COMMUNAUTÉ FRANÇAISE DE BELGIQUE
ACADÉMIE UNIVERSITAIRE WALLONIE-EUROPE
UNIVERSITÉ DE LIÈGE – GEMBLOUX AGRO-BIO TECH

Estimation of diet digestibility and intake by grazing ruminants through near infrared reflectance spectroscopy analysis of faeces. Application in various contexts of livestock production

Virginie Decruyenaere

Essai présenté en vue de l'obtention du grade
de docteur en sciences agronomiques et ingénierie biologique

Promoteur : Yves Beckers (Gembloux AGRO-BIO TECH)

Co-promoteur : Pierre Dardenne (CRA-W)

2015

COMMUNAUTÉ FRANÇAISE DE BELGIQUE
ACADÉMIE UNIVERSITAIRE WALLONIE-EUROPE
UNIVERSITÉ DE LIÈGE – GEMBLOUX AGRO-BIO TECH

Estimation of diet digestibility and intake by grazing ruminants through near infrared reflectance spectroscopy analysis of faeces. Application in various contexts of livestock production

Virginie Decruyenaere

Essai présenté en vue de l'obtention du grade
de docteur en sciences agronomiques et ingénierie biologique

Promoteur : Yves Beckers (Gembloux AGRO-BIO TECH)

Co-promoteur : Pierre Dardenne (CRA-W)

2015

This research was supported by the Walloon agricultural Researches Center (CRA-W).

Virginie Decruyenaere (2015). Estimation of diet digestibility and intake of grazing ruminants through near infrared reflectance spectroscopy analysis of faeces. Application in various contexts of livestock production. (PhD Dissertation in English). Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium, 163 p., 39 tabl., 13 fig.

Abstract

Grazing is the most economical feeding scheme for ruminants. Grazing management, however, is often difficult for breeders, particularly because of a lack of knowledge about grass availability and quality. There are methods for assessing the quantitative and qualitative characteristics of grass, but they are difficult to apply in the case of grazing ruminants. Near infrared reflectance spectroscopy (NIRS) is based on the absorption of infrared light by organic matters to provide NIRS spectra. These NIRS spectra can be correlated with the chemical or biological composition of samples in order to develop calibrations that can be used as predictive models. The primary objective of this PhD thesis was to study the potential of NIRS applied to faeces (FNIRS) in order to predict the characteristics of the diets of grazing herbivores. The particular focus was on the *in vivo* organic matter digestibility, voluntary intake and botanical composition of ingested diets.

The main results of the study show that FNIRS has great portential for estimating *in vivo* digestibility and voluntary intake by grazing ruminants and that faeces are a good indicator of ingested diets. Based on both large or small and varied databases, the results suggest that FNIRS spectral libraries could be developed for characterising ruminant feed intake. The accuracy of the FNIRS models in estimating *in vivo* digestibility and voluntary intake is similar to or better than that of other methods usually used to assess these parameters. FNIRS could also be used to predict ruminants' diet composition in terms of plant species. These predictions should be used only for ranking, however, because of the current lack of accurate procedures for determining diet selection individually.

NIRS applied to faeces can be used to predict the *in vivo* characteristics of forage with sufficient accuracy. The prediction error of NIRS calibrations depends on the accuracy and precision of the reference data. The prediction of *in vivo* digestibility and intake is sufficiently repeatable compared with the procedure using the reference method. Intake is more difficult to predict with sufficient precision and is more closely linked to animal variability and to uncertainty of the FNIRS models.

The major difficulty in using this method lies in generating the diet-faecal pairs as reliably as possible. FNIRS calibrations for predicting *in vivo* diet characteristics are derivative calibrations. The sample analysed for reference values (diet samples) differs from the samples submitted to NIRS analyses (faeces). With regard to research on forages, *in vivo* trials with animals confined in pens or digestibility crates appears to be the best reference method for generating FNIRS calibrations.

Future work will involve developing FNIRS calibrations for predicting independent datasets and using them to create decision-support tools for improving diverse grazing management schemes. The major focus should be to compare different feeding strategies rather than to obtain an exact estimate of feed intake values. As a low-cost and rapid prediction technique, FNIRS could contribute significantly to the development of a methodology that would help improve our knowledge of forage and animal variability.

Virginie Decruyenaere (2015). Estimation de la digestibilité et de l'ingestion des ruminants au pâturage par analyse des matières fécales en spectrométrie dans le proche infrarouge. Application à des contextes variables. (Thèse de doctorat en anglais). Gembloux Agro-Bio Tech, Université de Liège, Gembloux, Belgique, 163 p., 39 tabl., 13 fig.

Résumé

Pour les élevages de ruminants, le pâturage est un système d'alimentation très économique. Néanmoins, les éleveurs s'approprient mal le pâturage, lequel est souvent perçu comme difficile à mettre en oeuvre. La méconnaissance de la disponibilité de l'herbe et de sa qualité en est peut être la cause, d'autant plus que des méthodes simples d'estimation de ces paramètres au pâturage font défaut. La spectromètrie de réflectance dans le proche infrarouge (NIRS) est une méthode d'analyse rapide basée sur l'absorption de la lumière infrarouge par la matière. Les spectres infrarouges ainsi générés peuvent être mis en relation avec la composition chimique ou les caractéristiques biologiques des échantillons analysés pour développer des étalonnages infrarouges alors utilisables comme modèles prédictifs. Le principal objectif de cette thèse est de déterminer le potentiel de la spectroscopie de réflectance dans le proche infrarouge appliquée aux matières fécales (FNIRS) pour prédire les caractéristiques de la ration des ruminants. Les paramètres plus précisément étudiés sont la digestibilité *in vivo* de la matière organique, l'ingestion volontaire et la composition botanique de la ration.

Les résultats soulignent le bon potentiel de cette méthode pour estimer la digestibilité *in vivo* de la ration des animaux au pâturage et leur niveau d'ingestion. Selon nos résultats, des bases de données de spectres fécaux peuvent être développées pour caractériser la ration des ruminants au pâturage. La précision des modèles développés à partir des spectres fécaux est similaire ou supérieure à celle d'autres méthodes habituellement utilisées pour l'estimation de ces paramètres. La composition de l'ingéré, en terme de proportion de graminées ou de légumineuses, apparaît moins facile à prédire par analyse NIRS des matières fécales, probablement en raison de l'absence de méthodes précises de détermination de la sélection alimentaire des animaux au pâturage.

L'analyse NIRS des matières fécales permet donc une estimation des caractéristiques *in vivo* des fourrages avec une bonne précision. Cette estimation est suffisamment répétable au vu de la difficulté à obtenir de telles informations avec les méthodes de références. L'ingestion reste cependant un paramètre difficile à prédire.

Une des principales difficultés de cette méthode d'estimation est de générer les paires 'rations – matières fécales' aussi fiables que possible en vue de développer des calibrations suffisamment précises. Sur base des recherches menées dans ce domaine, les bilans *in vivo* réalisés sur des animaux confinés en box individuel ou en cage à métabolisme semblent être la meilleure méthode de référence pour générer ces paires et développer les bases de données spectrales.

Un des futurs développements de cette méthode d'estimation réside dans sa mobilisation au sein d'outil d'aide à la décision visant à améliorer la gestion du pâturage. Une fois les bases de données spectrales développées, cette technique de prédiction rapide et peu couteuse pourrait être appliquée à un large ensemble de spectres fécaux et ainsi contribuer à l'amélioration de la valorisation des fourrages et à appréhender la variabilité individuelle des ruminants.

Acknowledgements

At the term of this PhD, I would like to thank sincerely all the people who helped me to carry it out successfully.

My first thanks go to my supervisors, Pierre Dardenne and Yves Beckers for their support and for their wise advices. I also thank André Théwis. I send a particular thought to André Buldgen who did not see the realization of this work.

I would like to extend my acknowledgement to my PhD committee which, year after year, trusted me and knew how to direct this work and my PhD jury for its constructive remarks.

I wish to thank José Wavreille and to Didier Stilmant who always supported me throughout this work. I also send one thank to Philippe Lecomte who, few years ago, proposed me a master thesis which, a few years later, was the basis of my PhD.

A PhD is also a team work. Special thanks go to the teams of the CRA-W and, more particularly the staff of the 'Haute Belgique' and 'Bertrand Vissac' for their presence, their involvement and the working atmosphere. In Libramont and in Liroux, I thank warmly the team of the 'garage' and the team of the 'vaches' for the realization of the trials on animals; the team of the "labo" for reference analyses; the students who, within the framework of their master thesis works, contributed to this PhD. Many thanks to Claudine for all infrared analyses, the treatment and the building of databases, our productive discussions.

I would also thank the Belgian and other colleagues (France, Netherlands, Australia) which believed in this method of analysis and which contributed, for some, to its development.

Finally, I thank all those who 'encouraged' me. Many thanks to the members of my family, my husband, my children. Guy, thank you for your patience, for your understanding. I thank all those whom I did not quote.

Remerciements

Arrivée au terme de cette thèse, je tiens à remercier sincèrement toutes les personnes qui, de près ou de loin, m'ont aidée à la mener à bien.

Mes premiers remerciements vont à mes promoteurs, Pierre Dardenne et Yves Beckers. Merci de votre soutien et de vos conseils avisés. Merci également, dans ce cadre, à André Théwis. J'adresse une pensée particulière à André Buldgen qui n'a pas vu la concrétisation de ce travail.

Je remercie également mon comité de thèse qui, année après année, m'a fait confiance et a su orienter ce travail ainsi que mon jury de thèse pour ses remarques constructives.

Merci à José Wavreille et à Didier Stilmant qui m'ont toujours soutenue tout au long de ce travail. J'adresse également un merci particulier à Philippe Lecomte qui, il y a bien longtemps déjà, m'a proposé le travail de fin d'études devenu, quelques années plus tard, la base de ma thèse.

Une thèse c'est également un travail d'équipe. Un grand merci aux équipes du CRA-W et, plus particulièrement, au personnel des bâtiments de Haute Belgique et du Bertrand Vissac pour leur présence, leur implication et l'ambiance de travail. A Libramont et à Liroux, je remercie chaleureusement l'équipe du 'garage' et l'équipe des 'vaches' pour la réalisation des essais sur animaux; l'équipe du 'labo' pour les analyses de référence; les étudiants qui, dans le cadre de leur travail de fin d'études, ont contribué à ce travail. Mille mercis à Claudine pour toutes les analyses 'infrarouge', le traitement et le montage des bases de données, nos nombreuses et fructueuses discussions.

Je remercie les collègues belges et étrangers (France, Pays Bas, Australie) qui ont cru en cette méthode d'analyse et qui ont contribué, pour certains, à son développement.

Enfin, je remercie tous ceux qui m'ont encouragée. Merci aux membres de ma famille, mon mari, mes enfants. Guy, merci de ta patience, de ta compréhension. Merci à tous ceux que je n'ai pas cités.

Virginie Decruyenaere

Table of contents and lists

Table of contents

List of abbreviations

ADFD, *in vivo* ADF digestibility
ADFom, acid detergent fibre excluding residual ash
ADL, acid detergent lignin
al, ad libitum
ANOVA, analysis of variance
APM, animal performance method
BW, body weight
$BW^{0.75}$, metabolic weight
C-DMI, concentrate dry matter intake
CEL, cellulose
C-OMD, concentrate *in vivo* organic matter digestibility
C-OMI, concentrate organic matter intake
CP, crude protein
CPD, *in vivo* CP digestibility
CRAW, centre wallon de recherches agronomiques
CV, coefficient of variation
CTI, corrected total intake
d, day
D-DMI, diet dry matter intake
DM, dry matter
DMI, dry matter intake
DMVI, dry matter voluntary intake
D-OMD, diet *in vivo* organic matter digestibility
D-OMI, diet organic matter intake
FNI, faecal nitrogen indicator
FNIRS, near-infrared reflectance spectroscopy applied to faeces
FOM, faecal organic matter
G-DMI, grass dry matter intake
GGI, grazed grass intake
GLM, general linear model
G-OMD, grass *in vivo* organic matter digestibility
G-OMI, grass organic matter intake
H, Mahalanobis standardized distance
HL, Holcus lanatus
INRA, Intistitut national de Recherches agronomiques
LCTI, lactating cow total intake
LP, Lolium perenne
M, maintenance
MP, milk production
MPLS, modified partiel least square
MSerror, residual mean square error of the ANOVA
N, nitrogen
NDF, neutral detergent fibre
NE, net energy requirement
NEBW, NE for body weight changes
NEC, energy supplied by concentrate
NEG, NE for grazing activity

NEGr, net energy concentration of grass
NEL, NE for milk production
NEM, NE for mainteance
NEW, NE for walking
NIR, near infrared reflectance
NIRS, near infrared reflectance spectroscopy
OM, organic matter
OMD, *in vivo* organic matter digestibility
OMDcel, cellulase enzyme; *in vitro* organic matter digestibility
OMI, organic matter intake;
OMVI, organic matter voluntary intake
P, paddock
PCA, principal components analysis
PLS, partial least square procedure
r, coefficient of correlation (Pearson coefficient)
R, reflectance
R^2, coefficient of determination
RMS, root mean square
RPD, ratio between the standard deviation of the reference population and SEC
RT, ratio technique
SBW, standard body weight
SD, standard deviation
sd_r, repeatability standard deviation
SE, standard error of regression
SEC, standard error of calibration
SECV, standard error of cross-validation
SEM, standard error of mean
SEM, standard error of the mean
SEP, standard error of prediction
SEPc, standard error of prediction corrected for bias
SNV-D, standard normal variate and detrend
s_r, standard error of repeatability
s_r^2, repeatability variance
SP, sample presentation
STI, sheep total intake
TR, Trifolium repens
VI, voluntary intake

List of tables

Chapter I – Article I

Chapter III – Article II

Chapter III – Article III

Chapter III – Article IV

Chapter III – Article V

Chapter IV – Article VI

Chapter IV – Article VII

Chapter IV – Article VIII

List of figures

Chapter I - Article I

Chapter II

Chapter III - Article II

Chapter III - Article IV

Chapter IV - Article VI

Chapter IV - Article VII

Chapter IV - Article VIII

General introduction

The main objective of this PhD thesis was to explore the potential of near infrared reflectance spectroscopy applied to faeces (FNIRS) for predicting the characteristics of the diets of grazing herbivores. In particular, it focused on the ability of FNIRS libraries to predict the *in vivo* organic matter digestibility (OMD), organic matter and dry matter voluntary intake (OMVI and DMVI) and composition of ingested diets in temperate grazing situations.

For maximum cost-effectiveness, grass should be the basis of ruminant feeding. For example, where pasture-based systems are predominant, as in New Zealand or Ireland, milk production tends to be more economical (Dillon et al., 2008). The acquisition of nutritional information about grazed forage in real time and continuously is a key issue in livestock management (Stuth et al., 2003). The difficulty in obtaining and monitoring this information has been the subject of several reviews and is probably linked to the lack of adequate assessment methods. In terms of nutritional information, although OMD, OMVI and DMVI are essential components of 'feeding value', estimating them under grazing conditions poses a problem.

Measurements of *in vivo* digestibility and intake of forage are usually obtained through digestibility trials and over a short period of time. Although this is the reference method, its application is limited, it requires a large quantity of forage and it is time consuming. Over the past 30 years, in parallel with the development of computers, the potential of NIRS to characterize the nutritive value of forage has been widely demonstrated (Norris et al., 1976; Biston et al., 1989; De Boever et al., 1996; Corson et al., 1999). NIRS is an indirect method based on the development of calibration databases or spectral libraries linking NIRS spectra (light absorbencies at NIR wavelengths) to reference values. The most highly developed NIRS calibrations can estimate the chemical characteristics of a large range of forages with good accuracy (Dardenne et al., 1996).

Pasture is a heterogeneous environment and, with the selective behaviour of herbivores, the analysis of grass sampled from the field is not enough to characterize ingested diets. In order to address this problem, approaches based on NIRS analysis of faeces (FNIRS) have been proposed. Faeces contain diet residues and therefore provide information on the characteristics of ingested forage, as well as on the physiological status of the ruminant, that can be detected by NIRS (Dixon and Coates, 2009). If samples selected for calibration are representative of the entire population to be analysed (Shenk et al., 1992, cited by Sinneave et al., 1994), FNIRS can be used to manage diet under grazing conditions. The overall aim of this study was to develop and validate the potential of FNIRS to predict the *in vivo* digestibility, intake and botanical composition of the diets of grazing herbivores. Within this framework, digestibility trials constituted the reference method for generating NIRS spectral libraries.

This manuscript draws on published articles and is divided into five chapters. A review of the literature, presented in Chapter I (Article I), describes the factors affecting digestibility and intake and the methods commonly used for their estimation. Chapter II describes the research strategy of this study, with more detail on this given in subsequent chapters. Chapter III describes the building of forage and faeces NIRS spectral libraries, the methodology applied to generate these spectral NIRS databases and the subsequent NIRS calibrations for predicting diet characteristics (Articles II to IV). As the values of NIRS calibrations are closely linked to the repeatability of the reference values, this point is also studied in Article V. Chapter IV summarises the validation of FNIRS analysis for predicting the diet characteristics of a group

of grazing herbivores (sheep and dairy cows) in temperate or tropical environments. The FNIRS results are compared with other methods commonly used for this type of estimation (Articles VI to VIII). In Chapter V there is a general discussion of the study results, a look at future prospects and an overall conclusion.

References

Biston, R., Dardenne, P., Demarquilly, C., 1989. Determination of forage *in vivo* digestibility by NIRS. In: Proceedings of the XVI International Grassland Congress, Nice, France, 895-896.

Corson, D.C., Waghorn, G.C., Ulyatt, M.J., Lee, J., 1999. NIRS: Forage analysis and livestock feeding. Proc. New Zealand Grassland Association 61, 127–132.

Dardenne, P., Sinnaeve, G., Bollen, L., Agneessens, R., 1996. NIR-NIT Calibration list, Libramont, Station de Haute Belgique.

De Boever, J.L., Cottyn, B.G., De Brabander, D.L., Vanacker, J.M., Boucqué, C.V., 1996. Prediction of the feeding value of grass silages by chemical parameters, in vitro digestibility and near-infrared reflectance spectroscopy. Anim. Feed. Sci. Technol., 60, 103-116.

Dillon, P., Hennessy, T., Shalloo, L., Thorne, F., Horan, B., 2008. Future outlook for the Irish dairy industry: a study of international trade reform and requirement for change. International J. Dairy Technol. 61, 16-29.

Dixon, R., Coates, D.B., 2009. Near infrared reflectance spectroscopy of faeces to evaluate the nutrition and physiology of herbivores. J. Near Infrared Spectrosc. 17, 1-31.

Norris, K.H., Barnes, R.F., Moore, J.E., Shenk, J.S., 1976. Predicting forage quality by infrared reflectance spectroscopy. J. Anim.Sci., 43, 889-897.

Shenk et al., 1992, cited by Sinneave, G., Dardenne, P., Agneessens, R., 1994. Global or local? A choice for NIR calibrations analyses of forage quality. J. Near Infrared Spectrosc., 2, 163-175.

Stuth, J., Awuma, K., Tolleson, D., Ossiya, S., Ebong, C., Mnene, W., Mwilawa, A., Ebro, A., Byenkya, S., Jama, A., Kaitho, R., 2003. Infusing nutritional profiling technology in sub-Saharan Africa for free ranging livestock. Research Brief 03-04-Lews, April 2003.

Chapter I: Intake by grazing ruminants

Article I: Factors affecting intake by grazing ruminants and related intake assessment methods: a review

Intake by grazing ruminants

From an economical point of view, the sustainability of livestock production is strongly influenced by feed costs and production price volatility (milk or meat). Linked to the intensification of the production schemes and increases in production costs, especially feed costs, it is clear that pastures have an important role in the future. Several studies have shown that production systems based on grazing, as in Ireland or in New Zealand, are very competitive. Optimizing the management of livestock systems based on grazing is difficult for many producers, however, mainly because of the lack of appropriate techniques for monitoring the evolution of diet characteristics.

The term ‘diet characteristics’ integrates two concepts: ‘voluntary intake’ (the quantity that an animal can ingest without constraints) and ‘nutritive value’ (the concentration of nutrients in the ingested feed). Digestibility and intake are therefore very interdependent.

The literature review in Chapter I focuses on the factors affecting intake by grazing ruminants and on related intake assessment methods. It suggests that intake is a multi-factorial phenomenon. Under grazing conditions, the selective behaviour of animals, the post-ingestive feedback of intake, characteristics of grazed plants and grazing environment are all factors that can explain some of the variations in intake and digestibility. Few studies have been conducted on intake estimation and few methods for measuring intake during grazing have been developed. Currently, there are two techniques that can simultaneously estimate the intake level and digestibility of an ingested diet: one is the *n*-alkanes technique, based on the presence of natural indigestible markers in the cuticular waxes of plants; and the other is near infrared reflectance spectroscopy (NIRS) analysis. Recent studies suggest that NIRS applied to faeces (FNIRS) could be particularly promising and might be a good alternative for monitoring the diets of grazing and free-ranging ruminants

Factors affecting intake by grazing ruminants and related intake assessment methods: a review

Article I. – adapted from Decruyenaere et al. (2009)
Biotechnology, Agronomy, Society and Environment, **13** (4), 559-573.

Decruyenaere V.[(1)]*, Buldgen A.[(2)], Stilmant D.[(1)]

[(1)] Walloon Agricultural Research Centre (CRA-W). Production and Sectors Department, Chemin de Liroux, 8. B-5030 Gembloux (Belgium)

[(2)] ULg – Gembloux Agro-Bio Tech. Animal Science Unit. Passage des Déportés, 2. B-5030 Gembloux (Belgium)

*corresponding author – E-mail: v.decruyenaere@cra.wallonie.be

Abbreviation : BW, body weight; DM, dry matter; FNIRS, near infrared reflectance spectroscopy applied to faeces; I, intake; OM, organic matter; NIRS, near infrared spectroscopy

Abstract

This review discusses the factors affecting the intake of grazing ruminants and the main methods used to quantify intake. The level of intake depends on many factors linked, for example, to gut capacity, an animal's requirements and forage quality. The post-ingestive feedback of the intake, the morphological characteristics of grazed plants and environmental factors such as climate and feed resource characteristics also contribute to intake variation.

Intake is a multi-factorial phenomenon, and few studies have focused on assessing it. The methods and techniques that have been developed to measure intake are often laborious, expensive and inaccurate, and sometimes do not represent actual grazing conditions. Currently, *n*-alkanes, which are natural markers present in the plants, appear to be one of the best ways to simultaneously predict the intake and digestibility of an ingested diet. This method, however, is difficult to apply over long periods of time and in free-range systems. With sufficiently robust databases and calibrations, Near Infrared Spectroscopy (NIRS) appears to be a promising technique for rapidly predicting the intake and digestibility of grazed grass. Recent studies have shown that faecal NIRS appears to be particularly promising and could be a good alternative for assessing the quality and quantity of the diet of grazing and free-range ruminants.

Keywords : Intake, ruminants, grazing, estimation methods

Résumé

L'objectif de cette étude est de discuter les principaux facteurs de variation de l'ingestion des ruminants au pâturage ainsi que les principales méthodes d'estimation de ce paramètre. Le niveau d'ingestion dépend, simultanément, de nombreux facteurs liés, par exemple, à la capacité du tube digestif de l'animal, à la couverture de ses besoins en nutriments, à la concentration des éléments nutritifs des plantes fourragères. Les aspects post-ingestifs interviennent également, ainsi que les caractéristiques morphologiques des plantes broutées. L'environnement dans lequel évolue l'animal, par le biais de l'abondance des ressources alimentaires, du climat, des processus d'apprentissage, peut également influencer le niveau d'ingestion.

L'aspect multi-factoriel du contrôle de l'ingestion limite le nombre d'études sur l'estimation de ce paramètre en situation de pâturage. Les méthodes les plus couramment utilisées sont souvent lourdes à mettre en œuvre, coûteuses en temps et en argent et parfois peu représentatives des conditions réelles de pâturages. De plus, elles manquent souvent de précision. Actuellement, la méthode des *n*-alcanes, marqueurs internes présents dans les cires cuticulaires des plantes, apparaît comme l'une des meilleures voies pour estimer simultanément l'ingestion et la digestibilité de l'herbe pâturée. Toutefois, cette méthode reste difficile à appliquer sur de longues périodes et en situation de pâturage extensif. Si des bases de données suffisamment solides sont mises en place, la spectroscopie dans le proche infrarouge (NIRS) pourrait se révéler être une technique intéressante pour estimer rapidement la consommation d'herbe par les ruminants au pâturage. Plus spécialement, le NIRS appliqué aux matières fécales semble prometteuse dans le cadre de l'estimation de ce paramètre. Cette analyse rapide pourrait être considérée comme une alternative intéressante pour caractériser, qualitativement et quantitativement, le régime alimentaire des ruminants au pâturage.

Mots-clés: Ingestion, ruminants, pâturage, méthodes d'estimation

I. Introduction

Grass is the most economical feed for herbivores during the grazing season. It is therefore worth obtaining an estimate of *in vivo* digestibility and voluntary intake simultaneously in order to optimise the production of grazing ruminants.

In recent years, digestibility has become a widely studied parameter. The *in vivo* digestibility of forages is usually obtained by conducting digestibility trials on adult sheep (Demarquilly et al., 1995). This method is costly, labour-intensive and time-consuming and is used only for assessing the digestibility of unknown feedstuffs. There are other well-documented methods for estimating *in vivo* digestibility that are easier and more rapid. Some are based on regressions between *in vivo* digestibility and forage characteristics, such as cellulose content (Lecomte et al., 1992), plant morphological characteristics (Demarquilly and Jarrige, 1981) and plant physiological stage (Valente et al., 2000). These methods are accurate enough for estimating the *in vivo* digestibility of pure grass swards, but they are less suitable for mixed forages because of the presence of various plant species that differ in chemical composition and morphological development stage. In order to address this problem, *in vitro* techniques have been developed (Adegosan et al., 2000). The 'rumen fluid pepsin' method developed by Tilley and Terry (1963) is the oldest, but its reproducibility is poor (Wainman et al., 1981, cited by Adegosan et al., 2000). In order to address this problem, enzymatic mixtures that simulate ruminal activities have replaced rumen fluid (Jarrige and Thivend, 1969; De Boever et al., 1988; Aufrère and Graviou, 1996; De Boever et al., 1996; Aufrère et al., 2007). The cellulase method is now the most commonly used one for estimating the *in vivo* digestibility of a large range of forages. The gas test method (Menke et al., 1979), which measures the volume of gas produced by the fermentation of forages in the presence of rumen fluid, is also an interesting tool for estimating *in vivo* digestibility, particularly of tropical forages (Stern et al., 1997; Babatounde, 2005). Some indirect methods, based on faeces characteristics, are easy and accurate enough for predicting the *in vivo* digestibility of grazed grass. For example, linear or quadratic equations linking faecal nitrogen concentration and *in vivo* digestibility have been developed for temperate (Bartiaux-Thill and Oger, 1986; Peyraud, 1998) and tropical forages (Boval et al., 1996; Bouazizi and Majdoub, 1999). Similarly, indigestible internal plant markers such as lignin (Fahey and Jung, 1983), indigestible acid detergent fibre (Sunvold and Cochran, 1991) and *n*-alkanes, naturally present in the cuticular waxes of plants (Dove and Mayes, 1991, 1996) and therefore in faeces, are also used for estimating *in vivo* digestibility in grazing animals.

Few studies have been conducted on the voluntary intake of grazing ruminants because this is a complex parameter that is difficult to estimate with sufficient precision. When such studies have been done, intake is usually measured for one ruminant species and one type of pasture. Although, according to the definition given by Baumont et al. (2000) 'intake is the maximum quantity of feed that can be eaten by an animal when this is supplied *ad libitum* as the sole feed' and therefore would appear to be easy to quantify, its study is complex. According to Illius and Jessop (1996), intake can be considered as a 'psychological' phenomenon, involving the integration of many signals and reflecting the flexibility of a biological system evolved to cope with variability in food supply, composition and animal states. Plant properties (associated with, for example, taste, smell and the presence of toxins) are important parameters affecting the diet selection and ingestive behaviour of grazing ruminants and therefore their levels of intake (Provenza et al., 2003b).

This review discusses the factors affecting the intake and feed choice of grazing ruminants and the main methods used to quantify them.

II. Intake variations in grazing ruminants

II.1. Intake expression

There are many ways of expressing level of intake. Usually, forage dry or organic matter intake is expressed in weight unit per animal and per day, but this approach cannot be used to compare animal species or forages. For this reason, intake can be expressed by kg of body weight raised to an exponent that can vary between 0.54 and 1.00 (Meissner and Paulsmeier, 1995). The choice of the exponent is a function of forage's quality. With low-quality forage, an animal's intake capacity appears to be more closely linked to gut capacity and the rate of passage of forage. For such forages, the exponent is 1.00 and intake is expressed per kg of body weight or in percentage of body weight (Demment and Van Soest, 1985).

The intake of good-quality forage seems to be more controlled by physiological mechanisms and is usually expressed per kg of metabolic weight (body weight raised to 0.75). The assumption is that intake is linked to energy requirements that are proportional to 0.75 power of body weight (Klieber, 1961, cited by Allison, 1985).

A study by Sauvant et al. (2006) showed that, when comparing intake levels across forages and animal species, the best unit was the dry matter intake in percentage of body weight (DMI, % BW). On this basis, the relationship between intake and particle passage rate through the rumen or energy digestibility appears to be independent of animal species. This is not the case when intake is expressed per kg of metabolic weight.

II.2. Level of intake at grazing

Tables 1 and 2 summarize some examples of intake levels for ruminants (dairy cow, beef cattle, small ruminants) grazing various forages. An initial observation is that intake level is highly variable among ruminants and is linked to the characteristic of the forages.

According to INRA (2007), the *ad libitum* intake of a reference grass (15% crude protein, 77% organic matter digestibility on a dry matter basis) is 75, 95 and 140 g of dry matter per kg of metabolic weight for a standard sheep, a standard heifer and a standard lactating dairy cow, respectively. On this basis, it is possible to calculate the 'Fill Unit' ('*unité d'encombrement*') of various forages.

Intake level variability also occurs, however, between breeds and between individuals within a breed (Scott and Provenza, 1999; Pearson et al., 2005). For example, Dorper sheep are less selective grazers, consume more shrubs and bushes and ingest a greater number of plant species than Merino sheep (Brand, 2000).

The approach used to express intake level also contributes to this variability. As noted by Sauvant et al. (2006), if DMI, expressed in percentage of body weight, appears to be higher for small ruminants (sheep and goats) than for cattle (dairy or suckler cattle), the reverse is true when intake is expressed in terms of metabolic weight.

Table 1.
Some examples of the intake levels of grazing cattle and sheep

Reference	Animal specie	Type of forages	BW	Originally unit	Range of grass DMI (comparable unit)			
			kg		kg (% BW)$^{-1}$	kg (% BW)$^{-1}$	g kg BW$^{-0,75}$	g kg BW$^{-0,75}$
Jarrige *et al.* (1986)	cattle, heifers	Temperate grasses	300	DM - g kg BW$^{-0.75}$	2.2	2.7	92.0	114.0
Lippke *et al.* (2000)	steers	temperate grasses	189	OM - g kg BW$^{-0.75}$	4.6		168.9	
		temperate grasses + supplement	189		4.2		155.6	
Sprinkle *et al.* (2000)	suckler cow, dry	tropical grasses, early summer	420	OM - g kg BW^{-1}	3.5	3.7	158.4	165.5
		tropical grasses, late summer	437		2.7	3.8	125.5	171.7
	suckler cow, lactating	tropical grasses, early summer	356		4.2	4.6	180.5	197.8
		tropical grasses, late summer	350		4.1	5.1	176.8	218.6
Boval *et al.* (2007)	cattle heifers	tropical grasses	208	OM - kg day^{-1}	1.8	3.1	67.8	116.4
Kloppenburg *et al.* (1995)	steers	tropical grasses, spring	248	DM - kg day^{-1}	2.1	2.7	84.8	105.6
		tropical grasses, summer	277		2.6	3.0	104.6	123.7
		tropical grasses, fall	332		1.6	2.2	68.1	92.6
Arthington and Brown (2005)	steers	tropical grasses, 4 weeks growth	256	OM - kg (% BW)$^{-1}$	1.6	2.1	63.6	84.4
		tropical grasses, 10 weeks growth	256		1.4	1.8	54.7	73.8
		tropical grasses, 4 weeks growth	256		2.0	2.4	81.8	97.3
		tropical grasses, 10 weeks growth	256		1.3	1.9	52.0	77.8
Jarrige *et al.* (1986)	sheep	temperate grasses	60	DM - g kg BW$^{-0.75}$	2.4	3.2	66.0	90.0
Pasha *et al.* (1994)	sheep	temperate grasses	44	DM - g kg BW$^{-0.75}$	3.0	3.5	78.0	91.0
Penning *et al.* (1994)	sheep, ewes	temperate grasses, first rotational grazing	77	OM - g kg BW^{-075}	5.6		167.0	
		temperate grasses, second rotational grazing	77		3.5		102.7	
		temperate grasses, third rotational grazing	77		3.2		94.4	
		temperate grasses, continuous grazing	77		3.7		110.3	
Delaby *et al.* (2007)	sheep	temperate grasses, spring	55	DM - g kg BW$^{-0.75}$	2.3		62.0	
		temperate grasses, early summer	55		2.9		80.0	
		temperate grasses, late summer	55		3.0		83.0	
		temperate grasses, fall	55		2.7		74.0	
		temperate grasses, spring	55		2.5		67.0	
		temperate grasses, early summer	55		2.9		79.0	
		temperate grasses, late summer	55		3.0		81.0	
		temperate grasses, fall	55		3.0		81.0	
Delaby and Pecatte (2003)	sheep	temperate grasses, first cycle	55	DM - g kg BW$^{-0.75}$	2.4		65.0	
		temperate grasses, second cycle	55		2.9		79.0	
		temperate grasses, third cycle	55		2.1		58.0	
Decruyenaere *et al.* (2008)	sheep	temperate grasses	53	OM - g kg BW^{-075}	1.6	3.2	44.2	85.4
			53		2.0	2.9	52.7	79.1
			53		1.6	3.1	41.9	83.6
			53		2.1	2.9	56.4	79.1
			53		2.4	3.2	64.3	87.3
			53		2.0	3.9	52.7	104.7
			53		2.6	3.1	70.9	83.1
			53		2.4	3.7	63.3	98.3

Table 2.
Some examples of the intake levels of grazing dairy cows

Reference	Animal specie	Type of forage	BW	Originally unit	Range of grass DMI (comparable unit)			
			kg		kg (% BW)$^{-1}$	kg (% BW)$^{-1}$	g kg BW$^{-0,75}$	g kg BW$^{-0,75}$
Jarrigue *et al.* (1986)	dairy cows	temperate grasses	600	DM - g kg BW$^{-0.75}$	2.3	2.7	114.0	136.0
O'Donovan and Delaby (2005)	dairy cows	temperate grasses	546	DM - g kg BW$^{-0.75}$	3.0	3.5	145.2	169.1
Parga *et al.* (2002)	dairy cows	temperate grasses	598	OM - g kg BW$^{-0.75}$	2.6	3.1	130.5	153.4
Ribeiro *et al.* (2003)	dairy cows	temperate grasses	609	OM - g kg BW$^{-0.75}$	1.9	2.6	95.5	127.0
Hristov *et al.* (2005)	dairy cows	temperate grass, with or without supplements	613	DM - kg day^{-1}	2.6	4.9	129.9	242.7
Vazquez and Smith (2000)	dairy cows	temperate grasses	401	DM - kg day^{-1}	1.6	2.6	72.6	116.2
			479		2.3	2.7	107.4	126.0
			374		2.5	3.8	111.7	168.1
			423		1.7	5.0	76.2	228.6
			398		3.5	3.7	155.0	166.2
			543		2.1	2.7	102.3	132.6
			560		1.6	2.4	78.2	117.3
			378		1.6	3.3	72.4	146.0
			375		2.6	4.3	112.7	191.3
			410		2.8	4.2	126.3	187.8
			400		1.9	3.7	83.9	166.7
			335		2.0	2.5	85.6	108.6
	dairy cows	temperate grasses with supplement	427		1.5	2.5	69.2	112.8
			532		2.0	2.8	97.5	136.3
			514		2.3	3.3	111.2	156.7
			494		2.2	3.1	105.0	147.9
			577		1.8	2.6	90.0	128.3
			576		1.5	2.4	74.0	117.4
			575		0.5	2.2	22.1	106.5
			505		3.1	3.4	146.4	159.6
			424		2.2	3.8	100.6	174.4
			447		2.1	2.3	98.8	103.9
			477		1.7	3.4	79.4	158.7
			540		0.8	1.4	38.4	67.8
			574		2.2	2.6	106.7	126.3
			637		2.3		114.4	
			637		2.5	2.6	124.6	130.9
Berzaghi *et al.* (1996)	dairy cows	temperate grasses	554	DM - kg day^{-1}	2.6		126.5	
		temperate grasses with supplement	554	OM - kg day^{-1}	2.0		95.4	
Holden *et al.* (1994)	dairy cows	temperate grasses with supplement	596	DM - kg day^{-1}	1.9	2.6	96.2	129.3

BW = body weight; DM = dry matter; OM = organic matter

The voluntary intake of tropical forages is often lower than temperate forages, but not by much (2.03 kg DM per % BW *vs* 1.95 kg DM per % BW for temperate and tropical forages, respectively), as confirmed in the meta-analysis reported by Assoumaya et al. (2007). From a chemical point of view, temperate forages often contain more protein and less fibre. The difference decreases when the crude protein content of both types of forage is similar. In order to explain these differences, Assoumaya et al. (2007) reported that tropical forages are usually chewed for longer than temperate ones, leading to greater reduction of forage particle size, which compensates for their apparent lower nutritive value.

III. Factors affecting intake regulation

The regulation of intake is multi-factorial (Rhind et al., 2002; Forbes, 2003). It depends on plant characteristics in relation to: gut capacity; an animal's requirements and the nutrient content of forages; the post-ingestive feedback of the intake and the learning process; the morphological characteristics of grazed plants; and environmental factors such as climate and the abundance and availability of feed resources. Figure 1 illustrates the complexity of forage intake regulation (Baumont et al., 2000).

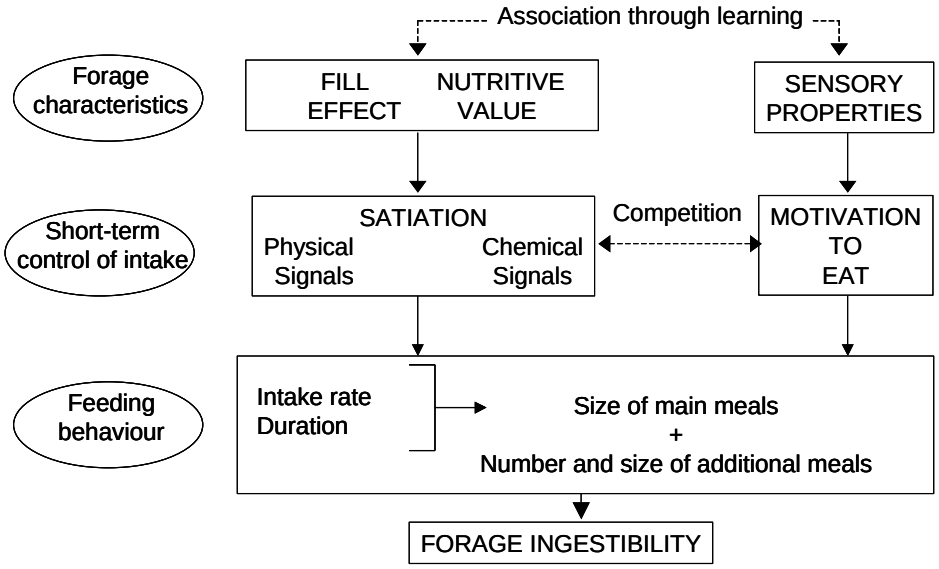

Fig. 1. Factors affecting forage ingestibility (from Baumont et al., 2000)

III.1. Role of the ruminal fill

'Gut fill capacity', in relation to forage characteristics, can be considered as one of the main factors regulating voluntary intake. Intake appears to be limited by the maximal volume that the digestive tract can take (Allison, 1985; Allen, 1996), even where herbivores are able to progressively modify the volume of their rumen and to increase the transit rate of digesta when the quality of forage decreases (Johnson and Combs, 1991; 1992; Van Soest, 1994 cited by Schettini et al., 1999). This has been confirmed by experiments where tennis balls, water filled bags or artificial fibres were introduced into the rumen. The bulkier the ruminal ballast in terms of volume or weight, the lower the intake was, with or without digestibility modification (Schettini et al. 1999). Gregorini et al. (2007) confirmed that ruminal fill can

affect grazing behaviour in terms of bite mass, bite depth and bite area, and thus it is short-term intake that is affected by ruminal fill.

Related to ruminal capacity, forage dry matter content can affect voluntary intake. If forage dry matter is lower than 20%, as in young grazed grass, the volume of water in the rumen increases and has a depressive effect on intake level, despite high forage digestibility (Pasha et al., 1994; Meissner and Paulsmeier, 1995).

The age of plant regrowth is also a factor of variation. As plants age, protein content decreases and cell walls and tissue lignification increase, resulting in an increase in forage retention time in the rumen, which limits voluntary intake (Jung and Allen, 1995; Baumont et al. 2000; Arthington and Brown, 2005). Parga et al. (2002) reported that the daily herbage intake of lactating dairy cows fell by 8.4% when comparing short and long time of grass growth. Jung and Allen (1995) and Vazquez and Smith (2000) confirmed that the level of DMI by dairy cows grazing on temperate grass was negatively correlated to hemicellulose and cellulose (NDF) content, but as indicated by the low coefficient of correlation between these two parameters (r = -0.65 and = -0.31) NDF alone appears to be a poor predictor of intake.

III.2. Role of animal nutriment requirements and forage nutrient content

The nutrient content of forage plays a role in the regulation of food intake. According to 'requirement theory', an animal eats in order to maximise its production potential within certain constraints such as gut volume and diet quality (Yearsley et al., 2001). Intake regulation is therefore based on meeting energy needs (Van Wieren, 1996; Kyriazakis, 2003). Peyraud et al. (1996) and Faverdin et al. (2007) demonstrated that intake is positively linked to the body weight and level of production of dairy cows and therefore to animal requirements. In a meta-analysis conducted by Vazquez and Smith (2000), factors linked to dairy cow needs and performance, such as animal body weight, change in body weight and milk yield, explained 71% of the total variation observed in DMI. Hristov et al. (2005) confirmed that DMI is strongly correlated to both nutrient digestibility and animal requirements. Ruminants eating very fibrous forage are therefore generally unable to meet their energy needs (Jung and Allen, 1995).

With regard to meeting energy needs, an animal's physiological state appears to be an important factor regulating voluntary intake. For example, lactating dairy or suckler cows, with their higher energy requirements, graze more selectively (favouring green grasses) and more intensively (grazing for longer periods of time) than dry cows (Gibb et al., 1999; Farruggia et al., 2006). As reported by Johnson and Combs (1991, 1992), it exists a critical ruminal fill level above which DMI is limited. This level can vary depending on the physiological status of animals. Introducing ballast into the rumen of dairy cows when their energy requirements are high (beginning of lactation) can lead to a significant decrease in intake level (0.043-0.099 kg dry matter per litre of added bulk). In order to meet their energy needs, cows in early lactation compensate for this by increasing their ruminal volume, reducing the digesta volume and increasing the digesta passage rate. When the same experiment was conducted with dairy cows later in their lactation, when energy requirements are lower, intake did not seem to be affected by introducing ballast into the rumen.

Pregnancy stage is also a factor in the regulation of voluntary intake. In the predictive equation developed by Faverdin et al. (2006), the intake capacity of a dairy cow is proportional to the lactation stage, age and maturity of the cow and to a 'pregnancy indicator' that explains the reduced intake during the last weeks of pregnancy.

Animals can also regulate their intake in terms of forage nutrient content. Cooper and Kyriazakis (1995) showed that, in cafeteria trials, when sheep had the choice between two diets with different energy levels, they chose the diet with the highest energy density. When they did not have this choice, they adapted their intake until their requirements were met.

The energy-protein balance of a diet can also influence level of intake and diet selection. In lambs that were able to select between pairs of diets where the crude protein content varied from 7.8 to 23.5%, the maximal level of intake was based on diets where the crude protein content varied between 14.1 and 17.2% (Kyriazakis and Oldham, 1993). These observations suggest that judicious forage supplementation could improve the nutritional balance of diets and thus increase total voluntary intake (Berzaghi et al. 1996; Lippke et al. 2000; Vazquez and Smith, 2000).

III.3. Role of the intake's peri and post-ingestive feedback

The level of intake can be affected by other characteristics of forage, such as flavour (taste and smell), appearance, texture and the post-ingestive feedback that occurs after intake. Thus, 'if forage tastes good, animals tend to eat it more' (Baumont, 1996). The flavour-feedback interaction depends directly on feed chemical characteristics, animal nutritional status and animal past or recent experiences. Provenza (2003a) noted that animals can be trained in various ways: 'Animals learn from their mother, before and after their birth, they learn from their pairs, they learn by testing new food, accepting them or rejecting them according to the consequences induced by this intake.' This could explain why preferences are never fixed. Post-ingestive feedback can evolve in relation to new situations experienced by an animal (Atwood et al., 2001).

Similarly, ruminants learn about the toxicity of feed. For grazing and, particularly, browsing ruminants, many plants contain secondary metabolites such as tannins and terpenes that can affect digestibility, have an emetic effect and thus reduce voluntary intake. Ruminants are able to use this information to modulate or avoid the intake of such plants if necessary. For example, browsing goats tend to reduce their preference for browse plants sprayed with lithium chloride, a chemical component associated with highly negative post-ingestive feedback (Ginane et al., 2005; Duncan et al., 2006). Tannins can reduce the grazing of some forage legumes, such as *Lotus pedunculatus*, by negatively affecting ruminal fermentation (Reed, 1995), and terpenes can inhibit the cellulolytic activity of ruminal micro-organisms and therefore limit the intake of such plants (Nagy and Tengerdy, 1968, cited by Provenza et al., 2003b).

Intake also appears to be regulated by various signals in the form of metabolites and hormones emitted by the central nervous system and peripheral organs such as the liver, pancreas and intestinal tracts, which can be regarded as appetite mediators (Rhind et al., 2002). The roles of leptin (a hormone secreted by fatty cells), cholocystokinine (a hormone secreted by the intestinal mucous membrane) and insulin in controlling satiety in ruminants have been widely documented (Forbes, 1996, 2003). Similarly, the ruminal environment (pH and osmolarity) during digestion can also contribute to variation in voluntary intake (Faverdin, 1999).

As demonstrated by Cooper and Kyriazakis (1995), sheep are able to make short-term changes in diet selection in order to maintain good ruminal fermentation and the sensation of well-being.

III.4. Role of plant morphological characteristics

Sward characteristics in terms of blade morphology, such as hair occurrence, cuticle thickness (Loney et al., 2006) leaf size (Barre et al., 2006), stem physical properties and dead materials ratio can stimulate or inhibit animal foraging behaviour (Provenza, 2003a). In particular, these parameters have a great influence on bite size and intake rate. Hodgson (1985), cited by Prache and Peyraud, (1997), reported that, in terms of grass characteristics, bite size can vary from 10 to 400 mg OM for sheep and from 70 to 610 mg OM for cattle. Under grazing conditions, there is a close relationship between leaf proportion (Parga et al., 2002; O'Donovan and Delaby, 2005), green leaf mass (Penning et al., 1994; Smit et al., 2005a), sward density (Prache and Peyraud, 1997) and DMI.

There are many factors explaining the influence of plant morphological characteristics on intake. Benvenutti et al. (2006) reported that stems can have a barrier effect on bite size and instantaneous intake rate. The higher the stem density, the smaller the bite area and the slower the biting rate, leading to a reduction in the instantaneous intake rate. Boval et al. (2007) confirmed that stem length and proportion in the sward have a negative impact on biting rate, with correlations of -0.67 and -0.40, respectively.

Sward composition, in terms of plant species, can also influence intake level. Compared with grasses, legumes such as white clover have often been associated with higher levels of intake (Ribeiro et al., 2003). Penning et al. (1995), Baumont (1996) and Assoumaya et al. (2007) reported that forage legumes were reduced to small particles more quickly than grasses and less time was needed to take and masticate a bite of clover than a similar bite of grass.

III.5. Role of the environmental factors

Intake during grazing does not depend only on diet quality. Short-term intake rates can also be directly correlated to forage distribution and availability (Garcia et al., 2003). This partly explains the lower level of intake observed under tropical rangeland, where forage resources can be scattered and/or heterogeneous, reducing biting frequency and intake rate due to the time animals spend moving from one favoured site to another (Roguet et al., 1998). Animals can compensate for this reduced biting rate to some extent by increasing their grazing time. Gibb et al. (1999) reported that when sward availability decreases (measured by sward height), cows increase their total grazing time, jaw movement and number of bites in order to maintain their daily intake. These observations were confirmed by Boval et al. (2007) for tropical forages.

Climatic conditions can also play an important role. Ruminants graze mainly during daylight and, in temperate climates, can have six to eight meals, with the two main ones at the start and end of the day. If the temperature is higher than 25°C, they adapt their grazing behaviour (grazing early morning, late evening or night grazing) in order to avoid the warmest period, which can reduce the time spent grazing and thus the daily intake (Baumont et al., 2000).

A herbivore's learning about the environment also plays an important role in resource utilization. Animals can retain a memory of food allowance, location and distribution, as reported in the review by Dumont and Gordon (2003). Rearing practices are also a factor. For example, the ability of cows to graze a specific environment, such as mountain slopes, is linked more to their rearing than their breed (Meuret et al., 2006).

The interaction between animals in a herd is sometimes cited as an explanation for differences in animal grazing behaviour. Sibbald et al. (2000) reported that where the vegetation is

homogeneous, total time spent grazing by Scottish blackface sheep was higher when the space allowance was high (200 m² per head vs 50 m² per head), without affecting herbage intake level or digestibility. They concluded that the relationship between time spent grazing and space allowance could be used to explain the extra activity required to maintain group cohesion when space allowance increases.

IV. Intake measurement during grazing

For many years, intake during grazing has been estimated using various methods, which can be grouped into two categories: direct or indirect.

IV.1. Direct measurements

Direct methods are based mainly on herbage mass measurement. In most cases, intake is estimated by the method of difference, as reported by Macoon et al. (2003) and Smit et al. (2005b). This method requires knowledge of herbage mass before and after grazing. Herbage mass is usually estimated by cutting and weighing the grass harvested from a defined area. A 'sward height meter', 'rising plate meter' or 'disk meter', which measure compressed sward surface height, can also be used to estimate grass density and quantity. The difference method is easy to apply and produces reliable results if the grazing period is short (1 or 2 days maximum) and the stocking rate is high (ideally, all grass in the grazing area should be consumed). If the grazing period is longer, the error of estimation linked to grass re-growth during this period is the major disadvantage of that method. In order to estimate the effect of grass regrowth, herbage mass and regrowth is then measured in cages that exclude grazing animals (exclosure cages). Through successive cuttings, grazing is simulated and herbage mass accumulation is measured. Without urine and dung restitution, however, and without specific defoliation linked to the grazing, the measured grass accumulation is often very different in grazed and non-grazed areas (Frame, 1993). The precision of the cutting methods is based mainly on the sampling methodology and good precision is required at all steps of the protocol in order to avoid additional errors of measure. The difference method is used mainly to measure the intake of animal herds (Smit et al., 2005b).

Instantaneous intake can also be measured directly through liveweight differences (Coates and Penning, 2000). With this method, it is possible to measure intake only over a very short period (e.g., 1 hour). The accuracy of the measurement depends greatly on the precision of the scales and the weight loss related to dung and urine excretion during the measurement period.

Another method of intake measurement is based on the hypothesis that knowledge of an animal's requirements and performance gives a good indication of the nutritive value of the ingested diet. This method is often used to determine the intake potential of dairy cows, as described by Faverdin et al. (2007). For grazing-supplemented dairy cows, Macoon et al. (2003) considered that determining grass intake from animal performance was reliable and less expensive than other methods. For beef cattle, Minson and Mc Donald (1987) estimated intake from liveweight and rate of growth with good accuracy (residual standard deviation of 8.7% of the mean). The difficulty with this the method is, specifically, the determination of actual herbivore requirements, particularly in tropical rangelands where many external factors, such as displacement and feed resources localisation must be involved (Allison, 1985).

IV.2. Indirect measurements

The intake of grazing ruminants can be estimated by indirect methods, such as marker techniques, ratio techniques, recording animal behaviour and other empirical models.

The marker technique is based on determining natural indigestible plant components such as lignin, alkanes or insoluble ashes that are excreted in faeces. The *n*-alkanes method developed by Mayes et al. (1986) appears to be the most suitable for estimating intake in grazing systems. Based on determining the concentration of natural odd chain and even chain *n*-alkanes in plant and faeces, this method enables intake to be calculated from:

$$I = (Fi/Fj) \times Dj / (Hi - (Fi/Fj) \times Hj)$$

where I = intake, Fi and Hi = concentration of natural odd chain *n*-alkanes in faeces and forage, Dj = dose rate of synthetic even chain *n*-alkanes, Fj and Hj = concentration of even chain *n*-alkanes in faeces and forage.

The ratio technique is based on determining two parameters: forage digestibility and faecal output. This method enables intake to be estimated from (Lippke, 2002):

$$\text{If } D = 100 \times ((I - F) / I)$$

$$I = F / (1-D/100).$$

where D = forage digestibility coefficient (%), I = intake (weight unit per day), F = total faecal excretion (weight unit per day).

Methods developed to estimate digestibility are numerous and well documented, as reported in the review conducted by Adegosan et al. (2000).

There are several methods for determining faecal output. Among them is the total collection of faeces, but this is difficult to apply during grazing. In order to collect faeces, animals need to be tethered or equipped with a faecal bag, both of which are likely to disturb their grazing behaviour (Lippke, 2002). In addition, an amount of faeces, difficult to estimate, can escape from the collection bags, and this can be a source of error in intake estimation (Adegosan et al., 2000).

Another method for estimating faecal output is based on using indigestible external markers such as chromium oxide and ytterbium. With regard to the total collection of faeces, the dosing of the markers requires a daily manipulation of the animal, which can be a problem during grazing. The development of the controlled release device technique, which automatically pulses a daily amount of external markers into the rumen throughout the trial period, limits the animal manipulation required and appears to be accurate enough to produce a good estimate of faecal output (Compère et al., 1992; Berry et al., 2000; Ferreira et al., 2004).

In both the marker and ratio techniques, one difficulty is that the sampling of forage needs to be as representative as possible of the ingested diet because it is from this sample that indigestible natural markers or digestibility are determined. For the *n*-alkanes, the fact that plant organs and plant species have different *n*-alkane profiles (Cortes et al., 2005) is a major source of error in intake estimation. Similarly, if digestibility is determined from a non-representative sample of grazed grass, the intake estimation will be biased. The hand plucking method, which simulates a herbivore's biting action, can be used to sample grazed

grass. The reproducibility of this measurement, however, is linked to the calibration between animals and operator observations. This calibration is easier to set up with cattle than with sheep or goats, whose grazing behaviour is more selective (Wallis DeVries, 1995). The use of oesophageally fistulated animals is not favoured for animal welfare reasons, and it can modify herbivore behaviour, as reported in several studies (Coates et al., 1987; Jones and Lascano, 1992).

Intake can be indirectly estimated by studying grazing behaviour. Intake is the product of three parameters: grazing time, biting rate and bite mass. Grazing time and biting rate can be measured by visual observation (Rook et al., 2004). The method is easy to apply and does not require costly equipment. The presence of an observer, however, can disturb a grazing animal and animals therefore need to become accustomed to the presence of observer if behaviour modification is to be avoided (Agreil and Meuret, 2004).

The recording of animal activities such as displacement, rumination and intake times has been widely tested and used to determine grazing time and biting rate (Laca and Wallis DeVries, 2000). These recording methods require expensive materials and harnessing an animal with recording apparatus, which can disturb its behaviour. The methods are difficult to apply with wild herbivores and on heterogeneous rangeland. As with some of the other methods, the major source of error with these methods lies in determining the biting mass, which can be estimated using oesophageally fistulated animals.

Another approach is the micro-histological analysis of the content of plant residues in faeces or in the stomach and intestinal tract. This method is often used to assess intake in wild ruminants. Its main disadvantages are that, apart from faeces collection, it requires the slaughter of the animal and that identifying the ingested plant fraction, at the species level, is very difficult because of the digestion process (Holecheck et al., 1982a). In addition, estimating the quantity of the various ingested plant fractions is seldom reliable because the quantity of plant fragments in faeces or stomachs is not directly proportional to the quantity of the ingested plant fractions, due to differences in digestibility.

Over the past 30 years, many empirical models have been developed for estimating forage intake in pasture. They are based on multiple regression between intake level and plant characteristics (e.g., OM yield, fibre content, digestibility, part of legume), animal characteristics (e.g., liveweight, average daily gain, milk production, stage of lactation, milk composition, pregnancy) and environmental factors (e.g., temperature, rainfall). Most of these models derive from dairy cow experimental data (Holter et al., 1997; Delagarde and O'Donovan, 2005) and are often specific to the relatively short range of grazing and experimental conditions used to develop them.

IV.3. Potential of Near Infrared Reflectance Spectroscopy (NIRS)

As noted earlier, estimating the intake of grazing ruminants is difficult. One solution could be to use NIRS. Over the past 20 years, NIRS analyses have been widely used to characterize the nutritive value of grazed grass. This indirect method is based on establishing calibration databases linking NIRS spectra (light absorbencies at different wavelengths) to values such as chemical or biological composition obtained by reference measurements in the laboratory. The most developed NIRS calibrations allow *in vivo* digestibility then intake from various organic substrates, such as forage, oesophageal extruda and faeces, to be estimated. In most cases, the digestibility and intake reference values stem from chemical analyses of forages or from animal trials.

As reported in many earlier studies (Norris et al., 1976; Holecheck et al., 1982b; Lippke et al., 1989; Biston et al., 1989; De Boever et al., 1996), NIRS analysis of forage appears to be as accurate as other methods (e.g., chemical, rumen fluid and enzymatic methods) for predicting the *in vivo* digestibility of a large range of forages. The size and representativity of the databases regrouping various forage species, sward conditions and locations, or growing stages explain the robustness of NIRS technology.

Studies have also shown that NIRS applied to faeces (FNIRS) could be as accurate as classical methods, if not more so, in predicting the diet characteristics of grazing ruminants (Stuth et al., 1989; Coleman et al., 1989; Coleman and Murray, 1993; Leite and Stuth, 1995; Decruyenaere et al., 2002). The chemical composition of faeces can reflect the biological and chemical characteristics of ingested forage, as well as the physiological status of the ruminant, two parameters that regulate intake. This chemical composition can be detected by NIRS and linked to intake and digestibility. For example, the importance of fat wavelength in estimating digestibility and intake could be linked to greater microbial growth in the rumen and therefore to a higher proportion of microbes linked to the faecal forage residues (Decruyenaere et al., 2008).

Stuth et al. (1989) and Lyons and Stuth (1992) showed that the *in vivo* grass digestibility of grazing ruminants in rangelands can be estimated by applying FNIRS with the same accuracy as that obtained using conventional analysis methods (standard error of calibration = 0.033). Garnsworthy and Unal (2004) concluded that predicting the DMI of dairy cows by applying FNIRS or using *n*-alkanes methods have had similar levels of accuracy (error of estimation = 0.36 and 0.44 kg DM per day from *n*-alkanes and 0.48 kg DM per day from FNIRS). Boval et al. (2004), Landau et al. (2004), Li et al. (2007) and Decruyenaere et al. (2008) confirmed the importance of FNIRS in assessing the diet characteristics of cattle, dairy goats and sheep.

The main constraint of the NIRS technique is the cost of the analytical equipment and the need to develop large reference databases that need to be updated frequently in order to develop robust calibrations that cover the range of field situations. In addition, because calibration robustness is directly linked to the accuracy of the method used to obtain the reference values, special attention needs to be paid to this aspect. The digestibility trials and the *n*-alkane techniques would be the most suitable for producing *in vivo* digestibility and intake reference values to match with faeces or forage NIRS spectra (Coates and Penning, 2000). The need for an independent set of samples in order to validate the calibrations is also a disadvantage of the NIRS technique. For small sample sets, however, the cross-validation technique can be used to evaluate the accuracy of the model (De Boever et al., 1995). In order to improve the potential of FNIRS for estimating the *in vivo* digestibility and intake of grazing ruminants, it will be necessary to create larger databases that regroup the greatest number of references in terms of forages and animal species.

V. Conclusion

The voluntary intake of grazing ruminants depends on many factors linked to herbage and animal characteristics. Given the complexity of the phenomenon, it is very difficult to estimate it continuously throughout a grazing season and with sufficient accuracy. Knowledge of grazing ruminants' intake level and diet quality is essential, however, for improving herd management by balancing sward availability with animal needs. Ideally, the methods developed to achieve this objective should be accurate, applicable at the individual or herd level and easy to use. This objective has not yet been achieved. The methods and techniques that have been developed to measure diet quality are often laborious, expensive

and inaccurate and sometimes do not represent actual grazing conditions (Lippke, 2002) because of the many factors affecting the digestibility and intake parameters.

Currently, the *n*-alkanes technique appears as one of the best for simultaneously predicting the intake and digestibility of ingested diets, but it is difficult to apply over a long period of tiem and in free-range systems. If sufficiently robust databases and calibrations are developed, FNIRS is a rapid and non-destructive technology that can simultaneously predict the digestibility and intake of a large set of similar samples. Recent studies have shown that FNIRS is promising and could be considered as a good alternative for assessing the diet, quantitatively and qualitatively, of ruminants in grazing or free-range systems.

VI. References

Adegosan, A.T., Givens, D.I., Owens, E., 2000. Measuring chemical composition and nutritive values in forages. In :'t Mannetje L.and Jones R.M., eds. Field and laboratory methods for grassland and animal production research. CAB International, 263-278.

Agreil, C., Meuret, M., 2004. An improved method for quantifying intake rate and ingestive behaviour of ruminants in diverse and variable habitats using direct observation. Small Ruminant Res., 54(1-2), 99-113.

Allen, M.S., 1996. Physical constraints on voluntary intake of forages by ruminants. J. Anim. Sci., 74, 3063-3075.

Allison, C.D., 1985. Factors affecting forage intake by range ruminants : a review. J. Range Manage., 38(4), 305-311.

Arthington, J.D., Brown, W.F., 2005. Estimation of feeding value of four tropical forage species at two stages of maturity. J. Anim. Sci., 83, 1726-1731.

Assoumaya, C., Sauvant, D., Archimède, H., 2007. Etude comparative de l'ingestion et de la digestion des fourrages tropicaux et tempérés. INRA Prod.Anim., 20(5), 383-392.

Atwood, S.B, Provenza, F.D., Wiedmeier, R.D., Banner, R.E., 2001. Influence of free-choice vs mixed-ration diets on food intake and performance of fattening calves. J. Anim. Sci., 79, 3034-3040.

Aufrère, J., Graviou, D., 1996. Prévision de la digestibilité des fourrages et aliments concentrés et composés des herbivores par une méthode enzymatique pepsine-cellulase. Rapport BIPEA Fourrages 14, 6p.

Aufrère, J., Baumont, R., Delaby, L., Peccate, J.R., Andrieu, J., Andrieu, J.P., Dulphy, J.P., 2007. Prévision de la digestibilité des fourrages par la méthode pepsine-cellulase. Le point sur les équations proposées. INRA Prod. Anim., 20(2), 129-136.

Babatounde, S., 2005. Etude de prédiction de la valeur alimentaire de graminées et de légumineuses fourragères en zone tropicale humide du Bénin. Thèse de doctorat : Faculté Universitaire des Sciences agronomiques de Gembloux (Belgique).

Barre, P., Emile, J.C., Betin, F., Surault, A., Ghesquière, M., Hazard, L., 2006. Morphological characteristics of perennial ryegrass leaves that influence short-term intake in dairy cows. Agron. J., 98, 978-985.

Bartiaux-Thill, N., Oger, R., 1986. The indirect estimation of the digestibility in cattle of herbage from Belgian permanent pasture. Grass Forage Sci., 41, 269-272.

Baumont, R., 1996. Palatabilité et comportement alimentaire chez le ruminant. INRA Prod. Anim., 9(5), 349-358.

Baumont, R., Prache, S., Meuret, M., Morhand Fehr, P., 2000. How forage characteristics influence behaviour and intake in small ruminants : a review. Livestock Prod. Sci., 61(1), 15-28.

Benvenutti, M.A., Gordon, I.J., Poppi, D.P., 2006. The effect of the density and physical properties of grass stems on the foraging behaviour and instantaneous intake rate by cattle grazing an artificial reproductive tropical sward. Grass Forage Sci., 61, 272-281.

Berry, N.R., Scheeder, M.R.L, Sutter, F., Krober, T.F., Kreuzer, M., 2000. The accuracy of intake estimation based on the use of alkane controlled-release capsules and faeces grab sampling in cows. Ann. Zootech., 49, 3-13.

Berzaghi, P., Herbein, J.H., Polan, C.E., 1996. Intake, site, and extent of nutrient digestion of lactating cows grazing pasture. J. Dairy Sci., 79(9), 581-1589.

Biston, R., Dardenne, P., Demarquilly, C., 1989. Determination of forage *in vivo* digestibility by NIRS. In : Proceedings of the XVI International Grassland Congress, Nice, France, 895-896.

Bouazizi, A., Majdoub, A., 1999. Prédiction des quantités ingérées et de la digestibilité du régime sélectionné par des ovins sur parcours semi-aride tunisien. Fourrages, 157, 77-87.

Boval, M., Peyraud, J.L., Xande, A., Aumont, G., Coppry, O., Saminadin, G., 1996. Evaluation d'indicateurs fécaux pour prédire la digestibilité et les quantités ingérées de Dichanthium sp par des bovins créoles. Ann. Zootech., 45, 121-134.

Boval, M., Coates, D.B., Lecomte, P., Decruyenaere, V., Archimède, H., 2004. Faecal Near Infrared Reflectance Spectroscopy (NIRS) to assess chemical composition, *in vivo* digestibility and intake of tropical grass by Creole cattle. Anim. Feed Sci. Technol., 114(1), 19-29.

Boval, M., Fanchone, A., Archimède, H., Gibb, M.J., 2007. Effect of structure of a tropical pasture on ingestive behaviour, digestibility of diet and daily intake by grazing cattle. Grass Forage Sci., 62, 44-54.

Brand, T.S., 2000. Grazing behaviour and diet selection by Dorper sheep. Small Ruminant Res., 36(2), 147-158.

Coates, D.B., Schachenmann, P., Jones, R.J., 1987. Reliability of extrusa samples collected from steers fistulated at the oesophagus to estimate the diet of resident animals in grazing experiments. Aust. J. Exp. Agric., 27, 739-745.

Coates, D.B., Penning, P., 2000. Measuring animal performances. In : 't Mannetje L. and Jones R.M., eds. Field and Laboratory Methods for grassland and Animal Production Research. CAB International, 353-402.

Coleman, S.W., Stuth, J.W., Holloway, J.W., 1989. Monitoring the nutrition of grazing cattle with near-infrared analysis of faeces. In : Proceedings of the XVI International Grassland Congress, Nice, France, 881-882.

Coleman, S.W., Murray, I., 1993. The use of near-infrared reflectance spectroscopy to define nutrient digestion of hay by cattle. Anim. Feed Sci. Technol., 44, 237-249.

Compère, R., Valdivia, J., Hellemans, P., Guns, M., Vandenbyvang, P., 1992. Comparaison de deux méthodes utilisant le Cr2O3 pour estimer la quantité de fèces émises par les moutons : pellets de luzerne et bolus CAPTEC-CRD. Bull. Rech. Agron. Gembloux, 27, 365-383.

Cooper, D.B., Kyriazakis, I., 1995. Diet selection in sheep: the role of the rumen environment in the selection of a diet from two feeds that differ in their energy density. Br. J. Nutr., 74, 39-54.

Cortes, C., Damanesco, J.C., Bechet, G., Prache, S., 2005. Species composition of ryegrass (Lolium perenne) and tall fescue (Festuca arundinacea) mixtures using various combinations of *n*-alkanes. Grass Forage Sci., 60, 254-261.

De Boever, J.L., Cottyn, B.G., Andries, J.I., Buysse, F.X., Vanacker, J.M., 1988. The use of pepsin cellulase technique to predict digestibility metabolizable and net energy of forages. Anim. Feed. Sci. Technol., 19, 247-260.

De Boever, J.L., Cottyn, B.G., Vanacker, J.M., Boucque, C.V., 1995. The use of NIRS to predict the chemical composition and the energy value of compound feeds for cattle. Anim. Feed Sci. Technol., 51, 243-253.

De Boever, J.L., Cottyn, B.G., De Brabander, D.L., Vanacker, J.M., Boucqué, C.V., 1996. Prediction of the feeding value of grass silages by chemical parameters, in vitro digestibility and near-infrared reflectance spectroscopy. Anim. Feed. Sci. Technol., 60(1-2), 103-116.

Decruyenaere, V., Stilmant, D., Lecomte, P., Buldgen, A., Dardenne, P., 2002. Improvement and validation of the NIRS analysis applied to faeces to measure grass intake in pasture. In : Durand J.L., Emile J.C., Huygue Ch. and Lemaire G., eds. Multi-function grasslands. Quality forages, animal products and landscapes. Grassl. Sci. Eur., 7, 196-197.

Decruyenaere, V., Lecomte, Ph., Demarquilly, C., Aufrere, J., Dardenne, P., Stilmant, D., Buldgen, A., 2008. Evaluation of green forage intake and digestibility in ruminants using neaur infrared reflectance spectrscopy (NIRS): Developing a global calibration. Anim Feed Sci. Technol., doi 10.1016/j.anifeedsci.2008.03.007.

Delaby, L., Pecatte, J.R., 2003. Valeur alimentaire des prairies d'association ray-grass anglais /trèfle blanc utilisées entre 6 et 12 semaines de repousse. Renc. Rech. Ruminants, 10, 389.

Delaby, L., Pecatte, J.R, Aufrère, J., Baumont, R., 2007. Description et prévision de la valeur alimentaire de prairies multi-espèces. Premiers résultats. Renc. Rech. Ruminants, 14, 249.

Delagarde, R., O'Donovan, M., 2005. Les modèles de prévision de l'ingestion journalière d'herbe et de la production laitière des vaches au pâturage. INRA Prod. Anim., 18(4), 241-253.

Demarquilly, C., Jarrige, R., 1981. Panorama des méthodes de prévision de la digestibilité et de la valeur énergétique des fourrages. In : Demarquilly C., ed. Prévision de la valeur nutritive des aliments des ruminants. INRA, France, 41-59.

Demarquilly, C., Chenost, M., Giger, S., 1995. Pertes fécales et digestibilités des aliments et des rations. In : Jarrigue R., Ruckenbusch Y., Demarquilly C., Farce M-H., Journet M., eds. Nutrition des ruminants domestiques, ingestion et digestion. INRA, France,601-647.

Demment, M.W., Van Soest, P.J., 1985. A nutritional explanation for body-size patterns of ruminant and non-ruminant herbivores. Am. Nat., 125, 641-672.

Dove, H., Mayes, R.W., 1991. The use of plant wax alkanes as marker substance in studies of nutrition herbivores: a review. Aust. J. Agric. Res., 42, 913-952.

Dove, H., Mayes, R.W., 1996. Plant wax components: a new approach to estimating intake and diet composition in herbivores. J. Nutr., 126, 13-26.

Dumont, B., Gordon, I.J., 2003. Diet selection and intake within sites and across landscapes. In : Herrera-Camacho C.A. and Sandoval-Castro J., eds. VIth International Symposium on the Nutrition of Herbivores, Merida, Mexico, 175-194.

Duncan, A.J., Ginane, C., Elston, D.A., Kunaver, A., Gordon, I.J., 2006. How do herbivores trade-off the positive and negative consequences of diet selection decisions? Anim. Behav., 71(1), 93-99.

Fahey, G.C., Jung, H.G., 1983. Lignin as marker in digestion studies : a review. J. Anim Sci., 57, 220-225.

Farruggia, A., Dumont, B., D'hour, P., Egal, D., Petit, M., 2006. Diet selection of dry and lactating beef cows grazing extensive pastures in late autumn. Grass Forage Sci., 61, 347-353.

Faverdin, P., 1999. The effect of nutrients on feed intake in ruminants. Proc. Nutr. Soc., 58, p. 523-531.

Faverdin, P., Delaby, L., Delagarde, R., 2006. Prévision de l'ingestion des vaches laitières au cours de la lactation. Renc. Rech. Ruminants, 13, .85-88.

Faverdin, P., Delaby, L., Delagarde, R., 2007. L'ingestion d'aliments par les vaches laitières et sa prévision au cours de la lactation. INRA Prod.Anim., 20(2), 151-162.

Ferreira, L.M.M., Olivan, M., Rodrigues, M.A.M., Osoro, K., Dove, H., Dias-Da-Silva, A., 2004. Estimation of feed intake by cattle using controlled-release capsules containing *n*-alkanes or chromium sesquioxide. J. Agric. Sci., 142, 225-234.

Forbes, J.M., 1996. Integration of regulatory signals controlling forage intake in ruminants. J. Anim. Sci., 74, 3029-3035.

Forbes, J.M., 2003. The multifactorial nature of food intake control. J. Anim. Sci., 81(E. Suppl. 2), 139-144.

Frame, J., 1993. Herbage mass. In : Davies A., Baker R.D., Grand S.A., Laidlaw A.S., eds. Sward Measurement Handbook, 2nd edition. ABGS publications, 39-68.

Garcia, F., Carrère, P., Soussana, J.F., Baumont, R., 2003. How do severity and frequency of grazing affect sward characteristics and the choices of sheep during the grazing season? Grass Forage Sci., 58, 138-150.

Garnsworthy, P.C., Unal, Y., 2004. Estimation of dry-matter intake and digestibility in group-fed dairy cows using near infrared reflectance spectroscopy. Anim. Sci,. 79, 327-334.

Gibb, M.J., Huckle, C.A., Nuthall, R., Rook, A.J., 1999. The effect of physiological state (lactating or dry) and sward surface height on grazing behaviour and intake by dairy cows. Appl. Anim. Behav. Sci., 63(4), 269-287.

Ginane, C., Duncan, A.J., Young, S.A., Elston, D.A., Gordon, I.J., 2005. Herbivore diet selection in response to simulated variation in nutrient rewards and plants secondary compounds. Anim. Behav., 69(3), 541-550.

Gregorini, P., Gunter, S.A., Masino, C.A., Beck, P.A., 2007. Effect of ruminal fill on short-term intake rate and grazing dynamics of beef heifers. Grass Forage Sci., 62, 346-354.

Hodgson, J., 1985, cited by Prache, S., Peyraud, JL., 1997. Préhensibilité de l'herbe pâturée chez les ovins et les bovins. INRA Prod. Anim., 10(5), 377-390.

Holden, L.A., Muller, L.D., Fales, S.L., 1994. Estimation of intake in high producing Holstein cows grazing grass pasture. J. Dairy Sci., 77, 2332-2340.

Holechek, J.L., Vavra, M., Piepper, R.D., 1982a. Botanical composition determination of range herbivores diets : a review. J. Range Manage., 35(3), 309-315.

Holechek, J.L., Shenk, J.S., Vavra, M., Arthun, D., 1982b. Prediction of forage quality using near-infrared reflectance spectroscopy on oesophageal fistula samples from cattle on mountain range. J. Anim. Sci., 55, 971-975.

Holter, J.B., West, J.W., Mc Gilliard, M.L., 1997. Predicting ad-libitum dry matter intake and yield of Holstein cows. J. Dairy Sci., 80, 2188-2199.

Hristov, A.N., Price, W.J., Shafii, B., 2005. A meta-analysis on the relationship between intake of nutrients and body weight with milk volume and milk protein yield in dairy cows. J. Dairy Sci., 88, 2860-2869.

Illius, A.W., Jessop, N.S., 1996. Metabolic constraints on voluntary intake in ruminants. J. Anim. Sci., 74, 3052-3062.

INRA, 2007. Alimentation des bovins, ovins et caprins. Besoins des animaux – valeurs des aliments. Eds., Quae, 307p.

Jarrige, R., Thivend, P., 1969. Action d'une cellulose fongique sur les membranes et son intérêt pour prévoir la digestibilité des plante fourragères. Ann. Biol. Anim. Bioch. Biophys., 9, 171-190.

Jarrige, R., Demarquilly, C., Dulphy, J.P., Hoden, A., Robelin, J., Beranger, C., Geay, Y., Journet, M., Malterre, C., Petit, M., 1986. The INRA 'fill unit' system for predicting the voluntary intake of forage-based diet in ruminants : a review. J. Anim. Sci., 63, 1737-1758.

Johnson, T.R., Combs, D.K., 1991. Effects of prepartum diet, inert rumen bulk, and dietary polyethylene glycol on dry matter intake of lactating dairy cows. J Dairy Sci., 74, 933-944.

Johnson, T.R., Combs, D.K., 1992. Effects of inert rumen bulk on dry matter intake in early and midlactation cows fed diets differing in forage content. J. Dairy Sci., 75, 508-519.

Jones, R.J., Lascano, C.E., 1992. Oesophageal fistulated cattle can give unreliable estimates of the proportion of legume in the diets of resident animals grazing tropical pastures. Grass Forage Sci., 47, 128-132.

Jung, H.G., Allen, M.S., 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J. Amin. Sci., 73, 2774-2790.

Klieber, M., 1961, cited by Allison C.D., 1985. Factors affecting forage intake by range ruminants : a review. J. Range Manage., 38(4), 305-311.

Kloppenburg, P.B, Kiesling, H.E., Kirksey, R.E., Donart, G.B., 1995. Forage quality, intake and digestibility of yearlong pastures for steers. J. Range Manage., 48, 542-548.

Kyriazakis, I., 2003. What are ruminant herbivores trying to achieve through their feeding behaviour and food intake. In : Herrera-Camacho C.A. and Sandoval-Castro J., eds. VIth International Symposium on the Nutrition of Herbivores, Merida, Mexico, 153-173.

Kyriazakis, I. Oldham, J.D., 1993. Diet selection in sheep : the ability of growing lambs to select a diet that meets their crude protein (nitrogen x 6.25) requirements. Br. J. Nutr., 69, 617-629.

Laca, E.A., Wallis, de Vries, M.F., (2000). Acoustic measurement of intake and grazing behaviour of cattle. Grass Forage Sci.. 55. 97-104.

Landau, S., Glasser, T., Dvash, L., Perevolotsky, A., 2004. Faecal NIRS to monitor diet of mediterranean goats. S. Afr. J. Anim. Sci., 34(1), 76-80.

Lecomte, Ph., Dardenne, P., Agneessens, R., 1992. Prédiction de la digestibilité de la matière organique des fourrages verts par la méthode enzymatique à la pepsine cellulase et par spectromètrie dans le proche infrarouge. Rev. Agric., 45(1), 77-81.

Leite, E.R., Stuth, J.W., 1995. Fecal NIRS equations to assess diet quality of free ranging goats. Small Ruminant Res., 15(3), 223-230.

Li, H., Tolleson, D., Stuth, J., Bai, K., Mo, F., Kronberg, S., 2007. Faecal near infrared reflectance spectroscopy to predict diet quality for sheep. Small Ruminant Res., 68(3), 263-268.

Lippke, H., Barton II, F.E., Ocumpaugh, W.R., 1989. Near infrared reflectance spectroscopy for estimation of digestible organic matter intake and body weight gain. In : Proceedings of the XVI International Grassland Congress, Nice, France, 893-894.

Lippke, H., Forbes, T.D.A., Ellis, W.C., 2000. Effect of supplement on growth and forage intake by stocker steers grazing wheat pasture. J. Anim. Sci., 78(6), 1625-1635.

Lippke, H., 2002. Estimation of forage intake by ruminants on pasture. Crop Sci., 42(3), 869-872.

Loney, P.E., Mc Arthur, Y.C., Potts, B.M., Jordan, G.J., 2006. How does ontogeny in a Eucalyptus species affect patterns of herbivory by Brushtail Possums? Funct. Ecol., 20(6), 982–988.

Lyons, R.K., Stuth, J.W., 1992. Feacal NIRS equations for predicting diet quality of free-ranging cattle. J. Range Manage., 45, 238-244.

Macoon, B., Sollenberg, L.E., Moore, J.E., Staples, C.R., Fike, J.H., Portier, K.M., 2003. Comparison of three techniques for estimating the forage intake of lactating dairy cows at pasture. J. Anim. Sci., 81, 2357-2366.

Mayes, R.W., Lamb, C.S., Colgrove, P.M., 1986. The use of dosed and herbage *n*-alkanes as markers for determination of herbage intake. J. Agric. Sci. Camb., 107, 161-170.

Meissner, H.H., Paulsmeier, D.V., 1995. Plant compositional constituents affecting between-plant and animal species prediction of forage intake. J. Anim. Sci., 73, 2447-2457.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Schneider, W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from gas production when they are incubated with rumen liquor in vitro. J. Agric. Sci, Camb., 93, 217-222.

Meuret, M., Débit, S., Agreil, C., Osty, P.L., 2006. Eduquer ses veaux et ses génisses : un savoir empirique pertinent pour l'agroenvironnement en montagne? Nature Sciences Sociétés, 14, 343-352.

Minson, D.J., Mc Donald, C.K., 1987. Estimating forages intake from the growth of beef cattle. Trop. Grasslands, 21, 116-122.

Nagy, J.G., Tengerdy, R.P., 1968 cited by Provenza F.D., Villalba J.J., Dziba L.E., Atwood S.B., Banner R.E., 2003b. Linking herbivore experience, varied diets, and plant biochemical diversity. Small Ruminant Res., 49, 257-274.

Norris, K.H., Barnes, R.F., Moore, J.E., Shenk, J.S., 1976. Predicting forage quality by infrared reflectance spectroscopy. J. Anim.Sci., 43, 889-897.

O'Donovan, M., Delaby, L., 2005. A comparison of perennial ryegrass cultivars differing in heading date and grass ploidy with spring calving dairy cows grazed at two different stocking rate. Anim. Res., 54, 337-350.

Parga, J., Peyraud, J.L., Delagarde, R., 2002. Age of regrowth affects grass intake and ruminal fermentations in grazing dairy cows. In : Durand J.L., Emile J.C., Huyghe C. and Lemaire G., eds. Multi-function grasslands. Quality forages, animal products and landscape. Grassl. Sci. Eur., 7, 256-257.

Pasha, T.N., Prigge, E.C., Russell, R.W., Bryan, W.B., 1994. Influence of moisture content of forage diets on intake and digestion by sheep. J. Anim. Sci., 72(9), 2455-2463.

Pearson, R.A., Archibald, R.F., Muirhead, R.H., 2005. A comparison of the effect of forage type and level of feeding on the digestibility and gastrointestinal mean retention time of dry forages given to cattle, sheep, ponies and donkeys. Br. J. Nutr., 95(1), 88-98.

Penning, P.D., Parsons, A.J., Orr, R.J., Hooper, G.E., 1994. Intake and behaviour responses by sheep to changes in sward characteristics under rotational grazing. Grass Forage Sci., 49, 476-486.

Penning, P.D., Parsons, A.J., Orr, R.J., Harvey, A., Champion, R.A., 1995. Intake and behaviour responses by sheep, in different physiological states, when grazing monocultures of grass or white clover. Appl. Anim. Behav. Sci., 45(1-2), 63-78.

Peyraud, J.L., Comeron, E.A., Wade, M.H., Lemaire, G., 1996. The effect of daily herbage allowance, herbage mass and animal factors upon herbage intake by grazing dairy cows. Ann. Zootech., 45(3), 201-217.

Peyraud, J.L., 1998. Techniques for measuring faecal flow, digestibility and intake of herbage in grazing ruminants. Techniques for investigating intake and ingestive behaviour by farm animals. IN : Proccedings of the IXth European Intake Workshop, IGER North Wyke, UK, 39-48.

Prache, S., Peyraud, J.L., 1997. Préhensibilité de l'herbe pâturée chez les ovins et les bovins. INRA Prod. Anim., 10(5), 377-390.

Provenza, F.D., 2003a. Foraging behavior: managing to survive in a world of change. USDA eds. 63p.

Provenza, F.D., Villalba, J.J., Dziba, L.E., Atwood, S.B., Banner, R.E., 2003b. Linking herbivore experience, varied diets, and plant biochemical diversity. Small Ruminant Res., 49, 257-274.

Reed, J.D., 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. J. Anim. Sci., 73, 1516-1528.

Rhind, S.M., Archer, Z.A., Adam, C.L., 2002. Seasonality of food intake in ruminants : recent developments in understanding. Nutr. Research Rev., 15, 43-65.

Ribeiro, H.M.N., Delagarde, R., Peyraud, J.L., 2003. Inclusion of white clover in strip-grazed ryegrass swards: herbage intake and milk yield of dairy cows at different ages of sward regrowth. Anim. Sci., 77, 499-510.

Roguet, C., Dumont, B., Prache, S., 1998. Sélection et utilisation des ressources fourragères par les herbivores : théories et expérimentations à l'échelle du site et de la station alimentaires. INRA Prod.Anim., 11(4), 273-284.

Rook, A.J., Harvey, A., Parsons, A.J., Orr, R.J., Rutter, S.M., 2004. Bite dimensions and grazing movements by sheep and cattle grazing homogeneous perennial ryegrass swards. Appl. Anim. Behav. Sci, 88, 227-242.

Sauvant, D., Assoumaya, C., Giger-Reverdin, S., Archimède, H., 2006. Etude comparative du mode d'expression du niveau d'alimentation chez les ruminants. Renc. Rech. Ruminants, 13, 103.

Schettini, M.A., Prigge, E.C., Nestor, E.L., 1999. Influence of mass and volume of ruminal contents on voluntary intake and digesta passage of forage diet in steers. J. Anim. Sci., 77, 1896-1904.

Scott, L.L., Provenza, F.D., 1999. Variation in food selection among lambs : effects of basal diet and foods offered in a meal. J. Anim Sci., 77, 2391-2397.

Sibbald, A.M., Shellard, L.J., Smart, T.S., 2000. Effect of space allowance on the grazing behaviour and spacing of sheep. Appl. Anim. Behav. Sci., 70(1), 49-62.

Smit, H.J., Tas, B.M., Taweel, H.Z., Tamminga, S., Elgersma, A., 2005a. Effects of perennial ryegrass (Lolium perenne L.) cultivars on herbage production, nutritional quality and herbage intake of grazing dairy cows. Grass Forage Sci., 60, 297-309.

Smit, H.J., Taweel, H.Z., Tas, B.M., Elgersma, A., 2005b. Comparison of techniques for estimating herbage intake of grazing dairy cows. J. Dairy Sci., 88, 1827-1836.

Sprinkle, J.E., Holloway, J.W., Warrington, B.G., Elis, W.C., Stuth, T.D.A., Greene, J.W., 2000. Digesta kinetics, energy intake, grazing behaviour, and body temperature of grazing beef cattle differing in adaptation to heat. J. Anim. Sci., 78, 1608-1624.

Stern M.D., Bach A., Calsamiglia S., 1997. Alternative techniques for measuring nutrient digestion in ruminants. J. Anim. Sci., 75, 2256-2276.

Stuth, J.W., Kapes, E.D., Lyons, R.K., 1989. Use of near infrared spectroscopy to assess nutritional status of cattle diets on rangeland. In : Proceedings of the XVI International Grassland Congress, Nice, France, 889-890.

Sunvold, G.D., Cochran, R.C., 1991. Technical note: Evaluation of acid detergent lignin, alkaline peroxide lignin, acid insoluble ash, and indigestible acid detergent fiber as internal markers for prediction of alfalfa, bromegrass, and prairie hay digestibility by beef steers. J. Anim. Sci., 69, 4951-4955.

Tilley, J.H.A, Terry, R.A., 1963. A two stage technique for 'in vitro' digestion of forage crops. Grass Forage Sci., 18, 104-111.

Valente, M.E., Borreani, G., Peiretti, P.G., Tabacco, E., 2000. Codified morphological stage for predicting digestibility of Italian Ryegrass during spring cycle. Agron. J., 92, 967-973.

Van Soest, P.J., 1994, cited by Schettini, M.A., Prigge, E.C., Nestor, E.L., 1999. Influence of mass and volume of ruminal contents on voluntary intake and digesta passage of forage diet in steers. J. Anim. Sci., 77, 1896-1904.

Van Wieren, S.E., 1996. Do large herbivores select a diet that maximizes short-term energy intake rate? Forest Ecol. Manage., 88, 149-156.

Vazquez, O.P., Smith, T.R., 2000. Factors affecting pasture intake and total dry matter intake in grazing dairy cows. J. Dairy Sci., 83, 2301-2309.

Wainman et al., 1981, cited by Adegosan, A.T., Givens, D.I., Owens, E., 2000. Measuring chemical composition and nutritive values in forages. In : 't Mannetje L. and Jones R.M., eds. Field and laboratory methods for grassland and animal production research. CAB International, 263-278.

Wallis DeVries, M.F., 1995. Estimating forage intake and quality in grazing cattle: a reconsideration of the hand-plucking method. J. Range Manage., 48, 370-375.

Yearsley, J., Tolkamp, B.J.N., Illius, A.W., 2001. Theoretical developments in the study and prediction of food intake. Proc. Nutr. Soc., 60, 145-156.

Chapter II: Research strategy

Context

Grazed grass is the most cost-effective feed for ruminants in temperate and tropical areas. Grazing management, however, is often difficult for breeders. A major weakness of grazed systems is the lack of knowledge about grass availability and quality. Better knowledge about 'diet characteristics' is therefore a key issue in the improving the use of grazed pasture. The main parameters of interest in 'diet characteristics' are *in vivo* organic matter digestibility (OMD), organic and/or dry matter voluntary intake (OMVI and DMVI) and the botanical composition of ingested diets. Monitoring the evolution of these parameters in grazed systems, however, in real time and continuously, poses a problem.

Estimations of *in vivo* digestibility and intake are usually obtained through digestibility trials, with adult sheep housed in digestibility crates or individual pens (Demarquilly et al., 1995; Andueza et al., 2011a). This method is expensive, time consuming and labour-intensive. Alternative methods have been proposed for estimating these parameters more rapidly. There are well-documented methods for estimating *in vivo* digestibility. For example, the 'rumen fluid pepsin' method (Tilley and Terry, 1963), the gas test method (Menke et al., 1979) and enzymatic mixtures that simulate ruminal activities (Jarrige and Thivend, 1969; De Boever et al., 1996; Aufrère et al., 2007) have been developed in order to estimate *in vivo* digestibility from a forage sample. The potential of using near infrared reflectance spectroscopy (NIRS) to characterize the nutritive value of forages has been widely demonstrated (Norris et al., 1976; Holecheck et al., 1982; Biston et al., 1989; Sinneave et al., 1994; De Boever et al., 1996).

Few studies have been conducted on estimating the voluntary intake of grazing ruminants, probably because this is a multifactorial parameter. According to Illius and Jessop (1996), intake can be considered as a 'psychological' phenomenon, involving the integration of many signals and reflecting the flexibility of a biological system evolved to cope with variability in food supply, composition and animal status. Plant characteristics, resource availability, animal species and physiological state are important parameters affecting diet selection and ingestive behaviour under grazing conditions and therefore the level of intake (Provenza et al., 2003). Consequently, it is difficult to assess the intake of grazing ruminants from the analysis of an average sward sample with sufficient precision.

Faeces are composite materials that contain digestion residues and thus reflect the biological and chemical characteristics of ingested diets, as well as the physiological status of the animal. Indirect methods based on faecal characteristics such as nitrogen (Bartiaux-Thill and Oger, 1986; Peyraud, 1998; Boval et al., 1996; Bouazizi and Majdoub, 1999), indigestible compounds such as lignin, (Fahey and Jung, 1983; Sunvold and Cochran, 1991) and *n*-alkanes (Dove and Mayes, 1991, 1996) can successfully predict the *in vivo* digestibility of grazed forages. Several studies have also shown the potential of applying NIRS to faeces (FNIRS) in order to predict both *in vivo* digestibility and intake of forage (Stuth et al., 1989; Coleman et al., 1989; Coleman and Murray, 1993; Leite and Stuth, 1995; Boval et al., 2004; Garnsworthy and Unal, 2004; Li et al., 2007; Fanchone et al., 2009; Dixon and Coates, 2009; Tran et al., 2010; Andueza et al., 2011b). As described by Walker et al. (2002) and Dixon and Coates (2009), FNIRS analysis can also be used for estimating the botanical composition of grazed diets. Most of the published studies focus on tropical rangelands and describe small or specific FNIRS databases. With regard to forage, the main constraint of FNIRS is the need to develop calibrations that are robust enough to cover a range of field situations.

Aim of the thesis

The aim of this thesis is twofold. First, it focuses on the development of FNIRS spectral libraries and calibrations that can be used to estimate the *in vivo* organic matter digestibility (OMD), voluntary intake (OMVI and DMVI) and botanical composition of ingested diets under grazing conditions. An original aspect of this approach is to link the spectral NIRS information contained in faeces to diet *in vivo* characteristics obtained through continuous digestibility trials. Second, the thesis seeks to validate these estimations over a diverse range of grazing conditions.

Structure of the thesis

This thesis draws on eight published scientific articles. Initially, it reviews factors affecting intake and intake assessment methods (Chapter I). This is followed by an outline of the research strategy used (Chapter II). It is then divided into two main chapters (Chapter III and IV), each corresponding to a specific objective (Figure 1).

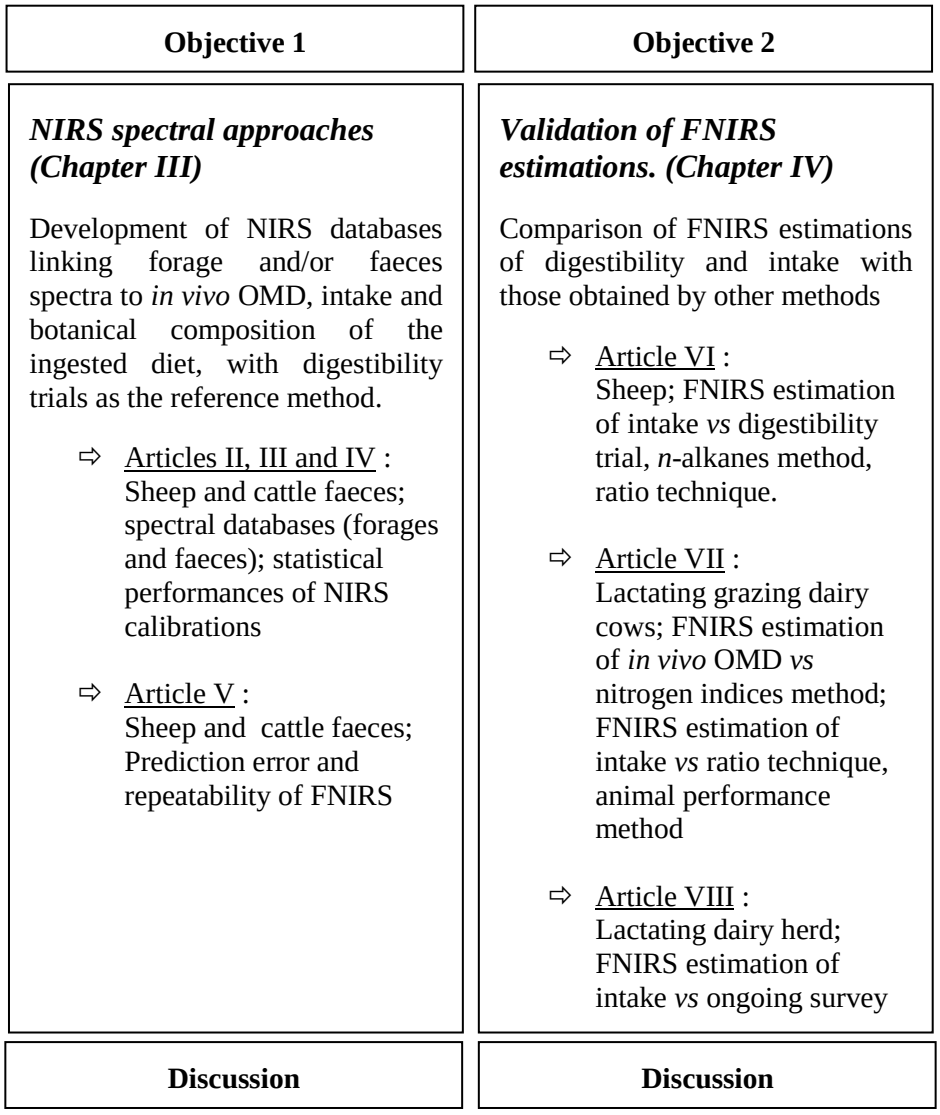

Fig. 1. Structure of Chapters III and IV

Chapter III describes the methodological approach for building the forage and faeces NIRS databases and the statistical performances of NIRS calibrations in predicting the *in vivo* digestibility, intake and botanical composition of the ingested diets of sheep or cattle (Articles II, III and IV). Given that the robustness of the calibration and the prediction error are directly linked to the accuracy of the reference values (Sørensen, 2002; Dryden, 2003), special attention needs to be paid to this aspect, as noted in Article V.

Chapter IV focuses on the validation of FNIRS predictions under grazing conditions. The validation of NIRS predictions usually involves comparing predicted values and reference values and then calculating the standard error of prediction (SEP). Under grazing conditions, it is difficult to obtain reference values. In this situation, the ability of FNIRS calibrations and libraries to predict OMD, OMVI and DMVI can be evaluated by comparison with other estimation methods (Articles VI, VII and VIII). The intake estimation methods described in Article VI are the reference method (digestibility trials with sheep), the *n*-alkanes method and the ratio technique. Those described in Article VII are the ratio technique and the animal performances method. For OMD, Article VII compares the nitrogen indices method with FNIRS predictions. Faecal NIRS was also applied on commercial dairy farms on La Réunion Island in order to assess grass intake at the herd scale (Article VIII). After this validation, FNIRS was used in a general study of the variation in milk composition on La Réunion (Bony et al., 2005).

Chapters III and IV conclude with an overall discussion. The final part of the thesis consists of a general discussion that puts the study results into perspective, followed by an outline of future prospects and an overall conclusion.

References

Andueza D., Picard F., Pradel Ph., Egal D., Hassoun P., Pecatte J.R., Beaumont R., 2011a. Reproductibility and repeatability of forage *in vivo* digestibility and voluntary intake of permanent grassland forages in sheep. Livestock Sci., 140, 42-48.

Andueza D., Picard F., Jestin M., Andrieu J., Baumont R., 2011b. NIRS prediction of the feed value of temperate forages efficacy of four calibration strategies. Animal 5:7, 1002-1013.

Aufrère J., Baumont R., Delaby L., Peccate J.R., Andrieu J., Andrieu J.P., Dulphy J.P., 2007. Prévision de la digestibilité des fourrages par la méthode pepsine-cellulase. Le point sur les équations proposées. INRA Prod. Anim., 20(2), 129-136.

Bartiaux-Thill N., Oger R., 1986. The indirect estimation of the digestibility in cattle of herbage from Belgian permanent pasture. Grass Forage Sci., 41, 269-272.

Biston R., Dardenne P., Demarquilly C., 1989. Determination of forage *in vivo* digestibility by NIRS. In : Proceedings of the XVI International Grassland Congress, Nice, France, 895-896.

Bony J., Contamin V., Gousseff M., Metais J., Tillard E., Juanes X., Decruyenaere V., Coulon J.B., 2005. Facteurs de variation de la composition du lait à la Réunion. INRA Prod. Anim., 18, 255-263.

Bouazizi A., Majdoub A., 1999. Prédiction des quantités ingérées et de la digestibilité du régime sélectionné par des ovins sur parcours semi-aride tunisien. Fourrages, 157, 77-87.

Boval M., Peyraud J.L., Xande A., Aumont G., Coppry O., Saminadin, G., 1996. Evaluation d'indicateurs fécaux pour prédire la digestibilité et les quantités ingérées de Dichanthium sp par des bovins créoles. Ann. Zootech., 45, 121-134.

Boval M., Coates D.B., Lecomte P., Decruyenaere V., Archimède H., 2004. Faecal Near Infrared Reflectance Spectroscopy (NIRS) to assess chemical composition, *in vivo* digestibility and intake of tropical grass by Creole cattle. Anim. Feed Sci. Technol., 114(1), 19-29.

Coleman S.W., Stuth J.W., Holloway J.W., 1989. Monitoring the nutrition of grazing cattle with near-infrared analysis of faeces. In : Proceedings of the XVI International Grassland Congress, Nice, France, 881-882.

Coleman S.W., Murray I., 1993. The use of near-infrared reflectance spectroscopy to define nutrient digestion of hay by cattle. Anim. Feed Sci. Technol., 44, 237-249.

De Boever J.L., Cottyn B.G., De Brabander D.L., Vanacker J.M., Boucqué C.V., 1996. Prediction of the feeding value of grass silages by chemical parameters, in vitro digestibility and near-infrared reflectance spectroscopy. Anim. Feed. Sci. Technol., 60(1-2), 103-116.

Demarquilly C., Chenost M., Giger S., 1995. Pertes fécales et digestibilités des aliments et des rations. In : Jarrigue R., Ruckenbusch Y., Demarquilly C., Farce M-H., Journet M., eds. Nutrition des ruminants domestiques, ingestion et digestion. INRA, France, 601-647.

Dixon R., Coates D.B., 2009. Near infrared reflectance spectroscopy of faeces to evaluate the nutrition and physiology of herbivores. J. Near Infrared Spectrosc. 17, 1-31.

Dove H., Mayes R.W., 1991. The use of plant wax alkanes as marker substance in studies of nutrition herbivores: a review. Aust. J. Agric. Res., 42, 913-952.

Dove H., Mayes R.W., 1996. Plant wax components: a new approach to estimating intake and diet composition in herbivores. J. Nutr., 126, 13-26.

Dryden G.McL., 2003. Near infrared spectroscopy: applications in deer nutrition. RIRCD Publication n° W03/007, 38p.

Fahey, G.C., Jung, H.G., 1983. Lignin as marker in digestion studies: a review. J. Anim Sci., 57, 220-225.

Fanchone A., Archimède H., Boval M., 2009. Comparison of fecal crude protein and fecal near-infrared reflectance spectroscopy to predict digestibility of fresh grass consumed by sheep. J. Anim Sci. 87, 236-243.

Garnsworthy P.C., Unal Y., 2004. Estimation of dry matter intake and digestibility in group-fed dairy cows using near infrared reflectance spectroscopy. Anim Sci. 79, 327-334.

Holechek J.L., Shenk J.S., Vavra M., Arthun D., 1982. Prediction of forage quality using near-infrared reflectance spectroscopy on oesophageal fistula samples from cattle on mountain range. J. Anim. Sci., 55, 971-975.

Illius A.W., Jessop N.S., 1996. Metabolic constraints on voluntary intake in ruminants. J. Anim. Sci., 74, 3052-3062.

Jarrige R., Thivend P., 1969. Action d'une cellulose fongique sur les membranes et son intérêt pour prévoir la digestibilité des plantes fourragères. Ann. Biol. Anim. Bioch. Biophys., 9, 171-190.

Leite E.R., Stuth J.W., 1995. Fecal NIRS equations to assess diet quality of free ranging goats. Small Ruminant Res., 15(3), 223-230.

Li H., Tolleson D., Stuth J., Bai K., Mo F., Kronberg S., 2007. Faecal near infrared reflectance spectroscopy to predict diet quality for sheep. Small Ruminant Res., 68(3), 263-268.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Schneider, W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from gas production when they are incubated with rumen liquor in vitro. J. Agric. Sci, Camb., 93, 217-222.

Norris, K.H., Barnes, R.F., Moore, J.E., Shenk, J.S., 1976. Predicting forage quality by infrared reflectance spectroscopy. J. Anim.Sci., 43, 889-897.

Peyraud, J.L., 1998. Techniques for measuring faecal flow, digestibility and intake of herbage in grazing ruminants. Techniques for investigating intake and ingestive behaviour by farm animals. IN : Proccedings of the IXth European Intake Workshop, IGER North Wyke, UK, 39-48.

Provenza, F.D., Villalba, J.J., Dziba, L.E., Atwood, S.B., Banner, R.E., 2003. Linking herbivore experience, varied diets, and plant biochemical diversity. Small Ruminant Res., 49, 257-274.

Sinneave G., Dardenne P., Agneessens R., 1994. Global or local? A choice for NIR calibrations analyses of forage quality. J. Near Infrared Spectrosc., 2,163-175.

Sørensen L.K., 2002. True accuracy of near infrared spectroscopy and its dependence on precision of reference data. J. Near Infrared Spectrosc. 10, 15-25.

Stuth J.W., Kapes E.D., Lyons R.K., 1989. Use of near infrared spectroscopy to assess nutritional status of cattle diets on rangeland. In : Proceedings of the XVI International Grassland Congress, Nice, France, 889-890.

Sunvold, G.D., Cochran, R.C., 1991. Technical note: Evaluation of acid detergent lignin, alkaline peroxide lignin, acid insoluble ash, and indigestible acid detergent fiber as internal markers for prediction of alfalfa, bromegrass, and prairie hay digestibility by beef steers. J. Anim. Sci., 69, 4951-4955.

Tilley J.H.A, Terry R.A., 1963. A two stage technique for 'in vitro' digestion of forage crops. Grass Forage Sci., 18, 104-111.

Tran H., Salgado P., Tillard E., Dardenne P., Nguyen X.T., Lecomte P., 2010. Global and local predictions of dairy diet nutritional quality using near infrared reflectance spectroscopy. J. Dairy Sci., 93, 4961-4975.

Walker J.W., Mc Coy S.D., Launchbaugh K.L., Fraker M.J., Powel J., 2002. Calibrating fecal NIRS equations for predicting botanical composition of diets. J. Range Manag. 55, 374-382.

Chapter III: Near infrared reflectance spectroscopy applied to faeces in order to characterize forage intake: spectral libraries and calibrations

Article II: Evaluation of green forage intake and digestibility in ruminants using near infrared reflectance spectroscopy (NIRS): developing a global calibration

Article III: Faecal Near infrared reflectance spectroscopy for ruminant feed intake prediction

Article IV: Near infrared spectroscopy applied to faeces to predict botanical composition of sheep intake

Article V: Prediction error and repeatability of near infrared reflectance spectroscopy applied on faeces samples to predict voluntary intake and digestibility of forages by ruminants

Near infrared reflectance spectroscopy applied to faeces in order to characterize forage intake: spectral libraries and calibrations

Near infrared reflectance spectroscopy (NIRS) is physical method of analysis that links the characteristics of samples obtained by the reference method to the reflectance spectrum (light absorbance values at NIRS wavelengths). In order to estimate the 'feeding value' of a diet, the most analysed substrate currently is feed and the predicted parameters are usually chemical composition (e.g., crude protein, crude fibres), enzymatic digestibility and, sometimes, *in vivo* digestibility. It is generally not possible to predict the intake or composition of an ingested diet from feed analysis because of the complexity of these parameters. Using NIRS applied to faeces (FNIRS) in order to predict diet characteristics appears to be an interesting approach. Faeces contain undigested feed and microbial residues that can be detected by NIRS and related to the intake and feeding values of an ingested diet.

The Chapter III describes how the FNIRS spectral libraries are developed, which spectral treatments are applied and how diet-faeces pairs are generated. The prediction error and repeatability of FNIRS predictions are also evaluated.

Evaluation of green forage intake and digestibility in ruminants using near infrared reflectance spectroscopy (NIRS): developing a global calibration

Article II – Decruyenaere et al. (2009)
Animal Feed Science and Technology 2009 148, 138-156

Decruyenaere V. [(1)*], Lecomte Ph. [(2)], Demarquilly C. [(3)], Aufrere J. [(3)], Dardenne P.[(4)], Stilmant D.[(1)], Buldgen A.[(5)]

[(1)] Centre Wallon de Recherches Agronomiques (CRA-W) – Département Productions et Filières, 100 rue du Serpont, B-6800 Libramont-Chevigny, Belgium

[(2)] Centre de Coopération Internationale de Recherches Agronomiques pour le Développement – Pôle Elevage – CIRAD REUNION - Chemin Irat – Ligne Paradis, 97410 Saint-Pierre

[(3)] Institut National de Recherche Agronomique (INRA) – Centre de Clermont Ferrand / Theix, 63122 Saint Genès Champanelle, France

[(4)] Centre Wallon de Recherches Agronomiques (CRA-W) – Département Qualité des Productions Agricoles, 24 Chaussée de Namur, B-5030 Gembloux, Belgium

[(5)] Faculté Universitaire des Sciences Agronomiques de Gembloux (FUSAG) – Unité de Zootechnie, 2 passages des Déportés, B-5030 Gembloux, Belgium

*corresponding author – E-mail : v.decruyenaere@cra.wallonie.be

Abbreviations: BW, body weight; CEL, cellulose; CP, crude protein; DM, dry matter; H, standardized distance; ADL, acid detergent lignin; NIRS, near infrared reflectance spectroscopy; OM, organic matter; OMDcel, *in vitro* organic matter digestibility coefficient; OMD, *in vivo* organic matter digestibility coefficient; OMVI, organic matter voluntary intake; PCA, principal components analysis; R, reflexion; R^2, coefficient of determination; RPD, standard error of reference database / standard error of cross validation; SD, standard error of reference database; SE, standard error of regression; SEC, standard error of calibration; SECV, standard error of cross validation.

Abstract

The objective of this study was to evaluate the potential of near infrared reflectance spectroscopy (NIRS), applied to forage and/or faeces, to estimate the *in vivo* organic matter digestibility (OMD) and the organic matter voluntary intake (OMVI, g/kg metabolic weight [$BW^{0.75}$]) for a wide range of temperate forages. Two different databases, in terms of forage species and development stages were studied. The first one included two grass species and two forage mixtures for which OMD and OMVI were continuously measured during the grass-growing seasons (spring and summer). The second one contained a large set of grass and legume species and forage mixtures (142 trials) for which OMD and OMVI were measured.

Forage and faeces samples were submitted to NIRS analysis and predictive calibrations were developed from forage spectra, faeces spectra, forage and faeces subtracted spectra, and faeces and forage concatenated spectra. Working on faecal spectra (alone or concatenated) enabled to develop the best calibration equations for both OMD and OMVI estimations. The

coefficient of determination (R^2) was greater than 0.8. The standard error of cross validation (SECV) for OMD and OMVI was 0.021 and 4.51 g/kg $BW^{0.75}$, respectively, and the accuracy was similar to that obtained with other predictive methods. With regard to the faecal spectra (second derivative mode), the fat absorbency at wavelengths of 1730, 2310 and 2350 nm was higher when the corresponding forage was highly digestible and ingestible.

In conclusion, applying NIRS to faeces is a rapid and easy analytical method that could be an interesting tool for managing grazing ruminants and optimising their performance.

Keywords: NIRS, faecal spectra, grass quality, feeding management tool

I. Introduction

The performances of herbivores when grazing depend directly on forage digestibility and intake. Measuring these parameters for herd management at pasture can be difficult, costly, time consuming, labour intensive and not suitable over long periods. The digestibility of ingested grass is usually estimated by chemical analyses of samples collected in the field. This is based on determining the regression between forage parameters defined in laboratory and *in vivo* measurements. Common laboratory analysis methods include that described by Tilley and Terry (1963) and *in vitro* enzymatic digestibilities (Bartiaux Thill and Oger, 1986; De Boever et al., 1988; Aufrère and Demarquilly, 1989). With these methods, accuracy is generally good, with a residual error of prediction of 0.015- 0.030 units of digestibility (Peyraud, 1998). However, the accuracy of such regressions is a function of the method used to collect field samples that are as representative as possible of the ingested diet. To overcome this difficulty, ingested diet can be obtained by using oesophageal-fistulated animals (Ward et al., 1982; Holechek et al., 1982; Forbes and Beattie, 1987; Stuth et al., 1989), but this practice has an adverse effect on animal welfare. In addition, on heterogeneous pasture such sampling methods are not always representative of diets selected by animals. Coates et al. (1987) showed that diet legume percentages of extrusa collected from oesophageal-fistulated steers, non-resident on pasture, were poorly correlated with those ingested by non-fistulated steers resident on pasture (R^2=0.127). Jones and Lascano (1992) suggested that this difference could be linked to the sampling strategy of extrusa (difference between morning and afternoon extrusa) or to behavioural differences in diet selection between resident and non-resident cattle. For instance, fasted or satiated oesophageal-fistulated cattle introduced in pasture do not have the same diet selection. Other methods have been developed to estimate grass digestibility by measuring such chemical faecal parameters as nitrogen (Bartiaux-Thill et al., 1985; Peyraud, 1998) or indigestible fibre (Lippke et al., 1986; Sunvold and Cochran., 1991). According to Peyraud (1998), the nitrogen faecal index is fairly accurate for assessing digestibility, but the relationship between faecal nitrogen and digestibility are strongly linked to pasture characteristics in terms of botanical composition or localisation and therefore lacks a universal application (Holloway et al., 1981). In addition, on rangeland or with tropical forages, the chemical composition of faeces does not appear to be a good indicator of forage quality because of the diversity of forage species ingested and the occurrence of some anti-nutritional factors, such as tannins and phenolic compounds that precipitate protein and lead to higher nitrogen faecal concentration than that observed in the initial diet (Wofford et al., 1985). More recently, the alkane recovery rates in faeces have been used to assess the digestibility of grazed forage. Dove and Mayes (1991; 1996) and Sandberg et al. (2000) suggest that these methods, based on analysing the natural alkanes in the cuticular waxes of plants and the dosed alkanes, reflect more accurately the digestibility of temperate or tropical herbivore diets.

Voluntary intake quantification requires measuring both digestibility and faecal output obtained by total faeces collection (Holechek et al., 1986) or using indigestible markers such as chromium oxide (Bartiaux-Thill et al., 1988; Compère et al., 1992), and ytterbium (Brandiberry et al., 1991; Galyean, 1993, Mambrini and Peyraud, 1997). The *n*-alkanes method is also used to estimate individual voluntary intake. Mayes et al. (1986), Malossini et al. (1996) and Dove et al. (2000) found that a good estimation of intake could be obtained by using C_{32} as a dosed alkane and C_{33} as a herbage alkane, but, as for digestibility determination, these techniques are difficult to apply under grazing conditions. The main source of variation in these methods remains the collection of a representative herbage sample (Smit et al. 2005). For example, grazing animals can select some plant species or parts of

plants in which the *n*-alkane profiles differ from the averaged grass sampled in the field (Dove et al., 1996).

To address this problem, approaches based on near infrared reflectance spectroscopy (NIRS) applied to faeces and/or forage have been developed to analyse the diet quality of grazing animal intake (Stuth et al., 1989; Coleman et al., 1989; Coleman and Murray, 1993; Leite and Stuth, 1995; Lyons et al., 1995; Coates, 2000; Decruyenaere et al., 2002, Stuth et al., 2003). Lyons and Stuth (1992) found that monitoring forage diet quality and intake using NIRS scanning of faecal samples appeared promising. They demonstrated that grass *in vivo* digestibility can be estimated by NIRS applied to faeces with the same accuracy as that obtained with conventional analysis methods. If there are appropriate calibration equations, NIRS is a rapid and non-destructive technology that could predict the digestibility and intake of a large set of similar samples.

The aim of this study is to evaluate the potential of NIRS, applied to forage and/or faeces, for determining the *in vivo* organic matter digestibility (OMD) and the organic matter voluntary intake (OMVI) obtained from *in vivo* feeding trials as reference values.

II. Materials and methods

The potential of NIRS to estimate *in vivo* organic matter digestibility (OMD) and organic matter voluntary intake (OMVI, g/kg $BW^{0.75}$) of fresh grass was evaluated using an

Table 1
Digestibility and intake reference databases

	Nature	Year	N[a]	feeding level[b]	OMD range	OMVI range
CRA-W						
1	Rye grass 4n (Meltra)	1992	148	al	0.584 – 0.841	40.64 - 64.44
2	Rye grass 2n (Talbot)	1992	148	al	0.526 – 0.822	44.98 - 65.65
3	Mixed forage without clover	1993	90	al	0.570 – 0.763	47.81 - 76.40
4	Mixed forage with clover	1993	90	al	0.550 – 0.775	41.38 - 73.80
5	Mixed forage without clover	1993	104	150 M	0.545 – 0.841	39.92 - 55.72
6	Mixed forage with clover	1993	104	150 M	0.542 – 0.849	43.62 - 54.21
7	Mixed forage without clover	1993	208	M	0.601 – 0.842	28.93 - 38.80
			892			
INRA						
1	Natural pastures		43	al	0.583 – 0.760	47.08 - 70.68
2	Cocksfoot		34	al	0.535 – 0.744	37.43 - 74.65
3	Tall fescue		9	al	0.570 – 0.742	50.37 - 70.63
4	Timothy		5	al	0.677 – 0.778	57.41 - 77.94
5	Rye grass		42	al	0.566 – 0.815	47.10 - 93.54
6	Lucerne		4	al	0.599 – 0.763	63.29 - 74.20
7	Red clover		5	al	0.625 – 0.802	56.55 - 87.74
			142			

OMD: *in vivo* organic matter digestibility; OMVI (g/kg $BW^{0.75}$): organic matter voluntary intake.
[a] N = number of forage and faeces samples based on 6-day moving averages calculated for the CRA-W trials, number of forage samples, as mean of the trial, and faeces samples, as mean of 6 sheep over the trial, for the INRA trials.
[b] feeding level: M: maintenance = 23 g OM digestible/kg $BW^{0.75}$; 150 M: 1.5 * maintenance; al: *ad libitum*.

important *in vivo* database obtained from feeding trials performed at Libramont (49°58’ N, 5°38’E, 440 m above sea level) in the Farming Systems Section of the Walloon Agricultural Research Centre (CRA-W) in Belgium, and at Clermont-Ferrand in the experimental farm of Theix (45°43’ N – 3°01’E, 890 m above sea level) of the National Institute of Agricultural Research (INRA) in France. The CRA-W database held data from digestibility trials carried

out in 1992 (CRA-W 1-2) and 1993 (CRA-W 3 to 7) during the plant vegetative growth phase. The INRA database held data from 142 digestibility trials conducted in the early 1980s (Table 1).

II.1. Forage and animal management

II.1.1. CRA-W trials

Seven trials were conducted as a continuous measurement of digestibility and intake during the main grass-growth seasons (spring, summer). The trials lasted 20–60 days in order to cover the widest range of digestibility and intake variations.

The forage tested came from temporary pastures sown in 1990 (CRA-W 1-2) and 1992 (CRA-W 3 to 7) and harvested in 1992 (CRA-W 1-2) and 1993 (CRA-W 3 to 7), in their second or first year of production. The sampled grasslands for CRA-W 1-2 consisted of pure ryegrass (diploid and tetraploid), whereas CRA-W 3 to 7 consisted of two mixed swards. The composition of these mixed swards was determined by manual sorting and was, for the first sward, ryegrass (625 g/kg DM), timothy (250 g/kg DM) and white clover (125 g/kg DM) and, for the second sward, ryegrass (715 g/kg DM) and timothy (285 g/kg DM).

The fertilisation levels were 88 units of P_2O_5/ha and 176 units of K_2O/ha, with a nitrogen application rate of 80 units/ha after each cut.

To perform the digestibility trials, the forage was supplied fresh, at different feeding levels, to sheep (castrated males weighing 45–60 kg) confined in individual digestibility crates.

The feeding levels were calculated according to the OMD of grass. This parameter was estimated daily by NIRS on a microwave-dried grass sample (Biston et al., 1989). Between two and six sheep were individually fed at maintenance level (23 g digestible organic matter /kg $BW^{0.75}$), at 150% of maintenance level or at *ad libitum*.

In each trial the forage was cut daily at 08.00 h, chopped to a length of 4–5 cm, stored at 6°C and distributed to sheep the following day. Throughout the trial period the sheep were fed twice daily, at 09.00 h and 16.30 h, and had continuous access to water and salt licks. The daily forage supply, refusals and faeces were individually weighed and sampled.

II.1.2. INRA trials

A total of 142 averaged samples were obtained from short digestibility trials (each trial lasting 6 days). The forage consisted of a wide range of fresh grass, including natural pastures, pure gramineous species (ryegrass, timothy, cocksfoot, tall fescue) and pure legume species (red clover, lucerne) in their first, second or third growth cycle.

The forage was cut daily at 08.00 h, chopped to a length of 4–5 cm and provided fresh, at *ad libitum*, twice daily (09.00 h and 16.30 h) to six sheep (castrated males 45–60 kg) housed in individual digestibility crates. The forage, refusals and faeces were individually weighed and sampled each day.

II.2. Sample management and calculation of reference values

All the forage, refusals and faeces samples collected in the CRA-W and INRA trials were oven dried (65°C for 36 h), roughly ground in a hammer mill and then ground again in a Cyclotec mill with a 1 mm screen.

The forage samples from INRA were bulked over the trial period and individual faecal samples were bulked daily for the six sheep in the trial to provide averaged samples. Ground samples of forage, refusals and faeces were stored in hermetically sealed plastic boxes until NIRS analysis.

For both the INRA and CRA-W trials, the OMD was calculated according to Demarquilly et al. (1995), whereas the OMVI was calculated according to the difference between organic matter supplied and organic matter refused.

To determine the organic matter content of supplied and refused forage and faeces, ash (g/kg DM) was estimated using NIRS (Table 2). Other chemical characteristics of forage, such as crude protein (CP, g/kg DM), cellulose (CEL, g/kg DM according to the Weende method, NF V 03-040 (1977)) and *in vitro* organic matter digestibility (OMDcel as described by De Boever et al. (1988)), and faeces, such as CP (g/kg DM) and CEL (g/kg DM), were also estimated using NIRS based on calibrations previously developed at CRA-W (Table 2).

Table 2.
NIRS calibrations to estimate the chemical composition of forage and faecal samples

Parameters	*N*	Mean	SD	SEC	R^2	SECV
Forage						
Ash (g/kg DM)	2468	96.9	25.7	9.7	0.86	9.9
CP (g/kg DM)	2765	147.7	59.0	8.6	0.98	8.6
CEL (g/kg DM)	2494	266.6	54.1	13.3	0.94	13.5
OMDcel	1598	0.771	0.102	0.022	0.95	0.022
Faeces						
Ash (g/kg DM)	115	196.0	91.0	9.9	0.99	11.5
CP (g/kg DM)	78	166.0	22.9	7.9	0.88	10.3
CEL (g/kg DM)	57	147.6	23.9	6.6	0.92	9.8

SD: standard deviation of the reference database; R^2: coefficient of determination of NIRS equations; SEC: standard error of calibration; SECV = standard error of cross validation; DM: dry matter; OMDcel: *in vitro* organic matter digestibility as described by De Boever et al. (1988); CP: crude protein; CEL: cellulose.

II.3. NIRS measurements, spectral treatments and calibrations

Faecal and forage samples from the CRA-W and INRA trials were submitted to NIRS scanning (NIRS system monochromator 5000 - 1100 to 2498 nm of wavelength by 2 nm steps) at the Farming Systems Section in 1989 (INRA), 1992 (CRA-W 1-2) and 1993 (CRA-W 3 to 7). The absorbency data were expressed as log 1/R.

For the CRA-W trials, in order to compare CRA-W and INRA databases, a moving average over 6 days was calculated for the reference values and the corresponding forage and faecal daily spectra, wavelength by wavelength. Thus, the day *i* (d_i) value equalled the 6-days mean from d_{i-2} to d_{i+3}.

For the whole database (CRA-W and INRA), a concatenation of faeces and forage spectra was made to extend the spectral information. This involved juxtaposing faeces and forage spectra by merging data in the same file that doubled the number of absorbency values. Similarly, the differences between forage and faeces spectra were calculated. These differences were expected to give a better representation of the digestive utilisation of the forage.

The NIRS calibrations were developed to estimate the OMD and OMVI (g/kg $BW^{0.75}$) from faeces spectra, forage spectra, concatenated spectra and spectra obtained by calculating the differences. The NIRS models were set up with a modified partial least square (PLS)

procedure with cross validation in WINISI® 1.50 software (Naes et al., 2002). For each parameter tested, 64 calibration equations varying in terms of derivative, gap, smooth and scatter correction were performed. The best predictive model was obtained using the second derivative mode spectrum with scatter correction using standard normal variate and detrend (SNV-D). The population boundaries for calibration were set with a maximum standardized *H* (distance between a sample and the centroid of the group) value of 3.0 (Shenk and Westerhaus, 1991).

To identify the wavelengths highly correlated to OMD and OMVI, the CRA-W and INRA databases were merged, sorted initially by ascending OMD and then by ascending OMVI. These databases were then divided into four equal groups per parameter. Each group was averaged to provide one NIRS spectrum associated with the corresponding reference values. The four averaged spectra corresponding to the ascending OMD or OMVI were visually compared using the Plot Spectra and Score procedure in WINISI® 1.50 software.

II.4. Statistical analysis

The performance of the NIRS calibration equations was expressed in terms of coefficient of determination (R^2), standard error of calibration (SEC) and standard error of cross validation (SECV). The RPD calculated as the ratio of the standard deviation of the original data to the standard error of cross validation (Williams, 2004), was also used to evaluate calibration performance.

In order to estimate the OMD and OMVI, a multiple regression analysis was performed (GLM procedure – Statistica 1999). The independent variables were the CP (g/kg DM) and CEL (g/kg DM) content of forage and faeces.

The number of days elapsed since 1 January was tested only for the CRA-W databases to evaluate the OMD and OMVI evolution during the vegetative growth period.

III. Results

III.1. Forage characteristics

The CRA-W forages tested in 1992 (CRA-W 1-2) were quite different from that tested in 1993 (CRA-W 3 to 7) and from the INRA forages, especially for CP and ash which were lower for 1992 forages (CP = 71.2 *vs* 132.5 and 149.6 g/kg DM and ashes = 80.7 *vs* 104.8 and 107.4 g/kg DM, in average, respectively, for the CRA-W 1-2, CRA-W 3 to 7 and INRA databases). The CEL content was similar over years and between databases, whereas the OMDcel was higher for forages tested in 1992 (Table 3).

Table 3.
Average and range of the chemical composition and enzymatic *in vitro* digestibility of CRA-W and INRA forage, estimated by NIRS

	CRA-W 1 - 2	CRA-W 3 – 7	INRA
OMDcel	0.780 (0.647–0.923)	0.746 (0.642–0.860)	0.721 (0.528–0.873)
CP (g/kg DM)	71.2 (44.8–107.8)	132.5 (102.1–185.9)	149.6 (62.0–242.0)
CEL (g/kg DM)	259.1 (181.3–327.6)	286.7 (230.0–338.7)	266.7 (168.0–355.0)
Ash (g/kg DM)	80.7 (73.5–90.7)	104.8 (98.3–118.3)	107.4 (75.3–142.7)

DM: dry matter; OMDcel: *in vitro* organic matter digestibility as described by De Boever et al. (1988); CP: crude protein; CEL: cellulose

With regard to plant growth, the OMD from the CRA-W trials decreased linearly from the first week of May until last week of June (OMD = -0.00454 x day since 1 January + 1.41 R^2 = 0.79; SE = 0.0257). Thus, in order to maintain the defined level of intake, the OMVI increased throughout the measurement period from 29.4 to 35.6 g/kg $BW^{0.75}$ for sheep fed at maintenance level and from 41.9 to 52.0 g/kg $BW^{0.75}$ for sheep fed at 150% of maintenance level. For sheep fed *ad libitum*, the OMVI decreased linearly from 65.3 at the beginning of the trial period to 46.6 g/kg $BW^{0.75}$ at the end of this period (OMVI = -0.5556 x day since 1 January + 143.14; R^2 = 0.66; SE = 4.17 g/kg $BW^{0.75}$).

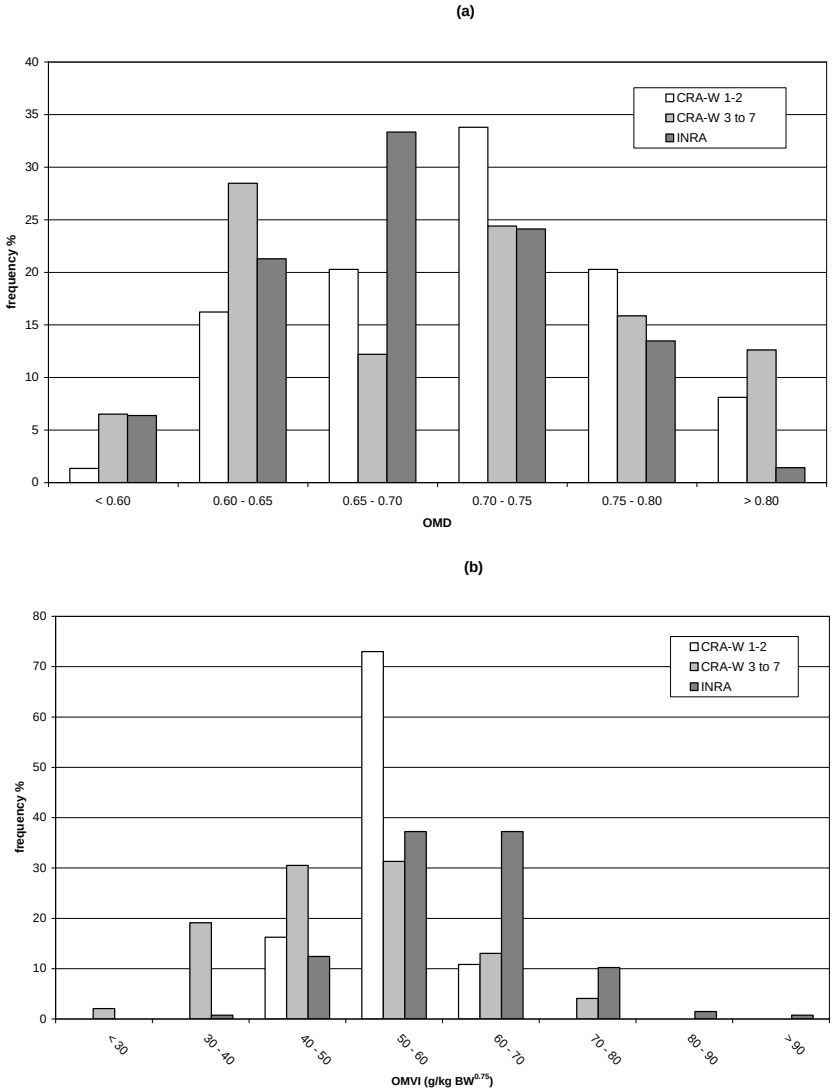

Fig.1. Frequency distribution of forage OMD (a) and OMVI (b) for INRA and CRA-W databases

For both databases, more than 65% of the OMD reference values were between 0.6 and 0.75 (CRA-W 1-2: 70%; CRA-W 3-7: 65%; INRA: 78%) (Fig. 1a). More than 70% of the INRA OMVI values were between 50 and 70 g/kg $BW^{0.75}$ whereas 60% of the CRA-W 3 to 7 OMVI values were between 40 and 60 g/kg $BW^{0.75}$. Again, CRA-W 1-2 differed, with more than 70% of the population in only one class of intake (Fig. 1b).

III.2. Estimation of OMD and OMVI from the chemical characteristics of forage and faeces

Table 4 shows the correlations between the CP and CEL content of forage or faeces and the OMD and OMVI. For the entire CRA-W database, the OMD averaged across sheep was well correlated to the CP and CEL content of forage or faeces (R^2 = 0.76; SE = 0.027 and R^2 = 0.78; SE = 0.026). It seemed impossible to estimate the OMVI respectively from forages or faeces chemical characteristics (R^2 = 0.06; SE = 10.42 and R^2 = 0.25; SE = 9.37 g/kg $BW^{0.75}$).

Table 4.
Relationship between OMD or OMVI and forage or faeces CP and CEL

Database	Parameter	Relation	R^2	N	SE	*F*	*P*
CRA-W	OMD	0.916+0.0081 Cpforage – 0.0099 CELforage	0.76	236	0.0272	(2,233) 380.1	< 0.001
	OMVI	72.18 – 0.72 CPforage – 0.45 CELforage	0.06	236	10.42	(2,233) 9.3	< 0.001
	OMD	1.294 – 0.0137 CPfaeces – 0.0169 CELfaeces	0.78	236	0.0265	(2,233) 409.5	< 0.001
	OMVI	-129.06+6.72 CPfaeces+3.95 CELfaeces	0.25	236	9.37	(2,233) 39.0	< 0.001
INRA	OMD	0.952+0.0010 CPforage – 0.0105 CELforage	0.65	141	0.0344	(2,138) 131.7	< 0.001
	OMVI	59.77+0.98 CPforage – 0.51 CELforage	0.34	137	7.18	(2,134) 35.9	< 0.001
	OMD	0.608+0.0118 CPfaeces – 0.0052 CELfaeces	0.81	141	0.0253	(2,138) 300.9	< 0.001
	OMVI	16.74+2.42 CPfaeces+0.47 CELfaeces	0.31	137	7.33	(2,134) 31.7	< 0.001

OMD: *in vivo* organic matter digestibility; OMVI (g/kg $BW^{0.75}$): organic matter voluntary intake; CP (g/kg DM): crude protein; CEL (g/kg DM) = cellulose

The same results were observed for the INRA trials. The OMD was well correlated with the grass CP and CEL (R^2 = 0.65; SE = 0.034). Similarly, the grass CP and CEL explained only 34% of the OMVI variability (R^2 = 0.34; SE = 7.18 g/kg $BW^{0.75}$). The relationship between OMD or OMVI and faeces composition was very highly significant (R^2 = 0.81; SE = 0.0253 and R^2 = 0.31; SE = 7.33 g/kg $BW^{0.75}$, respectively, for OMD and OMVI) but, again, only the OMD could be estimated with sufficient accuracy.

III.3. NIRS calibrations to estimate OMD and OMVI

For each database viewed separately (Table 5), the estimations of OMD by NIRS applied to forage or faeces were relatively good. The R^2 were greater than 0.85 and SECV ranged between 0.020 and 0.018 for CRA-W forage or faeces calibration. For the INRA forage, the NIRS models developed from forage or faeces spectra showed similar accuracy (SECV = 0.021). For both the CRA-W and INRA databases, the best NIRS model for estimating OMD was obtained with concatenated spectra (R^2 = 0.95; SECV = 0.016 and R^2 = 0.91; SECV = 0.019, respectively, for CRA-W and INRA). Working with subtracted spectra led to less accurate NIRS models only for the INRA database (R^2 = 0.93; SECV = 0.019 and R^2 = 0.83; SECV = 0.029, respectively, for CRA-W and INRA).

With the CRA-W forage samples, it was not possible to develop a robust calibration to measure OMVI (R^2 = 0.23; SECV = 7.00 g/kg $BW^{0.75}$). The OMVI estimations from faecal

NIRS calibration were more efficient (R^2 = 0.89; SECV = 3.58 g/kg $BW^{0.75}$). The NIRS equation statistics obtained with the INRA forage database to estimate OMVI (R^2 = 0.52, SECV = 6.24 g/kg $BW^{0.75}$) showed performances similar to those obtained from faecal spectra (R^2 = 0.66, SECV = 6.05 g/kg $BW^{0.75}$). The use of subtraction between forage and faeces spectra did not improve the NIRS equation statistics for the OMVI, as illustrated by the higher SECV values. Similarly, developing an NIRS equation with the concatenated spectra did not really improve the accuracy of the model (Table 5).

Table 5.
NIRS calibration performance in relation to the nature of the spectra (forage or faeces) and the origin of samples (INRA or CRA-W databases)

NIRS spectra	Parameters	*N*	Mean value	SD	SEC	R^2	SECV	RPD
CRA-W database								
Forage	OMD	190	0.733	0.0541	0.0197	0.87	0.0198	2.73
	OMVI	180	53.70	7.17	6.27	0.23	7.00	1.03
Faeces	OMD	886	0.707	0.0711	0.0171	0.94	0.0177	4.03
	OMVI	884	49.01	10.39	3.46	0.89	3.58	2.90
Subtracted	OMD	887	0.707	0.0713	0.0185	0.93	0.0191	3.73
	OMVI	887	49.09	10.47	3.82	0.87	3.95	2.65
Concatenated	OMD	817	0.714	0.0708	0.0154	0.95	0.0159	4.45
	OMVI	806	49.78	9.97	3.54	0.87	3.60	2.77
INRA database								
Forage	OMD	138	0.685	0.058	0.0186	0.90	0.0214	2.70
	OMVI	132	60.29	8.01	5.56	0.52	6.24	1.28
Faeces	OMD	140	0.687	0.0575	0.0195	0.88	0.0213	2.70
	OMVI	137	60.68	8.83	5.15	0.66	6.05	1.46
Subtracted	OMD	140	0.687	0.0575	0.024	0.83	0.0290	1.98
	OMVI	136	60.68	8.87	5.09	0.67	6.34	1.40
Concatenated	OMD	140	0.687	0.0575	0.0172	0.91	0.0191	3.00
	OMVI	137	60.68	8.83	4.96	0.68	6.15	1.44

OMD: *in vivo* organic matter digestibility; OMVI (g/kg $BW^{0.75}$): organic matter voluntary intake; R^2: coefficient of determination of NIRS equations; SEC: standard error of calibration; SECV: standard error of cross validation; SD: standard deviation of the reference database; RPD: SD/SECV.

As mentioned earlier, the databases studied differed in terms of forage species and chemical composition. Fig.2 illustrates spectral variability of the databases according to the first two axes of a PCA analysis of the faecal spectra. Ryegrass diploid and tetraploid cut in 1992 (CRA-W 1-2) had lower CP and ash content and higher OMDcel, and on a faecal basis they were completely different from the CRA-W 3-7 and INRA databases. This observation was confirmed by standardised *H* distances between the databases (Table 6). CRA-W 1-2 differed considerably from INRA (*H*=24.73) and from CRA-W 3-7 (*H*=22.33). The INRA database was far more variable than CRA-W 3-7 (*H*=14.35). In contrast, CRA W 3-7 appeared to be well integrated into the INRA database, with *H* lower than 3 (*H*=1.91).

Table 6.
Standardised H distance between the INRA, CRA-W 1-2 and CRA-W 3 to 7 databases, on a faecal spectra basis

Spectra files / PCA files	INRA	CRA-W 1-2	CRA-W 3 to 7
INRA	–	49.80	14.35
CRA-W 1–2	24.73	–	22.33
CRA-W 3–7	1.91	17.59	–

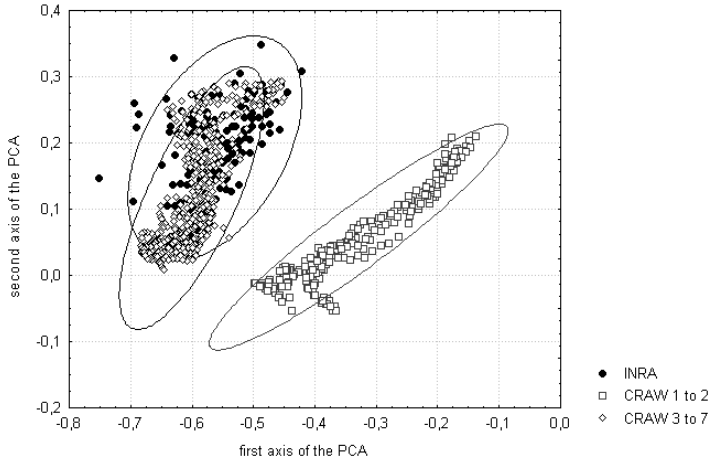

Fig. 2. Spectral variability of the databases in relation to the first two axes of a PCA analysis of NIRS faecal spectra

These results suggested it was not possible to estimate the OMD or OMVI of INRA faecal samples with CRA-W faecal NIRS equations. In order to increase the variability, the databases were merged to develop new global NIRS calibrations (Table 7). The global calibration equations developed using faeces spectra had an intermediate accuracy (SECV = 0.021 and 4.53 g/kg $BW^{0.75}$, respectively, for OMD and OMVI) compared with the calibrations obtained with the individual databases (Table 5).

Table 7.
NIRS calibration results from the merged database (INRA and CRA-W)

NIRS spectra	Parameters	*N*	Mean value	SD	SEC	R^2	SECV	RPD
Forage	OMD	328	0.713	0.0606	0.0226	0.86	0.0231	2.62
	OMVI	323	57.06	8.73	7.29	0.30	7.47	1.16
Faeces	OMD	951	0.710	0.0698	0.0200	0.92	0.0207	3.35
	OMVI	936	51.27	10.46	4.28	0.83	4.53	2.31
Subtracted	OMD	943	0.710	0.0694	0.0210	0.90	0.0224	3.10
	OMVI	925	51.21	10.43	4.13	0.84	4.40	2.37
Concatenated	OMD	953	0.709	0.0701	0.0174	0.94	0.0185	3.77
	OMVI	942	51.35	10.42	3.85	0.86	4.13	2.52

OMD: *in vivo* organic matter digestibility; OMVI (g/kg $BW^{0.75}$): organic matter voluntary intake; SEC: standard error of calibration; R^2: coefficient of determination of NIRS equations; SECV: standard error of cross validation; SD: standard deviation of the reference database; RPD: SD/SECV.

However, the accuracy of the faecal NIRS models was higher than that observed for regressions developed on the basis of the CP and CEL forage or faeces content, as reported in Table 4. As observed for the separate databases, it was not possible to estimate the OMVI from forage NIRS analysis (R^2 = 0.30; SECV = 7.74 g/kg $BW^{0.75}$). Working with concatenated spectra improved the calibration performances slightly for both OMD and OMVI. Compared with the other faecal NIRS equations, OMD estimation from forage-faeces subtracted spectra appeared less accurate as illustrated by higher SECV value.

III.4. Relevant NIR wavelengths

The visual comparison of the four averaged faecal spectra (second derivative mode) indicated a strong absorption in the wavelength region characteristics of fat (λ=1730; λ=1764; λ=2310;

λ=2350), fibres (λ=2078 to 2110; λ=2268) and protein (λ=2058; λ=2166), as defined by Bertrand (2002). For fat and protein wavelengths, the NIRS absorbencies decreased with OMD values, as illustrated in Fig. 3. Conversely, the absorbencies were higher in the region of fibres for faecal samples obtained after the intake of low digestibility forage. Wavelengths characteristic of fat content were very important for quantifying forage OMVI on the basis of the corresponding faecal spectra. The CP and CEL wavelengths did not appear to be so relevant.

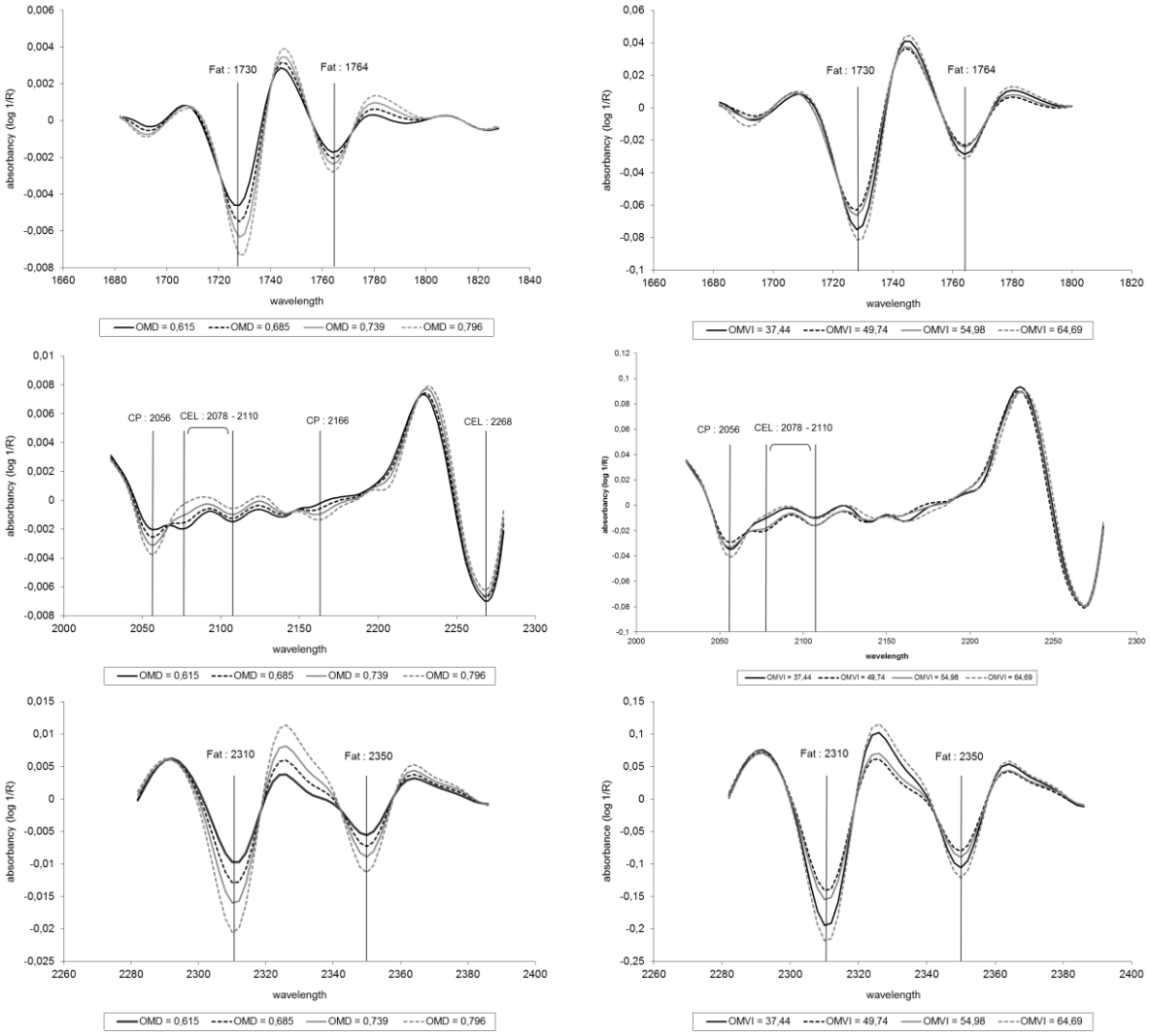

Fig. 3. Second derivative mode of the faecal spectral region in relation to four ascending OMD and OMVI (g/kg $BW^{0.75}$) values

IV. Discussion

The efficiency of NIRS equations can be evaluated using various statistical parameters, such as R^2, SECV and the RPD ratio. To be acceptable, the NIRS equations must have an R^2 higher than 0.80, a SECV close to the SEC and an RPD ratio higher than 3 (Williams, 2004).

Calibration equations developed to estimate OMD gave an excellent R^2 (R^2 > 0.90) and appeared sufficiently robust, with an RPD higher than 3 for all faecal databases (faeces spectra alone: RPD = 3.35; subtracted spectra: RPD = 3.10; concatenated spectra; RPD = 3.77). With the SECV varying from 0.021 to 0.018 for faecal and concatenated

databases, respectively, the faecal NIRS appeared to be a good tool to estimate the OMD of temperate forage. The efficiency of NIRS applied to forage or faeces to assess diet quality had also been confirmed by earlier studies.

Based on forage analysis, De Boever et al. (1996) reported that the *in vivo* OMD of 36 grass silages had a better correlation with NIR-estimated OMD (r = 0.89) than with *in vitro* enzymatic OMD (r = 0.83), rumen fluid OMD (r = 0.81) or ADL content (r = -0.73). Similarly, De La Roza et al. (2000) reported that the correlation between *in vivo* OMD and *in vitro* OMD was poor (R^2 = 0.51 and SE = 0.050) compared with NIRS performance in quantifying the *in vivo* OMD (R^2=0.86 and SECV=0.028). They concluded that NIRS spectra provided more information than *in vitro* enzymatic digestibility.

Norris et al. (1976) and Lippke et al. (1989) also showed that digestibility could be quantified from the NIRS analyses of forage harvested in the field or obtained from oesophageal fistula, with the SEC varying between 0.032 and 0.036. Compared with these results, the NIR model developed in the merged forage database (CRA-W and INRA) gave a better SEC (0.023). The use of faecal NIRS to estimate OMD tended to improve the performances of the models, as illustrated by our results and some earlier studies. Coleman et al. (1989) confirmed that faecal NIRS equations obtained from a wide range of forage species or forage mixtures to assess dry matter digestibility were precise enough to manage the nutrition of grazing herds. Similarly, Stuth et al. (1989) showed that the NIRS analyses of faeces could estimate the OMD of rangeland grazing ruminant diets with better accuracy than calibration equations developed from oesophageal extrusa (SEC = 0.033 and 0.051 for NIRS equations developed from faeces and oesophageal extrusa, respectively).

The accuracy of faecal NIRS models in estimating OMD was similar to or better than that obtained using other predictive methods, as reported by our results and several other studies. For instance, the faecal nitrogen index (faecal N) commonly used in linear, quadratic or hyperbolic functions could estimate the OMD of grazed temperate or tropical forage with a similar accuracy (Greenhalgh and Corbett, 1960; Bartiaux-Thill and Oger, 1986; Comeron and Peyraud, 1993; Boval et al., 1996; Bouazizi and Majdoub, 1999). Although the *n*-alkanes ratio appeared to be one of the best methods for estimating OMD at pasture, the results obtained using these techniques could be highly variable due to the lack of precision of the analytical procedure and to the partial digestibility of some *n*-alkane chains (Sandberg et al., 2000, Moshtaghi Nia and Wittenberg, 2002).

It was not possible to estimate the OMVI from the NIRS measurements of forage with sufficient accuracy (R^2 = 0.30, SEC = 7.29 g/kg $BW^{0.75}$; SECV = 7.47 g/kg $BW^{0.75}$). Comparable results were obtained by Norris et al. (1976). Minson et al. (1983) reported that voluntary dry matter intake could be measured by NIRS analyses of forage samples, with the SEC between 7 and 9 of g/kg $BW^{0.75}$. Ward et al. (1982) estimated the OMVI with similar accuracy (SEC = 9.6 g/kg $BW^{0.75}$). Working on faecal spectra (alone, subtracted or concatenated) improved the statistics of the NIRS models. The faecal calibration equations developed to analyse the OMVI had an R^2 between 0.80 and 0.90, and an SECV lower than 5 g/kg $BW^{0.75}$, and led to RPD values between 2.31 and 2.52.

The NIRS equations developed from concatenated databases seemed more suitable for estimating the OMVI (SEC = 4.13 g/kg $BW^{0.75}$). Stuth et al. (1989) estimated the dry matter intake with an accuracy of 17.3 g/kg $BW^{0.75}$. The calibration equations developed in our study from faeces and from concatenated spectra were more accurate, with an SEC 3 times smaller. More recently, Boval et al. (2004) and Landau et al. (2004) confirmed the potential

of faecal NIRS to characterise the diet attributes of ruminants (cattle and goats) grazing tropical grasslands.

Compared with other methods, faecal NIRS appeared to be accurate enough for estimating the OMVI. For instance, the *n*-alkanes ratio technique was as accurate as faecal NIRS for estimating the intake of different ruminants, such as sheep, cattle and goats (Mayes and Dove, 2000). However, it was difficult to use it for long periods because *n*-alkane dosing needs to be regular.

One explanation of the relevance of faecal NIRS for estimating OMD and OMVI was that these parameters also depended on physiologic and metabolic parameters, such as the digestion rate in the rumen (Illius and Jessop, 1996), plant characteristics (Jung and Allen, 1995; Allen, 1996) and animal behaviour (Faverdin, 1999; Provenza et al., 2003). Linked to animals, these factors were difficult or impossible to quantify only by analysing forage samples (Coelho et al., 1988). Faeces reflect biological and chemical characteristics of the forage consumed by animals as well as the physiological status of the herbivore. This chemical composition can be detected by NIRS and successfully correlated to the OMD and OMVI. This could explain why the NIRS analysis of faeces was as efficient, or more efficient, than the NIRS analysis of forage for assessing diet characteristics.

In our study, on the second derivative spectra of faeces from forage with low digestibility and a low intake level, there were higher peaks in the wavelength region of fibres (2078 to 2110 nm, 2268 nm). As confirmed by Leite and Stuth (1995), this peak was higher in faeces when the supplied forage was old. Similarly, Coleman and Murray (1993) showed that faecal spectra were negatively correlated with digestibility at 2100 nm. This peak was characteristic of the OH and CO groups such as sugar, starch and cellulose (Bertrand, 2002). It could be explained by the accumulation of more fibre residues in faeces when digestibility decreased. The relevant wavelength regions related to the OMD and OMVI were also similar to those selected on the forage spectra by Norris et al. (1976) and Lippke and Barton (1988).

With regard to faeces spectra, the negative peak centred at 1730, 1764, 2310, 2350 nm could be associated with the presence of fat. Peaks in these spectral regions were higher when plant digestibility and plant intake were high (Fig. 3). This observation was confirmed by Leite and Stuth (1995) who showed, with goat faeces, the highest absorption at 2301 nm for high quality forage. One explanation of the relevance of fat peaks in faeces could be related to the presence of endogenous residues linked to microbial activity in the rumen. According to Lecomte (1995), microbial contamination of forage nylon bag residues (measurement of *in situ* degradability) could be successfully estimated using NIRS. On these samples the absorbency peaks appeared clearly at 1722 and 2306 nm, characteristic wavelengths of the O-H link, representative of fatty acid. Moreover, Lecomte et al. (1994) have shown that rumen microbes contained a high proportion of stearic acid (532 g/kg DM). The relevance of fat wavelength to estimate OMD and OMVI could be linked to higher microbial growth in the rumen in relation to the high forage quality, as well as to a higher proportion of microbes linked to the faecal forage residues. Similarly, when grass came to maturity, the balance between protein and energy nutrients available for the rumen micro-organisms became negative. This led to a decrease in the cellulolytic activities of rumen bacteria, a decrease in digestibility and finally a decrease in bacterial contamination of forage residues. With such unbalanced diets, the retention time of the forage in the rumen was longer and the level of intake was lower. Another explanation of the fat wavelengths relevance could be linked to the presence in faeces of cuticular wax with a plant origin, such as *n*-alkanes commonly used to estimate digestibility and intake (Coleman and Murray, 1993). Cortes et al. (2005)

reported that the total *n*-alkane concentration of ryegrass and tall fescue decreased during the plant growth. This could explain the importance of fat peaks in faecal spectra when young forage was consumed.

V. Conclusion

This work underline the high potentialities of NIRS applied to faeces or faeces and forage, in this case on concatenated spectra, to estimate grazed grass digestibility. The accuracy of the NIR model to estimate OMVI is similar to or better than the accuracy of the others methods of estimation. We suggest that the accuracy achieved is acceptable in view of the difficulty to obtain this dietary parameter.

However, NIRS analysis of faeces can provide estimates of both OMD and OMVI only if the database variability, used to develop the calibrations, is high enough to include the diversity of field conditions. Future work will involve the validation of the performances of the faecal calibrations on independent data sets, under diverse grazing management schemes and its mobilisation to develop decision support system aiming to improve grazing management.

VI. References

Allen, M.S., 1996. Physical constraints on voluntary intake of forages by ruminants. J. Anim. Sci. 74, 3063-3075

Aufrère, J. Demarquilly, C., 1989. Predicting organic matter digestibility of forage by two pepsin-cellulase methods. XVI International Grassland Congress, Nice, France, 877-878.

Barthiaux-Thill, N., Biston, R., Dardenne, P., François, E., Thewis, A., Gielen, M., Limbourg, P., 1985. Mesure de l'ingestion d'herbe par la vache laitière au pâturage ou au moyen de la méthode des index fécaux. Rev. Agric. 2, 317-325.

Bartiaux-Thill, N. Oger, R., 1986. The indirect estimation of the digestibility in cattle of herbage from Belgian permanent pasture. Grass and Forage Sci. 41, 269-272.

Bartiaux-Thill, N., Oger, R., François, E., Théwis, A., 1988. Courbes d'excrétion fécale de l'oxyde de chrome administré aux vaches laitières au pâturage. Définition d'un échantillonnage bi-quotidien des fèces. Reprod. Nutr. Dev. 28, 87-88.

Bertrand, D. 2002. La spectrométrie dans le proche infrarouge et ses applications dans les industries de l'alimentation animale. INRA Prod. Anim. 15, 209-219.

Biston, R., Dardenne, P., Demarquilly, C., 1989. Determination of forage *in vivo* digestibility by NIRS. XVI International Grassland Congress, Nice, France, 895-896.

Bouazizi, A., Majdoub, A., 1999. Prédiction des quantités ingérées et de la digestibilité du régime sélectionné par des ovins sur parcours semi-aride tunisien. Fourrages 157, 77-87.

Boval, M., Peyraud, J.L., Xande, A., Aumont, G., Coppry, O., Saminadin, G., 1996. Evaluation d'indicateurs fécaux pour prédire la digestibilité et les quantités ingérées de Dichanthium sp par des bovins créoles. Ann. Zootech. 45, 121-134.

Boval, M., Coates, D.B., Lecomte, P., Decruyenaere, V., Archimède, H., 2004. Faecal Near Infrared Reflectance Spectroscopy (NIRS) to assess chemical composition,

in vivo digestibility and intake of tropical grass by Creole cattle. Anim. Feed Sci. Technol., 114, 19-29.

Brandyberry, S.D., Cochran, R.C., Vanzant, E.S., Harmon, D.L., 1991. Technical note : effectiveness of different methods of continuous marker administration for estimating fecal output. J. Anim. Sci. 69, 4611-4616.

Coates, D.B., Schachenmann, P., Jones, R.J., 1987. Reliability of extrusa samples collected from steers fistulated at the oesophagus to estimate the diet of resident animals in grazing experiments. Aust. J. Exp. Agric. 27, 739-745.

Coates, D.B., 2000. Faecal NIRS - What does it offer today's grazier? Tropical Grasslands 34, 230-239.

Coelho, M., Hembry, F.G., Barton, F.E., Saxton, A.M., 1988. A comparison of microbial, enzymatic, chemical and near-infrared reflectance spectroscopy methods in forage evaluation. Anim. Feed Sci. Technol. 20, 219-231.

Coleman, S.W., Stuth, J.W., Holloway, J.W., 1989. Monitoring the nutrition of grazing cattle with near-infrared analysis of faeces. XVI International Grassland Congress, Nice, France, 881-882.

Coleman, S.W., Murray, I., 1993. The use of near-infrared reflectance spectroscopy to define nutrient digestion of hay by cattle. Anim. Feed Sci. Technol. 44, 237-249.

Comeron, E.A., Peyraud, J.L., 1993. Prediction of herbage digestibility ingested by dairy cows. Rev. Argent. Prod. Anim. 13, 23-30.

Compère, R., Valdivia, J., Hellemans, P., Guns, M., Vandenbyvang, P., 1992. Comparaison de deux méthodes utilisant le Cr2O3 pour estimer la quantité de fèces émises par les moutons : pellets de luzerne et bolus CAPTEC-CRD. Bull. Rech. Agron. Gembloux 27, 365-383.

Cortes, C., Damanesco, J.C., Bechet, G., Prache, S., 2005. Species composition of ryegrass (Lolium perenne) and tall fescue (Festuca arundinacea) mixtures using various combinations of n-alkanes. Grass and Forage Sci. 60, 254-261.

De Boever, J.L., Cottyn, B.G., Andries, J.I., Buysse, F.X., Vanacker, J.M., 1988. The use of pepsin cellulase technique to predict digestibility metabolizable and net energy of forages. Anim. Feed. Sci. Technol. 19, 247-260.

De Boever, J.L., Cottyn, B.G., De Brabander, D.L., Vanacker, J.M., Boucqué, Ch.V., 1996. Prediction of the feeding value of grass silages by chemical parameters, in vitro digestibility and near-infrared reflectance spectroscopy. Anim. Feed. Sci. Technol. 60, 103-116.

De La Roza, B., Martinez, A., Modrono, S., Flores, G., Argamenteria, A, 2000. A comparison of near infrared spectroscopy with neutral detergent cellulase techniques to predict the *in vivo* digestibility of grass silage. In A.M.C. Davies and R. Giangiacomo (Eds), Proceedings of the 9th International conference - Verona Italy, pp.661-665.

Decruyenaere, V., Stilmant, D., Lecomte, P., Buldgen, A., Dardenne, P., 2002. Improvement and validation of the NIRS analysis applied to faeces to measure grass intake in pasture. In J.L. Durand, J.C. Emile, Ch. Huygue and G. Lemaire (Eds.), Multi-function grasslands. Quality forages, animal products and landscapes. Grassl. Sci. Eur. 7, pp.196-197.

Demarquilly, C., Chenost, M., Giger, S., 1995. Pertes fécales et digestibilités des aliments et des rations. In Jarrigue, R, Ruckenbusch, Y., Demarquilly, C., Farce, M-H., Journet, M., (Eds), Nutrition des ruminants domestiques, ingestion et digestion. INRA, France, pp.601-647.

Dove, H., Mayes, R.W., 1991. The use of plant wax alkanes as marker substance in studies of nutrition herbivores: a review. Aust. J. Agric. Res. 42, 913-952.

Dove, H., Mayes, R.W., 1996. Plant wax components: a new approach to estimating intake and diet composition in herbivores. J. Nutr. 126, 13-26.

Dove, H., Mayes, R.W., Freer, M., 1996. Effects of species, plant part and plant age on the n-alkanes concentrations in the cuticular wax of pasture plants. Aust. J. Agric. Res. 47, 1333-1347.

Dove, H., Freer, M., Foot, J.Z., 2000. The nutrition of grazing ewes during pregnancy and lactation: a comparison of n-alkane-based and chromium/in vitro-based estimates of herbage intake Aust. J. Agric. Res. 51, 765-777.

Faverdin, P., 1999. The effect of nutrients on feed intake in ruminants. Proc. Nutr. Soc. 58, 523-531.

Forbes, T.D.A., Beattie, M.M., 1987. Comparative studies of ingestive behaviour and diet composition in oesophageal-fistulated and non-fistulated cows and sheep. Grass Forage Sci. 42, 79-84.

Galyean, M.L., 1993. Technical note: an algebraic method for calculating fecal output from a pulse dose of an external marker. J. Anim. Sci. 71, 3466-3469.

Greenhalgh, J.F.D., Corbett, J.L., 1960. The indirect estimation of digestibility of pasture herbage. 1. Nitrogen and chromogen as faecal index substance. J. Agric. Sci., Camb. 55, 371-376.

Holechek, J.L., Shenk, J.S., Vavra, M., Arthun, D., 1982. Prediction of forage quality using near-infrared reflectance spectroscopy on oesophageal fisstula samples from cattle on mountain range. J. Anim. Sci 55, 971-975.

Holechek, J.L., Wofford, H., Arthun, D., Galyean, M.L., Wallace, J.D., 1986. Evaluation of total fecal collection for measuring cattle forage intake. J. Range Manage. 39, 2-4.

Holloway, J.W., Estell, II R.E., Butts, W.T. Jr, 1981. Relationships between fecal components and forage consumption and digestibility. J. Animal Sci. 52, 836-848.

Illius, A.W., Jessop, N.S., 1996. Metabolic constraints on voluntary intake in ruminants. J. Anim. Sci. 74, 3052-3062.

Jones, R.J., Lascano, C.E., 1992. Oesophageal fistulated cattle can give unreliable estimates of the proportion of legume in the diets of resident animals grazing tropical pastures. Grass Forage Sci. 47, 128-132.

Jung, H.G., Allen, M.S., 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J. Amin. Sci. 73, 2774-2790.

Landau, S., Glasser, T., Dvash, L., Perevolotsky, A., 2004. Faecal NIRS to monitor diet of mediterranean goats. South African J. Anim. Sci. 34, (suppl 1), 76-80.

Lecomte, Ph., Kamoun, M., Agneessens, R., Beckers, Y., Dardenne, P., François, E., 1994. Approach on the use of near infrared spectrometry for the estimation of microbial nitrogen contamination of forages incubated in nylon bag. Proc. Soc. Nutr. Physiol. 3, p44.

Lecomte, Ph., 1995. Gestion intégrée des ressources fourragères naturelles et de l'élevage dans la savane guinéenne de basse altitude. PhD Thesis, FUSAGx, 317 p.

Leite, E.R., Stuth, J.W., 1995. Fecal NIRS equations to assess diet quality of free ranging goats. Small Ruminant Res. 15, 223-230.

Lippke, H., Ellis, W.C., Jacobs, B.F., 1986. Recovery of indigestible fiber from feces of sheep and cattle on forage diets. J. Dairy Sci. 69, 403-412.

Lippke, H., Barton II, F.E., 1988. Near infrared relectance spectroscopy for predicting intake of digestible organic matter by cattle. J. Dairy Sci. 71, 2986-2991.

Lippke, H., Barton II, F.E., Ocumpaugh, W.R., 1989. Near infrared reflectance spectroscopy for estimation of digestible organic matter intake and body weight gain. XVI International Grassland Congress, Nice, France, pp.893-894.

Lyons, R.K., Stuth, J.W., 1992. Feacal NIRS equations for predicting diet quality of free-ranging cattle. J. Range Manage. 45, 238-244.

Lyons, R.K., Stuth, J.W., Angerer, J.P., 1995. Technical note Fecal NIRS equation field validation. J. Range Manage. 48, 380-382.

Malossini, F., Bovolenta, S., Piasentier, E., Piras, C., Martillotti, F., 1996. Comparison of n-alkane and chromium oxide methods for estimating herbage intake by grazing dairy cows. Anim. Feed Sci. Technol. 61, 155-165.

Mambrini, M., Peyraud, J.L., 1997. Retention time of feed particles and liquids in the stomachs and intestines of dairy cows. Direct measurements and calculations based on faecal collection. Reprod. Nutr. Dev. 37, 427-442.

Mayes, R.W., Lamb, C.S. and Colgrove, P.M., 1986. The use of dosed and herbage n-alkanes as markers for determination of herbage intake. J. Agric. Sci. Camb. 107, 161-170.

Mayes, R.W., Dove, H., 2000. Measurement of dietary nutrient intake in free ranging mammalian herbivores. Nutr. Res. Review 13, 107-138.

Minson, D.J., Butler, K.L., Grummitt, H.N., Law, D.P., 1983. Bias when predicting crude protein, dry matter digestibility and voluntary intake of tropical grasses by near-infrared reflectance. Anim. Feed Sci. Technol. 9, 221-237.

Moshtaghi Nia, S.A., Wittenberg, K.M., 2002. Evaluation of n-alkanes as markers for estimation of dry matter intake and digestibility in steers consuming all-forage or forage-concentrate diets. Can. J. Anim. Sci. 82, 419-425.

Naes, T., Isaksson, T., Fearn, T., Davies, T., 2002. A user friendly guide to multivariate calibration and classification. NIR Publications, Chichester UK, 30-33.

Norris, K.H., Barnes, R.F., Moore, J.E., Shenk, J.S., 1976. Predicting forage quality by infrared reflectance spectroscopy. J. Anim.Sci. 43, 889-897.

NF V 03-04 (1977). Détermination de l'indice d'insolubilité dit "cellulosique" méthode générale. In Recueil de normes françaises, 2éme édition (1985), eds AFNOR.

Peyraud, J.L., 1998. Techniques for measuring faecal flow, digestibility and intake of herbage in grazing ruminants. Techniques for Investigating Intake and Ingestive Behaviour by Farm Animals. IXth European Intake Workshop. IGER North Wyke UK pp.39-48.

Provenza, F.D., Villalba, J.J., Dziba, L.E., Atwood, S.B., Banner, R.E., 2003. Linking herbivore experience, varied diets, and plant biochemical diversity. Small Ruminant Res. 49, 257-274.

Sandberg, R.E., Adams, D.C., Klopfenstein, T.J., Grant, R.J., 2000. N-alkane as an internal marker for predicting digestibility of forages J. Range Manage. 53, 159-163.

Shenk, J.S., Westerhaus, M.O., 1991. Population definition, sample selection and calibration procedures for near-infrared reflectance spectroscopy. Crop Sci. 31, 469-474.

Smit, H.J., Taweel, H.Z., Tas, B.M., Elgersma, A., 2005. Comparison of techniques for estimating herbage intake of grazing dairy cows. J. Dairy Sci. 88, 1827-1836.

Stuth, J.W., Kapes, E.D., Lyons, R.K., 1989. Use of near infrared spectroscopy to assess nutritional status of cattle diets on rangeland. XVI International Grassland Congress, Nice, France, 889-890.

Stuth, J., Awuma, K., Tolleson, D., Ossiya, S., Ebong, C., Mnene, W., Mwilawa, A., Ebro, A., Byenkya, S., Jama, A., Kaitho, R., 2003. Infusing nutritional profiling technology in sub-Saharan Africa for free ranging livestock. Research Brief 03-04-Lews, April 2003.

Sunvold, G.D., Cochran, R.C., 1991. Technical note : evaluation of acid detergent lignin, alkaline peroxide lignin, acid insoluble ash and indigestible detergent fiber as internal markers for prediction of alfalfa, bromegrass and prairie hay digestibility by beef steers. J. Anim. Sci. 69, 4951-4955.

Tilley, J.H.A, Terry, R.A, 1963. A two stage technique for 'in vitro' digestion of forage crops. Grass Forage Sci. 18, 104-111.

Ward, R.G., Smith, G.S., Wallace, J.D., Urquhart, N.S., Shenk, J.S., 1982. Estimation of intake and quality of grazed range forage by near infrared reflectance spectroscopy. J. Anim. Sci. 54, 399-402.

Williams, P., 2004. Near-infrared technology, getting the best out of light. A short course in the practical implementation of Near-infrared spectroscopy for the user. PDK Grain, Nanaimo, British Colombia, and Winnipeg, Manitoba Canada, pp.12-13.

Wofford, H., Holechek, J.L., Galyean, M.L., Wallace, J.D., Cardenas, M., 1985. Evaluation of fecal indices to predict cattle diet quality. J. Range Manage. 38, 450-454.

Faecal Near infrared reflectance spectroscopy for ruminant feed intake prediction

Article III - Adapted from Decruyenaere et al. (2004)
Grassl. Sci. Eur. 9, pp.1034-1036.

Decruyenaere V.[(1)]*, De Visser M.[(2)], Stilmant D.[(1)], Clément C.[(1)] and Sinnaeve G.[(3)]

(1) Centre of Agricultural Researches - Farming Systems Section, 100 Rue du Serpont B-6800 Libramont, Belgium

(2) Animal Sciences Group, Wageningen UR - Applied Research, P.O.Box 2176, 8203AD Lelystad, The Netherlands

(3) Centre of Agricultural Researches - Departement of Agricultural Product Quality 24 Chaussée de Namur, B-5030 Gembloux, Belgium

corresponding auhtor – E-mail : v.decruyenaere@cra.wallonie.be

Abbreviations: DM, dry matter; DMI, dry matter intake; FNIRS, Near infrared refelctance spectroscopy applied to faeces; HL, Holcus lanatus; LP, Lolium perenne; R^2, coefficient of determination; RPD, standard error of reference database / standard error of cross validation; SD, standard error of reference database; SE, standard error of regression; SEC, standard error of calibration; SECV, standard error of cross validation; SEP, standard error of prediction; TR, Trifolium repens.

Abstract

The aim of the research was to test the potential of near infrared reflectance spectroscopy applied to faeces (FNIRS) for predicting both diet intake and characteristics of ruminant's diet. The lack of information on the intake of producing animals, especially concerning grazing animals, was the weakness of the system. The intake characteristics for improving the management of the grazing herd were (1) total dry matter intake (DMI); (2) botanical composition of intake (grass and clover proportions). Under grazing conditions, the latter may differ substantially from sward composition. The FNIRS database consisted of sample sets from three feeding trials: dairy cow, suckling cattle and sheep. For each trial, animals were kept in stable and were offered measured grass clover dominated rations. The database was expanded through repeated spectroscopy measurement under different sample grindings.

The FNIRS calibration of total dry matter intake (DMI, g/kg $BW^{0.75}$) was developed from a mixed suckling and dairy cow database. The statistics of calibrations are satisfactory (n=139, R^2 = 0.98, standard error of cross validation (SECV) = 6.78 g/kg $BW^{0.75}$). According to the result of calibrations on botanical composition (dairy cow and sheep database), it appears possible to predict botanical composition of diet with relatively good accuracy (SECV = 3.99 % and 4.59 %, respectively, for clover and grasses percentage). Results with the small but compound databases show that high quality NIRS calibrations on feed intake of ruminants are attainable.

keywords : ruminant faeces, NIRS, feed intake prediction, botanical composition

I. Introduction

Grazing animal diet characterisation provides for important management information and for efficient forage based livestock production. However, the estimation of dry matter intake (DMI) of grazing animals remains difficult, even if, the development of the *n*-alkanes methodology realised real progress (Dove and Mayes 2003). *N*-alkanes are long indigestible carbon chains naturally present in most of grazed plants. For predicting intake, the *n*-alkanes method is based on the analysis of the concentration, in plant and faeces, of natural (odd-chain) and synthetic (even-chain) *n*-alkanes. Unfortunately, this method can't be applied routinely as it needs the use of dosed synthetic alkanes. Other difficulty, linked to the use of *n*-alkanes, is to obtain a representative sample of ingested herbage, especially if mixed swards are offered to animals or if animals are under grazing. Methods for determining botanical composition of diet, as microhistological analysis, are laborious and have lower precision (Holecheck et al., 1982).

During the last 30 years, the potential of near infrared reflectance spectroscopy applied to faeces (FNIRS) to predict chemical composition and digestibility of feeds was well demonstrated (Boval et al., 2004; Landau et al., 2004, Li et al. 2007). Likewise, the potential of FNIRS to predict diet characteristics has been shown (Coates 2000, Coates and Dixon, 2008, Fanchone et al., 2007).

The aim of this study is to develop FNIRS calibrations to predict and to characterise intake of grazing ruminants. The robustness of NIRS calibrations integrating faeces of different ruminant's species (sheep and cattle) has been tested.

II. Materials and methods

Faecal samples databases are derived from feeding trials conducted on suckling cows (summer 1997) and sheep (summer and autumn 2001) fed with green fresh forage (CRA-W – Station de Haute Belgique, Libramont (B); 49°58' N – 5°38'E, 440 m above sea level) and on dairy cows fed with mixed diets (Animal Sciences Group, Wageningen UR Lelystad (NL); 52° 31' N, 5° 29' E, 1 m above sea level).

II.1. Feeding trials

II.1.1. Suckling cow and sheep trials

A first feeding trial was conducted on Belgian Blue White suckling cows (n = 3) housed in individual boxes. Cows were fed only with fresh grass cut every morning during fifteen days. The fresh grass was a mixed forage of white clover (Trifolium repens, TR), timothy (Phleum pratense) and rye grass (Lolium perenne, LP), offered at *ad libitum* level twice a day. Individual dry matter intake (DMI) was obtained by difference between offered and refused diet.

For the sheep experiment, Texel x Bleu du Maine castrated adult sheep (n = 9) were fed individually with three forage plant species, ray grass (Lolium perenne, LP), yorkshire fog (Holcus lanatus, HL) and white clover (Trifolium repens, TR), offered *ad libitum* in separate feeding troughs. These plant species, that could be selected freely, were offered in different proportions (table 1). During ten days, each forage species were cut daily, stored at 4°C and offered in 2 meals the next day. Sward purity (percentage of weeds), for each forage species, was determined daily. In this way, botanical composition of sheep intake was obtained in terms of clover and grasses percentages on a dry matter basis.

Table 1.
Diet composition (% of DM) of sheep trial (summer and autumn 2001)

Diet	Number of sheep	LP	HL	TR
Diet 1	2	50	25	25
Diet 2	2	25	50	25
Diet 3	3	30	30	40
Diet 4	1	55	30	15
Diet 5	1	30	55	15

II.1.2. Dairy cow trial

Six lactating Red Holstein dairy cows were offered *ad libitum* grass-clover mixture forage, three kilogram of concentrate and three levels of maize silage supplementation in a latin square design. Treatments were zero, 2.5 and 5.0 kg dry matter (DM) maize silage per cow per day. Cows produced on average about 20 kilogram milk per day. In three periods of four days each, the intake of concentrates, maize silage and grass clover mixture was measured. During one measuring period, the grass-clover forage was cut daily in one paddock in order to have a constant quality between days. The clover percentage in the cut herbage dry matter was from period one, two and three 19, 24 and 57 %, respectively.

II.2. NIRS acquisitions and calibrations

Along trials, suckling cows and sheep faeces were sampled 3 times a day on the pen floor, oven dried (65°C until constant weight). Sheep faecal samples (N= 133) were ground in a hammer mill (1 mm screen). To increase de variability of the FNIRS database, suckling cows faecal samples (N = 135) were ground according different screens size and mills (hammer mill, 1 mm screen (N = 45); hammer mill, 1.5 mm screen (N=45) and hammer mill, 1 mm screen followed by Cyclotec mill, 1 mm screen (N=45)).

Dairy cows faeces were sampled twice a day (6.00 AM and 5.00 PM). The eight samples taken during one period were mixed up proportionally per cow, resulting in a total of 18 faecal samples along with the same number of records on feed intake data.

Dried and ground faecal samples were submitted to NIRS analysis (NIRS system monochromator 5000). All individual spectra were recorded in the range of 1100 to 2500 nm by 2 nm steps. Dairy cow faecal samples were measured in triplicate to increase the number of spectral references.

Parameters for FNIRS calibrations were: (1) total dry matter intake (DMI, g per kg metabolic weight (kg $BW^{0.75}$) on each separate FNIRS database and by combining suckling cows and lactating dairy cows FNIRS databases and (2) percentages of legume and grasses in ingested diet on each separate FNIRS database and by combining sheep and lactating dairy cows FNIRS databases. As described by Decruyenaere et al. (2009), for suckling cow trial, a moving average over 6 days was calculated for the reference values and the corresponding faeces daily spectra, wavelength by wavelength. Thus, the day *i* (d_i) value equalled the 6-day mean from d_{i-2} to d_{i+3}. Characteristics of reference databases were summarized in table 2.

Table 2.
Characteristics of FNIRS reference databases

Parameters	Animal	Mean	Minimum	Maximum
DMI g/kg $BW^{0.75}$	Dairy cow	136.3	105.9	156.6
	Suckling cow	80.3	57.6	105.6
Grass %	Dairy cow	48.9	23.9	69.3
	Sheep	65.7	45.7	76.5
Legume %	Dairy cow	24.3	12.5	47.1
	Sheep	34.3	23.4	54.3

Calibrations were developed according to the partial least square procedure (PLS) with cross validation of the ISI software (WINISI 1.5, FOSS Tecator Infrasoft International LCC, Hillerød, Denmark). Calibration performances were done through the coefficient of determination (R^2), standard error of calibration (SEC) and standard error of cross validation (SECV). To evaluate the robustness of these calibrations, the ratio between the standard deviation (SD) of the reference population and the SECV (RPD ratio) was calculated. RPD values of 2.5 – 3.0 are considered adequate for a qualitative samples screening but values of at least 3.0 – 5.0 are required for quality assurance (Williams and Soberig 1992).

III. Results

Statistics of FNIRS calibrations are listed in table 3. From these results, it was not possible to predict DMI only from dairy cow FNIRS analyses. Though the R^2 of this calibration appeared satisfactory (R^2 = 0.85), the standard error of estimation was too high (SECV = 9.28 g/kg $BW^{0.75}$) and with a RPD ratio lower than 3. This lack of precision was probably due to the low number of available dairy cow samples (N = 18) and to the relatively small variability of the DMI reference values (coefficient of variation = 10 %). The NIRS measurement of faeces samples in triplicate didn't increase this variability of the database. Statistics of calibrations developed from suckling cow faecal spectra were better (R^2 = 0.96; SECV = 3.15 g/kg $BW^{0.75}$). To increase the variability of FNIRS databases through grinding seemed efficient. Combining the dairy cow and suckling cow faeces databases allowed to increase the NIRS variability and improved the calibration statistics (R^2 = 0.98; SEC = 4.88 g/kg $BW^{0.75}$; SECV = 6.78 g/kg $BW^{0.75}$; RPD = 4.56).

Table 3.
Statistics of NIRS calibrations for predicting diet attributes

	Faeces	N	Mean	SD	SEC	R^2	SECV	SD/SECV
DMI g/kg $BW^{0.75}$	Dairy cow	54	136.29	14.40	5.66	0.85	9.28	1.55
	Suckling cow	84	80.37	13.60	2.57	0.96	3.15	4.31
	Global	139	100.94	30.92	4.88	0.98	6.78	4.56
Grass %	Dairy cow	53	48.62	13.69	3.45	0.94	4.24	3.23
	Sheep	127	44.98	9.28	3.26	0.88	4.40	2.10
	Global	177	46.12	10.94	3.45	0.90	4.59	2.38
Legume %	Dairy cow	54	24.32	12.86	2.87	0.95	3.24	3.96
	Sheep	122	22.00	5.67	2.01	0.87	2.91	1.95
	Global	177	22.73	8.60	2.99	0.88	3.99	2.15

N= number of observations with outliers (standardised distance (H) > 3) excluded; SEC: standard error of calibration; R^2: coefficient of determination of NIRS equations; SECV: standard error of cross validation; SD: standard deviation of the reference database; RPD: SD/SECV.

RPD ratios associated to the calibration of botanical composition of intake were higher than 3, especially for dairy cows FNIRS calibrations. This means that botanical composition of grazed grass could be predicted by FNIRS with a relatively good accuracy. According to the

calibration statistics, the error of estimation (SECV) was about 4 to 4.5 % for both proportion of clover and proportion of grass in ingested diet. In opposition to the DMI, the addition of sheep database did not improve calibration performances.

IV. Discussion

The knowledge of intake and botanical composition of grazing ruminant diet appears as a key point of the grazing management, both for suckling and dairy production. Compared to grasses, legumes are often more digestible and voluntary intake of legumes is often higher due to the animal preference for legumes and due to the probable lower resistance to breakdown during chewing and rumination (Assoumaya et al. 2007). Despite the importance of these parameters for ruminant nutrition under grazing, measurements of intake and botanical composition of diet are laborious and sometimes, estimations are associated with a lack of accuracy. Did FNIRS solve the problem but with which success? Several studies have demonstrated that NIRS can predict the chemical composition of temperate or tropical forage with a good accuracy. FNIRS has also been used to predict intake and digestibility of temperate and tropical forages (Decruyenaere et al., 2002; Boval et al. 2004). Dixon and Zhu (2006) have tested FNIRS for predicting the species and leaf content of tropical grass diets of ruminants.

According to our results and probably due to the size of the database (*N*=18), the DMI calibration developed from dairy cow's faeces did not be used in a quantitative way (Williams, 2001). The same results were observed by Keli et al (2007) with FNIRS calibration developed on 16 sheep faecal samples had high SECV (11.56 g/kg $BW^{0.75}$). The RPD ratio (RPD = 1.12) indicated that the calibration was not useful for an efficient determination of DMI. Combining suckling and dairy cow's faecal spectra in a whole database increased the range of DMI values. The FNIRS calibration developed from this database to estimate DMI had excellent R^2. With RPD ratio ranged between 3.1 and 4.9, the DMI calibration appeared sufficiently robust to make a good screening. The error of estimations was acceptable. So, Tran et al. (2010) predicted DMI of dairy cows fed mixed diet with success (SEP = 1.46 to 1.70 kg DM/day for a dairy cow weighting 605 kg). For a same cow's weight, with our small database, SECV reached 0.827 kg DM/day.

NIRS analyses have been used to predict botanical composition of forage. The most NIRS models for predicting botanical composition of forage were developed on forage spectra associated with hand separation as reference data (Leconte et al., 1999, Locher et al., 2005). When mixed forages were submitted to NIRS analyses, according to the calibrations of Locher et al. (2005), the estimation error for the determination of legume proportion in mixed forages was lower than 4 %. The same level of accuracy was reached for our FNIRS determination of legumes in diet.

There are very few references on the determination of botanical composition of herbivore diets. According to Walker et al. (2010), researches to quantify the diet composition of herbivores could be hindered by a lack of adequate methods, except FNIRS. Most of studies using FNIRS concern free-ranging herbivores under tropical conditions. In the review of Walker et al. (2010), it appeared that SECV associated with prediction of percentage of grass and lucerne in diet were respectively 4.3 % and 3.8 %. The FNIRS calibration developed by Keli et al. (2007) predicted the proportion of lucerne in the diet with a similar accuracy (SECV = 5.52 %) and, as for our small database, RPD ratio was largely higher than 3 (RPD = 6.98). The FNIRS calibrations developed from dairy cows samples presented similar SECV respectively for grass and clover percentage in diet.

V. Conclusion

On one side, results based on the small but varied databases, show that FNIRS calibrations with good statistical performances could be developed to characterise ruminants feed intake. It can be concluded that, once we will have enough reference observations and sufficient variability in databases, we will be able to develop a FNIRS calibration that will integrate animal species variability.

On the other hand, we demonstrate that FNIRS can predict animal diet composition, in terms of plant species. Statistical performance of FNIRS calibrations are equivalent to the one of NIRS calibrations developed to predict species composition from sward samples. Unfortunately, the compound calibrations on grass and legume part didn't meet the quality assurance (RPD > 3) probably due to the high difference in composition between sheep and dairy cow faeces. In such way, FNIRS predictions of botanical composition should be used only for ranking.

Faecal NIRS seems an interesting method to estimate diet quality but the major concern is the development of accurate reference database covering the range of variation that could be met for the different herbivores species in the field. So, the use of FNIRS to predict diet characteristics of grazing animal must be linked to the development of reference data as robust and as diverse as possible.

VI. References

Assoumaya, C., Sauvant, D., Archimède, H., 2007. Etude comparative de l'ingestion et de la digestion des fourrages tropicaux et tempérés. INRA Prod.Anim., 20(5), 383-392.

Boval, M., Coates, D.B., Lecomte, P., Decruyenaere, V., Archimède, H., 2004. Faecal Near Infrared Reflectance Spectroscopy (NIRS) to assess chemical composition, *in vivo* digestibility and intake of tropical grass by Creole cattle. Anim. Feed Sci. Technol., 114(1), 19-29.

Coates, D.B. (2000) Faecal NIRS – What does it offer today's grazier ? Tropical grasslands, 34, 230-239.

Coates, D.B., Dixon, R.M, 2008. Development of near infrared analysis of faeces to estimate non-grass proportions in diets selected by cattle grazing tropical pastures. J. Near Infrared Spectrosc, 16, 471-480.

Decruyenaere, V., Stilmant, D., Lecomte, P., Buldgen, A., Dardenne, P., 2002. Improvement and validation of the NIRS analysis applied to faeces to measure grass intake in pasture. In : Durand J.L., Emile J.C., Huygue Ch. and Lemaire G., eds. Multi-function grasslands. Quality forages, animal products and landscapes. Grassl. Sci. Eur., 7, 196-197.

Decruyenaere, V., Lecomte, Ph., Demarquilly, C., Aufrere, J., Dardenne, P., Stilmant, D., Buldgen, A., 2009. Evaluation of green forage intake and digestibility in ruminants using neaur infrared reflectance spectrscopy (NIRS): Developing a global calibration. Anim Feed Sci. Technol., 148, 138–156.

Dixon, R.M., Zhu, G., 2006. Measurement of the leaf content of tropical grasses with near infrared reflectance spectroscopy. In: P. Flinn ed. Near infrared reflectance spectroscopy :

proceedings of the 12th Australian Near infrared reflectance spectroscopy conference, p48, Rockhampton, Australia.

Dove, H., Mayes, R.W. (2003) Wild and domestic herbivore diet characterization. In Proceedings of the ISNH6 satellite meeting, Merida, Mexico 17 to 19 october , 88 pp.

Fanchone, A., Boval, M., Lecomte, Ph., Archimède, H., 2007. Faecal indices based on near infrared spectroscopy to assess intake, *in vivo* digestibility and chemical composition of herbage ingested by sheep (crude protein, fibres and lignin content). J. Near Infrared Spectrosc. 15, 107-113.

Holechek, J.L., Vavra, M., Piepper, R.D., 1982. Botanical composition determination of range herbivores diets : a review. J. Range Manage., 35(3), 309-315.

Keli, A., Andueza, D., Baumont, R., Bechet, G., de Vega, A., 2007. The use of NIRS for prediction of intake, digestibility, diet composition and faecal concentration of n-alkanes in sheep fed different proportions of lucerne and rye grass (Lolium rigidum). Options Mediterranéennes, Séries A, N° 74, 237-241.

Landau, S., Glasser, T., Dvash, L., Perevolotsky, A., 2004. Faecal NIRS to monitor diet of mediterranean goats. S. Afr. J. Anim. Sci., 34(1), 76-80.

Leconte, D., Dardenne, P., Clement, C., Lecomte, Ph., 1999. Near infrared determination of the morphological structure of rye grass swards. Proceedingd of the 9th International Conference of Near Infrared spectroscopy, Eds Davies A.M.C. and Giangiacomo R., NIR Publications, Chichester, UK, p. 41-44.

Li, H., Tolleson, D., Stuth, J., Bai, K., Mo, F., Kronberg, S., 2007. Faecal near infrared reflectance spectroscopy to predict diet quality for sheep. Small Ruminant Res., 68(3), 263-268.

Locher, F, Heuwinkel, H., Gutser, R., Schmidhalter, U., 2005. Development of near infrared reflectance spectroscopy calibrations to estimate legume content of multispecies legume–grass mixtures. Agron. J. 97:11–17.

Tran, H., Salgado, P., Tillard, E., Dardenne, P., Nguyen, X.T., Lecomte, P., 2010. "Global" and "local" predictions of dairy diet nutritional quality using near infrared reflectance spectroscopy. J. Dairy Sci. 93, 4961-4975.

Walker, J.W., Campbell, E.J., Kott, R.W., Landau, S.Y., Lupton, C.J., Scott, C.B., Surber, L., Taylor Jr, C.A., Whitworth, W.R., 2010. Fecal NIRS for predicting botanical composition of herbivore diets. In Walker J and Tolleson D. Eds. Shining light on manure improves livestock and land management, Texas Agrilife Research and Society for Range Management, Technical bulletin, 53-65.

Williams, P., Soberig, D. (1992) In Near Infrared Spectroscopy : Brigding the gap between data analysis and NIR application. In : K. Hildrun, T. Isaksson, T. Naes and A. Tandberg (eds) Proceedings of the 5th ICNIRS, Ellis Horwood LTD, Chichester, Haugesund, Norway, pp. 441-446.

Williams P.C., 2001. Implementation of near-infrared technology. In Williams P. and Norris K. (Eds). Near-infrared technology in the agricultural and food industries (2nd edn). Americain Association of Cereal Chemist, Inc. St. Paul, MN, 171-186.

Near infrared spectroscopy applied to faeces to predict botanical composition of sheep intake

Article IV – Decruyenaere et al. (2004)
http://www.nirpublications.com

Decruyenaere V.[(1)*], M. Peters M.[(1)], Stilmant D.[(1)], Dardenne P.[(2)], Buldgen A.[(3)]

[(1)] Farming Systems Section, Centre of Agricultural Researches, 100 rue du Serpont, B-6800Libramont (Belgium)

[(2)] Department of Agricultural Product Quality, 24 chaussée de Namur, B-5030 Gembloux (Belgium)

[(3)] Faculté Universitaire des Sciences agronomiques, Zootechny Unit, 2 Passage des Déportés, B-5030 Gembloux (Belgium)

corresponding auhtor – E-mail : v.decruyenaere@cra.wallonie.be

Abbreviations: NIRS, Near infrared refelctance spectroscopy; HL, *Holcus lanatus*; LP, *Lolium perenne*; $BW^{0.75}$, metabolic weight; R^2, coefficient of determination; R, reflectance; SEC, standard error of calibration; SEP, standard error of prediction; SECV, standard error of cross validation; TR, *Trifolium repens*.

I. Introduction

Diet selection is a key process affecting both animal productions and pasture structure. This is particularly relevant when pasture is composed of several plant communities. By preferring some plants in a rangeland or in a common pasture, ruminants have a great impact on the ecosystem and the development of some plant populations (Archer and Smeins, 1991; Belsky, 1992). Actually adequate evaluation procedure for determining diet selection on a large number of individuals and on grazing situation is not available. Study of animal behaviour with conventional methods as direct observations of the animal, utilization techniques, automatic recording of animal movements, oesophageal fistula techniques, microhistological analysis of faeces or digestive tract content is labour intensive and time consuming (Holechek et al., 1982). Recent developments with indigestible plant cuticular wax components, especially *n*-alkanes method, have opened new techniques to estimate herbage intake and botanical composition of ingested diet of free grazing animals (Dove and Mayes, 1996; Dove et al., 2000; Hendricksen et al. 2002). Nevertheless this methodology is limited by the number of items researched.

Near infrared reflectance spectroscopy (NIRS) may become an interesting tool to assess diet preferences at pasture. These last years, NIRS has been used successfully to determine species composition of mixed forage samples (Coleman et al., 1990; Garcia-Criado et al., 1991; Pitman et al., 1991; Mika et al., 1998) and oesophageal fistula samples (Volesky and Coleman, 1996). NIRS has been also used to assess the plant composition in terms of stems and leaves (Leconte et al. 1999; Stilmant et al., 2001). Finally NIRS analyses of faeces were

developed to estimate both nutritive value of ingested grass and dietary nutrient content of free ranging ruminants (Leite and Stuth, 1995; Coates, 2000).

Indeed faeces are composite materials that contain undigested residues and in this way, they can provide NIR spectral information highly correlated with the nature of diet. Recently, Walker et al. (1998; 2002) have demonstrated that NIRS applied to faeces can predict the proportion of respectively leafy spurge (*Euphorbia esula L.*) and mountain big sagebrush (*Artemisia tridentate Nutt. Ssp. vaseyana (Rydb) Beetle*) in sheep diet on rangeland situation.

The objective of this study is to evaluate the potential of NIRS applied to faeces to predict botanical composition of diet ingested by sheep grazing temperate pasture.

II. Materials and methods

This research was conducted in summer 2001 (August and September) at the Agricultural research centre of Gembloux, Farming Systems section located in Libramont (Ardenne, Belgium) (altitude 480 m; raining 1550 mm for the year 2001). Faecal materials and diet samples were obtained from indoor and outdoor feeding trials.

II.1. Experiment 1: development of faecal NIR data base

This experiment was conducted on nine castrated Texel × Bleu du Maine sheep (82.2 ± 8.1 kg of live weight). During the experiment sheep were housed in individual box with continuously available water and were fed *ad libitum*. Three forage plant species (*Lolium perenne* (LP), *Trifolium repens* (TR) and *Holcus lanatus* (HL)) were offered in separate feeding trough. These plant species, which could be selected freely, were proposed in different proportions (Table 1).

At the beginning of the experimental period, each sheep was weighed and during the trial, each forage species was cut daily, stocked at 4°C and offered to sheep the next day. During the experimental period (seven days of diet adaptation and seven days of data collection), sheep were fed twice daily and forage species were individually weighed and subsampled for moisture (oven dried at 60°C during 48 hours) and for purity of sward determinations. In each plant species sward, weeds proportions were determined every day by hand separation. So that botanical composition of sheep intake was obtained in terms of legume, grass and weeds (proportion in weight on a dry matter basis). Individual forage residues were collected daily, weighted and samples were oven dried (60°C during 48 hours) for moisture determination. Dried forage and residues samples were finally ground in a hammer mill and in a cyclotec mill (1 mm) screen. Faeces were collected individually three times a day on the pen floor. Faecal samples were oven dried (60°C during 48 hours) and ground in a hammer mill (1 mm screen). Each sample of ground forage, residues and faeces were stored in plastic bags for future analysis.

Table 1.
Theoretical plant composition (% of DM) of studied diet

Diet	Number of sheep	LP	HL	TR
Diet 1	2	50	25	25
Diet 2	2	25	50	25
Diet 3	2	30	30	40
Diet 4	3	33.3	33.3	33.3

II.2. Experiment 2: prediction of diet composition at pasture

The second experiment was conducted on six castrated Texel × Bleu du Maine sheep (83.2 ± 6.1 kg live weight) grazing a pasture divided in three paddocks (6 × 45 m). The first one was a LP sward, the second a TR sward and the third a HL sward. Sheep were conducted in a one day grazing system, each day a new part of the three paddocks was taked at sheep's disposal. During the experimental period of eight days, faeces were sampled individually at morning and afternoon. Faecal samples were oven dried (60°C during 48 hours), ground (hammer mill 1 mm screen) and stored into plastic bags for future analyses. Forage species were individually sampled for moisture (oven dried at 60°C during 48 hours) and for purity of sward determinations. In each plant species sward, weeds proportions were determined every day by hand separation.

II.3. NIR data acquisition

Offered forages, forages residues and faeces were submitted to NIRS analysis (NIRS system monochromator 5000). In experiment 1, all individual spectral data (three spectra by day by sheep) in the range of 1100–2500 nm by 2 nm steps were recorded as log 1/R and correlated with the proportion of grasses, legume and weeds ingested by sheep one day before or two days before. Calibrations were developed according to the MPLS procedure with cross validation of ISI software. Before calibrations, each faecal spectrum was transformed with a (2,5,5) derivative and scatter correction was done with the standard normal variance and detrend procedure. Evaluation of calibrations was done with the coefficient of determination (R^2), standard error of calibration (SEC), standard error of cross-validation (SECV) and standard error of prediction (SEP). In Experiment 2, individual selected diets were predicted using faecal calibration developed in Experiment 1.

III. Results and discussion

III.1. Level intake and diet composition at feeding trough (experiment 1)

Over the experimental period, voluntary intake averaged 57.4 g/kg $BW^{0.75}$ with a maximum of 73.5 g/kg $BW^{0.75}$ and a minimum of 41.3 g/kg $BW^{0.75}$. Concerning diet composition, values varied respectively between 30.7% and 65.2% for grasses (LP + HL) and between 12.8% and 34.6% for the legume (TR). Voluntary intake decreased when the proportion of HL increased in offered diet (Table 2). The same results have been observed by Morton et al. (1992). HL is a softly hairy grass less palatable than LP or TR (Watt, 1978) and Penning et al. (1997) showed that Clover and Ryegrass are preferred by sheep, when they can choose.

Table 2.
Level (g/kg $BW^{0.75}$) and composition (% DM) of intake

Diet	LP	HL	TR	Weeds	Voluntary intake
Diet 1	37.0	14.3	16.0	32.8	58.4
Diet 2	20.3	35.9	17.8	26.0	55.7
Diet 3	22.5	18.7	26.6	32.8	56.7
Diet 4	21.6	14.0	27.1	37.3	57.7

III.2. Faecal NIR calibrations

Prior to develop final NIRS calibrations, data from diet 1, 2 and 3 were analysed to determine which lag time (24 hours or 48 hours) between diet consumption and faecal spectra provided the best calibrations (Table 3).

Table 3.
Lag time (24–48 hours) between NIR spectra and reference values

Variable		24 hours				48 hours				
(% DM)	N	Mean	SEC	R^2	SECV	N	mean	SEC	R^2	SECV
HL	97	22.8	4.9	0.74	6.7	73	23.7	4.2	0.84	5.6
LP	99	27.4	3.9	0.80	5.5	74	28.1	3.7	0.81	5.9
TR	95	19.1	1.7	0.87	2.5	75	18.9	2.1	0.83	3.1
Weeds	100	30.1	2.2	0.76	3.3	75	29.1	1.6	0.88	2.5

N = number of observations with outliers excluded (H, Mahalonobis distance >3); SEC: standard error of calibration; R^2: coefficient of determination of NIRS equations; SECV: standard error of cross validation.

According to the results described in Table 3, it was not possible to distinguish the two graminae. Indeed, statistics of calibration for HL and LP were poor with high SECV and low coefficients of determination. TR could be predicted with a relatively good accuracy. It appeared that statistics of TR calibrations were better for the 24 hours lag time. Walker et al. (1998) reported that for both sheep and goats, the best calibration results were found between NIR analysis of faeces and percent of leafy spurge in diets consumed 48 hours earlier. Lyons et al. (1995) found the same delay. In these studies, species proposed to animals were fibrous and less digestible than temperate grass. In our experiment, temperate forage proposed to sheep stayed probably less long in the digestive tract and a lag time of 24 hours seemed more appropriate. On the total database, faecal NIRS analysis can predict species composition of diet succesfully (table 4). Coefficients of determination were good with $R^2 = 0.87$ for TR and $R^2 = 0.88$ for grass (LP+HL). Calibration was less accurate to predict the parts of other plants in the diets. When a set of independent samples (n = 20) was predicted, correlations between predicted values and measured values were sufficient except for weeds ($R^2 = 0.52$). For the other tested parameters, SEP were close to SEC with a good slope (Table 4).

Walker et al. (1998) could predict leafy spurge in diet of sheep and goat with a comparable accuracy ($R^2 = 0.85$ and SEC = 6.4%). On forage samples, Pitman et al. (1991) and Mika et al. (1998) developed NIRS models to predict percent of grasses, legumes and forbs from analysis of forage samples. Statistics of their calibrations were close to those obtained from faecal samples and percentages of grasses, legumes and herbs were predicted with SEP lower than 6%. Faecal NIRS could predict species composition of ingested forage with the same accuracy. Moreover, under grazing situation, necessity to have a representative sample of ingested diet remains a real problem. Consequently working on faecal samples to predict composition of diet ingested could better reflect the consumed diet.

Table 4.
Statistics of faecal NIR calibrations

Variable	Calibration					Validation				
(% DM)	N	mean	SEC	R^2	SECV	N	SEP	R^2	Slope	bias
TR	122	22.0	2.0	0.87	2.9	20	2.7	0.80	0.92	-0.05
LP+HL	127	45.0	3.3	0.88	4.4	20	3.6	0.90	1.09	1.93
Weeds	122	33.1	2.3	0.74	2.6	20	4.3	0.52	1.09	-2.21

N = number of observations with outliers excluded (H, Mahalonobis distance >3); SEC: standard error of calibration; R^2: coefficient of determination of NIRS equations; SECV: standard error of cross validation; SEP: standard error of prediction.

III.3. Estimation of diet composition at pasture

According to the experimental procedure (availability of grasses = 66.6%, availability of legume = 33.3%) and to the purity of sward determined by hand separation (LP = 72%; HL = 69.7% and TR = 85.2%), theoretical intake of sheep should be 28% of legume, 47% of grasses and 24% of weeds. Over an eight days period, NIRS predicted forage mixtures ingested by sheep contained 60.5 ± 14.1% of grasses; 14.0 ± 8.4% of legumes and 28.8 ± 7.8% of weeds and if predicted percent of grasses, legumes and weeds were summed, total percentage was 103.4 ± 5.2% which was close to 100%. TR proportion predicted by faecal NIRS should be lower but grasses and weeds predicted values should be close to theoretical values. To test the robustness of NIRS models, predicted values of morning and afternoon sampled faeces were correlated. There was a very good relation between morning and afternoon predicted values as illustrated at Fig 1. Therefore, those predicted values could be considered as a good estimation of diet truly consumed.

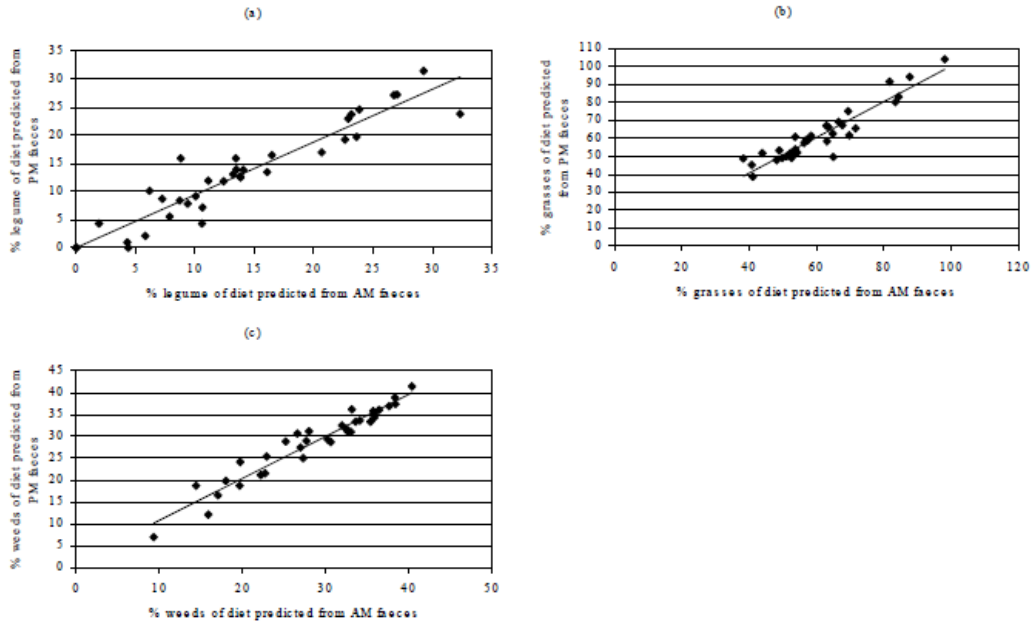

Fig. 1. Relation between morning and afternoon predictions for each species of ingested diet (a) legume; (b) grasses; (c) weeds

IV. Conclusions

NIRS applied to faeces gives good results in the prediction of animal choice at pasture. A major drawback of this method is the development of robust calibrations. Indeed, the value of a NIRS calibration is directly linked to the accuracy of the reference method and actually, the problem is mainly the acquisition of reference values because accurate procedures for determining diet selection individually were not available. To be robust, such calibrations need the integration of reference data as diverse as possible to be applied in conditions as diverse as possible.

V. References

Archer, S., Smeins, F.E., 1991. Grazing management : an ecological perspective. Ed by R.K. Heitschmidt and J.W. Stuth. Timber Press Inc., Portland, OR, USA, p. 109.

Belsky, A.J., 1992. Effects of grazing competition disturbance and fire on species composition and diversity in grassland communities. J. Veg. Sci. 3, 187-200.

Coates, D.B., 2000. Faecal NIRS - What does it offer today's grazier? Tropical Grasslands 34, 230-239.

Coleman, S.W., Christiansen, S., Shenk, J.S., 1990. Prediction of botanical composition using NIRS calibrations developed from botanical pure samples. Crop Sci. 30, 202-207.

Dove, H., Mayes, R.W., 1996. Plant wax components : a new approach to estimating intake and diet composition in herbivores. J. Nutr. 1, 13-26.

Dove, H., Freer, M., Foot, J.Z., 2000. The nutrition of grazing ewes during pregnancy and lactation: a comparison of alkane-based and chromium/in vitro-based estimates of herbage intake. Aust. J. Agric. Res., 51, 765-777.

Garcia-Criado, B., Garcia-Ciudad, A., Perez-Corona, M.E, 1991. Prediction of botanical composition in grassland herbage samples by near infrared reflectance spectroscopy. J. Sci. Food Agric. 57, 507-515.

Hendricksen, R.E., Reich, M.M., Roberton, R.F., Reid, D.J., Gazzola, C., Rideout, J.A., Hill, R.A., 2002. Estimating the voluntary intake and digestibility of buffel-grass and lucerne hays offered to Brahman-cross cattle using n-alkanes. Animal Sciences. 74, 567-577.

Holechek, J.L., Shenk, J.S., Vavra, M., Arthum, D., 1982. Prediction of forage quality using near-infrared reflectance spectroscopy on oesophageal fistula samples from cattle on mountain range. J. of Animal Sci. 55, 971-975.

Leconte, D., Dardenne, P., Clement, C., Lecomte, Ph., 1999. Near infrared determination of the morphological structure of rye grass swards. Proceedingd of the 9th International Conference of Near Infrared spectroscopy, Eds Davies A.M.C. and Giangiacomo R., NIR Publications, Chichester, UK, p. 41-44.

Leite, E.R., Stuth, J.W., 1995. Fecal NIRS equations to assess diet quality of free ranging goats. Small Ruminant Res. 15, 223-230.

Lyons, R.K., Stuth, J.W., Angerer, J.P., 1995. Technical note Fecal NIRS equation field validation. J. Range Manage. 48, 380-382.

Mika, V., Nerusil, P., Smital, F., 1998. The use of NIR spectroscopy for the prediction of botanical composition of grassland. In G. Nagy and K. Peto (eds), : Ecological aspect of grassland management, Proceedings of the 17th EGF Meeting, 479-481.

Morton, J.D., Bolton, G.R., Hodgson, J., 1992. The comparative performance of holcus lanatus and lolium perenne under sheep grazing in the Scottish uplands. Grass and Forage Science 47, 143-152.

Penning, P.D., Newman, J.A., Parsons, A.J., Harvey, A., Orr, R.J., 1997. Diet preferences of adult sheep and goats grazing rye grass and white clover. Small Ruminants Research 24, 175-184.

Pitman, W.D., Piacitelli, C.K., Aiken, G.E., Barton, F.E., 1991. Botanical composition of tropical grass-legume pastures estimated with near infrared reflectance spectroscopy. Agronomy Journal, 83, 103-107.

Stilmant, D., Delagarde, R., Clément, C., Meunier, B., Leconte, D., Lecomte, Ph., Dardenne, P., 2001. La SPIR – Un outil pour déterminer la composition spécifique et morphologique des couverts prairiaux. In Nouveaux regards sur le pâturage, Actes des Journées de l'AFPF, Paris,France, A7.

Volesky, J.D., Coleman, S.W., 1996. Estimation of botanical composition oesophageal extrusa samples using near infrared reflectance spectroscopy. J. Range Manage. 49, 163-166.

Walker, J.W., Clarck, D.H., McCoy, S.D., 1998. Fecal NIRS for predicting percent leafy spurge in diets. J. Range Manage. 51, 450-455.

Walker, J.W., McCoy, S.D., Launchbough, K.L., Fraker, M.J., Powell, J., 2002. Calibrating fecal NIRS equations for predicting botanical composition of diets. J. Range Manage. 55, 374-382.

Watt, T.A., 1978. The biology of holcus lanatus L. (Yorkshire fog) and its significance in grasslands. Common Wealth Bureau of Pastures and Field Crops 48, 195-204.

Prediction error and repeatability of near infrared reflectance spectroscopy applied to faeces samples in order to predict voluntary intake and digestibility of forages by ruminants

Article V – Decruyenaere et al. (2015)
Animal Feed Science and Technology (accepted-04/2015;
DOI:10.1016/j.anifeedsci.2015.04.011)

Decruyenaere V.[(1)], Planchon V.[(2)], Dardenne P.[(3)], Stilmant D.[(2)]

[(1)] Walloon Agricultural Research Centre (CRA-W) – Productions and Sectors Department. 8 Rue de Liroux, B-5030 Gembloux, Belgium

[(2)] Walloon Agricultural Research Centre (CRA-W) – Agriculture and Natural Environment Department. 9 Rue de Liroux, B-5030 Gembloux and 100 rue du Serpont, B-6800 Libramont, Belgium

[(3)] Walloon Agricultural Research Centre (CRA-W) – Valorisation of Agricultural Products Department. 24 Chaussée de Namur, B-5030 Gembloux, Belgium

corresponding auhtor – E-mail : v.decruyenaere@cra.wallonie.be

Abbreviations: ANOVA, analysis of variance; DMVI, dry matter voluntary intake; FNIRS, near infrared reflectance spectroscopy applied to faeces; NIRS, near infrared reflectance spectroscopy; OMD, in vivo organic matter digestibility; OMVI, organic matter voluntary intake; RMS, root mean square; SEC, standard error of calibration; SECV, standard error of cross-validation; SEM, standard error of the mean; SEP, standard error of prediction; CV, coefficient of variation

Abstract

The study examined the prediction error of near infrared reflectance spectroscopy of faeces (FNIRS) in estimations of *in vivo* organic matter digestibility (OMD) and dry and organic matter voluntary intake (DMVI and OMVI) of forages by ruminants in established calibration datasets from our laboratories. It also examined the repeatability of the FNIRS measurements of these parameters and the proportion of grass and clover in ingested forage. The prediction error of NIRS calibrations depends on the accuracy and precision of reference data. In this study, the variability in reference data for OMD, DMVI and OMVI was less than 10% of the mean. Correction for the error in the reference method almost halved the standard error of prediction (SEP) for OMD to 0.0155. For DMVI and OMVI, the corrected SEP was 8-9 g/kg $BW^{0.75}$, similar to the apparent SEP. These results suggested that the FNIRS calibrations were precise enough to predict OMD adequately, but probably not DMVI or OMVI.

The repeatability of FNIRS spectra and predictions assessed by repeated measurements of the same sample was satisfactory for all tested parameters. For OMD, DMVI, OMVI and the grass and clover proportion of the ingested forage, prediction repeatability was lower than, or similar to, the standard error of cross-validation (SECV) of the FNIRS calibration. The study showed that FNIRS could be a reliable method for predicting the OMD of ruminants, but the

prediction of voluntary intake with acceptable error was less satisfactory because of uncertainties in the FNIRS calibration models.

Keywords: NIRS, faeces, repeatability, uncertainty, digestibility, intake, botanical composition

I. Introduction

The nutrition and performance of grazing herbivores depends directly on the capacity of the animal to select a diet that has high digestibility and to have a high voluntary intake, which depends in turn on the quality and availability of pasture sward. The classical method of determining *in vivo* organic matter digestibility (OMD) and dry or organic matter voluntary intake (DMVI or OMVI) is based on measuring the quantity and quality of the diet ingested and the faeces excreted, and usually requires housing the animal in digestibility crates (Demarquilly et al., 1995; Andueza et al., 2007). This method is time consuming, however, and assumes that the harvested forage fed to the animal is representative of the forage selected by the grazing animal. It is difficult, therefore, to measure sward digestibility, voluntary intake and intake composition in pasture or rangeland, especially over long periods of time. In order to address these problems, methods linking *in vivo* digestibility or/and intake to a range of laboratory measurements have been developed. The 'rumen fluid pepsin' method developed by Tilley and Terry (1963) is probably the oldest method for estimating *in vivo* digestibility, but its reproducibility can be poor (Wainman et al., 1981, cited by Adegosan et al., 2000). Subsequently, in order to improve precision and eliminate the need for surgically prepared animals to provide rumen fluid, enzymatic mixtures simulating rumen activities such as the cellulase-method (De Boever et al., 1988; Aufrère et al., 2007) were developed and have been used to estimate the *in vivo* digestibility of a large range of forages. Estimating voluntary intake and the composition of diets ingested by grazing ruminants is more difficult because of diet selection. This key process affects both animal production and the effects of grazing on the pasture, particularly when that pasture comprises a number of plant communities (Walker et al., 2010). Conventional methods for studying animal behaviour and, indirectly, diet selection have included direct observation of the animals while grazing, analysis of samples obtained from oesophageal fistulae, digestive tracts or faeces (e.g., using microhistological analysis) and the automated or manual recording of animal movements or activities. A major disadvantage of all these methods is that they are labour intensive and generally have poor repeatability (Holechek et al., 1982). In recent decades, an alternative method has been developed for measuring the digestibility, intake and botanical composition of ingested forage by grazing herbivores. It is based on measuring plant waxes in forage and faeces, such as alkanes, which are indigestible or have low digestibility (Mayes et al., 1986; Dove and Mayes 1996, 2005; Ferreira et al., 2007; Pérez-Ramirez et al., 2012), but it can pose major difficulties. In conclusion, despite a lot of work over many decades, there is a consensus that there is no entirely satisfactory method for determining diet selection and nutrient intake by grazing herbivores with the accuracy and precision required by ruminant nutritionists.

Another approach involves applying near infrared reflectance spectroscopy to faeces (FNIRS) in order to predict the composition and attributes of forage diets. Over the past three decades several studies have highlighted the capacity and role of FNIRS in measuring the diet selected and managing the nutrition of grazing ruminants (Coates, 2004; Dixon and Coates, 2009). Using FNIRS technology is similar to using NIRS forage analyses for predicting diet composition and attributes. FNIRS calibrations have been developed to measure herbivore diet quality and intake under tropical and sub-tropical conditions (Coleman et al., 1989; Stuth et al., 1989; Coleman and Murray, 1993; Leite and Stuth, 1995; Boval et al., 2004; Dixon and Coates, 2009; Coates and Dixon, 2011) Mediterranean (Keli et al., 2008; Landau et al., 2008) and in temperate environments (Decruyenaere et al., 2002, 2004a, 2004b, 2009; Andueza et al. 2011b).

The first objective of NIRS calibration is to predict a parameter of interest as accurately and precisely as possible, and to do so over a wide, or at least known, range of circumstances. With most NIRS technologies, the precision and accuracy of predictions from calibration equations depend greatly on the quality of the reference values. Errors in reference values are likely to increase the error associated with the NIRS predictions (Dryden, 2003; Sørensen, 2002). Numerous studies have shown that feeding trials with animals housed in metabolism crates or pens where the forage ingested and the faeces are satisfactorily measured and sampled, can be used successfully to develop FNIRS calibration equations. Many of these studies were summarized by Coates and Dixon (2010) and Dixon and Coates (2009). In general, if NIRS is used to estimate a component of forages, such as crude protein content or *in vitro* digestibility, appropriately developed NIRS predictions can be as precise as other laboratory methods (Coates, 2002; Garrido-Varo, 2006). In these situations, the repeatability and reproducibility of NIRS measurements are often similar to, or better than, those obtained with current alternative methods of laboratory analysis (Genot et al., 2011). If the parameter to predict, however, is related to the interactions between a herbivore and its feeding resources, such as forage *in vivo* digestibility, intake and diet selection, prediction uncertainty appears to be higher (Sørensen, 2002). This is especially true if NIRS and reference analyses are not performed on the same materials, which is the case with FNIRS (Coates and Dixon, 2010). In this situation, detecting errors in reference values is far more difficult.

The precision of an FNIRS calibration equation is commonly expressed through the standard error of prediction (SEP). The SEP in FNIRS measurements has three components: (i) errors associated with the capacity of the calibration model to predict an attribute from the spectral measurements; (ii) errors associated with the measurement, and its repeatability, of the reference values; and (iii) errors associated with the measurement, and its repeatability, of the NIR spectra.

The aim of this study was to determine the prediction errors associated with these three forms of errors in FNIRS predictions. More specifically, the study sought to measure errors associated with the OMD, DMVI and OMVI of forage diets ingested by sheep or cattle as reference measurements and to evaluate the errors associated with repeatability of the NIRS spectral measurements.

II. Material and methods

The errors associated with predicting OMD, OMVI and DMVI from FNIRS calibrations were evaluated using the calibration datasets described by Decruyenaere et al (2003, 2004a, 2004b, 2009) for sheep or cattle fed freshly harvested forage consisting of temperate grass with or without temperate legume pasture species.

II.1. Repeatability of animal measurements used as reference for FNIRS.

The repeatability of the reference method was calculated for three parameters – OMD, OMVI (g/kg $BW^{0.75}$) and DMVI (g/kg $BW^{0.75}$) – measured for three FNIRS calibration datasets that had been established in our laboratories. The three forages used to assess repeatability were harvested each day during the vegetative stage of plant growth and comprised tetraploid (4 n) or diploid (2 n) perennial rye grass (*Lolium perenne*) as described by Decruyenaere et al. (2009), as well as a mixed sward forage comprising white clover (*Trifolium repens*), perennial rye grass (*Lolium perenne*) and Yorkshire fog (*Holcus lanatus*) as described by Decruyenaere et al. (2003). The raw characteristics of these forages are listed in Table 1.

As described by Decruyenaere et al. (2009), the reference values for the FNIRS database were measured in digestibility trials where sheep were housed in digestibility crates (n = 3 or 4 per forage) and fed at various levels from maintenance to *ad libitum*. Each mean measurement used to build the FNIRS database was a moving average over 6 days. Thus, the day 'i' (d_i) value equalled the 6-day mean from d_{i-2} to d_{i+3}. The repeatability standard deviation (sd_r) of the reference data was calculated for each sheep on a 6-day moving interval. The global sd_r of the reference measurements was the quadratic mean of all individual sheep sd_r for all 6 days.

Table 1.
In vivo characteristics of green forages (raw data from digestibility trial)

Nature	Year of trial	N	Feeding level	OMD range	OMVI range	DMVI range
Rye grass 4 n (Meltra)	1992	168	al	0.471 – 0.877	35.40 – 69.63	36.32 – 77.60
Rye grass 2 n (Talbot)	1992	168	al	0.146 – 0.908	32.24 – 76.31	34.47 – 81.78
Mixed forage	2001	48	al	0.628 – 0.889	35.54 – 63.04	41.30 – 73.47

N: number of raw data (4 sheep over 42 days on rye grass 4 n and rye grass 2 n; 3 sheep over 16 days on mixed forage); al: *ad libitum*; OMD: *in vivo* organic matter digestibility; OMVI: organic matter voluntary intake (g/kg $BW^{0.75}$); DMVI: dry matter voluntary intake (g/kg $BW^{0.75}$).

II.2. Standard error of prediction of FNIRS

The precision of the FNIRS models was evaluated using the SEP obtained from six independent validation sets. These sets (n = 71-194) were extracted successively from the entire FNIRS database (n ≈ 950) described by Decruyenaere et al. (2009) (Table 2). The NIR spectra of faecal samples had been measured using a NIRSystem Model 5000 monochromator (1100 to 2498 nm wavelengths in 2 nm steps, FOSS Electric, Hillerød, Denmark). Experiments were conducted in 1989 (INRA), 1992 (CRA-W 1 and 2) and 1993 (CRA-W 3 to 7). Each validation set comprised a separate and independent CRA-W or INRA experiment in which digestibility and intake were measured. Six calibrations were developed with the samples remaining after the removal of each validation set in order to predict the OMD, OMVI (g/kg $BW^{0.75}$) and DMVI (g/kg $BW^{0.75}$), and were then used to predict each of the six validation sets. The characteristics of the validation and calibration datasets are given in Table 2. Each FNIRS calibration was calculated (WINISI® 1.50 software, Naes et al., 2002) using the second derivative of the spectra and with scatter correction using standard normal variate and detrend and as a modified partial least square model with cross-validation. The population boundaries for the calibration were set with a maximum standardized H (distance between a sample and the centroid of the group) value of 3.0 (Shenk and Westerhaus, 1991). Finally, the FNIRS predicted and reference values of each independent validation set were pooled in order to calculate the final SEP.

Table 2.
In vivo digestibility (OMD) and voluntary intake (OMVI, DMVI) validation and calibration datasets

Validation dataset	Nature	Feeding level[2]	N[1]	OMD mean (SD)	OMVI mean (SD)	DMVI mean (SD)
CRA-W 1	Rye grass 4 n (Meltra)	al	148	0.732 (0.062)	53.46 (4.64)	58.15 (5.30)
CRA-W 2	Rye grass 2 n (Talbot)	al	148	0.695 (0.059)	54.11 (5.12)	57.80 (5.69)
CRA-W 3 and 5	Mixed forage without clover	al and 1.5 M	194	0.695 (0.082)	52.88 (8.36)	59.12 (9.49)
CRA-W 4 and 6	Mixed forage with clover	al	194	0.693 (0.072)	55.02 (7.90)	61.82 (8.88)
CRA-W 7	Mixed forage without clover	M	155	0.716 (0.068)	33.88 (2.51)	38.14 (2.21)
INRA 1-7	Natural pasture (22) cocksfoot (18); tall fescue (5) timothy (1); rye grass (21) lucerne (2); red clover (3)	al	71	0.676 (0.083)	59.84 (9.63)	66.62 (11.32)
Calibration dataset without						
CRA-W 1		al – 1.5 M - M	884	0.698 (0.072)	50.19 (11.75)	55.76 (13.00)
CRA-W 2		al – 1.5 M - M	884	0.704 (0.074)	50.08 (11.68)	55.82 (12.99)
CRA-W3 and 5		al – 1.5 M - M	838	0.705 (0.069)	50.15 (11.55)	55.41 (12.68)
CRA-W4 and 6		al – 1.5 M - M	838	0.706 (0.072)	49.65 (11.45)	54.78 (12.51)
CRA-W7		al – 1.5 M - M	928	0.702 (0.072)	52.54 (10.02)	58.12 (11.20)
INRA		al – 1.5 M - M	961	0.705 (0.071)	49.99 (10.87)	55.34 (11.94)

OMD: *in vivo* organic matter digestibility; OMVI (g/kg $BW^{0.75}$): organic matter voluntary intake; DMVI (g/kg $BW^{0.75}$): dry matter voluntary intake.
[1] N: number of forage and faeces samples based on 6-day moving averages calculated for the CRA-W trials, number of forage samples, as mean of the trial, and faeces samples, as mean of six sheep over the trial, for the INRA trials (Decruyenaere et al., 2009).
[2] Feeding level: M: maintenance = 23 g OM digestible/kg $BW^{0.75;}$ 150 M: 1.5 maintenance; al: *ad libitum*.

II.3. Prediction and spectra repeatability

Four faecal samples were selected from an independent database generated during the 2006 grazing season. Samples 1 and 2 comprised faeces from Swifter breed ewes, and samples 3 and 4 faeces from Belgian Blue White heifers. Samples 1, 2 and 3 were from groups of sheep and cattle grazing together. Sample 4 was from a leader-follower grazing scheme. The stocking rates were 3.6 and 3.8 livestock units per hectare and the sheep-cattle ratios (number sheep/number heifers) were 1.3 and 1.5 for the mixed and leader-follower grazing schemes, respectively. Fresh faeces were sampled from the field twice a week during the grazing season, oven dried (65°C for 36 h) and then ground through a 1 mm screen in a hammermill.

The factors affecting the NIR spectra included the mode used to fill the small ring cups and the NIRS prediction calibrations (Dardenne, 1990; Genot et al., 2011). In order to evaluate spectral and prediction repeatability, the NIR spectra of each of the four faecal samples was measured with each of two presentation modes using an NIRSystem Model 5000 monochromator (FOSS Electric, Hillerød, Denmark). In the first mode (SP-1), 10 ring cups (36 mm inside diameter) were filled with the homogenized sample. In the second mode (SP- 2), the ring cup was filled with the sample, scanned and then transferred 10 times to other cups in order to generate 10 scans of the sample. Thus, 80 spectra of faeces were generated (4 samples x 2 cup presentations x 10 repetitions). From each spectrum, the OMD, OMVI (g/kg $BW^{0.75}$), DMVI (g/kg $BW^{0.75}$) and the composition of ingested forage (grass, clover and other plants) were predicted using the FNIRS calibration models that had been developed in our laboratories (Table 3). First, the OMD and OMVI predictions were obtained from sheep FNIRS calibrations published by Decruyenaere et al. (2009). In order to test the impact of the NIR model on the OMD and OMVI predictions, the same FNIRS database was used in a LOCAL calibration procedure (WINISI® 1.50 software). The LOCAL NIRS models were set up with a modified partial least square, using the second derivative mode spectrum with scatter correction using standard normal variate and detrend. The number of

samples selected in the spectral libraries ranged from 70 to 200. Second, DMVI was predicted using a cattle FNIRS calibration (Decruyenaere et al., 2004a) and the composition of ingested forages was predicted from sheep FNIRS calibrations (Decruyenaere et al., 2004b).

Table 3.
FNIRS models used for *in vivo* digestibility (OMD), voluntary intake (OMVI, DMVI) and botanical composition of ingested forage prediction in order to quantify the repeatability of FNIRS predictions

Parameters	N	Mean value	R^2	SECV	Type of faeces	Reference
OMD	951	0.710	0.92	0.0207	sheep	Decruyenaere et al., 2009
OMVI	936	51.27	0.83	4.53	sheep	
DMVI	139	100.94	0.98	6.78	dairy and suckling cows	Decruyenaere et al., 2004a
Grass proportion	127	45.0	0.88	4.4	sheep	Decruyenaere et al., 2004b
Clover proportion	122	22.0	0.87	2.9	sheep	
Weed proportion	122	33.1	0.74	2.6	sheep	

OMD: *in vivo* organic matter digestibility; OMVI: organic matter voluntary intake (g/kg $BW^{0.75}$); DMVI: dry matter voluntary intake (g/kg $BW^{0.75}$); grass, clover and weed proportions in percent on a dry matter basis.

II.4. Statistical analysis

II.4.1. Repeatability of animal measurements used as reference values for FNIRS

Before determining repeatability, the OMD, OMVI and DMVI raw reference values (per sheep and per day; n = 42 values per sheep) were evaluated for outliers using the Cochran and Grubbs tests (ISO 5725-1, 1994). The Cochran test identifies outliers between replicated objects (amplitude), whereas the Grubbs test detects outliers based on values with a large deviation from the mean. The sd_r was then calculated per sheep and over a 6-day moving average interval. The moving average interval at d_i was considered to begin at d_i and finish at d_{i+6}.

$$\bar{x} = \frac{1}{6}\sum_{i=1}^{6} x_i$$

$$s_r^2 = \frac{1}{6}\sum_{i=1}^{6} (x_i - \bar{x})^2$$

$$sd_r = \sqrt{s_r^2}$$

where x = OMD, OMVI or DMVI value; $\bar{x}$ = mean over a 6-day interval; s_r^2 = repeatability variance over a 6-day interval; and sd_r = repeatability standard deviation over a 6-day interval.

A global mean was then calculated per trial. The global repeatability standard deviation (Global sd_r) was the quadratic mean of each sd_r per forage and for the three tested forages.

II.4.2. Prediction uncertainty

The uncertainty of the FNIRS prediction was assessed by determining the corrected SEP (Fernandez Pierna et al., 2003; Faaber, 2005).

$$\text{apparent SEP} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(x - x_{est})^2}$$

$$\text{corrected SEP} = \sqrt{(\text{apparent SEP}^2 - \text{Global SEM}_{6d}{}^2)}$$

$$\text{SEM}_{6d} = \sqrt{\frac{{s_r}^2}{6}}$$

where x = reference value; x_{est} = FNIRS predicted value; n = number of observations; SEM_{6d} = standard error of the mean of the reference value over a 6-day interval; s_r^2 = repeatability variance of reference value over a 6-day interval. The Global SEM_{6d} was the quadratic mean of SEM_{6d}.

II.4.3. Repeatability of the measurement of the spectra of faecal samples

The repeatability of the NIR spectra was evaluated for each of the four measured faecal samples through the root mean square (RMS) of the absorbance unit, wavelength by wavelength, for the 10 repeated spectra. The RMS was calculated from the 10 repeated raw spectra (i.e., without mathematical treatment) using the WINISI® 1.50 software procedure to calculate the RMS of a subsample.

II.4.4. Repeatability of predicted values

The repeatability of predicted values was estimated through two analyses of variance (ANOVA – General Linear Model procedure of Statistica 8.0 – Statsoft, France) performed after outliers detection (Cochran and Grubbs tests, ISO 5725-1, 1994).

For predictions of the DMVI and the grass and legume composition of ingested forage, the factors of variance taken into account for the ANOVA were sample (n=4) and sample presentation (SP-1 vs SP-2, n=2). The sample factor was random, whereas sample presentation was fixed and these factors were crossed. For the OMD and OMVI predictions, the factors of variance were sample (n=4), sample presentation (SP-1 vs SP-2, n=2) and NIRS prediction technique (GLOBAL vs LOCAL, n=2). These last two factors were fixed and also crossed for the ANOVA. The repeatability standard error was deduced from the residual error of these ANOVA (Genot et al. 2011).

$$s_r = \sqrt{MS_{error}}$$

where MS_{error} = residual mean square error of the ANOVA; s_r = standard error of repeatability.

III. Results

III.1. Repeatability of animal measurements used as reference values for FNIRS

The mean values and repeatability of the OMD, OMVI and DMVI reference values are shown in Table 4. The Cochran test results indicated that there were no outliers for the three parameters tested or for the three forages. The Grubbs test, however, indicated that there were four outliers for the OMD of diploid rye grass (2 n), two outliers for the DMVI of tetraploid rye grass (4 n) and three outliers for the DMVI and OMVI of the mixed forage. In all these cases, the values identified as outliers were the lowest in the dataset. These outliers were discarded in the determination of the sd_r of the OMD, OMVI and DMVI.

The averaged OMD values were 0.733, 0.696 and 0.746 for tetraploid rye grass (4 n), diploid rye grass (2 n) and mixed forage, respectively. The range of the sd_r was larger for the diploid than for the tetraploid rye grass, and for these forages the Global sd_r ranged between 0.048 and 0.066. The global coefficient of variation (CV) of OMD ranged from 6.4 - 9.5% of the mean.

The DMVI and OMVI of the three forages were quite similar, ranging from 57 to 58 g/kg $BW^{0.75}$ for DMVI and from 50 to 53 g/kg $BW^{0.75}$ for OMVI. The sd_r ranges were similar for the three forages, with the Global sd_r reaching 4-5 g/kg $BW^{0.75}$ for both these parameters. The DMVI and OMVI Global sd_r was close to, or lower than, 5 g/kg $BW^{0.75}$. Finally, for the three tested parameters, the global CV was lower than 10% of the mean and quite similar (8.01, 8.28 and 8.63 for OMD, DMVI and OMVI, respectively).

Table 4.
Repeatability (sd_r) of reference values obtained from digestibility trials

	N	mean range	Global mean	sd_r range	Global sd_r	Global CV
	Rye grass 4 n (Meltra)					
OMD	168	0.621 – 0.815	0.733	0.030 – 0.072	0.051	7.01
DMVI	166	48.82 – 66.35	58.08	1.88 – 5.36	3.85	6.62
OMVI	168	45.53 – 60.73	53.34	2.10 – 5.69	4.02	7.55
	Rye grass 2 n (Talbot)					
OMD	164	0.612 – 0.794	0.696	0.029 – 0.104	0.066	9.50
DMVI	168	48.02 – 65.65	57.91	3.51 – 7.56	5.06	8.73
OMVI	168	44.94 – 60.54	53.81	3.24 – 6.86	4.66	8.67
	Mixed forage					
OMD	48	0.710 – 0.774	0.746	0.042 – 0.053	0.048	6.39
DMVI	45	55.33 – 60.10	57.62	2.11 – 7.83	5.22	9.05
OMVI	45	48.09 – 51.89	49.91	1.62 – 6.31	4.35	8.72
	For the 3 forages					
OMD	/	/	0.719	/	0.061	8.01
DMVI	/	/	57.95	/	4.80	8.28
OMVI	/	/	53.15	/	4.58	8.63

OMD: *in vivo* organic matter digestibility; DMVI: dry matter voluntary intake (g/kg $BW^{0.75}$); OMVI: organic matter voluntary intake (g/kg $BW^{0.75}$); N: number of daily individual reference values after outlier elimination (Cochran and Grubbs tests); mean range: range of 6 days mobile means; Global mean: mean for the whole trial period; sd_r range: range of 6 days mobile repeatability standard deviation; Global sd_r: repeatability standard deviation for the whole trial period; CV: coefficient of variation= 100 x sd_r/mean.

III.2. Prediction uncertainty

The FNIRS calibration statistics are presented in Table 5. The statistics for the entire database calibration were very similar to those for each of the six calibrations used to calculate the apparent SEP. The apparent SEP of the OMD was as high as 0.0283, which was

very similar to the SEC and SECV of the entire FNIRS database calibration. The Global SEM_{6d} for OMD was also similar to the SEP. The corrected SEP was as high as 0.0155, which was about half the apparent SEP. The SEC and SECV of the entire FNIRS calibrations for the DMVI and OMVI were fairly similar and close to 5 g/kg $BW^{0.75}$.

Table 5.
Statistics for *in vivo* organic matter digestibility (OMD) and voluntary intake (OMVI, DMVI) FNIRS calibrations, standard error of the mean reference measure, and apparent and corrected standard error of prediction

	FNIRS calibration statistics						Variability of reference values		Uncertainty of FNIRS predictions	
	N	mean	SD	SEC	R^2	SECV	Global sd_r	Global SEM_{6d}	Apparent SEP	Corrected SEP
Entire FNIRS database calibration										
OMD	951	0.710	0.0698	0.0200	0.92	0.0207	0.061	0.0236	0.0283	0.0155
DMVI	1012	55.87	12.01	5.32	0.80	5.53	4.80	2.01	9.11	8.89
OMVI	936	51.27	10.46	4.28	0.83	4.53	4.58	1.86	8.42	8.21
Entire FNIRS database without CRA-W 1										
OMD	875	0.700	0.0700	0.0217	0.90	0.0230	/	/	0.0286	/
DMVI	861	55.40	12.65	5.96	0.78	6.22	/	/	5.52	/
OMVI	866	50.01	11.61	5.30	0.79	5.51	/	/	4.77	/
Entire FNIRS database without CRA-W 2										
OMD	872	0.706	0.0720	0.0212	0.91	0.0215	/	/	0.0303	/
DMVI	854	55.36	12.61	5.49	0.81	5.72	/	/	8.92	/
OMVI	853	49.69	11.38	4.80	0.82	5.06	/	/	8.14	/
Entire FNIRS database without CRA-W 3 and 5										
OMD	827	0.707	0.0668	0.0210	0.90	0.0215	/	/	0.0292	/
DMVI	816	55.05	12.32	5.34	0.81	5.57	/	/	11.08	/
OMVI	817	48.89	11.29	4.76	0.82	4.92	/	/	10.53	/
Entire FNIRS database without CRA-W 4 and 6										
OMD	827	0.707	0.0694	0.0210	0.91	0.0217	/	/	0.0291	/
DMVI	807	54.12	11.85	4.81	0.84	5.14	/	/	10.48	/
OMVI	807	49.09	10.95	4.32	0.84	4.50	/	/	8.77	/
Entire FNIRS database without CRA-W 7										
OMD	918	0.703	0.0699	0.0214	0.91	0.0220	/	/	0.0235	/
DMVI	910	57.90	10.89	5.59	0.74	5.82	/	/	8.35	/
OMVI	907	52.37	9.78	4.97	0.74	5.15	/	/	7.74	/
Entire FNIRS database without INRA										
OMD	947	0.706	0.0706	0.0205	0.92	0.0210	/	/	0.0290	/
DMVI	932	54.84	11.51	5.61	0.76	5.73	/	/	8.72	/
OMVI	936	49.62	10.58	5.01	0.78	5.08	/	/	7.58	/

OMD: *in vivo* organic matter digestibility; DMVI ((g/kg $BW^{0.75}$): dry matter voluntary intake; OMVI (g/kg $BW^{0.75}$): organic matter voluntary intake; SECV: standard error of cross validation; SEP: standard error of prediction; R^2: coefficient of determination; SD: standard deviation of the reference database; sd_r: standard deviation; SEM: standard error of the mean.

The SEC and SECV of the intake calibration equations were similar to the variability observed in the reference value measurements (sd_r = 4.8 and 4.6 g/kg $BW^{0.75}$ for DMVI and OMVI respectively), but higher than the Global SEM_{6d} (2.5 g/kg $BW^{0.75}$). The difference between the SECV and the corrected SEP was high (more than 3 g/kg $BW^{0.75}$). The true

precision of the FNIRS predictions of DMVI and OMVI expressed by corrected SEP were close to the apparent SEP.

III.3. Repeatability of the measurement of the spectra of faecal samples

Table 6 summarises the repeatability of the measurement of the NIR spectra of faeces.

Table 6.
Repeatability of faecal spectra measurements

		Sample presentation	Raw spectrum
			RMS (log 1/R)
sample 1	sheep	SP-1	0.00212
sample 2	sheep	SP-1	0.00131
sample 3	heifer	SP-1	0.00101
sample 4	heifer	SP-1	0.00171
sample 1	sheep	SP-2	0.00322
sample 2	sheep	SP-2	0.00239
sample 3	heifer	SP-2	0.00188
sample 4	heifer	SP-2	0.00242

1: 10 small ring cups filled with the homogenized sample; 2: one small ring cup filled with the sample that was then transferred, 10 times, into other cups; log 1/R = absorbance unit; RMS: root mean square obtained using WINISI® 1.50 software.

For both sample presentations, the RMS of raw spectra was lower than 0.005 absorbance units, which was lower than the limit of 0.03 absorbency units set by WINISI® 1.50 software. The spectral differences between repeated spectra were higher for the SP-2 mode, which involved transferring the contents of a ring cup into other ring cups 10 times.

III.4. FNIRS prediction repeatability

The results of the Cochran and Grubbs tests on the FNIRS predicted values indicated that there were no outliers for OMD, DMVI, OMVI or the grass and legume contents of the ingested forages. Table 7 summarizes the FNIRS predictions of OMD, OMVI, DMVI and grass and legume contents of ingested forage. No effect of sample presentation mode (SP-1 or SP-2) on the predictions was observed ($P>0.05$). The GLOBAL and LOCAL FNIRS models gave similar predicted values for OMD (0.745 and 0.726, respectively) and OMVI (67.8 and 73.1 g/kg $BW^{0.75}$, respectively) ($P>0.05$).

The variability in the DMVI and the grass and clover proportion predictions was similar, with a CV ranging between 2.5 and 3. Only the weed proportion had a CV lower than 2 (Table 8). The CV for the OMD repeatability was lower than that for the OMVI (1.12 and 2.28, respectively). Variability in the prediction of DMVI was also higher than for OMVI (s_r = 3.36 and 1.61, respectively). For all predicted parameters the s_r was lower than the SECV of the corresponding FNIRS calibration equations (Table 8). In all cases, the s_r values were lower than those obtained with the reference method (sd_r) for the corresponding parameter (Table 4).

Table 7.
In vivo organic matter digestibility (OMD), voluntary intake (OMVI, DMVI) and grass, clover and weed proportion of ingested diet estimated using FNIRS, variations linked to samples presentation mode (SP) and FNIRS predictive model (Global or Local)

		Sample				Presentation mode		FNIRS model	
		1	2	3	4	SP-1	SP-2	Global	Local
OMD[1]	mean	0.781	0.712	0.690	0.758	0.742	0.729	0.745	0.726
	SD	0.008	0.030	0.021	0.010	0.035	0.046	0.031	0.047
OMVI[1]	mean	66.89	74.90	69.26	70.63	70.76	70.08	67.76	73.08
	SD	7.09	3.87	6.14	8.46	7.07	7.29	5.35	7.77
DMVI[2]	mean	136.06	76.23	91.97	118.99	105.62	106.00	/	/
	SD	2.83	3.71	3.27	3.53	23.62	23.86	/	/
Grass	mean	41.3	55.0	53.5	45.9	49.1	48.7	/	/
proportion[3]	SD	1.3	1.4	1.1	1.3	6.0	5.6	/	/
Clover	mean	28.5	16.3	20.2	26.9	22.8	23.1	/	/
proportion[3]	SD	0.9	0.6	0.5	0.6	5.2	4.9	/	/
Weed	mean	31.4	32.4	31.8	35.0	32.3	33.0	/	/
proportion[3]	SD	0.6	0.5	0.6	0.4	1.6	1.4	/	/

OMD: *in vivo* organic matter digestibility; OMVI: organic matter voluntary intake (g/kg $BW^{0.75}$); DMVI: dry matter voluntary intake (g/kg $BW^{0.75}$); grass, clover and weed proportion in percent on a dry matter basis, SD: standard deviation.
[1] predicted from Decruyenaere et al. (2009) as described in Table 3
[2] predicted from Decruyenaere et al. (2004a) as described in Table 3
[3] predicted from Decruyenaere et al. (2004b) as described in Table 3

Table 8.
Repeatability of predicted *in vivo* organic matter digestibility (OMD), voluntary intake (OMVI, DMVI) and botanical composition of ingested forage

	SECV	mean	s_r	CV
OMD[1]	0.0207	0.736	0.0083	1.12
OMVI[1]	4.53	70.42	1.61	2.28
DMVI[2]	6.78	105.81	3.36	3.18
Grass proportion[3]	4.4	48.92	1.24	2.54
Clover proportion[3]	2.9	22.97	0.69	3.00
Weeds proportion[3]	2.6	32.64	0.41	1.25

OMD: *in vivo* organic matter digestibility; OMVI: organic matter voluntary intake (g/kg $BW^{0.75}$); DMVI: dry matter voluntary intake (g/$kg^{0.75}$); grass, clover and weed proportions in percentages on a dry matter basis, s_r: standard error of the repeatability obtained from the residual error of the ANOVA; CV: coefficient of variation= 100 x s_r/mean; SECV: standard error of cross-validation of the FNIRS calibration
[1] Predicted from Decruyenaere et al. (2009) as described in Table 3
[2] Predicted from Decruyenaere et al. (2004a) as described in Table 3
[3] Predicted from Decruyenaere et al. (2004b) as described in Table 3

IV. Discussion

NIRS is a physical method of analysis linking the reflectance spectra of samples to their reference values in order to develop predictive models or calibrations. The reference values are usually obtained from standardised laboratory analyses of the diet or faeces or from measurements of dietary attributes in the animal, such as *in vivo* digestibility and voluntary intake. As discussed by Dryden (2003), the precision of an NIRS predictive model is strongly influenced by the error in the reference measurements. This error of measurement can be expressed by the repeatability of measurement or by the SEM. The SEM can be considered as an estimation of the standard deviation linked to the error of the measurement. Few studies have described the repeatability of measurements of diet *in vivo* digestibility and intake as reference values for predictive models. Andueza et al. (2007, 2011a) showed that the sd_r relative to inter-animal variation reached 0.015 for OMD and was 6.00 g/kg $BW^{0.75}$ or less for DMVI for hay. In that study, the reproducibility standard deviation, linked to the environment (sites where the digestibility trials were performed), was similar for digestibility (0.018 for OMD), but somewhat higher for intake (more than 7.00 g/kg $BW^{0.75}$ for DMVI). In our study, the OMD sd_r was higher than in these previous studies, with variability linked to the sheep and the 6-day measurement interval ranging between 0.029 and 0.104. Our experimental design indicated that intake repeatability was better or similar, with sd_r values ranging between 1.62 and 7.83 g/kg $BW^{0.75}$ for DMVI or OMVI. Smaller sd_r values were observed at the start of the trial period. At that point, fresh forage was highly digestible and the measured voluntary intake was also higher, but there was some variability between sheep and between days. Intake variability increased with the maturity of the fresh forage. In our study, the Global SEM_{6d} was particularly low for voluntary intake parameters (OMVI and DMVI), probably because in our experimental design voluntary intake was measured daily and per sheep over a long period. Intake measurement variability reached 10% of the mean, as was the case in studies reported by Andueza et al. (2007, 2011a). Those authors concluded that it was not possible to estimate intake below this level of precision. In our study, OMD variability reached 8% of the mean, which was higher than that reported by Andueza et al. (2007, 2011a). Once again, this higher OMD variability expressed by the Global sd_r of the three studied forages could be explained by our experimental design. In order to cover a grass growth cycle, the length of our measurement periods was 42 and 16 days for rye grass 4 n and 2 n and for mixed forage, respectively. The Global sd_r of OMD, DMVI and OMVI was calculated on a 6-day moving average during this first cycle of plant development, whereas the usual measurement interval when determining OMD, DMVI and OMVI is 6 days (Demarquilly et al., 1995). In this scheme, the OMD, DMVI and OMVI variations could probably be explained by the modification of plant chemical composition during plant growth. The maturing of the plant reduced the nutritive value of the forage (Decruyenaere et al., 2009) and, in the 6-day mobile mean scheme, gave higher inter-animal and inter-day variability, especially for OMD. The variability in OMD, DMVI and OMVI could be also explained by the ability of sheep to select their diet when they were fed *ad libitum* or when complex forages were used (Andueza et al., 2011a). This was not observed in our study. The OMD, DMVI and OMVI variability observed with pure rye grass was similar to that observed with mixed forage.

Compared with the usual chemical parameters, the sd_r values of the *in vivo* characteristics were higher because of the variation between animals. This was particularly true for OMD. The forage chemical characteristics also had a significant influence on reference value repeatability and on the precision of predictive models. Sørensen (2002) reported that for *in vitro* OMD (Tilley and Terry method) there was greater imprecision in the reference values

at low digestibility levels than at high digestibility levels. This could explain why OMD variability increased with plant maturity.

The lower the error in reference values, the greater the precision required in a predictive model. As reported by Dryden (2003), samples included in NIRS calibration equations can be corrupted by laboratory errors or for FNIRS by mismatching errors. For FNIRS calibrations, the choice of the reference method appears to be very important. Lyons (1990, cited by Stuth et al., 2003) showed that when intake was estimated using an indigestible marker, such as ytterbium acetate, it was not possible to develop a reliable FNIRS calibration. This is not unexpected because using an indigestible marker to measure intake is associated with error in laboratory analysis, which would increase error associated with the reference method. The standardisation of chemical analysis is known to reduce error in reference values, but it was more difficult to establish *in vivo* characteristics because of animal variability. Working with animals confined in individual pens or digestibility crates, as in our study, provided more accurate reference values and allowed the most reliable diet-faecal pairs (Coates and Dixon, 2010).

High repeatability of reference values should reduce the uncertainty of the FNIRS predictions. As noted by Sørensen (2002), the true accuracy of an NIR model is closely related to the spectroscopic measurement without the reference method uncertainty. The SECV and SEP of an NIRS calibration have been identified as indicators of the predictive ability of an NIRS calibration equation and are usually similar to SEC (Stuth et al., 2003). In addition, SEP should not be higher than twice the laboratory standard error (Westerhaus, 1985, cited by Stimson et al., 1991). For OMD, the uncertainty linked to the reference values, expressed by the Global SEM_{6d}, was similar to the SECV of the calibration. Our results showed that, for OMD, apparent SEP was similar to the SEC of the calibration equation and lower than twice the Global SEM_{6d}. As discussed by Coates (2002), our results suggested that OMD FNIRS prediction was probably as accurate as the reference measurement. For OMVI and DMVI, corrected SEP and apparent SEP were very similar, but twice as high than either SEC or SECV. For these intake parameters, apparent SEP was greater than Global SEM_{6d}. These results suggested that the error of prediction was probably not due to error in the references values. Reference intake value variability expressed by sd_r was close to the SECV of the FNIRS calibration for DMVI and OMVI. This suggested that, for DMVI and OMVI, a large part of the prediction error was probably linked to the FNIRS models. These results accord with the report by Coleman (2010) that crude protein could be predicted best, followed by digestibility, but the prediction of intake by FNIRS was uncertain.

The factors affecting FNIRS have not been well studied (Walker, 2010). Some have been identified by Andueza et al. (2011b). One is that digestibility and intake are not really chemical entities, but are instead correlated with chemical properties of the diet. Another factor concerns the natural variation introduced by forage heterogeneity. As discussed by Coleman (2010), voluntary intake is more difficult to predict because it is a multi-factorial phenomenon and is influenced not only by forage characteristics, but also by many aspects of the physiology of the animal. Our FNIRS spectral database includes different levels of intake (from maintenance to *ad libitum*) obtained from a small sheep population and from fresh forage obtained at different stages of the plant growth cycle (Decruyenaere et al., 2009). Using this experimental design, it is possible that the variability of intake reference values was too small for a sufficiently variable spectral database to be developed. This was not the case for OMD, probably because of the more important decrease in digestibility during plant growth. As confirmed by Williams (2001, cited by Walker, 2010), five or six growing

seasons were needed to represent grain variability adequately, and this was probably the same for FNIRS libraries.

In order to develop adequate spectral libraries, another important challenge in developing FNIRS calibration databases is to avoid mismatching errors. As noted by Coates and Dixon (2010), FNIRS calibrations for predicting OMD, DMVI or OMVI are derivative calibrations because the sample analysed for reference values (diet samples) differs from the samples submitted to NIRS analyses (faeces). The major problem with FNIRS lies in generating reliable diet-faecal pairs, especially when the diet-faecal sample pairs are obtained from oesophageally fistulated animals or using manual plucking. This should not be a problem when the diet-faecal pairs are obtained from animals fed in pens. Finally, improving the precision of FNIRS models should depend, *inter alia*, on the measures taken to minimize mismatching errors and to increase the heterogeneity of the spectral database.

The repeatability of the spectral measurements was in line with the WINISI® 1.50 software recommendations. Repeated measurements of the same sample led to an RMS lower than the fixed limit of 0.03 absorbance units. The sample presentation induced variations that were explained by the sub-sampling for SP-1 and by the sedimentation of sample particles in the cup for SP-2 (Dardenne, 1990).

For all the tested FNIRS calibration equations, FNIRS prediction repeatability appeared to be similar for OMD, DMVI, OMVI and the botanical composition of ingested forages. No outliers were detected and, as reported by Genot et al. (2011), these results suggested that FNIRS prediction was sensitive enough for all studied parameters. All the s_r values in our FNIRS predictions were lower than the SECV of the respective calibration equation. For OMVI, however, there was a small difference between GLOBAL and LOCAL predictions (5.3 g/kg $BW^{0.75}$ on average), but this difference was minimal and similar to the SECV of the entire FNIRS model. These results highlight the difficulty of building FNIRS databases for predicting intake level.

V. Conclusion

NIRS applied to faeces can be used to predict the *in vivo* characteristics of forage. In our study, the OMD prediction was sufficiently repeatable and accurate in terms of performing the measurement using the reference method. Intake was more difficult to predict with sufficient precision and appeared to be more closely linked to the uncertainty of the FNIRS models. Future work will be needed to improve the precision of intake prediction, where the major difficulty lies in generating diet-faecal pairs that are as reliable as possible and FNIRS libraries that are more representative of various field conditions.

VI. References

Adegosan, A.T., Givens, D.I., Owens, E., 2000. Measuring chemical composition and nutritive values in forages, in: ’t Mannetje L. and Jones R.M. (Eds.), Field and laboratory methods for grassland and animal production research. CAB International, pp. 263-278.

Andueza, D., Picard, F., Pecatte, J.R., Gallard, Y., Hassoun, Ph., Viudes, G., Egal, D., Pradel, Ph., Trocquier, O., Thomas, D., Delaby, L., Agabriel, J., Baumont, R., 2007. Variability within and among laboratories of *in vivo* digestibility and voluntary intake of two hays evaluated in sheep. Renc. Rech. Rum., 14, 247 (in French).

Andueza, D., Picard, F., Pradel, Ph., Egal, D., Hassoun, P., Pecatte, J.R., Beaumont, R., 2011a. Reproducibility and repeatability of forage *in vivo* digestibility and voluntary intake of permanent grassland forages in sheep. Livest. Sci., 140, 42-48.

Andueza, D., Picard, F., Jestin, M., Andrieu, J., Baumont, R., 2011b. NIRS prediction of the feed value of temperate forages efficacy of four calibration strategies. Animal, 5, 1002-1013.

Aufrère, J., Baumont, R., Delaby, L., Peccate, J.R., Andrieu, J., Andrieu, J.P., Dulphy, J.P., 2007. Prévision de la digestibilité des fourrages par la méthode pepsine-cellulase. Le point sur les équations proposées. INRA Prod. Anim., 20, 129-136.

Boval, M., Coates, D.B., Lecomte, P., Decruyenaere, V., Archimède, H., 2004. Faecal near infrared reflectance spectroscopy (NIRS) to assess chemical composition, *in vivo* ingestibility and intake of tropical grass by Creole cattle. Anim. Feed Sci. Technol., 114, 19–29.

Coates, D.B., 2002. Is near infrared spectroscopy only as good as the laboratory reference values? An empirical approach. Spectrosc. Eur., 14, 24-26.

Coates, D.B., 2004. Faecal NIRS – Technology for improving nutritional management of grazing cattle. Final Report of Project NAP3.121. Meat and Livestock Australia, Sydney (available at www.mla.com.au).

Coates, D.B., Dixon, R.M., 2010. Fecal NIRS calibration for predicting protein and digestibility in the diet of cattle: fistulated and pen feeding procedures for generating diet-fecal pairs, in: Walker, J. and Tolleson, D. (Eds.), Shining light on manure improves livestock and land management. Texas Agrilife Research and Society for Range Management, Technical Bulletin, pp. 23-41.

Coates, D.B., Dixon, R.M., 2011. Developing robust faecal near infrared spectroscopy calibrations to predict diet dry matter digestibility in cattle consuming tropical forages. J. Near Infrared Spectrosc. 19, 507-519.

Coleman, S.W., Stuth, J.W., Holloway, J.W., 1989. Monitoring the nutrition of grazing cattle with near-infrared analysis of faeces. XVI International Grassland Congress, Nice, France, 881-882.

Coleman, S.W., Murray, I., 1993. The use of near-infrared reflectance spectroscopy to define nutrient digestion of hay by cattle. Anim. Feed Sci. Technol., 44, 237-249.

Coleman, S.W., 2010. Historic overview for fecal NIRS analysis, in: Walker, J. and Tolleson, D. (Eds.), Shining light on manure improves livestock and land management. Texas Agrilife Research and Society for Range Management, Technical Bulletin, pp. 9-22.

Dardenne, P., 1990. Contribution de la spectromètrie dans le proche infrarouge pour l'étude de critères de qualité des céréales et des fourrages. Thèse Faculté universtaire des Sciences agronomiques de Gembloux, 173p.

De Boever, J.L., Cottyn, B.G., Andries, J.I., Buysse, F.X., Vanacker, J.M., 1988. The use of a cellulase technique to predict digestibility metabolizable and net energy of forages. Anim. Feed. Sci. Technol., 19, 247-260.

Decruyenaere, V., Stilmant, D., Lecomte, P., Buldgen, A., Dardenne, P., 2002. Improvement and validation of the NIRS analysis applied to faeces to measure grass intake in pasture, in: Durand, J.L., Emile, J.C., Huygue, C. and Lemaire, G. (Eds.), Multi-function grasslands, Quality forages, animal products and landscapes. Grassl. Sci. Eur. 7, pp. 196-197.

Decruyenaere V., Peters M., Stilmant D., Lecomte Ph., Dardenne P., 2003. Near infrared reflectance spectroscopy applied to faeces to predict dry matter intake of sheep under grazing, comparison with n-alkanes and direct biomass measurement methods. Tropical and Subtropical Agroecosyst., 3, 471-476.

Decruyenaere, V., De Visser, M., Stilmant, D., Clément, C., Sinnaeve, G., 2004a. Faecal Near Infrared Reflectance Spectroscopy for ruminant feed intake prediction, in: Lüscher, A., Jeangros, B, Kessler, W., Huguenin, O., Lobsiger, M., Millar, N., Suter, D. (Eds.), Land use systems in grassland dominated regions. Grassl. Sci. Eur. 9, pp.1034-1036.

Decruyenaere V., Peters M., Stilmant D., Lecomte Ph., Dardenne P., 2004b. Near infrared spectroscopy applied to faeces to predict botanical composition of sheep intake. http://www.nirpublications.com.

Decruyenaere, V., Lecomte, Ph., Demarquilly, C., Aufrere, J., Dardenne, P., Stilmant, D., Buldgen A., 2009. Evaluation of green forage intake and digestibility in ruminants using near infrared reflectance spectroscopy (NIRS): Developing a global calibration. Anim. Feed Sci. Technol., 148, 138-156.

Demarquilly, C., Chenost, M., Giger, S., 1995. Pertes fécales et digestibilités des aliments et des rations, in : Jarrigue, R, Ruckenbusch, Y., Demarquilly, C., Farce, M-H., Journet, M., (Eds.), Nutrition des ruminants domestiques, ingestion et digestion. INRA, France, pp. 601-647.

Dixon, R., Coates, D., 2009. Review: Near infrared spectroscopy of faeces to evaluate the nutrition and physiology of herbivores. J. Near Infrared Spectrosc. 17, 1-31.

Dove, H., Mayes, R.W., 1996. Plant wax components: a new approach to estimating intake and diet composition in herbivores. J. Nutr., 126, 13-26.

Dove, H., Mayes, R.W., 2005. Using n-alkanes and other plant wax components to estimate intake, digestibility and diet composition of grazing/browsing sheep and goats. Small Rumin. Res., 59, 123-139.

Dryden, G.McL., 2003. Near infrared spectroscopy: applications in deer nutrition. RIRCD Publication No. W03/007, 38p.

Faaber, K., 2005. Multivariate prediction uncertainty. Chemometrics, Editorial Aug. 2005, 4p. http://www.chemometrics.se/Editorial.

Fernandez Pierna, J.A, Jin, L., Wahl, F., Faber, N.M., Massart, D.L., 2003. Estimation of partial least squares regression prediction uncertainty when the reference values carry a sizeable measurement error. Chemom. Intell. Lab. Syst., 65, 281-291.

Ferreira, L.M.M., Oliván, M., Celaya, R., Garcia, U., Rodrigues, M.A.M., Osoro, K., 2007. The use of the alkane technique to estimate diet selection of sheep grazing grass–clover/heather-gorse vegetation communities. J. Sci. Food Agric., 87, 274–285.

Garrido-Varo, A., 2006. La spectrométrie dans le proche infrarouge : une technologie d'appui pour un "service intégral" en alimentation animale, in : Bertrand D. and Dufour E. coord. La spectromètrie infrarouge et ses applications. Tec & Doc Eds, pp. 537-559 (in French).

Genot, V., Colinet, G., Bock, L., Vanvyve, D., Reusen, Y., Dardenne, P., 2011. Near infrared reflectance spectroscopy for estimating soil characteristics valuable in the diagnosis of soil fertility. J. Near Infrared Spectrosc. 19, 117-138.

Holechek, J.L., Shenk, J.S., Vavra, M., Arthum, D., 1982. Prediction of forage quality using near-infrared reflectance spectroscopy on oesophageal fistula samples from cattle on mountain range. J. Anim. Sci. 55, 971-975.

ISO 5725-1, 1994. Accuracy (trueness and precision) of measurement methods and results. Part 1: General principles and definitions. International Organisation for Standardization, Geneva, Switzerland, 17p.

Keli, A., Andueza, D., de Vega, A., Guada, J.A. 2008. Validation of the n-alkane and NIRS techniques to estimate intake, digestibility and diet composition in sheep fed mixed lucerne: ryegrass diets. Livest. Sci. 119, 42-54.

Landau, S., Giger-Reverdin, S., Rapetti, L., Dvash, L., Dorleans, M., Ungar, E.D., 2008. Data mining old digestibility trials for nutritional monitoring in confined goats with aids of near-infrared spectroscopy. Small Rumin. Res., 77, 146-158.

Leite, E.R., Stuth, J.W., 1995. Fecal NIRS equations to assess diet quality of free ranging goats. Small Rumin. Res. 15, 223-230.

Lyons, R.K., 1990. Fecal indices of nutritional status of free ranging cattle using near infrared reflectance spectroscopy. PhD. Dissertation, Texas A&M University, College Station, TX, USA, 140p.

Mayes, R.W., Lamb, C.S., Colgrove, P.M., 1986. The use of dosed and herbage n-alkanes as markers for determination of herbage intake. J. Agric. Sci. Camb., 107, 161-170.

Naes, T., Isaksson, T., Fearn, T., Davies, T., 2002. A user-friendly guide to multivariate calibration and classification. NIR Publications, Chichester UK, 30-33.

Pérez-Ramirez, E., Peyraud, J.L., Delagarde, R., 2012. N-alkanes *v.* ytterbium/faecal index as two methods for estimating herbage intake of dairy cows fed on diets differing in the herbage: maize silage ratio and feeding level. Animal, 6, 232–244.

Shenk, J.S., Westerhaus, M.O., 1991. Population definition, sample selection and calibration procedures for near-infrared reflectance spectroscopy. Crop Sci. 31, 469-474.

Sørensen, L.K., 2002. True accuracy of near infrared spectroscopy and its dependence on precision of reference data. J. Near Infrared Spectrosc. 10, 15-25.

Stimson, C., Kellaway, R.C., Tassell, R.J., Ison, R.L., 1991. Prediction of the nutrient content of botanical fractions from annual legumes by near-infrared reflectance spectroscopy. Grass Forage Sci., 46, 99–105.

Stuth, J.W., Kapes, E.D., Lyons, R.K., 1989. Use of near infrared spectroscopy to assess nutritional status of cattle diets on rangeland. XVI International Grassland Congress, Nice, France, 889-890.

Stuth, J., Jama, A., Tolleson, D., 2003. Direct and indirect means of predicting forage quality through near infrared reflectance spectroscopy. Field Crop Research, 84, 45-56.

Tilley, J.H.A, Terry, R.A., 1963. A two-stage technique for 'in vitro' digestion of forage crops. Grass Forage Sci., 18, 104-111.

Wainman, F.W., Dewey, P.J., Boyne, A.W., 1981. Compound feedingstuffs for ruminants. Third report, feedingstuffs evaluation unit, Rowett Research Institute, Aberdeen, UK, 49p.

Walker J.W., 2010. Primer on Near Infrared Spectroscopy, in: Walker, J and Tolleson, D. (Eds.), Shining light on manure improves livestock and land management. Texas Agrilife Research and Society for Range Management, Technical Bulletin, pp. 1-7.

Walker, J.W., Campbell, E.J., Kott, R.W., Landau, S.Y., Lupton, C.J., Scott, C.B., Surber, L., Taylor Jr, C.A., Whitworth, W.R., 2010. Fecal NIRS for predicting botanical composition of herbivore diets, in: Walker, J and Tolleson, D. (Eds.), Shining light on manure improves livestock and land management. Texas Agrilife Research and Society for Range Management, Technical Bulletin, pp. 53-65.

Westerhaus, M.O., 1985. Interpretation of regression statistics, in: Marten, G.C., Shenk, J.S., and Barton, E.F. (Eds.), Near infrared reflectance spectroscopy (NIRS): analysis of forage quality. Agricultural Handbook No. 643, United States Department of Agriculture.

Williams, P.C., 2001. Implementation of near-infrared technology, in: Williams, P. and Norris, K. (Eds.), Near-infrared technology in the agricultural and food industries (2^{nd} edn). Americain Association of Cereal Chemist, Inc. St. Paul, MN, pp. 171-186.

Discussion of the Chapter III

As shown in the results presented in Chapter III, the forage and faeces NIRS calibrations for estimating *in vivo* organic matter digestibility (OMD) provided excellent statistics ($R^2 > 0.90$) and were sufficiently robust, with an RPD ratio (standard deviation [SD]/standard error of cross-validation [SECV]) higher than 3 (William, 2004). In the NIRS analysis of forage, the SECV was 0.023. De Boever et al. (1996) and De La Roza et al. (2000) reported that the OMD of grass silage was better correlated with NIR-estimated OMD than with *in vitro* enzymatic OMD or rumen fluid OMD. Norris et al. (1976) and Lippke et al. (1989) also showed that digestibility could be quantified from the NIRS analysis of forage sampled in the field or obtained from oesophageal fistula, with the standard error of calibration (SEC) varying between 0.032 and 0.036. With the SECV varying from 0.021 to 0.018, calibrations developed from all faeces databases (faeces spectra alone, substracted spectra and concatenated spectra) were efficient enough to estimate the OMD of temperate fresh forage. This accuracy of FNIRS prediction was confirmed by Coleman et al. (1989) and Stuth et al. (1989).

As suggested by our results, the precision of FNIRS models for estimating OMD was similar to or better than that obtained using other predictive methods, such as the faecal nitrogen index (FNI) (Greenhalgh and Corbett, 1960; Bartiaux-Thill and Oger, 1986; Comeron and Peyraud, 1993; Boval et al., 1996; Bouazizi and Majdoub, 1999). Although the *n*-alkanes ratio was one of the best methods for estimating OMD at pasture, the precision of the OMD prediction obtained using this technique could be influenced by the lack of precision of the analytical procedure and by the partial digestibility of some *n*-alkanes chains (Sandberg et al., 2000; Moshtaghi Nia and Wittenberg, 2002). As the *n*-alkanes ratio was based on the analysis of both forages and faeces, forage sampling was a key issue. It should be as representative as possible of the ingested diet if an accurate estimation is to be obtained.

Under our experimental conditions, estimating the voluntary organic matter intake (OMVI) from an NIRS analysis of forage was difficult ($R^2 = 0.30$, SEC = 7.29 g/kg $BW^{0.75}$; SECV = 7.47 g/kg $BW^{0.75}$). Similar results were obtained by Norris et al. (1976), Ward et al. (1982) and Minson et al. (1983). These studies reported that voluntary dry matter intake could be measured by NIRS analyses of forage samples but with the SEC ranged between 7 and 9 g/kg $BW^{0.75}$. Working on faecal spectra improved the statistics of the NIRS models. Based on sheep faeces databases, FNIRS calibrations developed to estimate the OMVI presented an R^2 that varied between 0.80 and 0.90 and a SECV less than 5 g/kg $BW^{0.75}$, leading to RPD values that were between 2.31 and 2.52. The NIRS equations developed from concatenated databases seemed more suitable for estimating the OMVI (SECV = 4.13 g/kg $BW^{0.75}$). For concatenated faeces-forage spectra, however, the weak point remains the forage sampling, which could differ from that of ingested forage. FNIRS calibration developed from cattle faeces (dairy and suckling cows) databases to estimate DMI had an excellent R^2. The estimation error was acceptable (SECV = 6.78 g/kg $BW^{0.75}$). With the RPD ratio ranging between 3.1 and 4.9, the DMI calibration was robust enough for a good screening. Compared with the earliest studies in this area (Stuth et al., 1989; Coleman et al., 1989), our calibration equations developed from faeces (sheep or cattle origin) and from concatenated spectra were more accurate. Their performances were similar to those obtained by Boval et al. (2004) and Landau et al. (2004) with ruminants (cattle and goats) grazing tropical grasslands. Compared with other methods, such as the *n*-alkanes or ratio

techniques, FNIRS was just as accurate for estimating the intake of different ruminant species, such as sheep, cattle and goats (Mayes and Dove, 2000).

In order to improve the management of grazing ruminants, knowledge of the botanical composition of ingested diets is important. Determining the composition of ingested diets in terms of forage species or legume proportions could be useful for adjusting supplementation and for behavioural studies. Very few studies have focused on determining the botanical composition of grazing diets, probably because of the lack of adequate methods for measuring this parameter (Walker et al., 2010). FNIRS appeared to be a good method for estimating diet composition. Several studies have shown that an NIRS analysis of forage can predict the proportion of legumes in forage samples with a prediction error of less than 4% (Mika et al., 1998; Leconte et al., 1999; Stilmant et al., 2001; Locher et al., 2005). In our study, the same level of accuracy was reached with FNIRS (SECV = 3.99% for the prediction of the clover proportion in the diet), but for our small database the RPD ratio was lower 3 (RPD = 2.15). The FNIRS predictions were therefore as accurate as other methods used to predict the composition of ingested diets.

A question emerging from our results was why the NIRS analysis of faeces was as efficient, if not more so, as the NIRS analysis of forage for assessing diet characteristics. OMD, DMVI and OMVI depend on various parameters, such as plant characteristics (Jung and Allen, 1995; Allen, 1996), digestion rate in the rumen (Illius and Jessop, 1996) and animal behaviour (Faverdin, 1999; Provenza et al., 2003). These factors, directly linked to the animal, are difficult or impossible to quantify only by analysing forage samples (Coelho et al., 1988). Faeces can contain biological and chemical information on forage or diets consumed by animals, as well as on their physiological status. Our results showed that, on the second derivative spectra of faeces from forage with low digestibility and a low intake level, there were higher peaks in the wavelength region of fibres (2078 to 2110 nm, 2268 nm). As confirmed by Coleman and Murray (1993) and Leite and Stuth (1995), fibre peaks were higher in faeces when the supplied forage was old. This could be explained by the accumulation of more fibre residues in faeces when digestibility decreases. In particular, negative peaks centred at 1730, 1764, 2310 and 2350 nm could be associated with fat. Peaks in these spectral regions were higher when plant digestibility and intake were high, as confirmed by Leite and Stuth (1995). The importance of fat peaks in faeces could be related to the presence of endogenous residues directly linked to microbial activity in the rumen. Thus, Lecomte (1995) reported that on forage nylon bag residues (measurement of *in situ* degradability) NIRS absorbency peaks appeared clearly at 1722 and 2306 nm, characteristic wavelengths of the O-H link, representative of fatty acid. As the rumen microbes contained a high proportion of stearic acid (532 g/kg DM) (Lecomte et al., 1994), the importance of these wavelengths for estimating OMD and OMVI could be linked to higher microbial growth in the rumen in relation to high forage quality, as well as to a higher proportion of microbes linked to the faecal forage residues. The relevance of fat wavelengths could also be linked to the presence in faeces of cuticular waxes with a plant origin, such as *n*-alkanes commonly used to estimate digestibility and intake (Coleman and Murray, 1993). The chemical composition of undigested feed and the microbial activity can therefore be detected by NIRS and successfully correlated with OMD and DMVI or OMVI.

NIRS is a predictive method of analysis linking reflectance spectra to reference values. In most cases, the reference values result from standardised laboratory analysis. The repeatability of reference values or the standard error of the mean (SEM) is often used to describe the error in the measurement of reference value. As discussed by Dryden (2003), the precision of NIRS predictive models is strongly influenced by this error in the reference

measurements. It is therefore necessary to determine the prediction error of FNIRS models and the repeatability of FNIRS measurements.

Our FNIRS spectral libraries were built from *in vivo* reference values (OMD, DMVI, OMVI and botanical composition of ingested diets) obtained through digestibility trials. Few studies have described the repeatability of measurements of *in vivo* digestibility and intake as reference values for predictive models. Andueza et al. (2007, 2011) showed that, for hay, the repeatability standard deviation (sd_r) relative to inter-animal variation reached 0.015 for OMD and 6.00 g/kg $BW^{0.75}$ or less for DMVI. In our results, the OMD sd_r was higher, with variability linked to the sheep and to the 6-day measurement interval, ranging between 0.029 and 0.104. Intake repeatability was similar or better, with sd_r values ranging between 1.62 and 7.83 g/kg $BW^{0.75}$ for intake (DMVI or OMVI).

As in the case of all indirect methods of prediction, the precision and accuracy of FNIRS is linked to the repeatability of the reference values. Such errors can be reduced by the standardisation of chemical analysis, but it was more difficult to establish for the *in vivo* digestibility, intake or botanical composition of ingested forages because of inter-animal and temporal variability of forage. Inter-animal variability is difficult to reduce, except by increasing the number of animals. Working with animals confined in individual pens or digestibility crates, however, as in our study, provided more accurate reference values for generating reliable diet-faecal pairs (Coates and Dixon, 2010). FNIRS calibrations for predicting OMD, DMVI or OMVI are derivative calibrations because the sample analysed for reference values (diet samples) differs from the samples submitted to NIRS analyses (faeces) (Coates and Dixon, 2010). An important challenge in developing FNIRS calibration databases, therefore, is to avoid mismatching errors.

The precision of an FNIRS calibration equation is commonly expressed through the standard error of prediction (SEP). This SEP has three components: (i) errors associated with the capacity of the calibration model to predict the attribute; (ii) errors associated with the measurement, and its repeatability, of the reference values; and (iii) errors associated with the measurement, and its repeatability, of the NIR spectra.

As noted by Sørensen (2002), the true accuracy of NIRS models is closely related to the spectroscopic measurement without the reference method uncertainty (standard error of the mean [SEM] of the reference measurement). The SECV and SEP have been identified as indicators of the predictive ability of an NIRS calibration equation and are usually similar to SEC (Stuth et al., 2003). For OMD, the uncertainty linked to the reference values (Global SEM_{6d}) was similar to the SECV of the calibration. In addition, the apparent SEP of the OMD was similar to the SEC of the calibration. As in the results reported by Coates (2002), our results suggested that OMD FNIRS prediction was probably as accurate as the reference measurement. For OMVI and DMVI, corrected SEP and apparent SEP were very similar, but twice as high as either SEC or SECV (apparent SEP = 9.11 and 8.42 g/kg $BW^{0.75}$ for DMVI and OMVI, respectively), whereas global SEM_{6d} was close to 2 g/kg BW0.75 for both DMVI and OMVI. These results suggested that the error of prediction was probably not due to a lack of accuracy of the references values (SEM was lower than the SEP), but to a lack of variability in the intake reference values. These results agree with the report by Coleman (2010) that intake is more difficult to predict because it is a multi-factorial phenomenon influenced not only by the chemical characteristics of forage, but also by animal grazing behaviour. Our FNIRS intake database was probably not representative enough of the intake variability in the independent datasets. Williams (2001, cited by Walker, 2010) confirmed that five or six growing seasons were needed to represent grain variability adequately. It is

probably the same for FNIRS. Increasing the heterogeneity of the FNIRS databases would therefore probably improve the precision of intake prediction. Our reference intake value variability expressed by the repeatability standard deviation (sd_r), however, was close to the SECV of the FNIRS calibration for DMVI and OMVI. This suggested that a large part of the prediction error was probably linked to the FNIRS models.

Finally, spectra repeatability was studied through the repeated measurement of four faeces samples under two presentation modes. The repeatability of the spectral measurements was in line with the WINISI® 1.50 software recommendations. The FNIRS prediction repeatability (s_r) was estimated through two analyses of variance (the factors of variance were sample and sample presentation mode for DMVI predictions and grass and legume proportion of ingested diet prediction; sample, sample presentation mode and NIRS prediction technique for OMD and OMVI predictions). The FNIRS prediction repeatability was adequate for the OMD, OMVI, DMVI and botanical composition of ingested forages. No outliers were detected and, as reported by Genot et al. (2011), these results suggested that the FNIRS prediction was sensitive enough for all studied parameters. All the s_r values in our FNIRS predictions were lower than the SECV of the respective FNIRS calibration equation. For OMVI, however, there was a small difference between the NIRS prediction techniques (on average, 5.3 g/kg $BW^{0.75}$), but this difference was minimal and similar to the SECV of the FNIRS model. These results highlight the difficulty in building a sufficiently representative FNIRS database for predicting intake.

References

Allen, M.S., 1996. Physical constraints on voluntary intake of forages by ruminants. J. Anim. Sci. 74, 3063-3075.

Andueza, D., Picard, F., Pecatte, J.R., Gallard, Y., Hassoun, Ph., Viudes, G., Egal, D., Pradel, Ph., Trocquier, O., Thomas, D., Delaby, L., Agabril, J., Baumont, R., 2007. Variability within and among laboratories of *in vivo* digestibility and voluntary intake of two hays evaluated in sheep. Renc. Rech. Rum. 14, 247 (in French).

Andueza D., Picard F., Pradel Ph., Egal D., Hassoun P., Pecatte J.R., Beaumont R., 2011. Reproductibility and repeatability of forage *in vivo* digestibility and voluntary intake of permanent grassland forages in sheep. Livestock Sci., 140, 42-48.

Bartiaux-Thill, N. Oger, R., 1986. The indirect estimation of the digestibility in cattle of herbage from Belgian permanent pasture. Grass and Forage Sci. 41, 269-272.

Bouazizi, A., Majdoub, A., 1999. Prédiction des quantités ingérées et de la digestibilité du régime sélectionné par des ovins sur parcours semi-aride tunisien. Fourrages 157, 77-87.

Boval, M., Peyraud, J.L., Xande, A., Aumont, G., Coppry, O., Saminadin, G., 1996. Evaluation d'indicateurs fécaux pour prédire la digestibilité et les quantités ingérées de Dichanthium sp par des bovins créoles. Ann. Zootech. 45, 121-134.

Boval, M., Coates, D.B., Lecomte, P., Decruyenaere, V., Archimède, H., 2004. Faecal Near Infrared Reflectance Spectroscopy (NIRS) to assess chemical composition, *in vivo* digestibility and intake of tropical grass by Creole cattle. Anim. Feed Sci. Technol., 114, 19-29.

Coates D.B., 2002. Is near infrared spectroscopy only as good as the laboratory references values? An empirical approach. Spectroscopy Europe, 14, 24-26.

Coates D.B., Dixon R.M., 2010. Fecal NIRS calibration for predicting protein and digestibility in the diet of cattle : fistulated and pen feeding procedures for generating diet-fecal pairs. In Walker J and Tolleson D. Eds. Shining light on manure improves livestock and land management, Texas Agrilife Research and Society for Range Management, Technical bulletin, 23-41.

Coelho, M., Hembry, F.G., Barton, F.E., Saxton, A.M., 1988. A comparison of microbial, enzymatic, chemical and near-infrared reflectance spectroscopy methods in forage evaluation. Anim. Feed Sci. Technol. 20, 219-231.

Coleman, S.W., Stuth, J.W., Holloway, J.W., 1989. Monitoring the nutrition of grazing cattle with near-infrared analysis of faeces. XVI International Grassland Congress, Nice, France, 881-882.

Coleman, S.W., Murray, I., 1993. The use of near-infrared reflectance spectroscopy to define nutrient digestion of hay by cattle. Anim. Feed Sci. Technol. 44, 237-249.

Coleman S.W., 2010. Historic overview for fecal NIRS analysis. In Walker J and Tolleson D. Eds. Shining light on manure improves livestock and land management, Texas Agrilife Research and Society for Range Management, Technical bulletin, 9-22.

Comeron, E.A., Peyraud, J.L., 1993. Prediction of herbage digestibility ingested by dairy cows. Rev. Argent. Prod. Anim. 13, 23-30.

De Boever, J.L., Cottyn, B.G., De Brabander, D.L., Vanacker, J.M., Boucqué, Ch.V., 1996. Prediction of the feeding value of grass silages by chemical parameters, in vitro digestibility and near-infrared reflectance spectroscopy. Anim. Feed. Sci. Technol. 60, 103-116.

De La Roza, B., Martinez, A., Modrono, S., Flores, G., Argamenteria, A, 2000. A comparison of near infrared spectroscopy with neutral detergent cellulase techniques to predict the *in vivo* digestibility of grass silage. In A.M.C. Davies and R. Giangiacomo (Eds), Proceedings of the 9th International conference - Verona Italy, pp.661-665.

Dryden, G.McL., 2003. Near infrared spectroscopy : applications in deer nutrition. RIRCD Publication n° W03/007, 38p.

Faverdin, P., 1999. The effect of nutrients on feed intake in ruminants. Proc. Nutr. Soc. 58, 523-531.

Genot, V., Colinet, G., Bock, L., Vanvyve, D., Reusen, Y., Dardenne, P., 2011. Near infrared reflectance spectroscopy for estimating soil characteristics valuable in the diagnosis of soil fertility. J. Near Infrared Spectrosc. 19, 117-138.

Greenhalgh, J.F.D., Corbett, J.L., 1960. The indirect estimation of digestibility of pasture herbage. 1. Nitrogen and chromogen as faecal index substance. J. Agric. Sci., Camb. 55, 371-376.

Illius, A.W., Jessop, N.S., 1996. Metabolic constraints on voluntary intake in ruminants. J. Anim. Sci. 74, 3052-3062.

Jung, H.G., Allen, M.S., 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J. Amin. Sci. 73, 2774-2790.

Landau, S., Glasser, T., Dvash, L., Perevolotsky, A., 2004. Faecal NIRS to monitor diet of mediterranean goats. South African J. Anim. Sci. 34, (suppl 1), 76-80.

Lecomte, Ph., Kamoun, M., Agneessens, R., Beckers, Y., Dardenne, P., François, E., 1994. Approach on the use of near infrared spectrometry for the estimation of microbial nitrogen contamination of forages incubated in nylon bag. Proc. Soc. Nutr. Physiol. 3, p44.

Lecomte, Ph., 1995. Gestion intégrée des ressources fourragères naturelles et de l'élevage dans la savane guinéenne de basse altitude. PhD Thesis, FUSAGx, 317 p.

Leconte, D., Dardenne, P., Clement, C., Lecomte, Ph., 1999. Near infrared determination of the morphological structure of rye grass swards. Proceedingd of the 9th International Conference of Near Infrared spectroscopy, Eds Davies A.M.C. and Giangiacomo R., NIR Publications, Chichester, UK, 41-44.

Leite, E.R., Stuth, J.W., 1995. Fecal NIRS equations to assess diet quality of free ranging goats. Small Ruminant Res. 15, 223-230.

Lippke, H., Barton II, F.E., Ocumpaugh, W.R., 1989. Near infrared reflectance spectroscopy for estimation of digestible organic matter intake and body weight gain. XVI International Grassland Congress, Nice, France, 893-894.

Locher, F, Heuwinkel, H., Gutser, R., Schmidhalter, U., 2005. Development of near infrared reflectance spectroscopy calibrations to estimate legume content of multispecies legume–grass mixtures. Agron. J. 97:11–17.

Mayes, R.W., Dove, H., 2000. Measurement of dietary nutrient intake in free ranging mammalian herbivores. Nutr. Res. Review 13, 107-138.

Mika, V., Nerusil, P., Smital, F., 1998. The use of NIR spectroscopy for the prediction of botanical composition of grassland. In G. Nagy and K. Peto (eds), : Ecological aspect of grassland management, Proceedings of the 17th EGF Meeting, 479-481.

Minson, D.J., Butler, K.L., Grummitt, H.N., Law, D.P., 1983. Bias when predicting crude protein, dry matter digestibility and voluntary intake of tropical grasses by near-infrared reflectance. Anim. Feed Sci. Technol. 9, 221-237.

Moshtaghi Nia, S.A., Wittenberg, K.M., 2002. Evaluation of n-alkanes as markers for estimation of dry matter intake and digestibility in steers consuming all-forage or forage-concentrate diets. Can. J. Anim. Sci. 82, 419-425.

Norris, K.H., Barnes, R.F., Moore, J.E., Shenk, J.S., 1976. Predicting forage quality by infrared reflectance spectroscopy. J. Anim.Sci. 43, 889-897.

Provenza, F.D., Villalba, J.J., Dziba, L.E., Atwood, S.B., Banner, R.E., 2003. Linking herbivore experience, varied diets, and plant biochemical diversity. Small Ruminant Res. 49, 257-274.

Sandberg, R.E., Adams, D.C., Klopfenstein, T.J., Grant, R.J., 2000. N-alkane as an internal marker for predicting digestibility of forages J. Range Manage. 53, 159-163.

Sørensen, L.K., 2002. True accuracy of near infrared spectroscopy and its dependence on precision of reference data. J. Near Infrared Spectrosc. 10, 15-25.

Stilmant, D., Delagarde, R., Clément, C., Meunier, B., Leconte, D., Lecomte, Ph., Dardenne, P., 2001. La SPIR – Un outil pour déterminer la composition spécifique et morphologique des couverts prairiaux. In Nouveaux regards sur le pâturage, Actes des Journées de l'AFPF, Paris,France, A7.

Stuth, J.W., Kapes, E.D., Lyons, R.K., 1989. Use of near infrared spectroscopy to assess nutritional status of cattle diets on rangeland. XVI International Grassland Congress, Nice, France, 889-890.

Stuth, J., Awuma, K., Tolleson, D., Ossiya, S., Ebong, C., Mnene, W., Mwilawa, A., Ebro, A., Byenkya, S., Jama, A., Kaitho, R., 2003. Infusing nutritional profiling technology in sub-Saharan Africa for free ranging livestock. Research Brief 03-04-Lews, April 2003.

Walker, J.W., 2010. Primer on near infrared spectroscopy. In Walker J and Tolleson D. Eds. Shining light on manure improves livestock and land management, Texas Agrilife Research and Society for Range Management, Technical bulletin, 1-7.

Walker, J.W., Campbell, E.J., Kott, R.W., Landau, S.Y., Lupton, C.J., Scott, C.B., Surber, L., Taylor Jr, C.A., Whitworth, W.R., 2010. Fecal NIRS for predicting botanical composition of herbivore diets. In Walker J and Tolleson D. Eds. Shining light on manure improves livestock and land management, Texas Agrilife Research and Society for Range Management, Technical bulletin, 53-65.

Ward, R.G., Smith, G.S., Wallace, J.D., Urquhart, N.S., Shenk, J.S., 1982. Estimation of intake and quality of grazed range forage by near infrared reflectance spectroscopy. J. Anim. Sci. 54, 399-402.

Williams P.C., 2001. Implementation of near-infrared technology. In Williams P. and Norris K. (Eds). Near-infrared technology in the agricultural and food industries (2nd edn). Americain Association of Cereal Chemist, Inc. St. Paul, MN, 171-186.

Williams, P., 2004. Near-infrared technology, getting the best out of light. A short course in the practical implementation of Near-infrared spectroscopy for the user. PDK Grain, Nanaimo, British Colombia, and Winnipeg, Manitoba Canada, pp.12-13.

Chapter IV: Near infrared reflectance spectroscopy applied to faeces in order to characterize forage digestibility and intake: validation

Article VI: Near infrared reflectance spectroscopy applied to faeces to predict dry matter intake of sheep under grazing, comparison with *n*-alkanes and direct biomass measurement methods

Article VII: Faecal near infrared reflectance spectroscopy (NIRS) compared with others techniques for estimating the *in vivo* digestibility and dry matter intake of lactating grazing dairy cows

Article VIII: Methodological approach in La Reunion Island on the value of faecal near infrared reflectance spectroscopy (NIRS) to assess grazing intake and diet quality of the dairy cows

Near infrared reflectance spectroscopy applied to faeces in order to characterize forage digestibility and intake: validation

The validation of NIRS predictions usually involves comparing predicted values and reference values and calculating the standard error of prediction (SEP). In our study, the selected reference method for building spectral libraries was the digestibility trial. Thus, a known quantity of forage was distributed to animals, with refusals and faeces weighed in order to calculate *in vivo* digestibility and intake. The faeces were sampled for NIRS analysis and *in vivo* characteristics and faeces pairs were generated. Under grazing conditions it is difficult to obtain such reference values. In order to validate the robustness of faecal NIRS predictions, we therefore decided to test the ability of faecal NIRS calibrations to predict digestibility and voluntary intake of different types of grazing ruminants fed with heterogeneous fresh forage through comparison with other current predictive methods. Chapter IV presents the results of these validation tests.

Near infrared reflectance spectroscopy applied to faeces to predict dry matter intake of sheep under grazing, comparison with *n*-alkanes and direct biomass measurement methods

Article VI - Adapted from Decruyenaere et al. (2003) Tropical and Subtropical Agroecosystems, 3, 471-476.

Decruyenaere V. [(1)*], Peters M. [(3)], Stilmant D. [(1)], Lecomte Ph. [(2)], Dardenne P. [(4)]

[(1)] Centre Wallon de Recherches Agronomiques (CRA-W) – Département Productions et Filières, 100 rue du Serpont, B-6800 Libramont-Chevigny, Belgium

[(2)] Centre de Coopération Internationale de Recherches Agronomiques pour le Développement – Pôle Elevage – CIRAD REUNION - Chemin Irat – Ligne Paradis, 97410 Saint-Pierre

[(3)] Faculté Universitaire des Sciences Agronomiques de Gembloux (FUSAG) – Unité de Zootechnie, 2 passages des Déportés, B-5030 Gembloux, Belgium

[(4)] Centre Wallon de Recherches Agronomiques (CRA-W) – Département Qualité des Productions Agricoles, 24 Chaussée de Namur, B-5030 Gembloux, Belgium

*corresponding author – E-mail : v.decruyenaere@cra.wallonie.be

Abbreviations: BW, body weight; NIRS, near infrared reflectance spectroscopy; VI, voluntary intake

Abstract

Estimating animal intake under grazing remains a problem. Its measurement by conventional method is costly and labour intensive. This study would evaluate the potential of near infrared spectroscopy (NIRS) applied to faeces to predict dry matter intake of sheep grazing on temperate grasslands. More especially, faecal NIRS have been compared to direct biomass measure (feeding trough trial) and to *n*-alkanes methodology.

Dry matter intake estimated by faecal NIRS is very comparable to *in vivo* intake (56.6 and 61.3 g/kg metabolic weight (body weight$^{0.75}$ (BW$^{0.75}$)) *vs* 56.9 g/kg BW$^{0.75}$ for the *in vivo* measurement), while *n*-alkanes methodology underestimates *in vivo* intake at about 30 %. Causes of this underestimation can be multiple. One of these lies in the difficulty to obtain a sample of forage truly representative of diet really ingested.

Keywords: Intake, faecal NIRS, *n*-alkanes, sheep, fresh grass

I. Introduction

Today, there is not simple and rapid method to estimate animal intake under grazing. Measurements of intake by conventional methods are costly and labour intensive. Indeed, voluntary intake determination requires measurement of faecal output and diet digestibility.

Digestibility can be approached by several techniques such as chemical or near infrared spectroscopy (NIRS) analyses of grass sampled on field or obtained by oesophageal fistulated animals (Forbes and Beattie, 1987; Ward et al., 1982; Holechek et al., 1982). The major disadvantage of these techniques is that sampled forages does not necessarily represent the diet really ingested (Jones and Lascano, 1992).

Recent development with indigestible plant cuticular wax components, especially *n*-alkanes method, has opened new techniques to estimate herbage intake of free grazing animals (Mayes et al., 1986; Dove and Mayes, 1991, 1996; Dove et al., 2000; Hendricksen et al., 2002).

Different studies have also shown that near infrared reflectance spectroscopy (NIRS) applied to faeces had some potential to predict voluntary intake and diet quality of free ranging herbivores (Decruyenaere et al., 2002; Coates, 2000; Lyons et al., 1995; Coleman and Murray, 1993; Leite and Stuth, 1995; Stuth et al., 1989; Coleman et al., 1989). Indeed, faeces are composite materials that contain undigested residues of rumen fermentations, and, as consequence, faeces can provide NIRS spectral information highly correlated with diet intake and diet's digestibility.

Objective of this study was to evaluate the potential of NIRS applied to faeces to predict dry matter intake of sheep grazing on temperate grasslands through the comparison of the results obtained with this method, on one side, and with direct biomass measurement (reference method) and *n*-alkanes methods, on the other side.

II. Materials and methods

II.1. Animals and diets

The experiment was conducted on 6 castrated sheep (83.5 ± 9.5 kg of body weight (BW)). During the experiment sheep were housed in individual box with continuously available water. Three forage plant species (*Lolium perenne* (LP), *Trifolium repens* (TR) and *Holcus lanatus* (HL)) have been offered in separate feeding trough. These plant species, that could be selected freely, were offered at a total level of intake of 70 g dry matter (DM)/kg metabolic weight ($BW^{0.75}$) but in different proportions (Table 1).

Table 1.
Plant composition (% of DM) of diet tested

Diet	Number of sheep	LP	HL	TR
Diet 1	2	50	25	25
Diet 2	2	25	50	25
Diet 3	2	30	30	40

At the beginning of the experimental period, each sheep was weighed and dosed, using a balling gun, with a *n*-alkanes CRD (CAPTEC Ltd, Auckland, New Zealand) designed for an animal from 25 to 80 kg BW. Each capsule contained 1 g of *n*-dotriaconate (C_{32}) and 1 g of *n*-hexatriaconte (C_{36}) and was designed to release approximately 50 mg of both *n*-alkanes (44.9 mg of C_{32} and C_{36} per day as indicated by the supplier), each day, for 20 days.

During the trial, each forage species was cut daily, stocked at 4°C and offered to sheep the next day. Along the experimental period (7 days of diet adaptation and 7 days of data collection), sheep were fed twice daily and forage species were individually weighed and sub-sampled for moisture determination (oven dried at 60 °C during 48 hours) and for purity of sward composition. Dried samples were finally ground in a hammer mill and in a cyclotec mill (1 mm) screen.

Individual forage residues were collected daily, weighed and samples were oven dried (60 °C during 48 hours) for moisture determination and ground in a hammer mill and a cyclotec mill (1mm screen).

Faeces were collected individually 3 times a day on the pen floor. Faecal samples were oven dried (60°C during 48 hours) and ground in a hammer mill (1 mm screen). Each sample of ground forage, residues and faeces were stored in plastic bags for future analyses.

II.2. Calculation and estimation of voluntary dry matter intake (VI)

II.1.2.1. *In vivo* measurement

Dry matter voluntary intake (VI, g/kg $BW^{0.75}$) was obtained by *in vivo* trials and calculated from the *in vivo* data by the difference between offered forages and forage residues divided by the metabolic weight ($BW^{0.75}$) of sheep (VI_{vivo}).

II.1.2.2. NIRS applied to faeces

Offered forages, forage residues and faeces were submitted in small ring cup to NIRS analysis (NIRS system monochromator 5000 – 1100 to 2500 nm of wavelength by 2 nm steps) analyses. VI was firstly predicted from a faecal NIRS calibration (VI_{NIRS}) previously established (n = 1011 samples of sheep faeces fed on fresh forage, standard deviation of the database (SD) = 11.77 g/kg $BW^{0.75}$; R^2 = 0.84; standard error of calibration (SEC) = 4.83 g/kg $BW^{0.75}$; standard error of cross validation (SECV) = 5.11 g/kg $BW^{0.75}$). VI was secondly predicted from the same database but through the application of the local procedure of WINISI® 1.50 software ($VI_{NIRSloc}$).

II.1.2.3. N-alcanes method

N-alkanes profiles of forages species and faeces were determined on 4 g ground samples according to the method of Mayes et al. (1986) but without saponification. Quantification of *n*-alkanes were performed using a gaz chromatograph (Helwett 5890 Packard serie II) fitted with a capillary column (Fused silica OPTIMA 1-DF 0.35 - 30x0.32 mm ID) coupled to a FID detector. A mix of 4 commercial synthetic (even-chain) *n*-alkanes (C_{28}; C_{30}; C_{32} and C_{36}) dissolved in heptane was used to establish *n*-alkanes retention times. The carbon chain lengths were deduced from their retention time relative to know *n*-alkanes.

VI was firstly estimated from 2 ratio of the *n*-alkanes markers C_{31}/C_{32} and C_{33}/C_{32} ($VI_{C31/C32}$; $VI_{C33/C32}$) according to the formula of Dove et al. (2000):

$$VI = \left(\frac{Fi}{Fj}\right) \times Dj \,/\left(Hi - \left(\frac{Fi}{Fj}\right) \times Hj\right)$$

where VI = voluntary intake (g DM/day), Fi and Hi = concentration of natural odd-chain *n*-alkanes in faeces and forage, Dj = dose rate of synthetic even chain *n*-alkanes, Fj and Hj = concentration of even-chain *n*-alkanes in faeces and forage.

VI was secondly estimated from ratio technique (VI_{C36}) (Lippke, 2002). Faecal output was calculated using C_{36} as external marker. The C_{36} recovery was determined by digestibility trials and reached 94%.

$$D = 100 \times \left(\frac{(VI - F)}{VI}\right)$$

$$VI = \frac{F}{(1 - \frac{D}{100})}$$

where D = forage digestibility coefficient (%) predicted by NIRS analysis of forage, VI = voluntary intake (g DM/day), F = total faecal excretion (g DM/day).

II.3. Statistical analyse

VI of each sheep obtained by direct measurement and estimated by faecal NIRS, by *n*-alkanes method and from faecal output were analysed according to a 3 ways ANOVA. The three factors took into account were the day of measurement (random – 7 levels), the method (fix – 6 levels) and the diet (fix – 3 levels). Means values were compared with a reference (direct measurement) using a Dunett test and, between them, using a Newman and Keuls test. (GLM procedure – Statistica 1999)

For NIRS, the standard error of prediction (SEP) was used to evaluate the accuracy of the intake prediction. The correspondence of spectrum and the spectral database was evaluated through the Mahalanobis distance (H).

III. Results

Over a 7 days period and for the 6 sheep, VI_{vivo} measured at feeding trough was 57.1 g DM/kg $BW^{0.75}$ with a maximum of 68.2 g DM/kg $BW^{0.75}$ and a minimum of 45.6 g DM/kg $BW^{0.75}$. VI_{vivo} decreased significantly ($F_{(2,12)}$ =14.8 P=0.001) when the proportion of HL increased in offered diet (Table 2).

Table 2.
Level (g DM/kg $BW^{0.75}$) and composition (% DM) of intake

Diet	LP	HL	TR	Other*	VI_{vivo}
Diet 1	37.0	14.3	16.0	32.8	58.4
Diet 2	20.3	35.9	17.8	26.0	55.7
Diet 3	22.5	18.7	26.6	32.8	56.7

* Other = in each specie group, plant others than HP, TR and LP

According to results described in the Fig. 1, C_{31} was the most abundant *n*-alkanes in LP. HL had the lowest concentration while TR presented an intermediate *n*-alkanes profile.

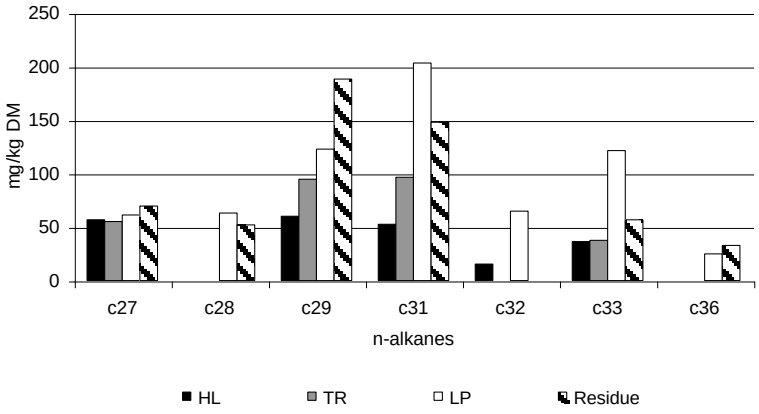

Fig. 1. Concentration of plant wax *n*-alkanes (mg/kg DM) in *Holcus lanatus* (HL), *Lolium perenne* (LP), *Trifolium repens* (TR) and residue, averaging on measurement period

Estimations of VI according to the different methods of calculation were listed in Table 3. There was a significant ($F_{(5,30)}$=55.2; P<0.000) method effect on the estimation of voluntary dry matter intake. The *n*-alkanes methods ($VI_{C31/C32}$; $VI_{C33/C32}$; VI_{C36}) led to an under-estimation of 32.4% of the VI_{vivo}. The Newman and Keuls test have shown that estimation of VI by *n*-alkanes techniques were similar between them and significantly different from VI_{vivo}. According to the NIRS prediction, VI_{NIRS} was very close from VI_{vivo} while $VI_{NIRSloc}$ over-estimated VI_{vivo} by 8 %. The NIRS predictions of intake were statistically similar to VI_{vivo}. In the analyse of variance, the 'diet – method' interaction was significant ($F_{(10,60)}$=6.2; P<0.000). For the diet 1, the under-estimation of VI by *n*-alkanes methods ($VI_{C31/C32}$ and $VI_{C33/C32}$) was only of 20 %, the difference was higher than 30 % for diet 2 and diet 3. No consistent correlation was observed between VI_{NIRS} and VI obtained by *n*-alkanes.

Finally, at a NIRS point of view, when the VI_{vivo} was compared to VI_{NIRS}, the standard error of prediction (SEP) reached respectively 8.1 and 7.8 g DM/kg $BW^{0.75}$ for VI_{NIRS} and $VI_{NIRSloc}$. The averaged H value (5.6 and 4.8 respectively for VI_{NIRS} and $VI_{NIRSloc}$) was close but higher than 3.

Table 3.
VI (g/kg $BW^{0.75}$) estimation according to the 6 tested methods

Method	N	VI
VI_{vivo}	42	56.9a
$VI_{C31/C32}$	42	40.2b
$VI_{C33/C32}$	42	37.8b
VI_{C36}	42	37.4b
VI_{NIRS}	42	56.6a
$VI_{NIRSloc}$	42	61.3a

In a same column, values quoted with different letters were different at α=0.05

IV. Discussion

The voluntary intake under grazing is influenced by several factors (Decruyenaere et al., 2009). More specialy some sward characteristics as leaf morphology, hair occurrence, leaf size (Barre et al., 2006) and stem physical properties were known to stimulate or limit animal foraging behaviour (Provenza, 2003). When the proportion of *Hocus lanatus* in diet increased, the voluntary intake tended to decrease. Similar results have been observed by

Morton et al. (1992). The low palatability of *Holcus lanatus* was probably due to the presence of hair on leaves and stems (Watt, 1978). Moreover, Penning et al. (1997) have shown that, in choice situation, White clover and Rye grass were preferred by sheep, as in our study.

N-alkanes method under-estimated voluntary intake. According to Dove et al. (2000) and in the context of our experiment, four major sources of error explained the less of accuracy of intake estimation through the *n*-alkanes method. The intake estimation could be affected by an incomplete faecal recovery of the marker. In our experiment, the recoveries of *n*-alkanes were determined by digestibility trials on sheep. The faecal recoveries of C_{31}, C_{32}, C_{33}, and C_{36} were respectively 82, 86, 90 and 94 %, that were similar to the results of others studies (Dove et al., 2000; Mayes et al., 1988). The release rate of *n*-alkanes CRD could be different that those indicated by the supplier (44.9 mg of C_{32} and C_{36} per day). For example, if the release rate of external markers was 55 mg/day (15 days releasing in place of 20 days), estimated intakes get 50.6 g/kg $BW^{0.75}$ for $VI_{C31/C32}$ and 47.7 g/kg $BW^{0.75}$ for $VI_{C33/C31}$, which was higher. Method of *n*-alkanes extraction (without preliminary saponification) could be not appropriate to determine the real content of odd-chain alkanes in faecal substrate. Finally, according to Mayes et al. (1986), concentration of different *n*-alkanes in herbage could vary greatly among species and within species. In the same way, the *n*-alkanes distribution varied with the part of plant, as observed by Bechet (2001). In our study, forage species were distributed in separate feeding troughs and *ad libitum*. Sheep had the possibility to select plant species or some part of plants. The knowledge of odd-chain *n*-alkanes concentrations in ingested diet was determinant for an accurate estimation of intake by *n*-alkanes method and for mixed diet, as in our study, it was difficult to obtain a representative sample of really ingested diet. The significant interaction 'diet – method' could be linked to a more stable release rate of C_{32} and C_{36} in the 'diet 1' sheep. Diet 1 contained also more *Lolium perenne* (higher C_{31} and C_{33} concentrations). So it could be hypothesised that higher were C_{31} and C_{33} diet contents, better could be intake estimation.

VI estimated by NIRS applied to faeces appeared close to VI_{vivo} but not correlated to *n*-alkanes methods. Faecal NIRS spectra appeared not too far from the faecal NIRS predictive database. As related by the H values higher but close than 3, the correspondence of our faecal spectra and the predictive database were sufficient. The forage proposed to sheep contained *Holcus lanatus*, a grass species not present in the predictive database, such difference could be normal. While the VI_{vivo} and VI_{NIRS} appeared statistically similar, the accuracy of the prediction (SEP) was about 8 g DM/kg $BW^{0.75}$. This level of accuracy appeared similar to those obtained by others studies. Stuth et al. (1989) estimated the dry matter intake with an accuracy of 17.3 g/kg $BW^{0.75}$. According to Garnsworthy and Unal (2004), by dairy cows, the accuracy of the intake prediction was better (SEP = 3.8 g/kg $BW^{0.75}$). Our results were intermediate and indicated the potential of NIRS applied to faeces for estimating diet characteristics at grazing.

V. Conclusion

NIRS applied to faeces gave good results in the prediction of animal intake of fresh grassland plant species. A major disadvantage of this method is the development of robust calibrations. Indeed, the value of a NIRS calibration depends to the accuracy of the reference method. To be robust, such calibration needs the integration of reference intake data as diverse as possible to be applied in conditions as diverse as possible.

In our study, *n*-alkanes method did not give valid results and led to a constant under-estimation of real intake. This difference could be explained by an insufficient extraction of natural *n*-alkanes of the faecal substrate or by real release rate of *n*-alkanes CRD different from the one indicated by bolus supplier. Moreover, in grazing or choice situation the necessity to have a representative sample of ingested diet remains a real problem.

VI. References

Barre, P., Emile, J.C., Betin, M., Surault, F., Ghesquiere, M., Hazard, L., 2006. Morphological characteristics of perennial ryegrass leaves that influence short-term intake in dairy cows. Agron. J., 98, 978-985.

Bechet, G., 2001. Variations des teneurs en n-alcanes du dactyle et du ray-grass. Nouveaux regards sur le pâturage. In: Actes JOUNÉES DE L'AFPF – 21 e 22 Mars 2001, p. A13.

Coates, D.B., (2000). Faecal NIRS - What does it offer todays grazier? Tropical Grasslands 34, 230-239.

Coleman, S.W., Murray, I., 1993. The use of near-infrared reflectance spectroscopy to define nutrient of hay by cattle. Anim. Feed Sc. Technol. 44, 237-249.

Coleman, S.W., Stuth, J.W., Holloway, J.W., 1989. Monitoring the nutrition of grazing cattle with near-infrared analysis of feces. . XVI International Grassland Congress, Nice, France, pp.881-882.

Decruyenaere, V., Stilmant, D., Lecomte, Ph., Buldgen, A., Dardenne, P., 2002. Improvement and indirect validation of the NIRS analysis applied to faeces to measure grass intake in pasture. In Durand J.L., Emile J.C., Huygue Ch. and Lemaire G. (Eds.), Multi-function grasslands. Quality forages, animal products and landscapes. Grassl. Sci. Eur. 7, pp.196-197.

Decruyenaere, V., Buldgen, A., Stilmant, D., 2009. Factors affecting intake by grazing ruminants and related quantification methods: a review. Biotechnol. Agron. Soc. Environ. 13, 559-573.

Dove, H., Mayes, R.W., 1991. The use of plant wax alkanes as marker substances in studies of nutrition of herbivores: a review. Aust. J. Agric. Res. 42, 913-952.

Dove, H., Mayes, R.W., 1996. Plant wax components: a new approache to estimating intake and diet composition in herbivores. J. Nutrition. 1, 13-26.

Dove, H., Freer, M., Foot, J.Z., 2000. The nutrition of grazing ewes during pregnancy and lactation : a comparison of alkane-based and chromium/*in vitro*-based estimates of herbage intake. Aust. J. Agric. Res. 51, 765-777.

Forbes, T.D.A., Beattie, M.M., 1987. Comparative studies of ingestive behaviour and diet comosition in oesophageal-fistulaed and non-fistulated cows and sheep. Grass Forage Sci. 42, 79-84.

Garnsworthy, P.C., Unal, Y., 2004. Estimation of dry matter intake and digestibility in group-fed dairy cows using near infrared reflectance spectroscopy. Anim Sci. 79, 327-334.

Hendricksen, R.E., Reich, M.M., Roberton, R.F., Reid, D.J., Gazzola, C., Rideout, J.A., Hill, R.A., 2002. Estimating the voluntary intake and digestibility of buffel-grass and lucerne hays offered to Brahman-cross cattle using n-alkanes. Anim. Res. 74, 567-577.

Holechek, J.L., Shenk, J.S., Vavra, M., Arthum, D., 1982. Prediction of forage quality using near-infrared reflectance spectroscopy on oesophageal fistula samples from cattle on mountain range. J. Anim. Sci. 55, 971-975.

Jones, R.J., Lascano, C.E., 1992. Oesophageal fistulated cattle can give unreliable estimates of proportion of legume in the diets of resident animals grazing tropical pastures. Grass Forage Sci. 47, 128-132.

Leite, E.R., Stuth, J.W., 1995. Fecal NIRS equations to assess diet quality of free ranging goats. Small Rumin. Res. 15, 223-230.

Lippke, H., 2002. Estimation of forage intake by ruminants on pasture. Crop Sci., 42, 869-872.

Lyons, R.K., Stuth, J.W., Angerer, J.P., 1995. Technical note Fecal NIRS equation field validation. J. Range Manage. 48, 380-382.

Mayes, R.W., Lamb, C.S., Colgrove, P.M., 1986. The use of dosed and herbage n-alkanes as markers for the determination of herbage intake, J. Agric. Sci. 107, 161-170.

Mayes, R.W., Lamb, C.S., Colgrove, P.M., 1988. Digestion and metabolism of dosed even-chain and herbage odd-chain n-alkanes in sheep. Proc 12th Gen. Meeting European Grassland Fed. Dublin, July 1988, 159-163.

Morton, J.D., Bolton, G.R., Hodgson, J., 1992. The comparative performances of Holcus lanatus and Lolium perenne under sheep grazing in the Scottish uplands. Grass Forage Sci. 47,143-152.

Penning, P.D., Newman, J.A., Parsons, A.J., Harvey, A., Orr, R.J., 1997. Diet preferences of adult sheep and goats grazing ryegrass and white clover. Small Rumin. Res. 24, 175-184.

Provenza, F.D., 2003. Foraging behavior: managing to survive in a world of change. Washington, DC: USDA, 63p.

Stuth, J.W., Kapes, E.D., Lyons, R.K., 1989. Use of near infrared spectroscopy to assess nutritional status of cattle diets on rangeland. XVI International Grassland Congress, Nice, France, pp.889-890.

Ward, R.G., Smith, G.S., Wallace, J.D., Urquhart, N.S., Shenk, J.S., 1982. Estimation of intake and quality of grazed range forage by near infrared reflectance spectoscopy. J. Anim. Sci. 54, 399-402.

Watt, T.A., 1978. The biology of Holcus lanatus L. (Yorkshire fog) and it s significance in grassland. Common Wealth Bureau of Pastures and Field Crops 48, 195-204.

Faecal near infrared reflectance spectroscopy (NIRS) compared with other techniques for estimating the *in vivo* digestibility and dry matter intake of lactating grazing dairy cows

Article VII : Decruyenaere et al. (2012)
Animal Feed Science and Technology. 73, 220-234.

Decruyenaere V.[(1)]*, Froidmont E.[(1)], Bartiaux-Thill N.[(1)], Buldgen A.[(2)], Stilmant D.[(3)]

(1) Walloon Agricultural Research Centre (CRA-W) – Production and Sectors Department, 8 Rue de Liroux, B-5030 Gembloux, Belgium

(2) ULG – Gembloux – Agro Bio Tech – Unité de Zootechnie, 2 Passages des Déportés, B-5030 Gembloux, Belgium

(3) Walloon Agricultural Research Centre (CRA-W) – Agriculture and Natural Environment, 100 rue du Serpont, B-6800 Libramont-Chevigny, Belgium

corresponding auhtor – E-mail : v.decruyenaere@cra.wallonie.be

Abbreviations: ADFom, acid detergent fibre excluding residual ash; APM, animal performance method; BW, body weight; CP, crude protein; CV, coefficient of variation; d, day; DM, dry matter; DMI, dry matter intake; C-DMI, concentrate dry matter intake; G-DMI, grass dry matter intake; D-DMI, diet dry matter intake; FNI, faecal nitrogen indicator; FNIRS, near-infrared reflectance spectroscopy applied to faeces; NE, net energy requirement; NIRS, near-infrared reflectance spectroscopy; OMDcel, cellulase enzyme organic matter digestibility; OMD, *in vivo* organic matter digestibility; C-OMD, concentrate *in vivo* organic matter digestibility; D-OMD, diet *in vivo* organic matter digestibility; G-OMD, grass *in vivo* organic matter digestibility; OM, organic matter; FOM, faecal organic matter; OMI, organic matter intake; C-OMI, concentrate organic matter intake; G-OMI, grass organic matter intake; r, coefficient of correlation; RT, ratio technique; SD, standard deviation of the database; SE, standard error of regression; SEC, standard error of calibration; SECV, standard error of cross validation; SEP, standard error of prediction; SEPc, standard error of prediction corrected for bias.

Abstract

The objective of this study was to validate near-infrared reflectance spectroscopy (NIRS) applied to faeces (FNIRS) for estimating the grass *in vivo* organic matter digestibility (G-OMD) and the grass dry matter intake (G-DMI, kg/d) of concentrate-supplemented grazing dairy cows. The G-OMD estimates from one FNIRS model were compared with two estimates using faecal nitrogen indicator (FNI) methods. Similarly, two FNIRS models were compared with the ratio technique (RT) and with three animal performance methods (APM) for estimating G-DMI. The results were analyzed at cow and herd level in two grazed paddocks (P1 and P2) in a rotational grazing scheme.

For both G-OMD and G-DMI, the FNIRS estimations were correlated ($P<0.05$) with other predictive methods ($r = 0.61$ for G-OMD and $r = 0.63$ to 0.88 for G-DMI). Depending on the estimation method, the G-OMD varied from 0.689 (FNIRS) to 0.773 (FNI). The FNI estimates were generally higher and were similar to the G-OMD estimates obtained from NIRS analyses of grass sampled in the field. The FNIRS and FNI estimates were biased at cow and paddock level by 0.01 to 0.1 digestibility units ($P<0.001$).

Depending on the estimation method, the G-DMI estimates varied from 11.9 to 16.4 kg/d. FNIRS and APM produced similar estimates of G-DMI at both cow and herd level. The RT

estimates of G-DMI were 3 kg/d higher than the FNIRS and APM estimates ($P<0.05$). The G-DMI estimated by RT methods was particularly high for P2, with a mean value of 18.5 kg/d, which seemed too high in terms of the maximum intake capacity of supplemented grazing dairy cows.

For both G-OMD and G-DMI and for all the estimation methods, inter-cow and intra-paddock variations, expressed through the coefficient of variation (SD/mean), ranged from 0.05 to 0.40. As the accuracy of the FNIRS models, expressed through the standard error of cross validation (SECV), was lower than these inter-animal and intra-paddock variations, we suggest that FNIRS could be used to record, quickly and easily, the evolution of grass digestibility and the intake of grazing dairy cows. These estimates could be implemented through decision-support systems aimed at improving the management of grazing dairy herds.

Keywords: NIRS, faeces, estimation, grass intake, grass digestibility

I. Introduction

In many countries, dairy farming is a prominent economic activity (Rohner-Thielen, 2008). Intensive milk production accounts for a significant consumption of concentrate feeds and conserved forages that can be a substitute for grazed grass. In this context, ensuring the economic sustainability of dairy systems requires rigorous control of production costs. Where pasture-based systems predominate, such as in New Zealand or Ireland, milk production tends to be more sustainable (Dillon et al., 2008), but milk production where grazed grass is the basal diet is difficult for many producers, mainly due to the lack of techniques for quantifying grass intake and the nutritional value of ingested grass.

There are many techniques for estimating grass intake, but they are often difficult to apply on pasture. In most cases, intake estimations are based on measuring herbage mass disappearance during grazing (Meijs et al., 1982; Macoon et al., 2003; Smit et al., 2005). Intake can also be estimated through animal performance measurements that link intake to animal energy requirements (CVB, 1999; NRC, 2001). These methods are easy to apply to dairy cattle, where the milk amount is often known.

Indirect methods, including using indigestible markers such as *n*-alkanes (Mayes et al., 1986), using the ratio technique (RT) (Lippke, 2002) to measure forage characteristics and/or animal behaviour or applying mathematical models, can also be used to estimate intake (Coleman, 2005). The *n*-alkanes method currently appears to be the best one for estimating intake during grazing (Coates and Penning, 2000). The RT method requires to determine forage digestibility and faecal output in order to estimate intake. According to Adegosan et al. (2000), there are many methods for estimating grass digestibility, based on grass or faeces analysis, but, as noted by Biston et al. (1988) and Lippke (2002), sampled grass can differ from ingested grass and therefore estimating digestibility from forage analysis is very dependent on sampling quality. At pasture level, the determination of faecal output can be based on weighing total excreted faeces (Lippke, 2002) or, more often, on using external indigestible markers such as chromium oxide (Compère et al., 1992; Berry et al., 2000; Ferreira et al., 2004). All these methods are difficult to apply with grazing ruminants.

Over the past 20 years, near-infrared reflectance spectroscopy (NIRS) techniques have been developed to characterize the nutritional value of forage. Currently, NIRS calibrations developed from grass or oesophageal extruda or faeces spectra can be used to predict both grass *in vivo* digestibility and grass intake. Boval et al. (2004), Landau et al. (2004), Li et al. (2007), Fanchone et al. (2007, 2009) and Decruyenaere et al. (2009) confirm the potential of NIRS applied to faeces (FNIRS) for assessing the diet of cattle, dairy goats and sheep fed with tropical or temperate forages. In most cases, FNIRS databases focus on animals fed only with grass. There are few data on supplemented grazing dairy cows.

The objectives of this study were to validate the potential of FNIRS for estimating the grass *in vivo* organic matter digestibility (G-OMD) and grass dry matter intake (G-DMI) of grazing lactating dairy cows supplemented with energy and protein concentrates. The FNIRS estimates were compared with faecal nitrogen indicator (FNI) estimates for G-OMD and with the RT and animal performance method (APM) estimates for G-DMI.

II. Materials and methods

The experiment was conducted in summer 2002 (5-13 August) at the Productions and Sectors Department of the Walloon Agricultural Research Centre (CRA-W) in Gembloux (50° 33' N, 4° 41' E), Belgium.

II.1. Experimental dairy cows

The experimental dairy herd consisted of 44 Holstein cows (12 first lactating cows, 15 cows in second lactation and 17 cows in third lactation or more). The cows had an average annual milk production of 7495 kg.

Thirteen lactating cows (6 primiparous and 7 multiparous) were selected from the dairy herd for the experiment. At the beginning of the experiment, the average cow was 180 days in milk, weighed 603 kg and produced 21.8 kg of milk per day (Table 1). The cows were selected to be as different as possible in terms of milk production and days in milk in order to introduce heterogeneity into the digestibility and intake values.

The cows were milked twice a day (06:30 and 16:30) and milk production was recorded individually after each milking. The milk was sampled individually at milking every day.

II.2. Experimental pasture

The grazing area was a permanent pasture grazed by the whole dairy herd (n = 13 selected cows and 31 remaining cows) in a rotational grazing system (13 paddocks, 4th grazing cycle, 34 days of grass regrowth at the beginning of the experiment). Over the 9 days of the experimental period, two paddocks (P1 = 1.2 ha and P2 = 1.3 ha) were successively grazed. P1 was initially grazed for 6 days, until the residual sward surface height, measured with graze height meter (30 x 30 cm aluminium plate weighing 2.122 kg/m²) was 8 cm. The dairy herd was then moved to P2.

II.2.2. Sward measurements

Grass heights (n=60) were measured with the graze height meter daily throughout the experimental period. The grass was sampled by randomly cutting six quadrats (delimited area of 40 x 40 cm) daily in the grazed paddock. This grass was then bulked, weighed and sampled for analysing.

To estimate grass availability in the grazed paddock, a specific linear relation was defined between the averaged grass height and the averaged grass yield measured in quadrats each day of the experiment:

$$\text{Grass yield (kg DM/ha)} = 161.6 \times \text{grass height (cm)} + 316.4$$

$$R^2 = 0.704,\ SE = 455.45,\ n = 8$$

To assess grass regrowth during the grazing period, the undisturbed herbage accumulation was estimated using the LINGRA model (Schapendonk et al., 1998).

The ingested grass per cow was then calculated using the difference technique based on the Linehan relationship, as described by Smit et al. (2005), and divided by the instantaneous stocking rate (36.1 and 34.9 cows/ha respectively for P1 and P2, respectively):

$$\text{G-DMI (kg/d)} = \frac{[\,(\text{herbage mass} - \text{residual}) \times \frac{\log(\text{herbage mass} + \Delta\ \text{regrowth}) - \log(\text{residual})}{\log(\text{herbage mass}) - \log(\text{residual})}]}{\text{instantaneous stocking rate}}$$

Finally, the proportion of grasses, legumes and other plants in the sward was determined using a hand-separation method on day 1 for P1 and on day 6 for P2.

II.3. Experimental diet

The basal diet consisted of grazed grass supplemented by dehydrated sugar beet pulp (1.80 kg DM/cow/d) and was intended to support a milk production of 19 kg milk/d. The dehydrated sugar beet pulp was placed in the feeding trough twice a day after milking, when cows were blocked at the feed barrier. Cows producing more than 19 kg milk received a commercial production concentrate and a commercial high protein concentrate. The amount of production concentrate was 0.87 kg DM per 2.5 kg of milk above basal production. The maximum amount of high protein concentrate given was 1.40 kg DM/cow/d.

The commercial concentrates were dispensed to the animals throughout the day using automatic feeders in the barn. Individual daily concentrate intakes were automatically recorded. The concentrate feeds were sampled at the beginning of the experiment for analysis.

II.4. Grass in vivo organic matter digestibility estimation

Two methods were compared to estimate the G-OMD: FNIRS and FNI (Fig. 1).

Table 1
Characteristics of dairy cows at the beginning of the experiment

Cow	Body weight (kg)	Number of calving	Days in milk	Milk production (kg/d)	Milk fat (g/kg)	Milk protein (g/kg)	Standardized milk production (kg/d)
1	678	3	299	24.8	44.5	34.7	25.8
2	613	2	203	20.4	31.8	31.4	20.5
3	505	1	251	16.4	37.7	30.0	17.4
4	562	1	170	20.2	33.8	29.0	19.4
5	588	1	245	13.2	34.6	30.9	13.0
6	530	1	100	22.4	36.1	30.8	20.6
7	521	1	140	10.8	33.6	29.5	10.6
8	527	1	88	18.4	46.7	28.9	18.9
9	650	3	132	32.6	38.4	30.0	30.5
10	669	2	165	26.6	33.8	29.6	26.1
11	658	5	175	29.6	35.4	30.3	25.5
12	633	3	109	29.2	38.3	30.3	32.1
13	703	4	259	19.2	32.2	29.4	17.8
Mean ± SD	603 ± 68.0		180 ± 67.3	21.8 ± 6.53	36.7 ± 4.51	30.4 ± 1.50	21.4 ± 6.36

SD = standard deviation of the mean
Standardized milk production = [0.337 + (0.116 x milk fat/10) + (0.06 x milk protein/10)] x milk production (De Brabander, 1993)

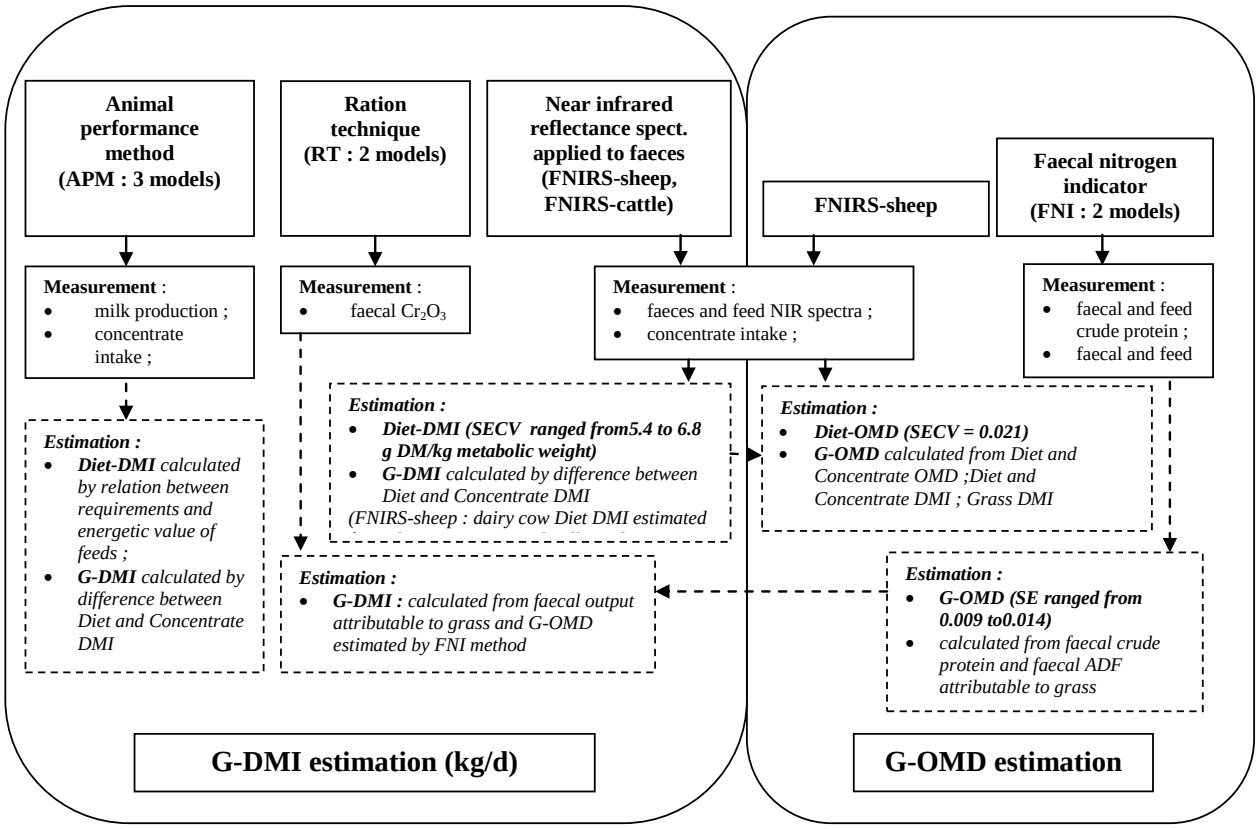

Fig. 1. Estimations of organic matter digestibility of grass (G-OMD) and grass dry matter intake (G-DMI), chart flow (full line = measurement; dotted line = estimation)

II.4.1. FNIRS method

One FNIRS model was tested. It estimated the *in vivo* organic matter digestibility of the diet (D-OMD) using a calibration developed from the faecal spectra of sheep (FNIRS-sheep; n=951, as described by Decruyenaere et al., 2009). The G-OMD then was derived from the D-OMD using the formula:

$$\text{D-OMD} = \frac{(\text{C-OMD} \times \text{C-OMI}) + (\text{G-OMD} \times \text{G-OMI})}{(\text{C-OMI} + \text{G-OMI})}$$

$$\text{G-OMD} = \frac{(\text{D-OMD} \times (\text{C-OMI} + \text{G-OMI}) - (\text{C-OMD} \times \text{C-OMI})}{(\text{G-OMI})}$$

where D-OMD = *in vivo* OM digestibility of the diet predicted by FNIRS; C-OMD = *in vivo* OM digestibility of the concentrates; C-OMI = concentrate OM intake (kg/d); G-OMD = *in vivo* OM digestibility of grass; and G-OMI = grass OM intake predicted by FNIRS (kg/d).

II.4.2. FNI method

Two equations were used to predict G-OMD. The first one was a quadratic equation based on faecal crude protein (CP) and developed in the 1980s for the CRA-W pastures (FNI-1: summer model by Bartiaux and Oger, 1986). The second was a multi-linear equation based on faecal CP, forage CP and faecal acid detergent fibre (ADFom) (FNI-2: model by Peyraud et al., unpublished data, cited by Ribeiro Filho et al., 2003). Both these models were developed for estimating the G-OMD of unsupplemented grazing animals. For supplemented

dairy cows, it was necessary to calculate the CP and ADFom excretion attributable to concentrates in order to calculate, based on differences, the CP and ADFom excretion attributable to grass. As described by Delagarde et al. (1999), the amounts of faecal CP and ADFom attributable to the concentrate were calculated from the concentrate CP and ADFom content and from the digestibility of these components. Faecal and forage CP and faecal ADFom were predicted by NIRS using the calibrations described in Table 2.

Table 2
Statistical performances of NIRS calibrations for estimating the chemical composition of grass, concentrate and faeces (CRA-W data)

	Parameters	N	mean	SD	SEC	R^2	SECV	SD/SECV
Grazed grass	Ash (g/kg DM)	2517	97.0	25.9	10.0	0.84	10.4	2.49
	CP (g/kg DM)	2765	148	59.0	8.5	0.98	8.6	6.86
	CEL (g/kg DM)	2494	267	54.1	13.3	0.94	13.5	4.01
	OMDcel[1]	1598	0.77	0.10	0.022	0.95	0.023	4.43
	G-OMD[2]	328	0.71	0.06	0.023	0.86	0.023	2.62
Concentrate	Ash (g/kg DM)	1199	84.4	22.5	10.9	0.75	11.2	2.01
	CP (g/kg DM)	2186	208	54.5	8.8	0.97	9.1	5.99
	Fat (g/kg DM)	965	45.4	21.1	4.4	0.95	4.6	4.58
	CEL (g/kg DM)	1237	104	44.4	10.5	0.94	10.9	4.07
	OMDcel[3]	582	0.88	0.04	0.017	0.78	0.018	2.17
Faeces	Ash (g/kg DM)	77	223	44.2	15.3	0.77	21.2	2.08
	CP (g/kg DM)	78	164	22.9	7.9	0.80	10.2	2.24
	ADFom (g/kg DM)	57	248	28.4	6.8	0.79	12.9	2.20

N: number of samples in the NIR database; SD: standard deviation in the reference database; SEC: standard error of calibration; R^2: coefficient of determination of NIR calibration; SECV : standard error of cross validation; DM: dry matter; CP: crude protein; CEL: cellulose (AFNOR, 1993); OMDcel[1]: cellulase enzyme organic matter digestibility (De Boever et al., 1988); G-OMD[2] : *in vivo* organic matter digestibility of grass (Decruyenaere et al., 2009); OMDcel[3]: cellulase enzyme organic matter digestibility (De Boever et al., 1986); ADFom = acid detergent fibre expressed exclusive of residual ash (AFNOR, 1997).

II.5. Grass dry matter intake estimation

Three methods were used to estimate the G-DMI : FNIRS, RT and APM (Fig.1).

II.5.1. FNIRS method

The FNIRS models used were able to estimate the diet DM intake (D-DMI). The first one was based on a calibration developed from faecal sheep spectra (FNIRS-sheep; n=925, as described by Decruyenaere et al., 2009). The FNIRS-sheep estimation of D-DMI was converted into dairy cow D-DMI, using the model developed by Dulphy et al. (1987), as described by Decruyenaere et al. (2006):

$$\text{D-DMI} = \frac{(36.7 + 0.942 \times \text{D-OMI}/(1\text{-Diet ash}) + 1.48 \times \text{standardized milk}) \times \text{BW}^{0.75}}{1000}$$

where D-DMI = diet DM intake (kg/d); D-OMI = diet OM intake predicted by FNIRS (g/kg $BW^{0.75}$); and BW = body weight (kg).

The second model was based on a calibration developed from faecal dairy cow and suckler cow spectra (FNIRS-cattle; n = 139, as described by Decruyenaere et al., 2004). The FNIRS-cattle calibration made it possible to estimate each dairy cow's D-DMI directly.

For both FNIRS models, G-DMI was obtained by subtracting C-DMI from D-DMI.

II.5.2. RT method

The RT method required measuring *in vivo* digestibility and faecal output in order to estimate intake (Lippke, 2002):

$$\text{OMD} = \frac{\text{OMI} - \text{FOM excretion}}{\text{OMI}}$$

$$\text{OMI} = \frac{\text{FOM excretion}}{\text{1-OMD}}$$

$$\text{DMI} = \frac{\text{OMI}}{1 - \text{ash}}$$

where OMD = *in vivo* OM digestibility; OMI = OM intake (kg/d); FOM = faecal OM excretion (kg/d); and DMI = DM intake (kg/d).

Two different RT estimates of intake were tested. The first one (RT-1) was based on the G-OMD estimated using the FNI-1 method, and the second one (RT-2) on the G-OMD estimated using the FNI-2 method. For RT-1 and RT-2, faecal output was estimated from the dilution of an external marker, chromic oxide (Cr_2O_3), in the faeces. For this procedure, each of the 13 selected cows was dosed with a Cr_2O_3 ruminal release bolus (CAPTEC – Cr_2O_3, Captec [NZ] Ltd, New Zealand; release yield: 1.42 g Cr_2O_3/d) 7 days before the start of the experiment.

FOM excretion attributable to grass was calculated by subtracting the indigestible OM attributable to concentrates from the total estimated FOM excretion (Delagarde et al., 1999). Based on the ash content and the C-OMD, the indigestible organic matter attributable to concentrates was estimated to be 149, 170 and 132 g/kg DM for dehydrated sugar beet pulp, commercial production concentrate and commercial high protein concentrate, respectively.

II.5.3. APM

The APM was used to calculate D-DMI from animal requirements and milk production. Three models were tested. The first one (APM-1) was developed in Belgium for Holstein dairy cows (De Brabander, 1993):

$$\text{D-DMI} = 3.4 + (0.3 \text{ x standardized milk production}) + (0.011 \text{ x BW})$$

$$\text{G-DMI} = \text{D-DMI} - \text{C-DMI}$$

where D-DMI = diet DM intake (kg/d); BW = body weight (kg); standardized milk production (kg); G-DMI = grass DM intake (kg/d); and C-DMI = concentrate DM intake (kg/d).

The second model (APM-2), developed in the Netherlands, was described by Smit et al. (2005) and calculated from the total net energy requirement for maintenance and milk production and from the net energy content of grass and concentrate:

$$\text{NE} = 6.9 \text{ x } [(42.4 \text{ x } \text{BW}^{0.75} + 442 \text{ x standardized milk production}) \text{ x } (1 + (\text{standardized milk production} - 15) \text{ x } 0.00165)]$$

$$\text{G-DMI} = \frac{\text{NE-NEC}}{\text{NEGr}}$$

where NE = net energy requirement for maintenance and milk production (VEM/d); BW = body weight (kg); standardized milk production (kg/d); NEC = net energy supplied by concentrate (VEM/d); and NEGr = net energy concentration of grass (VEM/kg DM).

The third model (APM-3) was derived from the NRC system (NRC, 2001) and was calculated as described by Macoon et al. (2003):

$$NE = NEM + NEL + NEBW + NEW + NEG$$

$$G\text{-}DMI = \frac{NE\text{-}NEC}{NEGr}$$

where NE = net energy requirement (Mcal/d); NEM = NE for maintenance; NEL = NE for milk production; NEBW = NE for body weight changes; NEW = NE for walking; NEG = NE for grazing activity; NEC = energy supplied by concentrate; and NEGr = energy concentration of grass (Mcal/kg DM).

Because the cows were weighed once in the middle of the experimental period and the automatic feeders were placed in the barn, it was difficult to estimate NEBW and NEW and therefore these values were not included in calculation of total NE.

II.6. Sample analysis and statistics

II.6.1. Sampling and analysis

Individual milk sampled at milking was bulked and the fat and protein content was estimated using NIRS (Milkoscan FT6000, FOSS Electric, Hillerød, Denmark) in order to calculate standardized milk production, as described by De Brabander (1993).

The daily grass and concentrate feed samples were oven dried (65°C, 36 h) in order to determine the DM content. Dried samples were ground initially in a hammer mill (1 mm screen) (Waterleau, BOA, Belgium) and then in a Cyclotec mill (1 mm screen) (FOSS Electric, Hillerød, Denmark).

Faeces were sampled individually (rectal grabbing) twice a day at each milking. The faecal samples were oven dried (65°C until constant weight) and then ground in a hammer mill (1 mm screen) (Waterleau, BOA, Belgium).

The grass, concentrate feed and faeces samples were presented in small ring cups to an NIRS-system 5000 monochromator spectrometer (FOSS Electric, Hillerød, Denmark), and the absorption data recorded as log 1/R from 1100 to 2498 nm, every 2 nm (WINISI 1.5, FOSS Tecator Infrasoft International LCC, Hillerød, Denmark).

The chemical composition and cellulase enzyme OMD (OMDcel) of grass and concentrates, such as faeces chemical composition, were estimated using NIRS calibrations developed at CRA-W (Table 2).

The ADFom and lignin (sa) of the concentrates were determined as described by Robertson and Van Soest (1981) and according to the AFNOR (1997). The C-OMD was estimated from the OMDcel (C-OMD = 0.969 OMDcel – 0.0355; De Boever et al., 1986).

The Cr_2O_3 content of faeces was determined using the simplified method described by François et al. (1978). This analysis, in two steps, requires destroying the OM using a

mixture of nitric and perchloric acids, followed by oxidation of the Cr(+III) to Cr(+VI). The Cr(+VI) was then titrated with Mohr's salt.

II.6.2. Statistical analysis

For both the G-OMD and G-DMI estimates, Pearson coefficients were calculated to test the correlation between the estimation methods. In order to compare the different estimation methods, the G-OMD and G-DMI estimates averaged per cow were submitted to a paired t-test (Statistica 8.0 – Stat Soft, France).

For NIRS analysis, the correspondence between the dairy cows' faeces spectra and the FNIRS database was evaluated using the Mahalanobis distance (H). As recommended by Shenk and Westerhaus (1991), H must be lower than 3 for accurate predictions.

To determine the potential of FNIRS, the G-OMD and G-DMI predicted by FNIRS were compared with other estimates, assumed to be reference values, using the MONITOR procedure in WINISI 1.5 (FOSS Tecator, Infrasoft International LCC, Hillerød, Denmark). The accuracy of the estimation was determined using three parameters: the bias between the NIRS predicted value and the reference value; the standard error of prediction (SEP); and the SEP corrected with bias (SEPc), as described in the following equations:

$$\text{bias} = \frac{1}{n}\sum(\text{Y} - \text{FNIRSest.})$$

$$\text{SEP} = \sqrt{\sum \frac{(\text{Y}-\text{FNIRSest.})^2}{\text{n}}}$$

$$\text{SEPc} = \sqrt{\sum \frac{[(\text{Y}-\text{FNIRSest.})-\text{bias}]^2}{(\text{n}-1)}}$$

where n = number of observations; Y = reference value; and FNIRSest. = FNIRS predicted value.

III. Results

III.1. Herbage allowance and diet characteristics

The chemical characteristics and botanical composition of the P1 and P2 grass and the characteristics of concentrates are shown in Tables 3 and 4. From a botanical point of view, P1 and P2 were fairly similar at the start of grazing, with more than 20 percent of clover and more than 60 percent of grasses in the sward. Plants other than clover and grasses accounted for less than 10 percent of the sward. The P2 grass had a higher CP content than the P1 grass, but similar DM, fibre and ash content. Based on OMDcel, the grass from P1 appeared to be just as digestible as the grass from P2. In contrast, the G-OMD estimated by NIRS analysis of the grass sampled in the field appeared to be higher for P1.

Table 3
Characteristics of concentrates (chemical composition and digestibility)

	Dehydrated sugar beet pulp	Production concentrate	High protein concentrate
DM (g/kg)	867	874	852
Ash (g/kg DM)	71.1	78.1	114.1
CP (g/kg DM)	63.7	224.1	433.3
Fat (g/kg DM)	7.9	52.9	26.8
ADFom (g/kg DM)	242	241	104
Lignin (sa) (g/kg DM)	38.5	33.9	16.5
CEL (g/kg DM)	213.8	111.5	100.3
OMDcel	0.958	0.879	0.914
OMD	0.84	0.82	0.85
CPD	0.62	0.70	0.90
ADFD	0.90	0.75	0.85

DM: dry matter; CP: crude protein; ADFom :acid detergent fibre expressed exclusive residual ash (AFNOR, 1997); Lignin (sa) determined by solubilisation of cellulose with sulphuric (AFNOR, 1997); CEL: cellulose (AFNOR, 1993) ; OMDcel: cellulase enzyme organic matter digestibility digestibility (De Boever et al., 1986); OMD: *in vivo* OM digestibility estimated from De Boever et al. (1986); CPD: *in vivo* CP digestibility (INRA, 1989); ADFD: *in vivo* ADF digestibility (Demarquilly et al., 1995).

Table 4
Chemical composition and botanical characteristics of herbage at the start of grazing (day 1 for P1 and day 6 for P2)

	Paddock	
	P1	P2
Chemical composition		
DM (g/kg)	135.7	130.6
CP (g/kg DM)	178.9	190.7
CEL (g/kg DM)	268.6	274.2
NDF (g/kg DM)	487.0	489.2
ADFom (g/kg DM)	305.9	311.3
Lignin (sa) (g/kg DM)	34.8	36.1
Ash (g/kg DM)	113.5	123.0
OMDcel	0.798	0.786
G-OMD estimation		
From OMDcel	0.738	0.726
From NIRS applied to grass	0.720	0.693
Botanical composition		
Grasses	0.694	0.638
Clover	0.224	0.288
Other	0.082	0.074

DM: dry matter; CP: crude protein; CEL: cellulose (AFNOR, 1993); NDF: neutral detergent fibre (Van Soest et al., 1991; AFNOR, 1997); ADFom :acid detergent fibre expressed exclusive residual ash (AFNOR, 1997); Lignin (sa) determined by solubilisation of cellulose with sulphuric (AFNOR, 1997); OMDcel: *in vitro* organic matter digestibility (method of De Boever et al., 1988); G-OMD: *in vivo* organic matter digestibility estimated from NIRS analysis of forage according to Decruyenaere et al. (2009).

Herbage availability, calculated regrowth and derived G-DMI, C-DMI and standardized milk production for each day of the experiment are given in Table 5. On the first day of grazing in each paddock (day 1 for P1 and day 6 for P2), the herbage allowance was 3416 and 3842 kg DM/ha in P1 and P2, respectively. Herbage availability per cow was therefore higher in P2 (106.5 kg DM/cow) than in P1 (97.8 kg DM/cow).

In P1, the herd G-DMI estimated using the Linehan relationship (Smit et al., 2005) decreased throughout the grazing period (days 1 to 5) without an increase in concentrate consumption.

Table 5
Herbage availability and regrowth (kg DM/ha/d), grass intake (G-DMI, kg DM/d), concentrate intake (C-DMI, kg DM/d), standardized milk production (kg/d) during the experiment

Paddock	Day	Herbage availability[1]	Regrowth[2]	G-DMI[3]	C-DMI	Standardized milk production
P1	1	3416	29.6	16.3	5.4	21.4
P1	2	2872	56.0	12.0	4.8	22.7
P1	3	2115	64.1	11.2	4.0	21.8
P1	4	2172	49.2	11.3	4.9	20.5
P1	5	1822	50.2	7.8	5.4	20.2
P1	6	1596	59.0			
P2	6	3842	54.8	25.4	3.7	20.2
P2	7	2977	67.4	25.0	4.4	20.0
P2	8	2121	68.9			

[1] estimated from grass height; [2] according to Schapendonk et al. (1998); G-DMI[3]: Grass dry matter intake calculated from Smit et al. (2005), C-DMI : concentrate dry matter intake

Averaged standardized milk production decreased during P1 grazing and was stable during P2 grazing. For herbage allowance, the estimated G-DMI in P2 appeared to be higher than in P1. Recorded concentrate intake was lower for P2 grazing.

III.2. In vivo organic matter digestibility of grass, comparison between methods

As shown in Table 6, the highest G-OMD estimates were obtained from the FNI method and the lower G-OMD values from the FNIRS-sheep method. For both methods, the G-OMD estimates decreased throughout the grazing period for P1 and, for the FNI method, increased when cows were moved to P2. For both methods, the G-OMD estimate decreased with increasing concentrate supplementation; for example, the rate of decrease of G-OMD was 0.008 and 0.01 digestibility unit per kg of concentrate supplementation for the FNIRS-sheep and FNI methods, respectively. The G-OMD variation (CV=SD/mean) at cow level was lower than 0.07 for both the FNIRS-sheep and FNI methods. For the FNI estimates, the G-OMD variation in the paddocks, reflecting grass quality and availability as well as cow selectivity, was higher in P1 than in P2. The CV in P2 was notably lower for FNI methods. The G-OMD estimates obtained using FNI methods were well correlated between the methods ($r=0.99$, $p<0.001$), but differed in terms of the result of the paired t-test ($t=10.25$; $P<0.001$). The FNIRS-sheep estimates were correlated with the FNI-1 estimates ($r=0.61$, $p<0.05$), but not with the FNI-2 estimates ($r=0.50$; $P>0.05$) (Table 7). The relationship and bias between the FNIRS-sheep and FNI estimates of the G-OMD are shown in Fig. 2.

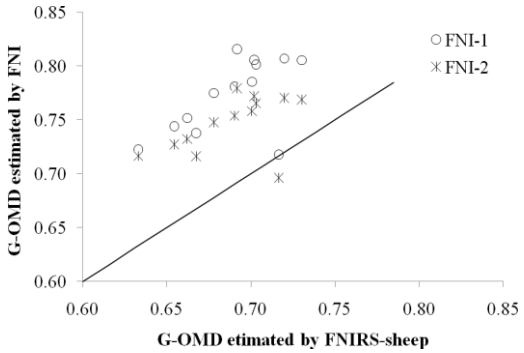

Fig. 2. Organic matter digestibility of grass (G-OMD): correlations between NIRS analyses of faeces (FNIRS-sheep) and faecal nitrogen indicator (FNI-1 and FNI-2) estimations.

Table 6
Grass *in vivo* organic matter digestibility (G-OMD) estimated using NIRS analyses of faeces (FNIRS-sheep) and faecal nitrogen indicator (FNI-1 ; FNI-2), variations linked to cows and to paddocks (n=91); variation in concentrates intake (mean and standard deviation) throughout the experimental period

Cow		FNIRS-sheep	FNI-1	FNI-2	Concentrates intake (kg DM/d)
1		0.662	0.752	0.732	4.2 ± 1.3
2		0.701	0.785	0.758	2.7 ± 1.0
3		0.720	0.807	0.770	0.4 ± 0.6
4		0.730	0.805	0.769	0.4 ± 0.7
5		0.702	0.805	0.772	0.4 ± 0.6
6		0.678	0.775	0.748	2.2 ± 1.4
7		0.703	0.801	0.765	1.4 ± 1.0
8		0.692	0.816	0.779	0.0 ± 0.0
9		0.654	0.744	0.727	4.9 ± 3.5
10		0.667	0.738	0.716	5.3 ± 1.7
11		0.717	0.718	0.696	5.2 ± 1.6
12		0.633	0.722	0.717	6.7 ± 0.5
13		0.690	0.781	0.754	0.6 ± 1.2
Cow	Mean ± SD[1]	0.689 ± 0.0391	0.773 ± 0.0505	0.747 ± 0.0410	/
	CV	0.0568	0.0653	0.0549	/
Paddock	Day	FNIRS-sheep	FNI-1	FNI-2	/
P1	2	0.706	0.788	0.766	/
P1	3	0.713	0.775	0.752	/
P1	4	0.694	0.747	0.733	/
P1	5	0.679	0.732	0.709	/
P2	6	0.674	0.787	0.755	/
P2	7	0.675	0.794	0.758	/
P2	8	0.680	0.792	0.756	/
P1	Mean ± SD[2]	0.698 ± 0.0401	0.761 ± 0.0604	0.740 ± 0.0513	/
	CV	0.0575	0.0794	0.0694	/
P2	Mean ± SD[2]	0.677 ± 0.0347	0.791 ± 0.0250	0.756 ± 0.0178	/
	CV	0.0512	0.0316	0.0236	/

SD: standard deviation of the mean : [1] across days and paddocks (n = 91); [2] across cows and days (n = 52 for P1, n = 39 for P2); CV: coefficient of variation (SD/mean); Concentrates intake = high protein concentrate + production concentrate

Table 7
Comparison between estimates of grass *in vivo* organic matter digestibility (G-OMD) : Pearson correlation coefficient (r) and paired t-test results (t value)

		FNIRS-sheep	FNI-1
FNI-1	*r*	0.608*	
	t value	10.79***	
FNI-2	*r*	0.502	0.989***
	t value	7.68***	10.25***

For Pearson coefficient (r) and paired t-test (t value): *P<0.05; **P<0.01; ***P<0.001; FNIRS : NIRS analyse of faeces; FNI : faecal nitrogen indicator.

Based on these results, the FNIRS-sheep and FNI estimates were biased. The bias between the FNIRS-sheep and FNI estimates was approximately 0.08 and 0.06 digestibility units for the FNI-1 and FNI-2 methods, respectively. For all comparisons, SEPc was low, with an error of prediction of less than 0.03 digestibility units for all tested methods (Table 8).

Table 8
Grass *in vivo* organic matter digestibility (G-OMD) estimated by NIRS analyse of faeces (FNIRS-sheep) compared with faecal nitrogen indicator (FNI-1; FNI-2) as reference values (Bias, SEP, SEPc)

	FNIRS-sheep		
	SEP	Bias	SEPc
FNI-1	0.0889	0.0847	0.0283
FNI-2	0.0637	0.0580	0.0272

SEP: standard error of prediction; SEPc: standard error of prediction corrected with bias

III.3. Grass DM intake, comparison between methods

As shown in Table 9, the G-DMI estimations ranged from 11.9 to 16.4 kg/d. The inter-cow variation was high, with the CV ranging between 0.15 and 0.37, depending on the estimation method. The variation in the evolution of sward during grazing was similar to the inter-cow variation for all tested methods. At paddock level, all estimation methods showed a decrease in the G-DMI linked to a decrease in herbage allowance. As observed for herbage allowance, the G-DMI estimated using the FNIRS-cattle and RT methods was higher in P2 than in P1. With the G-DMI higher than 20 and 17 kg/d in P2, respectively, it was possible that the RT values for G-DMI were overestimated.

Table 9
Estimated grass dry matter intake (G-DMI, kg/d) by NIRS analyse of faeces (FNIRS-sheep; FNIRS-cattle), ratio technique (RT-1 ; RT-2) and animal performance method (APM-1; APM-2 ; APM-3), variation linked to cows and to paddocks (n=91)

Cow		FNIRS-sheep	FNIRS-cattle	RT-1	RT-2	APM-1	APM-2	APM-3
1		14.7	15.8	17.2	15.7	12.7	12.4	12.5
2		12.8	12.1	20.3	17.9	12.7	12.2	13.2
3		12.1	12.9	18.0	15.1	12.6	12.2	13.9
4		13.4	14.2	20.5	17.2	13.6	13.3	14.8
5		13.6	15.6	18.9	16.1	12.5	11.0	12.9
6		12.0	13.0	18.2	16.0	11.8	11.6	13.1
7		10.6	13.4	10.8	9.1	9.8	7.3	9.3
8		13.1	13.5	14.3	11.8	13.3	12.9	14.6
9		15.0	14.2	17.3	15.4	14.2	16.0	15.5
10		11.7	9.9	12.9	11.7	11.3	10.2	11.4
11		11.7	9.8	12.1	10.8	11.7	11.6	11.4
12		10.9	9.9	10.4	10.1	10.3	10.1	10.2
13		17.1	17.0	22.8	20.2	14.6	13.5	14.0
Mean ± SD[1]		13.0 ± 2.28	13.2 ± 3.56	16.4 ± 6.02	14.4 ± 4.91	12.4 ± 1.90	11.9 ± 2.64	12.8 ± 2.46
CV		0.175	0.269	0.365	0.340	0.153	0.222	0.191
Paddock	Day	FNIRS-sheep	FNIRS-cattle	RT-1	RT-2	APM-1	APM-2	APM-3
P1	2	13.5	12.2	18.0	16.2	12.8	12.4	13.3
P1	3	13.2	11.7	13.9	12.4	13.1	13.1	13.9
P1	4	12.4	10.2	11.5	10.6	11.9	11.4	12.2
P1	5	11.8	11.0	11.9	10.8	11.3	10.4	11.4
P2	6	13.8	15.6	20.5	17.5	12.4	11.7	12.8
P2	7	13.9	16.3	20.1	17.0	12.9	12.3	13.4
P2	8	12.6	15.2	19.6	16.5	12.5	11.8	12.9
		Mean ± SD[2]						
P1		12.7 ± 2.07	11.3 ± 2.66	13.8 ± 5.19	12.4 ± 4.54	12.2 ± 1.78	11.8 ± 2.43	12.7 ± 2.34
	CV	0.163	0.235	0.377	0.364	0.146	0.205	0.184
P2		13.4 ± 2.48	15.7 ± 2.99	20.0 ± 5.16	17.0 ± 4.15	12.6 ± 2.05	11.9 ± 2.92	13.1 ± 2.61
	CV	0.184	0.190	0.257	0.244	0.163	0.245	0.200

SD: standard deviation of the mean; [1] across cows (n=91); [2] across days (n=52 for P1 and n=39 for P2); CV: coefficient of variation (SD/mean).

Based on the Pearson coefficients, there was correlation between the FNIRS-sheep and FNIRS-cattle estimates ($r=0.79$; $P<0.01$), as well as between the RT-1 and RT-2 estimates

(r=0.99; P<0.001) and the APM-1, APM-2 and APM-3 estimates (r ranged from 0.90 to 0.93; P<0.001). The FNIRS-sheep estimates were correlated with the RT (r=0.75 and 0.78; P<0.01) and APM estimates (r=0.88, P<0.001; r=0.74, P<0.01 and r=0.67, P<0.05). The FNIRS-cattle estimates were not correlated with the APM-2 or APM-3 estimates (r=0.39 and r=0.50; P>0.05) (Table 10). The relationships between FNIRS and all other tested methods are shown in Fig. 3.

Table 10
Comparison between grass dry matter intake (G-DMI) estimates: Pearson correlation coefficient (r) and paired t-test results (t value)

Method		FNIRS-sheep	FNIRS-cattle	RT-1	RT-2	APM-1	APM-2
FNIRS-cattle	*r*	0.790**					
	t value	0.49					
RT-1	*r*	0.745**	0.694**				
	t value	4.32***	4.07**				
RT-2	*r*	0.778**	0.670*	0.988***			
	t value	2.26*	1.77	8.79***			
APM-1	*r*	0.880***	0.635*	0.802***	0.790**		
	t value	2.42*	1.60	4.93***	3.01*		
APM-2	*r*	0.739**	0.392	0.617*	0.633*	0.912***	
	t value	2.84*	1.96	5.23***	3.51**	1.96	
APM-3	*r*	0.672*	0.503	0.736**	0.696**	0.928***	0.905***
	t value	0.36	0.58	4.49***	2.29*	2.15	4.00**

For Pearson coefficient (r) and paired t-test (t value): *P<0.05; **P<0.01; ***P<0.001; FNIRS : NIRS analyses of faeces; RT : ratio technique; APM : animal performance method

Based on the results of the paired t-test, FNIRS-sheep and FNIRS-cattle and FNIRS-sheep and APM-3 gave similar G-DMI estimates (t=0.49 and t=0.36; P>0.05). The FNIRS-cattle estimates differed only from the RT-1 estimates (t=4.07; P<0.01) (Table 10). Based on these observations, it appeared that the RT methods tended to overestimate G-DMI, with the exception of the FNIRS-sheep – RT-2 pair.

Table 11
Grass dry matter intake (G-DMI, kg/d) estimated by NIRS analyses of faeces (FNIRS-sheep;FNIRS-cattle) compared with ratio technique (RT-1; RT-2) and animal performance method (APM-1; APM-2; APM-3) as reference values (Bias, SEP, SEPc)

	FNIRS-sheep			FNIRS-cattle		
	SEP	Bias	SEPc	SEP	Bias	SEPc
FNIRS-cattle	1.36	0.19	1.40	/	/	/
RT-1	4.43	3.46	2.89	4.30	3.27	2.90
RT-2	2.59	1.41	2.25	2.69	1.22	2.49
APM-1	1.04	-0.59	0.88	1.87	-0.78	1.77
APM-2	1.76	-1.12	1.42	2.65	-1.31	2.40
APM-3	1.43	-0.15	1.48	2.04	-0.34	2.10

SEP: standard error of prediction ; SEPc: standard error of prediction corrected with bias

For G-DMI, if the FNIRS methods were well correlated with other estimation methods, bias was suspected (Table 11). The bias between the APM estimates and the FNIRS-sheep or FNIRS-cattle estimates was lower than 1.5 kg DM/d (12 g DM/kg metabolic weight). The bias between the FNIRS-sheep and FNIRS-cattle estimates was low (0.2 kg DM/d or 1.6 g DM/kg metabolic weight). There was a higher bias when RT-1 was taken as the reference method (bias ranged from 3.3 to 3.5 kg DM/d; 27-29 g DM/kg metabolic weight). The RT estimates in P2, in particular, appeared to be biased and probably overestimated. When the FNIRS estimates were compared with the APM estimates, the SEPc was at a maximum of 2.4

kg DM/d (20 g DM/kg metabolic weight). When the FNIRS estimates were compared with the RT estimates, the SEPc was higher, with a maximum of 2.9 kg DM/d (24 g DM/kg metabolic weight).

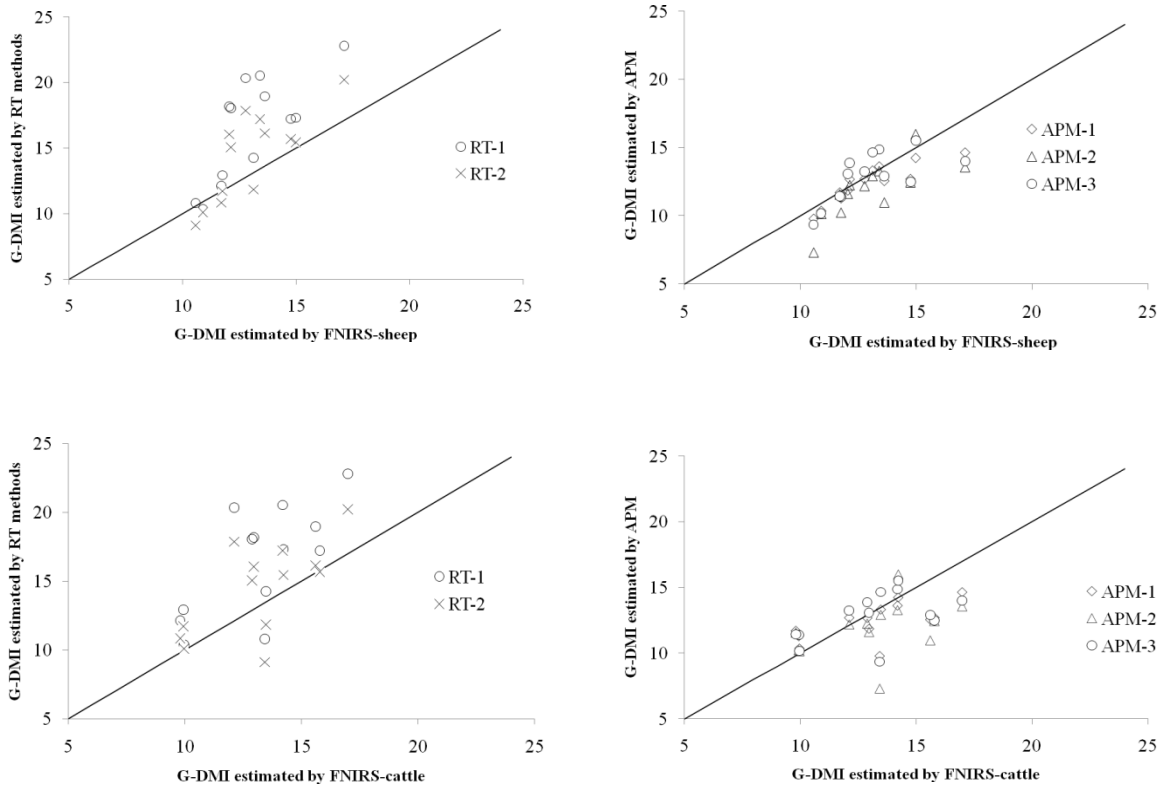

Fig. 3. Grass dry matter intake (G-DMI, kg/d): correlations between NIRS analyses of faeces (FNIRS-sheep and FNIRS-cattle), ratio technique (RT-1 and RT-2) and animal performance method (APM-1, APM-2 and APM-3) estimations

IV. Discussion

IV.1. G-OMD and G-DMI estimates

A good method for estimating the digestibility or intake by grazing ruminants needs to be accurate, repeatable and easily applied to individual animals or herds. Based on this study, the G-OMD estimates using the FNIRS-sheep method (0.706 in d2-P1 and 0.674 in d6-P2) were lower than the G-OMD estimates using NIRS analyses of grass sampled in the field (0.720 in P1 and 0.693 in P2 on the first day of paddock grazing). Similarly, the G-OMD estimates using OMDcel (0.738 and 0.726 for P1 and P2, respectively) was higher than the FNIRS estimates. The G-OMD estimates obtained using the FNI method were also higher (d2-P1 = 0.788 and 0.766, and d6-P2 = 0.787 and 0.755, for FNI-1 and FNI-2, respectively) than the FNIRS estimates. As reported by Biston et al. (1988) and Lippke (2002), G-OMD estimates based on forage analysis, as in the case of NIRS applied to grass and OMDcel, are very dependent on the grass sampling. The difference could be explained by the difference between the grass analysed and the grass ingested by cows.

Compared with the FNI estimates, the FNIRS-sheep method gave lower G-OMD estimates. If the FNI estimates are taken as the reference, this could mean that the FNIRS-sheep method underestimated the G-OMD. The FNIRS-sheep database contained only OMD obtained from sheep digestibility trial, with sheep housed in digestibility crates and fed exclusively on fresh grass. In this context, it is possible that the faeces spectra of supplemented dairy cows did not correspond exactly to the range of spectra of the FNIRS-sheep database, as reflected by the average standardized H distance being higher than 3 (H = 5.32; three quarts of 91 samples with H lower than 6). This hypothesis was confirmed by Leite and Stuth (1995), who reported that faecal calibrations developed for cattle were not appropriate for estimating the diet characteristics of goats. They suggested that this was linked to the chemical difference between goat and cattle faeces. Another explanation for the higher H value could be linked to the grinding. The FNIRS-sheep samples described by Decruyenaere et al. (2009) were initially ground in a hammer mill and then in a Cyclotec mill, whereas the dairy cow faecal samples were ground only in a hammer mill.

As the FNIRS-sheep method produced D-OMD estimates, and the G-OMD was derived from D-OMD through calculation, it is possible that this method of estimation led to an underestimation of G-OMD because diet digestibility was not always the weighted average of the digestibility of each component of the diet (Demarquilly et al., 1995). If the FNIRS estimates of G-OMD were underestimated, then, in contrast, the FNI estimates could have been overestimated. Both the FNIRS and FNI methods were calibrations linking NIRS spectra to OMD obtained from digestibility trials with sheep (Decruyenaere et al., 2009) and linking faecal nitrogen to OMD obtained from steers housed in individual stalls (Bartiaux and Oger, 1986) and from unsupplemented grazing dairy cows (Peyraud et al., unpublished data). It is also possible that the specificity of the databases used for developing the FNIRS and FNI models in terms of sward characteristics and animal species could explain the difference between estimates. As reported by Lukas et al. (2005), FNI models are generally very specific and related to one type of pasture (single-species sward in most cases) and animal. The FNI models would therefore be difficult to apply when ruminants graze more heterogeneous pastures or have a mixed diet. In addition, the FNI as described by Bartiaux et al. (1986) and Peyraud et al. (unpublished data) were linear or quadratic models, which tended to overestimate G-OMD when there were higher values for faecal CP (Fanchone et al. 2009).

With regard to the G-DMI, there appeared to be good correlation between the FNIRS and APM estimates. The very good correlation between the APM and FNIRS-sheep estimates was probably due to milk production, which was used in both models to estimate G-DMI. The FNIRS and APM estimates were lower than those obtained using the RT and sward cutting methods. For the supplemented grazing dairy cows, the average G-DMI generally varied between 11 and 15 kg/d (Delagarde et al., 1999; Soder et al., 2006; Morrison and Patterson, 2007). At cow level, the FNIRS, RT and APM estimates of G-DMI were close to these values. At paddock level, the RT estimates of G-DMI were very high during P2 grazing, but the RT estimates, although higher, were close to those reported by Bartiaux et al. (1985). These higher values could be explained by the use of Cr_2O_3 to estimate faecal output. Macoon et al. (2003) found that the use of an external marker (Cr_2O_3) as a pulse dose marker led to higher intake estimates. In our study, it is possible that the higher RT estimates were related to an overestimation of both G-OMD and faecal output. The G-OMD values obtained using the FNI methods have already been discussed. The faecal output estimates in this study were, on average, 4.9 kg DM/d. Even where faecal excretion varied with the growth stage of the grass and with the animal (Demarquilly et al., 1995), our estimated faecal output was 10 percent higher than that reported by Delagarde et al. (1999) for supplemented grazing dairy cows. One explanation could be the use of the dilution of Cr_2O_3 in faeces for estimating

faecal output. The intra-ruminal Cr_2O_3 device, as used in our study, was recommended for limiting the diurnal variation of marker excretion, but it was sometimes associated with an underestimation of Cr_2O_3 concentration in faeces (Lippke, 2002), which led an overestimation of faecal output (Hollingsworth et al., 1995). Faecal sampling in the field or grab sampling could also be a source of error. Biston et al. (1988) showed that grab sampling led to an underestimation of the faecal Cr_2O_3 concentration and therefore to a higher estimation of faecal output. In addition, the release of Cr_2O_3 in the rumen could be influenced by diet or supplementation (Hollingsworth et al., 1995; Lippke, 2002). The recovery rate of Cr_2O_3 could be incomplete, which might also lead to an overestimation of the faecal output and, for a same digestibility, of the intake. In order to adjust faecal output estimation, several studies have suggested weighting the total faecal excretion of one or two cows fed with the same diet and receiving a Cr_2O_3 marker (Hollingsworth et al., 1995; Lippke, 2002; Lukas et al., 2005). Another issue is the possible Cr_2O_3 interference with the NIRS analysis. According to Acamovic et al. (1992), the maximum of absorbance of Cr_2O_3 was in the visible part of the spectrum, linked mainly to the green colour of this marker. Our FNIRS models were developed only in the NIR part of the spectrum (1100 to 2498 nm). Therefore, if this interference exists, it must be low.

IV.2. Accuracy of G-OMD and G-DMI estimates

Boval et al. (2004) and Fanchone et al. (2007) demonstrated that FNIRS was accurate enough for assessing the digestibility of tropical herbage grazed by creole heifers or sheep. The methods of estimating G-OMD based on faecal analyses using FNIRS or FNI could be more accurate than the methods based on grass analyses, because faecal methods take into account the selectivity of the grazing animals. To predict the D-OMD, the accuracy of the FNIRS-sheep method, expressed by the SECV of calibrations, was 0.021 digestibility units, which was close to values reported in previous studies using FNIRS to predict digestibility (Leite and Stuth, 1995; Fanchone et al., 2007; Fanchone et al., 2009). With regard to the inter-cow variation (CV ranged from 0.05 to 0.07 digestibility units) and inter-paddock variation (CV ranged from 0.02 to 0.08 digestibility units), these levels of accuracy appeared to be sufficient for recording the digestibility variations related to cows and to grass evolution during the grazing period.

As reported by Bartiaux and Oger (1986) and Peyraud et al. (unpublished data), the FNI-1 and FNI-2 models appeared to be more accurate than the FNIRS models for predicting G-OMD. Both models produced G-OMD estimates with an SE of 0.014 and 0.009 unit of digestibility, respectively. This level of accuracy was similar to that generally reported by other studies using the same methodology (Comeron and Peyraud, 1993; Boval et al., 1996; Bouazizi and Majdoub, 1999; Lukas et al., 2005; Fanchone et al., 2009). It is possible that the accuracy of the FNI methods as applied in our study was not exactly the same as that reported by Bartiaux and Oger (1986) and Peyraud et al. (unpublished data). In both cases, the FNI methods were used for estimating the G-OMD of unsupplemented grazing animals according linear or quadratic correlations. With a low level of concentrate supplementation (lower than 3.7 kg/cow/d), Bartiaux et al. (1985) showed that an increase in the faecal CP was proportional to an increase in the diet CP. In this case, the relationship between digestibility and faecal CP could provide an estimate of the digestibility of the total diet. Where the level of concentrate is higher, as in our study, Delagarde et al. (1999) suggested that it was better to distinguish the proportion of faecal CP and faecal crude fibres attributable to grass and to concentrate before using the FNI model. The difficulty lay in accurately estimating the proportion of faecal CP and fibres attributable to the supplement, especially when it was a commercial formulation. If the faecal CP attributable to concentrate was underestimated,

faecal CP attributable to grass was overestimated and the G-OMD would probably be overestimated. The number of estimations used to calculate G-OMD increases the likelihood of estimation errors and therefore reduces the precision of the model. In addition, when dairy cows are supplemented, this could enhance the microbial synthesis in the rumen and lead to an increase in protein residues in the faeces. With the positive and linear relationship between OMD and faecal CP, any increase in faecal CP would lead to an increase in predicted G-OMD (Lukas et al., 2005; Fanchone et al., 2009).

For the FNIRS-sheep and FNIRS-cattle G-DMI estimates, the error of estimation, commonly expressed by SECV or SEP, was 5.4 and 6.8 g/kg metabolic weight, respectively (SECV = 0.7 and 0.8 kg DM/d, respectively). Boval et al. (2004) achieved similar accuracy when predicting OM intake (SECV = 5.3 g/kg metabolic weight). In a study by Garnsworthy and Unal (2004), the accuracy of the D-DMI calibration, expressed by SEP, was also similar (SEP = 3.8 g/kg metabolic weight). The very good performances of faecal NIRS calibrations developed by Garnsworthy and Unal (2004) could be explained by the characteristics of the calibrated set of samples that represent the current and sufficiently wide variability in dairy cow intake, as in the case of the FNIRS-cattle database.

The results reported by Andueza et al. (2007) indicated that the intake variability linked to animals was always high, and in most cases, for the same forage, CV could be close to 0.1. These authors concluded that it was not possible to estimate intake below this level of precision. Our results showed that, with a specific FNIRS database, as in the case of the FNIRS-cattle database, the SECV was close to 1 kg DM/cow/d, representing a variation of 0.1 around the mean value. The FNIRS models could therefore be used to determine variations between cows or relating to grass evolution over time.

Smit et al. (2005) reported that the variability in sward cutting method estimates depended on the cutting material or the operator. Errors related to herbage accumulation under grazing could be important. In addition, it was impossible to use this technique to estimate the individual intake of a group of cows grazing the same paddock (Macoon et al., 2003).

It was difficult to determine the level of precision of the RT or APM techniques. The accuracy of the RT method depended on the error of estimation for both digestibility and faecal output (Lippke, 2002). The APM, based on animal requirements and diet characteristics, were interesting for herd feeding management, but less so for determining individual variations in intake. In addition, estimating the requirements related to cow movement and grazing was difficult (Macoon et al., 2003); when the total requirements were known, they needed to be divided by the nutritional value of the diet in order to calculate intake. Again, with grazing ruminants, the determination of the nutritional value of the truly ingested diet remained a major problem and a potential source of error, as reported by Smit et al. (2005).

As reported by Fanchone et al. (2009), the main advantage of FNIRS compared with other faecal analysis methods was that it took into account the entire chemical composition of faeces through the use of the full NIRS spectrum. For example, Decruyenaere et al. (2009) have shown that fat wavelengths can be used to reflect the intensity of microbial synthesis in the rumen. Therefore, taking into account all wavelengths of the FNIRS spectrum could improve the accuracy of predicting both digestibility and intake. With regard to the ease of using the NIRS analytical procedure and the accuracy of the prediction, FNIRS, as a predictive method for estimating digestibility and intake, could be used as a tool for managing the feeding of grazing ruminants at individual and herd level.

V. Conclusion

With regard to the high inter-animal variation and the evolution of grass quality during grazing, the accuracy of the FNIRS and others tested methods was good for estimating both grass digestibility and the intake of grazing ruminants. The results showed that FNIRS can estimate digestibility and intake of grazing dairy cows with enough accuracy to differentiate the cows and the evolution of grass quality and quantity at paddock level in a rotational grazing system. FNIRS is easier to apply in grazing situations because only the analysis of dried and ground faecal samples is required to estimate intake and digestibility without animal manipulation or heavy analytical procedures. Using the NIRS faecal spectrum to predict digestibility and intake in a dairy cow herd provides more information on diet really ingested than diet analysis. These FNIRS estimations could be used in decision-support systems for improving the management of grazing dairy herds.

Our results also underline the difficulty of developing a spectral database that is as representative as possible of field data diversity, while maintaining a high degree of accuracy. The accuracy and choice of reference method used to develop the calibration is a key factor in determining the accuracy of the NIRS model. Under these conditions, it is probable that digestibility and intake values provided by *in vivo* trials remain the best way of obtaining a robust calibration.

VI. References

AFNOR, 1993. Agricultural and food product. Determination of crude fibre. General method. French standard, NF V03-040, Paris, October 1993 (in French).

AFNOR, 1997. Animal feeding stuffs. Determination of sequential cell-wall. Method by treatment with neutral and acid detergent and sulfuric acid. French standard, NF V18-122, Paris, August 1997 (in French).

Acamovic, T., Murray, I., Paterson, R.M., 1992. The use of NIR in predicting chromic oxide in diets. In Murray I. and A.I., eds. Making light work. Advances in near infrared spectroscopy. Ian Michael Publications, pp 250-252.

Adegosan, A.T., Givens, D.I., Owens, E., 2000. Measuring chemical composition and nutritive values in forages. In: 't Mannetje L. and Jones R.M., eds. Field and laboratory methods for grassland and animal production research. CAB International, pp 263-278.

Andueza, D., Picard, F., Pecatte, J.R., Gallard, Y., Hassoun, Ph., Viudes, G., Egal, D., Pradel, Ph., Trocquier, O., Thomas, D., Delaby, L., Agabriel, J., Baumont, R., 2007. Variability within and among laboratories of *in vivo* digestibility and voluntary intake of two hays evaluated in sheep. Renc. Rech. Rum. 14, 247 (in French).

Bartiaux-Thill, N., Biston, R., Dardenne, P., François, E., Théwis, A., Gielen, M., Limbourg P., 1985. Mesure de l'ingestion d'herbe par la vache laitière au pâturage au moyen de la méthode des index fécaux. (Eng. abstr.). Rev. Agric., 2, 38, 317-326.

Bartiaux-Thill, N., Oger, R., 1986. The indirect estimation of the digestibility in cattle of herbage from Belgian permanent pasture. Grass Forage Sci. 41, 269-272.

Berry, N.R., Scheeder, M.R.L, Sutter, F., Krober, T.F., Kreuzer, M., 2000. The accuracy of intake estimation based on the use of alkane controlled-release capsules and faeces grab sampling in cows. Ann. Zootech., 49, 3-13.

Biston, R., Bartiaux-Thill, N., Théwis, A., François, E., Limbourg, P., Gielen, M., Dardenne, P., 1988. Prévision de la quantité d'herbe ingérée par la vache laitière au pâturage. Comparaison de methodes d'estimation de la digestibilité et du mode d'échantillonnage des fèces. (Eng. abstr.). Reprod. Nutr. Dévelop., 28 Suppl 1, 61-62.

Bouazizi, A., Majdoub, A., 1999. Prédiction des quantités ingérées et de la digestibilité du régime sélectionné par des ovins sur parcours semi-aride tunisien. (Eng. abstr.). Fourrages 157, 77-87.

Boval, M., Coates, D.B., Lecomte, P., Decruyenaere, V., Archimède, H., 2004. Faecal Near Infrared Reflectance Spectroscopy (NIRS) to assess chemical composition, *in vivo* digestibility and intake of tropical grass by Creole cattle. Anim. Feed Sci. Technol., 114, 19-29.

Boval, M., Peyraud, J.L., Xande, A., Aumont, G., Coppry, O., Saminadin, G., 1996. Evaluation d'indicateurs fécaux pour prédire la digestibilité et les quantités ingérées de Dichanthium sp par des bovins créoles. (Eng. abstr.). Ann. Zootech. 45, 121-134.

Coates, D.B., Penning, P., 2000. Measuring animal performances. In: L. 't Mannetje, R.M. Jones (Eds), Field and Laboratory Methods for grassland and Animal Production Research. CAB International, pp. 353-402.

Coleman, S.W., 2005. Predicting forage intake by grazing ruminants. 16th Florida Ruminant Nutrition Symposium, 1-12 February, Gainsville, Florida, pp 72-90.

Comeron, E.A., Peyraud, J.L., 1993. Prediction of herbage digestibility ingested by dairy cows. Rev. Argent. Prod. Anim. 13, 23-30.

Compère, R., Valdivia, J., Hellemans, P., Guns, M., Vandenbyvang, P., 1992. Comparaison de deux méthodes utilisant le Cr2O3 pour estimer la quantité de fèces émises par les moutons : pellets de luzerne et bolus CAPTEC-CRD. (Eng. abstr.). Bull. Rech. Agron. Gembloux, 27, 365-383.

CVB, 1999. Manual of calculating the feeding value of roughages. Centraal Veevoeder Bureau, Lelystad, The Netherlands (in Dutch).

De Boever, J.L., Cottyn, B.G., Buysse, F.X., Wainman F.W., Vanacker, J.M., 1986. The use of an enzymatic technique to predict digestibility, metabolizable and net energy of compound feedstuffs for ruminants. Anim. Feed. Sci. Technol. 14, 203-214.

De Boever, J.L., Cottyn, B.G., Andries, J.I., Buysse, F.X., Vanacker, J.M., 1988. The use of pepsin cellulase technique to predict digestibility metabolizable and net energy of forages. Anim. Feed. Sci. Technol. 19, 247-260.

De Brabander, D. 1993. Feeding of dairy cows. Eds: Ministère de l'Agriculture, administration de l'agriculture et de l'horticulture, Service Information; 4éme édition (in French), 71p.

Decruyenaere, V., De Visser, M., Stilmant, D., Clément, C., Sinnaeve, G., 2004. Faecal Near Infrared Reflectance Spectroscopy for ruminant feed intake prediction. In A. Lüscher, B.

Jeangros, W. Kessler, O. Huguenin, M. Lobsiger, N. Millar, D. Suter (Eds.), Land and use systems in grassland dominated regions. Grassl. Sci. Eur. 9, pp.1034-1036.

Decruyenaere, V., Bony, J., Grimaud, P., Lecomte, Ph., 2006. A methodological approach in La Reunion Island on the value of faecal Near Infrared reflectance Spectroscopy (NIRS) to assess grazing intake and diet quality of the dairy cow. In C.A. Sandoval-Castro, F.D. DeB Hovell, J.F.J. Torres-Acosta and A. Ayala-Burgos (Eds), Herbivores the assessment of intake, digestibility and the roles of secondary compounds. BSAS publication 34, pp 93-99.

Decruyenaere, V., Lecomte, Ph., Demarquilly, C., Aufrere, J., Dardenne, P., Stilmant, D., Buldgen, A., 2009. Evaluation of green forage intake and digestibility in ruminants using near infrared reflectance spectroscopy (NIRS): Developing a global calibration. Anim Feed Sci. Technol., 148, 138-156.

Delagarde, R., Peyraud, J.L., Delaby, L., 1999. Influence of carbohydrate or protein supplementation on intake, behaviour and digestion in dairy cows strip-grazing low-nitrogen fertilized perennial ryegrass. Ann. Zootech. 81, 81-96.

Demarquilly, C., Chenost, M., Giger, S., 1995. Pertes fécales et digestibilités des aliments et des rations. In Jarrigue, R, Ruckenbusch, Y., Demarquilly, C., Farce, M-H., Journet, M.(Eds), Nutrition des ruminants domestiques, ingestion et digestion. INRA, France, pp.601-647.

Dillon, P., Hennessy, T., Shalloo, L., Thorne, F., Horan, B., 2008. Future outlook for the Irish dairy industry: a study of international trade reform and requirement for change. International J. Dairy Technol. 61, 16-29.

Dulphy, J.P., Faverdin, Ph., Micol, D., Bocquier, F., 1987. Révision du système des Unités d'Encombrement (UE). (Eng. abstr.). Bull. Tech. C.R.Z.V. Theix, INRA 70, 35-48.

Fanchone, A., Boval, M., Lecomte, Ph., Archimède, H., 2007. Faecal indices based on near infrared spectroscopy to assess intake, *in vivo* digestibility and chemical composition of herbage ingested by sheep (crude protein, fibres and lignin content). J. Near Infrared Spectrosc. 15, 107-113.

Fanchone, A., Archimède, H., Boval, M., 2009. Comparison of fecal crude protein and fecal near-infrared reflectance spectroscopy to predict digestibility of fresh grass consumed by sheep. J. Anim Sci. 87, 236-243.

Ferreira, L.M.M., Olivan, M., Rodrigues, M.A.M., Osoro, K., Dove, H., Dias-Da-Silva, A., 2004. Estimation of feed intake by cattle using controlled-release capsules containing n-alkanes or chromium sesquioxide. J. Agric. Sci., 142, 225-234.

François, E., Thill, N., Théwis, A., 1978. Méthode rapide de dosage de l'oxyde de chrome dans les aliments, les féces et les contenus digestifs par titrage après oxidation nitro-perchlorique. (Eng. abstr.). Ann. Zootech. 27, 355-361.

Garnsworthy, P.C., Unal, Y., 2004. Estimation of dry matter intake and digestibility in group-fed dairy cows using near infrared reflectance spectroscopy. Anim Sci. 79, 327-334.

Hollingsworth, K.J., Adams, D.C., Klopfenstein, T.J., Lamb, J.B., Villalobos, G., 1995. Supplement and forage effects on fecal output estimates from intra-ruminal marker device. J. Range Manage. 48, 137-140.

INRA, 1989. Ruminant nutrition, recommended allowances and feeds tables. In R. Jarrige (ed). Inra, John Libbey, Paris.

Landau, S., Glasser, T., Dvash, L., Perevolotsky, A., 2004. Faecal NIRS to monitor diet of mediterranean goats. S. Afr. J. Anim. Sci., 34, 76-80.

Leite, E.R., Stuth, J.W., 1995. Fecal NIRD equations to assess diet quality of free-ranging goats. Small Rumin. Res., 15, 223-230.

Li, H., Tolleson, D., Stuth, J., Bai, K., Mo, F., Kronberg, S., 2007. Faecal near infrared reflectance spectroscopy to predict diet quality for sheep. Small Ruminant Res., 68, 263-268.

Lippke, H., 2002. Estimation of forage intake by ruminants on pasture. Crop Sci., 42, 869-872.

Lukas, M., Südekum, H. H., Rave, G., Friedel, K., Susenbeth, A., 2005. Relationship between fecal crude protein concentration and diet organic matter digestibility in cattle. J. Anim Sci., 83, 1332-1344.

Macoon, B., Sollenberg, L.E., Moore, J.E., Staples, C.R., Fike, J.H., Portier, K.M., 2003. Comparison of three techniques for estimating the forage intake of lactating dairy cows on pasture. J. Anim Sci. 81, 2357-2366.

Mayes, R.W., Lamb, C.S., Colgrove, P.M., 1986. The use of dosed and herbage n-alkanes as markers for determination of herbage intake. J. Agric. Sci. Camb., 107, 161-170.

Meijs, J.A.C., Walters, R.J.K., Keen, A., 1982. Sward methods. In J.D. Leaver (ed), Herbage intake handbook. British Grassland Society, pp16-36.

Morrison, S.J., Patterson, D.C., 2007. The effects of offering a range of forage and concentrate supplements on milk production and dry matter intake of grazing dairy cows. Grass For. Sci. 62, 332-315.

NRC, 2001. Nutrient requirements of dairy cattle. 7th rev. ed. National Research Council, National Academy Press, Washington, DC.

Ribeiro Filho, H.M.N., Delagarde, R., Peyraud, J.L. 2003. Inclusion of white clover in strip-grazed perennial ryegrass swards: herbage intake and milk yield of dairy cows at different ages of sward regrowth. Anim. Sci. 77, 499-510.

Rhoner-Thielen, E, 2008. From grass to glass; a look of the dairy chain. Eurostat, Statistics in Focus,76/2008, 8p.

Robertson, J.B., Van Soest, P.J., 1981. Permanganate and sulphuric acid lignin, ADF, hemicellulose, cellulose, cutin and Si. In: James, W.P.T. and Theander, O. (Eds.), The Analysis of Dietary Fibre in Food., Ch. 9, The detergent system of analysis., Marcel Dekker, N.Y. and Basel, pp. 123-158.

Schapendonk, A.H.C.M., Stol, W., van Kraalingen, D.W.G., Bouman, B.M.A., 1998. LINGRA, a sink/source model to simulate grassland productivity in Europe. European J. Agronomy. 9, 87-100.

Shenk, J.S., Westerhaus, M.O., 1991. Population definition, sample selection, and calibration procedures for near infrared breflectance spectroscopy. Crop Sci. 31, 469-474.

Smit, H.J., Taweel, H.Z., Tas, B.M., Elgersma, A., 2005. Comparison of techniques for estimating herbage intake of grazing dairy cows. J. Dairy Sci. 88, 1827-1836.

Soder, K.J., Sanderson, M.A., Stack, J.L., Muller, L.D., 2006. Intake and performance of lactating cows grazing diverse forage mixtures. J. Dairy Sci., 89, 2158-2167.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. J. Dairy Sci. 74, 3583-3597.

Methodological approach in La Reunion Island on the value of faecal near infrared reflectance spectroscopy (NIRS) to assess grazing intake and diet quality of the dairy cows

Article VIII – Adapted from Decruyenaere et al. (2006). In: Sandoval-Castro, C.A., DeB Hovell, F.D., Torres-Acosta, J.F.J., Ayala-Burgos, A. (Eds.), Herbivores the Assessment of Intake, Digestibility and the Roles of Secondary Compounds, 34. BSAS Publication, 93–99.

Decruyenaere, V.[(1)], Bony, J.[(2)], Grimaud, P.[(4)], Lecomte, Ph.[(1,3)]

[(1)] Farming Systems Section, Walloon Centre of Agricultural Researches, 100 rue du Serpont, B-6800Libramont (Belgium)

[(2)] National Institute for Agricultural research, Herbivores Research Unit (INRA-URH), Clermont-Ferrand Theix; on secondment to CIRAD, Pole Elevage, Saint Pierre, La Réunion Island, France

[(3)] Centre International in Agricultural Research for Development (CIRAD-EMVT), Animal Productions program, Montpellier, France

[(4)] Centre International in Agricultural Research for Development (CIRAD-EMVT), Pole Elevage, Saint Pierre, La Réunion Island, France

corresponding auhtor – E-mail : v.decruyenaere@cra.wallonie.be

Abbreviation : BW, body weight; DM, dry matter; GGI, grazed grass intake; LCTI, lactating cow total intake; OMD, *in vivo* organic matter digestibility; OMI, organic matter intake; MP, milk production; NIRS, near infrared reflectance spectroscopy; PLS, partial least square; SD, standard deviation; SBW, standard body weight; SEC, standard error of calibration; SECV, standard error of cross-validation

Abstract

To test the applicability of faecal NIRS to real conditions, an experimental approach was undertaken across several representative dairy farms (N = 30) located in 'La Réunion' Island. From an ongoing survey, this approach consists to characterize the nutritional value of all feeds (grazed fresh forage, hay, silages and supplementary feeds) offered to the lactating herds, and to predict ingested diet from faecal NIRS models previously developed on a large experimental sheep faeces reference database.

The methodological objective was to evaluate if such a spectral database could be a useful reference to estimate dairy cow total dry matter intake and diet quality, and so predict the grazed grass intake with reasonable accuracy. According to preliminary results, the NIRS estimated total intake varied between 13.7 and 19 kg DM/day and *in vivo* organic matter digestibility ranged from 51.7 to 74.8 % with an average value of 66 %. The estimated grass intake varied between 0 to nearly 10 kg DM/day. On a spectral basis, dairy cows faeces were quite different from the sheep faeces reference database, with an averaged standardised distance (H) upper of 3.0 (H = 9.1; H_{min} = 2.08 – H_{max} = 19.22) but predicted intake appeared valid. Indeed, according to the feeding value of diets and lactating cow requirements, the NIRS predicted total intakes were well correlated to the level of milk production. Moreover, for four particular situations, the fresh grass was cut, distributed at the trough and total intake

really measured. The correlation between predicted and measured values was high with R^2 = 0.94 and standard error of regression = 0.469 kg DM/day. These initial results appear quite encouraging, although the methodology is still exploratory and needs to be validated across a larger set of data. As a low cost and rapid prediction technique, NIRS appears to be a potential methodology that could find many useful developments in the improvement of the knowledge of forage use in tropical conditions.

Key words : NIRS, faeces, dairy cows, intake, digestibility

I. Introduction

In 'La Réunion' Island (Indian Ocean), 20,000 tons of milk are produced every year. The standardisation of the quality (fat, protein) of the milk delivered for industrial processing is of major concern to all producers and processors. Due to the highly diversified agroecological systems, extending from tropical lowland to temperate altitude conditions, the livestock feeding systems are equally diversified. Preliminary studies showed important variations in milk quality parameters across the territories and between the seasons. Among other determinants, intake and quality of feeds and forage offered to the dairy herds are an important element which characterise milk quality variation that could be linked to feeding practices. To monitor these parameters, along with the collection of data on the feeds and forages distributed at the trough, it is necessary to estimate the grass ingested by animals during their daily outside grazing period. Measuring this component remains the most difficult aspect of range nutrition (Wofford et al., 1985). Recently, approaches based on NIRS analysis of forage and faeces to predict intake were developed to assess diet quality of free ranging or grazing herbivores (Coleman et al. 1989; Coleman and Murray, 1993; Coleman et al., 1999; Coates, 2000; Decruyenaere et al., 2002). NIRS applied to faeces appears to be an interesting tool for rapid and low cost evaluation of diet digestibility and dry matter intake.

To test the faecal NIRS applicability to real conditions, an experimental approach was undertaken across several representative dairy farms located in island agro-ecological contexts. The approach was to monitor the nutritional value of all forages (grazed fresh forage, hay, silages) and supplementary feeds offered to the lactating herds, and to predict diet intake from faecal NIRS models previously developed at CRA-W (Libramont, Belgium) on a large experimental sheep faeces reference database.

The methodological objective of the approach was to evaluate whether such a spectral database could be a useful reference with which to estimate dairy cow total dry matter intake (DMI) and diet quality, and so derive the grazed grass intake with reasonable accuracy. This paper describes the methodological approach and preliminary results of an ongoing survey.

II. Materials and methods

Within a large milk quality survey across 30 dairy farms, a monthly feeding practices follow up protocol has been developed. For the farms which combine grazing and indoor complementary feeding, a representative mean faecal sample of the lactating herd was collected randomly across 10 fresh dungings, inside the grazed pasture. Forage and feeds offered to dairy cows were sampled to define their nutritional value. The daily ration consumed by the herd from the trough was determined by weighing the offered and refused ration constituents. The actual average milk production (MP) of the lactating herd was recorded. The forage, feed and faecal samples were oven-dried at 60°C for 48 h., ground (screen: 1 mm) and submitted to NIRS scanning (NIRsystem 6500, 1108 – 2498 nm by 2 nm steps). The absorption data recorded as log 1/R. Feed and forage quality parameters were predicted according to classical models (Dardenne et al., 1996). Faecal NIR absorbency data were introduced into a general PLS cross-validated model based on a large set of sheep faecal spectra associated with *in vivo* organic matter intake (OMI) and digestibility (OMD) reference experimental data (Table 1).

Table 1
Parameters of the PLS NIRS model predicting standardised sheep organic matter intake (OMI) and *in vivo* organic matter digestibility (OMD)

	n	Mean	SD	SEC	R^2	SECV	Scatter correction.	Math. treatment	PLS terms
OMD %	913	70.69	7.13	1.92	0.93	2.05	SNVD	2 5 5	11
OMI g/kg $BW^{0.75}$	901	49.28	10.60	4.07	0.85	4.26	SNVD	2 5 5	12

BW = body weight; n = number of spectra; SD = standard deviation of reference database; R^2 = coefficient of determination; SEC = standard error of calibration; SECV = standard error in cross validation; PLS = partial least square

OMI data (g/kg $BW^{0.75}$) were then corrected for the ash content of the diet (ash, coefficient) and to a standard body weight (SBW, kg); the lactating cow total intake (LCTI, kg DM/day) being then estimated according to the model of Dulphy et al. (1987):

$$LCTI = \frac{(36.7 + 0.942 \times OMI/(1\text{-}ash) + 1.48 \times MP) \times SBW^{0.75}}{1000}$$

where MP = milk production,

Grazed Grass Intake (GGI, kg DM/day) was then estimated by subtracting the estimated intake at trough from NIRS estimation of the total intake, as described in Fig. 1.

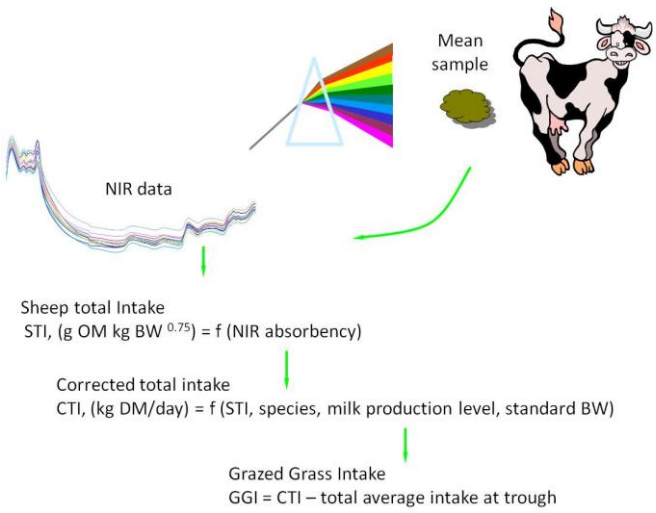

Fig.1. Grass intake estimation procedure

According to feeds and forages predicted nutritional value, the total diet calculation provides an indication on the coherence of the estimated intakes.

III. Results and discussion

Across a panel of 21 situations already observed for cows averaging 620 kg SBW, and milk production ranging between 12.1 to 22.6 l/day, the NIRS estimated total intake varied between 13.7 and 19 kg DM/day/cow. *In vivo* organic matter digestibility ranged from 51.7 to 74.8 % with an average value of 66 %. Total quantities of concentrates and forages distributed at trough varied between 7 and 18 kg DM/day/cow. Under these conditions, the estimated GGI varied between 0 to nearly 10 kg DM/day/cow. These results illustrated the

dairy farm diversity on 'La Réunion' Island. As a proportion, grazed grass ranged between almost 0 up to 60 % of the total intake (Fig. 2).

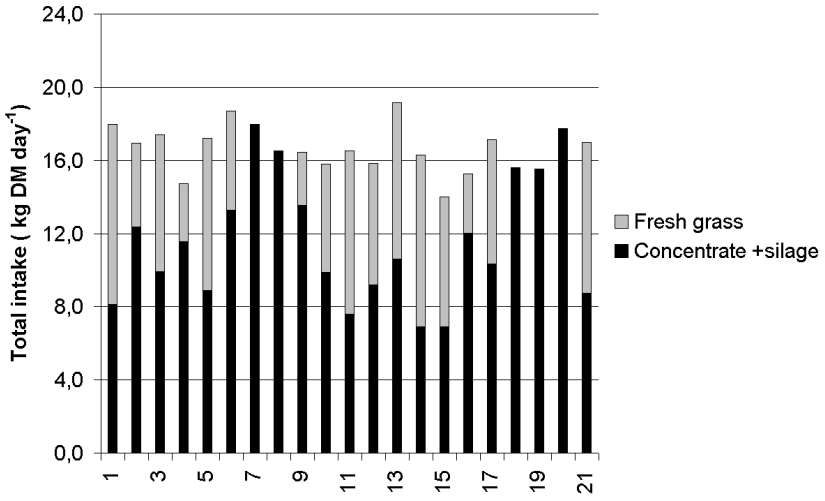

Fig.2. Total intake (kg DM/day) and proportion of constituents in the daily ration

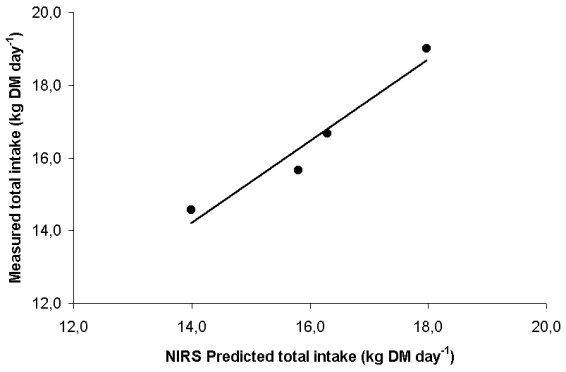

Fig. 3. Relation between NIRS predicted and measured total intake

On a spectral basis, dairy cows faeces were quite distinct from the sheep faeces reference database, with an averaged standardised distance (H) upper of 3.0 (H = 9.1; H_{min} = 2.08 – H_{max} = 19.22).

Nevertheless, according to the feeding value of diet, constituents and lactating cow requirements, the NIRS predicted total intakes appear to be valid. As shown in Fig. 3, a good correlation (R^2 = 0.92) exists between total NIR predicted and measured intakes. Indeed, for four particular situations, according to the farmers' practices, the fresh grass was cut, distributed at the trough and weighed (offered – refusal), total dairy cow intake was in this case, effectively measured and the deviation between predicted and measured values was less than 1 kg per animal per day.

IV. Conclusions

These initial results appear quite encouraging, although the methodology is still exploratory and needs to be validated across a larger set of data. Even if analysed dairy cow faecal samples are quite distinct from the NIR spectral reference base, intake predicted levels appear relevant and well correlated with effectively measured intake. Considering that the major concern here is to be able to compare different feeding strategies rather than to estimate exact intake values, the use of the same NIRS model as a relative predicted indicator across different situations is an interesting approach. As a low cost and rapid prediction technique, NIRS contributes here to a potential methodology that could find many useful developments in the improvement of the knowledge of forage use in tropical conditions.

V. References

Coates, D.B., 2000. Faecal NIRS - What does it offer today grazier? Tropical Grasslands, 34 (3-4): 230-239.

Coleman, S.W., Stuth, J.W., Holloway, J.W., 1989. Monitoring the nutrition of grazing cattle with near-infrared analysis of feces. XVI International Grassland Congress, Nice, France: pp 881-882.

Coleman, S.W., Murray, I., 1993. The use of near-infrared reflectance spectroscopy to define nutrient of hay by cattle. Anim. Feed Sci. Technol., 44: 237-249.

Coleman, S.W., Lippke, H, Gill, M., 1999. Estimating the nutritive potential of forages. In: Nutritional ecology of herbivores. Jung H-J.G and Fahey G.C. (eds), Vth International Symposium on the Nutrition of Herbivores. American Society of Animal Science, Savoy, IL, USA pp 647-695.

Dardenne, P., Sinnaeve, G., Bollen, L., Agneessens, R., 1996. NIR-NIT Calibration list, Libramont, Station de Haute Belgique.

Decruyenaere, V., Stilmant, D., Lecomte, Ph., Buldgen, A., Dardenne, P., 2002. Improvement and indirect validation of the NIRS analysis applied to faeces to measure grass intake in pasture. In: J.L. Durand, J.C. Emile, Ch. Huygue and G. Lemaire (Eds.), Multi-function grasslands. Quality forages, animal products and landscapes. Grassl. Sci. Eur. 7, pp.196-197.

Dulphy, J.P., Faverdin, Ph., Micol, D., Bocquier, F., 1987. Révision du système des Unités d'Encombrement (UE) Bull. Tech. C.R.Z.V. Theix, INRA 70 : 35-48.

Wofford, H., Holechek, J.L., Galyean, M.L., Wallace, J.D., Cardenas, M., 1985. Evaluation of faecal indices to predict cattle diet quality. J. Range Manag. 38, 450-454.

Discussion of the Chapter IV

The validation of NIRS predictions usually involves comparing predicted values and reference values and calculating the standard error of prediction (SEP), which should be close to the SECV of the calibration. The reference method used in this study to generate the reference values for FNIRS was the digestibility trial. It is difficult to calculate SEP exactly with grazing animals because digestibility and intake are not precisely known. We therefore decided to test the ability of FNIRS calibrations to predict the voluntary intake and digestibility of different ruminant species fed with heterogeneous fresh forages in comparison with other current methods of estimating these parameters.

In the first experiment, the DMVI of sheep housed in individual boxes and fed with freshly cut grass was estimated using FNIRS. Unknown FNIRS spectra were well integrated in the FNIRS spectral library, even when the average H value was higher than 3. The SEP was about 8 g/kg $BW^{0.75}$. This level of accuracy was similar to that reported in Chapter III, where the corrected SEP of the FNIRS database was 8.89 g/kg $BW^{0.75}$ for DMVI. The average FNIRS prediction was close to the average reference values (55.6 and 55.9 g/kg $BW^{0.75}$ for FNIRS and reference value, respectively), but differed from those obtained using the *n*-alkanes method. Compared with reference values, the *n*-alkanes method underestimated DMVI. Our findings, like those reported by Dove et al. (2000), suggested that four major sources of error could explain these results. The first one was an incomplete faecal recovery of the marker. The second was that the release rate of *n*-alkanes could differ from that indicated by the supplier, leading to an error of estimation. The third was analytical, in that the method of *n*-alkane extraction (without preliminary saponification) might not be appropriate for determining the real content of odd-chain alkanes in faecal substrate. The fourth was probably the error linked to the forage sampling for the determination of *n*-alkane concentration. The concentration and distribution of *n*-alkanes in herbage could vary among and within species (Mayes et al., 1986; Bechet, 2001) and, given the selective behaviour of grazing ruminants (sheep were fed *ad libitum*), the *n*-alkane profile of analysed forage could differ from that of ingested forage.

In the second experiment, the OMD and DMVI of grazing lactating dairy cows were estimated by different methods and FNIRS. Compared with the faecal nitrogen index (FNI) estimates, the FNIRS-sheep method gave lower grass OMD (G-OMD) estimates. The FNI methods for estimating the G-OMD are linear or quadratic correlations developed from unsupplemented grazing animals. With the positive and linear relationship between OMD and faecal nitrogen content, any increase in faecal nitrogen would lead to an increase in predicted OMD (Lukas et al., 2005; Fanchone et al., 2009). Similarly, the FNIRS-sheep spectral library contained OMD obtained from the sheep digestibility trial, with sheep fed exclusively on fresh grass. In this context, it is possible that the faeces spectra of supplemented dairy cows did not correspond exactly to the range of spectra of the FNIRS-sheep database, thus probably inducing greater uncertainty of the prediction. With regard to the grass DMVI (G-DMVI), the FNIRS predictions and animal performance methods (APM) were well correlated, but less so than those obtained using the ratio technique and sward cutting method.

With regard to the accuracy of the OMD and DMI estimates, Boval et al. (2004) and Fanchone et al. (2007) demonstrated that FNIRS was accurate enough for assessing the digestibility of tropical herbage grazed by creole heifers or sheep. Methods of estimating G-OMD based on faecal analysis using FNIRS or FNI might be more accurate than those

based on grass analyses because faecal methods take account of the selectivity of the grazing animals. In order to predict OMD, the accuracy of the FNIRS-sheep calibration, expressed by the SECV, was 0.021 digestibility units, which was close to values reported in earlier studies using FNIRS (Leite and Stuth, 1995; Fanchone et al., 2007; Fanchone et al., 2009). With regard to inter-animal variation (CV ranged from 0.05 to 0.07 digestibility units) and inter-paddock variation (CV ranged from 0.02 to 0.08 digestibility units), these levels of accuracy were sufficient for recording the digestibility variations related to cows and to grass evolution during the grazing period.

For the FNIRS-sheep and FNIRS-cattle DMVI estimates, the SECV was 5.4 and 6.8 g/kg $BW^{0.75}$, respectively. Boval et al. (2004) achieved similar accuracy when predicting OM intake (SECV = 5.3 g/kg $BW^{0.75}$). In the results reported by Andueza et al. (2007), intake variability linked to animals was always high and, in most cases, for the same forage the CV could be close to 0.1. These authors concluded that it was not possible to estimate intake below this level of precision. Our results showed that, with a specific FNIRS database, as in the case of the FNIRS-cattle database, the SECV was close to 1 kg DM/cow/d, representing a variation of 0.1 around the mean value. The FNIRS models could therefore be used to determine variations between cows or relating to grass evolution over time.

Our results indicated that FNIRS is a non-destructive and rapid method that could be used to define the diversity of a feeding system across a territory. Thus, in a milk quality survey across 30 dairy farms on La Réunion, feeding practices were followed and intake measured precisely on four farms. For each farm, the OMD and DMVI of dairy cows were estimated from FNIRS analyses.The correlation between FNIRS-predicted and measured intakes was high (R^2 = 0.92) and the diffference between predicted and measured intake values was less than 1 kg DM per cow per day.

As reported by Fanchone et al. (2009), the main advantage of FNIRS over other faeces analysis methods is that it takes account of the entire chemical composition of faeces through the use of the full NIRS spectrum. With regard to the ease of using the NIRS analytical procedure and the accuracy of the prediction, FNIRS, as a predictive method for estimating digestibility and intake, could be used for managing the feeding of grazing ruminants at individual and herd level.

References

Andueza, D., Picard, F., Pecatte, J.R., Gallard, Y., Hassoun, Ph., Viudes, G., Egal, D., Pradel, Ph., Trocquier, O., Thomas, D., Delaby, L., Agabril, J., Baumont, R., 2007. Variability within and among laboratories of *in vivo* digestibility and voluntary intake of two hays evaluated in sheep. Renc. Rech. Rum. 14, 247 (in French).

Bechet, G., 2001. Variations des teneurs en n-alcanes du dactyle et du ray-grass. Nouveaux regards sur le pâturage. In: Actes JOUNÉES DE L'AFPF – 21 e 22 Mars 2001, p. A13.

Boval, M., Coates, D.B., Lecomte, P., Decruyenaere, V., Archimède, H., 2004. Faecal Near Infrared Reflectance Spectroscopy (NIRS) to assess chemical composition, *in vivo* digestibility and intake of tropical grass by Creole cattle. Anim. Feed Sci. Technol., 114, 19-29.

Dove, H., Freer, M., Foot, J.Z., 2000. The nutrition of grazing ewes during pregnancy and lactation: a comparison of n-alkane-based and chromium/in vitro-based estimates of herbage intake Aust. J. Agric. Res. 51, 765-777.

Fanchone, A., Boval, M., Lecomte, Ph., Archimède, H., 2007. Faecal indices based on near infrared spectroscopy to assess intake, *in vivo* digestibility and chemical composition of herbage ingested by sheep (crude protein, fibres and lignin content). J. Near Infrared Spectrosc. 15, 107-113.

Fanchone, A., Archimède, H., Boval, M., 2009. Comparison of fecal crude protein and fecal near-infrared reflectance spectroscopy to predict digestibility of fresh grass consumed by sheep. J. Anim Sci. 87, 236-243.

Leite, E.R., Stuth, J.W., 1995. Fecal NIRS equations to assess diet quality of free ranging goats. Small Ruminant Res. 15, 223-230.

Lukas, M., Südekum, H. H., Rave, G., Friedel, K., Susenbeth, A., 2005. Relationship between fecal crude protein concentration and diet organic matter digestibility in cattle. J. Anim Sci., 83, 1332-1344.

Mayes, R.W., Lamb, C.S. and Colgrove, P.M., 1986. The use of dosed and herbage n-alkanes as markers for determination of herbage intake. J. Agric. Sci. Camb. 107, 161-170.

Chapter V: General discussion, future prospects and conclusion

General discussion

The performances of grazing ruminants depend directly on forage quality and availability. *In vivo* digestibility and intake and the composition of ingested diets are the main parameters of interest for optimizing grazed pasture utilisation. Measuring these parameters under grazing conditions, for herd management and in real time, is difficult because of grass growth and the selective behaviour of animals. Several studies have shown that laboratory methods can be used to estimate *in vivo* digestibility from forage samples. These methods focus on *in vitro* rumen fluid digestibility (Tilley and Terry, 1963) or *in vitro* enzymatic digestibilities (Bartiaux Thill and Oger, 1986; De Boever et al., 1988; Aufrère and Demarquilly, 1989). With these methods, accuracy of prediction is generally good, with a residual error of prediction of 0.015 to 0.030 digestibility units (Peyraud, 1998). Similarly, NIRS analyses of forage are accurate enough for predicting *in vitro* or *in vivo* digestibility (Norris et al. 1976; De Boever et al., 1996), with an accuracy close to 0.03. The results of our study showed that, based on heterogeneous spectral databases, NIRS analyses of forage can predict *in vivo* digestibility with the same accuracy (Decruyenaere et al., 2009). The use of forage analysis to characterize the diet of grazing ruminants, however, assumes that the forage sampled for the analysis is the same as that ingested by the animal. In order to avoid such problems, *in vivo* digestibility can be estimated using methods that measure some chemical parameters of faeces, such as nitrogen (Bartiaux-Thill et al., 1985; Boval et al., 1996; Peyraud, 1998) or indigestible fibre (Lippke et al., 1986; Sunvold and Cochran, 1991). Peyraud (1998) and Fanchone et al. (2009) found that the faecal nitrogen index (FNI) is fairly accurate for assessing the *in vivo* digestibility of grazing ruminants. The *n*-alkanes method, (Dove and Mayes, 1991; Dove et al., 1996) based on analysing natural alkanes present in the cuticular waxes of plants and the dosed alkanes in the faeces, can also accurately reflect the digestibility of temperate or tropical herbivore diets (Sandberg et al., 2000). Faecces are a good indicator of ingested diets and, as illustrated in several studies, NIRS analyses of faeces (FNIRS) can be used to define the diet quality of grazing animals (Stuth et al., 1989; Coleman et al., 1989; Lyons and Stuth, 1992; Coleman and Murray, 1993; Leite and Stuth, 1995; Lyons et al., 1995; Coates, 2000; Stuth et al., 2003; Keli et al., 2007; Tran et al., 2010; Andueza et al., 2011b). All these studies demonstrated that grass *in vivo* digestibility can be estimated using FNIRS with the same accuracy as that obtained using conventional analysis methods, if there are appropriate calibration equations. Our results support these observations. Based on heterogeneous spectral databases, calibrations developed from all faeces databases (faeces spectra alone, substracted spectra and concatenated spectra) were efficient enough to estimate the OMD of temperate fresh forage (SECV varied from 0.021 to 0.018) (Decruyenaere et al., 2009). This level of accuracy is similar to that obtained by other current estimation methods. Based on our results, FNIRS and FNI estimates of OMD are biased (Decruyenaere et al., 2012), probably because of the specificity of the relationship between OMD and faecal nitrogen content. The weakness of the FNI, therefore, is that regressions are strongly linked to pasture characteristics in terms of botanical composition or localisation and therefore cannot be used universally (Holloway et al., 1981). In addition, most of these regressions were developed from unsupplemented grazing ruminants, whereas we worked with supplemented grazing dairy cows (Decruyenaere et al., 2012).

Voluntary intake is more difficult to estimate. Intake depends of various factors linked to animals, to diet resources and to the animals' environment. In most cases, intake estimation is indirect and requires two parameters, digestibility and faecal output. Faecal output can be obtained by total faeces collection (Holechek et al., 1986) or using indigestible markers, such as chromium oxide (Bartiaux-Thill et al., 1988; Compère et al., 1992) and ytterbium

(Brandiberry et al., 1991; Galyean, 1993; Mambrini and Peyraud, 1997). It is difficult to achieve total faecal collection with grazing or free-ranging herbivores. With regard to estimating *in vivo* digestibility, the *n*-alkanes method is used to estimate individual or herd voluntary intake (Mayes et al., 1986; Malossini et al., 1996; Dove et al., 2000). The main source of variation in the *n*-alkanes method is forage sampling (Smit et al. 2005). Given the selective behaviour of grazing animals, the *n*-alkanes profiles of ingested grass can differ from those of averaged grass sampled in the field (Dove et al., 1996). Another weakness of the *n*-alkanes method is the dosing of synthetic alkanes, which needs to be regular. Introducing *n*-alkane CRD capsules into the rumen is a solution. Our results showed, however, that the DMVI of sheep in confinement estimated by FNIRS was close to the reference values, but differed from those obtained using the *n*-alkanes method (Decruyenaere et al., 2003). Compared with the reference values, the *n*-alkanes method underestimated the DMVI. Dove et al. (2000) suggested that factors responsible for the difference could include: error linked to the forage sampling for determining *n*-alkane concentrations; incomplete faecal recovery of the marker; different *n*-alkane release rates in the rumen; and the analytical method of *n*-alkane extraction (with or without saponification).

In our results, the FNIRS calibrations developed estimate the OMVI of sheep produced an SECV below 5 g/kg $BW^{0.75}$ (Decruyenaere et al., 2009). Similarly, the FNIRS calibration developed from cattle faeces (dairy and suckling cows) databases to estimate DMVI had an acceptable SECV (6.78 g/kg $BW^{0.75}$) (Decruyenaere et al., 2002). This suggests that FNIRS analysis could be an interesting alternative for estimating intake by grazing ruminants (Decruyenaere et al., 2006; 2012). If our results are compared with those from the earliest studies in this area (Stuth et al., 1989; Coleman et al., 1989), the FNIRS calibration equations developed from faeces (sheep or cattle origin) obtained through digestibility trials were more accurate. The performances of our FNIRS calibrations were similar to those reported by Boval et al. (2004) and Landau et al. (2004) with ruminants (cattle and goats) grazing tropical grasslands.

In order to improve the management of grazing ruminants, knowledge of the botanical composition of ingested diets can be important. For example, determining the composition of ingested diets in terms of proportions of forage species or legumes can be useful for adjusting supplementation or for behavioural studies. Very few studies have focused on determining the botanical composition of grazing diets, probably because of a lack of adequate methods for measuring this parameter (Walker et al., 2010). The micro-histological analysis of plant residues in faeces is among these methods. Faeces contain indigested residues of ingested forages and FNIRS can be considered as a good method for estimating diet composition. Our results have shown that the proportion of legumes in a diet can be predicted with an SECV close to 4%, but, due to the size of the database, this level of accuracy can be used only for a very rough screening (Decruyenaere et al., 2004a, 2004b). Overall, FNIRS predictions were as accurate as other methods for predicting the composition of ingested diets.

As noted by Coates and Dixon (2010), FNIRS calibrations are derivative calibrations because the sample analysed for reference values (diet samples) differs from the samples submitted to NIRS analyses (faeces). The major problem with FNIRS lies in the generation of reliable diet-faeces pairs, especially when the diet samples are obtained from oesophageally fistulated animals using manual plucking. This should not be a problem when the diet-faecal pairs are obtained from animals fed in confinement with a total collection of faeces, as in our study. Our FNIRS spectral libraries were built from *in vivo* reference values (OMD, DMVI, OMVI and botanical composition of ingested diets) obtained through digestibility trials. As discussed by Dryden (2003), the precision of NIRS predictive models is strongly influenced

by this error in the reference measurements. Few studies have described the repeatability of measurements of *in vivo* digestibility and intake as reference values for predictive models. Andueza et al. (2007; 2011a) showed that, for hay, the repeatability standard deviation (sd_r) relative to inter-animal variation reached 0.015 for OMD and was 6.00 g/kg $BW^{0.75}$ or less for DMVI. In our results, the OMD sd_r was higher, with variability linked to the sheep and to the 6-day measurement interval, ranging between 0.029 and 0.104. Intake repeatability was better or similar, with sd_r values ranging between 1.62 and 7.83 g/kg $BW^{0.75}$ for intake (DMVI or OMVI). These reference values were repeatable enough to develop accurate FNIRS calibrations. Although FNIRS calibration is accurate enough for OMD predictions, intake is more difficult to predict with sufficient precision and is more closely linked to the uncertainty of the FNIRS models (Decruyenaere et al., 2015).

Future prospects

FNIRS is an easy method for predicting the diet characteristics of grazing herbivores under various conditions. As noted in the review by Bastianelli (2013), NIRS analysis of pig or poultry faeces could be also used to assess feed digestibility. According to Bastianelli et al. (2015), FNIRS is a promising tool for large-scale digestibility evaluation in pigs. One possible future development would to build a large FNIRS spectral library that included faeces from all types of ruminants (sheep, suckling and dairy cattle, and goats) and/or monogastric animals (pigs and poultry) managed in temperate or tropical environments, fed by grazing or in barns, supplemented or unsupplemented. Another future development would be to monitor news of NIRS chemometric models in order to extract faecal information from large and heterogeneous databases. In addition, new FNIRS libraries could be developed for predicting the botanical composition of intake and the main nutriments of ingested diets (energy or protein) in order to optimize the use of local resources.

Another advantage of FNIRS is that it enables work to be done at the individual level. It is possible to use FNIRS to evaluate inter-animal variability, which is an important parameter in genetic studies. A recent study by Mehtiö et al. (2014) showed that FNIRS estimates of OMD could be used for assessing inter-cow variability. These authors suggested that it was possible to use FNIRS to improve dairy cattle digestibility by animal breeding. Another advantage of FNIRS is that it enables digestibility and intake to be measured under real farm conditions. In this context, and as demonstrated by Bony et al. (2005) and Decruyenaere et al. (2006), FNIRS analysis could be used in surveys conducted to understand the efficiency of diet utilisation in real conditions (Bastianelli, 2013).

FNIRS could be used in discriminant analysis, which can be applied in contrasting situations and would therefore be an interesting tool for studying wild animals in their environment. For example, Tolleson et al. (2005) used discriminant analysis of faecal NIRS spectra for classifying deer species. It is also possible to discriminate physiological status, such as pregnancy, as demonstrated by giant pandas (Wiedower et al., 2012) and marsupial folivores (Windley et al., 2013). FNIRS spectra have also been used for detecting tick infestations (Tolleson et al., 2007). The diagnosis of parasite infestations of grazing ruminants or digestive problems obtained from an NIRS analysis of faeces could be very useful in improving herd management. Thus, enlarged FNIRS databases, associated with multiple variables characterizing faeces, diet and animal status, could be used to develop decision-support tools that could be mobilised in precision livestock farming.

Conclusion

Our results underline the great potential of FNIRS in estimating grazed grass digestibility, intake level and the botanical composition of ingested diets. The accuracy of the NIR model in estimating digestibility or intake was similar to or better than the accuracy of the other estimation methods tested. Results based on large or small but varied databases show that appropriate FNIRS spectral libraries could be developed for characterizing ruminant feed intake. We showed that faecal NIRS can predict, albeit roughly, animal diet composition in terms of plant species. The statistical performance of faecal calibrations is equivalent to that of NIRS calibrations developed to predict species composition from sward samples.

FNIRS could be used to predict the *in vivo* digestibility, intake and composition of ingested diets with sufficient accuracy. The prediction was repeatable enough with regard to the difficulty in conducting measurements using the reference method. The major difficulty lay in generating the diet-faeces pairs as reliably as possible. With regard to research on forages, the *in vivo* trials with animals confined in pens or digestibility crates was the best alternative to generating diet-faeces pairs in order to develop robust spectral databases. In estimating the botanical composition of intake, the problem also lies in the acquisition of reference values because of the lack of accurate procedures for determining diet selection at the individual level.

The accuracy achieved was therefore acceptable in view of the difficulty in obtaining this dietary parameter under field conditions. The NIRS analysis of faeces can provide estimates of *in vivo* parameters only if the spectral library includes a wide diversity of field conditions. Future work will involve validating the performance of the faecal calibrations on independent datasets and using these calibrations to develop decision-support tools for improving diverse grazing management schemes. A major objective would be to compare different feeding strategies rather than to obtain exact intake values. In this way, using the FNIRS spectrum to predict digestibility and intake could provide more information on ingested diets than diet analysis. As a low-cost and rapid prediction technique, FNIRS could contribute to a methodology that would have many uses in efforts to improve our knowledge of the diets of grazing ruminants.

References

Andueza, D., Picard, F., Pecatte, J.R., Gallard, Y., Hassoun, Ph., Viudes, G., Egal, D., Pradel, Ph., Trocquier, O., Thomas, D., Delaby, L., Agabril, J., Baumont, R., 2007. Variability within and among laboratories of *in vivo* digestibility and voluntary intake of two hays evaluated in sheep. Renc. Rech. Rum. 14, 247 (in French).

Andueza D., Picard F., Pradel Ph., Egal D., Hassoun P., Pecatte J.R., Beaumont R., 2011a. Reproductibility and repeatability of forage *in vivo* digestibility and voluntary intake of permanent grassland forages in sheep. Livestock Sci., 140, 42-48.

Andueza D., Picard F., Jestin M., Andrieu J., Baumont R., 2011b. NIRS prediction of the feed value of temperate forages efficacy of four calibration strategies. Animal 5:7, 1002-1013.

Aufrère, J. Demarquilly, C., 1989. Predicting organic matter digestibility of forage by two pepsin-cellulase methods. XVI International Grassland Congress, Nice, France, 877-878.

Barthiaux-Thill, N., Biston, R., Dardenne, P., François, E., Thewis, A., Gielen, M., Limbourg, P., 1985. Mesure de l'ingestion d'herbe par la vache laitière au pâturage ou au moyen de la méthode des index fécaux. Rev. Agric. 2, 317-325.

Bartiaux-Thill, N. Oger, R., 1986. The indirect estimation of the digestibility in cattle of herbage from Belgian permanent pasture. Grass and Forage Sci. 41, 269-272.

Bartiaux-Thill, N., Oger, R., François, E., Théwis, A., 1988. Courbes d'excrétion fécale de l'oxyde de chrome administré aux vaches laitières au pâturage. Définition d'un échantillonnage bi-quotidien des fèces. Reprod. Nutr. Dev. 28, 87-88.

Bastianelli, D., 2013. NIRS as a tool to assess digestibility in feeds and feedstuffs. In International Congress on Advancements in Poultry Production in the Middle East and African Countries, 21-25 october 2013, Antalya, Turkey, 10 p.

Bastianelli, D., Bonnal, L., Jaguelin-Peyraud, Y., Noblet, J., 2015. Predicting feed digestibility from NIRS analysis of pig faeces. Animal, 9, 781-786.

Bony J., Contamin V., Gousseff M., Metais J., Tillard E., Juanes X., Decruyenaere V., Coulon J.B., 2005. Facteurs de variation de la composition du lait à la Réunion. INRA Prod. Anim., 18, 255-263.

Boval, M., Peyraud, J.L., Xande, A., Aumont, G., Coppry, O., Saminadin, G., 1996. Evaluation d'indicateurs fécaux pour prédire la digestibilité et les quantités ingérées de Dichanthium sp par des bovins créoles. Ann. Zootech. 45, 121-134.

Boval, M., Coates, D.B., Lecomte, P., Decruyenaere, V., Archimède, H., 2004. Faecal Near Infrared Reflectance Spectroscopy (NIRS) to assess chemical composition, *in vivo* digestibility and intake of tropical grass by Creole cattle. Anim. Feed Sci. Technol., 114, 19-29.

Brandyberry, S.D., Cochran, R.C., Vanzant, E.S., Harmon, D.L., 1991. Technical note : effectiveness of different methods of continuous marker administration for estimating fecal output. J. Anim. Sci. 69, 4611-4616.

Coates, D.B., 2000. Faecal NIRS - What does it offer today's grazier? Tropical Grasslands 34, 230-239.

Coates D.B., Dixon R.M., 2010. Fecal NIRS calibration for predicting protein and digestibility in the diet of cattle : fistulated and pen feeding procedures for generating diet-fecal pairs. In Walker J and Tolleson D. Eds. Shining light on manure improves livestock and land management, Texas Agrilife Research and Society for Range Management, Technical bulletin, 23-41.

Coleman, S.W., Stuth, J.W., Holloway, J.W., 1989. Monitoring the nutrition of grazing cattle with near-infrared analysis of faeces. XVI International Grassland Congress, Nice, France, pp 881-882.

Coleman, S.W., Murray, I., 1993. The use of near-infrared reflectance spectroscopy to define nutrient digestion of hay by cattle. Anim. Feed Sci. Technol. 44, 237-249.

Compère, R., Valdivia, J., Hellemans, P., Guns, M., Vandenbyvang, P., 1992. Comparaison de deux méthodes utilisant le Cr2O3 pour estimer la quantité de fèces émises par les

moutons : pellets de luzerne et bolus CAPTEC-CRD. Bull. Rech. Agron. Gembloux 27, 365-383.

De Boever, J.L., Cottyn, B.G., Andries, J.I., Buysse, F.X., Vanacker, J.M., 1988. The use of pepsin cellulase technique to predict digestibility metabolizable and net energy of forages. Anim. Feed. Sci. Technol. 19, 247-260.

De Boever, J.L., Cottyn, B.G., De Brabander, D.L., Vanacker, J.M., Boucqué, Ch.V., 1996. Prediction of the feeding value of grass silages by chemical parameters, in vitro digestibility and near-infrared reflectance spectroscopy. Anim. Feed. Sci. Technol. 60, 103-116.

Decruyenaere, V., Stilmant, D., Lecomte, P., Buldgen, A., Dardenne, P., 2002. Improvement and validation of the NIRS analysis applied to faeces to measure grass intake in pasture. In J.L. Durand, J.C. Emile, Ch. Huygue and G. Lemaire (Eds.), Multi-function grasslands. Quality forages, animal products and landscapes. Grassl. Sci. Eur. 7, pp.196-197.

Decruyenaere V., Peters M., Stilmant D., Lecomte Ph., Dardenne P., 2003. Near infrared reflectance spectroscopy applied to faeces to predict dry matter intake of sheep under grazing, comparison with n-alkanes and direct biomass measurement methods. Tropical and Subtropical Agroecosyst., 3, 471-476.

Decruyenaere, V., De Visser, M., Stilmant, D., Clément, C., Sinnaeve, G., 2004a. Faecal Near Infrared Reflectance Spectroscopy for ruminant feed intake prediction, in: Lüscher, A., Jeangros, B, Kessler, W., Huguenin, O., Lobsiger, M., Millar, N., Suter, D. (Eds.), Land use systems in grassland dominated regions. Grassl. Sci. Eur. 9, pp.1034-1036.

Decruyenaere V., Peters M., Stilmant D., Lecomte Ph., Dardenne P., 2004b. Near infrared spectroscopy applied to faeces to predict botanical composition of sheep intake. http://www.nirpublications.com.

Decruyenaere, V., Bony, J., Grimaud, P., Lecomte, Ph., 2006. A methodological approach in La Reunion Island on the value of faecal Near Infrared reflectance Spectroscopy (NIRS) to assess grazing intake and diet quality of the dairy cow. In C.A. Sandoval-Castro, F.D. DeB Hovell, J.F.J. Torres-Acosta and A. Ayala-Burgos (Eds), Herbivores the assessment of intake, digestibility and the roles of secondary compounds. BSAS publication 34, pp 93-99.

Decruyenaere, V., Lecomte, Ph., Demarquilly, C., Aufrere, J., Dardenne, P., Stilmant, D., Buldgen, A., 2009. Evaluation of green forage intake and digestibility in ruminants using neaur infrared reflectance spectrscopy (NIRS): Developing a global calibration. Anim Feed Sci. Technol., 148, 138–156.

Decruyenaere, V., Froidmont, E., Bartiaux-Thill, N., Buldgen, A., Stilmant, D., 2012. Faecal near-infrared reflectance spectroscopy (NIRS) compared with other techniques for estimating the in vivo digestibility and dry matter intake of lactating grazing dairy cows. Anim. Feed Sci. Technol., 173, 220-234.

Decruyenaere, V., Planchon, V., Dardenne, P., Stilmant, D., 2015. Prediction error and repeatability of near infrared reflectance spectroscopy applied to faeces samples in order to predict voluntary intake and digestibility of forages by ruminants. Anim. Feed Sci. Technol., 205, 49-59.

Dove, H., Mayes, R.W., 1991. The use of plant wax alkanes as marker substance in studies of nutrition herbivores: a review. Aust. J. Agric. Res. 42, 913-952.

Dove, H., Mayes, R.W., Freer, M., 1996. Effects of species, plant part and plant age on the n-alkanes concentrations in the cuticular wax of pasture plants. Aust. J. Agric. Res. 47, 1333-1347.

Dove, H., Freer, M., Foot, J.Z., 2000. The nutrition of grazing ewes during pregnancy and lactation: a comparison of n-alkane-based and chromium/in vitro-based estimates of herbage intake Aust. J. Agric. Res. 51, 765-777.

Dryden, G.McL., 2003. Near infrared spectroscopy : applications in deer nutrition. RIRCD Publication n° W03/007, 38p.

Fanchone, A., Archimède, H., Boval, M., 2009. Comparison of fecal crude protein and fecal near-infrared reflectance spectroscopy to predict digestibility of fresh grass consumed by sheep. J. Anim Sci. 87, 236-243.

Galyean, M.L., 1993. Technical note: an algebraic method for calculating fecal output from a pulse dose of an external marker. J. Anim. Sci. 71, 3466-3469.

Holechek, J.L., Wofford, H., Arthun, D., Galyean, M.L., Wallace, J.D., 1986. Evaluation of total fecal collection for measuring cattle forage intake. J. Range Manage. 39, 2-4.

Holloway, J.W., Estell, II R.E., Butts, W.T. Jr, 1981. Relationships between fecal components and forage consumption and digestibility. J. Animal Sci. 52, 836-848.

Keli, A., Andueza, D., Baumont, R., Bechet, G., de Vega, A., 2007. The use of NIRS for prediction of intake, digestibility, diet composition and faecal concentration of n-alkanes in sheep fed different proportions of lucerne and rye grass (Lolium rigidum). Options Mediterranéennes, Séries A, N° 74, 237-241.

Landau, S., Glasser, T., Dvash, L., Perevolotsky, A., 2004. Faecal NIRS to monitor diet of mediterranean goats. South African J. Anim. Sci. 34, (suppl 1), 76-80.

Leite, E.R., Stuth, J.W., 1995. Fecal NIRS equations to assess diet quality of free ranging goats. Small Ruminant Res. 15, 223-230.

Lippke, H., Ellis, W.C., Jacobs, B.F., 1986. Recovery of indigestible fiber from feces of sheep and cattle on forage diets. J. Dairy Sci. 69, 403-412.

Lyons, R.K., Stuth, J.W., 1992. Feacal NIRS equations for predicting diet quality of free-ranging cattle. J. Range Manage. 45, 238-244.

Lyons, R.K., Stuth, J.W., Angerer, J.P., 1995. Technical note Fecal NIRS equation field validation. J. Range Manage. 48, 380-382.

Malossini, F., Bovolenta, S., Piasentier, E., Piras, C., Martillotti, F., 1996. Comparison of n-alkane and chromium oxide methods for estimating herbage intake by grazing dairy cows. Anim. Feed Sci. Technol. 61, 155-165.

Mambrini, M., Peyraud, J.L., 1997. Retention time of feed particles and liquids in the stomachs and intestines of dairy cows. Direct measurements and calculations based on faecal collection. Reprod. Nutr. Dev. 37, 427-442.

Mayes, R.W., Lamb, C.S. and Colgrove, P.M., 1986. The use of dosed and herbage n-alkanes as markers for determination of herbage intake. J. Agric. Sci. Camb. 107, 161-170.

Mehtiö, T., Rinne, M., Nyholm, L., Sairanen, A., Pitkänen, T., Mäntysaari, E., Lidauer, M., 2014. Variability of cow-specific organic matter digestibility within lactation and across cows based on NIRS scans from fecal samples. In Proceedings, 10th world congress of genetics applied to livestock production, August 17-22, Vancouver, Canada, 3p.

Norris, K.H., Barnes, R.F., Moore, J.E., Shenk, J.S., 1976. Predicting forage quality by infrared reflectance spectroscopy. J. Anim.Sci. 43, 889-897.

Peyraud, J.L., 1998. Techniques for measuring faecal flow, digestibility and intake of herbage in grazing ruminants. Techniques for Investigating Intake and Ingestive Behaviour by Farm Animals. IXth European Intake Workshop. IGER North Wyke UK pp.39-48.

Sandberg, R.E., Adams, D.C., Klopfenstein, T.J., Grant, R.J., 2000. N-alkane as an internal marker for predicting digestibility of forages J. Range Manage. 53, 159-163.

Smit, H.J., Taweel, H.Z., Tas, B.M., Elgersma, A., 2005. Comparison of techniques for estimating herbage intake of grazing dairy cows. J. Dairy Sci. 88, 1827-1836.

Stuth, J.W., Kapes, E.D., Lyons, R.K., 1989. Use of near infrared spectroscopy to assess nutritional status of cattle diets on rangeland. XVI International Grassland Congress, Nice, France, pp 889-890.

Stuth, J., Awuma, K., Tolleson, D., Ossiya, S., Ebong, C., Mnene, W., Mwilawa, A., Ebro, A., Byenkya, S., Jama, A., Kaitho, R., 2003. Infusing nutritional profiling technology in sub-Saharan Africa for free ranging livestock. Research Brief 03-04-Lews, April 2003.

Sunvold, G.D., Cochran, R.C., 1991. Technical note : evaluation of acid detergent lignin, alkaline peroxide lignin, acid insoluble ash and indigestible detergent fiber as internal markers for prediction of alfalfa, bromegrass and prairie hay digestibility by beef steers. J. Anim. Sci. 69, 4951-4955.

Tilley, J.H.A, Terry, R.A, 1963. A two stage technique for 'in vitro' digestion of forage crops. Grass Forage Sci. 18, 104-111.

Tolleson, D.R., Randel, R.D., Stuth, J.W., Neuendorff, D.A., 2005. Determination of sex and species in red and fallow deer by near infrared reflectance spectroscopy of the faeces. Small Rumin. Res. 57, 141-150.

Tolleson, D.R., Teel, P.D., Stuth, J.W., Strey O.F., Welsh, Jr, T.H., Carstens, G.E., 2007. Faecal NIRS: detection of tick infestations in cattle and horses. Veterinary Parasitilogy 144, 146-152.

Tran, H., Salgado, P., Tillard, E., Dardenne, P., Nguyen, X.T., Lecomte, P., 2010. "Global" and "local" predictions of dairy diet nutritional quality using near infrared reflectance spectroscopy. J. Dairy Sci. 93, 4961-4975.

Walker, J.W., Campbell, E.J., Kott, R.W., Landau, S.Y., Lupton, C.J., Scott, C.B., Surber, L., Taylor Jr, C.A., Whitworth, W.R., 2010. Fecal NIRS for predicting botanical composition of herbivore diets. In Walker J and Tolleson D. Eds. Shining light on manure improves livestock and land management, Texas Agrilife Research and Society for Range Management, Technical bulletin, 53-65.

Wiedower, E.E., Kouba, A.J., Vance, C.K., Hansen, R.L., Stuth, J.W., Tolleson, D.R., 2012. Fecal near infrared spectroscopy to discriminate physiological status in giant pandas. PloS one (www.plosone.org), 7, e38908, 10p.

Windley, H.R., Wallis, I.R., De Gabriel, J.L., Moore, B.D., Johnson, C.N., Foley, W.J., 2013. A faecal index of diet quality that predicts reproductive success in a marsupial folivore. Oecologia, 173, 203-212.