Near-Infrared Reflectance Spectroscopy as a Method to Predict Chemical Composition of Breast Meat and Discriminate Between Different n-3 Feeding Sources

P. Berzaghi,*[,1] A. Dalle Zotte,* L. M. Jansson,† and I. Andrighetto*

*Department of Animal Science, Agripolis, Viale dell'Università 16, 35020 Legnaro (PD), Italy; and †Department of Food Science, Swedish University of Agricultural Sciences, Box 7051 SE-750 07 Uppsala, Sweden

ABSTRACT The objective of this study was to evaluate near-infrared reflectance spectroscopy (NIRS) as a tool to predict the physicochemical composition of breast meat samples of laying hens fed 4 different diets, a control and 3 diets enriched with different sources of n-3 polyunsaturated fatty acids: marine origin, extruded linseed, and ground linseed. Furthermore, NIRS was used as a tool to classify meat samples according to feeding regimen. Samples were analyzed chemically for DM, ash, protein, lipids, and fatty acid profile. Absorption spectra were collected in diffuse reflectance mode between 1,100 and 2,498 nm every 2 nm. The calibration results for the 72 meat samples were accurate in predicting DM, protein, lipids, and major fatty acids. Poor results were obtained for the calibration equations for ash, pH, color, and lipid oxidation parameters. Partial least squares discriminant analysis was developed to differentiate the breast meat samples that originated from hens fed the different diets. The performance of the discriminant models showed 100% correct classification between the control and the enriched diets. It was concluded that NIRS could be used for quality control predicting chemical composition of poultry meat and possibly some dietary treatments applied to the chickens.

(*Key words*: near-infrared reflectance spectroscopy, laying hen, meat, dietary n-3 polyunsaturated fatty acid, chemical composition)

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INTRODUCTION

During the last 20 yr, consumption of poultry meat has steadily increased. Much of its popularity is due to the consumer's perception of quality attributes such as flavor, texture, taste, and especially for its pale color. Chicken is a neutral protein source accepted in all world cultures and religions, and increasingly seen as an environmentally friendly source of nutrition (Hoogenkamp, 1999). Generally poultry contain a higher proportion of polyunsaturated fatty acids, compared with meats from polygastric animals. Fatty acid (FA) chain composition greatly varies according to the diet. Considering the positive effects of the highly unsaturated FA of the n-3 series, namely eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), on human health, they or their precursors are being introduced in animal feedstuffs of monogastric animals, poultry included, because lipids are adsorbed and deposited mainly in the form in which they are consumed.

In recent years, food of animal origin suffered several problems related to human safety (hormones, β-agonists, *Salmonella*, antibiotics, dioxins, bovine spongiform encephalopathy), which have affected consumer confidence in poultry meat. As the wholesomeness of meats is an indispensable requirement of the consumer, researchers must investigate the introduction of new methods for meat quality evaluation, in an attempt to guarantee the safety and quality of meat products. Because near-infrared reflectance spectroscopy (NIRS) is a rapid, nondestructive, and safe technique that is useful for simultaneous analysis of components in organic substances, it could be an advantageous surveying tool in poultry meat production.

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[1]To whom correspondence should be addressed: paolo.berzaghi@unipd.it.

Abbreviation Key: DHA = docosahexaenoic acid; EL = extruded linseed; EPA = eicosapentaenoic acid; FA = fatty acids; GL = ground linseed; MPLS = modified partial least squares; MSC = multiplicative scatter correction; NF = Nordos Fat W3 (highly unsaturated FA of marine origin); NIRS = near-infrared reflectance spectroscopy; PLS = partial least squares regression; SEC = standard error of calibration; SECV = standard error of cross-validation; SNV-DT = standard normal variate and detrending; TBARS = thiobarbituric acid-reactive substances; 1-VR = coefficient of determination of cross validation.

Near-infrared reflectance spectroscopy is widely applied for quantitative analysis of chemical constituents such as protein content, moisture, and fats in products of animal and vegetable origin (Burns and Ciurczak, 1992). Applications of NIRS for the prediction of functional properties and quality variables in foods have emerged (Molette et al., 2001; Windham and Morrison, 1998). Hildrum et al. (1994) reported that the near-infrared reflectance spectra of beef muscles changed during aging, and Park et al. (1998) showed that NIRS might be able to predict variation in tenderness of beef steaks. Furthermore, discriminant analysis makes it possible to use NIRS for identification and control of sample purity/quality (Murray et al. 2001). Near-infrared reflectance spectroscopy has, for example, been applied to the authentication of orange juice (Evans et al., 1993), olive oil (Bertran et al., 2000), and Basmati rice (Osborne et al., 1993), as well as meat speciation (McElhinney et al., 1999; Thyholt et al., 1998), and classification (Hervás et al., 1994; Fumière et al., 2000).

The primary objective of this research was to investigate whether the NIRS data could be used to predict the chemical and physical composition of the meat of laying hens. The second objective was to investigate if, by analyzing the meat by NIRS, it would be possible to use discriminant analysis to distinguish the 4 administered diets, which differed in n-3 FA source.

MATERIALS AND METHODS

Birds, Housing, and Experimental Diets

Seventy-two laying hens, 24 wk of age, were divided into 4 groups of 18 hens each and housed in pairs in laying cages of 483 cm^2 floor space per hen. Each group of hens was fed (ad libitum) 1 of the 4 isonitrogenous diets for a period of 60 d. The control diet (C) was a commercial diet and the other 3 experimental diets were prepared by adding to the C diet the following polyunsaturated FA supplements: extruded linseed (EL), ground linseed (GL), or a product rich in n-3 highly unsaturated FA of marine origin (NF)[2] at 10.0, 10.0, and 3.4% of the control diet, respectively. Table 1 shows the chemical composition of the experimental diets.

Sampling

At the end of the trial, the 72 hens were slaughtered and their breast muscles (pectoralis superficialis) were removed and weighed. A portion of each was immediately frozen and freeze-dried, with the remaining part over-wrapped in oxygen-permeable PVC film, physicochemically analyzed at 24 h postmortem, stored at 5°C under 1,000 lx illumination for up to 7 d, and analyzed again. The freeze-dried samples were ground through a 1-mm screen and scanned by NIRS.

[2]Nordos Fat W3, Trouw Nutrition, Highland, IL.
[3]InfraSoft International, Port Matilda, PA.

Laboratory Analysis

On the wet meat samples, pH and L*a*b* color (CIE, 1976) were measured at 24 h and 7 d postmortem.

On the freeze-dried samples, moisture, ether extract, and ash were determined and protein was calculated by difference in accordance with standards of the Association of Official Analytical Chemists (AOAC, 1984). Fatty acid profile was analyzed by GC, after Folch extraction (Folch et al., 1957). Cholesterol content was determined according to Casiraghi et al. (1994). Lipid oxidation was determined, after pooling the breast meat of the 2 hens sharing the same cage (n = 36), as thiobarbituric acid-reactive substances (TBARS) index by HPLC (Bergamo et al., 1998) and expressed in micrograms of malondialdehyde per gram of fresh tissue.

Diets were analyzed for chemical composition (DM, ash, CP, crude fiber) and for FA profile according to AOAC methods (1984).

NIRS

Measurement of the NIR spectra was performed using a Foss NIRSystems 5000 system, with small ring cup cells. Measurements were made in reflectance mode between 1,100 and 2,498 nm every 2 nm. All samples were scanned in duplicate. The 2 subsamples were compared by root mean square and if the root mean square corrected for bias was too large, 2 new subsamples were scanned. The average spectrum was used for NIR analysis. The resulting spectra were stored as log (1/R) on WINISI II version 1.02 software.[3]

Calibrations and Statistical Analyses

The WINISI software was used for the data analysis. Calibrations were performed by modified partial least squares (MPLS) regression. To optimize calibration accuracy, the data were subjected to a variety of derivative transformations using common mathematical treatments and scatter correction treatments. The 3 mathematical treatments were log (1/R), its first and second derivative. These are denoted 0,0,1; 1,4,4; and 2,10,4, respectively. The first number indicates the derivative order and the second number denotes the number of data points in the segment used to calculate the derivative. The third numbers denote the number of data points over which running average smoothing is conducted.

Cross validation was performed during model development, whereby one-fourth of the calibration samples at a time were temporarily removed from the calibration set. The optimal number of factors (MPLS terms) for the different constituents was that which produced a minimum in overall error between modeled and reference values (standard error of cross validation). Samples with large cross validation residuals (T values above 2.5) were omitted, and the cycle was performed a second time, that is, the computer program removed outliers twice before completing the final calibration.

TABLE 1. Chemical composition of experimental diets

	Control (C)	Extruded linseed (EL)	Ground linseed (GL)	n-3 HUFA[1] of marine origin (NF)
	%			
Inclusion level	—	10.0	10.0	3.4
Moisture	9.0	8.6	8.8	8.7
Crude protein	17.5	17.6	17.8	17.8
Ether extract	5.6	6.6	5.9	6.0
Ash	12.8	12.0	10.1	13.9
Crude fiber	2.9	3.9	4.2	4.7
	% of total fatty acids			
Total PUFA[2]	32.2	58.4	62.9	39.9
PUFA n-3:				
C18:3 n-3	1.87	31.41	30.40	5.64
C20:5 n-3	0.00	0.00	0.00	1.86
C22:6 n-3	0.00	0.00	0.00	2.24

[1]HUFA = highly unsaturated fatty acids.

[2]PUFA = polyunsaturated fatty acids.

The scatter corrections were performed by standard normal variate (SNV) and detrending (DT) used separately or in combination (SNV-DT) or by standard multiplicative scatter correction (MSC). The best one was selected for each constituent based on the highest R^2 (multiple correlation coefficient in calibration) and the lowest standard error of calibration and cross-validation (SEC and SECV, respectively).

Discriminant Analysis

The discriminant analysis was performed using partial least squares regression (PLS). In the PLS calibration, the spectral files from the 4 different groups were entered, and the program set up a calibration matrix with "dummy variables" (ones and twos) against the different file names. The calibration was then conducted by regressing the wavelength data on the groups defined as 1 or 2. Cross validation was used to test the accuracy of the model. Principal component analysis was used to calculate scores and produce scatter plots of the different feeding groups.

RESULTS AND DISCUSSION

Analyses

Chemical composition, cholesterol content, and TBARS index of the samples of freeze-dried breast muscle (n = 70) used for calibration are shown in Table 2.

The lipid content had the largest CV and ranged from 1.85 to 11.8 g/100 g of DM, with an SD of 2.3. Because of the tight relationship with the content of lipids, protein had similar SD (SD = 2.4% DM) and range of about 10 units (min = 83.0; max = 93.5% of DM). On the other hand, DM and ash had a much smaller SD of 0.7% of DM. Compared with meat from hens at the end of lay (Lee et al., 2003), the samples used in this study had a lower lipid content and greater content of protein and ash, which would agree with the younger age of the birds used in this trial.

Table 3 summarizes the results obtained for the FA contents of the meat lipid extract. The main saturated FA were C16:0 (0.43 to 3.07% of DM) and C18:0 (0.17 to 1.14% of DM), corresponding to an average of 25.2 and 9.7% of total FA, respectively. The main unsaturated FA were

TABLE 2. Statistical overview of chemical composition, cholesterol, and TBARS levels[1] of freeze-dried pectoralis superficialis muscles[2]

Constituent	Mean	Minimum	Maximum	SD	CV (%)
DM[3]	93.8	91.8	94.8	0.7	0.7
Crude protein[4]	89.3	83.0	93.5	2.4	2.6
Lipids	5.6	1.9	11.8	2.3	41.5
Ash	5.1	4.0	7.5	0.7	14.3
Cholesterol	223	185	272	19	8.5
TBARS	0.31	0.11	0.49	0.11	3.5

[1]TBARS = thiobarbituric acid-reactive substances.

[2]Units in g/100 g of DM. Cholesterol unit in mg/100 g of DM and TBARS unit in μg of malondialdehyde/g; n = 70 for DM, crude protein, lipids, ash, and cholesterol; n = 35 for TBARS.

[3]DM = residual DM after freeze-drying.

[4]Protein is calculated by difference (100 – ash – lipids) and, therefore, the value includes carbohydrates.

TABLE 3. Statistical overview of the fatty acid (FA) composition of freeze-dried pectoralis superficialis muscle (values in g/100 g of DM, n = 70)

Constituent	Mean	Minimum	Maximum	SD	CV (%)	% TFA[1]
C10:0	0.02	0.00	0.26	0.04	218	0.31
C12:0	0.02	0.00	0.34	0.05	240	0.39
C14:0	0.07	0.01	0.22	0.05	62	1.34
C15:0	0.04	0.00	0.34	0.06	154	0.65
C16:0	1.41	0.43	3.07	0.58	41	25.3
C17:0	0.02	0.00	0.21	0.03	137	0.37
C18:0	0.54	0.17	1.14	0.23	42	9.77
C24:0	0.05	0.00	0.21	0.05	95	0.94
Total saturated FA	2.17	0.69	4.43	0.88	40	39.0
C14:1	0.03	0.00	0.14	0.04	128	0.50
C16:1	0.07	0.01	0.17	0.04	59	1.20
C17:1	0.01	0.00	0.08	0.02	137	0.23
C18:1	1.39	0.44	2.99	0.57	41	25.0
Total monounsaturated FA	1.50	0.50	3.23	0.61	41	26.9
C18:2 n-6	0.72	0.26	1.58	0.29	41	13.0
C18:3 n-3	0.07	0.01	0.33	0.07	95	1.29
C20:2 n-6	0.01	0.00	0.03	0.01	159	0.07
C20:3 n-6	0.01	0.00	0.07	0.02	113	0.24
C20:4 n-6	0.76	0.25	1.69	0.34	44	13.6
C20:5 n-3	0.02	0.00	0.12	0.02	118	0.38
C22:6 n-3	0.30	0.07	1.03	0.22	72	5.46
Total polyunsaturated FA	1.90	0.67	3.75	0.76	40	34.1
Total unsaturated FA	3.40	1.16	6.86	1.35	40	61.0
n-6	1.50	0.54	3.25	0.63	42	26.9
n-3	0.40	0.08	1.15	0.25	63	7.13

[1]Percentage of total FA.

C18:1 (0.44 to 2.99% of DM), C18:2 n-6 (0.26 to 1.58% of DM), C20:4 n-6 (0.25 to 1.69% of DM), and DHA (0.07 to 1.03% of DM), corresponding to approximately 25.0, 13.1, 13.5, and 5.4% of total FA, respectively. Values of pH were similar between recordings at 24 h (range = 5.57 to 5.85) and 7 d (range = 5.51 to 5.86), both averaging 5.66. pH showed a very narrow range of variation with SD of only 0.05 to 0.06.

NIRS Calibration Results

For the calibration, 70 breast meat samples were used. The most suitable of the different mathematical treatments was applied for each constituent. The dispersion was reduced using MSC, SNV, DT, or SNV-DT. Generally the highest coefficient of determination (R^2) and lowest SECV were obtained using the first derivative. The statistical parameters of the calibration equations for each component are presented in Tables 4, 5, and 7.

Calibration for lipids had an R^2 of 0.99 and SEC of only 0.19% of DM. These performances were also carried after a cross-validation test confirming the ability of NIRS to predict total fat content. Similarly, Abeni and Bergoglio (2001) obtained SEC = 0.20, R^2 = 0.98, SECV = 0.24 and 1–VR = 0.97, for calibration of lipids on 39 freeze-dried chicken breast samples. Cozzolino et al. (1996) obtained an R^2 of 0.95 for lipid calibration on minced samples of chicken breast.

Despite the narrow range of DM content of our samples, the calibration equation had excellent performances [SEC = 0.14; R^2 = 0.96; SECV = 0.19; 1–VR (coefficient of determination of cross validation) = 0.91]. Other researchers found it difficult to calibrate for DM of freeze-dried samples because all samples have similar moisture content (Abeni and Bergoglio, 2001). Although moisture has strong absorption at the bands around 1,450 and 1,930 nm (Shenk et al., 1992), accuracy of prediction of moisture may be compromised because of the reference method in relation to the loss of volatiles during oven drying (Shenk et al., 1992). Also, dried samples may change moisture content in relation to variation of relative humidity in the air. If samples are not stored in airtight containers, residual moisture analysis and NIRS spectra collection should be performed at the same time to ensure best accuracy of the calibration equation.

Near infrared reflectance spectroscopy showed very good performances of calibration (SEC = 0.71; R^2 = 0.91; SECV = 0.74; 1–VR = 0.91) for CP. Protein is the main constituent of meat and averaged 89.3% of DM in these samples. That means that error of prediction (SECV = 0.74) was less than 1% of the content of protein, confirming the good predicting capacity of NIRS. In fact, protein bands can be seen at 2,174 nm (N-H second overtone, C-H stretch/C=O stretch combination band, C=O stretch/N-H amid combination band), 2,056 nm (N-H stretching vibrations of various types), at 1,516 nm due to N-H stretch first overtone, and at 2,468 nm (C-N-C stretch first overtone). These results confirm those obtained by Sindic et al. (1993), who reported similar accuracy of predictions for protein in broiler breast meat.

Calibration equations for cholesterol and TBARS had much poorer performances that were not reliable for accurate prediction. It should be noted that TBARS had an R^2 of calibration of 0.73, which would not be sufficient for quantitative determination but would provide good qualitative information. That there were only 35 samples avail-

TABLE 4. Calibration and validation statistics for chemical components in hen pectoralis superficialis muscle near-infrared reflectance spectroscopy

Constituent	Scatter correction and math[1]	T[2]	N[3]	SEC[4]	R^2 [5]	SECV[6]	1-VR[7]
DM	DT 1d	5	68	0.14	0.96	0.19	0.91
Crude protein	SNV-DT 1d	3	70	0.71	0.91	0.74	0.91
Lipids	DT 1d	5	69	0.19	0.99	0.24	0.99
Ash	SNV-DT 1d	1	69	0.64	0.05	0.65	–0.004
Cholesterol	SNV-DT 2d	4	67	0.11	0.54	0.14	0.34
TBARS[7]	MSC 2d	4	35[1]	0.06	0.73	0.08	0.53

[1]DT = detrending, SNV-DT = standard normal variate and detrending, MSC = multiplicative scatter correction.

[2]Number of modified partial least squares (MPLS) factors used to build the calibration.

[3]Number of samples in calibration (after outlier elimination).

[4]Standard error of calibration.

[5]Coefficient of determination in calibration.

[6]Standard error of cross validation.

[7]Coefficient of determination of cross validation.

[8]TBARS (thiobarbituric acid-reactive substances) were measured on a mixture of meat from the 2 hens sharing the same cage.

able for TBARS may have been a limitation in the development of good predicting calibration, and because of the limited number of samples, performances after the cross-validation may have been greatly reduced.

The calibrations were most accurate for the major FA (Table 5), i.e., the saturated FA, C16:0 and C18:0, and the unsaturated FA, C18:1, C18:2 n-6, C20:4 n-6, and DHA. Total saturated, monounsaturated, polyunsaturated, unsaturated, and n-6 FA obtained high R^2 values (0.97, 0.95, 0.98, 0.98, and 0.94, respectively). C18:3 n-3 had an R^2 = 0.85, similar to that for DHA (0.87), and a lower SECV (0.03 vs. 0.11).

According to Gonzàlez-Martìn et al. (2002), the structure of FA can be appreciated in special spectral character-

TABLE 5. Calibration and validation statistics for fatty acids (FA) in pectoralis superficialis muscle of hens by near-infrared reflectance spectroscopy

FA	Scatter correction and math[1]	T[2]	N[3]	SEC[4]	R^2 [5]	SECV[6]	1-VR[7]
C10:0	MSC 1d	1	65	0.01	0.16	0.014	0.16
C12:0	MSC 1d	1	65	0.01	0.21	0.014	0.17
C14:0	SNV-DT 1d	1	66	0.03	0.30	0.034	0.26
C15:0	SNV-DT 1d	3	67	0.03	0.27	0.034	0.11
C16:0	DT 1d	3	69	0.10	0.97	0.102	0.97
C17:0	SNV-DT 2d	1	67	0.01	0.10	0.012	0.01
C18:0	SNV-DT 1d	3	69	0.05	0.98	0.052	0.95
C24:0	SNV-DT 2d	2	69	0.01	0.34	0.015	0.31
Total saturated FA	DT 1d	8	69	0.15	0.97	0.160	0.97
C14:1	SNV-DT 2d	1	68	0.02	0.12	0.026	0.02
C16:1	SNV-DT 2d	3	68	0.02	0.65	0.027	0.53
C17:1	MSC 1d	1	65	0.01	0.11	0.010	0.06
C18:1	SNV-DT 1d	4	69	0.12	0.98	0.139	0.94
Total monounsaturated FA	DT 1d	4	69	0.14	0.95	0.150	0.94
C18:2 n-6	DT 1d	7	69	0.06	0.96	0.094	0.91
C18:3 n-3	MSC 1d	7	67	0.02	0.85	0.031	0.73
C20:2 n-6	SNV-DT 1d	3	69	0.01	0.49	0.005	0.37
C20:3 n-6	SNV-DT 1d	1	71	0.02	0.33	0.015	0.30
C20:4 n-6	DT 1d	3	69	0.11	0.90	0.117	0.89
C20:5 n-3	DT 1d	5	68	0.01	0.62	0.015	0.53
C22:6 n-3	SNV-DT 1d	8	69	0.07	0.87	0.111	0.69
Total polyunsaturated FA	DT 1d	5	68	0.11	0.98	0.135	0.97
Total unsaturated FA	DT 1d	3	68	0.19	0.98	0.201	0.98
n-6	DT 1d	3	69	0.15	0.94	0.165	0.94
n-3	DT 1d	5	68	0.11	0.78	0.120	0.73

[1]DT = detrending, SNV-DT = standard normal variate and detrending, MSC = multiplicative scatter correction.

[2]Number of modified partial least squares (MPLS) factors used to build the calibration.

[3]Number of samples in calibration (after outlier elimination).

[4]Standard error of calibration.

[5]Coefficient of determination in calibration.

[6]Standard error of cross validation.

[7]Coefficient of determination of cross validation.

TABLE 6. Calibration and validation statistics for 3 polyunsaturated fatty acids (FA) in pectoralis superficialis muscle of hens by near-infrared reflectance spectroscopy (zero values not included in the calculations)

Constituent	Scatter correction and math[1]	T[2]	N[3]	SEC[4]	R^{2} [5]	SECV[6]	1-VR[7]
C20:2 n-6	SNV-DT 1d	2	28	<0.01	0.75	0.003	0.67
C20:3 n-6	SNV-DT 1d	1	35	0.01	0.64	0.011	0.57
C20:5 n-3	SNV-DT 1d	4	43	<0.01	0.85	0.011	0.71

[1]SNV-DT = standard normal variate and detrending.
[2]Number of modified partial least squares (MPLS) factors used to build the calibration.
[3]Number of samples in calibration (after outlier elimination).
[4]Standard error of calibration.
[5]Coefficient of determination in calibration.
[6]Standard error of cross validation.
[7]Coefficient of determination of cross validation.

istics. Fatty acids show absorption at 1,680, 2,150, and 2,190 nm, attributable to the –CH bond joined to a *cis*-unsaturation. The absorption bands between 1,600 and 1,800 nm and those between 2,100 and 2,200 nm are related to the length of the chain and the double *cis*-bond, respectively (Gonzàlez-Martìn et al., 2002). The first overtone stretch bands from $–CH_3$ can be seen around 1,700 nm, and the stretch combination bands of the $–CH_2$-bonds are located around 1,722 and 1,760 nm. These results agreed with those reported by Pedro et al. (1992, cited by Windham and Morrison, 1998), who obtained reliable predictions for the major FA (C16:0, C18:0, C18:1, and C18:2 n-6) in Iberian swine carcasses, but found it difficult to quantitatively determine minor FA. Molette et al. (2001) reported successful results of NIRS calibration equations for the FA composition of goose fatty livers. In their study, the determinations of C14:0, C16:0, C:16:1, C18:0, C18:1 n-9, and C18:2 n-6 were accurate, with R^2 similar to those found in the present work (0.85, 0.89, 0.94, 0.97, 0.99, and 0.91, respectively).

From our data and the literature it appears that good relationships between spectral and chemical information for FA can be obtained with concentrations above of 0.5 g/100 g of DM. Plotting 1–VR against the concentration of each FA and FA group, it can be observed that as the concentration of the FA becomes greater, 1–VR rapidly increases and reaches values of 0.9 or above only when the concentration is greater than 0.5 g/100 g of DM. Among the FA at low concentration, it seems that the n-3 family has the better R^2-obtaining performances, which would satisfy qualitative evaluation. For the polyunsaturated FA at the lowest concentration (C20:2 n-6, C20:3 n-6, and C20:5 n-3), 50 to 65% of the samples did not have a detectable concentration of FA. The calibration had several samples that didn't carry information about that specific FA. For these 3 FA, calibration was rerun using only the samples with detectable concentrations of FA, improving all of the calibration performances (Table 6). The improvement may be attributed to an artificial increase in the average concentration of the data set because of deleting samples with zero values. If FA profile is an important component of quality of animal product, it may be possible to improve NIRS predictions by using actual fat samples that have greater concentrations of FA instead of meat samples such as those used in this study.

The statistical summary of calibration and prediction by cross-validation for pH, based on data from measurements 24 h postmortem and after 7 d, is presented in Table 7. The R^2 (0.67 vs. 0.64) and SECV (0.04 vs. 0.05) were similar for the 2 measurements, which could be expected because the data from 24 h and 7 d were rather similar. The large differences in performance between the

TABLE 7. Calibration and validation statistics for pH in pectoralis superficialis muscle of hens by near-infrared reflectance spectroscopy

Constituent	Scatter correction and math[1]	T[2]	N[3]	SEC[4]	R^{2} [5]	SECV[6]	1-VR[7]
pH 24 h	DT 1d	6	71	0.03	0.67	0.04	0.40
pH 7 d	SNV-DT 2d	5	69	0.04	0.64	0.05	0.41

[1]DT = detrending, SNV-DT = standard normal variate and detrending.
[2]Number of modified partial least squares (MPLS) factors used to build the calibration.
[3]Number of samples in calibration (after outlier elimination).
[4]Standard error of calibration.
[5]Coefficient of determination in calibration.
[6]Standard error of cross validation.
[7]Coefficient of determination of cross validation.

TABLE 8. Discriminant analysis of pectoralis superficialis muscle from hens submitted to 4 diets (standard normal variate and detrending correction and math treatment 0.0.1.1.)

	Prediction			
Diets	C	NF	GL	EL
Control (C)	17	0	0	0
n-3 of marine origin (NF)	0	16	2	1
Ground linseed (GL)	1	1	12	2
Extruded linseed (EL)	0	1	4	15
Total	18	18	18	18
Misclassified	1	2	6	3

SEC and SECV indicate the difficulties in the prediction of this parameter (R^2 = 0.67 and 1–VR = 0.40 for pH at 24 h). Better calibration models for pH have been obtained in intact and homogenized pork meat (R^2 = 0.73 to 0.79; Andersen et al., 1999). The low variability of pH and the different substrate used for pH measurement and NIR scan might contribute to the poor result of the calibration performances.

Discriminant Analysis

The modified partial least squares (MPLS) calibration was conducted on the combined data set of 72 samples. These consisted of 18 samples from each of the different feeding groups (C, NF, EL, and GL). The SNV-DT applied to 0,0,1 math using 14 MPLS terms yielded the best results. The SEC was 0.06, R^2 = 0.69, SECV = 0.15, and 1–VR = 0.20. The result of the discriminant analysis is presented in Table 8.

The meat from the C group was easiest to distinguish, followed by the NF group. The meats from the 2 linseed diets (EL and GL) were difficult to distinguish from each other. Spectral differences between the diets can be seen in the plot of the first derivative mean spectra between 2,200 and 2,400 nm (Figure 1). The absorption bands in this region are related to the stretch combination bands of the $–CH_2$- bonds (fat). Chemically, the meat samples differed mainly in FA composition, and that may have caused the differences in absorption in this region. Fumière et al. (2000) suggested that the FA composition of chicken meat was a discriminant criterion between "slow-growing" and "industrial" chicken strains. Because of the spectral differences in this region, and the similarities between the EL and GL meat, a new calibration was conducted using only the region between 2,200 and 2,490 nm and with EL and GL samples considered as one group. Here, the SNV-DT applied to 0,0,1 mathematics using 9 MPLS terms gave the best results. The results of the new discriminant analysis are presented in Table 9. By changing the performance it became more difficult to distinguish the NF samples from the EL and GL samples, whereas the C samples were 100% correctly classified.

The principal component score plot of the 72 samples (Figure 2) shows similarities between the NF samples and

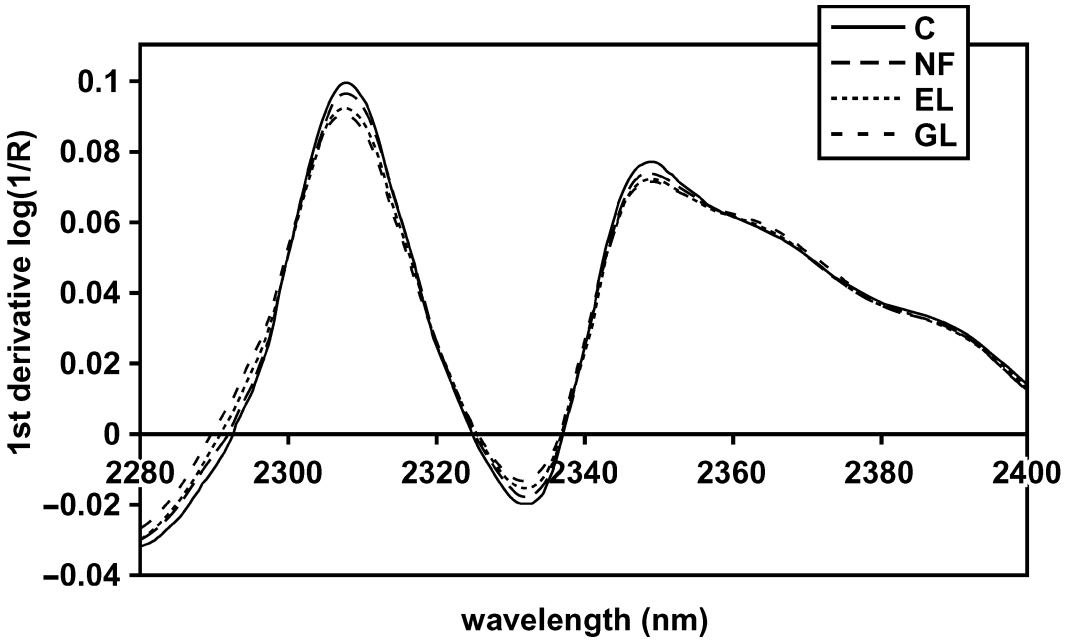

FIGURE 1. Pooled average (n = 18) near infrared reflectance spectra of pectoralis superficialis muscle samples from hens fed 4 diets differing in fatty acid source. C = control, NF = highly unsaturated fatty acids of marine origin, EL = extruded linseed, and GL = ground linseed.

TABLE 9. Discriminant analysis of pectoralis superficialis muscle from hens fed 4 diets (standard normal variate and detrending correction and math treatment 0.0.1.1.). Wavelengths between 2,200 and 2,490 nm

	Prediction		
Diets	C	NF	EL + GL
Control (C)	18	0	0
n-3 of marine origin (NF)	0	12	2
Extruded and ground linseed (EL + GL)	0	6	34
Total	18	18	36
Misclassified	0	6	2

the linseed samples, which can explain the difficulties in discriminating between these groups.

Results showed that NIRS could be used to determine the major chemical constituents of hens' freeze-dried breast muscle. The ability of NIRS to predict the contents of lipids, protein, and DM of chicken meat was confirmed, but ash prediction was not possible. The content of total FA and the major FA (C16:0, C18:0, C18:1, C18:2 n-6, and DHA) could be accurately determined, whereas the performances for the minor FA were poorer. The failure to determine some individual FA may be due to their low concentration in the muscle An exclusion of the samples not containing a certain FA (C20:2 n-6, C20:3 n-6, and EPA) improved the performances. Prediction of cholesterol, pH, and lipid oxidation was not possible for the freeze-dried meat. Partial least squares discriminant analysis of the NIR spectra successfully (100%) distinguished between the control meat samples and the meat from hens fed with polyunsaturated FA supplements, whereas it seemed more difficult to distinguish between the different dietary polyunsaturated FA sources. The discrimination is probably based on the FA composition of the meat.

Because of its speed of analysis and low operation costs, NIRS could be used for prediction of chemical composition and profile of the major fatty acids defining the quality of chicken meats.

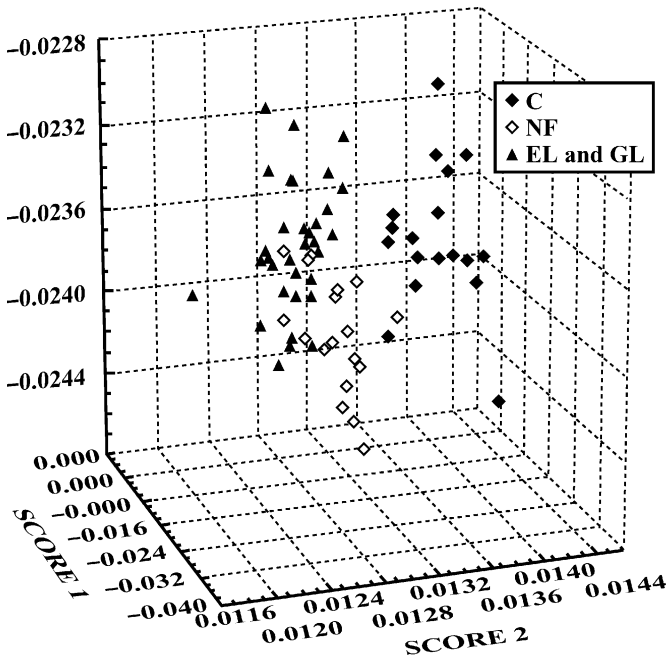

FIGURE 2. Principal component analysis score plot of the 72 samples of pectoralis superficialis muscle from hens fed 4 diets differing in fatty acid source. C = control, NF = highly unsaturated fatty acids of marine origin, EL = extruded linseed, and GL = ground linseed.

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