cm^{-1} and broad weak bands in the range 1870 to 1400 cm^{-1} can be assigned to water vapour in the breath. Finally, a carbon dioxide band was observed around 2350 cm^{-1}.

The instrument had previously been calibrated for

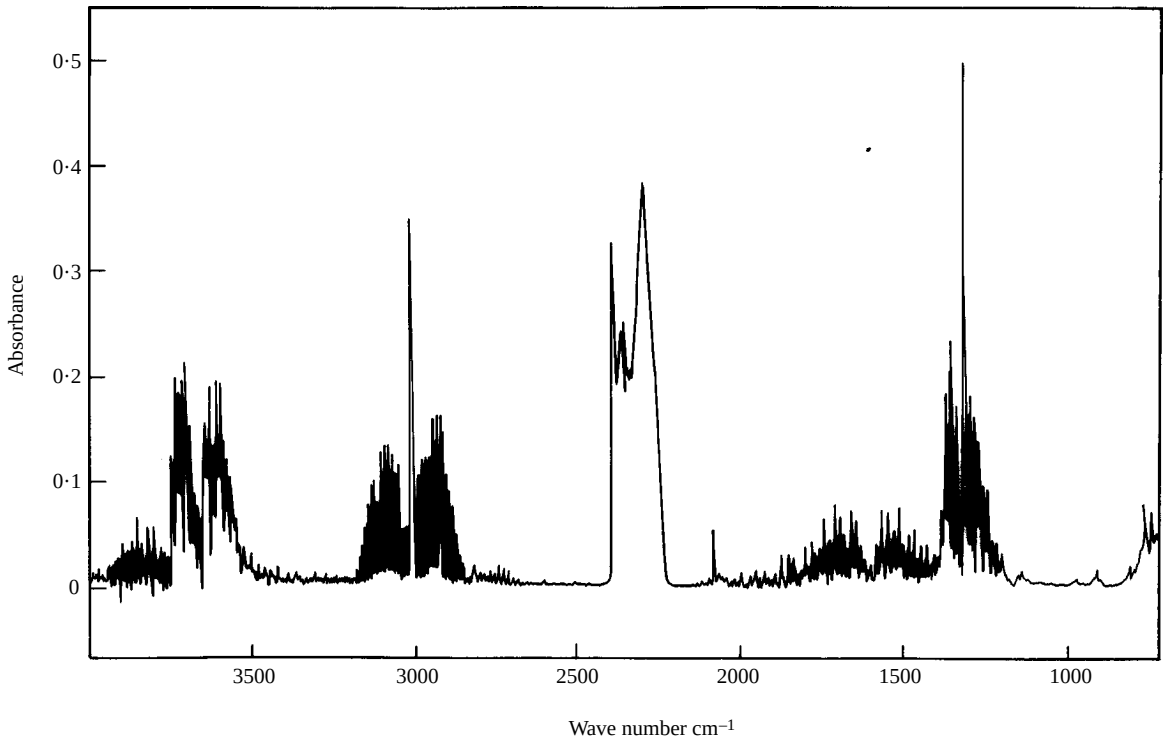

Fig. 3. Typical FTIR spectrum of cow breath sample from herd D over the range 4000–740 cm^{-1}. Bands centred around 3000 cm^{-1} and 1310 cm^{-1} arise through CH_4 absorption. Bands above 3500 cm^{-1} and in the range 1870–1400 cm^{-1} are due to H_2O. The CO_2 absorption peak is observed at 2350 cm^{-1}

methane concentration using a mixture of methane and nitrogen. From this, it was calculated that two cows from herd D exhaled breath with a 500 p.p.m. methane concentration. A cudding cow in the same herd produced a breath sample with a 70 p.p.m. methane concentration. Samples from herd E confirmed the presence of high concentrations of methane on the breath.

3.3. *Odour sensing*

A typical sensor response pattern to breath is shown in *Fig. 4.* The individual sensors in the array responded differently both in amplitude and rate. Breath sample response signals were recorded at the peak values on the response curves as the maximum change of either the sensor voltage (V_{odour}) or sensor conductance (G_{odour}) in odour, from the corresponding value in air, V_{air} or G_{air}. A fractional difference model (Eqn 1) can be applied when dilute odours of fixed concentration or small changes in concentration are presented to metal oxide sensor arrays.[11] However, because large, non-linear signals were obtained from a 0.5 l gas specimen, the responses were linearised (Eqn 2). Autoscaling was also used to give an equal weighting to each sensor response in the data matrix and so remove inadvertent weighting on extremely large values[12] (Eqn 3). The response parameter $x_{ij}^{(k)}$ and sensor probability distribution N are defined as follows.

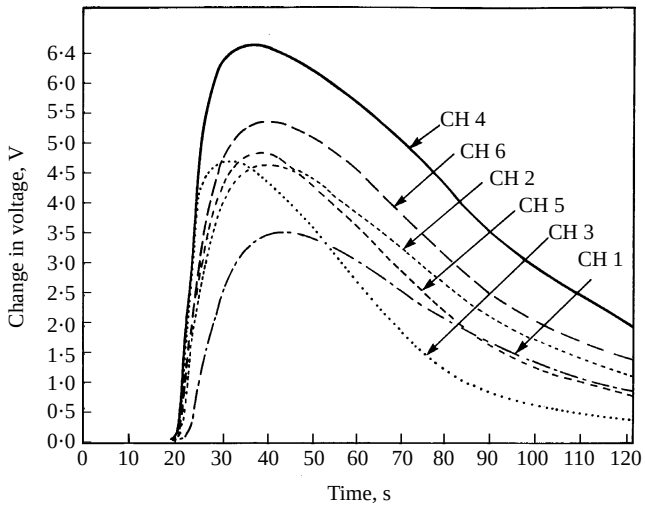

Fig. 4. Typical plot of the output from each of the six sensors when responding to a sample of cow's breath. The change in sensor voltage is shown with respect to time. The six sensors, CH1 to CH6, correspond to the sensors identified in Table 1. In each case the maximum value of the change in voltage was used in the subsequent analysis to calculate G_{odour}

Linear model:

$$x_{ij}^{(1)} = (G_{odour} - G_{air})/G_{air} \quad (1)$$

Non-linear model:

$$x_{ij}^{(2)} = \ln (G_{odour}/G_{air}) \quad (2)$$

Autoscaling:

$$N(\mu_i, \sigma_i^2) \rightarrow N(0, 1) \quad (3)$$

where μ_i and σ_i^2 are the mean and standard deviation of the responses of sensor i to all odours, respectively.

The preprocessed array of data was then analysed using the methods of principal components analysis (PCA) and hierarchical cluster analysis (CA).[12] *Figure 5* shows the first and second principal components of the response from a six-element electronic nose for breath samples tested for two cows in herd E, 21 to 120 d after calving. Only the first two principal components were required because 98.1% of the variance was found in these. The other principal components presented a virtually identical picture. Due to missing data for sensor 6, only five out of the six possible

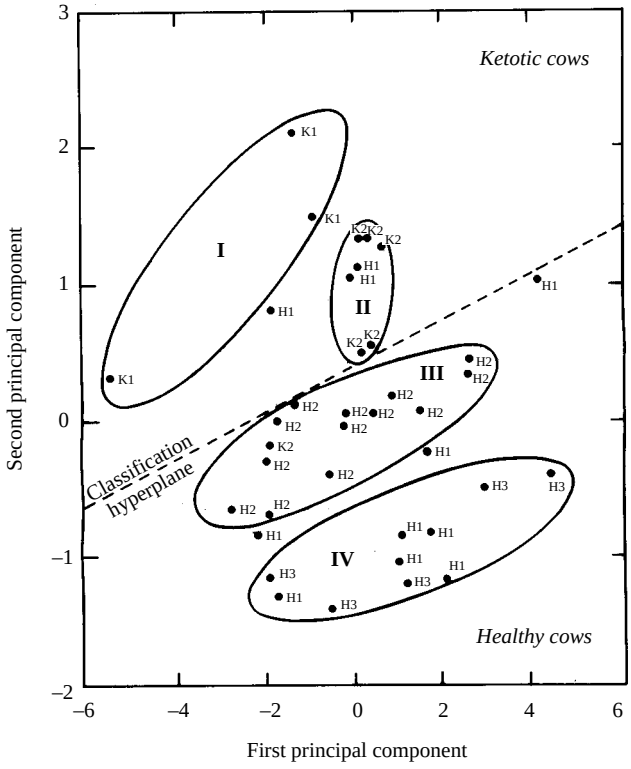

Fig. 5. Principal components analysis for breath sample classification in herd E using five of the six possible sensors in the electronic nose: first and second principal components of the autoscaled log(G_{odour}/G_{air}) response matrix are plotted. Four subsets are identified: I (K1 samples), II (K2 samples), III (H2 samples), and IV (H3 samples). The H1 samples show considerable variance

sensors were used in the analysis and a similar result was obtained using only three sensors. It was not apparent (from a knowledge of the origin of the samples) that individual cows produce different breath odours from each other. This was advantageous here, since variation among healthy individuals would reduce discrimination. Samples are classified by the state of health in each animal investigated, the symbol K signifying cows with serum 3-hydroxybutanoate (β-HB) levels outside the normal reference range (0 to 1.2 mmol/l). Samples labelled with an H indicate healthy levels of β-HB. The dashed diagonal line represents a jack-knife hyperplane boundary between specimens from healthy and ketonemic cows with an 89% success rate for classifying ketotic cows (labelled K). The confusion matrix for the data-set is given in Table 3. The method correctly predicted 34 out of 38 which is an accuracy of 89.5%. There was one false positive (FP), and three false negatives (FN). Table 4 collates the blood analysis with sample labels used in the PCA plot. In addition to the general separation of ketotic and healthy samples, it was reasonable to suggest that four distinct groups, subsets I, II, III and IV, had emerged. The spread of points in these clusters had no relationship with the time (after calving) of sampling. The success rates for this arbitrary classification of individual samples are given in Table 4. However, there are incorrectly assigned samples within the groups, e.g. one K2 data point within subset III which should be all H2 samples.

4. Discussion

The results from all three analytical techniques indicate that there was much variation amongst breath samples, which is not unexpected since there are a number of variable factors that can be influential to the sample, such as herd, health, lactation interval, diurnal changes and also cow to cow variation.

Although we made the assumption that bovines are compulsive nostril breathers it is still unknown whether the sample was cross-contaminated with rumen gases. Detection of methane by FTIR spectroscopy on breath may have been indicative of this (the non-polar capillary chromatography column used in the GC-MS apparatus exhibits a separating bias which excludes detection of hydrocarbons lower than C_3). However, it is apparent, from this study, that methane will always be present when sampling breath from the nostril. Release of methane could originate from, either the rumen (through belching), or the blood, where gaseous exchange takes place at the surface of the lungs, or possibly through both processes. Comis[13] stated that up to 350 l methane per cow per day can be produced depending upon diet. The exact origin of methane however was uncertain, since measurements were made with an array of outdoor sampling tubes, on grazing cattle in a 576 m^2 enclosure. Methane could therefore be a source of experimental interference in odour sensing measurements undertaken here, as metal oxide gas sensors are sensitive to hydrocarbons, and typically we observed that the NFI 1813 sensor (Table 1) showed the largest response to breath specimens. The sensor helped discrimination but was not essential as discrimination was still possible excluding this sensor. However, it is clearly better to design an array of sensors that does not react strongly with methane. Gas sensors based on conducting polymer materials may be advantageous here, as they are inert to small hydrocarbons. The detection of ethanoic acid may suggest contamination of nasal breath, but its presence due to normal metabolite excretion via the lungs cannot be ruled out.

Another cause of variation in the samples depends upon the breathing behaviour of the cow and indeterminate sampling error. It was uncertain whether the sample was consistently exhaled breath (deep breath) or a variable mixture of exhaled breath and surrounding air (shallow breath). Occasionally the exhalation cycle of the cow could not be predicted from whisker movement or breath mist if sampling took place in a strong breeze. Obvious improvements to the sampling device would include the incorporation of a breath flow sensor, and this development is in progress.

Table 3
Sample confusion matrix for two classes: ketotic and healthy (not-ketotic)

	Class 'ketotic'	*Class 'healthy'*	*Total predicted*
Predicted ketotic	8 (TP)	3 (FN)	11 (R+)
Predicted healthy	1 (FP)	26 (TN)	27 (R−)
Total in class	9 (C+)	29 (C−)	38

TP true positive; TN true negative; FP false positive: FN false negative; C+ class positive: C− class negative: R+ predicted positive: R − predicted negative

Table 4
Classification of breath samples: samples labelled in accordance with state of health defined by β-HB concentrations in blood serum

Sample label	*β-HB concentration / mmol l^{-1}*	*Subset*	*Success rate for discriminating groups of data %*	*Number of correct samples within subset*	*Number of incorrect samples within subset*
H1	0–0.5	—	—	—	—
H2	0.5–1.0	III	100	13	1
H3	1.0–1.2	IV	100	5	5
K1	1.2–2.0	II	83	5	2
K2	>2.0	I	100	3	0

The FTIR and GC-MS findings suggest that some of the water soluble breath components were lost through condensation or chemical decomposition occurring during storage of the sample. The FTIR spectrum shown in *Fig. 3* shows lack of water in the sample compared with samples collected from herd E. It was expected that cows breath would be saturated with water vapour. Although relative humidity was monitored, no attempt was made to control humidity or characterise sensor response to water vapour as it was anticipated that this would be conducted in a future controlled trial. Compounds such as butan-2-one, detected in herd A, with GC-MS would be expected to give strong absorption bands in the region, 1730 to 1700 cm^{-1} of the FTIR spectra. Their notable absence in all FTIR measurements may have been due to condensation processes but alternatively, the absorptions could have been concealed because of the very high concentrations of methane in the sample.

The preliminary observations in this work may suggest an inverse relationship between concentration of acetone and concentration of dimethyl sulphide on the breath, and further detailed studies are required to confirm this. The absence of GC-MS detectable VOCs in herd D could also be due to decomposition of samples. Dimethyl sulphide is chemically unstable in air[7] thus highlighting the advantage of rapid portable devices for odour measurement. Concentrations of acetone on the breath of clinically ketotic cows were previously unknown. Cow 7 (Table 2), diagnosed by the veterinarian as clinical, provided concentration values that coincided with values obtained from Dutch cows, in as yet unpublished work, in an investigation of the correlation between acetone on the breath with acetone and acetoacetate in milk for cows with induced ketosis.[14]

In the PCA scattergram (*Fig. 5*) there is a significant spread of samples designated H1. There would appear to be overlap of H1 samples with samples labelled H2, H3, and K2 and we suggest that this was due to sensor drift. Samples of subset III appear to be best assigned to a particular group. These breath samples correspond to serum β-HB values below the lower limit of subclinical ketosis (1.0 mmol/l) defined by Andersson.[15] The following chemical equilibrium exists in the blood:

$$\beta\text{-HB} \rightleftharpoons \text{acetoacetate} \rightleftharpoons \text{acetone}$$

It is notable that samples designated H2 (subset III) are in closer proxomity than samples H3 (subset IV), on the PCA plot, to samples K1 and K2 (subsets I and II). Therefore the location of samples on the scattergram would suggest non-linear correlation of serum β-HB with breath samples, analysed by the sensor array. There may be a stage where β-HB becomes exhausted in the blood, as the aforementioned equilibrium shifts to the right where increased amounts of acetone is manufactured and appears on the breath. This may have been the case for samples in the H2 regime. It was also of great significance that our only classification scheme relies on the biochemical analysis of serum, where the subsequent post-treatment of sample was unknown to us, e.g. time delay between bleeding and analysis, and temperature changes etc. Moreover, diurnal changes in the cow's metabolism due to feed[15] may result in poor matching between breath sample and blood sample analyses. Although the relative humidity and temperature, at which the sampling took place, were recorded, they appear to have little bearing on the outcome of the odour sensing studies.

The electronic nose is a cheap device, which may have potential as a tool to support diagnosis of disease in dairy cattle. It may be regarded as portable when compared with conventional analytical techniques, and we have shown that it is possible to work under non-ideal conditions, e.g. the gas analysis could be carried out by operating the apparatus from a mobile trailer on a farm. This method is not restricted to cows

and could perhaps be developed for application with other livestock, e.g. pregnancy toxaemia in sheep. In theory, all pathological disorders could be diagnosed when performing breath measurements. However, all nasal breath contaminants, depending upon, age, hunger state, and food debris will have an influencing effect on the environment of the nasal cavity. Our experiment has not permitted strict dietary and environmental controls to be executed and thus a more controlled trial is needed.

5. Conclusions

A non-intrusive method of obtaining cow breath for chemical analysis using a gas collection device has been demonstrated. The device is portable and sampling is possible even whilst the cow's head is in motion. Analysis of samples of cow breath using GC-MS show that dimethyl sulphide, butan-2-one, ethanoic acid, di(ethyleneglycol), methyl benzene and acetone are all present. In the case of ketotic animals, concentrations of acetone on the breath of the order of 10 p.p.m. were observed. Although more complex and expensive to carry out than the electronic nose measurements, GM-MS analysis may be useful for the calibration of gas sensor arrays. FTIR analysis of the breath samples shows that water and methane are always present, the latter in varying concentrations.

Small volume (0.5 l) breath specimens gave large sensor responses in a metal oxide gas sensor array. Principal components analysis of the sensor array response to breath samples gave an 89% success rate for the classification of the cows as healthy or ketotic and the confusion matrix for the data-set correctly predicted 34 out of the 38 samples. Discrimination between samples in this preliminary trial, conducted on the farm, therefore indicates potential for using an electronic nose as a non-invasive technique to support the diagnosis of ketosis. The specificity of the technique could be evaluated by using an improved sampling device in a more controlled trial and this work is in progress. In addition, the application of electronic noses based upon arrays of conducting polymer gas sensors may offer an advantage since these materials are less sensitive to hydrocarbons such as methane.

Acknowledgements

The authors would like to thank T. Cumby and E. Nigro, of Silsoe Research Institute, and H. Rutt and C. Mansfield, of University of Southampton for carrying out several IR measurements at different sites, ADAS Bridgets Farm and Shuttleworth College for access to cows, and Alpha MOS SA for the supply of software. This work was funded by the BBSRC and MAFF.

References

[1] **Nix J** Farm management pocketbook, 26th ed., Wye College Press: University of London, 1995

[2] **Bruss M L** Ketogenesis and ketosis, in clinical biochemistry of domestic animals, 4th ed. pp. 86–105. Academic Press Incorporated, 1989.

[3] **Murphy C** Olfactory psychophysics, in neurobiology of taste and smell, (Eds Finger, T E and Silver W L). Pp. 251–273. John Wiley and Sons: New York, 1987.

[4] **Gardner J W; Bartlett P N; Dodd G H; Shurmer H V** The design of an artificial olfactory system, in chemosensory information processing. Vol. 39 (Ed Schild, D.), NATO ASI series H: cell biology. Pp. 131–173. Springer-Verlag, 1990.

[5] **Pearce T C; Gardner J W; Friel S; Bartlett P N; Blair N** Electronic nose for monitoring the flavour of beers. Analyst 1993, **118**, 371–377.

[6] **Gardner J W; Shurmer H V; Tan T T** Application of an electronic nose to the discrimination of coffee, sensors and Actuators B, 1992, **6**, 71–75

[7] **Gardner J W; Bartlett P N** Intelligent chemSADs for artificial odor sensing of coffees and lager beers, in olfaction and taste XI, (Eds Kurihar K; Suzuki N; Ogawa H). Pp. 690–693. Springer-Verlag, Tokyo, 1994

[8] **Hobbs P J; Miselbrook T H; Pain B F** Assessment of odours from livestock wastes by a photoionization detector, an electronic nose, olfactometry and gas chromatography-mass spectrometry. Journal of Agricultural Engineering Research, 1995, **60**, 137–144

[9] **Mottram T T** Teat inspection device (Olfactory), U.K. Patent Application, 1992, GB 9224404.5

[10] **Bartlett P N; Gardner J W** Odour sensors for an electronic nose, in sensors and sensory systems for an electronic nose, Vol. 212. (Eds. Gardner J W; Bartlett P N), NATO ASI series E: applied sciences. Pp. 31–51. Kluwer Academic Publishers, 1992

[11] **Gardner J W** Detection of vapours and odours from a multisensor array using pattern recognition Part 1. Principal Component and Cluster Analysis, Sensors and Actuators B, 1991, **4**, 109–115

[12] **Gardner J W; Bartlett P N** Pattern recognition in gas sensing, in techniques and mechanisms in gas sensing, (eds. Moseley P; Norris J O; Williams D E). Pp. 347–380. Adam Hilger: Bristol, 1991.

[13] **Comis D** Monitoring methane. Agricultural Research 1995, **43**(6), 12–14

[14] **Dobbelaar P; Mottram T T; Nyabadza C; Hobbs P J; Elliott-Martin R J; Schukken Y H** Detection of ketosis in dairy cows by analysis of exhaled breath. Veterinary Quarterly, 1996, **18**(4), 151–152

[15] **Andersson L** Subclinical ketosis in dairy cows, veterinary clinics of North America. Food Animal Practice 1988, **4**(2), 233–251