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Effets de la défaunation de ruminants sur les performances de production, en fonction de la ration ingérée. Etude des variations de la protéosynthèse et de la cellulolyse microbienne ruminale

Maguy Eugène

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Maguy Eugène. Effets de la défaunation de ruminants sur les performances de production, en fonction de la ration ingérée. Etude des variations de la protéosynthèse et de la cellulolyse microbienne ruminale. Sciences du Vivant [q-bio]. INAPG (AgroParisTech), 2002. Français. NNT : 2002INAP0015 . pastel-00000348

HAL Id: pastel-00000348

https://pastel.hal.science/pastel-00000348v1

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INSTITUT NATIONAL AGRONOMIQUE PARIS-GRIGNON
ECOLE DOCTORALE ABIES

THESE

Pour obtenir le grade de

DOCTEUR DE L'INSTITUT NATIONAL AGRONOMIQUE PARIS-GRIGNON

Présentée et soutenue publiquement
Par

Maguy EUGENE

Le 5 juillet 2002

Effets de la défaunation de ruminants sur les performances de production, en fonction de la ration ingérée. Etude des variations de la protéosynthèse et de la cellulolyse microbienne ruminale.

JURY

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REMERCIEMENTS

Je tiens à remercier la Région Guadeloupe qui a cofinancé ce projet de thèse avec le département de Génétique de l'INRA. Ce travail a été réalisé à l'Unité de Recherches Zootechniques (URZ) du Centre de Recherches Agronomiques des Antilles-Guyanne (CRAAG).

Je remercie particulièrement Harry Archimède, pour son encadrement tout au long de ma thèse, pour la confiance qu'il m'a accordée pour la réussite de ce projet, sa rigueur scientifique et sa grande disponibilité.

Je tiens à remercier M. Daniel Sauvant pour avoir accepté d'être mon directeur de thèse, je le remercie également pour l'intérêt qu'il a porté à ce travail durant ces trois ans et demi, pour m'avoir guidée et pour ses encouragements.

Je suis très honorée et tiens à remercier les membres du jury :

M. D. Sauvant, pour m'avoir acceptée au sein de l'école doctorale ABIES et accepté de présider le jury

M. M. Doreau, J.P. Jouany, pour avoir accepté de lire et de faire l'analyse critique de ce mémoire de thèse en tant que rapporteurs.

M. A. Bâ et C Poncet pour avoir accepté d'être les examinateurs de ce travail de thèse.

Je suis reconnaissante aux membres de mon comité de thèse :

B. Michalet-Doreau, pour m'avoir accueillie chaleureusement au sein de son laboratoire afin d'acquérir les techniques de mesure d'activité enzymatique et pour ses nombreux conseils.

G. Fonty, pour son accueil dans son laboratoire afin d'acquérir les techniques de microbiologie, l'intérêt qu'il a porté dans la conduite des études de microbiologie, ses conseils et son soutien.

J.P, Jouany, pour son accueil au sein de son laboratoire, ses conseils dans la conduite des essais avec des animaux défaunés et son analyse critique des résultats.

Je tiens encore à remercier M Sauvant et Archimède pour avoir animé ce comité de thèse.

La réalisation de cette thèse n'aurait pas été possible sans l'investissement des membres de l'Unité Expérimentale en Production et Santé Animale du CRAAG, ainsi que des membres du Laboratoire d'analyse des Aliments du CRAAG. Je tiens à leur présenter à toutes et à tous mes sincères remerciements.

Je tiens plus particulièrement à remercier J.L. Weisbecker, directeur de l'UEPSA ainsi que toute l'équipe petits ruminants : M. Arjournin, O. Batrin, G. Désirée, P. Despois, M. Jean Bart, D. Jacquet, F. Periacarpin, F. Pommier, F. Nipeau, sans oublier J. Laroche (merci Titine). Votre bonne humeur, votre travail et votre investissement m'ont été d'un très grand réconfort. Je garderai un très bon souvenir de vous.

Ainsi que : G. Saminadin, responsable du laboratoire des aliments du CRAAG, toute son équipe : B. Calif, L. Phillibert, Chantal, Katia, Maryse, et toutes les autres. Vous m'avez chaleureusement accueillie au labo et je vous remercie infiniment pour l'ensemble de votre travail et de vos conseils.

Je tiens également à remercier tous les chercheurs pour leur appui scientifique et leur aide : M. A. Xandé président du CRAAG, G. Alexandre, M. Boval, N. Mandonnet, M. Mahieu, M. Naves, D. Renaudeau et l'ensemble du personnel de l'URZ, pour leur aide.

Je garde un excellent souvenir de mes passages dans les laboratoires suivants et remercie:
-dans le laboratoire de Microbiologie de Theix G. Fonty, E. Forano pour m'avoir accueillie, L. Millet, G. Andant, G. Chavarrot, B. Gaillard-Martinie, F. Durand, Brigitte, Faïza.
-dans le laboratoire de la Digestion Microbienne de Theix, B Doreau, C Martin, L Genestoux, M. Fabre
-dans le laboratoire de physiologie de la Nutrition et Alimentation de l'INA-PG, V. Berthelot, M.P. Poulain, F. Ude.

Je remercie tous les stagiaires qui ont participé de près ou de loin à ce travail de thèse, Christophe, Hélène, Nicolas, Olivier, Magalie. Vous avez contribué à mettre une bonne ambiance de travail gage de notre réussite. Je remercie Les Stagiaires, thésards, le personnel du CRAAG, du CIRAD-EMVT et de Theix que j'ai pu côtoyer pour leur aide, sympathie, amitié : Maguy, Isabel E., Euzébio, Isabelle F, David, Caroline, Arlette, Mélanie.

Je ne vous oublie pas non plus les trois drôles de dames, Maria, Sandrine Sophie et Patrick.

Je remercie toute ma famille pour m'avoir si chaleureusement accueillie en Guadeloupe, et mes parents pour leur soutien moral.

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INTRODUCTION GENERALE

Les données de la FAO (1997) indiquent que les populations des pays de la zone tropicale sont en progression (1.5 à 3 % par an). En conséquence, l'augmentation de la production de protéines animales (viande, lait, œuf) est l'un des objectifs majeurs des politiques agricoles des ces pays en réponse à l'augmentation des populations. L'élevage de ruminants constitue un réel enjeu en matière de développement. En effet, les ruminants permettent de transformer des biomasses végétales très diversifiées, notamment les matières fibreuses, en protéines animales.

Le développement de l'élevage des ruminants en zone tropicale fait appel à différentes sciences (nutrition, reproduction, génétique, santé) qu'il faut conduire en parallèle, de façon intégrée dans un système d'élevage. Les conditions du milieu d'élevage (températures, humidité, pathologies, qualité des fourrages...) sont difficiles et limitent les performances individuelles (production de lait et de viande). L'alimentation des ruminants d'élevage en zone tropicale a fait l'objet de nombreux travaux et plusieurs approches ont été développées.

La première approche a porté sur l'amélioration de la qualité de la ration de base. La faible valeur nutritionnelle des fourrages tropicaux est un des principaux facteurs qui limite les performances animales (Leng, 1990). Divers travaux ont été réalisés afin d'améliorer la qualité nutritive des fourrages, ainsi, de nombreuses variétés furent diffusées. L'amélioration de la digestibilité et de l'ingestibilité des fourrages, par des traitements physico-chimiques fut aussi étudiée (Chenost, 1993; Kayouli, 1994). Cependant, l'ingestibilité de ces fourrages, leur valeur protéique et énergétique, demeuraient inférieurs à ceux utilisés en zone tempérée.

Des travaux plus récents, menés à l'Unité de Recherches Zootechniques (URZ) à l'INRA de Guadeloupe par le groupe Alimentation et Systèmes Pâturés (ASP), font l'hypothèse que plus que le choix des génotypes végétaux, c'est d'abord leur gestion (âge de repousse, fertilisation, irrigation…) qui conditionne leur valeur nutritive (Aumont et al., 1995). Exploités très jeunes (moins d'un mois d'âge de repousse), les fourrages dits 'améliorés', comme les fourrages indigènes, ont des valeurs nutritives identiques à celles des fourrages tempérés (Archimède et al., 2000). Cependant, la valorisation des fourrages

au pâturage (stades jeunes), pose de nombreux problèmes (parasitisme gastro-intestinal, faible productivité des fourrages à l'hectare). Les stratégies visant à valoriser les fourrages tropicaux à des stades plus jeunes restent encore à définir.

La seconde voie d'amélioration envisagée a été la complémentation des fourrages. D'une part, il s'agit d'une complémentation catalytique dont l'objectif est de mieux couvrir les besoins des microorganismes afin d'optimiser la dégradation enzymatique ruminale. D'autre part, il convient d'apporter à l'animal les nutriments complémentaires, absents ou en faibles quantités dans le fourrage. Cependant, la complémentation catalytique n'a montré d'intérêt que dans le cas des fourrages âgés (Archimède et al., 1999). Par ailleurs, la principale limitation de l'utilisation de l'apport de concentré reste son coût.

Dans le cadre de ce présent travail de thèse, nous proposons une autre stratégie afin d'améliorer la nutrition des ruminants : **orienter la quantité et le profil des nutriments absorbés par l'élimination des protozoaires du rumen (Défaunation)**. En effet, de nombreux travaux (Bird et al., 1979; Bird and Leng, 1978; Bird and Leng, 1985; Demeyer et al., 1982; Eadie and Gill, 1971) indiquent que la croissance d'animaux défaunés (sans protozoaires) ingérant des rations fibreuses, peut être accrue jusqu'à 30% comparativement à celle des animaux conventionnels. Ce gain serait dû principalement à l'augmentation du flux intestinal d'azote microbien (diminution de la prédation des bactéries par les protozoaires) et secondairement à celle de la cellulolyse (augmentation de la population de bactéries et de champignons cellulolytiques). Cependant, ces résultats sont controversés (Abou Akkada and El-Shazly, 1965; Borhami et al., 1967).

L'examen de la bibliographie indique que la nature des rations étudiées ainsi que le potentiel de production des animaux seraient des facteurs de variation des effets de la défaunation. Le rapport azote / énergie de l'ingéré semble être un facteur de variation supplémentaire des effets de la défaunation. En effet, l'impact de la défaunation sur les performances animales serait d'autant plus significatif que le rapport azote / énergie ingéré de la ration serait faible et les besoins en protéines des animaux seraient élevés. La nature de la matière organique fermentée dans le rumen est aussi un facteur important. En effet, aucun ou peu d'effet n'est observé avec des rations riches en amidon contrairement aux rations riches en fibres et/ou en sucre. Ainsi, la défaunation devrait permettre d'augmenter la production d'animaux ingérant des aliments (fourrages

tropicaux, canne à sucre...), qui caractérisent les systèmes d'alimentation dominants de la zone tropicale.

Le principal objectif de ce travail de thèse est **d'évaluer la défaunation comme technique permettant une meilleure nutrition des ruminants. Le contexte du milieu tropical humide caractérisé par des fourrages pâturés à un âge tardif et d'aliments déséquilibrés en azote / énergie sera analysé.**

Nous nous sommes intéressés à l'effet de la défaunation sur la croissance en relation avec le type de ration ingérée, ainsi que sur la protéosynthèse et la cellulolyse microbienne. Notre hypothèse de travail est que le rapport azote / énergie des rations ingérées est le principal facteur explicatif de la réponse animale à la défaunation. L'étude de la digestion devrait nous permettre d'expliquer les variations des réponses observées.

Dans un premier temps, afin de déterminer les principaux effets de la défaunation sur la production des ruminants (viande, lait, laine) et sur certains paramètres de la digestion (digestibilité, flux de nutriments, produits fermentaires), nous avons réalisé une synthèse bibliographique des données de la littérature. Une base de données a été élaborée à partir des essais traitant des effets de la défaunation sur les productions (viande, lait et laine) et/ou sur la digestion et les produits fermentaires, en réponse à différentes rations. L'analyse quantitative de cette base de données avait pour but d'établir **les lois générales de la réponse animale à la défaunation**, en fonction des principaux facteurs explicatifs (quantités ingérées, teneur en azote / énergie de la ration, pourcentage de concentré de la ration).

Dans un second temps, nous avons mis en place deux études expérimentales à l'Unité Expérimentale en Production et Santé Animale de l'INRA de Guadeloupe (UEPSA). Les objectifs étaient de mesurer les effets de la défaunation sur les performances de croissance (essais zootechniques) et sur certains paramètres de la digestion (essais de digestion) en fonction de la ration. Pour établir **des lois de réponse animale à la défaunation, spécifiques du contexte tropical**, nous avons fait varier le type de ration et le gradient en azote / énergie de ces rations. Ainsi, ces lois pourraient être extrapolables à la large gamme de rations utilisées classiquement en zone tropicale humide. D'une part, nous avons testé des rations composées uniquement d'un fourrage tropical (*Digitaria decumbens*) en faisant varier l'âge de repousse de 14, 28, 42 à 56 jours afin de générer le gradient azote / énergie. D'autre part, un second type de rations composées de *Digitaria*

decumbens (28 jours) complémenté avec un mélange de concentré (maïs et tourteau de soja) fut testé. Nous avons fait varier les quantités de concentré, ainsi que la proportion de maïs comparativement à celle du tourteau de soja dans le concentré, afin de créer un gradient en azote / énergie. Nous nous sommes intéressés aux principaux paramètres de la digestion, ainsi qu'aux **facteurs explicatifs de la réponse animale observée : la protéosynthèse et la cellulolyse microbienne.**

La première partie de ce document sera consacrée à l'étude bibliographique des données de la littérature (Publication 1, soumise à Livestock Production). La deuxième partie de ce document sera consacrée aux résultats de l'étude expérimentale 1 (Publications 2 et 3, soumises à Animal Research) et aux résultats de l'étude expérimentale 2 (Publications 4 et 5, soumises à Animal Feed Science and Technology). Enfin, la dernière partie de ce document sera consacrée à la discussion générale des résultats, et aux principales conclusions et perspectives.

ETUDE BIBLIOGRAPHIQUE

LA DIGESTION CHEZ LES RUMINANTS : CONTRIBUTION DES PROTOZOAIRES.

Les différents composants de la ration sont réduits en petites particules, lors de la mastication ingestive qui est suivie d'une mastication mérycique. Cette réduction de la taille des particules contribue à augmenter la surface spécifique des particules afin qu'elles soient mieux dégradées par l'action des enzymes (Poppi et al., 1980). C'est la mastication mérycique qui permet la vidange du rumen et cette activité est accrue par la dégradation enzymatique ruminale (Jarrige, 1978).

Le système digestif des ruminants, hormis la bouche, a la particularité d'être composé de trois compartiments en amont de l' estomac fonctionnel (caillette), qui sont le rumen, le réseau (réticulum) et le feuillet (omasum) (Jarrige, 1978) (Figure 1). Ces différents dispositifs anatomiques ralentissent le passage des particules alimentaires au travers du tractus digestif et augmentent ainsi leur temps de séjour (Wallace, 1991). Elles y subissent une dégradation mécanique (mastication, contractions digestives) et une dégradation chimique (enzymes microbiennes du rumen).

Le rumen est le compartiment le plus volumineux puisqu'il peut contenir de 70 à 75% du contenu total de l'appareil digestif. La majeure partie des constituants digestibles de la ration est dégradée dans le rumen. La part de la dégradation ruminale des protéines, dans la digestion totale, varie de 70 à 85% et celle de la cellulose varie de 80 à 90 %. Celle des hémicelluloses est inférieure, tandis que la lignine est indigestible (Jarrige, 1978).

1. LES DIFFERENTS MICROORGANISMES DU RUMEN.

Les ruminants sont les meilleurs transformateurs de la biomasse végétale parmi les animaux domestiques. Ils utilisent très efficacement les glucides des parois végétales grâce à la présence d'un écosystème microbien ruminal performant. Le rumen constitue un écosystème anaérobie strict, dont les conditions de milieu favorisent le développement d'une microflore et d'une microfaune extrêmement importante et diversifiée (Hungate, 1966a)

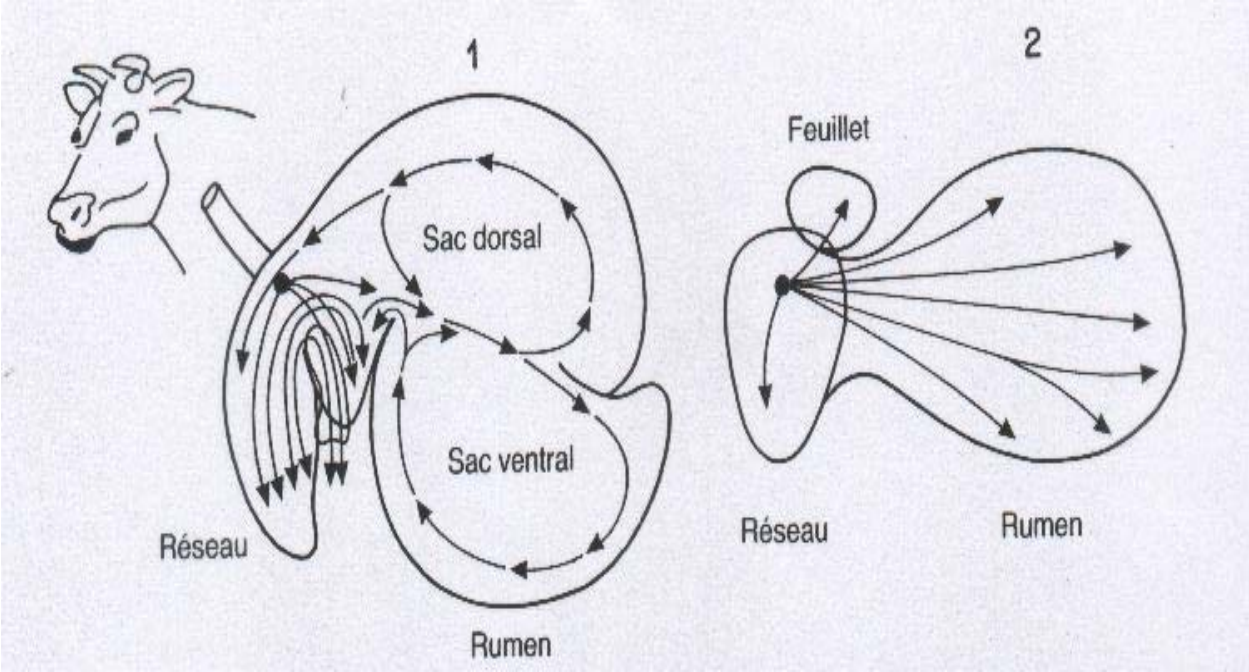

Figure 1 : Représentation schématique du rumen et du réseau dans un plan vertical (1) et un plan horizontal (2). Les flèches indiquent les mouvements du contenu. (d'après Waghorn et Reid, 1977).

Trois principaux types de microorganismes coexistent chez la plupart des ruminants domestiques : les bactéries, les protozoaires et les champignons anaérobies.

1.1. Les bactéries

Les bactéries du rumen représentent la moitié de la biomasse microbienne (Tableau 1). Elles constituent l'ensemble le plus diversifié (60 espèces recensées), et leurs concentrations varient de 10^{10} à 10^{11} cellules / ml (Fonty et al., 1995). De nouvelles techniques de biologie moléculaire ont émergé ces dernières années permettant la caractérisation des espèces, en plus des techniques classiques de dénombrement et de culture en anaérobiose (Hungate, 1966a). Ainsi le séquençage des acides nucléiques, et notamment de l'ARN ribosomal (16 S) contribue à l'amélioration de la caractérisation des espèces bactériennes.

La colonisation du tractus digestif des ruminants par les bactéries est rapide. Ainsi à l'âge d'un jour, les premières bactéries apparaissent (*Escherichia coli*, streptocoques). Les bactéries cellulolytiques apparaissent dès l'âge de 4 jours chez 75 % des jeunes ruminants (Fonty et al., 1987). Les trois principales espèces bactériennes cellulolytiques sont : *Fibrobacter succinogenes*, *Ruminococcus albus* et *Ruminococcus flavefaciens*. Elles sont présentes à des concentrations variant de 10^{8} à 10^{9} cellules / ml (Coleman, 1975). Les différentes bactéries ne sont pas distribuées de façon homogène dans le contenu ruminal, comme le suggère la stratification des digesta du rumen (Hungate, 1966b). Ainsi les bactéries associées à la phase solide du rumen, bactéries solidement adhérentes aux particules alimentaires, sont plus abondantes (50 – 75% des bactéries) que les bactéries faiblement associées ou libres de la phase liquide (Cheng and Costerton, 1980; Forsberg and Lam, 1977).

1.2. Les protozoaires

Les protozoaires sont des organismes eucaryotes unicellulaires, dont on distingue deux types : les flagellés et les ciliés (Tableau 2). Les ciliés représentent près de la moitié de la biomasse microbienne et leur concentration varie de 10^{4} à 10^{6} protozoaires / ml (Jouany, 1978). On distingue deux groupes : les holotriches et les entodiniomorphes. Les holotriches appartiennent à la classe des *Vestibulifera*, à l'ordre des *Trichostomatida*, et à la famille des *Isotrichidae*. On distingue les genres *Isotricha* et *Dasytricha*.

Espèce	Gram	Morph.	% G+C /ADN	Produits : **majeurs** et (mineurs)	Type
Prevotella ruminicola	-	bâton	49-50	**acétate, succinate** (formate, propionate, isobutyrate, butyrate, isovalérate, lactate)	hémicellulose, protéine
Ruminobacter amylophilus	-	bâton	40-42	**formate, acétate, succinate** (lactate)	amidon
Fibrobacter succinogenes	-	bâton	47-49	**acétate, succinate** (formate, propionate, isovalérate)	cellulose
Selenomonas ruminantium	-	croissant	54	**lactate, propionate, acétate, H_2, CO_2**	protéines, sucres
Butyrivibrio fibrisolvens	-	bâton courbé	36-41	**formate, butyrate, acétate, H_2, CO_2** (lactate, succinate)	répandue, cellulolytique
Anaerovibrio lipolytica	-	bâton		**propionate, succinate, acétate, H_2, CO_2** (lactate)	lipides
Vibrio (Wolinella) succinogenes	-	vibrion	47	**succinate, H_2, CO_2**	baisse H_2
Succinivibrio dextrinosolvens	-	vibrion		**acétate, succinate** (formate, lactate)	dextrines
Treponema bryantii	-	hélice	35-37	**formate, acétate, succinate**	sucres
Megasphaera elsdenii	-	coque	53-54	**acétate, butanol, pentanol, H_2** (isovalérate, valérate, isobutyrate, acétate, succinate, isobutanol, formate, propionate)	lactate
Veillonella parvula	-	coque	38-41	**acétate, propionate, H_2** (lactate)	lactate
Succinomonas amylolytica	-	coque ou bâton		**succinate** (acétate, propionate)	amidon
Ruminococcus albus	+	coque	42-46	**acétate, éthanol, CO_2** (formate, lactate)	cellulose
Ruminococcus flavefaciens	+	coque	39-44	**acétate, succinate, H_2** (formate, lactate)	cellulose
Streptococcus bovis	+	coque	37-39	**lactate, CO_2** (formate, acétate, éthanol)	amidon
Lachnospira multiparus	+	bâton		**formate, acétate, lactate, H_2, CO_2** (succinate, éthanol)	pectine
Eubacterium ruminantium	+	bâton		**formate, butyrate, lactate, CO_2** (acétate, propionate)	xylanes, sucres
Eubacterium oxidoreducens	+	bâton	36	**lactate, H_2** (formate, acétate, succinate, butyrate)	molécules aromatiques
Eubacterium limosum				**butyrate, acétate, CO_2**	hydrogène, formate
Lactobacillus ruminis	+	bâton	44-47	**lactate**	sucres
Lactobacillus vitullinus	+	bâton	34-37	**lactate**	sucres
Clostridium polysaccharolyticum	+	bâton		**formate, butyrate, acétate, H_2** (propionate, éthanol)	cellulose, amidon
Methanosarcina sp.			39	**CH_4**	méthanol, méthylamines, acétate (peu dans le rumen)
Methanomicrobium sp.			49	**CH4**	formate
Methanobrevibacter ruminantium			31	**CH_4**	formate
Methanobrevibacter sp.				**CH_4**	formate
Methanobacterium formicicum			41	**CH_4**	formate

Tableau 1 : Caractéristiques de quelques bactéries du rumen (d'après Mathieu, 1996 ; adapté de Stewart, 1991)

Phylum	Subphylum	Classe	Ordre	Famille	Sous-famille	*Genre espèce*
Mastigophorae	Metamastigophora	Retormonadea				*Chilomastix caprae*
	Parabasala	Trichomonadae				*Monocercomonas ruminantium* *Tetratrichomonas buttreyi* *Pentatrichomonas hominis*
Ciliophora	Prostomata	Prostomatea				*Buetschlia* sp.
		Vestibuliferea	Trichostomatida	Isotrichidae		*Isotricha prostoma* *Isotricha intestinalis* *Dasytricha ruminantium*
				Blepharocorythidae		*Charonina ventriculi*
			Entodiniomorphida	Ophryoscolecidae	Entodiniidae	*Entodinium* sp.
					Diplodiniidae	*Diplodinium* sp. *Eodinium* sp. *Eremoplastron* sp. *Eudiplodinium* sp. *Ostracodinium* sp. *Metadinium* sp. *Diploplastron* sp. *Enoploplastron* sp. *Elytroplastron* sp. *Polyplastron* sp.
					Epidiniidae	*Epidinium* sp. *Epiplastron* sp.
					Ophryoscolecinae	*Ophryoscolex* sp.

Tableau 2 : Classification systématique des protozoaires du rumen (Bohatier, 1991, adapté de De Puytorac et al, 1987)

Les entodiniomorphes appartenant à la classe des *Vestibulifera*, à l'ordre des *Entodiniomorphida*, la famille des *Ophryoscolecidae* et les quatre sous familles *Entodiniidae*, *Diplodiniidae*, *Epidiniidae* et *Ophryoscolecinae*. On retrouve fréquemment les genres *Entodinium*, *Diplodinium*, *Eudiplodinium*, *Diploplastron*, *Polyplastron Epidinium* et O*phryoscolex* (De Puytorac et al., 1987; Jouany, 1996).

L'implantation des protozoaires dans le rumen du jeune animal (ou dans un rumen dépourvu de protozoaire) ne peut se faire que par contact direct (salive) ou très proche avec un animal conventionnel (Coleman, 1979). Les ciliés du genre *Entodinium* apparaissent au bout de 15 jours après la naissance des agneaux (Fonty et al., 1984). Puis les holotriches s'implantent, suivis par les grands entodiniomorphes (Eadie and Hobson, 1962). La population des ciliés s'accroît constamment jusqu'à 60 jours (5.7×10^{5} protozoaires / ml). Bien qu'ils représentent une part importante de la biomasse microbienne, les ciliés du rumen ne sont pas indispensables à la vie des ruminants, contrairement aux bactéries (Fonty et al., 1995; Jouany, 1991a) .

Le type et la composition de la ration conditionnent fortement les populations de protozoaires (Jouany and Ushida, 1998). Ainsi, la population de protozoaires est modulée par la proportion de concentré de la ration. La population des entodiniomorphes (*Entodinium* 1.2×10^{6} protozoaires / ml) augmente quand le pourcentage d'amidon de la ration augmente jusqu'à 60% (Christiansen et al., 1963; Eadie et al., 1970). En ce qui concerne la population des holotriches, elle augmente (7×10^{4} protozoaires / ml) quand le pourcentage de sucres solubles de la ration augmente jusqu'à 40% (Jouany and Ushida, 1998). De même, on observe des variations des populations de protozoaires au cours du nycthémère. Ainsi, les holotriches se déplacent (chimiotactisme) du réseau vers le rumen après le repas (Abe et al., 1981). Les travaux de Yang (1992) indiquent qu'une partie importante des protozoaires est retenue dans le rumen. Cependant, la part de l'azote microbien, provenant des protozoaires, quittant le rumen, ne serait que de l'ordre de 20%.

1.3. Les champignons

Les champignons du rumen n'ont été découverts que tardivement (Orpin, 1975). Les principaux genres et espèces figurent dans le tableau 3. Leur cycle de développement est composé de l'alternance d'une phase mobile constituée de zoospores flagellées mobiles et d'une forme végétative. Cette dernière, fixée sur les tissus végétaux, est constituée d'un sporocyste pourvu de rhizoïdes (Orpin and Joblin, 1988).

Type de thalle	Nombre de flagelles sur la zoospore	Genre	Espèce	Origine	Référence
Monocentrique avec rhizoïdes filamenteux	>4	*Neocallimastix*	*N. frontalis* *N. patriciarum* *N. hurleyensis*	Rumen Rumen Rumen	Orpin (1975) Orpin et Munn (1986) Webb et Theodorou (1991)
	>4	*Piromyces*	*P. communis* *P. mae* *P. dumbonica* *P. rhizinflata*	Rumen Cæcum de cheval Cæcum d'éléphant Fæces d'âne	Orpin (1977a) Li *et al* (1990) Li *et al* (1990) Breton *et al* (1991)
Monocentrique avec rhizoïdes bulbeux		*Caecomyces*	*C. communis* *C. equi*	Rumen Cæcum de cheval	Orpin (1976) Gold *et al* (1988)
Polycentrique	>4	*Orpinomyces*	*O. joyonnii* *O. bovis*	Rumen Rumen	Breton *et al* (1989) Barr *et al* (1989)
	<4	*Anaeromyces* *Ruminomyces*	*A. mucronatus* *R. elegans*	Rumen Rumen	Breton *et al* (1990) Ho *et al* (1990)

Tableau 3 : Principales espèces de champignon anaérobies isolés du tube digestif des herbivores (Fonty et al, 1995).

La population fongique est estimée à 10^3 et 10^5 /ml, soit environ 10% de la biomasse microbienne (Fonty and Joblin, 1991). Cette estimation n'est pas très précise car les méthodes de dénombrement sont difficiles à réaliser. En effet, l'association étroite des rhizoïdes fongiques avec les végétaux, ainsi que la production irrégulière des sporocystes, rend le dénombrement incertain. De nouvelles techniques d'hybridation moléculaire à l'aide de sonde d'ADN spécifiques, notamment du genre majeur *Neocallimastix* (Brownlee, 1989), devraient permettre l'émergence de nouveaux travaux sur le dénombrement plus précis de la population fongique du rumen et de sa fonction (Brownlee, 1989; Fujino et al., 1998; Kim et al., 2002).

2. ROLE DES DIFFERENTS MICROORGANISMES DU RUMEN DANS LA DIGESTION

Les trois principales populations microbiennes possèdent l'équipement enzymatique nécessaire à la dégradation de l'ensemble des constituants de la ration, notamment des parois cellulaires des végétaux. Cependant, plusieurs facteurs conditionnent la dégradation ruminale des parois végétales : l'accessibilité des substrats aux microorganismes, leur adhésion aux particules et leur activité enzymatique (Cheng et al., 1991). Le rumen est le siège de nombreuses interactions entre les microorganismes qui doivent subsister dans cet environnement complexe (Fonty et al., 1995).

2.1. Le métabolisme azoté

Les protéines ingérées par l'animal vont être dégradées par les bactéries et les protozoaires du rumen. Cette dégradation implique deux étapes : la protéolyse, qui produit des peptides et des acides aminés et la désamination, qui dégrade les acides aminés en ammoniaque (NH3) et squelettes carbonés (AGV) (Figure 2).

L'activité protéolytique des bactéries est associée à leur paroi cellulaire (Nugent and Mangan, 1981). Ainsi, la première étape dans la dégradation des protéines est l'interaction entre les microorganismes et le substrat. Les protéines solubles sont principalement dégradées par les bactéries (Nugent and Mangan, 1981) , tandis que la contribution des protozoaires à cette dégradation est faible. Les protozoaires ingèrent les particules protéiques ainsi que des bactéries (Coleman, 1975; Ushida et al., 1986).

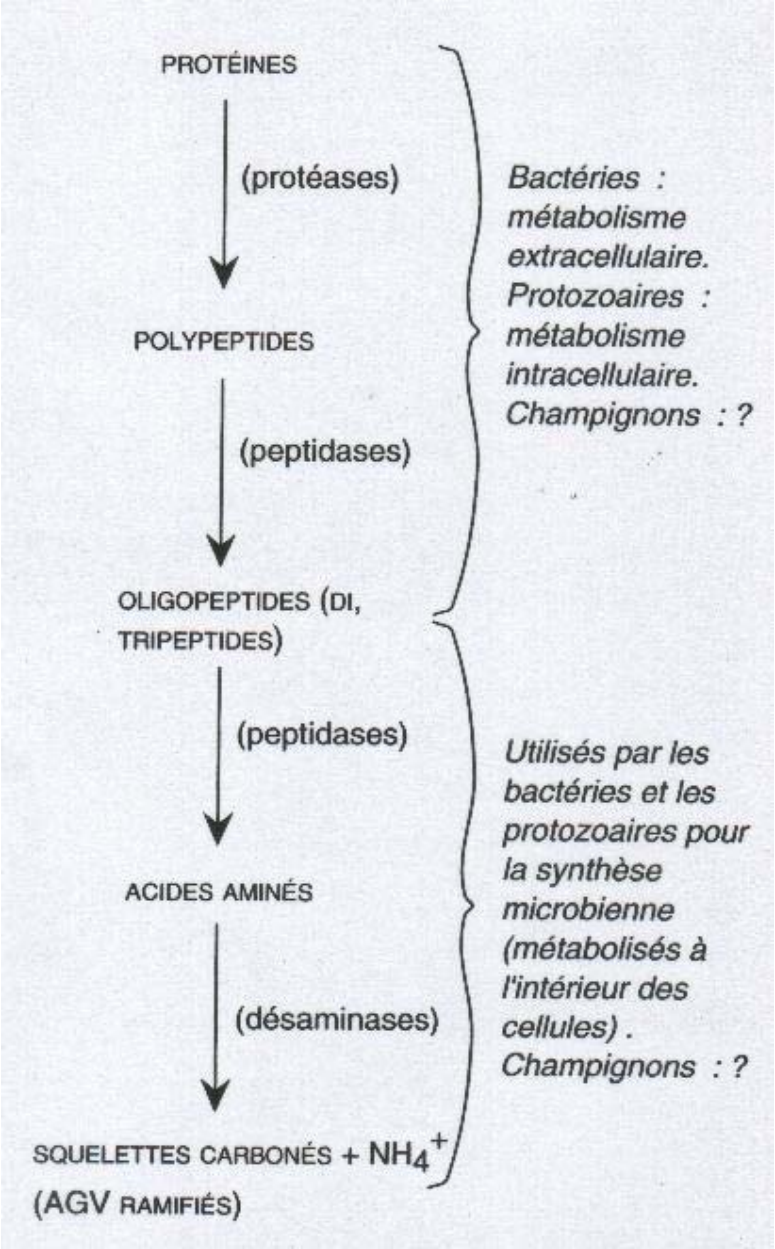

Figure 2 : Dégradation des composés azotés dans le rumen (Jouany, 1994)

Peu de champignons anaérobies du rumen possèdent une activité protéolytique importante, par conséquent leur contribution est variable (Wallace and Joblin, 1985) .

Les principales bactéries protéolytiques sont présentées dans le tableau 4. Les protéases bactériennes sont principalement des cystéine – protéases, mais les serine - protéases et les métallo protéases sont aussi présentes (Brock et al., 1982). Aucune des espèces bactériennes ne possède d'activité spécifique, mis à part *Streptococcus bovis* qui possède une très forte activité leucine amino-peptidase. Les protozoaires possèdent principalement des cysteine et aspartique–protéases. *Entodinium sp*. possède la plus forte activité, et beaucoup de protozoaires ont des protéases intracellulaires (Coleman, 1983).

Les peptides produits sont plus rapidement utilisés que les acides aminés libres correspondants (Wright, 1967). Ce sont principalement les bactéries du rumen qui les dégradent. Les produits de dégradation des peptides sont principalement extracellulaires, bien que la plupart des enzymes soient associées à la cellule (Wright, 1967). Les oligopeptides de faibles poids moléculaires sont préférentiellement intégrés aux protéines bactériennes, après avoir été hydrolysés en acides aminés au sein de la cellule bactérienne. Les acides aminés produits sont en faible concentration dans le rumen. Ils sont désaminés pour produire de l'ammoniaque et des acides gras volatils (AGVs) (Mackie and White, 1990). Les protozoaires jouent un rôle primordial dans la mesure où leur activité spécifique de désamination des acides aminés est 3 fois supérieure à celle des bactéries (Hino and Russel, 1985).

L'ammoniaque produit est issu de l'activité protéolytique, uréolytique et de désamination (acides aminés) des microorganismes (Fonty et al., 1995). Le recyclage de l'ammoniaque via l'urée dans le rumen est très important. De même, *in vitro*, le recyclage de l'azote bactérien, issu de la prédation des bactéries par les protozoaires peut représenter 90g de matière sèche / j chez le mouton (Coleman, 1975). L'ammoniaque est essentiel pour la croissance de nombreuses espèces bactériennes, qui l'assimilent grâce à une NAD/NADP glutamate deshydrogénase dans les conditions normales du rumen (Wallace and Cotta, 1988). La très forte affinité des bactéries, vis à vis de l'ammoniaque, leur confère la capacité de se développer à partir d'une concentration ruminale de NH3 minimale, variable selon les auteurs, d'environ 50 mg NH3-N / ml (Satter and Slyter, 1974).

Gram négatives	Gram positives
Prevotella ruminicola	*Butyrivibrio fibrisolvens*
Bacteroides amylophylus	*Streptococcus bovis*
Megasphaera elsdenii	*Lachnospira* sp.
Selenomonas ruminantium	*Eubacterium* sp.
Succinivibrio sp.	*Clostridium* sp.
	Propionibacterium sp.

Tableau 4 : Principales bactéries protéolytiques du rumen (Mackie and White, 1990).

2.2. Le métabolisme glucidique

Les bactéries cellulolytiques participent activement à la dégradation des polymères des plantes (Figure 3). Cette dégradation nécessite l'action d'enzymes spécialisées qui sont distribuées parmi les différents microorganismes fibrolytiques (Doré and Gouet, 1991). Ces différentes enzymes sont souvent regroupées au sein de complexes de hauts poids moléculaire (cellulosome) qui leur permettent sûrement d'être plus efficaces (Fonty and Forano, 1999). L'hydrolyse de la cellulose se fait par l'action de cellulases classées en trois groupes: les endoglucanases, les exoglucanases et les béta – glucosidases. De même, les hémicelluloses sont hydrolysées par trois groupes d'enzymes : des endoxylanases, des exoxylanases et des béta – xylosidases (Forano et al., 1996). Les protozoaires ciliés et les champignons possèdent aussi ce potentiel enzymatique (Fonty and Joblin, 1991; Grenet et al., 1989; Jouany and Ushida, 1990).

Les ciliés (larges entodiniomorphes) peuvent ingérer des particules alimentaires et les digérer à l'intérieur de vacuoles (Ushida , 1994). Ils possèdent une plus grande activité cellulasique que les ciliés *Entodinium*. L'activité cellulasique est associée à la fraction cytoplasmique des protozoaires. Leur activité cellulasique semblerait plus élevée que celle des bactéries (Conchie, 1954). Cependant, il est difficile de distinguer la contribution des bactéries fixées aux particules ingérées, de celle des protozoaires dans la dégradation de la cellulose (Fonty et al., 1995). Les hémicelluloses sont aussi activement dégradés par les larges entodiniomorphes, tandis que les holotriches ont un rôle mineur dans la cellulolyse. Ce sont les trois principales bactéries cellulolytiques, *Ruminococcus albus*, *Ruminococcus flavefaciens* et *Fibrobacter succinogenes* qui contribuent le plus à l'activité cellulolytique des microorganismes adhérant aux particules du rumen (Fonty et al., 1995) (Tableau 5).

Les produits de la dégradation des polyholosides sont des oligosaccharides solubles et des sucres, qui sont ensuite fermentés pour générer des molécules d'ATP utilisées par les différents microorganismes. La plupart des holotriches fermentent les sucres solubles, tandis que peu d'entodiniomorphes en sont capables (Jouany and Ushida, 1998). Cependant, certains sucres solubles ne sont pas fermentés par les holotriches (mannose, glucosamine).

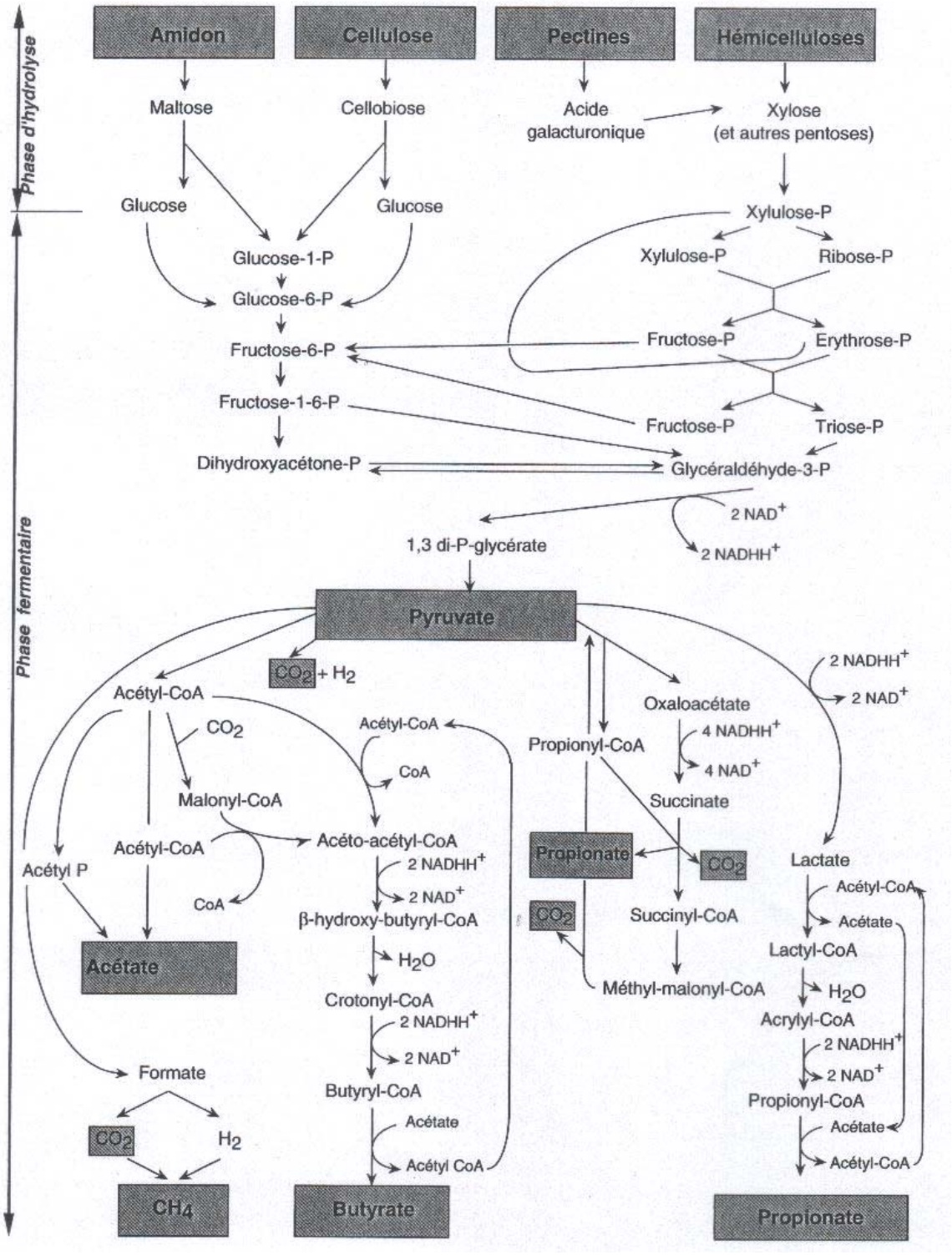

Figure 3 : Voies du métabolisme des glucides dans le rumen (Jouany, 1995)

L'amidon est ingéré sous forme de granules par les ciliés, surtout par *Isotricha* qui possède la plus grande activité amylolytique parmi l'ensemble des ciliés. Ceci contribue à limiter la diminution du pH, liée à la dégradation de l'amidon. Les espèces bactériennes amylolytiques du rumen, attachées aux grains d'amidon, sont souvent ingérées par les protozoaires. Les produits de la dégradation de l'amidon par les protozoaires sont : l'acide butyrique, H_2, CO_2 ainsi que de plus faibles quantité d'acide propionique et d'acide lactique.

	Métabolites fermentaires											
	Formate	Acétate	Propionate	Butydrate	Valérate	Caproate	Ethanol	Succinate	Lactate	H_2	CO_2	CH_4
Fibrobacter succinogenes	+	+						+				
Ruminococcus albus	+	+					+			+	+	
Ruminococcus flavefaciens	+	+						+		+	+	
Butyrivibrio fibrisolvens	+	+		+							+	
Prevotella ruminicola	+	+	+					+				
Selenomonas ruminantium		+	+						+			
Streptococcus bovis		+	-						+			
Ruminobacter amylophilus	+	+						+				
Succinomonas amylolytica		+	+					+				
Eubacterium ruminantium	+	+		+							+	
Succinivibrio dextrinosolvens	+	+						+	+			
Megasphaera elsdenii		+		+		+				+	+	
Lachnospira multiparus	+	+					+		+	+	+	
Anaerovibrio lipolytica		+	+					+				
Veillonella alcalescens			+									
Methanobacterium ruminantium												+
Wolinella succinogenes								+				
Eubacterium limosum		+		+								

Tableau 5 : Substrats dégradés et fermentés par les principales espèces bactériennes du rumen. La majorité des souches peuvent assurer cette fonction (+) ou (-) (Fonty et al ; 1995).

SYNTHESE BIBLIOGRAPHIQUE : EFFETS DE LA DEFAUNATION SUR LA CROISSANCE, L'INGESTION ET LA DIGESTION DE RUMINANTS

Les conclusions des effets de la défaunation sur les performances de croissance et sur la digestion des ruminants restent controversés dans la littérature. La ration semble être le principal modulateur des effets observés entre les différents essais.

Le premier objectif de ce travail de thèse est de déterminer les effets de la défaunation en rapport avec la composition de la ration. Une base de données bibliographiques (exhaustive) des essais de la littérature traitant des effets de la défaunation sur la croissance, l'ingestion, la digestion et les paramètres fermentaires du rumen a été constituée. L'analyse des données de cette base par une méthode statistique quantitative, appelée la méta-analyse (St-Pierre, 2001), nous a permis: 1) de déterminer les principaux effets de la défaunation sur les performances de production et les paramètres de la digestion de ruminants; 2) d' établir des lois de réponse animale à la défaunation (Publication 1).

Publication 1

Quantitative meta-analysis on the effects of defaunation of the rumen on growth, intake and digestion in ruminants

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(Livestock production)

Abstract :

A quantitative meta-analysis was applied on 90 papers and 169 trials dealing with defaunation of the rumen (removal of protozoa from the rumen) in order to point out the major quantitative effects of defaunation and identify interfering factors. Generally speaking defaunation significantly increased average daily gain (11% on average, 64 trials) but didn't affect dry matter intake. As a consequence, defaunation improved food conversion efficiency. These different effects were especially obvious as diets were both, high on forage and low on nitrogen and as animals had a low growth potential. Defaunation significantly altered organic matter digestibility (-1.7 units, 52 trials) and cell wall carbohydrate digestion (-5.7 units, 15 trials). The same trend was observed for rumen digestibilty. On the other hand, duodenal nitrogen flow, expressed as a rapport of nitrogen intake, was enhanced by defaunation. Duodenal microbial nitrogen flow, expressed as the percentage of live-weight, increased by 21% after defaunation. The latter two results may account for the lower ammonia concentration in the rumen (-50.3 mgNNH3/L, 75 trials) and the higher microbial synthesis efficiency (11.8 g microbial N / Kg OMDr -OM apparently degraded in the rumen- 19 trials) observed for the cilate-free (defaunated) animals. The level of concentrate in the diet strongly interfered with the effect of defaunation on the pH in the rumen. pH in the rumen was increased by defaunation with diets containing a high level of concentrate, whereas it decreased with diets containing a low level of concentrate. The molar proportion of propionic acid was enhanced by defaunation, whereas the molar proportion of butyric acid was lowered by defaunation, in the ruminal pool of Volatile Fatty Acids (VFA). Finally, defaunation increased the ruminal volume and the liquid phase outflow rate, but to a lesser extent.

We consider that these results strongly suggest a more efficient use of nutriments in cilate-free animals, especially when they are given poor diets limiting animal production.

Key-words *:* Meta-analysis ; Defaunation ; Ruminants ; Animal production ; Digestion ; Intake

Abbreviations used

ADG : average daily gain; DMI : dry matter intake; Dt : total tract digestibility; Dr : rumen digestibility; NH3 : ammonia concentrations in the rumen; VFA : Volatile Fatty Acids concentrations in the rumen; NI : Nitrogen intake ; Duo. NAN: duodenal non ammonia Nitrogen flow; Duo.MN : duodenal microbial Nitrogen flow.

1. Introduction

Domestic ruminants can valorise fibrous vegetal resources unused by men and other livestock animals. As primary consumers and transformers of this plant biomass, they represent an important source of animal protein for men. The microflora and microfauna play an important role in this process. The main role of this complex microbial ecosystem is to hydrolyse and ferment cell wall carbohydrates into available nutrients for the hosts they inhabit (Hungate, 1966). Protozoa can use most of the soluble carbohydrates, starch and cell wall carbohydrates as carbon and energy sources, after hydrolysis and fermentation in the rumen (Fonty et al., 1995). Dietary protein and bacteria are the main protein sources of protozoa which are degraded into AA, to be incorporated into their own proteins.

Many studies have tried, for many decades, to specify the interactions between the microbial population of the reticulo rumen (Hungate, 1955). Studies on defaunation of the rumen are hard to handle especially because it is difficult to keep the animals free of protozoa. As a consequence, as far as protozoa are concerned, *in vivo* studies have privileged the analysis of the differences in digestion and performances between conventional (faunated) and cilate-free animals. Despite numerous studies on the effects and the zootechnical interest of defaunation (Nolan et al., 1981; Bird and Leng, 1985; Bird, 1989; Jouany, 1991; Jouany and Ushida, 1998) conclusions remain controversary. The existing reviews on the topic, used a qualitative approach to analyse published data. The aim of the present work is to achieve a quantitative review of the published results of defaunation effects, with more data and especially using the meta-analysis (St-Pierre, 2001) which is a statistical method.

2. Material and methods

Ninety papers, comprising 169 comparisons, were compiled into a database. In view to be integrated into this database, data on production (animal growth, milk or wool production) had to be available and/or data on digestion, obtained on defaunated and conventional animals, in similar diets and environments. Most of the trials (60%) were dealing exclusively with digestion and 40% were dealing with production data (animal growth) and sometimes digestion. All three animal species were represented in studied animals, cattle, sheep and goat. Most of the animals were ovine males (80%), there were few goats. Growing animals represent 64% of the overall amount. Generally, for digestion trials adult animals were chosen (36%) with well-established ruminal flora and fauna.

Diets studied were diversified. Most of them (81%) were mixed diets, that's to say composed of forage complemented with concentrates. Seven percent of the diets were only composed of concentrate whereas 12% were only composed of forage. Forages studied were distributed as follows: hay (37%), straw (26%), roughage by-products (19%, corn cobs, oaten chaffs), pasture (13%), silage (6%). Concentrates were composed of classical cereals (33%, corn, barley, wheat), proteaginous and oleaginous seed (21%, soya, groundnut, peas), cereal by-products (6%, bran, soja meal) and of less classical supplements (40%, molasses, milk, urea, amino-acid infusion).

Chemicals (64%, Manoxol OT, alkanates, teric GN9, calcium peroxyde, copper sulfate), ruminal manipulation (11%, emptying the rumen), natural dietary compounds (9%, plant saponin, concentrate given ad libitum, milk, vegetable oil) and isolation of new born animals from their mother (16%) were used in order to achieve defaunation of the animals. Trials where the effects of additives, other than defaunating, were significant, were discarded.

The average daily gain (ADG, g/d) and the wool production (g/d) were selected as the two major productions measures (Table 1). For the study of intake, the dry matter intake (DMI, g DM/d) and the nitrogen intake (NI, g/d) were selected. To compare results, the latter were expressed as a percentage of body live-weight (%LW). Feed conversion efficiency (FCE) was considered (DMI / ADG, g/g), for trials where DMI differences were observed between defaunated and faunated animals.

Parameters	Nb data	Mean		sd	References[c]
		Defaunated	Faunated		
Diet caracteristics, on DM[a]					
organic matter (OM, %)	222	90.6	90.62	3.24	1-8;11-27;31;33-35;38-40;42-44; 48;49;51-57;60;62;66;68-74; 77-81;83-88
Crude protein (CP=N*6.25, %)	328	14.17	14.18	4.01	1 à 90
Neutral detergent fiber (NDF, %)	306	45.69	45.69	16.5	1 à 90
Percentage of concentrate (PCO, %)	328	46.14	46.16	32.74	1-44;46-49;51-57;60-90
Production, on LW[b]					
Daily live-weight gain (LWG, %)	128	0.444	0.401	0.319	1-4;6-9;11-34;42-44;85
Daily wool growth (Wool, %)	56	6.23	5.48	2.66	7-9;10-12;23;38;39;42-44
Intake, on LW					
Dry matter intake (DMI, %)	294	2.53	2.51	1.02	1-4;6-9;11-44;46;48-52; 54-56;60-74;76-90
Organic matter intake (OMI, %)	232	2.29	2.28	0.89	1-4;6-9;11-35;38-44; 51-55;57-74;76-90
Nitrogen intake (NI, %)	264	6.02	6.02	4.01	1-4;6-57;60-90
Digestibilities					
OM total tract digestibility (DtMO, %)	104	65.89	67.54	11.83	11;12;16;17;27;31-38;48; 52-61;66-78;81;83;87;90
NDF total tract digestibility (DtNDF, %)	30	55.37	61.09	6.70	16;27;31;48;54;66;69; 71;74;78;81;83;87
Nitrogen total tract digestibility (DtN, %)	76	68.68	69.92	9.71	16;27;31;35;48;53;54;56;58; 60-62;66-78;87;89;90
OM ruminal digestibility (DrMO, %)	38	41.58	48.21	8.39	12;48;49;60;62;68; 70-72;77;78;80-82;90
NDF ruminal digestibility (DrNDF, %)	22	46.77	51.90	9.39	48;51;54;60;66;71;77;79;81

DM [a] : Dry matter, LW[b] : Live weight (Kg), References[c] : see References used in the database

Table 1 : Effects of defaunation of the rumen on growth, intake and digestion in ruminants. Mean values of parameters analysed in the bibliographic database.(Part I)

Usual analyses of the diet determined were : dry matter (DM), organic matter (OM), total crude protein (CP = Nx6.25) contents of the diets and cell wall components, NDF, ADF and ADL.

Digestion parameters selected were OM total tract digestibility (DtOM) or ruminal digestibility (DrOM), Cell wall (DtNDF, DrNDF), nitrogen (DtN) digestibilities. Fermentative parameters of the rumen were rumen volatile fatty acids concentrations (VFAs, mM), ammonia concentrations in the rumen (NH3, mgN/L), pH in the rumen. Duodenal nitrogen daily flows considered were non-ammonia nitrogen (Duo. NAN, g/d), microbial nitrogen (Duo. MN, gN/d). Urinary and faecal nitrogen daily flows were also computed (Table 2).

As experimental design was unbalanced due to the gathering of various data in the base, the interpretation of data has favoured the comparison two by two of parameters studied, between defaunated and faunated animals given the same diet. In a first step, systematic graphical analysis has allowed to put in evidence the global relationships observed between the literature data. A primary comparison of results between defaunated and faunated animals on the same trial, considered as blocks, was conducted, by analysis of variance using GLM (Minitab, State College, PA).

$Y_{ij} = \mu + E_i + D_j + e_{ij}$ [model 1]

Y_{ij} is the observed character in trial i, μ the global mean, E_i the effect of the trial i, D_j Defaunation effect within a trial i (1 DF), and e_{ij} represents the unexplained residual error.

When another measured character (X) appeared to interfere with defaunation effect, it was introduced as a covariate using GLM, following model 2:

$Y_{ij} = \mu + E_i + D_j + a\ Xij + e_{ij}$ [model 2]

a is the regression coefficient of Y on the covariable X ; all other symbols are as defined in model 1.

Parameters	Nb data	Mean		sd	References[c]
		Defaunated	Faunated		
Nitrogen flows					
Duodenal non-ammonia Nitrogen (Duo.NAN, gN / d)	44	31.16	26.89	14.66	27;48;49;53;60;62;66;68; 70-72;77;78;80-90
Duodenal microbial Nitrogen flow (Duo.MN, gN / d)	38	17.58	15.75	8.71	48;49;60;62;66;68;70-72; 77;78;80;89;90
Urinary Nitrogen flow(Nu, gN / d)	16	27.59	31.77	22.87	31;66;73;78;90
Fecal Nitrogen flow (Nfec, gN / d)	64	13.00	12.67	14.99	16;17;27;31;35;48;53-56; 60-62;66-71;73-78;87-90
Digestion in the rumen					
ruminal pH (pH unit)	88	6.28	6.33	0.32	2;14;17;19;26;27;32;36;40; 48-55;61-69;72-84;86-90
Volatile Fatty Acids concentration (VFA, mM)	136	85.90	89.15	18.19	1;12;18-36;40-57;60-90
Acetate molar proportion (C2, %)	118	62.78	64.22	8.62	1;6;19-27;32;33;37;40;44-57;61-90
Propionate molar proportion (C3, %)	120	23.05	20.20	5.99	1;6;19-27;32;33;37;40;44-57;61-90
Butyrate molar proportion (C4, %)	116	10.94	12.57	5.49	1;6;19-27;32;33;37;40;44-57;61-90
Ammonia concentrations (NH3, mgN / L)	150	117.36	167.65	69.33	1;13-22;27-39;40-48; 51-57;60-72;74-90
Microbial synthesis efficiency (MSE, g MN / Kg OMDr)	38	39.96	28.14	13.29	27;48;49;60;62;66;68;70; 71;77;78;80;90
OM apparently degraded in the rumen (OMDr, g)	38	442	506	451	12;48;49;60;62;68;70-72; 77;78;80-82;90
Ruminal volume and turn-over					
Ruminal volume (Vr, %LW)	34	11.12	10.46	1.49	41;51;54;55;57;60;62; 70;78-81;83;88;90
Fractional turn-over rate					
Liquid phase (Kl, %/h)	36	8.19	8.26	2.65	41;54;55;57;62;70;78-80;83;87;88;90
Solid phase (Kp, %/h)	30	5.36	5.17	2.07	41;54;55;60;62;66;70;79;83;87;88

DM [a] : Dry matter, LW[b] : Live weight (Kg), References[c] : see References used in the database

Table 2 : Effects of defaunation of the rumen on growth, intake and digestion in ruminants. Mean values of parameters analysed in the bibliographic database.(Part II)

In order to be more specific and to better interpret interactions between some factors, analyses were also conducted on the outcome for measured variable Y according to the following model 3 :

$Y_i = \mu + E_i + a_j X + ej$ [model 3]

Y_{ij} is the difference between parameter Y for defaunated and faunated groups observed in trials i. It is considered as the response to defaunation (Res.); μ is the global mean, the E_i effect among studies i, X is the covariable, a_j is the regression coefficient of Y on X and e_j represents the unexplained residual error.

3. Results

3.1. Effect of defaunation on animal growth

Mean average daily gain measured in the database, expressed as a percentage of live-weight (ADG %LW), was 0.4223 ± 0.3187%LW. The first analysis conducted, considering available data from 64 trials, revealed a significant increase in average daily gain +0.040 ($p<0.01$) after defaunation (0.444 *vs* 0.401, s.e.m. = 0.095 %LW). A similar effect ($p<0.01$) was observed when average daily gain was expressed as g/Kg of metabolic weight (ADGMW, 10.67 *vs* 9.68, s.e.m. = 2.15 g/kg MW).

A second analysis was conducted, taking into account the level of dry matter intake (DMI%LW, 3.22 ± 0.98 %LW). Figure 1 indicates intra and global relationship between ADG %LW and DMI%LW. A significant effect of the covariate DMI%LW, with a regression coefficient of 0.198 ± 0.023 %LW was observed. In that case the effect of defaunation still remained significant and of similar amplitude (0.440 vs 0.404, s.e.m. = 0.082 %LW, $p<0.02$).

A third analysis of residual variations of the preceding model was conducted in order to point out the possible interfering factors. Two characteristics of the diet interfered significantly, the percentage of concentrate (PCO, 38.43 ± 28.61%) and the N%NDF ratio of the diet (6.40 ± 4.18 %). Hence, an analysis was conducted integrating DMI%LW and PCO as covariates into the model; and trial, defaunation and defaunation interaction with PCO as qualitatives factors. All the effects, except PCO effect ($p=0.09$), were highly significant ($p<0.001$), s.e.m. was 0.073 %LW.

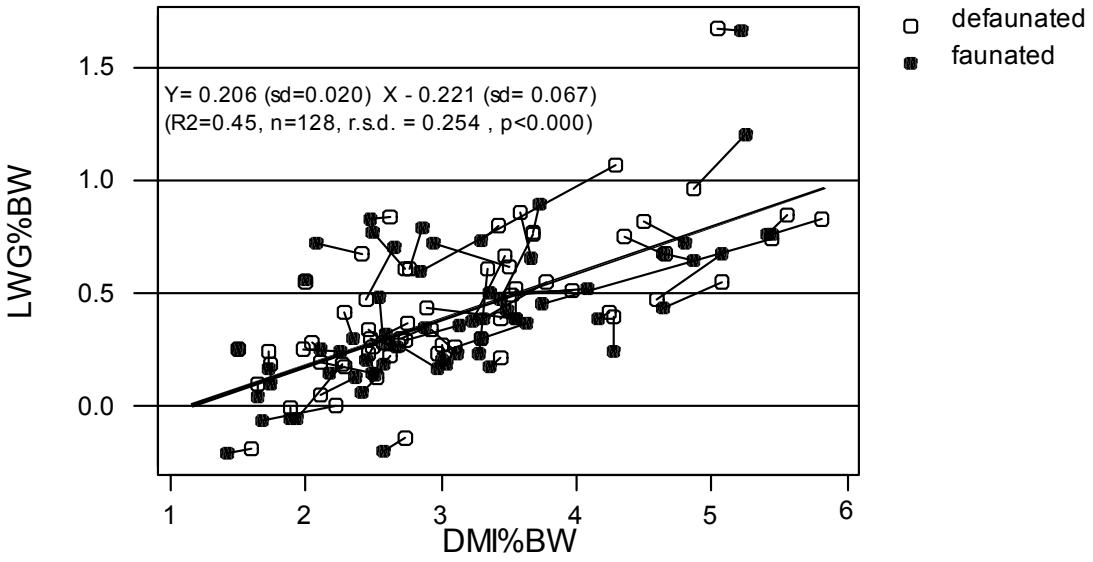

Figure 1 : Effect of defaunation on average daily gain (ADG%LW) of ruminants in relation to dry matter intake (DMI%LW). Synthesis of the literature data. BW : body weight.

In that case, the regression coefficient of covariate DMI%LW was 0.211 ± 0.034 %LW and that of PCO was 0.090 ± 0.053 % versus 0.092 ± 0.053 % for defaunated and faunated groups respectively. This result indicates that the effect of defaunation on average daily gain was more pronounced with diets containing high forage levels. Another analysis was conducted integrating DMI%LW and N%NDF ratio as covariates into the model; and trial, defaunation and defaunation interaction with N%NDF ratio as qualitatives factors. All the effects, except N%NDF ratio effect (p=0.42), were highly significant (p<0.001), s.e.m. was 0.073 %LW. In that second case, the regression coefficient of covariate DMI%LW was 0.226 ± 0.036 %LW and that of N%NDF ratio was -0.167 ± 0.194% versus -0.160 ± 0.194% for defaunated and faunated groups respectively. The effect of defaunation still remained comparable to preceding approaches and was 0.035 (0.440 *vs* 0.405%LW). This result indicates that the effect of defaunation is more obvious for diets low on nitrogen compared to cell wall contents (NDF). To sum up these interactions, a multiple regression of the response to defaunation of average daily gain %LW on either the response of DMI %LW, PCO and N%NDF ratio was calculated (Figure 2) :

Res. ADG%LW = 0.128 - 0.0074 N%NDF - 0.0013 PCO + 0.213 Res. DMI%LW
(n = 62, R = 0.68, r.s.d. = 0.099)

All the coefficients were significant, except for N%NDF ratio (p=0.08).

3.2. Effect of defaunation on voluntary intake

An analysis of variance of DMI%LW was conducted, considering only trials where intake was not limited and where there was a difference between faunated and defaunated groups (83 among 146 trials). Even with these trials no effect of defaunation on voluntary dry matter intake appeared (p=0.37).

3.3. Effect of defaunation on food conversion efficiency

Food conversion efficiency (FCE = 8.44 ± 4.02 g/g, n = 115) was reduced by 1.41 g /g (7.78 *vs* 9.19 g/g) after defaunation. This effect of defaunation was more pronounced with trials where ADG %LW was low, especially when it was inferior to 0.5%LW (figure 3). There was no difference between ovine and bovine responses to defaunation.

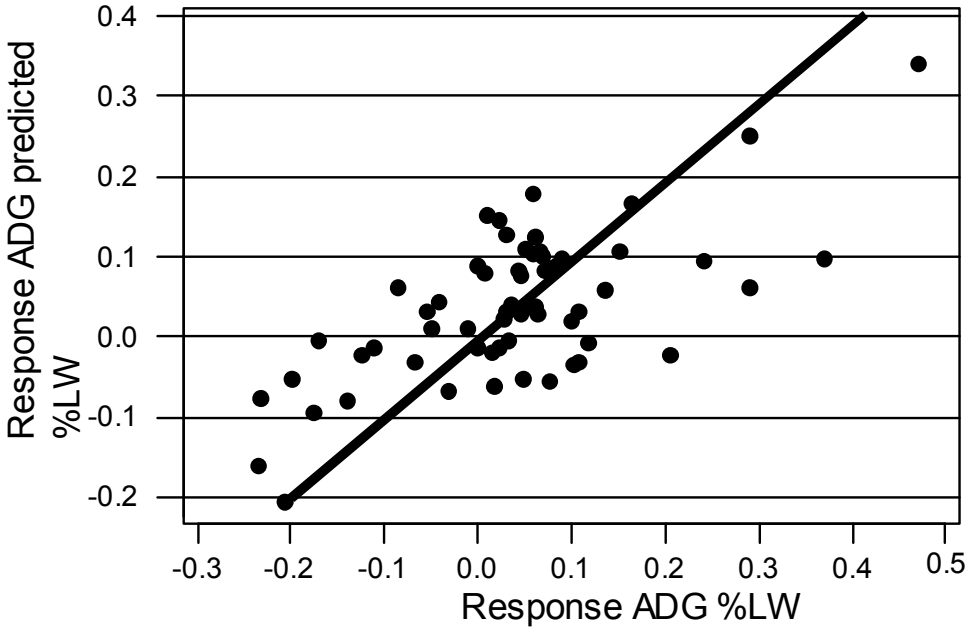

Figure 2 : Prediction of average daily gain response to defaunation (Res. ADG%LW predicted) in relation to dry matter intake (DMI %LW), percentage of concentrate (PCO) and nitrogen over energy ratio (N%NDF) in the dietary regimen. Synthesis of the literature data. Res. ADG%LW : observed response.

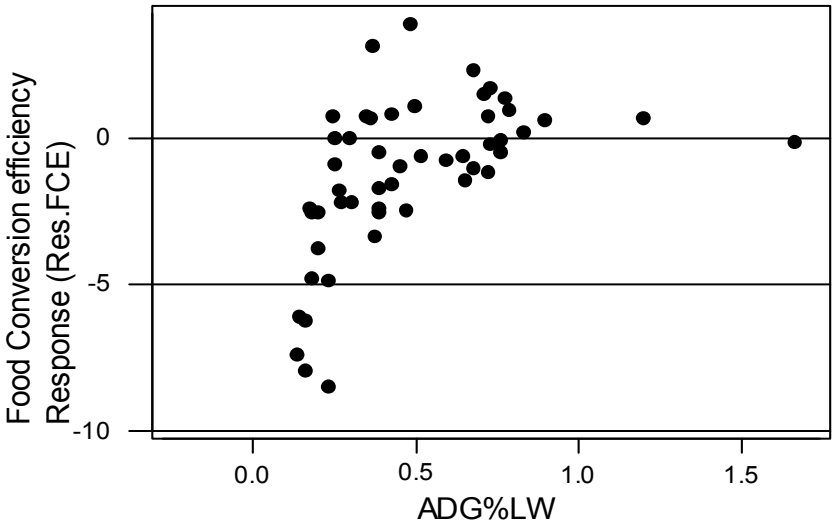

Figure 3 : Defaunation and average daily gain (ADG%LW) effects on the response of food conversion efficiency (Res. FCE) of ruminants.
Synthesis of the literature data.

3.4. Effect of defaunation on wool production

An analysis of variance of wool production was conducted with 28 trials. A highly significant increase ($p<0.001$) of this production appeared after defaunation (6.23 vs 5.48, s.e.m.= 0.56 g/j). Furthermore, response of wool production (Res. Wool) is higher with high level of intake and low level of N%NDF ratio ($p=0.003$) :

Res. Wool = 0.36 + 0.46 DMI%LW – 0.130 N%NDF (n= 25, R^2 = 0.77, r.s.d. = 0.41).

3.5. Effect of defaunation on digestibilities and digestive flows

3.5.1 Total tract digestibilities

An analysis of variance integrating 52 trials was applied to total tract OM digestibility (DtOM, 66.7±11.8 %), total tract cell wall digestibility (DtNDF, 58.2 ± 6.7 %) and total tract nitrogen digestibility (DtN, 66.9± 9.7 %). Analysis of 52 trials led to the conclusion that there was a significant depression ($p=0.001$) of DtOM by 1.7 units after defaunation (65.9 *vs* 67.5, s.e.m. = 2.3%). The trials indicating DtNDF values were less numerous (15), nevertheless, depression of DtNDF due to defaunation appeared to be more important (5.7 units) than that of DtOM and significant ($p<0.001$, 55.4 *vs* 61.09, s.e.m. = 2.6%).
When DtOM and DtNDF were measured simultanously (n = 15), DtOM appeared to decrease by 0.26 ± 0.13 unit while DtNDF dropped by 1 unit. Only a tendency ($p=0.07$) towards the decrease in DtN appeared after defaunation (38 trials, 68.68 *vs* 69.92, s.e.m. = 2.9%). Examination of residual variations of these different models revealed no major interfering factors with digestibilities.

3.5.2 Digestibilities in the reticulo-rumen

An analysis of variance was applied to apparent OM digestibility in the rumen (DrOM, 66.7±11.8 %), and apparent cell wall digestibility in the rumen (DrNDF, 49.3 ± 9.4 %). The analysis of DrOM through 19 trials led to the conclusion that there was a significant depression ($p=0.001$) of DrOM by 7.31 units after defaunation (41.6 *vs* 48.9, s.e.m. = 3.1%). Trials indicating DrNDF values were less numerous (12), depression of DrNDF due to defaunation appeared nearly significant ($p<0.07$) and lower (4.67 units)

than that of DrOM (46.8 *vs* 51.4, s.e.m. = 5.1). For these trials the percentage of OM digestible in total tract which is degraded in the rumen was significantly lower (p = 0.002) for defaunated animals (62.3 *vs* 70.5, s.e.m. = 5.1%).

3.5.3 Duodenal, urinary and faecal nitrogen outflows

For all the analyses on duodenal nitrogen outflows, nitrogen intake, expressed as %LW, was taken into account as a covariate in statistical models, except for duodenal outflows of nitrogen (Duo.N%NI) and crude protein (Duo.CP/Kg DMI).

An analysis of variance of 21 trials indicates duodenal non-ammonia nitrogen outflow values (expressed as %LW) was performed. Defaunation induced a significant (p < 0.001) increase in the latter (4.70 *vs* 4.00 ± 0.3 %LW). Duo.N %NI (n=44, Duo.N%NI = 103,39 ± 24.67%) significantly increased (p<0.001%, 22 trials) after defaunation (111.3 *vs* 91.8, s.e.m. = 9.8%). Twenty-two trials have allowed us to calculate duodenal crude protein /Kg DMI outflows (n=44, Duo.CP=143.2 ± 38.2 g/kgMSI). Defaunation significantly (p<0.001) increased this flow (155.5 *vs* 130.8, s.e.m. = 9.8 g/kgDMI). Examination of residual variations of these different models revealed no major interfering factors with digestibilities.

Among the 22 trials mentioned above, 18 indicated both duodenal non-ammonia and microbial nitrogen outflows. The analysis of the latter trials indicated that defaunation induced a significant (p=0.007) increase in duodenal microbial nitrogen outflow (2.82 *vs* 2.31, s.e.m. = 0.45 %LW). The ratio of duodenal microbial nitrogen outflow / duodenal non-ammonia nitrogen outflow wasn't affected by defaunation (59.58 ± 2.4%, p=0.910).

The analysis of the too few (8) trials indicating urinary nitrogen excretion enabled to point out a meaningless decrease (p=0.079) of the latter after defaunation (1.64 *vs* 1.92, s.e.m. = 0.17 %LW). On the contrary, defaunation induced a highly significant (p=0.003, n=30) increase in faecal nitrogen excretion (1.5 *vs* 1.4, s.e.m. = 0.7 %LW).

3.6. Fermentations in the rumen

3.6.1 Acidity in the rumen

The 68 trials concerning VFA allowed to point out a significant decrease (p=0.024) of VFA concentration in rumen juice (85.9 *vs* 89.1, s.e.m. = 8.2 mM) for defaunated animals. Furthermore, an analysis of the residuals of the preceding statistical model indicated that the DMI level interfered with the effects of defaunation on the decrease in VFA concentrations. Figure 4 represents this aspect; it indicates that the decrease in VFA concentration is more obvious with trials in which the level of intake was high (about superior to 3%LW).

No significant effect of defaunation on pH in the rumen was observed (6.28 *vs* 6.33, s.e.m. = 0.16) after analysis of 44 concerned trials, whereas defaunation significantly (p<0.009) decreased pH, when VFA accounted as a covariate in the model (5.6 vs 6.2, s.e.m. = 0.15). Moreover, a highly significant interaction appeared between the response of pH to defaunation (Res.pH, p=0.001) and the percentage of concentrate in the diet. That is why there is a significant negative regression link between the latter two parameters (figure 5).

Res.pH = 0.31 – 0,006 PCO (n = 44, R^2 = 0.34, r.s.d. = 0.21)

With low levels of concentrate, the acidity in the rumen decreased after defaunation, whereas inverse effect appeared with high levels of concentrate. This response of pH is also negatively and logically linked to the response of VFA to defaunation. As a consequence the above equation can be significantly enhanced:

Res.pH = 0.17 – 0,005 PCO – 0.005 Res.AGV (n = 34, R^2=0.41, r.s.d. = 0.18)

3.6.2 Molar proportions of the VFAs

Defaunation induced a decrease in the molar proportion of acetate in the rumen (%C2, p=0.08) after analysis of 59 concerned trials (62.8 *vs* 64.2, s.e.m. = 4.4%). A significant increase in the molar proportion of propionate in the rumen by 2.85% (p=0.006) was observed after defaunation (%C3, 23.05 *vs* 20.20, s.e.m. = 5.43%). Finally defaunation induced a significant decrease by 1.63% (p=0.009) of the molar proportion of butyrate in the rumen after defaunation (%C4, 10.94 *vs* 12.57, s.e.m.= 3.23%) as was shown by analysis of the 57 concerned trials.

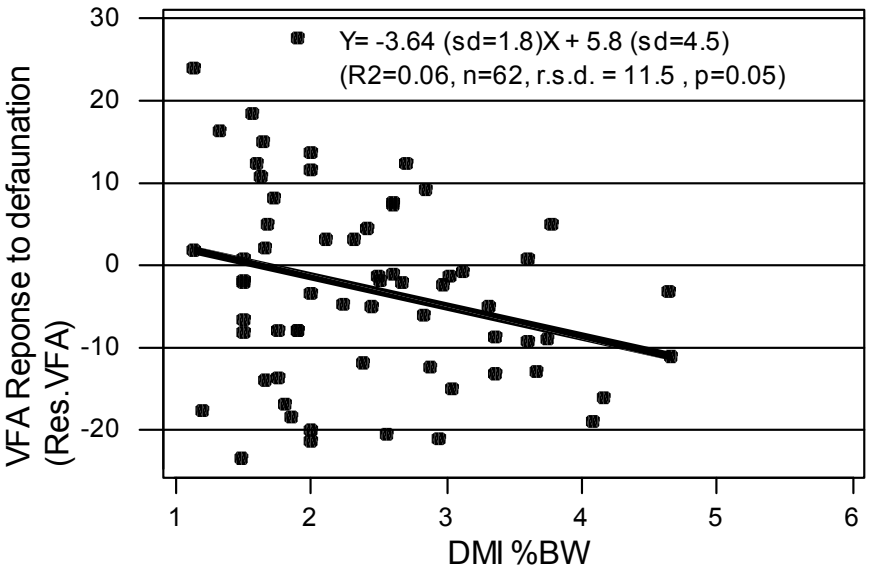

Figure 4 : Defaunation and dry matter intake influences upon volatile fatty acids concentration (VFA) in the ruminal fluid. Synthesis of the literature data.

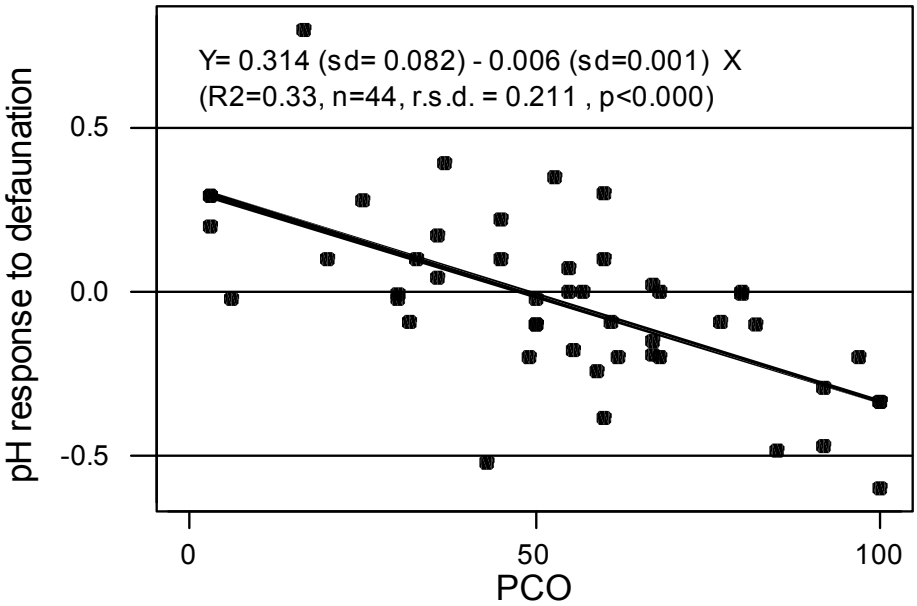

Figure 5 : Effect of the percentage of concentrate (PCO) on the response of pH to defaunation (Res.pH). Synthesis of the literature data.

Ratios of the fermentation pathway C2/C3, C2+C4/C3 and C3/C4 were calculated. The analysis of the 58 concerned trials, integrating the significant covariate DMI%LW (p=0.005), concluded that there was a significant decrease (p=0.001) of C2/C3 ratio (2.96 *vs* 3.49, s.e.m. = 0.72) after defaunation. A similar decrease (p=0.040) was observed for 53 trials where C2+C4/C3 ratio was available (3.40 *vs* 4.20, s.e.m. = 0.95). As concerning C3/C4 ratio, a highly significant (p=0.001) increase appeared after defaunation (2.65 *vs* 1.82, s.e.m. = 1.1).

Total concentration and molar proportion of VFA allowed us to calculate the different concentrations of each VFA. Acetate and propionate concentrations in the rumen juice weren't affected by defaunation. Butyrate concentration was significantly reduced (p=0.001) after defaunation (52 trials, 8.3 *vs* 9.9, s.e.m. = 2.5 mmol/L).

3.6.3 Ammonia concentration

For faunated groups a global positive inter-study relationship appeared between NH3 concentration in the rumen and percentage of total crude protein in the diet (CP%DM).

NH3 = 30.9 + 9.7 CP%DM (n = 75, R = 0.49, r.s.d. = 62.8)

A highly significant (p<0.000) decrease in NH3 in the rumen juice by 50.3 mgN/L appeared after defaunation (117.4 vs 167.7, s.e.m. = 30.0mgN/L), as indicated by analysis of the 75 concerned trials. Taking the preceding relationship into account, defaunation would amount to a 5 % increase in the diet crude protein content. No covariate had significant effect on ammonia in the rumen. However, effect of defaunation on NH3 decreased with diets low in CP%DM contents. Thus for 9 trials, with CP%DM contents inferior to 10%DM, NH3 adjusted values were 58.3 and 94.3 mgN/L for defaunated and faunated group respectively, a difference of 36.0 mgN/L (figure 6).

3.7. Microbial growth

Microbial synthesis efficiency (M.S.E., expressed in g microbial N (MN) / kg OM apparently digested in the rumen (OMDr)) had significantly (p=0.001) increased after defaunation (19 trials, 39.96 *vs* 28.14, s.e.m. = 6.26 g MN/kg OMDr).

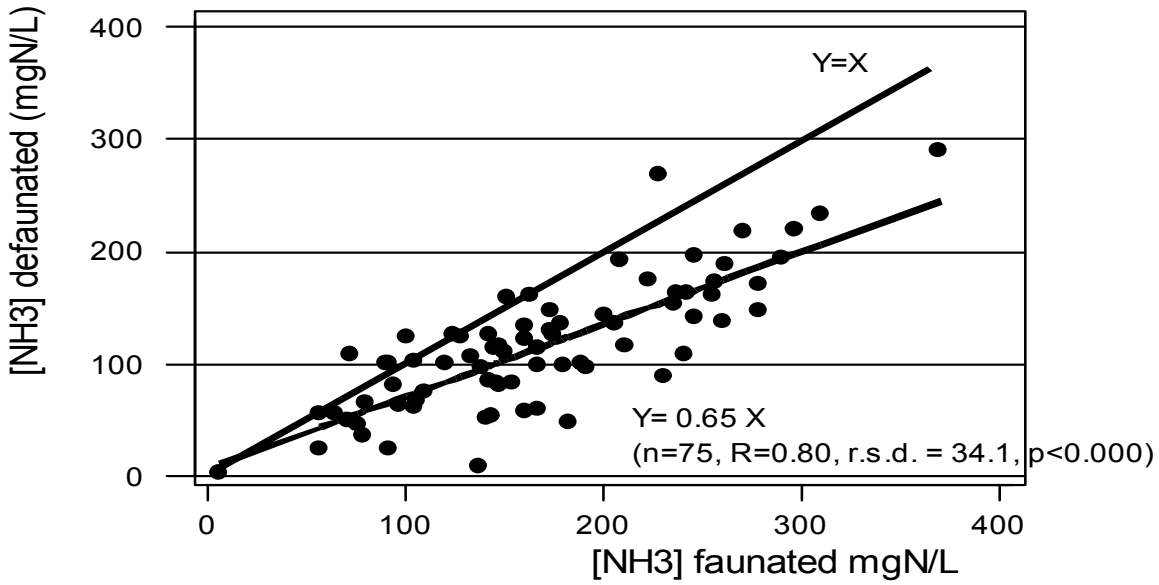

Figure 6 : Effect of defaunation on ammonia concentration (NH3). in ruminal fluid. Synthesis of the literature data.

Fifteen trials indicated both duodenal microbial nitrogen flow and M.S.E. and their analysis revealed a significant ($p<0.001$) increase in duodenal MN flow (15.5 *vs* 11.8, s.e.m. 1.9 gN/d). In parallel with that, analysis of variance of OMDr (474 ± 451 g/kg DM) conducted with the same 15 trials, led to the conclusion that there was a significant decrease in OMDr after defaunation (343 *vs* 400, s.e.m. = 27 g). The latter two results account for ($p<0.001$) M.S.E. increase after defaunation (44.7 *vs* 30, s.e.m. = 5.1 g Nm/kg MODr).

3.8. Ruminal volume, solid and liquid phase turn over rates in the rumen

DMI %LW was taken into account as a covariate in all analysis of variance related to ruminal volume and turn-over rates. As to compare results, weight and/or volume of the rumen were expressed as %LW (Vr = 10.8 ± 1.5 %LW). An analysis of variance of 17 trials indicated increased ($p=0.12$) ruminal liquid volume after defaunation (11.1 *vs* 10.5, s.e.m. = 1.1 %LW). Fractional turn-over rate in the rumen of the liquid phase (Kl = 8.2 ± 2.6 %/h) and solid phase (Kp = 5.3 ± 2.1 %/h) were measured in 18 and 15 trials respectively. Those two parameters were positively correlated when simultanously measured ($R^2 = 0.43$, n = 22). Kl slowly but significantly ($p=0.021$) increased after defaunation (8.4 *vs* 8.3, s.e.m. = 0.7 %/h) whereas Kp's increase wasn't significant ($p=0.527$) after defaunation (5.4 *vs* 5.1, s.e.m. = 1.0 %/h). On the other hand, response of Kp to defaunation was significantly and negatively correlated to response of ruminal volume ($Y = 0.58 – 0.63 X$; n = 12, $R^2 = 0.50$, r.s.d. = 1.1 %/h). This relationship indicates that particles flow tends to remain constant within a similar trial and that variations of these two parameters correspond to compensatory phenomena between ruminal volume and turn-over rate.

4. Discussion

Meta-analysis confirmed most of the reported results of defaunation and brought to light original conclusions on subjects, which were still conflicting. Furthermore, this work contributed to reveal interfering factors and statistical relationships, in order to elaborate or validate mechanistic ruminal digestion models. Such a meta-analysis appears to be more powerful than a single study to reveal some new effects of defaunation of the rumen and mainly new interfering factors.

Average daily gain and dry matter intake results explain the better food coefficient efficiency for the cilate-free animals. Furthermore, the positive effects of defaunation on average daily gain appeared obvious, especially as diets were poor and average daily gain was low. Bird (1989) thought that the level of concentrate in the diet might influence the outcome of defaunation on live-weigh gain. Our results confirmed this hypothesis, and further developed it. Indeed defaunation effects appeared more pronounced when the ratio N%NDF was roughly inferior to 6%, for diets with high level of straw or roughage. In the same way, they were higher when the percentage of concentrate in the diet was roughly inferior to 38%. Nevertheless, results obtained for diets with high level of concentrate must be carefully analysed. Indeed, providing high levels of concentrate to conventional animals decreased the number of protozoa in the rumen and even removed them all (Eadie et al., 1970), thus no significant differences betwen animals with reduced fauna and ciliatre free animals have been observed.

As defaunation has no effect on intake and as cilate-free animals have lower cell-wall carbohydrate and OM digestibility, absorbed energy has to be lower than it is in conventional animals. Thus, the better food conversion efficiency after defaunation may be mainly attributed to the higher yield of metabolic use of absorbed nutriments, for maintenance and growth. It confirms Bird's hypothesis (Bird, 1989). This higher yield may partly be explained by the higher nitrogen and essential AA absorption in the duodenum, as is suggested by the high increase in duodenal nitrogen flow (18%) reported by our results. Increase in duodenal nitrogen flow is explained, above all, by increase in duodenal microbial nitrogen flow, as reported by other authors (Demeyer et al., 1982; Ushida et al., 1990). On average, the latter increased by 22% versus 13% for duodenal non microbial nitrogen flow. The economy of dietary protein, suggested by some authors (Rowe et al., 1985; Ushida et al., 1986) was partially confirmed by the analysis of our database, may be because few data on nitrogen balance were available.

The high efficiency of nutriments metabolic use could also be due to the shape of energetic nutriments in the rumen. Indeed, defaunation undoubtely decreased VFAs, while the ratio (C2+C4)/C3 increased. Jouany et al. (1988) had already hypothesised that defaunation promoted propionate production, whatever the diet responsible for the shape of VFAs in the rumen. Increase in the molar proportion of propionate is in favour of a higher metabolic use of the nutriments (Kreuzer et al., 1986), although this hypothesis is

not shared by other authors (Ørskov et al., 1979; Demeyer and Van Nevel, 1986). Finally, as lipid concentration is higher in bacteria than in protozoa cell, especially for attached bacteria which are more numerous, the increased absorption of long chain fatty acids may partially account for higher metabolic use of absorbed nutriments in cilate-free animals. Thus Sauvant and Bas (2002) indicate that mean fatty acid concentration of adherent bacteria, free bacteria and protozoa are on average equal to 15-20, 6-10 and 2-4 %DM, respectively.

Our results confirmed that protozoa may prevent the abrupt drop of the pH in the rumen, in some instances, and are in agreement with other results (Chamberlain et al., 1983; Coleman, 1988; Ushida et al., 1991). Besides, we pointed out an interaction between the effect of defaunation on pH, and the level of concentrates in the diet. Thus, in diets containing less than 50% of concentrate, ruminal liquid is less acid in cilate-free animals than in conventional animals. Higher buffering capacities of conventional animals, given diets very rich in concentrate, may be explained as protozoa can store particles, starch grains and other carbohydrates inside their cells. Furthermore, protozoa can degrade lactic acid.

As reported by numerous authors, ammonia concentrations in the rumen were by far much lower in cilate-free animals compared to control (Ushida et al., 1986; Ivan et al., 1992; Jouany and Ushida, 1998; Santra and Karim, 2000). Part of these results should, on the one hand, be attributed to higher microbial synthesis, and on the other hand, to less bacteria proteolysis when protozoa are missing (Coleman, 1975; Ueda et al., 1975; Onodera et al., 1977; Demeyer and Van Nevel, 1979). To a lesser extent, absence of protozoal lysis, which occurs during nycthemeral phases could be responsible for those results (Abou Akkada and Howard, 1962). Furthermore, ruminal dietary protein fraction is less degraded in defaunated animals. In conventional animals, global nitrogen excess of the ecosystem in the rumen, explains relative nitrogen availability, higher than the requirements of the present microorganisms. Higher microbial protein production observed in cilate-free animals shows that part of free nitrogen compounds in excess in ruminal fluid, reported above, is engaged towards bacterial cell growth. As observed by Jouany (1996) and Eugène et al. (unpublished data) our analysis shows a lower nitrogen urinary excretion in cilate-free animals, despite the fact that the differences were not significant.

Higher nitrogen efficiency of ruminal ecosystem of cilate-free animals may explain this result. Thus nitrogen nutritive value of the diet is higher in cilate-free animals.

Our results demonstrate that the higher microbial synthesis efficiency is on the one hand, explained by a better microbial proteosynthesis (31%) and on the other hand by a lower OM intake digestion in cilate-free animals. Several aspects may account for higher microbial synthesis efficiency observed in the rumen of cilate-free animals. Moreover, a decrease in energetic requirements for maintenance of rumen microorganisms may occur in ciliate-free animals, in which the microbial biomass is reduced by about half (Jouany, 1978). Furthermore, ecological characteristics and dynamic of the fauna and flora in the rumen are different. Protozoa residence time in the rumen are more than 4 times higher than that of bacteria, according to studies on N15 binding of bacteria and protozoa (Tarakanow et al., 1984). Considering the rumen as a chemostat, energetic requirements for maintenance may presumably be higher for protozoa, since the major part of protozoa is sequestred and recycled inside the rumen. Thus, protozoa may negatively affect the biological efficiency of the rumen ecosystem. Finally, defaunation presumably affects molecular hydrogen metabolism. Indeed, the C3/C4 ratio highly increased as a consequence of defaunation, which may reveal an excess of hydrogen (Sauvant and Van Milgen, 1995). When methanogenesis decreases, hydrogen production may be disposed of in propionic acid production, at the expense of methane production (Demeyer and Van Nevel, 1986). These conclusions are shared by other authors (Jouany et al., 1981; Whitelaw et al., 1984; Kreuzer et al., 1986; Vermorel and Jouany, 1989; Williams and Coleman, 1992). They reported that defaunation decreased methane production. This suggests that higher OM digestibility, and therefore higher energy digestibility, observed in the rumen and in the total tract in ciliate-free animals is partly lost in methane emission.

The increase in weight of ruminal contents after defaunation is presumably due to the fill effect of the lower OM digestibility in the rumen. On the contrary, no obvious global law can be drawn from the effects of defaunation on the liquid phase and the solid phase fractional turnover rate of rumen contents. These results confirmed, with more data, those reported by Demeyer (1988).

The significant increase in wool production after defaunation is well admitted by all authors in the literature. Only one exception has occured : inverse results on wool

production reported by Cottle (1988a; 1988b) may be explained by the very special characteristics of the diet (percentage of concentrate >90%). These results are coherent with the higher nitrogen nutritive value of the diet for ciliate-free animals. Furthermore it seems that defaunation enhanced sulphur amino acids at the duodenum. Few data on milk production were available, therefore we could not use quantitative analysis to study the effects of defaunation. Further investigations have to be carried out in order to test the effects of defaunation on milk production, even if apparently the response to defaunation for meat and milk production efficiency should not be different.

Conclusion : As a whole the results indicate a more efficient use of diets in ciliate-free animals, especially when poor diets, limiting growth, are given. Thanks to these results we can screen diets and production systems, with which defaunation potentially better valorise food and enhance animal nutririon. That effect of defaunation is pointed out, despite lower parietal constituents and OM digestibility of the diet. Hence, better zootechnical efficiency of ciliate-free animals results mainly from more efficient metabolical use of absorbed nutriments. The factors put forward and which may account for higher efficiency of nutrients use, are the higher amino acids supply to the host animal, a more balanced VFAs and an enhanced microbial fatty acids synthesis.

Acknowledgements

The authors would like to thank C.Césaire for his help in collecting the data for the database.

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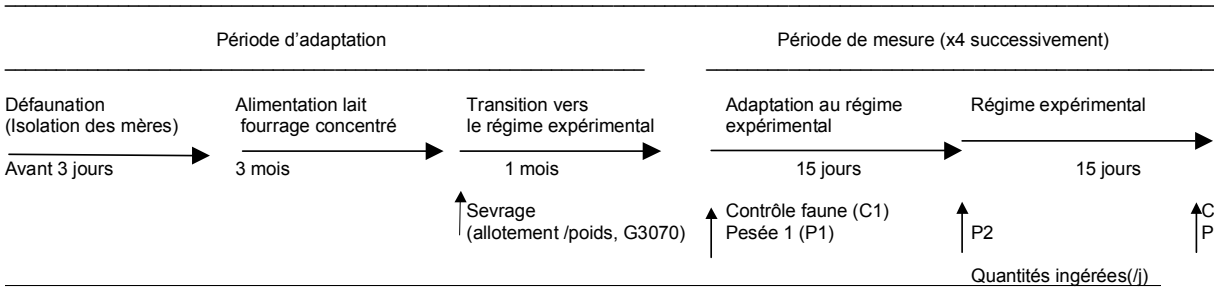
ETUDE EXPERIMENTALE

Le second objectif de ce travail de thèse est de déterminer les contextes alimentaires caractéristiques des pratiques d'alimentation en zone tropicale humide, pour lesquels la défaunation permet d'augmenter les performances animales. Afin de couvrir une large gamme de rations utilisées en zone tropicale humide, trois grands types de rations représentatives ont été choisis : des rations composées uniquement de fourrage (*Digitaria decumbens*); des rations mixtes composées de *Digitaria decumbens* et d'un complément (concentré énergétique et/ou protéique), des rations composées de canne à sucre et de farine de riz.

Nous ne sommes pas en mesure de présenter les résultats portant sur ces dernières rations. En effet, suite à un problème sanitaire survenu au cours de l'essai de croissance sur les agneaux, nous avons dû repousser la programmation de cette étude, au-delà des délais requis pour la présentation de la thèse.

Les figures 1 et 2 représentent les dispositifs expérimentaux et les mesures effectuées au cours des essais de croissance et de digestion.

20 Agneaux défaunés :



20 Agneaux faunés :

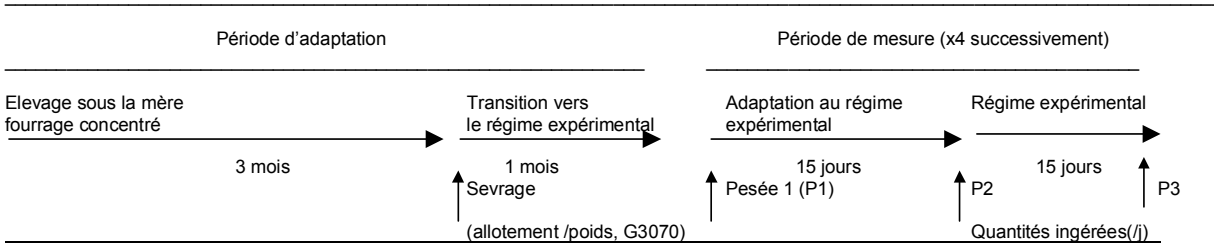


Figure 1 : Dispositif expérimental des agneaux en croissance

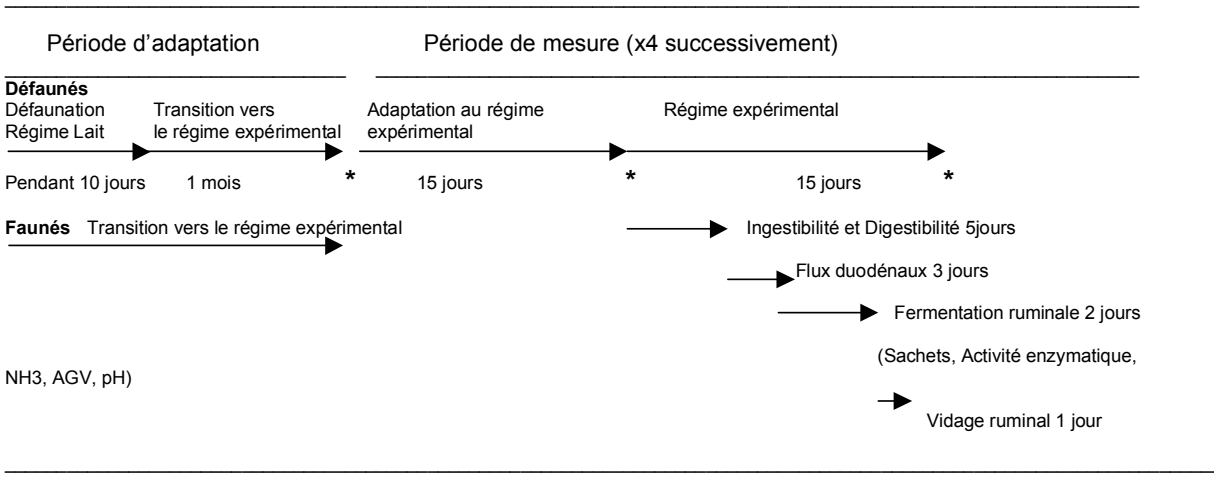


Figure 2 : Dispositif expérimental des béliers en cage à bilan.

1. INTRODUCTION A L' ETUDE EXPERIMENTALE 1

Au cours de la première étude expérimentale un essai zootechnique sur des agneaux en croissance a été mené, en parallèle à un essai de digestibilité avec des béliers adultes. Nous avons testé des rations mixtes composées d'un fourrage tropical (*Digitaria decumbens*) complémenté avec un mélange de concentré (maïs et tourteau de soja). Le gradient azote / énergie de la ration a été généré en faisant varier les quantités de concentré, ainsi que la proportion de maïs comparativement à celle du tourteau de soja dans le concentré. Quatre rations furent constituées avec un fourrage (*Digitaria decumbens*) de 28 jours d âge repousse, complementé avec différentes quantités de concentré, générant respectivement des teneurs en azote / énergie de 140, 120, 100, et 80 g PDIN / UF.

Nous présenterons: 1) les effets de la défaunation, en interaction avec la ration mixte, sur les performances de croissance d'agneaux, sur la digestion et les flux de nutriments azotés mesurés sur des béliers adultes (Publication 2, soumise à Animal Research).

2) L'impact de la défaunation sur les activités microbiennes, les produits fermentaires du rumen ainsi que sur la dynamique des populations microbiennes cellulolytiques (Publication 3, soumise à Animal Research).

Publication 2

Effects of defaunation on digestion of mixed diet (fresh *Digitaria decumbens* grass and concentrate) and growth of lambs

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(soumise à Animal Research)

Abstract —The effects of defaunation on growth and digestion have been measured on sheep consuming four mixed diets of *Digitaria decumbens* and concentrate differentiated through their protein to energy level of contents in the diet. Conventional and ciliate-free animals were conducted in 4 x 4 factorial Latin square designs, in parallel. On the one hand two Latin square designs were conducted with 20 faunated lambs and 20 defaunated lambs to measure growth. On the other hand, two others Latin square designs were conducted with 8 conventional mature rams and 8 ciliate-free mature rams to measure digestion parameters. The average daily gain of faunated lambs was significantly lower ($P < 0.03$) compared with defaunated lambs (165.9 *vs* 184.1, se 9.3 g. / d.). The evolution of the differences of growth between faunated and defaunated animals with the protein to energy ratio of the diet, tended to be curvilinear ($P > 0.09$). Feed conversion efficiency tended to be higher with conventional lambs compared with ciliate-free lambs (6.78 *vs* 6.28, se 0.38 g / g, $P < 0.09$). The same trend was observed with energy conversion efficiency. The differences of growth between conventional and ciliate-free animals have been explained by the higher availability of protein in ciliate-free animals. Duodenal non-ammonia nitrogen (NAN) flow was significantly increased ($P < 0.01$) by defaunation (144 *vs* 172, se 5.5 g NAN / d.). The differences of protein flows observed between defaunated and faunated animals increased ($P < 0.01$) with the protein to energy ratio. A better microbial synthesis efficiency and a lower digestion of dietary nitrogen for the defaunated animals, have explained the differences of nitrogen availability between the animals.

digestion / growth / defaunation / sheep

Résumé — Les effets de la défaunation sur la digestion de rations mixtes (herbe de *Digitaria decumbens* et concentré) et la croissance de moutons. Quatre régimes mixtes composés de *Digitaria decumbens* et de concentré, différenciés par le rapport protéine / énergie dans le régime, ont été constitués. Des animaux conventionnels et des animaux dépourvus de protozoaires ont été utilisés dans des dispositifs en carré latin 4 x 4 menés en parallèle. D'une part, deux dispositifs en carré latin ont été conduits avec 20 agneaux faunés et 20 agneaux défaunés afin de mesurer leur croissance. D'autre part, deux autres dispositifs en carré latin ont été conduits avec 8 béliers adultes conventionnels et 8 béliers dépourvus de protozoaires, afin de mesurer les paramètres de

la digestion. Le gain de poids moyen quotidien des agneaux faunés était significativement inférieur ($P < 0.03$) à celui des agneaux défaunés (165.9 *vs* 184.1 ± 9.3 g / j). L'évolution des différences de croissance entre agneaux défaunés et faunés, en fonction du rapport protéine / énergie de la ration, était de type curvilinéaire ($P > 0.09$). L'indice de consommation de l'aliment tendait a être supérieur pour les agneaux faunés comparativement aux agneaux défaunés (6.78 *vs* 6.28 ± 0.38 g / g, $P < 0.09$). La même tendance a été observée en ce qui concerne l'indice de transformation de l'énergie. Les différences de croissance entre les animaux faunés et défaunés ont été expliquées par une plus grande disponibilité en protéine des animaux défaunés. Le flux duodénal d'azote non ammoniacal (NAN) a été significativement augmenté par la défaunation (144 vs 172 ± 5.5 g NAN / j, $P < 0.01$). Les différences de flux de protéines observées entre animaux faunés et défaunés ont augmenté ($P < 0.01$) avec le rapport protéine / énergie de la ration. Une meilleure efficacité de la synthèse microbienne ajoutée à une moindre digestion de l'azote almimentaire en faveur des animaux défaunés ont expliqué les différences de disponibilité en azote des animaux.

digestion / croissance / défaunation / ovin

1. Introduction

The different literature syntheses dealing with the effect of defaunation on animal performance do not lead to concluding results [3,10,18]. Some studies report negative effects of defaunation on animal growth [1,8,9,16] whereas others indicate absence of any effect or positive effect [2,4,7,12] The nature of the diet may strongly account for the different results observed [18]. The main conclusion is that no effect is observed with forage high in nitrogen contents whereas positive effect could appear with poorer roughage [5].

Nevertheless this classification fails to explain some results of the literature. The nitrogen / (ruminal digestible energy) ratio would mainly be responsible for the observed results. Moreover, total amounts of protein and energy relatively to animal requirements would be another major factor of variation. The objective of this study is 1) to evaluate the effect of defaunation on growth of lambs consuming mixed diets formulated to provide increasing protein / (ruminal energy) ratio in the diet 2) to point out some parameters of the digestion which may explain the observed effects of defaunation.

2. Materials and Methods

2.1. Location

The research was carried out (in 1999) at the animal experimental station of the National Institute of Agricultural Research, in Guadeloupe, French West Indies, (latitude 16° 16' N, and longitude 61° 30'). Average temperatures ranged from 21°C to 31°C. The rainfall on the experimental site is 3000 mm a year.

2.2. Experimental design, animals and diets

2.2.1. Growth trial

Twenty Black Belly lambs (average live weight: 28.3 ± 5.9 kg) were defaunated by isolation from their dam, 1 day after their birth. They received colostrum, artificial milk, concentrate, and fresh grass. They were weaned at 3 months simultaneously with 20 fellow lambs living under their dam. After weaning all lambs received a similar diet (fresh grass and concentrate) during one month. Faunated and defaunated rams, isolated with a punch cloth, were housed in individual pens in the same room. Four groups of 5 defaunated lambs and 4 groups of 5 faunated lambs were constituted after allotting animals according to weight at the beginning of the trial and individual growth between 1 and 2 months of age. Faunated and defaunated animals were conducted following 2 parallels factorial 4 x 4 Latin square designs. Each period last one month. All lambs were fed 4 diets. The diet D140 was a 28 days age of regrowth *Digitaria decumbens* grass distributed *ad libitum* with 100 g of soja meal. In diets D 120, D 100 and D 80, the *Digitaria decumbens* of 28 days age of regrowth was complemented with 230 g (80g of soya meal + 150g of corn); 350 g (50g soya meal +300g of corn) and 600 g (600g of corn) of concentrate respectively. The chemical composition of the diet is presented in Table 1.

2.2.2. Digestion trial

Sixteen (16) Black Belly rams (average live weight: 45.1 ± 7.7 kg, 2 years old), each containing surgically implanted rumen and duodenum cannulae were used in this trial. Half of them were defaunated using a milk diet, which is a technique adapted from the method of [14].

Table 1 : Chemical composition (g / kg) of, organic matter (OM), Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and crude protein (CP) of the mixed diets composed of *Digitaria decumbens* grass (28 days age of regrowth) and 4 concentrates: Concentrate A (100% of corn), B (14% soya meal + 86 % of corn), C (35 % of soya meal + 65 % of corn) and D (100% of soya meal) were used on diets D80, D100, D120 and D140 respectively.

	OM	CP	NDF	ADF	ADL	PDI / UF*
Digitaria decumbens	930.0	82.0	725.0	351.0	41.0	
Concentrate A	982.0	104.0	130.0	35.0	7.0	80
Concentrate B	974.0	164.0	131.0	43.0	7.0	100
Concentrate C	962.0	249.0	133.0	53.0	6.9	120
Concentrate D	927.0	516.0	140.0	87.0	6.8	140

*The diet D80, D100, D120 and D140 were formulated to provide 4 protein / energy ratios of 80, 100, 120, and 140 PDI / UF level of contents in the diet respectively.

During the defaunation process the diet was gradually changed from a fresh young grass to milk powder diet within 10 days. Then animals were fed with milk alone during 7 days. The diet was gradually changed from milk to fresh grass. The milk powder was mixed with 400 ml of a 37° C water, and poured into the rumen via ruminal cannula.

The 16 rams were conducted following two parallel Latin square designs (faunated *vs* defaunated). Defaunated and faunated rams, placed in metabolism cages and housed in the same room, were isolated with a punch cloth. They were fed the same diet than in lambs trial but the amount of concentrate were twice as high than that of lambs. Each period was composed of 14 days of adaptation to the diet, 5 days of intake and total tract digestibility measurements, 3 days of duodenal sampling.

As with lambs, mineralised salt blocks were in free access. The blocks' composition was Ca (60.0), P (20.0), Mg (10.0), Na (280.0), Zn (17.5), Mn (5.5), Fe (1.5), I (0.03), Co (0.03) and Se (0.01 g / kg).

2.3. Experimental procedures

2.3.1.Growth trial

Ruminal contents (obtained via oesophageal tube) from all defaunated lambs were examined at the end of each experimental period and found to be free of protozoa. Intake was determined from daily weighing of the amounts of feed offered and refused. Representative samples of feed (3 samples / day of the distributed feed) were constituted for chemical analysis. A double weighing (24 hours of interval) of the rams were performed every week. The average daily growth was calculated on the last 15 days of each period.

2.3.2. Digestion trial

Intake and apparent digestibility were determined from daily weighing of the amounts of feed offered, feed refused and the faeces. Representative samples of feed (3 samples / day of the distributed feed) and faeces (10% / day of the amounts excreted) were constituted for chemical analysis. During the 5 days of intake measurements, daily excretions of urine were recorded. The urine was daily collected in drums containing sulphuric acid H_2SO_4 (approximately 1 vol. H_2SO_4 / 100 vol. urine). Daily samples of urine were pooled during 5 days for nitrogen determination. Nitrogen retention was estimated as nitrogen intake – (nitrogen urinary loss + nitrogen faecal loss).

Duodenal flows were estimated according to the double-marker method of Faichney [13] using indigestible lignin (IADL) and polyethylene glycol (PEG) as markers of the particulate and aqueous phases, respectively. The mean daily duodenal digesta sample was constituted by taking twelve 100 ml samples over three consecutive days at (day 1) 0, 6, 12 and 18 hours, (day 2) 2, 8, 14 and 20 hours, (day 3) 4, 10, 16 and 22 hours after the morning meal distribution. Fifty millilitres of this sample were kept to represent whole digesta (DG) and 50 ml were divided into a liquid-rich phase (LR) and a particle-rich fraction (PR) by squeezing the digesta through a nylon filter of 150 µm pore size. Then DG and PR were freeze-dried, prior to chemical analysis for crude protein (CP), microbial nitrogen, organic Matter (OM), Neutral Detergent Fibre (NDF), Acid detergent fibre (ADF) and Acid detergent lignin (ADL).

At the end of each period, the rumen of each animal was manually emptied and the total content weighed, 3 hours after the morning meal. The morning meal was interrupted after 3 h of free access. The rumen turnover rate (% / h) of the pangola hay has been estimated as: ((amount of lignin excreted in faeces daily) / 24) / (amount of lignin in the rumen 3 h after the morning meal)). Bacterial samples were isolated by carrying out two successive centrifugations of LR (800 *g* and 27000 *g*) as described by Yang [26].

2.4. Chemical analysis

Dry matter was determined by drying to a constant weight at 60°C in a forced draught oven. The samples were ground (1 mm) prior to chemical determination. The OM content was determined as the difference between dry and ash weight. Samples were burnt for 10 h at 550°C. The Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and Acid detergent lignin (ADL) were estimated following the method of Van Soest et al. [25]. Nitrogen concentration was determined using the Dumas technique. Microbial nitrogen was estimated from the purine bases to non-ammonia nitrogen ratio in samples of reconstituted duodenal digesta and ruminal bacteria. The purine base was analysed according to Zinn and Owens [27].

2.5. Statistical analyses

2.5.1. Growth trial

Dry matter intake (DMI) , feed and energy conversion efficiency and average daily growth (ADG) data were analysed by ANOVA procedure [23]. The statistical model used to analyse DMI data took into account, as fixed effects: defaunation (defaunated *vs* faunated), the diet, the period, the animal (inside of fauna status), the interaction between defaunation effect and the diet effect. The live-weight of the animals (at the beginning of each experimental measurements period) as a covariate. Concerning the ADG the statistical model used took into account defaunation, the diet, the period, the animal (inside of fauna status), the interaction between defaunation and the diet as the fixed effects. The DMI and the animal live-weight (inside of the fauna status) were integrated in the model as covariates. Concerning feed and energy conversion efficiency the preceding model used for ADG was performed, with the percentage of concentrate in the diet instead of diet effect as a supplementary covariate, because it was more significant than the diet effect.

2.5.2. Digestion trial

ANOVA procedures were used in all statistical analyses [20]. For DMI the model fixed effects were fauna status (defaunated *vs* faunated), the diet, the period, the animal (inside of fauna status) and the interaction between defaunation and the diet. The ruminal and total tract digestibilities, were analysed using the same model and the DMI was integrated as a supplementary covariate. The DMI was substituted by nitrogen intake as a covariate in statistical analyses of the ruminal ammonia concentrations and the duodenal nitrogen flow.

3. Results

3.1. Growth trial

The average daily gain (ADG, g / d) of faunated lambs was significantly lower ($P < 0.03$) compared with defaunated lambs (165.9 *vs* 184.1, s.e. 9.3g / d). There was no significant interaction between defaunation and the diet effects. However evolution of the differences of growth between faunated and defaunated animals tended ($P = 0.09$) to be curvilinear with the protein to energy ratio of the diet as illustrated by Table 2. The differences of growth increased while the protein to energy ratio of the diet increased from 80 to 100. Then it decreased and finally disappeared when the protein to energy ratio increased from 120 up to 140.

Table 2 : Effect of defaunation on intake, growth and feed conversion efficiency of lambs consuming mixed diets composed of *Digitaria decumbens* grass (28 days age of regrowth) and 4 concentrates mixture (soja meal + ground corn). The diets were formulated to provide 4 protein / energy ratios (PDI / UF) of 80, 100, 120 and 140 on diet D80, D100, D120 and D140 respectively.

	D80		D100		D120		D140			Significance		
	F	D	F	D	F	D	F	D	s.e.	Def.	Diet	Def.×Diet
DM intake (g / LW $^{0.75}$) of :												
Forage	38.9	32.1	60.1	52.4	65.4	59.0	70.2	65.7	1.87	***	***	
Total DM	85.5	77.3	87.0	79.1	82.3	76.1	77.9	71.5	1.87	***	***	
Energy intake (UF / day)	1.04	0.95	0.92	0.85	0.83	0.77	0.71	0.67	0.85	***	***	
Protein intake (g / d) (1)	81.6	72.7	93.1	85.8	95.7	89.9	94.2	89.3	1.83	***	***	
Protein / Energy Ratio (2)	77.8	76.4	100.8	100.2	117.4	117.5	131.8	137.0	1.18		***	*
Growth (g / day)	164.3	172.9	153.7	211.6	173.6	183.1	172.1	168.8	14.8	*		
Conversion efficiency of :												
Feed (g / g)	6.58	7.07	6.93	4.80	6.50	5.81	7.13	7.45	1.13			
Energy (UF / g 10^{-3})	6.3	7.1	6.2	4.3	5.7	5.1	6.0	6.6	1.0			*

D : defaunated animals F: faunated animals; Def. : Defaunation effect; (1) PDI ; (2) PDI / UF.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

The total dry matter intake was significantly higher with conventional lambs compared with cilate-free lambs ($P < 0.001$, 83.2 *vs* 76.0, s.e. 14.2 g / kg LW $^{0.75}$). Similar hierarchy has been observed with roughage intake ($P < 0.001$, 52.3 *vs* 58.6, s.e. 1.15 g / kg LW $^{0.75}$). There was no interaction ($P = 0.90$) between defaunation and diet effects (Tab. 2). Therefore total intake of energy (UF / kg LW $^{0.75}$) and protein (PDIN, g / kg LW $^{0.75}$) were also logically higher with faunated lambs compared with defaunated lambs: 0.074 *vs* 0.068 UF / kg LW $^{0.75}$ and 7.75 *vs* 7.20 PDIN, g / kg LW $^{0.75}$ respectively. No interaction between defaunation and diet effects appeared (Tab. 2).

Feed conversion efficiency (6.79 *vs* 6.28, s.e. 0.38 g / g, $P = 0.09$) and energy conversion efficiency (6.1 *vs* 5.8, s.e. 0.3 10^{-3}UF / g, $P = 0.07$) tended to be higher with conventional lambs compared with ciliate-free lambs. There was a significant interaction ($P < 0.05$) between defaunation and diet effects on the energy conversion efficiency (Tab. 2).

3.2. Digestion trial

3.2.1. Intake

Total dry matter intake (g / Kg LW $^{0.75}$) was significantly increased ($P < 0.011$) by defaunation (faunated *vs* defaunated): 75.1 *vs* 84.2 s.e. 2.3 g. / Kg LW $^{0.75}$ (Tab. 3). The major factor of the latter increase was the increase of intake of *Digitaria decumbens* (48.4 *vs* 53.1 g / Kg LW $^{0.75}$) after defaunation.

3.2.2. Total tract and ruminal digestibilities

Total tract digestibility of organic matter (DtOM) and fibre (DtNDF) tended to be higher with defaunated rams compared with faunated rams ($P = 0.19$). On the contrary, defaunation decreased both OM apparent ruminal digestibility (DrOM, $P = 0.12$) and NDF ruminal digestibility (DrNDF, $P = 0.08$) (Tab. 3). Crude protein total tract digestibility (DtCP) was significantly higher with defaunated rams than with faunated rams (70.1 *vs* 66.2, s.e. 0.9 %). DtCP differences between defaunated and faunated animals decreased ($P < 0.013$) while the soya meal level in the diet increased (Tab. 3).

3.2.3. Particle mean retention time

Table 3 : Effect of defaunation on dry matter intake and apparent digestibility in the total tract of organic matter (OM), cell wall (NDF) and crude protein (CP) of rams consuming mixed diets composed of *Digitaria decumbens* grass (28 days age of regrowth) and 4 concentrates mixture (soja meal + ground corn). The diets were formulated to provide 4 protein / energy ratios (PDI / UF) of 80, 100, 120 and 140 on diet D80, D100, D120 and D140 respectively.

	D80		D100		D120		D140			Significance		
	F	D	F	D	F	D	F	D	s.e.	Def.	Diet	Def.×Diet
DM intake (g / $LW^{0.75}$) of:												
Forage	26.6	37.3	50.3	40.8	50.1	56.4	57.9	74.9	2.1	*	***	*
Total DM	76.5	89.2	85.4	76.7	73.0	82.4	68.3	88.0	2.1	**		*
Total tract digestibility												
OM	0.742	0.741	0.746	0.773	0.723	0.722	0.648	0.705	0.01		***	
NDF	0.485	0.468	0.636	0.663	0.668	0.658	0.664	0.682	0.02		***	
CP	0.542	0.631	0.651	0.727	0.729	0.730	0.726	0.717	0.09	**	***	*
Rumen digestibility												
OM	0.604	0.478	0.524	0.502	0.461	0.408	0.466	0.439	0.02			
NDF	0.437	0.233	0.498	0.502	0.527	0.475	0.604	0.542	0.03		***	
Particle retention time												
In rumen (Hours)	37.3	36.9	33.1	26.8	41.8	20.5	46.0	35.1	2.2	**		

D: defaunated animals F: faunated animals Def.: Defaunation effect.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

The retention time of fibre particle in the rumen was significantly higher ($P < 0.01$) with faunated rams compared with defaunated rams (40 *vs* 30, se 2 h.).

3.2.4. Nitrogen Flows

Duodenal non-ammonia nitrogen (NAN) flow was significantly increased ($P < 0.001$) by defaunation (23.07 *vs* 27.58, se 0.9 g NAN / d). The differences observed between defaunated and faunated animals increased ($P = 0.8$) while the amount of soya meal in the diet increased up to 35% (D120) as indicated in table 4. At the highest level of soya meal in the diet (D140) the smallest differences were recorded. The duodenal NAN flow decreased with the increase of protein to energy ratio of the diet ($P < 0.001$).

The duodenal microbial nitrogen (MN) flow was increased by defaunation (12.3 *vs* 14.9, se 0.5 g MN / d, $P < 0.001$) and the differences between faunated and defaunated animals increased ($P = 0.4$) with the increasing amount of soya meal in the diet, up to diet D120, as indicated in table 4. The duodenal MN flow decreased with the increase of protein to energy ratio of the diet.

3.2.5. Faecal and urinary nitrogen excretions

The urinary nitrogen excretion decreased significantly ($P < 0.05$) with defaunation (7.26 *vs* 5.72, se 0.49 gN / d). The urinary nitrogen excretion logically decreased ($P < 0.05$) while the amounts of soya meal in the diet decreased. There was no interaction ($P = 0.80$) between defaunation and the diet (Tab. 4).

The faecal nitrogen excretion was significantly higher ($P < 0.05$) with conventional animals compared with ciliate-free animals (8.8 *vs* 7.7, se 0.36 gN / d). There was a significant interaction ($P < 0.05$) between defaunation and the diet (Tab. 4).

3.2.6. The efficiency of microbial synthesis

The efficiency of microbial synthesis (g MN synthesised per kg of organic matter apparently degraded in the rumen (OMADr)) increased ($P < 0.001$) with defaunation (20.3 *vs* 31.0 se 2.2 gMN / Kg OMADr). No significant interaction between defaunation and the diet appeared (Tab. 4).

Table 4 : Effect of defaunation on nitrogen intake, duodenal nitrogen flow, microbial synthesis efficiency, faecal and urinary excretion of rams consuming mixed diets composed of *Digitaria decumbens* grass (28 days age of regrowth) and 4 concentrates mixture (soja meal + ground corn). The diets were formulated to provide 4 protein / energy ratios (PDI / UF) of 80, 100, 120 and 140 on diet D80, D100, D120 and D140 respectively.

	D80		D100		D120		D140			Significance		
	F	D	F	D	F	D	F	D	s.e.	Def.	Diet	Def.×Diet
Nitrogen intake (g N / d)	22.3	21.5	28.0	25.7	28.6	28.0	28.3	30.2	0.5		***	
Nitrogen duodenal flow (g / day)												
Non ammonia Nitrogen	28.2	31.8	24.1	28.6	21.7	27.8	17.1	20.5	0.88	***	***	
Microbial nitrogen	16.4	18.7	12.9	16.3	10.8	14.5	8.5	9.2	0.51	***	***	
Faecal nitrogen (g N / day)	8.8	7.4	10.2	6.7	8.1	7.5	8.1	9.2	0.73	*		*
Urinary nitrogen (g N / day)	5.93	3.99	6.01	4.71	8.02	5.83	9.08	8.38	0.5	*	*	
Microbial synthesis Efficiency (g MN / kg OMADR)	31.0	36.8	17.9	30.0	17.2	40.5	14.9	16.9	2.2	***	**	

D: defaunated animals F: faunated animals Def: Defaunation effect.

OMADR: OM apparently degraded in the rumen; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Nevertheless the differences between faunated and defaunated animals increased (P = 0.06) with the increasing amount of soya meal in the diet, up to diet D120, as indicated in table 4.

4. Discussion

The theoretical potential of growth of the experimental lambs is 250 g / day. Then the energy and protein requirements are 1.05 UF and 98 g N / day respectively. In this experiment the amount of energy decreased with the increase of the protein to energy ratio in the diet. The consumption of energy was always lower than the requirements of animals, except with diet D80 for faunated lambs. The global evolution of growth with faunated lambs was an increase with the increasing amount of protein in the diet, which was presumably the first limiting factor of the experimental diets. Concerning the growth of the ciliate-free lambs, an increase was observed between diets D80 and D100 due to the increasing amount of proteins in the diet. Above diet D100, the level of energy, which became the first limiting factor at that time, could explain the decrease of growth observed with the ciliate-free lambs. In this context, it is not easy to test the interaction of diet and defaunation effects on growth. Nevertheless, the hypothesis that the effect of the protein to energy ratio interacted with the effects of defaunation on growth performance seems plausible.

The evolution of the difference of growth between lambs with the ratio of protein to energy of the diet, was curvilinear, despite the interaction between defaunation and the diet was not significant (P = 0.09). Similar results have been indicated previously [4,6,12]. The difference of growth between the two groups of lambs could be explained by the additional amounts of microbial and dietary protein with defaunation, in a context where protein is the first limiting factor relatively to energy [12,18]. The difference of growth between faunated and defaunated lambs increased on the diet D100 because the true protein to energy ratio of diet was higher with the defaunated animals while the amount of energy permitted a growth of 210 g / d. The microbial protein contributed to increase the protein value of the diet in a context where protein was the first limiting factor, as was already observed before [15,19,22]. Similar growth was observed between faunated and defaunated animals consuming the D140 diet, whereas intake of energy and protein was significantly higher with faunated animals. Globally, the consumption of protein and energy of ciliate-free animals seemed low relatively to the growth recorded. Presumably, the

supply of energy and protein of ciliate-free lambs was underestimated because of the overflow amount of dietary and microbial proteins in the intestine of defaunated animals compared with faunated ones.

The results of the two trials, on the effect of defaunation on intake are contradictory. The review of the literature data (Eugène et al, unpublished data) do not indicate any significant influence of defaunation on intake, because of varying effects of defaunation among the trials. Our experiment underlined this variability. As the mean level of intake has been similar between the two trials, comparisons between the growth and digestion trials could be considered, taken into account the intake as a covariate in the statistical analyses.

The results of the digestion trial underlined those of the growth trial. They confirmed the hypothesis of a better intestinal protein flow with defaunated animals compared with faunated animals. This result is in agreement with the global tendency observed in the literature data [17,21,24]. The higher dietary and microbial nitrogen flows may explain the better intestinal protein flow observed in defaunated animals. The contribution of these two sources of protein to the increase nitrogen flow at the duodenum was similar. On the one hand, the lower ruminal digestion of dietary protein can explain the increase of the intestinal dietary nitrogen flow with defaunation. Indeed the proteolytic activity towards dietary protein of the microbial population of defaunated animals decreased because of the absence of protozoa. On the other hand, the increase of the microbial nitrogen duodenal flow is explained by a better microbial synthesis efficiency, mainly due to a decrease of protozoa predation upon bacterial nitrogen [11].

The depressing effect of defaunation on the ruminal digestion of organic matter and fibre, even if it is not significant, decreased the energy availability of the cilate-free animals. Nevertheless, thanks to a compensatory digestion in the intestine, the nutritional impact of defaunation on digestion was low. Moreover there is a better use of the energy ingested as illustrated by the better feed and energy conversion efficiencies of defaunated animals.

5. Conclusions

The positive effect of defaunation on growth of lambs has been clearly established in this experiment. The hypothesis that the protein to energy ratio of the diet is the major

factor of variation of the effect of defaunation on animal growth performance seems plausible.

Acknowledgements

The authors would like to thank L. Philibert, S. Calif, P. Despois, C. Césaire and F. Nipeau, for their technical collaboration to this work. This work was supported by the “Région Guadeloupe” and the European Economic Community (FEOGA).

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Publication 3

Effects of defaunation on microbial activities in the rumen of rams consuming mixed diet (fresh *Digitaria decumbens* grass and concentrate).

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Abstract — The effects of defaunation of the rumen on ruminal microbial activities of sheep were assessed in the present in vivo digestion trial. The microbial activities were estimated by the in sacco degradation technique and enzymatic assays while the major cellulolytic bacterial population was enumerated by specific 16S rRNA targeted hybridisation probes. Eight faunated rams and eight defaunated rams, with ruminal cannula were used. Each animal was fed with 4 different mixed diets, *Digitaria decumbens* grass supplemented with 4 mixtures of soya meal and ground maize. The nitrogen (PDIN) to energy (UF) ratios in the diets were 80 (D80), 100 (D100), 120 (D120) and 140 (D140). Defaunation decreased the in situ DM degradation of *Digitaria decumbens* ($P = 0.06$) and this decrease was significantly enhanced while the PDIN / UF ratio decreased. Defaunation also decreased ($P < 0.05$) activities of fibrolytic enzymes, and activities of amylolytic enzymes tended to decrease ($P = 0.06$). The diet effect interacted ($P < 0.05$) with defaunation effect on carboxymethyl cellulase activity. Ruminal fermentative products ammonia (NH3) and volatile fatty acids (VFA) decreased significantly ($P < 0.05$) after defaunation. Defaunation significantly decreased the molar proportion of butyrate ($P < 0.01$). Defaunation increased the number of total bacteria ($P = 0.07$), and no interaction appeared. From theses results defaunation clearly decreased microbial activities in the rumen. Despite reduced enzymatic activities, a lower bacterial proteolysis and a more profitable ruminal VFA profile in defaunated animals induced a better use of nutrients by defaunated animals.

enzymatic activities / microbial population / defaunation / fiber degradation / sheep.

Résumé — Les effets de la défaunation sur les activités microbiennes du rumen de moutons ingérant des rations mixtes (*Digitaria decumbens* en vert plus du concentré). Les acivités microbiennes du rumen ont été estimées par la technique de dégradabilité in sacco et par des tests enzymatiques, tandis que les principales populations de bactéries cellulolytiques ont été dénombrées à l'aide de sondes spécifiques d'hybridation ciblant l'ARNr 16S. Huit béliers faunés et 8 béliers défaunés munis de cannules ruminales ont été utilisés. Chaque animal était alimenté par 4 régimes mixtes différents composés d'herbe de *Digitaria decumbens* complémentée avec 4 mélanges de tourteau de soja et de maïs broyé. Le niveau de proteine (PDIN) / énergie (UF) du régime était de 80 (D80), 100 (D100), 120 (D120) et 140 (D140). Ainsi, les effets de la

défaunation de type curviliniare ont pu être détectés. La défaunation a diminué la dégradation in sacco de la matière sèche ($P = 0.06$), de plus cette diminution s'est accrue significativement avec l'augmentation du pourcentage de maïs ingéré. La défaunation a aussi diminué les activités des enzymes fibrolytiques ($P < 0.05$) et les activités des enzymes amylolytiques ont eu tendance à décroître ($P = 0.06$). L'effet du régime a significativement interféré ($P < 0.05$) avec l'effet de la défaunation sur l'activité de la carboxymethyl cellulase. La défaunation a significativement diminué ($P < 0.05$) les concentrations des produits de la fermentaion ruminale, l'ammoniaque (NH3) et les acides gras volatiles (AGV). La proportion molaire de butyrate a été significativement diminuée par la défaunation ($P < 0.01$). Le nombre de bactéries totales a été augmenté après la défaunation ($P = 0.07$), mais aucune interaction n'est apparue. A partir de ces résultats il apparait clairement que la défaunation diminue les activités microbiennes du rumen. La réduction des activités enzymatiques est contrebalancée par la moindre protéolyse bactérienne et le profil des AGV plus avantageux dans le cas des animaux défaunés. En conséquence une meilleure utilisation des nutriments peut etre escomptée pour les animaux défaunés.

Activité enzymatique / population microbienne / défaunation / dégradabilité / mouton

1. Introduction

The effects of defaunation of ruminants have been investigated for many decades now, as an attempt to enhance animal production and valorise local resources or by-products [2,4,15,25]. Defaunation effects on growth performance have been contradictory, Bird [3,6,7] reported increased growth performance after defaunation whereas others report inverse effects [1,8,18]. Hence, to find out the factors which may explain the observed results several authors conducted digestion trials in order to measure ruminal degradation, mainly with the in sacco or in the vivo digestibilities techniques. Consequently few studies, on the effects of defaunation on the enzymatic activities in the rumen are available, and on the determination, with modern tools, of the microbial population involved. As Bird and Leng [5], we hypothesise that the nature of the diet may be the major factor of variation of the effect of defaunation on animal performance, especially through the resulting nitrogen to energy balance of the absorbed nutriments.

The aim of this study was to investigate the effect of defaunation and its interaction with the diet on the evolution of the microbial population, the enzymatic microbial activities and the fermentative products in the rumen.

2. Materials and Methods

2.1. Location

The research was carried out (in 2000) at the animal experimental station of the National Agricultural Research Institute of the French West Indies, Guadeloupe (latitude 16° 16' N, longitude 61° 30'). Average temperatures ranged from 21°C to 31°C. The rainfall on the experimental site is 3000 mm a year.

2.2. Experimental design, animals and diets

Sixteen (16) Black Belly rams (average live weight : 45.1, S.D 7.7kg; 2-years old), each containing surgically implanted rumen cannula were used in this trial. Half of them were defaunated using a milk diet. During the defaunation process the diet was gradually changed from a fresh young grass to milk powder diet within 10 days. Then animals were fed with milk alone during 7 days. The diet was gradually change from milk to grass fresh forage. The milk powder was mixed with 400 ml of a 37° C water, and poured into the rumen via rumen cannula.

The 16 rams were conducted following two parallel Latin Square designs (faunated *vs* defaunated). Defaunated and faunated rams, placed in metabolism cage and housed in the same room, were isolated with a punch cloth. Care was taken to prevent accidental inoculation of the rams with protozoa. Each week ruminal fluid of defaunated animals was examined in order to control that they remained free of protozoa. They were fed 4 diets. The diet D140 was a 28 days age of regrowth *Digitaria decumbens* grass distributed *ad libitum* with 200 g of soja meal. In diets D 120, D 100 and D 80, the *Digitaria decumbens* of 28 days age of regrowth was complemented with 460 g (160g of soya meal + 300g of corn); 700 g (100g soya meal + 600g of corn) and 1000 g (1000g of corn) of concentrate respectively. Each period was composed of 14 days of adaptation to the diet, 5 days of intake measurements, 4 days of ruminal content sampling and 7 days of nylon bags incubation.

The chemical composition of the diet is shown in table 1. Mineralised salt blocks were in free access. The blocks' composition was: Ca (60.0), P (20.0), Mg (10.0), Na (280.0), Zn (17.5), Mn (5.5), Fe (1.5), I (0.03), Co (0.03) and Se (0.01 g / kg).

2.3. Experimental procedures

The degradation of the forage was measured by the nylon bag technique. Surface of nylon bags was 50 cm² over a pore size of 50 µm (Blutex, Tissage Tissus Technique, Villeneuve La Garenne 92390, France). The nylon bags were filled with 15 g of the fresh 28 days *Digitaria decumbens* grass, cut at about 30 mm. The same forage, stored at – 20° C, was used during the four periods. The nylon bags were placed in the rumen after feeding and the time of incubation was 24, 48 and 96 hours. 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 every time) 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.

Samples of rumen liquor were taken from the sheep, immediately before the morning meal, and at 3, 6 and 12 hours after food was first available, during two consecutive days. The pH was measured immediately after sampling. Samples of rumen fluid were frozen (-20°C) after adding a mixture of H_3PO_4 and $HgCl_3$ (1 vol. / 10 vol.) prior to VFA determination. Rumen fluid was stored (4°C) with H_2SO_4 (1 vol. / 50 vol.) prior to ammonia determination.

Nine millilitres of rumen fluid were collected before and 3 hours after the morning meal. They were added to 1 ml of preservatives (50% Glycerol, 48% distilled water and 2% formaldehyde). Protozoa population was determined with a Dolfüss counting cell (Elvetec Services, Clermont ferrand, France) according to the method described by Jouany and Sénaud [22].

Representative samples of rumen contents (300g) were collected 3 hours after the morning meal (rumen total emptying) from faunated and defaunated animals fed with D80 and D140 diet and then freeze-dried in order to determine bacterial population.

Two representative samples of rumen contents (300g) were collected, one just before and the second 3 hours after the morning meal, in order to measure enzyme activities from the solid-adherent microorganisms.

Table 1 : Chemical composition (g / kg) of, organic matter (OM), Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and crude protein (CP) of the mixed diets composed of *Digitaria decumbens* grass (28 days age of regrowth) and 4 concentrates: Concentrate A (100% of corn), B (14% soya meal + 86 % of corn), C (35 % of soya meal + 65 % of corn) and D (100% of soya meal) were used on diets D80, D100, D120 and D140 respectively.

	OM	CP	NDF	ADF	ADL	PDIN / UF *
Digitaria decumbens	930.0	82.0	725.0	351.0	41.0	
Concentrate A	982.0	104.0	130.0	35.0	7.0	80
Concentrate B	974.0	164.0	131.0	43.0	7.0	100
Concentrate C	962.0	249.0	133.0	53.0	6.9	120
Concentrate D	927.0	516.0	140.0	87.0	6.8	140

*The diet D80, D100, D120 and D140 were formulated to provide 4 protein / energy ratios of 80, 100, 120, and 140 PDIN / UF level of contents in the diet respectively.

The measurements were carried out on two consecutive days, on 4 defaunated and 4 faunated animals. Samples were then strained through a 100µm nylon filter to collect the solid phase, under anaerobic conditions (CO_2 flows).

2.4. Chemical analysis

Dry matter was determined by drying to a constant weight at 60°C in a forced draught oven. The samples were ground (1 mm) prior to chemical determination. The OM content was determined as the difference between dry and ash weight. Samples were burnt for 10 h at 550°C. The Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL) were estimated following the method of Van Soest et al. [38]. Nitrogen concentration was determined using the Dumas technique. Ruminal volatile fatty acids were measured in the rumen liquid by gas chromatography [34]. Ammonia concentration was estimated in rumen liquor by distillation and titration.

The structure of the cellulolytic bacterial community has been investigated using specific oligonucleotide probes targeting 16S rRNA, on ruminal total digesta sampled 3 hours after the morning meal then freeze-dried. Stahl et al. method [36] has been adapted. Rumen digesta was then freeze-dried (50mg) and mixed with 1ml of RNAzol (Bioprobe System, Montreuil, France) and with 0.3 g zirconium beads (Biospec products, INC) in order to disrupt microorganisms. A Chloroform / Isopropanol / ethanol extraction was performed in order to recover total microbial RNA. Mix is heated to 100°C during 6 minutes then is shaken during 2 minutes. After two chloroform extractions (475µl), centrifugation at 14000 *g*, at room temperature, during 15 minutes was performed. The pellet was discarded and the supernatant was added to an equal volume of isopropanol to purify the pellet. After a second centrifugation at 14000 *g* at room temperature, the pellet was rinsed with ethanol 75°C (800µl) at 4°C. The supernatant is then discarded and 100µL of DEPC water is added to pelleted total microbial RNA before storage at –20°C. Oligonucleotide probes were labelled with gamma ATP (^{32}P) (Eurogenetec) with specific activity of 4000 curies / mmole at 5'P end using a T4 kinase (Eurogenetec). Four Oligonucleotide probes were used : the universal probe S-D Bact-0338-a-A-18 targeting the bacterial domain (Eub 338), probe S-S-F.suc-0650-a-A-20 targeting *Fibrobacter succinogenens* (*Fs*), probe S-S-R.alb-0196-a-A-18 targeting *Ruminococcus albus* (*Ra*) and probe S-S-R.fla-1269-a-A-20 targeting *Ruminococcus flavefaciens* (*Rf*). After denaturation with glutharaldehyde (2% phosphate buffer 50mM, pH=7) RNA extracts ,

between 25 and 100 ng RNA, were blotted on a nylon membrane (Hybond N+, Amersham Pharmacia Biotech, Saclay, France) using a dot blot apparatus (Biorad). Membrane was air dried at room temperature and RNA was fixed. Membrane were incubated for 1 hour with 100µl / cm ^{2} buffer (0.9M NaCl, 50mM $NaPO_4$, 5 mM EDTA, Denhart solution 10X and 0.5 mg PolyA / ml , pH=7.0) at 45°C in an hybridisation oven. Labelled probes were added to each membrane (at about 2x10 ^{5} cpm per sample dot) and were incubated for 5H at 45°C in an hybridisation oven. Membranes were washed for 20 minutes with SSC buffer (150mM NaCL, 150mM Sodium Citrate) and 1% SDS at temperature specific to each probe, at which duplex DNA-RNA is dissociated. Membrane were then air dried and exposed to a screen (Storm, Molecular Dynamics) before bound probes were quantified using Image Quant Software (Molecular Dynamics). RNA quantity in samples of ruminal contents was expressed as a ratio to RNA quantity obtained with eubacteria probe (EUB338). Results of each cellulolytic bacteria species were expressed as a percentage of total cellulolytic RNA (sum of all predominant cellulolytic bacteria, *Ra*, *Rf* and *Fs* 85) in the rumen.

Enzymatic manipulations were performed under anaerobic conditions. Enzymes extraction from the solid adherent microorganisms were achieved according to the procedure detailed by Martin and Michalet-Doreau [29]. Enzymes from solid-adherent microorganisms were measured using the assay procedures detailed by Nozière et al. [32] and Nozière and Michalet-Doreau [33]. Polysaccharides activities were determined by detecting spectrophotometrically at 410 nm [28], the amount of reducing sugars released from the purified substrates after incubation (60min 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 [35]. Enzyme activities were expressed as micromoles of reducing sugar (for polysaccharides) or nanomoles of p-nitrophenol (for glucosidase) released per milligram of protein per hour (specific activity) or per gram of DM per hour (total activity).

2.5. Statistical analyses

The ANOVA procedures (GLM) were used in all statistical analyses [31]. For the nylon bag degradation, the enzymatic activities and the pH, the statistical model fixed

effects used were: defaunation (faunated *vs* defaunated), the diet, the period, the animal (inside of defaunation), the interaction between defaunation and the diet. The statistical analysis of the ruminal ammonia concentrations was performed with the same preceding model, with the nitrogen intake considered as a covariate. The nitrogen intake was substituted by the total digestible organic matter in the same statistical analyses of the concentrations and the molar proportions of VFA. The number of bacteria was analysed using GLM procedure with: defaunation (faunated *vs* defaunated), the diet, the period and defaunation interaction with diet, considered as the fixed effects. The number of protozoa was analysed using GLM procedure with: the percentage of maize intake, the period and the animal, considered as the fixed effects.

3. Results

3.1. Microbial population in the rumen

3.1.1. Protozoa concentration in faunated animals

The number of protozoa, before the morning meal, raised up while the percentage of maize intake increased up to 38% (9.4×10^5 / ml s.e. = 1.8×10^5 / ml). Then it dropped when the percentage of maize intake reached 63% (6×10^5 / ml s.e. = 1.8×10^5 / ml). The same significant ($P < 0.05$) evolution was observed three hours after the morning meal.

3.1.2. Bacteria concentration

The amount of the total bacteria population in the rumen was increased ($P = 0.07$) after defaunation (Tab. 2). No interaction between defaunation and the diet appeared. The amount of *Ruminococcus flavefaciens* was too low to be detectable, so the results of this species are not presented. Concerning the amount of the predominant cellulolytic bacteria population (*Ruminococcus albus* and *Fibrobacter succinogenens*), defaunation had no effect on their number. Despite no significant interaction between defaunation and the diet effects occurred, the number of the cellulolytic bacteria increased ($P = 0.08$) after defaunation with the D80 diet, whereas an inverse effect was observed for the D140 diet.

Maize intake, %DMI				D80 63		D140 0			Effect		
PDIN / UF	F	D	s.e.	F	D	F	D	s.e.	Def.	Diet	D×Diet
Total bacteria	60.4	107.5	16.3	53.5	67.1	92.6	122	24.4			
Ruminococcus albus (R.a)	1.19	1.76	0.3	0.7	2.1	1.6	1.4	0.41			
Fibrobacter succinogenens (F.s)	2.2	1.6	0.4	1.2	0.9	3.2	2.2	0.55			
Cellulolytic bacteria[b]	3.6	3.7	0.5	1.8	3.8	5.3	3.7	0.79			
% Ra	1.89	2.08	0.6	1.8	3.1	1.9	1	0.89			
% Fs	2.9	1.7	0.5	2.3	1.4	3.5	2	0.72			
% Celluloytic bacteria	4.89	4.01	0.9	4.1	4.9	5.7	3.1	1.31			

Cellulolytic bacteria[b] : sum of the three major cellulolytic bacteria in the rumen (*Ra*,*Fs*, and *R. flavefasciens*)
Def.: Defaunation effect; * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Table 2 : Effect of defaunation on some cellulolytic bacteria, in the rumen of rams consuming mixed diets composed of *Digitaria decumbens* grass (28 days age of regrowth) and 4 concentrates mixture (soja meal + ground corn). The diets were formulated to provide 4 protein / energy ratios (PDIN / UF) of 80, 100, 120 and 140 % on diet D80, D100, D120 and D140 respectively. Amount of bacteria (µg microbial RNA / g DM rumen digesta).

Defaunation decreased (P = 0.50) the percentage of these cellulolytic bacteria (Tab. 2). No significant interaction between defaunation and the diet appeared. Nevertheless defaunation effect was decreased as the PDIN / UF level of the diet increased.

3.2. Enzymatic activity

Whatever the sampling time considered, defaunation decreased both fibrolytic enzymes (polysaccharidases) total activity (µmol reducing sugars / g DM*h) and specific activity (µmol p-nitrophenol / mg protein*h), which are responsible for the degradation of plant fiber (Tab. 3). Defaunation effect on total and specific activity of carboxymethyl cellulase (CMCase) increased while the PDIN / UF level of the diet increased up to 120, then it decreased when the PDIN / UF level of the diet reached 140. Significant interaction (P < 0.05) between defaunation effect and the diet effect only appeared when the total activity of the carboxymethyl cellulase was measured before the morning meal. Whatever the sampling time, defaunation effect on total and specific activity of xylanase globally increased while the PDIN / UF level of the diet increased, but no significant interaction appeared.

Concerning amylolytic activity, defaunation tended to decrease both specific and total activities of amylase (P = 0.10) before the morning meal (Tab. 3). Three hours after the morning meal defaunation decreased total activity of amylase, whereas it increased amylase specific activity. No significant interaction appeared between defaunation and the diet. Whatever the sampling time, defaunation effect on total and specific activity of amylase decreased while the PDIN / UF level of the diet increased up to 120, then it increased above 120, but no significant interaction appeared. Total activity of α-D glucosidase significantly decreased after defaunation (P < 0.01), at 0h and 3h after the morning meal (Tab. 4). Defaunation also decreased (P = 0.09) α-D glucosidase specific activity at 0h and 3h after the morning meal.

The main glucosidases enzymes that participate to fibre degradation are presented in Table 4. Concerning the glucosidases enzymes that exhibit the highest activities, β-D-glucosidase total activity, decreased significantly (P < 0.01) after defaunation, whatever the sampling time. This decrease was enhanced by the increased amount of PDIN / UF in the diet, up to 120. The same trend was observed with β-D-galactosidase total activity, but the effects were not significant 3 hours after feeding.

Table 3 : Effect of defaunation on enzymatic activity (polysaccharidases) in rumen solid-associated microorganisms, in rams consuming mixed diets composed of *Digitaria decumbens* grass (28 days age of regrowth) and 4 concentrates mixture (soja meal + ground corn). The diets were formulated to provide 4 protein / energy ratios (PDIN / UF) of 80, 100, 120 and 140 on diet D80, D100, D120 and D140 respectively.

PDIN / UF				D80		D100		D120		D140			Effect		
Maize intake, %DMI				63		38		20		0					
	F	D	s.e.d.	F	D	F	D	F	D	F	D	s.e.d.	Def.	Diet	D×Diet
Item and time after feeding															
Total activity (µmol reducing sugar / (gDM*h))															
CMCase															
0h	21.4	6.2	3.9	3.5	1.1	20.5	13.5	51.9	4.2	9.5	6.0	7.6	*	*	*
3h	23.9	8.3	3.9	9.6	2.1	14.2	4.6	47.2	15.3	24.8	11.2	7.6	*	*	
Xylanase															
0h	168	55.7	20.6	20.5	0.7	144	42.4	265	93.7	243	86.0	40.2	**	**	
3h	138	29.3	13.9	28.8	6.7	130	17.4	174	41.4	217	51.7	27.2	***	**	
Amylase															
0h	33.5	8.6	9.6	51.5	-2.9	15.4	9.0	19.8	15.9	47.4	12.5	19.0			
3h	48.2	25.6	11.3	59.5	5.4	37.0	27.6	30.0	32.9	66.0	36.6	18.7			
Specific activity (µmol reducing sugar / (mg protein*h))															
CMCase															
0h	2.7	1.1	0.5	0.3	0.3	3.0	2.2	5.2	0.8	2.0	1.0	0.9	*	*	
3h	2.8	2.2	0.5	1.7	2.3	2.2	1.1	4.3	2.4	3.1	2.8	1.02			
Xylanase															
0h	24	12.1	3.6	-3.2	0.9	23.8	10.3	35.8	16.0	39.4	21.2	6.9	*	**	
3h	18.1	9.49	2.7	0.5	1.8	21.6	10.1	24.7	8.2	25.7	17.9	5.25	*	**	
Amylase															
0h	4.48	1.3	1.3	7.5	-0.1	2.7	2.2	2.1	2.0	5.7	1.0	2.46			
3h	6.38	10.9	1.8	7.4	6.2	6.1	15.5	5.9	8.9	6.2	12.8	3.5			

Def.: Defaunation effect; D : defaunated animals; F : Faunated animals ; * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Table 4 : Effect of defaunation on enzymatic activity (glucosidases) in rumen solid-associated microorganisms, in rams consuming mixed diets. Diets were composed of *Digitaria decumbens* grass (28 days age of regrowth) and 4 concentrates mixture (soja meal + ground corn). The diets were formulated to provide 4 protein / energy ratios (PDIN / UF) of 80, 100, 120 and 140 on diet D80, D100, D120 and D140 respectively.

PDIN / UF				D80		D100		D120		D140			Effect		
maize intake, %DMI				63		38		20		0					
	F	D	s.e.	F	D	F	D	F	D	F	D	s.e.	Def.	Diet	D×Diet
Item and time after feeding															
Total activity (µmol p-nitrophenol / (gDM*h))															
β-D glucosidase															
0h	66.8	38.7	5.1	35.6	16.3	67.6	39.5	89.9	50.3	74.2	48.5	9.9	**	**	
3h	68.4	29.7	4.8	37.2	10.6	60.7	27.1	75.2	34.9	101	46.3	9.3	***	***	
β-D xylosidase															
0h	88.0	64.9	7.9	26.1	21.2	88.8	71.9	125	79.2	112	87.6	15.4		***	
3h	77.9	47.6	6.8	22.5	13.7	76.3	45.5	89.4	57.2	124	74.1	13.2	**	***	
α-D glucosidase															
0h	8.8	4.6	0.98	7.9	4.5	11.0	6.1	10.9	4.2	5.5	3.6	1.9	**		
3h	11.4	5.6	1.6	8.9	4.3	12.4	5.8	14.1	5.4	9.9	6.6	3.2	*		
Specific activity (µmol p-nitrophenol / (mg protein*h))															
β-D glucosidase															
0h	10.0	9.9	0.6	8.0	9.9	9.5	9.3	11.2	10.5	11.5	10.1	1.2			
3h	10.5	9.9	0.8	11.3	8.8	9.4	10.0	9.7	8.8	11.3	11.9	1.6			
β-D xylosidase															
0h	13.3	15.6	1.4	5.7	11.1	13.1	16.1	16.3	15.0	18.1	20.2	2.8		*	
3h	11.4	13.3	1.5	8.7	7.6	11.5	13.6	11.6	12.9	13.8	18.9	3.4			
α-D glucosidase															
0h	1.35	0.89	0.18	1.8	2.1	1.6	0.8	1.2	0.4	0.8	0.2	0.35		**	
3h	1.87	2.41	0.48	2.7	3.3	1.9	2.3	1.8	1.4	1.0	2.6	0.9			

Def.: Defaunation effect; D : defaunated animals; F : Faunated animals ; * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Generally speaking, specific activities of both β-D-glucosidase and β-D-galactosidase followed the same variations than their respective total activities.

3.3. In situ DM degradation

Defaunation reduced in situ nylon bag DM degradation of forage, when nylon bags were incubated for 24 hours in the rumen (47.8 *vs* 46.9, s.e. 1.5 %, $P = 0.24$) and for 48 hours (61.0 *vs* 63.7, s.e. 2.1 %, $P = 0.18$) but defaunation effect was not significant. Defaunation effect became significant after 96 hours incubation of the bags (74.0 *vs* 70.3, s.e. 0.2%, $P = 0.01$). Whatever the nylon bag incubation time in the rumen, the DM degradation decreased with the decreasing nitrogen to energy ratio in the diet , ($P < 0.01$), as indicated in table 5. Differences between faunated and defaunated animals increased while the level of PDIN / UF ratio in the diet decreased.

3.4. Ammonia concentrations

Ammonia concentrations in rumen liquid were always lower ($P < 0.03$) with defaunated animals, whatever the sampling time (Tab. 5) . Mean values of faunated *vs* defaunated rams were respectively 186 *vs* 147 mgN / L (s.e. 7.4). The higher differences between faunated and defaunated rams were observed 3 hours after the morning meal. The differences increased ($P < 0.01$) with the level of PDIN / UF ratio in the diet.

3.5. pH and VFA in the rumen.

3.5.1. pH

The pH of ruminal fluid remained lower with faunated rams than with defaunated rams, whatever the sampling time considered (Tab. 6). Defaunation effect became significant ($P < 0.01$) only for pH measured just before the morning meal (6.30 *vs* 6.53, s.e. 0.05).

3.5.2. VFA

The VFA concentrations measured in rumen fluid 0, 3 and 6 hours after the morning meal were significantly ($P < 0.03$) higher with conventional animals than with ciliate-free animals (Tab. 6).

Table 5 : Effect of defaunation on in situ dry matter (DM) degradation, and ammonia (NH3) in the rumen of rams consuming mixed diets. Diets were composed of *Digitaria decumbens* grass (28 days age of regrowth) and 4 concentrates mixture (soja meal + ground corn). The diets were formulated to provide 4 protein / energy ratios (PDIN / UF) of 80, 100, 120 and 140 on diet D80, D100, D120 and D140 respectively.

PDIN / UF				D80		D100		D120		D140					
Maize intake, %DMI				63		38		20		0			Effect		
	F	D	s.e.	F	D	F	D	F	D	F	D	s.e.	Def.	Diet	Def×Diet
Item and sampling time after feeding															
In situ degradation of DM															
24h	48.3	45.9	1.3	40.6	34.2	47.8	44.6	51.4	50.1	53.4	54.7	2.51		***	
48h	63.8	61.0	1.4	59.4	51.5	63.1	60.5	66.0	64.0	66.5	67.9	2.69		**	
96h	74.1	70.3	0.9	72.5	60.9	73.2	71.9	75.5	72.8	75.0	75.7	1.75	*	**	*
NH3 (mgN / L)															
0h	173	148	8.1	115	118	172	153	197	155	207	165	15.9	*	**	
3h	201	150	9.6	105	118	209	130	218	148	272	203	19	***	***	
6h	180	134	8.3	103	104	171	127	181	137	265	170	16.4	***	***	*
12h	190	157	9	125	101	180	150	210	183	244	193	17.8	*	***	
Mean NH3	186	147	7.4	112	110	183	140	202	156	247	183	14.6	***	***	

Def : Defaunation effect; D : defaunated animals F: faunated animals; * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Table 6 : Effect of defaunation on pH and Volatile Fatty Acids (VFA), in the rumen of rams consuming mixed diets. Diets were composed of *Digitaria decumbens* grass (28 days age of regrowth) and 4 concentrates mixture (soja meal + ground corn). The diets were formulated to provide 4 protein / energy ratios (PDIN / UF) of 80, 100, 120 and 140 on diet D80, D100, D120 and D140 respectively.

PDIN / UF				D80		D100		D120		D140					
Maize intake, %DMI				63		38		20		0			Effect		
	F	D	s.e.	F	D	F	D	F	D	F	D	s.e.	Def.	Diet	Def×Diet
Item and sampling time after feeding															
PH															
0h	6.30	6.53	0.06	6.09	6.32	6.31	6.42	6.41	6.71	6.38	6.68	0.11	**	**	
3h	6.06	6.13	0.04	5.86	5.99	5.93	5.97	6.17	6.10	6.28	6.47	0.08		***	
6h	5.91	6.00	0.05	5.73	5.88	5.74	5.83	5.96	5.88	6.24	6.42	0.09		***	
12h	5.99	6.09	0.04	5.87	5.88	5.92	6.09	6.00	6.13	6.21	6.28	0.08		***	
Mean pH	6.07	6.19	0.04	5.89	6.01	5.97	6.08	6.13	6.20	6.28	6.46	0.08	*	***	
Total VFA (mmol / L)															
0h	59.7	52.3	1.7	59.4	51	62.2	59.7	56.3	50.2	61.0	48.4	3.3	**		
3h	64.0	60.7	1.6	61.4	58.6	68.4	64.1	65.4	62.2	60.9	58.0	3.2			
6h	65.0	58.3	1.8	64.9	51.3	69.5	61.2	65.5	62.2	60.2	57.9	3.6	*		
12h	61.6	61.4	2	59.0	59.5	69.9	66.1	62.4	64.1	55.2	56.1	3.9		*	
Mean total VFA	62.6	58.2	1.2	61.2	55.1	67.5	63.0	62.4	59.7	59.4	55.1	2.3	*	*	
Molar proportions															
Acetate (C2, molar %)	63.1	64.2	0.8	59.6	60.8	62.0	64.4	63.7	64.9	66.9	66.8	1.49		**	
Propionate (C3, molar %)	20.3	20.6	0.4	20.7	21.3	22.1	18.7	19.9	21.7	18.3	20.5	0.86			*
Butyrate (C4, molar %)	12.2	10.1	0.4	13.6	10.7	11.9	12.2	12.7	9.0	10.5	8.5	0.78	***	*	
Ratios															
C2 / C3	3.2	3.2	0.09	2.9	2.9	2.9	3.6	3.3	3.1	3.7	3.3	0.19			*
C2 / C4	6.6	5.3	0.25	4.6	5.8	5.3	5.7	5.0	7.0	6.4	7.9	0.49	**	*	
C3 / C4	1.7	2.1	0.06	1.6	2.0	1.9	1.6	1.6	2.4	1.7	2.4	0.13	***		***
(C2+C4) / C3	3.8	3.7	0.1	3.6	3.4	3.4	4.2	4.0	3.5	4.3	3.7	0.2			*

Def : Defaunation effect; D : defaunated animals F: faunated animals; * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

There was no significant effect of defaunation 12 hours after the morning meal. The means values of VFA concentrations decreased significantly after defaunation ($P < 0.05$). No significant interaction between defaunation and the diet appeared. Defaunation decreased ($P = 0.06$) acetate and propionate mean concentrations and significantly decreased ($P < 0.001$) butyrate mean concentrations (7.6 *vs* 5.9, s.e. = 2.6 mM).

3.5.3. VFA profile

There was no defaunation effect on the molar proportions of acetate ($P = 0.29$) and propionate ($P = 0.64$) in the total VFA, whereas the molar proportion of butyrate was significantly decreased by defaunation (12.2 *vs* 10.1, s.e. 0.39 %, $P < 0.01$) (Tab. 6).

The acetate to propionate ratio was not significantly decreased by defaunation ($P = 0.96$). Nevertheless a significant interaction appeared between defaunation and the diet, defaunation increased the acetate to propionate ratio with diet D100. The acetate to butyrate ratio was significantly ($P < 0.01$) increased by defaunation (5.33 *vs* 6.58, s.e. 0.24). There was no interaction between defaunation and the diet. The propionate to butyrate ratio was significantly ($P < 0.01$) increased by defaunation (2.09 *vs* 1.69, s.e. 0.06). There was an interaction between defaunation and the diet, the ratio was decreased after defaunation with the D100 diet. There was no effect ($P = 0.49$) of defaunation on the (acetate + butyrate) to propionate ratio. Nevertheless a significant interaction appeared between defaunation and the diet, this ratio increased after defaunation with the diet D100.

4. Discussion

The present study indicate that defaunation decreased microbial activities in the rumen. Defaunation increased the number of total bacterial population in the rumen, as was already observed by classical bacterial viable counts [13,24,27]. It may be hypothesised that new ecological niches emerged as the protozoa disappeared, leading to increased number of bacteria. It is admitted that the three major cellulolytic bacteria in the rumen are composed of *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Fibrobacter succinogenens* [9], that is the reason why we studied these 3 species [14]. Despite their major role in fibre degradation, our results indicated that this cellulolytic population only represented 3 to 5 % of the total bacteria in the rumen. This result is in agreement with previous reports [36,40]. It may suggest that, on the one hand, interaction between microbial species are crucial and on the other hand, more than their number it is the

diversity of these population which is the first factor limiting fibrolytic degradation [16]. Contrary to the increased number of total bacteria, the number of cellulolytic bacteria remained unchanged after defaunation. As a consequence the percentage of the cellulolytic bacteria decreased by 20% after defaunation, nevertheless this decrease was not significant. This result is in agreement with Jouany and Ushida [23], they observed that this tendency may be explained by direct action of the protozoa on the cellulolytic bacteria or on enhancing the ruminal condition for their growth [27]. Moreover the percentage of the Ra specie remained unchanged after defaunation whereas that of Fs tended to decrease. As Fs is assumed to be the more numerous cellulolytic bacteria in the rumen [14], the decrease of the percentage of Fs may explain the decreased percentage of the cellulolytic population.

The activity of the fibrolytic enzymes decreased after defaunation. Similar results were obtained by Kurihara et al. [26]. The first factor responsible for the decrease of the fibrolytic activity may be attributed to the disappearance of the fibrolytic activity of protozoa after defaunation. The decreased percentage of the cellulolytic bacteria may also account for the decreased fibrolytic enzymes. Moreover Fs decrease may mainly account for the decrease of fibrolytic activity as it is assumed to have the higher fibrolytic enzyme activity [11]. The second factor that is reported to explain the decrease of fibrolytic activity is the pH [10,21,30]. Our results indicate that defaunation increased ruminal pH, so they are not in agreement with this previous statements. Increased pH after defaunation is coherent with previous results (Eugène et al., unpublished data) which indicate that defaunation effect on the pH varied and when the percentage of concentrate in the diet was inferior to about 50% defaunation increased the pH. Indeed in our study the percentage of concentrate was superior to 50% only with the D80 diet and this may therefore explain the increased pH after defaunation. Therefore in our study decreased fibrolytic activity may presumably be attributed to decreased fibrolytic microorganisms in the rumen.

Defaunation decreased in situ DM degradation and this decrease is reduced while the PDIN / UF ratio increased. Similar results were observed by Ushida et al. [37]. The low decrease by 4% of the in situ DM degradation has to be opposed to the rather high decrease (60%) in enzymatic activities, and is finally more coherent with the low decrease of the ruminal digestibility of NDF, by about 10%, as observed in a previous study [19].

Ammonia concentrations decreased after defaunation, as is classically observed in data literature. This decrease was enhanced as the PDIN / UF level of the diet increased. These results are mainly explained by the absence of predation of bacteria by protozoa after defaunation, consequently bacterial proteolysis tended to decrease after defaunation. Increased amounts of dietary nitrogen degraded in the rumen, while the PDIN / UF level of the diet increased, also explain the increased ammonia concentrations. All the more so since protozoa seem to digest both dietary and bacterial nitrogen at the same rate (Eugène et al. unpublished data) [20]. Thus dietary nitrogen degradation is reduced after defaunation. Increased use of ammonia by bacteria could have accounted for this decrease, as defaunation increased the number of total bacteria. Nevertheless Itabashi and Kandatsu [17] reported that the half life of NH3-N15 does not differ between defaunated and faunated animals, indicating that defaunation did not increased ammonia uptake by bacteria.

Defaunation decreased VFA concentration. This result is in agreement with the literature data with such diets [19]. This decrease (7%) could mostly be explained by decreased OM degradation in the rumen that may occur since our results indicate that in sacco digestibility in the rumen decreased after defaunation. Nevertheless this decrease is partly compensated for increased digestibility of OM, in the lower gut, thus limiting the decrease of energy availability for the animal after defaunation (Eugène et al. unpublished data). Defaunation also modified VFA profile in the rumen. Indeed higher C3 / C4 and C2 / C4 ratios were observed after defaunation in our study. This may lead to increased use of absorbed energy as the propionate metabolic use is more favourable than the acetate or butyrate ones [39]. The modification of microbial population of the rumen and consequently of their fermentative activity may be the main factor affecting the orientation of the ruminal fermentation. Several results are consistent with this statement. First of all, our results indicate that Fs, which is one of the major cellulolytic and succinate producers [12], decreased after defaunation. Moreover, amylolytic bacteria, which produced succinate, are reported to increase after defaunation [13,27]. Finally, as protozoa are the main butyrate producers compared to the predominant bacteria *Butyrivibrio fibrisolvens* [41], the decreased molar proportion of butyrate observed after defaunation is coherent with the elimination of protozoa after defaunation. In our study the diet had important effects, nevertheless it did not strongly interfered with defaunation effect, as was presumed.

5. Conclusion

Our results on the effect of defaunation on enzymatic microbial activities confirm previous studies, yet complementary analyses should be elaborated in order to find more relevant explanatory factor. Despite high variability of the values, the determination of the evolution of microbial population involved in the fibrolytic process, with the modern tools in order to monitor the effect of defaunation, led to satisfactory results. Nevertheless , as an attempt to determine more microbial predominant population in the rumen, more specific probes should be elaborated.

The decrease in the supply of energy after defaunation is moderate, because of the compensatory digestion of OM in the intestine. Furthermore the spare of nitrogen is high in defaunated animals. These latter results strongly account for the higher food conversion efficiency and thus, to the higher average daily gains observed in young defaunated animals compared to conventional animals, given similar diets.

Acknowledgements

The authors would like to thank L. Philibert, S. Calif , F. Pommier, M. Arjournin, and F. Periacarpin for their technical collaboration to this work. This work was supported by the “Région Guadeloupe” and the European Economic Community (FEOGA).

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2. INTRODUCTION A L' ETUDE EXPERIMENTALE 2

Au cours de la seconde étude expérimentale, un essai zootechnique et un essai de digestibilité ont été mené en parallèle, pour lesquels nous avons testé des rations composées uniquement du fourrage *Digitaria decumbens*. Dans cette étude le gradient azote / énergie des rations a été obtenu en faisant varier l'âge de repousse du fourrage de 14, 28, 42 à 56 jours. Pour l'essai de croissance sur les agneaux cela a généré des teneurs en azote / énergie respectives de 90, 70, 60 et 50 g PDIN / UF. Par suite de sévères conditions climatiques (sécheresse) l'essai des béliers à été décalé dans le temps. Par conséquent, les teneurs en azote / énergie ont respectivement varié de 120, 110, 90 et 70 g PDIN / UF.

Nous présenterons: 1.- les effets de la défaunation sur les performances de croissance d'agneaux et sur la digestion de béliers, ingérant uniquement du fourrage *Digitaria decumbens*, dans la publication 4 (soumise à Animal Feed Science and Technology).

2 - les effets de la défaunation sur les activités fibrolytiques microbiennes, sur les produits fermentaires du rumen ainsi que sur la dynamique des populations microbiennes fibrolytiques, dans la publication 5 (soumise à Animal Feed Science and Technology).

Publication 4

Effects of defaunation on digestion of fresh *Digitaria decumbens* grass and growth of lambs

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(soumise à Animal Feed Science and Technology)

Abstract

The effects of defaunation on growth and digestion have been measured on sheep consuming four diets of *Digitaria decumbens* fresh grass. The four diets were differentiated through their protein to energy level of contents in the diet, generated by four ages of regrowth of the grass (14, 28, 42 and 56 days of regrowth). Conventional and cilate-free animals were conducted in two parallel designs. On the one hand, 20 faunated lambs and 20 defaunated lambs were used to measure growth. On the other hand, 8 conventional mature rams and 8 cilate-free mature rams were used to measure digestion parameters. Defaunation had no global effect on the average daily gain of animals (P = 0.8). Nevertheless, the growths observed with the cilate-free lambs consuming the 56-days of regrowth fresh grass were significantly higher than those observed with the conventional lambs. Feed conversion efficiency tended to be higher with conventional lambs compared with cilate-free lambs (6.78 *vs* 6.28, ± 0.38 g / g, P = 0.14). The same trend was observed with energy conversion efficiency. The differences of growth between conventional and cilate-free animals have been explained by the higher availability of protein in cilate-free animals. Duodenal non-ammonia nitrogen (NAN) flow was significantly increased (P < 0.01) by defaunation (18.3 *vs* 20.1 se 0.5 g NAN / d.). The differences of nitrogen flows observed between defaunated and faunated animals increased (P < 0.01) with the protein to energy ratio. A better microbial synthesis efficiency and a lower digestion of dietary nitrogen for the defaunated animals accounted for the differences of nitrogen availability between the animals.

Keywords: Digestion; Growth; Defaunation; Sheep; *Digitaria decumbens*

1. Introduction

In tropical countries, one of the major factor that limits animal performance is the low content of nitrogen and energy of the forage given to the ruminants. Bird and Leng (1978) hypothesised that positive effect of defaunation on growth and milk production of ruminants consuming poor roughage could be expected, as an improvement of intestinal protein availability occurred after defaunation. Consequently defaunation could be a tool to improve the nutrition level of the animals. Nevertheless, the results of the effect of

defaunation on animal performances are still controversial (Bird, 1989; Coleman, 1988; Jouany and Ushida, 1998). Some studies report negative effects of defaunation on animal growth (Abou Akkada and El-Shazly, 1964; Borhami et al., 1967; Christiansen et al., 1963; Ivan et al., 1986) whereas others indicate absence of any effect or positive effect (Becker and Everett , 1930; Bird et al., 1979; Bird and Seccombe, 1998; Demeyer et al., 1982). The nitrogen / (ruminal digestible energy) ratio of the absorbed nutrients would mainly be responsible for the observed results. Moreover, total amounts of protein and energy relatively to animal requirements would be another major factor of variation.

The aim of the present trial is to evaluate defaunation effect on growth of lambs consuming tropical forages. Fresh *Digitaria decumbens* grass differentiated with 4 protein / (ruminal energy) ratios has been used as a model. A complementary digestion trial has been elaborated to try to explain the animal response.

2. Materials and Methods

2.1. Location

The research was carried out in 2001 at the animal experimental station of the National Institute of Agricultural Research, in Guadeloupe, French West Indies, (16° 16' N, and 61° 30' W). Average temperatures ranged from 21°C to 31°C. The average yearly rainfall on the experimental site is 3000 mm.

2.2. Experimental design, animals and diets

2.2.1. Growth trial

Twenty Black Belly lambs (average live weight: 26.1 ± 5.2 kg) were defaunated by isolation from their dam, 1 day after their birth. They received colostrum, artificial milk, concentrate, and fresh grass. They were weaned at 3 months simultaneously with 20 fellow lambs living under their dam. After weaning all lambs received a similar diet (fresh grass and concentrate) during one month. Faunated and defaunated rams, isolated with a punch cloth, were housed in individual pens in the same room. Two groups of lambs (20 defaunated versus 20 faunated lambs) were constituted after allotting animals according to weight at the beginning of the trial and individual growth between 1 and 2 months of age. Another group of 20 faunated lambs (the control) has been constituted on the same basis. Faunated and defaunated animals were conducted following 2 parallels designs. The trial

was composed of 4 periods, each period lasted one month. The control lambs have consumed *Digitaria decumbens* grass hay and concentrate during the four periods. The diet has been formulated to maximise the growth of the lambs. The lambs of the 2 others groups were fed with the same diet during each period. They have received successively the diet D90, D70, D60 and D50, corresponding to a 14, 28, 42, and 56 days of regrowth of a fresh *Digitaria decumbens* grass distributed *ad libitum,* during the periods 1, 2, 3 and 4 respectively. The chemical composition of the diet is presented in Table 1.

2.2.2. Digestion trial

Sixteen (16) Black Belly rams (average live weight: 43.7 ± 5.5 kg, 2 years old), each containing surgically implanted rumen and duodenum cannulae were used in this trial. Half of them were defaunated using a milk diet. During the defaunation process the diet was gradually changed from a fresh young grass to milk powder diet within 10 days. Then the animals were fed with milk alone during 7 days. The diet was gradually changed from milk to fresh grass. The milk powder was mixed with 400 ml of water at 37° C, and poured into the rumen via ruminal cannula. Because of climatic constraint (a very hard dry season) which limited the amount of grass, the digestion and the growth trial were not contemporaneous. The 16 rams were conducted following two parallel designs (faunated *vs* defaunated). Defaunated and faunated rams, placed in metabolism cages and housed in the same room, were isolated with a punch cloth. They were fed successively a 14, 28, 42 and 56-days of regrowth of a *Digitaria decumbens* during the 4 consecutive experimental periods. The chemical composition of the diets is given in Table 2 . Each period was composed of 14 days of adaptation to the diet, 5 days of intake and total tract digestibility measurements, 3 days of duodenal sampling.
As with lambs, mineralised salt blocks were in free access. The blocks' composition was Ca (60.0), P (20.0), Mg (10.0), Na (280.0), Zn (17.5), Mn (5.5), Fe (1.5), I (0.03), Co (0.03) and Se (0.01 g / kg).

2.3. Experimental procedures

2.3.1.Growth trial

Rumen contents, obtained via oesophageal tube from defaunated lambs, were examined at the end of each experimental period and found to be free of protozoa.

Table 1 : Chemical composition (g / kg) of, organic matter (OM), Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and crude protein (CP) of the diets given in lambs growth trial. The diets D90, D70, D60 and D50 were composed of fresh *Digitaria decumbens* grass at 14, 28, 42 and 56 days of age of regrowth respectively.

Digitaria decumbens	OM	CP	NDF	ADF	ADL	PDIN / UF *
Age of regrowth (d)						
14	927	107.7	749	372	31	90
28	907	75.2	718	352	35	70
42	919	62.6	775	400	44	60
56	926	46.9	738	375	32	50

*The diet D90, D70, D60 and D50 were formulated to provide 4 protein / energy ratios of 90, 70, 60 and 50 PDI / UF level of contents in the diet respectively.

Table 2. Chemical composition (g / kg) of, organic matter (OM), Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and crude protein (CP) of the diets given in rams digestion trial. The diets D120, D110, D90 and D60 were composed of fresh *Digitaria decumbens* grass at 14, 28, 42 and 56 days of age of regrowth respectively.

Digitaria decumbens	OM	CP	NDF	ADF	ADL	PDIN / UF*
Age of regrowth (d)						
14	856.2	147.8	711.1	347.5	58.7	120
28	871.3	120.5	721.6	346.6	56.8	110
42	859.1	119.8	716.0	350.1	42.2	90
56	924.5	57.1	713.1	354.5	39.7	60

*The diet D120, D110, D90 and D60 were formulated to provide 4 protein / energy ratios of 120, 110, 90 and 60 PDIN / UF level of contents in the diet respectively.

Intake was determined from daily weighing of the amounts of feed offered and refused. Representative samples of feed (3 samples / day of the distributed feed) were constituted for chemical analysis. A double weighing (24 hours of interval) of the rams were performed every week. The average daily growth was calculated on the last 15 days of each period.

2.3.2. Digestion trial

Intake and apparent digestibility were determined from daily weighing of the amounts of feed offered, feed refused and the faeces. Representative samples of feed (3 samples / day of the distributed feed) and faeces (10% / day of the amounts excreted) were constituted for chemical analysis. During the 5 days of intake measurements, daily excretions of urine were recorded. The urine was daily collected in drums containing sulphuric acid H_2SO_4 (approximately 1 vol. H_2SO_4 / 100 vol. urine). Daily samples of urine were pooled during 5 days for nitrogen determination. Nitrogen retention was estimated as nitrogen intake – (nitrogen urinary loss + nitrogen faecal loss). Duodenal flows were estimated according to the double-marker method of Faichney (1980) using indigestible lignin (IADL) and polyethylene glycol (PEG) as markers of the particulate and aqueous phases, respectively. The mean daily duodenal digesta sample was constituted by taking twelve 100 ml samples over three consecutive days at (day 1) 0, 6, 12 and 18 hours, (day 2) 2, 8, 14 and 20 hours, (day 3) 4, 10, 16 and 22 hours after the morning meal distribution. Fifty millilitres of this sample were kept to represent whole digesta (DG) and 50 ml were divided into a liquid-rich phase (LR) and a particle-rich fraction (PR) by squeezing the digesta through a nylon filter of 150 µm pore size. Then DG and PR were freeze-dried, prior to chemical analysis for crude protein (CP), microbial nitrogen, organic Matter (OM), Neutral Detergent Fibre (NDF), Acid detergent fibre (ADF) and Acid detergent lignin (ADL).

At the end of each period, the rumen of each animal was manually emptied and the total content weighed, 3 hours after the morning meal. The morning meal was interrupted after 3 h of free access. The rumen turnover rate (% / h) of the *Digitaria decumbens* grass has been estimated as: ((amount of lignin excreted in faeces daily) / 24) / (amount of lignin in the rumen 3 h after the morning meal)). Bacterial samples were isolated by carrying out two successive centrifugations of LR (800 *g* and 27000 *g*) as described by Yang (1992).

2.4. Chemical analysis

Dry matter was determined by drying to a constant weight at 60°C in a forced draught oven. The samples were ground (1 mm) prior to chemical determination. The OM content was determined as the difference between dry and ash weight. Samples were burnt for 10 h at 550°C. The Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and Acid detergent lignin (ADL) were estimated following the method of Van Soest et al. (1991). Nitrogen concentration was determined using the Dumas technique. Microbial nitrogen was estimated from the purine bases to non-ammonia nitrogen ratio in samples of reconstituted duodenal digesta and ruminal bacteria. The purine base was analysed according to Zinn and Owens (1986).

2.5. Statistical analyses

2.5.1. Growth trial

Dry matter intake (DMI), feed and energy conversion efficiency and average daily growth (ADG) data were analysed by ANOVA procedure (SAS, 1988). The statistical model used to analyse DMI data took into account, as fixed effects: fauna status (defaunated *vs* faunated), the diet , the animal (inside of fauna status), the interaction between defaunation effect and the diet effect. The live-weight of the animals (at the beginning of each experimental measurements period) was considered as a covariate. Concerning the ADG the statistical model used took into account as fixed effects : the fauna status, the diet, the animal (inside of fauna status), the interaction between defaunation and the diet. The DMI, the ADG during the second month after birth, the live-weight at the beginning of the experiment, were integrated in the model as covariates. Concerning feed and energy conversion efficiency the preceding model used for ADG was performed.

2.5.2. Digestion trial

ANOVA procedures were used in all statistical analyses (Minitab, 1998). For DMI the model fixed effects were : fauna status (faunated *vs* defaunated), the diet , animal (inside of fauna status) and the interaction between defaunation and diet. Animal weight at be beginning of each period was used as a covariable. The ruminal and total tract digestibilities were analysed using the same model and the DMI was integrated as a

supplementary covariate. The DMI was substituted by nitrogen intake as a covariate in statistical analyses of the duodenal nitrogen flows, the faecal and urinary nitrogen excretions and the microbial synthesis efficiency.

3. Results

3.1. Growth trial

The chemical composition of the diet has varied with the age of regrowth of the forage as illustrated in table 1. There was no effect of defaunation on the dry matter intake of lambs (64.8 vs 66.4, se = 2.2 g / kg LW $^{0.75}$). Neverthelesss significant interactions appeared between defaunation effect and diet effect (Tab. 3). Defaunation had no effect neither on energy intake (UF / d) nor on protein intake (PDIN, g / d) (Tab. 3).

The average daily growth (ADG, g/d) of the control group of lambs were 123 (± 17 g / d) 115 (± 17 g / d) 145 (± 17 g/d) and 125 (± 17 g / d) during the period 1, 2, 3 and 4 respectively. There was no significant differences between the periods.

Mean ADG of lambs decreased with the protein / energy ratio of the diet (Table 3). The mean ADG of faunated and defaunated rams were 75.7 and 76.8 (± 4.2 g / d). There was no significant difference of growth between faunated and defaunated rams except with the diet D50 (Table 3). For this last diet, the ADG of faunated rams was significantly ($P < 0.02$) lower than the one of defaunated rams (48.4 vs 25, s.e.= 13.4 g /d).

The feed conversion efficiency (14.4 *vs* 9.0 ± 1.2 / g, $P = 0.5$) and the energy conversion efficiency did not varied significantly after defaunation, but an interaction appeared between defaunation effect and diet effect (Table 3). The feed conversion and the energy conversion efficiency were significantly lower with the defaunated lambs compared with conventional lambs consuming diet D90, D70 and D50.

3.2. Digestion trial

3.2.1. Intake

Total dry matter intake (g / kg LW $^{0.75}$) was significantly ($P < 0.001$) higher with conventional rams compared with defaunated rams (76.4 *vs* 63.7 ± 1.6 g. / kg LW $^{0.75}$). Nevertheless there was no significant difference between animals with the diet D120 (Table 4).

Table 3. Effect of defaunation on intake, growth and feed conversion efficiency of lambs consuming diets composed of fresh *Digitaria decumbens* grass harvested at 14, 28, 42 and 56 days of age of regrowth. The diets were formulated to provide 4 protein / energy ratios (PDIN / UF) of 90, 70, 60 and 50 on diet D90 (14 days of regrowth), D70 (28 days of regrowth), D60 (42 days of regrowth) and D50 (56 days of regrowth) respectively.

	D90		D70		D60		D50			Significance		
	F	D	F	D	F	D	F	D	S.E.M.	Def.	Diet	Def.×Diet
DM intake (g / kg LW $^{0.75}$)	71.8	75.5	73.4	79.5	62.4	61.2	51.6	49.2	2.2		***	**
Energy intake (UF / d.)	0.57	0.57	0.58	0.60	0.50	0.48	0.38	0.36	0.02		***	
Nitrogen intake (g / d.) (1)	53.0	49.3	39.8	39.3	33.0	29.8	21.0	18.2	1.5		***	
Nitrogen / Energy Ratio (2)	92.9	92.9	69.6	69.6	61.7	61.7	47.6	47.6	0.2		***	
Growth (g / d.)	73.4	73.2	109.2	87.6	99.6	93.8	25.0	48.4	11.1		***	*
Conversion efficiency of :												
Feed (g / g)	8.9	5.9	9.7	8.1	11.9	9.7	27.1	12.3	1.2			
Energy (10^{-3} UF /g)	6.2	4.1	6.7	5.6	8.2	6.6	15.2	7.4	0.8			

D : defaunated animals F: faunated animals; Def. : Defaunation effect; (1) PDIN ; (2) PDIN / UF.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 4. Effect of defaunation on dry matter intake and apparent digestibility in the total tract of organic matter (OM), cell wall (NDF) and crude protein (CP) of rams consuming diets composed of fresh *Digitaria decumbens* grass harvested at 14, 28, 42 and 56 days of age of regrowth. The diets were formulated to provide 4 protein / energy ratios (PDIN / UF) of 120, 110, 90 and 60 on diet D 120 (14 days of regrowth), D110 (28 days of regrowth), D90 (42 days of regrowth) and D60 (56 days of regrowth) respectively.

	D120		D110		D90		D60			Significance		
	F	D	F	D	F	D	F	D	S.E.M.	Def.	Diet	Def.×Diet
DM intake (g / kg LW$^{0.75}$)	81.6	82.7	78.7	62.0	79.0	58.6	66.1	51.5	3.1	***	***	**
Total tract digestibility (%)												
OM	72.7	68.2	72.4	64.5	64.8	59.7	58.4	59.4	1.3	***	***	**
NDF	80.8	78.8	80.9	75.5	74.9	72.0	69.6	71.2	0.9	**	***	***
CP	79.3	76.3	74.9	69.8	73.7	72.0	42.1	40.7	2.0	*	***	
Rumen digestibility (%)												
OM	57.5	58.4	58.2	53.7	50.6	44.9	49.6	47.7	2.3		***	
NDF	74.3	74.0	74.6	69.6	69.3	65.6	62.8	61.3	1.4	*	***	
Particle mean retention time												
(Hours)	33.9	35.8	55.4	49.0	44.9	61.9	43.1	78.1	6.9		***	***

D: defaunated animals F: faunated animals Def.: Defaunation effect.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

3.2.2. Total tract and ruminal digestibilities

Total tract digestibility of organic matter (Dt OM) and fibre (Dt NDF) was significantly higher ($P < 0.01$) with conventional rams compared to cilate-free rams (Table 4). A significant interaction ($P < 0.01$) between defaunation and the diet appeared for both DtOM and DtNDF. Differences between conventional and cilate-free rams were not significant with diet D60 for which the ratio nitrogen/energy was the lowest (Table 4). Crude protein total tract digestibility (DtCP) was significantly higher with faunated rams than with defaunated rams (67.5 *vs* 64.7, ± 0.7 %).

Defaunation decreased both OM apparent ruminal digestibility (DrOM, $P = 0.12$) and NDF ruminal digestibility (DrNDF, $P = 0.05$) (Table 4), except for the diet D120 and D60 for which no difference was observed between animals.

3.2.3. Particle retention time

The mean retention time of fibre particles in the rumen was lower ($P = 0.10$) with conventional rams compared with defaunated animals, except with the diet D110 (Table 4).

3.2.4. Nitrogen Flows

Duodenal non-ammonia nitrogen (NAN) flow was significantly increased ($P < 0.05$) by defaunation (18.2 *vs* 20.1 ± 0.5 g NAN / d). There was no interactions between defaunation effect and the diet ($P = 0.56$) .

The duodenal microbial nitrogen (MN) flow did not increased ($P = 0.3$) after defaunation (10.3 *vs* 10.8, ± 0.3 g MN / d). Nevertheless, defaunation effect tended ($P < 0.15$) to vary with the diet (Table 5).

3.2.5. Faecal and urinary nitrogen excretions

The urinary nitrogen excretion decreased ($P < 0.01$) with the increased nitrogen /energy ratio of the forage (Table 5). The urinary nitrogen excretion decreased significantly ($P < 0.001$) after defaunation (7.7 *vs* 6.7 ± 0.5 gN / d).

Table 5 : Effect of defaunation on nitrogen intake, duodenal nitrogen flow, microbial synthesis efficiency, faecal and urinary excretion of rams consuming diets composed of fresh *Digitaria decumbens* grass harvested at 14, 28, 42 and 56 days of age of regrowth. The diets were formulated to provide 4 protein / energy ratios (PDIN / UF) of 120, 110, 90 and 60 on diet D120 (14 days of regrowth), D110 (28 days of regrowth), D90 (42 days of regrowth) and D60 (56 days of regrowth) respectively.

	D120		D110		D90		D60			Significance		
	F	D	F	D	F	D	F	D	S.E.M.	Def.	Diet	Def.×Diet
Nitrogen duodenal flow (g / day)												
Non ammonia Nitrogen	16.5	18.1	21.0	21.6	19.8	22.1	15.8	18.6	0.9	**	***	
Microbial nitrogen	10.8	10.3	8.3	10.2	12.2	12.5	10.0	10.3	0.6		***	
Faecal nitrogen (g N / day)	6.7	7.3	5.5	7.3	5.9	5.8	5.9	5.5	0.4	**	***	*
Intestinal digestion of Nitrogen (%)	60.2	59.3	68.2	62.2	68.2	76.3	59.3	64.2	3.7			
Urinary nitrogen (g N / day)	7.8	5.8	8.8	7.4	8.1	7.4	6.3	6.5	0.6	***	***	*
Microbial synthesis Efficiency												
(g MN / kg OMADR)	17.1	16.1	12.8	17.3	20.9	25.9	17.9	19.1	1.5	*	***	
(g MN / kg NDFR)	15.7	15.3	12.2	16.0	18.7	22.0	17.6	18.3	1.2	*	***	

D: defaunated animals F: faunated animals Def: Defaunation effect.
OMADR: OM apparently degraded in the rumen; NDFADR : NDF apparently degraded in the rumen
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

There was a significant interaction ($P < 0.01$) between defaunation and the diet (Table 5) : differences between faunated and defaunated rams decreased with the increased nitrogen / energy ratio of the diet, from diet D90.

The faecal nitrogen excretion was significantly lower ($P < 0.05$) with conventional animals compared with cilate-free animals (5.9 *vs* 6.7, ± 0.4 gN / d). There was a significant interaction ($P < 0.05$) between defaunation and the diet (Table 5).

3.2.6. The efficiency of microbial synthesis

The efficiency of microbial synthesis (g microbial nitrogen synthetised per kg of organic matter apparently degraded in the rumen (OMADr)) increased ($P < 0.05$) after defaunation (17.2 *vs* 19.6 ± 0.8 gMN / Kg OMADr). No significant interaction between defaunation and the diet appeared ($P = 0.17$) (Table 5). Similar tendencies were observed when the microbial synthesis was calculated on the basis of the fibre (NDF) apparently degraded in the rumen (NDFADR).

4. Discussion

The theoretical potential of growth of the experimental lambs is lower than 150 g / day as illustrated by the ADG of the control group. The theoretical protein (PDIN) / energy (UF) requirements for the growth observed with the conventional lambs consuming diets D90, D70, D60 and D50 were respectively 70, 70, 65 and 53 PDIN per UF (Bocquier and Thériez, 1989). Except for the diet D50, the amount of protein consumed relatively to the energy was sufficient. The energy was the first limiting factor of the growth.

Difference of growth between conventional and cilate-free lambs was only significant with the D50 diet, for which amount of protein was the first limiting factor. This result could indicate that positive effect of defaunation are observed only when the energy is not the first limiting factor. Really, defaunation potentially improved the amount of intestinal nitrogen. Defaunation generally decreased the amount of absorbed energy. However, the profile of nutrients absorbed allowed a better metabolic use of absorbed energy. The evolution of the difference of growth between lambs, with the ratio of protein to energy of the diet, was curvilinear as indicated by previous results (Bird et al., 1979; Bird and Leng, 1984; Demeyer et al., 1982).

Whatever the growth observed the feed and the energy conversion efficiency was better for cilate-free lambs. This result can be explained by the profile of absorbed nutrients, as is suggested by the digestion trial. The scale of variation of the nitrogen/energy of diet consumed during the growing trial is included in the one observed during the digestion trial.

There are good agreements between the results of the growth trial and the digestion trial. The intestinal nitrogen flow was higher with defaunated animals compared with fauned animals, feeding with the same level of forage. Moreover, the intestinal digestion of nitrogen is similar. Consequently, it can be hypothesised that absorption of protein was higher with the cilate-free lambs. On the contrary, with the same level of dry matter intake, the amount of absorbed energetic nutrients is probably lower with the cilate-free lambs compared with the conventional lambs. This can be attributed to the decrease of organic matter digestibility in the rumen and in the total tract. Nevertheless, the profile of energetic (VFA) observed on this diet (Eugène et al., unpublished data) allowed a better metabolic use of nutrients. Consequently, the estimation of the amounts of metabolic energy in ruminant, consuming the same amount of forage, could be different.

The significant increase by (+11%) of intestinal total protein flow, after defaunation is explained by the decrease of the proteolysis in the rumen and by an increase of the amount of dietary protein. The lower level of urinary nitrogen illustrated this decrease of proteolysis or a better metabolic use of nutriment with cilate-free ruminants. The absence of effect of defaunation on microbial nitrogen intestinal flow contrasts with the results generally observed in the litterature. Ushida et al. (1986) and Eugène et al. (unpublished data) indicated similar contribution of dietary and bacterial nitrogen in the increase of the intestinal protein flow after defaunation. Moreover, the results of microbial efficiency observed even with conventional animals were low. Archimède et al. (1999) , with similar forages, reported values of 31 to 32 g of nitrogen synthetised per kg of organic matter apparently digested in the rumen.

The depressing effect of defaunation on the ruminal and total tract digestion of organic matter and fibre is a classical result (Jouany and Ushida, 1998; Ushida K., 1994) . It was explained by the decease of the cellulolysis as the ciliates, which contribute to the cellulolytic capacity of the rumen (Conchie, 1954; Jouany and Ushida, 1998) , are eliminated. The increase of the mean retention time of the forage in the rumen (Diets D120, D110, D90, D60) did not permit to compensate for the decrease of the rumen

cellulolytic activity of cilate-free rams. On the other hand, the increase of the mean retention time of the fibrous particle in the rumen could limit the consumption of forage and consequently, energy intake. Nevertheless, more data are needed to confirm this effect of defaunation on particle transit time because contradictory results are observed in the litterature (Demeyer, 1988).

5. Conclusions

To conclude in favor of a positive effect of defaunation on growth of ruminant consuming tropical forage, additional data are needed. The animal response to defaunation would be varying according to the stage of regrowth of the tropical forage grass. No effect is observed with young forages whereas a positive effect appeared with older forages. This evolution could be linked to the nitrogen / energy ratio which decreases with the age of the forage. After defaunation, when ruminant consumed young tropical forage, the increased amount of protein due to defaunation effect, is not efficient on growth. With the consumption of older forages, for which the amount of protein is the first limiting factor, defaunation becomes efficient.

Acknowledgements

The authors would like to thank L. Philibert, S. Calif, P. Despois, N. Jalwan, M. Arjounin and F. Périacarpin, for their technical collaboration to this work. This work was supported by the “Région Guadeloupe” and the European Economic Community (FEDER, FEOGA).

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Publication 5

Effects of defaunation on microbial activities in the rumen of rams consuming fresh *Digitaria decumbens* grass.

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(soumise à Animal feed Science and Technology)

Abstract

The effects of defaunation on microbial enzyme activities have been measured on Black Belly rams consuming four diets of *Digitaria decumbens* at four different ages of regrowth. The diets were differentiated through their protein to energy level of contents (PDIN / UF). The diets D120 (PDIN / UF), D110, D90 and D 60 were composed of a 14, 28, 42 and 56 days of regrowth *Digitaria decumbens* grass respectively. Conventional and ciliate-free animals were conducted in two parallel designs. Eight conventional mature rams and 8 ciliate-free mature rams, with ruminal cannulae were used. They were fed the 4 diets on 4 successive periods. Defaunation decreased the number of total bacteria ($P < 0.05$), and a significant interaction between defaunation and the diet appeared. The percentage of cellulolytic bacteria was increased after defaunation ($P < 0.05$). Defaunation decreased activities of fibrolytic enzymes ($P < 0.05$), whatever the diet condidered. Defaunation also tended to decrease in situ DM degradation of *digitaria decumbens* ($P = 0.15$), whatever the diet and the sampling time considered. Defaunation had no effect on volatile fatty acids mean concentration (VFA), whatever the diet and the sampling time considered. Defaunation decreased the molar proportion of butyrate ($P < 0.001$) and propionate ($P < 0.05$) and these effects were more pronounced for diet D90

From theses results defaunation clearly decreased microbial activities in the rumen

Keywords: Enzymatic activities; Microbial population; Defaunation; Black Belly rams; *Digitaria decumbens*

1. Introduction

The effects of defaunation of ruminants have been investigated for many decades now, as an attempt to enhance animal production and valorise local resources or by-

products (Becker and Everett , 1930; Bird and Leng, 1978; Habib et al., 1989; Krebs et al., 1989). Defaunation effects on growth performance have been contradictory (Bird et al., 1978; Bird and Leng, 1985; Bird and Seccombe, 1998), they report increased growth performance after defaunation whereas other authors reported inverse effects (Abou Akkada and El-Shazly, 1964; Borhami et al., 1967; Ivan et al., 1986). The nature of the diet may be the major factor of variation of the effect of defaunation on animal performance, especially through the resulting nitrogen to energy balance of the absorbed nutriments (Bird and Leng, 1984).

It is hypothesised that defaunation could improve growth of ruminant consuming tropical forage (Eugène et al, in press) due to a better orientation of the digestion. To test this hypothesis, we conducted a digestion trial to measure digestion in the rumen. Particular attention was given on the balance of the products of rumen fermentation, on microbial degradation, on determination of the enzymatic activities and identification of the microbial population.

2. Materials and Methods

2.1. Location

The research was carried out in 2001 at the animal experimental station of the National Institute of Agricultural Research, in Guadeloupe, French West Indies, (16° 16’ N, and 61° 30’ W). Average temperatures ranged from 21°C to 31°C. The average yearly rainfall on the experimental site is 3000 mm.

2.2. Experimental design, animals and diets

Sixteen Black Belly rams (average live weight : 43.1 ± 5.5 kg; 2-years old), each containing surgically implanted rumen cannula were used in this trial. Half of them were defaunated using a milk diet. During the defaunation process the diet was gradually changed from a fresh young grass to milk powder diet within 10 days. Then animals were fed with milk alone during 7 days. The diet was gradually changed from milk to grass fresh forage. The milk powder was mixed with 400 ml of water at 37° C, and poured into the rumen via rumen cannula (Fujihara et al., 1994).

The 16 rams were conducted in parallel (faunated *vs* defaunated). Defaunated and faunated rams, placed in metabolism cage and housed in the same room, were isolated

with a punch cloth. Care was taken to prevent accidental inoculation of the rams with protozoa. Each week ruminal fluid of defaunated animals was examined in order to control that they remained free of protozoa. All the animal used remained free of protozoa troughout the experiment. The trial was composed of 4 periods of 1 month, during which the rams recieved the 14, 28, 42 and 56 days age of regrowth *Digitaria decumbens* grass, during each period successively. The diet D120 was the 14 days age of regrowth *Digitaria decumbens* grass distributed *ad libitum* . In diets D110, D90 and D60, the age of regrowth of the fresh *Digitaria decumbens* was 14, 28, 42 and 56 days respectively. Each period was composed of 14 days of adaptation to the diet, 5 days of intake measurements, 4 days of ruminal content sampling and 7 days of nylon bags incubation. The chemical composition of the diet is shown in table 1. Mineralised salt blocks were in free access. The blocks' composition was: Ca (60.0), P (20.0), Mg (10.0), Na (280.0), Zn (17.5), Mn (5.5), Fe (1.5), I (0.03), Co (0.03) and Se (0.01 g / kg).

2.3. Experimental procedures

The degradation of the forage was measured by the nylon bag technique. Surface of nylon bags was 50 cm² over a pore size of 50 µm (Blutex, Tissage Tissus Technique, Villeneuve La Garenne 92390, France). The nylon bags were filled with 15 g of the same fresh *Digitaria decumbens* grass corresponding to the forage given during each experimental period. Moreover the 14 days of regrowth forage, stored at – 20° C, was used during the four periods as a reference. The nylon bags were placed in the rumen just before the morning meal feeding and the time of incubation was 24, 48 and 96 hours. The bags serving as reference were incubated for 24 hours. 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 every time) 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.

Samples of rumen liquor were taken from the sheep, immediately before the morning meal, and at 3, 6 and 12 hours after food was first available, during two consecutive days. The pH was measured immediately after sampling.

Table 1 Chemical composition (g / kg) of, organic matter (OM), Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and crude protein (CP) of the diets. The diets D120, D110, D90 and D60 were composed of fresh *Digitaria decumbens* grass at 14, 28, 42 and 56 days of age of regrowth respectively.

Digitaria decumbens	OM	CP	NDF	ADF	ADL	PDIN / UF *
Age of regrowth (d)						
14	856.2	147.8	711.1	347.5	58.7	120
28	871.3	120.5	721.6	346.6	56.8	110
42	859.1	119.8	716.0	350.1	42.2	90
56	924.5	57.1	713.1	354.5	39.7	60

*The diet D120, D110, D90 and D60 were formulated to provide 4 protein / energy ratios of, 120, 110, 90and 60 PDIN / UF level of contents in the diet respectively.

Samples of rumen fluid were frozen (-20°C) after adding a mixture of H_3PO_4 and $HgCl_3$ (1 vol. / 10 vol.) prior to VFA determination. Rumen fluid was stored (4°C) with H_2SO_4 (1 vol. / 50 vol.) prior to ammonia determination.

Nine millilitres of rumen fluid were collected before and 3 hours after the morning meal. They were added to 1 ml of conservatives (50% Glycerol, 48% distilled water and 2% formaldehyde). Protozoa population was determined with a Dolfüss counting cell (Elvetec Services, Clermont ferrand, France) according to the method described by Jouany and Sénaud (1978).

Representative samples of rumen contents (300g) were collected 3 hours after the morning meal (rumen total emptying) from faunated and defaunated animals fed with D120 and D60 diet and then freeze-dried in order to determine bacterial population.
Two representative samples of rumen contents (300g) were collected, one just before and the second 3 hours after the morning meal, in order to measure enzyme activities from the solid-adherent microorganisms. The measurements were carried out on two consecutive days, on 4 defaunated and 4 faunated animals. Samples were then strained through a 100µm nylon filter to collect the solid phase, under anaerobic conditions (CO_2 flows).

2.4. Chemical analysis

Dry matter was determined by drying to a constant weight at 60°C in a forced draught oven. The samples were ground (1 mm) prior to chemical determination. The OM content was determined as the difference between dry and ash weight. Samples were burnt for 10 h at 550°C. The Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL) were estimated following the method of Van Soest et al. (Van Soest et al., 1991). Nitrogen concentration was determined using the Dumas technique. Ruminal volatile fatty acids were measured in the rumen liquid by gas chromatography (Ottenstein and Supina, 1974). Ammonia concentration was estimated in rumen liquor by distillation and titration.

The structure of the cellulolytic bacterial community has been investigated using specific oligonucleotide probes targeting 16S rRNA, on ruminal total digesta sampled 3 hours after the morning meal then freeze-dried. Sthal et al. method (Stahl et al., 1988) has been adapted. Rumen digesta was then freeze-dried (50mg) and mixed with 1ml of RNAzol (Bioprobe System, Montreuil, France) and with 0.3 g zirconium beads (Biospec products, INC) in order to disrupt microorganisms. A Chloroform / Isopropanol / ethanol

extraction was performed in order to recover total microbial RNA. Mix is heated to 100°C during 6 minutes then is shaken during 2 minutes. After two chloroform extractions (475µl), centrifugation at 14000 *g*, at room temperature, during 15 minutes was performed. The pellet was discarded and the supernatant was added to an equal volume of isopropanol to purify the pellet. After a second centrifugation at 14000 *g* at room temperature, the pellet was rinsed with ethanol 75°C (800µl) at 4°C. The supernatant is then discarded and 100µL of DEPC water is added to pelleted total microbial RNA before storage at –20°C. Oligonucleotide probes were labelled with gamma ATP (^{32} P) (Eurogenetec) with specific activity of 4000 Cu / mmole at 5'P end using a T4 kinase (Eurogenetec). Four Oligonucleotide probes were used : the universal probe S-D Bact-0338-a-A-18 targeting the bacterial domain (Eub 338), probe S-S-F.suc-0650-a-A-20 targeting *Fibrobacter succinogenens* (*Fs*), probe S-S-R.alb-0196-a-A-18 targeting *Ruminococcus albus* (*Ra*) and probe S-S-R.fla-1269-a-A-20 targeting *Ruminococcus flavefaciens* (*Rf*). After denaturation with glutharaldehyde (2% phosphate buffer 50mM, pH=7) RNA extracts , between 25 and 100 ng RNA, were blotted on a nylon membrane (Hybond N+, Amersham Pharmacia Biotech, Saclay, France) using a dot blot apparatus (Biorad). Membrane was air dried at room temperature and RNA was fixed. Membrane were incubated for 1 hour with 100µl / cm^2 buffer (0.9M NaCl, 50mM $NaPO_4$, 5 mM EDTA, Denhart solution 10X and 0.5 mg PolyA / ml , pH=7.0) at 45°C in an hybridisation oven. Labelled probes were added to each membrane (at about 2x10 ^{5} cpm per sample dot) and were incubated for 5H at 45°C in an hybridisation oven. Membranes were washed for 20 minutes with SSC buffer (150mM NaCL, 150mM Sodium Citrate) and 1% SDS at temperature specific to each probe, at which duplex DNA-RNA is dissociated. Membrane were then air dried and exposed to a screen (Storm, Molecular Dynamics) before bound probes were quantified using Image Quant Software (Molecular Dynamics). RNA quantity in samples of ruminal contents was expressed as a ratio to RNA quantity obtained with eubacteria probe (EUB338). Results of each cellulolytic bacteria species were expressed as a percentage of total cellulolytic RNA (sum of all predominant cellulolytic bacteria, *Ra*, *Rf* and *Fs* 85) in the rumen.

Enzymatic manipulations were performed under anaerobic conditions. Enzymes extraction from the solid adherent microorganisms were achieved 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 (60min at 39°C) with enzyme preparation. Glycosidase 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 glycosidase) released per milligram of protein per hour (specific activity) or per gram of DM per hour (total activity).

2.5. Statistical analyses

The ANOVA procedures were used in all statistical analyses (Minitab, 1998). For the nylon bag degradation, the enzymatic activities and the pH, the statistical model (GLM) fixed effects used were: defaunation (faunated *vs* defaunated), the diet, the animal (inside of defaunation), the interaction between defaunation and the diet. The statistical analysis of the ruminal ammonia concentrations was performed with the same preceding model, with the nitrogen intake considered as a covariate. The nitrogen intake was substituted by the total digestible organic matter in the same statistical analyses of the concentrations and the molar proportions of VFA. The number of bacteria was analysed using GLM procedure with: defaunation (faunated *vs* defaunated) and the diet, considered as the fixed effects. The number of protozoa was analysed using GLM procedure with the diet and the animal, considered as the fixed effects.

3. Results

3.1. Microbial population in the rumen

3.1.1. Protozoa concentration in faunated animals

The number of protozoa, before the morning meal tended to be lower than 3 hours after the morning meal ($P > 0.15$). No significant ($P > 0.60$) effect of the diet on protozoa number was observed before the morning meal (4.1×10^4 / ml $\pm$ 1.2×10^4 / ml) or 3 hours after the morning meal (8.2×10^4 / ml $\pm$ 2.7×10^4 / ml).

3.1.2. Bacteria concentration

The amount of the total bacteria population (µg rRNA / g DM) in the rumen was decreased ($P < 0.05$) after defaunation (Table. 2). A significant interaction between defaunation and the diet appeared ($P < 0.05$). Defaunation decreased the amount of the total bacteria by 72% on diet D60, whereas defaunation increased it by 7% on diet D120. The amount of *Ruminococcus flavefaciens* was too low to be detectable on diet D60 for the defaunated animals, so the results was not estimated. Concerning the amount of the predominant cellulolytic bacteria population (*Ruminococcus albus* and *Fibrobacter succinogenens*), defaunation tended ($P > 0.45$) to decrease their number. The number of the cellulolytic bacteria tended to decrease ($P > 0.05$) after defaunation (3.4 *vs* 3.2 ± 0.6 µg / gDM). A significant interaction appeared ($P < 0.05$) between defaunation and the diet. On diet D120 defaunation increased the number of cellulolytic bacteria whereas an inverse effect was observed on diet D60.

Defaunation significantly increased ($P < 0.05$) the percentage of these cellulolytic bacteria (Table 2). No significant interaction between defaunation and the diet appeared.

3.2. Enzymatic activity

Whatever the sampling time considered, defaunation decreased both fibrolytic enzymes (polysaccharidases) total activity (µmol reducing sugars / g DM*h) and specific activity (µmol p-nitrophenol / mg prtotein*h), which are responsible for the degradation of plant fiber (Table 3). Total activities of the fibrolytic enzymes tended ($P>0.05$) to be higher before the morning meal than 3 hours after the morning meal.

Whatever the sampling time defaunation effect on total and specific activity of carboxymethyl cellulase (CMCase) were higher on diets D 90 and D110 compared to diets D60 and D120. Nevertheless no significant interaction ($P > 0.05$) between defaunation effect and the diet appeared.

Whatever the sampling time, defaunation effect on total activity of xylanase were higher on diets D90 and D110 compared to diets D60 and D120, but no significant interaction appeared ($P > 0.05$). Concerning specific activity of xylanase, defaunation effects were higher on diets D60 and D120 compared to diets D90 and D110.

Table 2 : Effect of defaunation on some cellulolytic bacteria, in the rumen of rams consuming 4 diets. The diets D120, D110, D90 and D60 were composed of fresh *Digitaria decumbens* grass at 14, 28, 42 and 56 days of age of regrowth respectively.

				D120		D60					
PDIN / UF,	F	D	SEM	F	D	F	D	S.E.M.	Def.	Diet	D×Diet
Total bacteria	94.67	66.84	8.4	102.2	109.3	87.1	24.3	11.8	*	***	*
Ruminococcus albus (R.a)	1.98	1.80	0.20	2.36	2.59	1.61	1.01	0.29		**	
Fibrobacter succinogenens (F.s)	1.39	0.81	0.49	1.13	1.18	1.61	0.48	0.60			
Ruminococcus flavefasciens (R.f)	0.2	N.E.		0.5	NE	1.8	N.E.	0.40			
Cellulolytic bacteria[b]	3.4	3.2	0.6	3.0	4.9	3.7	1.5	0.8			*
% Ra	2.25	3.23	0.25	2.28	2.34	2.22	4.13	0.04	*		*
% Fs	1.32	1.58	0.55	0.99	1.20	1.64	1.97	0.65			
% Rf	0.02	NE		0.004	NE	0.2	NE				
% Celluloytic bacteria	3.5	5.3	0.5	2.8	4.5	4.2	6.1	0.7	*	*	

Cellulolytic bacteria[b] : sum of the three major cellulolytic bacteria in the rumen (*Ra*, *Fs* and *R. flavefasciens*)
NE : Non estimated. Def.: Defaunation effect; * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Table 3 : Effect of defaunation on enzymatic activity (polysaccharidases) in rumen solid-associated microorganisms, in rams consuming 4 diets. The diets D120, D110, D90 and D60 were composed of fresh *Digitaria decumbens* grass at 14, 28, 42 and 56 days of age of regrowth respectively.

PDIN / UF,				D120		D110		D90		D60					
	F	D	s.e.m.	F	D	F	D	F	D	F	D	s.e.m.	Def.	Diet	D×Diet
Item and time after feeding															
Total activity (µmol reducing sugar / (gDM*h))															
CMCase															
0h	29.0	13.9	2.6	5.7	0.1	60.4	41.9	44.6	13.0	5.4	0.5	5.2	***	***	
3h	24.1	15.8	1.6	4.1	-0.3	57.7	49.2	28.5	14.6	5.9	-0.3	3.2	**	***	
Xylanase															
0h	231.8	125.7	20	226.9	68.0	205.7	127.6	329.1	256.5	165.4	50.8	39.9	**	**	
3h	174.3	130.0	11.8	127.1	67.2	156.1	156.5	269.3	247.0	144.8	49.4	24	*	***	
Amylase															
0h	6.4	5.7	2.3	5.0	3.7	6.1	8.8	16.5	10.4	-0.8	-1.2	4.5		*	
3h	29.5	16.4	4.4	53.1	33.1	29.6	15.7	35.0	17.0	0.3	-0.3	8.7		***	
Specific activity (µmol reducing sugar / (mg protein*h))															
CMCase															
0h	3.7	1.9	0.3	1.2	0.01	7.8	5.6	4.7	1.7	1.0	0.1	0.6	***	***	
3h	2.6	1.6	0.3	0.5	-0.02	5.0	4.6	2.8	1.7	2.0	-0.01	3.2	*	***	
Xylanase															
0h	40.6	27.1	3.2	66.9	32.2	25.0	18.8	34.5	36.5	36.1	20.9	62.8	**	**	
3h	28.3	23.1	2.8	28.1	25.5	13.6	14.9	27.8	29.0	43.6	23.0	5.6		*	
Amylase															
0h	0.8	1.0	0.4	1.0	1.7	0.7	0.9	1.7	1.4	-0.1	-0.2	0.8			
3h	5.1	2.7	0.9	13.1	10.9	3.0	-0.2	3.9	0.5	0.3	-0.3	1.8		***	

Def.: Defaunation effect; D : defaunated animals; F : Faunated animals ; * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Concerning amylolytic activity, defaunation did not influence both total and specific activities of amylase (*P* > 0.80) before the morning meal (Table 3). Three hours after the morning meal, for which the activities of amylase were the highest (P > 0.05), defaunation decreased (*P* > 0.08) both total and specific activity of amylase. No significant interaction appeared between defaunation and the diet. Total activity of α-D glucosidase decreased after defaunation (*P* > 0.15), before the morning meal (Table 4), no effect was observed 3H ater the morning meal. Whereas defaunation increased (*P* > 0.25) α-D glucosidase specific activity at 0h and 3h after the morning meal.

The main glycosidases enzymes that participate to fibre degradation are presented in Table 4. Concerning the glycosidases enzymes that exhibit the highest activities, β-D-glucosidase total activity, decreased significantly (*P* < 0.001) after defaunation, whatever the sampling time. This decrease was especially marked 3h after the morning meal for diet D60, as was outlined by the significant interaction between defaunation and diet (*P* < 0.05). The same tendency was observed (*P* < 0.05) with β-D-glucosidase specific activity 3 hours after the morning meal. Whereas before the morning meal defaunation increased (*P* > 0.05) β-D-glucosidase specific activity. No effect of defaunation on total activity of β-D-xylosidase was observed, whatever the sampling time. Whereas defaunation significantly (*P* < 0.05) increased the specific activity of β-D-xylosidase, whatever the sampling time considered. An inverse effect of defaunation appeared (*P* < 0.05) before the morning meal for diet D120.

3.3. In situ DM degradation

Defaunation had no effect on in situ nylon bag DM degradation of forage, when nylon bags were incubated for 24 hours in the rumen (51.2 *vs* 50.6, faunated *vs* defaunated ± 0.8 %, *P* = 0.62) for 48 hours (64.1.0 *vs* 63.0, ± 0.9 %, *P* = 0.37) and for 96 hours (72.1 *vs* 68.8 ± 1.5%, *P* = 0.15). No significant interaction appeared between defaunation and diet effects, as indicated in table 5.

Defaunation had no effect on in situ nylon bag degradation of the forage of 14 days of regrowth, used as a reference, incubated for 24 hours (52.5 ±.1.2%). Degradation of the reference forage DM was significantly higher for diet D120. No significant interaction appeared between defaunation effect and the diet.

Table 4: Effect of defaunation on enzymatic activity (glycosidases) in rumen solid-associated microorganisms, in rams consuming 4 diets. The diets D120, D110, D90 and D60 were composed of fresh *Digitaria decumbens* grass at 14, 28, 42 and 56 days of age of regrowth respectively.

PDIN / UF,				D120		D110		D90		D60			effect		
	F	D	s.e.m	F	D	F	D	F	D	F	D	s.e.m	Def.	Diet	D×Diet
Item and time after feeding															
Total activity (µmol p-nitrophenol / (gDM*h))															
β-D glucosidase															
0h	67.2	45.2	3.7	55.6	30.1	56.3	52.4	94.1	71.1	62.5	27.2	7.4	***	***	
3h	75.3	48.5	3.5	62.9	33.8	73.7	65.4	84.5	70.8	79.9	24.0	7.0	***	**	*
β-D xylosidase															
0h	75.4	67.1	5.2	77.4	49.5	84.4	84.3	88.8	95.9	51.0	38.6	10.3		**	
3h	65.3	68.1	4.2	60.1	50.5	79.8	89.3	77.0	96.2	44.1	36.3	8.5		***	
α-D glucosidase															
0h	6.1	4.5	0.7	7.7	2.8	4.6	5.9	6.4	7.2	5.6	2.1	1.5			
3h	4.9	5.0	0.3	1.8	1.4	6.4	7.8	7.3	8.1	4.2	2.5	0.6		***	
Specific activity (µmol p-nitrophenol / (mg protein*h))															
β-D glucosidase															
0h	11.9	13.9	0.9	17.6	11.8	6.8	10.5	9.6	13.3	13.5	19.9	1.7		**	*
3h	13.1	10.6	0.7	14.0	12.7	6.4	6.4	8.6	8.7	23.3	14.6	1.3	*	***	*
β-D xylosidase															
0h	14.0	19.9	1.0	24.5	20.9	10.3	15.2	9.3	16.9	11.6	26.6	1.9	***	***	**
3h	10.7	14.3	1.2	14.6	17.9	7.0	9.0	7.9	12.1	13.3	18.2	2.3	*	**	
α-D glucosidase															
0h	1.2	1.4	0.1	2.5	1.4	0.5	1.2	0.5	1.4	1.3	1.7	0.3		*	*
3h	0.8	1.0	0.1	0.5	0.4	0.6	0.9	0.8	1.1	1.2	1.6	0.2		**	

Def.: Defaunation effect; D : defaunated animals; F : Faunated animals ; * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Table 5: Effect of defaunation on in situ dry matter (DM) degradation, and Ammonia (NH3) in the rumen of rams consuming 4 diets. The diets D120, D110, D90 and D60 were composed of fresh *Digitaria decumbens* grass at 14, 28, 42 and 56 days of age of regrowth respectively.

PDIN / UF,				D120		D110		D90		D60			Effect		
	F	D	S.E.M	F	D	F	D	F	D	F	D	S.E.M	Def.	Diet	Def×Diet
Item and sampling time after feeding															
DM in situ degradation (%)															
24h	51.2	50.6	0.8	55.7	57.3	50.7	48.5	46.2	43.9	52.2	52.7	1.6		***	
48h	64.1	63.0	0.9	67.4	68.5	66.6	61.2	59.3	58.5	63.2	63.6	1.7		***	
96h	72.1	68.8	1.5	74.2	75.0	73.7	68.8	69.9	60.8	70.7	70.7	2.3		*	
24h (Reference)*	52.8	51.9	1.2	55.7	57.7	50.4	48.6	53.6	49.0	51.4	52.1	2.2		*	
NH3 (mgN / L)															
0h	118.9	100.5	5.0	155.8	147.8	132.4	87.8	149.1	123.6	38.4	42.7	13.8	*	***	
3h	151.2	114.8	4.5	199.6	153.0	166.7	115.3	189.5	155.4	48.9	35.5	12.9	***	***	
6h	147.3	117.0	4.5	184.7	146.9	149.8	100.4	199.7	164.7	55.1	55.7	12.7	***	***	
12h	123.8	111.5	3.3	178.1	159.2	123.4	99.8	161.1	155.3	32.6	31.8	9.0	*	***	
Mean NH3	135.3	110.9	3.5	179.5	151.7	143.1	100.9	174.9	149.7	43.7	41.5	9.6	***	***	

Def : Defaunation effect; D : defaunated animals F: faunated animals; * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$
*24h (reference) : 14 days of regrowth Digitaria decumbens degradation for 24 hours

3.4. Ammonia concentrations

Ammonia concentrations in rumen liquid were always lower ($P < 0.05$) with defaunated animals, whatever the sampling time (Table 5) . Mean values of faunated *vs* defaunated rams were respectively 135.3 *vs* 110.9 ± 3.5 mgN / L. The highest differences between faunated and defaunated rams were observed 3 hours after the morning meal. No significant interaction between defaunation and the diet appeared.

3.5. Rumen acidity

3.5.1. pH

The pH of ruminal fluid always remained lower with defaunated rams than with faunated rams, except when measured 12 hours after the morning meal (Table 6). A significant interaction between defaunation effect and the diet occured. Defaunation increased pH, measured just before the morning meal, for diet D60. On the contrary, defaunation decreased pH, measured 12H after feeding, for diet D90.

3.5.2. VFA

Defaunation had no effect on the VFA concentrations measured in rumen fluid 0, 3, 6 and 12 hours after the morning meal ($P > 0.10$, Table 6). The means values of VFA concentrations did not change after defaunation ($P > 0.44$). No significant interaction between defaunation and the diet appeared. Defaunation significantly decreased ($P < 0.05$) propionate mean concentrations (11.6 *vs* 11.0 ± 0.2 mM) and butyrate mean concentrations (6.1 *vs* 5.1 ± 0.2 mM). Defaunation had no effect ($P > 0.7$) on acetate mean concentrations (39.6 ± 0.6 mM).

3.5.3. VFA profile

Defaunation significantly increased ($P < 0.001$) acetate molar proportions (65.7 vs 67.9 ± 0.3%). Whereas the molar proportion of propionate and butyrate were significantly ($P < 0.05$) decreased by defaunation (19.4 *vs* 18.9. ± 0.2 % and 10.3 *vs* 8.8 ± 0.2% respectively) (Table 6).

Table 6: Effect of defaunation on pH and Volatile Fatty Acids (VFA), in the rumen of rams consuming 4 diets. The diets D120, D110, D90 and D60 were composed of fresh *Digitaria decumbens* grass at 14, 28, 42 and 56 days of age of regrowth respectively

PDI / UF,				D120		D110		D90		D60			Effect		
	F	D	s.e.m	F	D	F	D	F	D	F	D	s.e.m.	Def.	Diet	Def×Diet
Item and sampling time after feeding															
pH															
0h	6.65	6.56	0.03	6.69	6.45	6.68	6.49	6.62	6.51	6.62	6.77	0.07			*
3h	6.56	6.50	0.03	6.51	6.40	6.72	6.57	6.48	6.38	6.53	6.66	0.06		**	
6h	6.36	6.30	0.04	6.41	6.28	6.32	6.27	6.27	6.11	6.45	6.55	0.07		***	
12h	6.11	6.16	0.04	6.14	6.15	5.81	6.14	6.07	5.87	6.42	6.49	0.08		***	*
Mean pH	6.42	6.38	0.03	6.44	6.32	6.38	6.37	6.36	6.22	6.50	6.62	0.06		***	
Total VFA (mmol / L)															
0h	57.7	56.8	1.4	63.6	58.1	60.2	63.2	55.0	56.4	52.0	49.5	2.7		*	
3h	57.9	57.9	1.2	62.0	59.3	59.3	62.3	56.7	57.4	53.8	52.4	2.4			
6h	59.2	59.4	1.3	63.8	59.5	64.4	62.2	59.9	64.2	48.7	51.8	2.6		***	
12h	65.1	61.6	1.4	64.1	61.4	73.7	63.4	67.2	66.8	55.2	54.7	2.8		***	
Mean total VFA	60.0	58.9	0.9	63.4	59.6	64.4	62.8	59.7	62.1	52.4	52.1	1.8		***	
Molar proportions															
Acetate (C2, molar %)	65.7	67.9	0.3	63.3	65.1	67.0	69.9	67.7	70.7	64.9	65.9	0.6	***	***	
Propionate (C3, molar %)	19.4	18.9	0.2	20.8	20.0	19.6	18.6	18.7	18.5	18.4	18.3	0.3	*	***	
Butyrate (C4, molar %)	10.3	8.8	0.2	10.7	9.8	9.2	7.9	8.9	6.5	12.3	11.1	0.5	***	***	
Ratios															
C2 / C3	3.4	3.6	0.04	3.1	3.3	3.4	3.8	3.6	3.8	3.5	3.6	0.07	**	***	
C2 / C4	6.7	8.4	0.2	6.3	6.9	7.4	9.2	7.7	11.5	5.2	5.9	0.4	***	***	***
C3 / C4	1.9	2.3	0.04	2.0	2.1	2.2	2.4	2.1	3.0	1.5	1.6	0.1	***	***	***
(C2+C4) / C3	3.9	4.1	0.04	3.6	3.8	3.9	4.2	4.1	4.2	4.2	4.2	0.9	*	***	

Def : Defaunation effect; D : defaunated animals F: faunated animals; * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

The acetate to propionate ratio was significantly increased by defaunation ($P < 0.01$). The acetate to butyrate ratio was significantly ($P < 0.001$) increased by defaunation (6.7 *vs* 8.4 ± 0.2). There was a significant interaction ($P < 0.001$) between defaunation and the diet, the ratio was the highest on diet D90. The propionate to butyrate ratio was significantly ($P < 0.001$) increased by defaunation (1.9 *vs* 2.3 ± 0.4). There was a significant ($P < 0.001$) interaction between defaunation and the diet, the ratio was the highest on diet D90. Defaunation increased significantly ($P < 0.05$) the (acetate + butyrate) / propionate ratio (3.9 *vs* 4.1 ± 0.04). No interaction appeared between defaunation and the diet.

4. Discussion

The present study indicate that defaunation decreased microbial activities in the rumen, with sheep fed forages diet. Defaunation decreased the number of total bacteria population in the rumen. Similar results were reported (Jouany et al., 1981), but increased populations of total bacteria are usually reported in data literature (Eadie and Hobson, 1962; Kurihara et al., 1968). Nevertheless, a significant interaction occured between defaunation and the diet effect. Thus, on diet D120 the number of total bacteria increased after defaunation as was observed previously, using classical bacterial viable counts (Eadie and Hobson, 1962; Klopfenstein et al., 1966; Kurihara et al., 1968). The inverse effect observed on diet D60 may be explained by the unsufficient level of energy (Y ATP) available for the microbial growth. Moreover, the low level of NH3, inferior to 50 mgN / L, may be another limiting factor of microbial growth. The effect of defaunation on this diet were the lowest, whereas the number of protozoa was higher on diet D60 compared to diet D120.

It is admitted that the three major cellulolytic bacteria in the rumen are composed of *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Fibrobacter succinogenens* (Coleman, 1975), that is the reason why we studied these 3 species (Fonty et al., 1995). The number of the cellulolytic bacteria decreased slightly (6%) compared to the decrease of total bacteria (30%) after defaunation. Defaunation increased the number of cellulolytic bacteria on diet D120 whereas it decreased it on diet D60. Consequently defaunation increased the percentage of cellulolytic bacteria, and this increase was more pronounced on diet D60 than on diet D120. The percentage varied from 4 to 6% on diet D60, and is a slightly higher than the percentage reported on mixed diets (Stahl et al., 1988; Weimer et

al., 1999). This result may suggest that a more adapted microbial population may develop in response to the diet offered. Hence as the PDIN / UF content of the diet decreased, mainly because of the decreased crude protein content of the forage, more specif fibrolytic bacteria developped. The percentage of the *Ruminococcus albus* specie increased by 43% after defaunation and that of *Fibrobacter succinogenes* increased by 20%. Our results indicated that *Ruminococcus albus* is the predominant cellulolytic bacteria on forage diets. This result is contradictory to the results reported by some authors on the same diet rich in lignocellulosic materials (Bryant and Burkey, 1953; Fonty et al., 1995)Fonty RND88 Eugene SA JAS, where Fs is assumed to be the more active cellulolytic bacteria in the rumen.

The activity of the fibrolytic enzymes decreased after defaunation. Similar results were obtained by Kurihara et al. (Kurihara et al., 1978). The first factor responsible for the decrease of the fibrolytic activity may be attributed to the disappearance of the fibrolytic activity of protozoa after defaunation. Despite increased percentage of cellulolytic bacteria, their number decreased and consequently fibrolytic enzymatic activity decreased. This result may be explained by the less active enzymatic complex of *R. albus* compared to the enzymatic complex of *F. succinogenens* (Chesson and Forsberg, 1997; Dehority, 1993). Therefore in our study, decreased fibrolytic activity may presumably be attributed to decreased fibrolytic microorganisms in the rumen, especially *F. succinogenens*. Another factor that is reported to explain the decrease of fibrolytic activity is the pH (Coleman, 1985; Jouany and Martin, 1997; Martin et al., 1999). Our results indicate that defaunation had no effect on ruminal pH so they are not in agreement with this previous statement.

Defaunation decreased in situ DM degradation, however the differences are not significant whatever the incubation time considered. Similar results were observed by Ushida et al. (Ushida et al., 1990). The low decrease by 4.6% of the in situ DM degradation (96 hours of incubation) has to be opposed to the rather high decrease (40%) in enzymatic activities. This may suggest that enzymatic activity is not the first limiting factor of digestion in ruminants with tropical forage. This hypothesis seemed to be validated by the results of the in sacco degradation of the forage , serving as a reference, which indicated that DM degradation did not vary significantly between diet D150 and D90. These latter results was already observed on mixed diets, on which a different microbial feature established. Rather than a unique and fixed microbial population, the rumen ecosystem elaborate the most suitable combinations and interactions between species in

response to diet effect, to provide maximum microbial growth (Hungate, 1966), and consequently optimal degradation.

Ammonia concentrations decreased after defaunation, as is classically observed in data literature. The highest differences between defaunated and faunated animals were observed for diets high on PDIN / UF contents, suggesting that proteolysis was enhanced by nitrogen incorporation in the diet . Low level of NH3 in defaunated animals are mainly explained by the absence of predation of bacteria by protozoa after defaunation, consequently bacterial proteolysis tended to decrease after defaunation. Increased amounts of dietary nitrogen degraded in the rumen, while the PDIN / UF level of the diet increased, also explain the increased ammonia concentrations. Moreover protozoa seem to digest both dietary and bacterial nitrogen at the same rate (Eugène et al. unpublished data) (Jouany, 1996). Thus dietary nitrogen degradation is reduced after defaunation.

Defaunation had no significant effect on VFA concentrations, only a small decrease by 2% appeared. This result may mainly be explained by the slight decrease of DM degradation and consequently of OM observed in the rumen. The significant decrease of butyrate molar proportions after defaunation lead to significant increase of both acetate to butyrate ratio and propionate to butyrate ratio. Forage degradation leads to high production of acetate concentrations in the rumen compared to mixed diets. The modification of microbial population of the rumen and consequently of their fermentative activity may be the main factor affecting the decrease of the ruminal fermentation. Several results are consistent with this statement. A factor could be the decrease of protozoa population, especially the strain *Polyplastron multivesiculatum* after defaunation. Indeed Jouany and Sénaud (1979) indicated that this strain had a great capacity to degrade lignocellulosic materials. As the percentage of the cellulolytic bacteria increased after defaunation, it may be hypothesised that they are mainly responsible for the increase acetate production Finally, as protozoa are the main butyrate producers compared to the predominant bacteria *Butyrivibrio fibrosolvens* (Wolin and Miller, 1988), the decreased molar proportion of butyrate observed after defaunation is coherent with the elimination of protozoa after defaunation. In our study the effects of the diet interacted with defaunation in some instance, especially with VFA profiles.

5. Conclusion

Our results on the effect of defaunation on microbial activities, with sheep given forages diets indicate that the microbial enzymatic activities and in sacco DM degradation decreased. Defaunation leads to significant modifications of microbial population, the number of total bacteria decreased whereas the number of cellulolytic bacteria remained unchange. More numerous specific probes should be elaborated in the future, in order to monitor the principal variations of microbial population. It may then be possible to better understand the relationships between microbial species and the fermentative products, in in vivo trials. Defaunation also modified VFA profiles, thus the concentration and percentage of butyrate significantly decreased. The decrease in the supply of energy after defaunation is moderate, because of the compensatory digestion of OM in the intestine. Nevertheless, with the low PDIN / UF generated on this study, the limiting factor may be the energy rather than the nitrogen.

Acknowledgements

The authors would like to thank L. Millet, M. Hilaire, N. Jalwan, F. Pommier, M. Arjournin, F. Nipeau and F. Periacarpin for their technical collaboration to this work. This work was supported by the “Région Guadeloupe” and the European Economic Community (FEOGA).

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DISCUSSION GENERALE

1. CONTRAINTES ET LIMITES DU TRAVAIL DE THESE

L'objectif de ce travail de thèse était de tester l'intérêt zootechnique de la défaunation dans une large gamme de rations alimentaires représentatives des pratiques les plus utilisées en élevage. L'hypothèse de travail avancée était que le rapport azote / énergie des rations ingérées serait le principal facteur explicatif de la réponse animale (croissance) à la défaunation. Ainsi, l'étude de l'impact de la défaunation sur certains paramètres de la digestion devait nous permettre de comprendre les mécanismes mis en jeu lors des réponses observées. Une analyse quantitative des résultats de la bibliographie par une méthode statistique (la méta-analyse) nous a permis de dégager certaines lois générales quantitatives originales. Des essais expérimentaux ont été effectués afin de compléter la méta-analyse, particulièrement dans le contexte tropical et en fonction de notre hypothèse de travail.

Le rapport MAT / NDF des diverses rations de la synthèse des données bibliographiques (Publication 1) a été retenu afin d'estimer le rapport azote / énergie des rations. Ce critère est discutable car la teneur en azote des rations (MAT) ne nous informe pas de manière satisfaisante sur les quantités d'azote absorbées par l'animal, d'où le développement du système PDI (Verité and Peyraud J.L., 1988). Cependant, compte tenu que la défaunation modifie sensiblement la valeur PDI d'une ration, l'emploi des MAT pourrait présenter moins d'inconvénients que des valeurs PDI mal estimées. De plus, la teneur en parois cellulaires des rations (NDF) ne rend compte ni de la nature de l'énergie de la ration, ni des quantités d'énergie absorbée. Cependant, dans le cas de notre étude bibliographique, les données émanent de différentes équipes utilisant des systèmes de détermination de la valeur énergétique parfois différents. Par conséquent, ce critère (MAT / NDF) était le plus souvent disponible (ou estimable) et le plus « robuste ».

Les rations testées au cours des deux études expérimentales couvrent une gamme de rations caractérisées par des profils énergétiques et azotés différents. Le gradient des teneurs en azote sur énergie est reflété par le gradient du rapport PDIN / UF de la ration.

Les profils énergétiques sont définis par des teneurs en glucides intracellulaires (amidon) et en glucides pariétaux, très variables selon les rations. De même, les profils azotés résultent de sources d'azote protéique différentes : faiblement dégradable (maïs), moyennement (tourteau de soja) ou fortement dégradable (fourrage en vert). La formulation des rations, qui avait pour objectif de refléter des rations utilisées en élevage, n'a pas permis de faire évoluer de façon indépendante le gradient en azote et celui en énergie. De plus, l'étude de ration iso azotée, à base de canne à sucre (profil énergétique sucre) complémentée par de la farine de riz, programmée initialement dans le projet de thèse, n'a pas eu lieu (problème sanitaire, contrainte de durée d'un projet de thèse). Cette étude aurait permis de répondre au précédent objectif.

Les plans expérimentaux relatifs à l'étude de la défaunation sont particulièrement contraignants (ressources humaines, maîtrise des techniques de défaunation, contrôle des animaux). A ces contraintes s'ajoutent celles liées à la conduite d'essais zootechniques et de digestion (nombre d' animaux, santé...). Dans le cadre des études zootechniques la part de la variabilité animale, contribuant à la variabilité globale des résultats, est importante. Toutefois, il a été difficile de totalement prendre en compte cette variabilité animale, dans le cadre de nos études expérimentales. En effet, pour la limiter, il aurait été nécessaire de travailler en dispositif carré latin, avec le même animal alternativement fauné puis défauné (ou l'inverse) (Jouany, 1991b). Bien que techniquement réalisable, compte tenu des objectifs du projet de thèse (8 rations testées, 8 animaux / traitement) cela entraîne des complications expérimentales (longues périodes de mesure) de sorte qu'un dispositif en carré latin n'a pas pu être mis en place. Nous avons du travailler en lot (faunés *vs* défaunés). Pour obtenir la même puissance statistique qu'un carré latin, il aurait fallu augmenter considérablement le nombre d'animaux, ce qui aurait été difficilement gérable car le personnel technique devient très vite un facteur limitant. Afin de tenir compte à la fois des contraintes humaines et statistiques (degré de liberté) il a été nécessaire de réaliser un compromis qui a abouti à des résultats statistiques souvent en limite du seuil de signification.

2. LES PRINCIPAUX RESULTATS

2.1. Effet de la défaunation sur la croissance animale

D'une manière générale, l'étude de la bibliographie et les essais expérimentaux indiquent que, tous contextes alimentaires confondus, la défaunation est sans effet ou améliore la croissance des animaux. En effet, la défaunation tend à augmenter (P = 0.09) le gain moyen quotidien (GMQ, g/j) de l'ensemble des agneaux de nos deux études expérimentales (132.6 *vs* 118.7 ± 5.2 g/j) (Figure 1). Ces résultats sont en accord avec ceux de la synthèse bibliographique (Figure 2). En effet, pour des quantités ingérées moyennes (MSI %PV) voisines de 3% PV (± 1%) et des teneurs en azote / énergie moyennes de 40 MAT/NDF (± 26.3), la défaunation augmente significativement de 10% la croissance moyenne des agneaux (GMQ %PV). De plus, quand la proportion de concentré et la teneur en azote / énergie de la ration diminue, l'effet de la défaunation sur la croissance augmente. De même, dans nos études nous observons une interaction entre les effets du rapport PDIN / UF de la ration et de la défaunation sur la croissance. Ainsi, l'effet moyen de la défaunation sur la croissance est plus marqué pour la ration simple contenant la plus faible teneur en azote / énergie (50 PDIN / UF) (P < 0.05, 25 *vs* 48.4 g / j). De même, les écarts entre faunés et défaunés ont été plus grands pour la ration mixte à faible teneur en azote / énergie (100 PDIN / UF) (P < 0.05, 153.7 *vs* 211.6 g / j).

Le rapport MAT / NDF est un facteur explicatif des variations observées dans la synthèse bibliographique. Des interactions entre l'effet de la défaunation et la ration (PDIN / UF) ont été mises en évidence par les résultats expérimentaux. L'effet bénéfique de la défaunation n'est pas lié à une augmentation des quantités de matière digestible ingérée. L'effet de défaunation est en effet très variable sur les quantités ingérées, alors qu'elle n'affecte pas ou pénalise peu la digestibilité des glucides.

L'effet bénéfique de la défaunation sur la croissance des ruminants s'explique par une modification du profil des nutriments absorbés comme en témoigne l'amélioration des indices de consommation de la matière sèche et de l'énergie. Ainsi que l'amélioration du pourcentage de propionate. L'augmentation de l'absorption intestinale des protéines et la modification du profil d'AGV en sont les principaux facteurs explicatifs.

L'absence d'effet bénéfique de la défaunation sur la croissance des ruminants est associée à un déséquilibre en azote / énergie dans les rations, principalement quand l'énergie est absorbée en quantité insuffisante relativement aux besoins de croissance des animaux.

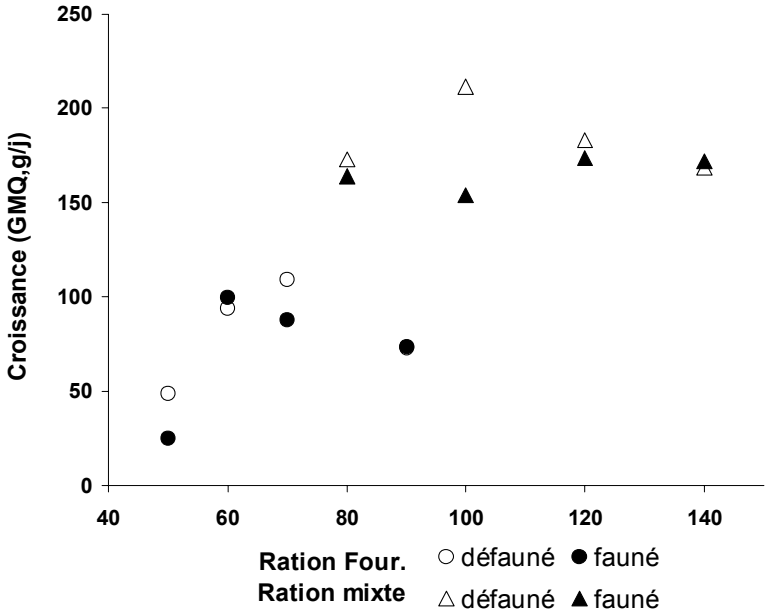


Figure 1 : Effets de la défaunation sur la croissance (GMQ, g/j) d'agneaux en fonction de la teneur en azote/énergie (g PDIN/UF) de rations composées de fourrage (*Digitaria decumbens*) sans concentré, ou avec concentré (mélange de mais et tourteau de soja). Moyennes ajustées des deux études expérimentales.

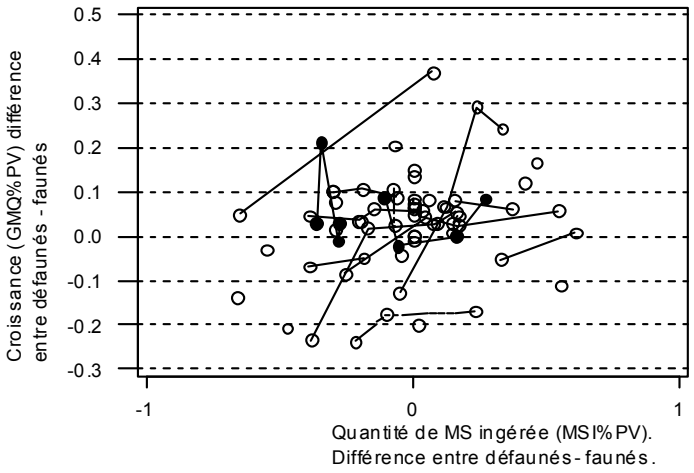


Figure 2 : Effets de la défaunation sur la croissance (GMQ, g/%PV) en fonction des quantités de MS ingérées (MSI, %PV). Différences entre défaunés-faunés. Comparaison de nos résultas expérimentaux ● avec ceux de la synthèse bibliographique des données de la litérature ❍.

Quand les rations couvrent les besoins des animaux la défaunation s'avère être sans intérêt.

Au regard des résultats obtenus, l'hypothèse du travail de thèse (le rapport azote sur énergie module les effets de la défaunation) demeure crédible, mais doit être reformulée. comme suit : **Le rapport azote / énergie est le principal facteur de variation de l'effet de la défaunation sur la croissance des animaux, quand l'ingestion d'énergie digestible n'est pas le premier facteur limitant**.

2.2. Effet de la défaunation sur les quantités de protéines absorbées

Dans le cas des rations mixtes, nos résultats indiquent que la défaunation augmente le flux duodénal d'azote total (non ammoniacal) de 21%. Le flux d'acides aminés absorbés est augmenté d'autant, si nous faisons l'hypothèse que la digestibilité intestinale de l'azote n'est pas modifiée. Ces résultats sont en accord avec ceux de la synthèse bibliographique qui indiquent également une augmentation moyenne de 21% de ce flux (Figure 3).

En premier lieu, ce résultat s'explique par une augmentation des quantités d'azote bactérien au niveau du duodénum. Cette dernière est attribuable à une diminution du taux de dégradation des protéines bactériennes (lyse bactérienne par les protozoaires). En second lieu, nos résultats expérimentaux et bibliographiques indiquent une augmentation équivalente des flux d'azote alimentaire et microbien chez les animaux défaunés. En effet, la part de l'azote microbien dans la quantité d'azote total au niveau du duodénum n'est pas modifiée par la défaunation. La dégradation moindre des protéines d'origine alimentaire dans le rumen, suite à la défaunation, contribuerait à l'augmentation des quantités d'azote alimentaire au niveau du duodénum (Ushida et al., 1986). Cette diminution de la dégradation protéique serait liée à la diminution de l'activité désaminase dans le fluide ruminal (Wallace, 1991) suite à l'élimination des protozoaires. En effet, ces derniers ont une activité désaminase 3 fois plus importante que celle des bactéries (Hino and Russel, 1985).

2.3. Impact de la défaunation sur l'excrétion fécale et urinaire d'azote

Les résultats de la défaunation observés sur la teneur en NNH3 du jus de rumen sont cohérents avec ceux de la litérature (Figure 4). Ils sont la conséquence des phénomènes décrits dans le paragraphe précédent.

Figure 3 : Effet de la défaunation sur les flux duodénaux d'Azote (non ammoniacal) gN/J. Comparaison entre les points expérimentaux (●) et les données de la synthèse bibliographique (❍).

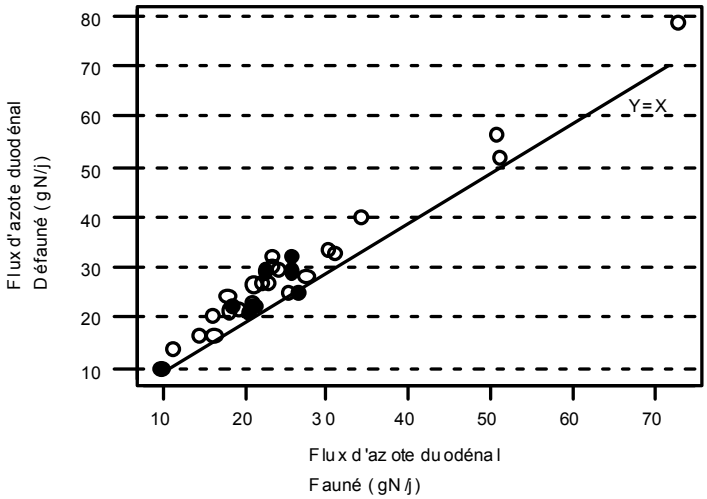
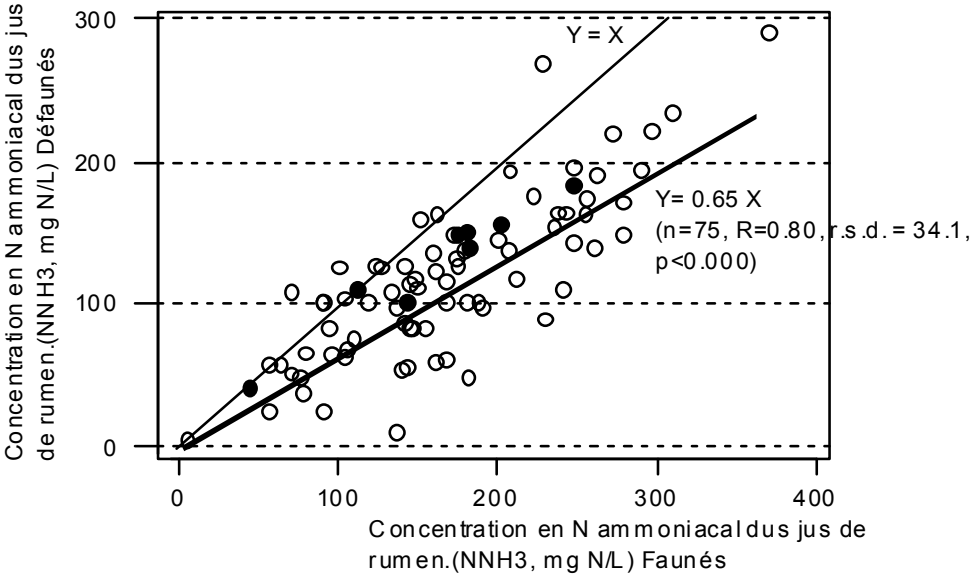


Figure 4 : Effet de la défaunation sur les concentration en ammoniaque du rumen ($\hat{N}NH_3$). Comparaison des points expérimentaux (●) avec les données de la synthèse bibliographique(❍)



D'autre part, nos résultats sur les quantités d'azote excrété sont cohérents avec ceux de NH3 du rumen. Peu de données bibliographiques sont disponibles sur l'effet de la défaunation sur l'excrétion urinaire d'azote (Ikwuegbu and Sutton, 1982; Punia et al., 1987; Whitelaw et al., 1984). Nos résultats indiquent que la défaunation entraîne une réduction significative des pertes azotées urinaires de 22% pour les rations mixtes et de 16% pour les rations de fourrages. Ces résultats sont en accord avec les données de la litérature (Figure 5). Ils suggèrent que les animaux défaunés ont des pertes en azote moindre liées vraisemblablement à l'absence de prédation des bactéries par les protozoaires et/ou à une modification du métabolisme azoté. De la même manière les pertes en azote fécal sont diminuées de 13% pour les rations mixtes. En revanche la défaunation entraîne une augmentation de 11% des pertes azotées fécales pour les rations de fourrages. Ces différences entre les 2 types de rations sont expliquées par les différences de digestibilité de l'azote .

2.4. Impact de la défaunation sur les quantités et la qualité de l'énergie absorbée

Il n'y a pas d'effet de la défaunation sur les quantités de matière sèche ingérées. La défaunation entraîne aussi une diminution des quantités de glucides digestibles ingérées conséquentes à une diminution de la cellulolyse ruminale, tandis que la digestibilité totale des glucides est peu affectée. Une compensation partielle ou totale a lieu au niveau intestinal pour les animaux défaunés. Cette tendance se retrouve au niveau de la synthèse bibliographique où la digestion ruminale de la MO et de NDF diminuent en moyenne respectivement de 7.3 et 4.7 points de digestibilité, après défaunation. Cependant, la diminution de la digestibilité de la MO n'est plus que de 1.7 points de digestibilité dans l'ensemble du tube digestif et elle s'accroît d'un point pour le NDF.

La diminution de la digestibilité ruminale des glucides pariétaux (Figure 6), observée expérimentalement après défaunation est attribuable à la baisse significative des activités enzymatiques glycolytiques des microorganismes du rumen (polyosidases et glucosidases). Toutefois, la modification du faciès microbien du rumen après l'élimination des protozoaires, entraîne un nouveau « profil bactérien » avec vraisemblablement l'augmentation des bactéries cellulolytiques à très fort potentiel enzymatique. Cependant, ce réarrangement des populations du rumen ne suffit pas à rétablir le potentiel enzymatique initial des animaux faunés.

Figure 5 : Effet de la défaunation sur l 'excrétion d 'azote urinaire (gN/j) selon les quantités d 'azote ingérées. Différence entre les animaux défaunés–faunés. Comparaison des points expérimentaux (●) avec les données de la synthèse bibliographique (❍)

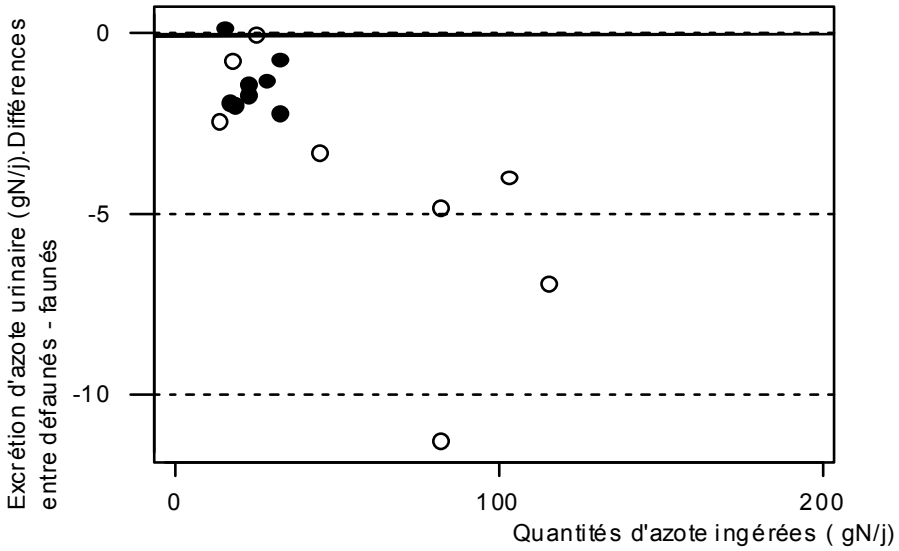
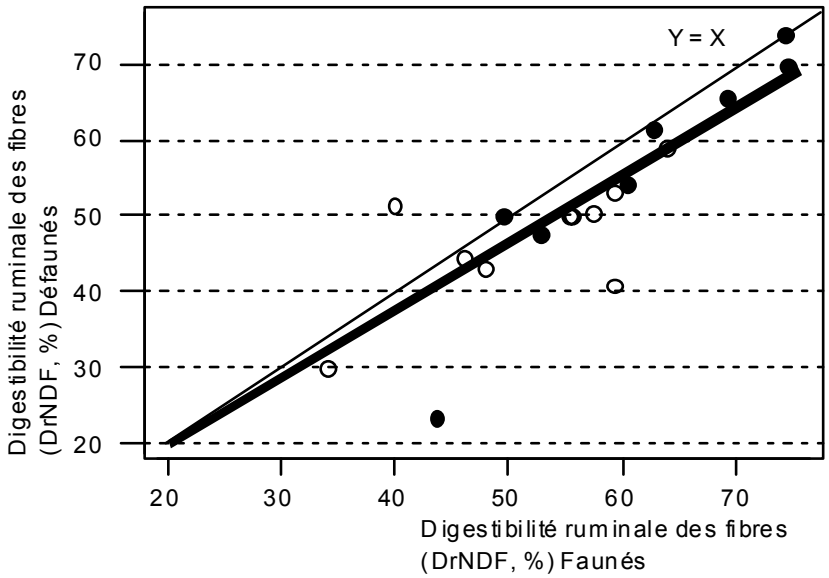


Figure 6 : Effet de la défaunation sur la digestibilité ruminale des fibres (DrNDF). Comparaison des points expérimentaux (●) avec les données de la synthèse bibliographique(❍)



L'impact modéré de la défaunation sur la digestion des fibres indiquerait que l'activité enzymatique du rumen, même dans le cas de fourrage de qualité moyenne à pauvre de la zone tropicale, ne serait pas le premier facteur limitant de la digestion.

Le profil des AGV absorbés semble être favorable pour les animaux défaunés comme l'indiquent nos résultats expérimentaux. Pour les rations mixtes, la défaunation contribue à augmenter la proportion de propionate dans le rumen au détriment de celles de l'acétate et du butyrate. Les proportions de propionate et d'acétate augmentent au détriment de celles du butyrate dans les rations simples de fourrage. Cette tendance confirme les résultats de la synthèse bibliographique où la proportion d'acétate + butyrate / propionate a fortement diminué avec la défaunation.

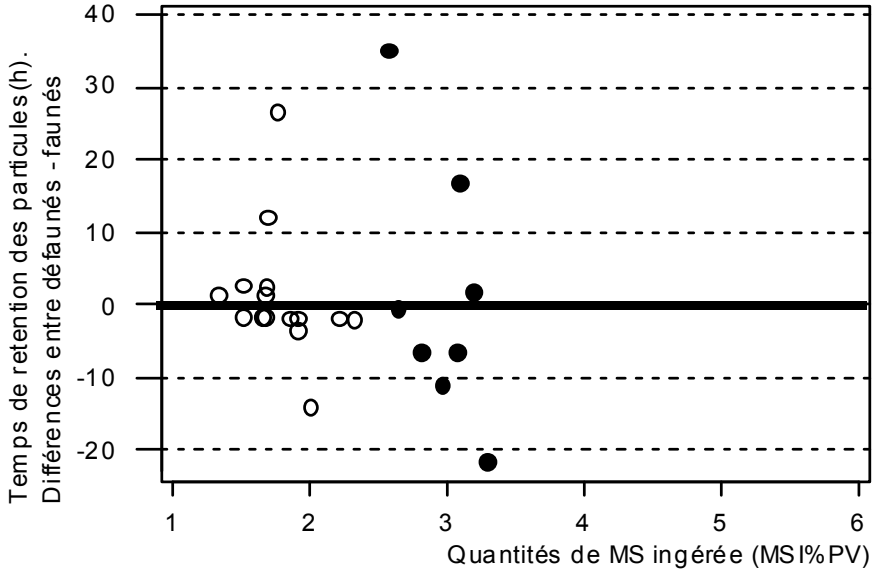
Cette modification du profil des AGV du rumen et par conséquent des AGV absorbés dans le sang, serait à mettre au profit du changement des orientations des fermentations ruminales après l'élimination des protozoaires du rumen. Le butyrate est l'AGV majoritairement produit à l'issue des fermentations des glucides du rumen par les principaux groupes de ciliés. La défaunation entraîne donc une diminution des proportions de butyrate qui ne semble pas être compensée par les produits issus de la fermentation par les autres populations microbiennes.

Le propionate étant le principal précurseur de glucose et ayant un effet insulinotrope et anabolisant chez le ruminant, l'augmentation de son pourcentage dans le pool d'AGV absorbés contribuerait à **l'amélioration de l'utilisation métabolique des nutriments énergétiques absorbés**. Cet aspect associé à une meilleure fourniture d'acides aminés essentiels (voir chapitre suivant), suggère que le rapport énergétique énergie nette / énergie métabolisable (E.Nette / E.M) du régime soit. vraisssemblablement meilleur chez les animaux défaunés.

2.5. Effet de la défaunation sur le temps de séjour moyen des particules dans le rumen.

Les données de la littérature sur le temps moyen de rétention des particules sont rares. De plus, les effets de la défaunation sont variables, ainsi on ne met pas en évidence d'effet moyen de la défaunation sur le temps de retention des particules dans le rumen. Nos résultats sont aussi variables que ceux de la bibliographie (Figure 7).

Figure 7 : Effet de la défaunation sur le temps de rétention des particules (h) selon les quantités de MS ingérées (MSI%PV). Différence entre les animaux défaunés–faunés. Comparaison des points expérimentaux (●) avec les données de la synthèse bibliographique(❍)



D'une part ils indiquent que la défaunation entraîne une diminution significative de 25% du temps de rétention des particules dans le rumen, pour les rations mixtes. En revanche, la défaunation augmente significativement le temps de rétention pour les rations de fourrages D60 et D50 uniquement, sinon elle est sans effet.

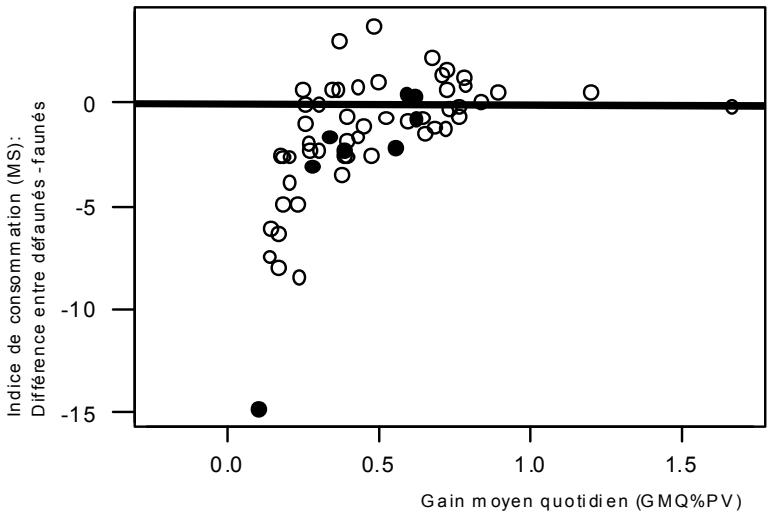
2.6. Effet de la défaunation sur l'indice de consommation

Bien que non significatif (*P* = 0.3), nos résultats indiquent que la défaunation diminue, l'indice de consommation de la matière sèche en moyenne de 0.5 g MS / g de croît pour les rations mixtes (complémentées). Une diminution de 1.5 g MS / g de croît est observée pour les rations de fourrage seul. Nos résultats sont en accord avec les résultats de la synthèse bibliographique qui indiquent une diminution hautement significative de l'indice de consommation de 1.4 g MS / g de croît sous l'effet de la défaunation (-15%).(Figure 8).

Les indices de consommation de matière sèche et de transformation de l' énergie des animaux défaunés sont meilleurs. L'amélioration de l'utilisation métabolique des nutriments énergétiques absorbés en serait la principale cause.

L'augmentation de l'absorption d'acides aminés contribue vraisemblablement à l'amélioration de l'utilisation métabolique des nutriments. Selon certains auteurs (Oldham and Tamminga, 1980) le profil des AA au niveau du duodénum est principalement lié au contenu en AA microbiens et non pas à la composition en AA de la ration. Cependant, d'autres auteurs (Bocquier, 1994; Han et al., 1996; Matras and Bartle, 2000) indiquent que la composition des protéines microbiennes peut ne pas être optimale, et présenter un déficit en méthionine et en lysine. Ceci est vrai pour les races à haut potentiel de production laitière des différentes espèces (vache, brebis et chèvre laitière). Pour ces dernières, la quantité de protéines microbiennes synthétisées dans le rumen est insuffisante (Robelin et al., 2000). Ainsi, la méthionine et la lysine sont souvent deux acides aminés limitants pour la production des protéines du lait (Bocquier, 1994; Rulquin, 1987)..

Figure 8 : Effet de la défaunation sur l' indice de consommation (MS) selon la croissance (GMQ %PV). Différence entre les animaux défaunés–faunés. Comparaison des points expérimentaux (●) avec les données de la synthèse bibliographique (❍).



2.7. Intérêt de la défaunation dans le contexte tropical

L'intérêt potentiel de la défaunation dans le contexte tropical reste encore à préciser. La défaunation ne présenterait pas d'intérêt pour les animaux ingérant de jeunes fourrages tropicaux. Les résultats des essais de croissance et de digestion vont dans le même sens. L'ingestion d'énergie, premier facteur limitant du potentiel de production des fourrages tropicaux, serait à l'origine de ces résultats qui méritent toutefois d'être confirmés. Il semblerait que dans le contexte tropical avec de jeunes fourrages pour lesquels l'énergie est limitante, la défaunation qui diminue la digestion, contribue à amplifier le déficit énergétique. Le fourrage canne à sucre est probablement le contre exemple compte tenu de sa carence en azote relativement à sa richesse en énergie. Les rations mixtes semblent répondre favorablement à la défaunation, surtout quand le rapport azote sur énergie est faible. Un pourcentage de concentré minimum de 30% semble suffisant. Toutefois, le coût de ce concentré devra être moins élevé que les gains financiers liés à la production escomptée.

CONCLUSIONS ET PERSPECTIVES

L'intérêt potentiel de la défaunation pour des ruminants ingérant des fourrages tropicaux ainsi que le cas particulier de la canne à sucre reste encore à préciser. De plus, la canne représente un modèle biologique intéressant. Il conviendrait de tester les rations à base de canne à sucre entière qui offre l'avantage d'être non limitant en énergie alors qu'elles sont déficientes en azote. Boodoo et al, (1978) a déjà montré qu'avec des régimes à base de canne à sucre, la défaunation entraîne des augmentations significatives de la croissance. Il serait donc intéressant d'envisager de poursuivre cette étude, afin de compléter la gamme de rations caractéristiques de la zone tropicale.

Seul le modèle croissance (extrapolable à tous les ruminants) a été étudié. La défaunation pourrait être bénéfique dans le cas de la production laitière. Cependant, cette hypothèse doit être confirmée d'autant plus que le profil de nutriments, nécessaire pour optimiser la production laitière, diffère de celui nécessaire pour la viande. Ainsi, Yang et Varga (1993) n'ont pas observé d'effet bénéfique de la défaunation sur la production laitière. Ce résultat s'expliquerait par l'utilisation de la race Holstein, à haut potentiel de production laitière, qui demande de très grandes quantités de protéines.

Cependant, afin de déterminer si la défaunation constitue une technique d'intérêt économique et facilement applicable, il convient de s'interroger sur la nécessité de maintenir les animaux totalement défaunés. En effet, la défaunation partielle, en maintenant la population de protozoaires à une concentration suffisamment basse, permettrait de s'affranchir des conditions très contraignantes des études expérimentales (difficilement applicables en élevage ouvert). De plus l'impact de la défaunation partielle sur les quantités d'énergie digestible devrait être réduit. La recherche de méthodes simples permettant d'atteindre cet objectif, tout en conservant les avantages décrits, serait une voie d' investigation intéressante.

La défaunation présente toutefois quelques contraintes. En effet, les protozoaires sont impliqués dans les mécanismes de détoxication (agents pathogènes, mycotoxine), ils

stabilisent le pH du rumen et sont des marqueurs biologiques de l'état de fonctionnement du rumen.

La défaunation, en dehors de l'aspect économique qu'elle peut représenter au niveau des productions animales, reste un bon modèle d'étude in vivo de l'impact des protozoaires dans la digestion ruminale. De plus, l'essor des techniques de biologie moléculaire (sonde d'ADN spécifique d'espèce ou de genre) devrait nous permettre de mieux caractériser l'écosystème microbien.

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Table 5 : Effect of defaunation on in situ dry matter (DM) degradation, and Ammonia (NH3) in the rumen of rams consuming 4 diets. The diets D120, D110, D90 and D60 were composed of fresh *Digitaria decumbens* grass at 14, 28, 42 and 56 days of age of regrowth respectively.

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LISTE DES ABREVIATIONS

AGV : Acide Gras Volatil

CMCase : Carboxyméthyl Cellulase

C_2 : Acétate

C_3 : Propionate

C_4 : Butyrate

D : Défauné

Def. : Effet de la défaunation

Dr : digestibilité ruminale

Dt :Digestibilité totale

F : Fauné

GMQ : Gain de poids moyen quotidien

IC : Indice de consommation

MAT : Matière azotée totale

MO : Matière organique

MSI : Matière sèche ingérée

N : Azote

N duo : Flux duodénal d'azote total non ammoniacal

NDF : Neutral Detergent fiber (résidu au détergent neutre)

NH3 : ammoniaque

NI : Azote ingéré

Nm duo : Flux duodénal d'azote microbien

PDIN : Protéines vraies digestibles dans l'intestin, permise par l'azote fermentescible de la ration.

PV : Poids vif

SR : Sucre réducteur

UF : Unité fourragère

Effect of defaunation of ruminants on production performances, according to the diet. Study of the variations of microbial proteosynthesis and cellulolysis.

The aim of this thesis was to evaluate defaunation as a technique, that allows a better nutrition of ruminants. Defaunation lead to an increase of non ammonia-nitrogen flow at the duodenum (NAN duo.). However, literature studies indicate that defaunation effect on animal production (meat, milk and wool) is varying. Our hypothesis is that the nitrogen / energy ratio (PDIN / UF) of the diet is the main factor responsible for the observed variyng effects of defaunation. The objectives were : 1) To determine the law of animal response to defaunation, generally speaking and in the specific context of the humid tropical area. 2) To determine the impact of defaunation on the parameters of digestion that may explain animal response to defaunation, especially microbial proteosynthesis and cellulolysis.

First of all, the statistical analysis (meta-analysis) of the literature data allowed us to establish the global laws of animal response to defaunation. Secondary, the study of four experimental diets composed of fresh forage (*Digitaria decumbens*) containing 50, 60, 70 and 90 g (PDIN / UF) was elaborated. In a second study, four other diets, composed of fresh forage (*Digitaria decumbens*) and concentrate (maize and soya meal) , and containing 80, 100, 120 and 140 g (PDIN / UF) were also tested. Meat production was determined by the measurements of average daily growth (ADG) on faunated and defaunated lambs , with the 8 dietary profiles (8 PDIN / UF ratios). Measurements of intestinal nitrogen flows were made on fistulated adult rams, faunated and defaunated, allowed us to estimate proteosynthesis. Microbial cellulolysis was estimated by both in sacco dry matter degradability technique, ruminal fiber (NDF) digestibility and ruminal glycolytic activity, on the same preceding rams.

The results of the bibliographic synthesis indicate that defaunation led to the increase of animal growth, on average by 10 % ($P < 0.01$, 132.6 *vs* 118.7 ± 5.2 g/d). In experimental studies defaunation led to an increase of ADG for mixed diets (PDIN / UF > 80) ($P < 0.03$, 184.1 vs 165.9 ± 9.3 g/d).No effect was observed on forage diets (ADG = 76 ± 3 g/j), except for diet contaning 50 PDIN/UF (25 vs 49 ± 11 g/d). The 20 % increase of intestinal nitrogen flow (microbial and dietary) after defaunation, contributes to the increased amounts of absorbed nitrogen nutrients. In experimental studies, defaunation led to a significant increase of this flow by 20% for mixed diets, and by 11% for forage diets. The bilbliographic synthesis indicated an enhancement of the shape of volatil fatty acids (VFAs) in the rumen (increase of propionate) after defaunation, whereas microbial cellulolysis decreased (- 4.7% DrNDF). The increased intestinal nitrogen flow and the enhanced shape of VFAs contribute to the enhanced metabolic use of energetic nutrients. Thus, defaunation led to an increase of food conversion efficiency (- 1.4 g DM / g growth). These results are confirmed by our experimental diets studies.

As a conclusion, the nitrogen /energy ratio of the diet modulate defaunation effect, while energy is not the growth limiting factor. The interest of defaunation in humid tropical area need to be confirmed, especially for young forage and sugar cane (low on nitrogen, high on glycoside).

Effet de la défaunation des ruminants sur les performances de production, en fonction de la ration ingérée. Etude des variations de la protéosynthèse et de la cellulolyse microbienne ruminale.

Cette thèse avait pour objectif d'évaluer la défaunation comme technique permettant une meilleure nutrition des ruminants. La défaunation entraîne une augmentation du flux d'azote (non ammoniacal) au niveau du duodénum. Cependant, des études de la littérature indiquent que l'effet de la défaunation sur les productions animales (viande, lait et laine) est variable. Notre hypothèse de travail est que le rapport azote / énergie (PDIN / UF) de la ration est le principal facteur de variation des effets observés. Nous avions pour objectifs : 1) Etablir les lois de la réponse animale à la défaunation en général, ainsi que dans le contexte spécifique de la zone tropicale humide. 2) Déterminer l'impact de la défaunation sur les paramètres de la digestion permettant d'expliquer la réponse animale à la défaunation, notamment sur la protéosynthèse et la cellulolyse microbienne.

En premier lieu, les lois générales des effets de la défaunation ont été établies à partir de l'analyse statistique d'une base de données de la littérature (méta-analyse). Dans un second temps, l'étude de quatre rations à base de fourrage (*Digitaria decumbens*) contenant 50, 60, 70 et 90 g PDIN /UF a été réalisée. Dans une seconde étude, quatre autres rations composées de fourrage avec complément (maïs et tourteau de soja) et contenant 80, 100, 120 et 140 g PDIN /UF, furent testées. La production de viande a été déterminée par mesures de gain de croît moyen quotidien (GMQ) d' agneaux faunés et défaunés, en fonction des huit profils alimentaires (8 rapports PDIN /UF). Des mesures de flux duodénaux d'azote sur des béliers adultes fistulés, faunés et défaunés, nous ont permis d'estimer la protéosynthèse. La cellulolyse microbienne a été estimé par les mesures de dégradabilité in sacco des fibres (NDF), de digestibilité ruminale des fibres (NDF) ainsi que par la mesure des activités enzymatiques glycolytiques du rumen, sur ces mêmes béliers.

Les résultats de la synthèse bibliographique indiquent que la défaunation entraîne une augmentation de la croissance des animaux en moyenne de 10% ($P < 0.01$, 132.6 *vs* 118.7 ± 5.2 g/j). Lors des essais expérimentaux, la défaunation a permis d'augmenter le GMQ en moyenne avec les rations mixtes (PDIN / UF > 80) ($P < 0.03$, 184.1 vs 165.9 ± 9.3 g/j). Aucun effet n'a été observé pour les rations de fourrage (GMQ = 76 ± 3 g/j), sauf pour la ration contenant 50 PDIN/UF (25 vs 49 ± 11 g/j). Dans la synthèse bibliographique, l'augmentation de 20 % du flux d'azote (microbien et alimentaire) intestinal après défaunation, contribue à l'augmentation des quantités de nutriments azotés absorbés. Pour les études expérimentales, après défaunation ce flux augmente significativement de 20% pour les rations mixtes, et de (+ 11%) pour la ration de fourrage. La synthèse indique une amélioration du profil des acides gras volatils (AGV) du rumen (augmentation du pourcentage de propionate) après defaunation, tandis que la cellulolyse microbienne diminue (- 4.7 % DrNDF). L'augmentation du flux d'azote intestinal et l'amélioration du profil des AGV contribuent à l'amélioration de l'utilisation métabolique des nutriments énergétiques. Ainsi, la défaunation améliore l'indice de consommation des animaux défaunés (-1.4 g MS / g de croît). L'ensemble de ces résultats est confirmé par l'étude de nos rations expérimentales.

En conclusion, le rapport azote / énergie de la ration module les effets de la défaunation, quand l'énergie n'est pas limitante. En zone tropicale humide, la défaunation ne présenterait un intérêt que pour les rations mixtes et les vieux fourrages. L'intérêt de la défaunation en ZTH reste à confirmer, notamment pour les jeunes fourrages et la canne à sucre (pauvre en azote, riche en sucre).