Comparaison de l'ingestion et de la digestion de moutons alimentés, à base de fourrage vert, à l'auge et au pâturage

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« Tiens-toi fermement à ton éducation, …

ne l'abandonne pas ; conserve-la,...

elle est ta vie »

Les proverbes 3, 13.

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LISTE DES PUBLICATIONS

Ce travail a été réalisé à l'Unité de Recherches Zootechniques (I.N.R.A., Petit Bourg, Guadeloupe), au sein de l'équipe « Alimentation et Systèmes Pâturés Tropicaux » (A.S.P.T) et il s'inscrit dans deux des six grands axes de recherches de l'équipe de l'U.R.Z. Il s'agit de l'étude des facteurs prairiaux limitant l'ingestion au pâturage et les relations herbe - animal, l'étude des facteurs ruminaux limitant l'ingestion des fourrages et l'optimisation de la digestion. Les différents travaux de recherches menés au cours de cette thèse ont fait l'objet des publications suivantes :

Articles scientifiques intégrés dans la thèse

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

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INTRODUCTION GENERALE

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Selon les prévisions des Nations Unies, la population mondiale devrait atteindre 2,5 milliards en 2050[1]. Cette augmentation sera principalement le fait des pays les moins développés (+ 46 %). Ainsi, la demande en produits végétaux et animaux dans ces zones qui est en progression régulière de 3% par an depuis 1980, devrait poursuivre sa progression d'ici à 2050. En conséquence, les productions agricoles et particulièrement, les protéines animales (viande, lait, œuf,…) doivent suivre cette évolution démographique (Delgado et al., 1999). De part leur capacité à transformer les biomasses fibreuses en protéines animales de haute qualité, et à valoriser les espaces non mécanisables et difficilement cultivables, les ruminants constituent un réel enjeu dans le développement de ces pays.

Ces dernières années, l'intensification de la production animale dans les pays développés a conduit à de nombreuses dérives : pollution (azote, pesticides, dioxine), surpâturage, zoonoses (maladie de la vache folle, maladie de la fièvre aphteuse…). L'accroissement de la production animale en zones tropicales bien qu'étant indispensable doit éviter ces dérives. De plus, les fortes attentes sociétales pour une meilleure gestion des ressources, la protection de l'environnement tout en produisant des aliments de qualité supérieure tendent à encourager une production moins « intensive ». Au même moment, dans les pays développés, le prix des céréales (maïs, blé, orge, …) qui constituent 55 à 80 % des ingrédients des aliments du bétail formulés par l'agro-industrie, ne cesse d'augmenter sur le marché international. Cette augmentation résulte d'une forte demande liée en partie à leur utilisation comme matière première dans la fabrication de biocarburants (OCDE, 2006). Cette nouvelle compétition pour les céréales suscite un véritable regain d'intérêt pour l'alimentation des ruminants à base d'herbe et de sous-produits (Buldgen, 2005). C'est dans ce contexte que les pays de l'Union Européenne ont décidé la mise en place d'une prime au maintien des systèmes d'élevage extensifs remplacés en 2003 par la « prime herbagère agro-environnementale » (Agreste, 2008)[2].

L'alimentation est un paramètre clé déterminant la production animale. L'amélioration de l'alimentation à base de fourrages et de sous produits, est une des voies d'accroissement de la production raisonnée, prônée par plusieurs organismes. L'alimentation à base de fourrages verts est perfectible mais complexe à évaluer et à améliorer, du fait de la grande hétérogénéité

[1] http://www.un.org/esa/population/publications/wpp2006/French.pdf
[2] http://agreste.agriculture.gouv.fr/IMG/pdf/R7208A01.pdf

des espèces fourragères et des systèmes de production. Par ailleurs, bien que les graminées tropicales en C4[3], génèrent des biomasses importantes, elles ont une qualité nutritionnelle généralement inférieure à celle des fourrages tempérés en C3[4], à des stades de repousse équivalents (Minson, 1990; Humphreys, 1991). Sous l'effet des températures élevées, les graminées tropicales en C4 maturent en effet rapidement que les graminées tempérées en C3, contribuant ainsi, à leur moins bonne valeur nutritive (Wilson, 1994) et à des digestibilités inférieures de 0.13 points (Leng, 1990; Assoumaya et al., 2007c).

Dans ce contexte, les recherches conduites jusqu'à ce jour, à l'U.R.Z, sur l'alimentation des ruminants ont eu pour objectifs la détermination de la valeur nutritive de diverses ressources fourragères, la compréhension du déterminisme de la digestion des graminées tropicales, la recherche de modalités de production et de valorisation de ces fourrages.

Les travaux réalisés à l'auge ont permis:

- de déterminer la valeur nutritive d'une grande variété d'espèces fourragères tropicales et de co-produits de la zone caraïbe et de la Réunion[5].
- de comprendre la dynamique digestive de fourrages en C4 consommés verts, de mesurer le rôle de l'activité cellulolytique du rumen et d'évaluer l'impact de la réduction de taille des particules sur l'ingestion et la digestion des fourrages verts[6],
- de tester des stratégies de valorisation de la biomasse fibreuse (défaunation, complémentation[7]).

Les travaux conduits au pâturage ont contribué à :

- évaluer l'impact de la conduite du pâturage sur l'alimentation, en faisant varier les âges de repousse, la fertilisation, ou les quantités proposées, la durée de pâturage ou le fractionnement des surfaces à pâturer ou la fauche des refus[8],
- apprécier l'impact de la structure prairiale sur le comportement, la digestibilité et l'ingestion, afin de mieux orienter le choix des fourrages à faire pâturer[9],
- mettre au point des méthodes de mesure de la digestibilité et de l'ingestion au pâturage, pour des bovins et des petits ruminants : index fécaux, spectroscopie dans le proche infrarouge (SPIR)[10].

[3] Le produit terminal de la photosynthèse est un composé à 4 carbones : l'oxaloactétate.
[4] Le produit terminal de la photosynthèse est un composé à 3 carbones : le 3-phosphoglycérate.
[5] Xandé (2005) ; Aumont et al. (1995)
[6] Archimède et al. (2000) ; Assoumaya (2007b,c)
[7] Eugène et al. (2004a,b) ; Archimède et al. (2001)
[8] Boval et al. (2000, 2002, 2007a) ; Ortega-Jimenez et al. (2005)
[9] Boval et al. (2007b)

Les divers résultats acquis mettent en évidence la marge de manœuvre qui existe pour améliorer l'alimentation à base de fourrages en zone tropicale, entre autres exploiter plus jeune les C4 tropicales, adapter le niveau de fertilisation et d'irrigation à la morphologie de la graminée, choisir l'espèce végétale en fonction du milieu de production, adapter la conduite du pâturage.

L'analyse de ces travaux et de la bibliographie (Minson, 1990; Van Soest, 1996) suggère l'existence de différences d'alimentation entre l'auge et le pâturage. A l'auge, l'alimentation des ruminants a été bien étudiée et des déterminants de l'alimentation ont été identifiés et hiérarchisés. Au pâturage, une partie des déterminants de l'alimentation mis en évidence à l'auge, sont aussi valables (Minson, 1990). Mais d'autres contraintes propres au pâturage et à la structure de la prairie influencent par ailleurs le comportement alimentaire et la ration consommée par les animaux. Ainsi, pour un même fourrage proposé, les digestibilités du fourrage consommé au pâturage ou à l'auge, sont parfois très éloignées. Les différences de digestibilité entre le fourrage proposé et celui pâturé, seraient de l'ordre de 0.05 à 0.10 points selon Van Soest (1996). De plus, à l'auge l'ingestion et la digestibilité sont généralement positivement corrélées (Minson, 1990; Ketelaars and Tolkamp, 1992; Archimede et al., 2000), alors qu'au pâturage, ce n'est pas toujours le cas (Hitchcock et al., 1990; Boval et al., 2007a). Par ailleurs, si à l'auge, des méthodes de référence pour la mesure de l'ingestion et de la digestion sont disponibles (Cochran and Galyean, 1994), elles ne sont pas transposables au pâturage compte tenu du comportement de sélection des animaux, de la repousse du fourrage durant la période de pâturage et des difficultés de caractériser le fourrage proposé et le refusé (Coleman, 2006).

Ce travail de thèse a pour principal objectif de comprendre le déterminisme de l'alimentation à base de fourrages verts quel que soit le mode d'alimentation (auge ou pâturage). Nous avons donc fait l'hypothèse que des différences d'alimentation existent entre l'auge et le pâturage et que l'origine de ces différences se situe dans le mode de présentation du fourrage à l'animal, couché à l'auge *vs* sur pied au pâturage. Pour valider ces hypothèses, nous avons convenu :

1) De mettre en évidence les différences d'alimentation entre l'auge et le pâturage lorsque le même fourrage est offert.
2) D'expliquer ces différences.

[10] Boval et al., (2004)

3) De contribuer à lever les freins méthodologiques à l'étude de l'alimentation au pâturage.

Nous avons donc mené des essais avec des béliers conduits simultanément à l'auge et au pâturage et alimentés avec un même fourrage issu de la même parcelle. Au préalable, des investigations méthodologiques ont été conduites. Nous avons dans un premier temps étudié le potentiel de la SPIR[11] pour prédire la composition chimique ainsi que les propriétés fonctionnelles (ingestion et digestibilité *in vivo*) de fourrages verts consommés par des ovins (**Etude méthodologique 1 : Fanchone et al., 2007**). Nous avons ensuite incrémenté la base de données ayant permis de réaliser les premières calibrations (**Etude méthodologique 2 : Fanchone et al., 2008a**). Une fois ces investigations méthodologiques réalisées, un premier essai visant à comparer l'auge et le pâturage à deux âges de repousse différents a été conduit (**Etude expérimentale 1 : Fanchone et al., 2008b**). Un second essai a été réalisé selon le même principe, avec deux niveaux de proposés simultanément à l'auge et au pâturage (Etude **expérimentale 2 : Fanchone et al., 2008c**). L'ensemble des données générées dans la thèse ont été ensuite compilées, et discutées (**Discussion générale**).

[11] Spectroscopie dans le proche infrarouge.

ETUDE BIBLIOGRAPHIQUE

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Nous avons synthétisé les principales connaissances de l'alimentation des ruminants à l'auge et au pâturage, à base de fourrages. Des facteurs communs sont évoqués dans les deux milieux, auxquels s'ajoutent au pâturage d'autres facteurs plus spécifiques. Les études visant à comparer l'auge et le pâturage sont peu nombreuses et ont été analysées et synthétisées. Enfin, une revue critique des moyens méthodologiques mis en œuvre pour mesurer l'ingestion (MOI) et la digestibilité (DMO) au pâturage a été réalisée, compte tenu des difficultés méthodologiques rencontrées au pâturage.

1. Facteurs déterminant l'alimentation à l'auge

La composition physico-chimique du fourrage ingéré est le principal facteur déterminant la MOI et la DMO à l'auge.

- **Teneur en matière sèche**

Lorsque la teneur en MS d'un fourrage est inférieure à 22 %, elle limite la MOI (Minson, 1990). Au-delà il n'y aurait pas d'effet de la teneur en MS sur la MOI. Un animal ingérant un fourrage humide, a tendance à l'avaler avant qu'il soit suffisamment réduit. Le temps de mastication mérycique s'accroît alors, induisant un accroissement du temps de séjour dans le rumen et une diminution de la MOI (Minson, 1990).

- **Teneur en MAT du fourrage**

La teneur en MAT du fourrage proposé est positivement corrélée à la DMO (Assoumaya, 2007). Elle constitue la principale source d'azote des microorganismes du rumen, notamment des bactéries cellulolytiques, impliquées dans la réduction de taille des particules alimentaires. Ainsi, lorsque la teneur en MAT est inférieure au seuil critique de 8 %, elle limite le développement de ces bactéries et par conséquent la DMO et la MOI (Moore et al., 1999).

- **Teneur en fibres et en lignine du fourrage**

Les teneurs en fibres du fourrage (NDF, ADF) sont négativement corrélées à la MOI et à la DMO. Les fibres sont plus résistantes à la digestion que les carbohydrates du contenu

cellulaire. De plus la digestion des particules fibreuses nécessite une réduction de taille par l'effet de la mastication mérycique (Jarrige, 1988). La lignine (ADL) est indigestible, elle n'est donc pas dégradée par les microorganismes du rumen et entrave la dégradation des autres constituants des parois végétales. La MOI et la DMO sont donc négativement corrélées à la teneur en ADL de la ration.

- **Teneur en minéraux du fourrage**

Les microorganismes du rumen ont besoin de minéraux, notamment de phosphore, de soufre, de magnésium, de cuivre, et de cobalt, pour leur bon développement. L'insuffisance de ces minéraux dans le rumen réduit l'efficacité de la cellulolyse (Leng, 1990) et par conséquent la MOI et la DMO.

- **Relations DMO / MOI**

A l'auge, la DMO est considérée comme un indicateur de la qualité du fourrage (mesure *in vitro* ou *in sacco* sur le fourrage, en conditions standards), ou comme un paramètre plus global de la qualité du régime, tenant compte de la variabilité liée à l'animal (Ketelaars and Tolkamp, 1992; Coleman and Moore, 2003). Elle est positivement corrélée à la MOI. Ainsi, une augmentation de la qualité du fourrage entraîne une diminution du temps de séjour des particules dans le rumen et une augmentation de la MOI.

Ainsi, tous les facteurs qui tendent à modifier la composition chimique du fourrage auront un effet sur la MOI et la DMO. Dans ce contexte, l'espèce fourragère (Aumont et al., 1995; Arthington and Brown, 2005), le stade de maturité du fourrage (Aumont et al., 1995; Archimede et al., 2000), la fertilisation (Monson and Burton, 1982) sont des facteurs de variation de la MOI et de la DMO.

2. Facteurs déterminant l'alimentation au pâturage.

La bibliographie rassemble peu de données sur l'ingestion et la digestion au pâturage et ces données ont été obtenues selon des méthodes très diverses et dans des conditions expérimentales multiples (Burns and Sollenberger, 2002). Néanmoins, les principaux facteurs déterminant l'alimentation à l'auge, mis en évidence précédemment, sont présents au pâturage. Toutefois, les relations obtenues sont moins bonnes et d'autres facteurs plus spécifiques au pâturage ont été rapportés dans la bibliographie.

Comportement de sélection des animaux

Les animaux conduits au pâturage sont caractérisés par leur comportement de sélection. Compte tenu du mode de présentation sur pied du fourrage et de la prise alimentaire par arrachage, les animaux au pâturage peuvent sélectionner un régime alimentaire de meilleure qualité (ingestibilité, digestibilité) que le fourrage offert (Burns and Sollenberger, 2002). Néanmoins, ce comportement de sélection a aussi été mis en évidence à l'auge, lorsque les quantités de fourrage proposées aux animaux dépassent plus de 10 % de la capacité d'ingestion des animaux (Zemmelink, 1980; Mbwile and Uden, 1997).

- **Facteurs fourragers ou facteurs prairiaux**

Des facteurs tels que la biomasse présente (Combellas and Hodgson, 1979; Ernst et al., 1980), l'hétérogénéité, la densité, la hauteur du couvert (Stobbs, 1973, 1975; Prache and Peyraud, 1997) ou la résistance au cisaillement (Inoue et al., 1993) viennent s'ajouter à la composition chimique du fourrage mise en évidence à l'auge. Ces facteurs influencent le comportement alimentaire de l'animal, notamment la taille des bouchées (Black and Kenney, 1984; Minson, 1990; Meuret, 1997). Ces caractéristiques déterminent la préhensibilité du fourrage pour l'animal. Dans ce contexte, il semble plus intéressant de parler au pâturage de facteurs prairiaux englobant à la fois les facteurs liés à la composition chimique du fourrage *sensu stricto*, et les facteurs liés à la structure du couvert.

3. Comparaison de l'alimentation à l'auge et au pâturage.

Des études visant à comparer l'auge et le pâturage existent. Les principaux facteurs étudiés sont l'ingestion (Keane and Allen, 1998; Zervas et al., 1999; Moniruzzaman et al., 2002; Misra et al., 2006; Raghuvansi et al., 2007), la digestibilité (Misra et al., 2006), le GMQ (Keane and Allen, 1998 ; Zervas et al., 1999 ; Fiems et al. 2002 ; Monniruzzaman et al. 2002 ; Misra et al., 2006 ; Raghuvansi et al. 2006), et le poids de la carcasse à l'abattage (Keane and Allen, 1998 ; Zervas et al., 1999). Cependant, dans la majorité de ces études, les auteurs comparent des systèmes d'alimentation : « intensif » à l'auge avec de forts pourcentages de concentré dans la ration *vs* « extensif » au pâturage souvent sans apport de concentré. Ainsi, les différences mises en évidence dans ces études sont souvent davantage liées à des différences de densité nutritionnelle de la ration, liées à l'utilisation de concentré dans la ration à l'auge, qu'à des différences strictes entre les deux modes d'alimentation. De plus, dans ces études, les fourrages distribués à l'auge et au pâturage diffèrent souvent. Dans

ce contexte, ces études mettent en avant des résultats divergents qui ne peuvent conduire à aucune loi générale de l'alimentation à base de fourrages verts. Ainsi, Keane and Allen (1998), montrent une meilleure ingestion et un meilleur GMQ et donc un meilleur poids à l'abattage pour des taurillons alimentés à l'auge avec de l'ensilage d'herbe et du concentré *ad libitum* comparativement à des animaux en pâturage rationnel. Zervas et al. (1999) montrent une meilleure ingestion sur pâturage non-irrigué comparativement à l'auge, pour des agneaux à l'engraissement consommant 200 g de foin plus du concentré. Cependant, dans cette même étude, le GMQ ainsi que le poids de la carcasse après abattage étaient supérieurs à l'auge. Parallèlement, Raghuvansi et al. (2006), rapportent une meilleure ingestion, une meilleure digestibilité et un meilleur GMQ pour des agneaux consommant à l'auge des pellets d'aliment complets, comparativement à des animaux au pâturage supplémentés. Misra et al. (2006) rapportent une meilleure ingestion, une meilleure digestibilité, et un meilleur GMQ, pour des veaux alimentés avec de la paille de blé traitée à l'ammoniaque ou pas, du son de riz ; de plus, les animaux à l'auge recevaient du maïs vert alors que les animaux au pâturage avaient un accès libre à une prairie naturelle. Fiems et al. (2002) rapportent un meilleur GMQ pour des taureaux conduits à l'auge et recevant de l'ensilage de maïs et du concentré alors que les animaux au pâturage avaient un accès libre à la prairie et recevaient 2 kg de pulpe de betterave par tête.

Monniruzzaman et al. (2002) est, à notre connaissance, la seule étude ayant comparé l'alimentation à l'auge et au pâturage avec le même fourrage offert et des niveaux de concentrés identiques entre les deux systèmes d'alimentation. Les principaux paramètres étudiés dans cette étude étaient la croissance, l'ingestion, et le comportement alimentaire. Ces auteurs montrent une meilleure ingestion au pâturage comparativement à l'auge, bien que la croissance des animaux était supérieure à l'auge comparativement au pâturage. Cependant, on peut s'interroger sur la fiabilité des méthodes de mesure de l'ingestion au pâturage.

Certaines études visent à apprécier l'effet du système d'alimentation (auge ou pâturage) sur la qualité du lait (Morand-Fehr et al., 2007), de la viande (Keane and Allen, 1998 ; Zervas et al., 1999, Fiems et al., 2002 ; Aurousseau et al., 2004 ; Nuernberg. et al, 2005 ; Popova, 2007) , ou du fromage (Soryal et al., 2004; Galina et al., 2007). Ces études rapportent souvent une meilleure qualité des produits au pâturage. Cependant, comme pour les études précédentes les animaux à l'auge et au pâturage reçoivent souvent des rations différentes. Ainsi, les différences de qualité énoncées dans ces études seraient plus liées à des différences de ration ingérées qu'à des différences strictes de mode d'alimentation.

4. Méthodologie de mesure de la MOI et de la DMO

4.1. Méthode de référence

A l'auge, la MOI et la DMO sont généralement estimées selon la méthode de référence (Streeter, 1969; Cochran and Galyean, 1994). Elle consiste globalement à peser quotidiennement et individuellement le fourrage proposé et refusé d'animaux alimentés en cages métaboliques. La quantité de fourrage proposé est fixée à 5 à 20 % de la capacité d'ingestion. L'excrétion fécale est mesurée journalièrement et des échantillons représentatifs des quantités de fourrage proposées et refusées et des fèces excrétés sont prélevés pour la détermination de la MS et les analyses chimiques (MO, MAT, NDF, ADF, ADL). La MOI se calcule de la manière suivante :

MOI = Quantité MO Proposée – Quantité MO Refusée

et la digestibilité :

DMO = (MOI – MOF) / MOI

Avec MOF la quantité de matière organique fécale excrétée.

4.2. Méthodologie d'estimation de la MOI et de la DMO au pâturage

Au pâturage, il est laborieux d'utiliser la méthode de référence compte tenu des difficultés à quantifier le fourrage proposé et le refusé (Coleman, 2006). Des méthodes indirectes d'estimation ont donc été mises au point.

- **Méthode des différences de poids vif**

La MOI peut être mesurée par la pesée de l'animal avant et après la distribution de la ration. La variation du poids vif correspond à la quantité de fourrage ingérée au cours des repas. La valeur d'ingestion doit alors être corrigée pour les pertes liées à l'excrétion (urine, fèces) l'évaporation et l'abreuvement (Penning and Hooper, 1985). Toutefois, son application demeure limitée car elle présente de nombreux biais. Notamment, elle ne tient pas compte de la productivité de l'animal.

- **Collecte de fourrage avant et après pâturage**

Cette méthode consiste à réaliser des mesures du fourrage avant et après une période de pâturage (Meijs, 1981). Il s'agit de mesurer les quantités de fourrage disparues, à condition que la croissance du fourrage soit négligeable entre l'entrée et la sortie des animaux. Cette technique nécessite des durées de pâture courtes, et de petites parcelles, où l'hétérogénéité (composition botanique et stade de croissance) est faible. Dans ce cas les quantités disparues peuvent être assimilées à la MOI. Pour des durées de pâturage plus longues, la pousse du fourrage peut-être mesurée dans des aires protégées du broutage, et être utilisée pour corriger la MOI (Minson, 1990). Le nombre de prélèvements et leur répartition doivent tenir compte de l'hétérogénéité du fourrage avant, et surtout après pâturage. Boval (1994) a montré que même dans ces conditions et avec des animaux conduits individuellement, cette méthode sous-estimait la MOI.

- **Le comportement alimentaire**

Selon Aldden and Whittaker (1970), l'ingestion est la résultante du temps de pâturage (TP), de la fréquence ou du nombre de bouchées (NB) et de la quantité prélevée par bouchée (QB).

  - **Mesure du temps de pâturage et de la fréquence des bouchées**

Le décompte des bouchées a été automatisé à partir de l'enregistrement des mouvements de mâchoire (Chacon et al., 1976; Rutter et al., 1997) ou des sons de cisaillement de l'herbe (Laca et al., 1992), ou encore à partir de la mesure conjointe des mouvements de mâchoire, de l'accélération du mouvement de la tête et de la position de celle-ci. Cette automatisation nécessite cependant une calibration avec des enregistrements visuels pour repérer les différents types de mouvements de mâchoire. De plus, la vérification des branchements plusieurs fois par jour rend fastidieux l'usage courant des appareils d'enregistrement.

Ces contraintes font que les observations visuelles (Stobbs and Cowper, 1973) sont plus utilisées, même si elles requièrent davantage de main d'oeuvre. Ces observations consistent à noter toutes les 5 min et durant 24 h, l'activité alimentaire en cours (ingestion, mastication mérycique, repos) des animaux observés. Les animaux doivent être préalablement adaptés à la présence des observateurs, afin que lors des mesures leur comportement ne soit pas perturbé. En parallèle, la fréquence des bouchées est estimée par comptage du nombre de bouchées réalisées sur une minute à partir du moment où l'animal commence à s'alimenter.

- **Mesure de la taille des bouchées et estimation des quantités ingérées**

La taille des bouchées peut être déterminée par simulation manuelle de prélèvements de fourrage effectués par l'animal, de façon à ce que ces simulations soient les plus proches possibles des prises naturelles des ruminants. Elles doivent simuler à la fois les quantités et le type d'organe prélevé par les animaux. L'observateur doit être capable d'identifier toutes les espèces végétales du pâturage et les animaux observés doivent être habitués à la présence de celui-ci. Outre sa lourdeur, cette méthode ne permet pas d'estimer de façon fiable les quantités ingérées car elle n'est pas répétable (Gordon, 1995). La simulation manuelle des bouchées surestime souvent la taille des bouchées et les quantités ingérées.

La masse des bouchées peut-être estimée par la technique des plateaux d'herbe. Elle consiste à peser le plateau avant et après pâturage avec correction des pertes d'eau (Laca et al., 1992). Cette technique permet aussi de déterminer la masse de la bouchée par le produit de son volume (profondeur×surface) et de la masse volumique de l'horizon pâturé (rapport entre la masse d'herbe par unité de surface et sa hauteur moyenne).

La taille des bouchées aussi peut être déterminée avec des animaux canulés de l'œsophage. Toutefois, les résultats sont biaisés par la contamination par la salive du bol prélevé et les particules dégluties. Pour tenir compte de la contamination salivaire, il est possible de prélever un échantillon fourrager et de le comparer avec l'échantillon prélevé dans l'œsophage, la différence en eau étant considérée comme la salive ajoutée au moment de l'ingestion (Le Du and Penning, 1982).

- **Méthodes basées sur l'excrétion fécale et la DMO**

A partir de la relation DMO = (MOI – MOF) / MOI énoncée précédemment on peut déduire la MOI (Streeter et al., 1969) :

MOI = MOF / (1 – DMO)

Dans ce contexte, en estimant MOF et DMO on peut déduire MOI.

- **Estimation de l'excrétion fécale**

L'excrétion fécale est estimée soit par la collecte totale des fèces ou l'utilisation de marqueurs externes.

L'excrétion fécale est déterminée *via* la collecte des fèces dans des sacs fixés sur les animaux. Ces sacs permettent une collecte totale quotidienne et individuelle. Les sacs, ajustés à l'animal doivent être posés quelques jours avant les mesures afin qu'ils s'y accoutument. Pour les femelles, l'équipement est plus complexe si les fèces doivent être séparés de l'urine.

Ainsi, des gazes absorbantes peuvent être placées dans le sac ou un tube en plastique fixé sur l'animal pour la détourner.

Des marqueurs externes sont largement utilisés pour quantifier la production fécale (Coleman, 2006). Ces marqueurs sont des substances indigestibles, non métabolisées, au cours des processus physiologiques de la digestion, non toxiques pour l'animal, et quantitativement récupérables et analysables dans les fèces. De plus, ils ne doivent avoir aucun effet sur la physiologie digestive de l'animal et sur l'action des micro-organismes du tractus digestif. Une fois administrés, ces marqueurs se retrouvent dans les fèces et doivent être facilement repérables et quantifiables. Cette technique est basée sur la dilution du marqueur dans les fèces. Dans les études de nutrition animale, de nombreux marqueurs indigestibles sont utilisés tels que l'oxyde de chrome (CR_2O_3), l'ytterbium (Yb) et le polyéthylène glycol (PEG). Ce dernier est de plus en plus utilisé de nos jours, au détriment de l'oxyde de chrome car il est peu onéreux, non polluant, précis, rapide à préparer (Hassoun et al., 2005). Le principal biais de cette méthode est lié à la difficulté d'obtenir des échantillons représentatifs compte tenu de la variation journalière de l'excrétion fécale (Coleman, 2006).

- **Estimation de la digestibilité**

Elle peut être estimée par la détermination de la dégradabilité ruminale des fourrages. Trois techniques sont utilisées pour la détermination de la dégradailité rumianle : la technique *in vivo*, sur des animaux fistulés du rumen (Torell, 1954), la technique *in vitro* qui implique l'utilisation de micro-organismes du rumen (Tilley and Terry, 1961) ou d'enzymes préparées (Minson, 1990) et la technique *in sacco* par l'utilisation de sacs de nylon déposés dans le rumen d'animaux canulés (Demarquilly and Jarrige, 1981).

La digestibilité peut être aussi déterminée à partir de régressions avec des constituants du fourrage et des fèces. L'azote est le marqueur interne le plus fréquemment utilisé. Les premières équations de prédiction de la DMO par l'azote fécal ont été publiées par Lancaster (1949). Cette méthode est basée sur le principe que la quantité de MAT excrétée est constante pour 100 g de MO ingérée. D'autres indicateurs de la digestibilité tels que le chromogène (Coleman, 2006), les fibres (NDF, ADF), la lignine (ADL), et les N-alkanes ont été proposés. La principale limite de cette méthode est la nécessité d'avoir des valeurs de référence à l'auge afin de réaliser l'équation de prédiction. De plus, ces relations sont locales, car elles sont liées au jeu de données (espèce fourragère, espèce animale, conduite du fourrage) à partir duquel elles ont été développées.

La spectrométrie dans le proche infra rouge (SPIR) permet aussi de mesurer la digestibilité et la composition chimique de l'ingéré (Coates, 1999; Landau et al., 2005). La SPIR est une méthode d'analyse dont le principe repose sur l'absorption de la lumière dans les longueurs d'onde du proche infra rouge (longueurs d'onde comprises entre 800 et 2 500 nm) par la matière organique, et plus précisément par les liaisons chimiques qui la composent. Les principaux avantages de cette méthode sont 1) la rapidité d'analyse (2 minutes pour la lecture d'un échantillon, 5 minutes pour l'application des équations de prédiction), 2) le faible coût des analyses, 3) la répétabilité de l'estimation, 4) l'absence de réactifs et de composés polluants. Toutefois, cette méthode nécessite elle aussi une phase préalable de calibration entre les données de référence réalisées à l'auge et les spectres. De plus, elle ne peut être utilisée dans des gammes de données de référence et de propriétés spectrales différentes de celles dans laquelle elle a été développée.

UTILISATION DE LA SPECTROSCOPIE DANS LE PROCHE INFRAROUGE POUR L'ETUDE DE L'ALIMENTATION AU PATURAGE

ETUDES METHODOLOGIQUES

1. *Utilisation de la spectroscopie dans le proche infrarouge pour l'étude de l'alimentation au pâturage*

1.1. Introduction à l'étude méthodologique 1

Fecal Indices based on near infrared spectroscopy to assess intake, *in vivo* digestibility and chemical composition of the herbage ingested by sheep (crude protein, fibres and lignin content)

Fanchone, A., Boval, M., Lecomte. Ph., and H. Archimède

La méthodologie est un frein à l'étude de l'alimentation (ingestion, qualité de l'ingéré) au pâturage. De nombreuses études ont montré le potentiel de la spectroscopie dans le proche infrarouge (SPIR) comme outil de prédiction de l'ingestion et de la qualité de l'ingéré (Lyons and Stuth, 1992; Leite and Stuth, 1995; Coates, 1999; Landau et al., 2006). Parallèlement, des équations de prédiction de l'ingestion et de la digestibilité à partir de spectres fécaux ont été développées dans nos conditions expérimentales pour des bovins (Boval et al., 2004). A notre connaissance, très peu d'informations sont disponibles dans la bibliographie sur des calibrations réalisées à partir d'échantillons fécaux de petits ruminants. De plus, les calibrations rapportées sont rarement basées sur des mesures *in vivo* (Li et al., 2007). L'objectif de cette première étude était d'évaluer le potentiel de la SPIR pour prédire l'ingestion, la digestibilité et la composition chimique du fourrage réellement ingéré mesuré *in vivo,* pour des ovins.

Ces investigations ont été possibles grâce à la constitution au début de la thèse, d'une base de données regroupant 9 essais réalisés à l'auge (6 essais avec des ovins, 1 avec des caprins, 2 avec des bovins) entre 1996 et 2002. Les données ont été compilées et homogénéisées et la base finale comprenait 584 lignes comportant chacune des variables mesurées par animal (données individuelles). Le fourrage distribué aux animaux dans l'ensemble des essais était du *Digitaria decumbens*. Les principales variables mesurées étaient : la composition chimique du fourrage, l'ingestibilité de la MS et de ses constituants (MO, MAT, NDF, ADF, et ADL), l'excrétion fécale, la digestibilité (gastro-intestinale et ruminale), les paramètres fermentaires du rumen (AGV, NH_3 et pH), et l'encombrement (volume moyen du rumen, granulométrie).

Au sein de cette base de données, une expérimentation a été retenue pour réaliser les calibrations car 1) l'espèce animale utilisée était l'ovin, 2), le nombre de données de référence (n = 84) était suffisant pour avoir des calibrations robustes, 3), les données de référence (ingestion, digestibilité, composition chimique du fourrage ingéré) étaient des mesures réalisées *in vivo*.

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Faecal indices based on near infrared spectroscopy to assess intake, *in vivo* digestibility and chemical composition of the herbage ingested by sheep (crude protein, fibres and lignin content)

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The aim of this study was to evaluate the potential of faecal indices based on near infrared (NIR) spectroscopy to assess chemical composition and functional properties (intake and *in vivo* digestibility) of fresh grass ingested by sheep. Reference data and faecal spectra were obtained from a pen experiment with 12 ewes individually housed and fed fresh *Digitaria decumbens* at varying stages of re-growth (14–63 days) during a period of 49 days. The amount of herbage offered, refused and faecal excretion were measured per ewe daily. Organic matter (OM) content, crude protein (CP) content, neutral and acid detergent fibre (NDF, ADF) and acid detergent lignin (ADL) content were dosed in offered, refused and faecal samples. OM digestibility (OMD), intake (OMI) and chemical composition of the herbage ingested (OMi, CPi, NDFi, ADFi, ADLi, % dry matter) were calculated per ewe and per seven days. Faecal samples were bulked within each seven days of measurement period, per ewe. Eighty four dried and milled faecal samples were scanned using a monochromator. Faecal spectra were used to calibrate and cross-validate equations for predicting the various parameters using the modified partial least square (MPLS) procedure. For the CP content of the herbage really ingested (CPi), derived standard error of cross-validation (*SECV*) and cross-validation R^2 (R^2cv) were 0.61% and 0.98. For NDFi, ADFi and ADLi, the values of *SEC-V* and R^2cv were, respectively, 1.64% and 0.45, 0.78% and 0.91 and 0.34% and 0.77. For OMD, the values of *SECV* and R^2cv were 2.02% and 0.77, whereas lower calibrations statistics were obtained for OMI (11.04 g kg BW–0.75 and 0.45). These values confirmed the potential of NIR Spectra of faeces as a technology for reliably predicting the *in vivo* digestibility and chemical quality of herbage really ingested and estimating the herbage intake by small ruminants.

Keywords: near infrared (NIR) spectroscopy, faeces, intake, *in vivo* digestibility, diet chemical composition, sheep, tropical fresh grass

Introduction

The amount of forage eaten is the most important parameter determining ruminant production from forage.[1] However, intake is the parameter which is the most variable and difficult to measure precisely. Although much time has been devoted to developing measurement techniques of intake and quality of diet, they often lack both accuracy and precision.[2] For grazing animals, the classically used method to assess intake requires a two-step process: the determination of total faecal output and the determination of digestibility of the diet consumed.[3,4] Total faecal output can be estimated by total collection of faeces [5] or by the use of an external indigestible marker.[6–9] Digestibility of grazed forage can be estimated by several indirect methods:[10,11] *in vitro* or *in sacco* degradability of hand plucked herbage,[12,13] oesophageal fistula[14] samples or by using internal markers.[15,16] These methods may provide bias in digestibility estimation as hand plucked herbage samples and, to a lesser extent, oesophageal fistula samples may not be exactly representative of the herbage grazed.

At the same time, faeces are the product of eroding and synthesising digestive processes and consist of residues of feed, plant tissue and components of microbial and animal origin. Therefore, faeces may contain information about characteristics of the diets and methods based on faecal indices may provide a more precise estimation of digestibility than those previously cited. Faecal indices based on chemistry, such as faecal nitrogen, afford consistent estimates of digestibility[17,18]

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but require local regression and analyses to determine the chemical composition of faeces. At the same time, faecal indices based on near infrared (NIR) reflectance spectroscopy are increasingly used in the agro industry to evaluate nutritional quality of human and animal foods. This method provides a rapid, environmentally-friendly and precise prediction compared to classical laboratory chemical procedures. In addition, it is an alternative to the difficulty of sampling the diet of grazing animals.[2] NIR spectroscopy has been successfully used to predict digestibility[11] and, to a lesser extent, the chemical composition of the herbage ingested for cattle.[19–21] Moreover, to our knowledge and as supported by Li *et al.*,[22] limited information is available using faecal samples to predict diet quality for small ruminants, which generally have lower digestibility and intake compared to cattle[23,24] and whose production is expected to develop strongly in the next years, notably in tropical areas.[25]

The aim of the study was to develop a technique to measure intake and quality of the herbage really ingested by grazing sheep from their faeces excreted in pasture. Thus we carried out a stall-fed experiment to collect *in vivo* data and to evaluate the potential of NIR spectroscopy applied on faecal samples to predict intake, digestibility and the chemical composition of tropical fresh grass really ingested by Martinik ewes: organic matter (OMi), crude protein (CPi), neutral detergent fibre (NDFi), acid detergent fibre (ADFi) and acid detergent lignin (ADLi) content.

Materials and methods

Location

The experiment was carried out at the experimental station of the National Agronomic Research Institute (INRA) in Guadeloupe (16°16′N, 61°30′W) from February to April, 2002. Temperatures ranged from 19 to 29°C and the mean rainfall was 93 mm per month.

Animal and sward management

Twelve Martinik ewes weighing 44 (±10 kg) were individually housed in metabolism cages to be fed fresh forage (*Digitaria decumbens*). Grass was cut daily, early in the morning and chopped (5 cm length) before being offered to animals. Animals received an amount of forage 1.1 times greater than the animal voluntary intake estimated during the adaptation period. The adaptation period lasted 14 days and animals received grass at 14 days of re-growth. Measurement period lasted 49 days during which the age of re-growth of the grass varied continuously from 14 to 63 days. Animals had free access to water and mineral blocks.

The experimental area was organised into two plots. On one side, a plot measuring 5000 m^2, intended to be exploited during the adaptation period at 14 days of growth (P14) and on the other side, another plot measuring 10,000 m^2 intended to be exploited during the measurement period at a varying stage of re-growth from 14 to 63 days (P14–63). Plot P14 was divided in seven sub-plots, each being cut 15 days before being harvested to feed the animals during one day. The plot P14–63 was entirely mown 15 days before the beginning of measuring period, then was divided into 49 sub-plots, each sub-plot being mown to feed the animals each day. At the time of mowing, plots P14 and P14–28 were fertilised with one kg N $ha\,ha^{-1}day^{-1}$ of re-growth of mineral fertiliser. Similar amounts of fertiliser were added to plot P29–63; the addition of quantities higher than 28 kg of N ha^{-1} would be inefficient.

Measurements and chemical analysis

From days 14 to 63 of the measurement period, the amounts of fresh forage offered and refused were weighed daily for each ewe. The total amount of faeces excreted each day were gathered for each ewe in racks fixed under the metabolism cages. For all the ewes, two sub-samples of herbage offered (300 g) were collected daily. For each ewe a sub-sample of refusal (300 g) and a sub-sample of faeces (10% of the total amount excreted) were collected daily. Dry matter (DM) content of forage and faeces sample were determined by drying to a constant weight at 65°C in a forced draught oven. Every seven days, the sub-samples of forage offered were pooled for all the ewes (300 g) while the sub-samples of forage refused and faeces were pooled per ewe (300 g and 200 g for forage refused and faeces, respectively). Pooled samples were then ground through a 0.75 mm screen before chemical and NIR analysis. The organic matter (OM) content was determined by ashing sub-samples for eight hours at 550°C. Neutral and acid detergent fibre (NDF and ADF) and acid detergent lignin (ADL) were estimated using the method of Van Soest.[26] Nitrogen content was estimated using the Kjeldahl method.[27] Crude protein (CP), OM, NDF, ADF and ADL of the grass offered and refused were determined for each ewe for each seven days. OM intake (OMI) and digestibility (OMD) were calculated. Chemical composition (OMi, CPi, NDFi, ADFi, ADLi) of the grass ingested was calculated from the amount and composition of the grass offered and grass refused.

NIR analysis

Absorbance spectra (log 1/*R*) of samples were recorded using an NIRSystem 6500 monochromator (NIRSystem 6500, Foss, Laurel, MD, USA). Samples were scanned at 2 nm intervals over the wavelength range 700–1100 and 1100–2500 nm. Only the spectra obtained from 1100 to 2500 nm were kept. Spectral data were processed using ISI software (Infrasoft International, Port Matilda, PA, USA).[28] Calibration of chemical composition of diet ingested, OMI and OMD were developed using the modified partial least square (MPLS) procedure as this technique proved to be superior in earlier research (Shenk and Westerhaus and Park *et al.*)[29,30,31] Among the first (1441) and second (2551) order of derivatisation of spectral data, the more precise math treatments were retained: standard error of calibration (*SEC*), standard error of cross-validation (*SECV*), the coefficient of determination (R^2) and the R^2 of cross-validation (R^2cv).

Table 1. OMI, OMD and chemical composition (OMi, CPi, NDFi, ADFi, ADLi) of the herbage ingested by ewes.

Grass regrowth stage (day)	OMi (%DM)	CPi (%DM)	NDFi (%DM)	ADFi (%DM)	ADLi (%DM)	OMD (%)	OMI (g kg $BW^{-0.75}$)
15–21	89.1	21.6	70.5	31.5	4.9	71.4	61.6
22–28	89.4	19.2	70.9	32.8	4.5	68.2	58.8
29–35	89.7	16.1	71.8	35.5	5.5	72.1	60.3
36–42	90.7	13.5	75.5	38.2	5.3	71.1	61.1
43–49	91.1	11.2	75.5	37.6	4.6	70.7	55.4
50–56	91.6	10.2	74.2	37.8	3.6	64.5	50.7
57–63	91.8	9.2	72.1	37.6	3.8	63.1	50.2
Standard error of the mean	0.113	0.483	0.261	0.281	0.781	0.469	1.53

Scatter corrections used were standard normal variate and detrend (*SNV-D*) and cross-validation, based on splitting the sample population into six groups in order to select the optimum number of terms (i.e. principal components or eigenvectors) without over-fitting.

Results and discussion

Chemical composition and nutritive value

Continuous variation in the re-growth stage over the measurement period provided a significant range in dietary attributes for use as reference values (Table 1). The nutritive value of the forage ingested decreased with the grass maturity. The main difference appeared in the CPi content, which decreased sharply in the diet between the 14th and the 39th day of re-growth, representing 70% of the general decrease (from 14 to 63 days). At the same time, OMD and OMI decreased daily, by approximately 0.16 digestibility unit day^{-1} and 0.23 g $LW^{-0.75}$, respectively. This decrease in OMD is close to the average value of 0.26 generally quoted for tropical grass.[1] For OMI, a greater decline was observed between days 39 and 53 of re-growth, representing about 91% of the general decrease (from days 14 to 69). These values in OMI, OMD and chemical composition of the herbage ingested, contributed to extending the range of reference values already suitable for calibration from NIR spectra applied on faeces and already published.[32] First, we worked with C4 forage for which one knows that the chemical composition differs from C3 forage (lower OMI, OMD and CP content and higher NDF, ADF and ADL content). Moreover, we worked with a continuous re-growth stage (63 days of re-growth) which allowed a further decrease in forage quality. Thus, our results, coupled to others in the tropical and temperate areas, contributed to the increase in variability in the sample set, in order to help in developing broadly-based robust equations.

NIR prediction

Prediction of diet quality

Equations derived from faecal spectra allowed precise estimations of the composition of the forage consumed (CPi, NDFi, ADFi and ADLi) ingested by the ewes. For CPi, we obtained very good calibration statistics [$SEC = 0.33\%$,

Table 2. Descriptive and equation statistics using modified partial least square regression for organic matter intake (OMI), organic matter digestibility (OMD) and chemical composition of the herbage ingested : organic matter (OMi), crude protein (CPi), neutral detergent fibre (NDFi), acid detergent fibre (ADFi), acid detergent lignin (ADLi).

Variable	*n*	Mean	*SD*	*SEC*	R^2	*SECV*	R^2cv
OMi (% DM)	84	90.5	1.02	0.15	0.98	0.23	0.95
CPi (% DM)	84	14.2	4.22	0.33	0.99	0.61	0.98
NDFi (% DM)	84	72.7	2.20	1.54	0.51	1.64	0.45
ADFi (% DM)	84	35.9	2.58	0.59	0.95	0.78	0.91
OMD (%)	84	68.8	4.12	1.78	0.81	2.02	0.77
OMI (g $kg^{-0.75}$)	84	56.5	13.81	6.64	0.77	11.04	0.45
ADLi (% DM)	84	4.6	0.72	0.24	0.88	0.34	0.77

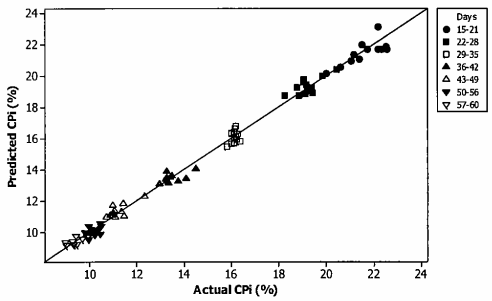

Figure 1. Actual and NIR predicted crude protein ingested (CPi) by ewes fed with *Digitaria decumbens* (the line Y=X represents agreement between predicted and observed CPi).

R^2=0.99 (Table 2, Figure 1)]. Our values compared favourably with others reported for sheep (*SEC* of 1.12% and 0.78% and R^2 of 0.94 and 0.97).[33,34] For goats fed with different ration of legume hay, concentrate and a combination of three browse species, *SECV* of 0.42% and R^2 of 0.98 were obtained.[35] For cattle fed with tropical grasses, comparable values with ours were found (*SEC*=0.33%; R^2=0.98).[21]

Acceptable *SEC* and lower R^2 were observed for NDFi [1.54% and 0.51 (Table 2)]. However, better calibration statistics than ours were reported for NDFi from NIR spectra also applied on faeces for goats[36] and cattle,[21] with *SEC* of 1.4 and 0.96 and R^2 of 0.94 and 0.88, respectively. However, our prediction precision is better than that reported from forage spectra, to predict NDF in the herbage proposed.[31,37,38]

Calibration statistics for dietary ADFi (Table 2) was more acceptable than for NDFi, with low *SEC* and relatively high R^2 values (0.59% and 0.95). Our calibration statistics are even better than those reported for cattle (*SEC*=0.81; R^2=0.89) fed with the same kind of forage.[20] Few other references exist on the prediction of ADF content in the diet consumed by ruminants. Compared to forage NIR, our *SEC* value is also lower than those reported which ranged from 1.0 to 2.8%.[39,40,41]

No published reports were found with which to compare our results in ADLi, but our cross-validation results were indicative of good predicted performance for this parameter. The Van Soest method is probably not the best method to estimate ADL[42] and the quality of reference-method analysis may have a crucial effect on the precision of NIR calibrations[43] but the Van Soest method is the most widespread in feeding experiments.

Thus, according to our calibrations, prediction of the chemical components of the herbage ingested is rather satisfactory for sheep. That is of great interest, more particularly in pasture where feeding can be very selective and where the knowledge of the herbage proposed is not enough to evaluate the quality of what is consumed. Thus, the knowledge of what CP is really consumed can make it possible to prevent a nitrogen deficit in the ration, which limits the cellulollytic activity in the rumen[1,44] and the performance of the animals. In the same way, because lignin is not degraded by rumen microorganisms and because it hides the degradation of the other components of vegetable walls, a rapid estimation of the amount of lignin consumed by ruminants would provide a better understanding of the digestion process. Thus, NIR spectroscopy is a technique of great interest in considering the chemical quality of the ingested herbage, because it must contribute to a better definition of the characteristics of the pasture to be produced, which is better for the animals.

Digestibility equations

For OMD (Table 2, Figure 2), the calibration statistics (*SEC*=1.78%; R^2=0.81) as well as the cross-validation statistics (*SECV*=2.02%; R^2cv=0.77) compared favourably with other studies aiming at predicting *in vivo* digestibility: for sheep fed with an array of forages, (*SEC*=2.26, R^2=0.94 and n=119);[45] for cattle, using a wide range of pasture grass and legume hay (*SEC*=2.5% and R^2=0.89)[46] and for cattle fed with tropical grasses (*SEC*=2.2% and R^2=0.72).[21] Our calibration statistics were also better than those reported in work aimed at the prediction of *in vitro* digestibility from NIR spectra of faeces.[32] Recently, *SEC* for *in vitro* digestibility for goats of 1.65% and 1.71% were reported.[35,36] Such a quality of prediction of *in vivo* OMD, higher than for *in vitro* digestibility, is very interesting, the more so as there can be differences from 0.05 to 0.10[47] between the digestibility of forage offered and digestibility of ingested forage. Such differences may have great implications, particularly in the grazing situation, due to the influence of morphological criteria affecting diet selection. This was due to the fact that *in vivo* values incorporated animal variation in this limited dataset.[46] In all events, it seems more advantageous to calibrate *in vivo* OMD from NIR spectra of faeces, even if the *in vitro* measurement of

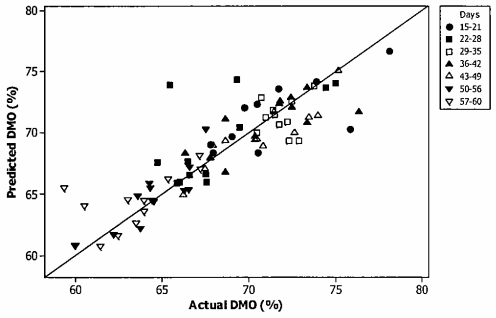

Figure 2. Actual and NIR predicted organic matter digestibility of diet ingested by ewes fed with *Digitaria decumbens* (the line Y=X represents agreement between predicted and observed digestibility).

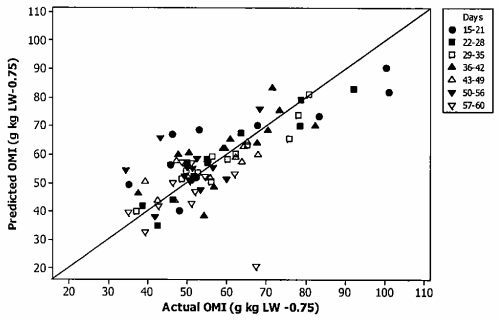

Figure 3. Actual and NIR predicted organic matter intake by ewes fed with *Digitaria decumbens* (the line Y = X represents agreement between predicted and observed intake).

OMD is easier, to better evaluate the nutrition of ruminants based on forage.

Intake equations

Our statistics for OMI calibrations (*SEC* of 6.64 g kg $LW^{-0.75}$ and $R^2 = 0.77$) and OMI prediction (*SECV* of 11.0 g kg $LW^{-0.75}$ and $R^2cv = 0.45$) are as good as those of other calibrations made from NIR spectra applied on faeces with sheep. Indeed, *SEC* ranging from 6.3 to 10.6 g kg $LW^{-0.75}$, when feeding is based on C3 and C4 grasses[48] or based on browse species [36]were reported. A similar range of calibration statistics from NIR spectra applied on faeces for cattle was also cited.[19,21] Among the functional properties we studied, the calibration of intake was less satisfactory (Figure 3) than for other studies with goats or cattle.[49,21,35] Compared to OMD, intake is more subjected to other animal factors, such as rumen size, physiological stage or feeding behaviour in addition to the usual effect of forage factors such as herbage allowance and sward characteristic. As intake is quantity and not a percentage like digestibility, it is not certain that variations in intake linked only to these animal factors are observable in faeces. Therefore, calibration of intake is less linear and precise than those of percentages of chemical contents or digestibility. It is also possible that a lack of precision in intake prediction arises from the application of the NIR-based procedure, which relies on multiple linear regressions for calibration, to phenomena that are not essentially linear.[35] Nevertheless, precision in intake prediction of 10% is acceptable in grazing systems.[1,50] The *SEC-V* of 10.8 g kg $BW^{-0.75}$ registered in our study represents approximately a 20% error of the mean value. Although calibration statistics for intake are high, according to the variability between animal which can range from 10 to 30% of the mean[2] and seeing the difficulty in assessing intake in grazing systems, equations with this level of precision may already be applied in farms. Moreover, with additional data, whose acquisition is currently in progress, it is almost certain that we will be able to improve the quality of the prediction.

Conclusion

NIR spectroscopy applied on faecal samples allow relatively good predictions of digestibility and chemical composition of the herbage ingested by sheep. These results show that, in addition to the prediction of the content of CP, NDF and ADF in the herbage ingested, the ADL content of the ingested herbage is also predictable. It is particularly interesting in the case of feeding based on tropical fresh grass, due to their higher lignin content. The prediction of intake is worse, but must be able to be improved. It is necessary to increase our set of reference values again, to improve the precision of the estimate and to widen the predictive potential of our equations to other situations. Further variation in the diet quality, by varying the species, the agronomic treatments of our tropical grass, or extending our data sets to C3 forages, have to be explored. Concurrently, calibration equations have to be tested in grazing experiments, where variables of production should allow an evaluation of the precision of our diet estimate.

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1.2. Principales conclusions de l'étude méthodologique 1

Cette étude a clairement montré que la SPIR basée sur des échantillons fécaux permet de bonnes prédictions de la digestibilité et de la composition chimique du fourrage réellement ingéré par des ovins. Une moins bonne équation de prédiction a été obtenue pour l'ingestion, néanmoins, cette équation peut être affinée notamment par concaténation de spectres fécaux et fourragers. Il s'avère toutefois nécessaire d'accroître la variabilité dans les données de références et dans les propriétés spectrales que nous avons utilisées, afin d'augmenter la précision de ces équations et d'élargir leur potentiel de prédiction. De plus, ces équations doivent être validées sur un jeu de données extérieur de manière à évaluer leur capacité de prédiction au pâturage.

COMPARAISON DE LA SPECTROSCOPIE DANS LE PROCHE INFRAROUGE ET DE L'AZOTE FECAL POUR L'ESTIMATION DE LA DIGESTIBILITE

2. Comparaison de la spectroscopie dans le proche infrarouge et de l'azote fécal pour l'estimation de la digestibilité.

2.1. Introduction à l'étude méthodologique 2

Comparison of fecal crude protein and fecal near infrared reflectance spectroscopy to predict digestibility of fresh grass consumed by sheep

Fanchone, A., Archimède, H., and M. Boval

Compte tenu des conclusions de l'étude méthodologique 1 et du nombre de données ovins présentes dans notre base de données, les données de référence ayant servi à réaliser l'étude méthodologique 1 (n = 84) ont été incrémentées et une nouvelle équation SPIR (n = 174) a été développée. Parallèlement, une équation de prédiction de la digestibilité à partir de la teneur en azote des fèces pour des ovins a été publiée (Boval et al., 2003) à l'U.R.Z. La base de données ayant servi à réaliser cette équation (n = 40) a elle aussi été incrémentée par celles présente dans notre base de données, et une nouvelle équation azote fécal (n = 174) a été développée.

Les différentes équations SPIR et azote fécal obtenues ont été appliquées sur un jeu de données indépendant, n'ayant pas servi à réaliser les calibration et pour lesquels nous disposions aussi des données de référence. Cette validation a permis d'apprécier le potentiel de prédiction de ces équations en vue de leur utilisation au pâturage.

L'objectif de cette seconde étude méthodologique était de comparer le potentiel d'équations azote fécal et SPIR, développées à partir de bases de données plus ou moins importantes, pour prédire la digestibilité d'un jeu de données indépendant.

Fecal indices methods to assess digestibility

Comparison of fecal crude protein and fecal near infrared reflectance spectroscopy to predict digestibility of fresh grass consumed by sheep[12]

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ABSTRACT

Organic matter digestibility (OMD), an essential criterion for the evaluation of the nutrition of ruminants, cannot be measured easily at pasture. Therefore, the objective of this study was to test and compare 2 methods of OMD prediction based on the fecal CP content (CPf) or near infrared spectroscopy (NIRS) applied to feces. Firstly, published equations derived from fecal N ($Eq1_{CP}$, n = 40) and from fecal NIRS ($Eq1_{NIRS}$, n = 84) were used to predict OMD of an independent validation data set (VDS) from which in vivo OMD, ranging from 58 to 74%, was measured for 4 regrowth stages of *Digitaria decumbens*. Secondly, to set up equations usable in grazing situations and to improve the efficiency of the predictions, new equations were calculated from a larger data set (LDS, n = 174) using CPf ($Eq2_{CP}$) or fecal NIRS ($Eq2_{NIRS}$). By applying the CPf method, $Eq2_{CPf}$ (OMD = 88.4 – 263.9 / CPf, Residual SD = 2.92, r^2 = 0.63) showed similar statistical parameters ($P < 0.01$) when compared to $Eq1_{CP}$ (OMD = 86.6 – 266.2/CPf, residual SD = 2.95, r^2 = 0.79). When using fecal NIRS, $Eq2_{NIRS}$ showed lower SE of calibration (SEC = 1.48) and of cross-validation (SECV = 1.75) and greater coefficient of determination of cross validation (R^2_{CV} = 0.85) than $Eq1_{NIRS}$ previously published (SEC = 1.78, SECV = 2.02, R^2_{CV} = 0.77). The validation of the 4 equations on the VDS was overall satisfactory with an average difference between the predicted and the observed OMD ranging from 0.98 to 2.8. The $Eq2_{NIRS}$ was nevertheless the most precise with a lower Residual SD of 2.53 and also the most accurate, as the standard deviation of the average difference between predicted and observed OMD was the lowest. Therefore, fecal NIRS provided the most reliable estimates of OMD and is thus a useful tool to predict OMD at pasture. However, an adequate number of reference data is required to establish a good calibration. Indeed, better calibration statistics were obtained by increasing the data set from

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84 ($Eq1_{NIRS}$) to 174 ($Eq2_{NIRS}$). In contrast, using fecal N on a set of 84 or 174 points did not improve the prediction. Both methods are useful for predicting OMD at pasture in certain circumstances, using fecal NIRS when a large data set (n > 80) is available and fecal CP with smaller data sets (n < 80).

Key words: digestibility, fecal crude protein, near infrared reflectance spectroscopy, sheep, tropical grass

INTRODUCTION

Digestibility of OM is 1 of the most important characteristics used to evaluate feed nutritional quality. For grazing animals, OM digestibility (OMD) cannot be determined directly, unlike in stall feedings where direct determination by quantitative measurements of ingested forage and fecal excretion can be accomplished. Therefore, several indirect assessment methods have been developed from forage or fecal samples. Methods based on forage samples, such as in vitro or in sacco degradability of hand plucked herbage, may introduce bias in digestibility estimation because hand plucked herbage may not be representative of the herbage grazed due to diet selection by grazing animals (Baumont et al., 2000; Schlegel et al., 2000). Methods based on esophageal fistula samples have then been used to overcome the inaccuracy of hand plucked samples (Le Du and Penning, 1982). However, these methods require the surgical alteration of experimental animals, which is both impractical in production situations and can be undesirable from an animal welfare point of view.

Methods to predict OMD based on fecal profiling, including regression with fecal CP (CPf) content (Wehausen, 1995; Boval et al., 2003) or near infrared reflectance spectroscopy (NIRS) applied to feces (Coates, 1999; Landau et al., 2006), have provided consistent estimates of OMD. Therefore, the aim of this study was to examine the potential of fecal indices based on fecal CP or fecal NIRS to predict OMD in grazing situations. A 2 step procedure was employed. First, existing published equations derived from fecal CP (Boval et al., 2003) and from fecal NIRS (Fanchone et al., 2007) were used to predict OMD of a small independent validation data set. Secondly, a larger calibration data set was created to further asses the applicability of these 2 fecal based methods to predict OMD in a variety of Pangola grass pastures.

MATERIALS AND METHODS

Care and use of animals were performed according to the Certificate of Authorization to Experiment on Living Animals issued by the French Ministry of Agriculture, fishing, and feeding.

Validation Data Set

The validation data set (VDS, n = 23) came from an independent trial carried out in 1996 at the animal experimental station of the "Institut National de la Recherche Agronomique" (INRA) in the French West Indies (Guadeloupe, latitude 16°16′N, longitude 61°30′W). This trial was designed to evaluate variations in nutritive values of Pangola grass (*Digitaria decumbens*) according to various stages of regrowth (Archimède et al., 2000). This data set was retained for validation because of its large range in values for OMD. Six adult Martinik rams (40.8 ± 0.6 kg) were individually housed in metabolic cages and fed 14, 28, 42 and 56 d old fresh regrowth of Pangola grass during 4 successive experimental periods. The regrowth stages 14, 28, 42, and 56 d were used during the period 1, 2, 3, and 4, respectively. The plots (n = 105) intended to be used at 14, 28, 42, and 56 d of regrowth were divided into 15, 30, 30, and 30 subplots, respectively. The first of the 15, 30, 30, and 30 subplots had been cut 15, 29, 43, and 57 d before the beginning of the experimental period 1, 2, 3, and 4, respectively. One subplot was cut per d, so that each subplot has 1 d more regrowth than the subplot cut the d before and 1 d less than the one cut the following day. Consequently, the regrowth stage of the subplot intended to be harvested daily was exactly 14, 28, 42, and 56 d in periods 1, 2, 3, and 4, respectively. For the 4 stages of regrowth (14, 28, 42, and 56 d), the concentration (DM basis) of OM was 84, 89, 90, and 88%; CP was 13.0, 7.9, 7.2, and 5.7%; NDF was 74, 78, 79, and 79%; and ADF was 38, 43, 44, and 44%, respectively (Archimède et al., 2000). In the 56 d treatment, 1 of the rams having a very low intake level (30% lower than the average intake of the group) was removed. Each experimental period consisted of 14 d of adaptation to the diet, followed by 5 d of intake and total tract digestibility measurements. The grass was cut daily at 3 cm height with a mowing machine (BCS S.p.A., Milan, Italy), early in the morning and chopped at 5-cm length using an electric chopper (DESSERTINE-HUPIN S.A., Buxieres les Mines, France) before being offered. The amount of forage provided was 1.15 x animal voluntary intake estimated during the adaptation period.

Digestibility was calculated per animal from daily intake and total amount of feces excreted. Daily intake per animal was measured on d 15 to 19 by weighing the daily amounts of forage offered, and the refusals. Total amount of feces excreted each day were gathered per ram in racks fixed under the metabolic cages on d 17 to 21 to help account for passage rate. Dry

matter content of fresh forage and refusal were determined daily by drying for 72 h at 60 °C (Cochran and Galyean, 1994). A representative sub-sample of feces excreted was obtained by pooling 10% of the daily amount of feces excreted per animal. Sub-samples of feces were stored at -20 °C until DM content determination. Dry matter content of fecal subsamples was determined in similar conditions as previously described for fresh forage and refusal. Samples were ground to a 0.75 mm particle size using a cross beater mill SK 100 (Retsch, Hann, Germany). Ground samples were then stored in closed plastic containers before chemical analyses. Organic matter content of forage and fecal samples was measured after an 8 h pyrolysis at 550 °C to estimate OMD according to the reference procedure of Cochran and Galyean (1994). Nitrogen concentration of feces was determined using the Dumas method (AOAC, 1990). Crude protein content of feces was calculated by multiplying the N concentration by 6.25. Approximately 2.5 g of ground fecal samples were packed in ring-cup sample cells with a near infrared, transparent, quartz cover glass (Foss, 2000). Cells were scanned 32 times using a scanning reflectance monocromator (NIRSystem 6500 Inc., Silver Springs, MD). Reflectance energy (Log [1/R], where R = Reflectance) was measured and averaged over the 32 scans. The average spectra of absorbance were recorded at 2-nm intervals over the wavelength range 700 to 1,100 and 1,100 to 2,500 nm. Only the near infrared region was used for calibration.

Calibration Data Set

A large data set (LDS, n = 174), was made from 4 digestibility trials carried out in stalls from 1997 to 2000. All trials were conducted at the animal experimental station at the "Institut National de la Recherche Agronomique" (INRA) in Guadeloupe. The effects tested through the different trials were mainly the leaf-stem proportion of the ration, the regrowth stage of the herbage, or the physiological stage of the animals (H. Archimède, unpublished data). In all trials, adult Martinik rams (45.1 ± 0.31 kg) were individually housed in metabolic cages and fed fresh Pangola.

Determination of OM content of forage and feces, estimation of in vivo OMD, calculation of CPf, as well as recording the absorbance spectra of fecal samples, were as previously described for the VDS.

Calculations and Statistical Analysis

Existing predicting equations of OMD, derived from CPf ($Eq1_{CP}$, Boval et al., 2003) and from fecal NIRS ($Eq1_{NIRS}$, Fanchone et al., 2007) were used to predict OMD of the VDS.

New equations, based on CPf ($Eq2_{CP}$) or fecal NIRS ($Eq2_{NIRS}$) were developed from the LDS and were used to predict OMD of the VDS. The equation $Eq2_{CP}$ was calculated according to a hyperbolic model (OMD = a – b / CPf) as proposed by Boval et al. (2003), because it has been observed to be more precise than linear or quadratic models. The MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) was used to account for the fixed effects of experiment (4 experiments) and the random effect of rams within each experiment (4 to 6 rams per experiment). The stage of regrowth was added as a covariate. The intercept and the slope of $Eq2_{CP}$ were compared with that of the equation of $Eq1_{CP}$ using the Neyman-Pearson test.

Before calibration, absorbance spectra of the LDS were transformed using standard normal variate and detrend scatter correction and 2 mathematical pre-treatments: 1.4.4. and 2.5.5. using ISI software (Infrasoft International, Port Matilda, PA), where the first number is the order of derivatisation of spectral data, the second number is the gap over which the derivative is to be calculated, and the third number is the smoothing factor. The mathematical treatment 2.5.5. yielded superior calibration statistics, namely lower SE of calibration (SEC), lower SE of cross validation (SECV), greater multiple coefficient of determination (R^2) and greater R^2 of cross validation (R^2cv), and was retained rather than 1.4.4. The SEC represents the variability in the difference between predicted values and reference values when the equation was developed from the calibration data set. The SECV represents the variability in the difference between predicted and reference values when the equation is applied sequentially to subsets of data from the calibration data set (Landau et al., 2006). Cross validation is often employed when an independent validation set is unavailable or when removal of samples from a calibration set results in too few samples for effective equation development. Briefly, this process involves removing a certain number of samples during the calibration procedure, e.g. 25%, and predicting these with the remaining 75%. This step is then repeated until all have served as validation samples. Cross validations were based on splitting the sample population into 6 groups to select the optimum number of terms (i.e. principal components or eigenvectors) without over-fitting. The combined SE for each of these steps is the SECV. The equation $Eq2_{NIRS}$ to predict OMD was derived by processing pre-treated fecal spectra of the LDS using modified, partial, least square regression (ISI, 1999) because this technique was proven to be superior than other methods (principal component regression or stepwise multiple linear regression) in earlier research (Shenk and Westerhaus, 1991; Park et al., 1997; 1998).

The predicted values of OMD for the VDS, starting from the 4 equations described above ($Eq1_{CP}$, $Eq1_{NIRS}$, $Eq2_{CP}$ and $Eq2_{NIRS}$), were compared with the observed values. The

precision of estimation was evaluated from the absolute difference (Dpo) between the predicted OMD and the observed OMD. Factorial analyses of variance was computed to determine the influence on Dpo of the main factors of variation in the VDS (Archimède et al., 2000), the regrowth stage (14, 28, 42 and 56 d), and animals using the GLM procedure of SAS, (SAS Inst. Inc., Cary, NC). The SECV of the fecal NIRS equations and the SD of the different equations were compared using a Fisher test.

Table 1: Descriptive statistics for OM digestibility (OMD) and fecal CP (CPf) in the validation data set (VDS), in the data set of Boval et al. (2003), in the data set of Fanchone et al. (2007) and in the large data set (LDS)

Data sets		OMD, %		CPf, %OM	
	N	Mean ± SD	Range	Mean ± SD	Range
VDS	23	66.6 ± 4.6	58.3 to 73.6	14.5 ± 2.9	11.1 to 20.4
$Eq1_{CP}$[1]	40	63.0 ± 1.8	59.0 to 71.0	11.7 ± 0.4	9.8 to 16.0
$Eq1_{NIRS}$[2]	84	68.7 ± 7.4	59.9 to 82.3	14.3 ± 2.6	10.0 to 20.3
LDS	174	67.1 ± 4.9	53.9 to 82.3	12.8 ± 2.6	7.9 to 20.3

[1]$Eq1_{CP}$: Fecal CP equation of Boval et al. (2003)

[2]$Eq1_{NIRS}$: Fecal NIRS equation of Fanchone et al. (2007)

Table 2: Predictive regressions of OM digestibility (OMD, %) from fecal CP content per unit of OM (CPf, %OM), calculated for sheep fed *Digitaria decumbens*

Item	n	Equation	Residual SD	r^2
$Eq1_{CP}$[1]	40	$OMD = 86.6_{(\pm 7.3)} - 266.2_{(\pm 83.1)} / CPf$	2.95	0.79
$Eq2_{CP}$[2]	174	$OMD = 88.4_{(\pm 4.72)} - 263.9_{(\pm 64.4)} / CPf$	2.92	0.63

[1]$Eq1_{CP}$: Equation of Boval et al. (2003)

[2]$Eq2_{CP}$: Fecal CP equation derived using the large data set (LDS)

RESULTS

In the VDS, OMD and CPf variation (about 26% and 84%, respectively) was mainly due to regrowth stage of the grass used in the study (Figure 1 and Table 1). In this set of data, OMD and CPf varied on broader range (15% and 9%, respectively), compared to the data used to establish $Eq1_{CP}$ (which varied on 12% and 6% for OMD and CPf, respectively). In addition, variation in OMD and CPf in the VDS were more reduced than the other 2 data sets used to establish $Eq1_{NIRS}$ and $Eq2_{NIRS}$. The LDS used to establish $Eq2_{NIRS}$ presented the most significant variation for the 2 parameters (range of approximately 28% for OMD and 12% for CPf). Considering all the data sets, there was greater variations in CPf values than in OMD values.

For CP equations, neither experiment, animal, nor stage of regrowth was significant ($P > 0.10$) on the equation $Eq2_{CP}$ ($P > 0.10$; Table 2). The a and b values of $Eq2_{CP}$ were not significantly different ($P > 0.05$) from those of the equation $Eq1_{CP}$ based on the Neyman-Pearson test. Similar residual SD were achieved for both equations ($P > 0.05$), whereas r^2 was lower for $Eq2_{CP}$.

For fecal NIRS equations derived from the LDS, better calibrations and cross-validation statistics (lower SEC and SECV, $P < 0.05$; greater R^2 and R^2cv, Table 3) were obtained for the equation $Eq2_{NIRS}$ than the $Eq1_{NIRS}$.

Using CPf equations to predict OMD from the VDS, we obtained a residual SD numerically greater with $Eq1_{CP}$ compared to $Eq2_{CP}$ (Table 4; Figure 2). The differences between the observed and predicted values were numerically lower with $Eq1_{CP}$ compared to $Eq2_{CP}$. Similarly, the SD of the difference was proportionally larger for $Eq1_{CP}$ compared to $Eq2_{CP}$. The effects of regrowth stage and animal were significant ($P < 0.05$) on the Dpo (Table 4) for the 2 equations $Eq1_{CP}$ and $Eq2_{CP}$. Using fecal NIRS to predict OMD of the VDS, we obtained a greater residual SD with the $Eq1_{NIRS}$ compared to the $Eq2_{NIRS}$ ($P < 0.09$; Table

Table 3: Descriptive statistics of the fecal near infrared reflectance spectroscopy (NIRS) equations to predict OM digestibility (OMD, %)

Item	n	Mean	SD	SEC[3]	R^2	SECV[4]	R^2 cv [5]
$Eq1_{NIRS}$[1]	84	68.8	4.12	1.78	0.81	2.02	0.77
$Eq2_{NIRS}$[2]	174	67.0	4.48	1.48	0.89	1.75	0.85

[1]$Eq1_{NIRS}$: Equation of Fanchone et al. (2007)

[2]$Eq2_{NIRS}$: Fecal NIRS equation derived using the large data set (LDS)

[3]SEC: SE of calibration

[4]SECV: SE of cross-validation

[5]R^2 cv: Coefficient of cross-validation

4 and Figure 3). For the $Eq1_{NIRS}$ the mean difference between predicted OMD and observed OMD was lower compared with $Eq2_{NIRS}$, but the SD was proportionally greater with $Eq1_{NIRS}$, compared with $Eq2_{NIRS}$ (Table 4). The effect of both stage of regrowth and animal, was highly significant (P = 0.02 and P = 0.003 for the effect of stage of regrowth and animal respectively) for the $Eq1_{NIRS}$; whereas, only the animal effect was significant on Dpo for the $Eq2_{NIRS}$ (P = 0.009). Considering the SECV of $Eq2_{NIRS}$ (Table 3) and the numerically lower SD of the equation predicting OMD by using $Eq2_{NIRS}$, this equation is more precise.

DISCUSSION

Fecal Crude Protein Equations

Fecal CP equations allow a precise prediction of in vivo OMD. The residual SD obtained using $Eq2_{CP}$ was numerically lower than that obtained with other methods aimed at predicting in vivo OMD from forage (from 3.2% to 5.1%, Kitessa et al., 1999; from 2.4 to 5.0%, Gosselink et al., 2004) and slightly higher than those of CPf equations using hyperbolic models (2.5%, Boval et al., 1996) or exponential models (2.7%, Lukas et al., 2005). By increasing the range of OMD and CPf using the LDS, we expected to increase the predictive ability of $Eq2_{CP}$ compared to the published $Eq1_{CP}$ of Boval et al. (2003) but that was not the case. The values of a and b were not significantly different between the 2 fecal CP equations. The similarity between the 2 CPf equations may be explained by the hyperbolic model retained to derive the 2 equations. This model is assumed to describe the biological relationship between OMD and CPf, as described by Lancaster (1949), and can be used outside of its range of establishment (Lancaster, 1949; Wehausen, 1995; Boval et al., 2003). Thus, although the range of variation of data from Boval et al. (2003) is less than the range of the LDS, $Eq1_{CP}$ was capable of adequately describing this biological relationship, giving a and b values valid throughout the entire range of OMD and CPf in the VDS. Hence, the

Table 4: Relationships between OM digestibility observed (OMD, %) in an independent validation set and predicted OMD, using four different equations, based on fecal CP ($Eq1_{CP}$ and $Eq2_{CP}$) or fecal near infrared reflectance spectroscopy NIRS ($Eq1_{NIRS}$ et $Eq2_{NIRS}$)

Predictions of OMD	Residual SD	Dpo[1]	SD	P-Value[2]	
				RS	A
OMD = 0.97 x OMD_{Eq1CP}[5]	3.04	1.65	3.02	0.010	0.013
OMD = 0.959 x OMD_{Eq2CP}[6]	2.80	2.79	2.79	0.047	0.009
OMD = 0.983 x $OMD_{Eq1NIRS}$[7]	3.15	0.98	3.21	0.002	0.003
OMD = 0.965 x $OMD_{Eq2NIRS}$[8]	2.53	2.40	2.55	0.453	0.009

[1]Dpo : Difference between predicted and observed OMD

[2]Factorial analysis of variance of Dpo, including the effects of regrowth stage (RS) and animal (A), †$P < 0.10$, *$P < 0.05$, **$P < 0.01$.

[3]RS : effect of regrowth stage

[4]A: Effect of animals

[5] OMD_{Eq1CP} = OMD predicted by the equation $Eq1_{CP}$ of Boval et al. (2003)

[6] OMD_{Eq2CP} = OMD predicted by the fecal CP equation $Eq2_{CP}$ derived using the large data set

[7] $OMD_{Eq1NIRS}$ = OMD predicted by the equation $Eq1_{NIRS}$ of Fanchone et al. (2007)

[8] $OMD_{Eq2NIRS}$ = OMD predicted by the NIRS equation $Eq2_{NIRS}$ derived using the large data set

similarity of our 2 equations based on CPf means that it is not absolutely necessary to develop local equations for Pangola grass, in a fixed range of variation, as reported by Le Du and Penning (1982) and Armstrong et al. (1989). In fact, different authors employed linear or quadratic models, which tended to over-estimate the OMD for high values of CPf; whereas, the hyperbolic or exponential models used by Wehausen (1995), Boval et al. (1996) and Lukas et al. (2005) better explain the biological relationship between OMD and CPf. Thus, equations established under a fixed range can have a wider range of application, and methods based on CPf may be more powerful than expected.

The prediction could be improved by using additional predictors, although Boval et al. (2003) and Lukas et al. (2005) explored, unsuccessfully, the fiber content of the herbage or feces and the CP content of herbage. Another possible improvement would be to better describe the biological relationship between OMD and CPf. Fecal CP is composed of 2 fractions: 1) the undigested dietary protein and 2) the metabolic fecal protein, including bacterial and endogenous protein (Lancaster, 1949; Wehausen, 1995; Ferri et al., 2003). These authors state that the biological relationship between OMD and CPf is linked to the metabolic fecal protein fraction. However, the opinions are divided concerning the respective role of bacterial and endogenous fractions and even on the proportion of these fractions in metabolic fecal protein. Therefore, an evaluation of the various sources of CPf and their respective relationship with OMD should improve the ability to predict OMD via fecal based calibrations. Further experiments are required to better understand the relationship between the different CPf fractions.

Fecal NIRS equations

The prediction of OMD by fecal NIRS using the LDS was as expected and confirmed the potential of this indirect method to assess in vivo OMD for grazing animals. Contrary to CPf equations, the precision of fecal NIRS equations increased by using a larger data set (Table 1). The $Eq2_{NIRS}$ calculated from the LDS was more precise than $Eq1_{NIRS}$ derived from a smaller data set. Enlarging the data set increased variability in spectral properties which led to an increase in precision of prediction. In addition, using the LDS expanded the prediction potential of the equation, particularly for low OMD values (lower than 0.60). For NIRS to be used successfully, it is essential that the sample and reference data cover all sources of variation likely to be encountered in routine analysis (Kitessa et al., 1999). Statistics of calibration for $Eq2_{NIRS}$ were thus better than those previously published. Indeed, SEC of 2.26

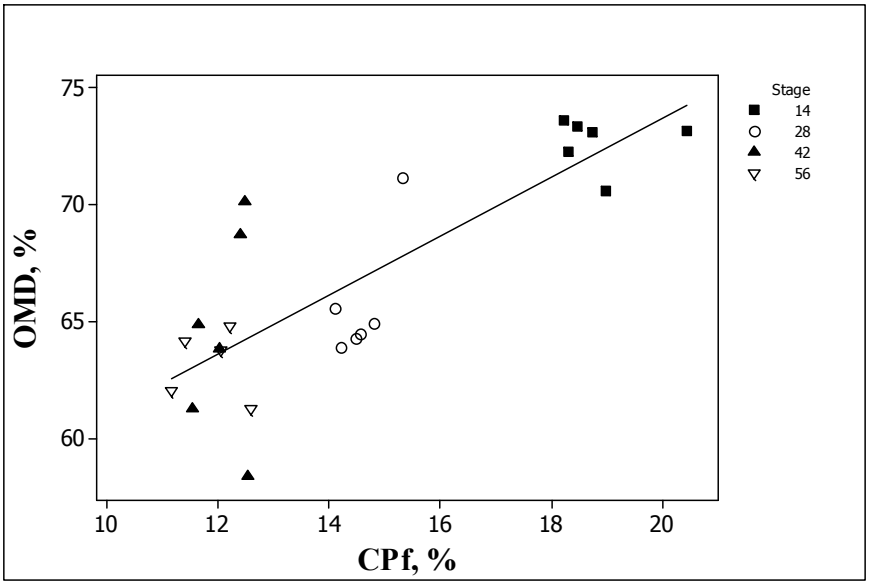

Figure 1 Evolution of OM digestibility (OMD) with fecal CP (CPf) content (% OM) for sheep fed with Pangola grass at various stages of regrowth (14, 28, 42, and 56 d) in the validation data set

and 2.2, and R^2 of 0.94 and 0.72 were reported, respectively, by Krachounov et al. (2000) for sheep fed with an array of forage, and Boval et al. (2004) for cattle fed tropical grasses.

Validation

The 2 CPf equations provided good estimates of in vivo OMD of the VDS. The precision of prediction using $Eq1_{CP}$ or $Eq2_{CP}$ on the VDS were close, although $Eq2_{CP}$ presents a residual SD slightly smaller. The variation of the difference between predicted and observed values was lower, indicating a more robust prediction. However, both CPf equations overestimated the low values of OMD measured for 42 and 56 d of regrowth (Figure 2). If studies agreed on the existence of a biological relationship between OMD and CPf, they also agreed on the fact that undigested dietary protein adversely affects this relationship (Lukas et al., 2005; Schlecht and Susenbeth, 2006). When the digestion of dietary CP is constrained, the undigested dietary protein fraction of CPf increases and induces an artificial increase in the OMD prediction. Particularly, tropical forages are known to be resistant to digestion because they mature rapidly. High fiber content of tropical forage may restrict CP digestion in the rumen leading to an increase of undigested dietary protein. For example, Archimède et al. (2000) reported a decrease in the apparent total tract CP digestibility from 0.67 to 0.32 for 14 and 56 d regrowth of Pangola grass, respectively. This lower CP digestibility for more mature forage can generate increased fractions of undigested dietary protein in feces and an over-estimate of the prediction of OMD by CPf method. Fecal NIRS equations provided more rewarding estimates of in vivo OMD of the VDS than CPf equations. The $Eq2_{NIRS}$ had the lowest residual SD of the 4 equations tested and appears to be the more precise. This equation was also the most reliable because the Dpo varied to a lesser extent than in $Eq1_{NIRS}$ and CPf equations and only the effect of animal was significant on Dpo. For the other models, both animal and stage of regrowth had a significant effect on Dpo. After increasing the variability in spectral properties and reference data by using the LDS, the entire range of variation of the VDS was covered, resulting in a gain in precision and reliability of prediction. The main advantage of the NIRS compared to the CPf technique is its ability to take indirectly into account several predictors in the calibration process, which improves the predictive ability. While the CPf method takes into account only 1 chemical component of feces to derive an equation, the NIRS technique provides 700 absorbances of light in wavelengths ranging between 1,100 and 2,500 nm, each one a potential indicator of diet characteristics. Therefore, the NIRS technique can retain absorbances related to CPf, as well as spectral absorbance

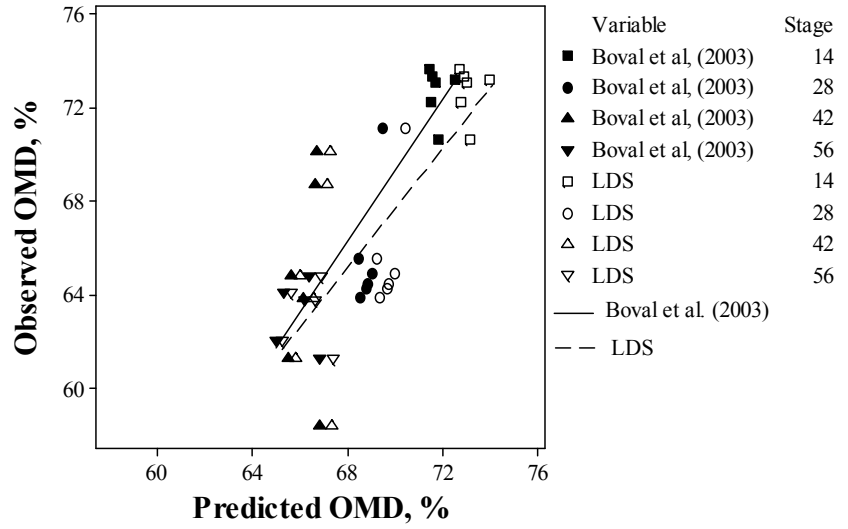

Figure 2. Observed and predicted OM digestibility (OMD) by fecal CP equations of Boval et al. (2003) or derived using a large data set (LDS; n = 174).

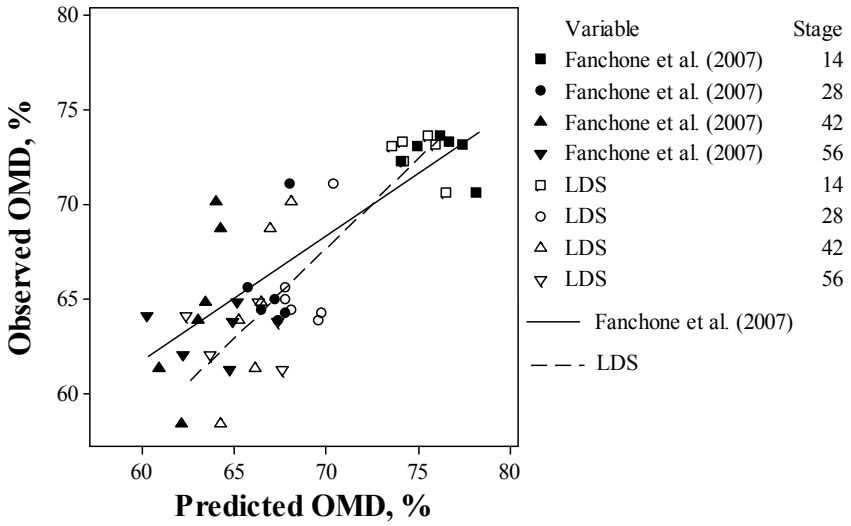

Figure 3. Observed and predicted OM digestibility (OMD) by fecal near infrared spectroscopy equations of Fanchone et al. (2007) or derived using a large data set (LDS; n = 174).

values for other constituents associated with OMD of the diet. Thus, fecal NIRS takes into account the microbial and endogenous fractions of CPf which are correlated to OMD.

Furthermore, other advantages of the NIRS technique compared with CPf are speed, repeatability of prediction, and it does not require repeated chemical analyses except for calibration (Stuth et al., 2003). In addition, NIRS calibration permits the estimation of several constituents from the scan of a single sample (Stuth et al., 2003). The main limitation of this approach is the difficulty of obtaining a sufficient number of samples, with values measured in vivo, to develop calibration equations (Deaville and Flinn, 2000). Nevertheless, this drawback is also shared by the CPf method.

However, given the similarity between $Eq1_{CP}$ and Eq2CP for the VDS, it appears possible to achieve satisfactory predictions with a small set of data. It is necessary, however, that this small data set covers a range of sufficient variation of CPf and that a suitable model, such as hyperbolic or exponential, is used (Lukas et al., 2005). When using NIRS, in contrast to the CPf method, the data set used by Boval et al. (2003) may not allow for consistent calibration because of a narrow range of spectral properties. Also, even if the NIRS has many advantages, the method of CPf can be useful with a small data set to derive reliable predictive equations of OMD. In fact, each of the 2 methods can be useful to estimate digestibility of pasture in different contexts.

This study has shown that CPf content is a reliable index to predict in vivo OMD for sheep. The hyperbolic model first proposed by Lancaster (1949) is of interest because it describes a biological relationship between OMD and CPf that allows reliable estimates using an independent data set with values outside the range of the originally modeled data. However, NIRS applied to fecal samples allows better estimation of in vivo OMD than the CPf method because it can take into account more indicators of digestibility (Andrés et al., 2005). An increase of variability of the reference data improved the precision of the estimated fecal NIRS equation. Varying the forage species, the agronomic treatment of tropical grass, or extending our data set to cool-season forages, should be explored to widen the predictive potential of our fecal NIRS equation and to further increase the precision of prediction. When a small reference data set ($n < 80$) is available, a hyperbolic equation based on CPf can be suitable to predict the OMD of grazing animals.

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2.2. Principales conclusions de l'étude méthodologique 2.

Cette étude a confirmé que la méthode d'estimation de la digestibilité basée sur l'azote fécal et notamment le modèle hyperbolique utilisé permettait une bonne estimation de la digestibilité pour un jeu de données indépendant. Parallèlement, l'augmentation de la variabilité dans les données de référence a induit un accroissement de la précision et de la robustesse de l'équation SPIR. De plus, la SPIR a permis, dans le cadre de ces données, une meilleure prédiction de la digestibilité que la méthode azote fécal.

COMPARAISON DE L'INGESTION ET DE LA DIGESTION DE MOUTONS ALIMENTES A BASE DE DIGITARIA DECUMBENS A L'AUGE ET AU PATURAGE, A DEUX AGES DE REPOUSSE

ETUDE EXPERIMENTALE

1. Comparaison de l'ingestion et de la digestion de moutons alimentés à base de *Digitaria decumbens* à l'auge et au pâturage, à deux âges de repousse.

1.1. Introduction à l'étude expérimentale 1.

Intake and digestibility of Digitaria decumbens fed by sheep indoors or at pasture, at two stages of regrowth.

Fanchone, A., Archimede, H., Delagarde, R., and M. Boval

Compte tenu des résultats de ces investigations méthodologiques nous disposions d'outils pour étudier les différences d'alimentation (ingestion, digestibilité) entre l'auge et le pâturage. La première étude expérimentale s'est déroulée sur des moutons de race « Martinik », canulés du rumen auxquels était offert simultanément à l'auge et au pâturage, du *Digitaria decumbens* à 2 âges de repousse. L'âge de repousse a été retenu comme facteur de variation dans cet essai compte tenu de sa capacité à discriminer la qualité du fourrage à l'auge (Archimede et al., 2000) comme au pâturage (Boval et al., 2007).

Nutrition of Indoor and grazing animals

Intake and digestibility of Digitaria decumbens fed by sheep indoors or at pasture, at two stages of regrowth.

A. Fanchone, H. Archimede, R. Delagarde and M. Boval

ABSTRACT

The effect of the feeding system on intake and digestion of fresh Digitaria decumbens grass were studied at two stages of regrowth. Twenty adult Martinik rams weighing on average 55 (± 10 kg) were randomly dispatched into four groups according to two simultaneous 2 x 2 Latin Square designs. In the first Latin Square, ten rams consumed a 21 d regrowth forage during two periods, indoor (5 rams) or at pasture (5 tethered rams). In the second Latin Square, ten other rams consumed a 35 d regrowth forage during two periods indoor (5 rams) or at pasture (5 tethered rams). Organic matter digestibility (OMD) were estimated using the fecal CP (CPf) and the fecal near infrared spectroscopy (NIRS) methods. The CPf method gave estimates of OMD more correlated (R^2 = 0.49) with in vivo OMD of rams fed indoor than the NIRS method (R^2 = 0.18). OM intake was calculated from fecal OM output and OMD estimated by using the CPf method. Organic matter digestibility estimated using the CPf methods (OMDCPf) was 2.4 % greater at pasture compared to indoor, whereas, OMICPf and digestible OM intake were greater by 13.7 % and 11.4 % respectively indoor compared to at pasture. From 21 to 35 d of regrowth, slight decrease in OMDCPf were found indoor compared to at pasture, whereas, for OMICPf, a greater decrease where found at pasture (13 g OM / kg BW0.75) compared to indoor (9 g OM / kg BW0.75). The greater ammonia concentration in the rumen coupled with the lower ruminating index measured illustrate the greater selective behavior of the rams and explained this difference in OMDCPf. This study point up that differences in OMDCPf and OMICPf exist between animals fed indoor and at pasture with the same forage, and that theses differences may vary according to the variation of the quality of the grass on offer.

Keywords: Feeding system, Stall, Grazing, Intake, Digestibility, Sheep

INTRODUCTION

Fresh forage is often the main resource for many ruminants in the world and intake and digestibility are the most important variable controlling ruminant production from forage. Indoor, nutrition has been widely studied and reference methods to measure nutrition in such conditions have been defined (Cochran and Galyean, 1994). In contrast, nutrition in grazing conditions has been less studied, mainly because the reference methods defined for indoor animals are not workable at pasture. Indeed, methods of nutrition measurement at pasture are still awkward, little precise, in spite of the many studies realised in this field (Cochran and Galyean, 1994; Penning, 2004). The assessment of nutrition at pasture is complicated by the diet selections by animals, the regrowth of forage during grazing and the difficulties of sampling forage and feces. Thus, general laws of animal feeding at pasture are often based on the extrapolation (while using correcting factors) of the results obtained indoor. Even though most of the factors identified to interact on intake and digestibility of animals fed indoor may also affect grazing animals, some factors mainly linked to morphological structure of grazed forage and diet selection by animals, appear to be specific to the grazed forage (Minson, 1990; Prache and Peyraud, 1997; Corbett and Freer, 2003). Thus, while several authors (Minson, 1990; Ketelaars and Tolkamp, 1992) reported positive correlation between organic matter intake (OMI) and organic matter digestibility (OMD) of indoor animals, such relationship may differ at pasture (Hitchcock et al., 1990; Boval et al., 2007a) and differences may occur between the two environments. Even though studies aiming at comparing nutrition between indoor and grazing animals are available (Keane and Allen, 1998; Zervas et al., 1999), they mainly compare production systems rather than discrete treatments. In these comparisons indoor animals often received diet with higher nutritional density including high supplement proportion, whereas grazing animals remained only at pasture. Moreover, these comparisons are seldom based on the same forage offered. Thus, the feeding differences between indoor and grazing animals reported in these studies would be more due to differences in the nutritional density of the diet than to the feeding system.

The aim of this study was, first, to point up and to explain the differences of intake and digestibility between animal fed with the same forage, indoor and at pasture. This comparison was led at two different stages of regrowth to test its constancy in various situations. By another way, we evaluated the ability of two indirect methods based on fecal index, i.e., fecal near infrared reflectance spectroscopy (NIRS) and fecal CP (CPf) to predict in vivo OMD measured indoor, and to be used afterwards at pasture.

MATERIAL AND METHODS

Care and use of animals were performed according to the Certificate of Authorization to Experiment on Living Animals issued by the French Ministry of Agriculture, fishing, and feeding. This study were carried out in 2006 at the animal experimental station of the "Institut National de la Recherche Agronomique" (INRA) at French West Indies (Guadeloupe, latitude 16°16′N, longitude 61°30′W).

Experimental design

Twenty adult Martinik rams weighing on average 55 (± 10 kg) were randomly dispatched into four groups according to two simultaneous 2 x 1 Latin Square designs. Treatments were two systems of feeding (indoor and at pasture) and two stages of regrowth (21 and 35 d of regrowth). In the first Latin Square, 2 groups were fed with a 21 d regrowth Pangola grass. One of 2 groups fed 21 d regrowth Pangola grass was conducted indoor, whereas the second was conducted at pasture. In the second Latin Square, the 2 other groups were fed with a 35 d regrowth Pangola grass. One of the 2 groups fed with 35 d regrowth Panola grass was conducted indoor, whereas the second were conducted at pasture. At the end of the first experimental period, groups fed with the same regrowth stage permuted between indoor and pasture within the same Latin Square. Each group were constituted of 5 rams including 2 fitted with rumen cannulae. Each experimental period lasted 26 d and consisted of 14 d adaptation to the diet, 5 d of intake and total tract digestibility measurements, and 7 d of rumen sampling on the rams fitted with rumen cannulea.

Pasture management

Two paddocks of a perennial Pangola grass pasture were divided each in 2 plots to have, during two successive 26-d periods, two 21-d and two 35-d Pangola regrowth stage plots. One of the 21-d plots were intended to be cut daily at 3 cm height to feed 5 Martinik rams indoor (I21), whereas the second were intended to be grazed daily during 24 h by 5 Martinik rams at pasture (P21). At the same time, one of the 35-d plots were intended to be cut daily at 3 cm height to feed 5 Martinik rams indoor (I35), whereas the second were intended to be grazed daily during 24 h by 5 Martinik rams at pasture (P35). The areas of plots I21, P21, I35, and P35 were 2400, 2600, 2500 and 2800 m² respectively and they were subdivided in 21, 22, 35 and 36 subplots, respectively for I21, P21, I35, and P35.

The first subplot of I21, P21, I35 and P35 has been cut 21, 22, 35 and 36-d, respectively, before the beginning of the experiment. One subplot has been mown daily, so that each subplot has one day more than the subplot mown the day before, and a day less than the subplot mown the following day. Consequently, the re-growth stage of the subplots intended to be harvest daily was exactly 21 days for I21 and P21 and 35 days for I35 and P35. After removal of grazing animals, the subplots were mown at 3 cm height to homogenize regrowth of grass. One kg/ha/regrowth age of mineral N fertiliser was applied on each subplot after mowing.

Animal and grazing management

Indoor, two groups of 5 rams were maintained in individual metabolism cages. Grass was cut daily at 0700 on I21 and I35 and chopped (5 cm) before being offered in two meals per day at 0800 and 1300. Animals received an amount of forage 1.1 times greater than animals voluntary intake measured during the 14-d of adaptation to the diet and they have free access to water.

At pasture, each group of 5 rams grazed daily on one subplot of P21 and P35. Each ram was tethered and had a defined circular area of pasture to graze within subplot. The rams were moved daily to fresh subplot at 0800 and had free access to water. To provide the same grass quality indoor and at pasture, the amount of forage allocated to grazing animals was calculated above the mowing height for indoor animals estimated at 3 cm during the 14 d adaptation period. The amount of forage allocated to grazing animals were determined by area of pasture available for each animal and thus by the length of the tether chain. The latter has been calculated from the herbage mass above 3 cm and the voluntary intake of the animals, estimated from weight of animals and a voluntary intake of 80 and 70 g/ kg BW0.75 estimated indoor from previous studies (Archimede et al., 2000; Assoumaya et al., 2007) at 21 and the 35 d of regrowth, respectively. The herbage mass above 3 cm were measured by weighing the amount of forage harvested to feed rams indoor, during the 14-d adaptation period. Moreover, to take into account the trampling of the sward by the animals, grazing animals received an amount of forage 20 % greater than the amount initially calculated.

Estimation of OMI and OMD

Indoor, in vivo OMI and OMD were measured per animal by weighing the daily amounts of food offered, refusals, and feces excreted (Cochran and Galyean, 1994). Two

sub-samples per regrowth stage of herbage offered (200 g) were harvested daily per ram. A sub-sample of forage refused (200 g) was collected daily per ram. The total amount of faeces excreted each day was gathered in individual bags. A representative sub-sample of feces was obtained by pooling for each animal 10% of the daily amount of faeces excreted. It was then stored at -20 ° C prior to DM content determination. Dry matter content of forage offered, refusal, and fecal samples were determined by drying samples at constant weight (for 72 h at 60°C) in a forced draught oven. Dried samples of forage, feces were then ground to 0.75 mm particle size prior to chemical analysis.

At pasture, the total amount of feces excreted was gathered, as indoor, in individual bags. Processing of fecal samples was performed as previously described for indoor animals. Organic matter digestibility was estimated using the CPf or the fecal NIRS equations of Fanchone et al, (2008) derived from a large data set (n = 174). Then, OMI was calculated from the OMD estimations and total fecal output (Streeter, 1969). The digestible OM intake (DOMI) was calculated by multiplying the estimations of OMI and OMD.

Characterisation of forage on offer

Indoor, chemical characterisation, i.e., OM, CP, NDF ADF and lignin contents, of forage offered was realised during all the measurement period. The total bulk density of forage offered was calculated by dividing the mean of the amount of grass offered in the two meals by the volume taken by the grass in the trough.

At pasture, the sward was characterised during 2 consecutive days in each measurement period within each of the circular areas intended to be grazed by rams. Sward height was measured with a rising-plate meter (Michell, 1982) at 5 sites per circular area. Extended lengths of ten random tillers per circular area were measured with a sliding ruler. Herbage mass was estimated at the same sites, by cutting the herbage under the plate over an area of 0.09 m², at ground level with hand-held electric clippers. Each of the 5 herbage samples cut was weighed fresh, and all of them were then pooled per circular area. A sub-sample of 200 g was kept to determine dry matter (DM) and were then ground to 0.75 particle size and conditioned prior to determination of chemical composition. The total herbage bulk density (kg OM / m3) before grazing was calculated by dividing the total herbage mass (kg OM / m²) by the mean height of the pasture (m).

Feeding Behaviour

The feeding behaviour was determined by visually observing the rams for 24 h, the third day of each measurement period. At night rams were observed with the aid of a flashlight. The observers recorded the current activity of each ram at 5-min intervals (Hodgson, 1982), categorized as eating (head down in the trough or on the pasture, searching for or biting herbage), ruminating or idling. Eating and ruminating indexes (min / g OMI) were defined as the times need to eat, or to ruminate per gram of forage, and were calculated by dividing the time spent eating (min) or ruminating (min), by the amount of forage eaten (g).

Rumen Characteristics

During the rumen sampling period, 100 ml of rumen liquid was collected on each ram fitted with rumen cannulea during two consecutive days at, 0, 3, 6, 12 h after the morning meal. pH were recorded immediately after the liquid collection, then samples were conserved at + 4 °C until ammonia determination. Then, two rumen empties (one per day) were carried out, 3 and 24-h after the meal. The times were chosen because the duration of the main morning meal of rams was 2.30 to 3 h. Moreover, previous observations (Archimede, unpublished data) have indicated, 1) that the time of 3 and 24-h represented the maximum and the minimum rumen filling respectively, 2) the mean of the two values is equivalent to the weighted mean of 3, 6, 12 and 24 h (Archimède, unpublished data). Rumen emptying was carried out manually on each animal fitted with rumen cannulea. The total content was weighed thoroughly mixed by hand and three sub-samples taken, two for dry matter (DM) determination and the third preserved (-20°C) until freeze-drying for chemical characterisation.

Laboratory analysis

Chemical characterisation (OM, CP, NDF, ADF, and lignin) of ground samples of forage offered, refusals and feces collected indoor and at pasture were carried out. Organic matter (OM) content of conditioned ground samples was measured after a 10 h ashing at 550°C. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were estimated following the method of (Van Soest et al., 1991). Nitrogen concentration was determined on forage and feces using the Dumas method (AOAC, 1990). Crude protein content of samples was calculated by multiplying the N

concentration by 6.25. The ammonia contents of rumen liquid were estimated by distillation. Organic matter, NDF, ADF, ADL and CP content of forage, refusals, and

Table 1 : Chemical composition of 21 and 35-d regrowth Digitaria decumbens grass offered to rams indoor or at pasture.

Items	21 d of regrowth		35 d of regrowth		SEM	Statistical analysis [a]
	Indoor	Pasture	Indoor	Pasture		
Allowance, kg OM / d	2.06 [w]	2.86 [x]	1.54 [y]	2.71 [x]	0.43	S*, W**
OM, %	89,96[w]	87.52 [w x]	89.06 [w]	86.42 [x]	1.92	W**
CP, %	11.91 [w]	11.28 [w]	11.93 [w]	11.29 [w]	1.05	NS
NDF, %	70.59 [w]	76.65 [x]	75.56 [x]	76.33 [x]	1.79	S**, W**, SxW**
ADF, %	35.68 [w]	38.77 [x]	38.26 [x]	39.09 [x]	1.22	S**, W**, SxW**
Lignin, %	6.41 [w]	8.98 [x]	7.48 [w]	8.84 [x]	1.18	S**
Total Bulk density, kg DM / m^3	12.31 [w]	2.90 [x]	9.25 [y]	2.40 [x]	1.61	W**
Total mass, t DM / ha	.	1.51 [w]	.	2.51 [x]	0.33	S**
Sward height, cm	.	5.78 [w]	.	10.82 [x]	1.36	S**
Tiller Length, cm	.	14.7 [w]	.	29.9 [x]	12.11	S*

[a] From an analysis of variance with a general linear model including the effects of regrowth stage (S), way of feeding (W) and interaction between regrowth stage and way of feeding (SxW). Statistical significance: ** $P < 0.01$, * $P < 0.05$, NS = Non significant.

[w,x,y] In a row, for each main effect, means without a common superscript letter differ ($P < 0.05$).

feces were expressed on laboratory DM basis, obtained by drying the corresponding samples during 24 h at 103°C. For recording of absorbance spectra of feces, approximately 2.5 g of ground, fecal samples were packed in ring-cup sample cells with a near infrared, transparent, quartz cover glass (FOSS, 2000). Cells were scanned 32 times using a scanning reflectance monocromator (NIRSystem 6500 Inc., Silver Springs, MD). Reflectance energy (Log [1/R], where R = Reflectance) was measured and averaged over the 32 scans. The average spectra of absorbance were recorded at 2 nm intervals over the wavelength range 700 to 1,100 and 1,100 to 2,500 nm.

Calculation and statistics

A total of 40 (10 rams per 2 periods per 2 Latin Square) observations were gathered for each measured variable, except for the ruminal characteristic, for which 16 observations were collected. The MIXED procedure of SAS (SAS Inst. Inc., Cary, NC,) was used for calculation to account for the fixed effect of stage of regrowth (21 vs 35 days), way of feeding (indoors vs pasture), period of measurements (1st vs 2nd period) and stage of re-growth X way of feeding interaction. The random effect of the rams within each Latin Square was also taken into account.

The relationships between OMI, OMD and OMDI and the forage characteristics were examined by calculating correlation coefficients using the COR procedure of SAS (SAS Inst. Inc., Cary, NC). The similarities between the spectra used to derive the fecal NIRS equation (Fanchone et al., 2008) and the spectra recorded in this study were evaluated using generalised Mahalanobis distance (Dardenne, 1990). A Mahalanobis distance lower than 3 showed that the equation is usable for prediction.

RESULTS

Allowance and herbage characteristics

Indoor, mean amount of forage offered to animals was 1.8 kg OM / d. The amount of forage offered at 21 d of regrowth was greater ($P < 0.05$) by 1.3 x that offered at 35 d of regrowth. The mean CP, NDF, ADF and lignin content of forage offered was 11.9, 73.1, 37.0, and 6.9 % whatever the stage of regrowth. The NDF and ADF contents following 35 d of regrowth were greater by 1.07, and 1.07 ($P < 0.05$) than that at 21 d of regrowth, whereas, the CP and lignin content did not differ significantly between the two regrowth

Table 2: Correlation between in vivo organic matter digestibility (OMD, %), in vivo organic matter intake (OMI, g/kg $BW^{0.75}$) and OMD (%) and OMI (g/kg $BW^{0.75}$) predicted using the fecal crude protein (CPf) method, and OMD (%) and OMI (g/kg $BW^{0.75}$) predicted using the fecal near infrared reflectance spectroscopy (fecal NIRS) method.

Item	OMD_{CPf}	OMI_{CPf}	OMD_{NIRS}	OMI_{NIRS}
In vivo OMD	0.489	0.032	0.183	0.188
	0.046	0.902	0.481	0.469
In vivo OMI	0.685	0.769	0.615	0.478
	0.034	0.0003	0.002	0.052

Table 3 : Total fecal output, organic matter digestibility (OMD), organic matter intake (OMI) and digestible organic matter intake (DOMI) estimated for Indoor or grazing animals by fecal crude protein (CPf).

Item	21		35			
	Indoor	Pasture	Indoor	Pasture	SEM	Statistical analysis [a]
Fecal output, g OM / kg $LW^{0.75}$	26.15 [w]	23.05 [x]	24.69 [wx]	20.16 [y]	2.82	S*, W**
OMD_{CPf}, %	69.93 [w]	71.25 [x]	68.05 [y]	69.93 [w]	1.00	S**, W**
OMI_{CPf} g/kg $LW^{0.75}$	78.41 [w]	71.66 [w]	69.24 [wx]	58.78 [x]	9.97	S**, W*
$DOMI_{CPf}$ g/kg $LW^{0.75}$	54.88 [w]	51.09 [w]	47.18 [x]	41.05 [x]	7.27	S**, W*

[a] From an analysis of variance with a general linear model including the effects of regrowth stage (S), way of feeding (W) and interaction. Statistical significance: ** $P < 0.01$, * $P < 0.05$, NS = Non significant.

[w,x,y] In a row, for each main effect, means without a common superscript letter differ ($P < 0.05$).

stages (Table 1). The mean total bulk density was 10.8 kg DM / m3 and was greater ($P < 0.001$) by 1.3 at 21 d of regrowth than at 35 d of regrowth.

At pasture, the mean amount of forage offered was 2.8 kg OM / d and was on average 1.5 x that offered indoor. The amount of forage offered at 21 d of regrowth did not differ significantly ($P = 0.44$) to that offered at 35 d of regrowth. The mean CP, NDF, ADF, and lignin content of the grass offered at pasture was 11.3, 76.5, 38.9, and 8.9, and was 0.95, 1.05, 1.05, and 1.29 x that registered indoor. The CP, NDF, ADF, and lignin contents of the grass after 21 d of regrowth were not significantly different ($P = 0.99$) to that offered at 35 d of regrowth. The mean total bulk density was 2.65 kg DM / m3 and was 4.1 x lower ($P < 0.001$) that offered indoor. Total bulk density at 21 d was not significantly different to that at 35 d of regrowth. The total herbage mass, the sward height and the tiller length increased ($P < 0.05$) by 1.7, 1.9, and 2.0 respectively, from the 21-d sward to the 35-d sward.

Comparison of methods to estimate in vivo OMD, and OMI

In vivo OMD (64.9 %) was overestimated ($P > 0.05$) by both the CPf (68.9 %) and the fecal NIRS (75.2 %) equations of Fanchone et al. (2008). Greater correlations were found between in vivo OMD and OMD predicted using the CPf methods (OMDCPf) than between in vivo OMD and OMD predicted by the fecal NIRS methods (OMD_{NIRS}, and Table 2). At the same time, the range of variation of CPf in the large data set used by Fanchone et al. (2008) to derive the CPf equation (from 7.9 to 20.3 % OM) include all the CPf variation encountered in this study, for both indoor (from 12.6 to 16.8 % OM) and grazing animals (from 13.8 to 18.4 % OM). The average Mahalanobis distance measured between the spectra recorded in this study and those used to derive the fecal NIRS equation, were 11.6. The estimate of in vivo OMI from total fecal collection using OMD_{CPf} ($r = 0.77$ and OMI_{CPf} = 73.8 g / kg BW0.75) were better correlated ($P > 0.05$) to in vivo OMI (73.7 g / kg BW0.75) than using OMD_{NIRS} ($r = 0.48$, OMI_{NIRS} = 110.8, and Table 2). Thus, OMD_{CPf} and OMI_{CPf} were retained to compare nutrition between indoor and grazing animals.

Nutrition Indoor and at Pasture

Indoor, mean OMD_{CPf} and OMI_{CPf} values of 68.9 % and 73.8 g/kg BW0.75 were calculated. From 21 to 35 d of regrowth, OMD_{CPf} and OMI_{CPf} decreased by 0.14 digestibility unit / d of regrowth and 0.65 g/kg $BW^{0.75}$/d of regrowth, respectively.

Table 4: Feeding behaviour of rams fed indoor and at pasture 21, and 35 d regrowth Digitaria decumbens

Item	21		35			
	Indoor	Pasture	Indoor	Pasture	SEM	Statistical analysis [a]
Eating time, min	512.13 [w]	491.87 [w]	337.46 [x]	411.98 [x]	77.7	S**
Ruminating time, min	486.84 [w]	393.71 [x]	553.39 [w]	437.71 [y]	65.4	S*, W**
Idling time, min	441.02 [w]	554.41 [w]	549.14 [w]	590.30 [w]	114.1	NS
Eating index, min/g OMI	0.368 [wx]	0.369 [w]	0.282 [x]	0.410 [w]	0.078	W*, SxW*
Intake rate, g OMI/ min	2.72 [wx]	2.71 [w]	3.56 [x]	2.44 [w]	0.530	W*, SxW*
Ruminating index, min/g OMI	0.739 [wx]	0.668 [x]	0.745 [wx]	0.842 [w]	0.087	S**

[a] From an analysis of variance with a general linear model including the effects of regrowth stage (S), way of feeding (W) and interaction between regrowth stage and way of feeding (SxW).. Statistical significance: ** $P < 0.01$, * $P < 0.05$, NS = Non significant.

[w,x,y] In a row, for each main effect, means without a common superscript letter differ ($P < 0.05$).

Mean resulting $DOMI_{CPf}$ was 51.0 g/kg BW0.75. At 21 d of regrowth, $DOMI_{CPf}$ was 1.16 x that at 35 d of regrowth.

At pasture, the mean OMD_{CPf} value was 70.5 % and was 1.02 higher ($P < 0.05$) on average compared to the mean value estimated indoor (Table 3). From 21 to 35 d of regrowth, OMD_{CPf} decreased daily ($P < 0.05$) by approximately 0.09 digestibility unit / d. Such daily decrease in OMD_{CPf} was 0.64 x that registered indoor. The mean calculated OMI_{CPf} value was 65.2 g/kg BW0.75, and was 0.88 x that registered indoor. From 21 to 35 d of regrowth, OMI_{CPf} decreased daily ($P < 0.05$) by approximately 0.92 g/kg BW0.75. Such decrease in OMI of grazing animals was 1.4 x that registered indoor. Mean resulting DOMI was 46.1 and was lower ($P < 0.05$) by 0.9 that registered indoor. Digestible OM intake at 21 d of regrowth was 1.2 x that at 35 d of regrowth.

Animal Feeding Behaviour

Indoor, mean eating time was 425 min (Table 4). Eating time at 21 of regrowth was 1.52 greater ($P < 0.05$) than that at 35 d of regrowth. Mean ruminating time was 520 min. Ruminating time at 21 d of regrowth did not differ significantly to ($P = 0.11$) of that at 35 d of regrowth. Mean idling time was 495 min. Idling time at 21 d of regrowth did not differ significantly to ($P = 0.14$) that at 35 d of regrowth. Mean eating index was 0.325 min / g OMI and did not differ significantly ($P = 0.09$) between the two stages of regrowth. Mean ruminating index was 0.742. Ruminating index at 21 d of regrowth did not differ significantly ($P = 0.10$) to that at 35 d of regrowth. The mean intake rate was 3.13 g OMI / min. The intake rate at 21 d of regrowth did not differ significantly from that at 35 d of regrowth.

At pasture, mean eating time was 452 min and was 1.06 x that measured indoor. Eating time at 21 d of regrowth was 1.19 greater ($P < 0.05$) that registered at 35 d of regrowth and did not differ significantly ($P = 0.68$) to that registered indoor, at 21 d of regrowth. Eating time at 35 d of regrowth was 1.2 lower ($P < 0.05$) than that registered indoor, at the same stage of regrowth. Mean ruminating time was 415.7 min and was lower (by 1.2) than that registered indoor ($P < 0.001$). Ruminating time at 21 d of regrowth accounted for 0.9 and 0.8 that at 35 d of regrowth and that registered indoor at 21 d of regrowth, respectively (Table 4). Ruminating time at 35 d of regrowth accounted for 0.8 that registered indoor at the same regrowth stage. Mean idling time was 572.4 min and 1.16 that registered indoor. Idling time at 21 d of regrowth did not differ significantly ($P = 0.51$) to that at 35 d of regrowth. Mean eating index was 0.389 and was 1.2 x that

Table 5: Ruminal ammonia and ruminal pH 0, 3, 6, 12 after the morning meal and Rumen fill 3 and 24 h after the morning meal, lignin passage rate of rams fed indoor and at pasture 21, and 35 d regrowth Digitaria decumbens

Item	21		35			
	Indoor	Pasture	Indoor	Pasture	SEM	Statistical analysis [a]
Rumen pH						
0 h	6.76[w]	6.85[w]	6.70[w]	6.72[w]	0.211	NS
3 h	6.41[w]	6.56[w]	6.46[w]	6.41[w]	0.157	NS
6 h	6.37[w]	6.15[w]	6.31[w]	6.34[w]	0.210	NS
12 h	5.74[w]	5.70[w]	5.93[w]	5.90[w]	0.230	NS
0 to 12 h	6.32[w]	6.32[w]	6.35[w]	6.34[w]	0.121	NS
Rumen ammonia						
0 h	181.4[w]	209.7[w]	182.8[w]	167.6[w]	32.23	NS
3 h	206.9[w]	225.3[w]	174.2[w]	196.1[w]	34.41	NS
6 h	187.7[w]	183.9[w]	136.3[w]	176.02[w]	50.09	NS
12 h	164.9[w]	181.7[w]	143.4[w]	174.7[w]	46.34	NS
0 to 12 h	185.2[w]	200.2[w]	159.2[w]	178.6[w]	36.37	NS
Rumen fill						
DM 3 h, g DM	1464.4[w]	1284.9[w]	1346.9[w]	1488.8[w]	255.9	NS
DM 24 h, g DM	1069.9[w]	916.1[w]	1097.8[w]	937.6[w]	174.1	NS
Mean DM, g	1267.1[w]	1100.5[w]	1222.3[w]	1213.2[w]	189.1	NS
Lignin 3 h, g	194.5[w]	213.7[w]	238.0[w]	291.5[w]	52.02	NS
Lignin 24 h, g	138.6[wx]	126.7[w]	180.3[x]	177.5[wx]	37.25	S P<0.03
Mean Lignin, g	168.7[w]	170.9[w]	209.3[w]	234.4[w]	38.7	S P<0.03
Lignin passage rate, % / h	0.061[w]	0.054[wx]	0.037[wx]	0.024[x]	0.018	S P<0.04

[a] From an analysis of variance with a general linear model including the effects of regrowth stage (S), way of feeding (W) and interaction between regrowth stage and way of feeding (SxW).. Statistical significance: ** $P < 0.01$, * $P < 0.05$, NS = Non significant.

[w,x,y] In a row, for each main effect, means without a common superscript letter differ ($P < 0.05$).

registered indoor ($P < 0.05$). Eating index at 21 d of regrowth did not differ significantly ($P = 0.26$) to that at 35 d of regrowth. Mean ruminating index was 0.755 and did not differ significantly to that registered indoor ($P = 0.14$). Ruminating index at 21 d of regrowth were 1.26 lower ($P < 0.05$) than that at 35 d of regrowth. The mean intake rate was 2.57 g OMI / min. The intake rate at 21 d of regrowth did not differ significantly neither to that at 35 d of regrowth at pasture, nor to that at 21 d of regrowth indoor. Intake rate at 35 d of regrowth at pasture was 1.46 lower ($P < 0.001$) than that registered indoor at the same stage of regrowth.

Rumen characteristics

Indoor, the mean ammonia concentration in the rumen was 172.2 mg / l. The mean rumen pH was 6.3 and the mean amount of DM in the rumen was 1244.7 g DM (Table 5). No significant differences were found between the ammonia concentration, rumen pH, and amount of DM in the rumen, at 21 and 35 d of regrowth, respectively (Table 5). Mean lignin in the rumen was 189 g. The amount of lignin in the rumen at 21 d of regrowth did not differ significantly ($P = 0.25$) to that at 35 d of regrowth. The mean lignin passage rate was 0.049 % / h. The lignin passage rate at 21 d of regrowth was 1.65 x that at 35 d of regrowth.

At pasture, the mean ammonia concentration in the rumen was 189.4 mg/l. Although no significant difference mean ammonia in the rumen was found between indoor and pasture ($P = 0.42$) and it was 10 % numerically greater at pasture compared to that registered indoor. At 21 d of regrowth, the mean rumen ammonia measured was numerically greater (1.12 x) than that at 35 d of regrowth. Rumen pH values were 6.3 and did not differ significantly that measured indoor. The mean amount of DM in the rumen was 1156.8 g DM and was 1.07 lower than that measured indoor. No significant differences were found between the mean amount of DM in the rumen neither between 21 and 35 d of regrwoth at pasture, nor between animals fed indoor or at pasture. Mean lignin in the rumen was 202.6 g. The amount of lignin in the rumen at 21 d of regrowth did not differ significantly ($P = 0.49$) to that at 35 d of regrowth. The mean lignin passage rate was 0.039 % / h and did not differ significantly ($P = 0.49$) to that that measured indoor. The lignin passage rate at 21 d of regrowth was 1.65 lower ($P < 0.05$) than that at 35 d of regrowth.

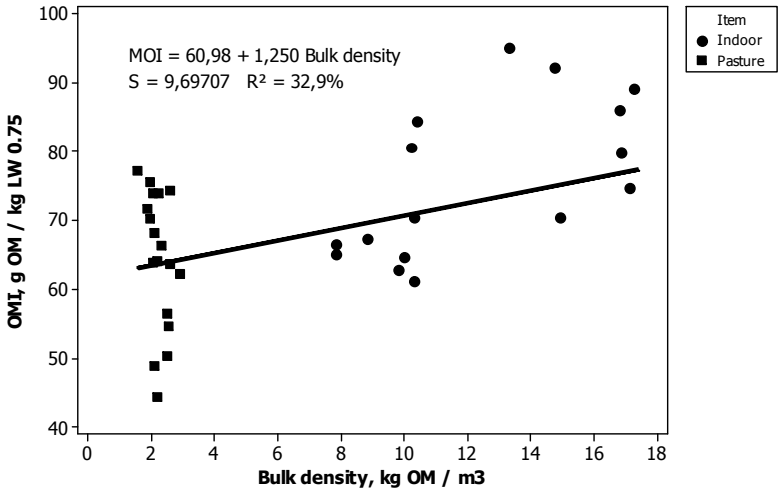

Figure 1: Relationship between OM intake (OMI, g OM / kg $LW^{0.75}$) and bulk density (kg OM /m^3) of rams fed with *Digitaria decumbens* indoor and at pasture

DISCUSSION

Allowance and characteristics of Pangola grass

The Pangola grass offered to rams indoor and at pasture were similar to other reports (Archimede et al., 2000; Assoumaya et al., 2007), having a CP content around 11.6 and a fibre content around 74.8, 37.9, and 7.9 for NDF, ADF, and lignin respectively. We measured, indoor, a slightly better grass quality, i.e., greater CP content coupled with lower fibre content compared to that measured at pasture. These differences may be related to the way of sampling grass between the two environments. Indoor, grass was sampled after cutting at 3 cm high. At pasture, grass was sampled at ground level with hand-held electric clippers. These three cm of differences were constituted mainly by stems, senescent and dead materials which have been proven to have lower quality i.e., lower CP, and greater fibre content, than green leafs (Minson, 1990; Moreira et al., 2004). The increase of the fibre and lignin contents of the grass offered as well as the decrease in CP content with the stage of regrowth, registered in this experiment are classical results previously observed indoor (Chenost, 1975; Aumont et al., 1995), and at pasture (Boval et al., 2007a). The ratio between herbage allowance at pasture and indoor was 1.5 and was greater than the 1.3 expected due to greater pasture productivity.

Estimation of OMD and OMI

We evaluated two methods to estimate in vivo OMD, i.e., CPf and fecal NIRS. Among these latter, the CPf method provided greater correlation between in vivo and predicted values of OMD and OMI and this method was thus retained to compare nutrition of indoor and grazing animals (Table 2). This greater correlation between OMD_{CPf} and in vivo OMD can be explained by the similarity in the range of variation of CPf in the data set used to derive the equation and that in this study, for both indoor and grazing animals. Moreover, it has been shown previously that this method may provide consistent estimates of in vivo OMD at pasture (Boval et al., 2003; Lukas et al., 2005; Schlecht and Susenbeth, 2006; Fanchone et al., 2008).

In contrast, the fecal NIRS method allowed a less accurate estimation of in vivo OMD and OMI with lower correlation (Table 2). However, this method gave accurate prediction of in vivo OMD in other situations (Krachounov and Kirilov, 2000; Boval et al., 2004; Fanchone et al., 2008). This worst correlation between in vivo OMD and OMD_{NIRS}, can be explained by the 11.6 average Mahalanobis distance measured between the spectra used to derive the fecal

NIRS equation (Fanchone et al,. 2008) and those registered in this study. Over the threshold of 3 of average Mahalanobis distance, prediction is not anymore possible (Dardenne, 1990). This method cannot be used outside of the spectral properties of the reference data used to develop the equation. Such results showed how the set of reference data used to derive the fecal NIRS equation need to be further expanded to be usable in all grazing situations (Fanchone et al., 2008).

Comparison of Nutrition Indoor and at Pasture

This study point up that, in our context, difference exists between nutrition indoor and at pasture. We measured at pasture on average 2.4 % greater OMD coupled with 13.7 % lower OMI and 11.4 % lower DOMI compared to that indoor.

The greater OMD measured at pasture may be related to the ability of animals to graze selectively, as reported by many authors (Van Soest, 1996). Indeed, in this study, contrary to indoor, OMD at pasture was not correlated with herbage characteristics (Table 4). That suggests that the herbage consumed at pasture was most likely different from that offered, because of the selective grazing of the rams. At pasture, the ruminating index was shorter, suggesting the consumption of a less fibrous, thus, less resistant to chewing forage, than that consumed indoor. At the same time, the ruminal concentration of ammonia, indicator of amount of nitrogen fermented in the rumen (Jarrige, 1980), was numerically greater at pasture compared to indoor, implying that a better forage has been consumed at pasture. Indoor, the ability of rams to select a better quality ration was restricted by offering a limited amount (1.1 animal voluntary intake) of a 5 cm length chopped, as it is a classical practice in indoor feeding experiments (Chenost and Demarquilly, 1982). It is probable that in other conditions i.e., with non chopped forage, or with other herbage allowance indoor or at pasture, one would have had other OMI and OMD values, although the same forage was on offer.

OMI was greater indoor compared to at pasture, contrary to OMD. The selective grazing animals implement at pasture responsible of greater OMD induce an increase of time for searching to the detriment of that for prehension (Hutchings and Gordon, 2001). Time for searching is difficult to assess but can greatly vary from 0.005 to 0.015 min / bite, respectively, for a low to a high quality sward (Parsons et al., 1994a; Woodward, 1997). At pasture, a greater eating index, on average 45 % longer was found, compared to indoor. Although ingestion of more fibrous and indigestible materials could increase eating index, we have demonstrated in the precedent lines that materials ingested at pasture had greater quality

compared with that ingested indoor. At pasture, the greater eating index and consequently, the lower intake rate was surely due to the time for searching at pasture, and to the greater bulk density indoor compared to at pasture. Hence, the amount taken per bite was surely different between indoor and pasture, inducing a weaker intake at pasture. Indoor, measurements of the characteristics of bite is difficult, the rams having always their head plunged in their trough. At pasture, it has been shown that bite mass is sensitive to variations in several characteristics of the grazed sward, such as the height, the herbage and leaf bulk density, or the total herbage mass (Hodgson et al., 1994; Cosgrove, 1997; Burns and Sollenberger, 2002). Among these characteristics, only the density of forage could be measured in the two feeding systems. Total bulk density largely (3.6 x) greater indoor compared to at pasture would not be a limiting factor of intake indoor, whereas, it was proved to affect bite mass at pasture. Thus, the difference in the forage density between the two systems of feeding (mown and packed in the trough, and up in situ at pasture), coupled to a reduced time devoted strictly for prehension, may explain the fall in OMI at pasture compared to indoor (Figure 1).

The divergent evolution of OMD and OMI between the two feeding systems illustrate well the concept of trade-off between diet quality and forage intake, deferred by various authors studying the ingestive behaviour at pasture (Parsons et al., 1994b; Thornley et al., 1994; Shipley et al., 1999; Wilson and Kerley, 2003): when grazing selectively, search time increase substantially and may come to limit intake rate, in addition to lower bite mass at pasture linked to lower bulk density. In these studies, intake rate was estimated on short times, and neither intake over the day, nor digestibility were measured. Thus the implication of such a trade-off on the nutrition at pasture is little known. Indeed, Chapman et al. (2007) for temperate pastures such as perennial ray-grass and white clover mixture, underline the little of experiments to explain the nutritional base of the diet selection. That is even truer with tropical pastures. This nutritional approach is essential because various feeding strategies can lead to similar digestible intake or not. In fact, at 21 d of regrowth the combination of a greater digestibility and a lower intake at pasture compared to indoor, did not induce a significant difference of DOMI (Table 3 and $P = 0.27$) between the two feeding systems. In contrast, at 35 days greater digestibility combined with a weaker intake at pasture, induced a 14.5 % weaker ($P < 0.005$) DOMI at pasture compared to indoor. Such differences between indoor and pasture may vary according to the characteristics of the herbage on offer and the diet selection that the rams implemented there.

This greater difference in DOMI between the two systems of feeding at 35 d can be explained by a larger variation in both OMD and OMI. From the 35 d to the 21 d swards,

OMD decreased both indoor and at pasture, but in a greater extent indoor. This decrease in OMD indoor, was related to the evolution of the chemical composition of the grass offered, i.e. mainly the increase in fibre content and lignin contents of the herbage consumed to which OMD was indeed significantly related. Such decrease in OMD were already reported for sheep fed indoor with tropical fresh grass increasingly old (Archimède et al., 2000; Assoumaya et al., 2007) and were also related to the evolution of the physicochemical structure of the grass.

As OMD, OMI also decreased between the two stages of regrowth, both indoor and at pasture. But the decrease in OMI between the 21 and the 35 d forage, was greater at pasture (13 g OM / kg BW0.75) compared to indoor (9 g OM / kg BW 0.75). At pasture, such decrease in OMI when the stage of regrowth of the grass was not consistent other reports (Hitchcock et al., 1990; Boval et al., 2007a). Slow fluctuation of bulk density was observed (Table 4 and P = 0.33). Moreover, whereas the sward height and the tiller length clearly varied from 21 to 35 d of regrowth, they were not correlated with intake. However, other parameters make suppose that the animals could not reach their level of feeding at pasture, at 35 d. Indeed, the smaller amount of fecal output and faecal lignin measured at 35 d of re-growth at pasture (20.16 and 170.4 g), were definitely weaker compared to the rams fed indoor with the same forage (24.7 g MOND/d and 209 g). By another way, the particle passage rate measured at pasture, at 35 d of regrowth, represented 0.76 of that measured with the same forage indoor (Table 2). That is coherent with the weaker intake at pasture according to the relation between particles passage rate and feed intake highlighted by Offer and Dixon (2000). At the same time, the better quality of the diet at pasture, as we showed, less fibrous and less resistant to chewing, should increase the particle passage rate. It is probable thus that a leafier fraction in the rumen induced phenomena of buoyancy and entanglement of the particles (Kennedy, 1995). That could limit the rate of passage of particles, increasing by this fact the time of reduction of the large particles in small particles.

Low differences were obtained in OMD in favour of pasture. Whereas, greater differences were achieved in OMI and DOMI in favour of animals fed indoor. Such gain in DOMI may have significant effect on production. To our knowledge, there exist little data of intake and digestibility for a similar diet measured simultaneously indoor and at pasture, with which to compare our results. As shown in our study, the difference between indoor and grazing animals may vary: no significant difference in DOMI at 21 d whereas a significant difference was found at 35 d of regrowth. Therefore, other situations have to be test, with other forage

offered, and using other management practice, to better understand the differences between this two feeding systems.

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1.2. Principales conclusions de l'étude expérimentale 1.

Cet essai a montré que des différences d'alimentation existent entre l'auge et le pâturage et que ces différences varient en fonction de la qualité du fourrage offert aux animaux. Ainsi, une digestibilité plus élevée a été mesurée au pâturage, alors que l'ingestion et la quantité ingérée digestible ont été plus importantes à l'auge. La plus forte digestibilité mesurée au pâturage serait liée à la capacité des animaux à sélectionner un meilleur fourrage dans ce milieu. La plus grande ingestion mesurée à l'auge serait liée à la plus grande densité du fourrage offert qui influerait la taille des bouchées. Les variations de la quantité ingérée digestible seraient davantage liées à des variations d'ingestion qu'à des variations de digestibilité. L'encombrement ne serait pas le premier facteur limitant au pâturage dans cet essai car les animaux n'ont pas atteint leur capacité d'ingestion dans ce milieu.

COMPARAISON DE L'INGESTION ET DE LA DIGESTION DE MOUTONS ALIMENTES A BASE DE DIGITARIA DECUMBENS A L'AUGE ET AU PATURAGE, A DEUX NIVEAUX DE PROPOSES.

2. *Comparaison de l'ingestion et de la digestion de moutons alimentés à base de Digitaria decumbens* à l'auge et au pâturage, à deux niveaux de proposés.

2.1. Introduction à l'étude expérimentale 2.

Intake and digestibility of Digitaria decumbens fed by sheep indoors or at pasture, at two herbage allowances.

Fanchone, A., Archimede, H., Baumont, R., and M. Boval

Dans l'essai 1, des différences d'ingestion, de digestibilité et de quantités ingérées digestibles ont été mises en évidence entre l'auge et le pâturage lorsque le même fourrage est offert. De plus, ces différences ont varié avec la qualité du fourrage offert. L'objectif de l'essai 2 était de tester ces différences d'alimentation entre l'auge et le pâturage à différentes quantités de fourrage proposé.

Nutrition of indoor and grazing animals

Intake and digestibility of fresh grass fed to sheep indoors or at pasture, at two herbage allowances[14]

A. Fanchone,* H. Archimede,* R. Baumont,† and M. Boval*[15]

ABSTRACT

The effect of the feeding system (indoors and at pasture) on intake and digestion of fresh 28 d regrowth *Digitaria decumbens* grass was studied at 2 herbage allowances. Sixteen adult Martinik rams weighing on average 52.4 (± 0.25 kg) were randomly assigned into 4 groups according to a 4 x 4 Latin Square design. Treatments were 2 systems of feeding (indoors and at pasture) and 2 herbage allowances, i.e., low herbage allowance (1.3 x animal voluntary intake), and high herbage allowance (1.5 x the low level). Two groups (8 rams) were fed indoors while the other 2 (8 rams) were fed at pasture. Of the 2 indoors groups, 1 was fed at low herbage allowance, and the other at high herbage allowance. Likewise, in the 2 grazing groups 1 group was fed at low herbage allowance, and the other at high herbage allowance. In vivo organic matter digestibility (OMD) was measured indoors from in vivo organic matter intake (OMI) and the total amount of feces excreted. In addition, OMD was estimated indoors and at pasture using the fecal CP (CPf) method (OMD_{CPf}). Then, OMI was estimated from fecal OM output and OMD estimated using the CPf method (OMI_{CPf}). Indoors, the CPf method gave precise estimates of in vivo OMD (r = 0.61 and $P < 0.001$) and OM intake (r = 0.79 and $P < 0.001$). At pasture, OMD_{CPf} was 2.8% greater compared to indoors, whereas, OMI_{CPf} and digestible OM intake estimated from OMD_{CPf} and OMI_{CPf} were greater by 23.8% and 20% respectively indoors compared to at pasture. Indoors, OMD_{CPf} increased from the low to the high herbage allowance, whereas it did not vary significantly at pasture. At the same time, indoors, OMI_{CPf} increased by 8.5 g/kg $BW^{0.75}$ from the low to the high herbage allowance, whereas it did not differ significantly at pasture. The greater ammonia concentration in the rumen illustrates the greater selective behavior of the rams and explained this difference in OMD_{CPf}, whereas, the difference in OMI_{CPf} was linked to differences in prehensibility of forage between the 2 feeding systems. This study shows that differences in

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OMD_{CPf} and OMI_{CPf} exist between animals fed indoors and at pasture with the same forage, and that these differences may vary according to the quantity of grass on offer, mainly due to variations indoors.

Key words: digestibility, grazing, herbage allowance, intake, sheep, stall

INTRODUCTION

Some studies suggest that differences in nutrition may occur between animals fed indoors and at pasture. For example, studies carried out indoors show a positive relationship between intake and digestibility (Minson, 1990; Ketelaars and Tolkamp, 1992; Archimede et al., 2000), whereas other studies have reported the opposite at pasture (Hitchcock et al., 1990; Van Soest, 1996; Boval et al., 2007). To our knowledge, studies that have attempted to compare nutrition indoors and at pasture (Keane and Allen, 1998; Zervas et al., 1999; Moniruzzaman et al., 2002; Misra et al., 2006; Raghuvansi et al., 2007), mainly compared production systems with high proportions of supplement in the diet indoors, whereas, animals at pasture did not receive anything other than the grazed grass. In addition, comparisons were seldom based on the same forage. Fanchone et al. (2008a) showed that feeding differences exist between animals fed indoors and at pasture when the same forage is on offer. They also showed that these differences may vary according to variation in the quality of the grass. At the same time, several studies have shown that herbage allowance influences intake indoors (Zemmelink, 1980; Mbwile and Uden, 1997) and at pasture (Combellas and Hodgson, 1979; Baker et al., 1981; Ribeiro Filho et al., 2005).

The aim of this study was to demonstrate and to explain the differences in OM intake (OMI) and OM digestibility (OMD) between animals fed with the same forage indoors and at pasture. This comparison used 2 different herbage allowances, to test its consistency in various situations.

MATERIAL AND METHODS

Care and use of animals were carried out according to the Certificate of Authorization to Experiment on Living Animals issued by the French Ministry of Agriculture, fishing, and feeding. This study was carried out in 2007 at the animal experimental station of the "Institut National de la Recherche Agronomique" (INRA) in the French West Indies (Guadeloupe, latitude 16°16′N, longitude 61°30′W).

Experimental Design

Sixteen adult Martinik rams (52.4 ± 0.25 kg) were randomly allocated into 4 groups according to a 4 x 4 Latin Square designs. Each group consisted of 4 rams, including 2 fitted with a rumen cannula, and was fed with a 28-d regrowth Pangola (*Digitaria decumbens*) grass diet. Treatments were 2 systems of feeding (indoors and at pasture) and 2 herbage allowances i.e., low herbage allowance (1.3 x animal voluntary intake), and high herbage allowance (1.5 x the low level). During each experimental period, 2 groups were fed indoors, whereas, the other 2 were fed at pasture. One of the 2 indoors groups was fed at low herbage allowance, whereas, the second was fed at high herbage allowance. At the same time, 1 of the 2 grazing groups was fed at low herbage allowance, whereas, the second was fed at high herbage allowance. Each experimental period lasted 28 d and consisted of 14 d adaptation to the diet, 5 d of OMI and OMD measurements, and 9 d of rumen sampling from the rams fitted with rumen cannula.

Pasture Management

One paddock of a perennial Pangola grass pasture was divided into 3 plots to give, during 4 successive 28 d periods, three 28 d Pangola regrowth stage plots. The first of the 3 plots (I) measuring 5,600 m², was subdivided into 28 subplots to be cut daily to feed 8 Martinik rams indoors at low (4 rams) and high (4 rams) herbage allowance. At the same time, the second subplot measuring 3,800 m² was subdivided into 29 subplots for 24 h grazing by 4 different Martinik rams at low herbage allowance (PL). The third of the 3 plots measured 5,800 m² and was subdivided into 29 subplots for 24 h grazing by 4 different Martinik rams at high herbage allowance (PH). The first subplot of I, PL, and PH was cut 28, 29, and 29 d before the beginning of the experiment. One subplot was cut each d, so that each subplot had 1 d more regrowth than the subplot cut the d before and a d less than the one cut the d after. Consequently, the regrowth stage of the subplots intended to be cut each d on plot I and to be grazed each d on plots PL and PH was exactly 28 d. After removal of animals, the grazed subplots were cut to homogenize regrowth of grass with a mowing machine (BCS S.p.A., Milan, Italy) set at a mowing height of 3 cm. One kg/(ha.d of regrowth) of mineral N fertilizer was applied on each subplot after mowing.

Animal Management

Indoors, 2 groups of 4 rams were maintained in individual metabolism cages. Grass was cut daily 3 cm above the ground with the same mowing machine described above (BCS

S.p.A., Milan, Italy) at 0700 h on 1 subplot of I. The forage was collected and chopped into 5 cm lengths using an electric chopper (DESSERTINE-HUPIN S.A., Buxieres les Mines, France) before being offered to animals in 2 meals per d at 0800 h and 1300 h. Animals at low herbage allowance received an amount of forage 1.3 times greater than their voluntary intake, whereas, animals at high herbage allowance received an amount of forage 1.5 times greater than animals at low herbage allowance. Voluntary intake of animals fed indoors was measured during the 14-d of adaptation to the diet. Animals had free access to water at all times.

At pasture, each group of 4 rams grazed daily on 1 subplot of PL and PH. Each ram was tethered and had a defined circular area of pasture to graze within the subplot. The rams were moved to a fresh subplot each d at 0800 h and had free access to water. Whatever the level of herbage allowance, grazing animals received 30% more forage than animals fed indoors, to take into account trampling of the sward by the animals. To provide the same grass quality indoors and at pasture, the amount of forage allocated to grazing animals was based on that above 3 cm from the ground. The herbage mass above 3 cm was measured by weighing the amount of forage harvested to feed rams indoors during the 14-d adaptation period.

Estimation of OMI and OMD

Indoors, in vivo OMD was measured per animal from in vivo OMI and the total amount of feces excreted. In vivo OMI per animal was measured on d 15 to 19 of each measurement period by weighing the amounts of forage offered daily, and the refusals. One sub-sample (200 g) of herbage offered and herbage refused was collected daily per ram. The DM content of fresh forage and refusals was determined daily by drying for 72 h at 60 °C (Cochran and Galyean, 1994). The total amount of feces excreted was gathered daily per ram in individual bags on d 17 to 21 to help account of passage rate. A representative sub-sample of feces was obtained by pooling 10% of the daily amount of feces excreted per animal. Sub-samples of feces were stored at –20 °C until DM content determination. The DM content of fecal sub-samples was determined in similar conditions as described for fresh forage and refusals. Dried samples of forage and feces were then ground to a 0.75 mm particle size using a cross beater mill SK 100 (Retsch, Hann, Germany). Ground samples were chemically analyzed (determination of OM, CP, NDF, ADF, and ADL content). In addition to the measurement in vivo, OMD was estimated from fecal CP content (OMD_{CPf}), using the equation of Fanchone et al. (2008b; n = 174). Afterwards, OMI was estimated from OMD_{CPf} (OMI_{CPf}) and total fecal output, according to the method of Streeter (1969). The digestible

OM intake (DOMI) estimated using the CPf method ($DOMI_{CPf}$) was calculated by multiplying OMD_{CPf} and OMI_{CPf}. In vivo OMD and OMI were then compared to values estimated using the CPf (OMD_{CPf} and OMI_{CPf}).

At pasture, individual collection of the total amount of feces excreted and processing of fecal samples were performed as previously described for indoors animals. As indoors, OMD_{CPf} was estimated from ground fecal samples using the CPf equation of Fanchone et al. (2008b; n = 174). Then, OMI_{CPf} was calculated from OMD_{CPf} and total fecal output (Streeter, 1969). Subsequently, $DOMI_{CPf}$ was calculated by multiplying OMD_{CPf} and OMI_{CPf}. Then, OMD_{CPf}, OMI_{CPf}, and $DOMI_{NIRS}$ were used to compare nutrition indoors and at pasture.

Characterization of Forage on Offer

Chemical characterization (i.e., determination of OM, CP, NDF ADF, and ADL content) of forage offered indoors was performed on d 15 to 28. The total bulk density of forage offered was calculated by dividing the mean of the amount of grass offered in the meals by the volume taken up by the grass in the trough.

At pasture, the sward was characterized on d 16 and 17 of each measurement period within each of the circular areas intended to be grazed by each ram. Sward height was measured with a rising-plate meter (Michell, 1982) at 5 sites per circular area. Extended lengths of ten random tillers per circular area were measured with a sliding ruler. Herbage mass was estimated at the same sites, by cutting the herbage under the plate over an area of 0.09 m², at ground level with hand-held electric clippers. Each of 5 herbage samples was weighed fresh, and the samples were then pooled per circular area. A sub-sample of 200 g was kept to determine DM and was ground to 0.75 mm particle size and conditioned before analysis of chemical composition. The total herbage bulk density (kg OM/m^3) before grazing was calculated by dividing the total herbage mass (kg OM/m^2) by the mean height of the pasture (m).

Feeding Behavior

Feeding behavior was determined by simultaneously observing the rams fed indoors and at pasture for 24 h on day 18. The observers recorded the current activity of each ram at 5-min intervals (Hodgson, 1982), categorized as eating (head down in the trough or on the pasture, searching for or biting herbage), ruminating or idling. At night rams were observed with the aid of a flashlight. Eating and ruminating indexes (min/g OMI), defined as the time

needed to eat, or to ruminate 1 g of forage, were calculated by dividing the time spent eating (min) or ruminating (min), by the amount of forage eaten (g).

Rumen Characteristics

On d 22 and 23, approximately 100 ml of rumen liquid was collected from each ram fitted with a rumen cannula, at 0, 3, 6, 12 h after the morning meal. The pH of the rumen liquid was recorded immediately after the collection of liquid, and then samples were conserved for 24 h at 4 °C until ammonia (NH_3) determination. Two rumen empties (1 on d 25 and 1 on d 28) were manually carried out 3 h and 24 h after the morning meal on each ram fitted with a rumen cannula. These times were chosen because previous observations (H. Archimede, unpublished data) indicated, 1) that 3 h and 24 h after the morning meal represented the maximum and the minimum rumen filling respectively, 2) the mean of these 2 values is equivalent to the weighted mean of the rumen fill at 3, 6, 12 and 24 h after the morning meal. The total content was weighed, thoroughly mixed by hand, and 3 sub-samples were taken: 2 of 200 g for DM determination and 1 of 250 g that was preserved at –20 °C until it was freeze-dried and ground into 0.75 mm particles for chemical characterization.

Laboratory Analysis

Chemical characterization (OM, CP, NDF, ADF, and ADL) of ground samples of forage offered, refusals, feces and rumen content collected indoors and at pasture were carried out. The OM content of ground samples was measured after a 10-h ashing at 550 °C. Neutral detergent fiber, ADF, and ADL were estimated following the method of Van Soest et al. (1991). The N concentration in forage, feces, and rumen samples was determined using the Dumas method (AOAC, 1990). The CP content of samples was calculated by multiplying the N concentration by 6.25. The NH_3 content of rumen liquid was estimated by distillation. The OM, NDF, ADF, ADL and CP content of forage, refusals, feces, and rumen samples were expressed on a laboratory DM basis, obtained by drying the corresponding samples for 24 h at 103 °C.

Statistical Analysis

All variables were averaged to generate period means for each ram, herbage allowance and way of feeding for statistical analysis. Sixty-four observations were made for all variables except those with repeated measures including ruminal pH and NH_3 (n = 32) and those measured exclusively indoors or at pasture (32 values).

Table 4 : Chemical composition of 28 d regrowth Digitaria decumbens grass offered to animal indoors or at pasture.

Items	Indoors	Grazing	SEM	Statistical analysis [a]
OM, %	90.66 [w]	89.31 [x]	1.67	W**
CP, %	9.64 [w]	7.85 [x]	0.95	W**
NDF, %	69.37 [w]	72.82 [x]	1.76	W**
ADF, %	34.88 [w]	36.30 [x]	1.08	W**
ADL, %	4.97 [w]	6.63 [x]	1.21	W**
Sward height, cm	.	21.06	11.3	NS
Total mass, t DM / ha	.	2.86	0.85	NS
Tiller Length, cm	.	90.08	3.94	NS

[a] From an analysis of variance with a general linear model including the effects of level of feeding (L), way of feeding (W) and interaction. Statistical significance: ** $P < 0.01$, * $P < 0.05$, NS = Non significant.

[w,x,y] In a row, for each main effect, means without a common superscript letter differ ($P < 0.05$).

Data collected simultaneously indoors and at pasture were analyzed as a 4 x 4 Latin Square using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included the fixed effects of period, herbage allowance, way of feeding, and herbage allowance x way of feeding, with the repeated subject being the animal nested within the period and herbage allowance. Compound symmetry provided the best fit to the data. Samples collected at fixed times after feeding (i.e., ruminal pH and NH_3) were analyzed using the REPEATED statement within the MIXED procedure of SAS. This model included the effects of period, herbage allowance, way of feeding, herbage allowance x way of feeding and time (expressed as 0 to 12 h of collection) and treatment x time. Each ram was used as the subject and compound symmetry was used as the covariance structure. Data collected either indoors or at pasture were analyzed as a 4 x 4 Latin Square. The model included the fixed effects of period and feeding, with the repeated subject being the animal. Statistical relationships between OMI, OMD, and DOMI and the forage characteristics were determined using the COR procedure of SAS.

RESULTS

Allowance and Herbage Characteristics

Table 1 presents the chemical composition (CP, NDF, ADF, and ADL content) of the grass offered indoors. The amount of forage offered was on average 2.55 kg/d. The amount of forage offered at the low herbage allowance was 30% lower ($P < 0.001$) than that offered at the high herbage allowance (Table 2). The total bulk density was on average 15.4 kg DM/m^3 and was 0.3 times lower ($P < 0.001$) at the low herbage allowance than at the high herbage allowance.

At pasture, the CP, NDF, ADF, and ADL content of the grass offered equated to 0.81, 1.04, 1.03, and 1.16 times that recorded for indoors (Table 1). The amount of forage offered above 3 cm and available for rams was on average 3.54 kg/d and was 1.4 times greater than that offered indoors (Table 2). The amount of forage offered at the high herbage allowance was 1.56 times greater than that offered at the low herbage allowance. The total bulk density was on average 3.74 kg DM/m^3 and was 70% lower than that offered indoors ($P < 0.001$). The total herbage mass, the sward height and the tiller length were 2.34 t DM/ha, 9.0 cm, and 21.1 cm respectively (Table 1).

Estimates of in vivo OMD and OMI

In vivo OMD and OMI measured indoors were on average 65.0% and 84.7 g/kg $BW^{0.75}$ respectively (Table 2). Strong correlations were found between in vivo OMD and

Table 5 : Herbage allowance, bulk density, fecal OM output, in vivo OM digestibility (OMD) and in vivo OM intake (OMI) measured indoors and estimated for indoors or grazing animals by the fecal CP (CPf) or near infrared reflectance spectroscopy (NIRS) equations of Fanchone et al. (2008b)

Item	Low		High			
	Indoors	Grazing	Indoors	Grazing	SEM	Statistical analysis[a]
Allowance, kg DM/d[b]	2.09^{w}	2.77^{w}	3.02^{x}	4.32^{y}	1.25	W**, L*, WL*
Bulk density, kg DM/m^3	12.48^{w}	3.74^{x}	18.26^{y}	3.74^{x}	1.62	W**, L*, WL**
Fecal OM output, g/kg BW$^{0.75}$	28.93^{w}	22.17^{x}	30.59^{y}	22.81^{x}	69.82	W**
In vivo OMD, %	64.8^{w}		65.2^{w}			NS
OMD_{CPf}, %	63.9^{w}	66.9^{x}	65.5^{y}	66.2^{xy}	1.87	W**, WL**
In vivo OMI, g/kg BW$^{0.75}$	81.77^{w}		87.59^{x}			
OMI_{CPf}, g/kg BW$^{0.75}$	79.4^{w}	67.2^{x}	87.9^{y}	67.9^{x}	9.01	W**
$DOMI_{CPf}$ g/kg BW$^{0.75}$	50.6^{w}	45.1^{x}	57.5^{y}	45.0^{x}	6.13	W**

[a] From an analysis of variance with a general linear model including the effects of level of feeding (L), way of feeding (W) and interaction. Statistical significance: ** $P < 0.01$, * $P < 0.05$, NS = Non significant.

[b] Herbage allowance measured above the 3-cm mowing height.

[w,x,y] In a row, for each main effect, means without a common superscript letter differ ($P < 0.05$).

OMD_{CPf} (r = 0.61; $P < 0.001$; data not shown) and between in vivo OMI and OMI_{CPf} (r = 0.78; $P < 0.001$; data not shown). The range of variation of CPf (from 7.9 to 20.3% OM) in the large data set (n = 174) used by Fanchone et al. (2008b) to derive the CPf equation included all the CPf variation encountered in this study, for both indoors (from 11.2 to 16.5% OM; data not shown) and grazing animals (from 10.4 to 15.0% OM; data not shown).

Nutrition Indoors and at Pasture

Indoors, OMD_{CPf} and OMI_{CPf} values were on average of 64.7% and 83.6 g/kg $BW^{0.75}$ respectively (Table 2). From the low to the high herbage allowance, OMD_{CPf} increased by 1.6 digestibility units. At the same time, OMI_{CPf} increased by 8.5 g/kg $BW^{0.75}$ and $DOMI_{CPf}$ by 6.9 g/kg $BW^{0.75}$ at the high herbage allowance compared to the low herbage allowance (Table 2).

At pasture, the OMD_{CPf} value was on average 66.5% and was 1.03 times greater ($P < 0.05$; data not shown) at pasture compared to indoors (Table 2). No significant variation ($P = 0.283$) was obtained in OMD_{CPf} at the 2 herbage allowances tested. The value of OMI_{CPf} was on average 67.5 g/kg $BW^{0.75}$, and was 24% lower than that recorded indoors. From the low to the high herbage allowance, OMI_{CPf} did not differ significantly ($P = 0.83$). Digestible OM intake was on average 45.0 g/kg $BW^{0.75}$ and was 19% lower ($P < 0.001$; data not shown) than that registered indoors. Like OMI, DOMI did not differ significantly between the 2 herbage allowances ($P = 0.99$).

Animal Feeding Behavior

Indoors, eating time, ruminating time and idling time were on average 404, 515, and 521 min, respectively (Table 3). Eating time did not differ significantly between the low and the high herbage allowance. Ruminating time decreased ($P < 0.05$) from the low to the high herbage allowance, whereas, idling time did not differ significantly between the 2 herbage allowances. The eating index and chewing index were on average 0.271 and 0.609 min/g OMI_{CPf}, respectively, and did not differ significantly ($P = 0.57$ and $P = 0.38$) between the 2 herbage allowances. The ruminating index was on average 0.345 and decreased by 1.2 from the low to the high herbage allowance. The intake rate was on average 3.85 g OMI_{CPf}/min and did not differ significantly ($P = 0.41$) between the 2 herbage allowances.

At pasture, eating time, ruminating time and idling time were on average 503, 468, and 469 min and were 1.2, 0.9, and 0.9 times that measured indoors, respectively (Table 3). Neither eating time ($P = 0.84$) nor ruminating time ($P = 0.76$) or idling time ($P = 0.92$) varied

Table 6: Animal feeding behaviour and rumen fill 3 and 24 h after the morning meal, ADL passage rate.

Item	Low		High			
	Indoors	Grazing	Indoors	Grazing	SEM	Statistical analysis[a]
Eating time, min	376.0^{w}	512.4^{x}	431.4^{yw}	493.1^{w}	77.2	W**
Ruminating time, min	541.8^{w}	465.5^{x}	487.6^{x}	471.2^{x}	94.05	NS
Idling time, min	522.3^{w}	462.1^{x}	521.0^{w}	475.6^{wx}	135.96	NS
Eating index, min/g OMI	0.264^{w}	0.432^{x}	0.278^{w}	0.413^{x}	0.066	NS
Ruminating index, min/g OMI	0.377^{w}	0.394^{w}	0.314^{x}	0.336^{w}	0.069	W**
Chewing index, min/g OMI	0.630^{w}	0.779^{x}	0.589^{w}	0.797^{x}	0.11	W**
Intake Rate, g OMI/min	3.96^{w}	2.57^{x}	3.74^{w}	2.54^{x}	0.65	W**

[a] From an analysis of variance with a general linear model including the effects of level of feeding (L), way of feeding (W) and interaction. Statistical significance: ** $P < 0.01$, * $P < 0.05$, NS = Non significant.

[w,x,y] In a row, for each main effect, means without a common superscript letter differ ($P < 0.05$).

significantly between the 2 allowances (Table 3). The eating index was on average 0.422 and was 1.6 greater ($P < 0.001$; data not shown) than that registered indoors. The eating index at high herbage did not differ significantly from that at low herbage allowance at pasture ($P = 0.98$), but was 1.5 times greater ($P < 0.01$) than that indoors at the same herbage allowance. The ruminating index was on average 0.365 and 1.05 times that registered indoors. The ruminating index did not differ significantly ($P < 0.58$) between allowances. The chewing index was on average 0.788 min/g OMI_{CPf} and was 29% greater ($P < 0.001$) than that recorded indoors. The intake rate was on average 2.55 g OMI_{CPf}/min and was 0.5 times lower ($P < 0.001$) than that registered indoors.

Rumen Characteristics

Indoors, the rumen pH was on average 6.28. The NH_3 and amount of DM in the rumen were on average 98.5 mg/l and 1174.1 g, respectively. Both pH and amount of DM in the rumen were lower ($P < 0.005$, Table 6) at the low herbage allowance compared to at the high herbage allowance. No significant difference was found between the NH_3 concentrations in the rumen at the low and high herbage allowances. The amount of ADL in the rumen was on average 111.8 g and was 1.14 times greater ($P < 0.05$) at the high herbage allowance compared to the low herbage allowance. The mean ADL passage rate was 0.034 %/h, and did not vary significantly with the herbage allowance.

At pasture, the rumen pH was on average 6.2 and did not differ significantly to that measured indoors. The NH_3 concentration in the rumen was on average 114.8 mg/l and was 17% greater than that recorded indoors. The rumen NH_3 did not differ significantly between the two allowances. The amount of DM in the rumen was on average 975.3 g and was 17% lower ($P < 0.05$; data not shown) than that measured indoors. No significant differences ($P = 0.62$) were found between the rumen pH, the rumen NH_3, the amounts of DM, and ADL in the rumen between the high and low herbage allowance. The amount of ADL in the rumen was on average 81.1 g. ($P > 0.05$; Table 4). The ADL passage rate was on average 0.033 %/h and did not differ significantly ($P = 0.95$; data not shown) to that measured indoors.

Table 7: Ruminal ammonia (NH_3) and ruminal pH, rumen fill 3 and 24 h after the morning meal, and passage rate in the rumen of rams fed indoors and at pasture a 28 d regrowth *Digitaria decumbens* at low and high herbage allowance.

Item	Low		High			
	Indoors	Grazing	Indoors	Grazing	SEM	Statistical analysis [a]
Rumen pH						
0 to 12 h	6.25^{w}	6.19^{w}	6.32^{x}	6.24^{w}	0.13	NS
Rumen NH_3						
0 to 12 h	91.4 ^{w}	123.4 ^{x}	105.6 ^{wx}	106.3 ^{wx}	26.74	NS
Rumen fill						
DM, g	1097.6^{w}	998.5^{x}	1250.6^{w}	952.1^{x}	212.71	W*
DM 3 h, g	1236.9^{w}	1072.1^{x}	1383.4^{y}	1042.7^{x}	305.18	W**
DM 24 h, g	954.6^{w}	924.6^{wx}	1127.7^{y}	859.8^{x}	184.74	NS
ADL, g	104.71^{w}	82.11^{x}	118.98^{y}	80.12^{x}	24.35	W**
ADL 3 h, g	116.7^{w}	87.12^{x}	129.36^{w}	79.35^{x}	30.97	W**
ADL 24 h, g	92.57^{w}	77.09^{w}	107.14^{y}	80.83^{x}	22.61	W*
ADL passage rate, % / h	0.0347^{w}	0.0344^{w}	0.0324^{w}	0.0323^{w}	0.0096	NS

[a] From an analysis of variance with a general linear model including the effects of level of feeding (L), way of feeding (W) and interaction. Statistical significance: ** $P < 0.01$, * $P < 0.05$, NS = Non significant.

^{w,x,y} In a row, for each main effect, means without a common superscript letter differ ($P < 0.05$).

DISCUSSION

The 28 d regrowth Pangola grass offered to rams indoors and at pasture had a similar composition to that in other reports at the same regrowth stage indoors (Archimede et al., 2000) and at pasture (Ortega-Jimenez et al., 2005). Indeed, Archimede et al. (2000) reported indoors CP, NDF, ADF, and ADL contents of 7.9, 77.4, 42.9, and 7.4% for Pangola grass, whereas, Ortega-Jimenez et al. (2005) reported CP, NDF, and ADF contents of 9.9, 72.4, and 35.6% for a tropical pasture dominated by *Dichanthium sp*. (39%) and Pangola grass (29%). As in a similar previous experiment (Fanchone et al., 2008b) the differences observed between the grass offered indoors and that offered at pasture were due to the method of sampling the grass in the 2 environments: above 3 cm indoors and at ground level at pasture. At the same time, by exploiting the length of the tethering chain to determine the amount of forage available at pasture, we achieved a similar ratio between the low and high allowance to that available indoors.

Whatever the level of herbage allowance tested, differences exist between nutrition indoors and at pasture. At pasture we measured on average 2.8% greater OMD coupled with 24% lower OMI and 19% lower DOMI compared to that indoors. These results compare favorably with the 2.4% greater OMD, 13.7% lower OMI, and 11.4% lower DOMI at pasture compared to indoors, reported by Fanchone et al. (2008a).

The greater OMD measured at pasture may be related to the ability of animals to graze selectively (Minson, 1990; Van Soest, 1996). Indeed, the ruminal concentration of NH_3, an indicator of the amount of nitrogen fermented in the rumen (Jarrige, 1980), was greater at pasture compared to indoors and was particularly significant at the low herbage allowance, implying that better quality forage was consumed at pasture. This better quality should induce a lower ruminating index at pasture compared to indoors, as noted previously (Fanchone et al., 2008a). However, this was not observed in this study, because the rumen was less full in the grazing rams than the rams fed indoors. In this case the larger rumination index at pasture reflects the lower effectiveness of rumination, rather than consumption of a lower quality ration.

Organic matter intake was lower at pasture compared to indoors, whereas, rams at pasture spent 20% more time eating. However the effectiveness of prehension was lower at pasture than indoors, with 60% more time needed for gripping of 1 g of MO. In fact, due to selective grazing by the rams, the time for prehension was negatively effected by searching time, as shown by Hutchings and Gordon (2001). In addition, due to the differences in bulk density between the 2 feeding systems (4.12 x greater indoors), the amount taken per bite at

pasture was surely lower than indoors, inducing a lower intake at pasture. Total bulk density is known to affect bite mass at pasture (Stobbs, 1975). Thus, the difference in the forage density between the 2 systems of feeding (cut and packed in the trough indoors, and in situ at pasture), coupled to the reduced time devoted strictly to prehension, may explain the fall in OMI at pasture compared to indoors.

The differences in nutrition measured between indoors and at pasture varied according to the herbage allowances. Hence, the difference in OMD between indoors and grazing animals was lower at the high herbage allowance compared to the low herbage allowance (Table 2). Indoors, at the high herbage allowance, OMD was greater than at the low herbage allowance due to the selection implemented by the animals. This result is consistent with other studies indoors (Zemmelink, 1980; Mbwile and Uden, 1997) and confirms that animals fed indoors are also able to select better quality forage, when the amount of forage offered is increased. Indoors, at the low herbage allowance, the ability of rams to select a better quality ration was restricted by offering a limited amount (1.3 times voluntary intake) of a 5 cm length chopped grass, as it is a classical practice in indoors feeding experiments (Chenost and Demarquilly, 1982). At pasture, by increasing the herbage allowance, we expected to increase the potential for grazing animals to further select a better quality ration, but this was not the case. Indeed, there was no significant difference between OMD at the low and high herbage allowances. These results are consistent with those reported by Boval et al. (2000) for heifers fed tropical grass at various herbage allowances. In contrast, differences in OMD were observed when the characteristics of the sward varied, for similar herbage allowances (Boval et al., 2002; 2007). This means that the OMD of grazing animals is not likely to change when there are no differences in structure or presentation. Contrary to OMD, for which differences between indoors and pasture decreased with the increase in herbage allowance, OMI and DOMI differences between indoors and pasture were amplified (Table 2). Indeed, indoors, we measured a rise in OMI from the low to the high herbage allowance, whereas at pasture, OMI did not differ significantly. Such a rise in OMI with herbage allowance indoors has also been reported by other authors (Mbwile and Udèn, 1997; Zemmelink et al., 2000) and was related to the ability of animals to select a better quality ration resulting in an increase in OMD and an increase in OMI. At pasture, a curvilinear relationship has been reported between herbage allowance and herbage intake (Le Du et al., 1979; Ribeiro Filho et al., 2005). However, several authors showed a positive effect of an increase in herbage allowance on herbage intake (Kim et al., 2001; Tharmaraj et al., 2003; Ribeiro Filho et al., 2005), and on milk production (Kim et al., 2001; Virkajarvi et al., 2002).In contrast, others showed that an

increase in herbage allowance had no significant effect either on intake (Boval et al., 2000; Sibbald et al., 2000), herbage digestibility (Boval et al., 2000; Sibbald et al., 2000) or on milk production (Kuusela and Khalili, 2002). These results suggest that herbage allowance is not necessarily the first factor limiting OMI at pasture. Hence, in our study, by offering a larger amount of forage of the same chemical composition and the same characteristics (same bulk density, same sward height and extended tiller length) we did not affect OMI. At the same time, given the smaller amount of non digestible OM intake, the amount of DM in the rumen and the amount of ADL in the rumen measured at pasture (22.4 g/kg BW0.75, 975.3, and 81 g) compared to indoors (29.76 g/kg BW0.75, 1950.6, and 111.8 g), rams were not able to reach their maximum level of feeding at pasture. Furthermore, the particle passage rate did not differ significantly between treatments, suggesting that it was not the rumen fill that limited OMI. Thus limitation of the OMI of grazing animals may occur before the arrival of the particle in the rumen. This suggests that others factors such as prehensibility may limit OMI at pasture and may be the first limiting factor of OMI in such a system.

While low differences were obtained in OMD in favor of pasture, greater differences were achieved in OMI and DOMI in favor of animals fed indoors. Such a gain in DOMI may have a significant effect on production and should be taken into account to manage feeding in stalls or at pasture. This study, like that of Fanchone et al. (2008a), confirms that differences exist between nutrition indoors and at pasture and that these differences may vary according to the situation studied. Moreover, it also suggests that the origin of these differences is related to the accessibility of forage for grazing animals. Other variables such as the bite mass and the frequency of mouthful should be studied to better understand the origin of these differences in nutrition between these 2 feeding systems.

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2.2. Principales conclusions de l'étude expérimentale 2

Cet essai a montré, tout comme l'essai 1, que des différences d'alimentation existent entre l'auge et le pâturage et que ces différences varient en fonction de la quantité du fourrage offert aux animaux. Ainsi, une plus grande digestibilité a été mesurée au pâturage, alors que l'ingestion et la quantité ingérée digestible sont supérieures à l'auge. La plus grande digestibilité mesurée au pâturage serait comme dans l'essai 1, liée à la capacité des animaux à sélectionner un meilleur fourrage. La meilleure ingestion mesurée à l'auge serait elle aussi liée à la plus grande densité du fourrage offert qui influerait la taille de bouchée. Les résultats de ce 2ème essai sont cohérents avec ceux du 1er essai. L'encombrement ne serait pas le premier facteur limitant au pâturage car les animaux n'ont pas atteint leur capacité d'ingestion dans ce milieu. La préhensibilité du fourrage serait le premier facteur limitant l'ingestion au pâturage.

DISCUSSION GENERALE

DISCUSSION GENERALE

L'objectif de ce travail était de mettre en évidence des différences d'alimentation entre l'auge et le pâturage et de les expliquer, afin de comprendre à terme le déterminisme de l'alimentation dans ces deux milieux. Une meilleure connaissance de ce déterminisme permettrait de mieux gérer l'alimentation en zone tropicale humide, à base de fourrages verts. Avant de conduire les essais, nous avons fait les hypothèses suivantes que nous nous proposions de tester : 1) des différences d'alimentation existent entre l'auge et le pâturage lorsque le même fourrage est offert, 2) l'origine de ces différences se situe dans le mode de présentation du fourrage à l'animal, couché à l'auge *vs* sur pied au pâturage. Par ailleurs, nous nous sommes proposés de lever les freins méthodologiques à l'étude de l'alimentation au pâturage.

1. Conditions expérimentales

L'étude des différences entre l'auge et le pâturage a nécessité de travailler de façon simultanée dans les deux systèmes. Ainsi, la programmation des mesures aux mêmes heures à l'auge et au pâturage a requis d'avantage de main d'œuvre que pour des protocoles classiques, n'étudiant qu'un seul mode d'alimentation. La recherche des facteurs digestifs déterminant l'alimentation dans ces deux systèmes nous a contraints à travailler avec des animaux canulés du rumen. Ces animaux, plus fragiles que les animaux non canulés, ont dû subir au pâturage, les effets directs du climat (soleil, pluie, …). Néanmoins, l'analyse de nos résultats ne montre pas de différences significatives entre animaux canulés et animaux non canulés, pour un même traitement.

Le choix des conditions de comparaison de l'auge et le pâturage n'a pas été aisé. Ainsi, nous avons décidé d'offrir aux animaux au pâturage dans l'essai 1, la même quantité de fourrage qu'à l'auge, soit 1.20 la capacité d'ingestion compte tenu de la méthode de référence (Cochran and Galyean, 1994). Selon Delagarde (données non publiées), l'alimentation au pâturage serait limitée en milieu tempéré lorsque les quantités proposées sont inférieures à 2.5 fois la capacité d'ingestion des animaux. Or, les résultats de l'essai 2 (Etude expérimentale 2) nous montrent qu'une augmentation de la quantité proposée au pâturage n'a pas eu d'effet significatif sur l'ingestion et la digestion des fourrages. Ainsi, les animaux au pâturage dans l'essai 1 n'étaient vraisemblablement pas limités par les quantités proposées. De plus, nous

avons choisi d'offrir à l'auge un fourrage haché en brins de 5 cm plutôt qu'un fourrage en brins longs, afin de tenir compte de la capacité des animaux au pâturage à prélever des brins plus courts par cisaillement du fourrage proposé.

Afin de mieux comprendre l'origine des différences entre l'auge et le pâturage nous avons convenu de comparer ces deux modes d'alimentation dans différentes situations. Des travaux antérieurs à cette thèse ont montré que l'âge de repousse du fourrage (Aumont et al., 1995; Archimede et al., 2000; Boval et al., 2007), la fertilisation azotée (Boval et al., 2002), et l'espèce fourragère (Assoumaya, 2007) permettaient de bien discriminer la qualité du fourrage offert et de faire varier l'ingestion et la digestibilité. Dans le cadre de ce travail de thèse, l'âge de repousse et le niveau de proposé ont été retenus comme facteurs de variations afin de faire varier d'une part, la qualité du fourrage proposé (facteur qualitatif), et d'autre part, les quantités de fourrage proposé (facteur quantitatif). Parallèlement, nous avons fait le choix de conduire les animaux au pâturage à l'attache. Ce mode de conduite, en plus de favoriser la contention des animaux pour les prélèvements d'échantillons et les mesures (collecte de fèces, vidage de rumen), nous permettait d'avoir des mesures individuelles plutôt que des mesures moyennes à l'échelle d'un troupeau.

2. Méthodologie.

Nos résultats ont clairement démontré le potentiel de la SPIR pour prédire l'ingestion, la digestibilité et la composition chimique des fourrages ingérés par des ovins au pâturage (Fanchone et al., 2007) comme déjà montré pour des bovins (Boval et al., 2004) et des caprins (Landau et al., 2005). Les bonnes statistiques de calibration et de validation obtenues pour la DMO, ont suggéré l'utilisation de la SPIR pour la prédiction de la DMO (Etudes méthodologiques 1 et 2). En revanche, la prédiction directe de la MOI est moins satisfaisante et nous avons donc eu recours à la méthode de Streeter (1969) en couplant une estimation de la DMO et l'excrétion fécale pour prédire la MOI.

Parallèlement, nos résultats confirment le potentiel de prédiction de la méthode basée sur l'azote fécal et plus particulièrement le modèle hyperbolique (DMO = a – b / MATf) pour prédire la DMO au pâturage. Ce modèle qu'avait énoncé Lancaster (1949) reflète bien une relation biologique entre la DMO et la MATf. Ce modèle résulte de l'hypothèse que la quantité de MATf est constante pour 100g de MO ingérée. Des modèles similaires ont aussi été publiés par d'autres auteurs (Lukas et al., 2005; Schlecht and Susenbeth, 2006).

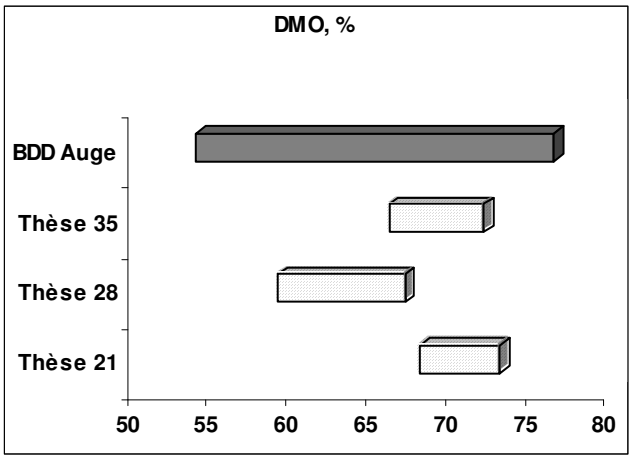

Figure 1.a Intervalles de variations de la digestibilité de la matière organique (DMO, %) dans la base de données de référence ayant servi à réaliser les calibrations (BDD Auge) et dans les données de la thèse à 21 (Thèse 21), 28 (thèse 28) et 35 (Thèse 35) jours de repousse.

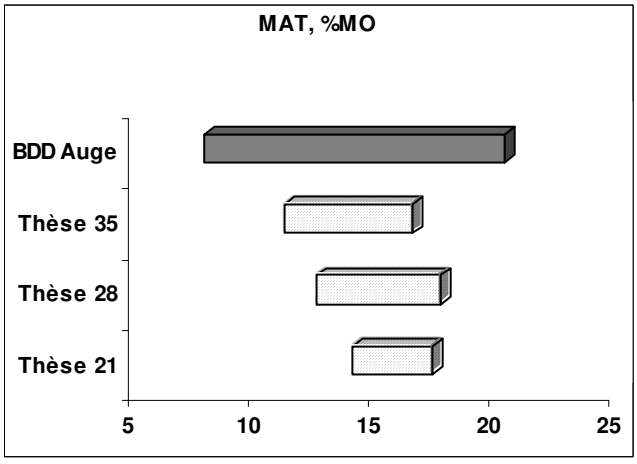

Figure 1.b Intervalles de variation de la teneur en matière azotée totale (MAT) fécale exprimée en fonction de la teneur en matière organique (MAT(MO)) dans la base de données de référence ayant servi à réaliser les calibrations (BDD Auge) et dans les données de la thèse à 21 (Thèse 21), 28 (thèse 28) et 35 (Thèse 35) jours de repousse.

De la comparaison des deux méthodes, azote fécal et SPIR, il ressort que la SPIR donne des estimations plus précises que l'azote fécal de la DMO pour un jeu de données indépendant, lorsque les données de références et les spectres ayant servi à faire l'équation englobe toute la variabilité observée dans la population à prédire (Etude méthodologique 2). A l'inverse, la méthode de l'azote fécal, bien que moins précise peut être utilisée dans des gammes de variation plus larges que celle dans laquelle il a été établi (Boval et al., 2003).

Les deux méthodes d'estimation de la DMO ont été combinées à la mesure de l'excrétion fécale, à l'auge et au pâturage. Des sacs collés à l'arrière des animaux nous ont permis d'obtenir une mesure fine de l'excrétion fécale par animal (données individuelles) et des échantillons représentatifs destinés aux analyses de composition chimique (azote fécal) et SPIR.

Bien que dans nos travaux méthodologiques préalables la SPIR permettait de prédire la DMO, la qualité de l'ingéré (MATi, NDFi, ADFi et ADLi), et indirectement la MOI, cette méthode n'a pas donné d'estimation précise de la DMO dans les essais réalisés dans la thèse. Or, les gammes de variation de la DMO, de la composition chimique du fourrage et des fèces dans les données de référence ayant servi à faire les calibrations couvraient celles des données de la thèse (Figure 1.a et 1.b). Ainsi, les différences de propriétés spectrales entre les 3 bases (données de références, données de l'essai 1 et données de l'essai 2) expliqueraient la mauvaise prédiction de la digestibilité. En effet, la distance de Mahalanobis qui quantifie la distance entre la moyenne des spectres de référence et celle de l'essai 1 était de 11.3, alors qu'elle était de 9.2 pour l'essai 2 (Figure 2). Selon Dardenne (1990), au dessus d'une valeur seuil de distance de Mahalanobis de 3, la prédiction n'est plus possible. Les facteurs susceptibles de faire varier les propriétés spectrales d'un échantillon sont des différences d'espèce fourragère, d'espèce animale, de pratique, de saison ou une dérive du spectromètre (Decruyenaere et al., 2008). Dans nos travaux, l'espèce animale (ovins), les pratiques (fertilisation, prise d'échantillons, broyage…), l'espèce fourragère (*Digitaria decumbens*) et les âges de repousses utilisés (21, 28, et 35 jours), étaient identiques à ceux de la base de données de référence. De plus, l'analyse de l'évolution des spectres d'échantillons témoins montre que les spectres de ces échantillons n'ont pas varié. Cela élimine ainsi la possibilité d'une dérive du spectromètre. Nous suspectons donc un effet saison et un effet année des essais par rapport à la base de calibration. Cette hypothèse devra être vérifiée par l'analyse d'une base plus large, englobant notamment des essais réalisés entre 2002 (derniers essais de la base de calibration) et 2006 (début des essais de thèse).

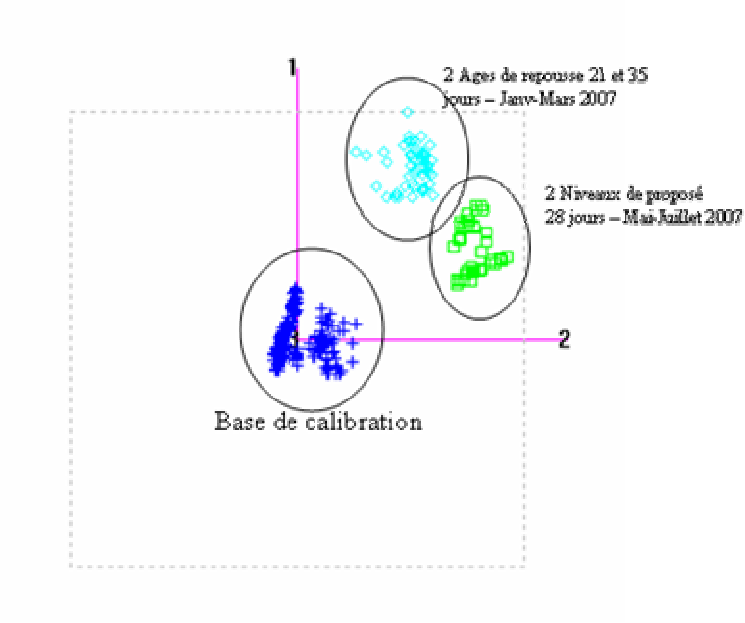

Figure 2. Représentation suivant les deux premières composantes principales des spectres de la base de calibration et des 2 essais (âges de repousses et niveaux de proposés) réalisés dans le cadre de la thèse.

En conclusion de ce travail méthodologique nous pouvons dire que la SPIR est une méthode intéressante pour l'étude de l'alimentation au pâturage, puisque qu'elle donne des calibrations relativement précises de la DMO notamment pour un jeu de données indépendant. De plus, cette méthode est, à notre connaissance, la seule qui permettrait d'estimer la qualité du fourrage réellement ingérée par l'animal. Cette mesure présente un grand intérêt, sachant qu'au pâturage le fourrage ingéré peut être très différent du fourrage offert. Néanmoins, la base de données de référence doit être incrémentée de manière à élargir le potentiel de prédiction de cette méthode. D'autre part, la méthode azote fécal a, en plus de sa capacité à estimer de manière précise la digestibilité, l'avantage de pouvoir être développée sur un nombre beaucoup plus restreint de données que la SPIR. En effet, l'accroissement de la variabilité dans les données de référence n'entraîne qu'un faible gain de précision de l'équation. De plus, la méthode azote fécal est utilisable dans des gammes de variation plus larges que celle dans laquelle elle a été établie. Les investigations visant à améliorer cette méthode devraient plutôt s'orienter vers une meilleure caractérisation de la relation biologique entre l'azote fécal et la DMO et notamment sur le rôle de l'azote endogène.

3. Valeurs mesurées de MOI et de DMO et facteurs de variation

La compilation des données des différents essais montre qu'à l'auge, les valeurs moyennes de DMO, de MOI et de MODI étaient de 65.0 %, 77.21 g /kg PM 50.13 g /kg PM (Tableau 1). Ces résultats ainsi que les gammes de variations obtenues sont comparables à d'autres études réalisées à l'U.R.Z. (Figure 3.a,b, et c) à l'auge avec du Pangola (Archimede et al., 2000; Assoumaya et al., 2007). L'encombrement du fourrage a donc varié de 0.8 à 1.4 sur la base d'un encombrement égal à 1 pour l'herbe de référence ingérée par le mouton à raison de 75 g / kg PM. Si l'on prend en compte la variabilité observée pour la DMO du fourrage, à l'auge, nous pouvons estimer que la valeur UF (UF breirem UF / kg MS = (2.36 MOD (g/kg) – 1.20 MO indigestible (g/kg)) / 1632) de nos fourrages expérimentaux à varié de 0.53 à 0.83 UF. La méthode UF breirem a été utilisée pour simplifier le calcul, toutefois nous sommes conscients qu'elle est plus sensible que les UFV à la fraction non digestible de la MO.

Au pâturage, les valeurs moyennes de DMO, de MOI, et de MODI étaient de 67.0 %, 64.4 g /kg PM, 43.3 g /kg PM, respectivement. Les gammes de variations obtenues couvrent celle d'autres études (Figure 3.a,b, et c) réalisées au pâturage à l'U.R.Z. avec du *Digitaria decumbens* (Ortega-Jimenez et al., 2005) et du *Dichantium spp.* (Boval et al., 2007).

Tableau 2 Moyenne, écart-type et intervalle de variation des principaux paramètres mesurés à l'auge et au pâturage durant la thèse.

		Auge				Pâturage			
	N	Moyenne	Ecart type	Minimum	Maximum	Moyenne	Ecart type	Minimum	Maximum
DMO	56	0,650 [a]	0,037	0,584	0,718	0,670 [b]	0,035	0,573	0,730
MOI	56	77,21 [a]	10,71 [a]	57,41	103,31	64,64 [b]	10,42	44,30	86,94
MODI	56	50,13 [a]	7,27	37,42	68,30	43,33 [b]	7,70	31,67	63,43
MONF	56	22,79 [a]	3,02	17,70	26,85	19,20 [b]	3,11	12,40	24,69
MATf	56	11,08 [a]	1,156	9,49	13,87	12,07 [b]	1,29	9,27	15,36
NDFf	56	65,64 [a]	4,26	56,79	72,79	65,72 [a]	4,26	58,50	73,90
TI	56	397,63 [a]	87,27	260	625	480,11 [b]	85,89	300,00	613,00
TR	56	519,34 [a]	118,23	310	895	518,11 [a]	135,29	290,00	825,00
TM	56	523,03 [a]	86,53	220	680	441,78 [b]	81,54	295,00	620,00
qMSp	56	2271,29 [a]	633,35	1295	3416,67	4083,17 [b]	2184,76	1753,52	12262,95
MATp	56	10,45 [a]	1,50	8,05	13,39	9,43 [b]	2,25	5,69	13,23
NDFPp	56	71,28 [a]	2,66	67,58	76,75	74,48 [a]	3,43	68,59	79,99
DensiH	56	13,52 [a]	3,70	7,73	20,16	2,62 [b]	0,96	1,21	5,33
NH3moy	20	123,37 [a]	50,842	53,71	256,34	134,15 [b]	46,33	81,38	241.28
Kp	20	0.040 [a]	0.019	0.020	0.108	0.039 [a]	0.019	0.02	0.098
CTRMS	20	1168.7 [a]	289.1	718.8	1649.3	985.3 [a]	146.7	707.7	1312.1
CTRADL	20	134.8 [a]	55.2	67.0	243.5	115.7 [b]	66.7	58.3	296.6

[a,b] Pour une même ligne les exposants différents indiquent que les moyennes ente auge et pâturage diffèrent significativement ($P < 0.05$)

L'encombrement du fourrage au pâturage a varié de 0.6 à 1.2 et sa valeur UF a varié de 0.51 à 0.85 UF. Les valeurs obtenues à l'auge comme au pâturage sont similaires à celles mesurées pour d'autres fourrages tropicaux (Aumont et al., 1995) et tempérés (Jarrige, 1988).

Ainsi, il apparaît que les fourrages tropicaux distribués seuls peuvent être de bonne qualité à l'auge comme au pâturage lorsqu'ils sont exploités à moins de 35 jours de repousse.

Lorsque l'âge repousse du fourrage augmente, la MOI diminue à l'auge comme au pâturage, dans nos données (Figure 4.a). Ces résultats sont cohérents à l'auge, avec d'autres études rapportées dans la bibliographie (Chenost, 1975; Aumont et al., 1995; Ichinohe et al., 1995; Archimede et al., 2000). Cependant, au pâturage, une augmentation de la MOI avec l'âge de repousse est rapportée pour des bovins (Hitchcock et al., 1990; Boval et al., 2007). Cette augmentation serait liée à un accroissement de la taille de bouchée avec l'âge de repousse, due à une augmentation de la hauteur et de la densité volumique du couvert prairial. Mais, dans nos essais, réalisés sur des ovins, la MOI n'a pas varié avec la hauteur de la prairie et sa densité volumique au pâturage.

Lorsqu'on compare l'auge et le pâturage, l'évolution de la DMO n'a pas été comparable à celle classiquement rapportée dans la bibliographie. En effet, la DMO obtenue à 28 jours de repousse était inférieure à celle observée à 35 jours alors qu'elle devrait théoriquement être intermédiaire entre la DMO à 21 jours et celle à 35 jours (Figure 4.a). Toutefois, une bonne hiérarchie de la DMO a bien été observée entre 21 et 35 jours de repousse. Cependant, nos essais n'ont pas été construits pour l'étude de l'effet de l'âge de repousse. Ainsi, les âges de repousse 21 et 35 jours ont été conduits en parallèle, en saison humide et en jours courts alors que l'âge de repousse 28 jours a été conduit seul, en saison sèche et en jours longs. En saison sèche et jours longs, pour une même biomasse produite, l'utilisation de l'azote est plus efficiente, induisant une croissance plus rapide avec des teneurs en azote plus faible dans la plante (Cruz, communication personnelle). Cet effet saison serait donc responsable des faibles teneurs en MAT observées à 28 jours comparativement au deux autres âges de repousse, et donc de la moins bonne DMO observée.

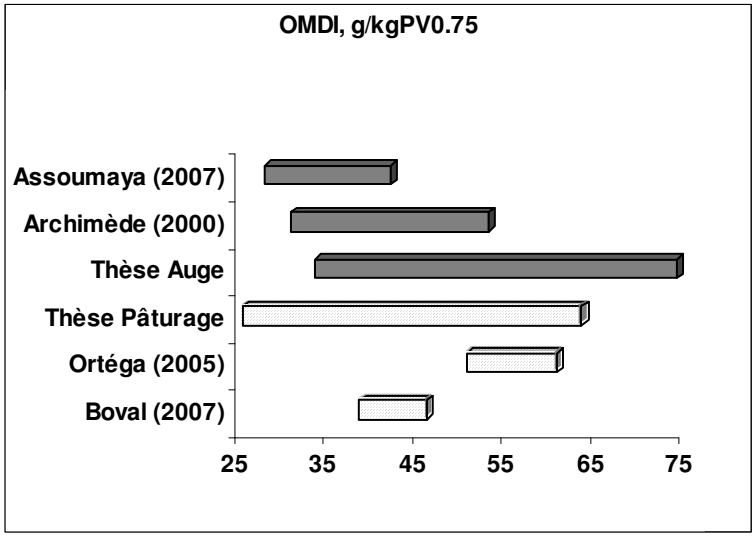

Figure 3.c. Intervalles de variation de la quantité de matière organique digestible ingérée (MODI) dans les données de thèse à l'auge (Thèse Auge) et au Pâturage (Thèse pâturage) et dans des essais à l'auge (Archimède et al., 2000 ; Assoumaya et al., 2007) et au pâturage (Ortega-Jimenez et al., 2005 ; Boval et al., 2007).

Au pâturage, la DMO du fourrage ingéré a été plus élevée qu'à l'auge (Figure 4.b). Ainsi, le fourrage ingéré au pâturage serait de meilleure qualité (plus riche en MAT et moins riche en fibres et en lignine) que le fourrage proposé. Or, la qualité de l'ingéré est un déterminant fort de l'alimentation à l'auge, et la MOI et la DMO y sont souvent positivement corrélés. Cet accroissement de la digestibilité au pâturage aurait donc pu s'accompagner d'un accroissement de la MOI. Ce n'est pas le cas dans nos données. En effet, la MOI, au contraire de la DMO, est plus élevée à l'auge comparativement au pâturage (Figure 4.b). Ces résultats suggèrent qu'au pâturage, un autre paramètre que la qualité du fourrage ingéré déterminerait l'alimentation au pâturage. Parallèlement, la MODI, comme la MOI, est plus élevée à l'auge qu'au pâturage (Figure 4.b). L'amplitude des variations observées sur la MOI étant plus grande que celles observées sur la DMO, leur résultante, la MODI est donc plus liée à la MOI qu'à la DMO. Or, la MODI est directement corrélée aux paramètres de production des animaux. Parallèlement, la DMO est le critère majeur pris en compte dans la majorité des études visant à sélectionner les fourrages destinés à l'alimentation animale. Nos données montrent qu'une sélection des fourrages basée sur l'ingestion et plus particulièrement sur la MODI serait plus pertinente pour accroître la production animale à l'auge comme au pâturage.

4. Facteurs explicatifs

4.1. Digestibilité

A l'auge, les facteurs les mieux corrélés à la DMO mis en évidence dans nos données sont classiquement connus. Ainsi, la teneur en MAT de l'ingéré exprimée en fonction de la MO (MATi(MO), Tableau 2), la teneur en MAT du proposé (MATp) et le rapport entre la MAT et le NDF du proposé (MATp/NDFp), sont les facteurs qui expliquent le mieux la DMO (Tableau 2). L'azote fermentescible issu de la dégradation de la MAT ingérée est indispensable au bon développement des microorganismes dans le rumen et notamment au développement des bactéries cellulolytiques impliquées dans la réduction de taille des particules alimentaires. La teneur en MAT du proposé constitue la principale source d'azote nécessaire à l'activité des microorganismes du rumen. Ainsi une augmentation de la MATp se traduit par une augmentation de l'activité cellulolityque du rumen et par une moins grande résistance à la mastication, d'où la bonne relation obtenue avec la DMO. Par ailleurs, à l'auge, les quantités proposées étant limitées à 1.20 fois la capacité d'ingestion afin de minimiser le

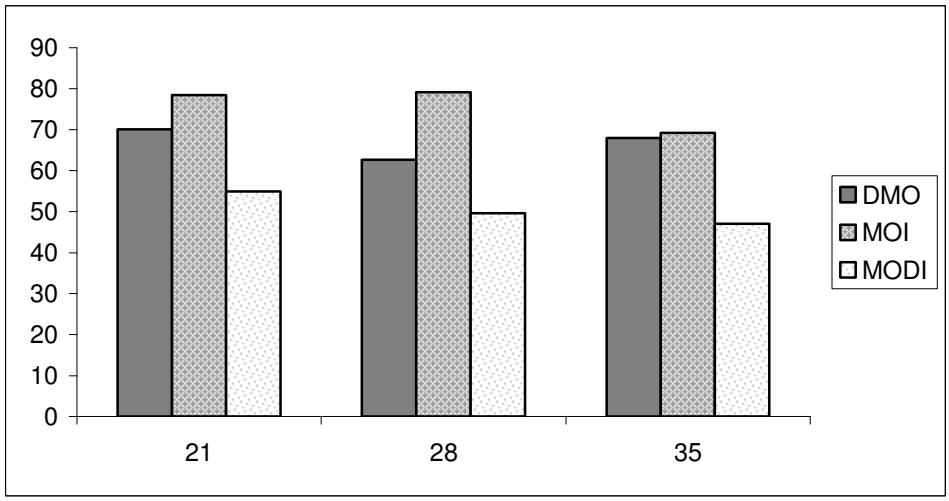

Figure 4.a. Evolution de la digestibilité de la matière organique (DMO, %), de la quantité de matière organique ingérée (MOI, g /kg PM), et de la quantité de matière organique digestible ingérée (MODI, g /kg PM) avec l'âge de repousse du (*Digitaria decumbens*), Données Auge et Pâturage.

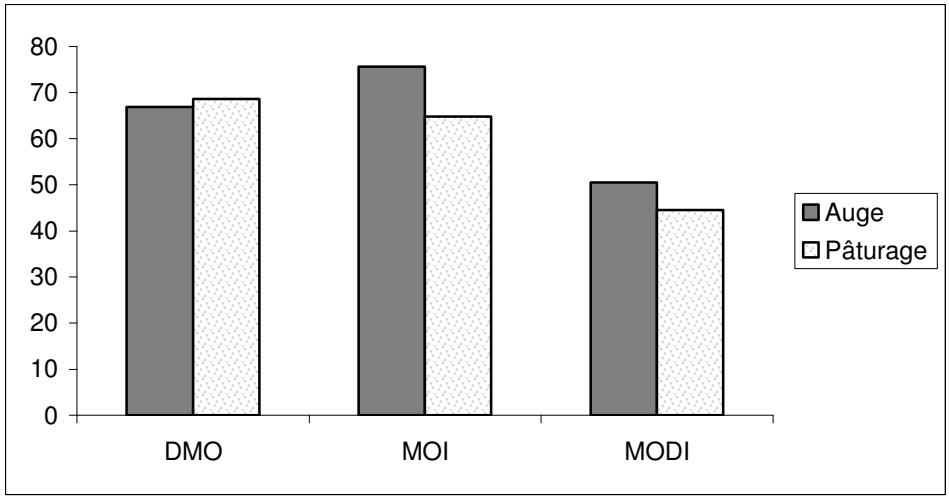

Figure 4.b. Evolution de a digestibilité de la matière organique (DMO, %), de la quantité de matière organique ingérée (OMI g /kg PM), et de la quantité de MO digestible ingérée (MODI, g /kg PM) entre l'auge et le pâturage.

tri, le fourrage ingéré est généralement très proche du fourrage proposé. Ainsi, les teneurs en MAT de l'ingéré peuvent s'apparenter et celles du proposé. Parallèlement, une bonne corrélation a été trouvée entre la DMO et la teneur moyenne en NH_3 du rumen (NH_{3moy}). La concentration en NH_3 dans le rumen est un bon indicateur de la concentration en azote fermentescible de l'ingéré (Jarrige, 1980). Elle reflète mieux l'azote disponible pour les microorganismes du rumen que la teneur en azote du fourrage (MATp). Cela explique la plus grande variation expliquée par la concentration en NH3 du rumen et la DMO que par la teneur en MAT du proposé (MATp) ou la teneur en MAT de l'ingéré (MATi).

Au pâturage, les facteurs mis en évidence dans nos données comme étant le mieux corrélés à la DMO sont aussi les facteurs classiquement connus à l'auge : la teneur en MAT du proposé (MATp, Tableau 2), le rapport entre la MAT et le NDF du proposé (MATp/NDFp), la teneur en NDF du proposé (NDFp). Cependant, au pâturage, les relations entre ces facteurs et la DMO sont moins significatives que celles obtenues à l'auge. Par ailleurs, un paramètre de comportement alimentaire des animaux (temps de mastication (TM)) apparaît comme étant bien corrélé à la DMO (DMO = 0.616 - 0.000109 TM ; Tableau 2). La pente négative de la relation entre TM et la DMO est cohérente, ainsi, une augmentation de TM qui traduit l'ingestion d'un fourrage de moins bonne qualité, entraînerait une diminution de la DMO. Comme à l'auge, la teneur moyenne en NH_3 du rumen, indicateur de la teneur en azote fermentescible de l'ingéré est le paramètre le mieux corrélé à la DMO au pâturage. Ainsi, cette bonne corrélation entre la DMO et NH_3 du rumen et les moins bonnes corrélations obtenues entre la DMO et les caractéristiques du fourrage proposé (MATp, MATp/NDFp, NDFp) suggèrent qu'au pâturage, la teneur en MAT du fourrage ingéré (MATi) serait différente de celle du fourrage proposé (MATp).

4.2. Ingestion

A l'auge, comme pour la DMO, les facteurs les mieux corrélés à la MOI sont classiquement connus. Ainsi, les durées unitaires de mastication (DUM), le temps d'ingestion (TI), les temps de rumination (TR), les teneurs en NDF et en ADF du proposé (NDFp et ADFp) sont les facteurs expliquant le mieux la MOI. La relation positive observée entre le TI et la MOI et les relations négatives obtenues entre le NDFp et ADFp et la MOI sont cohérentes. En effet, une augmentation de TI induit une diminution de TM, le temps de repos étant incompressible. Ainsi, une augmentation de TI traduit la consommation d'un fourrage de meilleure qualité qui sera donc mieux ingéré. Nous nous attendions à avoir une corrélation

Tableau 3. Relations entre la digestibilité de la matière organique (DMO) et les teneurs en matière azotée totale (MATp) et en parois (NDFp) du proposé, les teneurs en MAT de l'ingéré (MATi), les temps de mastications (TM), et les teneurs en NH3 du rumen.

Auge			Pâturage		
Equation	SE	R^2	Equation	SE	R^2
DMO = 0.566 + 0.000597 NH_{3moy}	0.024	0.617	DMO = 0.575 + 0.000635 NH_{3moy}	0.020	0.685
DMO = 0.478 + .0156 MATi(MO)	0.027	0.44	DMO = 0.5626 + 0.012 MATp	0.021	0.637
DMO = 0.456 + 0.01849 MATp	0.024	0.577	DMO = 0.551 + 1.0056 MATp/NDFp	0.022	0.610
DMO = 0.443 + 1.41 MATp/NDFp	0.024	0.559	DMO = 0.2474 + 0.00579 NDFp	0.028	0.328
			DMO = 0.616 - 0.000109 TM	0.037	0.138

positive entre le TM et la MOI, mais cela n'a pas été le cas dans nos donnés (Tableau 3). Parallèlement, les teneurs en fibres du fourrage proposé (NDFp et ADFp) sont des indicateurs du pouvoir encombrant du fourrage. Cela explique qu'elles soient négativement corrélées à la MOI. La relation entre la MOI et la quantité de MS proposée (qMSPro) est un biais lié à l'utilisation de la méthode de référence (Tableau 3). En effet, les qMSPro sont calculées en fonction de l'ingestion volontaire des animaux. Cependant, dans l'essai 2, nous avons offert 2 niveaux de proposés, et la relation entre la MOI dans cet essai (MOI_2) et la quantité proposée était beaucoup plus forte que dans l'essai 1. L'augmentation de la quantité proposée à l'auge permet, aux animaux de sélectionner une ration de meilleure qualité que le fourrage offert, et donc induit un accroissement de l'ingestion (Zemmelink, 1980). La durée unitaire de mastication (DUM) est un paramètre synthétique traduisant le travail masticatoire nécessaire pour valoriser un gramme de MO de fourrage. Ainsi, l'augmentation de la DUM traduit la réponse de l'animal à l'ingestion d'un fourrage de moins bonne qualité et induit une diminution de la MOI, ce qui explique la pente négative obtenue (MOI = 118 – 5.83 DUM, Tableau 3). Par opposition avec la relation entre TM et la MOI, la relation obtenue dans nos données entre la DUM et la MOI est donc cohérente.

Au pâturage, hormis la teneur en fibres du fourrage (NDFp, ADFp), les facteurs mis en évidence comme étant les mieux corrélés à la MOI sont les mêmes qu'à l'auge (DUM, TI, et TM ; Tableau 3). Le fait qu'au pâturage, les teneurs en fibres du fourrage proposé (ADFp et NDFp) ne soient pas corrélées avec la MOI, confirme que le fourrage ingéré au pâturage, peut être très différent du fourrage offert. Au pâturage, les corrélations obtenues entre la MOI, TI, et TM sont elles aussi cohérentes, toutefois, les relations obtenus sont moins significatives qu'à l'auge. Au pâturage, TI ne représente pas comme à l'auge, le temps de préhension mais incle aussi le temps de sélection, ce dernier pouvant limiter le temps de préhension et affaiblir la relation entre TI et la MOI.

A l'auge comme au pâturage, le taux de renouvellement des particules dans le rumen (Kp) n'est pas significativement corrélé dans nos données avec la MOI. Bien qu'ayant travaillé sur des âges de repousses différents (de 21 à 35 jours), les fourrages étaient toujours de bonne qualité (21 à 35 jours). Ainsi, les Kp ont varié dans nos données dans des gammes allant de 0.0024 à 0.0030 à l'auge, et de 0.0011 à 0.0027 au pâturage. Les âges de repousse utilisés dans la thèse n'ont pas induit assez de variabilité pour mettre en évidence un effet du Kp sur la MOI.

Tableau 3. Relations entre la quantité de matière organique ingérée (MOI) et les teneurs en matière azotée totale (MATp) et en parois (NDFp) du proposé, les teneurs en MAT de l'ingéré (MATi), les temps d'ingestion (TI), les temps de mastications (TM), les teneurs en les quantités proposé (qMSPro) dans l'essai 1 ($qMSPro_1$) et dans l'essai 2 ($qMSPro_2$), la durée unitaire de mastication (DUM), et la teneur en NH_3 du rumen.

Auge			Pâturage		
Equation	SE	R^2	Equation	SE	R^2
OMI = 47.45 + 0.072 TI	8.64	0.35	OMI = 39.017 + 0.0548 TI	9.995	0.18
OMI = 99.218 – 0.044 TM	9.37	0.24	OMI = 79.655 - 0.027 TM	10.408	0.12
OMI = 212.285 -1.8948 NDFp	9.55	0.22			
OMI = 150.067 -2.037 ADFp	10.14	0.12			
OMI = 56.326 + 0.009 qMSPro	9.09	0.29			
$OMI_1 = 62.35 + 0.0066\ qMSPro_1$	9.08	0.16			
$OMI_2 = 32.978 + 0.0226\ qMSPro_2$	7.42	0.62			
OMI = 118 – 5.83 DUM	7.94	0.46			

4.3. Capacité d'ingestion, MOND et encombrement

La quantité de matière organique non digérée (MOND) et la quantité de matière sèche dans le rumen (CTRMS) sont plus élevées à l'auge qu'au pâturage. Cela suggère que les animaux au pâturage n'atteignent pas leur capacité d'ingestion maximale alors qu'ils ingèrent une ration de meilleure qualité que les animaux à l'auge. Ainsi, ce n'est pas l'encombrement du rumen qui a limité la MOI des animaux au pâturage dans nos essais. En considérant les différences de densité de l'herbe observées entre l'auge et le pâturage, nous pouvons supposer que la plus faible densité du fourrage au pâturage a limité l'ingestion. A l'auge la densité n'est pas limitante et ce sont donc des facteurs classiques, liés à la composition physico-chimique (NDFp, ADFp) du fourrage et à la réduction de taille des particules dans le rumen (DUM) qui ont limité la MOI. Au pâturage, le premier facteur limitant la MOI serait plus lié à la structure de la prairie qui déterminerait la taille des bouchées via la hauteur et la densité volumique du fourrage, la proportion de biomasse verte et de feuille dans le couvert. L'hypothèse d'une diminution de la taille de bouchée entre l'auge et le pâturage, pourrait expliquer que les animaux au pâturage aient augmenté leur temps d'ingestion (Tableau 6.1). Toutefois, cette augmentation de TI ne leur a pas permis de compenser la taille plus réduite des bouchées et d'atteindre leur capacité d'ingestion.

CONCLUSIONS ET PERSPECTIVES

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Nos résultats indiquent : 1) que des différences d'alimentation existent entre l'auge et le pâturage pour un même fourrage offert ; 2) que ces différences varient en fonction de la qualité et de la quantité de fourrage proposé aux animaux ; 3) que ces différences seraient liées à la préhensibilité du fourrage et au mode de présentation de l'herbe qui différent entre les deux modes d'alimentation. Des travaux complémentaires sont nécessaires pour analyser cette dernière hypothèse. La taille de bouchée apparaît comme le paramètre supplémentaire le plus important à mesurer. En effet, ce paramètre se situe à l'interface entre l'herbe et l'animal. Il permettrait avec les autres paramètres du comportement alimentaire (temps d'ingestion, fréquence de bouchées), une meilleure compréhension du processus d'ingestion au pâturage. De plus, une caractérisation plus fine de la prairie est nécessaire, en mesurant en plus des paramètres classiques que nous avons mesurés, la composition morphologique (proportion feuille / tiges débris), et la composition chimique des différentes strates du couvert.

Nos résultats ont aussi mis en évidence que les variations des quantités de matières organiques digestibles ingérées sont davantage liées à celles de l'ingestion qu'à celles de la digestibilité. Or, la matière organique ingérée digestible est un paramètre très corrélé à la production. Il serait donc plus pertinent de caractériser les fourrages par leur ingestibilité que par leur digestibilité et leur composition chimique comme c'est le cas dans la majorité des programmes d'amélioration actuels.

L'âge de repousse n'a pas permis dans nos données de discriminer suffisamment la structure du couvert pour mettre en évidence des effets de la structure prairiale sur l'ingestion. Toutefois, dans nos essais, nous avons travaillé sur des fourrages relativement jeunes (21 à 35 jours).

Il serait intéressant d'élargir les gammes de variation en travaillant sur des âges de repousse plus importants afin de mieux discriminer la structure du couvert. Par ailleurs, l'espèce fourragère en travaillant sur des fourrages de port différents permettrait elle aussi de faire varier la structure du couvert. Mais, comme pour l'âge de repousse, la qualité du fourrage peut varier d'une espèce à une autre à un même âge. Dans ce contexte, un bon compromis serait d'étudier l'effet d'espèces fourragères différentes à un âge de repousse évoluant en continu, afin d'avoir une gamme suffisamment étendue. Bien que cet essai semble

lourd à mettre en place, nos données ont montré qu'il serait plus intéressant de focaliser sur la structure du couvert que sur le processus digestif pour comprendre les différences entre l'auge et le pâturage. Parallèlement, les quantités proposées sont un facteur important de variations pour accroître l'ingestion, néanmoins, nos données ont montrés, qu'au pâturage, il vaut mieux raisonner en quantités proposées accessibles pour l'animal, compte tenu de la relation entre la taille de bouchée et l'ingestion.

En plus de nos hypothèses de travail, nos résultats ont permis de montrer que la SPIR et les index fécaux sont des méthodes précises et robustes d'estimation de l'ingestion et de la qualité de l'ingéré au pâturage. Les bases de données SPIR doivent être incrémentées, notamment avec les données générées à l'auge dans le cadre de cette thèse afin d'accroître la robustesse des équations de prédiction.

Une meilleure compréhension de la relation biologique existante entre l'azote fécal et la digestibilité, et notamment entre la proportion d'azote endogène dans les fèces et la digestibilité permettait d'avoir des équations plus précises directement utilisables au pâturage.

Nos résultats confirment que l'alimentation doit être appréhendée différemment à l'auge et au pâturage. Il est important de raisonner des processus liés à la préhensibilité du fourrage. Une fois ingéré, les processus d'utilisation digestifs du fourrage sont vraisemblablement comparables quel que soit le système d'alimentation.

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LISTE DES ABREVIATIONS

ADF : Acid detergent fibre.

ADFi : Teneur en acide detergent fibre de l'ingéré.

ADL : Acid detergent lignin.

ADLi : Teneur en acide detergent lignin de l'ingéré.

CR_2O_3 : Oxyde de chrome.

CTRADL : Contenu total du rumen en lignine.

CTRMS : Contenu total du rumen en matière sèche.

DensiH : Densité du fourrage.

DMO : Digestibilité de la matière organique.

DUI : Durée unitaire d'ingestion.

DUM : Durée unitaire de mastication mérycique.

GMQ : Gain moyen quotidien.

Kp : Taux de renouvellement des particules dans le rumen.

MAT : Matière azotée totale.

MATf : Teneur en matière azotée totale des fèces.

MATi : Teneure en matière azotée totale de l'ingéré.

MATi(MO) : Teneur en matière azotée totale de l'ingéré exprimée en fonction de la matière organique.

MATp : Teneur en matière azotée totale du proposé.

MO : Matière organique.

MODI : Quantité de matière organique digestible ingérée.

MOF : Quantité de matière organique fécale excrétée.

MOI 2 : Quantité de matière organique ingérée dans l'essai 2.

MOI : Quantité de matière organique ingérée.

MOI1 : Quantité de matière organique ingérée dans l'essai 1.

MOND : Quantité de matière organique non digestible ingérée.

NB : Nombre de bouchées.

NDF : Neutral détergent fibre.

NDFf : Teneur en neutral detergent fibre des fèces.

NDFi : Teneur en neutral detergent fibre de l'ingéré .

NDFp : Teneur en neutral detergent fibre du proposé.

NH3 : Teneur en ammoniac.

NH3moy : Moyenne de la teneur en ammoniac sur des prélèvements 0, 3, 6, 12 après la distribution du repas.

PEG : Polyéthylène Glycol.

QB : Quantité prélevée par bouchée.

qMSPro : Quantité de matière sèche proposée.

qMSPro1 : Quantité de matière sèche proposée dans l'essai 1.

qMSPro2 : Quantité de matière sèche proposée dans l'essai 2.

SPIR : Spectrométrie dans le proche infra rouge.

TI : Temps d'ingestion.

TM : Temps de mastication mérycique.

TP : Temps de pâturage.

TR : Temps de repos.

U.R.Z : Unité de Recherches Zootechniques.

Yb : Ytterbium.