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Near infrared spectroscopy for enforcement of European legislation concerning the use of animal by-products in animal feeds

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The paper summarises the work done in the framework of two R&D projects aimed to demonstrate the contribution of Near Infrared Spectroscopy (NIRS) to help the enforcement of the European legislation governing the use of animal by-products in animal feeds. Three different types of animal feed products were studied: compound feeds (CFs), animal protein by-products meals (APBPs) and animal fats by-products (AFBPs). The quantitative and qualitative chemometric models produced with a large collection of compound feed samples (n = 1005 ground and 523 unground) have demonstrated, that NIRS can be used for the detection and quantification of the meat and bone meal (MBM) added to compound feeds. Discriminant models produced with unground samples produced 100% of correctly classified samples in two cloned instruments placed in two different locations. The results also show that two dimensions NIR spectra of Animal By-Products (ABP, animal meals and fats) may contain information about the animal species or group of species from which the ABPs were produced. However, further work is needed to enlarge the sample bank and the spectral libraries with well authenticated samples in order to increase the robustness of the quantitative and qualitative NIRS models. The paper opens expectations for using NIRS for the enforcement of legislation concerning the use of ABPs in animal feeds. More research and demonstration efforts have to be done in order to obtain more definitive and robust predictive models and for optimising its implementation either at-line, on-line and in-line in feed factories and inspection laboratories.
Keywords. Compound feeds, animal by-products, meat and bone meal, animal fats, NIRS, unground sample analysis.

Utilisation de la spectroscopie proche infrarouge pour renforcer l'application de la législation européenne en ce qui concerne l'utilisation des sous-produits animaux dans l'alimentation animale. L'article résume le travail réalisé dans le cadre de deux projets R&D visant à démontrer la contribution de la spectroscopie proche infrarouge (SPIR) au renforcement de l'application correcte de la législation européenne existante pour l'utilisation des sous-produits animaux dans l'alimentation animale. Trois types différents d'ingrédients animaux ont été utilisés : aliments composés (CFs), farines de protéines de sous-produits animaux (APBPs) et des graisses animales (AFBPs). Les modèles chimiométriques, quantitatifs et qualitatifs, construits en utilisant une grande collection de farines composées (n = 1005 broyés et 523 non-broyés) ont montré que la SPIR peut-être utilisée pour la détection et la quantification des farines animales ajoutées aux aliments composés. Des modèles dicriminants construits avec des échantillons non-broyés permettent de classer correctement 100 % des échantillons et cela en utilisant deux instruments clonés et installés dans deux laboratoires différents. Les résultats ont également montré que les spectres SPIR de sous-produits animaux (farines et graisses animales) peuvent contenir l'information concernant l'espèce ou le groupe d'espèces constituant le sous-produit animal. Toutefois, des recherches complémentaires sont nécessaires afin d'augmenter la banque d'échantillons et les librairies spectrales par l'ajout d'échantillons authentiques afin d'augmenter la robustesse des modèles SPIR quantitatifs et qualitatifs. L'article montre les perspectives de l'utilisation de la SPIR pour renforcer l'application correcte de la législation européenne existante pour l'utilisation des sous-produits animaux dans l'alimentation animale. Des recherches complémentaires doivent être menées afin d'obtenir des modèles définitifs et robustes ainsi que pour optimiser leur implémentation "at-line", "on-line" et "in-line" dans les fabriques d'aliments et les laboratoires.
Mots-clés. Aliments composés, sous-produits animaux, farine de viande et d'os, graisse animale, SPIR, analyse d'échantillons non-broyés.

1. INTRODUCTION

The use of animal by-products (ABPs) such as meat and bone meals (MBM) play a crucial role in feed manufacturing, but its safe and healthy use in animal feeds is of prime public concern in order to prevent the spread of BSE (Bovine Spongiform Encephalopathy). The ban on the use of animal origin meals in compound feeds (EC, 2000) is one of the measures carried out in the EU to stop the spread of the Bovine Spongiform Encephalopathy (BSE) and to prevent its re-occurrence. Further EU legislation (EU, 2002) addressed the intra-species recycling.

It is now clear that the existing legislation concerning the use of ABPs in animal feeds will be lifted only if analytical methods that ensure its enforcement are available. These methods have to be able to detect the presence of MBM, but also allow the identification of the animal species origin of the meal. Till now no current validated analytical test meets these requirements.

NIR spectroscopy is likely to be the most rapid method of testing feedingstuffs, allowing a substantial increase of the number of controlled samples and providing an instantaneous response to detect adulterated samples. NIRS is one of the favourite techniques for both quantitative and qualitative analyses of animal protein by-products (APBPs) and feed mixtures containing them (Murray *et al.*, 2001).

The objective of the present work is to summarise the work previously done in the framework of two R&D projects aimed to demonstrate the contribution of Near Infrared Spectroscopy to help the enforcement of legislation governing the use of animal by-products in animal feeds.

2. MATERIAL AND METHODS

Three types of animal feeds have been studied: compound feeds (CFs), animal protein by-products (APBPs) and animal fat by-products (AFBPs).

The samples were either coming from a "real-process" of production (feed mills and rendering plants) or deliberately produced under controled laboratory or pilot plant conditions. They have been collected in the framework of two R&D Projects (EU STRATFEED Project nºG6RD-2000-CT-00414 and MCYT-INIA-Spain Projects nºCAL 02-028-C2-2) and they were kept at the Sample Bank of the University of Córdoba (Spain).

2.1. NIR spectra analysis

Two scanning monochromator FOSS NIRSystems model 6500 SY-I and SY-II (FOSS NIRSystems, Silver Spring, MD, USA), equipped with a spinning module (for ground sample analysis) and a transport module (for unground sample analysis) respectively, were used to measure reflectance spectra from 400 to 2498 nm, with a spectral resolution of 2 nm. The spectra were recorded with the ISI NIRS 3 software ver.3.11 (Infrasoft International, Port Matilda, PA, USA).

Compound feeds (CFs) were analysed both in ground (Cyclotec Mill) and unground forms. In the first case the standard ring cups (3.75 cm diameter) were used. The unground samples were analysed using the rectangular cups (Natural Product Sample Cup IH-0331) with dimensions of 4.7 cm wide, 20 cm long and 4.3 cm depth.

The animal protein by-products (APBPs) meals were analysed in ground form, using the standard ring cups too.

Animal fats by-products (AFBPs) were analysed, after melting in microwave, in folded-transmission gold reflector cups, with a pathlength of 0.1 mm. Two spectra were measured per sample, using the mean spectrum for subsequent chemometric treatments.

2.2. Calibration/library sets

Several sets of compounds feeds (CFs), protein by-products meals (APBPs) and animal fat by-products (AFBP) were considered.

- CF1 set includes 1005 compound feeds (CFs). The reference data (inclusion percentage of meat and bone meal) of 560 of these samples were obtained from the formulation declared by feed company providers and the other data were estimated from optical microscopy. These samples were analysed after grinding (1 mm particle size).
- CF2 set includes 523 CF samples. The reference data were obtained from the feed formulation. The samples were analysed as they were provided (i.e. pellets, gross milling meals, extruded, etc.).
- CF3 set includes 18 CFs, collected from a feed manufacturer after the publication of the total MBM ban (EC, 2000). Ten samples were CFs for production animals and therefore free of MBM; while the eight others were pet foods containing different percentages of MBM. This set was analysed unground, using the natural cup, in two cloned NIR instruments placed at the two different laboratories.
- APBP1 set includes a total of 280 samples of animal protein by-products meals collected between October 2002 and April 2004 from different providers. Each sample was supplied with an identity form containing, among others, information about the raw materials used, that is, the percentage of meat of each animal species (i.e. pig, cow, poultry, ewe, etc.) used to produce the rendered meal sample.

– APBP2 set is made up of 1 blood meal sample, 8 fish meal samples, 1 hydrolised feather meal and 1 feather meal.
– APBP3 set consisted of 29 APBPs samples provided by the European Fat Processors and Renderers Association (EFPRA) as a blind set for external validation.
– AFBP1 set, with a total of 77 animal fat by-products collected from different rendering plants. Each sample was supplied with an identity form containing, among others, information about the raw materials used, that is, the percentage of meat of each animal species (i.e. pig, cow, poultry, sheep, etc.) used to produce the rendered fat.
– AFBP2 set, with a total of 5 fish oils, 48 rendered fats, 1 soybean oil and 1 olein.

2.3. NIRS data treatment

Different chemometric strategies are being evaluated on the various studied material (CFs, APBP and AFBP) in the framework of three PhD theses. However, only part of the results are described in this paper.

Compound feeds (CFs). Two different chemometric strategies were evaluated. The first one consisting in the developing of Modified Partial Least Square (MPLS) algorithm in order to build regression equations between the spectral data and the percentage of MBM of each sample in data sets CF1 and CF2.

The second one used also the MPLS algorithm, but instead of using the percentage of MBM as reference data, dummy variables (1 or 2 for CFs free and containing MBM, respectively) was used. In such a way a discrimination model is constructed. The software Win ISI ver. 1.05 was used to perform the MPLS regression equations and evaluate the calibration models. The statistics used to select the best equations were the coefficient of determination (r^2) and the standard error of cross validation (SECV) (ISI, 2000).

Animal protein by-products (APBPs). As for compound feeds, two different calibration strategies were evaluated. The first one consisting in the use of MPLS Regression algorithm to construct equations between the spectral data and the percentage of each species in the APBP1 samples.

The second one consisting in the use of Partial Least Square 2 discriminant analysis, using dummy variables, to discriminate between ruminant (1) and non-ruminant (2) APBPs.

Standard Normal Variate and Detrending (Barnes *et al.*, 1989) of log 1/R at each wavelength was also used to obtain two dimensional wavelengths spectral patterns of well identified samples belonging to set APBP1 and APBP2, and blind samples belonging to APBP3. The software Win ISI ver. 1.05 was used to perform the MPLS regression equations, Partial Least Square 2 for discriminant analysis and the Standard Normal Variate and Detrending Algorithms (ISI, 2000).

Animal fats by-products (AFBPs). As for AFBPs, Partial Least Square 2 discriminant analysis, using dummy variables, was performed with the APBP1 set to discriminate between ruminant poultry, pork and mixture (pork + poultry, cattle, sheep) fats. Standard Normal Variate and Detrending (Barnes *et al.*, 1989) of log 1/R at each wavelength was also used to obtain two dimensional spectral patterns of well identified samples provided by animal and vegetal fat producers belonging to set AFBP2.

3. RESULTS AND DISCUSSION

3.1. Compound feeds

The calibration sets CF1 (ground compound feeds) and CF2 (unground compound feeds) were used to obtain calibration equations for the prediction of the percentage of MBM. The mean, standard deviation (SD) and range of the MBM content in weight percentage and the other calibration statistics obtained are shown in **table 1**.

Calibration equations produced with ground and unground samples, explained a similar percentage (97% and 98%) of the variation existing in the percentage of MBM. However, the cross validation errors were lower when samples were analysed in unground form (SECV = 0.80) than in ground form (SECV = 0.94) (**Table 1**).

Table 1. Mean, range and standard deviation of the MBM percentage and calibration statistics for predicting the MBM percentage of compound feeds (sets CF1 and CF2) — *Moyenne, amplitude et déviation standard des pourcentages en farines de viande et d'os, et paramètres statistiques de calibrage pour la prédiction du pourcentage en farines de viande et d'os dans les aliments composés (lots CF1 and CF2)*.

	% MBM	
	Ground (set CF1)	**Unground (Set CF2)**
n	1005	523
Mean	3.24	3.09
Range	0.00–34.85	0.00–32.55
SD	5.50	5.23
SECV	0.94	0.80
r^2	0.97	0.98

n: number of samples in the calibration set; SD: standard deviation; SECV: standard error of cross validation; r^2: determination coefficient.

An in-house validation of the best calibration model (developed with CF2 set) was carried out with a completely external validation set (CF3) consisting of 18 CFs analysed unground in two matched NIR instruments. These validation samples, produced during 2003, were representative of those produced after the publication of the total ban. For the quantitative prediction of the MBM percentage, the results obtained in both matched instruments are quite similar (**Table 2**). Quantitative models produced 83.3% of correctly classified samples for instruments 1 and 2. All the samples containing MBM and seven out of the ten samples free of MBM were well predicted, resulting thus on three false positive samples. A specific feature of these three samples is that they contain animal fat. It seems that presence of animal fats may interfere in the prediction of the constituent MBM.

The second strategy evaluated was the use of models for predicting the presence/absence of MBM in compound feeds. The same strategy was successfully tested by Murray *et al.* (2001) to detect adulterations of fish meal with MBM. For this study, two discriminant equations, which had SECVs of 0.33% and 0.19 and r^2 of 0.54 and 0.86 for the ground and unground calibration sets (CF1 and CF2), respectively have been created. As for the quantitative models, the accuracy of the discriminant equations produced with unground samples was higher than with ground samples. The best discriminant model was applied to the blind test set of 18 CF3 samples analysed in the two cloned instruments. The results showed that discriminant models produced 100% correctly classified samples in both instruments (**Figure 1**). The three misclassified samples by the quantitative models are now well classified as being MBM free. It seems that qualitative NIR analysis is not affected by the possible interference caused by another animal ingredient present in the CF.

Figure 1 shows the score plots for the best qualitative model (unground). MBM-free compound feeds group together in one cluster around a score of 1.0, for the same samples scanned in the two instruments while CFs containing different levels of MBM cluster around 2.0. With a breakpoint score of 1.5, all samples were discriminated correctly. The same strategy was successfully tested by Murray *et al.* (2001) to detect adulterations of fish meal with MBM.

Table 2. Predicted % MBM versus reference data for the CF3 validation samples analysed with the two cloned instruments — *Pourcentages en farines de viande et d'os prédits par deux instruments clonés comparés aux données de référence pour les échantillons de validation du lot CF3*.

Sample reference	% MBM reference	%MBM NIRS Lab 1	Lab 2
13484	0	-0.19	-0.20
13485	0	-0.08	-0.24
13486	**0**	**1.32**	**1.57**
13487	0	-0.60	-0.88
13574	0	-0.09	-0.77
13581	**0**	**2.36**	**2.10**
13583	0	-1.42	-1.76
13584	0	-0.49	-0.40
13585	0	-0.29	-0.46
13586	**0**	**0.80**	**0.21**
13489	26.70	27.19	23.59
13490	27.40	28.35	24.86
13493	27.70	29.31	26.47
13580	27.8	22.37	19.78
13578	29.4	23.64	22.34
13488	32.1	30.67	30.47
13492	33.40	32.57	29.79
13571	34.95	28.53	25.70

3.2. Animal protein by-products meals

A total of 280 samples of processed animal protein meals (APBP) were collected between October 2002 and April 2004 from different providers. The animal meal samples were identified by the providers as being from pure poultry (n=56), pure pork (n=16), pure cattle (n=1), cattle-poultry mixture (n=1), cattle-pork mixture (n=3), poultry-pork mixture (n=3), blood (n=1), fish (n=8), hydrolysed feather meal (n=1), feather meal (n=1) and cattle-sheep-pork and poultry mixture (n=189). The spectra of the meal samples were used to build a NIRS spectral library of well-identified APBPs.

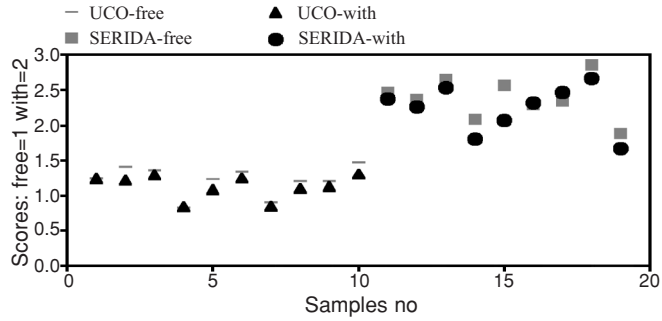

Figure 1. Classification results of the validation set (CF3) analysed in Lab 1 (UCO- Spain) and Lab 2 (SERIDA- Spain). Free: sample free of MBM; with: sample containing MBM — *Résultats de classification du lot de validation (CF3) analysé dans le laboratoire 1 (UCO-Espagne) et le laboratoire 2 (SERIDA-Espagne). Free : échantillon sans farine de viande et d'os, with : échantillon contenant de la farine de viande et d'os*.

MPLS regression equations were developed to predict the percentage of poultry, pork, cattle, ruminant and non-ruminant meat meals in APBPs. The mean, SD, range of each constituent and calibration statistics are given in **table 3**. The models obtained explained between 76 and 94% of the variance existing for the several "species" parameters in the complete spectral library. Despite that results are not shown, PLS2 discriminant models were also developed with set APBP1, using dummy variables (1=ruminant; 2=non-ruminant). The best model obtained explained the 82.7% of the total variance and it had a SECV value of 0.19.

These results show the potential of NIRS technology to identify the animal species in protein animal by-products. Collaboration with EFPRA and individual rendering plants around the world is needed for enlarging the library file in order to build models which can be applied to all the rendered protein meals circulating at intra and inter-European Community level.

Table 3. Statistics for the prediction of the percentage of poultry, pork, cattle, ruminant and non-ruminant meals in samples belonging to set APBP1 (n = 280) — *Statistiques de la prédiction du pourcentage de farines de poulet, de porc, de bovin, de ruminant et de non-ruminant pour les échantillons du lot APBP1 (n = 280)*.

Constituent (%)	**Mean**	**SD**	**Range**	**SECV**	**r^2**
Poultry meal	43.08	35.54	0-100	8.73	0.94
Pork meal	45.47	30.28	0-100	8.12	0.93
Cattle meal	9.2	7.8	0-26.6	3.5	0.81
Ruminant meal	9.8	8.1	0-28.5	3.9	0.76
Non-Ruminant meal	90.4	8.1	71.4-100	3.8	0.78

SD: standard deviation; SECV: standard error of cross validation; r^2: determination coefficient.

Figure 2 shows the mean spectrum of pure poultry meals (n=56), pure pork meals (n=16), pure cattle meals (n=1), cattle-poultry meal mixture (n=1), cattle-pork meal mixture (n=3), poultry-pork meal mixture (n=3), blood meal (n=1), fish meal (n=8), hydrolysed feather meal (n=1), feather meal (n=1) and cattle-sheep-pork and poultry meal mixture. There are visible differences in the region 1680–1760 nm, which may be of help to differentiate between the different types of APBPs.

Figure 3 confirms that the two-dimensional NIRS patterns allow to differentiate between animal species and to identify unknown spectra by comparison with the mean spectrum of well identified samples. That is the case, for instance, of the unknown EFPRA samples numbered as 32, 37, 38, 39 and 40, which may all be identified as being feather meals because their spectra patterns in the region 1694 to 1760, clearly differ from the spectra of all the identified APBP1 samples (with the exception of the feather meal sample). For the same reason the EFPRA samples 54 and 55 may be identified as blood and fish meals, respectively. The results obtained until now suggest that the spectra of APBPs contain information about the animal species, but technological factors may also affect the spectra. This suggests that spectral libraries may contain existing variability not only limited to the animal species but also due to differences in rendering process (i.e. wet/dry, continuous /batch system).

3.3. Animal fats

Inexpensive, fast, reliable and automated methodologies are needed for the complete traceability of feed grade fats and oils. It is crucial not only for enforcement of the present EU legislation concerning its use as feed, but also when looking for new alternatives of use of

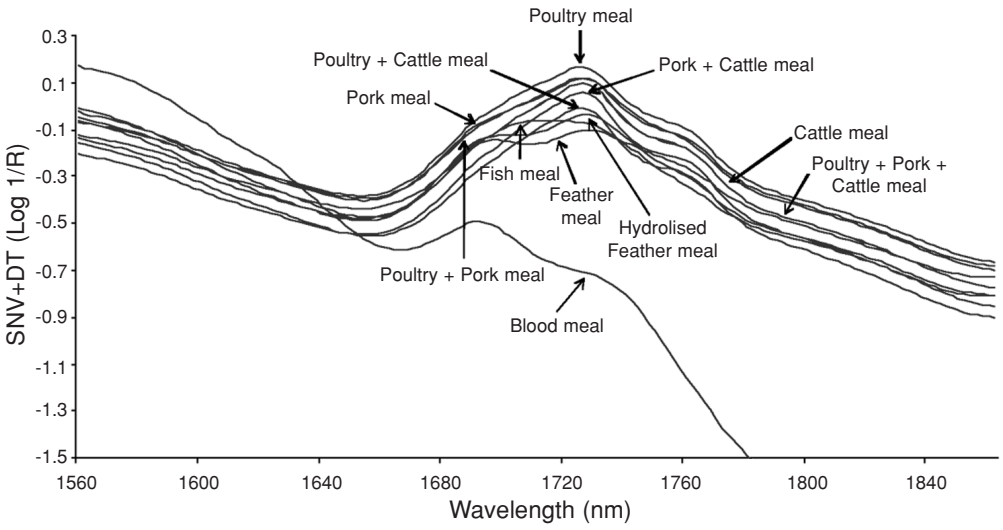

Figure 2. Relevant absorption features of the mean spectrum from well identified samples belonging to the library sets APBP1 and APBP2 — *Absorbances caractéristiques de spectres moyens d'échantillons provenant des lots APBP1 et APBP2.*

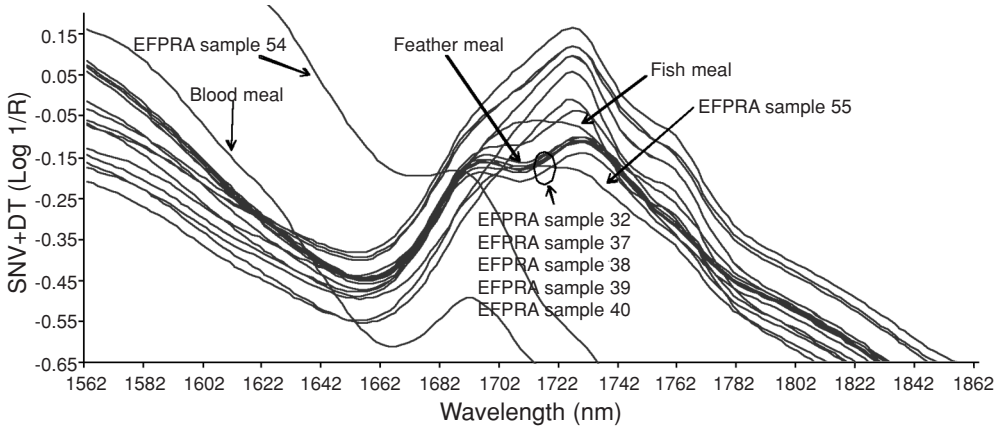

Figure 3. Relevant absorption features of APBPs spectra from samples of the Sample Bank versus unknown EFPRA samples (set APBP2) — *Absorbances caractéristiques de spectres de sous-produis animaux provenant de la banque d'échantillons y compris les échantillons inconnus de l'EFPRA (lot APBP2).*

feed ingredients grade fats and oils. In the framework of the previous mentioned project (MCYT-INIA-Spain Projects nº CAL 02-028-C2-2), an extensive R&D work is being undertaken not only for the chemical characterisation of fats by NIRS, but also work aimed to show how NIR spectra of animal and vegetable fats together with relevant chemical and technical processing conditions may offer an automatic and instantaneous method of fat classification according to its chemical composition/origin/class. Part of that research has been also undertaken in the framework of the STRATFEED Project nº G6RD-2000-CT-00414.

Table 4 and **figure 4** show results that underline the potential of NIRS technology for the enforcement of the existing and forthcoming legislation concerning the differential use of fats from different species and also from different origins and class (vegetal, terrestrian animals, fish, oleins, lard, etc).

As it can be observed in **table 4**, 15 samples of poultry fats were correctly classified, but 11 samples were misclassified as mixture samples. The 12 lard samples were correctly classified. Thirty seven out of the 39 fat mixtures were correctly classified and only two samples were misclassified as belonging to the poultry class. Some of the errors may be explained because the spectral library is still lacking some representative samples of certain species (i.e. pure beef, white pork, beef-pork mixtures, etc.). More work is in progress to enlarge the sample bank by adding samples coming from animal and vegetal feed fats providers.

Table 4. Results of the discriminant analysis for classification of animal fats (set AFBP1) between species categories (n=77) — *Résultats de l'analyse discriminante pour la classification de graisses animales (lot AFBP1) suivant différentes espèces (n= 77).*

	Classified as...		
Belonging to...	**Poultry**	**Pork**	**Mixture**[1]
Poultry	15	0	11
Pork	0	12	0
Mixture[1]	2	0	37

[1] (Poultry+ Pork + Cattle + Sheep)

Two dimensions NIRS patterns were used to detect regions of relevance for the types or classes of fat differentiation. **Figure 4** shows small band shifts in the vicinity of 1725 nm. Whatever their cause, the small shifts in wavelengths and inabsorbance value observed between the different fats and oils, highlight the discriminatory potential of NIRS technology. Thus, while the mean spectrum of fish oil shows a maximum near 1714 nm, the mean spectrum of animal fats (n = 48) appeared to be located fat around 1724 and 1726 nm. Two vegetable fats (soybean oil and olein) appeared in the middle (1700–1722 nm). Several authors have associated the peaks at 1710 and 1725 nm to differences in oleic and linoleic acids content and fat insaturation degree (Garrido *et al.*, 2004; Pérez-Marin *et al.*, 2004).

4. CONCLUSIONS

Near Infrared Spectroscopy provides a rapid, non-destructive and non-invasive technique for the detection and quantification of meat and bone meal in unground compound feeds. Near Infrared Spectroscopy has demonstrated that it is mature enough to be tested in a bigger collaborative study, once the validation protocol have been refined and agreed by laboratories with NIRS expertise in the analysis of compound feedingstuffs. However, other ingredients of animal origin could interfere in the prediction of MBM content, particularly animal fats. NIRS prediction models for these ingredients should be used together with MBM calibrations to get more accurate results.

The NIR spectra of animal protein and fat by-products contain relevant information related to the animal species from which they were obtained.

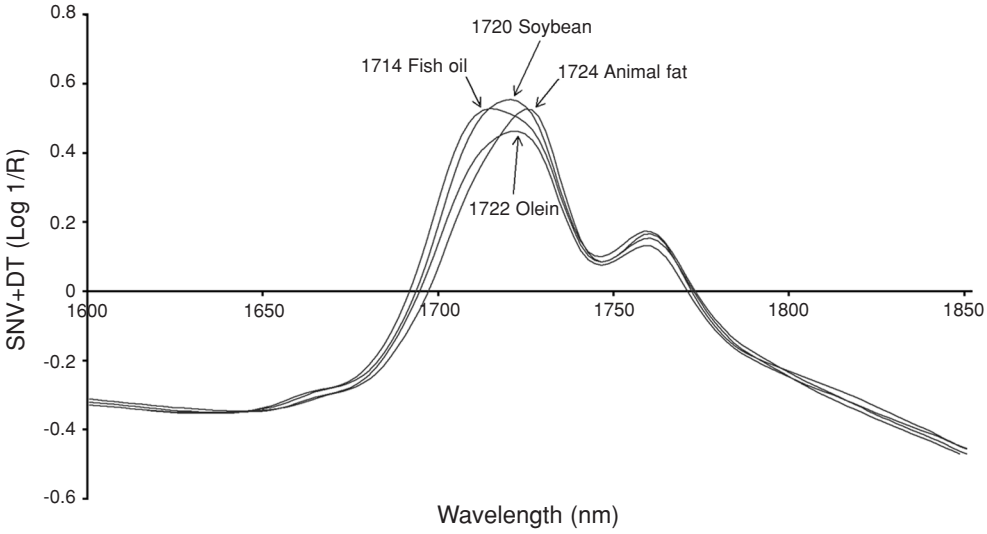

Figure 4. Relevant mean absorption features of spectra from well identified animal and vegetal fat samples from different providers (set AFBP2) — *Absorbances caractéristiques de spectres moyens d'échantillons de matières grasses animales et végétales provenant de différent fournisseurs (Lot AFBP2)*.

However further work is needed to enlarge the sample bank and the spectral libraries with well authenticated samples in order to increase the robustness of the quantitative and qualitative NIRS models. The success of a traceability system based on NIRS technology is clearly dependent on the collaboration of renderers and fat producers in the building of authenticated library files. The results may be of great help to the European Commission in order to allow derogations to the total ban and to accept the use of some categories of ABPs (i.e. feather meals or blood) for feeding of defined animal groups.

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Bibliography

Barnes RJ., Dhanoa MS., Lister SJ. (1989). Standard normal variate transformation and de-trending of Near Infrared Diffuse Reflectance spectra. *Appl. Spectrosc.* **43**, p. 772–777.

European Commission (2000). Commission Decision 2000/766/EC of 4 December 2000 concerning certain protection measures with regard to transmissible spongiform encephalophaties and the feeding of animal protein. *Off. J.* L**306**, 07/12/2000, p. 32–33.

European Commission (2002). Regulation EC Nº1774/2002 of the European Parliament and of the Council laying down health rules concerning animal by-products not intended for human consumption. *Off. J.* L **273**, 10/10/2002, p. 1–95.

Garrido-Varo A., García J., Pérez MD. (2004). Chapter XIX. Application in the analysis of fat and oils *In* C. Roberts, J. Workman, J. Reeves (Eds). *NIR spectroscopy in agriculture*. American Society of Agronomy (ASA), Crop Science Society of America (CSSA) and Soil Science Society of America (SSSA), p. 487–558.

ISI (2000). *The complete software solution using a single screen for routine analysis, robust calibrations, and networking. Manual. FOSS NIRSystems/TECATOR*. Sylver Spring MD, USA: Infrasoft International, LLC.

Murray I., Aucott LS., Pike IH. (2001). Use of discriminant analysis on visible and near infrared reflectance spectra to detect adulteration of fishmeal with meat and bone meal. *J. Near Infrared Spectrosc.* **9**, p. 297–311.

Pérez-Marín DC., Garrido-Varo A., Guerrero-Ginel JE., Gómez-Cabrera A. (2004). Near-Infrared Reflectance Spectroscopy (NIRS) for the mandatory labelling of compound feedingstuffs: chemical composition and open-declaration. *Anim. Feed Sci. Technol.* **116** (3–4), p. 333–349.

(7 ref.)