The use of near infrared reflectance spectroscopy (NIRS) for prediction of the nutritive value of barley for growing pigs

M.E.E. McCann[1,2]†, K.J. McCracken[1] and R.E. Agnew[2]

[1]*Department of Agricultural and Environmental Science, Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX*
[2]*Agri-Food and Biosciences Institute, Hillsborough, Co. Down BT26 6DR*

There is a need in the feed industry for a rapid means of evaluating the nutritive value of feeds and feed ingredients. Chemical analysis provides only basic information and most of the laboratory techniques take too long for this information to be of use in feed formulation at the feed mill. Near infrared reflectance spectroscopy (NIRS) has been proposed as an alternative means of predicting nutritive value. In this study, NIRS was used to predict the digestible energy (DE) concentration and *in vitro* ileal digestibility of crude protein (CP) and total-tract digestibility of energy of locally produced barley. The calibration and validation statistics were developed using modified partial least squares (MPLS). Derivatisation and scatter correction procedures were carried out to reduce interference from external effects. The correlations between actual and predicted DE values, based on both calibration (R^2 0.93) and validation (R^2 0.69), were strong with corresponding low standard errors of calibration (SEC) and cross validation (SECV) (SEC 0.128, SECV 0.279). Strong correlations were also observed between predicted and actual *in vitro* digestibility values for both calibration and validation exercises. It was noted that validation weakened the correlations (R^2 0.73 vs. 0.50 for *in vitro* ileal digestibility of CP and 0.80 vs. 0.68 for *in vitro* total tract digestibility of energy) and fractionally increased the standard errors (0.016 vs. 0.020 for *in vitro* ileal digestibility of CP and 0.018 vs. 0.024 for *in vitro* total tract digestibility of energy). The correlations obtained by cross validation of the lowest SECV equations were not significantly different to those obtained by the scatter correction treatments. The strong relationships and low standard errors obtained between the actual and predicted values indicates that NIRS may be of use in predicting the nutritive value of barley for growing pigs, although more research is required to include larger sample sets.

Keywords: Barley; near infrared reflectance spectroscopy; pigs

---

†Corresponding author: elizabeth.mccann@afbini.gov.uk

Introduction

Near infrared reflectance spectroscopy (NIRS) has been applied in the feed industry to predict a range of characteristics for a wide range of feed ingredients. In general, NIRS has been shown to be accurate in predicting the chemical composition of feeds and feed ingredients. Marten, Halgerson and Cherney (1983) used NIRS to predict the chemical composition of grain forages at diverse maturation stages and found that the predicted values (obtained via validation of 652 samples) for the concentrations of crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and for *in vitro* dry matter (DM) digestibility were highly correlated with analytical values (R^2 0.99, 0.94, 0.98 and 0.92, respectively). Kays, Barton and Windman (2000) conducted a study to investigate the potential of NIRS for the analysis of protein in a dataset that included products with numerous cereal grains processed by a number of methods. These workers reported that NIRS predicted the nitrogen concentration with a high correlation to chemical analysis (R^2 0.98). Garnsworthy, Wiseman and Fegeros (2000) predicted the chemical, nutritive and agronomic characteristics of wheat by NIRS and also reported that the predictions of chemical constituents were very accurate. The calibration coefficients (R^2) were 0.94 for DM, 0.90 for CP, 0.97 for ash, 0.78 for starch and 0.98 for oil. Agronomic characteristics were also predicted very accurately with the R^2 for grain hardness, bushel weight and thousand-grain weight being 0.98, 0.80 and 0.99, respectively. However, the prediction of nutritive value by NIRS was less accurate in that the correlation between predicted and actual values for digestible energy (DE) concentration and nitrogen digestibility were 0.17 and 0.22, respectively. This finding contradicted that of Aufrere *et al.* (1996), who used NIRS to predict chemical composition and DE concentration in pig diets and reported that there were weak predictions for concentrations of CP (standard error of calibration (SEC) 7.96 g/kg DM), starch (SEC 13.36 g/kg DM), NDF (SEC 13.19 g/kg DM) and ADF (SEC 6.17 g/kg DM), but that the accuracy of prediction for carbohydrate digestibility (SEC 0.97), energy digestibility (SEC 0.24) and organic matter (OM) digestibility (SEC 1.17) was very high. However, prediction of DE concentration with NIRS was no more accurate than the prediction based on chemical analysis (the standard error of prediction being 0.37 MJ/kg organic matter (OM) compared with 0.30 MJ/kg OM). George (2000) predicted the apparent metabolisable energy (AME) concentration of 30 wheat samples using NIRS and reported correlation coefficients between predicted and actual values of >0.90. However, when these results were validated using a set from the previous year, poor correlations were observed and it was concluded that NIRS was not an accurate means of predicting nutritive value.

Animal feeds are composed of a number of different feedstuffs combined in specific ratios to produce a final product that provides the optimum balance of amino acids and energy (Van Kempen and Simmons, 1997). The production of unbalanced feeds results either in energy being fed in relative excess to amino acids, which leads to undesirable fat accretion, or in amino acids being fed in relative excess to energy which leads to wastage of expensive amino acids and increased nitrogen pollution. It is therefore important to the feed industry to have a rapid and accurate means of evaluating the nutritive value of feedstuffs. The aim of this study was to examine the value of NIRS in predicting the DE concentration and *in vitro* digestibility of barley from which pig diets were formulated.

Materials and Methods

Pig diets containing 650 g/kg barley were formulated using 39 samples of locally produced barley. These diets were fed to growing pigs and the DE concentration of the barley was calculated (McCann, 2001). *In vitro* ileal digestibility of CP and total tract digestibility of energy were determined for the barley (McCann, 2001) by the techniques of Boisen and Fernandez (1995; 1997). Details of the barley samples are given in Table 1. The

Table 1. Digestible energy (DE) concentration (MJ/kg DM), *in vitro* ileal digestibility of crude protein (CP) and *in vitro* total-tract digestibility of energy of barley (McCann, 2001)

Variety	Location of production	Year of production	DE (MJ/kg)	*In vitro* ileal digestibility of CP	*In vitro* total tract digestibility of energy
Rivera	Coleraine	1998	16.4	0.757	0.807
Rivera	Ballymoney	1998	15.3	0.719	0.754
Rivera	Comber	1998	15.2	0.724	0.809
Rivera	Ballinderry	1998	14.8	0.732	0.762
Rivera	Londonderry	1998	15.5	0.711	0.766
Rivera	Killough	1998	15.6	0.697	0.798
Dandy	Coleraine	1998	15.1	0.607	0.747
Dandy	Ballymoney	1998	15.3	0.743	0.714
Dandy	Newtownards	1998	15.4	0.692	0.704
Dandy	Ballyclare	1998	15.1	0.723	0.728
Dandy	Donegal	1998	15.0	0.681	0.684
Dandy	Castlewellan	1998	15.3	0.773	0.742
Crusader	Cork (Ballycurra)	1998	15.6	0.750	0.787
Crusader	Backweston	1998	15.8	†	†
Crusader	Athenry	1998	15.8	0.728	0.793
Crusader	Port Laoise	1998	14.9	0.735	0.768
Crusader	Cork (North)	1998	15.0	0.725	0.782
Crusader	Donegal	1998	15.7	†	0.680
Lamba	Cork (Ballycurra)	1998	15.6	0.761	0.770
Lamba	Backweston	1998	15.5	0.756	0.760
Lamba	Athenry	1998	15.9	0.738	†
Lamba	Port Laoise	1998	15.6	0.769	0.748
Lamba	Cork (North)	1998	15.5	0.745	0.794
Lamba	Donegal	1998	14.6	0.675	0.778
Rivera	Castlewellan	1999	15.9	0.687	0.796
Rivera	Greyabbey	1999	16.1	0.711	0.794
Rivera	Armagh	1999	16.3	0.698	0.781
Rivera	Coleraine	1999	15.7	0.699	0.790
Rivera	Limavady	1999	16.1	0.713	0.805
Rivera	Comber	1999	15.5	0.611	0.803
Rivera	Donacloney	1999	15.4	0.699	0.784
Rivera	Londonderry	1999	16.3	0.641	0.780
Rivera	Killough	1999	15.4	0.717	0.818
Dandy	Coleraine	1999	15.7	0.687	0.814
Dandy	Ballyclare	1999	15.8	0.707	0.778
Dandy	Newtownards	1999	16.0	0.713	0.809
Dandy	Dundrod	1999	15.8	0.726	0.745
Dandy	Strabane	1999	15.8	0.703	0.791
Dandy	Crossnacreevy	1999	15.9	0.635	0.776
	Range		14.6–16.4	0.641–0.781	0.704–0.806

†Not determined due to lack of sample.

39 samples were milled through a 1 mm screen and dried overnight prior to scanning, with samples scanned in duplicate at 2 nm intervals over the visible and near infrared spectral range (400 to 2,500 nm) using a Foss NIRSystem 6500 instrument (Perstorp Analytical, Silver Spring, Maryland, USA). Samples were scanned and spectral data recorded as log{1/reflectance} values (log{1/R}).

The spectral data for the 39 samples (n = 78 in duplicate) were subjected to a range of mathematical treatments to develop the optimum prediction methods. Appropriate cross-validation was performed by removing six groups of spectra from the population and forming a calibration on the remaining spectra and using this to predict the excluded samples. This was done several times until all the spectra were used in the validation and the standard error of cross validation (SECV) was calculated.

Mathematical treatment of the spectral data was performed using ISI – NIRS version 4.0 software (Infrasoft International, Port Matilda, PA, and USA). Calibrations were developed for DE concentration, *in vitro* ileal digestibility of CP and total-tract digestibility of energy using modified partial least squares (MPLS) (Martens and Naes, 1989). Transformations of the spectral data through derivatisation and scatter correction procedures were examined as there is evidence that these can reduce interference from external effects (e.g. particle size) (Baker and Barnes, 1990). Equations were produced using log{1/R}, first and second derivatised data (Norris and Williams, 1984). The mathematical derivatisation of 1,4,4 was used in the first order and 2,10,5 was used in the second where the first digit indicates the order of the derivative, the second the interval in nanometres over which the calculation is performed and the third digit the number of data points in a running average. The scatter correction programmes included were; WMSC (weighted multiplicative scatter correction), SNVD (standard normal variate and detrend) and NMSC (normal multiplicative scatter correction) (Park *et al.,* 1997). The MPLS regression technique combined with the various scatter correction programmes (WMSC, NMSC, SNVD) were used to obtain the statistics.

Results

Table 2 shows the calibration and validation statistics for DE. Predicted DE values

Table 2. Calibration and validation statistics for the prediction of digestible energy (DE) (MJ/kg DM) concentration of barley using modified partial least squares

Derivative option	Transformation procedure[1]	Number of observations	Statistics[2]				Terms in model
			SEC	R^2	SECV	1 - VR	
1,4,4	WMSC	73	0.128	0.93	0.277	0.69	10
1,4,4	SNVD	74	0.157	0.91	0.282	0.70	10
2,10,5	SNVD	76	0.188	0.87	0.297	0.67	9
2,10,5	NMSC	76	0.188	0.87	0.299	0.67	9
2,10,5	WMSC	75	0.241	0.78	0.312	0.63	6
1,4,4	NMSC	77	0.166	0.90	0.322	0.62	10

[1]SNVD = Standard normal variate and detrend, NMSC = Normal multiplicative scatter correction, WMSC = Weighted multiplicative scatter correction.
[2]SEC = Standard error of calibration, SECV = Standard error of cross validation; VR = ratio of residual variance to total variance.

ranged from 14.4 to 16.6 MJ/kg DM. The 1,4,4 derivative combined with SNVD gave the best result in terms of SECV (0.277). The relationship for the calibration set was strong (R^2 0.93). With validation, the relationship was lower but still strong (R^2 0.69).

The calibration and validation statistics for *in vitro* ileal digestibility of CP are presented in Table 3. The range of predicted values was from 0.641 to 0.781. The 2,10,5 derivative combined with WMSC yielded the lowest SECV value (0.020)

and strong relationships for both the calibration and validation sets (R^2 0.80 and 0.68, respectively).

For *in vitro* total tract digestibility of energy, the range of predicted values was 0.704 to 0.806 (Table 4). The strongest relationships for calibration and validation (R^2 0.73 and 0.50, respectively) were found for the 1,4,4 derivative combined with WMSC with the SECV value being 0.024.

The samples were subjected to cross validation using the 1,4,4 derivatised data for DE concentration (Table 5). The cor-

Table 3. Calibration and validation statistics for the prediction of *in vitro* ileal digestibility coefficient of CP using modified partial least squares

Derivative option	Transformation procedure[1]	Number of observations	Statistics[2]				Terms in model
			SEC	R^2	SECV	1 – VR	
2,10,5	WMSC	68	0.016	0.80	0.020	0.68	5
1,4,4	SNVD	71	0.010	0.93	0.022	0.67	11
2,10,5	NMSC	70	0.014	0.85	0.023	0.60	8
2,10,5	SNVD	70	0.014	0.85	0.023	0.60	8
1,4,4	WMSC	73	0.022	0.68	0.027	0.53	5
1,4,4	NMSC	73	0.022	0.68	0.027	0.53	5

[1,2]See footnotes to Table 2.

Table 4. Calibration and validation statistics for the prediction of *in vitro* total tract digestibility coefficient of energy using modified partial least squares

Derivative option	Transformation procedure[1]	Number of observations	Statistics[2]				Terms in model
			SEC	R^2	SECV	1 – VR	
1,4,4	WMSC	70	0.018	0.73	0.024	0.50	6
1,4,4	NMSC	70	0.018	0.71	0.024	0.49	6
1,4,4	SNVD	72	0.020	0.67	0.026	0.44	6
2,10,5	NMSC	73	0.021	0.64	0.028	0.33	6
2,10,5	SNVD	73	0.021	0.64	0.028	0.33	6
2,10,5	WMSC	73	0.021	0.63	0.029	0.31	6

[1,2]See footnotes to Table 2.

Table 5. Statistics of cross validation for digestible energy (MJ/kg DM) concentration, ileal digestibility coefficient of crude protein (CP) and total-tract digestibility coefficient of energy of barley

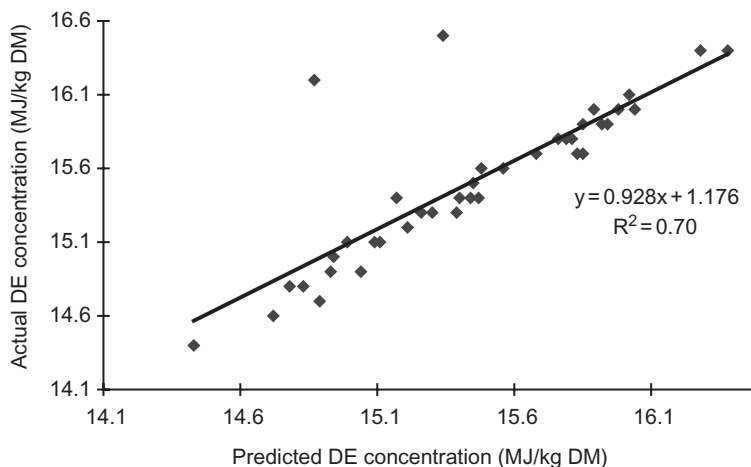|	Range	Mean	s.d.	SEP[†]	R^2
Digestible energy (MJ/kg DM)	14.4–16.4	15.4	0.47	0.29	0.70
Ileal digestibility of CP	0.641–0.781	0.715	0.0311	0.0253	0.59
Overall digestibility of energy	0.704–0.806	0.767	0.0291	0.0233	0.56

[†]Standard error of prediction.

Figure 1. Relationship between actual (y) and predicted (x) digestible energy (DE) concentrations (MJ/kg DM).

relation for calibration (R^2 0.70) between actual and predicted values was not as strong as that obtained using MLPS (Figure 1). Table 5 also shows the calibration and validation statistics for *in vitro* ileal digestibility of CP and overall digestibility of energy using the 2,10,5 and 1,4,4 derivatised data, respectively. The R^2 for correlations between actual and predicted values are shown in Figures 2 and 3.
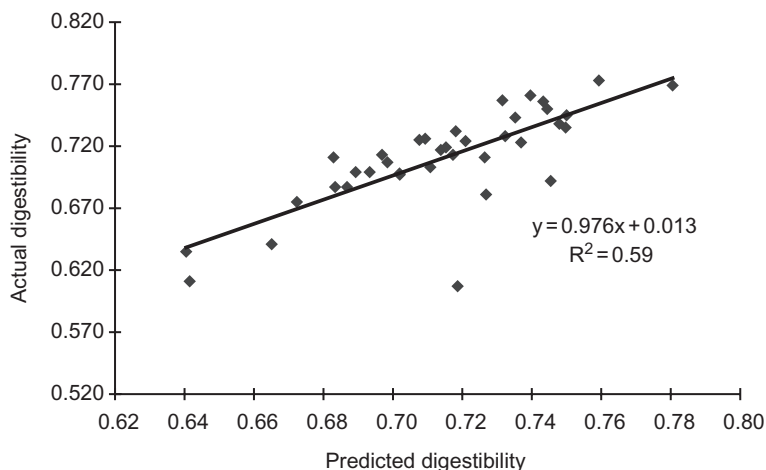

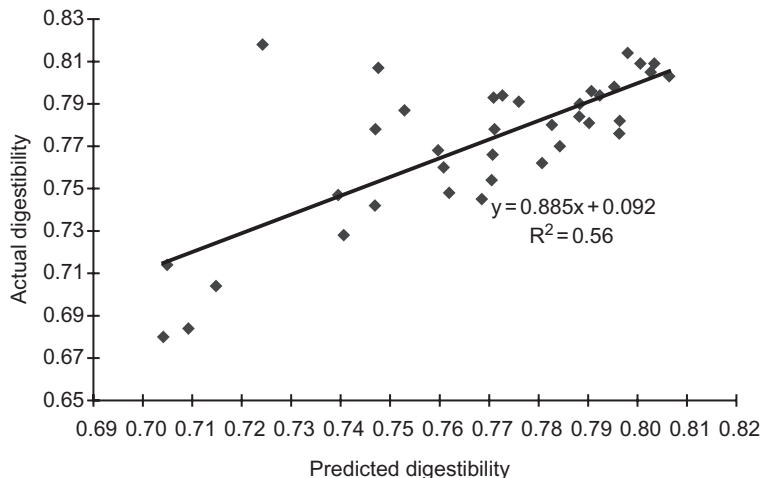*Figure 2. Relationship between actual (y) and predicted (x) in vitro ileal digestibility coefficient of crude protein.*

Figure 3. Relationship between actual (y) and predicted (x) in vitro total-tract digestibility coefficients of energy.

Discussion

The correlation between actual and predicted DE values for the calibration set was high and the correlation based on validation was also strong. High correlations between actual and predicted DE concentrations have been reported in the literature. For example, Aufrere *et al.* (1996) and Zijlstra *et al.* (1999) reported relationships of 0.87 and 0.96, respectively. However, these workers did not report the validation statistics. Xiccato *et al.* (1999) predicted the DE concentration of rabbit diets and reported the correlation between actual and predicted DE (via calibration) to be high (R^2 0.90). With validation, this relationship was weakened and the SECV increased. This effect has also been shown by George (2000) who studied the correlation between predicted and actual AME values of wheat for poultry and found that the R^2 declined dramatically (>0.90 to 0.09) between calibration and validation. This trend has been observed in the current study. However, it must be stated that the R^2 for validation is strong and the SECV relatively low. This high correlation for validation may be an effect of the small sample number as it is well known that small datasets produce high correlations due to less variability (Valdes and Leeson, 1992). Future work should include a greater number of samples in the regression equations, although this would require considerable resources to carry out the necessary *in vivo* studies.

In vitro digestibility is a means of predicting the nutritive value of feeds and feed ingredients without carrying out *in vivo* studies. There is a lack of information in the literature regarding the use of NIRS to predict *in vitro* digestibility in pigs. Van Kempen and Bodin (1998) compared the predictions obtained from NIRS for amino acid ileal digestibility and those obtained from nitrogen regression and reported that the relationship found with NIRS was stronger (R^2 0.76 vs. 0.48). Aufrere *et al.* (1996) used NIRS to predict *in vivo* digestibility of energy and reported a strong relationship between actual and predicted values with a low SEC (0.24).

However, neither of these workers carried out validation of the relationship and it is probable that these relationships would be weakened in a validation exercise. In this study, validation did weaken the relationship between actual and predicted values (R^2 0.73 vs. 0.50 for *in vitro* ileal digestibility of CP and 0.80 vs. 0.68 for *in vitro* overall digestibility of energy). However, the relationships remained relatively strong which may again be an effect of the small sample size.

The correlations obtained by the cross validation of the lowest SECV equations were similar to those obtained by the scatter correction treatments. This finding is in keeping with results of Park *et al.* (1997) who reported only slight differences in prediction using the two regression techniques. These workers found the relationship between predicted and actual organic matter digestibility and voluntary intake of silage to be 0.90 vs. 0.87 and 0.77 vs. 0.79, respectively.

It is concluded that the prediction of DE concentration, *in vitro* ileal digestibility of CP and total tract digestibility of energy using NIRS appears to be accurate as small SECV and strong correlations of validation were obtained. However, as the sample set included in the regressions was relatively small, more work is required in this area to enable firm conclusions to be drawn.

References

Aufrere, J., Graviou, D., Demarquilly, C., Perez, J.M. and Andrieu, J. 1996. Near infrared reflectance spectroscopy to predict energy value of compound feeds for swine and ruminants. *Animal Feed Science and Technology* **62**: 77–90.

Baker, C.W. and Barnes, R. 1990. The application of near infra-red spectroscopy to forage evaluation in the Agricultural Development and Advisory Service. In: Wiseman, J., Cole, D.J.A. (eds.). *Feedstuffs Evaluation*, Butterworth, London, pp. 337–354.

Boisen, S. and Fernandez, J.A. 1995. Prediction of the apparent ileal digestibility of protein and amino acids in feedstuffs and feed mixtures for pigs by *in vitro* analyses. *Animal Feed Science and Technology* **51**: 29–43.

Boisen, S. and Fernandez, J.A. 1997. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by *in vitro* analyses. *Animal Feed Science and Technology* **68**: 277–286.

Garnsworthy, P.C., Wiseman, J. and Fegeros, K. 2000. Prediction of chemical, nutritive and agronomic characteristics of wheat by near infrared spectroscopy. *Journal of Agricultural Science* **135**: 409–417.

George, J. 2000. *Studies on the relationships between physical and chemical parameters of wheat and their relevance to nutritive value*. PhD Thesis, Queen's University of Belfast.

Kays, S.E., Barton, F.E. and Windman, W.R. 2000. Predicting protein content by near infrared reflectance spectroscopy in diverse cereal food products. *Journal of Near Infrared Spectroscopy* **8**: 35–43.

Marten, G.C., Halgerson, J.L. and Cherney, J.H. 1983. Quality prediction of small grain forages by near infrared reflectance spectroscopy. *Crop Science* **23**: 94–96.

Martens, H. and Naes, T. 1989. *Multivariate Calibration*. Wiley, Chichester, UK 419 pages.

McCann, M.E.E. 2001. *Studies on the nutritive value of barley for growing pigs*. PhD Thesis, Queen's University of Belfast.

Norris, K.H. and Williams, P.C. 1984. Optimisation of mathematical treatment of raw near infrared signal in the measurement of protein in hard red spring wheat. 1. Influence of particle size. *Cereal Chemistry* **61:** 158–165.

Park, R.S., Gordon, F.J., Agnew, R.E., Barnes, R.J. and Steen, R.W.J. 1997. The use of Near Infrared Reflectance Spectroscopy on dried samples to predict biological parameters of grass silage. *Animal Feed Science and Technology* **68**: 235–246.

Valdes, E.V. and Leeson, S. 1992. Use of NIRS to measure ME in poultry feed ingredients. *Poultry Science* **71**: 1559–1563.

Van Kempen, T.A.T.G. and Bodin, J.C. 1998. Near infrared reflectance spectroscopy appears to be superior to nitrogen based regression as a rapid tool in predicting the poultry digestible amino acid content of commonly used feedstuffs. *Animal Feed Science and Technology* **76**: 139–147.

Van Kempen, T.A.T.G. and Simmons, P.H. 1997. Near infrared reflectance spectroscopy in precision feed formulation. *Journal of Applied Poultry Research* **6**: 471–477.

Xiccato, G., Trocino, A., Carrazzolo, A., Meurens, M., Maertens, L. and Carabano, R. 1999. Nutritive evaluation and ingredient prediction of compound feeds for rabbits by near infrared reflectance spectroscopy (NIRS). *Animal Feed Science and Technology* **77**: 201–212.

Zijlstra, R., Scott, T., Edney, M., Swift, M. and Patience, J. 1999. Measurements to predict swine digestible energy content of barley. *Journal of Animal Science* **77**(Supplement 1): 30.