NEAR INFRARED SPECTROSCOPY PREDICTION OF POLYUNSATURATED FATTY ACIDS AND BIOHYDROGENATION PRODUCTS IN PERIRENAL ADIPOSE TISSUE FROM CATTLE FED SUNFLOWER OR FLAXSEED

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Abstract – The objective of this study was to test the potential of near infrared reflectance spectroscopy (NIRS) to estimate the proportion of polyunsaturated fatty acids (FA) and their biohydrogenation products in perirenal adipose tissue (PrFat) from cattle fed sunflower or flaxseed. PrFat from 63 steers was collected immediately after skinning, scanned at 37 °C over a NIR spectral range from 400 to 2498 nm on benchtop equipment, and analysed for FA composition. NIR calibrations, tested by cross-validation, showed high predictability for most of the total n-3, conjugated linolenic acids, *t,t*-conjugated linoleic acids (CLA), non-CLA dienes and *trans*-monounsaturated FA, with R^2 and root mean square error of cross-validation (RMSECV, %) of 0.95 (0.07-0.08), 0.86-0.89 (0.01-0.09), 0.93 (0.07), 0.89 (0.46) and 0.92-0.93 (0.87-1.29), respectively. The results show NIRS to be a useful technique for the early, fast and relatively inexpensive estimation of proportion of FA with potential human health effects.

Key Words - NIRS, perirenal fat, fatty acid.

I. INTRODUCTION

Development of value-added beef products with enhanced levels of fatty acids (FA) beneficial to human health is of considerable interest to health conscious consumers. There have been many attempts to increase omega-3, rumenic (*c*9,*t*11-18:2) and vaccenic acids (*t*11-18:1) in beef due to their demonstrated health effects in animal models and their natural occurrence in ruminant animals [1,2]. To take FA enriched beef products to commercial endpoints, however, presents considerable additional challenges. Intramuscular fat content and composition can be variable between animals consuming the same diet, and there are no rapid analytical methods for sorting or quality control purposes.

An initial strategy might, therefore, be to target the harvesting of adipose tissue depots enriched with FA of interest, which can then be used in secondary value added products. One such depot is perirenal adipose tissue (PrFat). This fat tissue is easily accessible, naturally enriched with vaccenic and some omega-3 FA, and currently used for stuffing mixes, pie crusts, soup stocks, puddings, and has potential for use in many other products. Currently lacking is a rapid and relatively inexpensive method for measuring PrFat composition for sorting and quality control purposes.

Quantitative chemical techniques for the comprehensive determination of FA involves solvent extraction of total lipids, followed by conversion of FAs to their methyl esters and then analysis by GC and Ag+-HPLC [3], a time-consuming and costly process. Near infrared spectroscopy (NIRS) is a rapid and non-destructive method, neither requiring reagents nor producing waste. The structure of FA can produce individual spectral characteristics and they are, therefore, very accessible for detection by NIRS. Recently, researchers employing this technology have successfully predicted the FA content in subcutaneous fat from beef cows [4] and Iberian pigs [5].

Nevertheless, to our knowledge, there are no studies testing the ability of this technology to estimate the FA content in PrFat of steers, particularly those enriched with linoleic and linolenic acid biohydrogenation products. Hence, this study was conducted to examine the potential of NIRS to predict the proportion of FA with potential health effects in PrFat of steers. The steers used were part of an ongoing study

evaluating the effects of oilseed (sunflower seed or flaxseed) and forage type (hay or red clover silage) on enrichment of healthy FA in beef.

II. MATERIALS AND METHODS

A. Animals and diets

Sixty-four yearling steers with body weights averaging 533 ± 44.0 kg were used. Steers were fed at the Lacombe Research Centre, Alberta, Canada, and randomly assigned to one of four diets (15% flax + 70% red clover silage, 15% flax + 70% grass hay, 15% sunflower seed + 70% red clover silage, 15% sunflower seed + 70% grass hay) with an additional 15% made of ground barley, straw and vitamin/mineral supplement to balance estimated rates of gain between oilseed diets (all dry matter basis). Steers had *ad libitum* access to feed and water with 8 animals to a pen, two pens per diet and slaughtered at an average of 205 days on test. During the study one animal from the flaxseed and grass hay treatment was withdrawn due to lameness.

B. Slaughter and sample collection

Animals were slaughtered at the Lacombe Research Centre. Immediately after skinning, PrFat was collected and temperature was recorded. Approximately 200 g of PrFat was stored at −80 °C for subsequent FA determinations. Another 200 g was refrigerated at +2 ºC for solidification.

C. Fatty acid analysis

From the PrFat collected, 5g was sampled and 50 mg subsamples were freeze dried and used for FA analysis according to Aldai et al. [6].

D. Spectra collection

When PrFat was solidified enough to core (approx. 2 h), duplicate intact circular fat cores were obtained using a custom-constructed stainless steel device [4] to enable consolidation of fat and fat discs of an appropriate diameter (38 mm) and thickness (7 mm) to fit the ring cups of the NIRS machine. Each cold fat disc was placed in a ring cup, all visible air bubbles removed by squeezing, and the cup backed with thin black foam. Subsequently, the samples were placed in open plastic bags and heated in a water bath at 40 °C. A DuaLogR model 600–1050 (Barnant Company Barrington, USA) thermocouple was inserted into the centre of each fat sample for temperature monitoring during warming. As soon as the core sample reached that temperature of PrFat immediately after skinning (37 °C), samples were removed from the water bath and NIR spectra were collected. Each PrFat sample was scanned 32 times over the range (400–2498 nm) using a NIRSystems Versatile Agri Analyzer (SY-3665-II Model 6500, FOSS, Sweden) benchtop equipment and spectra were averaged by the equipment software. Two fat samples per animal were scanned using two different cells. The two reflectance spectra were visually examined for consistency and then averaged, with the mean spectrum being used to predict the FA proportions of each PrFat. The spectrometer interpolated the data to produce measurements in 2 nm steps, resulting in a diffuse reflectance spectrum of 1050 data points. Absorbance data were stored as log (1/*R*), where *R* is the reflectance. Instrument control and initial spectral manipulation were performed with WinISI II software (v1.04a; Infrasoft International, Port Matilda, MD).

E. Data analysis

Calibration and validation were performed using The Unscrambler program (version 8.5.0, Camo, Trondheim, Norway). Two passes of elimination of outliers (H and T) were allowed. Partial least square regression type I (PLSR1) was used for predicting FA proportions using NIR spectra as independent variables. Internal full cross-validation was performed in order to avoid over-fitting the PLSR equations. Thus, the optimal number of factors in each equation was determined as the number of factors after which the standard error of cross-validation no longer decreased. The accuracy of prediction was evaluated in terms of coefficient of determination (R^2) and root mean square error of cross-validation (RMSECV).

III. RESULTS AND DISCUSSION

There are few studies in the literature reporting the comprehensive FA composition of PrFat. In general terms, the percentages of FA of interest found in this study (Table 1) were higher than

those showed by Wistuba et al. [7] for steers fed a commercial diet (*t*11-18:1 = 5.83 vs. 2.84 %).

Table 1 Descriptive statistics for fatty acids (% total FA) in perirenal adipose tissue of steers (n = 63)

Fatty acid	Range	Mean	SD	CV (%)
n-3	0.21-0.99	0.48	0.192	40.20
C18:3n-3	0.18-0.90	0.43	0.183	42.98
C20:3n-3	0.00-0.03	0.02	0.007	42.13
C22:5n-3	0.01-0.08	0.04	0.016	42.48
CLNA	0.03-0.66	0.20	0.160	80.54
c9,*t*11,*t*15-18:3	0.01-0.09	0.04	0.025	68.00
c9,*t*11,*c*15-18:3	0.01-0.58	0.16	0.137	84.37
CLA	0.93-2.33	1.52	0.311	20.45
t,*t*-CLA	0.10-0.56	0.26	0.140	53.58
c,*t*-CLA	0.83-1.98	1.26	0.237	18.81
*c*9,*t*11-CLA	0.64-1.69	0.93	0.227	24.47
Non-CLA dienes	0.45-3.66	1.68	1.032	61.26
cis-MUFA	19.12-35.72	25.16	3.547	14.10
trans-MUFA	5.39-17.88	12.11	2.591	21.40
*t*11-18:1	2.55-10.23	5.83	1.666	28.56

CLNA: conjugated linolenic acids; CLA: conjugated linoleic acids; *t*,*t*-CLA: *t*12,*t*14 + *t*11,*t*13 + *t*10,*t*12 + *t*9,*t*11+ *t*8,*t*10 + *t*7,*t*9 + *t*6,*t*8-CLA; *c*,*t*-CLA: *t*12,*c*14 + *c*12,*t*14+ *t*11,*c*13 + *c*11,*t*13 + *t*10,*c*12 + *t*8,*c*10 + *t*7,*c*9 + *c*9,*t*11 + *t*9,*c*11-CLA; Non-CLA dienes: *t*11,*t*15-18:2 + *c*9,*t*13-/*t*8,*c*12-18:2 + *t*8,*c*13-18:2 + *c*9*t*12-18:2/*c*16-18:1 + *t*9*c*12-18:2 + *t*11*c*15-18:2 + *c*9*c*15-18:2 + *c*12*c*15-18:2; *cis*-MUFA: *c*9-14:1 + *c*9-15:1 + *c*7-16:1 + *c*9-16:1 + *c*10-16:1 + *c*11-16:1 + *c*13-16:1 + *c*9-17:1 + *c*9-*c*10-18:1 + *c*11-18:1 + *c*12-18:1 + *c*13-18:1 + *c*14-18:1 + *c*15-18:1 + *c*9-20:1 + *c*11-20:1; *trans*-MUFA: *t*9-16:1 + *t*11/*t*12-16:1 + *t*6-*t*8-18:1 + *t*9-18:1 + *t*10-18:1 + *t*11-18:1 + *t*12-18:1 + *t*13-*t*14-18:1 + *t*15-18:1 + *t*16-18:1; SD: standard deviation; CV: coefficient of variation.

As presented in Table 2, accurate NIRS predictions were found for total n-3 (R^2 = 0.95; RMSECV = 0.08%) and C18:3n-3 (R^2 = 0.95; RMSECV = 0.07%), showing ratios performance deviation (RPD = SD/RMSECV) of 2.43 and 2.66, respectively, suitable for screening purposes [8,9]. However, NIRS predictability was low for predicting minor n-3 FA (C20:3n-3 and C22:5n-3), the variance explained by the model being 6 and 8%, respectively.

Regarding the total conjugated linolenic acids (CLNA) and its two isomers *c*9,*t*11,*t*15-18:3 and *c*9,*t*11,*c*15-18:3, NIRS equations showed R^2 over 0.85 and low RMSECV (0.01-0.09%) compared to SD for these FA. Consequently, RPD statistics ranged from 1.84 to 2.12.

In relation to conjugated linoleic acids (CLA), only the total *t*,*t*-CLA could be estimated with accuracy (R^2 = 0.93; RMSECV = 0.07%; RPD = 2.09). Relatively low coefficients of variation (CV < 25%) for the rest of CLA could partly explain the unreliable prediction equations.

Non-CLA dienes were successfully predicted by NIRS (R^2 = 0.89; RMSECV = 0.46%; RPD = 2.22). The potential health effects of many non-CLA dienes are not known, but if sunflower and/or flaxseed are to be fed to ruminants at elevated levels, it will be important to ascertain if non-CLA dienes have any positive or negative effects on human or animal health and to estimate them quickly and accurately.

Table 2 Prediction of fatty acid proportions in perirenal adipose tissue of steers using NIR spectra*

Fatty acid	p	R^2	RMSEC	RMSECV	RPD
n-3	8	0.95	0.04	0.08	2.43
C18:3n-3	8	0.95	0.04	0.07	2.66
C20:3n-3	1	0.06	0.01	0.01	1.03
C22:5n-3	1	0.08	0.01	0.01	1.07
CLNA	7	0.86	0.06	0.09	1.84
*c*9,*t*11,*t*15-18:3	8	0.89	0.01	0.01	2.12
*c*9,*t*11,*c*15-18:3	7	0.86	0.05	0.07	1.84
CLA	7	0.76	0.15	0.21	1.43
t,*t*-CLA	8	0.93	0.04	0.07	2.09
c,*t*-CLA	7	0.70	0.12	0.17	1.32
*c*9,*t*11-CLA	7	0.79	0.10	0.19	1.24
Non-CLA dienes	7	0.89	0.34	0.46	2.22
cis-MUFA	5	0.73	1.86	2.22	1.63
trans-MUFA	7	0.92	0.74	1.29	1.97
*t*11-18:1	8	0.93	0.44	0.87	1.97

*: after eliminating two outliers; p: number of PLS terms utilized in the calibration equation; R^2: coefficient of determination of calibration; RMSEC: root mean square error of calibration; RMSECV: root mean square error of cross-validation; RPD: ratio performance deviation.

In the case of monounsaturated FA (MUFA), although the variance explained by the model was lower than 75% for *cis*-MUFA, the proportion of total *trans*-MUFA and *t*11-18:1 was predicted with accuracy (R^2 = 0.92 and 0.93; RMSECV = 1.29 and 0.87%; RPD = 1.97 and 1.97, respectively). The rapid and accurate estimation of vaccenic acid

by NIRS is again of great interest due to its potential health benefits [1].

To our knowledge, there are no studies testing NIRS ability to predict FA proportions in PrFat. Nevertheless, the current results are similar to our past results reported in Prieto et al. [4] for subcutaneous fat of beef cows fed flaxseed. However, some CLAs (e.g. *c*9,*t*11-CLA) in the present study were predicted with less accuracy than was found previously by Prieto et al. [4] (R^2 = 0.84; RMSECV = 2.24 $mg.g^{-1}$ fat) and Pérez-Juan et al. [5] in subcutaneous fat from pigs (R^2 = 0.92; RMSECV = 2 $mg.g^{-1}$ fat), probably due to a lower variability in the proportion of that FA in this study (CV = 25 vs. 60 and 128%, respectively).

IV. CONCLUSION

NIRS technology has the potential to quickly and accurately estimate the proportion of polyunsaturated FA and their biohydrogenation products in perirenal fat from steers, particularly when feeding diets with a high content of linoleic and linolenic acids. Accurate NIRS prediction was found for vaccenic acid, which has purported human health benefits. Further research will now be required to validate NIRS for FA analyses on-line in the abattoir.

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