PE4.72 Prediction of subcutaneous fatty acid composition from ham of CLA fed barrows and gilts using a NIRS fiber optic probe 256.00

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***Abstract*— The aim of this work was the use of NIR technology by direct application of a fiber optic probe on back fat to analyze the fatty acid composition of CLA fed boars and gilts. 265 animals were fed 3 different diets and the fatty acid profile of back fat from *Gluteus medius* was analyzed using gas chromatography and FT-NIR. Spectra were acquired using a Bruker Optics Matrix-F duplex spectrometer equipped with a fiber optic probe (IN-268-2). Oleic and stearic fatty acids were predicted accurately; myristic, vaccenic and linoleic fatty acids were predicted with lower accuracy, while palmitic and α-linolenic fatty acids were poorly predicted. The relative percentage of fatty acids and NIR spectra showed differences in fatty acid composition of back fat from pigs fed CLA which increased the relative percentage of SFA and PUFA while MUFA decreased. Results suggest that a NIR fiber optic probe can be used to predict total saturated and unsaturated fatty acid composition, as well as the percentage of stearic and oleic. NIR showed potential as a rapid and easily implemented method to discriminate carcasses from animals fed different diets.**

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Index Terms*— *Back fat, Boars, CLA, fatty acids, gilts, NIR, fiber optic probe.

I. INTRODUCTION

ONJUGATED linoleic acid (CLA) is a collective term Cto describe positional and geometric isomers of linoleic acid (*cis*-9,*cis*-12- octadecadienoic acid) with conjugated double bonds. The *cis*- 9, *trans*-11 and *trans*-10, *cis*-12 CLAs are the major CLA isomers in nature [1]. CLA supplementation of pigs has been suggested as an interesting approach to improve productive traits, such as feed conversion efficiency, decreasing backfat thickness [2], improving the technological quality of meat products (increasing fat firmness [3] and antioxidant properties [4], and increasing health benefits related to human consumption (anticarcinogenic function or antiobesity, [5]). Due to the interest of using CLA in animal feeding, some authors have analyzed its effect on fatty acid profile [6-9] and quality parameters on carcass and meat [10-12].

Near-infrared spectroscopy (NIRS) is a rapid and non destructive method requiring little or no sample preparation and its precision can be high. In contrast to traditional chemical analysis, no reagents are required and no waste is produced. Near-infrared reflectance spectroscopy has been used for determining meat composition or beef tenderness and has also been applied to study the fatty acid content of beef cuts, in bovine neck muscle and in Iberian pig carcasses [13]. The aim of this work was the use of NIR technology by direct application of a fiber optic probe on back fat to analyze the fatty acid composition of CLA fed boars and gilts.

II. MATERIALS AND METHODS

MATERIALS

A. Animals and Diets

265 animals, boars and gilts, were fed 3 diets with different composition: Diet 1 was control (commercial standard). Diet 2 contained citrics (flavonoids) as a growth promoter and antioxidants. Diet 3 was enriched with conjugated linoleic acid (CLA). Pigs were slaughtered at 115 kg live weight at a commercial slaughterhouse.

B. Back Fat Sample

A back fat sample of 10x6 cm with skin-on was removed from the *Gluteus medius* of each carcass at 24 h postmortem.

Back fat samples were vacuum packaged, transported to the Meat Laboratory of IRTA (Spain)

and kept refrigerated at 2±2ºC until NIR analysis which was conducted at 48 h from slaughter. After the NIR measurements, the samples were vacuum packaged in metallic aluminum bags and frozen at -20 ºC until fatty acids analysis by gas chromatography.

METHODS

C. *Near-Infrared Spectroscopy (NIRS)*

NIR adquisition were taken on the first back fat layer closer to lean muscle of the carcass. A longitudinal cut was performed on each sample to have a uniform layer (Figure 1). Then, NIR measurements were made in 3 different points along the sample. FT-NIR spectra were acquired using a Bruker Optics Matrix-F duplex spectrometer equipped with a solid difusse reflectance fiber optic probe (IN-268-2) over the range of 11000–4000 cm^{-1} (909–2500 nm). A ceramic plate was employed as probe reference. The spectral resolution was set at 8 cm^{-1}. Spectra were recorded performing 24 scans for both reference and samples.

All replicate spectra were subjected to preprocessing prior to data analysis. First, the second derivative of the spectra were calculated using the Savitzky-Golay approach utilising a 2 degree polynomial and a window size of 9 points. Subsequently, all spectra were corrected using Extended Multiplicative Signal Correction. The triplicate measurements were averaged, and the range of 9090–4150 cm^{-1} were used in the subsequent data analysis. All preprocessing and subsequent data analysis were performed using the Unscrambler software (version 9.8, CAMO PROCESS AS, Oslo, Norway).

D. *Fatty Acid Analysis*

Lipids from back fat samples were extracted following the chloroform-methanol procedure of Folch et al. [14], converted to fatty acid methyl esters (FAME) following the method ISO 5509-1978 (E) and analyzed in a gas chromatograph (Hewlett–Packard 5890 Series II GC, S.A, Barcelona, Spain) using tripentadecanoic acid (Sigma–Aldrich, Madrid, Spain) as internal standard. All samples were introduced by split injection in a fused silica capillary column (30m, 0.25mm ID, BPX 70; 0.25 □m film thickness; SGE, UK). Individual fatty acids were identified by retention time with reference to standards (Lipid Standard: FA methyl ester mixture 189– 19; Sigma–Aldrich, Madrid, Spain). Two injections of FAME per sample were analysed.

E. *Statistical analysis*

Fatty acid composition data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with feed source (control, citrics, CLA) in the model. Differences between treatment means were separated using least squares means procedure.

Principal component analysis (PCA) was performed on the 18 most abundant fatty acids as obtained by GC, and on the NIR spectra. All PCA models were validated using segmented cross-validation with segments of sample size 3. The samples of all segments were chosen randomly.

Partial Least-Squares Regression (PLSR) was used to develop multivariate regression models. The optimal number of PLSR factors was determined using segmented cross-validation with segments of sample size 3. The samples of all segments were chosen randomly. The regression results were evaluated based on the number of PLSR factors included, the estimation errors (RMSECV: Root mean square error of cross-validation) and the coefficients of determination (R^2).

III. RESULTS AND DISCUSSION

A. *Fatty acids analysis*

Table 1 shows the fatty acid composition of back fat samples from pigs fed the 3 diets (control, citrics, CLA). There were no differences (P>0.05) in fatty acid composition between the control and the diet enriched with flavonoids with the exception of myrisitic fatty acid which was higher for back fat from animals fed the citrics diet. Back fat from pigs fed CLA showed significant differences for all the fatty acids except for C18:2 n-6 and C18:3 n-3. CLA fed pigs showed higher percentage of myristic, palmitic, stearic and the two CLA isomers *cis*-9,*trans*-11 and *trans*-10,*cis*-12 and lower percentage of oleic and vaccenic compared with the control and the citrics diets. Back fat from CLA fed pigs showed higher percentage of total saturated and polyunsaturated fatty acids and lower total monounsaturated fatty acids compared with control and citrics diets. These results are in accordance with published data. Previous research has also shown that feeding pigs with CLA modifies the fatty acid profile, increasing the proportion of saturated fatty acids in different fat depots while decreasing the proportion of MUFA [2, 15]. This leads to less fluid and more consistent lards, which are considered as positive quality characteristics by meat processors [16]. Although the increase in saturated fatty acids could have negative health implications, there is a simultaneous increase in CLA isomers (polyunsaturated fatty acid) in adipose tissue of pigs fed with CLA-enriched diets, which could counteract this negative effect [16].

B. NIR

Figure 2 compares the score plots of PCA analyses of the most abundant fatty acids as obtained by GC analysis and the NIR spectra, respectively. The GC plot shows that the chemical information was able to differentiate 2 groups by the first principal component. The largest group included samples from animals fed the control and citrics diets. The fatty acid profiles of these samples were similar since the flavonoids present in the citrics diet act as growth promoters and antioxidants but do not seem to interfere significantly on fatty acid metabolism. The second smaller group corresponded to samples from animals fed CLA which showed a higher percentage of saturated and polyunsaturated fatty acids and a lower percentage of monounsaturated fatty acids compared with the other two diets (Table 1). These results agree with published data on the use of dietary CLA to modify tissue fatty acid composition [2, 15]. The same trend of separation is seen in the NIR plot, but to a lesser extent. PCA plot using chemical data shows that the first two components explain 94% of the variation in the data, while the PCA plot using NIR data explains 71% of the variation. However, these spectra were obtained from intact back fat samples, thus the results visualize the potential of using NIR for rapid screening of dietary effects in intact adipose tissue.

NIR regression results of selected fatty acids are provided in Table 2. The R^2 was 0.85 and 0.88 for oleic and stearic acid indicating good quantitative information [13]. Although the R^2 for CLA isomers *cis*-9,*trans*-11 and *trans*-10,*cis*-12 were 0.83 and 0.84, respectively indicating high accuracy of prediction, these high values are a consequence of having a group of animals with high CLA levels (CLA diet) and another group of animals with no CLA or traces of CLA in back fat (control and citrics diets). Therefore, CLA prediction from NIR data in this study cannot be considered as a good calibration since there is not enough variability in the data (Table 1) to achieve high accuracy of prediction. In summary, oleic and stearic fatty acids were predicted with high accuracy; myristic, vaccenic and linoleic were predicted with lower accuracy, while palmitic and α-linolenic were poorly predicted. Other authors used fat from iberian pigs for NIR calibrations of palmitic, palmitoleic, stearic, oleic and linoleic which were predicted accurately with R^2 between 0.78 and 0.91 [17]**.** The equations obtain had been successfully implemented at industry-wide level for paying farmers according to the three Iberian pork commercial categories ("Bellota", "Recebo" and "Cebo") [17].

When fatty acids were grouped, R^2 (Table 2) was 0.78 for saturated fatty acids, 0.89 for monounsaturated and 0.68 for polyunsaturated, and the Root mean square error of cross-validation (RMSECV) were 1.8, 1.5 and 1.5, respectively. Similar results were found in bibliography in a study about the potential of determining the fatty acid composition to predict fat consistency traits directly in pig fat without extracting the lard [18]. The proportion of saturated fatty acids, monounsaturated, and polyunsaturated fatty acids was validated with relative standard errors of prediction (SEP) of 0.9%, 1.6% and 4.7% and coefficients of determination (R^2) of 0.98, 0.88 and 0.96, respectively.

IV. CONCLUSION

Chemical data and NIR spectra showed differences in fatty acid composition of back fat from pigs fed CLA which increased the relative percentage of SFA and PUFA and decreased MUFA percentage compared with other diets. Results suggest that a NIR fiber optic probe can be used to accurately predict total saturated and unsaturated fatty acid composition, as well as the percentage of stearic and oleic. Myristic, vaccenic and linoleic fatty acids can be predicted with lower accuracy, while palmitic and α-linolenic fatty acids were poorly predicted. NIR showed potential as a rapid and easily implemented method to discriminate carcasses from animals fed different diets leading to different degree of back fat saturation.

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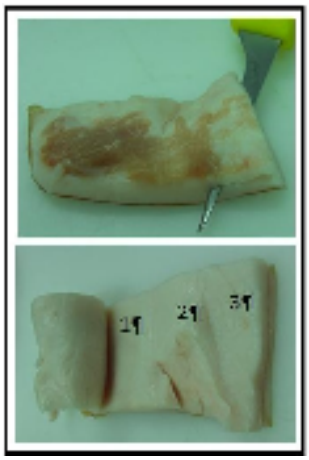

Figure 1. 10x6 sample from *Gluteus medius* fat. Numbers 1,2 and 3 indicates the points were NIR spectra were measured in each sample**.**

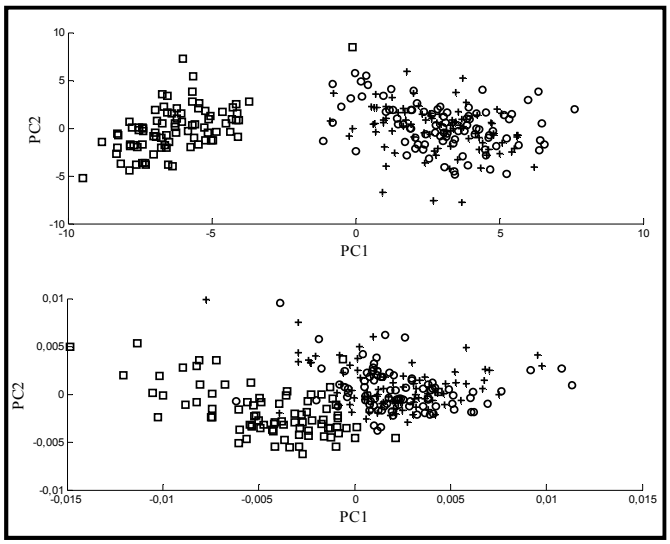

Figure 2. PCA score plot of abundant fatty acids obtained by GC analysis (upper plot, PC1 and PC2 explain 74 % and 20 %, respectively), and PCA score plot of NIR spectra of corresponding samples (lower plot, PC1 and PC2 explain 45 % and 26 %, respectively. Samples are separated by type of diet (diet 1: circles, diet 2: plus signs, and diet 3: squares).

Table 1. Relative percentage of fatty acids from back fat of boars and gilts fed with three different diets (diet 1: control, diet 2: with citrics and diet 3: with conjugated linoleic acid, CLA) determined by gas chromatography. Results are expressed as LSmeans ± Standard error).

	DIET		
FATTY ACIDS	CONTROL	CITRICS	CLA
C14:0 (myristic)	$1.31^{b} \pm 0.02$	$1.38^{c} \pm 0.02$	$1.81^{a} \pm 0.02$
C16:0 (palmitic)	$19.13^{b} \pm 0.16$	$19.48^{b} \pm 0.16$	$20.57^{a} \pm 0.17$
C18:0 (stearic)	$8.98^{b} \pm 0.11$	$9.01^{b} \pm 0.11$	$13.47^{a} \pm 0.11$
C18:1 n-9 (oleic)	$38.02^{a} \pm 0.14$	$37.82^{a} \pm 0.14$	$30.24^{b} \pm 0.14$
C18:1 n-7 (vaccenic)	$2.75^{a} \pm 0.02$	$2.79^{a} \pm 0.02$	$2.15^{b} \pm 0.02$
C18:2 n-6 (linoleic)	$22.51^{ab} \pm 0.20$	$22.17^{b} \pm 0.20$	$22.76^{a} \pm 0.20$
C18:3 n-3 (α-linolenic)	$1.59^{a} \pm 0.02$	$1.59^{a} \pm 0.02$	$1.57^{a} \pm 0.02$
CLA9_11	$0.02^{b} \pm 0.01$	$0.03^{b} \pm 0.01$	$1.63^{a} \pm 0.01$
CLA10_12	$0.00^{b} \pm 0.01$	$0.01^{b} \pm 0.01$	$0.90^{a} \pm 0.01$
SAT	$29.90^{b} \pm 0.24$	$30.37^{b} \pm 0.24$	$36.52^{a} \pm 0.25$
MUFA	$44.49^{a} \pm 0.16$	$44.38^{a} \pm 0.16$	$35.34^{b} \pm 0.16$
PUFA	$25.60^{b} \pm 0.22$	$25.25^{b} \pm 0.22$	$28.14^{a} \pm 0.23$

Letters indicates significant difference for each fatty acid among diets ($p<0.05$).

Table 2. Sample statistics and NIR regression results of selected fatty acids in back fat of boars and gilts fed with three different diets.

Fatty acids	Sample statistics			Regression results		
	Range (%)	Mean (%)	Stand. Dev.	R^2	RMSECV	#PLS components
C14:0 (myristic)	0.9 – 2.7	1.5	0.3	0.65	0.2	6
C16:0 (palmitic)	13.4 – 24.8	19.7	1.6	0.48	1.2	8
C18:0 (stearic)	6.9 – 15.6	10.4	2.3	0.85	0.9	6
C18:1 n-9 (oleic)	27.9 – 41.6	35.4	3.9	0.88	1.4	8
C18:1 n-7 (vaccenic)	1.7 – 3.3	2.6	0.4	0.62	0.2	9
C18:2 n-6 (linoleic)	16.0 – 29.4	22.5	2.0	0.60	1.3	9
C18:3 n-3 (α-linolenic)	1.1 – 2.2	1.6	0.2	0.38	0.1	9
CLA9_11	0.0 – 2.3	0.5	0.8	0.83	0.3	6
CLA10_12	0.0 – 1.3	0.3	0.4	0.84	0.2	7
SAT	24.2 – 43.3	32.2	3.8	0.78	1.8	6
MUFA	33.0 – 48.9	41.5	4.6	0.89	1.5	8
PUFA	18.2 – 34.7	26.3	2.6	0.68	1.5	9

R^2: coefficient of determination
RMSECV: Root mean square error of cross-validation
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