World Rabbit Science

World Rabbit Sci. 2011, 19: 203 - 208
doi:10.4995/wrs.2011.939

USE OF NEAR INFRARED SPECTROSCOPY FOR INTRAMUSCULAR FAT SELECTION IN RABBITS

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Abstract: The potential use of near infrared spectroscopy (NIRS) for the determination of intramuscular fat (IMF) content in rabbit selection programmes was evaluated. One hundred and thirty seven rabbits from 3 different synthetic lines slaughtered between 5 and 61 wk of age were used for NIR calibration. *Longissimus* muscles (LM) were homogenised, freeze-dried and scanned by NIRS reflectance and total lipid content was chemically analysed. Calibration equation parameters reported appropriate results for IMF (cross-validation standard error, SECV=0.07g/100g muscle; cross-validation coefficient of determination, R^2=0.98 and residual predictive deviation of cross-validation, RPD=7.57). Another 88 rabbits were used to study the suitability of NIR spectroscopy in selection programmes. Intramuscular fat was measured in LM using chemical and NIRS analyses. Descriptive statistics showed that NIRS could be a proper technique to average comparison, but regression analyses (R^2=0.92) and rank correlation measures, especially Kendall's tau-b correlation coefficient (0.83), indicated that NIRS may not be accurate enough to predict individual genetic values and produce ranking of animals. However, NIRS technique could be applied in truncated selection where the efficiency of the method is measured by the response to selection. Selection can be done on 2nd parities using the IMF value of 2 full sibs of 1st parities. Ten females and 5 males can be selected as parents to establish a new population of 40 females and 5 males. The IMF values were similar between animals selected on the basis of chemically-determined IMF and NIRS-predicted IMF content. Results of the experiment confirmed the potential of NIRS for the determination of IMF content in rabbit selection programmes instead of using laborious chemical methods.

Key Words: intramuscular fat, NIR spectroscopy, rabbit, selection.

INTRODUCTION

Intramuscular fat (IMF) content is an important meat trait because it is related to human nutrition and health and sensory meat properties (Wood *et al*., 2008). IMF can be improved by selection due to its high heritability and variability (Sellier 1998; Suzuki *et al*., 2005). Rabbit meat has a lower fat content, so selection to increase IMF could improve its meat quality. Rabbits are excellent experimental material for genetic studies due to their reduced generation interval and the low cost of the carcasses.

Conventional chemical methods to determine IMF content are laborious, expensive and time-consuming. Near infrared reflectance spectroscopy (NIRS) could be a suitable alternative to these conventional methods. Many studies have confirmed the ability of NIR spectroscopy to predict

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http://dx.doi.org/10.4995/wrs.2011.939

the IMF content in several species (reviewed by Prieto *et al*., 2009), although a few studies have been carried out in rabbits (Masoero *et al*., 1994; Pla *et al*., 2004; Bázár *et al*., 2007). Genetic programmes need a substantial amount of data, so it is necessary to have a fast, accurate and cheap analytical technique to estimate IMF in all individuals. However, to our knowledge there are no studies on the application of NIRS in rabbit selection.

The aim of this study was to evaluate the possibility of use NIRS instead of chemical methods for the determination of IMF content in rabbit selection programmes.

MATERIALS AND METHODS

Animals and samples

A total of 137 rabbits (58 females and 79 males) was used for NIR calibration. To ensure variability in the samples analysed, rabbits came from 3 different synthetic lines and were slaughtered between 5 and 61 wk of age.

Another 88 rabbits (44 females and 44 males) were used to study the application of NIRS in selection programmes. These rabbits came from one of the synthetic lines mentioned above and were slaughtered at 9 wk of age.

All rabbits were slaughtered by electrical stunning and exsanguination. After slaughter, the carcasses were stored at 3-5°C for 24 h and then *Longissimus* muscles (LM) were excised from the carcass. Meat obtained from LM was ground by a mincer, freeze-dried, vacuum packed and stored at –80°C until analysis.

Chemical analyses

Total lipids were determined by ether extraction (Soxtec 1043 extraction unit, Tecator, Höganäs, Sweden) (ISO-R-1443) with a previous acid hydrolysis (Soxcap 2047, Tecator Höganäs, Sweden) in triplicate from freeze-dried LM. Lipid content was expressed as grams per 100 g of fresh tissue; this value was obtained taking into account the dry matter content determined from the weight of minced LM (ISO-R-1442) before and after freeze-drying.

Throughout the paper will be consider as IMF values those obtained by the chemical method.

NIRS calibration for intramuscular fat

The 137 *Longissimus* muscle samples were scanned between 1100 and 2498 nm with a monochromator (model 5000, NIRSystem INC., Silver Spring, MD, USA) equipped with a transport module. Two round sample cups with 3.8 cm diameter quartz windows were filled with each sample and 2 spectra, rotating 90 degrees each cup, were recorded. The 4 reflectance spectra of each sample were averaged.

Calibrations were calculated using WINISI-4 ver. 1.60 chemometric software (Infrasoft International, LLC and FOSS). Prediction equations were obtained using MPLS (Modified Partial Least Squares) as regression method (Shenk and Weasterhaus, 1996). Cross-validation with 5 groups was performed to select the optimum number of factors and avoid overfitting. Just enough passes were performed to detect outliers. Critical values for "T" outliers (actual *vs*. predicted) was set at 2.5 and for "H" outliers (spectral distance from the population mean) was set at 10. Regression equations were obtained using several mathematical treatments by combining different derivative orders, different number of data points in the segment used to calculate the derivative, different number of data points over which running average smoothing was conducted and with or without scatter correction. The best equation was selected attending to determination coefficient of cross-validation (R^2), residual predictive deviation of cross-validation (RPD=SD/SECV), and range error ratio of cross-validation (RER=range/SECV), SD being the standard deviation of the reference data and SECV the standard error of cross-validation.

Statistical analyses.

Descriptive statistics, regression and nonparametric measures of association (Spearman rank-order and Kendall's tau-b correlation) were computed by the SAS statistical package (SAS, 2002).

RESULTS AND DISCUSSION

NIRS calibration for intramuscular fat

The parameters corresponding to the NIRS calibration (Table 1) indicate good prediction ability for IMF. Williams and Sobering (1996) considered that the residual predictive deviation (RPD) should ideally be at least 3 and the range error ratio (RER) at least 10. In our case, RPD and RER were higher than those limits. Results on the accuracy of calibration equation for predicting fat content are similar to previous works in rabbit (Masoero *et al*., 1994; Pla *et al.*, 2004), poultry (Abeni and Bergoglio, 2001), beef and pork meat (Prevolnik *et al.*, 2005).

Selection for increased intramuscular fat using NIRS

Table 2 shows descriptive statistics of IMF content measured by chemical analysis and predicted by NIRS. No significant differences were found between 2 methods of analyses ($P<0.05$) or between males and females ($P<0.05$). Mean, standard deviation and coefficient of variation were similar for both methods but the range was slightly different. These results show that NIRS could be a proper technique to compare different treatments or different populations.

The relationship between chemical and NIRS values for IMF can be observed in Figure 1. Regression statistics indicate that NIRS may not be an accurate enough technique to predict individual values (R^2=0.92). Predicted individual values can be used in genetic evaluation systems to produce ranking of animals in which the order is related to their economic value and a high accuracy in estimating genetic differences is required (Goddard and Wiggans, 1999). Therefore, the application of NIRS to rank individuals needs a complete concordance between rankings based on chemically-determined values and NIRS-predicted IMF. Two measures of rank correlation have been calculated to examine the agreement among chemical and NIRS rankings. Spearman rank correlation was proposed earlier as the ordinary correlation coefficient between the ranked values and is widely used because it is easy to compute. Kendall's tau-b

Table 1: Descriptive statistics of intramuscular fat (IMF g/100 g) content in rabbits used in calibration and statistical parameters of the best calibration equation to predict IMF by near infrared reflectance spectroscopy.

	No.	Mean	SD	Min-Max	SECV	R^2	RPD	RER
IMF	129	1.29	0.53	0.75-3.25	0.07	0.98	7.57	35.71

No.: number of samples, SD: standard deviation, SECV: cross-validation standard error, R^2: cross-validation coefficient of determination, RPD: residual predictive deviation=SD/SECV, RER: range error ratio=range/SECV.

Table 2: Descriptive statistics of intramuscular fat (IMF g/100 g) content measured by chemical analysis and predicted by near infrared reflectance spectroscopy (NIRS).

Method	No.	Mean	SE	SD	Min-Max	CV×100
Chemical	88	1.19	0.02	0.17	0.87-1.73	14.3
NIRS	88	1.22	0.02	0.16	0.93-1.67	13.1

No.: number of samples, SE: standard error, SD: standard deviation, CV: coefficient of variation.

correlation coefficient was proposed later as an alternative procedure for measuring rank correlation, although it is more difficult to compute. Kendall's tau-b correlation measures the accuracy of one of the rankings when considering the other one as the correct order classification (Snedecor and Cockran, 1980). According to Kendall (1952), Kendall's tau-b correlation coefficient offers a satisfactory measure of the compatibility of 2 rankings and has certain statistical advantages. In our case, there is not a complete concordance between rankings, as showed by Spearman rank and especially by Kendall's tau-b correlation coefficients (Table 3). Therefore, we concluded that rankings of animals based on IMF content will not be the same if NIRS or chemical analyses are used.

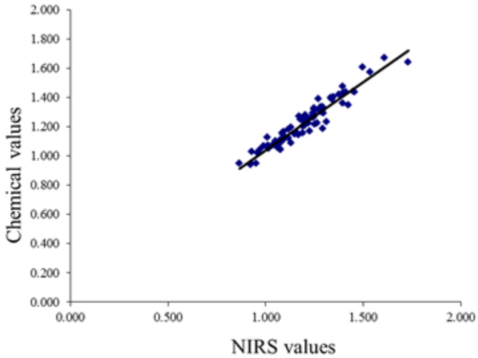

Figure 1: Regression between intramuscular fat (IMF) content in rabbits measured by chemical analysis and predicted by near infrared reflectance spectroscopy (NIRS). N=88 number of samples, SE=0.03 standard error of slope, RSD=0.05 residual standard deviation, R^2=0.92.

However, NIRS technique could be successfully used in truncated selection. This procedure is widely applied in rabbit, pig and poultry breeding. The population is divided at a point of truncation and all individuals above this value are selected. The relevant parameter is selection differential which determines the response to selection (Falconer and Mackay, 1996). Then, the possible application of NIRS in selection for IMF will depend on the response to selection using NIRS-predicted value which is similar to the response obtained using chemically-determined values.

In selection experiments for IMF, the data cannot be normally measured on the same individuals that will be used as parents and they have to be measured on relatives, so selection is based on the values of relatives. Selection can be done on the second parities using the IMF value of 2 full sibs (a male and a female) of the 1st parities. Ten females and 5 males can be selected as parents to establish a population of 40 females and 5 males in the next generation, corresponding to a

Table 3: Spearman rank and Kendall's tau-b correlation coefficients between intramuscular fat rank values measured by chemical analysis (chemical rank) and predicted by near infrared reflectance spectroscopy (NIRS rank).

	NIRS rank (Spearman rank correlation)	NIRS rank (Kendall's tau-b correlation)
Chemical rank	0.95** (No.=88)	0.83** (No.=88)

**Significant correlation at P<0.001.

Table 4. Descriptive statistics of intramuscular fat (IMF) content in parents selected using IMF values measured by chemical analysis and predicted by near infrared reflectance spectroscopy (NIRS).

	NIRS	Chemical
Females		
No.	10	10
Mean (g/100 g)	1.374	1.385
SE (g/100 g)	0.035	0.033
CV×100	8.2	7.6
Males		
No.	5	5
Mean (g/100 g)	1.442	1.447
SE (g/100 g)	0.057	0.056
CV×100	8.9	8.6

SE: standard error. CV: coefficient of variation.

selection pressure of 23% on females and 3% on males. Table 4 offers descriptive statistics of IMF content of parents selected on the base of chemically-determined or NIRS-predicted IMF values. No differences between the means of the 2 selection criteria were found for the IMF of selected parents (P<0.05 for females and males). Applying the same selection pressure, the average IMF content is only 0.6% higher (1.408 g/100 g *vs*. 1.416 g/100 g) in parents selected on the base of chemically-determined values than on the base of NIRS-predicted values.

CONCLUSIONS

A good NIRS calibration for predicting IMF in rabbit meat was obtained. NIRS is a suitable technique for average comparisons but it may not be accurate enough to predict individual genetic values for ranking animals where a higher accuracy is required. Chemical IMF values of selected animals on the basis of NIRS-predicted values are similar to those obtained when chemical criterion is applied. Thus, response to selection using NIRS should be similar to direct response using chemical analysis values. NIRS is a reasonable alternative for the determination of IMF content in rabbit selection programmes.

Acknowledgements: This work was supported by projects AGL2008-05514-C02-01 from the Spanish National Research Plan and PROMETEO/2009/125 from the Generalitat Valenciana. C. Zomeño acknowledges a Formación de Profesorado Universitario (FPU) grant from the Ministerio de Ciencia y Educación (MEC), Spain. The authors thank Verónica Juste and Federico Pardo for their technical assistance.

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