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# Comparison of 2 high-throughput spectral techniques to predict differences in diet composition of grazing sheep and cattle

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# Comparison of 2 high-throughput spectral techniques to predict differences in diet composition of grazing sheep and cattle[1]

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**ABSTRACT:** Diet composition can be estimated in free-ranging animals by the use of *n*-alkane and long-chain fatty alcohol concentrations in feces. However, this technique involves relatively laborious and costly analytical techniques. Two spectroscopy techniques were investigated as a way of determining whether dietary differences are likely, thus indicating whether the more expensive and labor-intensive techniques for more detailed analysis are justified. Fourier-transform infrared spectroscopy (FTIR) and front-face fluorescence emission spectroscopy ($\lambda_{excitation}$ = 380 nm, $\lambda_{emission}$ = 600 to 760 nm) were used to analyze fecal samples collected from 2 different breeds of cattle and sheep (4 groups in total, n = 6 per group) grazing moorland plants in 2 grazing sessions. These fecal samples were also analyzed for alkane and alcohol concentrations. Fourier-transform infrared spectra, particularly in the alkane regions, demonstrated clear separation between animal species. Fluorescence emission spectra showed similar separation; fluorophores were most likely chlorophylls and their derivatives. Multivariate analysis of all 3 data sets showed similar variation within and between groups of cattle and sheep, indicating differences in diet selection particularly between species, but also between breed and grazing session. Both spectroscopy methods showed utility in suggesting differences in diet composition that would be worth investigating using more detailed chemical analyses. Of the 2 techniques, the FTIR spectroscopy gave the better comparative results, being able to detect differences in sampling months that were detected with alkanes and alcohols that the fluorescence emission spectroscopy did not detect.

**Key words:** cattle, diet selection, fluorescence emission spectroscopy, Fourier-transform infrared spectroscopy, grazing, *n*-alkane



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## INTRODUCTION

Agricultural landscapes are increasingly regarded as supporting a variety of environmental and social functions beyond production purposes (Belenguer et al., 2005; Gibon, 2005; Orr, 2008). This requires an understanding of herbivore diet selection, which may be very different to the composition of the grazed plant community (Fraser et al., 2006, 2009b) leading to changes in sward structure that can affect animal productivity and habitat value.

One way to estimate diet composition uses plant-wax lipid compounds (including *n*-alkanes and long-chain fatty alcohols, hereafter referred to as alkanes and alcohols) as markers (Dove and Mayes, 1996; Ali et al., 2004; Fraser et al., 2006; Lin et al., 2009). Different plants and plant parts have different relative proportions of alkanes and alcohols, and the diet composition of an animal can be estimated from its fecal alkane and alcohol profile (Hameleers and Mayes, 1998; Fraser et al., 2006). However, a drawback is the time and expense of the chemical analyses. More rapid, greater-throughput analyses such as Fourier-transform infrared (**FTIR**) spectroscopy and fluorescence spectroscopy could potentially provide initial comparative information about diet choices of animals (Anderson et al., 1996; Parveen et al., 2008; Fraser et al., 2009b; Lee et al., 2009b). This could be used to determine the value

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of carrying out more detailed sample analysis (e.g., of alkanes and alcohols in feces and plants) to estimate diet composition.

The objective of this study was to investigate the use of rapid technologies to generate comparative information on diet composition. We hypothesized that analysis of feces by FTIR and fluorescence emission spectroscopies would separately generate comparative data about diet selection in free-ranging ruminants. We compared spectra with data generated after full extraction of alkanes and alcohols from feces obtained from 2 different ruminant species (cattle and sheep) and 2 breeds of each species.

## MATERIALS AND METHODS

All procedures using sheep and cattle in this experiment were licensed and regulated by the UK Home Office under the Animals (Scientific Procedures) Act 1986 and were authorized by the Institute of Biological, Environmental and Rural Sciences' Local Ethical Review Committee.

### *Sample Collection*

Fecal samples were collected as part of an experimental program investigating interactions between animal type, sward composition, and diet selection (Fraser et al., 2009a). This study was carried out with 2 separate sampling sessions, one commencing at the end of July 2005 and the other at the end of September 2005 at ADAS Pwllpeiran, Ceredigion, Wales. The experimental design followed that of earlier studies of comparative grazing (Grant et al., 1985, 1987; Fraser and Gordon, 1997). At the site, 2 adjacent 4-ha plots were prepared (an adaptation plot and a measurement plot) on an area with a sward comprising approximately 60% heather (*Calluna vulgaris*).

Sampling session plots were grazed by 2 breeds of sheep and 2 breeds of cattle. Sheep breeds were Welsh Mountain and Scottish Blackface, and cattle breeds were Welsh Black and a Holstein × Simmental and Holstein × Belgium Blue cross. Six mature, barren females of each breed grazed the plots (n = 24 animals in total), and these were selected from the main flock or herd at the start of the experiment on the basis of uniformity of production status, BW, and BCS. All had previous experience grazing hill areas, having been sourced from ADAS Pwllpeiran or ADAS Redesdale (Northumberland, UK), and were pastured on vegetation close to the experimental site for at least 2 wk before the start of each experimental session.

To ensure that the presence of type of animal did not influence the behavior of another (Arnold, 1984), the measurement plot was divided into 4 subplots. Each species/breed was randomly assigned to a subplot at the beginning of each measurement week. The animals rotationally grazed the 4 subplots, moving on to the next subplot each day. In this way, all 4 animal types separately grazed the same measurement area over the course of the measurement week. On 4 of the last 5 d of the measurement week, fecal grab samples were collected and bulked on an individual animal basis. A proportion (at least 1 g of fresh weight) was maintained at −20°C, before fluorescence emission spectroscopy, and the remainder was freeze-dried and milled through a 1-mm sieve before analysis. Although finer grinding (e.g., by a ball mill) may have been more suitable for analysis of the samples in the FTIR spectrometer accessory to achieve a more homogeneous material for analysis, the aim of the study was to investigate a quick and practical screening method that used samples prepared for standard alkane and alcohol analysis. Full details of the animal experimental protocol and associated results have been described previously (Fraser et al., 2009a,b).

### *Analysis of Alkanes and Alcohols*

Quantitative analysis of alkanes and alcohols in freeze-dried fecal samples was based on the methods of Mayes et al. (1986) and Ali et al. (2004). Heat treatment with ethanolic potassium hydroxide was followed by a biphasic liquid:liquid extraction. A series of fractionation and derivatization stages were then carried out, which resulted in replicate subsamples from each fecal sample containing the extracted hydrocarbons and fatty alcohols as trimethylsilyl-ether derivatives (Ali et al., 2004). Modifications to the Ali et al. (2004) method were that the extracted fraction containing the hydrocarbons and alcohols was dried down at a decreased temperature (50°C compared with 105°C), and the long-chain fatty alcohol trimethylsilyl-ether derivatives were prepared using decane instead of dodecane as part of the derivatizing reagent. Although the alkanes and alcohols were subsequently analyzed separately, both sample sets were analyzed by gas chromatography using the same chromatographic conditions. Analysis was by gas chromatography using a Varian CP3380 gas chromatograph (Varian Ltd., Yarnton, Oxford, UK) fitted with WCOT Varian FactorFour EZ-Guard VF-5ms column (30-m column length plus a 5-m integral guard column, 0.25 mm i.d., 0.25-μm film thickness). Sample delivery was by a Varian CP8400 autosampler, and detection was by flame ionization. The carrier gas was helium flowing at 2 mL/min. The temperature program consisted of an increase from 150°C at 25°C/min to 250°C followed by an increase at 15°C/min to 340°C, then held for 5 min. Total run time was 15 min. Quantification was enabled by internal standard using *n*-tetratriacontane ($C_{34}$; Aldrich Chemical Co. Ltd., Gillingham, UK) and *n*-pentacosanol ($C_{25}$-1-ol; Ultra Scientific, North Kingstown, RI) for the alkane and alcohol analyses, respectively.

### *FTIR Spectrometry*

A Golden Gate attenuated total reflection accessory (Specac Ltd., Orpington, Kent, UK) with a diamond

top plate fixed to an optical beam condensing unit (Bruker Optics Limited, Coventry, UK) was linked to a Bruker Equinox 55 spectrometer. Triplicate analysis of each freeze-dried fecal sample (approximately 10 mg of DM per analysis) was carried out. Resultant spectra were then averaged to produce 1 representative spectrum per sample to minimize analytical variability. Sample variability also was minimized by acquiring background spectra before each sample application to the diamond anvil of the attenuated total reflection accessory, and this was subtracted from the subsequent sample spectrum. Spectra were acquired over the range 4,000 $cm^{-1}$ to 370 $cm^{-1}$ in 2-$cm^{-1}$ intervals using OPUS version 4.2 software (Bruker Optics Limited).

### *Fluorescence Spectroscopy*

Fluorescence emission spectra were measured directly on the fresh feces. The samples (1 g of fresh weight) were placed into sample cuvettes, which exposed a disk with a diameter of 5 cm for the measurements. Fluorescence emission spectra were measured in the range 400 to 800 nm with excitation at 380 nm, using an optical bench system. Wavelengths were chosen based on the peak fluorescence responses for ruminant feces reported by Kim et al. (2003) and Lefcourt et al. (2005) when screening meat and fruit for fecal contamination. Excitation light was generated by a 300 W xenon light source (Oriel 6258, Oriel Corporation, Stratford, CT) and passed through a 10-nm bandwidth interference filter (Oriel 59920). The light was directed onto the samples at an angle of 45° with an exposure time of 5 s, and the temperature of the samples was maintained at 4°C. The fluorescence emission spectra were collected by an imaging spectrograph (Acton SP-150, Acton Research Corporation, Acton, MA) connected to a sensitive charge coupled device (CCD-camera, Roper Scientific NTE/CCD-1340/400-EMB, Roper Scientific, Trenton, NJ). A cut-off filter at 400 nm (Melles Griot 03FCG049) was positioned in front of the spectrograph slit to suppress excitation light reflected from the samples. All the samples were measured twice and an average was used in the analysis. The field of illumination was not perfectly homogeneous, so the samples were rotated 90° between each measurement to even out sample heterogeneity. To ensure stable illumination, emission intensity at 440 nm at excitation 382 nm (generated using an Oriel 59920 interference filter) was measured from a stable fluorescence standard of washable plastic (Ciba, Basel, Switzerland) before and after the measurements.

### *Data Analysis*

Data were analyzed in MATLAB (The Mathworks Inc., Cambridge, UK) and Genstat (VSN International Ltd., Hemel Hempstead, UK). The concentrations of all odd-chain length *n*-alkanes in the range C23 to C35 and all even-chain length long-chain fatty alcohols in the range C22 to C30 were used in the data analyses. Initial visual examination of vector-normalized FTIR spectra revealed clear separation of samples from sheep and cattle in 2 spectral regions (Figure 1), from approximately 2,960 to 2,800 $cm^{-1}$ and from approximately 1,700 to 1,300 $cm^{-1}$. Data from these regions were used in subsequent analyses. Fluorescence emission spectra in the spectral region 600 to 760 nm showed the greatest response within the larger examined spectral region of 400 to 800 nm and were used in subsequent analysis. The spectra contained detector artifacts in the regions at approximately 645 to 646 nm and 739 to 741 nm that were removed by interpolation before analysis (Figure 2).

Data from the selected regions of the FTIR and fluorescence emission spectra were subjected to principal components analysis (**PCA**) using the statistics toolbox of MATLAB. Visual inspection of scatter plots of principal component (**PC**) scores revealed similarities in the data from different chemical analyses of the samples. Principal components analysis of the fecal alkane and alcohol data at each of the sampling periods (July and September), with subsequent ANOVA of the first PC, was carried out by Fraser et al. (2009b). The same data were reanalyzed for this study, without separating the data from the sampling sessions. The ANOVA was also carried out on the first PC of the FTIR and fluorescence emission spectra: samples from individual animals were treated as replicates (n = 6 per group), and a treatment structure of animal group × sampling session was used with no blocking.

Procrustes analysis is a procedure that, through rotation, reflection, and scaling, minimizes the sum-of-squared differences (residuals) between 2 multivariate data sets (Peres-Neto and Jackson, 2001). It was performed in MATLAB to transform PC 1 and PC 2 of the FTIR data and separately to transform PC 1 and PC 3 of the fluorescence emission spectra, onto PC 1 and PC 2 of the alkanes and alcohols data. Principal components 2 and 3 were chosen for the FTIR and fluorescence emission data, respectively, because the objective of the study was to investigate a practical and rapid sample screening process and these PC were judged to give the most distinct patterns of data on the PC plots. The Procrustes goodness-of-fit criterion calculated by MATLAB was used as a measure of the dissimilarity of the transformed spectral data sets to the (untransformed) alkanes and alcohols data set: a smaller number represents a closer fit, with 0 being a complete match.

## RESULTS

One of the fluorescence emission spectra, for a sample collected from a Welsh Mountain sheep during the July sampling session, was visibly different to all the other spectra. Initial PCA of the fluorescence emission data set suggested this spectrum to be an outlier. Grubbs' test for outliers (Tietjen and Moore, 1972) in the third

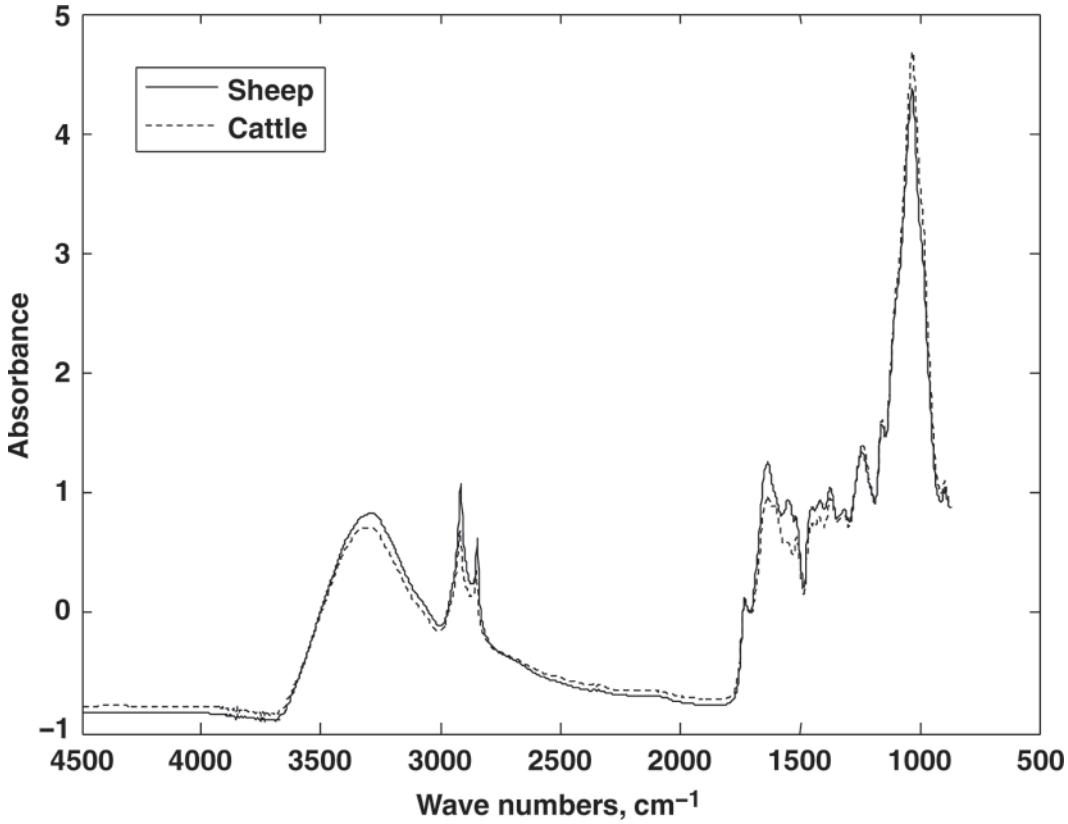


**Figure 1.** Mean Fourier-transform infrared spectra collected from feces from cattle and sheep using an attenuated total reflectance accessory.

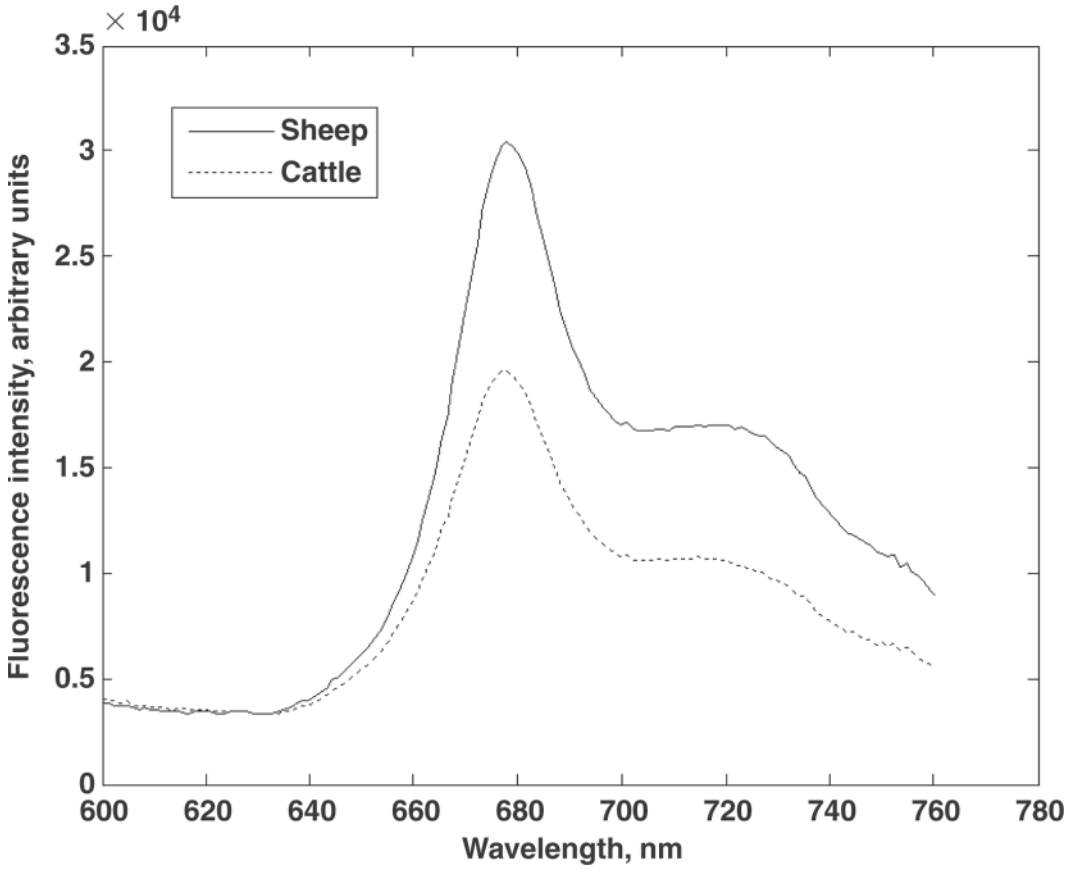


**Figure 2.** Mean fluorescence emission spectra of feces from cattle and sheep.

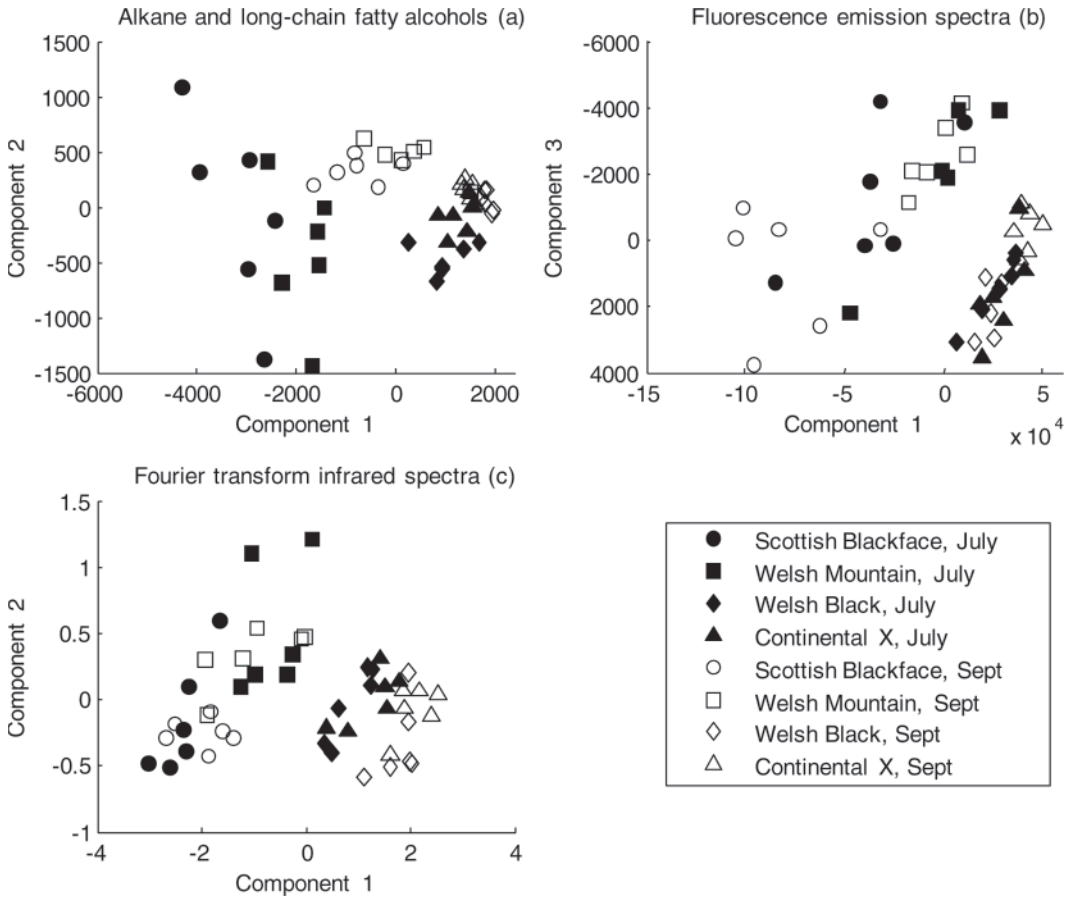


**Figure 3.** Principal components plots of data from analysis of *n*-alkane and long-chain fatty alcohols (panel a), fluorescence emission (panel b), and Fourier-transform infrared spectroscopy (panel c) of feces from 2 breeds of each of sheep (Scottish Blackface and Welsh Mountain) and cattle (Welsh Black and Continental X) grazing moorland pasture at 2 different times during the year. Note that the direction of the y-axis of the fluorescence data plot has been reversed to allow easier comparison with other plots.

PC of these data indicated that the data point originating from the suspect spectrum was significantly ($P < 0.05$) different from the others in the group, and it was therefore removed and not used in further analysis. No data points from the alkane and alcohol and the FTIR data sets were determined to be significant outliers. It was therefore assumed that there was an analytical error during the preparation of this fluorescence emission spectrum, although lack of sample meant that this analysis could not be repeated. Corresponding data from the alkanes and alcohols data set also were removed for the Procrustes analysis comparing them with the fluorescence emission data set.

Plots of PC from the 3 analytical data sets all showed similar patterns in terms of the positioning of individual data points (Figure 3). Note that because of the arbitrary nature of PC units, the y-axis of the fluorescence emission spectra PC plots has been reversed for comparative purposes. In particular, there was clear separation between the points corresponding to groups of sheep and cattle in each PC data plot. A significant ($P < 0.001$) effect of animal group (i.e., breed/species) was apparent after ANOVA of the FTIR and fluorescence emission spectra data (Table 1). However, although a significant ($P < 0.005$) effect of sampling month in the first PC of alkanes and alcohols data was also found in the first PC of the FTIR spectra, this was not found in the fluorescence emission spectra. Interaction effects were significant ($P < 0.05$) for all 3 data sets.

The Procrustes goodness-of-fit criteria between the alkanes and alcohols PC data and the transformed FTIR and fluorescence emission spectra PC data sets were 0.45 and 0.62, respectively. These values represent the dissimilarity between the 2 pairs of PC data sets (alkanes and alcohols vs. each of FTIR and fluorescence emission spectra) after they had been rotated, scaled, and reflected to best match one another. Therefore, following Procrustes rotation, the FTIR PC data were a better fit to the alkane and alcohol PC data than the fluorescence emission spectra PC data. Goodness-of-fit criterion following Procrustes analysis of the FTIR (PC 1 and 2) and fluorescence emission spectra (PC 1 and 3) was 0.38. Figure 4 shows the Procrustes superimposition plot for the alkanes and alcohols PC scores compared with the FTIR PC scores.

## DISCUSSION

This study used samples originally collected to estimate diet composition of free-ranging cattle and sheep using *n*-alkane and long-chain fatty alcohol profiles of feces, the results of which have been previously report-

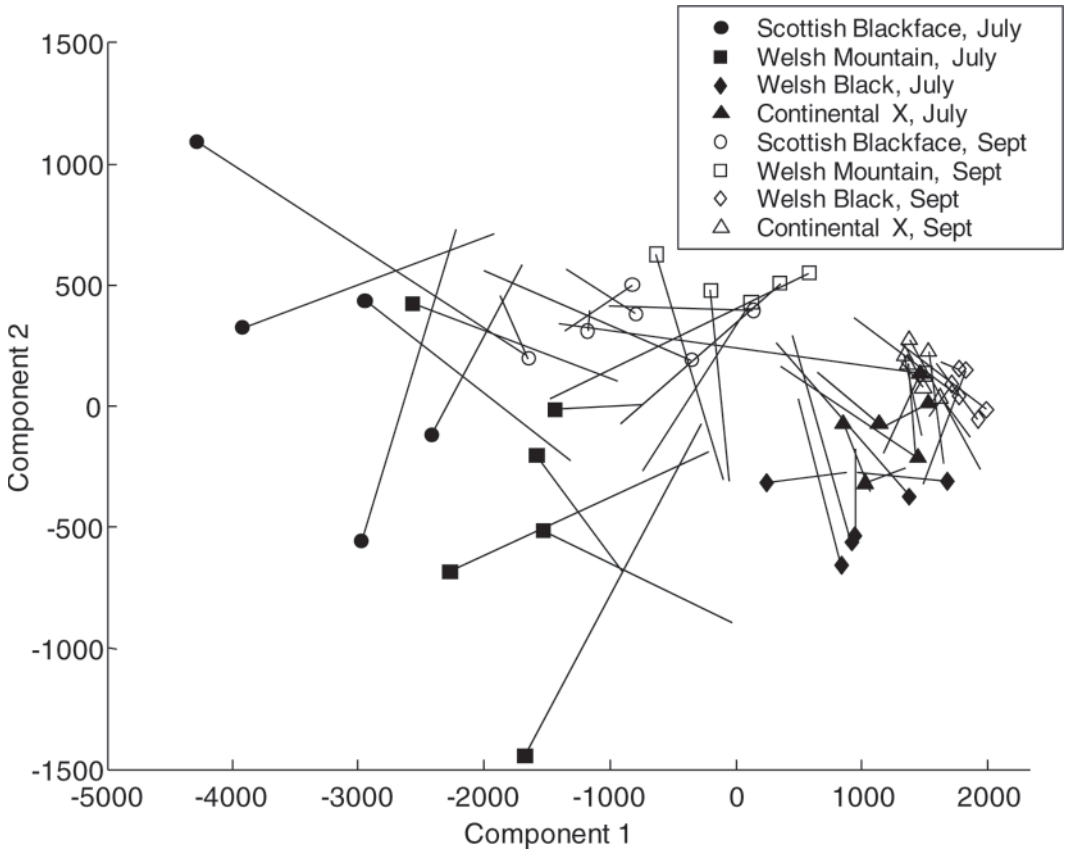


**Figure 4.** Procrustes superimposition plot of principal component scores for *n*-alkane and long-chain fatty alcohol data (markers) and Fourier-transform infrared data (end of solid lines). The solid lines represent the Procrustes residuals of both data sets.

ed (Fraser et al., 2009b). The objective of the current study was to determine whether other analyses, less expensive and less labor-intensive than those for the determination of alkane and long-chain fatty alcohol concentrations, could be used to evaluate whether the more detailed analyses are justified. Two such methodologies available to us were FTIR spectroscopy and front-face fluorescence spectroscopy. Recent reviews have reported some progress in the use of another methodology, near infrared (**NIR**) spectroscopy, for analysis of feces to determine the botanical composition of diets (Landau et al., 2006; Dixon and Coates, 2009), although generally such diets have consisted of a very limited number of components. The current study takes this approach forward and demonstrates the application of spectroscopy-based methods when animals are grazing complex swards. An advantage of the techniques evaluated in the current study (and also of NIR spectroscopy) is that the assay will not destroy the samples, allowing the material to be used for other chemical analyses. This is an important consideration in situations in which fecal material is difficult to collect. However, NIR spectroscopy generally requires a larger sample size than either FTIR or fluorescence spectroscopy, which limits its comparative versatility.

### *FTIR Spectroscopy*

In the FTIR spectroscopy, 2 spectral regions (2,960 to 2,800 $cm^{-1}$ and 1,700 to 1,300 $cm^{-1}$) separated the

**Table 1.** Probabilities of the effects of animal group (species and breed) and month of sampling, and their interaction, on the first principal component scores of data generated from feces analyzed for *n*-alkanes and long-chain fatty alcohols, by Fourier-transform infrared (FTIR) spectroscopy or by fluorescence emission spectroscopy

| | *P*-value | | |
|---|---|---|---|
| Data set | Animal group | Month | Interaction |
| Alkanes and fatty alcohols[1] | <0.001 | <0.001 | <0.001 |
| FTIR spectra | <0.001 | 0.005 | 0.014 |
| Fluorescence emission spectra | <0.001 | 0.173 | 0.005 |

[1]From Fraser et al. (2009b).

spectra of sheep feces from cattle feces. The mid-infrared region (4,000 to 600 $cm^{-1}$) can be broken down into several fingerprint regions, where strong absorption bands are directly related to specific components (Williams and Fleming, 1989; Harrigan and Goodacre, 2003; Bosch et al., 2006). The spectral region 3,050 to 2,800 $cm^{-1}$, where fatty acids predominate and thus referred to as the fatty acid region, is chemically characteristic of the carbon-hydrogen bond vibrations of functional groups and is dominated by alkanes, fatty acid hydrocarbon chains, and fatty alcohol hydrocarbon chains. Previous work has shown the ability of FTIR analysis of feces to discriminate between sheep offered closely related diets comprising a complex mixture of heathland plants (Parveen et al., 2008). In the current study, the regions of the FTIR spectrum that were used for analysis included absorption bands covering the alkanes and alcohols that also were analyzed using standard chemical techniques. It is, therefore, not surprising that similar patterns in the PC plots were seen for alkane and alcohol data and the FTIR spectral data.

All methodologies currently available for estimating diet composition are associated with potential sources of error. The analysis of alimentary tract contents and feces does not take into account variations in rate of digestion of different plant species or plant parts (Slater and Jones, 1971) and has been found to overestimate less digestible portions of the diet while underestimating more digestible portions (McInnis et al., 1983). Likewise, whereas Dove and Mayes (1996) concluded that plant alkanes are substantially indigestible, other studies have suggested that they are differentially digestible (Newman et al., 1998). Certain attributes of the animal, such as species, sex, pregnancy status, and parasite burden, have been shown to influence fecal NIR spectra (Dixon and Coates, 2009) and, therefore, may affect FTIR spectra. Such differences are likely to be confounded with diet because they are known to influence selectivity, and during the current study the experimental design sought to minimize the influence of all these factors apart from species and breed.

### *Fluorescence Spectroscopy*

Front-face fluorescence spectroscopy was used in the current study to investigate the fluorescence of chlorophylls and their associated breakdown products in fresh fecal samples. A related methodology, laser-induced fluorescence spectroscopy, has previously been shown to have potential in determining diet botanical composition in sheep (Anderson et al., 1996, 1998). They showed characteristic peaks within chloroform-extracted feces at 470 and 650 nm, which allowed the investigation of a broad separation of dietary constituents. More recently whole feces were shown to have maximum fluorescence at 675 nm (Kim et al., 2003), which has been attributed to chlorophyll and its metabolites (Ashby et al., 2003). The fluorescent characteristics of chlorophyll have been known, studied, and exploited for many decades (Govindjee, 1995). At room temperature, the emission spectrum of extracted chlorophyll-rich chloroplasts tends to have a main peak at about 685 nm, with a broad shoulder at 720 to 740 nm (Mohanty et al., 1972). The spectra collected from feces in this study are very similar to this, with a main peak at about 677 nm and a shoulder at about 722 nm. The PCA indicates at least 3 different spectral components, likely to be chlorophyll *a* and chlorophyll *b* coming from the diet as well as potentially other fluorochromes, probably breakdown derivatives of chlorophyll. These breakdown products, such as phaeophorbide *a* and phaeophorbide *b*, have specific spectral emission peaks in the same region as chlorophyll (Ashby et al., 2003). It is not possible to identify these contributing compounds from our current data. Previous studies by our group (Lee et al., 2009a) with sheep show that a diet with increased concentration of chlorophyll, such as fresh grass and clover, resulted in feces with similar high chlorophyll signals as obtained in the current study. This provides supporting evidence that the main variation in the chlorophyll signal from feces in the current study is connected to the diet of the animals. However, the fluorescence emission signal depends not only on the quantities of chlorophylls and their breakdown products in the sample, but also on the presence of other compounds that absorb energy in the same part of the spectrum being measured (Cerovic et al., 2002).

The raw fluorescence emission spectra and the PC scores suggest that chlorophyll concentrations were generally greater in sheep feces than in cattle feces and also that the variation in concentration within the sheep samples was much larger than in the cattle samples. The difference in fluorescence intensity between the 2 groups could be due to matrix effects or color effects; dark samples could reabsorb more of the fluorescence than lighter samples, and this would result in less emission intensity. However, the sheep feces were darker than the cattle feces (based on observation at the time of collection), and therefore, the results suggest that concentrations of chlorophyll and its breakdown products in sheep feces were actually greater than in cattle feces. Differences in diet selection would lead to differences in the consumption of chlorophylls and other UV-absorbing compounds from different plants and to the rate and extent of chlorophyll degradation to its derivatives depending on the digestibility of the plants and the rate of passage through the gut of an animal. This allows fluorescence of chlorophyll-based molecules in feces to act as a method for detecting differences in diet selection in free-ranging animals. This has been demonstrated to enable the discrimination between sheep offered closely related diets comprising a complex mixture of heathland plants (Lee et al., 2009b). The PC plot patterns in the current study based on fluorescence emission spectra were similar to those based on alkane and alcohol data (and also to those based on FTIR spectra), with separation of animal species in particular along the first PC axis, and a greater spread of

data, particularly from sheep, along the third (second in FTIR data) axis. This suggests that the variations in fecal chlorophyll and its derivatives (and possibly of other UV-absorbing compounds) correlate well with the variations in alkanes and alcohol data. Differences between the fecal fluorescence emission data of sheep and cattle are consistent with research findings that cattle are less selective grazers than sheep (Grant et al., 1985; Armstrong et al., 1997), leading to cattle diets containing a greater proportion of dead/senescent material, particularly when animals are selecting from extensively grazed heterogeneous swards. Dead plant material contains much less chlorophyll than live material (Hörtensteiner and Lee, 2007).

### *Technique Comparison*

Results obtained demonstrate that both FTIR spectroscopy and fluorescence emission spectroscopy have potential as rapid and inexpensive methodologies for generating comparative diet composition information. Both approaches gave initial information with regard to the similarity in diets consumed by different types of animal, and the degree of individual-animal variation. In situations in which quantitative data such as proportional composition are required, the results from an initial analysis could be used to identify appropriate samples to undergo more detailed and labor-intensive analyses. Thus, these novel applications of FTIR spectroscopy and fluorescence emission spectroscopy, when used in conjunction with traditional methodologies, offer researchers in the fields of grazing ecology and animal science the potential for improving flexibility and precision when gathering diet data. Further advantages of both spectroscopic methods are that only comparatively small sample sizes are required, and the analyses are nondestructive. This would also allow data to be obtained in situations when the collection of larger samples represents a particular challenge, such as when studying wild, small, or juvenile animals. A logical progression of this work would be to attempt to produce calibration sets to predict alkane and alcohol concentrations in feces and to use these to estimate diet composition.

In conclusion, 2 high-throughput nondestructive tests were shown to provide preliminary data that could be used to make a decision on whether to carry out more labor-intensive and expensive analyses on feces samples as part of a research project. Of the 2 techniques, the FTIR spectroscopy gave the better comparative results, being able to detect differences in sampling months that were detected with alkanes and alcohols that the fluorescence emission spectroscopy did not detect.

## LITERATURE CITED

Ali, H. A. M., R. W. Mayes, C. S. Lamb, B. L. Hector, A. K. Verma, and E. R. Ørskov. 2004. The potential of long-chain fatty alcohols and long-chain fatty acids as diet composition markers: Development of methods for quantitative analysis and faecal recoveries of these compounds in sheep fed mixed diets. J. Agric. Sci. 142:71–78.

Anderson, D. M., E. L. Fredrickson, P. Nachman, R. E. Estell, K. M. Havstad, and L. W. Murray. 1998. Laser-induced fluorescence (LIF) spectra of herbaceous and woody pre- and post-digested plant material. Anim. Feed Sci. Technol. 70:315–337.

Anderson, D. M., P. Nachman, R. E. Estell, T. Ruekgauer, K. M. Havstad, E. L. Fredrickson, and L. W. Murray. 1996. The potential of laser-induced fluorescence (LIF) spectra of sheep feces to determine diet botanical composition. Small Rumin. Res. 21:1–10.

Armstrong, H. M., I. J. Gordon, S. A. Grant, N. J. Hutchings, J. A. Milne, and A. R. Sibbald. 1997. A model of the grazing of hill vegetation by sheep in the UK. 1. The prediction of vegetation biomass. J. Appl. Ecol. 34:166–185.

Arnold, G. W. 1984. Spatial relationships between sheep, cattle and horse groups grazing together. Appl. Anim. Behav. Sci. 13:7–17.

Ashby, K. D., J. Wen, P. Chowdhury, T. A. Casey, M. A. Rasmussen, and J. W. Petrich. 2003. Fluorescence of dietary porphyrins as a basis for real-time detection of fecal contamination on meat. J. Agric. Food Chem. 51:3502–3507.

Belenguer, A., J. Balcells, J. A. Guada, M. Decoux, and E. Milne. 2005. Protein recycling in growing rabbits: Contribution of microbial lysine to amino acid metabolism. Br. J. Nutr. 94:763–770.

Bosch, A., D. Serra, C. Prieto, J. Schmitt, D. Naumann, and O. Yantorno. 2006. Characterization of *Bordetella pertussis* growing as biofilm by chemical analysis and FT-IR spectroscopy. Appl. Microbiol. Biotechnol. 71:736–747.

Cerovic, Z. G., A. Ounis, A. Cartelat, G. Latouche, Y. Goulas, S. Meyer, and I. Moya. 2002. The use of chlorophyll fluorescence excitation spectra for the non-destructive in situ assessment of UV-absorbing compounds in leaves. Plant Cell Environ. 25:1663–1676.

Dixon, R. M., and D. B. Coates. 2009. Near infrared spectroscopy of faeces to evaluate the nutrition and physiology of herbivores. J. Near Infrared Spectrosc. 17:1–31.

Dove, H., and R. W. Mayes. 1996. Plant wax components: A new approach to estimating intake and diet composition in herbivores. J. Nutr. 126:13–26.

Fraser, M. D., and I. J. Gordon. 1997. The diet of goats, red deer and South American camelids feeding on three contrasting Scottish upland vegetation communities. J. Appl. Ecol. 34:668–686.

Fraser, M. D., V. J. Theobald, D. R. Davies, and J. M. Moorby. 2009a. Impact of diet selected by cattle and sheep grazing heathland communities on nutrient supply and faecal microflora characteristics. Agric. Ecosyst. Environ. 129:367–377.

Fraser, M. D., V. J. Theobald, J. B. Griffiths, S. M. Morris, and J. M. Moorby. 2009b. Comparative diet selection by cattle and sheep grazing two contrasting heathland communities. Agric. Ecosyst. Environ. 129:182–192.

Fraser, M. D., V. J. Theobald, and J. M. Moorby. 2006. Determining diet composition on complex swards using *n*-alkanes and long-chain fatty alcohols. Ecol. Appl. 16:1901–1910.

Gibon, A. 2005. Managing grassland for production, the environment and the landscape. Challenges at the farm and the landscape level. Livest. Prod. Sci. 96:11–31.

Govindjee. 1995. Sixty-three years since Kautsky: Chlorophyll a fluorescence. Aust. J. Plant Physiol. 22:131–160.

Grant, S. A., D. E. Suckling, H. K. Smith, L. Torvell, T. D. A. Forbes, and J. Hodgson. 1985. Comparative studies of diet selection by sheep and cattle: The hill grasslands. J. Ecol. 73:987–1004.

Grant, S. A., L. Torvell, H. K. Smith, D. E. Suckling, T. D. A. Forbes, and J. Hodgson. 1987. Comparative studies of diet selection by sheep and cattle: Blanket bog and heather moor. J. Ecol. 75:947–960.

Hameleers, A., and R. W. Mayes. 1998. The use of *n*-alkanes to estimate herbage intake and diet composition by dairy cows

offered a perennial ryegrass white clover mixture. Grass Forage Sci. 53:164–169.

Harrigan, G. G., and R. Goodacre. 2003. Metabolic Profiling: Its role in biomarker discovery and gene function analysis. Kluwer Academic, London, UK.

Hörtensteiner, S., and D. W. Lee. 2007. Chlorophyll catabolism and leaf coloration. Pages 12–31 in Annual Plant Reviews: Senescence Processes in Plants No. 26. S. Gan, ed. Blackwell Publ., Oxford, UK.

Kim, M. S., A. M. Lefcourt, and Y.-R. Chen. 2003. Optimal fluorescence excitation and emission bands for detection of fecal contamination. J. Food Prot. 66:1198–1207.

Landau, S., T. Glasser, and L. Dvash. 2006. Monitoring nutrition in small ruminants with the aid of near infrared reflectance spectroscopy (NIRS) technology: A review. Small Rumin. Res. 61:1–11.

Lee, M. R. F., V. J. Theobald, M. K. Theodorou, A. Veberg Dahl, F. Lundby, and J.-P. Wold. 2009a. Spectroscopic imaging as a real-time solution for the detection of faecal contamination on carcasses. Pages 29–30 in Advancing Beef Safety Through Research and Innovation: An International Conference, Dublin, Ireland. Teagasc, Dublin, Ireland.

Lee, M. R. F., V. J. Theobald, J.-P. Wold, A. Veberg, F. Lundby, and M. D. Fraser. 2009b. Front face fluorescence spectroscopy as a rapid non-invasive tool for determining diet composition in free-ranging ruminants. Page 119 in Proc. BSAS Annu. Conf., Southport, UK. Cambridge Univ. Press, Cambridge, UK.

Lefcourt, A. M., M. S. Kim, and Y. R. Chen. 2005. A transportable fluorescence imagining system for detecting fecal contaminants. Comput. Electron. Agric. 48:63–74.

Lin, L. J., H. L. Luo, H. Wang, Y. J. Zhang, and B. Shu. 2009. Evaluation of long-chain alcohols and fatty acids, in combination with alkanes, as markers in the estimation of the composition of four herbages in mixtures. Grass Forage Sci. 64:19–25.

Mayes, R. W., C. S. Lamb, and P. M. Colgrove. 1986. The use of dosed and herbage *n*-alkanes as markers for the determination of herbage intake. J. Agric. Sci. Camb. 107:161–170.

McInnis, M. L., M. Vavra, and W. C. Krueger. 1983. A comparison of four methods used to determine the diets of large herbivores. J. Range Manage. 36:302–306.

Mohanty, P., B. Z. Braun, Govindjee, and J. P. Thornber. 1972. Chlorophyll fluorescence characteristics of system I chlorophyll α-protein complex and system II particles at room and liquid nitrogen temperatures. Plant Cell Physiol. 13:81–91.

Newman, J. A., F. Cribari-Neto, and M. J. Jensen. 1998. The sensitivity of *n*-alkane analysis to measurement error: Implications for use in the study of diet composition. J. Agric. Sci. 131:465–476.

Orr, H. 2008. Climate change in the uplands: Is this the end of cheap and free services. Aspects Appl. Biol. 85, Shaping a Vision for the Uplands:65–66.

Parveen, I., J. M. Moorby, W. M. Hirst, S. M. Morris, and M. D. Fraser. 2008. Profiling of plasma and faeces by FT-IR to differentiate between heathland plant diets offered to zero-grazed sheep. Anim. Feed Sci. Technol. 144:65–81.

Peres-Neto, P. R., and D. A. Jackson. 2001. How well do multivariate data sets match? The advantages of a Procrustean superimposition approach over the Mantel test. Oecologia 129:169–178.

Slater, J., and R. J. Jones. 1971. Estimation of the diets selected by grazing animals from microscopic analysis of the faeces—A warning. J. Aust. Inst. Agric. Sci. 37:238–240.

Tietjen, G. L., and R. H. Moore. 1972. Some Grubbs-type statistics for the detection of several outliers. Technometrics 14:583–597.

Williams, D. H., and I. Fleming. 1989. Spectroscopic methods in organic chemistry. McGraw-Hill Company (UK) Limited, London, UK.