Use of near-infrared spectroscopy to predict energy content of commercial dog food

M. Hervera,*2 C. Castrillo,† E. Albanell,‡ and M. D. Baucells*

*Animal Nutrition, Management and Welfare Research Group, Universitat Autònoma de Barcelona, Cerdanyola (Barcelona) 08193, Spain; †Departamento de Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, Zaragoza 50013, Spain; and ‡Ruminant Research Group, Universitat Autònoma de Barcelona, Cerdanyola (Barcelona) 08193, Spain

ABSTRACT: Near-infrared spectroscopy (NIRS) is used in the pet food industry for rapid assessment of several macronutrients and GE content, but there is little published data on its usefulness for evaluating GE and GE digestibility (GED) of commercial pet food. Using NIRS spectra of 71 commercial extruded dog foods and reference values determined with calorimetry and in vivo feeding trials, chemometric models were developed for GE, GED, and DE prediction. The SE and R² of cross-validation were 0.30 MJ/kg DM and 0.93 for GE, 2.10% and 0.82 for GED, and 0.53 MJ/kg DM and 0.92 for DE. The results indicated that NIRS provides GE, GED, and DE estimation values for dog food with an accuracy similar to that of the 2006 NRC proposed equations for use in pet food. Near-infrared spectroscopy is a fast and accurate method for predicting energy content in commercial extruded dog food, and is a useful and reliable tool to be used by the pet food industry when a wide enough calibration set is available.

Key words: digestible energy, dog food, energy digestibility, gross energy, National Research Council, near-infrared spectroscopy

INTRODUCTION

Near-infrared spectroscopy (NIRS) is useful for feed evaluation. It is fast and avoids chemical reagents and producing chemical waste. The NIRS technique can be used in feed formulation and for quality control to estimate total and available nutrients and energy content of ingredients and compound feeds. Also, NIRS can quantify a range of characteristics in many different ingredients (Leeson et al., 2000). A lack of comprehensive data on availability of various nutrients in feedstuffs and feeds has hampered the use of NIRS for estimating content of available nutrients for many species of animals. Several studies indicate that NIRS is useful for estimating nutritive and energetic content of feed ingredients and diets for poultry (Valdes and Leeson, 1992, 1994), swine (Aufrère et al., 1996; Van Barneveld et al., 1999), rabbits (Xiccato et al., 1999, 2003), and ruminants (Aufrère et al., 1996; De Boever et al., 2003). However, few studies have been published on using NIRS to assess composition and nutritive value of pet food, probably because in vivo data for a robust calibration are difficult to obtain. Castrillo et al. (2005) obtained precise calibration equations by using 56 commercial extruded dog foods to predict proximate analysis, nutrient digestibilities, and energy composition. More recently, Alomar et al. (2006) obtained calibration equations for proximate analysis and amino acid and trace element concentrations using 59 dry dog foods with accurate results. Further research is needed to determine the usefulness of this technique for predicting available energy in pet food. The current GE and DE prediction proposal in pet foods is based on the proximate analysis of food and, therefore, requires from chemical analysis. Moreover, its accuracy is limited in foods with high crude fiber (CF) content. Our aim for this study was to assess the usefulness of NIRS for predicting energy content of commercial extruded dry dog food.

MATERIALS AND METHODS

The protocol used in feeding trials suggested by the European Pet Food Industry Federation (FEDIAF, 2008) was approved by Universidad de Zaragoza.

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2Corresponding author: marta.hervera@gmail.com

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Reference Samples Analysis

A set of 71 commercial extruded compound dog foods were used in this study for calibration development. The apparent digestibility of energy of each food was determined from in vivo feeding trials according to the protocol suggested by FEDIAF (2008). Each feeding trial was performed using 6 healthy female Beagle dogs with ages ranging from 2 to 7 yr, BW ranging from 12 to 15 kg, and BCS ranging from 4 to 6 on a 9-point scale. Each trial consisted of a 10-d adaptation period followed by 5 to 7 d of total feces collection. Food was given at a fixed intake of 350 g/d, which represented 1.2 to 1.6 times maintenance energy requirements, depending on BW of the animal and the energy density of the food. Food refusals and feces were collected, weighed, and recorded daily. Feces were dried at 105°C for 48 h. Ash, CP, ether extract (EE) of the hydrolysate, and CF contents of foods were analyzed according to AOAC (2007) methods 942.05, 2001.11, 954.02, and 978.10, respectively, and N-free extract (NFE) was calculated by difference according the Weende method. Gross energy content of foods and feces was measured by adiabatic calorimeter (IKA C-4000; Janke-Kunkel, Staufen, Germany). The sample distribution of measured GE, GED, and DE from the calibration set is shown in Figure 1.

Models were developed to predict GE content (MJ/kg DM), GED (%), and DE content (MJ/kg DM). The accuracy of predictions from NIRS was compared with that obtained from using the prediction equations published in the latest edition of the NRC (2006) manual.

From the proximate analysis, GE, GED, and DE were estimated using the following NRC (2006) equations:

\[
\text{GENRC} = 23.8 \times \text{CP} + 39.7 \times \text{EE} + 17.1 \times (\text{NFE} + \text{CF}),
\]

\[
\text{GED}_{\text{NRC}} = 91.2 \times 1.43 \times \text{CF},
\]

\[
\text{DENRC} = \left( \frac{\text{GED}_{\text{NRC}}}{100} \right) \times \text{GENRC},
\]

in which \(\text{GENRC}\) is GE predicted from NRC (2006), \(\text{GED}_{\text{NRC}}\) is GE digestibility predicted from NRC (2006), \(\text{DENRC}\) is DE predicted from NRC (2006), and CP, EE, NFE, and CF are percentages.

Five samples from the whole set, with CF content over 8%, were not taken into account for \(\text{GED}_{\text{NRC}}\) and \(\text{DENRC}\) calculations, as recommended by the NRC (2006); therefore, estimated values from the NRC (2006) proposal were obtained from 66 foods of the whole pool of calibration used.

Near-Infrared Spectroscopy Analysis and Calibration Development

Fresh food samples were milled through a 1-mm sieve and were scanned twice in reflectance mode in the spectrophotometer (FOSS NIRSystem 5000; NIRSys-
Table 1. Chemical composition, GE content, apparent GE digestibility, and DE content determined by feeding trials of commercial extruded dog food set (n = 71) used for near-infrared spectroscopy calibration

<table>
<thead>
<tr>
<th>Item</th>
<th>Range</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical composition, g/100 g of food on DM basis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>15.80 to 39.60</td>
<td>27.96</td>
<td>3.84</td>
</tr>
<tr>
<td>Ether extract</td>
<td>5.89 to 26.14</td>
<td>15.04</td>
<td>4.89</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>0.61 to 10.15</td>
<td>2.67</td>
<td>2.27</td>
</tr>
<tr>
<td>Nitrogen free extract</td>
<td>31.76 to 64.50</td>
<td>47.01</td>
<td>6.38</td>
</tr>
<tr>
<td>Energy content, MJ/kg of food on DM basis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GE</td>
<td>19.37 to 24.66</td>
<td>21.37</td>
<td>1.13</td>
</tr>
<tr>
<td>DE</td>
<td>13.65 to 21.31</td>
<td>18.04</td>
<td>1.85</td>
</tr>
<tr>
<td>GE, %</td>
<td>68.76 to 90.87</td>
<td>84.25</td>
<td>4.85</td>
</tr>
</tbody>
</table>

Results and Discussion

Chemical Composition and Energy Value of Food Samples

Chemical composition and measured GE, GED, and DE of compound foods used for calibration are presented in Table 1. The chemical composition and energy content of the food samples extended over a broad range that covered most commercial compound extruded foods usually fed to pet dogs.

Spectral Characteristics of Samples

Figure 2 shows the average, minimum, and maximum spectra of the studied pet foods. Spectra show peaks at 1,726, 1,762, 2,308, and 2,348 nm, which is assumed to be related to absorption by C–H bond groups in lipids (Mark, 2001; Miller, 2001). In the set of foods used, samples with greater GE (over 22 MJ/kg DM), which are also the richest in fat, showed sharper peaks at those wavelengths (represented in the maximum spread spectrum of Figure 2) compared with the average spectrum. Spectra also showed peaks at 1,210 nm, corresponding to absorption by O–H groups in carbohydrate (Miller, 2001), which represent cereal flours and fibrous sources that are often included in variable proportions as ingredients in dog food. Commercial dog food protein content was reflected in a peak at around 2,062 nm, corresponding to N–H deformation and stretching modes in protein (Murray and Williams, 1987). Peaks corresponding to absorption bands for water occurred at 1,472 and 1,938 nm.

![Figure 2](image-url)
Development of Calibration Equations

Raw log 1/Rf data were corrected for the effects of scatter using standard normal variance (SNV) and detrend (DT) and transformed into second or third derivatives. The data processing system chosen was the one that optimized the SECrV. Many math processing combinations (derivate order, gap over which the derivative is calculated in nanometers, first smoothing interval in nanometers, and second smoothing interval) used in compound feeds and foods NIRS calibrations were tested, and the combinations used were those reaching the greatest R²CrV and lesser SECrV. Table 2 shows the statistics of selected equations and math processing used, the optimum MPLS factors used for prediction, and the number of outliers found during cross-validation. Optimum scatter corrections were obtained using SNV and DT in all calibrations performed.

The largest R²CrV and smallest SECrV were obtained with third derivative processing for GE and GED calibrations and with second derivative processing for DE calibration, and those transformations were fixed. However, similar results were obtained with second derivative processing in GE and GED calibrations, which were frequently used by different authors for calibration to predict GE and DE content of feeds with variable gap and smoothing intervals (Aufrère et al., 1996; Van Barneveld et al., 1999; Xiccato et al., 1999; Kays and Barton, 2002).

Three (for GE and GED) and 4 (for DE) spectrum data samples were identified as outliers by the residuals Student’s t-test during cross-validation and were not taken into account for the development of calibration equations. The MPLS factors used for the prediction of GE, GED, and DE were 3, 4, and 4, respectively, explaining 95, 89, and 94% of the spectral variation.

The MPLS loading plots show the regression coefficients of each wavelength to the constituent of interest and indicate which of these constituents are important in developing a model. Large intensities in the MPLS loading plots are suggested to be associated with areas of the spectrum of known chemical origin (Miller, 2001). The MPLS loadings for GE calibration had large intensities related to C–H groups from carbohydrates at 2,200 to 2,400 nm in all loads. In loads 1 and 3 (Figure 3) significant variation occurred at 1,724, 1,756, and 2,300, 2,340, and 2,356 nm related to first overtone of C–H stretch and combination peaks in lipids (Osborne and Fearn, 1983).

The GED MPLS loadings had the largest intensities in the same absorption regions; however, large peaks

![Figure 3. Loading near-infrared spectroscopy spectra for the optimum modified partial least squares factors of GE, GE digestibility (GED), and DE of cross-validation with 71 commercial extruded dog foods.](image-url)
at 2,332 nm occurred in all loads corresponding to the cellulose absorption band. In DE calibration, the largest intensities were observed at 1,724, 2,308, and 2,348 nm in all loads and at 1,212 nm in loads 1 to 3 (Figure 3) related to C–H stretch groups in lipids (Osborne and Fearn, 1983). Significant variations were also observed at 2,324 nm corresponding to starch absorption bands and at 1,980 and 2,052 nm in Load 2 (data not shown) corresponding to protein absorption bands (Murray and Williams, 1987). Large intensities were detected in all loads at 1,924, 2,332, and 2,364 nm, which correspond to a strong cellulose absorption band (Clark and Lamb, 1991; Coleman and Murray, 1993; Mark, 2001).

The MPLS loadings for energy content (GE and DE) revealed the relevance of wavelengths related to mainly starch and lipid absorption bands. This may be because EE contributes significantly more to GE of food than protein or carbohydrate (39.3 kJ/g for lipids vs. 23.8 kJ/g for protein and 17.1 kJ/g for carbohydrates; NRC, 2006) and are usually found in commercial dog food in large amounts (EE in our dataset was 15.04 ± 4.89 g/100 g DM; Table 1). Commercial dog food usually includes large amounts of cereals flours rich in starch as energy sources.

Large intensities of MPLS loadings were also found in spectra regions related to O–H and C–O groups in carbohydrates and to cellulose in DE calibration, which was probably due to the negative effect of fiber components on energy digestibility in dog food as shown in the literature (Castrillo et al., 2001; Laflamme, 2001; NRC, 2006). Protein, because it contributes to the caloric value of the food as well, was also shown to influence food energy content prediction although less intensely than the other food components.

These spectra results are consistent with those reported for compound feeds (Aufrère et al., 1996; Valdés and Leeson, 1992; Xiccato et al., 1999) and cereal food products (Kays and Barton, 2002) for other species, although previous research indicated that starch contributed to the DE prediction more than fat, which is likely a result of the greater starch and lesser fat content in compound feeds for animals other than dogs.

The overall SECrV and R2CrV, using 6 cross-validation groups, were 0.30 MJ/kg DM and 0.93 for GE, 2.10% and 0.82 for GED, and 0.53 MJ/kg DM and 0.92 for DE, respectively (Table 2). Obtained values for RER and RPD had an excellent prediction accuracy for GE and DE and good accuracy for GED according Shenk and Westerhaus (1991).

The SECrV and R2CrV for GE, GED, and DE were in the range of those reported for rabbit compound feeds (Xiccato et al., 2003), ruminant and swine compounds feeds (Aufrère et al., 1996), dog food (Alomar et al., 2006; Castrillo et al., 2005), and cereal products for human consumption (Kays and Barton, 2002).

In any case, NIRS prediction of GE, GED, and DE of dog foods gave satisfactory results, with large R2CrV and relatively small SECrV, and with RER and RPD ratios larger than the minimum recommended for prediction uses (RER over 10 and RPD over 3; Williams and Sobering, 1996) except for GED. The less precise digestibility estimation compared with GE is expected because prediction of the energy availability is more complex and depends on feed characteristics and animal response to the feed. The reduced accuracy obtained for GED values predicted from NIRS spectrum may also be explained by sample distribution. The set of samples used in this study reflected the commercial supply of pet food, which has a limited variability in digestibility. Even though samples with large CF content (from 6 to 10%) and consequently smaller GED values were included in this study (Table 1), most samples (84%) fell in a range of digestibility between 80 and 91%. The majority of spectra samples (70%) had H less than 0.6, which considers spectrums equivalent (Shenk and Westerhaus, 1991). A larger number of samples with more extreme compositions would allow good accuracy throughout the calibration range. With the samples used here, uncertain accuracy may be expected in predicting samples of extreme composition (outside the range). Xiccato et al. (2003) reported larger R2 (0.92 vs. 0.89) as well as larger RER and RPD ratios using a calibration set of samples with a wider range of GE content than in previous work (Xiccato et al., 1999) with a limited set of samples (SE of 0.7 vs. 0.3 MJ/kg DM).

Comparison of Predicted and In Vivo Data

The accuracy of NIRS prediction was compared with the accuracy of the prediction equations currently proposed for commercial extruded dog food (NRC, 2006). Table 3 shows the relationship between measured (obtained from in vivo trials) and predicted GE values (obtained either by the predicting equations using the chemical composition of feeds or by NIRS) and the resulting equations after fitting the values to a linear model.

The predicted GE values explained over 80% of the variation of measured GE with both NIRS analysis and the NRC (2006) approach. The slopes of the equations did not differ from the unit and the ordinates at the origin were also not different from 0. However, a greater R2 (0.88 vs. 0.81) and a lesser CV (1.89 vs. 2.52%) were obtained via NIRS analysis prediction compared with the NRC (2006) approach (Table 3). Predicted GE differed more than 2.5% from measured values in 38% of the samples using NRC (2006) approach and only in 7% of the samples using NIRS analysis.

The equations comparing GED (%) measured by in vivo feeding trials with estimated NRC (2006) values...
Once NIRS calibration for the prediction of GE, GED, and DE content of dog foods is developed, the determination of energy by NIRS for new dog food samples does not require further sample preparation other than grinding and sample disposal. The scanning process does not take more than 3 min for each sample analyzed. By contrast, an in vivo feeding trial takes 1 wk of feces collection plus the time to adapt the animals. Moreover, the NRC (2006) estimation approach involves chemical analyses of food, which requires several hours of work or, if based on label information, introduce a source of error as discussed elsewhere (Hill et al., 2009). Therefore, NIRS is a fast method and at least as accurate as the currently recommended predictive equations (NRC, 2006) for predicting GE, GED, and DE in commercial extruded dog food, and it can be considered a useful and reliable technology to be used by the pet food industry when an adequately broad calibration set is available.

Near-infrared spectroscopy is a rapid method, which does not consume reagents, for evaluating GE and DE of dry extruded dog food. In addition, estimates from NIRS methods are more accurate than estimations obtained using NRC (2006) proposed equations, which are the prediction methods currently recommended by FEDIAF and Association of American Feed Control Officials and used by the pet food industry. The main limitation of NIRS is the difficulty of obtaining a set of samples with a wide range of composition and uniform enough to obtain a robust calibration.

<table>
<thead>
<tr>
<th>Expression</th>
<th>n</th>
<th>R²</th>
<th>SE</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>GED(<em>{\text{NIRS}}) (MJ/kg DM) = 6.12 (± 0.78) + 0.69 DE(</em>{iv})</td>
<td>66</td>
<td>0.80</td>
<td>0.56</td>
<td>2.98</td>
</tr>
<tr>
<td>GED(_{\text{NRC}}) (%) = 6.11 (± 2.51) + 0.31 GED</td>
<td>66</td>
<td>0.63</td>
<td>0.90</td>
<td>1.02</td>
</tr>
<tr>
<td>DE(<em>{\text{NIRS}}) (MJ/kg DM) = 0.80 (± 0.85) + 0.95 DE(</em>{iv})</td>
<td>66</td>
<td>0.85</td>
<td>0.73</td>
<td>4.04</td>
</tr>
<tr>
<td>DE(<em>{\text{NRC}}) (MJ/kg DM) = 6.12 (± 0.78) + 0.69 DE(</em>{iv})</td>
<td>66</td>
<td>0.80</td>
<td>0.56</td>
<td>2.98</td>
</tr>
</tbody>
</table>

1^GE\(_{\text{NIRS}}\) = GE predicted from near-infrared spectroscopy calibration equations; GED\(_{\text{NIRS}}\) = GE digestibility predicted from near-infrared spectroscopy calibration equations; DE\(_{iv}\) = DE measured from feeding trials; DE\(_{\text{NRC}}\) = DE predicted from NRC (2006).

(from the equation GED = 91.2 – 1.43 × CF in DM basis) and with NIRS analysis resulted in a greater R² (0.79 for NIRS analysis vs. 0.63 for NRC approach) but a lesser CV (2.56% for NIRS analysis vs. 1.02% for NRC approach) for the NIRS analysis compared with the NRC (2006) approach (Table 3).

Linear regression of DE estimated according to the NRC (2006) equations and DE determined in vivo showed an ordinate at the origin different from 0 and a slope different from the unit (P < 0.001). Predicted NRC (2006) values overestimated in vivo values of those foods with GED below 89.6%, which represent 86% of the total data, and overestimated GED values of foods with GED above 89.6%. A similar trend was observed with NIRS predicted DE values when linear regression between predicted and measured values was performed, although the ordinate in the origin did not differ from 0 and the slope did not differ from the unit. Moreover, 27% of NRC (2006) predicted GED values differed from in vivo values over 5% compared with only 7% of NIRS predicted GED values.

Similar to what happened with GED, NIRS predicted DE values resulted in a greater R² (0.85 for NIRS analysis vs. 0.80 for NRC approach) but also a greater CV (4.04% for NIRS analysis vs. 2.98% for NRC approach) when compared with the NRC (2006) predicted values (Table 3). The NRC (2006) prediction tended to overestimate the DE content of those foods with DE content less than 19.74 MJ/kg DM and tended to underestimate the DE content of foods with greater DE content. This was not the case with NIRS prediction because the slope of the equation did not differ from the unit and the ordinate at the origin was also not different from 0. The 21 and 14% of predicted values from NRC (2006) approach and NIRS analysis, respectively, differed over 5% from in vivo values.

### LITERATURE CITED


Near-infrared spectroscopy for dog food


