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Determination of sex and species in red and fallow deer by near infrared reflectance spectroscopy of the faeces

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Abstract

Near infrared reflectance spectroscopy (NIRS) of faeces is a non-invasive analytical technique that has been used to determine physiological status of free-ranging herbivores. Sixty-eight faecal samples collected in April of 2000 and 2002 from adult animals maintained on ryegrass (*Lolium perenne* L.) were used to determine the ability of NIRS to distinguish faeces by sex and/or species in red (*Cervus elaphus*) versus fallow (*Dama dama*) deer. Two-block partial least squares procedures indicate a high degree of discrimination ($R^2 > 0.90$) between groups within year. Discriminant equations developed from samples within either year were ineffective in identifying sex or species in the other year's samples (<60% correct). Dissimilar forage conditions (i.e. diet quality) between the 2 years probably contributed the greatest amount of variation in faecal spectra, thus diminishing any possible animal related effects. Combining both years samples yielded a high degree of discrimination ($R^2 > 0.87$) and, when the resultant discriminant equations were applied to validation samples withheld from calibration, correctly identified 80–100% of faecal samples grouped by sex, species, sex within species, and species within sex. Fecal NIRS successfully discriminated sex and species in red and fallow deer maintained in unisex groups on a monoculture pasture. The use of faecal NIRS to characterize wildlife population demographics and non-invasively monitor individual animal physiology should now be explored in free-ranging animals.

Keywords: Faeces; Fallow deer; FNIRS; Near infrared reflectance spectroscopy; Red deer

1. Introduction

Free-ranging herbivorous ungulates normally excrete approximately 1% of body weight in faeces each day. Faeces largely consist of undigested food, bacteria, gastric secretions, metabolized hormones, sloughed gut tissue, water, and minerals. This is a material rich in organic matter, readily degradable and thus valuable for soil enhancement. It is also a material rich in information, not only about the animal's diet, but also about the animal itself. This fact has not been lost on biologists for they have often exploited it in their re-

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search endeavors. Historically, faeces have been used as indices to animal abundance (Bennett et al., 1940; Cochran and Stains, 1961), as a means of determining diet composition (Baumgartner and Martin, 1939; Adams et al., 1962; Anthony and Smith, 1974; Smith et al., 1998; Saint-Andrieux et al., 2000), or to make inferences about nutrient density (Hodgeman et al., 1986; Leite and Stuth, 1990; Irwin et al., 1993; Cook et al., 1994; Lewis, 1994; Plumptre and Harris, 1995; Kucera, 1997; Osborn et al., 1997; Osborn and Jenks, 1998; Osborn and Ginnett, 2001). More recently, faecal hormone assays have been used to monitor reproductive status (Brown et al., 2001; Graham et al., 2001; Li et al., 2001). Today, it is possible to identify individual animals through DNA analysis of faeces (Wasser et al., 1997; Ernest et al., 2002; Smith et al., 2002).

Near infrared reflectance spectroscopy (NIRS) is a rapid, non-invasive analytical technique based on the unique absorption of light in the 700–2500 nm range by chemical bonds, primarily those involving N, C, H, and O. Faecal NIRS has been used to monitor diet quality in domestic livestock (*Bos* spp.: Coleman et al., 1989; Coates, 1998; Lyons and Stuth, 1992; Lyons et al., 1993; Lyons et al., 1995; Whitley, 1996; Purnomoadi et al., 1998; Ossiya, 1999; Ksiksi et al., 2000; Gibbs et al., 2002; *Capra hircus*: Leite and Stuth, 1995; Ossiya, 1999; *Ovis aries*: Ossiya, 1999; Krachounov et al., 2000) as well as white-tailed deer (*Odocoileus virginianus*, Gallagher, 1990; Showers, 1997) elk (*Cervus elaphus*, Brooks et al., 1984; Keating et al., 2001), and roan antelope (*Hippotragus equinus*, Dorgeloh et al., 1998). Dietary tannin content (Ossiya, 1999; Tolleson et al., 2000a) has been determined as well as intake of pine (*Pinus ponderosa*) needle (Kronberg et al., 1998), leafy spurge (*Euphorbia esula*, Walker et al., 1998) and mountain big sagebrush (*Artemisia tridentata*, Walker et al., 2000) using this technique.

The potential for faecal NIRS to determine physiological status in grazing animals has been explored. For instance, gender has been successfully determined in cattle (Tolleson et al., 2000b), sheep (Godfrey et al., 2001) and several species of wildlife (Tolleson et al., 2001a). Likewise, both gender and age in white-tailed deer (Osborn et al., 2002) were categorized. Tolleson et al. (2000a) reported differences in faecal near infrared (NIR) spectra between pregnant and ovariectomized cattle. Godfrey et al. (2001) observed similar differences between pregnant and lactating sheep. Faecal NIRS has also been applied to pregnancy determination in cattle (Tolleson et al., 2001b,c), but was unsuccessful in predicting serum progesterone (Tolleson et al., 2001d). Additionally, differences in faecal NIR spectra have been observed in both cattle and horses (*Equus caballus*), which had an external parasite burden versus those which did not (Tolleson et al., 2000c, 2002).

In addition to the diet quality work mentioned previously, other studies have explored the application of NIRS to free-ranging herbivore ecology. Forage quality of two folivorous marsupials: the greater glider (*Petauroides volans*) and the common ringtail possum (*Pseudocheirus peregrinus*) has been predicted with NIRS (McIlwee et al., 2001). Wallis and Foley (2003) recently validated this procedure for the ringtail possum. Similar work has been reported for dietary nutritive value in the northern hairy nosed wombat (*Lasiorhinus krefftii*, Woolnough and Foley, 2002). Nutritional constituents of eucalypt species have been correlated with foraging strategies in koala (*Phascolarctos cinereus*, Foley et al., 2000). Greyling (2002) found differences in the NIR spectra of forage consumed by different sex/age classes of free-ranging elephants (*Loxodonta africanus*) in South Africa. Similarly, Lister et al. (1997) found differences in the NIR spectra of forages preferred versus not preferred by elephants in Kenya. The nutritional quality of sea-grasses consumed by dugongs (*Dugong dugon*, Aragones, 1996) was examined via NIRS, and Valdes et al. (1997) determined nutritional characteristics of fish fed to zoo animals by this technique. Foley et al. (1998) have eloquently argued the case for the use of NIRS in the field of wildlife ecology.

Morphological characteristics of red (*Cervus elaphus*) and fallow (*Dama dama*) deer fecal pellets have been studied (Alvarez, 1994) and can be used to identify species, sex and age classes. Morphological changes in pellets between year and season within a class could however, complicate the use of this method. Non-morphological characteristics could provide a more reliable method of identification. We hypothesized that non-morphological differences exist in the faeces of red and fallow deer, and between males and females of both species. Further, we proposed that these differences could be detected by NIRS. If true, faecal NIRS could be added to the repertoire of tools used by re-

source managers seeking reliable non-invasive methods to monitor the nutrition and physiology of free-ranging cervids.

2. Materials and methods

A total of 68 faecal grab samples (Table 1) were collected in April of 2000 and 2002 from individual male and female, red or fallow deer maintained on ryegrass (*Lolium perenne* L.) pastures at the Texas Agricultural Experiment Station in Overton, Texas, United States. In both years, samples were collected under similar (by visual observation) forage conditions. All animals were adults (>1.5 years) and females were pregnant (>90 days) at the time of sampling. Samples were stored at −40 °C until further processing. After thawing at room temperature, samples were dried at 60 °C for 12 h and ground to pass a 1 mm screen. Ground samples were re-dried at 60 °C for 12 h prior to analysis by NIRS. Near infrared spectra were obtained on a Foss 6500 scanning monochrometer in the 1100–2498 nm range.

Discriminant equations to determine group membership were developed using WinISI v. 1.04a® software. This procedure utilizes two-block partial least squares (Martens and Martens, 2001) to predict a set of indicator variables identified with the calibration spectra. Indicator variables in the calibration set are assigned thusly: a sample belonging to group A (e.g. male) would be represented as {2, 1} in the algebraic matrix; a sample belonging to group B (e.g. female) would be {1, 2}. A prediction of an "unknown" sample produces estimates for both indicator variables. The sample is assigned to the group with the higher predicted indicator variable value.

Table 1
Number of samples collected by year, species, and sex

Group	*N*
Year	
2000	39
2002	29
Species	
Red	35
Fallow	33
Sex	
Male	33
Female	35

For instance, if the predicted values for sample X are {1.8, 1.1}, membership would be assigned to group A. The closer the value to 2.0, the stronger the indication of group membership. In this study, a predicted indicator value greater than 1.5 was required for a "correct" determination of sex or species. The standard error (S.E.) for discriminant equations developed by this method represents the precision associated with prediction of these indicator variables. Standard errors for discriminant equations will be reported without units in subsequent tables. A predicted value plus or minus the S.E. defines the 95% confidence interval for that predicted value.

Due to the small number of samples, validation samples were not withheld from calibration for comparisons within year. In these instances, cross validation (Martens and Martens, 2001) was employed. For comparisons across years, in addition to cross validation, random samples were chosen and withheld from calibration. These samples were subsequently predicted with the appropriate discriminant equations. To determine if any spectral differences observed should be attributed to biological characteristics and not random chance, a discriminant equation was developed with all odd versus all even numbered samples, irrespective of sex or species. Faecal N was determined by NIRS (Tolleson et al., 2004). Analysis of variance procedures were used to identify differences in faecal N between groups.

3. Results

Results from discriminant equations developed with the 2000 data set are given in Table 2. Accuracy of an NIRS equation is usually judged against a laboratory reference method and is considered adequate if S.E. of prediction is less than 2× the S.E. of the reference method (Shenk and Westerhaus, 1994). In this case, visual gender determination is 100% accurate, so S.E. would be zero. The S.E. for this discriminant procedure would be associated with the numerical matrix predicted in the two-block PLS. So the S.E. of calibration (SEC) is indicative of how accurately the 1s and 2s are assigned in the matrix. An SEC of 0.15 indicates a 10% error around the 1.5 predicted value required for group membership.

Table 2
Discriminant equation performance statistics: year 2000

Comparison	N	R^2	SEC[a]
Year 2000			
Species	39	0.97	0.09
Sex	39	0.90	0.16
Red deer			
Sex	19	0.86	0.18
Fallow deer			
Sex	20	0.92	0.14
Male			
Species	20	0.97	0.08
Female			
Species	19	0.75	0.25

[a] Standard error of calibration.

Table 3
Discriminant equation performance statistics: year 2002

Comparison	N	R^2	SEC[a]
Year 2002			
Species	29	0.98	0.07
Sex	29	0.98	0.08
Red deer			
Sex	16	0.93	0.14
Fallow deer			
Sex	13	0.96	0.10
Male			
Species	13	0.82	0.21
Female			
Species	16	0.92	0.14

[a] Standard error of calibration.

Discriminant equation statistics (Table 2) indicate acceptable performance. When the equations derived from these samples were applied to the 2002 samples, however, very poor discriminations were observed. The majority of samples were classified as belonging to either fallow deer or females. To summarize, the year 2000-derived equations correctly classified 6.25% (1/16) of samples collected from red deer, 69.23% (9/13) from fallow deer, 0% (0/13) from males and 100% (16/16) from females. We propose that even though sampling occurred at visually similar forage conditions, diet quality between years was sufficiently different that the small calibration set was not able to accurately discriminate between samples from a different year. A cattle-derived NIRS equation for percent faecal N (Tolleson et al., 2004) indicated that percent faecal N in 2000 was 3.03 ± 0.10 S.E. versus 2.57 ± 0.07 in 2002 ($P < 0.001$).

Results from the discriminant equations developed with the 2002 data set are given in Table 3. Again, equation performance statistics were acceptable, but applying the 2002 equations to the 2000 samples yielded poor results. Just as occurred in 2000, most samples were classified as belonging to female fallow deer. To summarize, the year 2002-derived equations correctly classified 15.79% (3/19) of samples collected from red deer, 100% (20/20) from fallow deer, 0% (0/20) from males and 100% (19/19) from females. The data sets were then combined and a random set of validation samples (n = 5 per group) removed from the calibration set. The validation sets are admittedly small, but still provide some indication of equation performance while allowing adequate sample numbers for calibration. The results of all combined discriminant equations are given in Table 4, validation results in Table 5. Combining data across years resulted in equations which were able to account for differing forage conditions and still discriminate between species and gender.

Results of the odd versus even sample number discriminant equation were: R^2 = 0.051, SEC = 0.487. Validation results were two out of five (40%) correct for both the odd and even sample number groups.

Table 4
Discriminant equation performance statistics: years combined

Comparison	N	R^2	SEC[a]
Year 2000 and 2002			
Species	58	0.89	0.16
Sex	58	0.99	0.05
Red deer			
Sex	30	0.93	0.13
Fallow deer			
Sex	28	0.92	0.14
Male			
Species	28	0.88	0.17
Female			
Species	30	0.96	0.11

[a] Standard error of calibration.

Table 5
Discriminant equation validation results

Comparison	Group	# Correct/total
Year 2000 and 2002		
Species	Red deer	5/5
	Fallow deer	5/5
Sex	Male	5/5
	Female	4/5
Red deer		
Sex	Male	2/2
	Female	3/3
Fallow deer		
Sex	Male	2/2
	Female	3/3
Male		
Species	Red deer	3/3
	Fallow deer	2/2
Female		
Species	Red deer	2/2
	Fallow deer	3/3

Number of correct classifications by group.

4. Discussion

The results of this work illustrate clear differences in faecal chemistry between male and female, red and fallow deer. These findings agree with previous studies utilizing fecal NIRS in domestic livestock (Tolleson et al., 2000b; Godfrey et al., 2001) and white-tailed deer (Osborn et al., 2002). We have made similar observations between species (American bison (*Bos bison)* versus cattle, and mule (*O. hemionus*) versus white-tailed deer, Tolleson, unpublished data) residing on the same forage resource. That faecal chemical characteristics differ between species and sexes is not surprising. The physical appearance of faeces has historically been used to aid animal identification in the wild (Halfpenny and Biesot, 1986).

Fecal pellet morphology has been used to determine sex and species in red and fallow deer (Alvarez, 1994). In the Alvarez study, red deer were successfully identified in 80.8% of samples while in fallow deer the success rate was 77.8%. Male fallow deer were correctly identified in 77.8% of samples and females in 75.0%. Gender discrimination was not reported for red deer. When the number of classes to be discriminated increased to all four sex/species classes at once, the percent correct in each class decreased to less than 60% in all but female fallow deer (77.8%). A decision-tree type approach to NIRS discrimination between samples with two or more levels of classification has been described (Downey, 2000).

If such a decision-tree approach were applied to the discriminant equations developed in the present study, one would first determine species, for example, and then subject the resultant groups to the specific sex within species equations. The 100% correct identifications achieved in this study with farmed animals on a monoculture diet are not likely to be obtained in a free-ranging population. Assuming a 90% success rate for independent species and sex discriminations, an 81% overall success rate would result. One would not expect the physical attributes of faeces to be completely determined by anatomical characteristics of the animal in question. Differences in metabolism or in diet selection between species or sexes would result in chemical differences of excreted material as well.

Smaller species have faster digesta passage rates and thus greater faecal nutrient loss compared to larger ones (Arman et al., 1975; Demment and Van Soest, 1985; Gross et al., 1996; Owen-Smith, 1988; Robbins, 1993). Females have been reported to have greater faecal N losses than males (Beir, 1987; Bleich et al., 1997; Padmalal and Takatsukim, 1994). Osborn and Ginnet (2001) however, found no differences in either faecal N or 2,6-diaminopimelic acid between male and female white-tailed deer, perhaps due to the smaller degree of sexual dimorphism in this species compared to those in the previously mentioned studies. In the present study, females tended ($P = 0.12$) to have higher percent faecal N than males (2.93 ± 0.10 versus 2.73 ± 0.07, respectively) while percent faecal N of red deer was 2.77 ± 0.011 versus 2.89 ± 0.06 for fallow deer ($P = 0.35$).

Factors other than the relative amounts of nitrogen present in the faeces of each group are obviously affecting the spectra. Just what these factors are and to what degree each interacts with the others cannot be determined by these data and are fact beyond the scope of the experimental design. Nonetheless, much information is available in the literature to allow for speculation.

Many physical and chemical changes occur to a meal ingested by a ruminant animal during the digestive process. Any or all of these could affect faecal characteristics. Consequently, any consistent difference in one

or more of these processes due to sex or species could create the divergence in faecal NIR spectra reported in this study.

Differential use of habitat or diet selection between male and female cervids (Apollonio et al., 1998; Conradt et al., 2001; McShea et al., 2001), or between sympatric species (Telfer, 1994) has been documented. Although both are classified as intermediate feeders (Hoffman, 1985), red deer are larger than fallow deer and both species exhibit sexual dimorphism. In the wild, diet or habitat selection could play a major role in determining faecal chemistry and would serve to accentuate more subtle differences due to metabolic influences. The subjects of this study, however, were maintained as unisex groups in monoculture paddocks. Dietary preference beyond selection for relative proportions of leaf versus stem should not have been a factor in these results.

Gross et al. (1996) reported differences in digesta retention time between male and female nubian ibex (*Capra ibex nubiana*) but similar digestibility. These authors recorded greater mastication time for females, which could have compensated for the shorter retention times. They also observed a similar relationship between sheep and goats, i.e. differences in body size across species result in differences much like the sexual dimorphism exhibited in the ibex. Kay (1987) suggested that dental pattern affects intake in mature red deer since although bite width does not increase with age, length and gape of the jaw do, thus larger, less selective bites result. Gender differences in incisor length among red deer have been reported (male > female, Clutton-Brock et al., 1982), while Weckerly (1993) observed no difference in incisor breadth of black-tailed deer (*O. hemionus columbianus*) due to body mass within sex. Molars and pre-molars from male white-tailed deer were wider and exhibited more wear than those from females (Van Deelen et al., 2000).

Other factors related to the ingestion of food should also be considered. Body mass affects foraging behavior in cattle, with larger animals having greater bite size and grazing time (Erlinger et al., 1990). Red deer stags spent more time ruminating than hinds (Clutton-Brock et al., 1982). All of these determinants would be expected to primarily affect physical properties of ingested forage. How then would these effects be manifested in changes of faecal NIR spectra? Particle size and shape create scatter in NIR light (Olinger et al., 2001). Scatter is a property of the reflectance of NIR light and in addition to the population of chemical bonds mentioned previously, contributes to the shape of NIR spectra. Thus, differences in the actual physical action of acquiring and chewing food between animals differing in size, sex or species could contribute to unique spectral properties of faeces.

Nagy and Regelin (1975) found that fallow deer (27.2%) had a greater total digestive organ weight as a percent of body weight than red deer (23.6%) but reported the tissue weight of the ruminoreticulum as a percent of body weight to be similar between the two ($2.02 \pm 0.04\%$ versus $1.96 \pm 0.09\%$, respectively). Ruminoreticular volume was reported to be lower in fallow (14% BW) than red (23% BW) deer (Prins and Geelen, 1971). Fallow deer tended to have higher total volatile fatty acids and lower pH in the rumen than red deer (Prins and Geelen, 1971). Prins and Geelen (1971) also reported greater rumen fermentation rates for fallow compared to red deer due to the lower acetate:propionate ratio of the former (1.96 versus 3.09, respectively).

Coarse forage is contained in the rumen by rumen pillars and the reticulo-rumen fold. It is here that ruminal contractions and microbial action reduce particle size to that which can pass through to the omasum. Hofmann (1985) proposed that the reticulo-omasal orifice controls ruminal outflow, and due to it's location at the end of the muscular lips of the reticular groove, that its size can be regulated, thus affecting faecal particle size. Reticulo-omasal orifice form and function is thus a potential species dependant characteristic that would result in different products of microbial fermentation entering the omasum. Bulk or roughage feeders exhibit the highest level of omasum development, while conversely, concentrate selectors display the least. As intermediate feeders, these two cervids posses a medium sized omasum. Omasum tissue weight as a percentage of body weight was less for fallow than red deer ($0.15 \pm 0.01\%$ versus $0.19 \pm 0.01\%$, respectively), while there was no difference between species in percent of abomasum tissue on a body weight basis ($0.29 \pm 0.01\%$ versus $0.27 \pm 0.02\%$, respectively, Nagy and Regelin, 1975).

Hofmann (1985) found differences in rumen size or capacity between sexes in the species considered here, but were primarily due to season and or reproductive state. These factors were consistent between species in

this study as the males were not rutting and the females were pregnant at a similar stage (>90 days). Although the males were growing velvet antler, they were in a relatively low point in the yearly cycle of nutritional needs (compared to rutting), while females were in a moderate stage. This observation suggests that metabolic processes related to reproductive state could have contributed to the observed differences in faecal spectra.

Whereas the previous discussion provides no conclusive evidence for the observed differences in faecal NIR spectra between the sexes and species in question, we propose that any or all of the characteristics mentioned offer plausible explanations. Future studies should be conducted to systematically explore differences such as dentition, rumen architecture, or reproductive state in these and other herbivores, and how these changes may influence faecal chemistry. Armed with this knowledge, biologists will be better able to exploit the application of faecal NIRS by designing more comprehensive sampling regimes, as per the suggestion of Woolnough and Foley (2002).

The inability to discriminate groups across years in this study indicates that future calibrations to be applied in free-ranging populations should incorporate as much dietary diversity as would be expected to occur for the animals in question. Temporal variation should also be considered to allow for animal related effects due to changes in physiology (e.g. reproductive state). Combining the two data sets in this study appeared to overcome the effects of forage conditions on faecal spectra.

5. Conclusions

Non-invasive monitoring of free-ranging herbivores, such as that demonstrated by this research, could have far-reaching implications. Faecal analysis would seem to be a logical method for use in widely dispersed or herd animals and in situations where handling or capture is undesirable, impractical, or dangerous. If further development of sex and species discrimination by faecal NIRS yields results similar to those reported here, this technique could prove to be a valuable addition to the methodology currently employed by managers and biologists alike. Add to this the possibility of determining not only species and sex, but diet quality, age, and reproductive or parasite status, all from the same sample and the value of such a technique becomes even more evident. In addition to population demographic data, resource utilization and competition studies would also benefit from refinement of the faecal NIRS method. For instance, water source utilization or conflict between native and exotic species, or by sex and age classes within a species would seem to be a logical application of this analytical technique. No doubt there are many others.

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