Study of limiting factors of intake in small ruminants fed with tropical forages

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ETUDE DES FACTEURS LIMITANT L'INGESTIBILITE CHEZ LES PETITS RUMINANTS VALORISANT DES FOURRAGES TROPICAUX

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A mes fils Thomas et Samuel,
(Votre présence est un énorme réconfort)

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Ce travail de thèse a été réalisé à l'Unité de Recherches Zootechniques de l'INRA- Antilles Guyane. Il a donné lieu à l'écriture de huit projets d'articles (dont 2 déjà aceptés) qui forment la trame de cette thèse :

- **partie bibliographique** :

1. Etude comparative de l'ingestion et de la digestion des fourrages tropicaux et tempérés. Assoumaya C., Sauvant D., Archimède H. 2006. (soumis à Productions Animales)

- **partie expérimentale** :

2. Limits of exogenous fibrolytic enzymes to improve digestion and intake of a tropical grass. Assoumaya C., Boval M., Weisbecker JL., Saminadin G., Archimède H. (Australian-Asian Journal of Animal Science, *sous presse*)

3. Effect of frequency of meals on intake and digestion of tropical grass consumed by rams. Assoumaya C., Sauvant D., Pommier F., Boval M., Calif B. and Archimède H. (*soumis à Animal*)

4. Feeding behaviour and rumen load in related sheep receiving two equal meal of a tropical forage. Assoumaya C., Archimède H., Saminadin G., Periacarpin F., Pommier F., Sauvant D.

5. Intake and digestive processes in the rumen of rams fed with *Digitaria decumbens* harvested at four stages of grass regrowth age. Assoumaya C., Boval M., Sauvant D., Xandé A., Poncet C., Archimède H. (Australian-Asian Journal of Animal Science, *sous presse*)

6. Intake and digestive processes of rams fed *Panicum maximum* harvested at four stages of grass maturity. Assoumaya C., Sauvant D., Périacarpin F., Etienne T., Archimède H. (à *soumettre à Animal*)

7. Rumen fermentation in rams fed *Panicum maximum* harvested at four stages of grass maturity. Assoumaya C., Sauvant D., Philibert L., Deloumeau C. and Archimède H. (*à soumettre à Animal*)

8. Intake and digestive processes of rams fed *Digitaria decumbens* harvested at three stages of grass maturity. Assoumaya C., Sauvant D., Périacarpin F., Dumoulin P.J., T. Etienne and Archimède H. (à *soumettre à Animal*)

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LISTE DES ABREVIATIONS

ET GLOSSAIRE

a : soluble fraction

ADF: Acid Detergent Fiber

ADFf: teneur en ADF du fourrage

ADL: Acid Detergent Lignine

ADOMR : quantité de Matière Organique Apparemment Digérée dans le Rumen

AGV (VFA) : Acides Gras Volatils (Volatile fatty Acid)

b : potentially degradable fraction

c : degradation rate

C_3: première molécule formée au cours de la photosynthèse composée de 3 carbones (de type tempéré)

C_4: première molécule formée au cours de la photosynthèse composée de 4 carbones (de type tropical)

ChewT : Chewing Time

CODPHYESP : expérimentations ayant pris en compte le stade physiologique et l'espèce animale

Cov : covariable

CR (WRC) : Contenu total Ruminal (Whole rumen Content)

D : Effective degradability

D. decumbens : Digitaria decumbens

dDM: total tract digestibility of dry matter

Deg : Degradability

DL (DF): Degré de liberté (Degree of Freedom)

dMO (dOM): digestibilité totale de la matière organique (total tract digestibility of organic matter)

dNDF : digestibilité totale des parois (NDF)

DNint : Digestibilité intestinale de l'azote

drMO : digestibilité ruminale de la matière organique

drNDF : digestibilité ruminale des parois (NDF)

EEF (EFE) : enzymes exogènes fibrolytiques (exogenous fibrolytic enzyme)

Eff µ : Efficacité microbienne

IENDFruing : indice d'encombrement égal à la quantité de NDF du rumen rapportée sur le flux de NDF ingéré

IEMSru fec : indice d'encombrement égal à la quantité de matière sèche du rumen rapportée sur le flux de matière sèche fécale

IdMast (ChewId) : Indice de mastication (Chewing Index)

INA-PG : Institut National Agronomique- Paris-Grignon

INRA : Institut National de Recherche Agronomique

Kc : taux de comminution

Kp : transit des particules

LP : large particle

MAµDUOD : Quantités de matières azotées microbiennes au niveau du duodénum

MADUOD : Quantités de matières azotées totales au niveau du duodénum

MAT (CP) : Matières Azotées Totales (Crude protein)

MATf : teneur en MAT du fourrage

MO (OM): Matière Organique (Organic Matter)

MODI : quantités de matières organiques digérées

MODIPM : quantités de matières organiques digérées ramenées au Poids métabolique

MODI%PV : quantités de matières organiques digérées par pourcentage de poids vif

MS ou **DM**: Matière Sèche

MSI (DMI) : Matière Sèche Ingérée (Dry Matter Intake)

MSIPM (DMIMW) : Matière sèche ingérée ramenée au poids métabolique

MSIPV (DMILW): Matière sèche ingérée ramenée au poids vif

MSRum%PV : Contenu total ruminal exprimé en matière sèche par pourcentage de poids vif

NDF: Neutral Detergent Fiber

NDFf : teneur en NDF du fourrage

NDFI : NDF ingéré

NDFnd : NDF non digestible

NDFRum%PV : Contenu total ruminal exprimé en NDF par pourcentage de poids vif

NS : No significant

Pa : Paille

P. maximum : Panicum maximum

PEG : PolyEthylene Glycol

PM (MW) : poids métabolique ($PV^{0.75}$) (Metabolic Weight)

PV (LW) : poids vif (Live Weight)

SP : small particle

SRZ: Station de recherches Zootechniques

Te : fourrage Tempéré

Tr : fourrage tropical

MRT : Mean Retention Time

UEPSA: Unité Expérimentale de Production et Santé Animale

UF : Unité fourragère

URZ: Unité de Recherches Zootechniques

VS: very small particle

WRC-NDF : Whole rumen Content of NDF

Yb: Ytterbium

INTRODUCTION GÉNÉRALE

Introduction générale

Définition de la problématique et du contexte

Les herbivores d'élevage pourraient être nourris exclusivement avec de l'herbe bien que dans le monde « moderne », la tendance générale, depuis le début de la rationalisation de l'élevage au XIXe siècle, a été l'incorporation croissante d'aliments concentrés dans les rations. Cette tendance est justifiée par le « progrès génétique » d'animaux dont l'accroissement de performances individuelles de production (lait, viande) nécessitait l'ingestion de rations à forte densité nutritionnelle. Certains pays, tels la Nouvelle Zélande et l'Argentine, se singularisent en développant encore des systèmes d'élevage au pâturage avec la recherche d'une maximisation de la productivité animale à l'hectare.

Les crises récentes des filières animales (vache folle, crise de la dioxine, tremblante du mouton…), le développement de travaux de recherche sur la typicité des produits, la forte attente sociétale pour une meilleure gestion des ressources et la protection de l'environnement, des considérations éthiques ou philosophiques sur la compétition entre l'homme et l'animal vis à vis de certaines ressources (céréales…) ont créé un véritable regain d'intérêt pour la valorisation de l'herbe dans les systèmes d'élevage et sous différents climats.

La valorisation des fourrages a fait l'objet de nombreux travaux, aussi bien en zone tempérée que tropicale (Demarquilly 1981, Leng 1989, Minson 1990). Cependant, les connaissances accumulées sur les modèles tropicaux demeurent insuffisantes pour assurer une valorisation optimale des fourrages, d'autant plus que ces derniers demeurent dominants voire exclusifs dans les systèmes d'alimentation. Les recherches sur les graminées fourragères tropicales, souvent postérieures à celles entreprises en zones tempérées, se sont basées sur le socle de connaissances et de méthodologies acquises dans ces milieux et portant donc sur des modèles biologiques différents. Cependant, les chercheurs de la zone tropicale ont posé certains résultats comme des paradigmes universels, ce qui a contribué, d'une certaine façon, à limiter les champs d'investigation qui auraient du être plus ouverts compte tenu de l'originalité de certaines ressources biologiques. Pourtant la physiologie originale des graminées tropicales (plantes à photosynthèse en C4) est à l'origine de leur vieillissement accéléré comparativement aux graminées tempérées (plantes à photosynthèse en C3). Une des conséquences directes, est que l'optimisation de la valeur alimentaire et nutritionnelle des graminées tropicales ne peut probablement pas se faire aux mêmes âge et rythme calendaires que les graminées tempérées. Cependant, la plupart des études sur les fourrages tropicaux ont

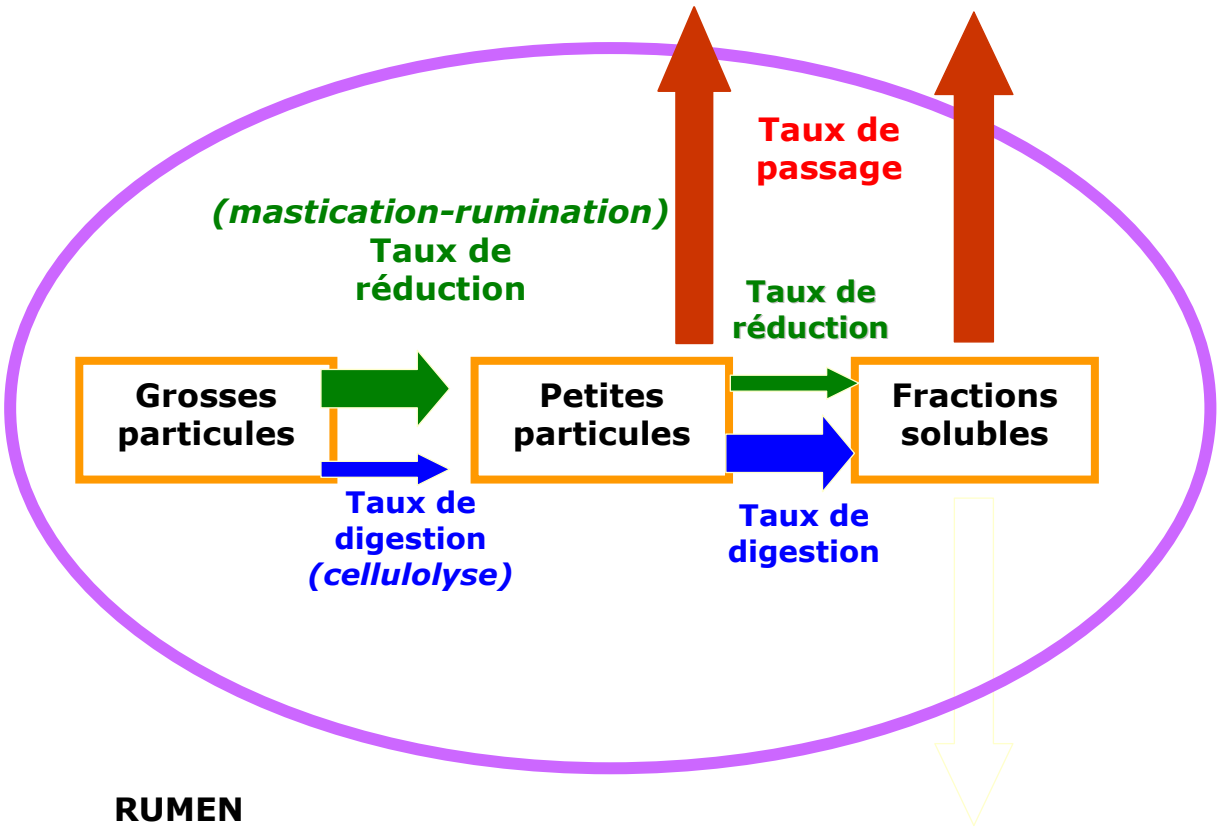


Figure 1. Phénomènes physiques et chimiques entrant en jeu dans la dégradation des particules fourragères dans le rumen.

ignoré cette particularité en étudiant par exemple la valeur des fourrages tropicaux aux mêmes âges de repousses que ceux retenus en zone tempérée.

La conclusion générale des recherches conduites (analyse biochimique des fourrages, étude de la digestion et des performances animales…) est que les fourrages tropicaux sont de qualité nutritive moyenne à médiocre. Ces fourrages sont présentés comme des ressources fourragères de valeur alimentaire intermédiaire entre les graminées herbacées et les pailles. L'analyse des bases de données de fourrages de la zone tropicale indique en effet de faibles valeurs de digestibilité et d'ingestibilité. La faible digestibilité des fourrages tropicaux a été expliquée (Leng and Preston, 1986 ; Leng, 1990 ; Minson, 1990) par leurs caractéristiques physico-chimiques qui pénalisent la capacité cellulolytique du rumen (difficulté à dégrader les constituants pariétaux, faible activité de la population microbienne). De plus, la faible ingestibilité de ces mêmes fourrages a été essentiellement reliée à leur faible digestibilité.

Des travaux (Wilson et al., 1989a, 1994) ont cependant apporté des connaissances nouvelles sur les graminées tropicales : la structure anatomique des feuilles des graminées tropicales se singularise de celles des graminées tempérées par un nombre élevé de cellules indigestibles. Ces données interrogent les chercheurs étudiant les ressources tropicales sur la pertinence de certains modèles de déterminisme de l'ingestion. Quel est le poids relatif des facteurs biochimiques, enzymatiques et mécaniques ?

Ces dernières connaissances nous ont conduits à nous interroger, dans le cadre de cette thèse, sur l'analyse des facteurs limitant l'ingestion des graminées tropicales.

Nous avons fait les hypothèses suivantes que nous nous proposons de tester:

1) valorisés à des stades physiologiques assez précoces, les caractéristiques physico-chimiques des fourrages tropicaux seraient tels que l'ingestiblité et la digestibilité pourraient être significativement améliorées,

2) la capacité cellulolytique du rumen ne serait pas le premier facteur limitant l'ingestion et la digestion des fourrages,

3) la résistance à la mastication et en conséquence la vitesse de réduction de taille des particules alimentaires (comminution) serait le premier facteur limitant l'ingestion.

Démarche générale de recherches

Notre modèle d'étude de l'ingestion des fourrages est résumé de façon schématique par la figure 1. Le fourrage ingéré par l'animal est constitué de particules de grande taille qui sont

réduites en petites particules sous l'effet des mastications ingestive et mérycique[1]. Dans notre cas, la discrimination entre ces particules a été effectuée par une grille de 1.18 mm d'ouverture. Les petites particules subissent l'action de 2 phénomènes simultanés: une évacuation hors du rumen et une digestion enzymatique microbienne dans le rumen, les grosses particules restent piégées dans le rumen (= effet d'encombrement), par contre elles subissent un début de solubilisation-digestion. Les fractions solubles sont fermentées sur place ou sont évacuées hors du rumen. La vitesse de disparition des particules alimentaires dans le rumen conditionne l'ingestion qui serait donc d'autant plus élevée que les taux de digestion et d'évacuation sont élevés. L'activité enzymatique est d'autant plus efficace que le nombre et la vitesse d'apparition des particules de petite taille sont élevés. En effet, l'augmentation de la surface spécifique des particules alimentaires favorise leur colonisation microbienne et leur dégradation enzymatique.

Le choix de ce modèle d'étude impliquait que les travaux soient réalisés sur des animaux expérimentaux porteurs de canules ruminale et duodénale afin de réaliser toutes les mesures nécessaires.

L'objectif du travail était d'avoir des résultats de portée générale. Pour cela nous avons effectué une analyse bibliographique quantitative à partir d'une vaste base de données sur la digestion, l'ingestion, la mastication… des fourrages tropicaux que nous avons comparés aux fourrages tempérés et aux pailles. A ce propos, nous avons fait l'hypothèse que les différences observées devraient avant tout être attribuées à la « dureté » des parois des fourrages tropicaux. Au niveau expérimental, il était impossible de travailler sur de nombreux fourrages. Deux fourrages ont été retenus parce qu'ils étaient largement utilisés dans la zone intertropicale et qu'ils représentaient un modèle de plante stolonifère (*Digitaria decumbens ou* Pangola*)* et un modèle de plante thallifère *(Panicum Maximum* ou Herbe de Guinée). Le Pangola et l'Herbe de guinée sont des fourrages bien référencés. De plus, ces deux types de fourrage présentaient une évolution de leur rapport feuilles /tiges différentes (rapide pour le pangola et lente pour l'herbe de Guinée) qui nous a permis de faire varier la qualité nutritionnelle du fourrage. De plus, nous avons aussi fait varier la qualité nutritionnelle du fourrage à travers l'âge de repousse (de 14 à 56 jours).

Le bilan de la digestion des parois végétales dans le rumen est la résultante d'au moins trois forces : l'action des enzymes, la réduction physique de la taille des particules alimentaires

[1] La mastication est l'activité physique buccale du ruminant qui permet de réduire les aliments en particules de petites tailles. Elle est dite ingestive quand elle se déroule pendant l'ingestion d'aliment. Elle est dite mérycique quand elle a lieu suite à la remontée d'un bol alimentaire *via* l'œsophage.

(qui augmente la surface spécifique accessible par les enzymes), et le temps de séjour[2] des particules alimentaires dans le rumen (qui limite plus ou moins le temps d'action des enzymes). A l'action de ces forces, il convient d'ajouter la dégradabilité intrinsèque des parois végétales qui varie d'un fourrage à l'autre et en fonction du stade physiologique. Nous avons utilisé ces indicateurs de la digestion dans nos expérimentations.

L'activité et la capacité cellulolytique du rumen ont été approchées par différentes méthodes en l'absence d'une méthode de référence. Ainsi, des sachets de nylon renfermant une matière première de référence (témoin) ont été mis à incuber dans le rumen.

En ce qui concerne la réduction de taille des particules, en absence de méthode standardisée, nous avons évalué le travail masticatoire par le temps de mastication (ingestion + rumination) d'une unité d'aliment ingéré. L'analyse de la cinétique d'évolution de la granulométrie des particules alimentaires dans le rumen a aussi été réalisée pour être confrontée aux résultats de mastication.

La thèse s'est déroulée à l'INRA, Station de Recherches Zootechniques. De nombreux travaux y ont été réalisés sur les fourrages de la zone tropicale humide valorisés en vert. Des tables de valeurs alimentaires (Xandé et Garcia-Trujillo, 1985 ; Aumont et al, 1991) et synthèses (Aumont et al, 1995) ont été publiées. La principale conclusion de ces travaux est que l'âge de repousse est le principal facteur expliquant les différences de valeur nutritive entre les fourrages tropicaux. La teneur en protéines des graminées tropicales est très variable mais excède rarement 12 % alors que les teneurs en ADF et NDF sont peu variables avec des valeurs moyennes de 360 et 670 g/ kg de matière sèche. Des travaux plus récents à l'URZ ont eu pour objectif l'amélioration de la valeur nutritive par des stratégies de supplémentation (énergie et azote fermentescible) pour stimuler les fermentations microbiennes (Archimède et al, 1999a) mais cette voie semblait peu prometteuse. D'autres travaux ont porté sur la gestion du fourrage (âge de repousse) comme méthode pour l'amélioration de la valeur nutritive (Archimède et al, 2000). La thèse a été programmée dans le prolongement de ces derniers travaux.

La première partie de ce document sera consacrée à l'étude bibliographique des données de la littérature (Publication 1). La deuxième partie de ce document sera consacrée aux résultats de l'étude expérimentale (Publications 2 à 8). Enfin, la dernière partie de ce document sera consacrée à la discussion générale des résultats, et aux principales conclusions et perspectives.

[2] Le temps de séjour correspond à la durée moyenne pendant laquelle une particule alimentaire est piégée dans le rumen, il est égal à l'inverse du taux fractionnaire de transit.

ETUDE BIBLIOGRAPHIQUE

META-ANALYSE BIBLIOGRAPHIQUE : ETUDE COMPARATIVE DE L'INGESTION ET DE LA DIGESTION DES FOURRAGES TROPICAUX ET TEMPERES

La comparaison de l'ingestion et de la digestibilité entre les fourrages tempérés et tropicaux, toute chose égale par ailleurs, n'est pas chose aisée. En effet, les conditions climatiques extrêmes de culture de ces deux types de fourrages rendent très difficiles, voire impossible, leur comparaison au sein d'un même laboratoire.

L'objectif principal de ce travail est de pouvoir comparer les fourrages tropicaux et tempérés à travers les lois générales explicatives les plus connues de la valeur alimentaire des fourrages. Une base de données bibliographiques (exhaustive) des essais de la littérature au sein desquels sont donnés des paramètres caractérisant l'ingestion et la digestion (composition chimique, quantités ingérées, digestibilité…), a été constituée. L'analyse des données de cette base par une méthode statistique quantitative, appelée méta-analyse (St-Pierre, 2001), nous a permis : 1) de comparer l'ingestion et la digestion des fourrages tropicaux et tempérés ; 2) d'avoir un début de réponse à nos hypothèses de travail.

PUBLICATION 1

Etude comparative de l'ingestion et de la digestion des fourrages tropicaux et tempérés

Assoumaya C., Sauvant D., Archimède H.

(soumis à Productions Animales en 2006)

La comparaison des valeurs alimentaires des fourrages tempérés et tropicaux n'est pas aisée. En effet, une des difficultés majeures réside dans le fait qu'il existe peu d'expériences comparatives réalisées au sein d'un même laboratoire et dans des conditions expérimentales satisfaisantes, qui se sont d'ailleurs limitées à des comparaisons de foin. L'objectif de ce texte est de pouvoir comparer via des méta-analyses d'une base de données, les fourrages tropicaux et tempérés à travers les lois générales explicatives les plus connues de la valeur alimentaire des fourrages.

Résumé

Des lois générales caractéristiques de l'ingestion et de la digestion des fourrages tempérés ont été appliquées aux fourrages tropicaux. Cependant, nous ne disposons pas d'étude permettant d'affirmer si ces lois établies sur fourrages tempérés sont extrapolables aux fourrages tropicaux. En effet, des comparaisons strictes de ces deux types de fourrages par la méthode expérimentale est difficilement réalisable du fait des différences de conditions de milieux et de physiologie. En conséquence, nous avons réalisé une méta-analyse d'une base de données regroupant des publications sélectionnées sur la mesure de certains critères (ingestion, digestibilité, temps de mastication, encombrement ruminal…). Les conclusions classiquement établies sur les différences entre fourrages tropicaux et tempérés sont confirmées. En effet, les fourrages tempérés étaient en moyenne plus riche en protéines brutes, moins fibreux, mieux ingérés et digérés qu'un fourrage tropical. De plus, l'indice de mastication était moins important avec les fourrages tempérés. Des informations originales ont été obtenues. Ainsi, à même teneur en protéines brutes et en paroi cellulaire, alors que les hiérarchies au niveau de l'ingestion et de l'indice de mastication étaient conservées, les différences de digestibilité disparaissaient. Ces résultats impliquent que chercher à mieux valoriser les fourrages tropicaux revient à se focaliser plutôt sur l'amélioration de l'ingestion que sur celle de la digestibilité. En effet, ils suggèrent la prépondérance de mécanismes physiques (mastication) relativement aux mécanismes biochimiques (capacités cellulolytiques du rumen) dans les dynamiques ingestives et digestives.

Abstract

Specifics laws characteristic of intake and digestion of temperate forages have been generalised to tropical grasses. However, no study has confirmed if laws established with temperate forages can be extrapolated and applied to tropical forages, because of the difficulty to compare these forages experimentally (climatic conditions, specific physiology).

Consequently, we conducted a meta-analysis of a data base by regrouping publications selected with some criteria (intake, digestibility, chewing time, rumen load…). General results established between tropical and temperate forages have been confirmed: crude protein, intake and digestibility were higher and cell wall and index chewing were lower with temperate forage than with tropical forage. More, it appeared that at same content in crude protein and NDF, the hierarchies in intake and index of chewing were the same, whereas differences in digestibility disappeared. This result implies that when trying to valorize tropical forage, we have to focus on the improvement of intake and not on the improvement of digestibility. Our work seems to indicate the preponderance of physical mechanisms (chewing) relative to the biochemical mechanisms (cellulolytic capacities in the rumen) in the ingestive and digestive dynamics.

Introduction

La comparaison de la valeur alimentaire des fourrages tempérés et tropicaux a déjà été l'objet de synthèses dans la littérature. (Leng 1990, Minson 1990, Van Soest *et al* 1991). L'une des difficultés majeures pour mener à bien cette comparaison concerne le faible nombre d'expériences comparatives réalisées au sein d'un même laboratoire et dans des conditions expérimentales satisfaisantes (tout chose égale par ailleurs). Dans ce contexte, la méta-analyse nous a semblé être un outil adéquat pour synthétiser la connaissance et aboutir à des comparaisons probantes grâce à la prise en compte d'un grand nombre d'essais publiés. De manière à pouvoir contrôler au mieux la variabilité inter-expérimentale, la comparaison entre les deux types de fourrages est effectuée à travers des lois générales connues de réponses de l'ingestion et de la digestion aux principales caractéristiques des régimes. L'objectif principal de l'étude était de vérifier si ces lois générales obtenues avec les fourrages tropicaux sont comparables à celles des fourrages tempérés et, dans l'affirmative, de permettre ainsi d'affiner leur comparaison.

Tableau 1. Répartition du nombre d'observations obtenu dans la base de données par type de fourrages (tempéré, tropical ou paille) pour les analyses de composition chimique, d'ingestion, de digestion totale et ruminale, de comportement alimentaire, de contenu ruminal et de transit des particules solides et liquides.

Fourrage	Tempéré	Tropical	Paille
Nb. Total d'observations	841	737	84
Expérimentations travaillant :			
sur le type fourrage	365	493	41
sur l'âge de repousse	109	349	.
sur le niv. d'ingestion	127	99	14
sur le trait. physique	100	15	.
sur le mode de conservation	70	3	12
sur la saison	56	51	.
Ovins à l'entretien	339	472	56
Ovins allaitants	0	16	0
Bovins à l'entretien	315	229	28
Bovins allaitants	187	20	0

1/ Construction de la base

Les articles de la base de données ont été retenus lorsqu'au moins l'une des caractéristiques suivantes étaient présente : durée de mastication, volume du rumen, transit digestif, digestibilité totale et/ou ruminale des principaux constituants (MO, NDF…). Les expérimentations dans lesquelles, les animaux étaient nourris en condition restreinte et /ou avec une teneur en concentré supérieure à 20% n'ont pas été prises en compte dans cette étude. De même, les fourrages broyés, les régimes mixtes (paille + fourrage) contenant plus de 15% de fourrages, ainsi que les animaux en lactation ont été exclus du présent traitement.

1-1/ Descriptif de la base de données

La base constituée contenait 1693 traitements conduits à travers 760 expérimentations issues de 360 publications scientifiques. Les expériences et les publications ont été codées. Des codages spécifiques ont été appliqués afin de discriminer la nature du fourrage (tempéré, tropical, paille) et le type d'animal (bovin ou petits ruminants). De plus, les principaux traitements expérimentaux étudiés ont été codés afin de pouvoir étudier leurs effets de façon spécifique. Il s'agit, par ordre décroissant de fréquence, du type de fourrage (graminées, légumineuses) (n = 899), du stade physiologique du fourrage (<28j, 28 à 35j, >35j) (n = 458), du niveau d'ingestion (à volonté, restreint) (n = 240), du traitement physique (haché, broyé, long) (n = 115), de l'influence de la saison (été, automne, printemps, hiver) (n = 107) et du mode de conservation (ensilage, foin, paille, vert, pâture) (n = 85). Le tableau 1 présente le nombre d'observations de ces différentes modalités expérimentales.

Afin d'extraire des lois générales applicables à tous les ruminants, les données d'ingestion ont été rapportées au poids vif ou métabolique. De même, les indices de mastication des ovins et caprins ont été divisées par 10 (rapport moyen des poids vifs entre bovins et petits ruminants) pour pouvoir les associer à ceux des bovins. Certains paramètres (indices d'encombrement, temps de séjour dans le rumen…) ont été calculés à partir des caractéristiques recueillies dans les publications. Les méthodes de calcul spécifiques appliquées sont explicitées avant de présenter les résultats correspondants.

Le modèle statistique utilisé avait pour principe d'intégrer les lois générales les plus connues, à l'aide d'une ou plusieurs co-variables (NDF, MAT du régime…), tandis que le type de fourrage et l'espèce animale étaient considérés comme des facteurs qualitatifs. Le maximum

Tableau 2. Valeurs moyennes de composition chimique, d'ingestion, de digestibilité, de dégradation ruminale, de comportement alimentaire, de contenu ruminal total et de transit des différentes classes de fourrage (tempéré, tropical ou paille).

Fourrage	Tempéré	Tropical	Paille
Données de composition chimique (%MS) :			
Neutral detergent fiber (NDF)	54.13 (n=695 ; σ=13.60)	70.13 (n=529 ; σ =8.84)	75.97 (n=54 ; σ =7.09)
Acid detergent fiber (ADF)	33.23 (n=650 ; σ =8.70)	39.76 (n=375 ; σ =7.74)	49.61 (n=45 ; σ =2.31)
Lignine (ADL)	4.88 (n=394 ; σ =2.60)	7.13 (n=353 ; σ =3.09)	7.04 (n=45 ; σ =2.31)
Protéine (MAT)	13.40 (n=796 ; σ =6.25)	9.75 (n=679 ; σ =4.54)	5.37 (n=71 ; σ =2.86)
Données d'ingestion/ PM exprimées en (g/kg $PV^{0.75}$) :			
Matière sèche	80.01 (n=775 ; σ=32.94)	59.13 (n=693 ; σ =18.35)	52.20 (n=73 ; σ =23.27)
Neutral detergent fiber	42.01 (n=640 ; σ =16.32)	42.32 (n=496 ; σ =14.37)	39.01 (n=62 ; σ =16.35)
Acid detergent fiber	26.25 (n=588 ; σ =9.92)	23.36 (n=355 ; σ =7.32)	25.14 (n=56 ; σ =9.15)
Lignine	3.74 (n=372 ; σ =2.11)	4.11 (n=325 ; σ =2.05)	3.68 (n=45 ; σ =2.10)
Protéine	13.17 (n=751 ; σ =18.54)	5.96 (n=544 ; σ =5.72)	5.44 (n=71 ; σ =7.77)
Données de digestibilité totale exprimées en (%):			
Matière Organique	65.57 (n=651 ; σ =10.52)	59.99 (n=620 ; σ =8.22)	51.60 (n=73 ; σ =6.16)
Neutral detergent fiber	60.23 (n=406 ; σ =12.30)	60.74 (n=336 ; σ =9.86)	53.17 (n=53 ; σ =9.05)
Acid detergent fiber	55.51 (n=257 ; σ =12.56)	59.04 (n=161 ; σ =10.99)	51.17 (n=34 ; σ =11.27)
Protéine	62.78 (n=407 ; σ =17.00)	55.79 (n=295 ; σ =19.13)	26.36 (n=48 ; σ =39.76)
Données de dégradation ruminale exprimées en (%):			
Matière Organique	47.17 (n=246 ; σ =11.71)	45.93 (n= 93 ; σ =7.79)	30.57 (n=23 ; σ =12.68)
Neutral detergent fiber	56.72 (n=128 ; σ =14.87)	54.88 (n= 61 ; σ =12.83)	52.28 (n= 4 ; σ =5.89)
Acid detergent fiber	46.15 (n= 63 ; σ =13.32)	52.01 (n= 38 ; σ =16.22)	38.73 (n= 6 ; σ =12.30)
Données de comportement alimentaire (min):			
Durées d'ingestion	313 (n=321 ; σ =144)	396 (n= 89 ; σ =101)	192 (n=8 ; σ =67)
Durées de rumination	463 (n=327 ; σ =121)	461 (n= 99 ; σ =89)	573 (n=8 ; σ =200)
Données de contenu ruminal total exprimées en (kg):			
Matière sèche *(bov. à l' ent.)*	8.44 (n=87 ; σ =3.68)	7.62 (n= 39 ; σ =1.89)	5.84 (n=21 ; σ =2.27)
Neutral detergent fiber *(bov. à l' ent.)*	4.16 (n= 32 ; σ =2.30)	6.83 (n= 10 ; σ =1.21)	5.57 (n=2 ; σ =0.66)
Acid detergent fiber *(bov. à l' ent.)*	0.93 (n= 9 ; σ =0.37)	1.67 (n= 4 ; σ =0.21)	.
Matière sèche *(ov. à l' ent.)*	1.04 (n=58 ; σ =0.41)	0.95 (n= 88 ; σ =0.47)	0.88 (n=16 ; σ =0.46)
Neutral detergent fiber *(ov. à l' ent.)*	0.71 (n= 34 ; σ =0.31)	0.76 (n= 51 ; σ =0.29)	0.58 (n=14 ; σ =0.20)
Acid detergent fiber *(ov. à l' ent.)*	0.41 (n= 18 ; σ =0.22)	0.46 (n= 25 ; σ =0.21)	0.41 (n=12 ; σ =0.10)
Données de transit (h^{-1}):			
Phase liquide	0.097 (n=236 ; σ =0.043)	0.065 (n= 106 ; σ=0.017)	0.057 (n=41 ; σ =0.029)
Particules solides	0.035 (n=329 ; σ =0.014)	0.032 (n= 218 ; σ =0.012)	0.030 (n=59 ; σ =0.015)

de données a toujours été pris en compte, cependant, lorsqu'un traitement ou une expérimentation était à l'évidence aberrant (au-delà de 3 écarts-types par rapport aux valeurs ajustées) pour un caractère ou une relation considérée elle a été retirée de l'analyse statistique. La méta-analyse des données a été effectuée suivant les recommandations de Sauvant *et al*, 2005 en utilisant le logiciel Minitab 2003. Le modèle statistique appliqué est le suivant :

$Y_{ijk} = \mu + \alpha_i + \beta_j + \Sigma A\ X_{ijk} + e_{ijk}$

Avec μ moyenne générale

αi effet fourrage (2DL)

βj effet bovins vs petits ruminants (1 DL)

$\Sigma\ A\ X_{ijk}$ covariable exprimant une loi générale connue

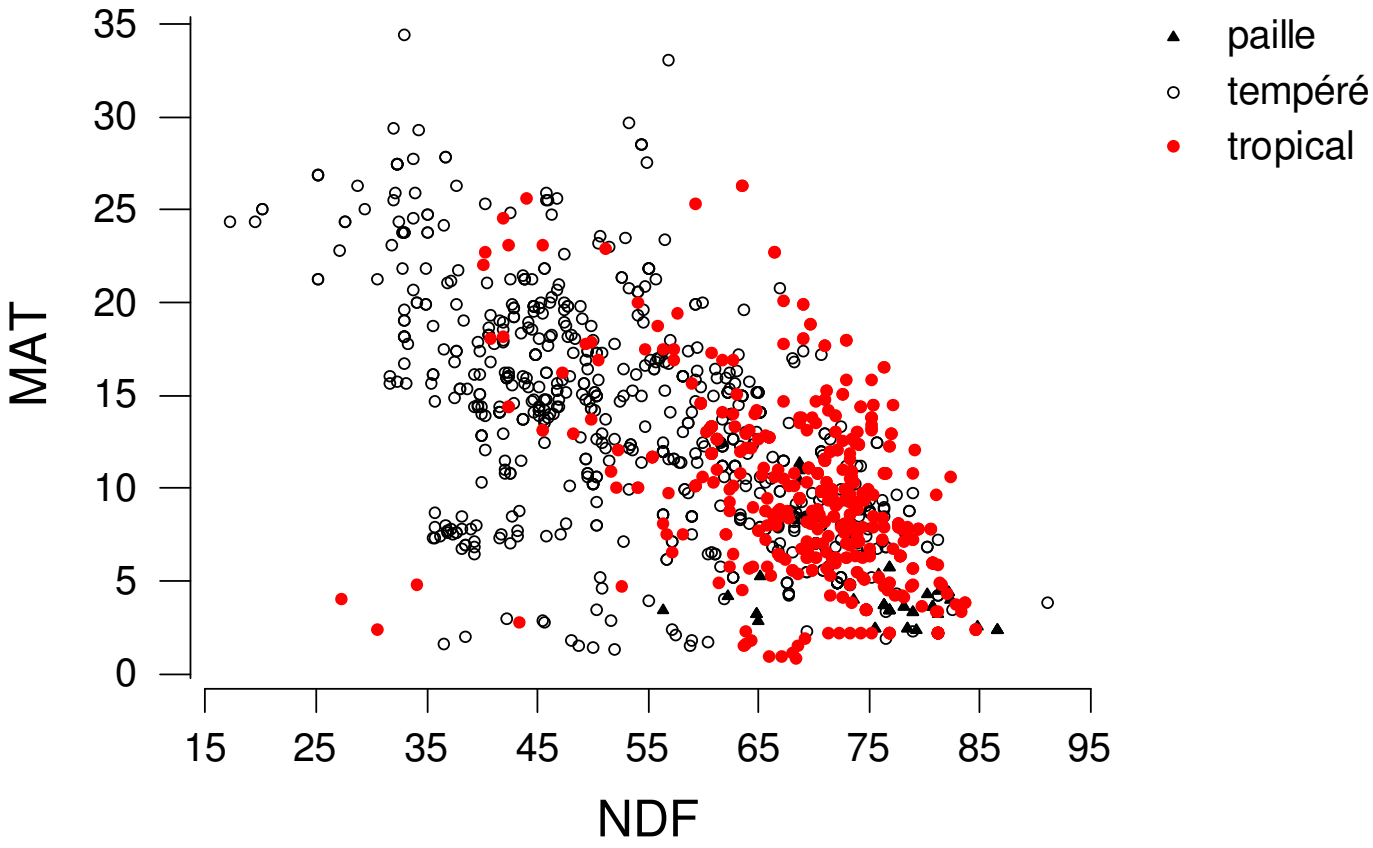
Les lois générales exprimées par les covariables sont rapportées dans le texte, il en est de même des caractéristiques moyennes des groupes non ajustées par des covariables. Par contre, les valeurs ajustées par les covariables sont présentées dans des tableaux.

2/ Résultats

2-1/ Composition chimique

Le tableau 2 présente les paramètres statistiques des principaux critères chimiques cités dans les publications. Il apparaît que, sur les échantillons considérés, les teneurs en constituants pariétaux des fourrages tempérés sont plus faibles que pour les fourrages tropicaux. Par contre, la comparaison entre les fourrages tropicaux et les pailles ne révèle pas de différence significative pour la teneur en NDF. Les teneurs en MAT des fourrages tempérés sont plus élevées que celles des tropicaux. Ces dernières sont plus élevées que celles des pailles.

Des relations lient, et parfois de façon étroite, certains des critères présentés. Il s'agit en particulier des constituants pariétaux, sachant que la différence entre les résidus NDF et ADF, qualifiée d'hémicelluloses, est très liée à la famille végétale. Ainsi, la teneur (% MS) en hémicelluloses des graminées est de 27.30 (n=759 ; σ = 7.18) et 12.12 (n=167 ; σ = 5.39) pour les légumineuses. La figure 1, qui discrimine les 3 types de fourrages, met en évidence une corrélation négative entre la MAT et le NDF tout en illustrant une forte variabilité de la MAT pour un même niveau de NDF. Le traitement statistique de la relation entre les critères MAT et NDF indique que la même régression s'applique aux fourrages tempérés et tropicaux. Ainsi, pour une même teneur moyenne en NDF (61.6%), les fourrages tempérés et tropicaux présentent une même teneur en MAT (11.5% et 11.7%), tandis que les pailles sont à un niveau inférieur (8.17%). Les régressions par groupe sont les suivantes :



MAT (%)= -0.26 NDF (%) + 28 (pour les fourrages tempérés et tropicaux) (n = 1171 ; σ = 4.63 ; P= 0.000 ; R^2 = 40%)

MAT (%)= -0.14 NDF (%) + 14.7 (pour les pailles) (n = 54 ; σ = 2.12 ; P= 0.002 ; R^2 = 17.4%)

Figure 1. Evolution de la teneur en protéines du fourrage en fonction de celle en Neutral detergent fiber (NDF), exprimées en pourcentage de matière sèche pour les différentes classes de fourrages (tempéré, tropical ou paille).

MAT (%)= -0.26 NDF (%) + 28 (pour les fourrages tempérés et tropicaux) (n = 1171 ; σ = 4.63 ; P= 0.000 ; R^2 = 40%)

MAT (%)= -0.14 NDF (%) + 14.7 (pour les pailles) (n = 54 ; σ = 2.12 ; P= 0.002 ; R^2 = 17.4%)

2-2/ Ingestion

Lorsque aucune covariable n'est prise en compte, des différences hautement significatives apparaissent entre les niveaux d'ingestion des différents types de fourrages, qu'ils soient exprimés par rapport au poids métabolique (MSIPM = gMSI/ kg $PV^{0.75}$) ou au poids vif (MSIPV= kg MSI/ % kg PV). Quel que soit le mode d'expression, la hiérarchie demeure identique. Pour MSIPM, les résultats sont respectivement pour les fourrages tempérés (Te), tropicaux (Tr) et pailles (Pa) 68.70a ; 57.83b ; 47.36c (n= 1216; σ=20.30; P<0.001 ; R^2= 8.61%). Pour MSIPV ils sont de 2.033a; 1.950b; 1.631c (n= 1172; σ=0.61; P<0.001 ; R^2 =1.92%). Les covariables ont été sélectionnées en testant des relations globales entre l'ingestion de matière sèche (MSI % PV ou MSI/g PM) et les teneurs en NDF ou MAT des mêmes fourrages. Les meilleures régressions ont été obtenues avec MAT:

MSIPM (g MSI /kg $PV^{0.75}$) = - 0.08 MAT^2 (%) + 2.76 MAT (%) + 44.65 (n = 1159 ; σ = 17.06 ; P<0.001 ; R^2 = 34.54%)

MSIPV (kg MSI/ %kg PV) = - 22.10^{-4} MAT^2 (%) + 0.08 MAT (%) + 1.31 (n = 1125 ; σ= 0.57 ; P<0.001 ; R^2 = 17.81%)

A composition comparable, la hiérarchie entre bovins et petits ruminants est inversée selon le mode d'expression de l'ingestion, ramenée au poids vif ou au poids métabolique (tableau 3). En ce qui concerne les fourrages, la hiérarchie de l'ingestibilité est la même selon le mode d'expression de l'ingestion mais les seuils de signification diffèrent. En effet, les fourrages tempérés sont plus ingestibles que les fourrages tropicaux et les pailles, cependant l'écart n'est significatif que lorsque la quantité ingérée est rapportée au PM (+4.48g MSI/kg PM entre fourrages tempérés et tropicaux) et en limite lorsqu'elle est rapportée au PV (tableau 3).

2-3/ Digestibilité totale (MO et NDF)

Lorsque aucune covariable n'est prise en compte, des différences hautement significatives séparent les valeurs de dMO des différents fourrages (Te=63.99a ; Tr=59.85b ; Pa= 51.49c ; avec n= 1112 ; σ=8.74 ; P<0.001 ; R^2= 10.93%). Pour la dMO, deux covariables majeures ont

Tableau 3. Estimation des paramètres d'ingestion et de digestion à même niveau de teneur en parois (covariables : MAT, NDF, MAT², NDF², ADL ou ADL²) ou à même niveau d'ingestion (covariables : MSIPM ou MSIPV)

	Tempéré	Tropical	Paille	P	Ruminants		P
					Petits	Gros	
Ingestion à même teneur en protéines brutes (covariables : MAT, MAT²) :							
Matière sèche ingérée (g MSI /kg $PV^{0.75}$)	67.43a	62.95b	58.14b	**0.000**	51.76a	73.92b	**0.000**
Matière sèche ingérée (kg MSI/ %kg PV)	1.969	1.916	1.801	**0.103**	2.044a	1.746b	**0.000**
Digestibilité totale de la matière organique et de la paroi (NDF) à même teneur en paroi et protéines brutes:							
- (covariables : NDF, NDF², MAT, MAT²)							
dMO (%)	61.91a	61.52a	57.39b	**0.004**	59.45a	61.10b	**0.002**
- (covariables : MAT)							
dNDF (%)	59.56a	61.82b	58.16a	**0.008**	58.84a	60.85b	**0.016**
- (covariables: MAT, ADL)							
dNDF (%)	59.17a	63.68b	55.79a	**0.000**	60.45a	58.64b	**0.035**
Ingestion de matière organique digestible exprimée sur le poids métabolique (MODIPM), à même teneur en protéine brute ou paroi :							
- (covariables : MAT, MAT²)							
MODIPM (g/kg $PV^{0.75}$)	39.87a	35.41b	30.59c	**0.000**	28.11a	42.47b	**0.000**
- (covariables : NDF²)							
MODIPM (g/kg $PV^{0.75}$)	38.83a	37.20a	28.78b	**0.000**	27.63a	42.24c	**0.000**

Figure 2

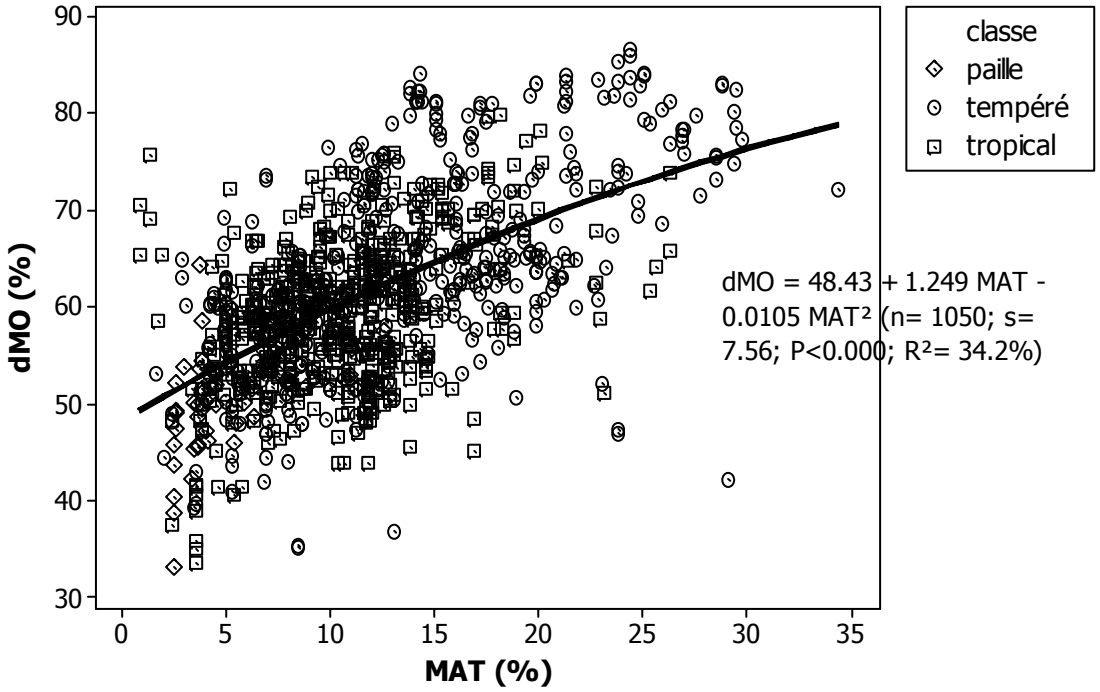
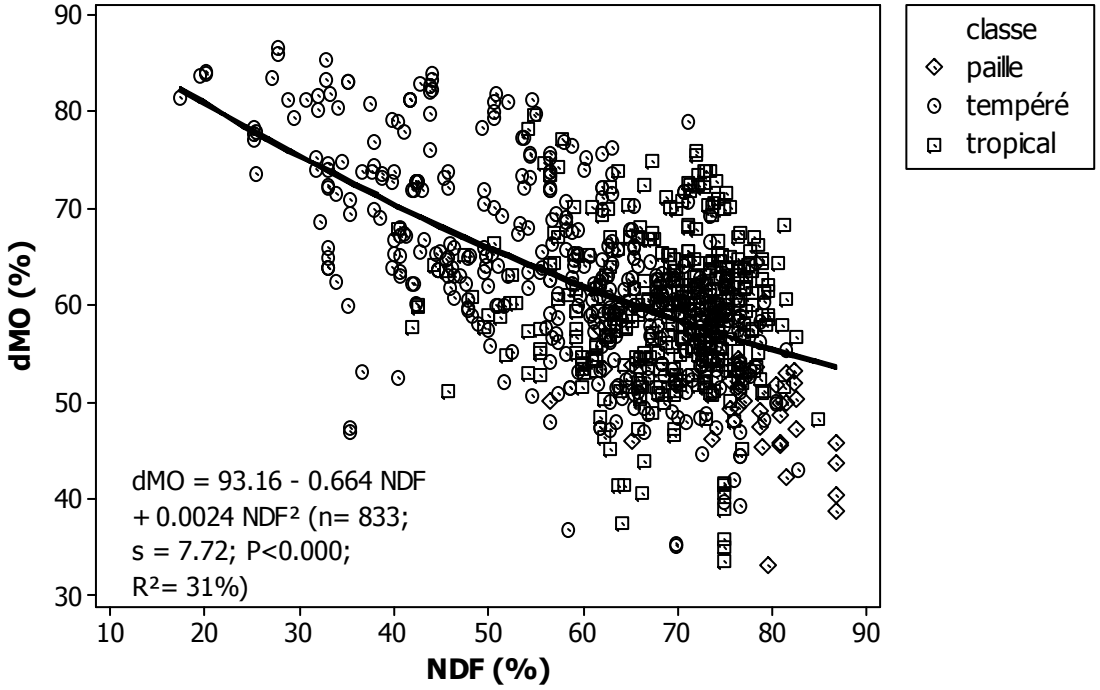


Figure 3



Figures 2 et 3. Digestibilité de la matière organique en fonction de la teneur en protéines brutes du fourrage (fig. 2) ou de celle en NDF (fig.3) pour les différentes classes de fourrage (tempéré, tropical ou paille).

été testées pour intégrer les relations présentées aux figures 2 et 3. La meilleure combinaison de celles ci intègre les termes quadratiques NDF² et MAT².

dMO (%)= 63.10^{-4}NDF² (%) - 30.10^{-3}MAT² (%) + 1.50MAT (%) – 0.89NDF (%) + 77.84 (n =800 ; σ = 6.99; P<0.005; R² =44.70%)

Dans cette analyse, il ressort qu'à même teneur en NDF et MAT, la dMO des fourrages tropicaux, est d'environ 1/2 point inférieure à celle des tempérés ; cette différence n'est pas significative (tableau 3). Par contre, la paille est significativement moins digérée que les deux autres fourrages.

Lorsqu'aucune covariable n'est prise en compte, les digestibilités des parois végétales des différents fourrages, quantifiées par le résidu NDF, sont significativement différentes en raison de la faible valeur de la paille (Te = 60.45a, Tr = 60.80a, Pa = 53.39b ; avec n= 651 ; σ=10.60 ; P<0.001 ; R² = 2.88%). Différents jeux de covariables ont été testés, contrairement à la teneur en NDF, celle de la MAT était significative :

dNDF (%)= 0.73 MAT (%) + 51.66 (n =639; σ =10.03; P<0.009 ; R² = 13.97%)

Avec cette covariable, la différence entre les fourrages de type tempéré et tropical a été significative à l'avantage de ces derniers (P<0.03) (tableau 3). Un autre jeu de covariables comprenant MAT et ADLignine a été testé. Bien que le nombre de traitements fût moindre, la précision du modèle d'ajustement était nettement meilleure :

dNDF (%)= - 1.46 ADL (%) + 0.72 MAT (%) + 61.40 (n =400; σ =7.9; P=<0.001 ; R² = 40.35%)

Avec ces covariables, les différences entre les fourrages ont été amplifiées (tableau 3). Il convient enfin de remarquer qu'avec ces données les bovins digèrent moins efficacement que les petits ruminants (tableau 3).

En ce qui concerne les niveaux d'ingestion de matière organique digestible exprimée sur le poids métabolique (MODIPM), lorsque aucune covariable n'est prise en compte, les différences entre fourrages sont hautement significatives (MODIPM : Te= 40.40a, Tr= 31.63b, Pa= 22.31c ; avec n= 1043 ; σ=13.34 ; P<0.001 ; R² = 13.28%)

Deux combinaisons significatives de covariables ont été testées sur la MODIPM :

MODIPM (g MODI /kg $PV^{0.75}$) = - 40.10^{-3} MAT² (%) + 1.82 MAT (%) + 20.85 (n =998; σ =11.05; P<0.001 ; R² = 41.13%)

MODIPM (g MODI /kg $PV^{0.75}$) = - 27.10^{-4} NDF² (%) + 46.14 (n =783; σ =11.36; P<0.001 ; R² = 38.47%)

Tableau 4. Estimation des indices de mastication, de l'encombrement ruminal et du temps de rétention moyen en fonction du type de fourrage (tempéré, tropical ou paille) et de l'espèce animale.

	Tempéré	Tropical	Paille	P	Ruminants		P
					Petits	Gros	
Indice de mastication à même teneur en paroi et en protéines brutes:							
- (covariables : NDF)							
IdMast (min/ kgMSI)	92.01a	98.70a	137.28b	**0.044**	92.48a	126.19b	**0.000**
- (covariables : NDF, MAT)							
IdMast (min/ kgMSI) **0.000**		87.82a	102.62b	129.63b **0.005**	89.52a		123.86b
Encombrement ruminal à même teneur en paroi ou même quantité d'ingéré en NDF par pourcentage de poids vif):							
- (covariable :NDF)							
MSRum %PV(kg/ %kg PV)	2.024a	2.181a	1.581b	**0.000**	2.015a	1.842b	**0.035**
NDFRum %PV(kg/ %kg PV)	1.206a	1.435b	1.074a	**0.000**	1.239	1.238	**0.983**
- (covariable :NDFI%PV)							
NDFRum %PV(kg/ %kg PV)	1.084a	1.469b	1.385b	**0.000**	1.343	1.282	**0.406**
- (covariables :NDF, NDF²)							
IENDFing (kgNDFru/kgNDFI)	1.151	1.240	1.386	**0.294**	1.120a	1.397b	**0.003**
- (covariables :NDF, NDF²)							
IEMSrufec (kgMSru/kgMSfec)	2.801ab	3.000a	2.435b	**0.026**	2.660	2.830	**0.191**
Transit à même quantité de MSI ramenée au poids métabolique ou au pourcentage de poids vif ou à même teneur en paroi :							
(covariable : MSIPM)							
MRT (h)	36.37a	36.82a	45.09b	**0.001**	32.20a	46.65b	**0.000**
(covariable : MSIPV, MSIPV²)							
MRT (h)	35.20a	36.75a	44.96b	**0.000**	36.11a	41.83b	**0.000**
(covariable : NDF, NDF²)							
MRT (h)	32.69a	36.87b	38.31b	**0.004**	32.32a	39.60b	**0.000**

Avec la première équation, les hiérarchies obtenues entre les trois fourrages sont globalement confirmées, cependant les écarts sont moins marqués (tableau 3). Avec la seconde équation les fourrages Te et Tr ne sont pas significativement discriminés.

2-4/ Critères masticatoires, d'encombrement et de transit digestif

a/ : Activités masticatoires (tableau 4)

Afin de rendre comparable les indices de mastication (IdMast), exprimées en min/kg MSI, entre gros et petits ruminants, une harmonisation des données a été réalisées : le rapport entre le poids vif des gros et petits ruminants correspondant à 10 (données de la base), les valeurs de quantités ingérées, utilisées pour le calcul des indices de mastication des petits ruminants ont donc été multipliées par 10. Lorsque aucune covariable n'était prise en compte, les indices de mastication des trois types de fourrages différaient significativement (Te = 83.02 a, Tr = 101.97b, Pa = 153.30c ; avec n= 217 ; σ=34.31 ; P<0.001 ; R^2= 13.75%).

Différentes covariables ont été appliquées. La teneur en NDF est la plus significative :

IdMast (min/kg MSI) = 1.13 NDF (%) + 40.10 (n = 160; σ = 29.78; P< 0.03 ; R^2= 35.16%)

Avec cette covariable les fourrages tempérés et tropicaux ne peuvent être discriminés alors que la paille demande, à NDF égal, un travail masticatoire bien plus important.

La combinaison de covariables la plus significative a associé les teneurs en NDF et en MAT de la ration :

IdMast (min/kg MSI) = 0.47 NDF (%) – 1.76 MAT (%) + 100.5 (n =154; σ =26.25; P<0.005 ; R^2= 46.25%)

A mêmes teneurs en NDF et MAT, les fourrages tropicaux nécessitaient un travail masticatoire plus important (+17%) que les fourrages tempérés. D'autre part, les rations contenant de la paille étaient plus mastiquées que les fourrages tropicaux (de l'ordre de 27 minutes), mais la différence n'était pas significative.

b/ Encombrement ruminal :

Plusieurs critères représentatifs de l'encombrement ruminal ont été calculés :

- les quantités de MS et de NDF du rumen rapportées au poids vif (MSRUM% PV ou NDFRUM%PV) ;

-les indices d'encombrement égaux aux quantités de matière sèche ou de NDF du rumen rapportés sur le flux fécal (IEMSrufec) ou ingéré du même constituant (IENDFing).

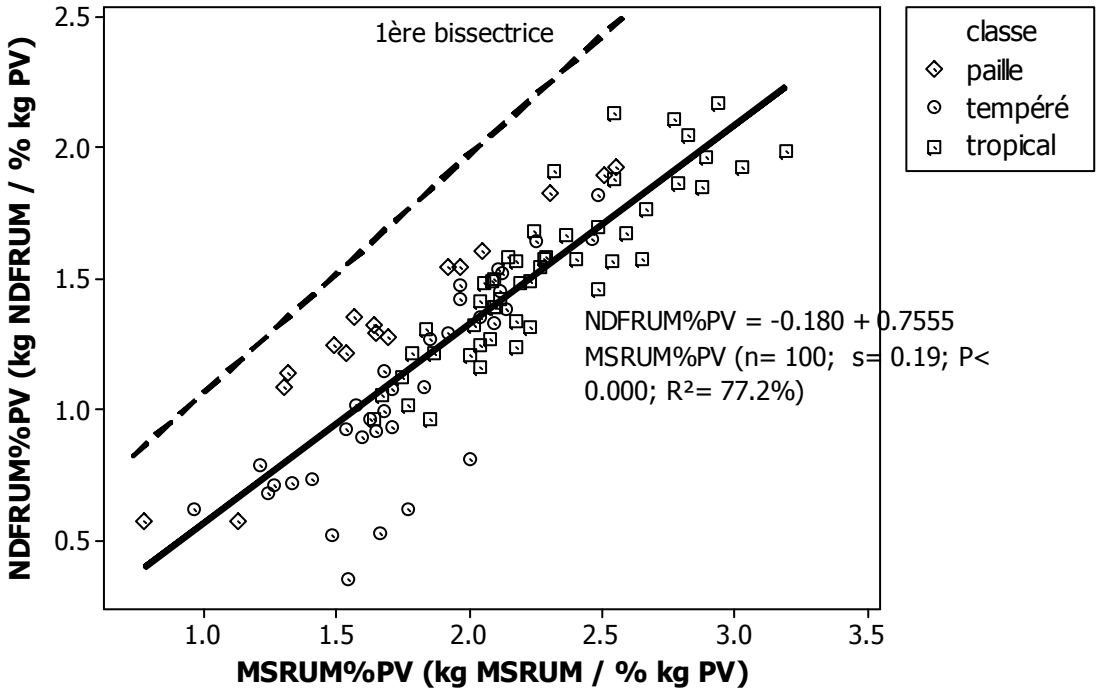


Figure 4. Evolution de l'encombrement ruminal exprimé en NDF par pourcentage de poids vif en fonction de l'encombrement ruminal exprimé en matière sèche par pourcentage de poids vif pour les différents types de fourrage (tempéré, tropical ou paille).

Ces derniers critères sont cohérents avec les durées de présence, exprimées en jours, des contenus digestifs dans le rumen. Lorsqu'aucune covariable n'était prise en compte, l'encombrement ruminal, rapporté au poids vif, était globalement plus élevé pour les fourrages tropicaux, comparativement aux fourrages tempérés et aux pailles (pour MSRUM%PV : Te= 1.903a; Tr=2.181b ; Pa=1.857a ; avec n =265; σ =0.64 ; P<0.001 ; R^2= 4.92% ; pour NDFRUM%PV : Te=0.999a; Tr=1.527b ; Pa=1.341b ; avec n =140; σ =0.34 ; P<0.001 ; R^2= 35.23%).

Les valeurs de l'encombrement ruminal, exprimées en MS ou en NDF (MSRUM%PV ou NDFRUM%PV), sont étroitement corrélées (figure 4). Les valeurs des coefficients de regression indiquent que la teneur en NDF des contenus ruminaux sont liés à ceux du fourrage ingéré. L'encombrement ruminal a été significativement et positivement influencé par la teneur en NDF de la ration ainsi que par le type d'animal considéré (P<0.001) :

MSRUM%PV (kg MSRUM / % kg PV) = 0.017 NDF (%) + 0.83

(n =243; σ =0.59; P<0.001 ; R^2= 16.75%)

L'analyse de covariance a indiqué qu'à même teneur en NDF dans la ration, l'encombrement ruminal en MS des fourrages tropicaux était plus important que celui des fourrages tempérés mais que la différence n'était pas significative, la paille étant nettement moins encombrante. Lorsque l'encombrement était calculé sur la base des quantités de NDF dans le rumen (NDFRUM%PV), la hiérarchie précédemment observée était respectée mais la différence entre Tr et Te devient alors significative (tableau 4). L'encombrement ruminal (NDFRUM%PV) a été plus significativement influencé par la quantité de NDF ingérée que par la teneur en NDF de la ration :

NDFRUM%PV (kg NDFRUM / %kg PV) = 0.19 NDFI%PV (kg NDFI / %kg PV) + 1.07 (n =124; σ =0.31; P<0.001 ; R^2 = 35.73%)

L'analyse de covariance de l'encombrement ruminal exprimé en NDFRUM%PV a confirmé que les fourrages tempérés étaient moins encombrants que les tropicaux et les pailles (tableau 4).

En absence de covariable, les indices d'encombrement des fourrages tempérés étaient plus faibles que ceux des tropicaux et des pailles (pour IEMSrufec : Te=2.629a; Tr=2.956b; Pa=3.048b; avec n =238; σ =0.92 ; P<0.02 ; R^2= 3.32% ; pour IENDFing : Te=0.947a ; Tr=1.209b ; Pa= 1.528c; avec n =127; σ =0.42 ; P<0.001 ; R^2= 17.15%). La teneur en NDF a présenté une influence quadratique sur ces critères d'encombrement :

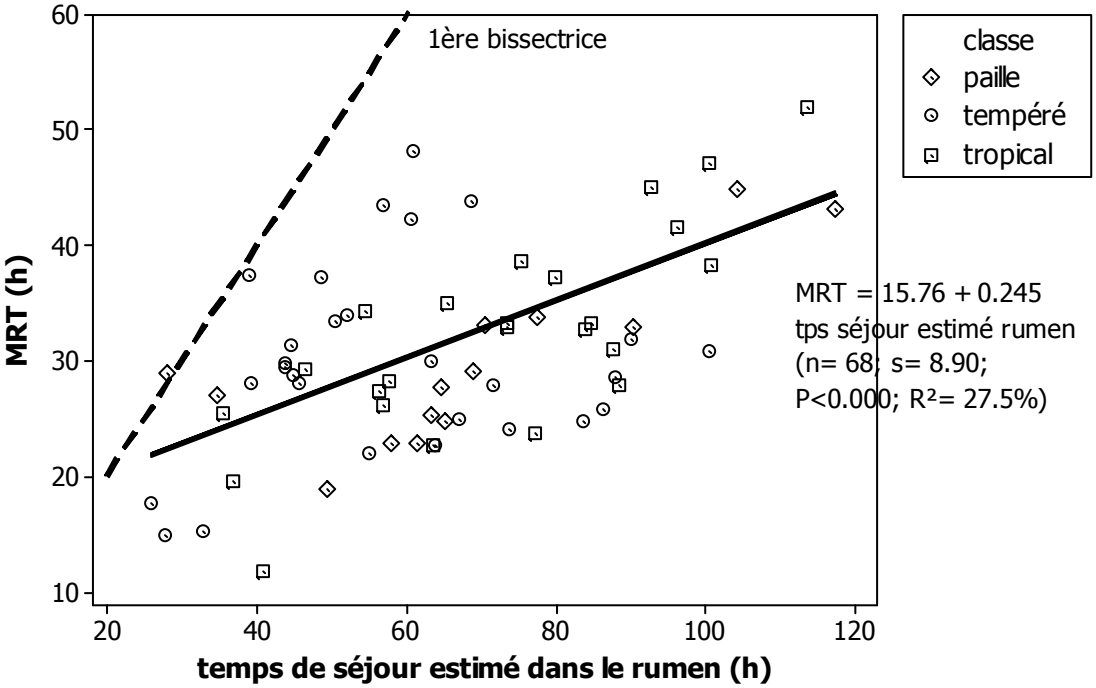


Figure 5. Evolution du temps de séjour de la matière sèche estimé par marqueurs (MRT,h) avec le temps de séjour du NDF estimé dans le rumen pour les différents types de fourrage (tempéré, tropical et paille)

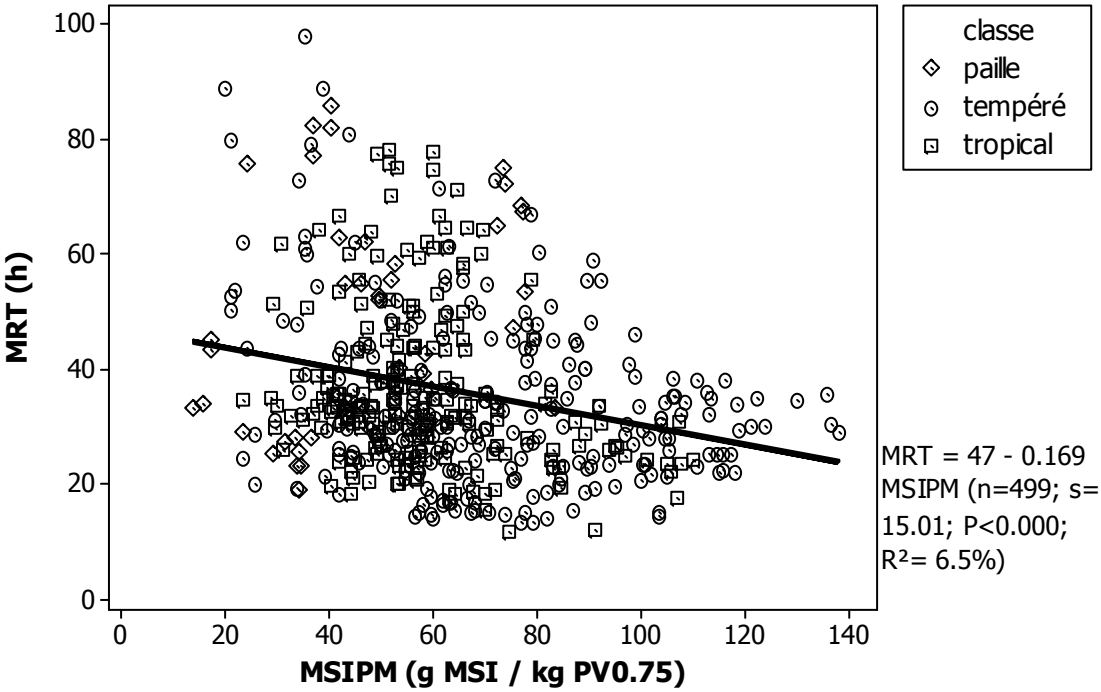


Figure 6. Evolution du temps de séjour des particules en fonction de la quantité de matière sèche ingérée par kg de poids métabolique pour les différentes classes de fourrage (tempéré, tropical ou paille).

IEMSrufec (kg MSRUM/ kg MSfec) = 11.10^{-4} NDF² (%) -0.106 NDF (%) +4.65 (n =216; σ =0.87; P<0.03 ; R²= 17.22%)

IENDFing (kg NDFRUM/ kg NDFI) = 13.10^{-5} NDF² (%) + 0.66 (n =127; σ =0.39; P<0.001 ; R²= 28.49%)

L'analyse de covariance avec ces covariables n'a pas mis en évidence de différence significative entre les fourrages sur le critère IENDFing, alors que des différences significatives apparaissaient entre les fourrages tropicaux et les pailles quand l'encombrement était exprimé en IEMSrufec.

c/ Transit digestif

Plusieurs variantes méthodologiques ont été utilisées. Les analyses ont été conduites sur des données de transit estimées par les temps de séjour dans le rumen, exprimés en heures (méthode des marqueurs). En absence de ces mesures, le transit était estimé à partir des quantités de MS (ou de NDF) dans le rumen, rapportées aux excrétions fécales journalières respectives. Les valeurs obtenues par cette dernière méthode étaient en général largement supérieures à celles obtenues par mesure directe à l'aide de marqueurs digestifs, cependant les tendances et les hiérarchies entre traitements sont demeurées comparables (figure 5).

En absence de covariable, la durée de transit, exprimée en heures, a été plus importante pour la paille (MRT : Te = 33.9a, Tr = 36.7a, Pa = 47.4b ; avec n= 513 ; σ=15.04; P<0.001 ; R² = 5.58%).

Ce paramètre était significativement influencé par les quantités de MS ingérées (rapportées au poids métabolique ou au pourcentage de poids vif) et par le type de ruminants (P< 0.001) (tableau 4 et figure 6).

MRT (h) = - 0.28 MSIPM (g MSI/kg $PV^{0.75}$) + 57.08 (n =499; σ =13.35; P< 0.002 ; R²= 26.43%)

MRT (h) = 1.78 MSIPV² (kg MSI/ %kg PV) - 15.23 MSIPV (kg MSI/ %kg PV) + 61.14 (n =499; σ =13.50; P<0.001 ; R²= 24.94%)

Lorsque les quantités ingérées étaient prises en covariable, aucune différence significative n'est apparue entre les fourrages tempérés et tropicaux (P >0.4), par contre les rations à base de paille présentaient un transit plus long. D'autre part, pour un même niveau d'ingestion exprimé sur le PM, les petits ruminants présentaient un transit ruminal plus rapide.

Tableau 5. Estimation des digestibilités ruminales exprimées en matière organique ou en NDF, quantité de matières azotées totale (MADUO%MSI) et de matières azotées microbienne (MAμDUO%MSI) dans le duodénum, efficacité microbienne (g d'azote microbien/ quantité de matière organique apparemment digérée dans le rumen) et digestibilité intestinale de l'azote en fonction des classes de fourrage et de l'espèce animale.

	Tempéré	Tropical	Paille	**P**	Ruminants		**P**
					Petits	Gros	
Digestibilité ruminale de la matière organique à même teneur en paroi, protéines brutes ou digestibilité totale de la MO:							
- (covariables : NDF², MAT):							
drMO (%)	45.95a	50.36b	33.02c	**0.000**	39.08a	47.14b	**0.000**
- (covariables : NDF, dMO)							
drMO (%)	46.17a	47.33a	36.20b	**0.000**	39.17a	47.29b	**0.000**
Digestibilité ruminale du NDF à même digestibilité totale en NDF:							
- (covariables : dNDF) :							
drNDF (%)	55.88	57.54	.	**0.121**	55.65a	57.77b	**0.041**
Bilan azoté à même teneur en paroi:							
- (*covariable : NDF*)							
MADUO%MSI (g/% gMSI)	12.60a	12.95a	9.79b	**0.024**	12.41a	11.15b	**0.043**
MAμDUO%MSI (g/% gMSI)	6.42	7.05	4.26	**0.236**	5.71	6.10	**0.423**
Effic μ (g/kgADOMR)	23.68	24.99	20.80	**0.682**	24.20	22.12	**0.208**
Digestibilité intestinale							
(cov NDF)							
DNint (%)	66.23a	61.18b	58.23b	**0.002**	64.39a	54.35b	**0.000**
(cov ADL)							
DNint (%)	68.73a	60.85b	56.68b	**0.000**	68.00a	56.17b	**0.000**

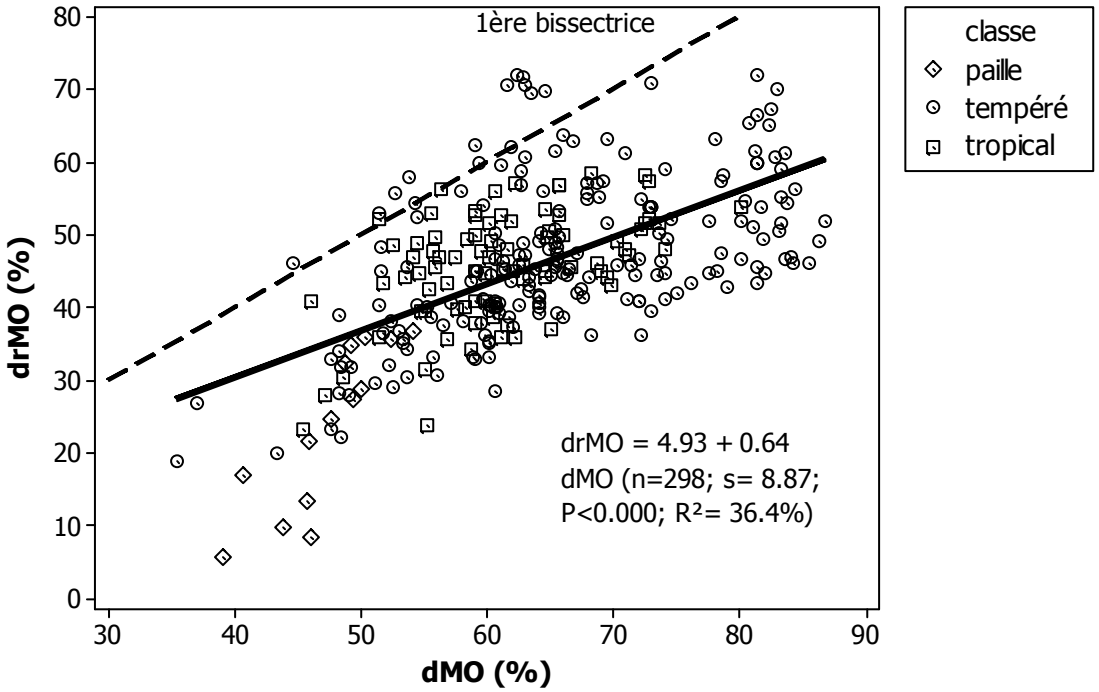


Figure 7. Variations de la digestibilité ruminale de la matière organique en fonction de sa digestibilité totale pour les 3 classes de fourrage (tempéré, tropical ou paille).

La teneur en NDF de la ration a aussi significativement influencé le transit digestif, vraisemblablement en relation avec son effet sur l'ingestion :

MRT (h) = 0.01NDF² (%) - 1.05 NDF (%) + 56.82 (n= 427 ; σ=12.32 ; P<0.005 ; R²= 18.39%)

Dans ce cas, l'analyse de covariance a mis en évidence que les fourrages tropicaux et les pailles présentaient un transit plus long que celui des fourrages tempérés. Par ailleurs, les petits ruminants présentaient un transit ruminal plus rapide.

2-5/ Digestion dans le rumen

a/ Digestibilité de la MO (tableau 5)

En absence de covariable, la paille a présenté une digestibilité plus faible que les autres fourrages (Te : 47.5a, Tr =45.9a, Pa = 23.8b ; avec n= 313; σ=10.08 ; P<0.001 ; R²= 18.99%).

La dMO ruminale a varié significativement avec les teneurs en NDF, et MAT de la ration:

drMO (%) = - 99.10^{-5} NDF² (%) + 0.29 MAT (%) + 43.29 (n =255; σ =9.27; P <0.001 ; R²= 33.23%)

L'analyse de covariance a indiqué, qu'à teneurs comparables en NDF et MAT, la digestibilité ruminale des fourrages tropicaux était supérieure à celle des fourrages tempérés tandis qu'elle était nettement inférieure pour la paille (tableau 5). Il apparaissait en outre que les petits ruminants digéraient significativement moins bien la ration ingérée dans le rumen.

La dMO dans l'ensemble du tube digestif est influencée par la drMO (figure 7). Dans ce cas, les précédentes covariables (MAT et NDF²) n'ont plus d'influence significative :

drMO (%) = 0.17 NDF (%) +0.75 dMO (%) – 13.88 (n =246; σ =7.20; P<0.001 ; R²= 59.17%)

L'analyse de covariance de la digestiblité ruminale de la MO a indiqué qu'il n'y avait pas de différence significative entre les fourrages tropicaux et tempérés, cependant la hiérarchie reste conservée entre les gros et les petits ruminants.

Figure 8

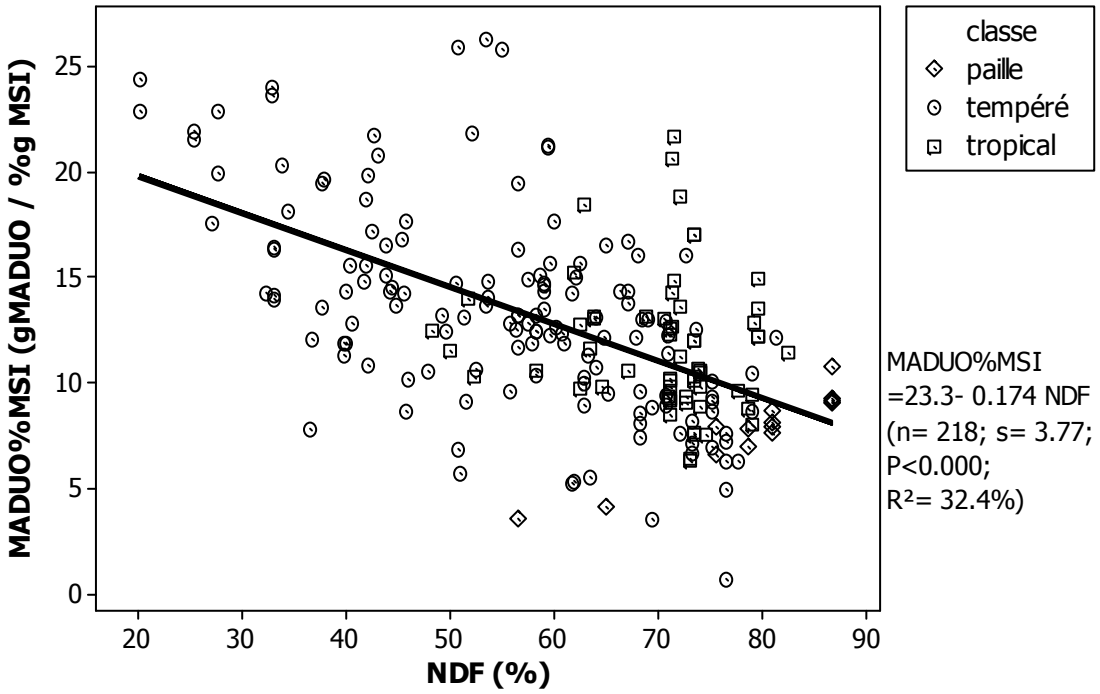
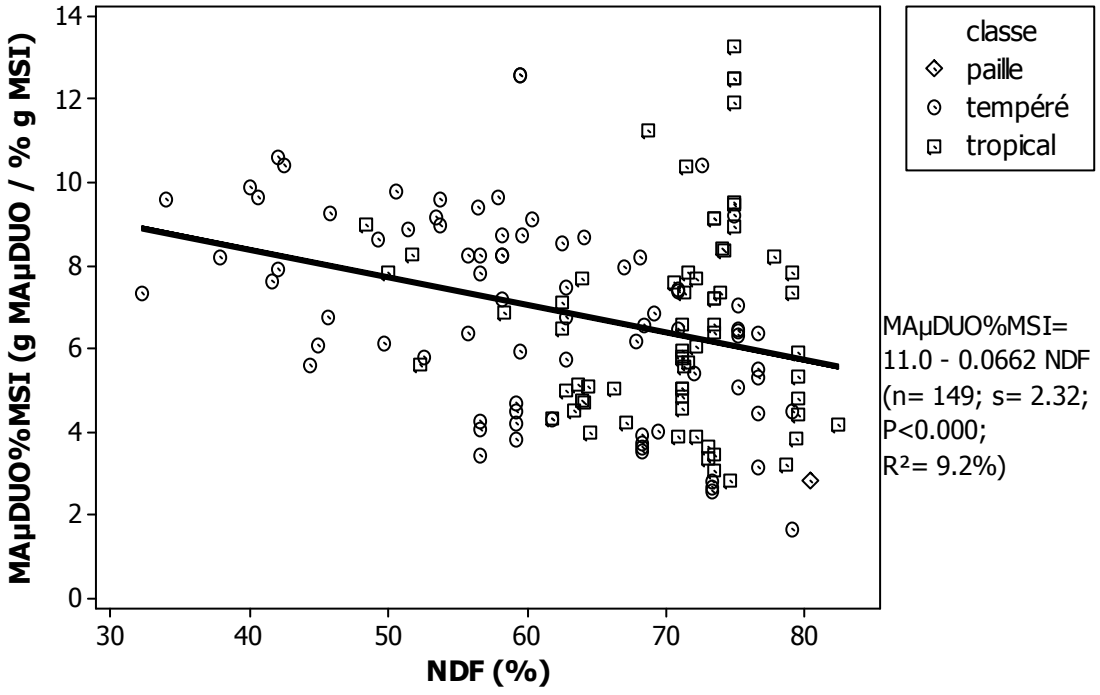


Figure 9



Figures 8 et 9. Flux de matière azotée totale et de matière azotée microbienne au duodénumen fonction de la teneur en NDF du fourrage pour les 3 classes de fourrage (tempéré, tropical ou paille).

b/ Digestibilité des constituants pariétaux (tableau 5)

Lorsque aucune covariable n'est prise en compte, il n'y a pas différence de digestibilités ruminales du NDF entre fourrages : Te = 57,10a, Tr = 54.90a, Pa = . (n= 171; σ =13.76 ; P=0.314 ; R^2= 0.60%).

La digestibilité du NDF dans l'ensemble du tube digestif a fortement été influencée par la digestibilité ruminale du NDF :

drNDF (%) = 1.012 dNDF (%) – 6.72 (n =161; σ =6.17; P<0.009 ; R^2= 81.16%)

L'analyse de covariance de la drNDF a indiqué une absence de différence significative entre les types de fourrage, par contre les petits ruminants digéraient moins bien que les gros ruminants.

c/ Flux azoté ruminal (tableau 5)

Le flux azoté ruminal a été estimé par le flux de MA au niveau du duodénum rapporté sur la MS ingérée (MADUO % MSI). En absence de covariable, le flux azoté duodénal de la paille a été significativement plus faible que celui des fourrages tropicaux dont le flux est significativement plus faible que celui des fourrages tempérés : (Te =13.73a, Tr =12.14b, Pa = 7.74c ; avec n= 262; σ= 4.47; P<0.001 ; R^2= 9.35%).

Le flux duodénal a été significativement influencé par la teneur en NDF de la ration (figure 8) :

MADUO%MSI (g MADUO / % g MSI) = - 0.17 NDF (%) + 23.3 (n = 218 ; σ= 3.77 ; P<0.000 ; R^2= 32.4%)

Lorsque la teneur en NDF du fourrage était prise en covariable, aucune différence significative n'était enregistrée entre les fourrages tempérés et tropicaux. Par contre le flux duodénal observé avec les petits ruminants était plus élevé qu'avec les bovins.

d/ Croissance microbienne (tableau 5)

Deux modes d'estimation de la croissance microbienne ont été testés :

-le flux de MA microbienne au niveau du duodénum, rapporté sur la matière sèche ingérée (MAμDUO% MSI) ;

- l'efficacité de la synthèse microbienne (Effic μ).

En absence de covariable, le flux de MA microbienne des fourrages tempérés était significativement plus élevé que celui des fourrages tropicaux (MAμDUO%MSI : Te =

7.73a ; Tr = 6.49b ; Pa = 2.86ab ; avec n= 176; σ = 2.99; P<0.02 ; R²= 5.15%). En ce qui concerne l'efficacité microbienne, la même hiérarchie a été observée (Effic µ : Te = 28.2a, Tr = 23.3b, Pa = 16.5b ; avec n = 183 ; σ = 11.66; P<0.001 ; R²= 8.29%).

La teneur en NDF du fourrage a eu une influence significative négative sur la production de proteines microbiennes (figure 9), quel que soit son mode d'estimation (MAµDUO%MSI ou Effic µ) :

MAµDUO%MSI (g MAµDUO / % g MSI) = - 0.07 NDF (%) + 11.00 (n= 149 ; σ = 2.32 ; P<0.000 ; R²= 9.2%)

Effic µ (g Nµ/ kg ADOMR)= - 0.31 NDF (%) + 42.31 (n =145 ; σ =7.91 ; P<0.001; R²= 20.90%)

Cependant, avec ces relations prises en covariable, aucune différence significative n'apparaissait entre les croissances microbiennes obtenues avec les différents types de fourrages.

2-6/ Digestibilité intestinale de l'azote (tableau 5)

En absence de covariable, la digestibilité intestinale de la matière azotée des fourrages tempérés était significativement plus élevée que celle des autres types de fourrages (dNint : Te = 67.5a, Tr = 62.3b, Pa = 60.5b ; avec n= 238 ; σ =11.30 ; P<0.003 ; R²= 5.34%).

La digestibilité intestinale a été significativement influencée par la teneur en NDF du régime :

dNint (%) = - 0.15 NDF (%) + 71.03 (n = 200 ; σ =9.51 ; P< 0.02 ; R²= 33.13%)

Dans ce cas, la digestibilité intestinale de l'azote, des fourrages tempérés était significativement plus élevée que celles des deux autres types de fourrages considérés dans notre étude.

La teneur en ADLignine du régime présentait également une influence significative sur la digestibilité intestinale de l'azote:

dNint (%) = - 1.54 ADL (%) + 70.70 (n = 179; σ =8.92 ; P< 0.001 ; R²= 42.84%)

L'analyse de covariance réalisée avec cette covariable a confirmé que la digestibilité intestinale des fourrages tempérés était significativement plus élevée que celles des deux autres types de fourrages. De plus, quelle que soit la covariable utilisée (teneurs en NDF ou

ADL du régime), la digestibilité intestinale des petits ruminants était plus importante que celle des gros ruminants.

3/ Discussion

3-1/ Considérations générales.

Plusieurs synthèses bibliographiques ont eu pour but de comparer les fourrages tropicaux aux fourrages tempérés (Leng 1990, Minson 1990...), cependant, à notre connaissance, ce travail est la première méta-analyse publiée sur le sujet. Le recours à la méta-analyse constitue certainement l'outil le plus approprié pour cette étude car la démarche expérimentale ne permet pas de comparer de façon satisfaisante des fourrages tempérés et tropicaux dans les mêmes conditions, surtout quand ils sont consommés en vert. Cette difficulté expérimentale a aussi des conséquences sur la puissance statistique de la méta-analyse car il est impossible de réaliser des comparaisons en intra-expériences qui auraient eu l'avantage de réduire la variabilité résiduelle. Cette contrainte a motivé le choix des modèles d'analyses basés sur l'étude des écarts entre types de fourrages en se calant sur des lois générales connues. Cette méthode semble satisfaisante dans la mesure où la plupart des modèles étudiés se sont révélés très significatifs. Cependant, la relation associant les critères de MAT et NDF ont limité l'interprétation des coefficients de régressions.

Une autre conséquence de cette contrainte a été la nécessité de travailler sur un nombre important de données. Nous avons ainsi essayé d'être exhaustif de la littérature disponible pour les critères considérés, sans pour autant être certain de l'avoir été car les travaux expérimentaux conduits sur les fourrages tropicaux sont parfois publiés dans des revues à caractère local qui sont difficiles à atteindre avec les moteurs de recherche bibliographiques automatisés.

Une autre difficulté de cette étude réside dans le déséquilibre du nombre de données entre les trois classes de fourrages retenues, et ceci d'autant plus que l'on s'intéressait aux critères digestifs les plus difficiles à mesurer (transit, durée de mastication…). Enfin les comparaisons des fourrages au niveau des critères de la digestion dans le rumen souffrent probablement des difficultés liées à la précision de certaines mesures (calcul des flux duodénaux) ; qui induisent de la variabilité qui n'a pas pu être « piégée » par un effet expérience.

3-2/ Composition chimique des fourrages

Cette méta-analyse confirme les conclusions déjà obtenues dans les différentes synthèses bibliographiques publiées sur la caractérisation chimique des fourrages : les graminées tropicales étudiées sont en moyenne plus riches en parois mais plus pauvres en matières azotées que les graminées tempérées. Cependant, on peut s'interroger sur la pertinence des critères classiques de la composition chimique utilisés pour comparer les fourrages entre eux car ils ne rendent pas forcément compte d'éléments de structure de la matière (arrangement structural, liaisons…) dont les conséquences peuvent être majeures sur la valorisation des fourrages (Wilson 1994).

Les comparaisons des teneurs en protéines brutes entre les graminées tropicales et tempérées doivent être analysées avec prudence, car d'une part, les différences disparaissent quand la comparaison entre graminées est réalisée à même niveau de NDF et d'autre part la gestion agronomique (fertilisation, irrigation) influence la composition des fourrages (composants pariétaux, MAT). Le niveau moyen d'intensification des fourrages n'est probablement pas le même dans les environnements tropicaux et tempérés, ce qui peut être à l'origine des biais dans les comparaisons (Minson 1990). Par conséquent la comparaison entre fourrages tempérés et tropicaux couvre probablement quelque chose d'autre que de simples réalités physiologiques (plantes en C4/plantes en C3).

3-3/ Ingestion, digestibilité totale, mastication et encombrement ruminal

Plusieurs auteurs ont déjà souligné le fait que l'ingestion volontaire des fourrages tropicaux était inférieure à celles des fourrages tempérés (Minson 1990, Leng 1990, Kennedy 1995). Nos résultats obtenus d'une part, à partir de traitements expérimentaux plus importants que dans les travaux cités et, d'autre part, sur la comparaison des fourrages à même niveau d'azote et de constituants pariétaux, confirment les observations de la littérature. Néanmoins, il est important de noter que l'analyse comparative de l'ingestibilité reste très globale. Par exemple, elle n'a pas traité spécifiquement l'influence majeure de l'âge du fourrage, bien que cette caractéristique soit piégée en partie ici avec des covariables telles que les teneurs en NDF et MAT. De plus, l'effet d'espèces fourragères n'a pas été introduit dans l'analyse à cause de la configuration (fréquence d'observations par espèce) de la base de données. Enfin, notre analyse ne prend pas en compte les différences entre légumineuses et graminées alors que la composition structurale des parois cellulaires de ces deux familles est différente avec de probables conséquences sur l'ingestion. Les légumineuses tempérées sont ingérées en plus

grande quantité que les graminées car elles offrent une moindre résistance à leur réduction qui a lieu durant les mastications ingestive et mérycique (Minson 1990**).** La plus faible résistance des légumineuses tempérées à la réduction de taille des particules alimentaires par la mastication a pour conséquence des temps de rétention dans le rumen plus courts que ceux des graminées. Les grosses particules des légumineuses sont aussi réduites plus rapidement dans le rumen que celles des graminées (Minson 1990). Cette différenciation entre légumineuses et graminées tempérées est probablement amplifiée pour les fourrages tropicaux car il n'y a aucune différence dans la structure de la paroi cellulaire entre des légumineuses tropicales et tempérées alors que la paroi cellulaire des graminées tropicales est plus dure que celle des fourrages tempérés. Dans un essai, McLeod *et al* 1990, ont estimé une augmentation moyenne de mastication (min / g de matière Sèche Ingérée) de 90% en comparant les légumineuses aux graminées.

Les différences d'ingestion observées en comparant les fourrages tempérés aux tropicaux, à même niveau d'N et de NDF, pourraient s'expliquer par l'organisation structurale de la paroi cellulaire (comme indiqué dans le précédent paragraphe). En effet, l'augmentation de l'ingestion exige une dégradation physique des fourrages par la mastication (réduction de taille des particules dans le rumen) corrélée avec un taux de passage des particules alimentaires plus rapide vers l'abomasum. Nos résultats discriminent significativement les fourrages tempérés des fourrages tropicaux sur les paramètres qui illustrent l'activité masticatrice (encombrement du rumen, index de mastication). En effet, en comparant les graminées tropicales aux tempérées, le travail masticatoire pour valoriser 1kg de MS de fourrage tropical augmente significativement (50% sur toute la base de données, 15% quand les niveaux de MAT et NDF sont pris en considération). Dans les essais comparant strictement les graminées tropicales et tempérées, Mac Leod *et al* 1990, ont rapporté une augmentation moyenne du temps de mastication de 20% pour les fourrages tropicaux relativement aux tempérés.

En considérant les 76 traitements pour lesquels les fourrages tropicaux distribués ad libitum en brins longs, le temps journalier de mastication, approximativement 1000 minutes, était proche du maximum possible journalier. Ce résultat met en évidence l'impact de contraintes physiques sur l'ingestion de graminées tropicales comme d'autres auteurs l'avaient déjà rapporté (Minson 1990, Kennedy 1995). Wilson 1991, a expliqué ces différences par la composition des parois cellulaires des graminées tempérées et tropicales.

L'encombrement ruminal d'animaux nourris avec du fourrage tropical est significativement plus élevé (49%) que celui d'animaux nourris avec du fourrage tempéré. Ce résultat est en

accord avec ceux illustrant l'activité masticatoire et le turn-over du rumen. Bien que la faiblesse du nombre de données concernant la granulométrie du contenu ruminal n'a pas permis d'analyses statistiques, nous supposons qu'à composition chimique comparable, le compartiment des grosses particules piégées dans le rumen est plus important avec les fourrages tropicaux à cause des difficultés du travail de mastication-comminution. Le niveau d'ingestion est étroitement associé avec la proportion de fibres indigestibles dans l'alimentation et au temps de rétention de ces fibres dans le rumen (Ulyatt *et al* 1986, Minson 1990). Le temps nécessaire à la réduction de taille des particules alimentaires et en conséquence au turn-over du rumen, conditionne l'ingestion. La digestion est inefficace sur le temps de réduction des particules (Wilson *et al* 1989) mais il ne peut être exclus un effet indirect par une fragilisation des tissus (Evans *et al* 1973) augmentant ainsi l'efficacité de la rumination sur la durée de la réduction de particules.

Les covariables prises en compte, teneurs en NDF et MAT du régime, permettent d'aboutir à des prédictions inter-expériences de la dMO assez précises. En outre, les relations globales observées ne sont pas différentes de celles extraites en intra-expériences, ce résultat est assez logique compte tenu du rôle essentiel des constituants pariétaux vis à vis de la digestibilité. Différents auteurs (Leng 1990, Minson 1990) ont évoqué le fait que les fourrages tropicaux pourraient être moins digestibles que les fourrages tempérés. A même teneur en paroi, nos résultats infirment cette conclusion, la différence de dMO n'est que de 1 point entre les deux types de fourrage. Elle n'est pas significative lorsque seuls des critères analytiques sont pris en compte. Nos comparaisons réalisées à même composition chimique, piègent partiellement les différences physiologiques entre fourrages tropicaux et tempérés et expliquent les différences observées avec la littérature. En fait, à cause de leur physiologie (photosynthèse en C4), les graminées tropicales croissent plus vite que les tempérées et présentent donc une maturation plus rapide. Ceci peut expliquer qu'à âge identique, les graminées tropicales soient moins digérées que les tempérées. Classiquement, les essais qui comparent ces deux types de fourrages prennent en compte l'âge calendaire mais jamais l'âge physiologique.

La conséquence des différences observées sur les niveaux d'ingestion et de digestibilité entre fourrages tempérés et tropicaux explique les différences observées dans l'ingestion de la matière organique digestible. Par conséquent, la quantité d'énergie disponible nécessaire aux animaux est plus faible avec du fourrage tropical qu'avec du fourrage tempéré.

La corrélation positive entre l'ingestion et la digestibilité est souvent analysée comme une relation mécanique. Cependant, la compilation des données de fourrages tropicaux et tempérés, l'absence de différences de digestibilité pour une même teneur en NDF alors que

des différences d'ingestibilité sont enregistrées plaident pour l'absence d'une relation mécanique étroite entre l'ingestion et la digestibilité. Par conséquent, ce résultat pourrait aussi indiquer que la cellulolyse n'est pas forcément un facteur limitant en matière d'ingestion alors qu'il peut l'être en matière de digestion. L'ingestibilité comme indiquée ci-dessus, serait davantage expliquée par des phénomènes de réduction de taille des particules.

3-4/ Digestibilités ruminale et intestinale

Les résultats de digestibilités ruminale et intestinale sont en adéquation avec les résultats globaux illustrant une absence de différence significative quand les fourrages tropicaux et tempérés sont comparés à même composition chimique. Toutefois, la différence de 2 points de digestibilité ruminale du NDF à même digestibilité totale, bien que non significative, pourrait traduire une plus faible digestibilité intestinale des parois. Ce résultat explique peut-être la plus faible digestibilité intestinale de l'azote des fourrages tropicaux, celle- ci étant liée aux parois.

3-5/ Valeur alimentaire

L'ensemble des résultats interpelle sur la pertinence de la valeur des paramètres utilisés pour estimer la valeur alimentaire des fourrages tropicaux avec le système INRA. Quel est l'impact de l'effort de mastication plus grand, induit par l'ingestion des fourrages tropicaux, sur les pertes énergétiques et en conséquence sur la valeur du coefficient de passage entre l'énergie métabolisable et nette. De même la moindre digestibilité intestinale des protéines alimentaires des fourrages tropicaux devrait aussi conduire à l'utilisation d'une valeur spécifique pour les fourrages tropicaux.

Conclusion

La méta-analyse a permis d'obtenir des informations nouvelles concernant l'étude comparative des fourrages tropicaux versus tempérés. Les nouveaux résultats obtenus sont liés à la méthodologie utilisée qui a permis de conduire les comparaisons entre types de fourrages sur les mêmes bases, chose quasiment impossible par la méthode expérimentale classique. La principale conclusion est que la différence majeure entre fourrage tempéré et tropical est la moindre ingestibilité (et non la digestibilité) de ce dernier à cause de contraintes

liées à un effort de mastication plus élevé. Par ailleurs la digestibilité intestinale de l'azote est moindre. Ces deux éléments de différenciation des fourrages devraient conduire à une révision des choix de la valeur des paramètres utilisés pour la détermination des valeurs alimentaires par le système INRA.

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PARTIE EXPERIMENTALE

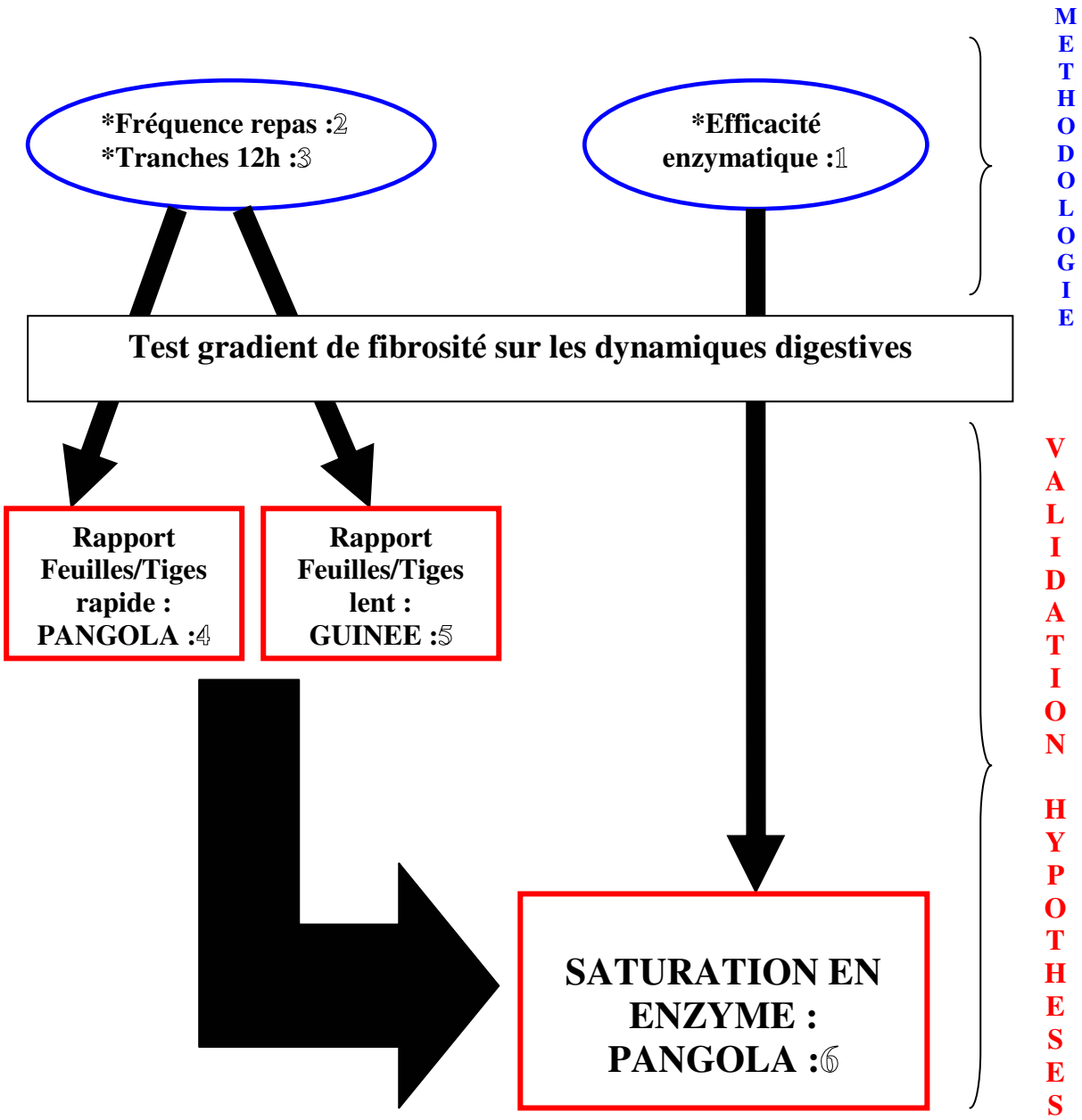

Figure 1. Démarche expérimentale de la thèse

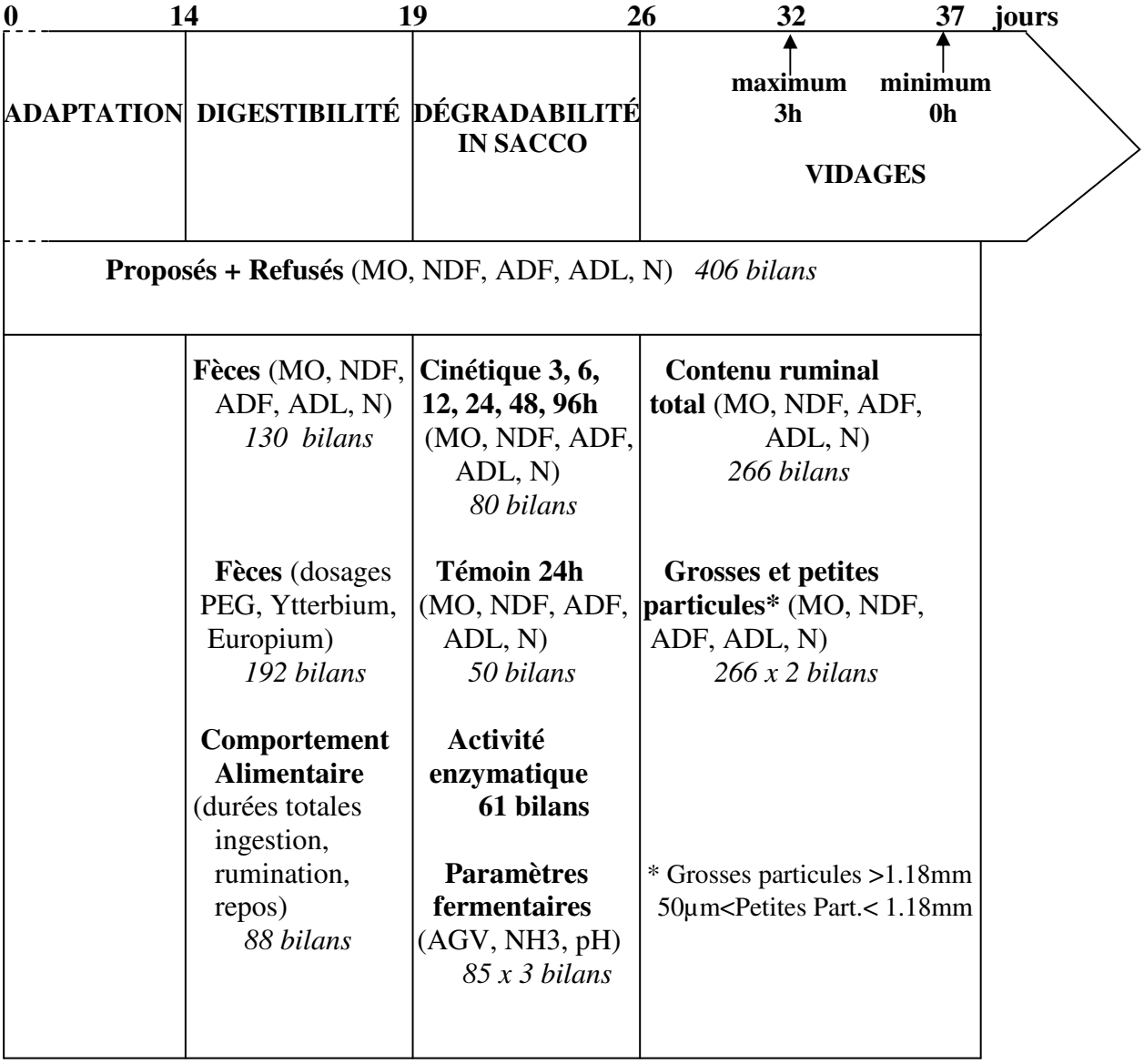

Figure 2. Déroulement schématique d'une période expérimentale et mesures effectuées au cours d'une période.

INTRODUCTION A L'ETUDE EXPERIMENTALE

Deux groupes d'expérimentations ont servi de trame à l'étude expérimentale (figure **1**).

Au sein du premier groupe, les protocoles (1, 2 et 3) relevaient de préoccupations méthodologiques et ont eu pour but de définir les conditions d'études du deuxième groupe d'expérimentations. Le deuxième groupe, constitué des protocoles 4, 5 et 6 avait pour objectif de répondre à la question principale de recherche, à savoir le déterminisme de l'ingestion.

L'étude expérimentale s'est déroulée sur des moutons de race Black-Belly, canulés du rumen auxquels étaient distribués du fourrage vert haché, à volonté (10% de refus). La figure **2** représente le déroulement schématique d'une période expérimentale type et des mesures effectuées au cours de cette période.

GROUPE EXPERIMENTAL A BUT « METHODOLOGIQUE »

INTRODUCTION AU GROUPE D'EXPERIMENTATIONS A BUT METHODOLOGIQUE

But du premier groupe d'expérimentations : définir les conditions d'études du deuxième groupe expérimental :

Une des expérimentations du deuxième groupe expérimental (protocole **6**) devait nous permettre de n'étudier que la part des phénomènes mécaniques. Pour ce faire, il a été question de travailler en condition enzymatique non limitante en apportant des enzymes exogènes. Ainsi, préalablement à cette expérimentation, le protocole **1** a eu pour objectif de vérifier l'efficacité de l'apport d'enzymes exogènes.

Classiquement dans les études portant sur les dynamiques digestives, les animaux sont nourris avec plusieurs repas par jour (jusqu'à 12), afin de réaliser certaines mesures nécessitant un certain état d'équilibre dans le rumen. Or, nous voulions nous situer dans des conditions qui soient les plus proches de celles de l'élevage, où deux repas sont distribués en moyenne par jour. Le deuxième protocole méthodologique (protocole **2**) a donc eu pour objectif d'étudier l'impact de la fréquence de distribution des fourrages sur les paramètres de dynamiques digestives.

Afin de tenter de simplifier les protocoles de mesures du deuxième groupe expérimental, nous avons fait l'hypothèse que sur la base de deux repas par jour distribués à 12 heures d'intervalle, une période de 12 heures était substituable à une autre. L'objectif du troisième protocole à but méthodologique (protocole **3**) était de comparer les activités d'ingestion et de digestion sur 2 périodes de 12 heures, en éclairage continu.

PUBLICATION 2

Limits of exogenous fibrolytic enzymes to improve digestion and intake of a tropical grass

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ABSTRACT: The effect of the addition of exogenous fibrolytic enzymes (EFE) on intake, total tract digestibility and nylon bag degradability of a chopped fresh *Digitaria decumbens* grass was studied using 2 stages of regrowth (21 and 56-day old grasses). Moreover, comparisons between ground and chopped grass were done using the nylon bag degradability method. DM intake (g/kg $W^{0.75}$) and organic matter total tract digestibility for control and enzyme treatments respectively were 69.1 *versus* 65.9 ($P > 0.05$) and 0.723 *versus* 0.727 ($P > 0.05$) with the 21-day old regrowth. Based on the same parameters, values for the 56-day old grass were 58.1 *versus* 52.7 ($P > 0.05$) and 0.621 *versus* 0.591 ($P > 0.05$). Nylon bag degradation rates at 24 hours of the dry matter for control *versus* enzyme treatments were 0.653 *versus* 0.70 ($P < 0.05$) and 0.644 *versus* 0.733 ($P < 0.0001$) for the 21-day old chopped and ground forage whereas with the 56-day old grass, they were 0.321 *versus* 0.392 ($P < 0.0001$) and 0.463 *versus* 0.481 ($P > 0.05$). The positive impact of EFE on degradation of the young and ground pangola grass may suggest that in some cases, enzyme accessibility to potentially digestible cell wall is a limiting factor in their digestion.

[Key Words : Digitaria decumbens, Sheep, Exogenous enzyme, Intake, Digestion]

INTRODUCTION

Tropical grasses are often characterised as low quality forages when compared to temperate ones because of their low intake and digestibility (Minson, 1990). Research into technology aiming at increasing digestibility and intake of tropical grass is a challenge.

Some researchers have shown that the use of exogenous fibrolytic enzymes (EFE) can enhance fibre degradation, in vitro (Forwood et al., 1990; Varel et al., 1993; Feng et al., 1996; Hristov et al., 1996;) and in situ (Lewis et al., 1996). While some authors (Beauchemin et al., 1999; Yang et al., 1999) have confirmed these results in vivo, others authors (Firkins et al., 1990; Varel and Kreikemeier, 1994; Kung et al., 2000) have found the opposite result . In a review, Wang and McAllister (2002) indicated that there is ample evidence that EFE exert dissimilar effects on different feed types and that each preparation reacts differently according to substrate. All published experiments have been done on temperate forage or straw. No results have been published on tropical grass which represents a specific and original biological model. Cell wall content is lower in temperate than tropical grasses. The latter are characterised by cell types with thickened secondary wall, such as in the vascular bundles, sclerenchyma strands, epidermis and parenchyma bundles (Wilson, 1994). Wang and McAllister (2002) indicated that commercially available enzyme preparations still lack the model activities that can overcome the factors limiting ruminal digestion of plant cell wall especially for low quality forage. The objective of this study was to test this hypothesis on tropical forage.

MATERIALS AND METHODS

Location

Research was carried out in 2004 at the experimental animal station at the National Agronomic Research Institute (INRA) in the French West Indies (Guadeloupe, latitude 16.16

N, longitude 61.30 W). Temperatures ranged on average from 21°C to 31°C. Mean rainfall on the experimental site was 3000 mm / year. Rainfall was regular during the experiment.

Experimental design, animals, diets and feeding

The harvest of a perennial *Digitaria decumbens* (pangola) pasture, divided into plots and subplots, was planned in such a way as to have 21- and 56-day old regrowth for 2 successive 30 day periods. Plots P21 and P56 were used during periods 1 and 2 respectively. Plots P21 and P56 were subdivided into 21 and 30 subplots respectively. The first subplot of both P21 and P56 was cut 22 and 57 days respectively before the beginning of experimental periods 1 and 2. One subplot was cut per day. One kg/ha/regrowth age of mineral nitrogen fertiliser was added to each subplot the same day. Areas of the subplots were 400 and 300 m² respectively for P21 and P56. Each subplot in P21 or P56 was cut once or twice. At the beginning of periods 1 and 2, the first subplot in P21 and P56 had 21 and 56 days of regrowth respectively. Within a plot, grass from subplot n had one day less regrowth than subplot n-1. Consequently, during experimental periods 1 and 2, regrowth age of the grass harvested daily was exactly 21 and 56 days old respectively. Eight Black-belly rams (mean liveweight: 51.28 ± 3.62 kg) were used in this experiment. They were fitted with ruminal cannulae and maintained in metabolism cages. The 30-day experimental period consisted of 14 days of adaptation to the diet, 5 days of intake and total tract digestibility measurements and 7 days of nylon bag incubation in the rumen. The pangola grass was cut early every morning and chopped (5 cm-length) before being offered. The amount of forage provided was 1.15 times greater than the animal voluntary intake estimated during periods of adaptation. Water and mineral blocks were offered for *ad libitum*.

Table 1. Composition and activities of the experimental fibrolytic enzyme

Ingredients	
Trichoderma reesei fermentation extracts	80 - 86%
Sorbitol	10 - 12 %
Salts	4 - 5 %
Preservatives	0.25-0.3 %
Cellulase activity (DNS-CMC)	5500 IU / g
Xylanase activity (DNS-ABX)	> 10000 IU/g

Trial 1: Intake and total tract digestibility

Eight rams were fed successively with 21-day and 56-day pangola regrowth. Four rams consumed chopped grass mixed with tap water without enzymes (one litre to 15 kg of fresh forage). Four other rams were fed with chopped forage mixed with a commercial fibrolytic enzyme solution (Nutreco Rumizyme-alpha). 15 ml of this enzyme were diluted with 100 times its volume in tap water. One litre of this diluted enzyme was mixed with 15 kg of fresh grass. The enzyme contained mainly xylase and cellulase activities (Table 1).

Trial 2: In sacco degradability

Three rams were fed with chopped forage mixed with tap water (1 litre to 15kg of fresh forage). Two different granulometry have been tested: chopped or ground forage. Nylon bags filled with fresh chopped or dried-ground forage mixed with tap water for ten minutes were incubated in their rumen for 24 or 48 hours. At the same time, three others rams were fed with chopped forage mixed with the diluted fibrolytic enzyme solution (1 litre to 15kg of fresh forage). Nylon bags filled with fresh chopped or dried-ground forage mixed with the same solution that the basal diet, for ten minutes, were incubated in their rumens for 24 or 48 hours.

Measurements

Intake and total tract digestibility were measured by weighing the daily amounts of food offered, refusals and faeces. Degradability of forage offered was measured using the nylon bag method. The nylon bags were 10 cm x 5 cm, with a pore size of 50 x 50 µm and filled with 15 g of fresh chopped forage or 3 g of freeze dried then ground forage in the basal diet. Fresh grass was manually cut into particles of 2 mm mean length. Frozen dry forage was ground (1mm). Incubation times in the rumen were 24 and 48 h.

Chemical analyses

DM content of fresh forage, refusals and residues of incubation was determined daily by drying at constant weight at 60°C in a forced-draught oven. DM content of faeces was determined in similar conditions using a representative sub-sample. The latter came from a sample obtained by pooling 10% of the daily amount of faeces excreted by each animal. It was then stored (-20°C). Samples were ground (1 mm) prior to chemical analysis. Organic matter (OM) content was measured after a 10 h pyrolysis at 550°C. Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) were estimated following the methods of Van Soest et al. (1991). Nitrogen concentration of forage was determined using the Dumas method.

Statistical analyses

Data were analysed using the General Linear Model procedure of SAS (1987). Concerning data on nylon bag degradation, the model included age of forage (D.F. 1), enzyme treatment (D.F. 1), forage granulometry (D.F. 1), and enzyme * granulometry interaction. With regard to intake and digestibility, the model took into account the effects of forage (D.F. 1), and enzyme treatment (D.F. 1) .

Table 2. Chemical composition (g/kg DM) of a 21- and 56-day old fertilized *Digitaria decumbens* grass

	Regrowth age (days)	
Constituents (g/kg DM)	21	56
Organic matter	909	900
Crude protein	151	64
Neutral detergent fibre	727	751
Acid detergent fibre	362	406
Acid detergent lignin	54	72

Table 3. Degradability of a 21- and 56-day old fresh chopped (G-) and frozen-dry ground (G+) *Digitaria decumbens* grass, sprayed with tap water (E-) and with a 1/100 diluted enzymatic solution (E+), in nylon bags incubated for 24 and 48 hours in the rumen of six rams feeding similar grasses

Age of regrowth (days)	21			56		
Treatment	E-	E+	S.E.	E-	E+	S.E
Degradability of Dry Matter (%):						
G- 24h	65.30^{a}	70.04^{b}	1.30	32.14^{f}	39.25^{g}	1.50
G+ 24h	64.42^{a}	73.30^{b}	1.30	46.27^{h}	48.10^{h}	1.26
G- 48h	78.68^{c}	83.32^{de}	1.30	49.26^{h}	49.43^{h}	1.42
G+ 48h	81.47^{cd}	85.80^{e}	1.26	59.19^{i}	57.02^{i}	1.26
Degradability of Neutral Detergent Fiber (%):						
G- 24h	58.01^{a}	63.13^{b}	1.65	19.24^{e}	26.20^{f}	1.90
G+ 24h	54.83^{a}	66.52^{b}	1.65	32.44^{g}	35.26^{g}	1.60
G- 48h	75.24^{c}	79.01^{cd}	1.65	40.24^{h}	35.57^{gh}	1.80
G+ 48h	77.57^{c}	81.92^{d}	1.60	49.80^{i}	46.51^{i}	1.60
Degradability of Acid Detergent Fiber (%):						
G- 24h	43.89^{ac}	52.24^{e}	2.00	15.13^{h}	22.71^{i}	2.28
G+ 24h	39.97^{ab}	58.39^{d}	2.00	29.28^{j}	34.63^{bk}	1.93
G- 48h	66.19^{f}	70.07^{f}	2.00	36.99^{bk}	31.52^{jk}	2.17
G+ 48h	70.78^{f}	76.65^{g}	1.93	48.81^{ce}	45.42^{c}	1.93

$^{a, b, c, d, e,\ldots}$ Means within each category of degradability of components lacking a common superscript letter differ ($P<0.05$)

RESULTS

Dietary composition

The mean composition of *Digitaria decumbens* was reported in Table 2. The 56-day old grasses were characterised by lower nitrogen and higher fiber contents as compared with the 21-day old grasses.

In sacco degradability

Values for *in sacco* degradability were reported in table 3. Dry matter degradability of young forage increased significantly with the enzyme supplement whatever the incubation time and the forage granulometry. The same tendency was observed with NDF and ADF although differences are not always significant. Concerning the old forage, significant positive effect of enzyme was registered only for the chopped forage that underwent 24 hours of incubation. Whatever the forage regrowth, the interaction between incubation time and enzymatic treatment was always significant ($P<0.001$).

The interaction between forage granulometry and enzyme supplement was not significant whatever the grass regrowth. Physical treatment improved the degradability of old forage whereas no difference was observed with young forage.

Intake

DM, OM, NDF and ADF intakes were reported in table 4. No difference was observed between control and enzyme treatments whatever the forage age and the DM component. Moreover, intake was higher with the younger forage.

Table 4. Dry matter, Organic matter, Crude protein, Neutral Detergent Fiber, Acid Detergent Fiber and Acid Detergent Lignin Intake and total tract digestibility of a 21- and 56-day old fresh chopped and frozen-dry ground *Digitaria decumbens* grass, sprayed with tap water (E-) and a 1/100 diluted enzymatic solution (E+) given *ad libitum* to eight Blackbelly rams

Age of regrowth (days)	21			56		
Enzyme treatment	E-	E+	S.E.	E-	E+	S.E.
Intake (g/day/$LW^{0.75}$):						
Dry matter	69.09[a]	65.96[a]	4.56	58.10[b]	52.72[b]	4.56
Intake (g/day):						
Dry matter	1258.82[a]	1284.54[a]	76.49	1108.04[ab]	1017.39[b]	76.49
Organic Matter	1155.52[a]	1179.42[a]	67.24	949.81[ab]	850.75[b]	86.37
Neutral Detergent Fiber	914.26[a]	928.96[a]	57.07	835.86[a]	763.84[a]	57.07
Acid Detergent Fiber	449.85[a]	455.23[a]	29.37	450.34[a]	410.15[a]	29.37
Total tract digestibility						
Dry Matter	0.663[a]	0.689[a]	0.019	0.560[b]	0.532[b]	0.019
Organic Matter	0.723[a]	0.727[a]	0.015	0.621[b]	0.591[b]	0.015
Neutral Detergent Fiber	0.688[a]	0.702[a]	0.023	0.557[b]	0.529[b]	0.023
Acid Detergent Fiber	0.652[a]	0.656[a]	0.022	0.549[b]	0.525[b]	0.022

[a,b]: Means within a row lacking a common superscript letter differ ($P<0.05$).

Total tract digestibility

DM, OM, NDF and ADF total tract digestibility of diets were reported in table 4. No difference was observed between control and enzyme treatments whatever the forage age and the dry matter component. Total tract digestibility was significantly lower with the older forage whatever the dry matter component.

DISCUSSION

The main objective of this work was to test the efficacy of EFE in increasing cell wall digestion in tropical grass. Due to large variation of feed value of these last grasses with their maturity, two extreme regrowth ages (21 versus 56-days) have been studied. Several studies have shown that EFE can improve the rate of feed digestion but their ability to increase the extent of digestion may be limited by a lack of the enzyme that breaks down the core structure of lignin-cellulosic complex (Wang and McAllister, 2002). In our experiment, we use grinding as a means of increasing the total diet particle surface and the accessibility of potential digestible cell wall to enzymatic activity in the rumen (Journet and Demarquilly, 1967).

In the present study where EFE were added to tropical grass both the rate of degradation and the extent of cell wall digestion (DM, NDF, ADF) decreased whereas the level of maturity of the forage increased. This adverse result has to be analysed taking into account the chemical composition of the plant cell wall. The chemical analysis of forage indicated an increase in the level of lignin with maturation and consequently an increase in the linkage between polysaccharide (cellulose and hemicelluloses) and lignin. This linkage limits the ability of the EFE which did not contain the enzymes (e.g., esterase) to break down the esterified bonds between lignin and carbohydrates. Our results for nylon bags (positive action of grinding on the degradation of the grass) illustrate limited enzyme accessibility to the potentially

digestible cell wall. In reality, the positive effect of grinding on dry matter (or NDF and ADF) degradation of the old pangola grass in the control treatment could be an indication that amount of microbial enzymes is not the first limiting factor in the digestion of old forage but rather the range of colonisation of feed particle by microbes. On the contrary, the lack of effect of grinding on dry matter degradation in the control treatment of the young forage could illustrate that maximal degradability was reached.

The lack of positive effect of EFE on the in *sacco* degradability (DM, NDF, ADF) of the 56-day pangola grass at 48 hours whatever the forage granulometry coincides strongly with the hypothesis of Wang and McAllister (2002). The positive effect of EFE on the in sacco degradability (DM, NDF, ADF) of the 56-day pangola grass, at 24 hours probably illustrates a higher rate of degradation with addition of EFE as is typically observed in other studies (Wang and McAllister, 2002). Concerning the 21-day pangola grass, the efficacy of EFE has to be explained by the nature of the cell wall. Our results are similar to those of Wang et al. (2004) who have studied the effect of alkali pre-treatment of straw on the efficacy of EFE. They found that EFE have a significant effect on both the rate and extent of degradation of the pre-treated straw whereas no significant effect was registered with control. They suggested that removal of the phenolic barriers that impede the microbial digestion of the feed may be an additional factor in the alkali treatment. In our study phenolic barriers are low for the young forage and high for the old one.

Our results concerning total tract digestibility for which no effect of EFE have been registered whatever the forage age differ significantly from those recorded for the *in sacco* degradation. This discrepancy could be explained by compensatory cell wall digestion in the large intestine with the young forage.

Concerning the impact of EFE on intake, our results, like those reported by Feng et al. (1996), Mc Allister et al. (1999), Kung et al., (2000), Wang et al. (2004) on temperate forage or straw, revealed that they have none significant effect.

CONCLUSION

In conclusion, in this study, applying EFE to tropical forage had no effect on intake and total tract digestibility whatever the age of the grass. Concerning rumen digestion, addition of EFE seems to have beneficial effects on degradation of young tropical forage (<28d) whereas no improvement was recorded for old forage. In order to confirm these preliminary results, further investigations on tropical forage will have to be carried out.

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PUBLICATION 3

Effect of frequency of meals on intake and digestion of tropical grass consumed by rams

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(*soumis à Animal*)

Abstract

Ten Black Belly rams (45.2kg) fitted with permanent ruminal cannulae were used in a 2 x 2 factorial design to determine the effects of feeding frequency and regrowth age on intake and digestion. Rams were fed with 21- or 35-day old fresh pangola grass offered ad libitum two or four times a day. Irrespective of the regrowth age, there was a tendency for intake to be positively correlated with increase in meal frequency. Differences were not significant ($P >$ 0.25). Significant effects of meal frequency were observed in NDF and ADF total tract digestibility of the 35-day grass (35-day grass) which decreases as the number of meals increases. Meal frequency had no visible effect on feeding behaviour. Total rumen content increased when animals were fed twice a day as opposed to four times a day. Similarly, an accumulation of small and very small particles was observed in the rumen of rams fed twice a day in comparison with those fed four times a day. These results suggest that studies of digestive dynamics performed at a steady state are not representative of the rumen loading observed in farm rams which have two important peaks of meal.

Keywords: feeding frequency, intake, feeding behaviour, digestive dynamics, tropical forage

Introduction

Digestible organic matter intake is a major factor influencing the feed value of tropical grass (Aumont *et al.*, 1995). To improve the nutrition of ruminants fed with tropical grass, it is important to increase their feed intake level by controlling influencing factors that could affect intake. These factors include the residence time of forage in the rumen which affects the quantity of forage consumed, particularly for low digestible forages (Poppi *et al.*, 1980, Poppi *et al*., 1981a b, Poppi *et al*., 1985); as well as the comminution and chemical degradation which are believed to influence the fill effect of tropical grass in ruminants (Wilson, 1994).

Studies on digestive dynamics are often cumbersome and complicated; therefore most experiments are carried out with animals fed frequently at regular time intervals on a daily basis to obtain a quasi-steady state situation in the rumen. These types of studies have provided interesting insights on the digestion process in the rumen, particularly cellulolysis and physical degradation of forage. However, under farms (pasture or stall) and in wild conditions, ruminants usually have two main meals per day. Consequently, it could be expected that feed intake and digestibility in ruminants, on-farm and in wild, may be different from those obtained with experimental steady-state type of studies. In this study, we evaluated the effect of frequency of meal on the process of intake, chewing and digestion of tropical grass in rams.

Material and methods

Location

The experiment was conducted in 2002 at the animal experimental station of the ‘‘National Institute of Agronomic Research" (INRA, French West Indies, Guadeloupe, latitude 16.16 N, longitude 61.30 W). The mean rainfall on the experimental site was 3000 mm/year and the

average monthly temperatures ranged from 21°C to 31°C. The entire experiment was carried out during the rainy season when rainfall and temperature remained almost constant.

Experimental design, animals, diets and feeding

The experiment was conducted using a cross-over design. Experimental treatments consisted of a 21- or 35-day old pangola grass offered ad libitum (1·15 times the animals' estimated voluntary intake) two or four times a day, at 12- and 6-hours interval, respectively. Ten Black-belly rams (mean liveweight (LW): 45.2 ± (s.d. 1.0) kg) were used for the experiment. The rams were fitted with rumen cannulae and maintained in individual metabolism cages, with free access to water and mineral blocks. Rams were weighed at the onset of the study and for each experimental period. For the 4 months trial, the animals were placed into two blocks (A and B). Group A were offered a 21-day old grass (21-day grass) twice a day, a 21-day grass four times a day, a 35-day old grass (35-day grass) twice a day and a 35-day grass four times a day in that order. The animals in group B were offered a 35-day grass twice a day, a 35-day grass four times a day, a 21-day grass twice a day old and a 21-day grass four times a day. Each period of the trial lasted 33 days which consisted of 14 days of adaptation, 5 days of intake and total tract digestibility estimation and 14 days of rumen emptying.

The harvesting of the perennial *Digitaria decumbens* (pangola) pasture was planned in order to have two plots (P21) and (P35) with pastures at 21 days and 35 days regrowth stages respectively, at each harvesting period. The P21 plot was divided into 21 subplots and the P35 plot into 35 subplots respectively. The first subplot of both P21 and P35 were cut at 21 and 35 days respectively before the beginning of the experiment and the following subplots were cut daily in successive order. As such, the grass in subplot n was one day older than that in subplot n+1. Consequently, during each experimental period the regrowth age of the grass harvested daily on the subplots of P21 and P35 was exactly 21 and 35 days old. Mineral

nitrogen fertiliser was applied every day after each harvest at 1kg/ha/regrowth age for each plot. The harvested grass was kept overnight at 4°C in a cold chamber in preparation for feeding the following day. In the morning, the pangola grass was chopped to an average length of 4 cm just before feeding.

Measurements

Intake and apparent digestibility were determined from daily weighing of the amounts of food offered, refusals and faeces. Dried and ground samples of grass, refusals and faeces were stored for chemical analyses.

Dry matter intake (DMI2-3h) during the morning main eating period was estimated as the amount of grass eaten during the two first hours following the morning distribution of the meal.

The transit time of the liquid phase was estimated using PolyEthylene Glycol (PEG) as the marker. Eight hours before the first collection of faeces, 30g of PEG were introduced directly into each rumen. The faeces were then harvested at 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 48, 52, 56, 62, 68, 80 and 104 hours after the introduction of the PEG. Transit time of indigestible fibre was estimated using lignin as the marker.

Feeding behaviour parameters (time spent eating, ruminating and idling) were determined for 24h per measurement period by an automatic recording system (Rutter *et al.*, 1997). Moreover, eating, ruminating and idling times were estimated during the first 6 hours after the distribution of the morning meal. Eating, ruminating and chewing indexes were estimated as follow:

- Eating index = (daily time spent eating) / (daily intake of dry matter or Neutral detergent fibre)

- Ruminating index = (daily time spent ruminating) / (daily intake of dry matter or Neutral detergent fibre)
- Chewing index = (daily time spent chewing) / (daily intake of dry matter or Neutral detergent fibre)

Kinetics of intake were estimated by dividing daily intake by 24 based on the assumption that hourly intake rate was identical.

Rumen degradation of forage offered was measured using the nylon bag method (Michalet-Doreau *et al.*, 1987). Nylon bags of 10 cm x 5 cm, with a pore size of 50 x 50 µm, were filled with 15g of fresh forage of the basic diet. The grass was cut manually into particles of a mean length of approximately 2 mm long. Incubation time in the rumen was 24 h.

During the rumen sampling period of animals consuming two diets per day, four total emptying of the rumen were carried out every 3 days, at 0, 3, 9 and 21 hours after the morning meal which was limited to 3 hours. On the day of the third emptying, the second meal was distributed just after the rumen emptying, while on the day of the last emptying, the animals only received the first meal which lasted 3 hours, thus fasting (without food) for 21 hours until the emptying. When rams consumed four diets daily, rumen emptying were carried out at 0, 1, 2, and 4 hours after the morning meal which was limited to 2 hours. After each emptying, the rumen content was weighed and mixed thoroughly by hand and four sub-samples were taken. Two of these sub-samples were used for dry matter determination, one sub-sample was freeze-dried and preserved at (-20°C) for chemical analyses and the last was used for determination of the digesta particle size. Rumen samples were separated into Large particle (LP), Small Particles (SP) and Very Small particle (VS), by wet sieving using gradual sieves. LP were defined as those retained in the 4 mm and 1.18mm sieves, SP were defined as

those that passed through 1.18 mm sieves but retained in 0.050 mm-sieves and particles that passed through the 0.050 mm were considered as VS.

Mean rumen load (WRC) was estimated with two different equations depending on the frequency of feeding as follow:

- when meal was distributed twice a day:

Mean WRC = (3 x WRC0h + 3 x WRC3h + 6 x WRC 9h + 12 x WRC 21h) /24

- when meal was distributed four times a day:

Mean WRC = (2 x WRC0h + 1 x WRC1h + 1 x WRC 2h + 2 x WRC 4h) /6

where WRC (0, 1, 2, 3, 4, 9, 21 h) corresponded to rumen load collected at 0, 1, 2, 3, 4, 9 and 21 hours respectively after the morning meal.

Different values of coefficients were applied (for example we used the mean time between the distribution of the meal and the rumen emptying) to calculate the mean WRC. Only minor differences were observed between the different methods of estimation and the range of effects was always the same.

The passage rate (%/h) of indigestible rumen fibre (kpADL) was estimated using the ratio: (faecal intake of lignin (g) / 24) / (mean total amount of lignin in the rumen (g)).

The mean retention time (h) of indigestible rumen fibre (MRT) was estimated as the ratio: 1/kpADL (%/h).

Because there were 4 rumen emptyings for animals receiving two meals per day, it was possible to fit the corresponding data to a bicompartimental model. The first compartment was for the large particles (LP > 1mm) and the second was for the small particles (SP < 1mm). It was assumed that LP was trapped in the rumen. They were comminuted into SP by rumination. The basic differential dynamic equations of this system were:

$$dLP/dt = - kc. LP$$

$$dSP/dt = kc.LP – kp.SP$$

In these equations kc was the fractional comminution rate of LP and kp the fractionnal outflow rate of SP. The 16 individual values of kc and kp and the initial values of LP (LPo) and SP (SPo) were obtained by fitting data with the software "Modelmaker" (version 3, Cherwell). The initial values for the flow of comminution (FLco) and transit (FLto) were calculated from LPo, Spo, kc and kp.

Chemical analyses

The DM content of the fresh forage and refusals was obtained daily by drying it to a constant weight at 60°C in a forced-draught oven. Samples were then ground to 1 mm prior to chemical analysis. Organic matter (OM) content was measured after a 10 h pyrolysis at 550°C. Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) were estimated using the methods of Van Soest *et al.* (1991). The nitrogen content was determined from a representative sample of dry forage using the Dumas method (AFNOR, 1988).

Statistical analyses

Data were analysed using the General Linear Model procedure of SAS (1987) including the forage, the frequency, the interaction forage * frequency and the animal effects.

Table 1. *Chemical composition (% dry matter) of a 21- and 35-day old fertilized Digitaria decumbens grass*

Regrowth age (days)	21	35
Organic matter	87.1	89.0
Crude protein	13.0	12.3
Neutral detergent fibre	72.0	74.2
Acid detergent fibre	37.7	40.0
Acid detergent lignin	7.0	7.0

Table 2. *Daily intake (dry matter, neutral detergent fibre, acid detergent fibre), dry matter intake during the principal morning meal of Black-belly rams given a 21- or a 35-day old Digitaria decumbens at two or four meals per day*

Age of regrowth (Age)	21		35			Main effects		
Frequency of meal (Freq)	4	2	4	2	s.e.	Age	Freq	Age* Freq
Intake								
Dry Matter (g/kg $W^{0.75}$)	81.8[a]	79.3[a]	67.2[b]	61.6[b]	9.2	***	NS	NS
Dry Matter (g/d)	1475.1[a]	1371.6[a]	1173.8[b]	1093.9[b]	151.9	***	NS	NS
Neutral detergent fibre (g/d)	1102.2[a]	986.1[a]	831.2[b]	837.1[b]	114.1	***	NS	NS
Acid detergent fibre (g/d)	586.5[a]	558.6[a]	426.1[b]	442.6[b]	63.0	***	NS	NS
DMI2-3h (g)†	243.7[a]	328.4[b]	232.0[a]	238.7[a]	7.79	***	***	***

† DMI2-3h: dry matter intake during the principal morning meal

a, b, c Values within rows with different superscripts are different ($P < 0.05$)

NS = not significant, * $P < 0.005$; ** $P < 0.001$; ****P<0.0001*.

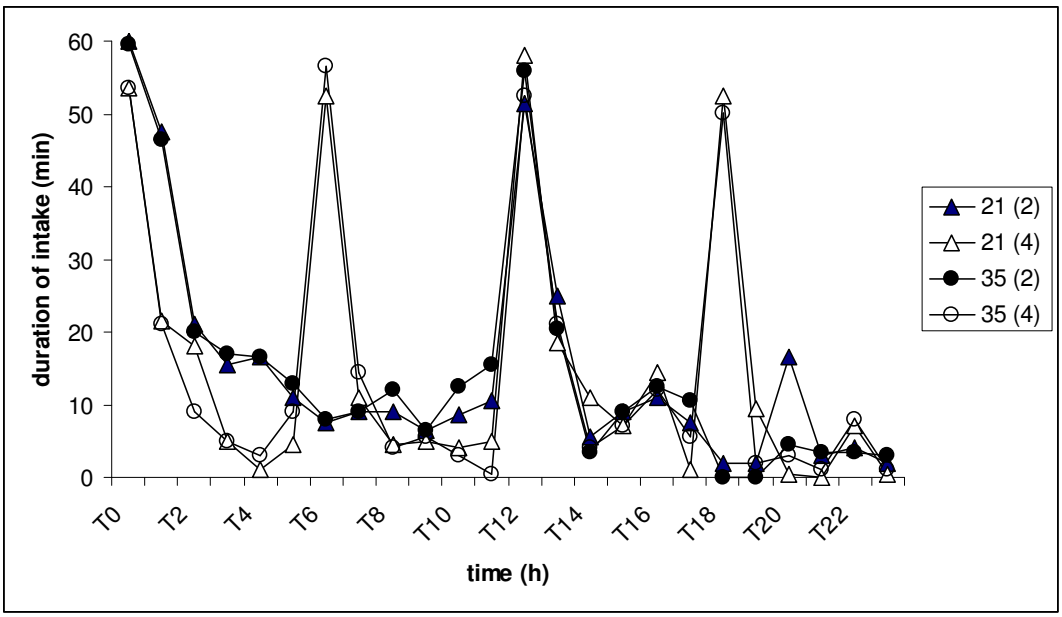

Figure 1a. Effect of feeding frequency on the feeding pattern of Black-belly rams given a 21- or a 35-day old *Digitaria decumbens* at a frequency of two or four meals per day.

21 (2): 21-day old, 2 meals; 21 (4): 21-day old, 4 meals; 35 (2): 35-day old, 2 meals; 35 (4): 35-day old, 4 meals.

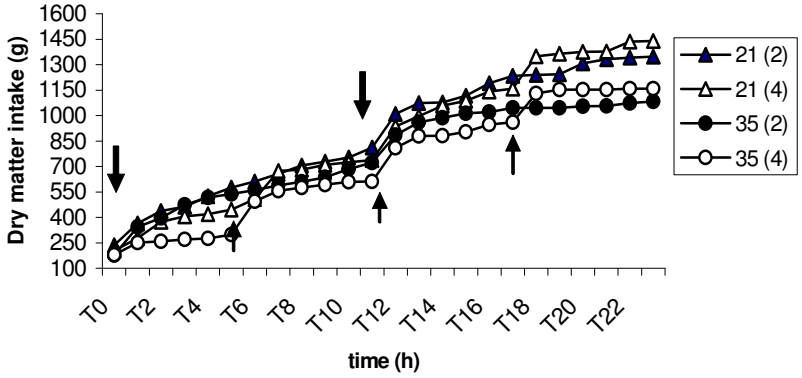

Figure 1b. Effect of feeding frequency on the dry matter intake of Black-belly rams given a 21- or a 35-old *Digitaria decumbens* at two or four meals per day.

21 (2): 21-day old, 2 meals; 21 (4): 21-day old, 4 meals; 35 (2): 35-day old, 2 meals; 35 (4): 35-day old, 4 meals

Table 3. *Ingestive behaviour parameters (idling, eating, ruminating and chewing time; eating, ruminating, and chewing index) in Black-belly rams given a 21- or a 35-day old Digitaria decumbens at two or four meals per day*

Age of regrowth (day)	21		35			Main effects		
Frequency of meal (Freq)	4	2	4	2	s.e.	Age	Freq	Age*Freq
Eating time (min)	371.9	372.0	337.0	367.5	42.8	NS	NS	NS
Ruminating time (min)	431.9	414.0	423.5	364.4	76.2	NS	NS	NS
Chewing time (min)	803.7	786.1	760.4	731.9	102.4	NS	NS	NS
Idling time (min)	636.2	653.9	679.6	708.1	102.4	NS	NS	NS
Eating time 6h†	103.7^{a}	171.2^{b}	111.2^{a}	170.0^{b}	24.5	NS	***	NS
Ruminating time 6h††	80.0	67.6	96.6	63.7	33.9	NS	NS	NS
Idling time 6h†††	176.3^{a}	121.2^{b}	152.2^{ab}	126.3^{b}	40.1	NS	**	NS
Eating index DM (min/gDMI)	0.25^{a}	0.27^{a}	0.29^{a}	0.34^{b}	0.04	***	*	NS
Ruminating index DM (min/gDMI)	0.29^{a}	0.31^{ac}	0.36^{b}	0.34^{bc}	0.04	**	NS	NS
Chewing index DM (min/gDMI)	0.54^{a}	0.58^{a}	0.65^{b}	0.67^{b}	0.06	***	NS	NS
Eating index NDF (min/gNDFI)	0.34^{a}	0.38^{ab}	0.41^{bc}	0.44^{c}	0.05	**	NS	NS
Ruminating index NDF (min/gNDFI)	0.39^{a}	0.43^{a}	0.51^{b}	0.44^{a}	0.06	**	NS	*
Chewing index NDF (min/gNDFI)	0.73^{a}	0.81^{ac}	0.92^{b}	0.88^{bc}	0.09	***	NS	NS

a, b, c Values within rows with different superscripts are different ($P < 0.05$)

NS = not significant, * $P < 0.005$; ** $P < 0.001$; *** $P<0.0001$

† Eating time during the first 6 hours after the distribution of the morning meal

†† Ruminating time during the first 6 hours after the distribution of the morning meal

††† Idling time during the first 6 hours after the distribution of the morning meal

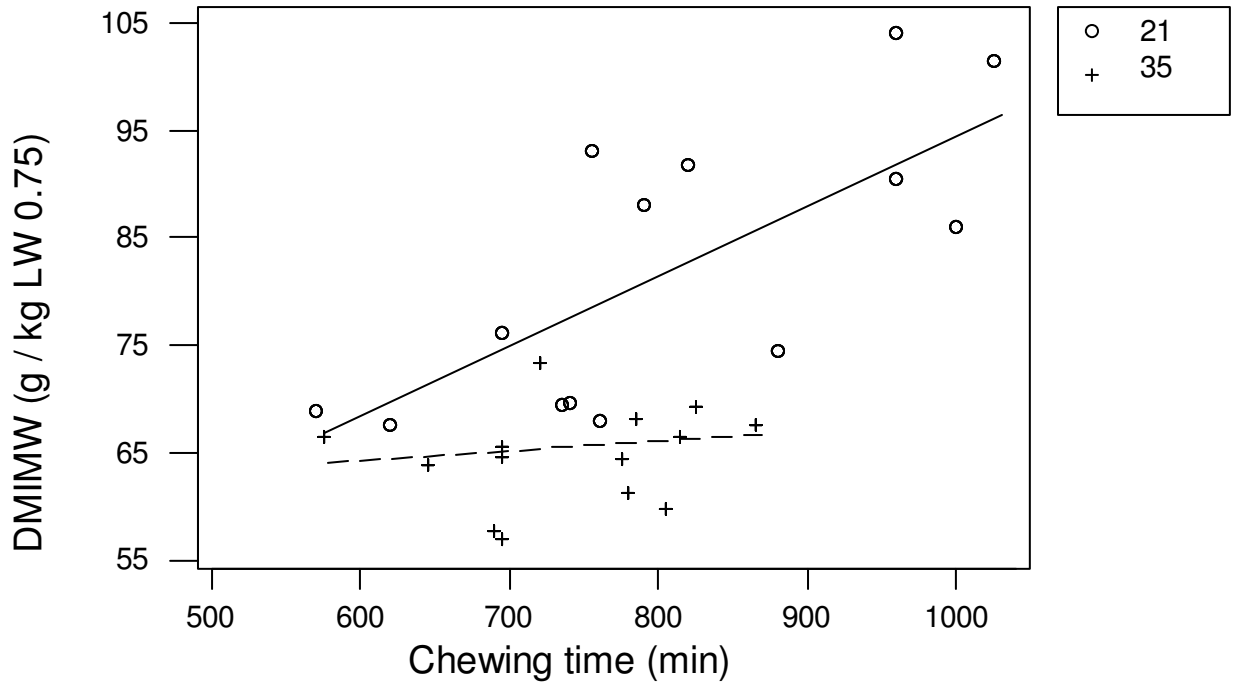

Figure 2. *Changes in dry matter intake (g DM / g $LW^{0.75}$) with the chewing time (min) in Black-belly rams given a 21- or a 35-old Digitaria decumbens at two or four meals per day.*

Results

Chemical composition and intake

Chemical composition of grass is presented in Table 1. Minor differences were observed between the roughages despite the difference in regrowth age. Values for dietary intake are reported in Table 2. Irrespective of the compound, intakes were significantly higher in the 21 than in the 35-day grass ($P < 0.0001$). Daily intake tended to be higher as meal frequency increased but the differences were not significant ($P > 0.1$).

Dry matter intake recorded just before rumen emptying (DMI2-3h) was significantly higher when animals fed with the 21-day-grass received two instead of four meals per day ($P = 0.000$) (Table 2). Moreover, when animals received meals twice a day, DMI2-3h was significantly higher with the 21-day than in the 35-day grass (Table 2). It also appeared that this quantity was positively related to daily dry matter intake (DMI):

DMI2-3h = 135 + 0.098 DMI (n = 120, $R^2 = 11.4\%$, rsd = 65, $P = 0.000$)

Feeding behaviour

Intake pattern is illustrated in Figures 1a and 1b. The number of main peaks in intake was equal to the number of meals. This was consistent with the above mentioned data on DMI2-3h, feeding frequency had an impact on the pattern of the first meal. Eating time 6h after the first distribution of meal was more significant when two meals were offered (Table 3 and Figures 1a, 1b).

Forage and frequency of diet had no significant effect on the daily time spent eating, ruminating, chewing and idling ($P > 0.15$; Table 3). The indexes of intake, ruminating and chewing increased with the 35-day grass in comparison with the 21-day grass ($P < 0.006$).

Dry matter intake (DMI) was positively related with chewing time (ChewT, minute) and the regression slope obtained with the 21-day grass was much more significant (Figure 2) as indicated with the following equations:

Table 4. *Total tract digestibility (dry matter, organic matter, crude protein, neutral detergent fibre, acid detergent fibre), in sacco DM degradability of a 21- or a 35-day old Digitaria decumbens (Deg21, Deg35), rumen turn over of liquid (kl) and fibrous particle (kpADL) in Black-belly rams given a 21- or a 35-day old Digitaria decumbens at a frequency of two or four meals per day*

Age of regrowth (Age)	21		35			Main effects		
Frequency of meal (Freq)	4	2	4	2	s.e.	Age	Freq	Age*Freq
Total tract digestibility (%)								
Organic Matter	76.0^{a}	75.6^{a}	66.7^{b}	65.3^{b}	1.6	***	NS	NS
Neutral detergent fibre	79.2^{a}	79.6^{a}	72.5^{b}	75.3^{c}	1.8	***	*	NS
Acid detergent fibre	79.2^{a}	81.0^{a}	70.2^{b}	74.6^{c}	1.9	***	***	NS
Crude Protein	73.3^{a}	74.3^{a}	61.1^{b}	61.9^{b}	2.3	***	NS	NS
In sacco DM degradability (%)								
Deg21	.	68.4^{a}	.	60.4^{b}	1.4	***	.	.
Deg35	.	67.0^{a}	.	62.7^{b}	2.4	***	.	.
kpADL (%/h)	3.9^{a}	3.3^{a}	4.3^{a}	2.9^{b}	1.0	NS	*	NS
kl (%/h)	8.4^{a}	7.1^{ab}	6.3^{b}	6.9^{ab}	1.42	*	NS	NS
Flow of liquid (g/h)	1301	1181	1354	1210	288	NS	NS	NS

a, b, c Values within rows with different superscripts are different ($P < 0.05$)

NS = not significant, * $P < 0.005$; ** $P < 0.001$; *** $P < 0.0001$

- 21-day grass

DMI (g/$LW^{0.75}$) = 27.8 + 0.07 ChewT

(n = 62; R^2 = 55.4; rsd = 9.31; P = 0.000)

- 35-day grass

DMI (g/$LW^{0.75}$) = 45.2 + 0.03 ChewT

(n = 60; R^2 = 18.1; rsd = 4.79; P = 0.001)

Thus, efficiency of chewing, which correspond to the regressions in Figure 2 was higher at 21-day old than at 35-day old. Overall, the eating index DM decreased when meal frequency increased but no difference was recorded for the 21-day grass ($P > 0.3$). No effect of meal frequency was recorded for the indexes of ruminating and chewing ($P > 0.3$).

Total tract digestibility

Total tract digestibilities are reported on Table 4. Values were highest with the youngest forage ($P < 0.0001$). The meal frequency had no effect on the total tract digestibility of OM and CP ($P > 0.1$). However, the NDF and ADF total tract digestibility of the 35-days old grass significantly decreased with the increase in number of meals ($P < 0.009$). This latter one has no effect on the total tract digestibility of the 21-day grass ($P > 0.09$).

Nylon bag degradation

Nylon bag DM degradation (24 h) of 21-day grass was significantly higher than the 35-day grass, incubated in rumen of animals respectively fed with 21-day and 35-day grasses ($P < 0.05$; Table 4). Furthermore, the levels of degradation of the 21- and 35-day grasses were higher when the nylon bags were incubated in the rumen of animals fed the 21-day diet ($P < 0.02$).

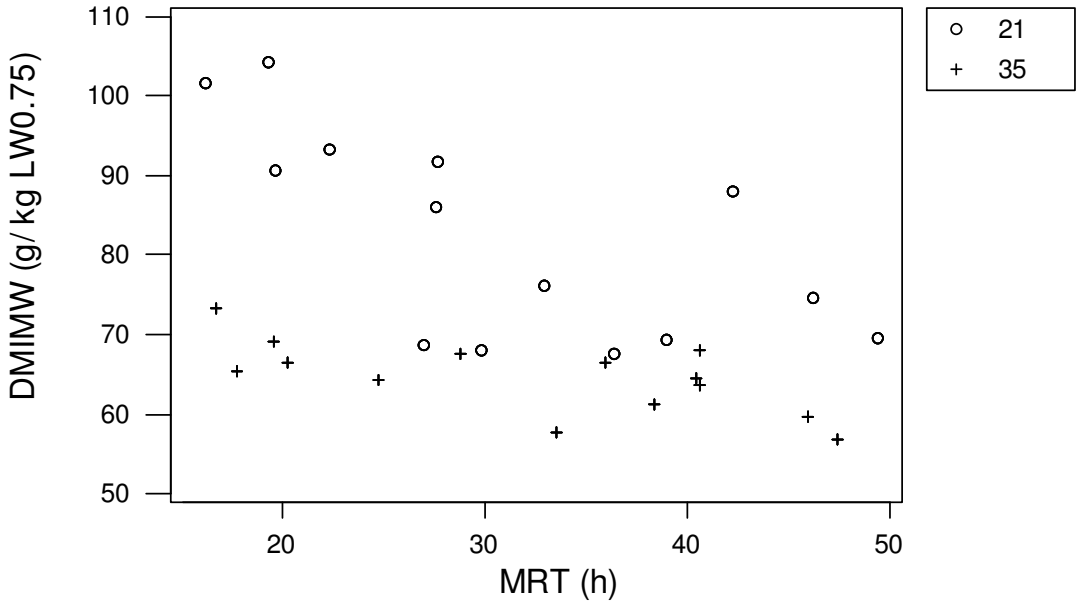

Figure 3. *Changes in dry matter intake (g DM / g $LW^{0.75}$) with the mean retention time (h) in Black-belly rams given a 21- or a 35-old Digitaria decumbens at a frequency of two or four meals.*

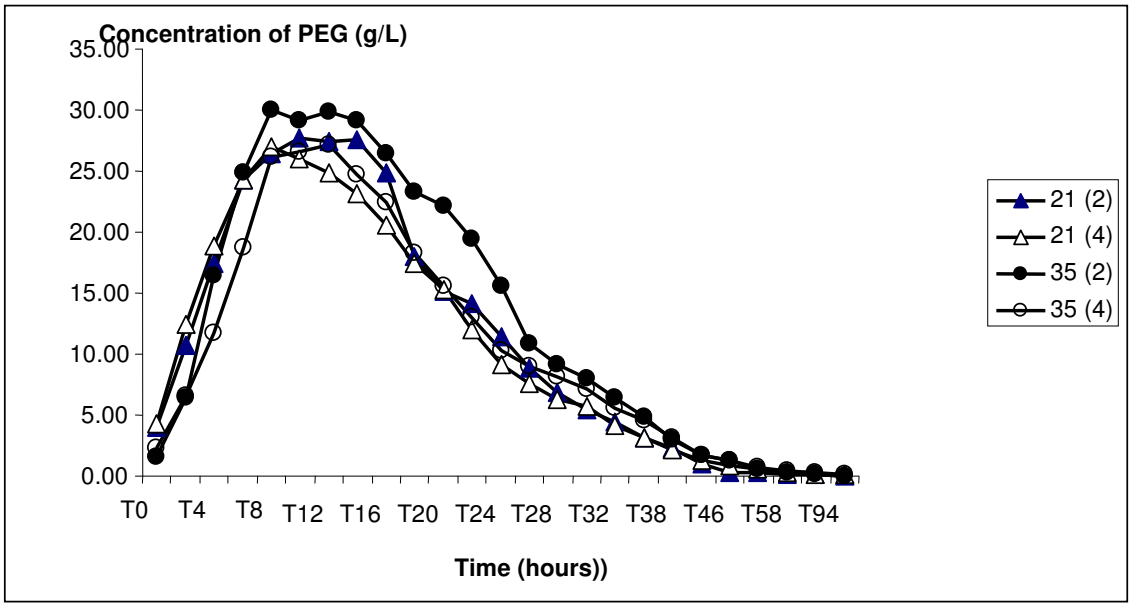

Figure 4. *Effect of frequency of meals on transit time of the liquid phase of rams given a 21- or a 35-day old Digitaria decumbens at a frequency of two or four meals per day.21 (2): 21-day of regrowth, 2 meals; 21 (4): 21-day of regrowth, 4 meals; 35 (2): 35-day of regrowth, 2 meals; 35 (4): 35-day of regrowth, 4 meals.*

Table 5. *Mean rumen load (WRC) of dry matter (DM), Neutral detergent fibre (NDF) and granulometry in Black-belly rams given a 21- or a 35-day old Digitaria decumbens at a frequency of two or four meals per day*

Age of regrowth (Age)	21		35			Main effects		
Frequency of meal (Freq)	4	2	4	2	s.e.	Age	Freq	Age* Freq
DM WRC (g)	710.0[a]	858.1[b]	665.0[a]	1011.6[c]	110.8	*	***	***
NDF WRC (g)	425.6[a]	490.8[a]	358.2[b]	611.8[c]	73.4	NS	***	***
Dry Matter WRC (g/g DM intake)	1.01[a]	0.77[b]	0.96[a]	1.03[a]	0.10	*	NS	***
NDF WRC (g/g NDF intake)	0.81[a]	0.58[b]	0.77[a]	0.80[a]	0.09	*	*	***
Large particle (g/g DM intake)	0.44[a]	0.21[b]	0.37[ac]	0.32[c]	0.04	NS	***	***
Large particle (g/g NDF intake)	0.49[a]	0.25[b]	0.43[ac]	0.36[c]	0.05	NS	***	***
Small particle (g/g DM intake)	0.23[a]	0.19[b]	0.18[b]	0.20[ab]	0.02	*	NS	***
Small particle (g/g NDF intake)	0.25[a]	0.21[b]	0.20[b]	0.22[b]	0.02	*	NS	***
Very Small particle (g/g DM intake)	0.34[a]	0.37[a]	0.41[a]	0.51[b]	0.05	***	**	NS

a, b, c Values within rows with different superscripts are different ($P < 0.05$)

NS = not significant, * $P < 0.005$; ** $P < 0.001$; *** $P < 0.0001$

Rumen turnover

Generally, the turnover of Acid Detergent Lignin (kpADL) increased positively with diet frequency ($P < 0.0005$) but the only notable difference was observed in animals offered two meals of a 35-day grass ($P < 0.0005$) (Table 4). Mean retention time (MRT, hour) was negatively related with dry matter intake as illustrated with the figure 3 and the following equations:

- 21-day grass

DM intake ($g/LW^{0.75}$) = 109 – 0.87 MRT

(n = 64; R^2 = 58.4; rsd = 8.85 g ; P = 0.000)

- 35-day grass

DM intake ($g/LW^{0.75}$) = 74.4 – 0.31 MRT

(n = 60; R^2 = 62.5; rsd = 3.24 g ; P = 0.000)

For the fractional outflow rate of liquid phase (kl) and the flow of liquid, no significant difference was reported with the diet frequency ($P > 0.5$; Table 4; Figure 4). In contrast, the turn over of the liquid phase decreased with the maturity of the forage whereas fibrous particle and flow of liquid did not vary ($P > 0.05$).

Rumen load

The mean rumen load of DM (g/g intake) varied significantly with respect to regrowth age of the forages ($0.03 < P < 0.02$) (Table 5). The pool of LP significantly increased ($P < 0.05$) with the regrowth age when the rams had two meals a day whereas no significant changes were observed with the four meals. The pool of SP significantly decreased ($P < 0.05$) with the regrowth age when the rams had four meals a day whereas no significant changes were observed with the two meals. The pool of VS significantly increased ($P < 0.05$) with the

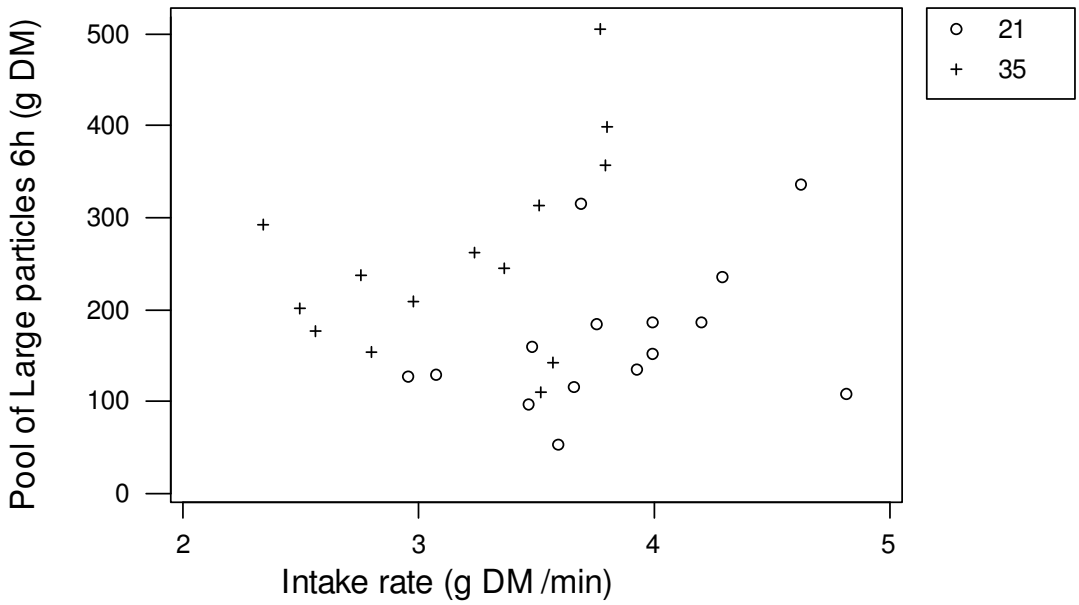

Figure 5. *Changes in the pool of large particles (g DM) registered 6 hours after meal distribution with rate intake (g DM/ min) in Black-belly rams given a 21- or a 35-day old Digitaria decumbens at a frequency of two or four meals per day.*

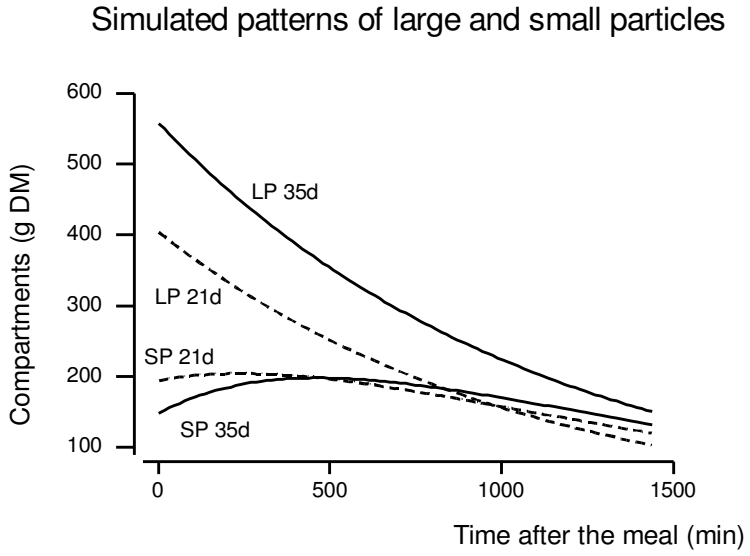

Figure 6. *Simulated patterns of large (LP) and small particles (SP) compartments, with time, in the rumen Black-belly rams given a 21- (21d) or a 35-day old (35d) Digitaria decumbens.*

regrowth age when the rams had two meals a day whereas no significant changes were observed with the four meals.

The meal frequency had a significant effect ($0.06 < P < 0.0001$) on the rumen load of DM (g/g intake). There was a significant interaction between the forage age and the meal frequency. The amount of LP (g/g intake) in the rumen content increased with the diet frequency ($P < 0.01$). The amount of SP (g/g intake) in the rumen content increased with the frequency of distribution of the 21-day grass ($P < 0.01$) whereas no significant effect was observed with the 35-day grass. With respect to the pool of VS, its amount decreased with the frequency of distribution of the 35-day grass ($P < 0.05$) whereas no significant effect was observed with the 21-day grass.

Assuming that hourly intake rate is constant, intake rate recorded 6 hours after the distribution of the morning meal was positively correlated with the pool of LP. Moreover, it appeared that, at the same intake rate there were more LP with the 35-day grass (Figure 5).

The figure 6, which results from kinetic adjustments, clearly shows that the regrowth age mainly influences the LP stasis in the rumen and that comminution is not able to compensate the higher LP fill with the 35-day grass.

Intake and digestive parameters correlations

Correlation analyses indicate that DMI (%LW) was mostly related with dry matter intake rate (0.82). It was also linked with organic matter total tract digestibility ($R = 0.68$) and chewing index ($R = -0.64$). In addition, intake rate was, linked with chewing index ($R = -0.80$) and organic matter total tract digestibility ($R = 0.61$) at a constant intake. Organic matter total tract digestibility was limited with the chewing index ($R = -0.66$). All these coefficients of correlation were significantly high. In contrast, age response of DMI (%LW) showed no significant link to total chewing duration, rumen load and lignin MRT.

Table 6. *Predictive equations of fractional comminution rate (kc), initial flow of transit (FLto), initial values of total large particle compartment (LPo), LPo with a 21-day old (LPo21) and 35-day old Digitaria decumbens (LPo35) from initial values of small particle compartment (SPo), LPo, dry matter intake during the main morning meal (DMI2-3h) and chewing index (ChewId) respectively*

	s.e.	P
kc = 0.075 – 0.00011*SPo	0.0079	***
FLto = 0.049*LPo	6.1	***
LPo = 229 + 0.479*DMI2-3h	95	*
LPo21 = 980 – 0.82*ChewId + 354	98	**
LPo35 = 980 – 0.82*ChewId + 608	98	**

* $P < 0.005$; ** $P < 0.001$; *** $P < 0.0001$

Individual variations of intake

The major relationship among animals was that individual DMI (%LW) was negatively related with lignin MRT (R = -0.87) and with organic matter total tract digestibility (R = -0.68). Whereas, DMI (%LW) was positively related with total chewing time (R = 0.84). Consistent relationships associated lignin MRT and organic matter total tract digestibility (R = 0.65) and chewing time (R = -0.79). The latter was negatively related with organic matter total tract digestibility (R = -0.44). All the coefficients of correlation, except the last one, were significant.

Modelling particle kinetics with two meals

The initial LP compartment (LP_0) increased significantly at 35-day grass *v.* 21-day grass (558 *v.* 404 g, rsd = 100g, $P < 0.02$). In contrast the SP_0 compartment decreased at 35-day grass *v.* 21-day grass, though not significantly (148 *v.* 194g, rsd = 62g, $P < 0.18$). The parameters kc (0.056±0.011 1/h) and kp (0.096±0.034 1/h) were not altered by any of the regrowth age. The initial flow of comminution decreased between the 35-day grass and 21-day grass, though not significantly (15.6 *v.* 23.4 g/h, rsd = 11.3 g/h, $P < 0.21$). Meanwhile, there was a significant increase in the flow of transit at 35-day grass *v.* 21-day grass (30.3 *v.* 16.1 g/h, rsd = 6.0 g/h, $P < 0.002$). There was no significant influence of rams on these elements.

Although, the parameters kp and kc were closely linked (R = 0.63, $P < 0.009$), the relationship was not influenced by either the ages of regrowth or the rams. The SPo compartment was negatively related to kc (Table 6). Another major relationship was the positive influence of LPo on flow of transit (Table 6).

The initial value of LP was significantly and positively influenced by DM intake before the first rumen emptying (DMI2-3h, Table 6). DMI2-3h was negatively linked with the daily level of DMI (see part of the Results – Diet composition and intake).

Table 6 shows that the chewing index (min/kgDMI), which was largely explained by the ram effect, was negatively related to LPo (P = 0.005). However, with a single ram, the influence of regrowth age was significantly positive (P = 0.001). Thus, inducing a larger LPo compartment for the same chewing index value. The two equations of this model of adjustment are presented in Table 6.

Discussion

Effect of regrowth age

The effect of regrowth age on intake and total tract digestibility in this study are consistent with previous studies (Chenost, 1975; Aumont *et al.*, 1995; Archimède *et al.*, 2000), illustrating negative correlations between forage age and the two main nutritive parameters (intake and total tract digestibility) of forage. Archimède *et al.* (2000) reported a mean daily decrease in intake and total tract digestibility with increasing regrowth age of pangola (14, 28, 42 and 56 days) at an average rate of 0.66g/kg $LW^{0.75}$ and 0.52% respectively. For this current study, with respect to the regrowth age of pangola at 21 and 35 days, the corresponding values for mean daily decrease in intake and total tract digestibility as regrowth age increases are 1.26 g/kg $W^{0.75}$ and 0.74% respectively. The low range between the two regrowth ages (14 days) and the younger stages of the grass in this study could explain the observed higher values for mean daily decrease in comparison to those observed by Archimede *et al.* (2000). These results could be closely linked with the physiology of pangola grass. Wilson, (1994) reported that C4 grasses have faster maturation compared to C3 grasses.

Considering the low chemical differences that we recorded between the two regrowth stages, it can be argued that, chemical composition alone may not necessarily be sufficient criterion for explaining all the effects of maturation. Rather structural changes in the cell wall characteristics could be better indicators.

The improvement observed in rumen degradation, with the nylon bag method, may strongly suggest that enzymatic microbial activity could also be a limiting factor of the forage digestion at 35-days grass *v.* 21-day grass.

In the one hand, for rumen fractional turnover, where rams were offered two meals, results obtained in our study were generally similar to those cited in previous studies (Poppi *et al.*, 1981a; Ichinohe *et al.*, 1995; Archimède *et al.*, 2000). On the other hand, where rams were offered four meals, our results showed little difference, which differs from previous studies. This showed that rumen fractional turnover decreased significantly with the maturity of the forage and we have no explanation for this inconsistency.

With respect to the pool of rumen content, our results are quite similar to the works referred to in the literature. Rumen content increased with the maturity of the forage. The amount of digesta trapped in the rumen as LP largely increased with regrowth age as illustrated by several authors (Poppi *et al.*, 1981b; Ichinohe *et al.*, 1995). Modelling the rumen emptying data of the two meal frequency clearly emphasized the increase of LP load throughout the day and the limited influence of chewing efficiency on rumen fill for aged forage.

Furthermore, the correlations between DMI (% LW) and the other parameters suggested that the limitation of intake capacity due to age increase was, firstly, chewing efficiency, appreciated with values of intake rate and chewing index. The second factor seemed to be the amount of indigestible matter contained in the forage which could limit the reticulo-omasal orifice capacity to treat larger flows of matter.

The correlations between individual variations revealed that animals having a higher capacity of intake also presented a shorter transit time and a lower digestibility capacity. Moreover, they also have longer mastication time per day. More work is required to assess the extent to which these individual variations and relationships are repeatable and linked to genotypic influence.

Effect of diet frequency

Few studies have dealt with the effect of diet frequency on intake and digestion with forage being the sole ingredient of the ration (Bunting *et al.*, 1987; Faichney, 1968; Burt and Dunton, 1967, Dulphy *et al.,* 1980). Moreover, none of these studies have dealt exclusively with fresh grass and tropical grass.

In our study, although it was not significant, an increase in intake was recorded with an increase in feeding frequency, with a higher incidence on the old forage. This tendency is the same as those found by Burt and Dunton, (1967) and Bunting *et al*. (1987), who worked with high quality forage. These authors have explained this increase of intake by an increasing rate of DM outflow from the rumen, as it was recorded in our experiment. In addition, like Ulyatt *et al*., 1984, we observed that the dynamics of digesta in the rumen was altered by feeding frequency. The highest reticulo-rumen pool sizes were recorded when feeding frequency decreased and this above all with the older forage (35 days). This variation could be explained by the lower ruminal turnover. The highest frequency of meals could accelerate ruminal motility and stimulate transit. Consequently, an increase of intake is recorded.

The negative effect of the increased rate of DM turnover from the rumen is the escape of potentially degradable fibre from the rumen of animals fed with low digestible grass. This phenomenon could explain the depressive effect of meal frequency on digestibility. Our results for impact of feeding frequency on total digestibility of DM, OM and cell wall

constituents do not differ from previous studies, where differential effects are reported according to forage quality. Bunting *et al*., (1987), when studying a high quality forage, noted that apparent tract digestibility of DM, OM and cell wall constituents were not significantly affected by frequency (2, 4, 8 times daily), which was also observed with our 21-day pangola grass. These authors concluded that the response of animals to frequent feeding would be highly dependent on forage quality. Our study confirms this hypothesis. Negative effect of increasing meal frequency on the total tract digestibility of NDF (and ADF) was recorded for the 35-day grass whereas no effect was recorded for the 21-day grass.

Conclusions

This experiment was performed to evaluate the pertinence of working in non steady conditions when studying intake and digestion of tropical forage. Increasing frequency of meals tends to increase intake whereas significant decrease of fibre digestion was recorded with the older forage. This result shows a faster evacuation of the rumen content mainly with older forage. These results underline the need to conduct studies with animals fed according to a pattern closely related to field conditions.

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PUBLICATION 4

Feeding behaviour and rumen load in related sheep receiving two equal meal of a tropical forage

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Abstract

The intake, feeding behaviour, and rumen load of fresh Digitaria decumbens grass were compared following the half a day. Four rams (mean liveweight: 50.96 ± 2.91 kg) received successively a 56-, 21- and 21-day respectively chopped, long and chopped old forage during three 30-day periods. Pangola grass were distributed ad libitum twice a day in equal quantities at 12-hours interval. Whatever the age of forage, dry matter intake and Neutral Detergent Fiber rumen load registered during morning meals tend to be higher but no significant difference has been registered. Ruminating and real mastication indice respectively tend to be and are more important the night for the young and the old forage. Amounts of NDF large particles (g/gNDFI) just after the principal meal, tend to be and are higher the night respectively for the old and the young forage. Just before the distribution of the main meal, quantities of NDF large particles (g/gNDFI) are significantly higher the night with the oldest forage whereas no significant difference was registered with the youngest forage. Consequently, there is an effect of the day-night cycle on several parameters of ingestive and digestive dynamics.

Keywords: Intake; Feeding behaviour; Rumen load; Tropical forage; Rams

1. Introduction

To improve the nutritive value of tropical forages, it is necessary to increase their intakes (Minson, 1990). Large variations of the latter have been registered (Aumont, 1995). The major factor controlling grass intake, particularly for forages of low digestibility, is the

retention time of fibrous constituents in the rumen (Poppi et al., 1985). Therefore, to flow out of the rumen, the forage particles have to be smaller in size, but denser. Previous studies (McLeod and Minson, 1988 and McLeod *et al.,* 1990) have shown that mainly, the breakdown of the forage large particles to small particles is achieved by chewing during eating and rumination and only a small part of the particle breakdown can be attributed to microbial degradation. Studies on the digestive dynamics are very complex. Therefore, classically, experimental trials are realised, feeding animal daily and at regular times, so that to achieve steady state conditions in the rumen. These studies have improved knowledge on digestion dynamics in the rumen, particularly concerning cellulolysis and physical degradation of forage. Nevertheless, normally, farm animals absorb two mains meals a day (Dulphy et al, 1980). Consequently, hypothesis could be made that under such conditions, dynamics of intake and digestion could be different than when registered under steady state conditions. In a previous study, we compared intake and digestion when animals were fed 2 or 4 times a day. In the present experiment, our objective is to analyze eventual differences in intake, feeding behaviour and rumen load when compared two half a day.

2. Materials and methods

2.1. Location

Research was carried out in 2004 at the experimental animal station at the National Agronomic Research Institute (INRA) in the French West Indies (Guadeloupe, latitude 16.16 N, longitude 61.30 W). Temperatures ranged on average from 21°C to 31°C. Mean rainfall on the experimental site was 3000 mm / year. Rainfall was regular during the experiment.

2.2. Experimental design, animals, diets and feeding

The harvest of a perennial *Digitaria decumbens* (pangola) pasture, divided into plots and subplots, was planned in such a way as to have 56- and 21- day old regrowth for 2 successive 30 days periods (periods 1 and 2) then 21-day old regrowth for a 30 days period (period 3). Plots P21 and P56 were subdivided into 21 and 30 subplots respectively. The first subplot of P21 and P56 were cut 22 and 57 days respectively before the beginning of experimental periods. One subplot was cut per day. One kg/ha/regrowth age of mineral nitrogen fertiliser was added to each subplot the same day. Areas of the subplots were 400 and 300 m² respectively for P21 and P56. Each subplot of P21 or P56 was cut twice or once. At the beginning of each period, the first subplot of P21 and P56 had 21 and 56 days of regrowth respectively. Within a plot, grass from subplot n had one day less regrowth than subplot n-1. Consequently during experimental periods, regrowth age of the grass harvested daily, was exactly 21 and 56 days old respectively. Four Black-belly rams (mean liveweight: 50.96 ± 2.91 kg) were used in this experiment. They were fitted with ruminal cannulae and maintained in metabolism cages with free access to water. The 30-days experimental periods consisted of 14 days of adaptation to the diet, 5 days of intake and total tract digestibility measurements and 11 days of rumen emptying. The pangola grass was cut every morning. During periods 1 and 3, the 56- and 21- old day regrowth were chopped (4 cm-length) just before being offered whereas during period 2, the 21-old day regrowth were offered long. The regrowth age and the physical treatment have been chosen to induce several level of resistance to mastication, which have significant impact on digestive and ingestive dynamics. Concerning the age of regrowth, hypothesis has been made that maximum mastication will be registered with 56 day regrowth and minimum with the chopped 21 day grass. Concerning the physical treatment, hypothesis has been made that maximum mastication will be registered with the long forage and minimum with the chopped 21 day grass. The amount of forage provided was 1.15 times greater than the animal voluntary intake estimated during periods of adaptation. This amount

was divided in two equal parts distributed at 12-hours interval, twice a day. The second part was kept at 4°C in cold chamber until feeding. Artificial light was switch on during night.

2.3. Measurements

Intake and total tract digestibility were determined from daily weighing of the amounts of food offered, refusals and faeces. Representative samples of grass, refusals and faeces were stored for chemical analyses.

The feeding behavioural parameters (time spent eating, ruminating and idling, percent of prehension) were determined for 24h per measurement period by automatically recording system (IGER recorders*). Apparent mastication indice is the sum of eating and ruminating indices. Real mastication indice is the sum of mastication ingestive and ruminating indices. In the same way, in order to establish a kinetic of intake, troughs were weighed hourly.

During the rumen sampling period, four total emptying of rumen were carried out every 3 days, 1h30 (rumen load max) and 12 hours (rumen load min) after the start of the morning meal and after the start of the evening meal. The time “1h30” and “12h” have been choosed because they represented respectively the end of the main diet after the distribution of meal and the time when level of dry matter in the rumen is minimum (previous observations). After sampling, the total rumen content obtained 12 hours after the start of the meal were stored in a hermetic recipient at 37°C. Animals were fed for 30 minutes, then total emptying of rumen were carried out (rumen load 30).

After every emptying, the rumen content was weighed and thoroughly mixed by hand. Four sub-samples were taken, two for dry matter determination, one was preserved (-20°C) until freeze-dried for chemical analyses determination, while the last was used to determine the digesta particle size. Rumen particles were separated into Large (LP) and Small Particles (SP), by wet sieving using gradual sieves of sizes 4 mm, 1.18 mm, 250 µm, and 50µm. LP

were defined as those retained in the 4 mm and 1.18mm sieves, SP were defined as those that passed through 1.18 mm sieves but retained in 0.050 mm-sieves and particles that passed through the 0.050 mm were considered as Very Small Particles (VS).

The daily passage rate (h^{-1}) of whole rumen fiber content (trADL) was estimated by the ratio: (daily intake of lignin (g) / 24) / (mean total amount of lignin in the rumen (g)).

The morning or evening passage rate (h^{-1}) of whole rumen fiber content (trADL) was respectively estimated by the ratio: (morning or evening intake of lignin (g) / 12) / (morning or evening amount of lignin in the rumen (g)).

2.4. Chemical analyses

DM content of fresh forage and refusals were determined daily by drying at constant weight at 60°C in a forced-draught oven. DM content of faeces was determined in similar conditions using a representative sub-sample. The latter came from a sample obtained by pooling 10% of the daily amount of faeces excreted by each animal. It was then stored (-20°C). Samples were ground (1 mm) prior to chemical analysis. Organic matter (OM) content was measured after a 10 h pyrolysis at 550°C. Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) were estimated following the methods of Van Soest et al, (1991). Nitrogen concentration of forage was determined using the Dumas method.

2.5. Statistical analyses

Data were analysed using the General Linear Model procedure of SAS (1987). The data has been analysed in two steps. First, data concerning the 21- and 56-day chopped grass were analysed. The model included age of forage (D.F. 1), the time of the day (D.F. 1) and age of forage * time of the day interaction. In a second step, we have compared the effect of the

physical treatment on the 21-old regrowth. The model included, the time of the day (D.F. 1), the physical treatment and the interaction between the physical treatment * time of the day.

Table1. Chemical composition (g/kg DM) of a 21- and 56-day old fertilized *Digitaria decumbens* grass.

Regrowth age (days)	**21**	**56**
Organic matter	908	910
Crude protein	144	68
Neutral detergent fibre	743	773
Acid detergent fibre	360	428
Acid detergent lignin	61	64

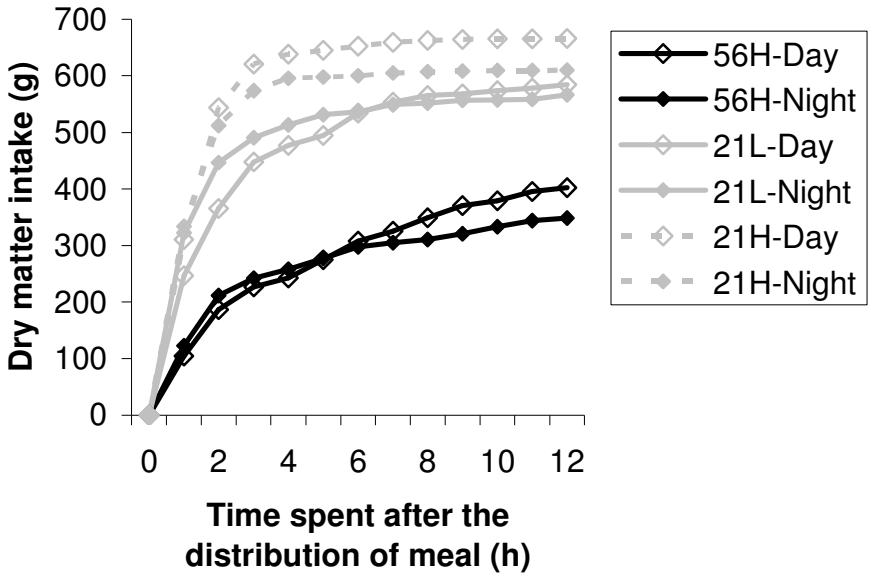

Figure 1. Comparison of kinetics of intake between day and night in rams fed with a chopped 56-day (56H), 21-day (21H) and a long 21-day (21L) regrowth age of *Digitaria decumbens* in 2 equals meals distributed at 7h00 and 19h00.

Table 2. Effect of time of the day (morning *versus* night) on dry matter intake (DMI) and feeding behaviour of rams consuming a chopped 21-day and 56-day regrowth age of *Digitaria decumbens* in 2 equals meals distributed at 7h00 and 19h00.

Day period	**Day**		**Night**			**Main effects**	
Age of regrowth	**21**	**56**	**21**	**56**	**SE**	**Regrowth age**	**Day period**
Intake							
DMI (g/day/$LW^{0.75}$)	34.85a	20.74b	31.89a	17.98b	1.05	<0.0001	0.0231
DMI 1h30 post feeding (g)	433.1a	146.4b	428.9a	166.6b	43.22	0.000	0.718
Rate of intake (%/h)	0.778a	0.242b	0.897a	0.440b	0.0907	0.0004	0.1142
Feeding behaviour (min)							
Intake time (min)	166.27a	213.30c	119.21b	204.40c	12.23	0.0004	0.0480
Rumination time (min)	219.63a	213.80a	301.33b	296.08b	19.02	0.7776	0.0020
Mastication time (min)	35.98	44.37	30.03	35.88	5.93	0.2601	0.2538
Apparent mastication time (min)	385.90a	428.61a	420.54a	509.58b	16.90	0.0036	0.0076
Real mastication time (min)	255.61a	258.18a	331.36b	331.96b	15.39	0.9201	0.0009
Idling time (min)	334.11a	288.40a	299.46a	222.92b	17.00	0.0058	0.0163
Feeding behaviour (index)							
Intake index (min/g DM)	0.248a	0.544b	0.194a	0.606b	0.038	<0.0001	0.9154
Rumination index (min/g DM)	0.328a	0.524a	0.497a	0.888b	0.081	0.0057	0.0097
Ingestive mastication index (min/g DM)	0.054a	0.112b	0.048a	0.105b	0.013	0.0019	0.6395
Apparent mastication index (min/g DM)	0.576a	1.072b	0.691a	1.522c	0.096	<0.0001	0.0166
Real mastication index (min/g DM)	0.382a	0.637b	0.545ab	0.993c	0.079	0.0016	0.0092
Idling index (min/g DM)	0.513a	0.743b	0.496a	0.668b	0.047	0.0022	0.3569

3. Results

3.1. Dietary composition

The mean composition of *Digitaria decumbens* is reported on Table 1. The 56-day old grass is characterised by lower nitrogen and higher fiber contents compared with the 21-day old grass.

3.2. Intake

Effect of day-night cycle according to the age of regrowth

The half a day DM intake is reported on table 2 and figure 1. Globally, the daily dry matter intake and intake registered 1h30 after meal were significantly higher with of the younger forage. The rate of intake is higher for the young forage. Pooling the two regrowth ages, the daily DM intake was significantly higher during the morning. Nevertheless, concerning the 56-day grass the intake during the main meal tended to be higher at night however no significant difference was registered with the 56- or 21-day grasses when the morning was compared with the night.

The tendencies registered with the other components were similar to those registered with dry matter (data not shown).

As illustrated on figure 1, the dynamics of intake were different according to day of regrowth of grasses. Fast intake (3 hours of intake) followed with idling was registered with the 21-day grass whereas slow and regular intake was observed with the 56-days one. Comparing the diurnal and nocturnal intake dynamics, differences appeared between the two regrowth ages. Concerning the 21-days, a faster rate of intake was registered after the distribution of the meals. With the 56 days regrowth significant amount of dry matter are ingested at the end of the night whereas no difference was registered at the beginning of the meal.

Table 3. Effect of time of the day (morning *versus* night) on dry matter intake (DMI) and feeding behaviour of rams consuming a long and chopped 21-day of *Digitaria decumbens* in 2 equals meals distributed at 7h00 and 19h00.

Day period	**Day**		**Night**			**Mean effect**	
Physical treatment	**chopped**	**long**	**chopped**	**long**	**SE**	**Physical treatment**	**Period of the day**
Intake							
DMI (g/day/$LW^{0.75}$)	34.85	31.31	31.89	30.32	1.56	0.1365	0.2371
DMI 1h30 post feeding (g)	433.1a	297.7b	428.9a	375.2ab	38.9	0.001	0.092
Rate of intake (%/h)	0.778a	0.524b	0.897a	0.850a	0.064	0.0434	0.0070
Feeding behaviour (min)							
Intake time (min)	166.27a	235.40b	119.21a	231.17b	15.80	0.0003	0.1391
Rumination time (min)	219.63ac	184.64a	301.33b	265.91bc	16.36	0.0599	0.0008
Mastication time (min)	35.98	40.41	30.03	43.56	7.37	0.2539	0.8530
Apparent mastication time (min)	385.90a	420.03ab	420.54ab	497.08b	25.76	0.0603	0.0583
True mastication time (min)	255.61ac	225.05a	331.36b	309.46bc	19.34	0.2080	0.0025
Idling time (min)	334.11a	300.01ab	299.46ab	222.93b	25.76	0.0603	0.0582
Feeding behaviour (index)							
Intake index (min/g DM)	0.248a	0.407b	0.194a	0.410b	0.021	<0.0001	0.2514
Rumination index (min/g DM)	0.328a	0.320a	0.497b	0.472b	0.031	0.6033	0.0006
Ingestive mastication index (min/g DM)	0.054	0.073	0.048	0.078	0.013	0.0953	0.9976
Apparent mastication index (min/g DM)	0.576a	0.727b	0.691ab	0.882c	0.038	0.0015	0.0060
Real mastication index (min/g DM)	0.382a	0.393a	0.545b	0.550b	0.036	0.8338	0.0016
Idling index (min/g DM)	0.513	0.519	0.496	0.397	0.058	0.4393	0.2593

Effect of day-night cycle according to the physical treatment

Half a day DM intake is reported on table 3 and figure 1. Globally, the DM intakes at the end of the main morning meal and the rate of intake are higher with the chopped grass compared to the long form grass. Nevertheless no significant difference was registered between physical treatments for the daily dry matter intake.

Comparing diurnal and nocturnal phases the intake at the end of the main meal and the rate of intake were higher in night. Nevertheless differences were significant only for the rate of intake.

The rate of intake of the long forage was significantly higher during the nocturnal phase compared to the diurnal whereas no significant difference was registered with the chopped forage.

3.3. Total tract digestibility

OM, NDF and ADF total tract digestibility of diets respectively registered with the 21- and the 56-old of regrowth were 71.06 vs 62.98 (s.e. 0.55), 66.18 vs 56.95 (s.e. 0.52) and 65.49 vs 55.84 (s.e. 0.47). Total tract digestibility was significantly lower with the older forage whatever the dry matter component.

3.4. Feeding behaviour

Effect of day-night cycle according to the age of regrowth

Daily eating and apparent mastication time were significantly higher with the older forage whereas idling time was significantly higher with the younger forage (table 2). Moreover, eating, ruminating, ingestive mastication, apparent mastication, real mastication and idling indices were significantly higher with the older forage.

Comparing nocturnal and diurnal phases, pooling all the regrowth age, ruminating, apparent mastication, real mastication time and indices were significantly higher at night. In contrast, intake time and idling time were significantly higher in the morning.

Ruminating, apparent and mastication indices were significantly higher for the older forage at night whereas no difference was registered with the younger grass.

Effect of day-night cycle according to the physical treatment

A significant effect of the physical treatment was registered on daily eating time indice and apparent mastication indice which were significantly higher with the long forage (table 3).

Pooling both physical treatments, time of rumination and real mastication and indices of rumination, real and apparent mastication were significantly higher at night.

Moreover, whatever the physical treatment, ruminating, apparent mastication, real mastication indices tended to be higher at night. Nevertheless, the difference was significant only for ruminating and real mastication with the chopped forage (table 3). With the long forage, all these indices were significantly higher at night.

Whatever the physical treatment, no significant difference was registered concerning eating, ingestive mastication and idling indices during the two nychtemeral phases.

3.5. Total Rumen load

Effect of day-night cycle according to the age of regrowth

Comparing regrowth age, the total amounts of NDF in the rumen before and at the end of the main meal do not differ. Moreover, no significant difference was registered when comparing the nycthemeral phases. Nevertheless, for a given emptying time the amount of NDF tends to be higher during the diurnal phase.

Table 4. Effect of time of the day (morning *versus* night) on the maximum (1h30 post feeding and minimum (12h00 post feeding) amount of Neutral Detergent Fiber in the reticulo-rumen of rams consuming a chopped 21-day and 56-day regrowth age of *Digitaria decumbens* in 2 equals meals distributed at 7h00 and 19h00.

Day period	**Day**		**Night**			**Main effects**	
Age of regrowth	**21**	**56**	**21**	**56**	**SE**	**Regrowth age**	**Day period**
Whole rumen content (NDF)							
Maximum NDF (g)	923.43	900.36	810.62	865.83	47.27	0.7417	0.1536
Minimum NDF (g)	811.74	811.83	684.69	787.29	42.17	0.2543	0.1058
Maximum NDF (g/g NDF intake)	1.830a	2.803b	1.877a	3.102b	0.172	0.0001	0.3406
Minimum NDF (g/g NDF intake)	1.601a	2.534b	1.595a	2.832b	0.157	<0.0001	0.3769
Whole rumen content (ADF)							
Maximum ADF (g)	447.78	512.00	454.52	481.25	25.11	0.1035	0.6439
Minimum ADF (g)	406.38a	466.53b	358.58a	484.05b	19.38	0.0010	0.4548
Maximum ADF (g/g ADF intake)	1.860a	2.982b	2.209a	3.214b	0.191	0.0004	0.1630
Minimum ADF (g/g ADF intake)	1.679a	2.724b	1.753a	3.243b	0.189	<0.0001	0.1501

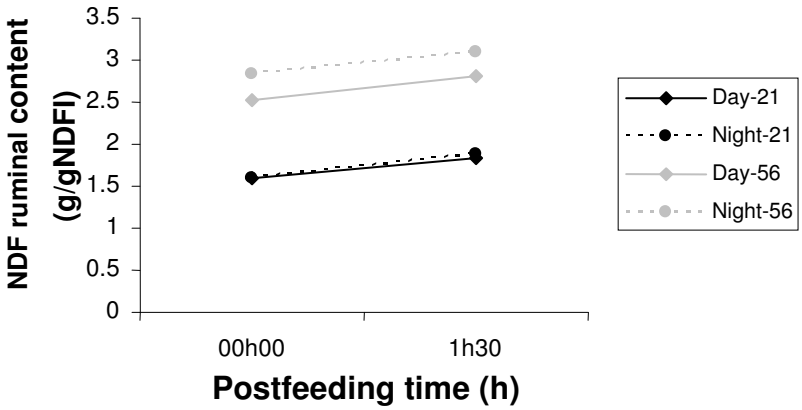

Figure 2. Evolution of NDF rumen content (g/gNDFI) according to the postfeeding time and the cycle day-night in rams consuming a chopped 21-day and 56-day regrowth age of *Digitaria decumbens* in 2 equals meals distributed at 7h00 and 19h00.

Table 5. Effect of time of the day (morning *versus* night) on the maximum (1h30 post feeding and minimum (12h00 post feeding) amount of Neutral Detergent Fiber in the reticulo-rumen of rams consuming a long and chopped 21-day of *Digitaria decumbens* in 2 equals meals distributed at 7h00 and 19h00.

Day period	**Day**		**Night**			**Main effects**	
Physical treatment	**chopped**	**long**	**chopped**	**long**	**SE**	**Physical treatment**	**Period of the day**
Whole rumen content (NDF)							
Maximum NDF (g)	923.43a	735.78b	810.62ab	939.35a	37.35	0.4506	0.2553
Minimum NDF (g)	684.69ab	584.89b	811.74a	655.09ab	46.91	0.0231	0.0648
Maximum NDF (g/g NDF intake)	1.830ab	1.656a	1.877ab	2.190b	0.124	0.5906	0.0442
Minimum NDF (g/g NDF intake)	1.601	1.444	1.595	1.342	0.093	0.0553	0.5795
Whole rumen content (ADF)							
Maximum ADF (g)	447.78a	446.01a	454.52a	555.97b	21.31	0.0441	0.0229
Minimum ADF (g)	358.58	319.42	406.38	360.35	26.38	0.1408	0.1270
Maximum ADF (g/g ADF intake)	1.860a	2.020a	2.209ab	2.623b	0.152	0.0903	0.0119
Minimum ADF (g/g ADF intake)	1.679	1.593	1.753	1.482	0.101	0.1109	0.8606

Concerning the amount of ADF, values registered before the meal was significantly higher for the 56 day of regrowth age.

Taking into account the intake of NDF or ADF, the amount of NDF or ADF were significantly higher for the 56-day compared with the 21-day of regrowth whatever the time of emptying.

Whatever the age of regrowth and the time of emptying, no significant difference of amount of NDF or ADF was registered between nycthemeral phases. Nevertheless, values of amounts of NDF or ADF tend to be higher during the nocturnal phase compared with the diurnal phase. This tendency is accentuated with the older grass (table 4 + figure 2).

Effect of day-night cycle according to the physical treatment (table 5)

Globally, the amount of NDF in the rumen registered before meals are higher with the chopped forage whereas no difference was registered after the meal.

Taking into account the level of intake, no significant difference was registered between physical treatments.

Whatever the physical treatment for the same time of ruminal emptying, there was not significant difference in the amount of NDF. Concerning the amount of ADF, it was higher at the end of the main morning meal.

Taking into account the intake, amounts of NDF or ADF registered at the end of the main meal were higher during the night compared to the diurnal phase.

Taking or not the intake into account, the amounts of NDF or ADF of rumen content, registered at the end of the main meal were higher at the night with the long forage.

Table 6 . Effect of time of the day (morning *versus* night) on the amounts of Large particle (LP), Small particle (SP) and Very Small Particles (VS) (gNDF/g NDF intake) or ADF(gADF/g ADF intake) in the reticulo-rumen content, 0h00 and 1h30 post feeding, with rams consuming a chopped 21-day and 56-day regrowth age of *Digitaria decumbens* in 2 equals meals distributed at 7h00 and 19h00.

Day period	**Day**		**Night**			**Main effects**	
Age of regrowth	**21**	**56**	**21**	**56**	**SE**	**Regrowth age**	**Period of day**
Rumen granulometry (NDF)							
LP NDF 00h00 (g/g NDF intake)	0.633a	0.894a	0.600a	1.353b	0.144	0.0066	0.1747
SP NDF 00h00(g/g NDF intake)	0.488a	0.974b	0.573a	0.933b	0.047	<0.0001	0.6453
VS NDF 00h00(g/g NDF intake)	0.479a	0.666b	0.422a	0.546ab	0.053	0.0164	0.1254
LP NDF 1h30 (g/g NDF intake)	0.841a	1.113b	1.134b	1.343b	0.075	0.0103	0.0065
SP NDF 1h30(g/g NDF intake)	0.511a	1.067b	0.657a	1.089b	0.077	0.0001	0.3039
VS NDF 1h30(g/g NDF intake)	0.479a	0.623ac	0.086b	0.670c	0.053	<.0001	0.0102
Rumen granulometry (ADF)							
LP ADF 00h00 (g/g ADF intake)	0.726a	1.030a	0.676a	1.569b	0.175	0.0077	0.1970
SP ADF 00h00 (g/g ADF intake)	0.533a	1.079b	0.620a	1.050b	0.049	<0.0001	0.5665
VS ADF 00h00 (g/g ADF intake)	0.420a	0.614ab	0.457ab	0.624b	0.058	0.0128	0.6954
LP ADF 1h30 (g/g ADF intake)	1.019a	1.227ab	1.386bc	1.513c	0.090	0.0954	0.0056
SP ADF 1h30(g/g ADF intake)	0.579a	1.138b	0.757a	1.162b	0.084	0.0003	0.2568
VS ADF 1h30(g/g ADF intake)	0.262a	0.616c	0.066b	0.539c	0.053	<.0001	0.0283
Acid Detergent Lignin turn over rate (%/h)	0.025a	0.014c	0.021b	0.013c	0.001	<0.0001	0.0481

Table 7. Effect of time of the day (morning *versus* night) on the amounts of Large particle (LP), Small particle (SP) and Very Small particles (VS) of NDF (gNDF/g NDF intake) or ADF(gADF/g ADF intake) in the reticulo-rumen content, 0h00 and 1h30 post feeding, with rams consuming a long and chopped 21-day of *Digitaria decumbens* in 2 equals meals distributed at 7h00 and 19h00.

Day period	**Day**		**Night**			**Main effects**	
Physical treatment	**chopped**	**long**	**chopped**	**long**	**SE**	**Physical treatment**	**Day period**
Rumen granulometry (NDF)							
LP NDF 00h00 (g/g NDF intake)	0.633a	0.431b	0.600a	0.383b	0.049	0.0020	0.4243
SP NDF 00h00(g/g NDF intake)	0.488	0.559	0.573	0.526	0.025	0.6500	0.3123
VS NDF 00h00(g/g NDF intake)	0.479a	0.454a	0.422a	0.433a	0.031a	0.8304	0.2390
LP NDF 1h30 (g/g NDF intake)	0.841a	0.762a	1.134b	1.001ab	0.086	0.2479	0.0125
SP NDF 1h30(g/g NDF intake)	0.511a	0.567ab	0.657b	0.670b	0.037	0.3737	0.0087
VS NDF 1h30(g/g NDF intake)	0.479a	0.326c	0.086b	0.519a	0.028	0.0008	0.0066
Rumen granulometry (ADF)							
LP ADF 00h00 (g/g ADF intake)	0.726a	0.502b	0.676a	0.440b	0.055	0.0025	0.3395
SP ADF 00h00 (g/g ADF intake)	0.533a	0.611b	0.620b	0.617b	0.024	0.1532	0.0824
VS ADF 00h00 (g/g ADF intake)	0.420	0.481	0.457	0.425	0.041	0.7454	0.8242
LP ADF 1h30 (g/g ADF intake)	1.019a	0.936a	1.386b	1.204ab	0.103	0.2300	0.0129
SP ADF 1h30(g/g ADF intake)	0.579a	0.643ab	0.757bc	0.786c	0.040	0.2769	0.0031
VS ADF 1h30(g/g ADF intake)	0.262a	0.442b	0.066c	0.633d	0.036	<.0001	0.9439
Acid Detergent Lignin turn over rate (%/h)	0.025a	0.021b	0.020b	0.016c	0.0011	0.0007	0.0062

3.6. Rumen granulometry

The low value registered for very small particles obtained at the end of the nocturnal main meal with the 21-day grass indicate a probable wrong value. Consequently, these results will not be discussed.

Effect of day-night cycle according to the age of regrowth

Data concerning rumen granulometry are reported and illustrated on table 6. Whatever, the time and the constituent, the amount of large and small particles are or tend to be lower with the 21-day grass compared to the 56-day one. Moreover, there are more large particles during the nocturnal phase compared with the diurnal phase at the end of the main meal. The same tendencies were registered for small particle. The day-night cycle has not significant effect on granulometry of the rumen registered before the distribution of the meal.

Effect of day-night cycle according to the physical treatment

The data are reported on table 7. Whatever the time and the constituent, the amount of large particles is or tend to be higher with the chopped forage. Moreover, there are more large particles during the nocturnal phase compared to the diurnal phase at the end of the main meal. The same tendencies were registered for small particle. The day-night cycle has not significant effect on granulometry of the rumen registered before the distribution of the meal.

3.7. Ruminal turnover

Effect of day-night cycle according to the age of regrowth

Globally, the turnover rate of ADL was significantly higher in the case of the 21-old regrowth compared with the 56-day (table 6). Pooling all the data, the turnover rate estimate for the

diurnal phases was significantly higher than in the case of night. Nevertheless, differences were significant only for the 21-day grass.

Effect of day-night cycle according to the physical treatment

Globally, the turnover rate of ADL was significantly higher for the chopped forage compared with the long forage (table 7). Pooling all the data, the turnover rate estimate for the diurnal phases was significantly higher than the one estimate for the nocturnal phases. Moreover, differences between diurnal and nocturnal phases were significant whatever the physical treatment (table 7).

4. Discussion.

Effect of the regrowth age and treatment

Whatever the parameters studied (intake, digestibility, animal behaviour, ruminal turnover, ruminal granulometry and ruminal load), our results are in good agreement with previous studies. Intake, and digestibility decrease with the regrowth age (Archimede et al., 2000, Dulphy et al., 1980, Ichinohe, 1995). Indices of intake, rumination and mastication, ruminal load and ruminal turnover increase with the regrowth age as reported by Archimede et al., (2000), Dulphy et al., (1980), Ichinohe, (1995).

Grinding has positive effect on intake, indice of mastication and rumination, ruminal turnover as reported by Minson, 1990.

The equal amounts of NDF in the rumen at the end of the main meal whatever the diet, illustrate rumen load. These results confirm previous studies indicating that intake is limited by rumen load (Bowman et al., 1991, Forbes,1994).

Effect of nychtemeral phases.

As far as we know, there is no publication of any study performed under the same kind of conditions as here. The main results of this study is that, under the above described conditions, there are differences in digestive and ingestive dynamics between nocturnal and diurnal phases. Nevertheless, differences can appeared with forage age or treatment.

Sheep ingest similar amounts of forage between nocturnal and diurnal phases, but for each phase the mechanisms are different. It seems that the animal feeding behaviour is the "mechanism" which is the most affected by the nychtemeral phases. Indeed, intake tends to be higher during the morning phase compared with night contrary to the rate of intake, for which opposite results have been observed. Moreover, time and indices of rumination and real mastication are higher during the nocturnal phases than during the diurnal phases. These results agree with those reported by Dulphy et al. (1980) indicating that time of intake is higher during the morning whereas time of rumination is higher during the nocturnal phases. Classically, these results are explained by circadian cycle (Gordon and Mc Allister, 1970, Metz, 1975). Light being continuously present, our results have to be explained by the fact that our experiments no took place in a room totally closed, so, animals have the impact of the circadian cycle (Baumont et al. 1997). In our experiment, the granulometry of the rumen content during nocturnal phase is characterised by a larger amount of large particle at night than during the day. The presence of large particles favours the initiation of rumination. Indeed, several authors have indicated that the rumen load in fibrous particles could initiate rumination (Jarrige et al., 1995). The higher index of mastication during the nocturnal phase, compared with the morning, confirms the higher need of mastication due to its specific granulometry.

5. Conclusion

The objective here was to analyse wether or not the intake, the rumen fill, the rumen turnover and the feeding behaviour was the same during nocturnal and diurnal phases, under the conditions that the sheep were fed at identical intervals with 2 similar meals during the day. In this paper we have shown that there is an effect of the day-night cycle on several parameters of ingestive and digestive dynamics. Consequently, it seems inopportune to simplify the protocol by choosing a fraction of a day to analyze ingestive and digestive dynamics of animals receiving two meals a day.

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GROUPE EXPERIMENTAL VISANT A VALIDER LES HYPOTHESES DE LA THESE

INTRODUCTION AU GROUPE D'EXPERIMENTATIONS VISANT A VALIDER LES HYPOTHESES DE TRAVAIL

Le deuxième groupe d'expérimentations avaient pour objectifs d'analyser l'impact relatif des facteurs enzymatiques et mécaniques sur l'ingestion et la digestion des fourrages :

- les protocoles **4** et **5** ont été conduits respectivement sur le Pangola et l'herbe de guinée en absence de tout traitement ;
- lors du protocole **6**, de l'enzyme a été ajoutée au pangola afin d'être en condition enzymatique non limitante et ainsi de mieux cerner les aspects mécaniques.

Tous ces protocoles se sont déroulés suivant les conditions définies au cours des protocoles à but méthodologique :

- l'efficacité de l'apport d'une enzyme fibrolytique exogène ayant été prouvée au cours du protocole **1,** l'étude des dynamiques digestives en condition enzymatique non limitante (protocole **6**) s'est déroulée en présence de cette enzyme ;
- afin de se situer dans des conditions qui soient les plus proches de celles de l'élevage, l'étude des dynamiques digestives (protocoles **4**, **5** et **6**) a été réalisée en distribuant le fourrage deux fois par jour (suite aux résultats du protocole **2**) ;
- les résultats obtenus au cours du protocole **3** ayant montré qu'il était impossible, dans nos conditions expérimentales, d'extrapoler les résultats obtenus sur 12 heures à 24 heures, les mesures effectuées au cours du deuxième groupe expérimental ont donc été réalisées en continu sur 24 heures.

PUBLICATION 5

Intake and digestive processes in the rumen of rams fed with *Digitaria decumbens* harvested at four stages of grass regrowth age

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(Australian-Asian Journal of Animal Science, *sous presse*)

ABSTRACT : This study was designed to measure the effect of regrowth age of *Digitaria decumbens (D. decumbens)* on the intake and dynamics of digesta in the rumen of rams. Six Black-belly rams (mean liveweight: 51·6 (s.d. 0·68) kg) fitted with rumen cannulae were fed a 14-, 28-, 42- and 56-day old fresh *D. decumbens* successively for 4 experimental periods. The daily dry matter intake decreased curvilineary from 75.2 to 48.5 (s.e. 2·0) g / kg.$BW^{0.75}$ as the age of the *D. decumbens* grass increased from 14 to 56 days. Dry matter intake for the first 3 hours after the morning meal was 863·6, 598·3, 576·4 and 401·5 (s.e. 55·6) g respectively for the 14-, 28-, 42- and 56-day old grasses.

The pool of NDF in the rumen at the end of the 3-hour feeding period did not vary significantly among the four diets. Twelve hours after the beginning of the morning meal, the pool of NDF increased with the forage regrowth age. Within the total pool of NDF, the pool of large particles tended to increase with the regrowth age.

It was concluded that high intake was associated with fast evacuation of NDF outside the rumen. Moreover, digestion (cellulolysis) rate and degree of particle reduction by rumination are highly correlated though speed of physical degradation of forage seems to be the driving force behind intake.

"Key Words: Tropical grass, Intake, Rumen digestion"

INTRODUCTION

Fresh forage is often the sole component of the diet of many ruminants in the humid tropics. Minson (1990), Aumont et al,(1995) recognised however that voluntary intake is a major factor limiting the nutritional value of tropical forage. Voluntary intake of a low quality forage is limited by rumen fill and the fractional disappearance rate of dry matter (DM) from the reticulo rumen (Bowman et al., 1991). The forage particles therefore need to be smaller in size but denser in order to flow out of the rumen. Previous studies (McLeod and Minson, 1988; McLeod et al., 1990) demonstrate that the breaking down of large forage particles to small particles is usually achieved by chewing during eating and rumination whereas only a small part of the particle breakdown (17%) can be attributed to microbial degradation.

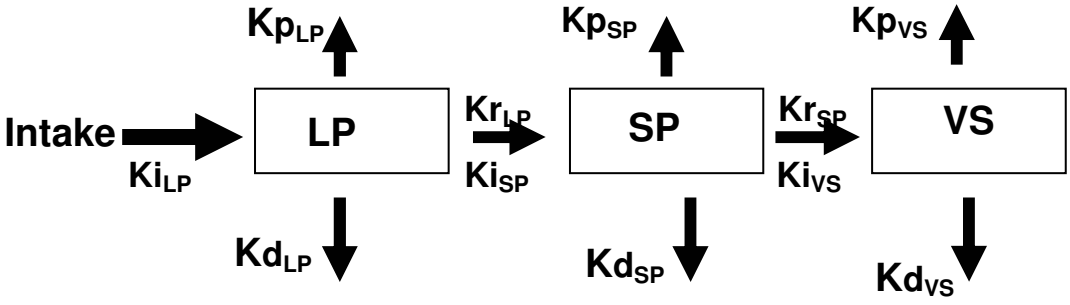
Regardless of the very high level of fibre and thick-walled bundle sheath in tropical grasses Wilson (1994) and Archimède et al. (2000) have hypothesised that in intensive systems in tropical areas where the forage is irrigated and fertilised, the low rate of reduction in size of large forage particles by chewing is the first limiting factor on intake, even with young forage. The experiment was designed (i) to evaluate variations in tropical forage grass intake according to regrowth age, (ii) to study the digestion kinetics of fiber fraction in the rumen of rams, (iii) to study the impact of cellulolysis and physical breakdown of grass particles on intake. To taken into account fast growth of tropical forages, this study has been performed with grass with state of regrowth age within bounds larger than those classically studied.

MATERIALS AND METHODS

Experimental design, animals, diets and feeding

The harvest of the perennial grass *D. decumbens* pasture was planned in such a manner as to obtain forage at 14, 28, 42 and 56 days of regrowth for four successive 42-day experimental periods (Archimède et al., 2000). Six Black-belly rams (mean liveweight: 51·6 (s.d. 0·68) kg were fed fresh 14-, 28-, 42- and 56-day regrowth successively for the experimental periods. Each experimental period consisted of 19 days of adaptation to the diet, 7 days of intake measurement, 7 days of nylon bag incubation and 3 days of rumen sampling. The grass was cut early every morning and chopped (5 cm-length) before being offered. The rams were fed twice at 12-hour intervals. The quantity offered was 1·15 times more than the animals' estimated voluntary intake during periods of adaptation. The rams used in this trial were fitted with rumen cannulae and kept in metabolism cages.

Figure 1. Flow diagram of size reduction, digestion and passage rate of digesta in the rumen. Kr_{LP}, Kr_{SP}, are particle size reduction rate of large and small particles respectively. Kd_{LP}, $Kd_{SP,}$ KdV_{VS}, are the digestion rate of large, small and very small particles respectively. Kp_{LP}, Kp_{SP}, Kp_{VS}, are the passage rate of large and small and very small particles respectively.



Measurements and calculation

Intake was measured daily by weighing the amount of forage offered as well as refusals.

The kinetic degradation rate of forage and of rumen granulometry were estimated using the nylon bag method. The nylon bags measured 10 cm x 5 cm, with a pore size of 50 x 50 µm, and were filled with 3 g of raw freeze-dried SP or LP. With respect to forage 15 g of green matter were introduced in the bag. SP and LP came from the rumens of 3 rams fed with the experimental diet and emptied manually 3 hours after the start of their morning meal. The rumen contents were not ground. The incubation time of the nylon bags in the rumen was 96 h.

During the rumen sampling period, two rumen empties (one per day) was carried out on each animal 3 hours after the morning meal and just before the evening meal. The times were chosen to represent minimum and the maximum rumen filling. There were 3 days between the emptying of the rumen. The total rumen content was weighed and mixed by hand. Four sub-samples were taken, two for dry matter determination, one preserved (-20°C) until freeze-dried for chemical analysis determination while the last was used to determine digesta particle size. Rumen particles were separated into Large Particles (LP) and Small Particles (SP), by wet sieving using gradual sieves of 4 mm, 2mm, 1.18mm, 0·75 mm, 0·250 mm and 0.05mm in size. LP were defined as those retained in the 4mm and 1.18 mm sieves, SP were defined as those that passed through 1.18mm sieves but retained in 0.050mm sieves and particles that passed through the 0.050 mm were considered as Very small Particles (VS).

NDF particle size in forage and duodenal content were measured in simultaneous experiments using a similar diet: all particles of forage were Large particles whereas for duodenal content, large, small and very small particles represented 1.33, 54.8 and 43.8 % of the pool of NDF with no difference between diets.

The total rumen content of NDF (WRC-NDF) was estimated multiplying the total rumen content of DM by its content in NDF.

To calculate reduction size, passage and digestion rate we use the same model for digestion in the rumen illustrated by Ichinohe et al. (1995) in figure 1.

The fractional rates (% per hour) of total disappearance, passage and digestion of total NDF, Large Particle of NDF (LP), Small Particle (SP) of NDF and very small particles (VS) particle of NDF were calculated as follows (Rinne et al., 2002):

- NDF rate of disappearance from the rumen (kiWR) = (1/24)*(intake NDF, g/d)/(mean rumen pool size of NDF, g)
- NDF rate of passage from the rumen (kpWR) = (1/24)*(ruminal outflow of ADL, g/d)/(mean rumen pool size of total ADL, g)
- NDF rate of digestion (kdWR) = kiWR – kpWR

Ruminal outflow was calculated using the mean coefficients of stomach digestibility recorded with similar forage (Archimède et al., 2000).

- LP entrance rate in the rumen (kiLP) = (1/24)*(intake NDF, g/d)/(rumen pool size of LP-NDF, g)
- LP reduction size rate in the rumen (krLP) = (1/24)*(intake of indigestible NDF, g/d)/(mean rumen pool size of indigestible LP-NDF, g)
- LP passage rate (kpLP) = (1/24)*(ruminal out flow of LP-NDF) /)/(mean rumen pool size of indigestible LP-NDF, g)
- LP-NDF digestion rate in the rumen (kdLP) = KiLP - krLP – kpLP
- SP-NDF entrance rate in the rumen (kiSP) = krLP.
- SP-NDF reduction rate in the rumen (krSP) = ((1/24)*(indigestible NDF intake, g/d)*krLP) /(mean rumen pool size of indigestible NDF-SP, g)
- SP-NDF passage rate from the rumen (kpSP) = (1/24)*(ruminal outflow of SP-NDF) /)/(mean rumen pool size of indigestible SP-NDF, g)
- SP-NDF digestion rate (KdSP) = kiSP – krSP - kpSP.
- VS-NDF entrance rate in the rumen (kiVS) = krSP.
- VS-NDF passage rate (kpVS) is deduced of the equation hypothesing that kpWR= mean weighed of kpLP, kpSP and kpVS
- VS-NDF digestion rate (KdVS) = kiVS – kpVS.

Indigestible NDF intake was determined using the mean coefficient of total tract indigestible NDF of each forage (Archimède et al., 2000). Ruminal outflow of NDF, LP-NDF and SP-NDF were calculated using the mean coefficient of stomach indigestible NDF of each forage and the proportion of each particle in the duodenal sample (Archimède et al., 2000). Indigestible LP-NDF and SP-NDF were determined using the coefficient of indigestible NDF obtained with the nylon bag method.

Table 1. Chemical composition (g/kgDM) of the 14, 28, 42 and 56-day old *Digitaria decumbens* grass consumed by six Black belly rams

	Regrowth age (days)			
Constituents (g/kgDM)	14	28	42	56
Organic matter	840	887	896	879
Crude protein	130	79	72	57
Neutral detergent fibre	740	777	790	790
Acid detergent fibre	380	429	442	441
Acid detergent lignin	71	74	78	78

Table 2. Effect of the regrowth age of a *Digitaria decumbens* grass (14-, 28-, 42- and 56-day old) on intake in the rumen of Blackbelly rams

	Age of regrowth (days)				
	14	28	42	56	sem
Intake (g /$BW^{0.75}$/day)					
Dry matter intake	75.2a	62.9b	52.6b	48.5c	2.0
NDF intake	55.6a	48.7b	41.5c	38.3c	1.6
Intake (g /day)					
Dry matter	1454.0a	1234.0b	1022.0c	915.0d	38.0
Organic matter	1221.0a	1095.0b	916.0c	803.0d	33.0
Crude protein	189.0a	97.0b	73.6c	52.1d	4.1
Neutral detergent fibre	1076.0a	959.0b	808.0c	723.0d	30.0
Acid detergent fibre	552.0a	530.0b	452.0c	403.0d	16.0
Intake (g / 3 hours)					
Dry matter	863.7a	598.3b	576.4b	401.5c	55.6
Neutral detergent fibre	639.1a	464.9b	455.3b	317.2c	41.8

Chemical analyses

The DM content of the fresh forage and refusals was obtained daily by drying it to a constant weight at 60°C in a forced-draught oven. Samples were then ground to 1 mm prior to chemical analysis. Organic matter (OM) content was measured after a 10 h pyrolysis at 550°C. Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) were estimated using the methods of Van Soest et al. (1991). The nitrogen content was determined from a representative sample of dry forage using the Kjeldahl method.

Statistical analyses

Data (intake, digestive parameters) were analysed using the General Linear Model procedure of SAS (1987) including forage (D.F. 3) and animal (D.F. 5) effects. The effect of periods was tested on the residues of the precedent model. It was not significant. Moreover, similarities among all measurement parameters were first shown by calculating correlation coefficients using the COR procedure of SAS. Regressions between intake and the digestive parameters were then calculated based on the REG procedure and using the MAXR (Maximum r^2 improvement).

RESULTS

Diet composition and intake

The composition and intake of *D. decumbens* are reported in Tables 1 and 2. DM ($g/BW^{0.75}$) intake was found to be significantly higher for the 14-day old forage than for the 28-, 42- and 56-day old grasses. The daily intake decreased curvilineary with regrowth age according to the following equation:

$DMI = 82.4 - 1{\cdot}379\ days + 0{\cdot}0104\ days^2$

($r^2 = 0.82$, SE = 5.2, n = 24, $p < 0.0001$)

The mean daily DM intake on the days of rumen emptying did not vary from ram to ram. The tendencies registered for intake of NDF are similar to those registered for DM.

The DM intake of 14-day grass for the first 3 hours after the morning meal (Table 2) was found to be significantly higher than either the 28-, 42- or 56-day old pangola grass.

Table 3. Nylon degradation (96h) of NDF fraction of forage, large particle (LP) and small particle (SP) of a 14-, 28-, 42- and 56-day old *Digitaria decumbens* grass fed to six Blackbelly rams

	Age of regrowth (days)				
	14	28	42	56	**s.e.**
Degradation 96 hours of forage	78.93a	73.7 b	67.4 c	60.30d	1.20
Degradation 96 hours of LP	51.60a	45.38b	36.96c	34.50d	0.97
Degradation 96 hours of SP	23.60	22.90	21.60	22.87	0.72

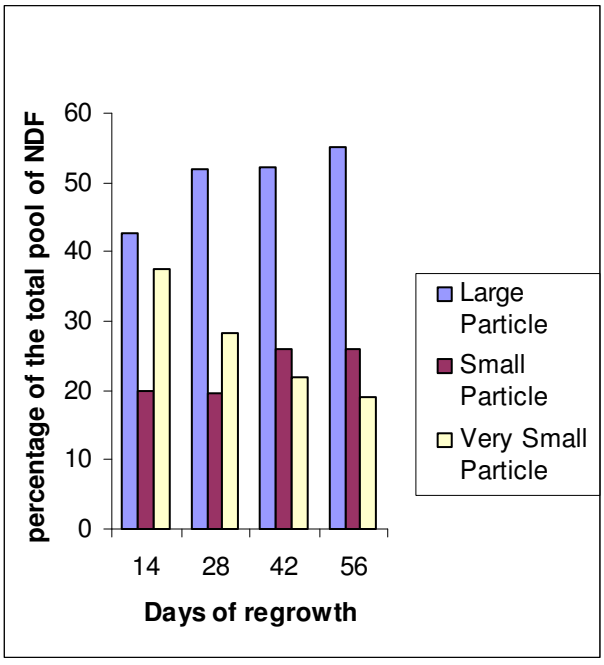
Table 4. Effect of the regrowth age of a *Digitaria decumbens* grass (14-, 28-, 42- and 56-day old) on the particle size distribution of NDF (g) in the rumen of Blackbelly rams, 3 and 12 hours after the morning meal

	Age of regrowth (days)				
	14	**28**	**42**	**56**	**s.e.**
NDF pool in Whole Rumen Content (g)					
3 hours	809·8	881.5	863.1	816.5	61.2
12 hours	491·5a	527·5 a	662·0 b	742·0 b	40·5
NDF pool in Large Particle (g)					
3 hours	346·5	462.0	451·2	469·2	38·5
12 hours	209·5 a	242·3 ab	297·7 bc	334·7 c	28·4
NDF pool in Small Particle (g)					
3 hours	159·7 a	173·8 a	223·8 b	211·2 b	15·3
12 hours	104·0 a	163·2 b	189·7 b	245·2 c	13·9
NDF pool in Very small Particle (g)					
3 hours	303·7 a	245·7 a	188·2 b	136·3 b	26·5
12 hours	178·0	122·0	174·7	162·2	22·3

Table 5 . Effect of the regrowth age of a *Digitaria decumbens* grass (14-, 28-, 42- and 56-day old) on the particle size distribution of NDF (g/1000g of NDF intake) in the rumen of Blackbelly rams, 3 and 12 hours after the morning meal.

	Age of regrowth (days)				
	14	**28**	**42**	**56**	**s.e.**
NDF pool in WRC (g/kg NDFI)					
3 hours	712.6 a	911.7 b	1079.7c	1147.3 c	45.3
12 hours	428.2 a	554.2 a	814.8 b	1037.8 c	51.5
NDF pool in LP (g/kg NDFI)					
3 hours	304.7 a	473.4 b	563.7 bc	631.0 c	36.9
12 hours	184.4 a	255.9a	366.7 c	459.3 c	34.3
NDF pool in SP (g/kg NDFI)					
3 hours	141·1 a	179·8 b	280·0 c	297·5 c	13.2
12 hours	89.7 a	170.3 b	232.6 c	337.8 d	15.4
NDF pool in Very small particle (g/kg NDFI)					
3 hours	266.7	258.3	236.0	218.8	30.7
12 hours	154.2 a	128·0 a	215.6 b	240.6 c	29.6

Figure 2. Effect of the pangola grass maturity (14, 28, 42 and 56 days regrowth) on the granulometry of the pool of NDF in the rumen at the end of the morning meal



In situ nylon bag degradation

In situ 96-hour degradation of the NDF fraction of forage and LP decreased as the regrowth age of the pangola increased (Table 3). No significant difference was registered for the SP fraction.

Rumen digestion

- **Digesta pool**

The regrowth age of the pangola grass induces no significant differences in rumen NDF pool (WRC-NDF) estimated 3 hours after the beginning of the morning meal (Table 4). Twelve hours after the beginning of the morning meal, significant differences appear between diets. The amount of NDF increased steadily for regrowth ranging from 14 to 56 days, the differences being largest however between 28- and 42-day old grasses.

Regarding intake level (Table 5), there was an increase in NDF (g/kg NDF intake) with the regrowth age whatever the time after the meal. The difference was again most notable for regrowth between 28 and 42 days old.

In general, 3 hours after the morning meal, granulometry of the rumen tended to have more LP and less VS particles when the regrowth age of the forage increased (figure 2). As was observed with the WRC-NDF (g), 3 hours after the beginning of the meal, the LP-NDF pool did not increase significantly with the regrowth age of the forage (Table 4). Nevertheless the pool registered with the 14-day grass tended ($0.06 < p < 0.09$) to be lower. Twelve hours after the meal, there was an increase in the pool of LP-NDF (g) and the differences were higher for 28- and 42- day old forage. Expressed per kg of NDF intake, 3 hours after the meal the pool of LP-NDF increased significantly with the forage regrowth age and this general increase was maintained 12 hours after (Table 5).

Overall, results concerning the pool of SP-NDF were similar to those registered with LP (Tables 4 and 5).

Variations in the pool of VS-NDF in the rumen after the morning meal (Tables 4 and 5) were generally opposite to those registered for LP and SP. After the morning meal, the amounts of VS-NDF (g) levels decreased with forage regrowth age. These differences disappeared 12 hours later. Expressed per kg of NDF intake, VS-NDF pools were not altered by forage maturity 3 hours after the meal. Twelve hours later, they increased for the two oldest forages.

Table 6. Effect of the regrowth age of a *Digitaria decumbens* grass (14-, 28-, 42- and 56-day old) on : disappearance rate, reduction rate, passage rate, digestion rate of whole rumen NDF, large particle of NDF, Small particle of NDF (SP-NDF) and NDF as very small particle fraction ; in the rumen of Blackbelly rams, 3 and 12 hours after the morning meal.

	Age of regrowth (days)				
	14	28	42	56	s.e.
Whole Rumen NDF					
Disappearance rate	7.47a	5.72b	3.81c	3.89c	0·25
Passage rate	4.49a	3.36b	2.63c	2.42c	0·14
Digestion rate	2.98a	2.36b	1.18c	1.47c	0·16
Large Particle NDF					
Reduction rate	9.78a	7.60b	5.10c	4.98c	0·32
Passage rate	0.13a	0.10b	0.07c	0.07c	0·00
Digestion rate	7.65a	3.87b	4.13bc	3.38c	0·25
Small Particle NDF					
Reduction rate	1.27a	0.91b	0.38c	0.33c	0·06
Passage rate	7.10a	6.59a	4.10b	3.65b	0·29
Digestion rate	1.41a	0.10b	0.617b	0.99a	0·28
Very small particle NDF					
Passage rate	4.35a	4.03a	2.55a	3.11a	0·68

- **Fractional rate of disappearance, reduction, passage and digestion**

The effects of grass regrowth age on digestion kinetics are presented in Table 6. The rates of disappearance, passage and digestion of WRC-NDF generally decreased with forage regrowth age.

Concerning LP-NDF, it was observed that the rate of particle size reduction decreased significantly as regrowth age increased (Table 6). The same tendency was also observed for the digestion rate. Logically, a negligible rate of passage was recorded for forage regrowth age. Decreasing for rate of reduction and passage were estimated for the SP-NDF in the case of forage regrowth age whereas little or no significant difference was noted for digestion rate. No significant difference was calculated for the rate of passage of the VS-NDF.

Intake and digestive parameters

Multivariate predictive regressions of DM intake from digestive parameters showed that total passage rate of NDF from the rumen was the main determining parameter for intake (R^2= 0.66). Moreover, passage rate is in close correlation with the WRC-NDF disappearance rate (r=0.97), the digestion rate of WRC-NDF (r=0.95), the reduction rate of SP-NDF (r=0.92), the reduction rate of LP (r=0.83), the digestion rate of LP-NDF (r=0.83). No significant correlation between passage of NDF and digestion rate of SP was recorded.

Simple predictive regression of intake from digestive parameters showed high and positive correlations between intake of DM (or NDF) with WRC-NDF passage rate (r=0.81), WRC-NDF disappearance rate (r=0.77), SP-NDF reduction rate (r=0.76), SP-NDF passage rate (r=0.72), LP-NDF passage rate (r=0.68), LP-NDF reduction size rate (r=0.68), LP-NDF digestion rate (r=0.68) and total NDF digestion rate (r=0.64). The correlations were not significant between intake, VS-NDF passage rate (0.18) and SP-NDF digestion rate (0.05).

DISCUSSION

Intake

Intake results for this experiment confirm those previously recorded by several authors: Chenost (1975); Aumont et al. (1995); Archimède et al. (2000). Large variations in the range of dry matter intake existed according to the regrowth age of the tropical grass. Intake decreased with older regrowth. Moreover, the intake of the 14-day old pangola grass was observed to be high when compared with that of young temperate forage. Nevertheless these

high levels of intake are registered with more mature temperate grass. This phenomenon is a consequence of the particular physiology associated C4 pathway of tropical grasses whose cell components mature rapidly in comparison to C3 grasses (Wilson, 1994). So a 6-week tropical grass is an old forage, and for this latter reason, when compared to a temperate grass forage, it must be valorised at a younger stage of regrowth, once the first objective is that of increasing dry matter intake as well as digestible dry matter intakes. In this experiment the 14-day old grass can be considered a reference, a grass with some of the characteristics of a temperate one.

Rumen digestion kinetic and intake

Estimates of rate of passage and digestion of NDF, reduction rate of Large Particles and Small particles and passage rate of Small particles are closely related to those reported by Poppi et al. (1981ab) and McLeod et al. (1990) for tropical grass and Ichinohe et al., (1995) and Mc Leod and Minson, (1988) for temperate forage.

In our experiment, dry matter intake of grasses appeared to be regulated by rumen fill and by problems in the evacuation processes of this content through digestion and passage rate (Forbes, 1994). NDF intake actually decreased with grass regrowth age, whereas the pool of NDF in the rumen at the end of the morning meal was similar. This shows that there is a limit to the amount of NDF that can accumulate in the rumen. Consequently, the faster the NDF disappears from the rumen, the higher the intake as illustrated by the positive correlation between intake and the disappearance rate of NDF. Moreover, the increase in the amount of NDF in the rumen per unit of NDF intake illustrates an accumulation of NDF in the rumen with older regrowth resulting from the decrease in the disappearance rate of the rumen pool of NDF. The disappearance rate of the NDF pool depends on physical breakdown of particles and cellulolysis (microbial digestion). The faster evacuation of NDF content in younger grass cannot be explained by cellulolysis. Microbial enzymatic digestion needs time to be efficient (Wilson et al, 1989a b) and the duration of the meal was only 3 hours. This is not sufficient to explain the reduction and then the evacuation of NDF outside the rumen. Our data concerning the rumen pool of NDF (corrected by intake) 3 hours after the beginning of the meal underscore the importance of the process of chewing during eating. Firstly, the mean amount of NDF in the rumen per unit of NDF intake is lower for the 14 days grass. This may be an indication that during ingestive mastication an important part of NDF has been made soluble and evacuated outside the rumen with the younger forage. Moreover, the higher granulometry fineness of the particle pool in the rumen with the 14-day regrowth forage results mainly from

a more efficient physical particle breakdown by chewing during eating. The more efficient ingestive mastication of young forage is illustrated by the values registered for the rate of size reduction. However, this also takes into account chewing during rumination. The increase in the LP-NDF pool (corrected with intake) with older regrowth also illustrates an increase in the resistance of the grass to physical breakdown. This result is closely related to the evolution of the reduction rate of LP-NDF which decreases with the grass regrowth age. Wilson et al. (1989) and Kennedy (1995) indicated that the tropical grasses have an inherent disadvantage with respect to particle breakdown that may be difficult to overcome. Our results illustrated a large variation in the resistance of the grasses to physical breakdown. Moreover, values for the reduction rate of LP-NDF remain relatively important in comparison to the rate of digestion which is relatively low and decreases dramatically. Our estimates of LP reduction rate are similar to those indicated for temperate grass (Bernard, 1992 and Ichinohe et al., 1995).

Digestion helps to elevate the density of particles to a level (Bernard, 1992) necessary (1.1-1.2) for them to be evacuated from the rumen. In our experiment, the low digestion rates for the older forage can partially explain the decrease in intake of older grass. Really, SP-NDF tends to accumulate in the rumen when the grass grows whereas these particles have theoretically the critical size to left the rumen. It can be assumed that their density limits their evacuation from the rumen.

Our results indicate a high correlation between parameters which estimate physical (reduction size) and chemical (enzymatic) degradation in the rumen except for Small particle. This can be explained by the fact that the breaking down of the grass increases particle surface which is necessary to maximise microbial cellulolytic activity. Some of the biochemical characteristics of cell-walls (bond lignin-cellulose) probably limit enzymatic activity as well.

Because of the link between physical breakdown and enzymatic digestion of grass in the rumen our results do not allow for any conclusion concerning the driving force behind intake.

CONCLUSIONS

Improved intake is a major objective in ruminant nutrition. A general analysis of the results indicates large variations in intake correlated with large variations in parameters of cellulolysis and physical breakdown. This experiment also indicates that grass management is an important tool to pilot intake. It displays for the management of tropical forage at young state of regrowth age (< one month) for which high level of intake is the objective.

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PUBLICATION 6

Intake and digestive processes of rams fed *Panicum maximum* harvested at four stages of grass maturity

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Abstract

This study examined the changes in physical digestion of guinea grass (*Panicum maximum)* in relation to forage maturity and the impact on intake. Six sheep (mean liveweight (LW): 37.12 ± 1.5kg) were fed 14, 21, 28 and 56-day old guinea grass during four successive 5-week periods. Between the 14 and 56 day-old of regrowth, the Dry Matter Intake (DMI) (g/kg $LW^{0.75}$) and the organic matter total tract digestibility (%) decreased from 93 to 49 and from 75 to 65 respectively. The ruminating index (min/g DMI) increased from 0.45 to 0.60 between the 14 and the 56 day guinea grass. Furthermore, the mean ruminating index was 20% higher than the mean time spent eating. The rumen fill for the 56 day regrowth was twice that of the 14 day regrowth. Compared with pangola grass, guinea grass showed a fast decline in intake and digestibility with it maturation. This fast decline could be attributed to the longer time the animal spent ruminating, and which, on becoming more pronounced with maturation, would have a much more negative effect on the rumen load and therefore on the intake.

Keywords: *Panicum maximum*, rams, intake, digestive parameters

Introduction

Tropical grasses generally have a problem of low intake compared to temperate grasses (Minson, 1990). This is partly associated with the high fibre content of tropical grasses compared with that of temperate grasses which is due to the photosynthetic physiology that differs between tropical and temperate grasses. In tropical grasses two principal morphogenetic types can be distinguished, the tuffed and the creeping grasses. Creeping grasses (*Digitaria decumbens*) present a fast growth of leaf/stem ratio. In contrast to the tuffed grasses (*Panicum maximum*) for which the leaf/stem ratio is slow. Also, the latter has a morphogenetic similarity with most temperate grasses (Lemaire *et al.*, 1999). It is known that

leafy grasses offer low resistance to chewing and possess high digestibility and energy values. Furthermore, temperate forages generally have higher voluntary intake and digestibility than tropical forages. However, studies of digestibility and voluntary intake (Minson, 1972) have shown that *P. maximum* belongs to forage group with rapidly declining intake and digestibility, whereas *D. decumbens* belongs to the group with very slow rates of decline. Nevertheless, these investigations were conducted with relatively old forage (28 to 105 days), for which only parameters of intake and total tract digestion were considered.

This study aims to examine the changes in physical digestion (feeding behaviour, transit time, rumen load...) of guinea grass (*Panicum maximum)* in relation to forage maturity and the impact on intake.

Material and methods

Location

The trial was conducted in 2005 at the animal experimental station of the ‘‘National Institute of Agronomic Research" (INRA, French West Indies, Guadeloupe, latitude 16.16 N, longitude 61.30 W). Average monthly temperature at the experimental site, ranged from 21°C to 31°C, and the mean annual rainfall was approximately 3000 mm/year.

Experimental design, animals, diets and feeding

The experimental design was conducted in four successive periods. Each experimental period was 35 days long and consisted of 14 days of adaptation to the diet, 7 days of intake and total tract digestibility estimation, 7 days of nylon bag incubation and ruminal content sampling and 7 days of rumen emptying. Five Black-belly lambs (mean live weight: 37.12 (s.d. 1.50) kg) were successively fed the fresh Guinea grass of 56, 28, 21 and 14 days of regrowth for the experimental periods 1, 2, 3 and 4 respectively. One kg/ha/regrowth age of mineral nitrogen

fertiliser was applied each day after the removal of the grass. After each harvest, the grass was kept at 4°C a night in a cold chamber until feeding. In the morning, the grass was chopped (approximately 4 cm) just before being offered. The lambs were fed twice a day at 12-hour intervals. The quantity was offered *ad libitum* (1.15 times more than the animal's estimated voluntary intake during periods of adaptation). The rams used in this trial were fitted with rumen cannulae and kept in metabolism cages.

Measurements

Intake and apparent digestibility were determined by daily weighing of the amounts of food offered, refusals and faeces. Dried and ground samples of grass, refusals and faeces were stored for chemical analyses.

The feeding behavioural parameters (time spent eating, ruminating and idling) were determined by 24h of visual observation. Also, to establish the kinetic of intake, refusals were weighed hourly.

During the rumen emptying period, firstly, two rumen empties (one per day) were carried out on each animal: (i) 3 hours after the morning meal (WRCmax) and (ii) just before the morning meal (WRCmin). The times were chosen to represent the maximum and the minimum rumen load respectively. There were 3 days between these two rumen evacuations. A third rumen emptying was carried out just after the second rumen emptying. Total rumen content collected just before the morning meal was stored in a hermetic recipient at 37°C. Animals were fed for one hour, then total rumen content was evacuated (WRCingmast). This last emptying was to enable the estimation of the impact of the ingestive mastication on the size reduction of forage. After each emptying, the total rumen content was weighed and thoroughly mixed by hand. Four sub-samples were taken, two for dry matter determination, one was preserved (-20°C) until freeze-dried for chemical analyses, while the last was used to

determine the digesta particle size. Rumen particles were separated into Large Particles (LP) and Small Particles (SP), by wet sieving using gradual sieves of 4 mm, 1.18mm, 0·75 mm, 0·250 mm and 0.050mm in size. LP were defined as those retained in the 4mm and 1.18 mm sieves, SP were defined as those that passed through 1.18mm sieves but retained in 0.050mm sieves and particles that passed through the 0.050 mm were considered as Very small Particles (VS).

The daily passage rate (h^{-1}) of whole rumen fibre content (trADL) was estimated by the ratio: (daily faecal of lignin (g) / 24) / (mean total amount of lignin in the rumen (g)).

Chemical analyses

Dry Matter (DM) of fresh forage, refusals, faeces and rumen content were determined each day by drying to a constant weight at 60°C in a forced-draught oven. Amount of LP, SP and VS were determined as a percentage of the total amount of rumen DM content. The organic matter (OM) content was measured after a 10 h pyrolysis at 550°C. Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) were estimated following the methods of Van Soest *et al*., 1991. Nitrogen content was determined on representative sample of dry forage using the Dumas method (AFNOR, 1988).

Statistical analyses

Data were analysed using the General Linear Model procedure of Minitab14 (2003) including the age of regrowth (D.F. 3) (α) and the animal (D.F. 4) (β).

$$Y_{ij} = \mu + \alpha_i + \beta_j + e_{ij}$$

The effect of periods was tested on the residues of the previous model, no significant difference was registered.

Table 1. Chemical composition (% DM) of a 14-, 21-, 28- and 56-day old fertilized *Panicum maximum* grass.

Constituents (%DM)	Regrowth age (days)			
	14	21	28	56
Organic matter	88.3	88.4	89.2	89.5
Crude protein	20.18	17.75	9.26	4.77
Neutral detergent fibre	67.35	67.28	73.93	78.88
Acid detergent fibre	31.40	34.49	42.53	44.26
Acid detergent lignin	3.51	4.84	4.87	5.51

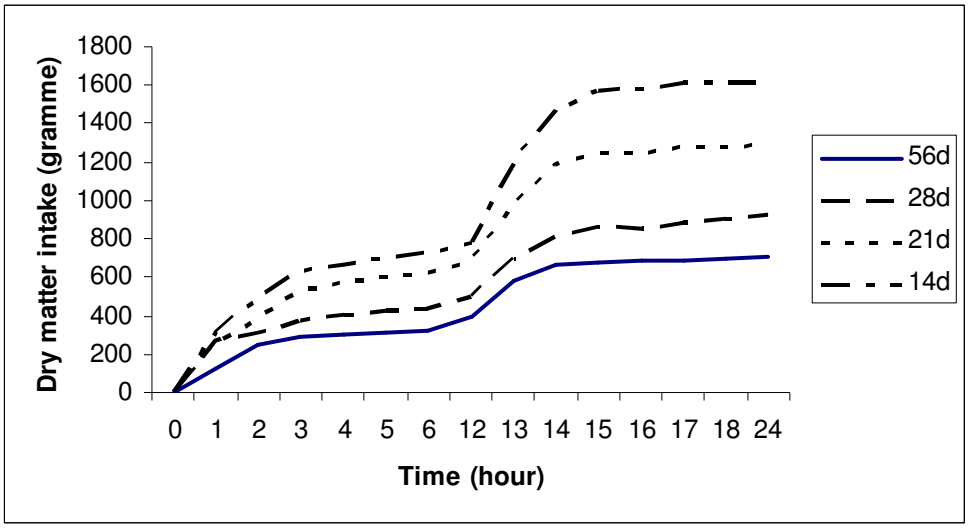

Figure 1. Effect of age of regrowth on kinetic of intake of rams given a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Table 2. Intake and Total tract digestibility of dry matter, organic matter, neutral detergent fiber, acid detergent fiber, crude protein, intake of the digestible organic matter and ruminal turnover rate of ADL in Blackbelly lambs given a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Item	Regrowth ages (days)				s.e.d.	Signifi-cance
	14	21	28	56		
Intake						
Dry Matter (g/j)	1616.2a	1288.5b	922.6c	709.3c	60.95	***
Dry Matter (g/kg $LW^{0.75}$)	92.80 a	87.16 a	65.70 b	49.32 c	3.10	***
Organic matter (g/kg $LW^{0.75}$)	82.11a	76.79a	58.72b	44.18c	5.73	***
Neutral Detergent Fiber (g/kg $LW^{0.75}$)	62.66a	57.60a	48.24b	38.67c	4.71	***
Acid Detergent Fiber (g/kg $LW^{0.75}$)	28.32a	28.95a	27.51a	21.05b	2.53	***
Crude Protein (g/kg $LW^{0.75}$)	16.38a	13.16b	3.85c	2.71c	1.04	***
Intake of the digestible OM (g)	1066.1a	771.8b	549.5c	407.5d	76.38	***
Total tract digestibility (%)						
Dry Matter	72.93 a	68.86 a	60.66 b	58.90 b	3.51	***
Organic matter	75.29a	67.93ab	62.48b	61.63b	4.08	***
Neutral Detergent Fiber	78.08a	72.09ab	63.31b	60.24b	3.78	***
Acid Detergent Fiber	70.40a	67.70ab	59.64bc	53.93c	4.16	***
Crude Protein	79.46a	70.81ab	62.42b	41.46c	6.83	***
Passage rate (%/h)	0.028a	0.027a	0.024a	0.010b	0.005	*

a, b, c, d: means within the same row with different superscripts differ (P< 0.05)
* $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$

Results

Diet composition, intake and total tract digestibility

Panicum maximum composition is presented in Table 1. Increases of 17% and 41% were registered between the 14 and 56 day old forage for the Neutral detergent fibre and the Acid detergent fibre respectively. Likewise, a decrease of 76% was registered for the crude protein. No significant change was registered for the Acid detergent lignin.

Intake and total tract digestibility are reported in Table 2. The DM (g/kg $LW^{0.75}$) intake values of the 14-day and 21-day old forage were found to be significantly higher than those registered for the 28-day and 56-day old grass (figure 1). In addition, intake of the 28-day old grass was significantly higher than the 56-day. The daily intake decreased curvilinearly according to the following equation:

DMI (g/kg $LW^{0.75}$) = 0.024 days² (± 0.015) – 2.76 (± 1.07) day + 129.32 (± 15.89) (P = 0.026; r^2 = 84.3%)

The trend observed for DM were similar to those of the dry matter components (OM, NDF, ADF, CP).

The DM (OM, NDF, ADF, CP) total tract digestibility values of the 14-day forage were found to be significantly higher than those registered for the 28-day and 56-day old grass (table 2). More, values of digestibility (DM, OM, NDF, ADF, CP) of the 21-day old grass tended to be or were significantly higher than the 28- and 56-day, and no significant difference was observed between the 28- and 56-day old forage, except for CP digestibility.

The mean decrease of total tract digestibility is 0.26 point per day. Nevertheless the general tendency of the decrease of total tract digestibility of dry matter (dDM) with day of regrowth is curvilinearly illustrated with the following equation:

dDM (%) = 0.019 days² (± 0.005) – 1.60 (± 0.39) day + 91.79 (± 5.81) (P <0.005; r^2 = 78.6%)

Table 3. Feeding behaviour parameters in Blackbelly lambs given a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Behaviour	Regrowth ages (days)				s.e.d.	Signifi-cance
	14	21	28	56		
Eating time (min)	386.3	422.4	418.5	419.4	66.26	NS
Ruminating time (min)	490.7	539.9	538.1	545.4	57.33	NS
Chewing time (min)	876.9	962.2	956.6	964.8	91.47	NS
Idling time (min)	563.1	477.0	483.3	475.4	91.47	NS
Eating index DM (min/gDMI)	0.33a	0.34a	0.38a	0.49b	0.034	***
Ruminating index DM (min/gDMI)	0.42a	0.42a	0.48a	0.64b	0.065	**
Chewing index DM (min/gDMI)	0.75a	0.76a	0.86a	1.13b	0.066	***
Eating index NDF (min/gNDFI)	0.49a	0.50a	0.51a	0.62b	0.046	*
Ruminating index NDF (min/gNDFI)	0.62	0.63	0.66	0.81	0.09	NS
Chewing index NDF (min/gNDFI)	1.11a	1.13a	1.17a	1.43b	0.086	***
Intake rate (gMSI/min)	4.16a	3.23b	2.19c	1.81c	0.30	***

a, b, c: means within the same row with different superscripts differ (P< 0.05
* $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$

Table 4. Amount of DM (total DM, Large particles, Small particles, and Very Small particles) in the rumen of black belly rams 0 (min) and 3 (max) hours after the morning meal of a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Rumen fill	Regrowth ages (days)				s.e.d.	Signifi-cance
	14	21	28	56		
Dry matter min (g)	676.6	752.4	869.8	816.4	60.99	NS
Dry matter max (g)	1123.5	984.4	1066.7	1090.1	52.52	NS
Dry Matter min (g / g DM intake)	0.622a	0.780ab	0.836b	1.056c	0.047	***
Dry Matter max (g / g DM intake)	0.773a	0.933ab	1.009b	1.552c	0.057	***
Large particle min (g / g DM intake)	0.113	0.143	0.153	0.124	0.011	NS
Small particle min (g / g DM intake)	0.241 a	0.321 ab	0.373 b	0.600c	0.026	***
Very Small particle min (g / g DM intake)	0.268	0.315	0.310	0.334	0.024	NS
Large particle max (g / g DM intake)	0.257a	0.283ab	0.316ab	0.399b	0.028	*
Small particle max (g / g DM intake)	0.242 a	0.326 ab	0.375 b	0.716c	0.023	***
Very Small particle max (g / g DM intake)	0.275 a	0.324a	0.318a	0.437b	0.019	***

a, b, c: means within the same row with different superscripts differ (P< 0.05)
* $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$

Voluntary intake of digestible organic matter (g/kg LW $^{0.75}$) decreased inversely with the age of regrowth (61.24, 52.14, 38.98 and 28.39 for the 14-, 21-, 28- and 56-old *P. maximum* respectively).

Ruminal Turnover Rate

The ruminal turnover rate (trADL) of the 14-, 21- and 28-day old forages were significantly higher than those registered with the 56-day old forage and no significant difference was observed between the 14-, 21- and 28-day old forages (table 2). A decrease of 64% was registered between the youngest and the oldest forage, and the highest decrease was registered between the 28- and the 56-day old forage.

Feeding Behaviour

No significant effect was registered for the age of regrowth on the times spent eating, ruminating, chewing and idling (table 3). The indexes of intake, rumination and chewing increased significantly with the 56- days grass compared to those obtained with forage less than one month old (table 3).

Intake rate decreased significantly with increasing age of regrowth (table 3). A decrease of 56% was registered between the youngest and the oldest forage.

Rumen Fill

The maturity of the guinea grass had no significant effect on rumen fill estimated just before the morning meal or 3 hours after the start of the morning meal (table 4). The analysis of the data taking into account the level of intake (g /g DMI) indicates that the amount of dry matter increased with grass maturity, irrespective of the time after the meal (table 4). The 56-day grass was significantly higher than the other grasses, and the 28-day forage was significantly higher than the 14-day old. Further more, no significant difference was recorded between the

Table 5. Total Rumen fill (RF), large, small and very small particles of Neutral Detergent Fiber collected 1h after a first emptying in Blackbelly rams given a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Rumen fill	Regrowth ages (days)				s.e.d.	Signifi-cance
	14	21	28	56		
NDF RF (g/g NDFI)	0.848a	0.962ab	1.097b	1.042b	0.090	*
Large NDF particle (%)	33.1a	41.1ab	46.4b	51.9b	3.317	*
Small NDF particle (%)	30.1 a	22.8b	24.1 b	28.8ab	1.384	***
Very Small NDF particle (%)	36.8 a	36.1a	29.5ab	19.2b	2.289	***

a, b, c: means within the same row with different superscripts differ (P< 0.05)
* $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$

14 and the 21 day old grasses or between the 21 and the 28 day old grasses, irrespective of the time after the meal.

Concerning the granulometry of the rumen content, the analysis of the data taking in account dry matter intake, showed that just before the morning meal, no significant difference was observed between the amount of large particle registered at the different age of regrowth, whereas 3 hours after the start of the morning meal, the amount of large particle increased with increasing age of regrowth (table 4). However, only the 56- and the 14-days old were significantly different. The amount of small particles of dry matter increased with maturity of the guinea grass irrespective of the time after the meal. Also, the 56-day old grass had significantly higher small particles than grasses of other regrowth ages, and the 28-day forage was significantly higher than the 14-day old. No significant difference was recorded between the 14 and the 21 day old forage or between the 21 and the 28 day old forage, irrespective of the time after the meal. With regard to the very small particles, no significant difference was registered with the increasing age of regrowth when they were collected just before the morning meal. Nevertheless, it appeared that very small particles obtained 3 hours after the morning meal were significantly higher with the 56-day old regrowth compared with forages less than one month.

Impact of the ingestive mastication

Results obtained when the rumen content were collected in order to estimate the impact of ingestive mastication indicated that, for NDF intake, rumen fill increased with the maturity of the grass (table 5). The 14-day old grass was significantly lower than the 28- and 56-day old grasses.

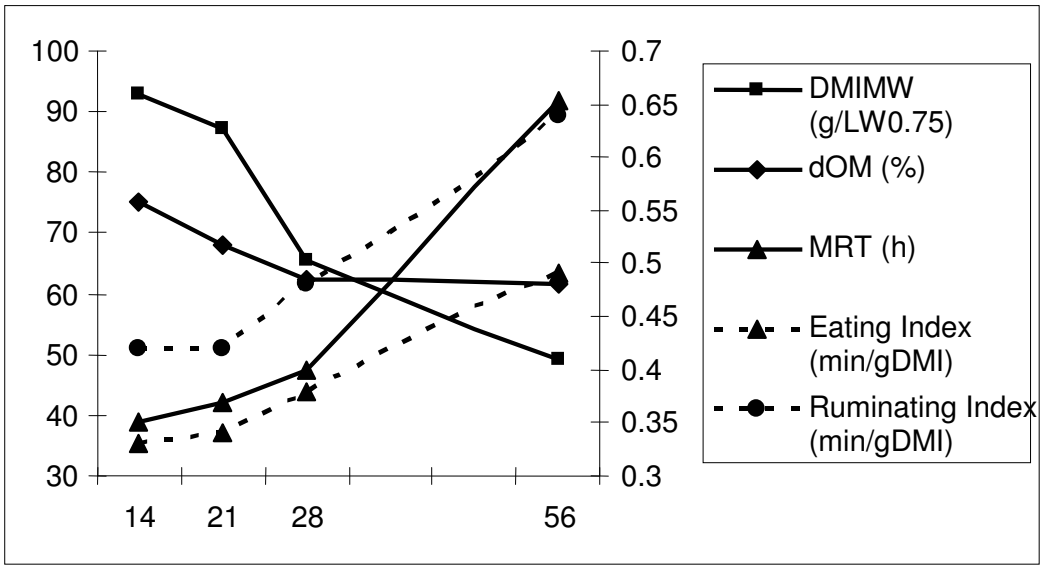

Figure 2. Effect of regrowth age (days) on intake, total tract digestibility of OM, eating index, ruminating index and mean transit time in the rumen of *Panicum maximum* fresh grass

	DMIMW	dOM	Eating Index	Rum. index	Chewing index	MRT	NDF RF max
dOM (%)	0,659						
	0,004						
Eating index (min/gDMI)	-0,753	-0,454					
	0,001	0,103					
Ruminating id (min/gDMI)	-0,733	-0,263	0,725				
	0,001	0,363	0,001				
Chewing index (min/gDMI)	-0,798	-0,392	0,908	0,947			
	0,000	0,165	0,000	0,000			
MRT (h)	-0,854	-0,498	0,823	0,795	0,878		
	0,000	0,084	0,001	0,001	0,000		
NDF RF max (g/NDFI)	-0,879	-0,592	0,780	0,726	0,806	0,929	
	0,000	0,016	0,001	0,002	0,000	0,000	
NDF RF min (g/NDFI)	-0,811	-0,490	0,580	0,549	0,604	0,792	0,862
	0,000	0,054	0,023	0,034	0,017	0,001	0,000

Cell Contents: Pearson correlation
P-Value

Table 6. Corelation between intake and digestive parameters.

Similarly, the percentage of large particle (table 5) increased with the grass maturity. Percentage of small particle was significantly higher with the 14- day old grass when compared to the 21-, 28- and 56-day old which were not significant different from each other. Percentage of very small particles decreased with increasing age of regrowth. Significant difference was observed between the oldest forage, and the 14- and 21-day old grasses.

Correlation between intake and digestive parameters

The figure 2 illustrates the changes in different parameters with the regrowth age of the guinea grass. Intake and total tract digestibility of OM decreased whereas transit time, ruminating and eating indexes increased with increasing regrowth age

Correlation analyses (table 6) indicate that intake (g/kg $LW^{0.75}$) was mostly related with maximum rumen fill (gNDF/gNDFI) (-0.88), mean retention time (-0.85), minimum rumen fill (gNDF/gNDFI) (-0.81), index of chewing (-0.80), index of eating (-0.75), index of ruminating (-0.73) and digestibility of OM (0.66).

Maximum rumen fill (gNDF/gNDFI) was related with mean retention time (0.93), index of chewing (0.81), index of rumination (0.73), digestibility of OM (-0.59), .

Minimum rumen fill (gNDF/gNDFI) was related with maximum rumen fill (gNDF/gNDFI) (0.86), mean retention time (0.79), index of chewing (0.60) index of rumination (0.55) and digestibility of OM (-0.49).

Mean retention time was related with Index of chewing (0.88), index of rumination (0.80) and digestibility of OM (-0.50).

Discussion

The observed chemical composition of guinea grass which shows a decrease in crude protein content and an increase in the cell wall content with increasing age of the regrowth is in agreement with previous results (Chenost 1975, Minson, 1990, Archimède *et a*l 2000 Niekerk *et al*, 2002). However, higher NDF was observed in this present study when compared to the results obtained with pangola grass under similar conditions (Archimede *et al.*, 2000; C. Assoumaya, unpublished data). Also, the ADF/NDF ratio tends to increase for guinea grass, whereas it is constant with the pangola grass. This result is unexpected, considering the low leaf/stem ratio of guinea grass when compared with the pangola grass. These observations could illustrate that guinea grass may have a high proportion of mechanical strengthening tissue in leaves as reported by Wilson *et al.* (1989). Consequently the cell wall of leaves in guinea grass could be tougher than that of the pangola grass.

The decrease in intake and digestibility with the increasing of regrowth age reported in this study is similar to previous observations. (Chenost, 1975; Ichinohe *et al.*, 1995; Archimede *et al*., 2000). However, the range of decrease recorded for intake with guinea grass is higher than that of pangola (1.02 vs 0.66 g/kg $LW^{0.75}$). This value is also higher than the mean value of 0.17 reported for tropical forage (Minson, 1990). The experimental range of stage of growth taking into account very young grass and as well as the curvilinear tendency of decrease of intake with time explain this discrepancy between our results and the literature. This result underlines the necessity to valorise tropical grass at young stage of regrowth. Concerning the difference registered between the guinea and the pangola grasses, it could be explained by the difference in the cell wall composition of the two grasses.

Like the result for intake, the decrease of total tract digestibility with increasing regrowth age is in accordance with previous studies (Aumont *et al.*, 1995, Eugène 2002, Babatounde, 2005). The mean rate of fall in dDM (0.22 points) is similar to the one registered by

Archimede *et al*. (2000) with pangola under similar conditions, and close to the mean value of 0.33 points reported by Minson (1972) with older guinea forage. Nevertheless, like for intake, the general tendency is curvilinear with a fast decrease for young stage of regrowth. The range of decrease of dDM between 14 to 56 day is low relative to intake (15 versus 46%) illustrating the major role of intake in variation of nutritive value of grass and the need to maximize this value.

Analysis of result of animal behaviour is vital to explaining the intake variation. Considering that the total time of chewing was close to the maximum value (1000 min) reported in the literature (Jarrige *et al.*, 1995), it could be hypothesized that irrespective of the stage of regrowth of the grass, the animals maximized the time spent eating and ruminating whereas the amount of dry matter ingested or masticated decreased with increasing regrowth age. Generally, voluntary intake of low quality forage is considered to be limited by the fill of dry matter and the clearance from the reticulo-rumen (Mc Leod *et al.*, 1990). Digesta can leave the reticulo-rumen when most of the particles have been reduce below a critical size (Poppi *et al.,* 1985) mainly by chewing (McLeod and Minson, 1988). Our results illustrated an increasing gradient of cell wall hardness of the guinea grass with maturation. This is in good agreement with the increasing ADF/NDF ratio of the guinea grass. Also, the increase of the intake rate, the eating and chewing indexes, are consequences of increasing resistance of cell wall to mastication. Consequently, the largest part of Large particles arrived in the rumen during the intake of forage and rumen turnover rate is reduced with the maturity of the forage. All these results underline a major impact of physical degradation of forage on intake. The results of the rumen pool and its granulometry are also informative on the constraint of the physical degradation of the grass. Rumen-fill increases with the forage maturation as illustrated by the decreasing range between maximum and minimum level of rumen content. This increasing fill with maturity is in good agreement with the decreasing turnover of fibrous

particle in the rumen. Forbes (1994) noted that intake is regulated by the rumen fill. Therefore, in this experiment, decreasing intake with the forage maturity can be partly explained by the turnover rate of the rumen and the intake rate. These parameters are correlated with the efficacy of reduction size of grass particle. The fact that the intake rate was more important with the youngest forage whereas the rumen fill was twice less important after the meal, tend to suggest that the turnover rate is more important with the youngest grass.

Comparing guinea grass with pangola grass (C. Assoumaya, unpublished data) the index of mastication is higher with guinea grass. Thus it appears that 1g of 14-day old P. maximum will require 40% more time of mastication by a ruminant than 1g of 21-day old D. Decumbens. In addition, values of rumen fill with guinea grass (at the end of the meal) are lower than those obtained with pangola grass under similar conditions (C. Assoumaya, unpublished data). These results enable us to hypothesize that guinea grass has a higher fill value compared with pangola grass.

Conclusions

Like previous observations with pangola grass, the present result underlines the impact of physical constraints on intake of guinea grass. Taking into account the morphological aspects of guinea grass, our results are unexpected. Also, guinea grass remained leafy during its growth in contrast to the pangola grass for which the leaf/stem ratio is fast. So, we expected a slow decrease in the intake of the guinea grass, with the increasing age of regrowth. These results enable us hypothesise that a structural differentiated arrangement of the guinea grass would have a negative impact on its chewing and intake when it matures. Studies in electronic microscopy should allow us to validate this hypothesis.

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PUBLICATION 7

Rumen fermentation in rams fed *Panicum maximum* harvested at four stages of grass maturity

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Abstract

The aim of this study was to consider the impact of forage maturity on microbial activities in the rumen and its consequences on intake and digestibility. Six sheep (mean liveweight: 37.12 ± 1.5kg) received a 14, 21, 28 and 56-day old guinea grass during four successive 5-week periods. Between the 14 and 56 days of regrowth, the degradation rate (%/h) and the effective degradability (%) decreased from 0.068 to 0.029 and from 59.29 to 32.16 respectively. The mean pH increased from 6.00 to 6.61 whereas the mean total Volatile Fatty Acids (VFA) (g/L) decreased from 87.23 to 65.05 between the youngest and the oldest forage. The mean NH3 (mgN/L) decreased from 353.01 to 85.19 and was consistently higher than 50 mgN/L. Furthermore, total or specific activities of polysaccharidases do not decrease with the age of forage. In conclusion, enzymatic activities do not vary with maturity of forage and consequently do not impact intake and digestibility.

Keywords: Panicum maximum, rams, ruminal fermentation, cellulolysis activity

Introduction

Fresh forage is often the sole component of the diet of many ruminant in the humid tropics. Minson (1990) and Aumont *et al.,* (1995) observed however that primarily, voluntary intake and then secondary total tract digestibility are major factors limiting the nutritional value of tropical forage. Moreover, voluntary forage intake is broadly related to the total tract digestibility of the fibre content of a feed. Nevertheless, there are significant differences in intake between grasses and legumes and also between different grass cultivars, even at the same level of fibre contents or digestibility (Minson, 1972, Burns *et al.,* 1997). Thus, indicating that probably there is no mechanical relationship between intake and digestibility. Digestion is a complex process which combines interdependent physical (size reduction of particle via mastication) and biochemical activities (microbial enzyme). We hypothesize that,

intake is mainly limited by physical constraints whereas, digestibility is limited by physical and biochemical constraints. Generally, fermentation of polysaccharide, the main component of forage (cell wall), is accomplished through activities of microbial enzymes present in the rumen. However, the structural complexity and inaccessibility of cell wall components limit the extent to which they are fermented in the rumen.

Our study was performed to evaluate the contributions of physical and biochemical activities on intake and digestion of tropical forage at different regrowth age. In a previous paper, we have described the impact of physical degradation on intake and total tract digestibility. As follow up, this current paper aims to consider the impact of the maturity of forage on microbial activities in the rumen and its consequences on intake and digestibility.

Material and methods

Location

The trial was conducted in 2005 at the animal experimental station of the ‘‘National Institute of Agronomic Research" (INRA, French West Indies, Guadeloupe, latitude 16.16 N, longitude 61.30 W). Temperatures ranged in average from 21°C to 31°C. The mean rainfall on the experimental site was 3000 mm/year. All the experimental period took place during the raining season where no significant trend has been registered for rain and temperature.

Experimental design, animals, diets and feeding

The experimental design is similar to that of Assoumaya *et al.,* (xxxx). Five Black-belly lambs (mean live weight: 37.12 (s.d. 1.50) kg were successively fed fresh grass of 56, 28, 21 and 14 days of regrowth for the experimental periods 1, 2, 3 and 4 respectively. The lambs were fed twice a day at 12-hour intervals. The quantity was offered ad libitum (1.15 times more than the animal’s estimated voluntary intake during periods of adaptation). The rams

used in this trial were fitted with rumen cannulae and kept in metabolism cages. Each experimental period lasted 35 days. During the fourth week, nylon bag incubation and ruminal sampling were carried out.

Measurements

The kinetic degradation rate of the forage offered was measured using the nylon bag method. Nylon bags of 10 cm x 5 cm, with a pore size of 50 x 50 µm (Blutex, Tissage Tissus Technique, Villeneuve La Garenne 92390, France), were filled with 15g of fresh forage of the basal diet. The grass was manually cut into particles of 2 mm mean length. The forage in the nylon bag was the same as those fed to the ram. Three nylon bags per incubation times and per animal, were placed in the rumen after feeding and the incubation times were 3, 6, 12, 24, 48 and 96 h. After removal from the rumen, the bags were rinsed under cold running water and were frozen (-20°C) until the end of the experiment. Then, the bags were washed (3 times, 10 min each) in water using a small automatic washing machine (Alternatic Calor, capacity:1 kg) to minimise contamination of diet residues by microbial population. A fourth washing (10 min) took place in an ultrasonic bath to remove adherent bacteria associated with diet residues. The relationship between DM disappearance from the nylon bags and incubation time for each animal was described by the model of Ørskov and McDonald (1979). Effective degradability was calculated from the parameters (a (fraction quickly extinct, essentially by solubilization), b (insoluble fraction but potentially degradable) of the model, and the estimated fractional outflow rates (c):

$$D\ (\%) = a + bc / (c + k)$$

Effective degradability was calculated using two values for “k”:

- the rate of renewal of the particles in the rumen (inverse of the retention time) estimated for each experimental diets (Assoumaya *et al.,* (xxxx)). Values registered with the 14-, 21-, 28- and 56-day forage were respectively 0.028, 0.027, 0.024 and 0.010 %/h.

- the mean value of 4% / h obtained in previous studies.

Furthermore, during each period, three nylon bags per animal, filled with 15 g of the 14 day fresh *Panicum maximum* grass were incubated for 24 hours as reference.

During this period of nylon bag degradation, two representative samples of rumen contents (300g) were removed in order to measure enzyme activities from the solid-adherent microorganisms. The samples were collected for two consecutive days, immediately before the morning meal and 3 hours after the morning meal. Samples were then filtered through a 100µm nylon filter to collect the solid phase, under anaerobic conditions (CO2 flows). Samples of rumen liquor used for pH, ammonia and Volatile Fatty Acids (VFA) determination were obtained by squeezing the ruminal digesta through a nylon filter of 150 µm pore size. The rumen liquor was removed for two consecutive days, immediately before the morning meal, and at 3, 6 and 12 hours after the morning meal. The pH was measured immediately after sampling. Samples of rumen fluid were frozen (-20°C) after adding a mixture of H3PO4 and HgCl2 (1 vol./ 10 vol.) prior to VFA determination. Rumen fluid was stored (4°C) with H2SO4 (1 vol./ 50 vol.) prior to ammonia determination.

Chemical analyses

Enzymatic manipulations were performed under anaerobic conditions. Enzyme extraction from the solid adherent microorganisms were conducted according to the procedure detailed by Martin and Michalet-Doreau (1995). Enzymes from solid-adherent microorganisms were measured using the assay procedures detailed by Nozière *et al.* (1996) and Nozière and Michalet-Doreau (1996). Polysaccharides activities were determined by detecting spectrophotometrically at 410 nm (Lever, 1977), the amount of reducing sugars released from the purified substrates after incubation (60 min at 39°C) with enzyme preparation.

Table 1. Chemical composition (% DM) of a 14-, 21-, 28- and 56-day old fertilized *Panicum maximum* grass.

Constituents (% DM)	Regrowth age (days)			
	14	21	28	56
Organic matter	88.3	88.4	89.2	89.5
Crude protein	20.18	17.75	9.26	4.77
Neutral detergent fibre	67.35	67.28	73.93	78.88
Acid detergent fibre	31.40	34.49	42.53	44.26
Acid detergent lignin	3.51	4.84	4.87	5.51

Glucosidase activities were obtained by measuring spectrophotometrically at 420 nm the rate of p-nitrophenol release from the appropriate p-nitrophenyl glycoside after incubation for 30 to 60 min at 39°C. The protein content of the enzyme preparations was determined according to Pierce and Suelter (1977). Enzyme activities were expressed as micromoles of reducing sugar (for polysaccharides) or nanomoles of p-nitrophenol (for glucosidase) released per gram of DM per hour (total activity). Ruminal volatile fatty acids were measured with the rumen liquid by gas chromatography (Ottenstein and Supina, 1974). Ammonia concentration was estimated with rumen liquor by distillation and titration.

Statistical analyses

The ANOVA data were analysed using the General Linear Model procedure of Minitab14 (2003) including the age of regrowth (D.F. 3) (α) and the animal (D.F. 4) (β).

$$Y_{ij} = \mu + \alpha_i + \beta_j + e_{ij}$$

The effect of periods was tested on the residues of the previous model, no significant difference was registered.

Intakes and total tract digestibilities of dry matter were predicted by multiple regressions on intake measured in vivo (Assoumaya *et al.,* xxxx)

Results

Diet composition

The chemical composition (Table 1) of diets were similar to those registered in Assoumaya *et al.* (xxxx).

Table 2. Degradability of dry matter and nitrogen in Blackbelly rams consuming a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Age of regrowth	14	21	28	56	s.e.d.	Significance
Incubation time after feeding						
DM Degradability (%)						
3h	31.82a	31.37a	23.58b	16.73c	1.57	***
6h	37.98a	42.20b	28.28c	18.77d	1.94	***
12h	52.97a	60.50b	39.86c	25.62d	3.41	***
24h	69.84a	74.32b	57.68c	36.71d	3.55	***
48h	79.54a	80.78a	67.48b	47.30c	1.98	***
96h	82.85a	83.84a	72.52b	56.91c	0.96	***
Soluble fraction	18.44a	14.99b	13.70bc	11.86c	1.46	***
Potentially degradable fraction	64.98a	67.99a	59.35b	47.94c	1.92	***
Degradation rate	0.068a	0.090b	0.052c	0.029d	0.004	***
Effective degrad. (%) k=0.04	59.29a	62.08b	47.14c	32.16d	1.112	***
Effect. Degrad. (%) k=tradl	64.44a	67.35b	54.20c	47.63d	1.031	***
Degrad. 24h (reference)	81.54a	82.08a	81.74a	77.19b	3.327	***
NDF Degradability (%)						
3h	20.50a	16.71b	12.26c	1.97d	1.84	***
6h	26.16a	30.77b	16.75c	6.44d	2.28	***
12h	46.41a	51.04b	29.87c	12.69d	4.05	***
24h	64.19a	66.97a	49.53b	25.32c	4.26	***
48h	74.43a	75.00a	61.22b	37.27c	2.39	***
96h	78.15a	78.99a	67.34b	49.48c	1.16	***
Soluble fraction (NDF)	5.14a	-1.58b	1.40ab	-2.62b	2.27	***
Potentially degradable fraction (NDF)	73.27a	78.87a	66.71b	55.94c	2.74	***
Degradation rate (NDF)	0.067a	0.088b	0.049c	0.028d	0.0082	***
NDF Effective degrad. (%) k=0.04	50.85a	52.74a	38.17b	20.45c	1.912	***
NDF Effective degrad. (%) k=tradl	56.62 a	58.85 a	46.20 b	38.58 c	1.845	***

N Degradability (%)						
3h	39.85a	46.60b	36.26c	43.12d	1.25	***
6h	45.69a	57.67b	37.95c	42.62d	1.58	***
12h	63.34a	77.20b	55.15c	45.48d	2.40	***
24h	84.13a	90.02b	75.13c	53.84d	2.03	***
48h	92.53a	95.32b	84.17c	67.36d	0.99	***
96h	93.91a	95.17b	88.48c	74.10d	0.49	***
Soluble fraction (N)	24.18ab	26.98a	22.50b	38.52c	1.47	***
Potentially degradable fraction (N)	70.78a	67.35b	66.52b	42.58c	1.52	***
Degradation rate (N)	0.07a	0.102b	0.058c	0.020d	0.0046	***
N Effective degrad. (%) k=0.04	69.18a	75.41b	61.85c	52.75d	1.097	***
N Effective degrad. (%) k=tradl	74.69 a	80.27 a	69.53 b	66.97 c	1.000	***

a, b, c, d: means within the same row with different superscripts differ (P< 0.05)
* $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$

Table 3. pH in Blackbelly rams consuming a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Age of regrowth	14	21	28	56	s.e.d.	Significance
Item and sampling time after feeding						
pH						
0h	6.21a	6.52ab	6.68b	6.60b	0.18	***
3h	6.02a	6.37b	6.58b	6.59b	0.23	***
6h	5.76a	6.21b	6.45bc	6.54c	0.21	***
12h	6.01a	6.29b	6.56c	6.70c	0.18	***
Mean pH	6.00a	6.35b	6.57c	6.61c	0.19	***

a, b, c, d: means within the same row with different superscripts differ (P< 0.05)
* $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$

Table 4. Volatile Fatty Acids (VFA) in Blackbelly rams consuming a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Age of regrowth	14	21	28	56	s.e.d.	Significance
Item and sampling time after feeding						
Total VFA (mmol / L)						
0h	81.60a	74.38ab	66.71b	65.32b	10.79	***
3h	92.17a	83.97a	69.43b	66.79b	6.91	***
6h	93.21a	88.58a	72.25b	68.50b	8.12	***
12h	81.95a	82.85a	70.92b	59.59c	6.43	***
Mean total VFA	87.23a	82.44a	69.83b	65.05c	8.28	***
Mean VFA (mmol / L)						
Acetate (C2)	62.89a	59.09a	51.06b	49.73b	6.51	***
Propionate (C3)	16.29a	15.36a	12.94b	10.76c	1.62	***
Butyrate (C4)	5.63a	5.78a	4.35b	3.69c	0.64	***
Iso butyrate (IsoC4)	0.57a	0.53a	0.44b	0.20c	0.14	***
Valerate (C5)	0.65a	0.55b	0.34c	0.23d	0.11	***
Isovalerique (IsoC5)	1.20a	1.13a	0.69b	0.45c	0.19	***
VFA pourcentage						
Acetate (C2, %)	72.16ab	71.61a	72.95b	76.35c	1.53	***
Proponiate (C3, %)	18.67a	18.59a	18.58a	16.60b	1.13	***
Butyrate (C4, %)	7.07a	7.72b	6.96a	6.00c	0.75	***
Valerate (C5, %)	2.09a	2.08a	1.51b	1.05c	0.34	***
Ratios						
C2/C3	3.88a	3.86a	3.97a	4.61b	0.33	***
C2/C4	10.32ab	9.35a	10.80b	12.99c	1.55	***
C3/C4	2.69a	2.44b	2.72a	2.81a	0.34	***
(C2+C4)/C3	4.26a	4.27a	4.34a	4.98b	0.34	***

a, b, c, d: means within the same row with different superscripts differ (P< 0.05)
* $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$

Kinetic in situ DM degradation

Generally, DM degradability per incubation time, DM effective degradability, the parameters of the fitting model of Orskov (soluble fraction, potential degradable fraction, degradation rate) decreased with maturity (table 2). The estimates of effective degradability obtained with k=0.04 were lower than those obtained using measured turnover rate. Also, the difference between the two estimates increased in value with the forage maturity. Concerning nitrogen, similar tendencies like those registered for DM were observed.

"Reference" degradability was significantly lower with the oldest forage whereas no significant difference was observed among the other 3 forages.

Rumen acidity pH.

The pH of the rumen significantly increased with the age of regrowth, irrespective of the sampling time (table 3). Irrespective of the age of regrowth, the pH decreased from 0 to 6 hours after the distribution of the meal, and then increased. Irrespective of the sampling time, values of the 14-day old were below 6.3. The pH is highly related with the VFA concentration in the rumen fluid.

pHmoy = 8.04 - 0.0218 VFA moy (S = 0.1281 R^2= 78.4% ; P = 0.000)

Volatile Fatty Acids.

The total VFA concentrations measured in rumen fluid significantly decreased with the age of regrowth, irrespective of the sampling time (P<0.000) (table 4). In contrast with pH, values of total VFA increased from 0 to 6 hours after the distribution of the meal and then decreased, irrespective of the age of regrowth. Irrespective of the fatty acid, concentrations significantly decreased with the increase of the age of regrowth. The proportion of acetate significantly increased with the age of regrowth whereas a significant decrease was registered with the other fatty acids.

Table 5. Ammonia concentrations in Blackbelly rams consuming a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Age of regrowth	14	21	28	56	s.e.d.	Significance
Item and sampling time after feeding **NH3 (mgN / L)**						
0h	296.7a	245.3b	124.8c	104.1c	34.12	***
3h	477.40a	348.83b	153.33c	94.16d	31.28	***
6h	406.16a	279.47b	104.08c	60.62c	52.04	***
12h	231.81a	259.71a	97.76b	81.92b	22.71	***
Mean NH3	353.01a	283.33b	120.00c	85.19d	35.18	***

a, b, c, d: means within the same row with different superscripts differ (P< 0.05)
* $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$

Table 6. Enzymatics activities (polysaccharidases) in rumen solid-associated microorganisms, in Blackbelly rams consuming a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Age of regrowth	14	21	28	56	s.e.d.	Significance
Item and time after feeding	**Total activity** (µmol reducing sugar / (gDM*h))					
Carboxymethylcellulase						
0h	7.3a	11.6ab	16.2bc	24.2c	3.4	***
3h	1.6a	21.7a	7.3a	14.1a	9.6	***
Xylanase						
0h	279.8a	345.1b	296.2ab	299.0ab	17.4	***
3h	173.5a	249.2b	259.0b	295.4b	22.5	***
	Specific activity (µmol reducing sugar / (mg protein*h))					
Carboxymethylcellulase						
0h	1.4a	6.0b	3.2a	5.5b	0.8	***
3h	0.26a	2.61ab	1.8ab	2.7b	2.1	***
Xylanase						
0h	50.6a	182.2b	59a	75.3a	12.2	***
3h	31.0a	143.8b	67.4cd	58.2ad	9.7	***

a, b, c, d: means within the same row with different superscripts differ (P< 0.05)
* $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$

Table 7. Enzymatics activities (glucosidases) in rumen solid-associated microorganisms, in Blackbelly rams consuming a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Age of regrowth	14	21	28	56	s.e.d.	Significance
Item and time after feeding	**Total activity** (µmol reducing sugar / (gDM*h))					
β-D- Xylosidase						
0h	87.4a	86.6a	110.5a	111.0a	11.2	***
3h	58.3a	68.3a	76.7a	107.2b	7.5	***
β–D-Galactosidase						
0h	31.0a	7.9b	20.0c	19.8c	3.4	***
3h	23.2a	8.0b	19.2c	25.0a	1.4	***
β–D-Glucosidase						
0h	25.2ab	23.8a	28.4ab	33.0b	2.9	***
3h	45.8a	43.0a	49.4a	72.8b	3.9	***
	Specific activity (µmol reducing sugar / (mg protein*h))					
β-D- Xylosidase						
0h	15.7a	44.3b	21.5ac	27.2c	3.5	***
3h	10.5a	38.0b	19.9c	21.2c	2.1	***
β–D-Galactosidase						
0h	5.7a	4.1b	3.7b	4.4b	0.4	***
3h	4.1a	4.5a	4.8a	5.0a	0.3	***
β–D-Glucosidase						
0h	4.5a	12.8b	5.5ac	8.0c	1.1	***
3h	8.2a	24.3b	12.5c	14.4c	1.1	***

a, b, c: means within the same row with different superscripts differ (P< 0.05)
* $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$

The acetate to propionate, acetate to butyrate, and (acetate + butyrate) to propionate ratios significantly increased with the age of regrowth whereas the propionate to butyrate ratio remained relatively stable.

VFA concentrations are explained by total tract digestion of organic matter (publication 6) as illustrated with the following equations :

VFA 0h = 20.6 + 0.763 dOM (s= 8.67750; R^2= 18.8%; P=0.063)
VFA3h = - 17.5 + 1.41 dOM (S = 10.2407; R^2= 36.1%; P=0.006)
VFA6h = - 18.7 + 1.46 dOM (S = 10.9665; R^2= 34.7% ; P= 0.008)
VFA12h = 5.1 + 1.01 dOM (S = 11.4166; R^2= 18.9%; P=0.063)
VFA moy = - 2.6 + 1.16 dOM (S = 9.57962; R^2= 30.5%; P= 0.014)

Ammonia concentrations

Ammonia concentrations in rumen liquid significantly decreased (P<0.000) with the age of regrowth irrespective of the sampling time (table 5). Mean values decreased curvilinearly from 353.0 to 85.2mgN/ L from the youngest to the oldest forage. Higher differences among these regrowth ages were observed 3 hours after the morning meal. Values of NH3 increased from 0 to 3 hours after the distribution of the meal and then decreased for the forages which were less than one month. Values of NH3 were relatively stable with the 56-days. No value was below 50 mg/N/L.

Enzymatic activity

Generally, total enzymatic activities (glucosidases and polysaccharidases) tended to increase or to remain stable with the age of regrowth (tables 6 and 7). Irrespective of the sampling time, the same tendency was observed for both the polysaccharidases (fibrolytic enzymes) and the glucosidases total activities (µmol reducing sugars /g DM*h) and specific activities (µmol p-nitrophenol/mg protein*h).

Table 8. Predictive equations of intake (n=17) (g DMI/kg $LW^{0.75}$) and total tract digestiblity (dDM) (n=15) (%).

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	SE	R^2	P
DMIMW = – 6.2 + 5.48 a	11.4996	65.3	0.000
DMIMW = – 47.2 + 2.01 b	10.4775	71.2	0.000
DMIMW = 35.1 + 645 c	12.3930	59.7	0.000
DMIMW = – 52.9 + 3.19a + 1.33b	7.71136	85.4	0.000
DMIMW = – 35.8 + 1.80 aNDF + 1.58 bNDF	8.43407	82.1	0.000
DMIMW = 39.1 + 2.15 aNDF + 581 cNDF	9.30878	78.2	0.000
DMIMW = – 31.7 + 1.82 aNDF + 1.49 bNDF +36cNDF	8.70692	82.1	0.000
dDM = 46.9 + 1.29 a	5.40108	32.1	0.014
dDM = 40.8 + 0.413 b	5.61220	26.7	0.028
dDM = 57.7 + 133 c	5.76643	22.6	0.046
dDM = 39.2 + 0.912 a + 0.220b	5.37530	36.9	0.032
dDM = 47.4 + 0.996 a + 63.9c	5.43152	35.6	0.037
dDM = 64.9 + 0.570 aNDF	6.46536	10.9	0.195
dDM = 28.6 + 0.527 bNDF	5.09104	44.8	0.003
dDM = 54.0 + 190 cNDF	5.36134	38.8	0.008
dDM = 29.4 + 0.480 aNDF + 0.510 bNDF	4.88814	52.5	0.005
dDM = 53.4 + 0.586 aNDF + 191 cNDF	4.99966	50.3	0.008

Prediction of intake and total tract digestibility

The parameters (slowly digestible fractions, soluble fraction and degradation rate) obtained by modelling in sacco dry matter degradation of forages enable us to observed relevant prediction of DM intakes (table 8). The slowly digestible fraction highly (R^2 = 0.712) explained the intake than the degradation rate (R^2=0.597) and the soluble fraction (R^2=0.653). Multiple regression with the soluble dry matter fraction and the potential fermentable fraction improved the accuracy of the regression significantly (R^2=0.85) whereas no improvement was observed with the rate of degradation. Similar tendencies were registered when Dry matter intake was predicted with these parameters modelling in sacco degradation of NDF. Concerning total tract digestion prediction (Table 8), using the same parameters like for intake, the models of prediction are less accurate ($0.22 < R^2 < 0.32$).

Discussion

The inverse relationship between forage maturity and digestibility measured by direct or indirect method are reported in literature (Chenost, 1975, Archimede *et al.*, 2000, Eugène, 2002; Arthington and Brown, 2005).

Values of solubility, level of degradation and degradation rate of nitrogen reported in this current study and their variation with forage maturity are similar to those reported by Babatounde (2005) on guinea grass and Aumont *et al*. (1994) with pangola grass. In contrast, our results for the effective degradability calculated with k = 0.04, or the estimated turnover rate are logically higher than those estimated by Babatounde (2005) who used a value of k=0.06 (usually used for temperate forage) in calculation.

From our results, increase in the differences with the maturity, between the 2 methods of estimates of the effective degradation (with k=0.04 or with the estimated turnover rate), can

be explained by the decreasing value of the turnover rate with the age of regrowth (Assoumaya *et al.,* xxxx). Practically, this means that old forage stay longer time in the rumen so fibrolytic activity of enzyme is most efficient. Considering that 90 % of digestion of the forage dry matter occurs in the rumen (Archimede *et al*., 1997) we hypothesize that the effective degradability was underestimated.

The tendency of pH to increase with the forage maturity agrees with the decreasing level of total VFA and the probable increasing buffering capacity of saliva resulting from higher index of mastication (Assoumaya *et al.,* xxxx). The wide range of variation in pH recorded with the forage maturity probably has some effect on the cellulolytic capacity of the rumen. It can be hypothesized that the growth of cellulolytic bacteria is undermines with the 14-days guinea grass. Thus, pH values of the rumen fluid of rams consuming the 14-day grass is always under 6.3. However, it is known that cellulolytic organism grow optimally at a pH of 6.7 and that the growth decreases at pH below 6.3 (Mould and Orskov, 1983). The low pH observed with the 14 day grass could explain the low nylon bag degradation recorded with the 14 day when compared with the 21 day one. Also, the low pH could explain the absence of significant difference observed with the "reference" grass.

The general decrease of Total VFA with the grass maturity could be explained by the decreasing amount of organic matter fermented in the rumen as registered in this experiment (Assoumaya *et al.,* xxxx). Previous studies showed variation in the proportion of the VFA with maturity of the forage. Generally, increase in the proportion of acetate, and decrease in the proportion of butyrate have been observed with increasing maturation of the forage, while the proportion of propionate is either stable or decrease slightly (Mc Collum et al, 1985; Relling et al, 2001a; Eugène, 2002; Niekerk et al, 2002). Similar trends were observed in our study, irrespective of the proportion of VFA considered.

Levels of ammonia reported in this trial were higher than results from previous studies with tropical grasses. These high levels of ammonia could be due to the level of nitrogen in the experimental guinea grass. Playne and Kennedy (1976) reported that rumen ammonia concentration is positively correlated (r=0.58) with the nitrogen content of the diet. However, Van Soest (1982) noted that it is not only a function of N content, but also of nitrogen solubility, energy content of the diet and level of intake. Considering the high level of protein in the grass used in this study, we can hypothesize that the main factor responsible for the observed high ammonia concentration is the nitrogen solubility as illustrated by the estimate of the soluble fraction. The level of ammonia is always higher than 50 mg /l, which indicates that this was never a limiting factor for microbial growth in the rumen. Indeed, Satter and Roffer (1975), suggested a reduced rumen microbial growth and activity if the rumen ammonia drops below 50 mg/l.

Like most published trials, estimates of enzymatic activities indicated some variabilities linked to the method (Eugène, 2002). Notwithstanding, the most important result is increase or stable enzymatic activities with the maturation of the forage. The global and indirect method of measurement of cellulolytic activities via the nylon bag method give similar results except with the oldest grass for which a low depression cellulolysis capacity is reported. Similar trends were reported by Eugène (2002) on *Digitaria decumbens* at 14 to 56 days of regrowth age. These results suggest that enzymatic activities may not be the primary limiting factor of digestion in ruminants with tropical forage. This hypothesis is in agreement with results of Eugène *et al.* (2004b) on the changes in bacteria population in the rumen with maturity of the forage. Generally, the amounts of total and cellulolytic bacterial 16S rRNA remain stable with forage between 28 and 56-days of regrowth, and, *F. succinogenes,*

considered as the most efficient against recalcitrant lignocellulosic substrates, was the dominant species. In addition, its proportion increased with the forage maturity.

The nylon bag method gives a good prediction of intake like already reported by other authors (Orskov et al, 1988, Madsen et al 1994) whereas prediction of total tract digestibility is low. These results underlight that relationship between intake and total tract digestibility are not impressive.

The best prediction of intake with slowly degradable fraction of nylon bag degradation compared with degradation rate will enable us to understand some mechanisms regulating intake. Really the slowly digestible fraction is an important component explaining the fill value of forages (Madsen et al., 1994). This last one is mainly limited by physical factors.

Conclusion

Our results indicate that with matured guinea grass, cellulolytic activities is not the main limiting factor of digestion. Consequently, explanations have to be found in mechanisms linked to physical degradation of grass.

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PUBLICATION 8

Intake and digestive processes of rams fed *Digitaria decumbens* harvested at three stages of grass maturity

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Abstract

This study examined the changes physical and microbial digestion of pangola grass (*Digitaria decumbens)* in relation with its maturity and the impact on intake. Moreover Exogenous Fibrolytic Enzyme (EFE) (mainly xylanase and cellulase activities, 15mL/15kg of fresh forage) . Twelve rams (mean liveweight (LW): 53.02 (s.d. 7.59) kg were fed 21, 28 and 56-day old pangola grass during three successive 5-week periods. Between the 21 and 56 day-old of regrowth, the Dry Matter Intake (DMI) (g/kg $LW^{0.75}$) and the organic matter total tract digestibility (%) decreased from 86 to 59 and from 70 to 56 respectively.

EFE had no significant effect on intake and digestion of the pangola grass. No significant evolution of cellulolytic activity in the rumen was observed during the forage maturity. The ruminating index (min/g DMI) increased from 0.715 to 1.250 between the 21 and the 56 day pangola grass. The rumen fill for the 56 day regrowth was twice that of the 21 day regrowth. The rumen turnover rate (%) of fibrous particle, small particle and liquid decreased from 3.5 to 1.9, 7.3 to 4.3 and 12.7 to 7.8 respectively. It has been concluded that when pangola grass matured, cellulolytic activities are not the main limiting factor of digestion.

Keywords: *Digitaria decumbens*, rams, intake, digestive parameters

Introduction

Tropical grasses generally have a low intake and total tract digestibility compared to temperate grasses (Leng, 1990; Minson, 1990). These characteristics are partly associated with the high fibre content of tropical grasses compared with that of temperate grasses. Bowman *et al* (1991) indicated that voluntary intake of low quality forage is limited by rumen fill and the fractional disappearance rate of dry matter (DM) from the reticulo rumen. The forage particles need to be smaller in size but denser in order to flow out of the rumen. Previous studies (McLeod and Minson, 1988; McLeod et al., 1990) demonstrated that the breaking down of large forage particles to small particles is usually achieved by chewing during eating and rumination whereas only a small part of the particle breakdown (17%) can be attributed to microbial degradation. Regardless of the very high level of fibre and thick-walled bundle sheath in tropical grasses, Wilson (1994) has hypothesised that in intensive systems in tropical areas where the forage is irrigated and fertilised, the low rate of reduction in size of large forage particles by chewing is the first limiting factor on intake, even with young forage. Nevertheless only few data has been accumulated inside of this area of research with tropical forage (Kennedy, 1995) and the respective contribution of cellulolysis and physical degradation as limiting factor of intake and digestibility remains an open question. The experiment was designed (i) to evaluate variations in tropical forage grass intake according to regrowth age, (ii) to study the impact of cellulolysis and physical breakdown of grass particles on intake. To taken into account fast growth of tropical forages, this study has been performed with grass with state of regrowth age within bounds larger than those classically studied. Moreover, we added an efficient exogenous fibrolytic enzymes (EFE) (Assoumaya et al xxxx) to be overcome interaction between physical and enzymatic degradation postulating that availability of fibrolytic enzyme in the rumen could be the first limiting factor of intake and digestion.

Material and methods

Location

The trial was conducted in 2005 at the animal experimental station of the ‘‘National Institute of Agronomic Research" (INRA, French West Indies, Guadeloupe, latitude 16.16 N, longitude 61.30 W). Average monthly temperature at the experimental site, ranged from 21°C to 31°C, and the mean annual rainfall was approximately 3000 mm/year.

Experimental design, animals, diets and feeding

The experimental design was conducted in three successive periods. Each experimental period was 35 days long and consisted of 14 days of adaptation to the diet, 7 days of intake and total tract digestibility estimation, 7 days of nylon bag incubation and ruminal content sampling and 7 days of rumen emptying. Twelve Black-belly rams (mean live weight: 53.02 (s.d. 7.59) kg) were successively fed the fresh pangola grass of 56, 28 and 21 days of regrowth for the experimental periods 1, 2 and 3 respectively. One kg/ha/regrowth age of mineral nitrogen fertiliser was applied each day after the removal of the grass. After each harvest, the grass was kept at 4°C a night in a cold chamber until feeding. In the morning, the grass was chopped (approximately 4 cm) just before being offered. The rams were fed twice a day at 12-hour intervals. The quantity was offered *ad libitum* (1.15 times more than the animal’s estimated voluntary intake during periods of adaptation). The rams were allotted in two groups balanced on the basis of the weight and their capacity of intake estimated on a 14-day pangola grass. Six rams were fed with chopped forage mixed with tap water (1 litre to 15kg of fresh forage). Six other rams were fed with chopped forage mixed with a commercial fibrolytic enzyme solution (Nutreco Rumizyme-alpha). 15 ml of this enzyme were diluted with 100 times its volume in tap water. One litre of this diluted enzyme was mixed with 15 kg of fresh grass. The enzyme contained mainly xylanase and cellulase activities.

The rams used in this trial were fitted with rumen cannulae and kept in metabolism cages.

Measurements

Intake and apparent digestibility were determined by daily weighing of the amounts of food offered, refusals and faeces. Dried and ground samples of grass, refusals and faeces were stored for chemical analyses.

The feeding behavioural parameters (time spent eating, ruminating and idling) were determined by 24h of visual observation. Also, to establish the kinetic of intake, refusals were weighed hourly.

The rumen turnover rate of the liquid phase and small particle were estimated using PolyEthylene Glycol (PEG) and ytterbium labelled small particle as markers. Eight and 12 hours before the first collection of faeces, 30g of PEG and 30 g of Ytterbium (Yb) labelled small particle were introduced directly into each rumen. The faeces were then harvested 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 48, 52, 56, 62, 68, 80 and 104 hours after the introduction of the markers. The rumen turnover rate of PEG and Yb were estimated modelling the faecal excretion curve of these two markers following the model Gamma (faecal markers concentration =a1*exp(-l1*time) + a2*exp(-l1*time)).

The kinetic degradation rate of the forage offered was measured using the nylon bag method. Nylon bags of 10 cm x 5 cm, with a pore size of 50 x 50 µm (Blutex, Tissage Tissus Technique, Villeneuve La Garenne 92390, France), were filled with 15g of fresh forage of the basal diet treated or not with EFE. Nylon bags filled with fresh chopped mixed for 2 minutes with tap water were introduced in rumen of rams fed with fresh forage mixed with tap water (one litre to 15 kg of fresh forage). Nylon bags filled with fresh chopped forage mixed for 2 minutes with the diluted fibrolytic enzyme solution were introduced in rumen of rams fed

with fresh forage mixed with the enzymatic solution (one litre to 15 kg of fresh forage). The grass was manually cut into particles of 2 mm mean length.

Three nylon bags per incubation time and per animal, were placed in the rumen just before the distribution of the meal and the incubation times were 3, 6, 12, 24, 48 and 96 h. After removal from the rumen, the bags were rinsed under cold running water and were frozen (-20°C) until the end of the experiment. Then, the bags were washed (3 times, 10 min each) in water using a small automatic washing machine (Alternatic Calor, capacity:1 kg) to minimise contamination of diet residues by microbial population. A fourth washing (10 min) took place in an ultrasonic bath to remove adherent bacteria associated with diet residues. The relationship between DM disappearance from the nylon bags and incubation time for each animal was described by the model of Ørskov and McDonald (1979). Effective degradability was calculated from the parameters (a (fraction quickly extinct, essentially by solubilization), b (insoluble fraction but potentially degradable) of the model, and the estimated fractional outflow rates (c):

$$D\ (\%) = a + bc / (c + k)$$

Effective degradability was calculated using two values for “k”:

- the rate of renewal of the particles in the rumen (inverse of the retention time) estimated for each experimental diets (Assoumaya *et al.,* (xxxx)). Values registered with the 14-, 21-, 28- and 56-day forage were respectively 0.028, 0.027, 0.024 and 0.010 %/h.

- the mean value of 4% / h obtained in previous studies.

Moreover, during each period, three nylon bags per animal, filled with 15 g of the 14 day fresh pangola grass were incubated for 24 hours as reference.

During this period of nylon bag degradation, two representative samples of rumen contents (300g) were removed in order to measure enzyme activities from the solid-adherent microorganisms. The samples were collected for two consecutive days, immediately before

the morning meal and 3 hours after the morning meal. Samples were then filtered through a 100µm nylon filter to collect the solid phase, under anaerobic conditions (CO2 flows).
Samples of rumen liquor used for ammonia (NH3) determination were obtained by squeezing the ruminal digesta through a nylon filter of 150 µm pore size. The rumen liquor was removed for two consecutive days, immediately before the morning meal, and at 3, 6 and 12 hours after the morning meal. Rumen fluid was stored (4°C) with H2SO4 (1 vol./ 50 vol.) prior to ammonia determination.

During the rumen emptying period, firstly, two rumen empties (one per day) were carried out on each animal: (i) 3 hours after the morning meal (WRCmax) and (ii) just before the morning meal (WRCmin). The times were chosen to represent the maximum and the minimum rumen load respectively. There were 3 days between these two rumen evacuations. A third rumen emptying was carried out just after the second rumen emptying. Total rumen content collected during the second rumen emptying was stored in a hermetic recipient at 37°C. Animals were fed for one hour, then total rumen content was evacuated (WRCingmast). This last emptying was to enable the estimation of the impact of the ingestive mastication on the size reduction of forage. After each emptying, the total rumen content was weighed and thoroughly mixed by hand. Four sub-samples were taken, two for dry matter determination, one was preserved (-20°C) until freeze-dried for chemical analyses, while the last was used to determine the digesta particle size. Rumen particles were separated into Large Particles (LP) and Small Particles (SP), by wet sieving using gradual sieves of 4 mm, 1.18mm, 0·75 mm, 0·250 mm and 0.050mm in size. LP were defined as those retained in the 4mm and 1.18 mm sieves, SP were defined as those that passed through 1.18mm sieves but retained in 0.050mm sieves and particles that passed through the 0.050 mm were considered as Very small Particles (VS).

The daily passage rate (h^{-1}) of whole rumen fibre content (trADL) was estimated by the ratio: (daily faecal of lignin (g) / 24) / (mean total amount of lignin in the rumen (g)).

Chemical analyses

Dry Matter (DM) of fresh forage, refusals, faeces and rumen content were determined each day by drying to a constant weight at 60°C in a forced-draught oven. Amount of LP, SP and VS were determined as a percentage of the total amount of rumen dry matter content. The organic matter (OM) content was measured after a 10 h pyrolysis at 550°C. Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) were estimated following the methods of Van Soest *et al*., 1991. Nitrogen content was determined on representative sample of dry forage using the Dumas method (AFNOR, 1988).

Enzymatic manipulations were performed under anaerobic conditions. Enzyme extractions from the solid adherent microorganisms were conducted according to the procedure detailed by Martin and Michalet-Doreau (1995). Enzymes from solid-adherent microorganisms were measured using the assay procedures detailed by Nozière *et al.* (1996) and Nozière and Michalet-Doreau (1996). Polysaccharides activities were determined by detecting spectrophotometrically at 410 nm (Lever, 1977), the amount of reducing sugars released from the purified substrates after incubation (60 min at 39°C) with enzyme preparation. Glucosidase activities were obtained by measuring spectrophotometrically at 420 nm the rate of p-nitrophenol release from the appropriate p-nitrophenyl glycoside after incubation for 30 to 60 min at 39°C. The protein content of the enzyme preparations was determined according to Pierce and Suelter (1977). Enzyme activities were expressed as micromoles of reducing sugar (for polysaccharides) or nanomoles of p-nitrophenol (for glucosidase) released per gram of DM per hour (total activity). Ammonia concentration was estimated with rumen liquor by distillation and titration.

Table 1. Chemical composition (% DM) of a 21-, 28- and 56-day old fertilized *Digitaria decumbens* grass.

Constituents (% DM)	Regrowth age (days)		
	21	28	56
Organic matter	88.9	91.0	91.3
Crude protein	16.4	10.7	7.6
Neutral detergent fibre	71.5	72.5	76.7
Acid detergent fibre	35.1	35.9	41.3
Acid detergent lignin	5.11	6.40	7.76

Table 2. Intake and Total tract digestibility of dry matter, organic matter, neutral detergent fiber, acid detergent fiber and crude protein of ADL in Blackbelly rams given a 56-, 28- or a 21-old *Digitaria decumbens.*

Item	Regrowth ages (days)				Significance	
	21	28	56	s.e.m.	age	treat.
***Intake*(g/kg LW$^{0.75}$)**						
Dry Matter	85.74a	79.52 a	53.99 b	2.28	0.000	0.791
Organic matter	76.22a	72.40a	49.30b	2.06	0.000	0.789
Neutral Detergent Fiber	61.33a	57.99a	40.39b	1.84	0.000	0.749
Acid Detergent Fiber	29.65a	28.07a	22.00b	0.94	0.000	0.627
Total tract digestibility (%)						
Organic matter	69.53a	68.46a	55.85b	1.02	0.000	0.723
Neutral Detergent Fiber	72.99a	73.71a	51.61b	1.09	0.000	0.414
Acid Detergent Fiber	69.96a	70.15a	47.93b	1.02	0.000	0.134

a, b: means within the same row with different superscripts differ (P< 0.05)

Statistical analyses

The ANOVA data were analysed using the General Linear Model procedure of Minitab14 (2003) including the age of regrowth (D.F. 2) (α), the enzyme treatment (D.F. 1) (β) and the animal (D.F. 11) (δ).

$$Y_{ij} = \mu + \alpha_i + \beta_j + \delta_k + e_{ijk}$$

The interactions between treatment and age were tested. There were no significant.

The effect of periods was tested on the residues of the previous model, no significant difference was registered.

Results

Effect of exogenous enzyme

Globally, there were no significant effects of the enzyme on the major criteria analysed in this trial. The main effect is an increase of the speed of degradation of forage (*in sacco* degradability) during the first 12 hours without significant effect on the balance of digestion. Consequently superscripts in tables indicate significant difference due to the age.

Diet composition, intake, total tract digestibility and degradability

Digitaria decumbens composition is presented in Table 1. Increases of 7% and 17% were registered between the 21 and 56 day old forage for the Neutral detergent fibre and the Acid detergent fibre respectively. Moreover the content of Acid detergent lignin increased with the maturity of the forage. Likewise, the level of protein of the 56-day regrowth grass is twice lower than the 21-day.

Intake and total tract digestibility are reported in Table 2. The DM intake (g/kg $LW^{0.75}$) decreased with the maturity of the forage but the differences were no significant between the 21 and 28-day old grasses. The mean daily intake decrease is 0.91 g/day.

Table 3. Estimates parameter of kinetic of degradation (model of Orskov) dry matter, neutral detergent fiber and nitrogen of a 56-, 28- or a 21-old *Digitaria decumbens* (nylon bags) incubated in rumen of Blackbelly rams consuming a 56-, 28- or a 21-old *Digitaria decumbens.*

	Regrowth ages (days)				Significance	
	21	28	56	s.e.m.	age	treat.
DM Degradability						
Soluble fraction (%)	17.06a	16.93a	18.22a	1.01	0.533	0.000
Potentially degradable fraction (%)	61.15a	56.95b	47.29c	0.95	0.000	0.000
Degradation rate (%/h)	0.064a	0.045b	0.034c	0.002	0.000	0.552
Effective degrade. (%) k=0.04	54.57a	47.44b	40.56c	0.61	0.000	0.004
Effect. Degrad. (%) k=tradl	56.47a	52.20b	48.65c	0.59	0.000	0.016
Reference 24-hour (%) degradability of a 14-day pangola grass	69.62a	70.67a	70.39a	0.93	0.714	0.133
N Degradability						
N Soluble fraction (%)	19.81a	26.38b	26.25b	0.94	0.000	0.000
N Potentially degradable fraction (%)	65.42a	52.60b	46.19c	0.88	0.000	0.000
N Degradation rate (%/h)	0.069a	0.040b	0.040b	0.002	0.000	0.564
N Effective degrad. (%) k=0.04	61.24a	53.31b	49.83c	0.49	0.000	0.002
N Effective degrad. (%) k=tradl	63.23 a	57.78b	57.58b	0.48	0.000	0.010
NDF Degradability						
NDF Soluble fraction ()	9.51a	6.94a	14.39b	1.12	0.000	0.000
NDF Potentially degradable fraction (%)	67.50a	64.30a	49.56b	1.08	0.000	0.000
NDF Degradation rate (%/h)	0.063a	0.047b	0.029c	0.0018	0.000	0.536
NDF Effective degrad. (%) k=0.04	50.68a	41.77b	35.51c	0.67	0.000	0.004
NDF Effective degrad. (%) k=tradl	52.79 a	47.22b	44.39c	0.65	0.000	0.015

a, b, c : means within the same row with different superscripts differ (P< 0.05)

Table 4a. Polysaccharidases activities in rumen solid-associated microorganisms, in Blackbelly ram consuming a 56-, 28- or a 21-old *Digitaria decumbens.*

	Regrowth ages (days)				Significance	
	21	28	56	s.e.m.	age	treat.
Item and time after feeding	**Total activity** (µmol reducing sugar / (gDM*h))					
Carboxymethylcellulase						
0h	17.16a	44.07b	18.68a	2.54	0.000	0.734
3h	12.58a	27.42b	17.80a	3.88	0.013	0.800
Xylanase						
0h	222.5a	353.3b	203.0a	14.55	0.000	0.785
3h	162.6a	293.9b	205.4a	16.13	0.000	0.712
	Specific activity (µmol reducing sugar / (mg protein*h))					
Carboxymethylcellulase						
0h	2.51a	5.03ab	7.35b	0.95	0.003	0.183
3h	1.47a	3.21a	5.02b	0.58	0.001	0.244
Xylanase						
0h	27.60a	37.69b	57.80c	2.71	0.000	0.221
3h	18.14a	32.27b	64.93c	3.99	0.000	0.036

a, b, c: means within the same row with different superscripts differ (P< 0.05)

Table 4b. Glucosidase activities in rumen solid-associated microorganisms, in Blackbelly ram consuming a 56-, 28- or a 21-old *Digitaria decumbens.*

	Regrowth ages (days)				Significance	
	21	28	56	s.e.m.	age	treat.
Item and time after feeding	**Total activity** (µmol reducing sugar / (gDM*h))					
β-D- Xylosidase						
0h	79.54	77.05	77.28	5.22	0.927	0.593
3h	70.80	71.34	80.80	5.80	0.319	0.150
β–D-Galactosidase						
0h	23.05a	18.77a	12.21b	1.81	0.000	0.107
3h	17.59a	16.58a	12.30b	1.45	0.017	0.510
β–D-Glucosidase						
0h	61.59	61.30	64.36	3.84	0.781	0.058
3h	42.32a	56.49b	74.00c	4.06	0.000	0.701
	Specific activity (µmol reducing sugar / (mg protein*h))					
β-D- Xylosidase						
0h	11.89a	10.35a	22.26b	1.01	0.000	0.041
3h	9.29a	9.77a	26.87b	1.46	0.000	0.013
β–D-Galactosidase						
0h	3.47a	2.44b	3.43a	0.26	0.005	0.276
3h	2.33a	2.28a	4.39b	0.28	0.000	0.126
β–D-Glucosidase						
0h	9.19a	8.18a	18.74b	0.70	0.000	0.275
3h	5.64a	7.71a	25.59b	0.71	0.000	0.371

a, b, c: means within the same row with different superscripts differ (P< 0.05)

The trend observed for DM was similar to those of the dry matter components (OM, NDF, ADF).

The total tract digestibility of OM, NDF and ADF (Table 2) decreased with the maturity of the forage but the differences were no significant between the 21 and 28-day old grasses. The mean daily digestibility decrease were 0.42, 0.66 and 0.68 points per day for OM, NDF and ADF respectively. The decrease observed for total tract digestibility with the maturity of the forage is relatively low compared to the one reported for intake.

Generally, DM (or NDF) degradability per incubation time (data no shown), DM (or NDF) effective degradability, the parameters of the fitting model of Orskov (potential degradable fraction, degradation rate) decreased with maturity (Table 3). The estimates of effective degradability obtained with k=0.04 were lower than those obtained using measured turnover rate. Also, the difference between the two estimates increased in value with the forage maturity. Concerning nitrogen, similar tendencies like those registered for DM were observed.

The degradability at 24 hours of the 14-day pangola grass, using as reference, was similar when nylon bags were incubated in rumen of rams consuming pangola grass from 21- to 56-days grass of regrowth.

Enzymatic activity

Generally, irrespective of the sampling time, total polysaccharidases activities tended to remain stable with the maturity whereas the specific activity increased (Table 4a). Values of total activities with the 28-days grass seem abnormally high.

Generally, irrespective of the sampling time, total glucosidases activities tended to remain stable with the maturity except for β-D-galactosidase and β-D-glucosidase 3h after the morning meal which decreased and increased respectively with the regrowth age (Table 4b).

Table 5. Ammonia concentrations in rumen liquid of Blackbelly ram consuming a 56-, 28- or a 21-old *Digitaria decumbens.*

	Regrowth ages (days)				Significance	
	21	28	56	s.e.m.	age	treat.
Item and sampling time after feeding **NH3 (mgN / L)**						
0h	278.3a	208.8b	127.0c	7.30	0.000	0.371
3h	389.1a	233.9b	72.3c	8.23	0.000	0.661
6h	325.5a	221.0b	80.41c	8.44	0.000	0.662
12h	260.9a	228.7b	107.7c	9.75	0.000	0.691
Mean NH3	314.0a	223.2b	96.3c	5.23	0.000	0.851

a, b, c: means within the same row with different superscripts differ (P< 0.05)

Table 6. Feeding behaviour parameters in Blackbelly rams given a 56-, 28- or a 21-old *Digitaria decumbens.*

	Regrowth ages (days)				Significance	
Behaviour	21	28	56	s.e.m.	age	treat.
Eating time (min)	334.2a	382.2ab	441.5b	24.20	0.004	0.401
Ruminating time (min)	500.3a	539.1a	406.3b	23.37	0.001	0.479
Chewing time (min)	834.5	921.3	847.8	30.60	0.168	0.899
Idling time (min)	605.5	517.9	592.1	30.53	0.161	0.908
Eating index NDF (min/gNDFI)	0.292a	0.451a	0.658b	0.050	0.000	0.866
Ruminating index NDF (min/gNDFI)	0.422a	0.603b	0.593b	0.038	0.001	0.215
Chewing index NDF (min/gNDFI)	0.715a	1.054b	1.250b	0.077	0.000	0.472
Rate intake (g/h)	59.1a	56.5a	44.1b	2.83	0.000	0.187

a, b: means within the same row with different superscripts differ (P< 0.05)

Table 7. Amount of NDF (total NDF, Large particle, Small particles, and Very Small particle), ruminal turnover rate of liquid, fibrous particle (ADL) and small particle in the rumen of black belly rams 0 (min) and 3 (max) hours after the morning meal of a 56-, 28-, 21- or a 14-old *Digitaria decumbens.*

	Regrowth ages (days)				Significance	
Rumen fill	21	28	56	s.e.m.	age	treat.
NDF min (g)	629.7a	586.7a	889.7b	54.76	0.000	0.854
NDF max (g)	978.0a	926.5a	1195.7b	75.15	0.017	0.437
NDF min (g / g NDF intake)	0.642a	0.706a	0.932b	0.062	0.002	0.242
NDF max (g / g NDF intake)	0.749a	1.004a	1.515b	0.091	0.000	0.076
Large particle min (g / g NDF intake)	0.228	0.216	0.273	0.024	0.182	0.174
Small particle min (g / g NDF intake)	0.284 a	0.415 b	0.464b	0.027	0.000	0.268
Very Small particle min (g / g NDF intake)	0.130ab	0.075a	0.196b	0.022	0.002	0.642
Large particle max (g / g NDF intake)	0.314a	0.478b	0.577b	0.031	0.000	0.127
Small particle max (g / g NDF intake)	0.228 a	0.392 b	0.574c	0.035	0.000	0.150
Very Small particle max (g / g NDF intake)	0.207a	0.135a	0.363b	0.037	0.000	0.082
Lignin passage rate (%/h)	3.50a	2.80b	1.90c	0.20	0.000	0.575
Liquid passage rate (%/h)	12.67a	11.08a	7.83b	0.88	0.000	0.950
Small Particles passage rate (%/h)	7.30a	6.35ab	4.27b	0.78	0.006	0.819

a, b, c: means within the same row with different superscripts differ (P< 0.05)

Concerning the corresponding specific activities the general tendency is an increase with the maturity of the grass.

Ammonia concentrations

Ammonia concentrations in rumen liquid significantly decreased ($P<0.000$) with the age of regrowth irrespective of the sampling time (Table 5). No value was below 50 mg N/ L.

Feeding Behaviour

Time spent eating increased with the forage regrowth age whereas idling and chewing remained stable (Table 6). The indexes of intake and chewing increased significantly with the maturity of the grass. Nevertheless, concerning the chewing the differences were no significant between the 28- and 56-days of regrowth. Intake rate decreased significantly with increasing age of regrowth (Table 6).

Rumen fill and turnover rate

Generally, the rumen fill estimated before and 3 hours after the start of the morning meal increased with the maturity of the pangola grass (Table 7). Concerning the granulometry of the rumen content, the analysis of the data taking in account NDF intake, showed that generally LP and SP increased or tended to increase in the rumen with the maturity of the forage. The relative proportion of each fraction of particle doesn't vary significantly with the regrowth age.

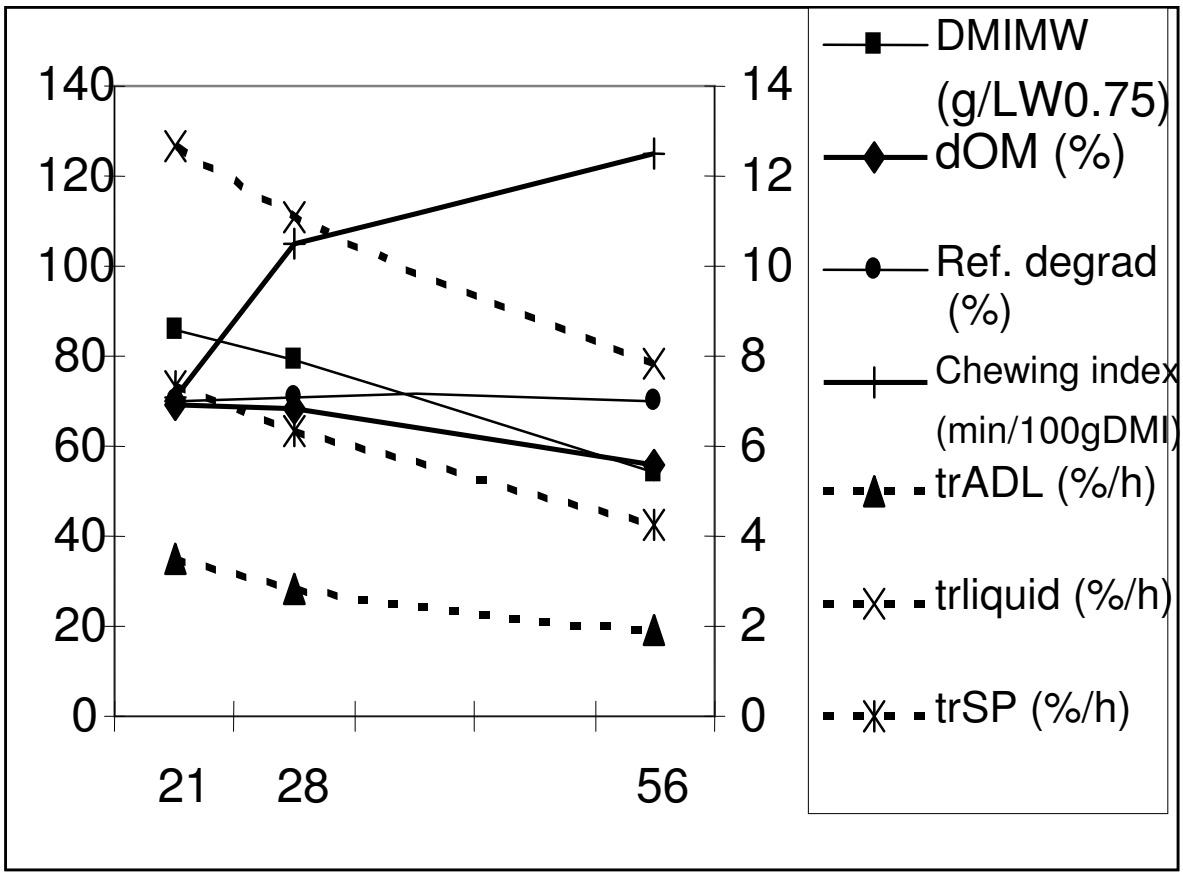
The ruminal turnover rate of fibrous particle (trADL) significantly decreased between the 21- and the 56-day old forage (Table 7). A decrease of 46% was registered between the youngest and the oldest forage. Concerning small particle (trYb) and liquid (trPEG), the mean turnover rate decreased of 42% and 38% respectively between the youngest and the oldest forage.

Table 8. Total Rumen fill (RF), large, small and very small particles of Neutral Detergent Fiber collected 1h after a first emptying in Blackbelly rams given a 56-, 28-, 21- or a 14-old *Digitaria decumbens.*

	Regrowth ages (days)				Significance	
Rumen fill	21	28	56	s.e.m.	Age	treat.
NDF RF (g/g NDFI)	0.805a	1.204b	1.260b	0.044	0.000	0.297
Large NDF particle (g/g NDFI)	0.532a	0.828b	0.798b	0.037	0.000	0.215
Small NDF particle (g/g NDFI)	0.192a	0.264 b	0.288b	0.015	0.000	0.141
Very Small NDF particle (g/g NDFI)	0.081a	0.091ab	0.173b	0.026	0.014	0.353

a, b: means within the same row with different superscripts differ (P< 0.05)

Figure 1. Effect of regrowth age (days) on intake, total tract digestibility, cellulolysis (nylon bag degradation of a reference forage) chewing index, rumen mean transit time of liquid, fibrous particle and small particle in rams consuming a 56-, 28- or 21-old *Digitaria decumbens.*



Impact of the ingestive mastication

The granulometry of the biomass in the rumen, emptying after 1-hour meal did not vary significantly with the forage maturity (Table 8). Nevertheless the ratio between forage ingested and the biomass collected in the rumen increased with the maturity of the grass illustrating that a fraction of the ingested forage moved out the rumen as small or very small particles.

Correlation between intake and digestive parameters

The figure 1 illustrates relationships between intake, total tract digestion of OM and main digestive parameters. Intake and total tract OM digestibility were highly correlated (R=0.842; P=0.000). Moreover, intake was highly correlated with parameters illustrating the physical degradation (rumen turnover of adl (R=0.763; P=0.000), indexes of intake (R=-0.753; P=0.000) and mastication (R=-0.716; P=0.000)) and the forage fill (potential degradable fraction (R=0.773; P=0.000), degradation rate (R=0.534; P=0.001)). Moreover the rumen turn over of adl is also highly correlated with the index of mastication (R=-0.795; P=0.000). Low correlations are reported between turnover rate of small particle and physical degradation of the forage (potential degradable fraction (R=0.539; P=0.002), degradation rate (R=0.494; P=0.006)).

Discussion

General observation

The main objective of this trial was to study the impact of cellulolysis and physical breakdown of forage particles on intake of tropical grass. These two biological mechanisms are the main factors explaining intake and digestion of forages. The main result of this experiment is, taken into account a large gradient of forage quality (intake, digestibility) there

is no significant variation of cellulolytic activities in the rumen whereas large variations are reported in physical degradation of the experimental grasses.

In this experiment, we used stage of regrowth age within bounds larger than those classically studied (<28-old forage) to create variability in forage composition, intake and digestibility representative to those observed with the tropical forages. Our results are representative of this variability. The observed chemical composition of pangola grass which shows a decrease in crude protein content and an increase in the cell wall content with increasing age of the regrowth are in agreement with previous results (Minson, 1990). The decreases in intake and digestibility with the increasing of regrowth age reported in this study are also similar to previous observations (Chenost, 1975; Ichinohe *et al.*, 1995; Archimede *et al*., 2000; Arthington and Brown, 2005). However, the rate of decrease (0.9 g/kgLW$^{0.75}$/day of regrowth) recorded for intake is higher than the mean rate (0.17 g/kgLW$^{0.75}$/day of regrowth) reported for tropical forages (Minson, 1990). The large experimental range of stage of growth taking into account very young grass explains this discrepancy between our results and the literature. This result underlines the necessity to valorise tropical grass at young stage of regrowth. Like the result for intake, the decrease of total tract digestibility with the forage maturity is in accordance with previous studies (Aumont *et al.*, 1995, Eugène 2002). The mean rate of fall in dry matter total tract digestibility (0.42 points) is higher to the one (0.22 points) registered by Archimede *et al*. (2000) with pangola under similar conditions. The range of decrease in dry matter total tract digestibility between 21 to 56 day is low relative to intake (15 versus 46%) illustrating the major role of intake in variation of nutritive value of grass and the need to maximize this value. The forage physical fill also increased with maturity like illustrated with the decrease its potential digestible fraction and degradation rate (Madsen *et al.,* 1994).

Evolution of enzymatic activities in the rumen with maturity of forage

We added an efficient exogenous fibrolytic enzymes (EFE) (Assoumaya et al xxxx) to be overcome interaction between physical and enzymatic degradation postulating that availability of fibrolytic enzyme in the rumen could be the first limiting factor of intake and digestion. The lack of positive effect of EFE on intake, total tract digestibility and other indicators like forage load estimated as the rumen turnover rate of fibrous particle is a first indication that availability of fibrolytic enzyme in the rumen should not be the first limiting factor of intake and digestion. Eugene et al., 2004b, hypothesised that with mature fertilized forage the cellulolytic capacity in the rumen (amount of microbial enzymes) were not the first limiting factor in the digestion of old forage but rather the range of colonisation of feed particle by microbes. Really, EFE (or microbial enzyme) can improve the rate of feed digestion but their ability to increase the extent of digestion may be limited by a lack of the enzyme that breaks down the core structure of lignin-cellulosic complex (Wang and McAllister, 2002). The accessibility of forage digestible tissues for microbial digestion necessitate breakdown of fibrous particle via rumination (McLeod and Minson, 1988; McLeod *et al.*, 1990).

The global (enzymatic activities) and indirect method (degradability of the reference 14-day grass) of measurement of cellulolytic activities gave similar results. Like most published trials, estimates of enzymatic activities indicated some variabilities linked to the method (Eugène, 2002). Notwithstanding, the most important result in this experiment is increase or stable enzymatic activities with the maturation of the forage. Similar trends were reported by Eugène (2002) and Assoumaya (unpublished) on *Digitaria decumbens* and *Panicum maximum* respectively growing from 14 to 56 days of age. These results suggest that microbial activities in the rumen may not be the first limiting factor of digestion in ruminants with tropical forage. This hypothesis is in agreement with results of Eugène *et al.* (2004) on

the changes in bacteria population in the rumen with maturity of the forage. Generally, the amounts of total and cellulolytic bacterial 16S rRNA remain stable with forage between 28 and 56-days of regrowth, and *F. succinogenes,* considered as the most efficient against recalcitrant lignocellulosic substrates, was the dominant species. In addition, the proportion of *F. succinogenes* increased with the forage maturity. The increase of the specific enzymatic activities observed in the present experiment could illustrate a modification of the microbial species in the rumen population with presence of more active bacteria. Moreover, the increase of the transit time of fibrous particle in the rumen with the maturity of the forage could be an advantage for the microbial digestion. Practically, this means that old forage stay longer time in the rumen so fibrolytic activity of enzyme is most efficient. The differences between estimates of effective degradation using k=0.04 or kpadl, and the low decrease of effective degradation estimated with kpadl illustrate this idea.

The level of ammonia is always higher than 50 mg /l indicates that this was never a limiting factor for microbial growth in the rumen whatever the regrowth age. Indeed, Satter and Roffer (1975), suggested a reduced rumen microbial growth and activity if the rumen ammonia drops below 50 mg/l.

Evolution of physical degradation of grass with its maturity

The high correlations between intake and rumen fill in one hand and the rumen turnover rate in the other hand illustrate that physical constraints of digestion should be the driving force of intake. Generally, voluntary intake of low quality forage is considered to be limited by the fill of dry matter and the clearance from the reticulo-rumen (Forbes, 1994; Mc Leod *et al.*, 1990). Digesta can leave the reticulo-rumen when most of the particles have been reduce below a critical size (Poppi *et al.,* 1985) mainly by chewing (McLeod and Minson, 1988).

The amount of NDF in the rumen at the end of the meal slowly varied relatively to intake of NDF. It could be hypothesized that irrespective of the stage of regrowth of the grass, the animals maximized the rumen fill. Moreover, analysing our results concerning animal behaviour, it could also be hypothesized that irrespective of the stage of regrowth of the grass, the animals maximized the time spent eating and ruminating whereas the amount of dry matter ingested or masticated decreased with increasing regrowth age. Really, the total times of chewing are close to the maximum values reported in the literature (Jarrige *et al.*, 1995). Consequently, the differences observed in forage intake have to be mainly explained by the rumen turnover rate. Moreover, the index of chewing (the driving force of particle reduction size) is highly and negatively correlated with intake. The rumen turnover rate decreased with the forage physical fill which increased the forage maturation. Nevertheless, the forage physical fill is only partly explained by the rate of particle reduction. In one hand, the decrease of the intake rate, the increase of the eating and chewing indexes, are consequences of increasing resistance of cell wall to mastication with the maturity of the forage. The results of the rumen pool and its granulometry are also informative on the constraint of the physical degradation of the grass. The increasing ADF/NDF ratio of the pangola grass observed with its maturity could illustrate chemical and physical modification explaining this increasing resistance of cell wall to mastication. Other anatomical modifications of the forage cell wall have to be taken into account also (Wilson, 1994). In the other hand, the decrease of Small particle and liquid turnover rate with the maturity can not be explained with the size of the particle. SP and VS are the size to left the rumen. Kennedy (1995) indicated that the likely major constraint to intake of C4 grasses is slow passage of small particle from rumen. He hypothesises that slow particle may result from properties of size and buoyancy which impede movement from the rumen. The lower content in C4 plants of thin-walled tissues with the greater length and volume of vascular bundles in leaves compared with C3 plants may give

rise to a larger size of particle in the small pool. The buoyancy characteristics of small C3 particles may more closely mach the optimum required for passage than for C4 particle of the same size.

Conclusions

Our results indicate that with matured pangola grass, cellulolytic activities are not the main limiting factor of digestion. Consequently, explanations have to be found in mechanisms linked to physical degradation of grass. The rate of reduction size of particle via chewing is a major constraint. Nevertheless, when fibrous particles are theoretically the size to left the rumen, physical properties of C4 grass could limit movement of small particles and consequently the rumen turnover and intake.

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DISCUSSION GÉNÉRALE

- CONCLUSIONS

L'objectif de l'étude était d'analyser l'impact relatif des phénomènes physiques, biologiques et biochimiques sur le déterminisme de l'ingestion des fourrages tropicaux.
Nous avons fait les hypothèses suivantes que nous nous proposions de tester: 1) valorisés à des stades physiologiques assez précoces, l'ingestion et la digestion des fourrages tropicaux pouvaient être significativement améliorées, 2) la capacité cellulolytique du rumen ne serait pas le premier facteur limitant l'ingestion et la digestion des fourrages, 3) la résistance à la mastication et en conséquence la vitesse de réduction de taille des particules alimentaires (comminution) serait le premier facteur limitant l'ingestion.

Une méta-analyse des résultats de données bibliographiques nous a permis dans un premier temps de dégager certains résultats originaux permettant de préciser les hypothèses de travail à tester. Dans un deuxième temps, des expérimentations ont permis d'améliorer nos connaissances sur les dynamiques digestives induites par l'ingestion des fourrages tropicaux.
Afin de conduire la discussion générale nous avons compilé les données des différents essais plutôt que de reprendre les essais un à un. Dans certains cas, nous avons travaillé avec les moyennes des traitements par essai. Dans d'autres cas, afin d'analyser la variabilité individuelle, nous avons travaillé avec les données individuelles. Les analyses statistiques ont été conduites en prenant ou non en compte l'effet essai. Ce dernier a logiquement amélioré la précision du modèle sans changer les tendances statistiques et les valeurs des coefficients des droites de prédiction, obtenues avec les valeurs moyennes. Les équations accompagnant les différents figures ne prennent pas en compte l'effet essai. Pour ce qui est des analyses conduites sur les données individuelles, la prise en compte de l'effet a généralement confirmé les tendances significatives. Cependant, lorsque nous étions à la limite des seuils de signification, la valeur des coefficients a été significativement modifiée. Dans certains cas, la répartition des données n'a pas permis de prendre en compte l'effet essai.

1. Contraintes et limites du travail de thèse

Nous avons du faire face concrêtement aux contraintes climatiques (alternance de sécheresse et importante pluviométrie) qui ont impacté sur la disponibilité du fourrage et la qualité de la repousse. De plus, le pangola a subi un épisode parasitaire (attaque de champignons) qui nous a contraint à avoir des expérimentations relativement longues par rapport aux programmations initiales et peut être éventuellement une confusion d'effet période et âge de repousse notamment dans l'expérimentation 6.

Les travaux expérimentaux sur la dynamique digestive imposent de travailler avec des animaux canulés du rumen et du duodénum. La variabilité de mesures liée aux contenus digestifs et à leurs flux est plus importante (celle de l'animal plus celle de la méthode) que celle qui peut être rencontrée dans des études classiques de digestibilité sur l'ensemble du tube digestif. Pour réduire la variabilité expérimentale, et mieux contrôler la variabilité individuelle en particulier, il aurait été souhaitable de travailler avec un dispositif carré latin. Ce dispositif, bien que séduisant sur le principe, présentait un grand risque expérimental, surtout avec les longues périodes de mesures imposées aux animaux. Ces animaux canulés sont fragiles et la probabilité de cumuler beaucoup de données manquantes était donc élevée et nous avons donc adopté des dispositifs plus « sécurisés ».

Suite aux résultats de l'expérimentation 2, nous avons choisi de travailler, contrairement à la plupart des travaux, dans des conditions de ''non équilibre'' pour analyser les dynamiques digestives. La multiplication du nombre de repas nécessaire pour atteindre les conditions d'équilibre n'est pas représentative des conditions réelles d'élevage et aboutit à des dynamiques digestives différentes. Ainsi, la digestibilité du NDF a diminué avec l'augmentation de la fréquence des repas principalement avec un vieux fourrage. De plus, le volume du contenu ruminal diminuait avec l'augmentation de la fréquence des repas. Une accumulation des petites et fines particules était observée dans le rumen des animaux ingérant deux repas (projet publication 3).

Les études sur les dynamiques digestives sont lourdes aussi, nous avons souhaité simplifier les mesures en travaillant sur une seule demi-journée (plutôt que sur 24 heures) avec des animaux, placés en condition d'éclairage permanent et recevant 2 repas par jours espacés de 12 heures. Les résultats de l'expérimentation 3 ne nous ont pas autorisés à simplifier les protocoles de mesures et prélèvements. En effet, les quantités ingérées et la biomasse ruminale tendent à être plus élevées dans la période diurne comparativement à la période nocturne. Les index de rumination et de mastication sont plus importants durant la période nocturne. De même, la granulométrie du rumen est plus grossière juste avant le repas du soir comparativement à celle du matin (projet publication 4).

Nous avons aussi dû faire des choix méthodologiques qui peuvent être contestables, cependant nous estimons que, pour l'essentiel, les comparaisons relatives gardent leur valeur.

Nous avons fait le choix d'apporter des enzymes exogènes fibrolytiques (EEF) dans le rumen afin de travailler en conditions enzymatiques non limitantes pour mieux apprécier l'impact des facteurs physiques tout en sachant que les EEF n'avaient pas la diversité d'actions de la population microbienne ruminale. Cependant, au cours de la première expérimentation, nous

avons démontré que l'apport d'enzymes exogènes était efficace sur les fourrages tropicaux (publication 2), comme déjà démontré pour la paille et les fourrages tempérés. Ainsi, le taux de dégradation, étudié par la méthode des sachets, était plus élevé en présence d'enzymes exogènes.

A propos de **la réduction physique de taille des particules (comminution),** nous avons évalué le travail masticatoire par le temps de mastication (ingestion + rumination) d'un kg de MS d'aliment ingéré. Le découplage du temps d'ingestion en temps de préhension et temps de mastication ingestive aurait permis de gagner en précision. Cette volonté a été à l'origine de l'utilisation de l'appareil de comportement de RUTTER au cours d'expérimentations préalables (projet de publication 3). Toutefois, nous avons été contraint de recourir aux observations visuelles vu les nombreux biais induits (diminution de l'ingestion) par la pose de l'appareil, même après une longue période d'adaptation.

L'analyse de la cinétique d'évolution de la granulométrie des particules alimentaires dans le rumen a aussi été réalisée. La réduction de taille des particules induite par la mastication ingestive a été obtenue par le prélèvement du contenu ruminal 30 minutes après le début de l'ingestion sur des animaux préalablement vidés. Cette méthode a été préférée à l'utilisation d'animaux canulés de l'œsophage classiquement utilisés pour ce genre de mesure, afin de limiter le nombre de canules posées sur les animaux.

L'activité et la capacité cellulolytique du rumen ont été étudiées par différentes méthodes car il n'existe pas de méthode de référence. Des sachets de nylon renfermant une matière première de référence (témoin) ont été mis à incuber dans le rumen. La limite de cette méthode est que le contenu du sachet n'est pas un témoin parfait des conditions physico-chimiques du rumen. Par ailleurs, le bilan de la dégradation du témoin est le reflet de l'activité cellulolytique d'une part, et de la propre résistance du témoin à la dégradation, d'autre part. L'appréciation de l'activité cellulolytique du rumen *via* le dosage des activités enzymatiques est aussi discutable car l'interprétation est encore moins aisée que celle des sachets. Le bilan des activités enzymatiques microbiennes est complexe et la dégradation des différents polymères en composés simples nécessite l'activité d'une multitude d'enzymes hydrolysant les chaînes de polymères ainsi que les liaisons des polymères entre eux. Ainsi l'hydrolyse complète de la cellulose en monomères requiert l'action synergique: 1) d'endoglucanases hydrolysant au hasard les liaisons situées à l'intérieur des chaînes ; 2)d'exoglucanases libérant du cellobiose dimère de glucose à partir d'une extrémité de chaînes ; 3) d'oligosacharidases et de β-glucosidases scindant le cellobiose. Pour les hémicelluloses, deuxième composante des

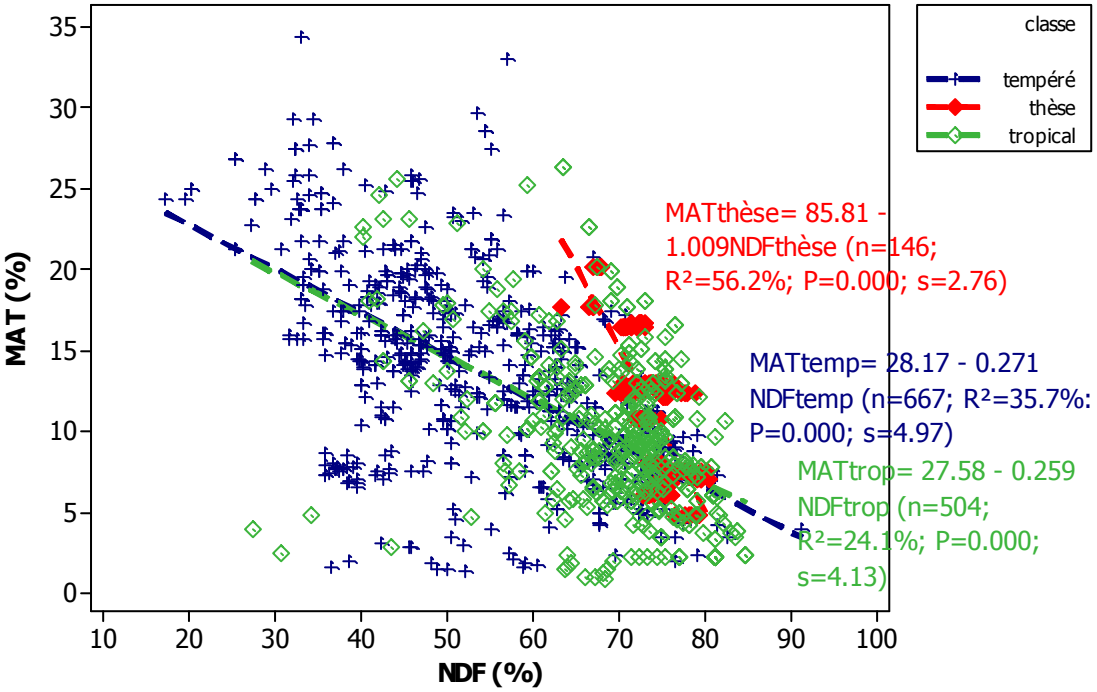
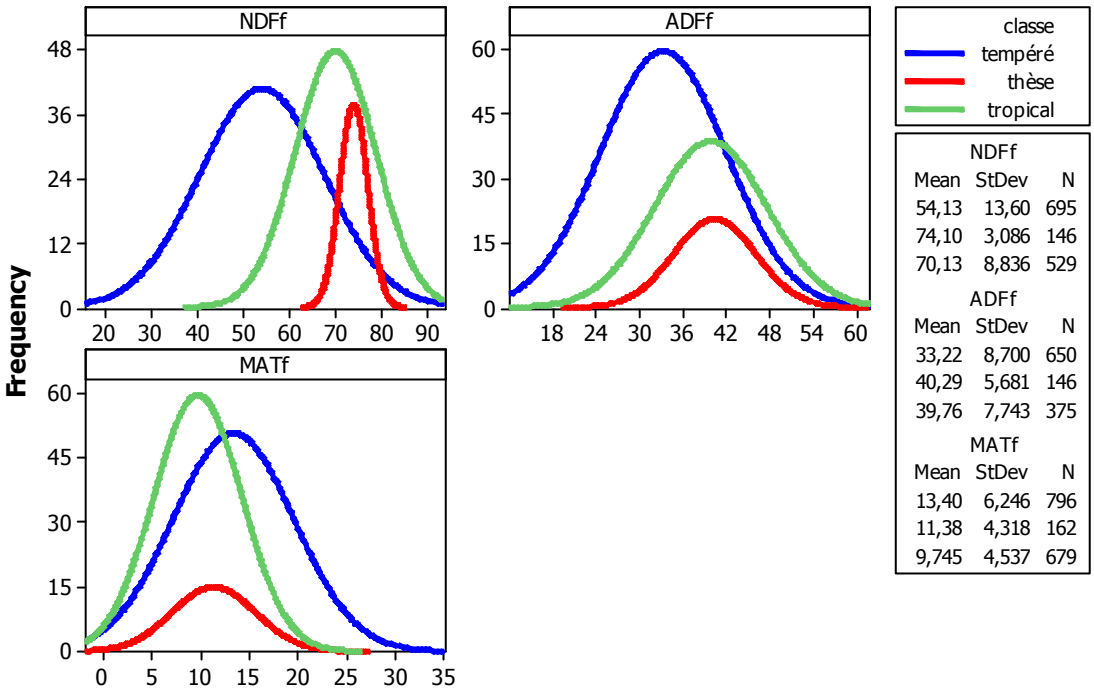


Figure 1. Evolution de la teneur en MAT en fonction de celle en NDF des différentes classes de fourrages (tempérée, tropicale ou « tropicale thèse »).



Figures 2a,b,c. Dispersion des teneurs en NDF, ADF et MAT en fonction de la classe de fourrages (tempérée, tropicale ou « thèse »).

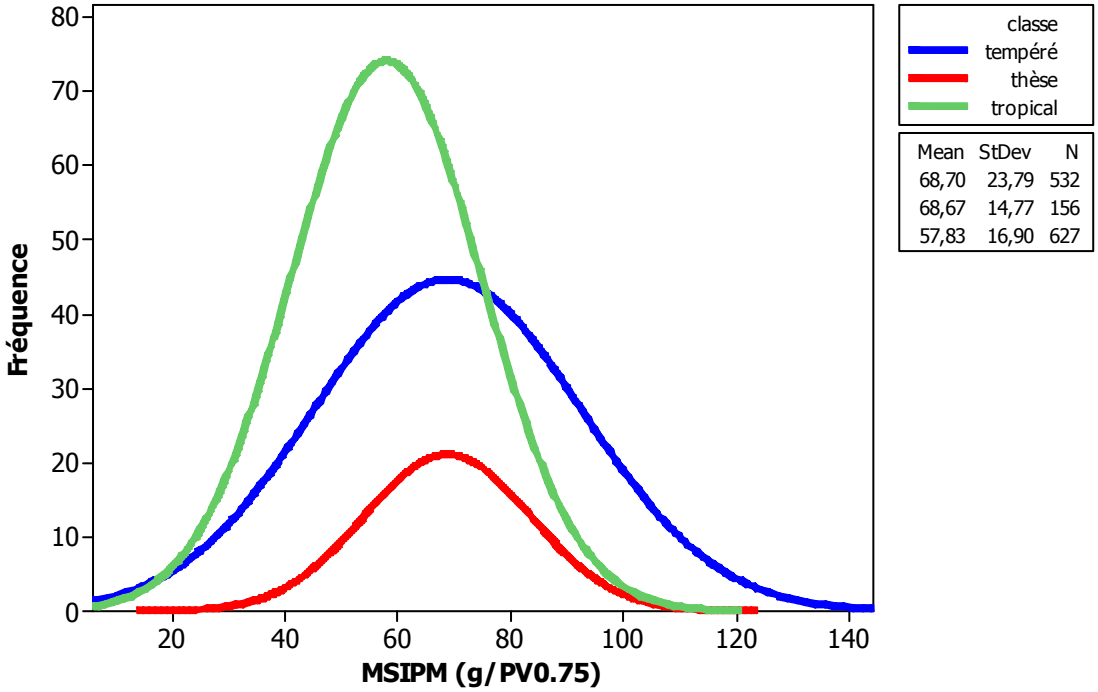


Figure 3. Dispersion des quantités de matières sèches ingérées (MSIPM) en fonction de la classe de fourrages (tempérée, tropicale, « thèse »).

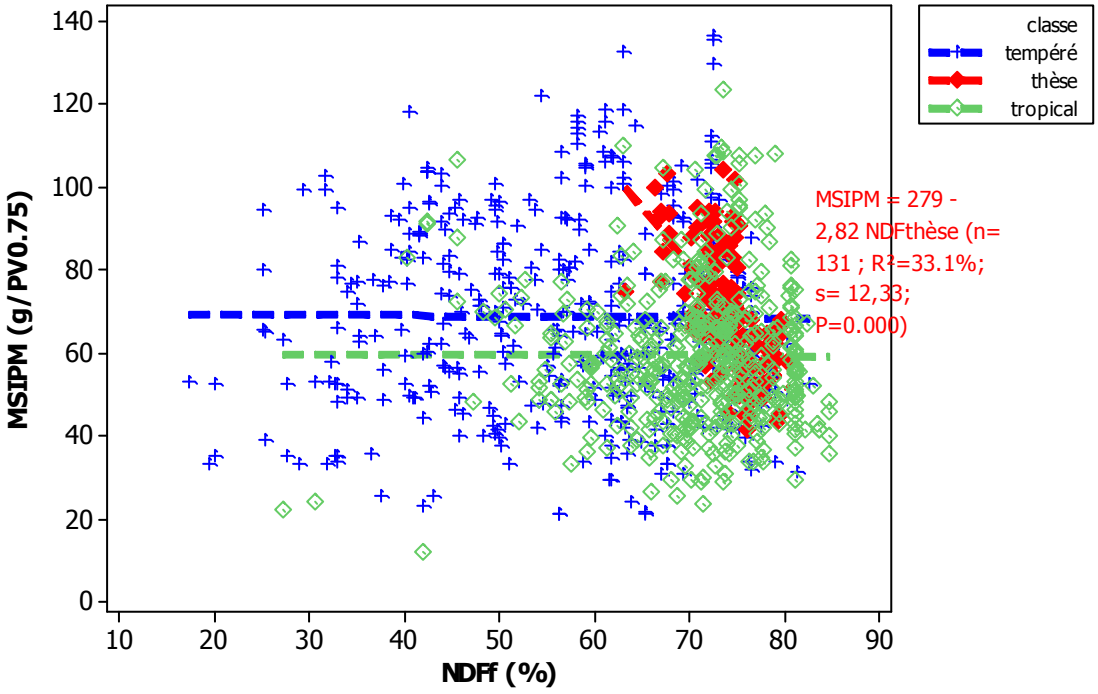


Figure 4a. Evolution des quantités ingérées (MSIPM) en fonction de la teneur en NDF des différentes classes de fourrages (tempérée, tropicale, « thèse »).

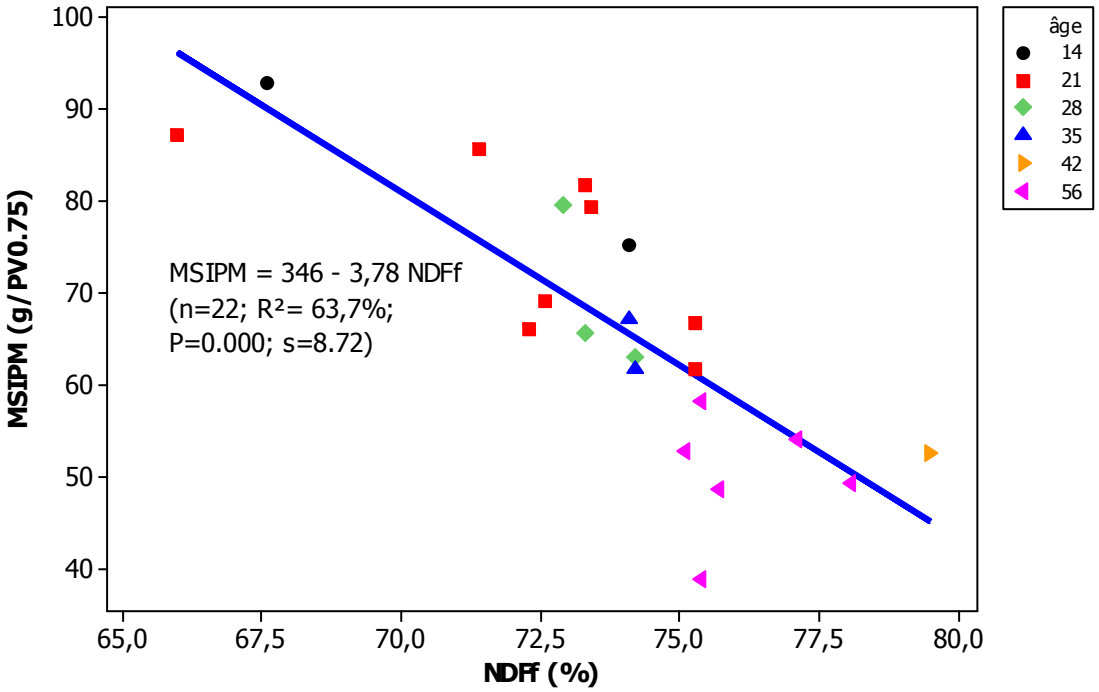


Figure 4b. Evolution des quantités ingérées (MSIPM) moyennes observées au cours de la thèse en fonction de la teneur en NDF du fourrage.

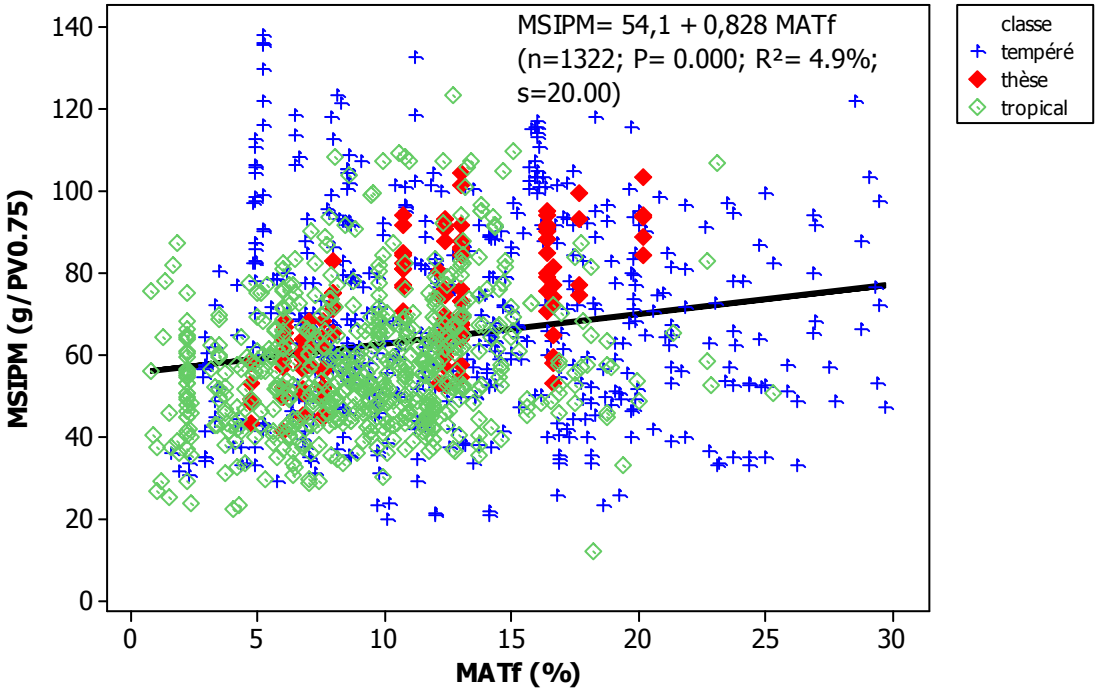


Figure 5a. Evolution des quantités ingérées (MSIPM) en fonction de la teneur en azote (MAT) des différentes classes de fourrages (tempérée, tropicale, « thèse »).

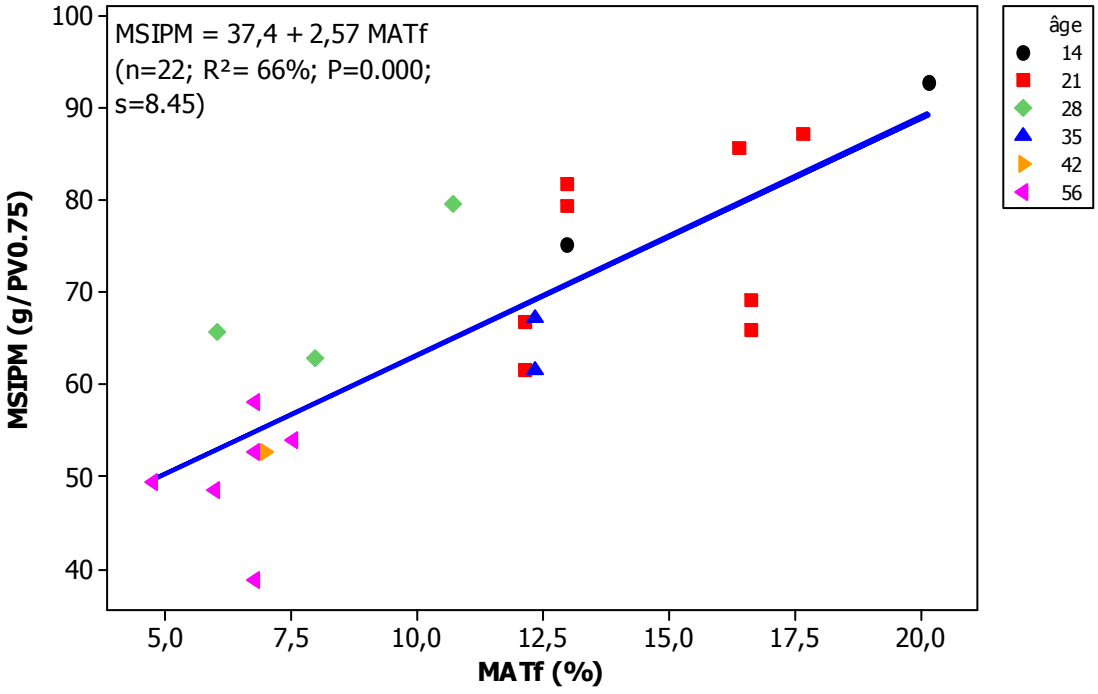


Figure 5b. Evolution des quantités ingérées (MSIPM) moyennes observées au cours de la thèse en fonction de la teneur en MAT du fourrage.

parois cellulaires, des enzymes de type endoxylanases, exoxylanases et xylosidases ainsi que des enzymes débranchantes de type estérases sont nécessaires. Ainsi un bilan des activités enzymatiques nécessiterait d'une part, la prise en compte de toutes ces enzymes, d'autre part, il faudrait que nous puissions hiérarchiser une action par rapport à une autre ce qui est difficile dans l'état actuel des connaissances. Par exemple, il est connu que l'action des estérases est très importante car elle permet de rompre les liaisons entre lignines et hémicelluloses, rendant ces dernières accessibles aux xylanases et augmente la digestibilité des parois végétales.

2. Représentativité des données de la thèse par rapport aux données tropicales

2.1. Composition chimique

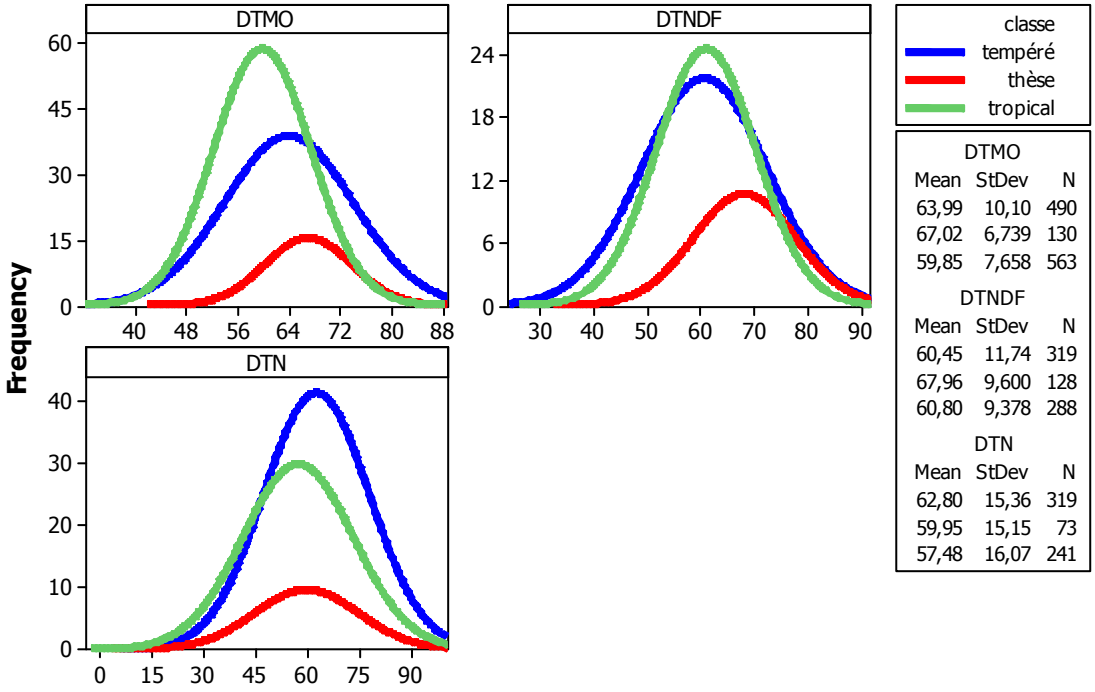
La figure 1 situe les teneurs en MAT et NDF obtenues au cours de la thèse vis-à-vis des références tropicales et tempérées, qui ont fait l'objet de l'étude bibliographique (projet article n°1). La compilation des différents essais expérimentaux a permis d'obtenir une population de fourrages représentative de la diversité tropicale. Les teneurs des différents composés chimiques sont proches de celles enregistrées pour les fourrages tropicaux (figures 2a, 2b et 2c). Cependant, les teneurs en protéines brutes des fourrages expérimentaux sont en moyenne plus élevées que celles des fourrages tropicaux à cause probablement des niveaux élevés de fertilisants que nous avons utilisé. La teneur moyenne en NDF des fourrages expérimentaux est aussi plus élevée et moins étendue que celle des fourrages tropicaux de la littérature, en dépit de la large gamme de variation des âges de repousse.

Nous confirmons par ailleurs que la population de fourrages expérimentaux, tout comme celle des fourrages tropicaux, se singularise des fourrages tempérés par des teneurs en NDF plus élevées et en azote en moyenne plus faible.

2.2. Ingestibilité

Nos données expérimentales concernant les quantités moyennes de matière sèche ingérée (g/ $P^{0.75}$) sont supérieures à celles généralement rapportées pour la zone tropicale, elles sont comparables à celles enregistrées en zone tempérée (figure 3). Ces résultats s'expliqueraient à priori par la composition de notre population expérimentale plus riche en jeunes fourrages (< 28jours d'âge de repousse) quelle celle de la bibliographie tropicale.

Nos données répondent aux lois générales connues de prédiction de l'ingestibilité par les teneurs en NDF et MAT du fourrage (4a, 4b et 5a, 5b). Cependant, par rapport à l'ensemble des données, les pentes que nous observons sont bien plus abruptes.



Figures 6 a, b et c. Evolutions des digestibilités de Matière Organique, de NDF et d'azote en fonction des différentes classes de fourrages (tempérées, tropicales, « thèse »).

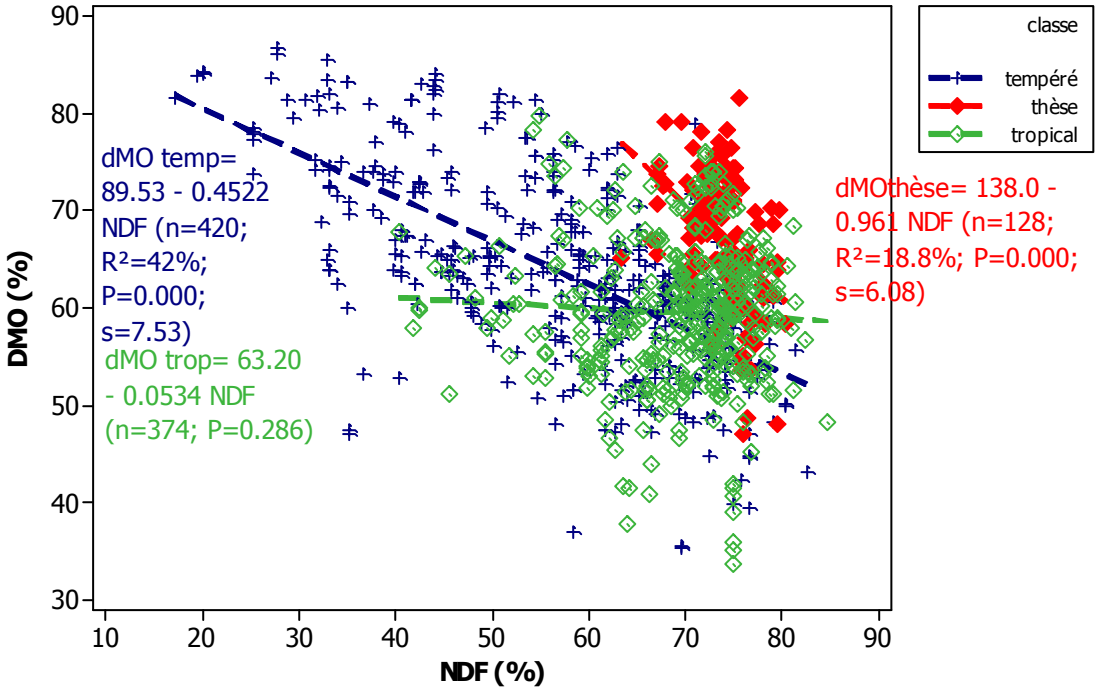


Figure 7a. Variation de la digestibilité totale du tractus digestif exprimée en MO avec la teneur en NDF des différentes classes de fourrage (temperée, tropicale, « tropicale thèse »).

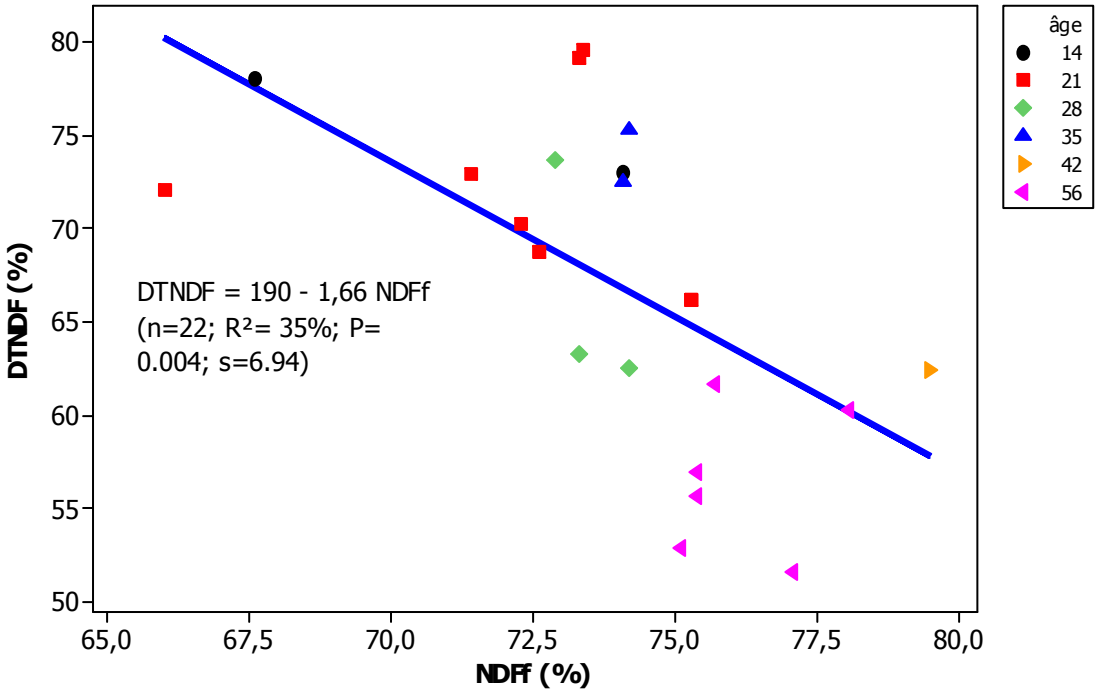


Figure 7b. Evolution des digestibilités totales moyennes exprimées en NDF (DNDF) observée au cours de la thèse en fonction de la teneur en NDF du fourrage.

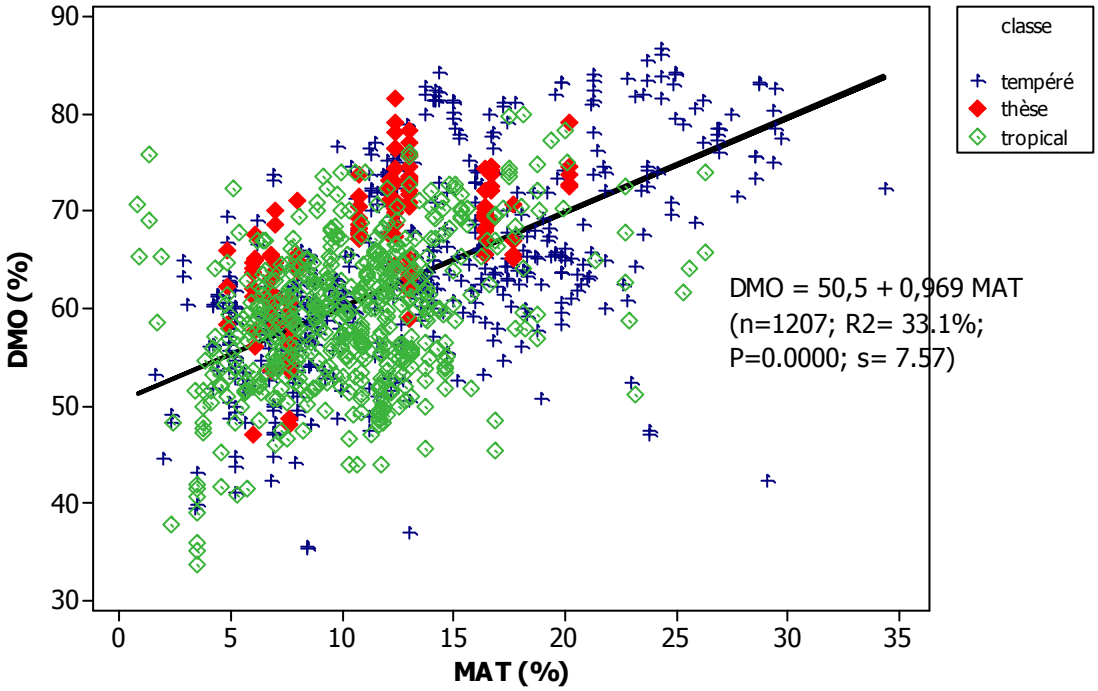


Figure 8a. Variation de la digestibilité totale du tractus digestif exprimée en MO avec la teneur en MAT des différentes classes de fourrage (tempérée, tropicale, « tropicale thèse »).

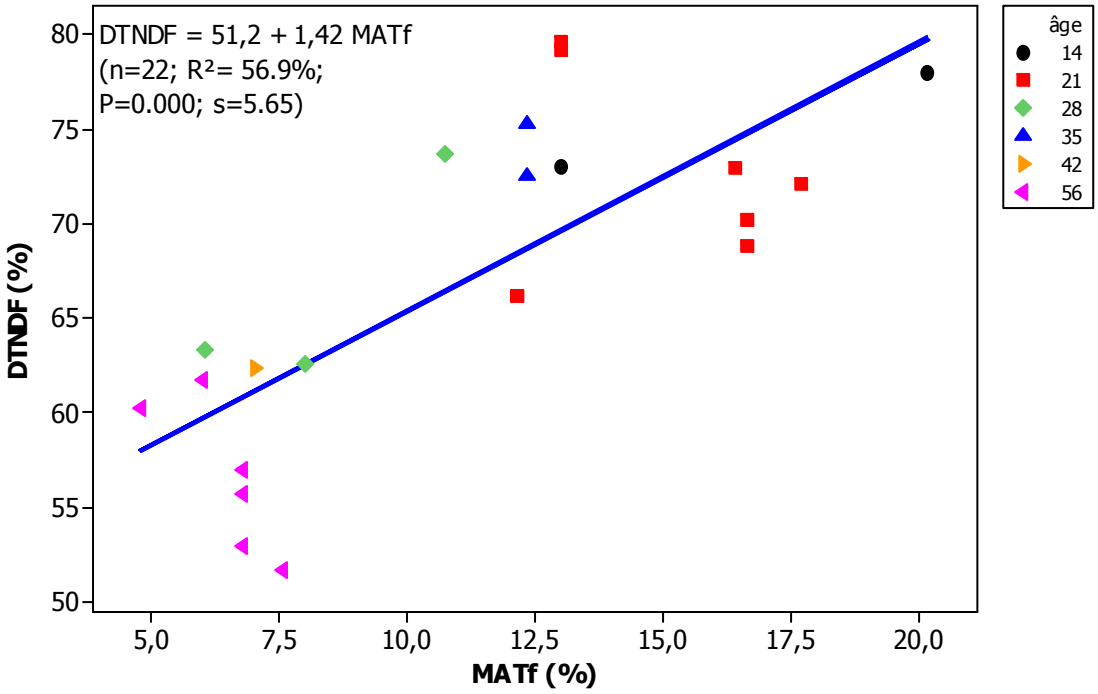


Figure 8b. Evolution des digestibilités totales moyennes exprimées en NDF (DNDF) observées au cours de la thèse en fonction de la teneur en MAT du fourrage.

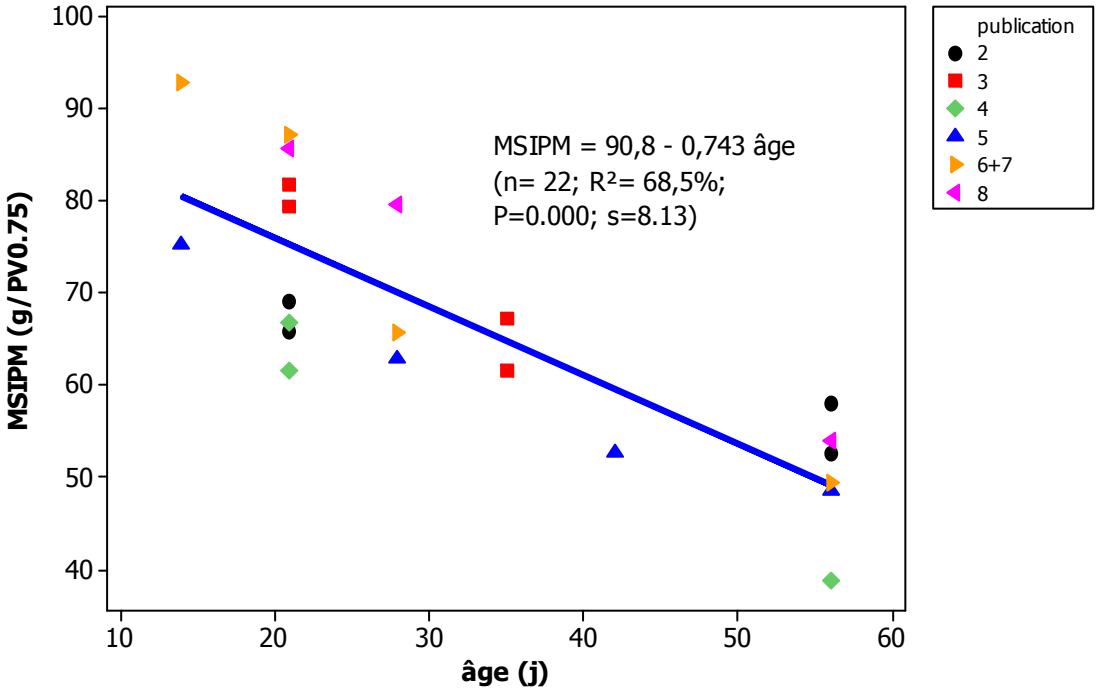


Figure 9. Evolution des quantités ingérées moyennes exprimées en MS (MSIPM) observées au cours de la thèse en fonction de l'âge de repousse du fourrage.

Tableau 1 . Synthèse des principaux résultats obtenus au cours des différentes expérimentations.

Publi-cation	âge	traitement	NDFf	MSIPM	DNDF	Degrad témoin	Id degrad temoin	BD Xylosidase-AS	Index ingestion MS	Index de rumination MS	Index de mastication MS	B	C	Id CRNDFmin	Id CRNDFmax	trADL
2	21	Enzyme -	72,6	69,09	68,8	*	*	*	*	*	*	*	*	*	*	*
2	21	Enzyme +	72,3	65,96	70,2	*	*	*	*	*	*	*	*	*	*	*
2	56	Enzyme -	75,4	58,1	55,7	*	*	*	*	*	*	*	*	*	*	*
2	56	Enzyme +	75,1	52,72	52,9	*	*	*	*	*	*	*	*	*	*	*
3	21	4 repas	73,3	81,8	79,2	*	*	*	0,25	0,29	0,54	*	*	*	0,87	3,9
3	21	2 repas	73,4	79,3	79,6	67,8	81,54	*	0,27	0,31	0,58	*	*	*	1,12	3,3
3	35	4 repas	74,1	67,2	72,5	*	*	*	0,29	0,36	0,65	*	*	*	0,76	4,3
3	35	2 repas	74,2	61,6	75,3	62,71	75,42	*	0,34	0,34	0,67	*	*	*	1,13	2,9
4	21	haché	75,3	66,74	66,18	*	*	*	0,22	0,41	0,63	*	*	0,76	0,97	4,6
4	56	haché	75,4	38,72	56,95	*	*	*	0,57	0,68	1,27	*	*	1,28	1,54	2,7
4	21	long	75,3	61,63	66,18	*	*	*	0,41	0,39	0,8	*	*	0,69	0,96	3,7
5	14	*	74,1	75,2	73,09	*	*	*	*	*	*	77,36	0,073	0,36	0,82	*
5	28	*	74,2	62,9	62,56	*	*	*	*	*	*	58,86	0,054	0,54	0,85	*
5	42	*	79,5	52,6	62,4	*	*	*	*	*	*	48,23	0,045	0,86	1,00	*
5	56	*	75,7	48,5	61,7	*	*	*	*	*	*	43,81	0,034	0,92	1,34	*
6+7	14	*	67,6	92,8	78,08	81,54	81,54	13,1	0,33	0,42	0,75	64,98	0,068	0,62	0,77	2,8
6+7	21	*	66	87,16	72,09	82,08	82,08	41,15	0,34	0,42	0,76	67,99	0,09	0,78	0,93	2,7
6+7	28	*	73,3	65,7	63,31	81,74	81,74	20,7	0,38	0,48	0,86	59,35	0,052	0,84	1,01	2,4
6+7	56	*	78,1	49,32	60,24	77,19	77,19	24,2	0,49	0,64	1,13	47,94	0,029	1,06	1,55	1
8	21	*	71,4	85,74	72,99	69,62	81,54	10,59	0,19	0,29	0,48	61,15	0,064	0,64	0,75	3,5
8	28	*	72,9	79,52	73,71	70,67	82,77	10,06	0,25	0,37	0,62	56,95	0,045	0,71	1,00	2,8
8	56	*	77,1	53,99	51,61	70,39	82,44	24,56	0,5	0,46	0,96	47,29	0,034	0,93	1,52	1,9

2.3. Digestibilité

Les observations relatives à l'ingestibilité des fourrages se retrouvent pour la digestibilité et nos explications sont les mêmes. Nos données expérimentales de digestibilité (MO, NDF) sont en moyenne plus élevées que celles enregistrées en zone tropicale ainsi qu'en zone tempérée (figures 6a et 6b). La digestibilité de la MAT est comparable à celle de la population tropicale de référence et inférieure à celle des fourrages tempérés (figure 6c).

Nos données répondent dans le même sens que les lois générales connues de prédiction de la digestibilité par les teneurs en NDF et MAT du fourrage (figures 7 a, 7b et 8a, 8b). Pour le NDF, notre pente moyenne est plus abrupte que pour l'ensemble des données de la littérature. En fait, il faudrait ne pouvoir se comparer qu'aux essais portant sur le vieillissement du fourrage. Pour l'influence de la teneur en MAT, l'ensemble de nos points se répartissent dans ceux de la littérature, par contre nos pentes sont nettement supérieures lorsque l'on se place en intra-publication.

3. Discussion des hypothèses de la thèse :

3.1. L'ingestion et la digestion des fourrages tropicaux sont significativement améliorées à des stades physiologiques assez précoces

Le tableau 1 résume les principales données de la thèse. L'ingestion du fourrage a varié de 38 à 92 g de MS / kg $P^{0.75}$. La digestibilité de NDF a varié de 0.51 à 0.79. L'encombrement du fourrage a donc varié de 0.8 à 2 sur la base d'un encombrement égal à 1 pour l'herbe de référence ingérée par le mouton à raison de 75 g / $P^{0.75}$. Si l'on prend en compte la variabilité observée pour la digestibilité de la matière organique du fourrage, 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 a varié de 0.38 à 0.98 UF (nous avons utilisé les UF breirem pour simplifier les calculs, nous sommes conscient qu'elles sont plus sensibles que les UFV à la fraction non digestible de la MO). Ainsi, il apparaît que les fourrages tropicaux peuvent être de très bonne qualité.

Le stade physiologique, donc l'âge de repousse a eu un effet déterminant sur la valeur alimentaire comme l'illustre les prédictions de l'ingestibilité par l'âge de repousse (figure 9), comme évoqué par de nombreux auteurs (Chenost, 1975; Ichinohe *et al.*, 1995; Arthington and Brown, 2005). Ces résultats vont dans le même sens que des données antérieures,

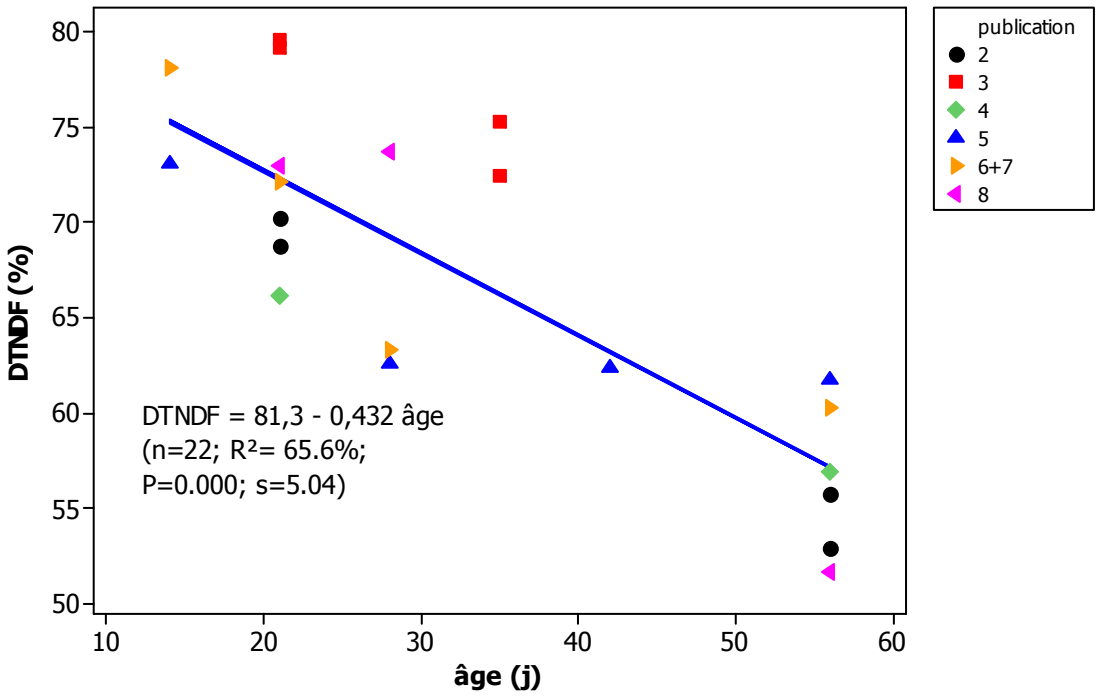


Figure 10. Evolution des digestibilités totales moyennes exprimées en NDF (DNDF) observées au cours de la thèse en fonction de l'âge de repousse du fourrage.

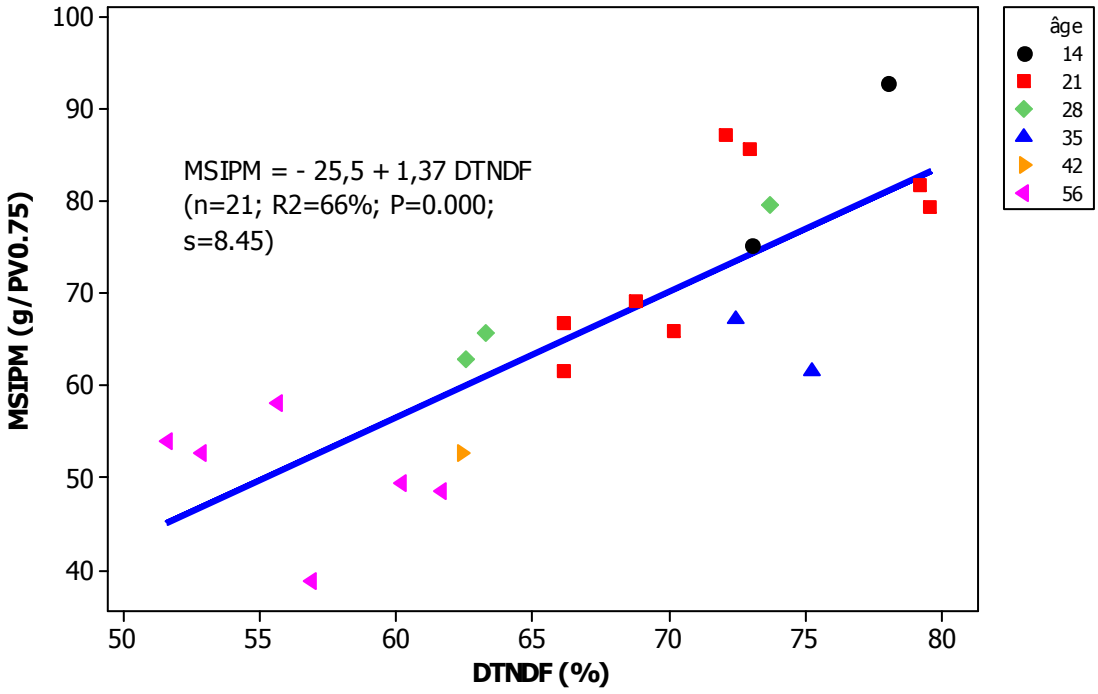


Figure 11. Evolution des quantités ingérées moyennes exprimées en MS (MSIPM) observées au cours de la thèse en fonction de la digestibilité totale exprimée en NDF.

obtenues à l'URZ. Archimède et al., 2000 travaillant sur du pangola âgé de 14 à 56 jours indiquaient une diminution d'ingestibilité du pangola de 0.66 g /PM / jour de repousse contre 0.72 enregistré sur les données de la thèse. Cependant, comme rapporté dans les projets d'articles 5 et 6, la relation entre l'ingestion et l'âge de repousse est de type curvilinéaire. Cette valeur est proche de la gamme de variation rapportée pour les fourrages tempérés variant de 0. 41 à 0.65 (Demarquilly, 1981). L'analyse de l'ensemble de la base de données de notre unité de recherche donne une valeur de diminution 0.13 g / jour, proche de 0.17g/j (valeur obtenue par Minson, 1990). L'une des différences essentielle de la base de données de l'URZ avec nos données est l'étendue de l'âge de repousse. Les études étaient réalisées avec des fourrages âgés de plus de 21 jours à plus de 77 jours. Les fourrages étudiés jusqu'alors à l'URZ étaient donc en moyenne plus vieux que les nôtres alors qu'il est maintenant connu que la maturation des fourrages tropicaux se fait très précocement (Wilson, 1994). Ce phénomène est accentué du fait de l'évolution curvilinéaire avec l'âge.

L'âge de repousse a eu un effet similaire sur la digestibilité du NDF avec une chute de 0.43 point par jour de repousse (figure 10) contre 0.18 pour la MS dans la base de l'URZ. Cette grande différence est là encore liée à la gamme des âges de repousse étudiés. La variation de digestibilité du NDF enregistrée avec les fourrages tempérés de 0.48 (Demarquilly, 1981) est assez proche de nos résultats.

Comme déjà rapporté par d'autres auteurs (Minson, 1990), nos résultats illustrent une bonne corrélation entre l'ingestion du fourrage et la digestibilité de sa fraction NDF (figure 11). Une relation étroite lie aussi l'ingestion à la teneur en NDF du fourrage consommé (figure 4b) alors que la relation est moins forte entre la digestibilité et la teneur en NDF de ce même fourrage (figure 7b). Ces derniers résultats pourraient témoigner de facteurs explicatifs différents pour les deux composantes importantes de la valeur alimentaire d'un fourrage que sont l'ingestibilité et la digestibilité.

Les conclusions globales issues de la compilation de nos expérimentations concernant l'effet de l'âge de repousse sont globalement cohérentes avec les conclusions partielles obtenues au sein de chaque expérimentation (projets de publications 5, 6 et 8). Les tableaux 2a et 2b ont été établis par des analyses de variance-covariance pour chercher à comparer les différents prédicteurs de l'ingestiblité et de la digestiblité des fourrages étudiés dans la thèse. Les données sont celles du tableau 1. Le facteur qualitatif a été la publication (3 à 6 DL). Les relations ont été linéaires et les interactions covariable*type de fourrage n'ont pas été testées dans le modèle. Ces régressions permettent de comparer les différents prédicteurs, sur la base de l'ETR ; l'âge est le critère le plus précis.

Tableaux 2a et 2b. Comparaison des prédicteurs de la MSIPM et de la digestiblité du NDF.

2a. Prédicteurs MSIPM

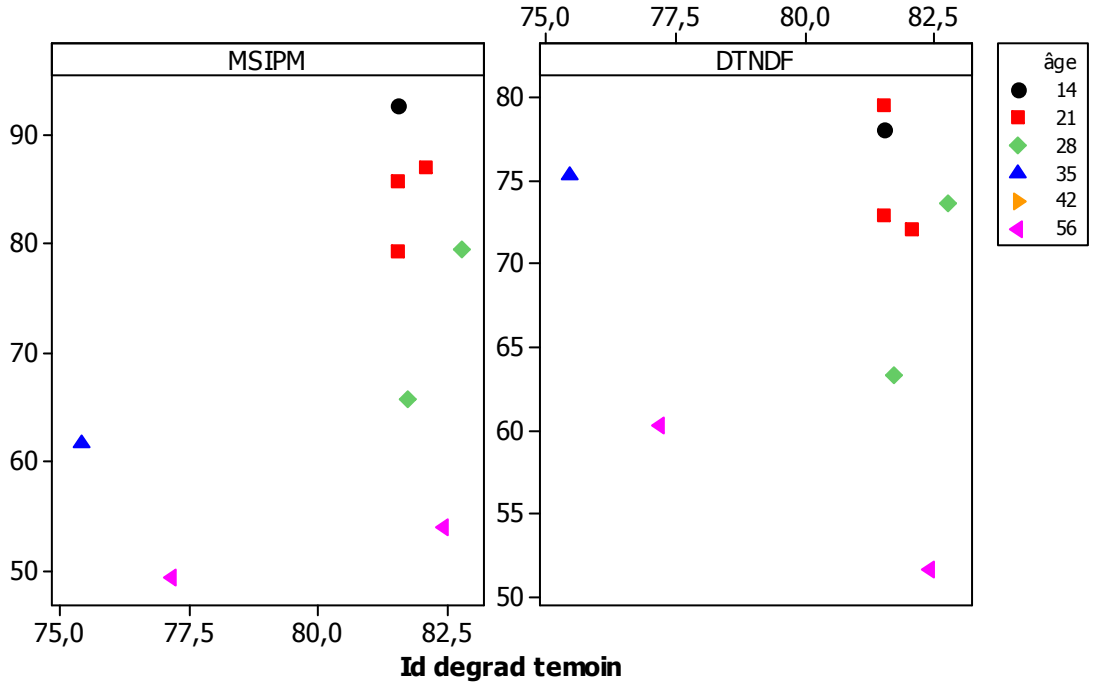
Critère (nb obs)	unité	constante	Regression/j	ETR	R^2	P
NDFf (22)	%	**350.38**	**-3.84**	**8.41**	**74.73**	**0.000**
MATf (22)	%	**38.61**	**2.46**	**7.46**	**80.13**	**0.000**
AGE (22)	j	**89.93**	**-0.72**	**5.97**	**87.28**	**0.000**
DNDF (22)	%	**-40.70**	**1.60**	**6.90**	**83.00**	**0.000**
NDFnd (22)	%msi	**114.04**	**-1.95**	**6.37**	**85.49**	**0.000**
Degrad tem (9)	%	**-290.90**	**5.04**	**15.33**	**39.46**	**0.134**
B-D Xylosidase (7)	µmol/ (mgprot*h)	**81.51**	**-0.4**	**20.49**	**5.23**	**0.664**
1-b (10)	%	**151.3**	**-1.88**	**6.8**	**89.42**	**0.001**
c (10)	%/h	**29.58**	**742.9**	**8.34**	**84.07**	**0.004**
crndfmax (18)	g	**147.43**	**-0.099**	**13.22**	**46.54**	**0.051**
idcrndfmax (18)	g/gmsi	**112.44**	**-43.03**	**8.17**	**79.59**	**0.000**
IdMast (14)	min/gmsi	**120.10**	**-66.87**	**7.44**	**84.50**	**0.000**
vitcom (14)	gmsi/min	**9.96**	**41.89**	**8.17**	**81.28**	**0.000**
tradl (14)	1/h	**18.88**	**16.32**	**9.47**	**74.88**	**0.002**

2b. Prédicteurs digestibilité

Critère (nb obs)	unité	constante	Regression/j	ETR	R^2	P
NDFf (22)	%	**206.28**	**-1.88**	**5.44**	**70.03**	**0.001**
MATf (22)	%	**51.57**	**1.37**	**3.93**	**84.33**	**0.000**
age (22)	j	**79.71**	**-0.39**	**3.56**	**87.12**	**0.000**
degradtem (9)	%	**-37.5**	**1.5**	**9.57**	**33.63**	**0.433**
B-D Xylosidase (7)	µmol/ (mgprot*h)	**75.63**	**-0.42**	**10.21**	**20.47**	**0.387**
1-b (10)	%	**100.48**	**-0.78**	**6.32**	**58.78**	**0.039**
c (10)	%/h	**50.68**	**294.7**	**6.90**	**50.94**	**0.070**
Idcrndfmax (18)	g/gmsi	**87.40**	**-18.51**	**5.02**	**72.83**	**0.002**
crndfmax (18)	g	**112.61**	**-0.05**	**6.06**	**60.51**	**0.022**
IdMast (14)	min/gmsi	**91.71**	**-30.13**	**4.72**	**79.33**	**0.002**
vitcom (14)	gmsi/min	**40.89**	**19.73**	**4.55**	**80.83**	**0.001**
tradl (14)	1/h	**48.75**	**6.49**	**6.14**	**65.04**	**0.026**

Tableau 3. Evolution des principaux paramètres en fonction de l'âge de la repousse.

Critère (nb obs)	unité	constante	Regression/j	ETR	Variation/sem	R^2	P
MSIPM (22)	g/kgPM	**89.9**	**-0.72**	**5.97**	**- 5.0**	**87.3**	**0.000**
NDFF (22)	%MS	**70.1**	**0.116**	**2.17**	**+0.81**	**62.1**	**0.002**
DNDF (22)	%	**79.7**	**-0.39**	**3.6**	**-2.7**	**87.1**	**0.000**
NDFnd (22)	%MSI	**13.81**	**0.33**	**2.91**	**+2.3**	**87.1**	**0.000**
IdMast (14)	min/gmsi	**0.385**	**0.012**	**0.07**	**+0.86**	**93.6**	**0.000**
Tr ADL (14)	1/h	**4.3**	**-0.040**	**0.47**	**-0.28**	**82.7**	**0.002**
IdCRNDFmax (18)	g/gmsi	**0.55**	**0.016**	**0.14**	**+0.11**	**80.1**	**0.000**
1-b (10)	%	**28.93**	**0.45**	**2.48**	**3.15**	**94.1**	**0.000**
c (10)	%/h	**0.084**	**-0.00094**	**0.01**	**-0.0069**	**74.2**	**0.010**
B-D Xylosidase (7)	µmol/(mgprot*h)	**13.7**	**0.194**	**11.1**	**0.97**	**31.4**	**0.511**
Degrad temoin (id) (9)	%	**83.1**	**-0.084**	**2.13**	**-0.45**	**56.8**	**0.154**



Figures 12a et 12b. Evolution des quantités ingérées moyennes exprimées en MS (MSIPM) et des digestibilités totales moyennes exprimées en NDF (DNDF) observées au cours de la thèse en fonction de l'indice de dégradabilité du témoin.

Les critères qui prédisent le mieux l'ingestion sont ensuite DNDF (ou 100-DNDF) et surtout la teneur en NDF non digestible du fourrage (NDFnd %MSI). Ce résultat met en évidence le rôle déterminant du travail nécessaire pour faire transiter la fraction indigestible des fourrages. L'Index de mastication est également assez précis, malheureusement il n'a pas été mesuré sur autant d'observations que les critères précédents.

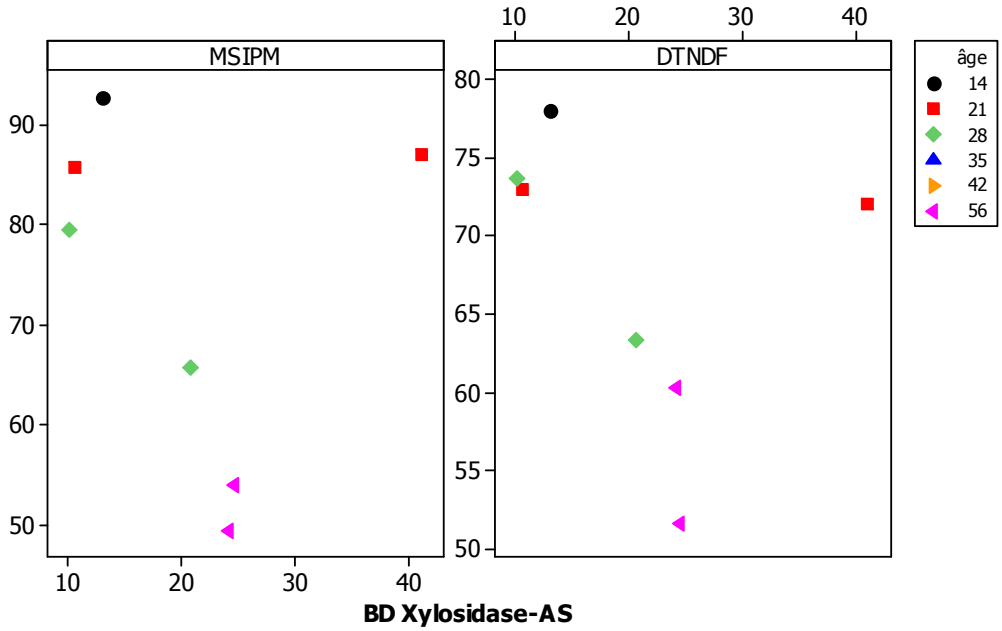
Les variables prédictrices les « moins bonnes » sont le trADL, l'encombrement maximal du rumen, la dégradabilité du témoin et l'activité enzymatique, représentée par la B-D Xylosidase. Cette hiérarchie tend à confirmer la dépendance de l'ingestion aux difficultés à faire transiter des flux de matières dans le tube digestif : au niveau buccal avec l'indice de mastication et dans le reste du tube digestif avec DNDF et NDFnd.

Concernant les prédicteurs de la digestibilité (tableau 2b), le plus précis est là encore l'âge et les moins précis sont aussi la dégradabilité du témoin et l'activité enzymatique.

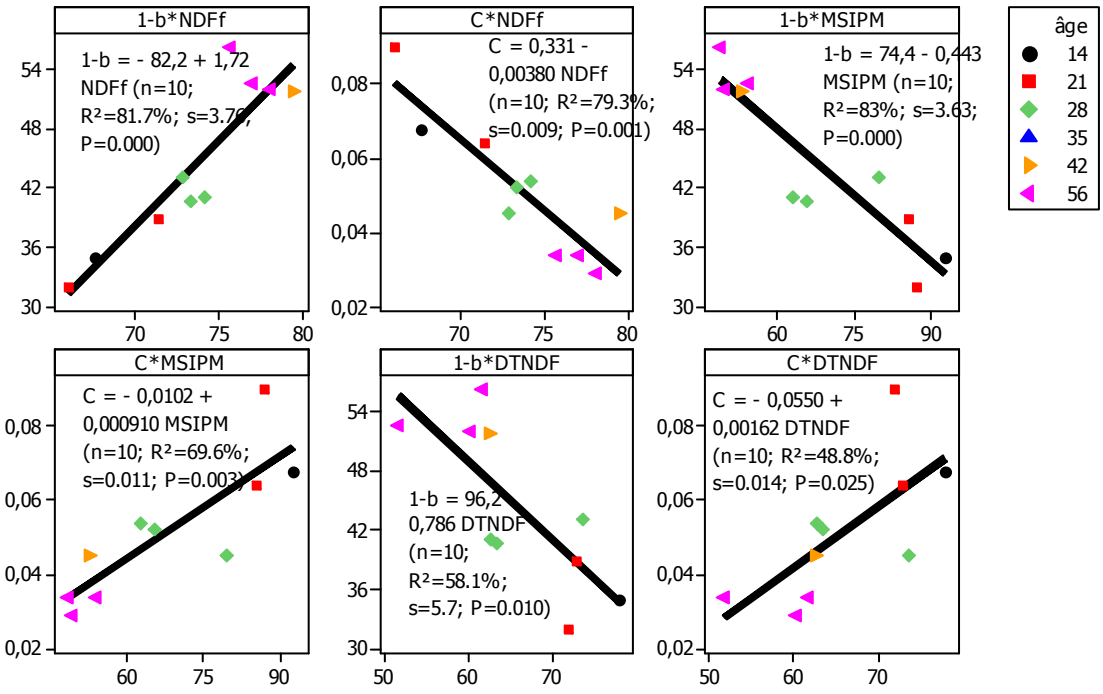
Le tableau 3 a été établi comme le précédent, à partir des données du tableau 1. Le facteur qualitatif a été la publication (3 à 6 DL). Il apparaît que l'âge de repousse a aussi des effets sur les prédicteurs de l'ingestibilité et de la digestibilité.

3.2. la capacité cellulolytique du rumen n'est pas le premier facteur limitant l'ingestion et la digestion des fourrages

Nous avons utilisé des sachets témoin (fourrages de 14 jours) en intra-essai pour évaluer le potentiel cellulolytique du rumen. Lors de la compilation des données, nous avons travaillé sur des indices, en prenant comme référence la plus haute valeur obtenue avec du 14 jours et en faisant l'hypothèse que dans les différents essais l'activité enzymatique n'était pas un facteur limitant pour les fourrages jeunes. Les figures 12a et 12b indiquent que le potentiel cellulolytique du rumen varie assez faiblement avec les différents fourrages expérimentaux et n'a pas d'effet significatif sur les quantités ingérées et sur la digestibilité (tableaux 2a et b). Cette relation globale confirme les observations partielles mises en évidence dans les différents essais (essais 2, 5 et 6). Des résultats similaires (dégradabilité in sacco de fourrage témoin de 14 jours) avaient déjà été obtenus à l'URZ lors d'essais où l'âge du fourrage avait varié de 14 à 56 jours Eugène (2002). Dans l'ensemble de ces essais, les fourrages témoins sont hachés et non broyés. On peut donc formuler l'hypothèse que les niveaux de dégradation observés étaient inférieurs au potentiel cellulolytique du rumen car la surface spécifique des particules du fourrage n'était pas assez importante pour assurer une bonne colonisation microbienne. De plus, dans notre contexte expérimental, les particules alimentaires ont séjourné entre 33 (les plus jeunes fourrages) et 62 (les fourrages les plus âgés) heures dans le



Figures 13a et 13b. Evolution des quantités ingérées moyennes exprimées en MS (MSIPM) et des digestibilités totales moyennes exprimées en NDF (DNDF) observées au cours de la thèse en fonction d'une activité enzymatique.



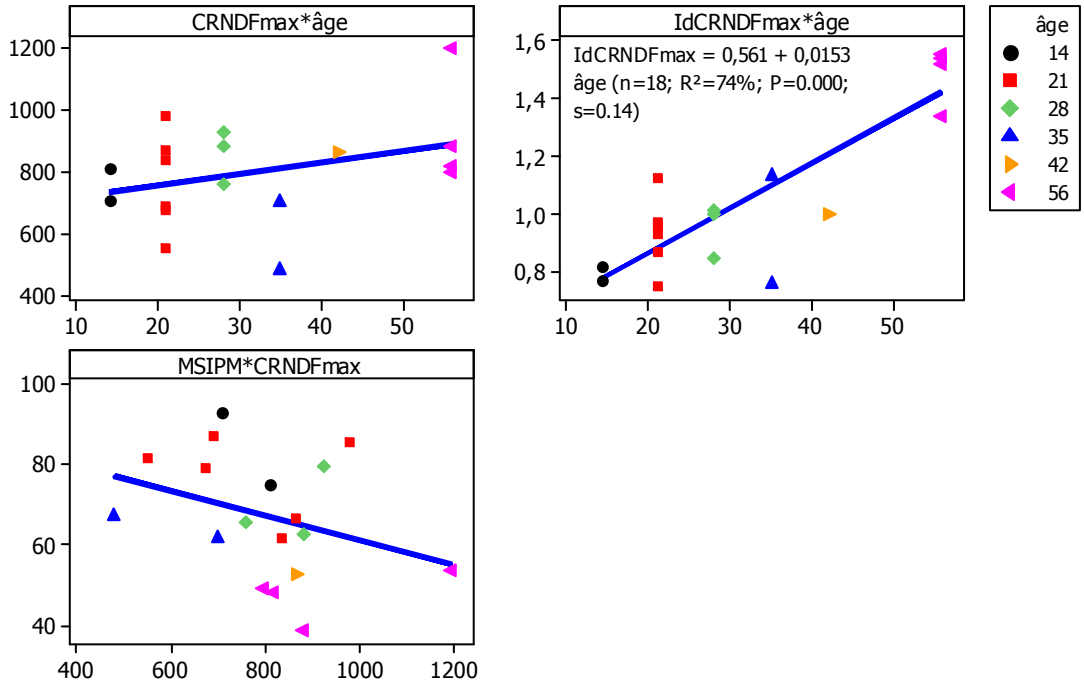
Figures 14 a, b, c, d, e, f. Evolution des valeurs moyennes de la fraction potentiellement digestible (B) et de la vitesse de dégradation (C) en fonction de la teneur en NDF du fourrage, des quantités ingérées (MSIPM) et de la digestibilité du NDF (DNDF).

rumen (le temps de séjour est estimé comme l'inverse du taux de renouvellement de l'ADL), alors que nos sachets témoins n'ont séjourné que 24 heures. La digestion microbienne est d'autant plus élevée que l'exposition est longue.

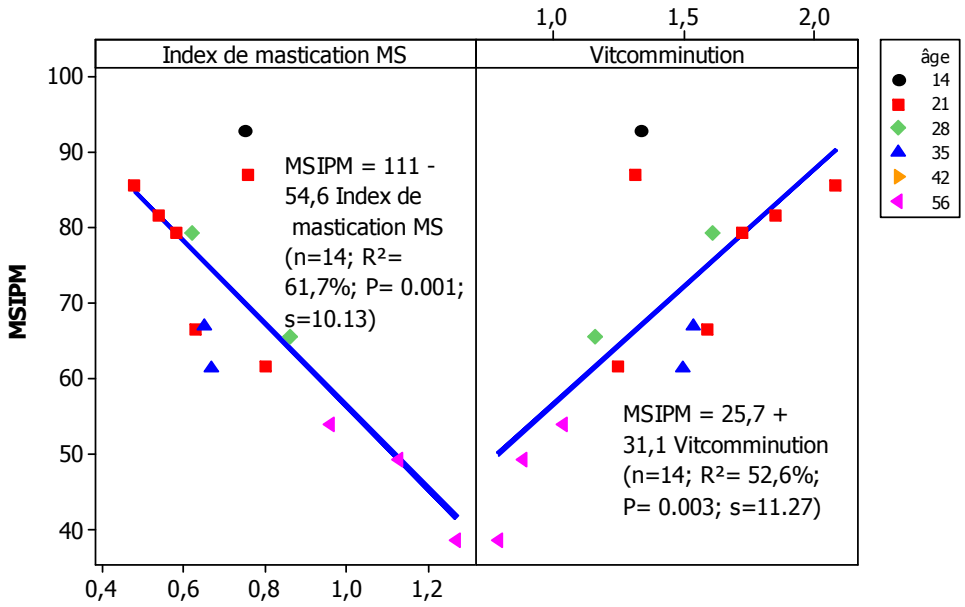
Par ailleurs, Satter et Roffer (1975), Hoover (1986) ont suggéré que la croissance et l'activité microbiennes dans le rumen diminuaient quand les concentrations en ammoniac dans le rumen étaient inférieures à 50 mg/l. Cette limite ne fut jamais atteinte lors de nos expérimentations (essais 4, 5 et 6) ce qui pourrait indiquer que la croissance microbienne et les activités enzymatiques associées n'ont pas été pénalisées avec l'âge du fourrage. C'est ce que semble confirmer les mesures directes d'activités enzymatiques dans le rumen (essais 5 et 6). En effet, quelque soit l'enzyme considérée, il n' y a pas eu d'effet significatif des activités enzymatiques sur les quantités ingérées et la digestibilité (figures 13a, 13b et tableaux 2a et b). De plus, il y a une relation négative entre l'ingestibilité et les différentes activités enzymatiques (tableau 2a). Ce résultat pourrait indiquer qu'il y a une adaptation de la population du rumen à la nature du fourrage avec notamment des espèces plus actives et /ou des activités plus importantes quand les fibres végétales sont « récalcitrantes ». Des résultats déjà obtenus à l'unité (Eugène et al.,1999) montraient une évolution de la composition de la population bactérienne du rumen en faveur des espèces connues pour leur plus grande activité fibrolytique avec le vieillissement du fourrage.

3.3. la résistance à la mastication et en conséquence la vitesse de réduction de taille des particules alimentaires (comminution) serait le premier facteur limitant l'ingestion.

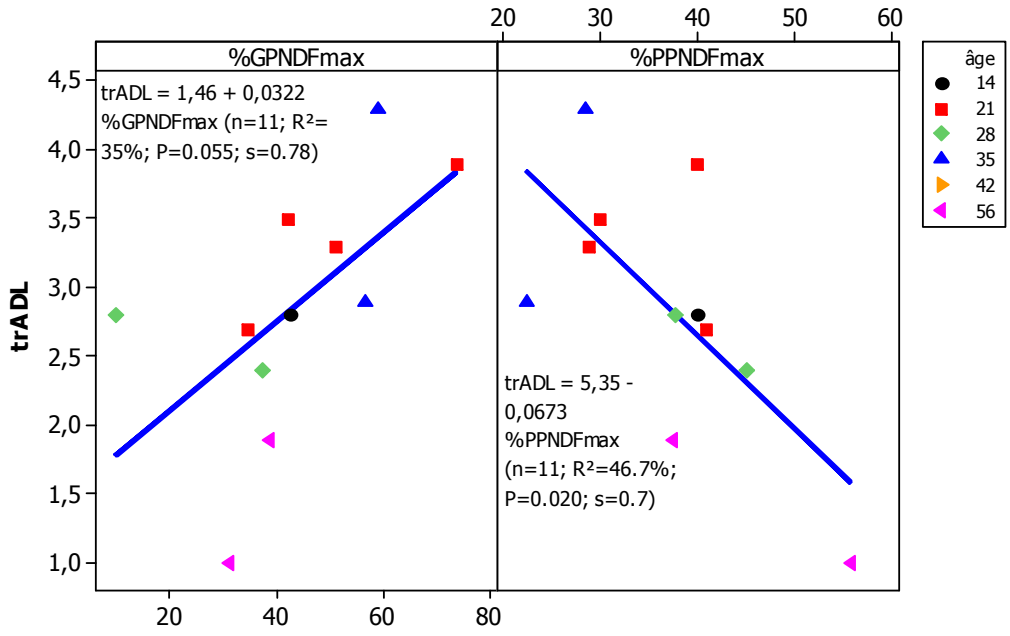
La résistance d'un fourrage à la mastication est un élément déterminant de son encombrement. L'encombrement des fourrages peut aussi être évalué par les résultats des cinétiques in situ. Lors de nos essais (projets de publication 5, 7 et 8) l'encombrement des fourrages a été estimé, par la fraction potentiellement indigestible (1-b) et le taux de dégradation (c) obtenus par les cinétiques de dégradation in sacco (Madsen *et al.,* 1994). L'indigestible NDF constitue aussi un indicateur de l'encombrement des fourrages et à ce titre nous l'avons utilisé dans l'analyse globale des résultats (Minson, 1990). Les figures 14a et 14b reflètent un encombrement croissant avec la maturité et la teneur en NDF des fourrages. De plus, les figures 14 c, 14d, 14e et 14f montrent que la fraction potentiellement indigestible ainsi que la vitesse de dégradation sont beaucoup plus fortement corrélées à l'ingestibilité qu'à la digestibilité. L'analyse statistique des données moyennes (tableau 2a) indique que les différents indicateurs de l'encombrement des fourrages, contrairement à ceux du ‘‘potentiel



Figures 15 a, b, c. Evolution des valeurs moyennes obtenues au cours de la thèse, exprimées en NDF, du volume ruminal brut (CRNDFmax) ou rapporté aux quantités ingérées (IdCRNDFmax) en fonction de l'âge du fourrage (15 a et b). Evolution des valeurs moyennes obtenues au cours de la thèse des quantités ingérées (MSIPM) en fonction du volume ruminal brut (CRNDFmax) (15 c).



Figures 16a et 16b. Evolution des valeurs moyennes obtenues au cours de la thèse, des quantités ingérées (MSIPM) en fonction de l'index de mastication et de la vitesse de communition.



Figures 17 a et b. Evolution des valeurs moyennes de trADL obtenues au cours de la thèse en fonction de la proportion de grosses particules (%GPNDFmax) et de petites particules (%PPNDFmax) ruminales.

cellulolytique'', expliquent fortement l'ingestion des fourrages. De plus, l'ingestion augmente significativement avec le taux de renouvellement du rumen (tableau 2a).

Globalement, les tendances observées entre les indicateurs de l'encombrement, ceux du potentiel cellulolytique et l'ingestion existent aussi avec la digestibilité (tableau 2b). Les fortes relations statistiques entre l'encombrement et l'ingestibilité plutôt que la digestibilité confirmerait un poids plus élevé des phénomènes de dégradation physique (comminution) relativement à l'activité microbienne (cellulolyse) sur l'écoulement des particules hors du rumen.

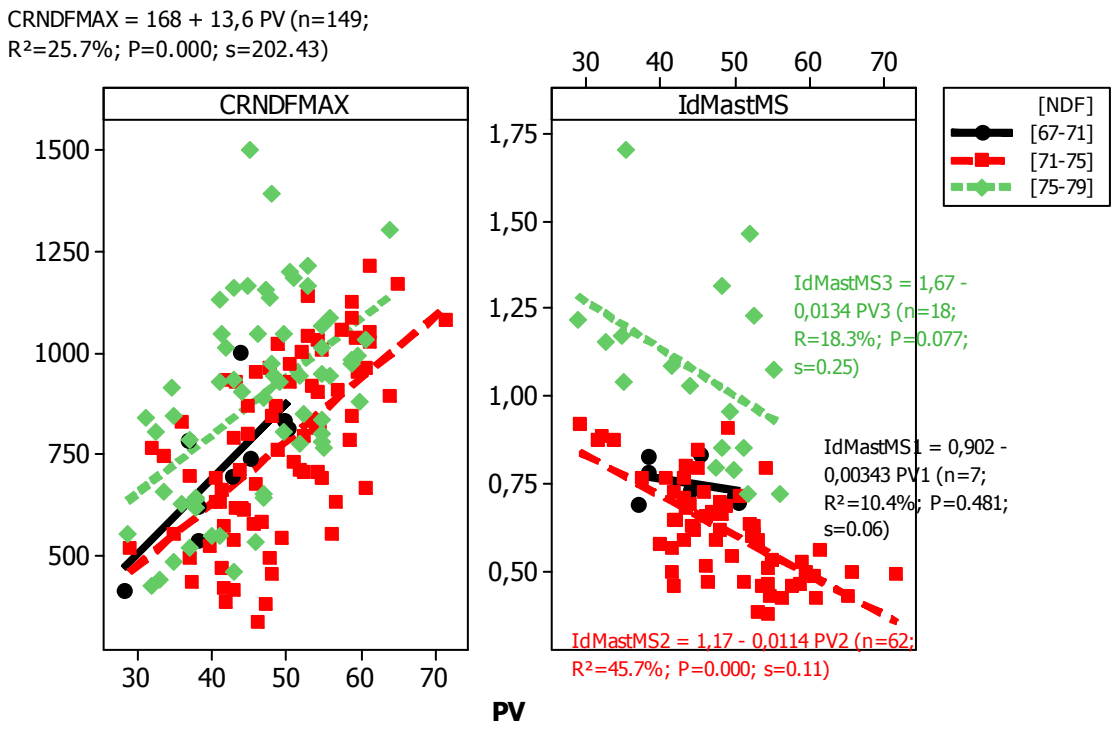
La figure 15a montre que les quantités maximales de NDF dans le rumen varient peu avec l'âge du fourrage ou sa teneur en NDF. La figure 15b indique que ces mêmes quantités rapportées aux quantités ingérées augmentent avec la teneur en NDF ou l'âge du fourrage. Ces résultats sont conformes aux données de la bibliographie. En effet, les quantités de fourrages volontairement ingérées par un ruminant sont principalement limitées par la quantité de biomasse fibreuse présente dans le rumen (figure 15c) et la vitesse de réduction de taille des particules alimentaires qui doivent atteindre une taille et une densité critique pour pouvoir quitter le rumen (McLeod and Minson, 1988). La force motrice majeure de la vitesse de réduction de taille des particules est l'efficacité de la comminution des particules via les mastications ingestive et mérycique (McLeod and Minson, 1988 ; Mac Leod et al, 1990, Ulyatt, 1986).

Les figures 16a et 16b, ainsi que le tableau 2a montrent que l'ingestion est fortement liée à l'index de mastication et à la vitesse de comminution calculée comme l'inverse de l'index de mastication. Ainsi, la vitesse de comminution pourrait être la force motrice de l'ingestion.

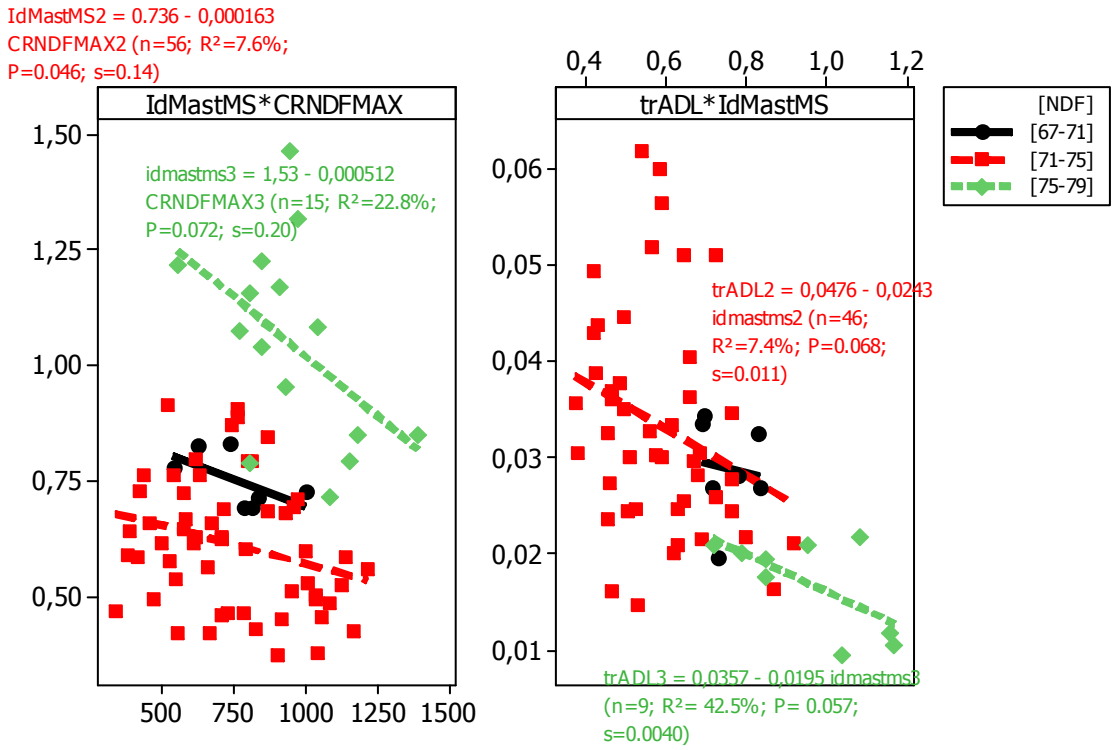
4. Relations entre la cellulolyse et la comminution des particules

L'analyse globale des données moyennes n'indiquent pas d'effet significatif d'indicateurs du potentiel cellulolytique sur l'ingestion, contrairement à ceux de l'encombrement. Toutefois, nous ne pouvons pas exclure un impact de la cellulolyse sur l'ingestion.

La figure 17a met en évidence une relation positive entre le taux de renouvellement et la proportion de grosses particules dans le rumen, alors que la figure 17b illustre une corrélation négative entre le taux de renouvellement et la proportion de petites particules dans le rumen. La relation positive entre le taux de renouvellement et les grosses particules pourrait s'expliquer par un effet de « chasse » lié à l'ingestion d'aliment. En ce qui concerne les petites particules, théoriquement éligibles pour quitter le rumen, nos résultats illustrent un phénomène d'accumulation des petites particules dans le rumen. Cela tient probablement à



Figures 18 et 19. Evolution des valeurs individuelles de volume ruminal (CRNDF max) et d'index de mastication obtenues au cours de la thèse en fonction du poids de l'animal.



Figures 20 et 21. Evolution des valeurs individuelles obtenues au cours de la thèse de l'index de mastication en fonction du volume ruminal (CRNDF max) et du taux de renouvellement de l'ADL.

des contraintes de densité des particules. La digestion microbienne intervient dans la modification de la densité spécifique fonctionnelle des particules (Siciliano-Jones et Murphy, 1991).

En effet, la digestion de la matrice fibreuse du tissu végétal induite par la colonisation microbienne, modifie la composition des groupes fonctionnels de la matrice et augmente la capacité échangeuse de cations (absorption d'eau) (Mc Burney et al 1981). Avec les fermentations microbiennes, des bulles de gaz sont synthétisées, remontent à la surface du liquide du rumen et favorisent la flottaison des grosses particules. Ainsi la cellulolyse pourrait impacter indirectement l'ingestion en ralentissant l'accroissement de densité des petites particules et en conséquence leur vitesse de sortie du rumen.

5. Variabilités individuelles

Dans chaque expérience les variations individuelles ont été importantes sur la plupart des paramètres mesurés. Une partie de celles ci sont liées aux différences de poids entre animaux. Les animaux les plus lourds ingèrent plus (0.24 kgMSI/10kgPV) et ont une capacité ruminale plus importante. Ainsi, la figure 18 indique la relation positive logique entre les volumes ruminaux et le poids. Par ailleurs, une relation négative lie aussi les index de mastication au poids des animaux (figure 19). Les animaux les plus lourds ont une puissance masticatoire plus importante. Logiquement, l'index de mastication est aussi négativement lié au volume ruminal (figure 20).

L'analyse statistique de ces données a été réalisée en prenant en compte des classes de teneurs en NDF du fourrage. Pour les deux classes les plus élevées en NDF, des relations significatives ont été mises en évidence entre index de mastication et poids d'une part, index de mastication et volume du rumen, d'autre part. En ce qui concerne les faibles teneurs en NDF, la relation n'a pas été significative, peut être en raison de la faible étendue de la variable explicative. Ces variations individuelles, liées au gabarit, s'accentueraient donc avec la teneur en NDF des fourrages.

L'explication à cette plus grande efficacité individuelle est à rechercher dans la plus grande capacité de stockage et son plus fort taux de renouvellement (figure 21). Au delà des différences liées au poids vif, il existe des variations individuelles intéressantes de la capacité d'ingestion, ramenée à l'unité de poids (MSI/PV ou MSI/PM), des animaux. En effet il apparaît que les plus gros mangeurs se caractérisent par une puissance masticatoire (MSI/min de mastication) accrue, un transit plus rapide des liquides et des particules et une meilleure

capacité digestive, donc moins de flux de matières à faire transiter. Les mécanismes impliqués et leur degré d'héritabilité mériteraient des approfondissements.

CONCLUSIONS ET PERSPECTIVES DE TRAVAIL

Nos résultats indiquent : 1) que les jeunes fourrages tropicaux ont des ingestibilité et digestibilité élevées donc de bonnes valeurs nutritives ; 2) qu'il n'y a pas de relation directe entre l'activité microbienne du rumen et l'ingestion du fourrage ; 3) que l'index de mastication semble déterminant dans l'ingestion des fourrages.

En plus de nos hypothèses de travail, nos résultats ont mis en évidence des contraintes physiques spécifiques aux fourrages tropicaux, liées à leur densité. En effet les fines particules qui ont la taille critique pour quitter le rumen tendent à s'y accumuler. La plus lente vitesse de dégradation des fourrages qui contribue à l'accroissement de la densité des particules, ainsi que la composition particulière des parois végétales seraient des éléments explicatifs de cette densité. Des travaux complémentaires sont utiles pour analyser cette hypothèse.

Par ailleurs, l'analyse individuelle de nos données semble indiquer une variabilité individuelle de l'ingestion liée à une plus grande efficacité de la mastication. Cette dernière serait positivement liée au format de l'animal et à son volume ruminal. Des investigations sont nécessaires pour approfondir cette piste qui peut avoir des conséquences sur les critères de sélection des animaux notamment ceux conduits au pâturage.

L'ensemble des résultats interpelle les tables de valeur alimentaire élaborées pour des fourrages tropicaux. Concernant celles proposées par l'INRA, une question est ouverte sur la pertinence des valeurs des paramètres utilisées pour estimer, les valeurs energétique et protéique des fourrages tropicaux. Quel est l'impact de la moindre digestibilité intestinale des protéines alimentaires des fourrages tropicaux ? Ne faudrait-il pas utiliser une valeur spécifique ? Quel est l'impact de l'effort de mastication plus grand, induit par l'ingestion des fourrages tropicaux, sur les pertes énergétiques et en conséquence sur la valeur du coefficient de passage entre l'énergie métabolisable et nette.

Par ailleurs, compte tenu du poids statistique important de critères tels l'âge de repousse, de la teneur en paroi dans l'estimation des différentes composantes de la valeur alimentaire, quel est l'intérêt réel d'un catologue exhaustif des nombreuses graminées ? N'y a-t'il pas là, une certaine redondance ? N'y a-t' il pas une plus-value à décrire moins d'espèces, choisies sur des critères physiologiques (exemple vitesse de lignification) et à être plus exhaustif sur leur contexte agronomique (âge, saison, fertilisation, irrigation) de production ? Compte tenu des

contraintes importantes de mastication, n'y a-t'il pas utilité à affiner certains critères de dureté des parois qui pourraient être utilisés en routine.

Nos résultats constituent une bonne transition pour les travaux en cours à l'URZ sur le déterminisme de l'ingestion au pâturage. Nous avons accumulé des données qui permettront de modéliser, en valorisant notamment les modèles conceptuels existants, l'ingestion à l'auge des fourrages tropicaux. La compréhension des contraintes digestives (compartiments ruminal et buccal) par ces études à l'auge complétées par celles des contraintes prairiales (études en cours) qui impactent sur la préhension des fourrages permettront à l'URZ de valider un modèle sur l'ingestion des fourrages au pâturage.

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Figure 1. Comparison of kinetics of intake between day and night in rams fed with a chopped 56-day (56H), 21-day (21H) and a long 21-day (21L) regrowth age of *Digitaria decumbens* in 2 equals meals distributed at 7h00 and 19h00.

Table 3. Effect of time of the day (morning *versus* night) on dry matter intake (DMI) and feeding behaviour of rams consuming a long and chopped 21-day of *Digitaria decumbens* in 2 equals meals distributed at 7h00 and 19h00.

Table 4. Effect of time of the day (morning *versus* night) on the maximum (1h30 post feeding and minimum (12h00 post feeding) amount of Neutral Detergent Fiber in the reticulo-rumen of rams consuming a chopped 21-day and 56-day regrowth age of *Digitaria decumbens* in 2 equals meals distributed at 7h00 and 19h00.

Figure 2. Evolution of NDF rumen content (g/gNDFI) according to the postfeeding time and the cycle day-night in rams consuming a chopped 21-day and 56-day regrowth age of *Digitaria decumbens* in 2 equals meals distributed at 7h00 and 19h00.

Table 5. Effect of time of the day (morning *versus* night) on the maximum (1h30 post feeding and minimum (12h00 post feeding) amount of Neutral Detergent Fiber in the reticulo-rumen of rams consuming a long and chopped 21-day of *Digitaria decumbens* in 2 equals meals distributed at 7h00 and 19h00.

Table 6 . Effect of time of the day (morning *versus* night) on the amounts of Large particle (LP), Small particle (SP) and Very Small Particle (VS) (gNDF/g NDF intake) or ADF(gADF/g ADF intake) in the réticulo-rumen content, 0h00 and 1h30 post feeding, with rams consuming a chopped 21-day and 56-day regrowth age of *Digitaria decumbens* in 2 equals meals distributed at 7h00 and 19h00.

Table 7. Effect of time of the day (morning *versus* night) on the amounts of Large particle (LP), Small particle (SP) and Very Small Particle (VS) of NDF (gNDF/g NDF intake) or ADF(gADF/g ADF intake) in the réticulo-rumen content, 0h00 and 1h30 post feeding, with rams consuming a long and chopped 21-day of *Digitaria decumbens* in 2 equals meals distributed at 7h00 and 19h00.

PUBLICATION 5

Table 1. Chemical composition (g/kg) of the 14, 28, 42 and 56-day old *Digitaria decumbens* grass consumed by six Black belly rams

Table 2. Effect of the regrowth age of a *Digitaria decumbens* grass (14-, 28-, 42- and 56-day old) on intake in the rumen of Blackbelly rams

Table 3. Nylon degradation of NDF fraction of forage, large particle (LP) and small particle (SP) of a 14-, 28-, 42- and 56-day old *Digitaria decumbens* grass fed to six Blackbelly rams

Table 4. Effect of the regrowth age of a *Digitaria decumbens* grass (14-, 28-, 42- and 56-day old) on the particle size distribution of NDF (g) in the rumen of Blackbelly rams, 3 and 12 hours after the morning meal

Table 5 . Effect of the regrowth age of a *Digitaria decumbens* grass (14-, 28-, 42- and 56-day old) on the particle size distribution of NDF (g/1000g of NDF intake) in the rumen of Blackbelly rams, 3 and 12 hours after the morning meal.

Figure 1. Flow diagram of size reduction, digestion and passage rate of digesta in the rumen. Kr_{LP}, Kr_{SP}, are particle size reduction rate of large and small particles respectively. Kd_{LP}, $Kd_{SP,}$ KdV_{VP}, are the digestion rate of large, small and very small particles respectively. Kp_{LP}, Kp_{SP}, Kp_{VP}, are the passage rate of large and small and very small particles respectively.

Figure 2. Effect of the pangola grass maturity (14, 28, 42 and 56 days regrowth) on the granulometry of the pool of NDF in the rumen at the end of the morning meal

Table 6. Effect of the regrowth age of a *Digitaria decumbens* grass (14-, 28-, 42- and 56-day old) on : disappearance rate, reduction rate, passage rate, digestion rate of whole rumen NDF, large particle of NDF, Small particle of NDF (SP-NDF) and NDF as very small particle fraction ; in the rumen of Blackbelly rams, 3 and 12 hours after the morning meal.

PUBLICATION 6

Table 1. Chemical composition (% DM) of a 14-, 21-, 28- and 56-day old fertilized *Panicum maximum* grass.

Table 2. Intake and Total tract digestibility of dry matter, organic matter, neutral detergent fiber, acid detergent fiber, crude protein, intake of the digestible organic matter and ruminal turnover rate of ADL in Blackbelly lambs given a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Table 3. Feeding behaviour parameters in Blackbelly lambs given a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Table 4. Amount of DM (total DM, Large particles, Small particles, and Very Small particles) in the rumen of black belly rams 0 (min) and 3 (max) hours after the morning meal of a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Table 5. Total Rumen fill (RF), large, small and very small particles of Neutral Detergent Fiber collected 1h after a first emptying in Blackbelly rams given a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Figure 1. Effect of age of regrowth on kinetic of intake of rams given a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Figure 2. Effect of regrowth age (days) on intake, total tract digestibility, eating index, ruminating index and mean transit time in the rumen of *Panicum maximum* fresh grass

Table 6. Correlation between intake and digestive parameters.

PUBLICATION 7

Table 1. Chemical composition (% DM) of a 14-, 21-, 28- and 56-day old fertilized *Panicum maximum* grass.

Table 2. Degradability of dry matter and nitrogen in Blackbelly rams consuming a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Table 3. pH in Blackbelly rams consuming a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Table 4. Volatile Fatty Acids (VFA) in Blackbelly rams consuming a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Table 5. Ammonia concentrations in Blackbelly rams consuming a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Table 6. Enzymatics activities (polysaccharidases) in rumen solid-associated microorganisms, in Blackbelly rams consuming a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Table 7. Enzymatics activities (glucosidases) in rumen solid-associated microorganisms, in Blackbelly rams consuming a 56-, 28-, 21- or a 14-old *Panicum maximum.*

Table 8. Predictive equations of intake (g DMI/kg LW0.75) and total tract digestiblity (%).

PUBLICATION 8

Table 1. Chemical composition (% DM) of a 21-, 28- and 56-day old fertilized *Digitaria decumbens* grass.

Table 2. Intake and Total tract digestibility of dry matter, organic matter, neutral detergent fiber, acid detergent fiber and crude protein of ADL in Blackbelly rams given a 56-, 28- or a 21-old *Digitaria decumbens.*

Table 3. Estimates parameter of kinetic of degradation (model of Orskov) dry matter, neutral detergent fiber and nitrogen of a 56-, 28- or a 21-old *Digitaria decumbens* (nylon bags) incubated in rumen of Blackbelly rams consuming a 56-, 28- or a 21-old *Digitaria decumbens.*

Table 4a. Polysaccharidases activities in rumen solid-associated microorganisms, in Blackbelly ram consuming a 56-, 28- or a 21-old *Digitaria decumbens.*

Table 4b. Glucosidase activities in rumen solid-associated microorganisms, in Blackbelly ram consuming a 56-, 28- or a 21-old *Digitaria decumbens.*

Table 5. Ammonia concentrations in rumen liquid of Blackbelly ram consuming a 56-, 28- or a 21-old *Digitaria decumbens.*

Table 6. Feeding behaviour parameters in Blackbelly rams given a 56-, 28- or a 21-old *Digitaria decumbens.*

Table 7. Amount of NDF (total NDF, Large particle, Small particles, and Very Small particle), ruminal turnover rate of liquid, fibrous particle (ADL) and small particle in the rumen of black belly rams 0 (min) and 3 (max) hours after the morning meal of a 56-, 28-, 21- or a 14-old *Digitaria decumbens.*

Table 8. Total Rumen fill (RF), large, small and very small particles of Neutral Detergent Fiber collected 1h after a first emptying in Blackbelly rams given a 56-, 28-, 21- or a 14-old *Digitaria decumbens.*

Figure 1. Effect of regrowth age (days) on intake, total tract digestibility, cellulolysis (nylon bag degradation of a reference forage) chewing index, rumen mean transit time of liquid, fibrous particle and small particle in rams consuming a 56-, 28- or 21-old *Digitaria decumbens.*

DISCUSSION

Figure 1. Evolution de la teneur en MAT en fonction de celle en NDF des différentes classes de fourrages (tempérée, tropicale ou « tropicale thèse »).

Figures 2a,b,c. Dispersion des teneurs en NDF, ADF et MAT en fonction de la classe de fourrages (tempérée, tropicale ou « thèse »).

Figure 3. Dispersion des quantités de matières sèches ingérées (MSIPM) en fonction de la classe de fourrages (tempérée, tropicale, « thèse »).

Figure 4a. Evolution des quantités ingérées (MSIPM) en fonction de la teneur en NDF des différentes classes de fourrages (tempérée, tropicale, « thèse »).

Figure 4b. Evolution des quantités ingérées (MSIPM) moyennes observées au cours de la thèse en fonction de la teneur en NDF du fourrage.

Figure 5a. Evolution des quantités ingérées (MSIPM) en fonction de la teneur en azote (MAT) des différentes classes de fourrages (tempérée, tropicale, « thèse »).

Figure 5b. Evolution des quantités ingérées (MSIPM) moyennes observées au cours de la thèse en fonction de la teneur en MAT du fourrage.

Figures 6 a, b et c. Evolutions des digestibilités de Matière Organique, de NDF et d'azote en fonction des différentes classes de fourrages (tempérées, tropicales, « thèse »).

Figure 7a. Variation de la digestibilité totale du tractus digestif exprimée en MO avec la teneur en NDF des différentes classes de fourrage (temperée, tropicale, « tropicale thèse »).

Figure 7b. Evolution des digestibilités totales moyennes exprimées en NDF (DNDF) observée au cours de la thèse en fonction de la teneur en NDF du fourrage.

Figure 8a. Variation de la digestibilité totale du tractus digestif exprimée en MO avec la teneur en MAT des différentes classes de fourrage (tempérée, tropicale, « tropicale thèse »).

Figure 8b. Evolution des digestibilités totales moyennes exprimées en NDF (DNDF) observées au cours de la thèse en fonction de la teneur en MAT du fourrage.

Figure 9. Evolution des quantités ingérées moyennes exprimées en MS (MSIPM) observées au cours de la thèse en fonction de l'âge de repousse du fourrage

Tableau 1 . Synthèse des principaux résultats obtenus au cours des différentes expérimentations.

Figure 10. Evolution des digestibilités totales moyennes exprimées en NDF (DNDF) observées au cours de la thèse en fonction de l'âge de repousse du fourrage

Figure 11. Evolution des quantités ingérées moyennes exprimées en MS (MSIPM) observées au cours de la thèse en fonction de la digestibilité totale exprimée en NDF.

Tableau 2. Comparaison des prédicteurs de la MSIPM et de la digestibilité du NDF.

Tableau 3. Evolution des principaux paramètres en fonction de l'âge de repousse.

Figures 12a et 12b. Evolution des quantités ingérées moyennes exprimées en MS (MSIPM) et des digestibilités totales moyennes exprimées en NDF (DNDF) observées au cours de la thèse en fonction de l'indice de dégradabilité du témoin

Figures 13a et 13b. Evolution des quantités ingérées moyennes exprimées en MS (MSIPM) et des digestibilités totales moyennes exprimées en NDF (DNDF) observées au cours de la thèse en fonction d'une activité enzymatique.

Figures 14 a, b, c, d, e, f. Evolution des valeurs moyennes de la fraction potentiellement digestible (B) et de la vitesse de dégradation (C) en fonction de la teneur en NDF du fourrage, des quantités ingérées (MSIPM) et de la digestibilité du NDF (DNDF).

Figures 15 a, b, c. Evolution des valeurs moyennes obtenues au cours de la thèse, exprimées en NDF, du volume ruminal brut (CRNDFmax) ou rapporté aux quantités ingérées

(IdCRNDFmax) en fonction de l'âge du fourrage (15 a et b). Evolution des valeurs moyennes obtenues au cours de la thèse des quantités ingérées (MSIPM) en fonction du volume ruminal brut (CRNDFmax) (15 c).

Figures 16a et 16b. Evolution des valeurs moyennes obtenues au cours de la thèse, des quantités ingérées (MSIPM) en fonction de l'index de mastication et de la vitesse de communition.

Figures 17 a et b. Evolution des valeurs moyennes de trADL obtenues au cours de la thèse en fonction de la proportion de grosses particules (%GPNDFmax) et de petites particules (%PPNDFmax) ruminales.

Figures 18 et 19. Evolution des valeurs individuelles de volume ruminal (CRNDF max) et d'index de mastication obtenues au cours de la thèse en fonction du poids de l'animal

Figures 20 et 21. Evolution des valeurs individuelles obtenues au cours de la thèse de l'index de mastication en fonction du volume ruminal (CRNDF max) (figure 20) et du taux de renouvellement de l'ADL (figure 21).

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Formation de base:

- DEA de Nutrition, Aspects Moléculaires et Cellulaires, Option Humaine, Université Saint-Jérôme de Marseille, Juin 2000.

Activités formatrices :

- Encadrement d'un stagiaire- Licence de Biologie Cellulaire et Physiologie. Université Bordeaux2. « Effet de l'apport d'enzyme sur l'ingestion et la digestion de Pangola (*Digitaria decumbens*) âgé de 21 et 56 jours chez le mouton « Martinik » ». INRA. , Centre de Recherches Antilles-Guyane. 3 Juillet au 28 Août 2006.

- Stage « Initiation à la modélisation systémique ». D. Sauvant, D. Wallach, O. Martin. INRA. , Centre de Recherches Antilles-Guyane, 3 au 6 Avril 2006.

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- Conseil Scientifique de la Station de Recherches Zootechniques, INRA. , Centre de Recherches Antilles-Guyane. Communication orale : « Etude des facteurs limitant l'ingestion et la digestion chez les petits ruminants. Valorisation des fourrages tropicaux ». Janvier 2006.

- Encadrement d'un stagiaire- 4ème année ISARA Lyon : « Comportement alimentaire et granulométrie du contenu ruminal de moutons recevant deux repas par jour ». INRA. , Centre de Recherches Antilles-Guyane. 6 Juin au 6 Septembre 2005.

- Encadrement d'un stagiaire « Ouvrier »- 1ere année ESCOM : « Participation à la préparation des échantillons destinés aux analyses physico-chimiques en production animale. » INRA. , Centre de Recherches Antilles-Guyane. 1er Juillet au 31 Août 2005.

- Production des Ateliers Démarche Qualité, INRA. , Centre de Recherches Antilles-

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- Université d'Eté Européenne, Université des Antilles et de la Guyane, Guadeloupe. Communication orale : « Evaluation de la dureté des parois : Adaptation d'une méthode d'enregistrement du comportement alimentaire ». 1er au 10 Juillet 2004.

- Formation aux statistiques expérimentales, INRA. , Centre de Recherches Antilles-Guyane. 15 au 24 Juin 2004.

- Cours d'anglais intensif, INA-PG. 14 au 16 Avril 2003.

- Rencontres INA-PG. INA-PG. 17 et 18 Avril 2003.

- Cours de Zootechnie- Module 22- Lycée Agricole- Guadeloupe. 13 Janvier au 19 Mai 2003.

- Introduction aux modèles linéaires mixtes, INRA. , Centre de Recherches Antilles-Guyane. 29 Avril au 3 Mai 2002.

- Conduite de projet, , INRA. , Centre de Recherches Antilles-Guyane. 19 et 20 Février 2002.