COMMUNAUTÉ FRANÇAISE DE BELGIQUE

UNIVERSITÉ DE LIÈGE – GEMBLOUX AGRO-BIO TECH

TREES AND SHRUBS INFLUENCE THE BEHAVIOUR OF GRAZING CATTLE AND RUMEN FERMENTATION

Sophie VANDERMEULEN

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

Promoteurs : Jérôme Bindelle

Carlos Alberto Ramírez-Restrepo (CSIRO, Australia)

Année civile : 2016

COMMUNAUTÉ FRANÇAISE DE BELGIQUE

UNIVERSITÉ DE LIÈGE – GEMBLOUX AGRO-BIO TECH

TREES AND SHRUBS INFLUENCE THE BEHAVIOUR OF GRAZING CATTLE AND RUMEN FERMENTATION

Sophie VANDERMEULEN

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

Promoteurs : Jérôme Bindelle

Carlos Alberto Ramírez-Restrepo (CSIRO, Australia)

Année civile : 2016

Copyright.

Aux termes de la loi belge du 30 juin 1994 sur le droit d'auteur et les droits voisins, seul l'auteur a le droit de reproduire partiellement ou complètement cet ouvrage de quelque façon et forme que ce soit ou d'en autoriser la reproduction partielle ou complète de quelque manière et sous quelque forme que ce soit. Toute photocopie ou reproduction sous autre forme est donc faite en violation de ladite loi et des modifications ultérieures.

Vandermeulen Sophie (2016). Trees and shrubs influence the behaviour of grazing cattle and rumen fermentation.

145 p., 18 tables, 3 figures.

Abstract

Hedgerows and woody strips have been used to enclose fields but declined due to the loss of a direct economic value, abandonment of traditional management techniques and agricultural intensification. Nowadays, shrubs and trees on pastures are promoted again through environment-friendly policies and the interest in using them as forage for ruminant increases in both temperate and tropical ecosystems. Woody plants on farmland could yield a wide range of ecosystem services and provide benefits for farmers and their animals such as forage supply to livestock, animal protection against severe weather and reduced parasitic infestation. Moreover, shifts in digestive physiology can be observed that will in turn affect the welfare and the performances of the animal and the production system as a whole. Therefore, in order to contribute to the development of sustainable systems using shrubs and trees as a feed component in its full right, the aim of this work was to investigate the influence of trees and shrubs on the behaviour of grazing cattle and their selectivity towards woody species, and to determine the changes induced by temperate and tropical shrub and tree species on rumen fermentation.

Firstly, the behaviour of grazing dairy heifers was recorded during the whole grazing season as well as their selectivity towards temperate woody species in a hedge. It was concluded that having access to a hedge influenced the behaviour of grazing cattle, as the animals ingested woody plants in each season but mostly when the available pasture biomass was lower. The most selected species were *Carpinus betulus, Corylus avellana, Cornus sanguinea* and *Crataegus monogyna*. Secondly, the chemical composition, *in vitro* rumen fermentation profile and protein precipitation capacity (PPC) of the temperate shrub and tree species were measured. The analyses showed that *Fraxinus excelsior* presented the most interesting profile in terms of chemical composition and *in vitro* fermentation production. Among the preferred species, *C. monogyna* and *C. avellana* produced lower CH_4 and the latest had the highest PPC. Thirdly, three newly-developed cultivars of the tropical *Desmanthus* genus were studied for their effects on *in vitro* rumen fermentation including potential to reduce CH_4. *Desmanthus*

leptophyllus and *D. bicornutus* had the highest anti-methanogenic potential, and *D. bicornutus* was more digestible.

In conclusion, both temperate and tropical shrub and tree fodder are promising to supplement cattle with good quality forage. Cattle can browse woody species voluntarily, however, further investigations are needed to provide relevant practical recommendations on how to manage this resource adequately in order to balance intake by the animal and regrowth capacity of the plant. The impact of management strategies relying on cutting and preservation should also be assessed. Moreover, benefits of shrubs and trees on pastures beyond the animal feeding and nutrition are still poorly characterized while, in an agroecological perspective, they can contribute to a significant improvement of the sustainability of ruminant production systems.

Keywords

Ruminant, woody forage, production system, selectivity, nutritive value, chemical composition, rumen digestion, methane, plant secondary compound, tannin.

Vandermeulen Sophie (2016). Les arbres et arbustes influencent le comportement de bovins au pâturage et les fermentations ruminales.

145 p., 18 tables, 3 figures.

Résumé

Les haies et bandes boisées ont toujours été utilisées pour délimiter les prairies mais ont peu à peu disparu du paysage agricole en raison des pertes économiques qu'elles engendrent, de l'abandon des modes de gestion traditionnels et de l'intensification agricole. A présent, l'implantation d'arbres et arbustes est à nouveau encouragée suite à la mise en place de mesures respectueuses de l'environnement, et l'intérêt de leur utilisation comme fourrage pour les ruminants est croissant aussi bien dans les écosystèmes tempérés que tropicaux. Au sein des systèmes de productions, les plantes ligneuses peuvent rendre de nombreux services écosystémiques et procurer des bénéfices pour les agriculteurs et leurs animaux tels que l'apport de fourrages, la protection contre le mauvais temps et une réduction de l'infestation parasitaire. De plus, lorsqu'ils sont ingérés, ces fourrages peuvent entraîner des changements de la physiologie digestive qui à leur tour, vont influencer le bien-être et les performances des animaux ainsi que le système de production dans son ensemble. Dès lors, afin de contribuer au développement de systèmes durables utilisant les arbres et arbustes comme composante fourragère, le but de ce travail a été d'évaluer l'influence de plantes ligneuses sur le comportement de bovins au pâturage, de déterminer leur sélectivité vis-a-vis de ces espèces ligneuses, ainsi que de mesurer les changements induits par des espèces tempérées et tropicales d'arbres et arbustes sur la fermentation ruminale.

Premièrement, le comportement de génisses laitières au pâturage a été observé pendant toute la saison de pâturage ainsi que leur sélectivité vis-à-vis des espèces ligneuses présentes dans une haie. Il a été conclu que l'accès à une haie a influencé le comportement des bovins au pâturage, puisque les animaux ont brouté des plantes ligneuses à chaque saison. Cela a été principalement observé lorsque la biomasse disponible de la prairie était faible. Les espèces les plus sélectionnées étaient *Carpinus betulus, Corylus avellana, Cornus sanguinea* et *Crataegus monogyna*. Deuxièmement, la composition chimique, le profil de fermentation ruminale *in vitro* et la capacité à précipiter les protéines (CPP) des espèces d'abres et arbustes tempérés ont été mesurés. Les analyses ont montré que *Fraxinus excelsior* présentait le profil

le plus intéressant en termes de composition chimique et de fermentation. Parmi les espèces les plus sélectionnées, *C. monogyna* et *C. avellana* ont produit moins de CH_4 et cette dernière espèce a montré la CPP la plus élevée. Troisièmement, trois cultivars du genre tropical *Desmanthus* ont été étudiés pour leurs effets sur les fermentations ruminales *in vitro* ainsi que leur potentiel à réduire la production de CH_4. *Desmanthus leptophyllus* et *D. bicornutus* ont présenté le meilleur potentiel anti-méthanogène, et *D. bicornutus* a présenté une plus grande digestibilité.

En conclusion, les fourrages d'arbres et d'arbustes aussi bien tempérés que tropicaux sont prometteurs pour complémenter les bovins avec des plantes de bonne qualité. Les animaux peuvent brouter les espèces ligneuses volontairement, mais des investigations supplémentaires sont nécessaires pour fournir des recommandations pratiques pertinentes sur la gestion adéquate de cette ressource de manière à déterminer le meilleur équilibre entre l'ingestion par l'animal et la capacité de la plante à repousser. L'impact des stratégies de gestion basées sur la coupe et la conservation des fourrages ligneux devrait également être évalué. De plus, à côté de la nutrition animale, les bénéfices qu'apportent les arbres et arbustes en prairie sont encore peu caractérisés, alors que, d'un point de vue agro-écologique, ils pourraient contribuer à une amélioration significative de la durabilité des systèmes de production de ruminants.

Mots-clés: Ruminant, fourrage ligneux, système de productions, sélectivité, valeur nutritive, composition chimique, digestion ruminale, méthane, composés secondaires végétaux, tanin.

Acknowledgments

First of all, I would like to deeply thank both of my supervisors, Jérôme Bindelle and Carlos Ramírez-Restrepo, for giving me the opportunity to complete this thesis. I really appreciate your support and all the time sharing your experience in animal nutrition, helping me during the experiments and correcting the articles. Jérôme, since I was a young student, you encouraged me and I thank you for all the things you have taught me. Carlos, I thank you for welcoming me in Australia and making this year an incredible and unforgettable experience.

I also thank Pr. André Théwis and Pr. Yves Beckers, respectively former and current Head of the Animal Science Unit. Your advices and reviewings of manuscripts always allowed me to think about my experiments and work in a way I haven't seen before.

Thank you to the valuable experience of the Committee and Jury of my thesis: Dr. Virginie Decruyenaere, Pr. Isabelle Dusfrane, Pr. Marie-Laure Fauconnier and Pr. Georges Lognay, Pr. Hugues Claessens and Pr. Georges Daube.

I acknowledge Mr. Christian Marche and all the technical staff of the Centre de Technologies Agronomiques (Strée, Belgium) for their support and help during the experiment with the dairy herd.

I also thank the CRA-W (Gembloux – Libramont, Belgium) and the team of Virginie Decruyenaere for the NIRS analyses.

I am grateful to the the National Fund for Scientific Research (FNRS, Brussels, Belgium) and University of Liège for offering a travel grant to the Commonwealth Scientific and Industrial Research Organization (CSIRO, Townsville, Australia). I thank all the scientists I have been working with in CSIRO: Dr. Sultan Singh (Indian Grassland and Fodder Research Institute, Jhansi, India), Dr.Rob Kinley, Dr. Ed Charmley, Dr. Ian Watson, Ms Mel Matthews. I also thank Mr. Christopher Gardiner and Pr. Joe Holtum from James Cook University (Townsville, Australia), Mr. Iain Hannah and Mr. Nick Kempe from Agrimix Pty Ltd (Eagle farm Australia).

I thank Julie Leblois for choosing to do her Master' thesis with me, and her very good work in the lab (and the nights in the farm!). I am sincerely grateful to all co-authors of the manuscripts writing during my thesis, for your precious experience in the lab and for reviewing scientific papers.

I thank all my collegues of the Animal Science Unit of GxABT, and notably Emilie Bera, Sylvie Mabille, Christelle Boudry, Cécile Russo, Bernadette Smet and François Debande for your help in the lab and with the cows. A special thanks to the ones in the library office for the good time and all the fun: Yannick, Julie, Emilie, Lauriane, Patrick, Pascale, Coraline, Bienvenu, Gaëtan, and all the others I could have forgotten.

Thanks to Carmen, Maria and Carlos for your warm welcoming in Townsville, and all the nice dinners we had together. Of course I thank all our mates for this amazing year: Julie, Marty, Céline, Arielle, Malo, Alexia, Dinouk, Sharon, Mel, Gonzalo, Rob, Ginny, Luna, Kenny and their boys Alex and Marty… I also thank all our friends in Belgium: Popo (best roommate ever!), Romain, Mathilde, Hada, Ludo, Manon, Barbara, and all the others…

I thank all the persons who contributed directly or indirectly to this thesis.

I deeply thank my family, and particularly my parents who gave me the opportunity to study in Gembloux which gave me this love of Science, and my sister. Last but not least, I thank Alex, for all your encouragements and understanding, and for giving me two beautiful baby kangaroos, Isaac and Victor…

I would like to finish with these few words…

I have a dream.

I have a dream that the forest will return;

that the farmers will plant trees;

that the land will be healed.

(Mead, 1995)

Table of content

Abstract i

Résumé iii

Acknowledgments v

Table of content vii

List of tables xii

List of figures xiv

Abbreviations xv

CHAPTER I 1

General introduction 2

1. Context 2

2. Objectives 4

3. Research strategy 5

4. References 6

CHAPTER II 12

Article 1. *Agroforestry for ruminants: a review of trees and shrubs as fodder in silvopastoral temperate and tropical production systems* 14

1. Abstract 15

2. Introduction 16

3. Silvopastoralism: Origin, practices and distribution throughout the world today 17

3.1. Agroforestry and silvopastoralism: concepts and definitions 17

3.2. Economic implications, benefits and limitations of woody plants on the whole farming system 19

3.3. Management of trees and shrubs as fodder in ruminant production systems 21

3.3.1. Direct browsing on plants 21

3.3.2. Pruned shrubs and trees (fresh fodder) 29

3.3.3. Preservation of shrub and tree forage 31

3.4. Integrating shrubs and trees into temperate ruminants production systems: contributions, obstacles and prospects 32

4. Conclusion 33

5. References 34

CHAPTER III 45

Article 2. *Compatibility of using TiO_2 and the faecal near-infrared reflectance spectrometry for estimation of cattle intake* 47

1. Abstract 48

2. Introduction 49

3. Materials and methods 49

4. Results and discussion 49

5. Conclusion 51

6. References 51

CHAPTER IV 53

Article 3. *Behaviour and browse species selectivity of heifers grazing in a temperate silvopastoral system* 55

1. Abstract 56

2. Introduction 57

3. Materials and methods 58

3.1. Location and experimental treatments 58

3.2. Plant measurements 59

3.3. Animal measurements 59

3.4. Laboratory analyses 61

3.5. Statistical analysis 61

4. Results 62

4.1. Pasture botanical composition and biomass 62

4.2. Chemical composition and *in vitro* organic matter digestibility of pasture and hedge fodder 62

4.3. Faecal chemical composition, *in vivo* organic matter digestibility and dry matter intake of the diet 64

4.4. Live weight 64

4.5. Heifers behaviour 66

4.6. Browsing selectivity 66

5. Discussion 70

6. Conclusion 73

7. References 73

CHAPTER V 79

Article 4. *Hedges and woody strips browsing by cattle on pasture in Wallonia* 81

1. Abstract 82

2. Introduction 83

3. Materials and methods 83

3.1. Grazing and browsing behaviour 83

3.2. Chemical composition and *in vitro* ruminal fermentation 83

4. Results and discussion 84

4.1. Grazing and browsing behaviour 84

4.2. Chemical composition and *in vitro* fermentation 84

5. Conclusion 87

6. References 87

Communication 5. *In vitro evaluation of protein precipitation capacity of temperate browse species* 88

1. Abstract 89

2. Introduction 90

3. Materials and methods 91
3.1. Plant samples preparation 91
3.2. Laboratory analyses 91
3.3. Statistical analysis 92
4. Results and discussion 92
5. Conclusion 93
6. References 93
CHAPTER VI 96
Article 6. *In vitro assessment of ruminal fermentation, digestibility and methane production of three species of Desmanthus for application in northern Australian grazing systems* 98
1. Abstract 100
2. Introduction 101
3. Materials and methods 102
3.1. Study site 102
3.2. Plant material harvesting and preparation 102
3.3. Chemical composition 103
3.3.1. Proximate analysis 103
3.3.2. Carbohydrate and protein fractinations 103
3.3.3. Phenols and tannins analyses 104
3.4. *In vitro* ruminal fermentation 104
3.4.1. Rumen inoculum preparation 104
3.4.2. *In vitro* rumen inoculation and incubation 104
3.4.3. Total gas and methane production 105
3.4.4. *In vitro* apparent digestibility of substrate organic matter and volatile fatty acids analysis 105
3.5. Statistical analysis 105

4. Results 106
4.1. Chemical composition 106
4.2. *In vitro* fermentation 109
5. Discussion 114
6. Conclusion 117
7. References 117
CHAPTER VII 125
General discussion and perspectives 126
1. Use of shrubs and trees in silvopastoral systems 126
2. Heifers behaviour and selectivity towards woody plants 129
3. Nutritive value and *in vitro* fermentation profile including methane production of woody species 130
4. Other services of shrubs and trees within silvopastoral systems 133
5. References 135
Author's publications related to this thesis 144
1. Articles 144
2. Conference 144
3. Posters 145

List of tables

Table 1. Main feeding methods, animals and woody species in silvopastoral systems. 22

Table 2. Examples of management and production characteristics of silvopastoral woody species and limitations of their use to feed ruminants. 26

Table 3. Chemical composition and nutritive value of browse species. 30

Table 4. Description of the recorded behavioural activities. 60

Table 5. Chemical composition (g/kg DM) and *in vitro* organic matter digestibility (IVOMD) of browse species in the experimental hedge and browsing group (BG) pasture samples at each season (n=3). 63

Table 6. Chemical composition (g/kg DM) of the faeces, dry matter intake (g/kg $LW^{0.75}$.d) and *in vivo* organic matter digestibility estimated by NIRS for both groups of heifers (n=6). 65

Table 7. Percentage of time spent displaying a behaviour in animals grazing a pasture with (browsing group; BG) and without (control pasture group; CPG) access to a hedge composed of shrubs and trees during the grazing season of 2013 (n=18). 67

Table 8. Proportions of woody species in the browsed diet and in the hedge and selectivity index during the 3 seasons of feeding behaviour observations in 2013 (n=18). 69

Table 9. Chemical composition (g/kg DM) and gross energy (MJ/kg DM) of herbaceous and woody species leaves and stems collected in May 2012. 85

Table 10. *In vitro* rumen fermentation parameters and molar ratio of acetic, propionic and butyric acids of herbaceous and browse species over 72 h and CH_4 production after 24 h of incubation (n=3). 86

Table 11. Shrubs and trees species collected in July 2013 from a hedge at CTA. 91

Table 12. Condensed tannins contents (g/kg depigmented sample) of herbaceous and woody species at 3 harvesting periods and protein precipitation capacity (%) of forage samples of July. 93

Table 13. Chemical composition (g/kg DM) of the *Desmanthus* species (n=3) and Rhodes grass (*Chloris gayana*) hay samples (n=3). 107

Table 14. Protein fractions of *Desmanthus* species and Rhodes grass (*Chloris gayana*) hay (n=3). 108

Table 15. Carbohydrate and its fraction in the legume cultivars and control grass (n=3). 109

Table 16. Phenols and tannins constituents (g/kg DM) of the leguminous and grass species samples (n=3). 110

Table 17. Effects of *Desmanthus* spp. on *in vitro* organic matter digestibility (IVOMD), total gas and methane (CH_4) production at 24, 48 and/or 72 hours of incubation. 112

Table 18. Comparative volatile fatty acids concentration (mmol/l) between *Desmanthus* spp. over 72 h of *in vitro* incubation with rumen fluid from grazing Brahman Zebu rumen-cannulated steers. 113

List of figures

Figure 1. Evolution of titanium dioxide (TiO_2) contents (mg/g) in the faeces of the three cows. 50

Figure 2. Means and standard deviation of crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents (g/kg DM) of the faeces and the dry matter intake (DMI; g/kg $LW^{0.75}$.d) and *in vivo* organic matter digestibility (OMD; g/kg) predicted by FNIRS along the experiment. 51

Figure 3. Graphical abstract aiming at describing several main components of silvopastoral systems and what was achieved during the thesis. 128

Abbreviations

A, maximum gas volume

ADF, acid detergent fiber

ADL, acid detergent lignin

AECM, agri-environmental and climatic measures

B, mid-fermentation time

BG, browsing group

BSA, bovine serum albumine

CF, crude fiber

CG, gas chromatography

CP, crude protein

CPG, control pasture group

CT, condensed tannins

CTA, Centre des Technlogies Agronomiques

DM, dry matter

DMI, dry matter intake

EE, ether extracts

FNIRS, faecal near-infrared reflectance spectrometry

GE, gross energy

H, Mahalanobis distance

HPLC, high performance liquid chromatography

HT, Hydrolysable tannins

IVOMD, *in vitro* organic matter digestibility

LW, live weight

NIRS, near-infrared reflectance spectrometry

NA, not applicable

ND, not detected

NTP, non-tannin phenols

OM, organic matter

OMD, organic matter digestibility

P, p-value

PPC, protein precipitation capacity

r, correlation coefficient

R_{max}, maximum rate of fermentation

SEM, standard error of the mean

STF, shrub and tree forage

TA, total ashes

TCD, thermal conductivity detector

TGP, total gas produced

T_{max}, the time when the maximum rate of fermentation is reached

TP, total phenols

TT, total tannins

UAA, utilized agricultural area

VFA, volatile fatty acids

CHAPTER I

General introduction

1. Context

Silvopastoralism is an agroforestry land-use system that combines woody perennials and animals on the same area (Nair 1991, 1993; Etienne 1996). The use of shrubs and trees as forage for goats, sheep and cattle is prevalent in both tropical and temperate extensive ecosystems (Moore *et al.* 2003; Mosquera-Losada *et al.* 2005; Dalzell *et al.* 2006). In tropical regions, relying on introduced browse species is a common practice to improve the use of natural grasslands (Cox and Gardiner 2013). This is well-documented in northern Australia where sown shrubby legumes such as *Leucaena leucocephala* (Lam.) de Wit, *Calliandra calothyrsus* Meisn., *Stylosanthes* Sw. spp., *Gliricidia sepium* (Jacq.) Kunth ex Walp. or *Desmanthus* Willd. spp. are widely distributed in beef cattle grazing systems to supply forage during the seasonal dry periods (Lefroy *et al.* 1992; Cook *et al.* 2005; Dalzell *et al.* 2006). In contrast, in Europe, the intensification of temperate livestock production systems has resulted in the reduction of the number of woody perennials in agricultural landscapes (Nerlich *et al.* 2013). However, recent European policies are promoting the implementation of indigenous shrubs and trees in pastures as hedges and woody strips (Walloon Government 2015, 2016). In these guidelines [i.e. agri-environmental and climatic measures (AECM)], some of the promoted species are for example *Acer* L. spp., *Crateagus monogyna* Jacq., *Corylus avellana* L., *Fraxinus* L. spp., *Poplar* L. spp. or *Quercus* L. spp..

Specifically in the southern part of Belgium (Wallonia), SPW (2010) reported 13 m of hedgerow per ha of utilized agricultural area (UAA) and 1 tree planted per 6.4 ha in 2007. In 2010, it reached 16 m of hedge per ha of UAA and 1 tree for 5.8 ha (SPW 2012). Hedges and woody strips are the most popular AECM and represented 33% of total AECM implemented by farmers in 2012, with 12 370 km of hedgerows in Wallonia (SPW 2014). Since the application of these AECM, farmers' participation has increased steadily, but from 2013, budget restrictions were limiting most AECM. However, lastest updates of policies related to aids granted for the plantation of live fences, linear coppices, orchards and tree alignments and for the maintainance of pollards, are promoting shrubs and trees on farmland via e.g. 20 % increase if the project supports directly an ecosystemic service (Walloon Government 2016).

Nowadays, woody resources are mainly integrated into extensive systems such as in the Mediterranean region, high mountains or northern silvopastures (Rigueiro-Rodríguez *et*

al. 2009). Shrubs and trees in silvopastoral systems have multifunctional potentials that can benefit farmers. They may play a role (i) in the reduction of parasitic infestation (Mupeyo *et al.* 2011); (ii) in welfare improvement by protecting livestock from wind and rain in winter and providing shade in summer (Gregory 1995; Liagre 2006; Van laer *et al.* 2015); and (iii) supplying fodder to animals (Moore *et al.* 2003; Kanani *et al.* 2006; Rangel and Gardiner 2009). Silvopastoral systems can then lead to the diversity of income through timber (i.e. wood and energy) and non-timber production (i.e. animal, fruit production). Shrubs and trees have been indeed demonstrated to contribute to ecosystemic services and environmental benefits (Jose 2009; Torralba *et al.* 2016). Hedges as live fences are instrumental in biodiversity conservation by providing habitat and refuge to indigenous birds, mammals, insects, amphibians, reptiles and plants (Liagre 2006; Pulido-Santacruz and Renjifo 2011). Shrubs and trees may contribute to nutrient cycling and above- and below-ground carbon storage, since trees fixes and stores carbon through photosynthesis and roots and litter decomposition provide biomass to the soil (Kaur *et al.* 2002; Montagnini and Nair 2004). Woody plants may also control wind and water erosion. Further, they have the potential to reduce greenhouse gas emissions including enteric methane (CH_4) (Ramírez-Restrepo *et al.* 2010a; Tomkins *et al.* 2014).

In parallel, shrub and tree foliage (STF) contain a lot of bio-active molecules such as condensed and hydrolysable tannins, saponins or alkaloids (Ramírez-Restrepo and Charmley 2015). Among them, condensed tannins (CT) exhibit anthelmintic potential (Ramírez-Restrepo *et al.* 2010b; Mupeyo *et al.* 2011), while those secondary compounds may influence rumen metabolism (Frutos *et al.* 2004; Tedeschi *et al*. 2014), notably protein digestion (Jones and Mangan 1977; Min *et al.* 2005). In the rumen, CT form stable complexes with dietary proteins (pH 5.5-7), whereas in the abomasum (pH 2-3) complexes dissociate, making proteins available for digestion and absorption in the small intestine (Jones and Mangan 1977; Waghorn *et al.* 1994). As less ammonia would be produced from dietary protein degradation in the rumen, it may result in a shift of nitrogen excretion from urine to faeces (Waghorn *et al.* 1987; Grainger *et al.* 2009) in which N is mainly organic and therefore less volatile (Carulla *et al.* 2005; Grainger *et al.* 2009). Relying on CT-containing woody plants that could balance the rumen function by decreasing ruminal N degradation and then reduce urinary N losses and N_2O emissions would be economically and environmentally interesting (de Klein and Eckard 2008). However, the influence of CT on rumen metabolism has been studied mainly with herbaceous CT-containing legumes such as *Lotus corniculatus* L. (Min *et*

al. 2005; Ramírez-Restrepo and Barry 2005) and *Lotus pedunculatus* Cav. (Barry and Duncan 1984; Waghorn *et al.* 1994), with less focus on shrubs and tree species.

Although there is a renewed interest in the use of shrubs and trees as forage for ruminants in Europe, the influence of access to the above mentioned temperate woody species on the behaviour of grazing cattle and their selectivity have not been deeply investigated. In an integrated approach of the scientific research questions, this work considered also tropical species in a different environment than that of temperate regions. Indeed, as there also is a lack of information about the nutritive value and influence on rumen metabolism of both temperate and tropical shrub and tree species, this thesis has been undertaken to address these issues.

2. Objectives

The aim of the thesis is to assess whether shrub and tree foliage could significantly influence ruminant feeding in temperate grass-based systems by (1) identifying to which extent cattle with access to temperate woody forage species in hedges would feed on this resource and which factor would influence their behaviour and their selectivity to plant species; and (2) determining the changes induced by temperate and tropical woody forage on rumen metabolism. In this context, five specific and related research questions have been defined to achieve this goal:

1. How are shrub and tree species integrated in ruminant production systems?
2. Which temperate woody forage species are consumed by grazing and browsing cattle?
3. When ingested, how will woody forage species modulate rumen fermentation?
4. What are the effects of secondary compounds of woody forages on the rumen protein metabolism?
5. How will tropical browse species influence rumen fermentation including methane production?

To answer these questions, a literature review, a preliminary methodologic experiment and four main experiments have been designed, validated and completed. These experiments and subsequent published or submitted articles that make up the body of this thesis are briefly depicted in the following section.

3. Research strategy

A literature review introduces the work undertaken in this thesis by describing the use of temperate and tropical woody species as forage in ruminant production systems. This review outlined how temperate and tropical trees and shrubs are currently integrated as fodder in ruminant diets, and the potential outputs and limitations of using woody forage in the whole ruminant system. Chapter 2 has been submitted for publication to *Animal Production Science* (Article 1) and is presently under (minor) revision. It was also presented at Global Farm Platform Conference 2016: Steps to Sustainable Livestock (Bristol, United Kingdom).

Prior to the main experiments, we conducted preliminary trials to test the compatibility of two methods which are commonly used to determine grazing cattle intake. The first technique is based on an indigestible marker, titanium dioxide (TiO_2), while the second is the faecal near-infrared reflectance spectrometry method (FNIRS). Chapter 3 (Article 2) has been published in *Grassland Science in Europe* (2014), 19, 625-627.

The second experiment of the thesis aimed to measure the behaviour and selectivity of grazing heifers in presence of a hedge composed of 11 temperate browse species, and to relate the behaviour and selectivity to the nutritive value of the silvopastoral forage components. The results of this experiment are reported in Chapter 4 and are published in Article 3 in *Agroforesty Systems* (in press).

Once ingested, woody forage species are available for fermentation by microorganisms in the rumen. The objective of the third experiment was to assess the chemical composition and *in vitro* rumen fermentation profile, using rumen fluid from Red-Pied cows (*Bos taurus*), including the kinetic parameters, methane and volatile fatty acids (VFA) production of 12 temperate shrub and tree species. The results are described in Article 4 which was adapted from the publication in *Revitalising Grasslands to Sustain Our Communities: Proceedings 22nd International Grassland Congress* (2013)*.' Eds. DL Michalk, GD Millar, WB Badgery, KM Broadfoot*, pp. 1011-1012. According to the results obtained in the previous experiments and the condensed tannins (CT) contents of the woody species, their protein precipitation capacity (PPC) was tested in a fourth experiment. The PPC was assessed in the woody samples using 2 proteins: bovine serum albumin (BSA) and casein. The correlation between PPC and CT contents was also established. This experiment was presented as a poster at *The 21th National Symposium on Applied Biological Sciences*

(2016) and was, in this thesis, adapted from it (Communication 5). Chapter 5 gathers the results of both third and fourth experiments (Article 4 and Communication 5, respectively).

In a holistic approach of the above-mentionned research questions, one experiment has been designed in the context of ruminant nutrition in a milieu that is environmentally and ecologically different compared to that in temperate regions. For this purpose, a collaboration has been developed with CSIRO (Townsville, QLD, Australia). In contrast with the renewed interest in temperate silvopastoral systems, using sown shrubby legumes such as *Desmanthus* spp. to improve pastures and supply forage for grazing cattle is indeed commonly practiced in northern Australia. However, finding species adapted to the edaphic and climatic conditions of this region is challenging, but the newly-developed *Desmanthus* cultivars, namely *D. leptophyllus* Kunth cv JCU1, *D. virgatus* (L.) Willd. cv JCU2 and *D. bicornutus* S. Watson cv JCU4, are among the few to persist. Although the agronomic traits of the *Desmanthus* genus have been investigated, little information about their nutritive value is known. Therefore, the goal of the fifth experiment was to evaluate the nutritive value of these three *Desmanthus* cultivars and their effects on *in vitro* rumen fermentation, including potential to reduce methane production using rumen fluid from grazing Brahman Zebu rumen-cannulated steers (*Bos indicus*). The information provided by this study contributed to improve knowledge about the use of diversified biomass and is presented in Chapter 6 (Article 6) which has been submitted for publication in *Crop and Pasture Science* and is currently under minor revision.

The thesis is then finalized by a general discussion leading to conclusions and perspectives.

4. References

Barry TN, Duncan SJ (1984) The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. *British Journal of Nutrition* **51**, 485–491.

Carulla J, Kreuzer M, Machmüller A, Hess H (2005) Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. *Crop and Pasture Science* **56**, 961–970.

Cook B, Pengelly BC, Brown S, Donnelly JB, Eagles D, Franco A, Hanson J, Mullen BF, Partridge I, Peters M, Schultze-Kraft R (2005) Tropical Forages: an interactive selection tool. [CD-ROM], CSIRO, DPI&F(Qld), CIAT and ILRI, Brisbane, Australia [Online]. Available at http://www.tropicalforages.info/ (verified 22 March 2016)

Cox K, Gardiner C (2013) Pasture legumes in Queensland–a new wave? In 'Proceedings of the Northern Beef Research Update Conference'. pp. 15-20. (Queensland North Australia Beef Research Council: Gympie, Qld)

Dalzell SA, Shelton HM, Mullen BF, Larsen PH, McLaughlin KG (2006) 'Leucaena: a guide to establishment and management.' (Meat & Livestock Australia Ltd.: Sydney, NSW)

de Klein CAM, Eckard RJ (2008) Targeted technologies for nitrous oxide abatement from animal agriculture. *Australian Journal of Experimental Agriculture* **48**, 14-20. doi:10.1071/EA07217

Etienne M (1996) Research on temperate and tropical silvopastoral systems: a review. In 'Temperate and Mediterranean Silvopastoral Systems of Western Europe'. (Ed Etienne M.), pp. 5–22 (INRA Editions: Paris).

Frutos P, Hervas G, García FG, Mantecón AR (2004) Review. Tannins and ruminant nutrition. *Spanish Journal of Agricultural Research* **2**, 191–202.

Grainger C, Clarke T, Auldist MJ, Beauchemin KA, McGinn SM, Waghorn GC, Eckard RJ (2009) Potential use of *Acacia mearnsii* condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. *Canadian Journal of Animal Science* **89**, 241–251.

Gregory NG (1995) The role of shelterbelts in protecting livestock: A review. *New Zealand Journal of Agricultural Research* **38**, 423–450. doi:10.1080/00288233.1995.9513146

Jones WT, Mangan JL (1977) Complexes of the condensed tannins of sainfoin (*Onobrychis viciifolia* Scop.) with fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. *Journal of the Science of Food and Agriculture* **28**, 126–136.

Jose S (2009) Agroforestry for ecosystem services and environmental benefits: an overview. *Agroforestry Systems* **76**, 1–10. doi:10.1007/s10457-009-9229-7

Kanani J, Lukefahr SD, Stanko RL (2006) Evaluation of tropical forage legumes (*Medicago sativa, Dolichos lablab, Leucaena leucocephala* and *Desmanthus bicornutus*) for growing goats. *Small Ruminant Research* **65**, 1–7. doi:10.1016/j.smallrumres.2005.04.028

Kaur B, Gupta SR, Singh G (2002) Carbon storage and nitrogen cycling in silvopastoral systems on a sodic in northwestern India. *Agroforestry Systems* **54**, 21–29.

Lefroy EC, Dann PR, Wildin JH, Wesley-Smith RN, McGowan AA (1992) Trees and shrubs as sources of fodder in Australia. *Agroforestry Systems* **20**, 117–139.

Liagre F (2006) 'Les haies rurales.' (France Agricole Editions: Paris)

Min BR, Attwood GT, McNabb WC, Molan AL, Barry TN (2005) The effect of condensed tannins from *Lotus corniculatus* on the proteolytic activities and growth of rumen bacteria. *Animal Feed Science and Technology* **121,** 45–58. doi:10.1016/j.anifeedsci.2005.02.007

Montagnini F, Nair PKR (2004) Carbon sequestration: an underexploited environmental benefit of agroforestry systems. *Agroforestry Systems* **61**, 281–295.

Moore KM, Barry TN, Cameron PN, Lopez-Villalobos N, Cameron DJ (2003) Willow (*Salix* sp.) as a supplement for grazing cattle under drought conditions. *Animal Feed Science and Technology* **104**, 1–11. doi:10.1016/S0377-8401(02)00326-7

Mosquera-Losada MR, McAdam JH, Rigueiro-Rodriguez A (2005) Silvopastoralism and Sustainable Land Management. (Eds Mosquera MR, McAdam J, Rigueiro A.) pp. 429 (CAB International: Wallingford).

Mupeyo B, Barry TN, Pomroy WE, Ramírez-Restrepo CA, López-Villalobos N, Pernthaner A (2011) Effects of feeding willow (*Salix* spp.) upon death of established parasites and parasite fecundity. *Animal Feed Science and Technology* **164**, 8–20. doi:10.1016/j.anifeedsci.2010.11.015

Nair PKR (1993) An Introduction to Agroforestry. pp. 499 (Kluwer Academic Publishers: Dordrecht)

Nair PKR (1991) State-of-the-art of agroforestry systems. *Forest Ecology and Management* **45**, 5–29.

Nerlich K, Graeff-Hönninger S, Claupein W (2013) Agroforestry in Europe: a review of the disappearance of traditional systems and development of modern agroforestry practices, with emphasis on experiences in Germany. *Agroforestry Systems* **87**, 475–492. doi:10.1007/s10457-012-9560-2

Pulido-Santacruz P, Renjifo LM (2011) Live fences as tools for biodiversity conservation: a study case with birds and plants. *Agroforestry Systems* **81**, 15–30. doi:10.1007/s10457-010-9331-x

Ramírez-Restrepo CA, Barry TN (2005) Alternative temperate forages containing secondary compounds for improving sustainable productivity in grazing ruminants. *Animal Feed Science and Technology* **120**, 179–201. doi:10.1016/j.anifeedsci.2005.01.015

Ramírez-Restrepo CA, Barry TN, Marriner A, López-Villalobos N, McWilliam EL, Lassey KR, Clark H (2010a) Effects of grazing willow fodder blocks upon methane production and blood composition in young sheep. *Animal Feed Science and Technology* **155**, 33–43. doi:10.1016/j.anifeedsci.2009.10.003

Ramírez-Restrepo CA, Pernthaner A, Barry TN, López-Villalobos N, Shaw RJ, Pomroy WE, Hein WR (2010b) Characterization of immune responses against gastrointestinal nematodes in weaned lambs grazing willow fodder blocks. *Animal Feed Science and Technology* **155**, 99–110. doi:10.1016/j.anifeedsci.2009.10.006

Ramírez-Restrepo CA, Charmley E (2015) An integrated mitigation potential framework to assist sustainable extensive beef production in the tropics. In 'Grassland: A Global Resource Perspective'. (Eds PK Ghosh, SK Mahanta, JB Singh, PS Pathak) pp. 417-436 (Range Management Society of India: Jhansi)

Rangel JH de A, Gardiner CP (2009) Stimulation of wool growth by *Desmanthus* spp. as a supplement in a Mitchell grass hay diet. *Tropical Grasslands* **43**, 106–111.

Rigueiro-Rodríguez A, Mosquera-Losada MR, McAdam JH (2009) Agroforestry in Europe: Current Status and Future Prospects. (Eds Rigueiro-Rodríguez, Antonio, McAdam, Jim, Mosquera-Losada, María Rosa) pp. 450 (Springer: Dordrecht).

SPW (2010) Tableau de bord de l'environnement wallon 2010. Rapport sur l'état de l'environnement wallon. Service public de Wallonie, Cellule Etat de l'environnement wallon, SPW – DGARNE – DEMNA - DEE (SPW: Jambes). Available at http://etat.environnement.wallonie.be/index.php?page=le-Table-de-bord-2010 (verified 22 December 2016)

SPW (2012) Key Environmental Indicators for Wallonia in 2012. Report on the State of the Environment in Wallonia. State of the Environment Directorate, SPW (Service public de Wallonie) - DGO3 - DEMNA - DEE (SPW Editions: Jambes). Available at http://etat.environnement.wallonie.be/index.php?page=archives (verified 13 February 2017)

SPW (2014) Les Indicateurs Clés de l'Environnement Wallon 2014. Service public de Wallonie, Direction de l'Etat Environnemental, SPW - DGO3 - DEMNA - DEE. (SPW Editions: Jambes). Available at http://etat.environnement.wallonie.be/index.php?page=icew-2014 (Verified 23 December 2016)

Tedeschi LO, Ramírez-Restrepo CA, Muir JP (2014) Developing a conceptual model of possible benefits of condensed tannins for ruminant production. *Animal* **8**, 1095–1105.

Tomkins NW, Denman S, McGavin S, Padmanabha J, McSweeney C (2014) Leucaena (*L. leucocephala*) Based Pastures Have the Potential to Reduce Methane Emissions for Beef Cattle. In 'Animal Production in Australia, Volume 30, Proceedings of the 30th Biennial Conference of the Australian Society of Animal Production: Harnessing the Ecology and Physiology of Herbivores'. (Eds Hatcher S, Krebs GL, Holman BWB) p. 284 (Australian Society of Animal Production: Canberra).

Torralba M, Fagerholm N, Burgess PJ, Moreno G, Plieninger T (2016) Do European agroforestry systems enhance biodiversity and ecosystem services? A meta-analysis. *Agriculture, Ecosystems and Environment* **230**, 150–161. doi:10.1016/j.agee.2016.06.002

Van laer E, Tuyttens FAM, Ampe B, Sonck B, Moons CPH, Vandaele L (2015) Effect of summer conditions and shade on the production and metabolism of Holstein dairy cows on pasture in temperate climate. *Animal* **9**, 1547–1558. doi:10.1017/S1751731115000816

Waghorn GC, Ulyatt MJ, John A, Fisher MT (1987) The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L. *British Journal of Nutrition* **57**, 115–126.

Waghorn GC, Shelton ID, McNabb WC (1994) Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 2. Nitrogenous aspects. *Journal of Agricultural Science (Cambridge)* **123**, 109–119.

Walloon Government (2015) Decree of 3 September 2015 on the granting of agri-environmental and climatic aids. Belgian Official Journal, 2 October 2015.

Walloon Government (2016) Decree of 8 September 2016 on the granting of aids for planting live fence, linear coppice, orchard and tree alignment, and for maintaining pollards, Belgian Official Journal, 29 September 2016.

CHAPTER II

Chapter 2 introduces the work undertaken during the PhD project and is therefore a review of the literature about the use of shrubs and trees in both temperate and tropical production systems with ruminants. This chapter describes how trees and shrubs are presently managed as forage in ruminant systems. It also discusses the potential benefits and limitations of using woody plants in the whole farming system. The contribution of shrubs and trees to temperate ruminant production systems is discussed, as well as the obstacles and requirements to their integration in these systems.

Article 1

Agroforestry for ruminants: a review of trees and shrubs as fodder in silvopastoral temperate and tropical production systems

Sophie Vandermeulen[1,2*], Carlos Alberto Ramírez-Restrepo[3], Yves Beckers[1], Hugues Claessens[4], Jérôme Bindelle[1,5]

[1]*University of Liège, Gembloux Agro-Bio Tech, Precision Livestock and Nutrition Unit, 2 Passage des Déportés, 5030 Gembloux, Belgium.* [*]*Email: vandermeulen.sophie@gmail.com*

[2]*Research Foundation for Industry and Agriculture, National Scientific Research Foundation (FRIA-FNRS), 5 Rue d'Egmont, 1000 Bruxelles, Belgium*

[3]*CSIRO, Agriculture, Australian Tropical Sciences and Innovation Precinct, James Cook Drive, Townsville, QLD 4811, Australia*

[4]*University of Liège, Gembloux Agro-Bio Tech, Forest and Nature Management, 2 Passage des Déportés, 5030 Gembloux, Belgium.*

[5]*AgricultureisLife Platform, 2 Passage des Déportés, 5030 Gembloux, Belgium.*

Short title: Woody forage in ruminant production systems

This article has been submitted to *Animal Production Science* and is presently under "minor revision". This present chapter is the revised form which was re-submitted on the 9th of February 2017.

1. Abstract

Among the oldest agroforestry systems, silvopastoralism uses shrubs and trees to feed ruminants. The practice is common in extensive livestock production systems, while the intensification of grass-based systems in the past century has led to the removal of woody species from agricultural temperate landscapes. In Europe however, woody species are promoted again on grasslands through environment-friendly policies due to the ecosystem services they provide such as carbon sequestration, control of soil erosion, limitation of air-borne pollutants and biodiversity conservation. Positive effects of browse on rumen digestion and parasite control have also been documented across different plant species and regions. Under optimal conditions, feeding ruminants from woody fodder sustains animal production. Nonetheless, limitations can restrict the use of woody forage into animal diets, such as the presence of anti-nutritive and toxic compounds. The incorporation of this resource in ruminant feeding systems raises the question of the management of the interface between the plant and the animal. Various management systems are practiced. Temperate species such as *Salix* spp. and *Populus* spp. are fed to sheep and cattle in fodder blocks or by pruning trees in New Zealand, and *Fraxinus* spp. or *Corylus avellana* in hedgerows supply forage to livestock in Belgium, while *Leucaena leucocepahala* and *Desmanthus* spp. browsing is common in Australia. Nowadays, ensiling and pelleting techniques are being developed as a way to store browse forage. As the renewed interest in using shrubs and trees to feed ruminants is recent, especially in temperate regions, additional research about introducing optimally this resource within systems is needed.

Keywords

Silvopastoralism, livestock husbandry, browse species, feeding, nutritive value.

2. Introduction

Silvopastoralism, a multifunctional land-use system that associates animals with shrubs or trees and pasture, is one of the most ancient agroforestry systems (Etienne 1996). The integration of shrubs and trees as fodder resources into grazing systems is practiced in the tropics (Abdulrazak *et al.* 1996; Hove *et al.* 2001; Dalzell *et al.* 2006), the Mediterranean area (Papachristou and Papanastasis 1994; Mosquera-Losada *et al.* 2012) and in highlands around the globe (Vandenberghe *et al.* 2007; Buttler *et al.* 2009). However, in temperate Europe, shrubs and trees have been progressively removed from agricultural landscapes due to the intensification of grass-based production systems (Nerlich *et al.* 2013). Unfortunately, although the interest for woody species as feed for livestock is rising again (Bestman *et al.* 2014; Smith *et al.* 2014; Vandermeulen *et al.* 2016), little is known about the use of fodder sources in more intensive ruminant production systems.

In silvopastoral systems, shrubs and trees can supply energy, protein and other nutrients to livestock (Papachristou and Papanastasis 1994; Kemp *et al.* 2001), while they may further become the only forage resource available during critical periods of grass shortages (Papachristou and Papanastasis 1994; Dalzell *et al.* 2006). They can also provide shelter against extreme environmental conditions (Hawkes and Wedderburn 1994; Liagre 2006; Van laer *et al.* 2015) and may improve reproductive performance (Pitta *et al.* 2005; Musonda *et al.* 2009), body growth (Abdulrazak *et al.* 1996; Gardiner and Parker 2012) and milk production (Maasdorp *et al.* 1999). Furthermore, depending on the browse species and their secondary compounds content (e.g. condensed tannins; CT), they may reduce internal parasite infestation (Mupeyo *et al.* 2011) and also methanogenesis (Ramírez-Restrepo *et al.* 2010). Nevertheless, besides all these beneficial effects, the incorporation of woody fodder in animal diets might be restricted due to low palatability (Kanani *et al.* 2006) or toxicity (Jones *et al.* 1976; Dalzell *et al.* 2006).

In modern silvopastoral systems, the interest in using trees as a supplementary fodder source for feeding animals is increasing although the woody plants may have originally been established for other purposes. For instance, willow (*Salix* spp.), planted for soil conservation in New Zealand (Pitta *et al.* 2005) and to produce wood chips for energy in United Kingdom (Smith *et al.* 2014), is used to feed livestock at the same time. Shrubs and trees may then be established within grazing systems for environmental purposes as silvopastoral systems are considered to supply ecosystem services, at a range of spatial and temporal scales (Jose 2009; Sharrow *et al.* 2009). Planting shrubs and trees on farmlands has been shown to improve air

and water quality. For example, hedgerows can mitigate undesirable livestock odors around the farm while wind and water erosion can be reduced by trees, leading to soil stabilization (Liagre 2006; Jose 2009). Shrubs and trees can play a significant role in carbon sequestration, above and below ground (Kaur *et al.* 2002) and improve the soil quality, e.g. through nutrient cycling. Shrub and tree legumes are particularly interesting for the fixation of atmospheric nitrogen used by the plant to produce protein while it can be cycled to the companion pasture plants (Dalzell *et al.* 2006; Cox and Gardiner 2013). Furthermore, feeding livestock with CT-containing woody fodder can enhance nitrogen recycling in the pasture by a shift from excretion in urine to faeces (Waghorn *et al.* 1987; Grainger *et al.* 2009), in which N is less volatile leading to lower risk of N_2O emissions and N losses (de Klein and Eckard 2008). Biodiversity conservation can also be promoted by shrubs and trees through a number of functions such as providing habitats for flora and fauna species (Liagre 2006; Pulido-Santacruz and Renjifo 2011) while the landscape aesthetics may be enhanced as well (Jose 2009).

Nowadays, the interest in such modern silvopastoral systems in which browse are used as an extra feed resource for ruminants from trees and shrubs primarily established for other purposes is growing (Pitta *et al.* 2005; Smith *et al.* 2014). However, more needs to be known about the different ways of sustainably introducing and managing woody forage in more intensive and sustainable temperate and tropical production systems. Therefore, this review aims at describing how trees and shrubs are currently integrated as fodder in ruminant diets. The potential outputs and limitations of using woody forage in the whole ruminant system are also discussed, as well as the contribution of shrub and tree species to temperate ruminant production systems, obstacles and requirements to their integration in these systems.

3. Silvopastoralism: Origin, practices and distribution throughout the world today

3.1. Agroforestry and silvopastoralism: concepts and definitions

Agroforestry is defined as a land-use system that combines on the same area woody perennials with crops and/or animal production (Nair 1991; Allen *et al.* 2011). Consequently, based on this combination, agroforestry is made up of silvoarable (i.e crops and trees), silvopastoral (i.e. animals and trees) and agrosilvopastoral (i.e. crops, animal and trees) systems.

Silvopastoral systems are encountered worldwide (Hove *et al.* 2001; Dalzell *et al.* 2006; Sharrow *et al.* 2009; Bestman *et al.* 2014) and reported as the most prevalent agroforestry system in developed countries (Sharrow 1999). They are diverse and complex: forest grazing or silvopastures (Sharrow *et al.* 2009), woodlands or wood-pastures (Rackham 2013), locally named as the *Dehesa* in Spain, *Montado* in Portugal (Dupraz and Liagre 2008) or *Streuobst* in some temperate European countries (Herzog 1998). In some systems, animals are set to graze the pasture growing under or beside the woody resource but are not fed with it, while in others, it is considered as feed, assuming that two types of fodder may be produced from shrubs and trees, the foliage (i.e. leaves and twigs) and the fruits (Liagre 2006). Usually foliage is the main fodder resource from which animals are fed, but sometimes, fruits can also be used in more specific cases. For example, chestnuts (*Castanea sativa*), honey locust pods (*Gleditsia triacanthos*) and acorns (*Quercus* spp.) may be fed to ruminants and sometimes monogastric animals (Liagre 2006). However, only shrub and tree foliage as a fodder resource will be discussed in this review.

Although a wide variety of systems exists according to objectives and management procedures, silvopastoral systems are commonly achieved in two ways, either by planting trees on an established pasture or by introducing livestock and/or forage production in a forestland (Peeters *et al.* 2014). In accordance with Etienne (1996) and Sharrow *et al.* (2009) at farm-scale level, three main silvopastoral structures can be considered in terms of plant composition:

- trees on pasture: woody perennials are planted widely-spaced on an already established sward in order to benefit from product diversification and/or from woody-herbaceous plants associations;
- grazed forest: an existing woodland or forest is thinned and sown to take advantage of the components interaction and/or diversification;
- forestry in a livestock farm or forested rangelands: trees and shrubs are planted at high density to diversify production at whole farm level.

Further distinction may include differences due to the animal species and breeds, the trees and shrubs species and cultivars, the pasture plants and other vegetation components, the soil, the climate, the land-use patterns and the planting arrangements (Calub 2003; Papanastasis *et al.* 2008). Considering ruminants, goats (Papachristou and Papanastasis 1994; Hove *et al.* 2001; Bestman *et al.* 2014), sheep (Pitta *et al.* 2005; Rangel and Gardiner 2009) and cattle

(Moore *et al.* 2003; Vandermeulen *et al.* 2016) are reported as being managed with shrub and tree fodder.

3.2. Economic implications, benefits and limitations of woody plants on the whole farming system

Besides the environmental benefits of trees and shrubs mentioned previously, the livestock component integrated in a global agricultural system will provide incomes in the short term while multipurpose shrubs and trees can ensure long-term profits through timber for example (Sharrow *et al.* 2009). The timber sector can lead to various outcomes as the wood may be used as softwood lumber, firewood or ramial chipped wood used as litter for livestock (Liagre 2006). Besides economic outputs, shrubs and trees in grasslands can contribute to animal welfare by offering shelter against extreme weather conditions, e.g. shade and protection from rain and wind (Hawkes and Wedderburn 1994; Gregory 1995; Liagre 2006).

Regarding the animal component, shrubs and trees have demonstrated that they can support the production during struggling periods by reducing weight loss (McWilliam *et al.* 2005a; Dalzell *et al.* 2006; Moore *et al.* 2003) and further, they may improve the animal performances (e.g. Abdulrazak *et al.* 1996; Musonda *et al.* 2009; Rangel and Gardiner 2009). In temperate areas, the use of willow as fodder is common in the East Coast regions of New Zealand to secure forage supply during summer and autumn droughts (Charlton *et al.* 2003; Moore *et al.* 2003; Pitta *et al.* 2005). This temperate browse has been widely investigated for its impacts on animal performances. It has been reported to improve reproductive rate, e.g. by 20 % units in ewes, with more births of twin lambs (Pitta *et al.* 2005) or by 17 lambs/100 hoggets mated as a result of increased oestrus activity and conception rates (Musonda *et al.* 2009), and reduce post-natal lamb mortality from 17.1 to 8.4 % compared to control group (McWilliam *et al.* 2005b). Full access to fodder blocks could lessen daily live weight (LW) loss by up to 60 % in sheep (McWilliam *et al.* 2005b; Pitta *et al.* 2005) and by 44 % when fresh willow prunings supplemented cattle grazing a summer dry pasture (Moore *et al.* 2003). Furthermore, willow is capable of reducing livestock parasitism e.g. by reducing nematodes fecundity (Mupeyo *et al.* 2011). Most of these effects have been associated with condensed tannins (CT), bio-active secondary metabolites found in many woody species (Hove *et al.* 2001; Kemp *et al.* 2001). These molecules influence the rumen metabolism in many different ways, with beneficial or detrimental effects depending notably on the compound, the ingested amount and the animal species (Jones *et al.* 1976; Frutos *et al.* 2004;

Bueno *et al.* 2015). The effects of CT on ruminant digestive metabolism have been extensively described (McLeod 1974; Makkar 2003; Frutos *et al.* 2004), and will not be detailed in this review.

In tropical ecosystems, the shrub legume leucaena (*Leucaena leucocephala*) is widely used to supply fodder to ruminants (Devendra 1989; Dalzell *et al.* 2006). In a study in the lowland semi-humid tropics of Kenya, this shrub fodder supplemented at 0, 4 and 8 kg level to *Pennisetum purpureum* lessened Ayrshire/Brown Swiss x Sahiwal crossbred cows LW loss (560, 235 and 175 g/day, respectively), increased daily milk production (7.3, 7.7 and 8.3 kg) and improved yield persistency (-370, -270 and -160 g loss per week) (Muinga *et al.* 1992). When consuming 4 kg DM of this legume per day (~35-40 % of the diet), 450 kg-steers could gain more than 1 kg of bodyweight per day, with LW gain reaching up to 1.6 kg/head.day for the best results obtained in Clermont (Queensland, Australia) for finishing steers with the legume (Dalzell *et al.* 2006). Steers fed *Pennisetum purpureum* diet increased the daily LW gain from 538 to 850 g when supplemented with leucaena and from 306 to 478 g/day with *Gliricidia sepium* (Abdulrazak *et al.* 1996). Among other legumes used as pasture supplementation, *Desmanthus* spp. appear promising since over a 3-month study during a dry winter in central Queensland, steers on a *Desmanthus*-buffel grass pasture gained an extra 40 kg of LW compared with steers grazing only buffel grass (Gardiner and Parker 2012). This plant improved also the wool yield of supplemented Merino wethers with production reaching up to 0.18 mg wool/cm²/day higher than that of control animals (Rangel and Gardiner 2009), while potentially reducing CH_4 emissions (Vandermeulen *et al.* unpublished data).

Although woody plants can deliver benefits to animal production systems, limitations will restrict their implementation within production systems such as the presence of toxic compounds (Hegarty *et al.* 1964; Dalzell *et al.* 2006). Negative impacts of integrating trees into pasture in terms of reducing pasture productivity have been mentioned (Sharrow 1999; Devokta *et al.* 2009). Shrubs and trees and pasture plants compete for above- and below-ground resources. Major effects on pasture production are shade, and the competition for moisture and nutrients, and these effects are tree and pasture species dependent (Sharrow 1999; Devokta *et al.* 2009). Managing the appropriate species in the system is crucial; for example, in temperate systems, planting nitrogen-fixing trees as *Alnus* spp. are expected to enhance nutrient cycling and increase soil fertility which may be beneficial to pasture plants (Smith and Gerrard 2015). However the lack of knowledge about the technical itinerary is a

significant barrier to the integration of shrubs and trees and their use of fodder for ruminants mainly in temperate systems.

3.3. Management of trees and shrubs as fodder in ruminant production systems

Irrespective of the feeding system (Table 1), woody perennials can be scattered or grouped, inside the land or on the edge (Peeters *et al.* 2014). However, the productivity and limitations of silvopastoral systems are variable due to species and cultivars, plant age and structure for feeding, growth status, harvesting period, environmental conditions and management (Table 2; Kemp *et al.* 2001; Douglas *et al.* 2003; Dalzell *et al.* 2006). Besides physical distribution, the use for ruminants can be done in different ways: direct browsing or pruning, with or without preservation of the forage.

3.3.1. Direct browsing on plants

Originally planted for soil conservation in New Zealand, the temperate species *Salix* and *Populus* spp. have been used to feed ruminants during summer and autumn droughts (Moore *et al.* 2003; McWilliam *et al.* 2005a; Pitta *et al.* 2005). Tree fodder from poplars (*Populus* spp.) and willows is obtained from cutting widely spaced trees that are used primarily for soil erosion management or from special purpose fodder blocks that may be coppiced or browed (Charlton *et al*. 2003; Douglas *et al.* 2003; Table 1). These intensively planted browse blocks are less widely used and generally comprise willows. In the willow block systems, shrubs are established at a higher density (1500-30000 stems/ha) than the ones used for soil conservation (Douglas *et al.* 2003). The browse blocks can be designed e.g. by planting the shrubs at 1.2 m × 1.2 m and managing them through controlled browsing and trimming every year to maintain the branches within animals' reach (Table 2). Different species and cultivars have been developed for the fodder block systems in New Zealand, such as *Salix* spp., *Populus* spp. and *Dorycnium rectum* (Oppong *et al.* 2001; McWilliam *et al.* 2005a; Ramírez-Restrepo *et al.* 2010). In terms of yield (Table 2), *Salix matsudana* x *alba* can produce up to 7.2 t DM/ha.year of which 15-19 % is edible, compared to a perennial ryegrass (*Lolium perenne*) pasture yielding 9.8-10.9 t DM/ha in total during the season (Douglas *et al.* 1996). In an experiment with ewes accessing willow fodder blocks during late summer and autumn, the voluntary feed intake was estimated at 2.1 kg DM/ewe.day with 0.29 kg accounting for woody foliage, while the control pasture intake was in the range of 0.7-1.66 kg DM/ewe.day (Pitta *et al.* 2007). Kemp *et al.* (2001) observed that cattle browsed 0.7-2.4 kg DM of trees/animal at 1.6-2.2 m high.

Table 1. Main feeding methods, animals and woody species in silvopastoral systems.

Feeding system	Type/Description	Animal	Trees/shrubs species	Examples of regions/countries where it is used	References
Browsing	Hedgerows	Cattle	*Leucaena leucocepahala*	Northern Australia	Dalzell *et al.* (2006)
			Various native species: e.g. *Fraxinus excelsior, Quercus* spp., *Corylus avellana, Acer* spp.	Europe e.g. France, Belgium	Baudry *et al.* (2000), Liagre (2006); Vandermeulen *et al.* (2016)
	Browse blocks	Sheep, cattle, goats	*Salix* spp., *Populus* spp.	New Zealand	Charlton *et al.* (2003), Douglas *et al.* (2003), Pitta *et al.* (2007)
			Quercus spp., *Corylus avellana, Robinia pseudoacacia,* etc.	Greece	Papachristou and Papanastasis (1994)
	Scattered trees and shrubs	Cattle	*Desmanthus* spp. + other woody legumes	Australia	Rangel and Gardiner (2009), Gardiner *et al.*(2013)
Pruned fodder	Traditional cut-and-carry systems	Cattle, sheep, goats	Various species e.g. *L. leucocepahala, Ficus* spp., *Populus* spp., *Chamaecytisus prolifer* var. *palmensis*	Asia e.g. Indonesia, Nepal, China; Africa	Devendra (1989), Calub (2003), Cook *et al.* (2005)
	Shredding	Cattle Sheep, goats	*Fraxinus excelsior Quercus* spp., *Fagus* spp.	Mediterranean region e.g. Greece	Papanastasis *et al.* (2009)
	Pollarding	Sheep, goats, cattle	*Morus alba, Fraxinus excelsior*, *Salix* spp., *Populus* spp., *Quercus* spp., *Fagus* spp.	Mediterranean region e.g. South of France, Greece; New Zealand	Charlton *et al.* (2003), Liagre (2006), Papanastasis *et al.* (2009)

	Coppicing	Sheep, cattle	*Salix* spp., *Populus* spp.	New Zealand	Charlton *et al.* (2003), Douglas *et al.* (2003)
Preserved forage	Dried	Sheep, cattle	*Fraxinus excelsior*	France	Liagre (2006)
	Silage	Goats, cattle	*Salix* spp.	Europe e.g. United Kingdom, The Netherlands	Bestman *et al.* (2014), Smith *et al.* (2014)
	Pellets	Cattle, goats, buffalo	*Morus alba, Leucaena leucocepahala, Tectona grandis*	India, Thailand	Anbarasu *et al.* (2004), Huyen *et al.* (2012), Hung *et al.* (2013)

In Europe, hedgerows and windbreaks (i.e. shelterbelts) aim primarily to enclose the fields and meadows (Baudry *et al.* 2000) and control erosion (Nerlich *et al.* 2013) respectively, while the shrubs and trees composing them may be browsed by animals (Vandermeulen *et al.* 2016; Table 1). The "*bocage"* in Brittany and Normandy in north-west of France is a typical example of hedgerow systems relying on lines of mid-stem e.g. *Carpinus betulus*, *Coryllus avellana*, *Acer campestre*, and high-stem trees species, such as *Castanea sativa*, *Fagus sylvatica* and *Quercus* spp. (Thenail *et al.* 2014). A large variety of other species can compose these hedgerow types e.g. *Fraxinus excelsior, Crataegus monogyna*, *Cornus sanguinea*, *Populus* spp. or *Salix* spp. (Baudry *et al.* 2000; Vandermeulen *et al.* 2016). The bocage landscapes are also found in northern Spain, Italy, Switzerland, Germany and Belgium (Baudry *et al.* 2000; Brootcorne 2011). However, this ancient agroforestry system has suffered from agricultural intensification during the second half of the 20th century with an important decrease in hedgerows numbers (Nerlich *et al.* 2013; Thenail *et al.* 2014; Vandermeulen *et al.* 2016); a situation that is trying to be reversed by the new establishment of hedgerows (Thenail *et al.* 2014).

In Belgium like in most European countries, woody perennials have been removed from farmland due to the intensification of production systems (Nerlich *et al.* 2013). However, due to new environmental policies [i.e. agri-environmental and climatic measures (AECM)], the integration of shrubs and trees is promoted again as farmers may receive annual subsidies for the establishment and maintenance of hedgerows and woody strips (25€/200 m) as well as individual shrubs, trees, bushes or groves (25€/20 units; Walloon Government 2015, 2016). Within this framework, several criteria must be met, such as the use of indigenous species e.g. *Fraxinus excelsior, Crataegus monogyna* or *Corylus avellana* (SPW 2010; Walloon Government 2016). In Wallonia in southern Belgium, it was reported that 13 m of hedgerow per ha of utilized agricultural area (UAA) and 1 tree per 6.4 ha have been newly planted into pasture (SPW 2010) while in 2010, it reached 16 m of hedge per ha of UAA and 1 tree for 5.8 ha (SPW 2012). Between 1999 and 2009, more than 100 km of hedgerows have been planted (SPW 2010). Among the AECM, hedges and woody strips are the most popular with 33% of total AECM newly implemented by farmers in 2012 (SPW 2014). The interest in the environment-friendly practices results in 12,370 km of hedgerows in total in Wallonia (SPW 2014). Overall, it is estimated that since the implementation of the program, farmers' participation has increased steadily. However, between 2013 and 2015, budget restrictions limited most AECM, while latest updates of policies related to aids

granted for the plantation of live fences, linear coppices, orchards and tree alignments and for the maintenance of pollards, are promoting shrubs and trees on farmland e.g. 20 % grants increase if the project supports directly an ecosystem service (Walloon Government 2016).

As pointed out earlier, the interest in integrating shrubs and trees in agricultural landscapes in Belgium is driven by environmental concerns, but their use as an extra fodder resource might result from it. Recent studies (Vandermeulen *et al.* 2016) found that grazing cattle may browse shrub and tree fodder integrated as hedgerows during the grazing season, from spring (i.e. April) to autumn (i.e. October). It is also interesting to see that current research projects in Europe are aiming to integrate woody forage in ruminant systems (Bestman *et al.* 2014; Smith *et al.* 2014; Van laer *et al.* 2015). Nevertheless, it should be stated that additional research is needed to better understand the sustainable productivity from introduced browsing plant species.

In the Tropics, continuous, rotational or seasonal grazing systems facilitate browsing practices using leucaena to support beef cattle industry in the north-east region of Australia (Dalzell *et al.* 2006; Cox and Gardiner 2013), where the plant is also aligned along hedgerows (i.e. live fences; Table 1). Leucaena productivity was reported to vary between 13.7 and 32.0 tons of dry matter (DM)/ha depending on the harvest interval and row spacing (Ferraris 1979; Table 2). Thus, to ensure plant survival and optimal productivity, plant height (i.e. 1.5 to 2.0 m) and age (i.e. 6 to 12 months after seeding) should be considered at the time of browsing (Dalzell *et al.* 2006). It is also reported that the stocking rate on leucaena-grass pastures in Queensland can range between 0.6 head/ha in leucaena-buffel grass (*Pennisetum ciliare*) pastures to 2.5 steers/ha in irrigated systems assuming that 450 kg steers would ingest 35 % of leucaena in their diet (Dalzell *et al.* 2006). Although leucaena is known to be palatable, nutritious, productive and widely established in Australia (Dalzell *et al.* 2006; Shelton and Dalzell 2007), its toxicity limits its introduction in ruminant systems (Table 2). Furthermore, this plant is considered as an environmental weed that can threaten the whole grassland ecosystem (Dalzell *et al.* 2006). Nevertheless, actions may be taken to deal with adverse outcomes e.g. inoculating the ruminal bacterium *Synergistes jonesii* (Allison *et al.* 1992) which is able to degrade mimosine to non-toxic end products or the implementation of preventive procedures to minimize the spread of unwanted plants (Dalzell *et al.* 2006).

Table 2. Examples of management and production characteristics of silvopastoral woody species and limitations of their use to feed ruminants.

Species	Ecological area	Utilization practices/management	Potential yield	Limitations	References
Calliandra calothyrsus	Humid to sub-humid tropics Mean T° between 18-28°C 700-4000 mm 0-1850 m altitude	Hedgerows: Seedlings planted 0.5-1.0 m apart in hedgerows spaced 3-4 m apart, Fodder banks spaced 0.5-1.0 m apart in a grid pattern Cut-and-carry	3-14 t of DM/ha.year[1]	Possibly due to high CT content (> 50 g/kg DM ; see Table 3)	Cook *et al.* (2005)
Corylus avellana	Eurasia (subatlantic-submediterranean trend) Up to 1700 m	Hedgerows	Not documented	Not documented	Rameau *et al.* (1989); Vandermeulen *et al.* (2016); Thenail *et al.* (2014)
Desmanthus virgatus	From continuously wet to lengthened dry season environments Up to 1800 m	Pure legume in cut-and-carry systems Used in legume-grass pasture in Queensland, Australia	Up to 7.6 t DM/ha[1]	Not documented	Cook *et al.* (2005); Jones and Brandon (1998)
Fraxinus excelsior	Europe (subatlantic trend) Up to 1400 m	Hedgerows Pollarding or shredding (fresh or dried)	40-60 kg of leaves/tree.year[2]	Not documented	Rameau *et al.* (1989); Liagre (2006)
Leucaena leucocephala	Sub-tropics Annual rainfall > 600 mm Optimum T°: 25-30°C	Hedgerows: sown 5-10 m apart	0.6-25.9 kg DM/tree[1] over a 2-year period	Mimosine content Considered as environmental weed	Cook *et al.* (2005); Dalzell *et al.* (2006); Mullen and Gutteridge (2002)
Populus deltoides x *nigra*	New Zealand notably	Coppicing or browsing of fodder blocks	1.6-18 kg DM/tree[2]	Not documented	Kemp *et al.* (2001)

Salix matsudana x *alba*	New Zealand notably	Coppicing or browsing of fodder blocks	< 1-22 kg DM/tree[2]	Not documented	Douglas *et al.* (2003); Kemp *et al.* (2001); Oppong *et al.* (2001)
Salix viminalis	Eurasia Up to 400 m	Coppicing or browsing of fodder blocks	Not documented	Not documented	Rameau *et al.* (1989)

[1] Total biomass including branches and leaves

[2]Edible biomass or leaves

Calliandra calothyrsus is another tropical shrub legume used in direct browsing or in cut-and-carry systems (Palmer and Schlink 1992; Maasdorp *et al.* 1999). The plant contains CT (> 50 g/kg DM; Table 3) which eaten in large quantities may reduce DM intake (DMI) and disrupt animal performance (Barry and Duncan 1984; Frutos *et al.* 2004). However, studies conducted by Hove *et al.* (2001) indicated that goats fed with a native pasture hay supplemented with *C. calothyrsus* (196 g CT/kg DM) had similar DMI of goats fed *Acacia augustissima* (33 g CT/kg DM) or *L. leucocepahala* (134 g CT/kg DM).

Beside *L. leucocepahala* and *C. calothyrsus*, *Stylosanthes* spp., *Sesbania sesban*, and *Gliciridia sepium* are among perennial woody legumes promoted in the north-east semi-arid region of Australia (Palmer and Schlink 1992; Cox and Gardiner 2013). Characterized by a seasonally dry period extending from April to October, grasslands of this region have been mixed with shrub or tree legumes to supply quality feed and to improve the total nutrient availability to grazing cattle during grass shortages (Cox and Gardiner 2013). In contrast with temperate areas, many genera and species used as pasture plants for cattle have been selected. *Leucaena leucocephala* previously mentioned is one example of a native shrub from America largely introduced in northern Australian grasslands, with about 150,000 ha reported in Queensland in 2007. In the specific context of semi-arid clay soils of northern Australia, the genus *Desmanthus* has also been selected for its persistence in this environment while other sown species did not survive (Gardiner and Swan 2008). *Desmanthus* spp. are palatable, non-toxic, non-thorny and protein rich trees (Gardiner *et al.* 2013). They are also well adapted to heavy grazing systems (Pengelly and Conway 2000). In this context, *D. bicornutus*, *D. leptophyllus* and *D. virgatus* have been particularly targeted (Gardiner *et al.* 2013) to improve paddock performance and sustain livestock production in dry tropics systems.

Although livestock are considered as a product, animals can be used as a tool to manage the woody plants by grazing the grass stratum and browsing shrubs and trees (Sharrow *et al.* 2009). Livestock, mainly goats, also have a role in reducing fire risk in Mediterranean systems by grazing and browsing the understorey vegetation (Papanastasis *et al*. 2008, 2009), and in weed control (Sharrow 2009). In plantation systems associating several browse species, it is preferable to include plants of similar palatability to avoid overbrowsing of the preferred ones (Papachristou and Papanastasis 1994). To insure optimal productivity, the browse height needs to be regulated and varies with the livestock species. In Australian beef cattle grazing systems, leucaena should be managed to remain between 2 to 3 m tall based on appropriate browsing pressure and cuttings (Dalzell *et al.* 2006). In contrast,

willow fodder blocks may be cut at 0.4 m above ground to be browsed by sheep (Douglas *et al.* 2003). The complementarity between different animal species might be exploited as it is the case in New Zealand with willows block systems browsed by sheep first and then by cattle to overcome excessive plant development (Pitta *et al.* 2007). However, care must be exercised to avoid irreversible damage from large animals (Eason *et al.* 1996; Vandenberghe *et al.* 2007). Although very little data was found in the literature, the sensitivity to browsing varies between species. For example, Eason *et al.* (1996) observed that *F. excelsior* suffered more from browsing by sheep than *Acer pseudoplatanus* which could be due to the difference in palatability and/or tree height. Greater browsing height of cattle (~ 1.8 m) can be destructive to some tree species.

3.3.2. Pruned shrubs and trees (fresh fodder)

Instead of direct browsing, woody forage can be cut and distributed to the animals at the stalls or eaten on-site (Charlton *et al.* 2003; Bestman *et al.* 2014) as practiced for example by French shepherds in the Pyrenees and the Massif Central with *Fraxinus excelsior* branches (Liagre 2006). The cut-and-carry practice has a long tradition in tropical silvopastoral systems (Calub 2003) and in temperate feeding systems (Baudry *et al.* 2000; Liagre 2006). Traditional cut-and-carry systems (Table 1) are widely used in many countries of Asia as in Indonesia with leucaena or in Nepal with *Ficus* spp., or in tropical Africa (Devendra 1989; Calub 2003).

Different pruning methods may be used to harvest the shrub and tree fodder e.g. shredding, pollarding and coppicing (Table 1). Shredding is achieved by cutting lower lateral branches resulting in a 5 to 7 m-trunk with branches longwise while pollarding produces multiple branches on the top of a short trunk of 1.5 to 2.5 m (Baudry *et al.* 2000; Charlton *et al.* 2003; Papanastasis *et al.* 2009), protecting trees from browsing. Both techniques in Greece are typically used in traditional silvopastoral systems with *Quercus* spp. and *Fagus* spp., but these management practices have been progressively abandoned (Papanastasis *et al.* 2009).

Table 3. Chemical composition and nutritive value of browse species.

Species	Plant part	Processing	CP (% DM)	NDF (% DM)	IVOMD	CT (%DM)	References
Calliandra calothyrsus	Leaves	Sun-dried	11.9[A]	53.4		19.6	Hove *et al.* (2001)
		Dried	25.3	39.6	0.409	0.4 – 12.7	Salawu *et al.* (1997)
Corylus avellana	Leaves (+ twigs)		9.1 – 18.1	43.2 – 53.5	0.384 – 0.535		Papachristou and Papanastasis (1994)
Leucaena leucocephala	Leaves	Sun-dried	20[A]	34.9		13.4	Hove *et al.* (2001)
			17[A]	27.8		3.8	McSweeney *et al.* (1999)
	Leaves + petioles	Oven-dried (65°C)			0.420 – 0.510		Edwards *et al.* (2012)
Populus deltoides x *nigra*	Leaves + edible stems (< 5mm diameter)		12.8 – 17.9			0.6 – 2.6	Kemp *et al.* (2001)
Robinia pseudoacacia	Leaves (+ twigs)		11.6 – 29.3	33.1 – 56.7	0.483 – 0.632		Papachristou and Papanastasis (1994)
Salix matsudana x *alba*	Leaves + edible stems (< 5 mm diameter)		11.7 – 15.5			1.8 – 4.2	Kemp *et al.* (2001)
Salix viminalis	Leaves + stems (< 8 mm diameter)	Dried	16.7	57.3	0.405		Smith *et al.* (2014)
		Silage	18.2	44.0	0.421	4.7	
	Leaves	Silage	21.9	28.7	0.511	10.3	

CP, crude protein; CT, condensed tannins; DM, dry matter; IVOMD, *in vitro* organic matter digestibility; NDF, neutral detergent fiber.

[A]CP = N × 6.25

Coppicing consists of producing a basal stump with growing branches (Baudry *et al.* 2000) by cutting trees to near ground level, for example at 0.3-0.5 m high (Charlton *et al.* 2003). The fodder is carried afterwards to the stall or left on-site for eating. This technique is commonly found in New Zealand with willows and poplars that are used originally for soil conservation (Charlton *et al.* 2003; Douglas *et al.* 2003). In this kind of system, Hereford-Friesian crossbred cows grazing a sparse pasture supplemented with unchopped pruned *Salix* spp. could ingest between 0.5 to more than 3 kg DM/cow.day (Moore *et al.* 2003). In browse fodder blocks also performed in New Zealand, coppicing may be performed as post-browsing shrub management (Douglas *et al.* 2003).

Plant material is usually harvested mechanically as carried out by dairy farmers in cut-and-carry systems in the Netherlands with willows, ash (*F. excelsior*) or hazels (*C. avellana*) (Bestman *et al.* 2014), but some operations can be manual such as topping *Salix* spp. trees to stumps in fodder blocks in New Zealand (Douglas *et al.* 2003; Pitta *et al.* 2007). The way of feeding can also vary between systems as the fodder can be distributed as whole plants (i.e. branches and leaves) or by separating leaves from branches while the forage can be kept intact (i.e. not fragmented) or shredded (Bestman *et al.* 2014). Once the fodder is harvested, it may be provided fresh to the animals. Alternatively a preservation method can extend the use of the harvested forage.

3.3.3. Preservation of shrub and tree forage

Preserving browse hay is notably practiced in Greece (Papanastasis *et al.* 2009), Norway and France (Baudry *et al.* 2000; Thiébault 2005) for winter. Nevertheless, browse preservation methods are time-consuming which explains why some have progressively disappeared (Liagre 2006).

Chemical composition and nutritive value of shrubs and trees fodder depend on the species and cultivars, composition of the plant material, growth status, harvesting period, environment and management (Papachristou and Papanastasis 1994; Kemp *et al.* 2001; Dalzell *et al.* 2006), but the processing of the forage, e.g. fresh, dried, silage or pellets, is a determinant as well (Palmer and Schlink 1992; Smith *et al.* 2014; Table 3). For example, it is suggested to distribute *C. calothyrsus* fresh in a minimum time after harvesting, instead of dried (Palmer and Schlink 1992; Maasdorp *et al.* 1999) as drying decreases DM digestibility of this species.

The fodder can be dried naturally in the sun (Hove *et al.* 2001) or force-dried in an oven (Palmer and Schlink 1992). Ash has been traditionally used as fresh fodder during summer drought or dried for winter in France, to feed ruminants (Liagre 2006). With a high concentration in Ca, this forage is particularly recommended for suckler and lactating cows. Nowadays, techniques that are commonly used to preserve herbaceous forage are being applied to browse. The ensilability of *Salix* spp. foliage was recently investigated in The Netherlands (Bestman *et al.* 2014) and the United Kingdom (Smith *et al.* 2014). However, the effects of the conservation method on the plants palatability (Bestman *et al.* 2014) and nutritive value are still unknown (Smith *et al.* 2014). Since tannins can prevent feed protein degradation in silages of some legumes (Albrecht and Muck 1991), woody legumes containing these bio-active compounds could yield high quality silage by preventing proteolysis.

In the tropics, pelleting leaves of e.g. *L. leucocephala* (Hung *et al.* 2013) or mulberry (*Morus alba*; Huyen *et al.* 2012) has been used to supplement ruminants. The pellets are prepared by mixing, in different proportions, the tree leaf meal with urea, molasses, cassava starch, salt, sulfur and a mineral mixture (Huyen *et al.* 2012; Hung *et al.* 2013).

3.4. Integrating shrubs and trees into temperate ruminants production systems: contributions, obstacles and prospects

Shrubs and trees have the potential to contribute to temperate ruminant production systems. They can secure forage supply to ruminants by supplementing pasture during summer and autumn droughts (Liagre 2006; Douglas 2003). Because shrubs and trees are usually considered as a forage security rather than a definite production, it is difficult to have a good overview of both the nutritive value of the different woody species and their productivity over the year (Liagre 2006).

In browsing systems where pasture biomass production might be sufficient to cover livestock requirements as in Belgium, other reasons than fodder supply might encourage herbivores to choose woody plants over grass. When mixing trees with grass, the animals have the opportunity to diversify their diet. This can satisfy the individual nutritional requirements and preferences but it also offers alternatives to better cope with toxins and parasites (Provenza *et al.* 2003; Manteca *et al.* 2008). Parasitized animals offered plant secondary compounds-containing feed are able to self-medicate, as it has been reported that lambs with parasitic burdens ingested more of the tannin-containing feed than unparasitized

animals (Lisonbee *et al.* 2009; Villalba *et al.* 2010). Feeding willow fodder to young sheep in New Zealand reduced nematodes burden and fecundity which has been associated to willow CT content (Mupeyo *et al.* 2011). Hence, taking care of animals according to the therapeutic or nutritive properties of some species is a frequent argument (Thiébault 2005). Fodder trees can also improve low-quality pasture or diet by delivering N and mineral supplement to animals (Leng 1992), and the plant feeding value can influence the choice between plants to ingest (Decruyenaere *et al.* 2009; Meier *et al.* 2014). Unfortunately, the selection of woody species to implement within a production system relies sometimes on local traditional knowledge or beliefs rather than proper scientific evaluation (Thiébault 2005).

Although trees and shrubs might contribute to ruminant livestock production systems, obstacles to their introduction within systems and their use as fodder have been reported. Farmers mentioned the additional labour, regulatory requirements and administrative constraints, cost of planting shrubs and trees, lack of training, interference with agriculture mechanization or pasture problems such as locally lower pasture production due to tree shade (Brootcorne 2011; Luske 2014). Regarding weed and disease control, some declared that hedges play a significant role in the transmission of beef cattle scabies (Brootcorne 2011). These constraints differ according to the systems; the management of tree regeneration seems more complex with goats than dairy cows (Luske 2014). This highlights the lack of knowledge about the technical itinerary in temperate production systems. Recent research evaluated how woody species can fit within the systems (Bestman *et al.* 2014; Luske 2014; Smith *et al.* 2014; Vandermeulen *et al.* 2016). However, there is still a need to deeper investigate the potential productivity of fodder woody species and the management of the access by animals to this forage resource (Luske 2014; Vandermeulen *et al.* 2016). It will also be determinant to measure the economic balance between investments, labour and profits (Bestman *et al.* 2014) to ensure that this fodder resource provides positive economic outcomes for farmers.

4. Conclusion

Feeding ruminants with browse species has been practiced in many regions while it has progressively declined in intensive production systems. Nevertheless, environment-friendly policies are promoting silvopastoral systems as multipurpose shrubs and trees are known to be able to deliver ecosystem services. Furthermore, woody fodder has been reported to improve ruminal protein digestion, reduce parasitic infestation or lessen methane emissions

but limitations such as toxins can restrict their use. Integrating this overlooked forage resource in ruminant husbandry can be achieved by direct browsing, cut-and-carry systems or conserving fodder. Several programs are studying the pelleting or ensiling of browse fodder. In optimal conditions, shrubs and trees sustain and further enhance animal production. As the renewed interest in using this fodder resource more intensively is rather young, further research is needed to more deeply investigate a wider range of systems and promising species, especially in temperate regions.

5. References

Abdulrazak SA, Muinga RW, Thorpe W, Ørskov ER (1996) The effects of supplementation with *Gliricidia sepium* or *Leucaena leucocephala* forage on intake, digestion and live-weight gains of *Bos taurus* × *Bos indicus* steers offered napier grass. *Animal Science* **63**, 381–388. doi:10.1017/S1357729800015265

Albrecht KA, Muck RE (1991) Proteolysis in ensiled forage legumes that vary in tannin concentration. *Crop Science* **31**, 464–469.

Allen VG, Batello C, Berretta EJ, Hodgson J, Kothmann M, Li X, McIvor J, Milne J, Morris, C, Peeters A, Sanderson M (2011) An international terminology for grazing lands and grazing animals: Grazing lands and grazing animals. *Grass and Forage Science* **66**, 2–28. doi:10.1111/j.1365-2494.2010.00780.x

Allison MJ, Mayberry WR, McSweeney CS, Stahl DA (1992) *Synergistes jonesii*, gen. nov., sp. nov.: A Rumen Bacterium That Degrades Toxic Pyridinediols. *Systematic and Applied Microbiology* **15**, 522–529.

Anbarasu C, Dutta N, Sharma K, Rawat M (2004) Response of goats to partial replacement of dietary protein by a leaf meal mixture containing *Leucaena leucocephala*, *Morus alba* and *Tectona grandis*. *Small Ruminant Research* **51**, 47–56. doi:10.1016/S0921-4488(03)00203-7

Barry TN, Duncan SJ (1984) The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. *British Journal of Nutrition* **51**, 485–491.

Baudry J, Bunce RG, Burel F (2000) Hedgerows: An international perspective on their origin, function and management. *Journal of Environmental Management* **60**, 7–22. doi:10.1006/jema.2000.0358

Bestman M, van Eekeren N, Luske B, Vonk M, Anssems E, Boosten M, van Bree M (2014) Introducing trees in dairy and poultry farms. Experiences dairy and poultry farmers' networks in The Netherlands. In '18th IFOAM Organic World Congress 2014', Istanbul, Turkey, 13-15 October 2014 [Online]. Available at http://orgprints.org/23894/9/23894.pdf (verified 22 March 2016)

Brootcorne N (2011) 'Analyse des facteurs d'adoption ou de rejets de l'agroforesterie dans le cadre d'un projet en région wallonne.' Master Thesis, The University of Liège (Campus of Arlon), Belgium.

Bueno ICS, Brandi RA, Franzolin R, Benetel G, Fagundes GM, Abdalla AL, Louvandini H, Muir JP (2015) *In vitro* methane production and tolerance to condensed tannins in five ruminant species. *Animal Feed Science and Technology* **205**, 1–9. doi:10.1016/j.anifeedsci.2015.03.008

Buttler A, Kohler F, Gillet F (2009) The Swiss mountain wooded pastures: patterns and processes. In 'Agroforestry in Europe: Current Status and Future Prospects'. (Eds A Rigueiro-Rodríguez, J McAdam, MR Mosquera-Losada) pp. 377–396. (Springer Science & Business Media B.V.: Dordrecht)

Calub BM (2003) Understanding silvopastoral systems. APA News, Asia-Pacific Agroforestry Newsletter No. 22, August 2003. (FAO: Bangkok)

Charlton JFL, Douglas GB, Wills BJ, Prebble JE (2003) Farmer experience with tree fodder. In 'Using trees on farms'. (Ed. JFL Charlton) Grassland research and practice series No. 10, pp. 7–16. (New Zealand Grassland Association: Wellington)

Cook B, Pengelly BC, Brown S, Donnelly JB, Eagles D, Franco A, Hanson J, Mullen BF, Partridge I, Peters M, Schultze-Kraft R (2005) Tropical Forages: an interactive selection tool. [CD-ROM], CSIRO, DPI&F (Qld), CIAT and ILRI, Brisbane, Australia [Online]. Available at http://www.tropicalforages.info/ (verified 22 March 2016)

Cox K, Gardiner C (2013) Pasture legumes in Queensland–a new wave? In 'Proceedings of the Northern Beef Research Update Conference'. pp. 15-20. (Queensland North Australia Beef Research Council: Gympie, Qld)

Dalzell SA, Shelton HM, Mullen BF, Larsen PH, McLaughlin KG (2006) 'Leucaena: a guide to establishment and management.' (Meat & Livestock Australia Ltd.: Sydney, NSW)

Decruyenaere V, Buldgen A, Stilmant D (2009) Factors affecting intake by grazing ruminants and related quantification methods: a review. *Biotechnology, Agronomy and Society and Environment* **13**, 559–573.

de Klein CAM, Eckard RJ (2008) Targeted technologies for nitrous oxide abatement from animal agriculture. *Australian Journal of Experimental Agriculture* **48**, 14-20. doi:10.1071/EA07217

Devendra C (Ed.) (1989) 'Shrubs and tree fodders for farm animals: Proceedings of a workshop in Denpasar, Indonesia, 24-29 July 1989.' (IDRC: Ottawa)

Devkota NR, Kemp PD, Hodgson J (2009) Relationship between tree canopy height and the production of pasture species in a silvopastoral system based on alder trees. *Agroforestry Systems* **76**, 363–374. doi:10.1007/s10457-008-9192-8

Douglas GB, Bulloch BT, Foote AG (1996) Cutting management of willows (*Salix* spp.) and leguminous shrubs for forage during summer. *New Zealand Journal of Agricultural Research* **39**, 175–184. doi:10.1080/00288233.1996.9513176

Douglas GB, Barry TN, Faulknor NA, Kemp PD, Foote AG, Cameron PN, Pitta DW (2003) Willow coppice and browse blocks: establishment and management. In 'Using trees on farms'. (Ed. JFL Charlton) Grassland research and practice series No. 10, pp. 41-51. (New Zealand Grassland Association: Wellington)

Dupraz C, Liagre F (2008) 'Agroforesterie - Des arbres et des cultures.' (France Agricole Editions : Paris)

Eason WR, Gill EK, Roberts JE (1996) Evaluation of anti-sheep tree-stem-protection products in silvopastoral agroforestry. *Agroforestry systems* **34**, 259–264.

Edwards A, Mlambo, Lallo CHO, Garcia GW (2012) Yield, Chemical Composition and *In Vitro* Ruminal Fermentation of the Leaves of *Leucaena Leucocephala*, *Gliricidia Sepium* and *Trichanthera Gigantea* as Influenced by Harvesting Frequency. *Journal of Animal Science Advances* **2**, 321–331.

Etienne M (1996) Research on temperate and tropical silvopastoral systems: a review. In 'Western European Silvopastoral Systems'. (Ed. M Etienne) pp. 5–19. (INRA: Paris)

Ferraris R (1979) Productivity of *Leucaena leucocephala* in the wet tropics of north Queensland. *Tropical Grasslands* **13**, 20–27.

Frutos P, Hervas G, García FG, Mantecón AR (2004) Review. Tannins and ruminant nutrition. *Spanish Journal of Agricultural Research* **2**, 191–202.

Gardiner CP, Swan SJ (2008) Abandoned pasture legumes offer potential economic and environmental benefits in semiarid clay soil rangelands. In: 'Proceedings of the 15th Biennial Conference of the Australian Rangeland Society. A climate of change in the rangelands'. 4 pp. (Australian Rangeland Society: Mount Lawley, WA).

Gardiner C, Parker AJ (2012) Steer liveweight gains on Progardes desmanthus/buffel pastures in Queensland. In 'Proceedings of Second Joint Conference of the New Zealand Society of Animal Production and the Australian Society of Animal Production'. pp. 1. (Lincoln University: Christchurch).

Gardiner C, Kempe N, Hannah I, McDonald J (2013). PROGARDES TM: a legume for tropical/subtropical semi-arid clay soils. *Tropical Grasslands-Forrajes Tropicales* **1**, 78–80.

Grainger C, Clarke T, Auldist MJ, Beauchemin KA, McGinn SM, Waghorn GC, Eckard RJ (2009) Potential use of *Acacia mearnsii* condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. *Canadian Journal of Animal Science* **89**, 241–251.

Gregory NG (1995) The role of shelterbelts in protecting livestock: A review. *New Zealand Journal of Agricultural Research* **38**, 423–450. doi:10.1080/00288233.1995.9513146

Hawkes MF, Wedderburn ME (1994) Microclimate changes under *pinus radiata* agroforestry regimes in New Zealand. *Agricultural and Forest Meteorology* **71**, 133–145.

Hegarty MP, Schinckel PG, Court RD (1964) Reaction of sheep to the consumption of *Leucaena glauca* Benth. and to its toxic principle mimosine. *Australian Journal of Agricultural Research* **15**, 153–167.

Herzog F (1998) Streuobst: a traditional agroforestry system as a model for agroforestry development in temperate Europe. *Agroforestry Systems* **42**, 61–80.

Hove L, Topps JH, Sibanda S, Ndlovu LR (2001) Nutrient intake and utilisation by goats fed dried leaves of the shrub legumes *Acacia angustissima*, *Calliandra calothyrsus* and *Leucaena leucocephala* as supplements to native pasture hay. *Animal Feed Science and Technology* **91**, 95–106.

Hung LV, Wanapat M, Cherdthong A (2013) Effects of Leucaena leaf pellet on bacterial diversity and microbial protein synthesis in swamp buffalo fed on rice straw. *Livestock Science* **151**, 188–197. doi:10.1016/j.livsci.2012.11.011

Huyen NT, Wanapat M, Navanukraw C (2012) Effect of Mulberry leaf pellet (MUP) supplementation on rumen fermentation and nutrient digestibility in beef cattle fed on rice straw-based diets. *Animal Feed Science and Technology* **175**, 8–15. doi:10.1016/j.anifeedsci.2012.03.020

Jones RJ, Blunt CG, Holmes HG (1976) Enlarged thyroid glands in cattle grazing leucaena pastures. *Tropical Grasslands* **10**, 113–116.

Jones RM, Brandon NJ (1998) Persistence and productivity of eight accessions of *Desmanthus virgatus* under a range of grazing pressures in subtropical Queensland. *Tropical Grasslands* **32**, 145–152.

Jose S (2009) Agroforestry for ecosystem services and environmental benefits: an overview. *Agroforestry Systems* **76**, 1–10. doi:10.1007/s10457-009-9229-7

Kanani J, Lukefahr SD, Stanko RL (2006) Evaluation of tropical forage legumes (*Medicago sativa*, *Dolichos lablab*, *Leucaena leucocephala* and *Desmanthus bicornutus*) for growing goats. *Small Ruminant Research* **65**, 1–7. doi:10.1016/j.smallrumres.2005.04.028

Kaur B, Gupta SR, Singh G (2002) Carbon storage and nitrogen cycling in silvopastoral systems on a sodic in northwestern India. *Agroforestry systems* **54**, 21–29.

Kemp PD, Mackay AD, Matheson LA, Timmins ME (2001) The forage value of poplars and willows. *Proceedings of the New Zealand Grassland Association* **63**, 115–120.

Leng (1992) 'Tree foliage in ruminant nutrition.' (FAO: Rome)

Liagre F (2006) 'Les haies rurales.' (France Agricole Editions: Paris)

Lisonbee LD, Villalba JJ, Provenza FD, Hall JO (2009) Tannins and self-medication: Implications for sustainable parasite control in herbivores. *Behavioural Processes* **82**, 184–189. doi:10.1016/j.beproc.2009.06.009

Luske B (2014) Initial Stakeholder Meeting Report: Fodder trees for cattle and goats in the Netherlands [Online]. 6 October 2014. Available at https://www.agforward.eu/index.php/en/fodder-trees-for-cattle-and-goats-in-the-netherlands.html (verified 30 January 2017)

Maasdorp BV, Muchenje V, Titterton M (1999) Palatability and effect on dairy cow milk yield of dried fodder from the forage trees *Acacia boliviana*, *Calliandra calothyrsus* and *Leucaena leucocephala*. *Animal Feed Science and Technology* **77**, 49–59.

Makkar HP (2003) Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Ruminant Research* **49**, 241–256. doi:10.1016/S0921-4488(03)00142-1

Manteca X, Villalba JJ, Atwood SB, Dziba L, Provenza FD (2008) Is dietary choice important to animal welfare? *Journal of Veterinary Behavior: Clinical Applications and Research* **3**, 229–239. doi:10.1016/j.jveb.2008.05.005

McLeod MN (1974) Plant Tannins - Their Role in Forage Quality. *Nutrition Abstracts & Reviews* **44**, 803–815.

McSweeney CS, Palmer B, Bunch R, Krause DO (1999) *In vitro* quality assessment of tannin-containing tropical shrub legumes: protein and fibre digestion. *Animal Feed Science and Technology* **82**, 227–241.

McWilliam EL, Barry TN, López-Villalobos N, Cameron PN, Kemp PD (2005a) Effects of willow (*Salix*) versus poplar (*Populus*) supplementation on the reproductive performance of ewes grazing low quality drought pasture during mating. *Animal Feed Science and Technology* **119**, 69–86. doi:10.1016/j.anifeedsci.2004.12.003

McWilliam EL, Barry TN, López-Villalobos N, Cameron PN, Kemp PD (2005b) Effects of willow (*Salix*) supplementation for 31 and 63d on the reproductive performance of ewes grazing low quality drought pasture during mating. *Animal Feed Science and Technology* **119**, 87–106. doi:10.1016/j.anifeedsci.2004.11.013

Meier JS, Liesegang A, Louhaichi M, Hilali M, Rischkowsky B, Kreuzer M, Marquardt S (2014) Intake pattern and nutrient supply of lactating sheep selecting dried forage from woody plants and straw offered in binary or multiple choice. *Animal Feed Science and Technology* **188**, 1–12. doi:10.1016/j.anifeedsci.2013.11.003

Moore KM, Barry TN, Cameron PN, Lopez-Villalobos N, Cameron DJ (2003) Willow (*Salix* sp.) as a supplement for grazing cattle under drought conditions. *Animal Feed Science and Technology* **104**, 1–11. doi:10.1016/S0377-8401(02)00326-7

Mosquera-Losada MR, Moreno G, Pardini A, McAdam JH, Papanastasis V, Burgess PJ, Lamersdorf N, Castro M, Liagre F, Rigueiro-Rodríguez A (2012) Past, Present and Future of Agroforestry Systems in Europe. In 'Agroforestry - The Future of Global Land Use'. (Eds PKR Nair, D Garrity) pp. 285–312. (Springer: Dordrecht)

Muinga RW, Thorpe W, Topps JH (1992) Voluntary food intake, live-weight change and lactation performance of crossbred dairy cows given ad libitum *Pennisetum purpureum* (napier grass var. Bana) supplemented with leucaena forage in the lowland semi-humid tropics. *Animal Production* **55**, 331–337. doi:10.1017/S0003356100021024

Mullen BF, Gutteridge RC (2002) Wood and biomass production of *Leucaena* in subtropical Australia. *Agroforestry systems* **55**, 195–205.

Mupeyo B, Barry TN, Pomroy WE, Ramírez-Restrepo CA, López-Villalobos N, Pernthaner A (2011) Effects of feeding willow (*Salix* spp.) upon death of established parasites and parasite fecundity. *Animal Feed Science and Technology* **164**, 8–20. doi:10.1016/j.anifeedsci.2010.11.015

Musonda K, Barry TN, McWilliam EL, López-Villalobos N, Pomroy WE (2009) Grazing willow (*Salix* spp.) fodder blocks for increased reproductive rates and internal parasite control in mated hoggets. *Animal Feed Science and Technology* **150**, 46–61. doi:10.1016/j.anifeedsci.2008.08.003

Nair PKR (1991) State-of-the-art of agroforestry systems. *Forest Ecology and Management* **45**, 5–29.

Nerlich K, Graeff-Hönninger S, Claupein W (2013) Agroforestry in Europe: a review of the disappearance of traditional systems and development of modern agroforestry practices, with emphasis on experiences in Germany. *Agroforestry Systems* **87**, 475–492. doi:10.1007/s10457-012-9560-2

Oppong SK, Kemp PD, Douglas GB, Foote AG (2001) Browse yield and nutritive value of two *Salix* species and *Dorycnium rectum* in New Zealand. *Agroforestry systems* **51**, 11–21.

Palmer B, Schlink AC (1992) The effect of drying on the intake and rate of digestion of the shrub legume *Calliandra calothyrsus*. *Tropical Grasslands* **26**, 89–93.

Papachristou TG, Papanastasis VP (1994) Forage value of Mediterranean deciduous woody fodder species and its implication to management of silvo-pastoral systems for goats. *Agroforestry systems* **27**, 269–282.

Papanastasis VP, Yiakoulaki MD, Decandia M, Dini-Papanastasi O (2008) Integrating woody species into livestock feeding in the Mediterranean areas of Europe. *Animal Feed Science and Technology* **140**, 1–17. doi:10.1016/j.anifeedsci.2007.03.012

Papanastasis VP, Mantzanas K, Dini-Papanastasi O, Ispikoudis I (2009) Traditional agroforestry systems and their evolution in Greece. In 'Agroforestry in Europe: Current Status and Future Prospects'. (Eds A Rigueiro-Rodríguez, J McAdam, MR Mosquera-Losada) pp. 89–109. (Springer: Dordrecht)

Peeters A, Beaufoy G, Canals RM, De Vliegher A, Huyghe C, Isselstein J, Jones G, Kessler W, Kirilov A, Mosquera-Losada MR, Nilsdotter-Linde N, Parente G, Peyraud J, Pickert J, Plantureux S, Porqueddu C, Rataj D, Stypinski P, Tonn B, van den Pol – van Dasselaar A, Vintu V, Wilkins RJ (2014) Grassland term definitions and classifications adapted to the diversity of European grassland-based systems. *Grassland Science in Europe* **19**, 743–750.

Pengelly BC, Conway MJ (2000) Pastures on cropping soils: which tropical pasture legume to use? *Tropical Grasslands* **34**, 162–168.

Pitta DW, Barry TN, López-Villalobos N, Kemp PD (2005) Effects on ewe reproduction of grazing willow fodder blocks during drought. *Animal Feed Science and Technology* **120**, 217–234. doi:10.1016/j.anifeedsci.2005.02.030

Pitta DW, Barry TN, López-Villalobos N, Kemp PD (2007) Willow fodder blocks—An alternate forage to low quality pasture for mating ewes during drought? *Animal Feed Science and Technology* **133**, 240–258. doi:10.1016/j.anifeedsci.2006.04.006

Provenza F, Villalba J, Dziba L, Atwood S, Banner R (2003) Linking herbivore experience, varied diets, and plant biochemical diversity. *Small Ruminant Research* **49**, 257–274. doi:10.1016/S0921-4488(03)00143-3

Pulido-Santacruz P, Renjifo LM (2011) Live fences as tools for biodiversity conservation: a study case with birds and plants. *Agroforestry Systems* **81**, 15–30. doi:10.1007/s10457-010-9331-x

Rackham O (2013) Woodland and wood-pasture. In 'Trees, Forested Landscapes and Grazing Animals: A European Perspective on Woodlands and Grazed Treescapes'. (Ed ID Rotherham) pp. 11–22. (Routledge: Abingdon, Oxon)

Rameau J-C, Mansion D, Dumé G (1989) 'Flore forestière française, Guide écologique illustré, Tome 1, Plaines et collines.' (Institut pour le développement forestier: Paris)

Ramírez-Restrepo CA, Barry TN, Marriner A, López-Villalobos N, McWilliam EL, Lassey KR, Clark H (2010) Effects of grazing willow fodder blocks upon methane production and blood composition in young sheep. *Animal Feed Science and Technology* **155**, 33–43. doi:10.1016/j.anifeedsci.2009.10.003

Rangel JH de A, Gardiner CP (2009) Stimulation of wool growth by *Desmanthus* spp. as a supplement in a Mitchell grass hay diet. *Tropical Grasslands* **43**, 106–111.

Salawu MB, Acamovic T, Stewart CS, Hovell FD (1997) Quebracho tannins with or without Browse Plus (a commercial preparation of polyethylene glycol) in sheep diets: effect on digestibility of nutrients *in vivo* and degradation of grass hay in sacco and *in vitro*. *Animal Feed Science and Technology* **69**, 67–78.

Sharrow SH (1999) Silvopastoralism: Competition and Facilitation Between Trees, Livestock, and Improved Grass-Clover Pastures on Temperate Rainfed Lands. In 'Agroforestry in Sustainable Agricultural Systems'. (Eds LE Buck, JP Lassoie, ECM Fernandes) pp. 111–130. (CRC Press: Boca Raton, Florida).

Sharrow SH, Brauer D, Clason TR (2009) Silvopastoral practices. In 'North American Agroforestry: An Integrated Science and Practice'. (Ed HE Garrett) pp. 105–131. (American Society of Agronomy: Madison, WI)

Shelton M, Dalzell S (2007) Production, economic and environmental benefits of leucaena pastures. *Tropical Grasslands* **41**, 174–190.

Smith J, Kuoppala K, Yáñez-Ruiz D, Leach K, Rinne M (2014) Nutritional and fermentation quality of ensiled willow from an integrated feed and bioenergy agroforestry system in UK [Online]. In 'Maataloustieteen Päivät 2014'. (Eds Ed. M Hakojärvi, N Schulman)

Suomen Maataloustieteellisen Seuran julkaisuja No. 30. pp. 1-9. Available at www.smts.fi (verified 8 February 2017)

Smith J, Gerrard C (2015) System Report: Agroforestry for Ruminants in England [Online]. 30 October 2015. Available at http://www.agforward.eu/index.php/en/agroforestry-with-ruminants-uk.html (verified 27 January 2017)

SPW (2010) Tableau de bord de l'environnement wallon 2010. Rapport sur l'état de l'environnement wallon. Service public de Wallonie, Cellule Etat de l'environnement wallon, SPW – DGARNE – DEMNA - DEE (SPW: Jambes)

SPW (2012) Key Environmental Indicators for Wallonia in 2012. Report on the State of the Environment in Wallonia. State of the Environment Directorate, SPW (Service public de Wallonie) - DGO3 - DEMNA - DEE (SPW Editions: Jambes).

SPW (2014) Les Indicateurs Clés de l'Environnement Wallon 2014. Service public de Wallonie, Direction de l'Etat Environnemental, SPW - DGO3 - DEMNA - DEE. (SPW Editions: Jambes).

Thenail C, Viaud V, Hongtao H (2014) Initial Stakeholder Meeting Report: Bocage agroforestry in Brittany, France [Online]. Available at https://www.agforward.eu/index.php/dk/bocage-agroforestry-in-brittany-france.html (verified 8 February 2017)

Thiébault S (2005) L'apport du fourrage d'arbre dans l'élevage depuis le Néolithique. *Anthropozoologica* **40**, 95–108.

Vandenberghe C, Freléchoux F, Moravie M-A, Gadallah F, Buttler A (2007) Short-term effects of cattle browsing on tree sapling growth in mountain wooded pastures. *Plant Ecology* **188**, 253–264. doi:10.1007/s11258-006-9160-1

Vandermeulen S, Ramírez-Restrepo CA, Marche C, Decruyenaere V, Beckers Y, Bindelle J (2016) Behaviour and browse species selectivity of heifers grazing in a temperate silvopastoral system. *Agroforestry Systems*. doi:10.1007/s10457-016-0041-x

Van laer E, Tuyttens FAM, Ampe B, Sonck B, Moons CPH, Vandaele L (2015) Effect of summer conditions and shade on the production and metabolism of Holstein dairy cows on pasture in temperate climate. *Animal* **9**, 1547–1558. doi:10.1017/S1751731115000816

Villalba JJ, Provenza FD, Hall JO, Lisonbee LD (2010) Selection of tannins by sheep in response to gastrointestinal nematode infection. *Journal of Animal Science* **88**, 2189–2198. doi:10.2527/jas.2009-2272

Waghorn GC, Ulyatt MJ, John A, Fisher MT (1987) The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L. *British Journal of Nutrition* **57**, 115–126.

Walloon Government (2015) Decree of 3 September 2015 on the granting of agri-environmental and climatic aids. Belgian Official Journal, 2 October 2015.

Walloon Government (2016) Decree of 8 September 2016 on the granting of aids for planting live fence, linear coppice, orchard and tree alignment, and for maintaining pollards, Belgian Official Journal, 29 September 2016.

CHAPTER III

Chapter 3 evaluates the compatibility of two methods that are commonly used to determine cattle intake: an indigestible marker, the titanium dioxide (TiO_2), and the faecal near-infrared reflectance spectrometry (FNIRS). Once this methodology trial finished, only the FNIRS method was used during the thesis since this method is easy, non-destructive and inexpensive conversely to the TiO_2 marker method which would have required dosing the animals with TiO_2 every day.

Article 2

Compatibility of using TiO_2 and the faecal near-infrared reflectance spectrometry for estimation of cattle intake

Sophie Vandermeulen[1,2], Virginie Decruyenaere[1,3] , Carlos Alberto Ramírez-Restrepo[4] and Jérôme Bindelle[1]

[1]*Gembloux Agro-Bio Tech, Animal Science Unit, University of Liège, 2 Passage des Déportés B-5030, Gembloux, Belgium*

[2]*National Fund for Scientific Research, 5 Rue d'Egmont, B-1000 Brussels, Belgium*

[3]*Walloon Agricultural Research Centre (CRA-W), Production and Sectors Department, 8 Rue de Liroux, B-5030 Gembloux, Belgium*

[4] *CSIRO, Animal, Food and Health Sciences, Australian Tropical Sciences and Innovation Precinct, James Cook University, Building 145, James Cook Drive, 4811 Townsville, QLD, Australia*

Acknowledgments

The authors are grateful to Walloon Agricultural Research Centre for the near-infrared reflectance spectrometry analyses. Sophie Vandermeulen, as a research fellow, acknowledges the support of the National Fund for Scientific Research (Brussels, Belgium).

This article in published in:

Grassland Science in Europe (2014), **19**, 625-627.

1. Abstract

Combining titanium dioxide (TiO_2) as indigestible marker to faecal near-infrared reflectance spectrometry (FNIRS) can be used to determine cattle feed intake and quality of ingested forage if FNIRS spectra are not modified by the marker. This study aimed at determining the compatibility of TiO_2 with FNIRS. Three dry cows were fed a standard hay-based diet for three weeks supplemented with a daily dose of 0.1 % (10 g) TiO_2 during the last two weeks of the experiment. Faeces samples were collected every day and analysed for TiO_2 and FNIRS. Results suggest that TiO_2 did not interfere with FNIRS analyses. The calculations of crude protein, neutral detergent fiber, acid detergent lignin contents, as well as dry matter intake did not change over time with increasing TiO_2 in the faeces ($P > 0.05$). Slight differences observed for other predicted parameters seemed to be independent from TiO_2. The higher Mahalanobis distance (H) for chemical composition ($H = 7.2$) independent from TiO_2 inclusion could indicate that faecal spectra did not correspond exactly to the prediction database. Although 0.1 % incorporation of TiO_2 seem not to interfere with FNIRS measurements, caution must be taken with higher levels of TiO_2 as nothing indicates that interference could not appear.

Keywords

Ruminant, titanium dioxide, faecal near-infrared spectrometry, intake, diet chemical composition.

2. Introduction

Methods used to determine feed intake and quality of consumed forage in grazing cattle are time-consuming, expensive and sometimes controversial in respect to animal welfare as they include different techniques such as sward clipping techniques or oesophageal fistulated animals (Decruyenaere *et al.* 2009). A combination of an indigestible marker to faecal near-infrared reflectance spectrometry (FNIRS) can provide a useful alternative providing that the marker fed daily to the animal does not interfere with FNIRS spectra (Titgemeyer *et al.* 2001; Decruyenaere *et al.* 2012). As previous studies report that chromium oxide (Cr_2O_3) is likely to interfere with NIRS calibration data (Decruyenaere *et al.* 2012), this study investigates if titanium dioxide (TiO_2) used as indigestible marker was compatible with FNIRS analysis.

3. Materials and methods

A three-week experiment was performed on three dry red-pied cows housed in free stalls in the Animal Science Unit of GxABT (Gembloux, Belgium). All cows received 7 kg/d of standard temperate hay and 2 kg/d of a mixed concentrate and had a free access to water. After an adaptation period of one week, 10 g of TiO_2 mixed with 50 ml of molasses was distributed every day to each cow until the end of the experiment. The faeces were collected every day, dried at 60°C, and ground to pass a 1-mm screen prior to TiO_2 and FNIRS analyses. TiO_2 dosage in faeces was performed according Myers *et al.* (2004). The FNIRS analyses were achieved as described by Decruyenaere *et al.* (2012). The chemical composition, dry matter intake (DMI) and *in vivo* organic matter digestibility (OMD) predicted from the FNIRS database were compared daily along the entire experiment using the MIXED procedure of SAS 9.2. with the 'cow×day' as experimental unit. The correlation between these parameters and TiO_2 content in the faeces was calculated with the CORR procedure of SAS 9.2.

4. Results and discussion

Figure 1 shows the evolution of TiO_2 contents in the faeces before and during the daily incorporation of 10 g of TiO_2 in the diet (day 6 being the first day of TiO_2 distribution). During the adaptation period without TiO_2, its faecal content was equal or close to zero before increasing and then reaching a plateau towards the end of the experiment; so the TiO_2 dosage did not face interference problems. Dietary TiO_2 did not interfere with the FNIRS analysis. Prediction of crude protein (CP), neutral detergent fibre (NDF) and acid detergent lignin (ADL) contents as well as the DMI (Figure 2) did not change over time ($P > 0.05$; $P = 0.0723$

for NDF content as the lowest P-value). Despite some changes along days for the acid detergent fibre (ADF, $P = 0.0381$) content and the *in vivo* OMD ($P = 0.0009$) (Figure 2), the slightly different values seemed to appear independently before or after ingestion of titanium dioxide. The DMI ($P = 0.4613$; Figure 2) which seemed more fluctuating along the experiment could be explained by individual differences of intake; for example, the 13th day of the experiment, the DMI of one cow reached 85.3 *vs* 47.5 g/kg metabolic weight (live weight $(LW)^{0.75}$) for another one. The standardized Mahalanobis distance (H) which evaluates the correspondence between the faeces spectra and the FNIRS database should ideally be lower than 3 for an accurate prediction (Shenk and Westerhaus, 1991). For OMD and DMI, the average distance H was below 3 for the DMI and the OMD ($H = 2.8$ and 2.87 respectively; two thirds of the samples being lower than 3) while it reached 7.2 for the chemical composition. This should probably not be due to TiO_2 inclusion in the diet, but rather to a discrepancy between the samples and the calibration dataset.

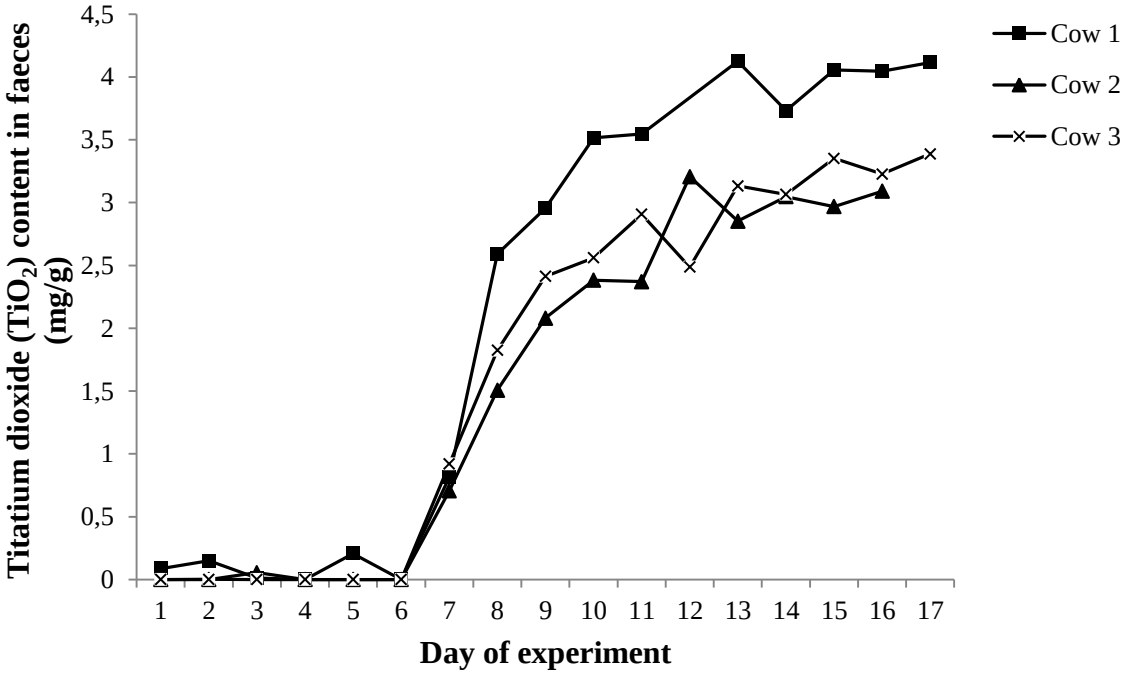

Figure 1. Evolution of titanium dioxide (TiO_2) contents (mg/g) in the faeces of the three cows.

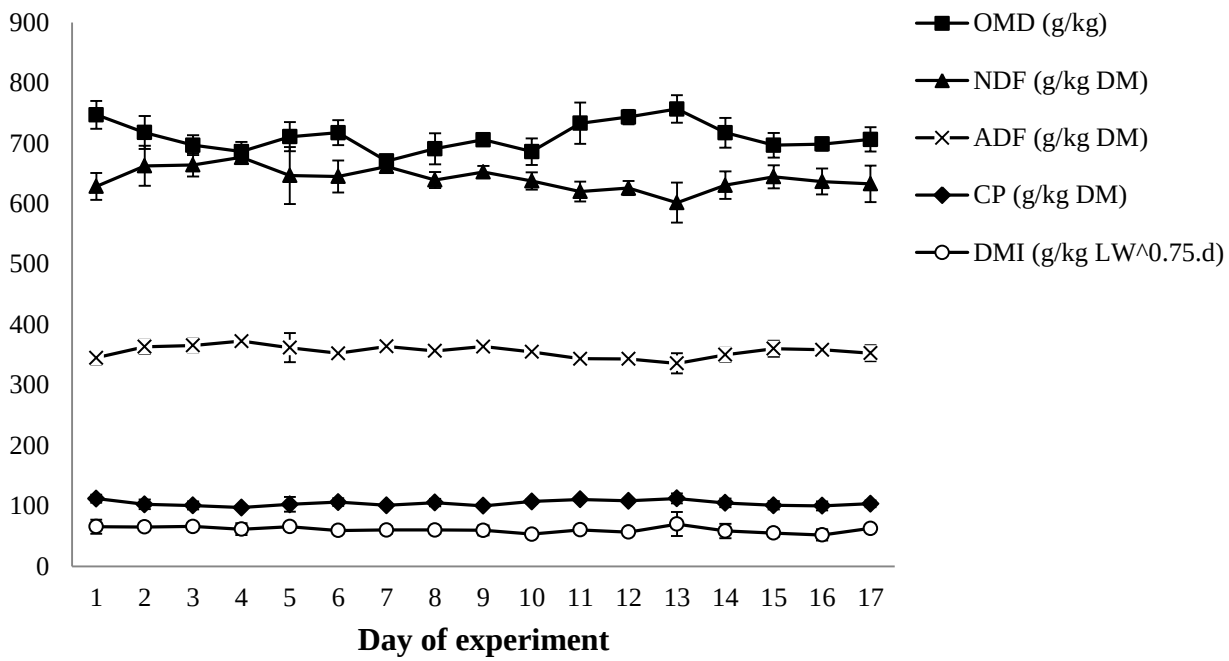

Figure 2. Means and standard deviation of crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents (g/kg DM) of the faeces and the dry matter intake (DMI; g/kg $LW^{0.75}$.d) and *in vivo* organic matter digestibility (OMD; g/kg) predicted by FNIRS along the experiment.

The results of correlation between the titanium dioxide content in the faeces and the parameters predicted by FNIRS should be considered with caution. Indeed most of parameters, as DMI or OMD, were not significatively correlated to the TiO_2 content ($P > 0.05$; $P = 0.1086$ and $r = 0.2321$ for CP as the highest P-value and correlation coefficient (r)) but other parameters were significatively correlated to TiO_2 content as total ashes (TA), NDF and ADF content ($P < 0.0001$ and $r = 0.5865$ for TA).

5. Conclusion

Feeding 10 g/d TiO_2 as indigestible marker in cattle (0.1 % incorporation level) did not interfere with FNIRS prediction. The use of these results should be done with caution as nothing indicates that interference could not appear when higher levels of TiO_2 are incorporated in the diets.

6. References

Decruyenaere V, Buldgen A, Stilmant D (2009) Factors affecting intake by grazing ruminants and related quantification methods: a review. *Biotechnologie, Agronomie, Société et Environnement* **13**, 559–573.

Decruyenaere V, Froidmont E, Bartiaux-Thill N, Buldgen A, Stilmant D (2012) Faecal near-infrared reflectance spectrometry (NIRS) compared with other techniques for estimating the *in vivo* digestibility and dry matter intake of lactating grazing dairy cows. *Animal Feed Science and Technology* **173**, 220–234.

Myers WD, Ludden PA, Nayigihugu V, Hess BW (2004) Technical Note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. *Journal of Animal Science* **82**, 179–183.

Shenk JS, Westerhaus MO (1991) Population definition, sample selection, and calibration procedures for near infrared reflectance spectroscopy. *Crop Science*. **31**, 469–474.

Titgemeyer EC, Armendariz CK, Bindel DJ, Greenwood,RH Löest CA (2001) Evaluation of titanium dioxide as a digestibility marker for cattle. *Journal of Animal Science* **79**, 1059-1063.

CHAPTER IV

Chapter 4 describes a study conducted in a temperate silvopastoral system (Belgium) that aimed at determining the influence of having access to shrubs and trees in a hedgerow on the behaviour of grazing heifers and their selectivity towards the woody species. The first objective was to prove that cattle do browse when they have an access to woody foliage and subsequently to understand what possible animal-, pasture- or shrubs-related drivers could influence this behaviour. The FNIRS method described and analysed in the previous article has been used during this experiment to quantify intake and diet quality of the grazing and browsing animals.

Article 3

Behaviour and browse species selectivity of heifers grazing in a temperate silvopastoral system

Sophie Vandermeulen[1,2], Carlos Alberto Ramírez-Restrepo[3], Christian Marche[4], Virginie Decruyenaere[5], Yves Beckers[1], Jérôme Bindelle[1]

[1]*University of Liège, Gembloux Agro-Bio Tech, Precision Livestock and Nutrition Unit, 2 Passage des Déportés, 5030 Gembloux, Belgium*

[2]*Research Foundation for Industry and Agriculture, National Scientific Research Foundation (FRIA-FNRS), 5 Rue d'Egmont, 1000 Bruxelles, Belgium*

[3]*CSIRO Agriculture, ATSIP, James Cook University, 145 James Cook Drive, Townsville, QLD 4811, Australia*

[4]*Centre des Technologies Agronomiques, 16 Rue de la Charmille, 4577 Strée, Belgium*

[5]*Walloon Agricultural Research Centre, Production and Sectors Department, 8 Rue de Liroux, 5030 Gembloux, Belgium*

Email : vandermeulen.sophie@gmail.com, phone: +3281622609, fax: +3281622115

Acknowlegments

The expert technical assistance of the staff of the Centre des Technologies Agronomiques is gratefully acknowledged. Dr Robert D. Kinley from CSIRO (Townsville, Australia) is thanked for his proofreading. Sophie Vandermeulen was funded by the Research Foundation for Industry and Agriculture (FRIA)-National Fund for Scientific Research (F.R.S.-FNRS).

This paper is adapted from the original article that has been accepted (2016) for publication in *Agroforestry Systems:*

http://link.springer.com/article/10.1007/s10457-016-0041-x

1. Abstract

In Belgium silvopastoral grazing systems associating trees and pasture are instrumental in enhancing dynamic trade-offs between ruminant farming and habitat improvement. A 130-day study was conducted in Belgium from April to September 2013 to evaluate the effects of browsing a combination of shrubs and trees (i.e. hedge) on the selective behaviour of cattle and to relate these observations to changes in forage nutritive value. Twelve Holstein dairy heifers (*Bos taurus*; 487 kg) were allocated to either a control ryegrass pasture (i.e. control pasture group; CPG) or a pasture plus unrestricted browsing (i.e. browsing group; BG) of a hedge composed of shrubs and trees. Behaviour and selectivity towards the woody species were recorded for 14h on a daily basis during 3 consecutive days over spring, early summer and late summer. Leaves and stems of woody species and faecal samples were collected during each season to analyse their nutritive value and predict the dry matter intake by means of near infrared reflectance spectroscopy. Integrating shrubs and trees along a pasture influenced the heifers' behaviour and BG heifers spent 19.3, 5.9 and 5.4% of their time browsing during spring, and early and late summer, respectively ($P < 0.001$). This behaviour was correlated to the pre-grazing pasture biomass ($r = 0.50$; $P < 0.001$). Compared with the summer seasons, the greater browsing activity in spring was associated with higher plant feeding value. Overall, the most ingested species were *Carpinus betulus, Cornus sanguinea, Corylus avellana* and *Crataegus monogyna.* It was concluded that cattle use a significant time budget for browsing on temperate ryegrass pasture but further research is required to investigate potential benefits of silvopastoral systems in Belgium.

Keywords

Agroforestry, ruminant, woody fodder, dietary preference, feeding value, NIRS.

2. Introduction

Browsing woody forage by ruminants is a common practice during the dry season in the tropics (Lefroy *et al.* 1992; Liagre 2006), in high mountains (Vandenberghe *et al.* 2007), and in the Mediterranean region (Le Houérou 2006; Papanastasis *et al.* 2008). Various reasons motivate ruminants to browse shrub and tree forage (STF), including strong seasonal shortages of herbaceous forage biomass (Liagre 2006; Lefroy *et al.* 1992) or low quality grass supplementation (Hirata *et al.* 2008; Leng 1992). Trees and shrubs with grasses growing underneath (i.e. silvopastoral system) supply energy, protein and other nutrients (Lefroy *et al.* 1992). They also provide shelter (Liagre 2006), improve livestock productivity (Murgueitio *et al.* 2011; Paciullo *et al.* 2011), offspring survival (Liagre 2006; Pollard 2006) and they can reduce internal parasite infestation (Nguyen *et al.* 2005; Ramírez-Restrepo *et al.* 2010a). Condensed tannins (CT)-forage legumes (i.e. herbaceous and trees) have been demonstrated to induce in sheep increased reproductive efficiency (Ramírez-Restrepo *et al.* 2005a), reproductive rate (Pitta *et al.* 2005), lamb growth and wool production (Ramírez-Restrepo *et al.* 2004, 2005a). They can lead to the expansion of immune cells subsets (Ramírez-Restrepo *et al.* 2010a), while lowering nematode parasite fecundity (Mupeyo *et al.* 2011), parasite counts, anthelmintic drenching (Ramírez-Restrepo *et al.* 2005b), and methanogenesis (Ramírez-Restrepo *et al.* 2010b).

In Belgium, trees and shrubs have been withdrawn from production systems during agricultural intensification (Nerlich *et al.* 2013). However, they are promoted again through the establishment of hedges and woody strips into pasture owing to new European agri-environmental policies (Walloon Government 2014). Unfortunately, little is known about cattle browsing behaviour under these conditions and its effects on nutrition and productivity. Consequently, farmers do not take advantage of the potential benefits of this forage resource. The first objective of this study was to measure the grazing behaviour of Holstein heifers in presence of a hedge composed of 11 temperate browse species planted along a mixed *Lolium perenne* (ryegrass)/*Poa trivialis* (rough meadow grass) pasture and to determine which woody species are consumed. The second objective was to determine if the behaviour and browsing selectivity of woody species are associated with the changes in nutritive value of the silvopastoral forage components throughout the seasonal periods.

3. Materials and methods

3.1. Location and experimental treatments

A grazing experiment was conducted at the Centre des Technologies Agronomiques (CTA), Strée, Belgium (50° 30' N, 18° 6' E) between 29 April and 5 September (130 days) of 2013. The experiment was designed and conducted following animal ethics guidelines issued by the Belgian Government. Twelve Holstein bred dairy heifers (*Bos taurus*) were weighed, balanced and randomly allocated to control pasture group (CPG; 1 ha grazing pasture; n=6, 489 ± 41 kg live weight (LW), mean ± SD) and browsing group (BG; 1 ha grazing pasture and browsing, n=6, 485 ± 67 kg). Heifers grazed for the first time (i.e. first year of grazing) during this experiment. Heifers had access to water *ad libitum* throughout the study. Pasture in both treatments was dominated by perennial ryegrass (*Lolium perenne*), rough meadow grass (*Poa trivialis*), dandelion (*Taraxacum* spp.) and white clover (*Trifolium repens*). Previously during the spring of 2005 the hedge was planted with 11 three-shrub species (i.e. woody forage). They were: *Acer campestre*, *A. pseudoplatanus*, *Carpinus betulus*, *Cornus sanguinea*, *Corylus avellana*, *Crataegus monogyna*, *Fraxinus excelsior*, *Populus nigra*, *Quercus robur*, *Robinia pseudoacacia* and *Sambucus nigra.* Shrubs and trees were pruned each year at 2.5 m high as a period maintenance.

The experiment was conducted in late spring (May), early summer (July) and late summer (September) and behaviour was monitored during 3 to 4 consecutive measurement days in each time block. An adaptation period of 14 days was implemented preceding measurement periods, and the heifers were allocated into a pasture with similar characteristics. At the initiation of the experimental periods, animals were weighed and moved to the experimental paddocks and behaviour measurements started 3 to 4 days later for 3 to 4 days (3 days per group). Once observations were finished, animals were weighed and maintained as a single grazing herd between measurement periods. The same groups of heifers allocated respectively to treatments BG and CPG in spring were kept in early and late summer. In total, for each season, animals were kept 20 to 22 days in separate experimental groups (i.e. BG and CPG treatments) and they were kept together 27 to 35 days between measurement periods. The experimental paddocks were the same during the whole experiment. Paddock rotation was performed to allow a resting period of 20 to 30 days according to the season. Lower paddock biomass results from low rainfall in July and September and subsequent shorter resting period compared to that in May. At the end of the

first season, the maximum height at which heifers browsed was measured at 15 sites along the hedge.

Weather data were collected daily by CTA. The monthly average temperature was 10.8, 19.6 and 14.2°C, and average rainfall was 36.3, 17.7 and 19.4 mm in spring, early and late summer, respectively.

3.2. Plant measurements

Pre-grazing pasture biomass was determined with a rising plate meter (RPM; 30*30*1.5 cm^3 aluminium plate 4.05 kg/m^2) deployed in a zigzag pattern with minimum 33 measurements in each paddock using an in-house calibration curve (Sanderson *et al.* 2001). Briefly, the rising plate meter was calibrated on 62 quadrats (0.090 m^2) cut to 1-cm above ground level along seven transects of 8 to 10 quadrats each taken on the same pasture. Grass samples were dried using a forced-air oven at 60°C for at least 72 h, in order to link the herbage height to the dry matter (DM) biomass. The calibration equation was: $y = 646.45 + 255.47\ x$, where y is herbage mass (kg DM/ha); x is herbage height (cm); $n = 62$ and $r^2 = 0.87$.

The pasture botanical composition was determined in spring before starting the measurement period, using 132 quadrats (0.090 m^2) set every 10 m along four transects (32 to 34 quadrats each) following the dry-weight rank method of Nijland (2000). The three most abundant species in each quadrat were classified as highest to lowest biomass by ranking from 1 to 3 according to their biomass contribution on a dry-weight basis. For each species, the sum for rank 1, 2 and 3 were multiplied by 3, 2 and 1, respectively to obtain the dominance percentage. The proportion of STF species in the hedge was measured by dividing the number of individuals by the total number of shrubs and trees present.

Before each measurement period, samples of mixed pasture forage (n=6; 3 in each paddock), perennial ryegrass (n=3) and white clover (n=3) were collected. Leaves and petioles of 3 individuals per woody species (n=3) were also sampled separately in the hedge. Pasture and woody forage samples were stored at -18°C until laboratory analyses. *Robinia pseudoacacia* was not collected because of its invasiveness (i.e. non-endemic species in Belgium; Halford *et al.* 2011).

3.3. Animal measurements

The heifers' behaviours in each group and season were recorded by two observers from 0600 to 2000h over 3 consecutive days. In the first morning, each observer was assigned to either

CPG or BG and they shifted groups on a daily basis to avoid bias. Each heifer was observed during 1 min every 20 min and her activity recorded classified (Table 4). Each behaviour activity was expressed as the proportion of the time spent displaying the behaviour relative to the total time. This was calculated per animal and per day for each measurement period. The woody species consumed were recorded to estimate the proportion of the species and selectivity of the browsed diet. Selectivity was defined as the proportion of a given woody species in the browsed diet relative to its proportion in the hedge.

The selectivity index of Jacobs (1974) was determined as follows:

$$S_i = \frac{(D_i - H_i)}{(D_i + H_i - 2D_iH_i)}$$

Where D_i is the proportion of the woody species in the browsed diet (between 0 and 1); H_i is the proportion of the species in the hedge (between 0 and 1) and the selectivity (S_i) can vary between -1 and 1. H_i proportion represents the STF availability for heifers; it is based on the number of STF in the hedge but not its actual biomass.

The total time for forage intake corresponded to grazing for the CPG and to summing up grazing and browsing behaviours for the BG (Table 4). For rumination and resting behaviours, the position (standing or lying) was recorded while total rumination and total rest were obtained by summing up the time spent in standing and lying position.

Faeces samples from fresh spontaneous emissions were collected from all heifers at the end of each measurement period and stored at -18°C until laboratory analysis.

Table 4. Description of the recorded behavioural activities.

Behaviour category	Description
Grazing	Eating herbaceous species on the pasture
Browsing	Eating ligneous species in the hedge
Search for food	Searching for food with the head downwards (grazing) or upwards (browsing) without ingestion
Rumination (standing/lying)	Chewing with the specific regular pattern for rumination, either lying or standing
Resting (standing/lying)	The heifer is still with open or closed eyes (standing or lying) or with the head backwards (lying)
Social activity	Playing, fighting or mounting another animal
Grooming	Licking oneself or another heifer
Walking	Walking or running (without eating/searching for food)
Drinking	Drinking at the common trough
Other activity	Any other activity not described before

3.4. Laboratory analyses

Forage and faeces samples were freeze-dried (min pressure 0.630 mbar; Delta 1-24 LSC, Martin Christ, Osterode, Germany) and ground to pass a 1mm mesh sieve (Cyclotec 1093 Sample Mill, FOSS Electric, Hillerød, Denmark) before being analysed by means of near infrared reflectance spectroscopy (NIRS) using a XDS monochromator spectrometer system (FOSS Electric, Hillerød, Denmark). The absorption data was recorded as log 1/R from 1100 to 2498 nm, every 2 nm (WINISI 1.5, FOSS Tecator Infrasoft International LCC, Hillerød, Denmark). The NIRS system used the equations of Meuret *et al.* (1993), Decruyenaere *et al.* (2009) and Decruyenaere *et al.* (2015) for the forage chemical composition (Organic matter (OM), crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL)) and the *in vitro* organic matter digestibility (IVOMD; i.e. based on a pepsin-cellulase method); the chemical composition of the faeces (OM, CP, NDF, ADF and ADL) and DM intake (DMI; g/kg $LW^{0.75}$.d) and *in vivo* OMD, respectively.

3.5. Statistical analysis

Data were analysed using fixed linear models in the MIXED procedure of SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

Pre-grazing herbage mass on pasture, faeces chemical composition, predicted DMI and OMD were compared between experimental groups (i.e. CPG or BG), seasons (i.e. late spring, early and late summer) and their interaction. Forage chemical composition and IVOMD considered the effects of forage type or species (i.e. herbaceous pasture, woody shrubs and trees), seasons and their interaction.

Differences in LW and behaviours were assessed considering the effects of seasons, groups and their interaction. For rumination and resting, the position (i.e. standing or lying) effect was also considered. Browsing was only compared across seasons. The proportion of woody species composing the browsed diet was compared using the effects of shrub and tree species, the seasons and their interaction. To explore inter-individual difference, the proportion of each species in the browsed diet considered the effects of heifers (i.e. 1 to 6), woody species and their interactions, while FNIRS predictions (i.e. faeces chemical composition, DMI and OMD) considered the effect of heifers. Correlation coefficients were obtained using the CORR procedure in SAS. Least square means were declared significantly different at $P \leq 0.05$ and tending to differ when $P \leq 0.10$.

4. Results

4.1. Pasture botanical composition and biomass

The CPG paddock was composed (%) of *Lolium perenne* (41.7), *Poa trivialis* (26.4), *Taraxacum* spp. (20.2), *Trifolium repens* (4.7) and other species (7.0), whilst following the same botanical order, the BG paddock comprised 46.2, 22.5, 23.2, 5.1 and 3.0%, respectively.

Irrespective of the treatment, pre-grazing herbage mass decreased from spring to summer ($P < 0.001$). In late spring, early and late summer 2025, 1863 and 1398 kg DM/ha, respectively, were recorded. However, the biomass tended ($P = 0.06$) to be marginally more abundant in the CPG paddock than in the BG paddock.

4.2. Chemical composition and in vitro organic matter digestibility of pasture and hedge fodder

Chemical composition and IVOMD of pasture and hedge forage varied between forage plants and seasons (Table 5; $P < 0.01$). Across woody plants, averaged CP decreased ($P < 0.001$) from spring to early and late summer (178, 142 and 133 g/kg DM, respectively) but was similar for pasture forage ($P > 0.05$). Averaged NDF (280, 320 and 317 g/kg DM), ADF (122, 165 and 165 g/kg DM) and ADL (56, 84 and 89 g/kg DM) concentrations in woody foliage increased between spring and late summer ($P < 0.001$), while the pasture ADF concentration was higher in spring (219 g/kg DM; $P < 0.01$) than in early (206 g/kg DM) and late summer (209 g/kg DM). The pasture NDF concentration was lower in early summer (399 g/kg DM; $P < 0.01$) than in spring and late summer (419 vs 427 g/kg DM).

The ADL concentration of STF was higher than that of pasture plants (77 *vs* 27 g/kg DM; $P < 0.001$), whilst NDF concentration in pasture forage was higher than STF (427 *vs* 317 g/kg DM; $P < 0.001$).

On average woody forage had higher IVOMD than pasture plants in spring (0.898 *vs* 0.840 g/kg DM; $P < 0.01$), but the opposite was observed in early (0.721 *vs* 0.872*;* $P < 0.001$) and late (0.699 *vs* 0.799; $P < 0.01$) summer. These values show that the overall STF IVOMD decreased over time ($P < 0.001$) while it was the highest in early summer for pasture ($P < 0.01$).

Table 5. Chemical composition (g/kg DM) and *in vitro* organic matter digestibility (IVOMD) of browse species in the experimental hedge and browsing group (BG) pasture samples at each season (n=3).

	OM	CP	CF	NDF	ADF	ADL	IVOMD
Early spring							
Herbaceous species - BG	906^{ef}	186^{cde}	192^{a}	424^{a}	220^{abc}	26^{qr}	0.836^{ghi}
Woody species†	910	178	135	280	122	56	0.898
A. campestre	905^{ef}	194^{c}	116^{lmn}	323^{hi}	101^{op}	60^{klm}	0.895^{cdef}
A. pseudoplatanus	911^{def}	194^{bc}	165^{cdefg}	322^{hi}	128^{n}	48^{mno}	0.954^{ab}
C. betulus	912^{cdef}	182^{cdef}	103no	307^{ij}	73^{r}	35^{pqr}	0.923^{abc}
C. sanguinea	883^{gh}	179^{cdefgh}	111^{mn}	269^{klmn}	118^{n}	47^{nop}	0.841^{ghi}
C. avellana	910^{def}	188^{cd}	150^{ghi}	282^{klm}	149^{lm}	67^{ijk}	0.853^{efghi}
C. monogyna	924^{bcd}	121^{jklmn}	136^{ij}	210^{p}	128^{n}	104^{abcd}	0.872^{defg}
F. excelsior	919^{cde}	200^{bc}	155^{efg}	259^{mno}	150^{lm}	63^{jk}	0.896^{cde}
P. nigra	918^{cde}	177^{cdefgh}	165^{cdef}	287^{kl}	172^{ijk}	74^{ghij}	0.869^{defgh}
Q. robur	913^{cdef}	196^{bc}	119^{klm}	303^{ij}	78^{qr}	26^{qr}	0.966^{a}
S. nigra	904^{ef}	152^{efghij}	131^{kl}	238^{o}	120^{n}	37^{opq}	0.910^{bcd}
Early summer							
Herbaceous species - BG	915^{cde}	206^{bc}	159^{efg}	389^{cdef}	198^{efg}	24^{r}	0.887^{cdefg}
Woody species†	911	142	147	320	165	85	0.721
A. campestre	907^{ef}	126^{ijklm}	154^{fgh}	365^{fg}	183^{ghij}	98^{bcd}	0.639^{pq}
A. pseudoplatanus	899^{f}	122^{ijklmn}	165^{cdefg}	396^{bcde}	175^{hijk}	76^{ghi}	0.667^{opq}
C. betulus	915^{cde}	155^{defghij}	110^{mn}	339^{gh}	113no	64^{ijk}	0.750^{klmn}
C. sanguinea	867^{ij}	119^{klmn}	89^{op}	282^{klm}	93^{pq}	51^{lmn}	0.756^{klmn}
C. avellana	912^{def}	97^{mn}	137^{ij}	295^{jk}	167^{k}	94de	0.674^{op}
C. monogyna	928^{abc}	110^{klmn}	134^{jk}	269^{klmn}	149^{lm}	110^{a}	0.770^{k}
F. excelsior	919^{cde}	148^{fghij}	164^{defg}	260^{lmno}	165^{kl}	74^{ghij}	0.769^{kl}
P. nigra	911^{def}	177^{cdefgh}	189^{ab}	341^{gh}	224^{ab}	93^{def}	0.708^{no}
Q. robur	942^{a}	106^{klmn}	162^{defg}	379^{ef}	189^{fgh}	109^{ab}	0.621^{q}
S. nigra	911^{def}	259^{a}	169^{cde}	272^{klmn}	187^{fghi}	80^{gh}	0.855^{efghi}
Late summer							
Herbaceous species - BG	912^{cdef}	184^{cdef}	184^{ab}	432^{a}	210^{bcde}	31^{qr}	0.783^{jk}
Woody species†	902	133	140	317	165	89	0.699
A. campestre	905^{ef}	93^{mn}	139^{hij}	341^{gh}	168^{jk}	107^{abc}	0.642^{pq}
A. pseudoplatanus	878^{hi}	97^{mn}	128^{kl}	384^{def}	148^{m}	83^{efg}	0.640^{pq}
C. betulus	898^{f}	138^{ijklm}	107^{mn}	328^{hi}	126^{n}	68^{hijk}	0.718^{lmno}
C. sanguinea	852^{j}	123^{ijklmn}	86^{p}	257^{mno}	100^{op}	61^{kl}	0.763^{klm}
C. avellana	910^{def}	89^{n}	130^{kl}	306^{ij}	168^{k}	95^{cd}	0.644^{pq}
C. monogyna	919^{cde}	111^{klmn}	131^{k}	295^{k}	159^{klm}	112^{a}	0.717^{mno}
F. excelsior	904^{ef}	145^{ghijk}	157^{efg}	253no	163^{klm}	79^{gh}	0.758^{klmn}
P. nigra	908^{ef}	157^{defghi}	188^{ab}	346^{gh}	231^{a}	10^{abcd}	0.654^{pq}
Q. robur	937^{ab}	144^{hijk}	175^{cbd}	389^{cdef}	203^{def}	104^{abcd}	0.638^{pq}
S. nigra	905^{ef}	230^{ab}	155^{efg}	272^{klmn}	188^{fghi}	81.2^{fg}	0.819^{hij}

SEM	1.77	4.44	2.89	5.89	4.21	2.83	0.010
P-value							
Forage	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Season	0.003	< 0.001	0.023	< 0.001	< 0.001	< 0.001	< 0.001
Forage×Season	0.002	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

ADL, acid detergent lignin; ADF, acid detergent fiber; BG, browsing group; CF, crude fiber; CP, crude protein; DM, dry matter; IVOMD, *in vitro* organic matter digestibility; NDF, neutral detergent fiber; OM, organic matter; SEM, standard error of the mean.

Within a column, values with different letters are significantly different ($P < 0.05$).

[†]Average of woody species was not included in statistical analysis.

4.3. Faecal chemical composition, in vivo organic matter digestibility and dry matter intake of the diet

Faecal chemical composition, DMI and *in vivo* OMD data are summarized in Table 6. Overall, the faecal NDF content was higher in late summer (468 g/kg DM; $P < 0.001$) than in spring (404 g/kg DM) and early summer (393 g/kgDM), while CP content was greater in early summer (203 g/kg DM; $P < 0.001$) compared to spring (173 g/kg DM) and late summer (160 g/kg DM). Faecal ADL content was higher ($P < 0.001$) for the BG than the CPG (119 and 98 g/kg DM, respectively). Although OMD did not differ between groups, the overall OMD decreased with time (spring, 0.780; early summer, 0.749 and late summer, 0.720; $P < 0.001$). Across seasons, predicted DMI for CPG heifers was higher than for BG heifers (91 *vs* 79 g/kg $LW^{0.75}$.d; $P < 0.01$). No differences on the above parameters were found between heifers ($P > 0.05$).

4.4. Live weight

Body weight increased with time over the grazing season ($P < 0.01$). Compared to the CPG heifers, BG had similar LW during spring (479 *vs* 479 kg), early (496 *vs* 497 kg) and late summer (535 *vs* 539 kg).

Table 6. Chemical composition (g/kg DM) of the faeces, dry matter intake (g/kg $LW^{0.75}$.d) and *in vivo* organic matter digestibility estimated by NIRS for both groups of heifers (n=6).

	Late spring		Early summer		Late summer		SEM	*P-value*		
	BG	CPG	BG	CPG	BG	CPG		Group	Season	Group×Season
Chemical composition										
OM	784^{ab}	685^{c}	803^{a}	795^{ab}	773^{ab}	763^{b}	7.89	< 0.001	< 0.001	0.001
CP	179^{b}	166^{bc}	206^{a}	200^{a}	157^{c}	162^{c}	3.91	0.301	< 0.001	0.342
NDF	417^{b}	392^{b}	385^{b}	402^{b}	474^{a}	463^{a}	7.85	0.558	< 0.001	0.307
ADF	300^{ab}	222^{c}	229^{c}	226^{c}	302^{a}	272^{b}	6.94	< 0.001	< 0.001	0.007
ADL	136^{a}	95^{c}	99^{c}	93^{c}	123^{a}	107^{bc}	3.62	< 0.001	0.005	0.031
DMI	66^{c}	75^{c}	97^{ab}	107^{a}	73^{c}	91^{b}	3.14	0.005	< 0.001	0.615
OMD	0.789^{a}	0.770^{ab}	0.746^{bcd}	0.752^{bc}	0.717^{d}	0.723^{cd}	0.006	0.783	< 0.001	0.451

ADF, acid detergent fiber; ADL, acid detergent lignin; BG, browsing group; CP, crude protein; CPG, control pasture group; DM, dry matter; DMI, diet dry matter intake; LW, live weight; NDF, neutral detergent fiber; OM, organic matter; OMD, diet *in vivo* organic matter digestibility; SEM, standard error of the mean.

Within a row, values with different letters are significantly different ($P < 0.05$).

4.5. Heifers behaviour

The BG heifers browsed throughout the duration of the experiment (Table 7). Time spent grazing varied between heifers groups and seasons ($P < 0.05$). Irrespective of the season, the CPG animals spent more time grazing than their counterparts ($P < 0.05$). Across seasons, grazing time increased ($P < 0.001$), while browsing time decreased ($P < 0.001$). Browsing behaviour was correlated to the pre-grazing herbage mass on pasture ($r = 0.50$; $P < 0.001$). The maximum browsing height reached by the BG was 2.14 ± 0.07 m. Total time for forage intake was lower ($P < 0.05$) in the CPG (49.6 %) than in the BG (53.2 %). In spring, CPG ingested for a shorter time (44.4 %; $P < 0.05$) than the BG (50.7 %) but this difference was not significant in both summer seasons (49.1 *vs* 50.8 % and 55.2 *vs* 58.0 % in early and late summer respectively; $P > 0.05$). The average intake increased from spring to late summer (47.5, 50.0 and 56. 6%; $P < 0.001$). In both groups, rumination time was influenced by the season ($P < 0.05$; Table 7). The CPG heifers (16.6 %) ruminated longer than the BG (13.5 %; $P < 0.01$). In early summer, rumination time was higher in the CPG than the BG ($P < 0.001$) while it was similar in spring and late summer ($P > 0.05$). The cumulated time for intake, searching for food and rumination was higher in the BG (61.6 %; $P < 0.05$) than in the CPG (54.9 %) in spring while it was similar during the summer months ($P > 0.05$).

Resting time was higher ($P < 0.05$) in spring (33.0 %) than in early (23.6 %) and late summer (22.0 %). BG heifers rested on average longer in lying position (16.7 %; $P < 0.01$) than CPG heifers (14.1 %), whilst the difference was the highest in early summer (18.4 *vs* 13.1 %). Social, grooming, drinking and all other activities were influenced neither by the treatment group nor by the season ($P > 0.05$).

4.6. Browsing selectivity

Each browse species was consumed at least once along the experimental programme (Table 8; $P < 0.001$), except *R. pseudoacacia*. Across seasons, *C. monogyna* represented the highest proportion of the browsed diet ($P < 0.01$). On average, *C. monogyna* (0.472), *C. avellana* (0.203), *C. betulus* (0.129), and *C. sanguinea* (0.101) accounted for the greatest proportion of the browsed plants ($P < 0.001$).

Table 7. Percentage of time spent displaying a behaviour in animals grazing a pasture with (browsing group; BG) and without (control pasture group; CPG) access to a hedge composed of shrubs and trees during the grazing season of 2013 (n=18).

	Late spring		Early summer		Late summer		SEM	*P-value*		
	BG	CPG	BG	CPG	BG	CPG		Group	Season	Group×Season
Grazing	31.4^{d}	44.4^{c}	44.9^{c}	49.1^{bc}	52.6^{ab}	55.2^{a}	1.11	< 0.001	< 0.001	0.030
Browsing	19.3^{a}	NA	5.9^{b}	NA	5.4^{b}	NA	1.11	NA	< 0.001	NA
Searching for food	1.7^{a}	1.5^{ab}	0.3^{c}	0.8^{bc}	0.8^{bc}	0.3^{c}	0.12	0.768	< 0.001	0.186
Rumination (total)	9.2^{d}	9.0^{d}	17.4^{b}	24.3^{a}	13.8^{c}	16.6^{bc}	0.71	0.003	< 0.001	0.018
Resting (total)	29.7^{b}	36.4^{a}	25.9^{bc}	21.3^{c}	21.6^{c}	22.4^{c}	0.92	0.538	< 0.001	0.013
Social activities	0.5	0.1	0.3	0.4	0.0	0.2	0.06	0.552	0.210	0.214
Grooming	1.2	1.1	1.6	1.0	1.7	1.5	0.14	0.335	0.456	0.742
Walking	6.6^{a}	6.0^{a}	2.5^{b}	2.4^{b}	2.9^{b}	2.4^{b}	0.29	0.394	< 0.001	0.898
Drinking	0.3	1.2	0.8	0.8	1.1	1.5	0.14	0.120	0.185	0.386
Other activities	0.1	0.3	0.3	0.0	0.0	0.0	0.04	0.432	0.104	0.072

BG, browsing group; CPG, control pasture group; NA, not applicable; SEM, Standard error of the mean.

Within a row, values with different letters are significantly different ($P < 0.05$).

In spring, heifers showed a greater preference for *C. monogyna, C. betulus* and *C. avellana* (Table 8). *C. sanguinea* was selected more in early and late summer while *A. pseudoplatanus* and *P. nigra* were only selected during these seasons. Independent of seasons only *C. sanguinea* (0.545), *C. avellana* (0.149) and *C. monogyna* (0.144) were on average always positively selected.

The proportion of STF species in the browsed diet varied between heifers ($P < 0.001$; data not shown). Out of 11 species composing the hedge, *C. avellana*, *C. betulus*, *C. monogyna* and *C. sanguinea* were browsed by all heifers, while *Q. robur* and *P. nigra* were only chosen by two heifers. One heifer ate all the species, except *R. pseudoacacia*, whilst 2 other heifers browsed only 4 species. One of these 2 heifers consumed mainly *C. monogyna* (0.710) and less *C. avellana* (0.040) while these species ranged respectively from 0.407 to 0.486 and 0.172 to 0.302 in the other heifers' diet.

Table 8. Proportions of woody species in the browsed diet and in the hedge and selectivity index during the 3 seasons of feeding behaviour observations in 2013 (n=18).

	Proportion in heifers' diet	Proportion in the hedge	Jacob's selectivity index
Late spring			
A. campestre	0.026^{fgh}	0.026	-0.011
A. pseudoplatanus	0.015^{gh}	0.026	-0.292
C. betulus	0.206^{cd}	0.136	0.245
C. sanguinea	0.019^{gh}	0.018	0.028
C. avellana	0.195^{cd}	0.158	0.128
C. monogyna	0.534^{a}	0.399	0.266
F. excelsior	0^{h}	0.123	-1
P. nigra	0^{h}	0.031	-1
Q. robur	0.003^{h}	0.009	-0.482
R. pseudoacacia	0^{h}	0.066	-1
S. nigra	0.003^{h}	0.009	-0.502
Early summer			
A. campestre	0.028^{fgh}	0.026	0.028
A. pseudoplatanus	0.038^{fgh}	0.026	0.190
C. betulus	0.083^{efg}	0.136	-0.271
C. sanguinea	0.132^{de}	0.018	0.790
C. avellana	0.188^{cd}	0.158	0.105
C. monogyna	0.488^{a}	0.399	0.178
F. excelsior	0.028^{fgh}	0.123	-0.657
P. nigra	0.016^{gh}	0.031	-0.332
Q. robur	0^{h}	0.009	-1
R. pseudoacacia	0^{h}	0.066	-1
S. nigra	0^{h}	0.009	-1
Late summer			
A. campestre	0.020^{gh}	0.026	-0.149
A. pseudoplatanus	0.012^{gh}	0.026	-0.388
C. betulus	0.098^{ef}	0.136	-0.186
C. sanguinea	0.152^{cde}	0.018	0.818
C. avellana	0.225^{c}	0.158	0.214
C. monogyna	0.394^{b}	0.399	-0.010
F. excelsior	0.043^{fgh}	0.123	-0.512
P. nigra	0.049^{fgh}	0.031	0.239
Q. robur	0.8^{gh}	0.009	-0.022
R. pseudoacacia	0^{h}	0.066	-1
S. nigra	0^{h}	0.009	-1
SEM	0.008	NA	Not determined
P-value			
Species	< 0.001	NA	Not determined
Season	1.000	NA	Not determined
Species×Season	0.003	NA	Not determined

NA, not applicable; SEM, standard error of the mean.

Within a column, values with different letters are significantly different ($P < 0.05$).

5. Discussion

This study aimed to assess the selective behaviour of heifers towards woody species on pasture and to relate their behaviour to the evolution of forage nutritive value over the grazing season. The main finding was that heifers browsed woody forage throughout the entire grazing season, from May to September ($P < 0.001$). They spent between 9 and 38 % of the observed intake time browsing the hedge. However, the constant daily observation period (i.e. 6AM-8PM) should be considered when comparing heifer's behaviour between seasons. The nocturnal behaviours were not recorded here and changes in day/night length between seasons might have interfered with the heifers' behaviour. *Carpinus betulus, C. sanguinea, C. avellana, C. monogyna* were the most browsed plants (proportion of the browsed diet of 0.129, 0.101, 0.203 and 0.472, respectively). Previous studies reported that cattle rely on STF when the availability of the herbaceous stratum declines, for example during prolonged droughts (Lefroy *et al.* 1992; Katjiua and Ward 2006; Liagre 2006). Interestingly, in the present study, the interaction did not follow the same pattern because heifers browsed for longer periods when pasture availability was greatest in late spring ($r = 0.50$; $P < 0.001$). Heifers browsed 19.3, 5.9 and 5.4 % of their time in spring, early and late summer, respectively, while the pre-grazing biomass on pasture was 2025, 1863 and 1398 kg DM/ha respectively. This probably emphasises heifers' ability to adapt to environmental changes and include those that functionally interact with selective browsing behaviour such as specific nutritional, sanitary or hedonic requirements, rather than merely by forage availability. For example, STF have been shown to supplement low quality herbage throughout the year, notably by providing highly digestible biomass, protein, energy, vitamins or minerals (Hirata *et al.* 2008; Leng 1992). In this study, STF in spring was on average more digestible than the pasture with similar CP contents, which may be one of the reasons why heifers browsed longer during this season.

There is also evidence that browsing does influence heifers' intake and nutrition since the DMI was reduced in the BG compared to CPG (79 *vs* 91 g/kg $LW^{0.75}$.d; $P < 0.01$). Browse species had higher ADL content than pasture forage (77 *vs* 27 g/kg DM; $P < 0.001$), an effect between treatments that was addressed by FNIRS (119 *vs* 98 g ADL/kg DM; $P < 0.001$). The woody foliage in spring was characterised by low ADL content, but greater IVOMD and CP concentration than in summer. Therefore, based on these results, it seems that the linear decline in STF nutritive value could explain the greater time spent browsing in spring

compared to the following seasons since the herbaceous samples did not show such a sharp decrease.

Although rumination time was similar in spring, the time for total intake (grazing for CPG treatment, and grazing and browsing for BG treatment) cumulated with the time for searching for food and for rumination was higher in BG (61.6 %; $P < 0.05$) than in CPG (54.9 %), with 15h and 13h, respectively in the context of a 24-h day. This greater cumulated time for BG, possibly driven by more digestible browse fodder, might result from the heifers' mouth structure that is less effective in consuming STF per bite (Gordon and Illius 1988) than grass when grazing. The CPG heifers ruminated on average longer than those of the BG (16.6 *vs* 13.5 %; $P < 0.01$). This result is particularly relevant because rumination is a physiological process that reduces forage particle size (Chai *et al.* 1988) and is regulated by physical and chemical composition of the diet (Welch and Smith 1970), feeding time (Schirmann *et al.* 2012) and grazing management (Gibb *et al.* 1997; Gregorini *et al.* 2012). As NDF and ADF contents of the most ingested species *C. betulus, C. sanguinea, C. avellana* and *C. monogyna* were lower than the pasture whatever the season, it could be assumed that the BG ruminate less than the CPG grazing exclusively because of the higher digestibility of the forage. Alternatively, this may indicate that STF allows for more efficient energy utilization since at the same time the overall DMI was reduced with similar levels of OMD of the diet in both groups. Nevertheless, the ADL content of STF was higher than the pasture. Furthermore, intake prediction from FNIRS has been previously reported to be unreliable (Coleman 2010; Decruyenaere *et al.* 2015) and in our study, the DMI predicted from a FNIRS methodology developed with a diet of a different type (grass and legume *vs* combination of pasture and browse species) should be considered cautiously. Therefore, additional research is needed to support these results and validate that the energy requirement might be a significant driver encouraging grazing cattle to browse shrubs and trees.

Along with decreasing feeding value of the browse species, it can be expected that bio-active secondary metabolites concentrations such as condensed tannins (CT) will increase with time (Feeny 1970; Makkar *et al.* 1991; Riipi *et al.* 2002), reducing the palatability of browse forage between seasons. Although CT contents of browse and pasture forage were not measured in the present study, Kamalak *et al.* (2004), Mebirouk-boudechiche *et al.* (2014) and Paolini *et al.* (2004) have reported CT concentrations of 20, 19 and 14 g/kg DM in *C. betulus, C. monogyna and C. avellana* respectively. This could explain why faecal CP content of BG heifers, although not significant ($P > 0.05$), was higher than CPG in spring (179 *vs* 166

g/kg DM). Condensed tannins bound to leaf protein after mastication and the CT-protein complexes are stable in the rumen at pH 5.5-7.0 (Jones and Mangan 1977), which protects the CT-bound molecules from microbial degradation (McLeod 1974; Min *et al.* 2005). However, the protein is released for hydrolysis and absorption in acidic conditions in the abomasum and small intestine (Jones and Mangan 1977). Consequently, a shift from N excretion in urine to faeces occurs (Waghorn *et al.* 1987; Grainger *et al.* 2009) since less ammonia is released from dietary protein fermentation in the rumen. There, N is mainly organic and so less volatile (Carulla *et al.* 2005; Grainger *et al.* 2009). Therefore, further research is required to quantify the urine N concentration to determine if browsing of ligneous fodder might lead to such change.

With the progressing season, heifers grazed longer as the herbage availability decreased, but did not seem to be influenced by outside weather conditions and temperature. Observational data indicate that with high temperature, grazing time decreases and conversely resting time increases (Hejcmanová *et al.* 2009). In this study, grazing time increased during summer months when the temperature was higher (on average 10.8°C in spring *vs* 19.6 and 14.2°C in early and late summer). Furthermore, in early summer, when temperature was the highest, our records showed that the BG rested longer (lying 18.4 % of their time) mainly in the shade along hedgerows while the CPG rested lying 13.1 % of their time. Those results are in agreement with research that assessed the effect of woody species' shade on grazing cattle welfare (Gregory 1995; Liagre 2006). However, as already mentioned, the nocturnal behaviours were not recorded in our study which might influence the results.

There is strong evidence (Villalba and Provenza 2009) that besides social learning, feeding behaviour is influenced by the individual's past experiences leading to postingestive feedbacks. Although it cannot be concluded from this study that past experiences influenced the selectivity by individuals, it clearly showed strong selectivity differences between animals. The selectivity of STF by heifers in our study was measured using the availability of woody plants in terms of numbers of plants in the hedge, which might have underestimated species with high biomass but not numerically important and vice versa. However, some heifers selected every species offered in the hedge, except *R. pseudoacacia*, whereas others rejected most of the STF species *a priori*. Typical research focuses rather on group averages than individuals, while food intake and preference of individuals within a group can differ strongly (Provenza *et al.* 2003; Manteca *et al.* 2008). Therefore, in agreement with Searle *et al.* (2010) the depth of understanding of differences in selectivity by livestock could be useful

to manipulate forage resources on pasture and develop animal selection indexes aimed at improving efficiency of silvopastoral systems.

6. Conclusion

The presence of a hedge composed of diverse temperate shrubs and trees species fencing a pasture influenced grazing heifers' feeding behaviour. Browsing woody species was observed throughout the grazing study with a peak in late spring when most browse plants displayed high nutritive value, but also when herbaceous forage was plentiful. Though almost all woody species have been ingested at least once during the experiment, *C. betulus*, *C. sanguinea*, *C. avellana* and *C. monogyna* were preferred and the selection towards the STF was markedly driven by individual choice. This study indicates that integrating trees and shrubs as an extra source of forage for the grazing herd could enhance the individual intake diversity, presumably leading to improved welfare and conversion efficiency of the available biomass. However, the different heifers activities spent during the night might have been underestimated in summer due to the contrasting light regime across seasons. Therefore, further research in such temperate silvopastoral systems are needed to integrate this resource properly in grazing managements combining pasture and STF.

7. References

Carulla J, Kreuzer M, Machmüller A, Hess H (2005) Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. *Crop and Pasture Science* **56**, 961–970.

Chai K, Milligan LP, Mathison GW (1988) Effect of muzzling on rumination in sheep. *Canadian Journal of Animal Science* **68**, 387–397.

Coleman S (2010) Historic Overview for Fecal NIRS Analysis. Technical Bulletin SANG-2010-0250, July 2010, Shining Light on Manure Improves Livestock and Land Management. pp. 9–22. Texas Agrilife Research and Society for Range Management (Brown Printing: Jefferson City)

Decruyenaere V, Lecomte P, Demarquilly C, Aufrere J, Dardenne P, Stilmant D, Buldgen, A (2009) Evaluation of green forage intake and digestibility in ruminants using near infrared reflectance spectroscopy (NIRS): Developing a global calibration. *Animal Feed Science and Technology* **148**, 138–156. doi:10.1016/j.anifeedsci.2008.03.007

Decruyenaere V, Planchon V, Dardenne P, Stilmant D (2015) Prediction error and repeatability of near infrared reflectance spectroscopy applied to faeces samples in order to predict voluntary intake and digestibility of forages by ruminants. *Animal Feed Science and Technology* **205**, 49–59. doi:10.1016/j.anifeedsci.2015.04.011

Feeny P (1970) Seasonal Changes in Oak Leaf Tannins and Nutrients as a Cause of Spring Feeding by Winter Moth Caterpillars. *Ecology* **51**, 565-581. doi:10.2307/1934037

Gibb MJ, Huckle CA, Nuthall R, Rook AJ (1997) Effect of sward surface height on intake and grazing behaviour by lactating Holstein Friesian cows. *Grass and Forage Science* **52** ,309–321.

Gordon IJ, Illius AW (1988) Incisor Arcade Structure and Diet Selection in Ruminants. *Functional Ecology* **2**, 15-22. doi:10.2307/2389455

Grainger C, Clarke T, Auldist MJ, Beauchemin KA, McGinn SM, Waghorn GC, Eckard RJ (2009) Potential use of *Acacia mearnsii* condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. *Canadian Journal of Animal Science* **89**, 241–251.

Gregorini P, DelaRue B, McLeod K, Clark CEF, Glassey CB, Jago J (2012) Rumination behavior of grazing dairy cows in response to restricted time at pasture. *Livestock Science* **146**, 95–98. doi:10.1016/j.livsci.2012.02.020

Gregory NG (1995) The role of shelterbelts in protecting livestock: A review. *New Zealand Journal of Agricultural Research* **38**, 423–450. doi:10.1080/00288233.1995.9513146

Halford M, Heemers L, Mathys C, Vanderhoeven S, Mahy G (2011) Socio-economic survey on invasive ornamental plants in Belgium. AlterIAS project, Final report February 2011. (Biodiversity & Landscape Unit, University of Liège-Gembloux Agro-Bio Tech: Gembloux)

Hejcmanová P, Stejskalová M, Pavlů V, Hejcman M (2009) Behavioural patterns of heifers under intensive and extensive continuous grazing on species-rich pasture in the Czech Republic. *Applied Animal Behaviour Science* **117,** 137–143. doi:10.1016/j.applanim.2009.01.003

Hirata M, Hasegawa N, Takahashi T, Chowdappa R, Ogura S, Nogami K, Sonoda T (2008) Grazing behaviour, diet selection and feed intake of cattle in a young tree plantation in southern Kyushu, Japan. *Tropical Grasslands* **42**, 170–180.

Jacobs J (1974) Quantitative measurement of food selection. *Oecologia* **14**, 413–417

Jones WT, Mangan JL (1977) Complexes of the condensed tannins of sainfoin (*Onobrychis viciifolia* Scop.) with fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. *Journal of the Science of Food and Agriculture* **28**, 126–136.

Kamalak A, Filho JMP, Canbolat O, Gurbuz Y, Ozay O, Ozkan CO (2004) Chemical composition and its relationship to *in vitro* dry matter digestibility of several tannin-containing trees and shrub leaves [Online]. *Livestock Research for Rural Development* **16**. Available at http://www.lrrd.org/lrrd16/4/kama16027.htm (Verified 20 February 2015)

Katjiua M, Ward D (2006) Cattle diet selection during the hot-dry season in a semi-arid region of Namibia. *African Journal of Range & Forage Science* **23**, 59–67. doi:10.2989/10220110609485887

Lefroy EC, Dann PR, Wildin JH, Wesley-Smith RN, McGowan AA (1992) Trees and shrubs as sources of fodder in Australia. *Agroforestry Systems* **20**, 117–139.

Le Houérou HN (2006) Agroforestry and sylvopastoralism: The role of trees and shrubs (Trubs) in range rehabilitation and development. *Sécheresse* **17**, 343–348.

Leng RA (1992) 'Tree foliage in ruminant nutrition' (FAO: Rome)

Liagre F (2006) 'Les haies rurales.' (France Agricole Editions: Paris)

Makkar HPS, Dawra RK, Singh B (1991) Tannin levels in leaves of some oak species at different stages of maturity. *Journal of the Science of Food and Agriculture* **54**, 513–519.

Manteca X, Villalba JJ, Atwood SB, Dziba L, Provenza FD (2008) Is dietary choice important to animal welfare? *Journal of Veterinary Behavior: Clinical Applications and Research* **3**, 229–239. doi:10.1016/j.jveb.2008.05.005

McLeod MN (1974) Plant Tannins - Their Role in Forage Quality. *Nutrition Abstracts & Reviews* **44**, 803–815.

Mebirouk-boudechiche L, Cherif M, Boudechiche L, Sammar F (2014). Teneurs en composés primaires et secondaires des feuilles d'arbustes fourragers de la région humide d'Algérie. *Revue de Médecine Vétérinaire* **165**, 344–352.

Meuret M, Dardenne P, Biston R, Poty O (1993) The use of NIR in predicting nutritive value of Mediterranean tree and shrub foliage. *Journal of Near Infrared Spectrometry* **22**, 45–54.

Min BR, Attwood GT, McNabb WC, Molan AL, Barry TN (2005) The effect of condensed tannins from *Lotus corniculatus* on the proteolytic activities and growth of rumen bacteria. *Animal Feed Science and Technology* **121**, 45–58. doi:10.1016/j.anifeedsci.2005.02.007

Mupeyo B, Barry TN, Pomroy WE, Ramírez-Restrepo CA, López-Villalobos N, Pernthaner A (2011) Effects of feeding willow (*Salix* spp.) upon death of established parasites and parasite fecundity. *Animal Feed Science and Technology* **164**, 8–20. doi:10.1016/j.anifeedsci.2010.11.015

Murgueitio E, Calle Z, Uribe F, Calle A, Solorio B (2011) Native trees and shrubs for the productive rehabilitation of tropical cattle ranching lands. *Forest Ecology and Management* **261**, 1654–1663. doi:10.1016/j.foreco.2010.09.027

Nerlich K, Graeff-Hönninger S, Claupein W (2013) Agroforestry in Europe: a review of the disappearance of traditional systems and development of modern agroforestry practices, with emphasis on experiences in Germany. *Agroforestry Systems* **87**, 475–492. doi:10.1007/s10457-012-9560-2

Nguyen TM, Binh DV, Ørskov ER (2005) Effect of foliages containing condensed tannins and on gastrointestinal parasites. *Animal Feed Science and Technology* **121**, 77–87. doi:10.1016/j.anifeedsci.2005.02.013

Nijland GO (2000) A variant of the dry-weight rank method for botanical analysis of grassland with dominance-based multipliers. *Grass and Forage Science* **55**, 309–313

Paciullo DSC, de Castro CRT, Gomide CA de M, Maurício RM, Pires M de FÁ, Müller MD, Xavier DF (2011) Performance of dairy heifers in a silvopastoral system. *Livestock Science* **141**, 166–172. doi:10.1016/j.livsci.2011.05.012

Paolini V, Fouraste I, Hoste H (2004) *In vitro* effects of three woody plant and sainfoin extracts on 3rd-stage larvae and adult worms of three gastrointestinal nematodes. *Parasitology* **129**, 69–77

Papanastasis VP, Yiakoulaki MD, Decandia M, Dini-Papanastasi O (2008) Integrating woody species into livestock feeding in the Mediterranean areas of Europe. *Animal Feed Science and Technology* **140**, 1–17. doi:10.1016/j.anifeedsci.2007.03.012

Pitta DW, Barry TN, Lopez-Villalobos N, Kemp PD (2005) Effects on ewe reproduction of grazing willow fodder blocks during drought. *Animal Feed Science and Technology* **120**, 217–234. doi:10.1016/j.anifeedsci.2005.02.030

Pollard JC (2006) Shelter for lambing sheep in New Zealand: A review. *New Zealand Journal of Agricultural Research* **49**, 395–404. doi:10.1080/00288233.2006.9513730

Provenza F, Villalba JJ, Dziba L, Atwood S, Banner R (2003) Linking herbivore experience, varied diets, and plant biochemical diversity. Small Ruminant Research **49**, 257–274. doi:10.1016/S0921-4488(03)00143-3

Ramírez-Restrepo CA, Barry TN, López-Villalobos N, Kemp PD, McNabb WC (2004) Use of *Lotus corniculatus* containing condensed tannins to increase lamb and wool production under commercial dryland farming conditions without the use of anthelmintics. *Animal Feed Science and Technology* **117**, 85–105. doi:10.1016/j.anifeedsci.2004.05.005

Ramírez-Restrepo CA, Barry TN, López-Villalobos N, Kemp PD, Harvey TG (2005a) Use of *Lotus corniculatus* containing condensed tannins to increase reproductive efficiency in ewes under commercial dryland farming conditions. *Animal Feed Science and Technology* **121**, 23–43. doi:10.1016/j.anifeedsci.2005.02.006

Ramírez-Restrepo CA, Barry TN, Pomroy WE, López-Villalobos N, McNabb WC, Kemp PD (2005b) Use of *Lotus corniculatus* containing condensed tannins to increase summer lamb growth under commercial dryland farming conditions with minimal anthelmintic drench input. *Animal Feed Science and Technology* **122**, 197–217

Ramírez-Restrepo CA, Pernthaner A, Barry TN, López-Villalobos N, Shaw RJ, Pomroy WE, Hein WR (2010a) Characterization of immune responses against gastrointestinal nematodes in weaned lambs grazing willow fodder blocks. *Animal Feed Science and Technology* **155**, 99–110. doi:10.1016/j.anifeedsci.2009.10.006

Ramírez-Restrepo CA, Barry TN, Marriner A, López-Villalobos N, McWilliam EL, Lassey KR, Clark H (2010b) Effects of grazing willow fodder blocks upon methane production and blood composition in young sheep. *Animal Feed Science and Technology* **155**, 33–43. doi:10.1016/j.anifeedsci.2009.10.003

Riipi M, Ossipov V, Lempa K, Haukioja E, Koricheva J, Ossipova S, Pihlaja K (2002) Seasonal changes in birch leaf chemistry: are there trade-offs between leaf growth and accumulation of phenolics? *Oecologia* **130**, 380–390. doi:10.1007/s00442-001-0826-z

Sanderson MA, Rotz CA, Fultz SW, Rayburn EB (2001) Estimating forage mass with a commercial capacitance meter, rising plate meter, and pasture ruler. *Agronomy Journal* **93**, 1281–1286

Schirmann K, Chapinal N, Weary DM, Heuwieser W, von Keyserlingk MAG (2012) Rumination and its relationship to feeding and lying behavior in Holstein dairy cows. *Journal of Dairy Science* **95**, 3212–3217. doi:10.3168/jds.2011-4741

Searle KR, Hunt LP, Gordon IJ (2010) Individualistic herds: Individual variation in herbivore foraging behavior and application to rangeland management. *Applied Animal Behaviour Science* **122**, 1–12. doi:10.1016/j.applanim.2009.10.005

Vandenberghe C, Freléchoux F, Moravie M-A, Gadallah F, Buttler A (2007) Short-Term Effects of Cattle Browsing on Tree Sapling Growth in Mountain Wooded Pastures. *Plant Ecology* **188**, 253–264. doi:10.1007/s11258-006-9160-1

Villalba JJ, Provenza FD (2009) Learning and Dietary Choice in Herbivores. *Rangeland Ecology & Management* **62**, 399–406. doi:10.2111/08-076.1

Waghorn GC, Ulyatt MJ, John A, Fisher MT (1987) The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L. *British Journal of Nutrition* **57**, 115–126.

Walloon Government (2014) Decree of 13 February 2014 on the granting of agri-environmental aids. Belgian Official Journal of 10 April 2014

Welch JG, Smith AM (1970) Forage Quality and Rumination Time in Cattle. *Journal of Dairy* Science **53**, 797–800.

CHAPTER V

Once the selectivity of grazing and browsing heifers towards the temperate woody species was measured, there was a need to study the nutritive value of these plants, and their potential to modulate *in vitro* rumen fermentation and capacity to precipitate the protein. The goal of Chapter 5 was therefore to determine these characteristics using laboratory methodologies.

Article 4

Hedges and woody strips browsing by cattle on pasture in Wallonia

Sophie Vandermeulen[1], Mathilde Pascal[1,2], Christian Marche[2], Arnaud Pilet[2], Yves Beckers[1], Jérôme Bindelle[1]

[1]*Gembloux Agro-Bio Tech, Animal Science Unit, University of Liège, Passage des Déportés 2 B-5030 Gembloux, Belgium. Email sophie.vandermeulen@ulg.ac.be*

[2]*Centre des Technologies Agronomiques, Rue de la Charmille, 16 B-4577 Strée-Modave, Belgium. www.cta-stree.be Email ctastree@yahoo.fr*

This article is adapted from:

Vandermeulen S, Pascal M, Marche C, Pilet A, Beckers Y, Bindelle J (2013) Hedges and woody strips browsing by cattle on pasture in Wallonia, Belgium. In: 'Revitalising Grasslands to Sustain Our Communities: Proceedings 22nd International Grassland Congress.' (Eds DL Michalk, GD Millar, WB Badgery, KM Broadfoot), pp. 1011-1012 (New South Wales Department of Primary Industry: Orange).

1. Abstract

Agri-environmental measures promote woody hedges along meadows in Wallonia, raising the interest in tree and shrub as forage in intensive mixed dairy temperate production systems. This study aimed at (1) determining the influence of an access to hedges on the grazing behavior of young cattle and (2) assessing the rumen fermentation characteristics of woody species promoted in Wallonia. Twelve heifers (577 ± 59 kg), divided in two groups, were set to graze a ryegrass and white clover-based pasture in May 2012. The first group had access to a hedge composed of 12 temperate tree and shrub species, and the second group grazed the pasture only. The grazing and browsing behavior of the animals was recorded for 4 weeks along with pasture biomass availability. Samples of the 12 tree species were fermented using rumen fluid and gas production kinetics, methane (CH_4) and volatile fatty acids production compared to ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). Results suggest that the feeding behavior was influenced by the hedge. Grazing time of the control group was higher than heifers that could browse woody foliage ($P < 0.05$). Browsing was especially noticeable when grass availability on pasture was lowest. *In vitro* ruminal fermentation kinetics differed among species ($P < 0.001$) with *Populus nigra* and *Fraxinus excelsior* showing a potential of fermentation similar to herbaceous forage. Woody species produced lower CH_4 than both ryegrass and clover. It can be concluded that due to the browsing by cattle some woody species could be interesting as complementary feeding. Besides their attributes regarding the agricultural landscape, their feeding qualities should be considered when planting the hedges.

Keywords

Silvopastoralism, ruminants, grazing behaviour, fermentation, feeding value.

2. Introduction

Shrub and tree forages are commonly used in animal production in many regions of the world. Nonetheless, in Western Europe, and especially in Wallonia in Belgium, hedges and woody strips have disappeared from the agricultural landscape over the past 60 years. Browse species are usually rich in plant secondary compounds such as condensed tannins which benefits lowering methane (CH_4) production and internal parasitism, improving productivity and modulating inmmune cell responses have been highlighted (Ramírez-Restrepo *et al.* 2010ab). Currently, agri-environmental measures taken by the Walloon government promote hedges and woody strips in pastures, raising the interest in browse species functionalities in ruminant production. This study aimed at (1) determining the influence of the access to a hedge on the behaviour of cattle on pasture and (2) evaluating the fermentability by rumen microbes of foliage of woody species promoted in the Walloon landscape.

3. Materials and methods

3.1. Grazing and browsing behaviour

Twelve dairy heifers, divided in 2 groups, were set to graze a ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture during 4 consecutive weeks in May 2012. The experimental group (580 ± 60 kg live weight, mean ± standard deviation) had a free access to a hedge composed of 12 tree and shrub species (Table 9) while the control group (575 ± 64 kg) did not. Pasture biomass availability was assessed once a week and the pasture area was adjusted weekly in order to ensure sufficient forage availability. The feeding behaviour (grazing and browsing) of each heifers group was recorded during 10 h/d replicated 2 d/week using the hand-plucking method as well as other activities (rumination, rest, watering, social activity and walking). The activities were compared per week using the MIXED procedure of SAS 9.2. and the daily observations on each cow as experimental unit (n = 12).

3.2. Chemical composition and in vitro ruminal fermentation

The leaves of the woody species found in the hedge harvested from 3 different plants (n = 3) in late May 2012 and a sample of pasture species (ryegrass and white clover) were freeze-dried and analysed for organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and gross energy (GE) contents.

Forage samples were also fermented *in vitro* in duplicate (n = 2) with bovine ruminal fluid for 72 h and gas production recorded (Menke and Steingass 1988). Short-chain fatty acids (SCFA) were analysed after 72 h by high performance liquid chromatography. Methane (CH_4) production was measured after 24 h of *in vitro* rumen fermentation using gas chromatography. The chemical composition and fermentation data including kinetic parameters of gas production after mathematical modelling (Groot *et al.* 1996), CH_4 and SCFA were compared using the MIXED procedure of SAS 9.4..

4. Results and discussion

4.1. Grazing and browsing behaviour

The feeding behaviour was influenced by the hedge. Grazing time of the control group (59.8 %) was on average higher than the heifers that could browse the woody forages (54.8 %; $P = 0.023$). The other activities (i.e. rumination, watering, social activities, rest and walking) were not influenced by the access to the hedge ($P > 0.05$). Significant browsing was noted only the second week of the experiment and reached 3.7 % of the total time. This happened when the biomass in the pasture was low (50 % less than the other weeks). During this week, grazing time of the control group tended to be higher than the experimental one (52.2 % *vs* 41.3 %; $P = 0.057$).

4.2. Chemical composition and in vitro fermentation

The chemical composition and GE showed striking differences between species (Table 9) as did their fermentation profile (Table 10). *Sambucus nigra* showed an outstandingly high CP content. *Prunus spinosa*, *Viburnum opulus*, *Fraxinus excelsior and Populus nigra* seem promising forages because they yielded higher or similar gas production and/or fermentation rates than ryegrass and clover and because, except for *Viburnum opulus*, their CP content is quite high. Woody species contained higher GE than both herbaceous species, with the exception of *Carpinus betulus.* Furthermore shrubs and trees produced less CH_4 than the herbaceous species. Among woody species, CH_4 production varied between 39.6 ml/g of dry matter (DM) produced by *P. spinosa* and 14.0 ml/g DM for *Quercus robur*, while the ratio was between 0.173 for *P. spinosa* and 0.096 in *C. betulus*. Species with high gas volume produced also higher CH_4 while lower gas seemed to be associated to lower CH_4. In terms of VFA production, the average acetate: propionate: butyrate molar ratio across all woody species was 75:18:7 (Table 10).

Table 9. Chemical composition (g/kg DM) and gross energy (MJ/kg DM) of herbaceous and woody species leaves and stems collected in May 2012.

Species	Chemical composition					GE
	OM	CP	NDF	ADF	ADL	
*Lolium perenne**	889	265	469	212	16.7	18.8
*Trifolium repens**	878	286	230	168	31.5	18.6
Acer campestre	946^{bc}	206^{cd}	327^{bc}	171^{ef}	43.1^{def}	19.1^{cd}
Acer pseudoplatanus	939^{cd}	213^{cd}	301^{bcd}	211^{b}	60.1^{b}	19.5^{bc}
Carpinus betulus	953^{ab}	161^{g}	270^{cd}	141^{g}	24.8^{g}	18.3^{e}
Cornus sanguinea	900^{h}	176^{ef}	185^{e}	117^{h}	23.5^{g}	17.8^{f}
Corylus avellana	933^{d}	170^{fg}	361^{ab}	201^{bcd}	50.3^{cd}	19.3^{bcd}
Crataegus monogyna	920^{ef}	162^{fg}	350^{a}	154^{fg}	42.3^{ef}	19.6^{bc}
Fraxinus excelsior	939^{cd}	245^{b}	341^{ab}	179^{de}	55.8^{bc}	20.3^{a}
Populus nigra	906^{gh}	217^{bc}	310^{bc}	204^{bc}	50.0^{cde}	19.1^{cd}
Prunus spinosa	914^{fg}	202^{cde}	240^{de}	136^{gh}	46.0^{def}	19.0^{d}
Quercus pubescens	958^{a}	185^{def}	395^{a}	238^{a}	87.2^{a}	19.7^{b}
Sambucus nigra	885^{i}	320^{a}	244^{de}	185^{cde}	38.6^{f}	19.3^{bcd}
Viburnum opulus	929^{de}	143^{g}	267^{cd}	173^{ef}	55.0^{bc}	19.4^{bcd}
SEM	3.73	8.04	10.99	5.96	2.79	0.11
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; GE, gross energy; SEM, standard error of means.

Within a column, values with different letters are significantly different ($P < 0.05$).

*Not included in the statistical analysis ($n = 1$).

Table 10. *In vitro* rumen fermentation parameters and molar ratio of acetic, propionic and butyric acids of herbaceous and browse species over 72 h and CH_4 production after 24 h of incubation (n=3).

Species	Parameters of *in vitro* fermentation kinetics				*In vitro* CH_4 (ml/g DM)	*In vitro* CH_4 ratio (ml CH_4/ml gas)	Molar ratio of acetic:propionic:butyric
	A (ml/g DM)	*B* (h)	R_{max} (ml g/DM.h)	T_{max} (h)			
*Lolium perenne**	267	7.30	22.3	2.69	41.1	0.187	73:19:9
*Trifolium repens**	270	6.02	27.5	2.53	43.5	0.190	72:19:9
Acer campestre	187^{gh}	11.3^{ab}	10.4^{d}	4.38^{ab}	18.3^{de}	0.123^{cde}	82:16:3
Acer pseudoplatanus	239^{de}	6.54^{f}	22.6^{b}	2.38^{de}	30.3^{bc}	0.148^{abc}	76:18:7
Carpinus betulus	216^{ef}	9.64^{cd}	13.9^{cd}	3.91^{bc}	16.6^{de}	0.096^{e}	80:16:5
Cornus sanguinea	194^{fgh}	8.92^{de}	13.8^{cd}	2.41^{de}	17.0^{de}	0.115^{de}	77:17:5
Corylus avellana	207^{fg}	10.8^{abc}	11.9^{d}	3.58^{bc}	19.4^{de}	0.121^{cde}	77:18:5
Crataegus monogyna	242^{cd}	12.1^{a}	12.5^{d}	5.17^{a}	24.4^{cd}	0.125^{bcde}	74:18:8
Fraxinus excelsior	272^{ab}	6.91^{f}	25.1^{ab}	1.98^{ef}	38.5^{a}	0.172^{a}	74:18:8
Populus nigra	247^{bcd}	5.45^{g}	28.6^{a}	1.38^{f}	34.0^{ab}	0.156^{ab}	73:19:8
Prunus spinosa	280^{a}	7.75^{ef}	22.5^{b}	2.98^{cd}	39.6^{a}	0.173^{a}	70:19:10
Quercus robur	178^{h}	10.3^{bc}	10.7^{d}	3.19^{cd}	14.0^{e}	0.101^{e}	78:18:4
Sambucus nigra	199^{fgh}	7.19^{f}	17.5^{c}	2.33^{de}	17.1^{de}	0.097^{e}	73:20:7
Viburnum opulus	267^{abc}	7.56^{f}	21.9^{b}	2.49^{de}	29.2^{bc}	0.132^{bcd}	75:16:8
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	NA
SEM	6.06	0.36	1.07	0.19	1.64	0.005	NA

DM, dry matter; NA, not applicable; SEM, standard error of the mean.

A, maximum gas volume; *B*, mid-fermentation time; R_{max}, maximum rate of fermentation; T_{max}, the time at which the maximum rate of fermentation is reached.

Within a column, values with different letters are significantly different ($P < 0.05$).

*Not included in the statistical analysis

5. Conclusion

It can be concluded that in the grazing conditions in Wallonia, browsing can also be considered as complementary forage for cattle in pastures with hedges. Some woody species seem interesting for ruminant nutrition as plain forage or to induce shifts in rumen fermentation patterns. These attributes should be better documented to allow proper advice when farmers plant hedges along pastures.

6. References

Groot JCJ, Cone JW, Williams BA, Debersaques FMA and Lantinga EA (1996) Multiphasic analysis of gas production kinetics for *in vitro* fermentation of ruminants feeds. *Animal Feed Science and Technology* **64**, 77-89.

Menke KH and Steingass H (1988) Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Development* **28**, 7–55.

Ramírez-Restrepo CA, Barry TN, Marriner A, López-Villalobos N, McWilliam EL, Lassey KR and Clark H (2010a) Effects of grazing willow fodder blocks upon methane production and blood composition in young sheep. *Animal Feed Science and Technology* **155**, 33–43.

Ramírez-Restrepo CA, Pernthaner A, Barry TN, López-Villalobos N, Shaw RJ, Pomroy WE, Hein WR (2010b) Characterization of immune responses against gastrointestinal nematodes in weaned lambs grazing willow fodder blocks. *Animal Feed Science and Technology* **155**, 99–110. doi:10.1016/j.anifeedsci.2009.10.006

Communication 5

In vitro evaluation of protein precipitation capacity of temperate browse species

Sophie Vandermeulen[1], Julie Leblois[1], Carlos Alberto Ramírez-Restrepo[2], Yves Beckers[1], Georges Lognay[3] and Jérôme Bindelle[1]

[1]*Precision Livestock and Nutrition Unit, Gembloux Agro-Bio Tech, University of Liège, 5030 Gembloux, Belgium*

[2]*CSIRO Agriculture, Australian Tropical Sciences and Innovation Precinct, James Cook University, Qld 4811, Townsville, Australia*

[3]*Laboratory of Analytical Chemistry, Gembloux Agro-Bio Tech, University of Liège, 5030 Gembloux, Belgium*

This article is adapted from a poster presented at *The 21th National Symposium on Applied Biological Sciences* (2016).

1. Abstract

European agri-environmental policies are promoting the establishment of shrubs and trees on grasslands. The use of browse as fodder requires knowledge on their nutritive value since intensive production systems are still relying on expensive and environment-costing protein sources. However, information on the influence of temperate condensed tannins (CT)-containing browse forage on rumen protein metabolism is elusive. The study aims to assess the protein precipitation capacity (PPC) of 10 temperate browse species and establish the correlation between PPC values and plants CT content, in comparison to perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). Extractable CT concentration of 3 individuals per woody plants collected at 3 different periods (i.e. May, July and September of 2013) was quantified by spectrophotometry. The PPC of foliage collected in July was measured using 2 model proteins: bovine serum albumin (BSA) and casein. The N content in protein solutions (4.6 g/l; pH=6.8) was determined before and after adding each forage sample. The PPC varied across plant species ($P < 0.001$). *Corylus avellana* had the highest ability to precipitate casein (52.4 %). In contrast, the BSA precipitation (18.3 %) of this plant was similar to *Cornus sanguinea* (12.7 %), *Quercus robur* (12.1 %) and *Crataegus monogyna* (11.0 %). Condensed tannins concentration ranged from 1.27 in *Fraxinus excelsior* in September to 82.7 g/kg of depigmented sample in *Corylus avellana* in July ($P < 0.001$). Both BSA ($r = 0.70$; $P < 0.001$) and casein PC ($r = 0.54$; $P < 0.01$) were correlated to CT concentration in woody plants. It was concluded that woody species could play a significant role in modifying protein metabolism, but further *in vivo* trials are required.

Keywords

Ruminants, shrubs, trees, foliage, protein digestion, condensed tannins.

2. Introduction

Shrubs and trees are nowadays promoted in temperate grassland-based systems due to the implementation of European agri-environmental policies. Although these environment-friendly practices are leading to a renewed interest in using shrub and tree foliage as fodder for ruminants (Bestman *et al.* 2014; Luske and van Eekeren 2014), little is known about the nutritive value of browse forage and their influence on protein metabolism in the rumen.

Protein is the most critical nutrient in livestock nutrition (Poppi and McLennan 1995), while intensive cattle feeding systems rely on expensive and environment-costing protein sources such as soybean in order to meet the animal's requirements (Cutrignelli *et al.* 2011). Although grasses may contain high levels of protein, this protein may be highly degradable in the rumen and grass fed ruminants usually excrete 60 to 90 % of the dietary (Calsamiglia *et al.* 2010). Thus, strategies to improve protein digestion and its utilization based on condensed tannin-rich legumes such as the perennial herbaceous *Lotus corniculatus* and *Hedysarum coronarium* have been proposed (Ramírez-Restrepo and Barry 2005). However, relying on shrubs and trees in hedges and woody strips seems more feasible, because keeping such herbaceous legume species under heavy grazing conditions as in Wallonia is challenging (de Buyl 2012).

Tree and shrub foliage is known to be rich in bio-active secondary metabolites such as polyphenols (McLeod 1974; Makkar 2003; Jayanegara *et al*. 2010). Those molecules like condensed tannins (CT) have a potential for modulating rumen fermentation and then influencing the availability of nutrients, especially protein, during the digestion process (Ramírez-Restrepo *et al.* 2006; Tedeschi *et al*. 2014). In the rumen, CT form complexes with dietary proteins that dissociate at the low pH of the abomasum (Jones and Mangan 1977). This effect increases the rumen escape of protein and the proportion of dietary protein reaching the small intestine (Bach *et al.* 2005; Waghorn *et al.* 1994). This mechanism can increase the microbial protein flow from rumen and feed utilization efficiency. Conversely to temperate herbaceous species, temperate woody species have not been deeply investigated for their secondary metabolites contents such as CT and their influence on protein digestion in the rumen. Therefore the study aims to assess the protein precipitation capacity (PPC) of 10 temperate shrub and tree species and establish the correlation between PPC values and plants CT content.

3. Materials and methods

3.1. Plant samples preparation

Leaves and petioles from shrub and tree species (Table 11) were harvested manually in May, July and September 2013 at Centre des Technologes Agronomiques (CTA, Strée, Belgium). Three independent samples of each species were collected from 3 different plants selected randomly within a hedge fencing a meadow. Besides browse species, 3 samples of leaves and stems of perennial ryegrass and white clover were randomly collected in the pasture. Samples were freeze-dried (Delta 1-24 LSC, Martin Christ, Osterode, Germany) before milling to pass a 1-mm diameter screen (Cyclotec 1093 Sample Mill, FOSS Electric, Hillerød, Denmark). Samples were then stored at room temperature and away from light until further treatment.

Table 11. Shrubs and trees species collected in July 2013 from a hedge at CTA.

Woody species	Common name
Acer campestre L.	Field maple
Acer pseudoplatanus L.	Sycamore maple
Carpinus betulus L.	Hornbeam
Cornus sanguinea L.	Common dogwood
Corylus avellana L.	Hazel
Crataegus monogyna Jacq.	Hawthorn
Fraxinus excelsior L.	Common ash
Populus nigra L.	Black poplar
Quercus robur L.	Pedonculate oak
Sambucus nigra L.	Elderberry

3.2. Laboratory analyses

The total CT content of the forage samples was measured according to the method of Porter *et al.* (1986) for the 3 harvesting periods. Protein precipitation capacity (PPC) of woody and herbaceous species collected in July was determined following the modified method of Amory and Schubert (1987), using casein (VWR International, USA) and bovine serum albumin (BSA; Sigma-Aldrich, USA) as protein sources. Forage sample (0.5 g with BSA; 1 g with casein) was mixed with 30 ml of phosphate buffer (pH 6.8; 4.6 g/l protein) and kept at 4°C overnight. Samples were centrifuged at 3220×g and 4°C for 15 min (5810R Centrifuge, Eppendorf, Germany) to stabilize the pellet, followed by a second centrifugation at 12,500×g (4°C for 15 min; Jouan KR22i Centrifuge, Jouan, France). The supernatant (5 ml) was analyzed using the Kjeldahl method to measure the N content in the supernatant. Samples

containing only the protein phosphate buffer (i.e. buffer with 4.6 g/l protein) and samples containing the forage and phosphate buffer (i.e. without protein) were also included in the experiment. The PPC was calculated as: $PPC = (X + Y) - Z / (X + Y)$; where X is the CP content in the phosphate buffer containing the protein (i.e. without the forage sample), Y is the CP content in the phosphate buffer with the forage sample (i.e. without the protein), Z is the CP content that remains in the protein buffer after incorporating the forage sample. In addition, PPC of tannic acid (i.e. 0.1 g with casein and 0.05 g with BSA buffers; ACS Reagent, Sigma-Aldrich) was measured to compare woody species PPC to that of standard tannic acid.

3.3. Statistical analysis

Data of CT content and PPC of BSA and casein were analyzed using SAS 9.4. (SAS Institute Inc., Cary, NC, USA) in a model considering the woody species as fixed factor. Pearson's correlation was calculated between PPC of both proteins (BSA and casein) and CT content of woody species harvested in July. Herbaceous species were not included in the statistical analysis.

4. Results and discussion

As shown in Table 12, CT concentration and PPC of both BSA and casein varied between woody species ($P < 0.001$). The CT in woody foliage ranged from 1.27 in *Fraxinus excelsior* in September to 82.7 g/kg of depigmented sample in *Corylus avellana* in July. High CT content species were *Corylus avellana* and *Crataegus monogyna* in all periods, and also *Quercus robur* in July. The high variation among individual plants of the same species might explain that the means of CT contents were classified only in two groups at this period.

As a comparison, the PPC of the tannic acid reference (i.e. quantity of acid tannic like a plant sample containing 10 % of tannic acid) was of 34.8 and 54.3 % with BSA and casein respectively. PPC of both proteins was positively correlated to CT content in woody species of July, and the relationship was stronger for BSA ($r = 0.70$; $P < 0.001$) than casein ($r = 0.54$; $P < 0.01$). However, high CT content was not always associated to low PPC. For example, *C. sanguinea* precipitated highly both casein and BSA but this species contained low CT. Although CT have previously demonstrated potential for precipitation of protein under ruminal conditions (Waghorn *et al.* 1994; Min *et al.* 2005), the only CT concentration did not explain the potential of each woody species to precipitate the protein.

Table 12. Condensed tannins contents (g/kg depigmented sample) of herbaceous and woody species at 3 harvesting periods and protein precipitation capacity (%) of forage samples of July.

Species	CT			PPC (%)	
	May	July	September	BSA	Casein
Lolium perenne[*]	1.09	1.24	1.48	0	1.03
Trifolium repens[*]	1.28	1.37	1.56	0	0
Acer campestre	2.15^{c}	21.9^{b}	36.5^{bcd}	8.38^{bc}	32.1^{c}
Acer pseudoplatanus	2.29^{c}	15.8^{b}	20.8^{cde}	0^{d}	3.44^{e}
Carpinus betulus	2.22^{c}	4.89^{b}	7.90^{de}	0.255^{cd}	13.6^{d}
Cornus sanguinea	1.55^{c}	1.92^{b}	3.99^{e}	12.7^{ab}	42.5^{b}
Corylus avellana	58.2^{b}	82.7^{a}	78.5^{a}	18.3^{a}	52.4^{a}
Crataegus monogyna	75.6^{a}	74.7^{a}	56.6^{ab}	11.0^{ab}	24.7^{c}
Fraxinus excelsior	1.48^{c}	1.40^{b}	1.27^{e}	0^{d}	1.68^{e}
Populus nigra	4.41^{c}	6.12^{b}	6.83^{de}	0^{d}	0^{e}
Quercus robur	3.30^{c}	82.4^{a}	39.0^{bc}	12.1^{ab}	29.2^{c}
Sambucus nigra	1.64^{c}	2.79^{b}	2.98^{e}	0^{ab}	0^{e}
SEM	4.87	6.83	5.37	1.44	3.44
P-value	<0.001	<0.001	<0.001	<0.001	<0.001

BSA, bovine serum albumin; CT, condensed tannins; PPC, protein precipitation capacity. SEM, standard error of the mean.

*Not included in the statistical analysis.

5. Conclusion

This *in vitro* study demonstrated that woody species influence the protein degradability in ruminal conditions and could play a significant role in protecting it from microbial degradation, but additional research are required to assess further effects on rumen protein metabolism and their consequences on the nutrition and subsequent performances of ruminants *in vivo*.

6. References

Amory AM, Schubert CL (1987) A method to determine tannin concentration by the measurement and quantification of protein—tannin interactions. *Oecologia* **73**, 420–424.

Bach A, Calsamiglia S, Stern MD (2005) Nitrogen metabolism in the rumen. *Journal of Dairy Science* **88**, E9–E21.

Bestman M, van Eekeren N, Luske B, Vonk M, Anssems E, Boosten M, van Bree M (2014). Introducing trees in dairy and poultry farms. Experiences dairy and poultry farmers' networks in The Netherlands. In '18th IFOAM Organic World Congress 2014', Istanbul, Turkey, 13-15 October 2014 [Online]. Available at http://orgprints.org/23894/9/23894.pdf (verified 22 March 2016).

Calsamiglia S, Ferret A, Reynolds CK, Kristensen NB, van Vuuren AM (2010) Strategies for optimizing nitrogen use in ruminants. *Animal* **4**, 1184-1196.

Cutrignelli MI, Calabrò S, Tudisco R, Infascelli F and Piccolo V (2011) Protein Sources in Ruminant Nutrition. In 'Recent Trends for Enhancing the Diversity and Quality of Soybean Products'. (Ed. D Krezhova) pp. 191–214. (InTech: Rijeka)

de Buyl R (2012) 'Influence du mode de présentation du couvert prairial sur le comportement alimentaire et l'ingestion du mouton au pâturage'. Master's Thesis, University of Liège-Gembloux Agro-Bio Tech, Belgium.

Jayanegara A, Goel G, Makkar HPS, Becker K (2010) Reduction in methane emissions from ruminants by plant secondary metabolites: Effects of Polyphenols and Saponins. In 'Sustainable Improvement of Animal Production and Health'. (Eds NE Odongo, M Garcia, GJ Viljoen) pp. 151–157. (FAO: Rome)

Jones WT, Mangan JL (1977) Complexes of the condensed tannins of sainfoin (*Onobrychis viciifolia* Scop.) with fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. *Journal of the Science of Food and Agriculture* **28**, 126–136.

Luske B, van Eekeren N (2014) Renewed interest for silvopastoral systems in Europe – an inventory of the feeding value of fodder trees. In 'Proceedings of the 4th ISOFAR Scientific Conference, 'Building Organic Bridges'' (Eds G Rahmann, U Aksoy) pp. 811–814. (Thuenen: Braunschweig)

Makkar HP (2003) 'Quantification of tannins in tree and shrub foliage: a laboratory manual.' (Kluwer Academics Publishers: Dordrecht, The Netherlands)

Min BR., Attwood GT, McNabb WC, Molan AL, Barry TN (2005) The effect of condensed tannins from *Lotus corniculatus* on the proteolytic activities and growth of rumen bacteria. *Animal Feed Science and Technology* **121**, 45–58. doi:10.1016/j.anifeedsci.2005.02.007

McLeod MN (1974) Plant Tannins - Their Role in Forage Quality. *Nutrition Abstracts & Reviews* **44**, 803–815.

Poppi D, McLennan S (1995) Protein and energy utilization by ruminants at pasture. *Journal of Animal Science* **73**, 278–290.

Porter LJ, Hrstich LN, Chan BG (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* **25**, 223–230.

Ramírez-Restrepo CA, Barry TN (2005) Alternative temperate forages containing secondary compounds for improving sustainable productivity in grazing ruminants. *Animal Feed Science and Technology* **120**, 179–201. doi:10.1016/j.anifeedsci.2005.01.015

Ramírez-Restrepo CA, Barry TN, López-Villalobos (2006) Organic matter digestibility of condensed tannin-containing *Lotus corniculatus* and its prediction *in vitro* using cellulase/hemicellulose enzymes. *Animal Feed Science and Technology* **125**, 61–71.

Tedeschi LO, Ramírez-Restrepo CA, Muir JP (2014) Developing a conceptual model of possible benefits of condensed tannins for ruminant production. *Animal* **8**, 1095–1105.

Waghorn GC, Shelton ID, McNabb WC (1994) Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 2. Nitrogenous aspects. *Journal of Agricultural Science (Cambridge)* **123**, 109–119.

CHAPTER VI

Since using shrubs and trees to supply forage to cattle is commonly practiced in tropical grassland-based systems of northern Australia, Chapter 6 was designed to complete the thesis with a study in a tropical ecosystem for which grass and shrub species as well as animals, with zebu instead of taurine cattle, differ from those in previous chapters (i.e. temperate systems). The aim of this last article was to study the chemical composition and *in vitro* fermentation profile including methane production of three newly-developed cultivars of *Desmanthus* spp.

Article 6

In vitro assessment of ruminal fermentation, digestibility and methane production of three species of Desmanthus for application in northern Australian grazing systems

Sophie Vandermeulen[1,2,3,§], Sultan Singh[1,4,§], Carlos Alberto Ramírez-Restrepo[1,8], Robert D. Kinley[1], Christopher P. Gardiner[5], Joseph A.M. Holtum[6], Iain Hannah[7] and Jérôme Bindelle[2]

[1]*CSIRO Agriculture, Australian Tropical Sciences and Innovation Precinct, Building 145, James Cook Drive, James Cook University, Townsville, 4811 QLD, Australia*

[2]*University of Liège, Gembloux Agro-Bio Tech, Precision Livestock and Nutrition Unit, 2 Passage des Déportés, 5030 Gembloux, Belgium*

[3]*Research Foundation for Industry and Agriculture - National Scientific Research Foundation (FRIA-FNRS), 5 Rue d'Egmont, 1000 Bruxelles, Belgium*

[4]*Indian Grassland and Fodder Research Institute, Plant Animal Relationship Division, Jhansi 284003, UP, India*

[5]*James Cook University, School of Veterinary and Biomedical Sciences, Building 87, James Cook Drive, Townsville, 4811 QLD, Australia*

[6]*James Cook University, Terrestrial Ecosystems and Climate Change, College of Marine and Environmental Sciences, Building 28, James Cook Drive, Townsville, 4811 QLD, Australia*

[7]*Agrimix Pty. Ltd., Eagle Farm, 19 Chapman Place PO Box 1045, 4009 QLD, Australia*

[8]*Corresponding author. Email: carlos.ramirez@csiro.au*

[§] *Joint first authors.*

Short title

In vitro ruminal fermentation of *Desmanthus* spp.

Acknowledgments

The authors acknowledge the FRIA-FNRS, CURAGx for provision of Scholarship and travel grants to Sophie Vandermeulen. Special thanks are extended to The Commonwealth Scientific and Industrial Research Organisation (CSIRO) for making the internship experience possible for Ms Vandermeulen. We are also grateful to The Australian Endeavour Fellowship programme for the financial support provided to Dr Sultan Singh, while in Australia. Appreciation is expressed to the Indian Grassland and Fodder Research Institute (IGFRI) for plant quality lab screening. Drs Shane Askew and Matt Vucko are also thanked for their guidance on analytical procedures and data analysis. We are grateful to Nick Kempe, Agrimix Pty. Ltd. and James Cook University (JCU). Finally, technical support provided by Wayne Flintham and Steve Austin at Lansdown Research Station cannot be forgotten.

This article has been submitted to *Crop and Pasture Science* and is currently under "minor revision". This present document is adapted from the revised form which was re-submitted on the 22nd of December 2016.

1. Abstract

Three species of *Desmanthus* adapted to the heavy clay soils of northern Australia were studied to determine their nutritive value and effects on *in vitro* fermentation with rumen fluid, compared to Rhodes grass (*Chloris gayana*) hay. Leaves and stems of *D. leptophyllus* cv. JCU 1, *D. virgatus* cv. JCU 2 and *D. bicornutus* cv. JCU 4 were collected in summer, winter and spring of 2014 and analysed for chemical composition. Apparent digestibility as *in vitro* organic matter digestibility (IVOMD) and fermentation parameters including methane (CH_4) production were measured during 72-h fermentations using rumen fluid from grazing steer donors. Relative to control Rhodes grass, *D. leptophyllus* reduced the CH_4 production up to 36% and produced higher volatile fatty acids (VFA) per g of organic matter fermented, but the legume showed also lower IVOMD. *Desmanthus bicornutus* showed also an anti-methanogenic potential and this species was more digestible. Overall, *Desmanthus* species produced lower *in vitro* CH_4 and lower volatile fatty acids concentration compared to the control grass. These effects may be due to presence of secondary compounds such as hydrolysable tannins, condensed tannins and/or their combination in *Desmanthus* species. This suggests that contrasting fermentative profiles in *Desmanthus* cultivars may offer the opportunity to reduce the carbon footprint of the beef industry. However, it is required to test *in vivo* the present results associated with productive farming practices to effectively minimise the environmental impacts of extensive pastoral systems in northern Australia.

Additional keywords

Digestion, greenhouse gas, legume, ruminant, tannins.

2. Introduction

Extensive grazing beef production systems in northern Australia are affected by annual dry periods extending usually from April to October, resulting in reduced animal productivity and farm profitability (Cox and Gardiner 2013). To mitigate the effect of low availability and low quality, the use of improved grass, shrub and legume species to increase nutritive quality of native pasture and beef productivity has been a common, but costly practice (Shelton *et al.* 1991; Dalzell *et al.* 2006; Hill *et al.* 2009). However, not all species adapt equally to variable soil and climatic conditions that prevail on rangelands (Vera and Seré 1985). Therefore, finding adapted herbaceous and shrub legumes that thrive on the heavy clay soils is challenging, but plants from the genus *Desmanthus* are among the few to be successfully adapted to those conditions in northern Australia (Pengelly and Conway 2000; Gardiner and Swan 2008; Gardiner *et al.* 2013).

Recently, new *Desmanthus* cultivars (cv. JCU 1 to 5) described by Loch (2015) Gardiner (2016) have become available. Among them, *D. leptophyllus* cv. JCU 1, *D. virgatus* cv. JCU 2 and *D. bicornutus* cv. JCU 4 have been marketed and planted as a blend of the three species (Gardiner *et al.* 2013). These *Desmanthus* cultivars comprise a wide range of early to late maturity types, herbaceous to suffruticose plant habits, and edaphic and climatic tolerances. These legumes have also been associated with improved animal production. In Queensland, according to Gardiner and Parker (2012) steers grazing a *Desmanthus*-buffel grass (*Cenchrus ciliaris*) paddock during 90 days (i.e. winter) achieved 40 kg more weight gain than steers only grazing buffel grass. Furthermore, a mixture of *Desmanthus* with Mitchell grass (*Astrebla* spp.) increased wool production by up to 34 % in grazing sheep (Rangel and Gardiner 2009).

In contrast, detrimental effects have been demonstrated in growing goats. Compared to *Leucaena leucocephala*, Kanani *et al.* (2006) showed reduced animal preference of *D. bicornutus* resulting in low liveweight gain. Although both species contain common secondary metabolites such as condensed tannins (CT; Gonzalez-V. *et al.* 2005; Tan *et al.* 2011) that mitigate pastoral methane emissions in large (Grainger *et al.* 2009) and small ruminant (Ramírez-Restrepo *et al.* 2010) systems, the toxic alkaloid mimosine is not present in *Desmanthus* spp. (Cook *et al.* 2005).

Therefore, it is obvious that improvements in productivity of low input beef pastoral systems require at least the association of palatable and productive grasslands to complement native forage communities (Department of Primary Industries Queensland 1988, 1999).

However, any attempt to achieve sustainable tropical pastoral production requires an integrated mitigation framework that considers profiles of plant-rumen fermentation characteristics as one of the key elements (Ramírez-Restrepo and Charmley 2015). This approach will help improve nutrition balance, health and cattle welfare (Provenza *et al.* 2007; Manteca *et al.* 2008; Provenza and Villalba 2010). In this respect, *Desmanthus* cultivars may contribute to improved nutrition for pastures and as an additional effect may mitigate methane emission from grazing cattle.

Agronomic attributes of members of the *Desmanthus* genus have been shown before (Jones and Brandon 1998; Pengelly and Conway 2000; Cook *et al.* 2005). However, given that forage quality varies markedly with seasons, there is not data that considers the effects of *Desmanthus* spp. on nutritive values and the *in vitro* anti-methanogenic characteristics using rumen fluid harvested from grazing donors, an important omission. The objective of the present study was to assess the nutritive value of three *Desmanthus* species and their effects on *in vitro* fermentation, including potential to reduce methane production using rumen fluid from grazing *Bos indicus* Brahman breed steers.

3. Materials and methods

3.1. Study site

The *in vitro* experimentation involving the use of the same *Bos indicus* Brahman breed rumen-cannulated steers (n = 4, 407 ± 9.45 kg liveweight) as ruminal fluid donors was conducted at CSIRO Agriculture at the Australian Tropical Sciences and Innovation Precinct (ATSIP) in Townsville and at the Lansdown Research Station (19°39' S, 146°50' E). The study followed CSIRO Animal Ethics Committee approved guidelines (A12/2014) and the Australian code of practice for the care and use of animals for scientific purposes (NHRMC 2013).

3.2. Plant material harvesting and preparation

Desmanthus leptophyllus (cv. JCU 1), *D. virgatus* (cv. JCU 2) and *D. bicornutus* (cv. JCU 4) were grown in pots under identical agronomic practices in a semi-enclosed greenhouse at the faculty of Agriculture of the University of Queensland. Each species was composed of 4 groups of 5 pots and all plants were cut back to approximately 10 cm on the 29th January 2014 (day 0). In parallel, one sample for each *Desmanthus* species was harvested by Agrimix Pty Ltd. (Eagle Farm, QLD, Australia) from the same number of pots at three different growth

stages, in summer (March – day 51), winter (August – day 189) and spring (October – day 273) of 2014. After collection, samples were immediately stored at -20 °C and freeze-dried. Rhodes grass (*Chloris gayana*) was representatively sampled from commercial local hay and oven-dried at 55°C to be used as the control. All substrates were ground to pass a 1-mm mesh screen using a Cyclotec 1093 Sample Mill (Foss Tecator, Hillerød, Denmark).

3.3. Chemical composition

3.3.1. Proximate analysis

Forage samples were analysed using standard methods of AOAC (1995) for their content in dry matter (DM; method 967.03), ash (method 942.05), N (method 984.13) and ether extract (EE; method 930.39). Neutral detergent fiber (NDF) without heat stable alpha-amylase, acid detergent fiber (ADF), cellulose and acid detergent lignin (ADL (sa)) were determined following the procedures of Van Soest *et al.* (1991). The NDF and ADF are expressed including residual ash.

3.3.2. Carbohydrate and protein fractinations

The carbohydrate and protein fractions have been evaluated using the analytical procedures described by Singh *et al.* (2011). Nine fractions have been measured; firstly, NDIP is neutral detergent insoluble protein, ADIP, acid detergent insoluble protein and NPN, non-protein nitrogen. Further, the protein is partitioned into three fractions: P_A, protein fraction A, is non-protein nitrogen (NPN × 6.25), P_B, protein fraction B, is a true protein and P_C, protein fraction C, is unavailable or lignin-bound protein. Protein fraction B is then divided into three sub-fractions: P_{B1}, P_{B2} and P_{B3} which represent proteins of rapid, intermediate and slow rate of rumen degradation respectively. Protein fractions A and P_{B1} are soluble in borate phosphate buffer, while P_{B2} is soluble in neutral detergent solution but insoluble buffer. Fraction P_{B3} is insoluble in neutral detergent solution but soluble in acid detergent. The fraction P_C is insoluble in acid detergent solution (ADIP) and includes protein bound with lignin, tannin–protein complex and Maillard products.

The total carbohydrates (TCHO), structural carbohydrates (SC) and non-structural carbohydrates (NSC) were determined. The carbohydrate fractions were further classified into two fractions according to the degradation rate: C_{B2}, slowly degradable cell wall, and C_C, unavailable/lignin-bound cell wall. The fractions C_A, rapidly degradable sugars, and C_{B1}, intermediately degradable starch and pectins, were not measured.

3.3.3. Phenols and tannins analyses

Prior to the phenolic fraction assays, pigments were removed from ground samples using diethyl-ether with acetic acid (99:1 v/v) according to the modified method of Ammar *et al.* (2004). Depigmented samples were oven-dried at 40°C to avoid deterioration of phenolic compounds, and phenol contents i.e. total phenols (TP), total tannins (TT) and CT were extracted using aqueous acetone (70:30 v/v; Makkar 2003).

The TP and non-tannins phenols (NTP) contents in the plant extract were measured by means of the Folin-Ciocalteu reagent method. The TT fraction was determined by the difference between TP and NTP (Makkar 2003). Total phenols, NTP and TT were expressed as tannic acid equivalent. The total CT fraction was expressed as leucocyanidin equivalent and estimated by the n-butanol-HCl method described by Porter *et al.* (1986). Hydrolysable tannins (HT) content in plant substrate extract were calculated as the difference between the TT and CT (Singh *et al.* 2005).

3.4. In vitro ruminal fermentation

3.4.1. Rumen inoculum preparation

Rumen-cannulated Brahman steers grazed together on *Cenchrus ciliaris*, *Chloris* spp., *Macroptilium* spp., *Panicum* spp., *Urochloa* spp., *Stylosanthes* spp., and other native pasture communities. On collection days (0600), liquid and particulate fractions of the rumen content were manually collected from the four quadrants of the rumen of the steers through fistula and conditioned as described by Ramírez-Restrepo *et al.* (2014) until further processing.

3.4.2. In vitro rumen inoculation and incubation

The inoculation and *in vitro* fermentation were performed following the methods described by Kinley *et al.* (2016). Briefly, the rumen fluid incubation medium was prepared by combining the strained rumen fluid with the Goering and Van Soest (1970) buffer at a ratio of 1:4 (v/v). Rumen media (125 mL) was added anaerobically to the 39°C incubation bottle containing 1 g of organic matter (OM) of forage sample. Further the inoculated bottles were purged with N_2 and capped gas tight with an Ankom RF1 gas production module (Macedon, NY, USA). Bottles were incubated at 39°C in an orbital incubator (Ratek, OM11, Boronia, VIC, Australia) at 85 rpm.

The experimental scheme yielded a total of 44 bottles and was replicated over three independent fermentation runs as follows:

[3 *Desmanthus* forages × 3 growing stages + 1 Rhodes grass hay control and 1 blank (rumen medium only)] × 4 bottles × 3 fermentation runs.

During each fermentation run, 1 bottle for each substrate was stopped after 24 and 48 h and the remaining 2 bottles were stopped after 72 h, yielding 3 replicates for measures taken at 24 and 48 h and 6 replicates for measurements done at 72 h, i.e. measurements of total gas, methane and volatile fatty acid (VFA) productions and *in vitro* apparent organic matter digestibility (IVOMD).

3.4.3. Total gas and methane production

The total gas production (TGP) was measured continuously over 72 h of incubation according to Kinley *et al.* (2016), with the cumulative pressure recorded every 20 min. The cumulative TGP was obtained by converting the pressure readings to ml/g OM and ml/g OM fermented.

In vitro methane (CH_4) concentration was measured in headspace samples collected into pre-vacuumed 10 ml vials at the predetermined time series points of incubation (i.e. 24, 48 and 72 h). Gas concentration was determined as described by Kinley *et al.* 2016 using gas chromatography (GC-2014, Shimadzu Corporation, Kyoto, Japan) equipped with a Restek (Bellefonte, PA, USA) ShinCarbon ST 100/120 micropacked column (2 m × 1 mm) and both flame ionization detector (FID) and thermal conductivity detector (TCD). Methane concentration in headspace was converted to ml/g OM and ml/g OM fermented using the TGP by application of the natural gas law.

3.4.4. In vitro apparent digestibility of substrate organic matter and volatile fatty acids analysis

The IVOMD and VFA analyses were performed as described by Kinley *et al.* (2016). Briefly, after gas sample collection, the *in vitro* fluid was filtered with a 0.5 cm layer of filtration sand. The solid residues were dried (105°C) and burned (550°C; 8h) to determine the IVOMD. The concentration in VFA in the *in vitro* fluid samples was measured using a Shimadzu GC-17A equipped with a FID and a Restek Stabilwax-DA fused silica column (30 m × 0.25 mm × 0.25 µm), and with ultra purity N as the carrier gas was . Peak detection and integration of VFAwere performed with the Shimadzu GC Solution Software.

3.5. Statistical analysis

All statistical analyses were performed using the Statistical Analysis System version 9.4 (SAS Institute, Cary, NC, USA). The UNIVARIATE procedure was used to determine the pooled

standard error of the mean (SEM) of the chemical composition of *Desmanthus* cultivars and Rhodes grass. The *in vitro* fermentation parameters (i.e. IVOMD; TGP and methane (ml/g OM fermented and mL/g OM); and VFA's (mmol/l and mmol/g OM fermented) were assessed using the MIXED procedure in a model that included the *Desmanthus* species (i.e. *D. leptophyllus* cv. JCU 1, *D. virgatus* cv. JCU 2 and *D. bicornutus* cv. JCU 4) and seasons (i.e. summer, winter and spring) as fixed factors and their interactions. To compare the legume species between them and the control grass hay, the treatment (i.e. *D. leptophyllus* in summer, *D. leptophyllus* in winter, *D. leptophyllus* in spring, *D. virgatus* in summer, *D. virgatus* in winter, *D. virgatus* in spring, *D. bicornutus* in summer, *D. bicornutus* in winter, *D. bicornutus* in spring and Rhodes grass hay) was used as factor in a fixed model. Pearson's correlations were used to determine the interaction between CH_4 production at different incubation time points and the phenolic compounds concentration in forage samples. Least squares means ± SEM were considered significantly different at $P < 0.05$ and tending to differ when $P \leq 0.10$.

4. Results

4.1. Chemical composition

As displayed in Table 13, the CP concentration was higher in *D. leptophyllus* in summer and winter while in spring, it was greater in *D. bicornutus*. *Desmanthus virgatus* had a higher NDF content in summer and winter, but this content was greater in *D. leptophyllus* in spring. Irrespective of the season, *D. virgatus* contained more ADF, cellulose and ADL than the other varieties.

Desmanthus leptophyllus in summer had higher ADIN, NPN, SP, P_A and P_C fractions (Table 14). Compared to the legume cultivars, Rhodes grass had on average lower NPN and SP but higher NDIN and P_{B3} fractions. The content in TCHO in *Desmanthus* species and the grass were in the same range (Table 15). The control showed lower NSC and C_C, and higher SC and C_{B2}.

Among the legumes, *D. virgatus* displayed the lowest contents in all secondary compounds presented in Table 16. Rhodes grass contained less TP, TT, CT and HT than the legumes, and furthermore CT was not detected in the grass (Table 16).

Table 13. Chemical composition (g/kg DM) of the *Desmanthus* species (n=3) and Rhodes grass (*Chloris gayana*) hay samples (n=3).

Species	Cultivar	Season	CP	EE	NDF	ADF	Cellulose	ADL
D. leptophyllus	JCU1	Summer	170	49.2	470	271	212	59.4
		Winter	189	32.4	495	240	184	64.9
		Spring	135	29.7	470	238	184	51.5
D. virgatus	JCU2	Summer	126	29.6	525	340	277	72.7
		Winter	112	18.8	584	340	271	76.1
		Spring	132	25.2	458	257	199	56.7
D. bicornutus	JCU4	Summer	139	41.7	478	273	197	47.9
		Winter	174	51.0	398	172	130	41.3
		Spring	182	46.6	403	171	128	43.7
C. gayana	NA	NA	135	22.4	658	342	297	27.6
Pooled SEM	NA	NA	8.47	3.68	24.9	20.0	18.4	4.72

ADF, acid detergent fiber; ADL, acid detergent lignin; CP, crude protein (N×6.25); DM, dry matter; EE, Ether extract; NA, not applicable; ND, not determined; NDF, neutral detergent fiber; SEM, standard error of the mean.

Table 14. Protein fractions of *Desmanthus* species and Rhodes grass (*Chloris gayana*) hay (n=3).

Species	Cultivar	Season	ADIN (%TN)	NDIN (%TN)	NPN (%TN)	SP (%TN)	P_A (%CP)	P_{B1} (%CP)	P_{B2} (%CP)	P_{B3} (%CP)	P_C (%CP)
D. leptophyllus	JCU1	Summer	20.0	34.7	39.8	47.3	18.8	28.4	34.6	14.7	3.40
		Winter	15.4	38.3	31.9	38.2	12.2	26.0	36.0	22.9	2.92
		Spring	12.4	47.0	35.2	36.8	13.0	23.8	26.9	34.7	1.67
D. virgatus	JCU2	Summer	15.7	48.5	33.6	39.4	13.3	26.1	25.8	32.9	1.96
		Winter	16.9	54.2	29.1	35.9	10.5	25.4	25.0	37.3	1.89
		Spring	13.4	37.0	38.3	41.7	16.0	25.7	32.9	23.6	1.75
D. bicornutus	JCU4	Summer	13.1	44.7	20.4	38.7	7.9	30.8	27.9	31.6	1.82
		Winter	17.3	35.9	24.7	33.1	8.64	24.4	45.3	18.6	3.00
		Spring	9.10	29.6	24.5	34.1	6.86	27.3	43.7	20.5	1.66
C. gayana	NA	NA	12.2	53.0	18.0	22.9	4.87	18.0	34.7	40.8	1.64

ADIN, acid detergent insoluble nitrogen; NA, not applicable; NDIN, neutral detergent insoluble nitrogen; NPN, non-protein nitrogen; P_A, protein fraction A; P_{B1}, protein fraction B1; P_{B2}, protein fraction B2; P_{B3}, protein fraction B3 ; P_C, protein fraction C; SP, soluble protein.

Table 15. Carbohydrate and its fraction in the legume cultivars and control grass (n=3).

Species	Cultivar	Season	TCHO (%DM)	NSC (%DM)	SC (%DM)	C_C (%TCHO)	C_{B2} (%TCHO)
D. leptophyllus	JCU1	Summer	69.4	23.4	41.1	20.5	29.6
		Winter	70.8	22.5	42.3	22.0	28.6
		Spring	76.4	30.5	40.6	16.2	32.0
D. virgatus	JCU2	Summer	76.5	25.1	46.4	22.8	30.8
		Winter	83.1	25.7	52.4	22.0	36.5
		Spring	76.2	31.1	41.0	17.9	30.3
D. bicornutus	JCU4	Summer	73.5	26.7	41.6	15.6	35.3
		Winter	69.0	30.2	33.6	14.4	27.8
		Spring	69.0	29.5	34.9	15.2	28.6
C. gayana	NA	NA	74.3	9.62	58.6	8.91	67.0

C_{B2}, carbohydrate fraction B2; C_C, carbohydrate fraction C; DM, dry matter; NA, not applicable; NSC, non-structural carbohydrates; SC, structural carbohydrates; TCHO, total carbohydrates.

C_A (i.e. carbohydrate fraction A) and C_{B1} (i.e.carbohydrate fraction B1) were not determined.

4.2. In vitro fermentation

Overall, *D. bicornutus* had higher IVOMD after 24 h of incubation in rumen fluid ($P < 0.05$; 0.615 ± 0.015) than *D. virgatus* (0.548 ± 0.015), *D. leptophyllus* (0.383 ± 0.015) and the control grass hay (0.548 ± 0.026; Table 17). This species was on average similar to the control at 48 h (0.688 ± 0.016 *vs* 0.656 ± 0.027 of IVOMD, respectively) and 72 h (0.698 ± 0.010 *vs* 0.696 ± 0.017 of IVOMD). Compared to *D. virgatus* and *D. leptophyllus* after 72 h of incubation for the summer, winter and spring harvests, IVOMD was greater for *D. bicornutus* ($P < 0.01$; Table 17). Irrespective of the season and time of fermentation, *D. leptophyllus* showed lower IVOMD than the other legumes (24 h, $P < 0.01$; 48 h, $P < 0.05$; 72 h, $P < 0.001$).

Table 16. Phenols and tannins constituents (g/kg DM) of the leguminous and grass species samples (n=3).

Species	Cultivar	Season	TP	NTP	TT	CT	HT
D. leptophyllus	JCU1	Summer	79.9	8.7	71.3	34.1	37.1
		Winter	78.2	7.4	70.8	36.7	34.1
		Spring	96.2	8.6	87.6	37.2	50.4
D. virgatus	JCU2	Summer	36.9	6.6	30.3	14.5	15.9
		Winter	40.8	4.9	35.9	23.0	12.9
		Spring	49.9	10.8	39.0	25.1	14.0
D. bicornutus	JCU4	Summer	60.7	5.2	55.5	34.7	20.8
		Winter	73.0	7.5	65.4	45.3	20.1
		Spring	73.2	9.3	63.9	34.2	29.6
C. gayana	NA	NA	7.8	6.1	1.7	ND	1.7
Pooled SEM	NA	NA	8.25	0.60	8.0	4.19	4.49

CT, condensed tannins; DM, dry matter; HT, hydrolysable tannins; NA, not applicable; ND, not detected; NTP, non-tannin phenolics; SEM, standard error of the mean; TP, total phenolics; TT, total tannins.

Total gas produced (TGP, mL/g OM; Table 17) by *D. leptophyllus* was lower than the other forage species after 72 h of incubation ($P < 0.05$). However, when this parameter was expressed relative to the digestibility (mL/g OM fermented), differences became smaller since, TGP of this legume species was similar to that of *D. virgatus* in summer and spring, and to that of *D. bicornutus* in winter. In terms of CH_4 production (mL/g OM; Table 17), irrespective to the season , *D. leptophyllus* produced less *in vitro* CH_4 compared to the other substrates after 72 h of incubation ($P < 0.05$). The *Desmanthus* species released less CH_4 than the control grass hay at 72 h of incubation ($P < 0.001$). The higher TGP reached by Rhodes grass ($P < 0.01$) was associated to greater CH_4 production ($P < 0.05$). When expressed to the OM fermented (mL CH_4/g OM fermented), *D. leptophyllus* and/or *D. bicornutus*, according to the season, displayed lower CH_4 production after 72 h ($P < 0.001$; Table 17). Both species produced less CH_4 per g of OM fermented than the control after 48 and 72 h ($P < 0.05$).

Methane production (mL/g OM) was negatively correlated to TP, with *r* values in the range -0.55 to -0.61 ($P < 0.01$). This was also observed with TT (r = -0.58 to -0.61; $P < 0.001$) but not with NTP. Correlation between CH_4 production (mL/g OM) and HT was higher

(r = -0.71 at 24 and 48 h; r = - 0.64 at 72 h; $P < 0.001$) than with CT ($P > 0.05$ at 24 and 48 h; r = -0.33 and $P < 0.05$ at 72 h). The same trend was also obtained with CH_4 per g of OM fermented at 24 (r = -0.33, $P < 0.1$ for CT *vs* r = -0.60, $P < 0.001$ for HT) and 48 h (r = -0.42, $P < 0.05$ for CT *vs* r = -0.49, $P < 0.05$ for HT) but with lower r-values. However, after 72 h of incubation the correlation was greater between CH_4 production per gram of OM fermented and CT (r = -0.62; $P < 0.001$ *vs* r = -0.44; $P < 0.01$ for HT).

The total VFA concentration was not different between *Desmanthus* species in summer, winter and spring seasons (Table 18 for 72 h of incubation; data not shown for 24 h and 48 h). Overall after 72 h of incubation, the total VFA concentration was higher for the control ($P < 0.05$; 125.9 ± 4.60 mmol/l) than the *Desmanthus* species (115.0, 113.1 and 105.7 ± 2.66 mmol/l for *D. virgatus*, *D. bicornutus* and *D. leptophyllus* respectively). Similar effect was obtained for acetate ($P < 0.05$) and propionate ($P < 0.001$) concentrations. However, when total VFA were expressed relative to the digestibility (mmol/g OM fermented; Table 18), the legume cultivars, with the exception of *D. bicornutus* in spring, produced similar amounts of VFA compared to Rhodes grass. The only cultivar that produced higher VFA that the control was *D. leptophyllus* ($P < 0.01$).

Table 17. Effects of *Desmanthus* spp. on *in vitro* organic matter digestibility (IVOMD), total gas and methane (CH_4) production at 24, 48 and/or 72 hours of incubation.

Species	Cultivar	Season	IVOMD			Total gas (ml/g OM fermented)			CH_4 (mL/g OM fermented)			Total gas	CH_4
												(ml/g OM)	
			24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	72 h	
D. leptophyllus	JCU1	Summer	0.369^{b}	0.423^{b}	0.454^{c}	203.8^{a}	229.2^{a}	247.3^{a}	17.50^{b}	27.56^{a}	30.71^{b}	111.1^{c}	13.64^{c}
		Winter	0.403^{c}	0.502^{b}	0.502^{c}	203.9^{b}	219.8^{b}	232.5^{b}	19.45^{b}	26.01^{b}	29.56^{c}	114.6^{c}	14.62^{b}
		Spring	0.378^{b}	0.492^{b}	0.514^{c}	235.8^{a}	248.6^{a}	259.5^{a}	22.53^{a}	29.81^{a}	33.13^{ab}	132.9^{b}	18.06^{b}
D. virgatus	JCU2	Summer	0.562^{a}	0.622^{a}	0.625^{b}	218.5^{a}	240.3^{a}	257.8^{a}	23.77^{a}	29.55^{a}	36.86^{a}	159.8^{a}	22.76^{a}
		Winter	0.507^{b}	0.577^{b}	0.597^{b}	250.7^{a}	279.0^{a}	287.5^{a}	28.74^{a}	37.95^{a}	39.90^{a}	169.8^{a}	23.54^{a}
		Spring	0.574^{a}	0.660^{a}	0.662^{b}	220.6^{a}	235.6^{a}	238.4^{ab}	26.16^{a}	33.52^{a}	34.76^{a}	157.6^{a}	23.00^{a}
D. bicornutus	JCU4	Summer	0.593^{a}	0.655^{a}	0.657^{a}	197.5^{a}	208.9^{a}	217.3^{b}	21.95^{ab}	27.80^{a}	28.71^{b}	142.7^{b}	18.87^{b}
		Winter	0.601^{a}	0.684^{a}	0.701^{a}	215.9^{ab}	227.3^{ab}	228.4^{b}	24.35^{ab}	30.52^{b}	32.94^{b}	159.3^{b}	22.99^{a}
		Spring	0.650^{a}	0.713^{a}	0.732^{a}	201.5^{a}	218.2^{a}	219.6^{b}	24.35^{a}	31.17^{a}	32.58^{b}	160.5^{a}	23.82^{a}
C. gayana†	NA	NA	0.547	0.656	0.698	245.3	276.2	260.7	27.13	38.09	38.75	183.9	27.35
Pooled SEM		Summer	0.020	0.035	0.008	10.76	21.04	6.66	1.69	2.12	1.16	3.37	0.75
		Winter	0.019	0.033	0.019	12.56	16.65	6.32	1.69	1.57	0.95	3.14	0.68
		Spring	0.034	0.019	0.016	11.85	12.99	6.81	1.19	1.36	0.64	2.40	0.52

Three (24 and 48 h) and six (72 h) samples were initially incubated for each forage. NA, not applicable; OM, organic matter; SEM, standard error of the mean.
Least squares means values among the legumes within the same column and per seasons followed by the same letters are not significantly different ($P < 0.05$). † Positive control (not included in the statistical analysis).

Table 18. Comparative volatile fatty acids concentration (mmol/l) between *Desmanthus* spp. over 72 h of *in vitro* incubation with rumen fluid from grazing Brahman Zebu rumen-cannulated steers.

Species	Cultivar	Season	Volatile fatty acids						A:P	A:B	Total VFA	
			Acetic	Propionic	*Iso*-butyric	Butyric	*Iso*-valeric	Valeric			mmol/l	mmol/g OM fermented
D. leptophyllus	JCU1	Summer	74.6^{a}	15.4^{a}	1.3^{a}	10.0^{a}	2.4^{a}	1.3^{a}	4.9^{a}	7.6^{a}	105.1^{a}	29.40^{a}
		Winter	75.1^{a}	15.3^{b}	1.4^{a}	10.5^{a}	2.6^{a}	1.3^{a}	4.9^{a}	7.4^{a}	106.2^{a}	27.13^{a}
		Spring	75.3^{a}	14.9^{b}	1.2^{b}	10.8^{a}	2.2^{b}	1.2^{b}	5.1^{a}	7.2^{a}	105.6^{a}	26.35^{a}
D. virgatus	JCU2	Summer	81.2^{a}	17.4^{a}	1.6^{a}	12.3^{a}	2.8^{a}	1.5^{a}	4.7^{a}	6.8^{a}	116.6^{a}	23.32^{b}
		Winter	78.5^{a}	17.7^{a}	1.4^{a}	12.6^{a}	2.5^{a}	1.3^{a}	4.4^{b}	6.3^{a}	114.0^{a}	23.91^{b}
		Spring	79.3^{a}	17.0^{a}	1.5^{ab}	12.4^{a}	2.8^{ab}	1.5^{ab}	4.7^{b}	6.5^{a}	114.4^{a}	21.61^{b}
D. bicornutus	JCU4	Summer	75.7^{a}	15.7^{a}	1.5^{a}	11.8^{a}	2.7^{a}	1.4^{a}	4.8^{a}	6.5^{a}	108.9^{a}	20.69^{b}
		Winter	81.6^{a}	17.1^{ab}	1.5^{a}	12.2^{a}	2.8^{a}	1.5^{a}	4.8^{ab}	6.8^{a}	116.7^{a}	20.79^{c}
		Spring	78.8^{a}	16.5^{a}	1.6^{b}	12.2^{a}	3.0^{a}	1.6^{a}	4.8^{ab}	6.6^{a}	113.8^{a}	19.45^{c}
C. gayana†	NA	NA	88.5	20.1	1.7	11.0	2.9	1.7	4.4	8.3	125.9	22.53
Pooled SEM		Summer	2.99	0.67	0.14	0.98	0.27	0.12	0.12	0.41	4.99	1.04
		Winter	3.30	0.69	0.15	1.00	0.28	0.13	0.14	0.41	5.25	0.94
		Spring	2.29	0.52	0.12	0.86	0.25	0.11	0.12	0.42	3.68	0.49

Six samples were initially incubated for each forage.

NA, not applicable; SEM, standard error of the mean; VFA, volatile fatty acids. A:P, acetic : propionic acid; A:B, acetic : n-butyric acid.

Least squares means values among the legumes within the same column and per seasons followed by the same letters are not significantly different ($P < 0.05$).

† Positive control (not included in the statistical analysis).

5. Discussion

The objective of the present study was to investigate the effects of *Desmanthus* spp. on *in vitro* fermentation profile including methane production using rumen fluid from grazing Brahman steers. The main finding was that, according to the season, *D. leptophyllus* and/or *D. bicornutus* exhibited the highest anti-methanogenic potential. Compared to the control Rhodes grass hay, *in vitro* CH_4 production per gram of OM fermented was reduced by 15 to 36 % with *D. leptophyllus*, and by 10 to 27 % wit *D. bicornutus*. This may be due to the presence of secondary compounds such as CT because both species contain the highest concentrations of these molecules and significant correlations were found. Previous studies on sheep and cattle demonstrated the potential of CT to lower CH_4 emissions, either with purified CT extracts (Grainger *et al.* 2009; Tan *et al.* 2011) or CT-containing forages (Tavendale *et al.* 2005; Hess *et al.* 2006; Ramírez-Restrepo *et al.* 2010). However, although the direct effect of HT on the fermentation was not assessed, the anti-methanogenic effect of HT or the combination of tannins present in *Desmanthus* biomass cannot be ruled out because the systemic interrelation amongst polyphenolic compounds, protein and carbohydrate fractions is complex (Tedeschi *et al.* 2014; Tedeschi and Fox 2016). Jayanegara *et al.* (2015) investigated the impacts of purified HT and CT on CH_4 emission and highlighted the anti-methanogenic effect of HT associated to less detrimental impact on digestibility, as demonstrated with CT (Kamalak *et al.* 2004; Animut *et al.* 2008). Our study supports the hypothesis that HT might play a considerable role on CH_4 mitigation as we found a significant negative correlation between HT concentration in *Desmanthus* forages and CH_4 emission per g of OM fermented (r = -0.60, $P < 0.001$ at 24 h; r = -0.49, $P < 0.05$ at 48 h and r = -0.44, $P < 0.01$ at 72 h). Indeed, hydrolysable tannins have been reported to decrease methanogenesis via direct effect as the inhibition of the growth and/or activity of methanogens and/or hydrogen-producing microbes (Bhatta *et al.* 2009; Jayanegara *et al.* 2010). Mechanisms involving HT in *Desmanthus* plants on methanogenesis need to be deeper investigated as this form of tannins is much less documented than CT (Bhatta *et al.* 2009). Furthermore, when examining a range of plant secondary compounds for their CH_4 reduction attributes, Jayanegara *et al.* (2015) suggested that measuring the biological activity of metabolites, e.g. via the assessment of bovine serum albumin protein precipitation capacity (e.g. Asquith and Butler 1985), is more accurate than the concentration of compounds. Thus, the assessment of the biological activity of the tannins in both *Desmanthus* species would be appropriate to confirm these preliminary results reporting the significant role of HT on CH_4 mitigation.

Results showed that, among the two species presenting an anti-methanogenic potential, *Desmanthus bicornutus* was more digestible. *Desmanthus leptophyllus* which presented reduced *in vitro* gas and CH_4 production (ml/g OM) had lower IVOMD irrespective of the season (Table 17). This observation resulted in a contrasting trend when gas and CH_4 are expressed per g of OM fermented. While HT appear to decrease CH_4 more through direct effect, CT would also act indirectly by a reduction in fiber digestion (Jayanegara *et al.* 2010; Tiemann *et al.* 2008). However, in a study achieved to separate the effects of CT and fiber on digestibility of tropical legumes containing CT, Tiemann *et al.* (2008) concluded that low quality and reduced CH_4 production of CT-rich legumes are also partly explained by the degradation of fiber in the rumen. Properties of fiber in highly tanniferous plants seem determinant because lignin prevented mainly hemicellulose from degradation through the formation of indigestible complexes, while it is suggested that the extent of degradation of hemicellulose have influenced CH_4 production. Moreover, in our study, *D. bicornutus* had lower detergent fiber contents than *D. leptophyllus*. Since this method does not consider soluble fiber fractions, it can be hypothesized that the content in highly fermentable soluble carbohydrates is higher for *D. bicornutus* and hence induced higher IV-OMD values. This increased fermentability resulted in higher CH_4 production, but expressed per g of fermented OM, CH_4 production in *D. bicornutus* was on average as low as that of *D. leptophyllus*, showing the high potential of this species in supplying high value forage with low CH_4.

Reduced CH_4 production accompanied with lower digestibility has been observed in previous research (Animut *et al.* 2008; Tan *et al.* 2011). Supplementing goats fed sorghum-sudangrass (*S. bicolor*) with different levels of the CT-containing legume *Lespedeza striata* (i.e. 1.00, 0.67, 0.33 and 0) reduced CH_4 emission, but at relatively low dietary CT levels this was not accompanied by considerable adverse effects on digestion such as total tract N digestibility (Animut *et al.* 2008). It is clear that CT bind protein in the rumen and the CT-protein complex remain stable at pH 5.5-7 (Jones and Mangan 1977), protected from microbial degradation (McLeod 1974; Min *et al.* 2005). Rumen bypass protein then becomes available for lower digestive tract utilization (Jones and Mangan 1977), which results in improved feed protein availability to the animal. Therefore, CT-containing *Desmanthus* spp. may have a beneficial impact on dietary N efficiency compared to Rhodes grass, but this should be considered within a framework of *in vivo* studies.

Compared to the control diet, the total VFA, acetate and propionate concentration after 72 h of incubation (Table 18) were overall reduced for the *Desmanthus* spp. Volatile fatty

acids are the main source of metabolizable energy for ruminants, resulting from the rumen microbial fermentation (Bergman 1990). As a result, a reduction in VFA production is undesired. As VFA result from the fermentation of the diet in the rumen, a lower fermentability may explain lower VFA production which was observed in this study. However, when VFA production was expressed relative to the IVOMD, *Desmanthus leptophyllus* that had the lowest IVOMD presented then the highest VFAs production (mmol/g OM fermented; Table 18). It is also known that plant secondary compounds such as tannins may modulate the microbial consortium of the rumen environment including notably modified microbial diversity and activity (McSweeney *et al.* 2001, 2002), and lead to a reduction in VFA production (Bhatta *et al.* 2009; Jayanegara *et al.* 2015). Polyphenolic secondary compounds in the legume species may explain once more the lower VFA concentration compared to Rhodes grass in which they were mostly lower or not found. In a study on tropical legumes with various CT concentration (Barahona *et al.* 2003), CT-containing *Flemingia macrophylla* and *Desmodium ovalifolium* had lower total VFA than the non-tanniferous legumes *Leucaena macrophylla* as it is observed in our study with *Desmanthus* forage containing CT compared to the non-tanniferous control grass. However, the relation with CT in not straightforward as in the study of Barahona *et al.* 2003, *Calliandra calothyrsus* which had similar or higher CT content produced greater VFA concentration than both *F. macrophylla* and *D. ovalifolium,* and *Leucaena leucocephala* produced higher VFA concentration with CT content in the same range than *D. ovalifolium.*

Although lower VFA production would imply less energy available for the animal, previous studies demonstrated that supplementing with *Desmanthus* spp. steers fed Buffel (*Cenchrus ciliaris*) and sheep fed Mitchell grasses (*Astrebla* spp.) increased steers weight gain (Gardiner and Parker 2012) and boosted sheep wool production (Rangel and Gardiner 2009). Furthermore, in our study, all *Desmanthus* cultivars contained less than 50 g CT/kg DM. It has been reported that high dietary CT concentration (i.e. > 50 g CT/kg DM) can lead to low feed intake (Barry and Duncan 1984; Waghorn *et al.* 1994; Bhatta *et al.* 2002), while low amounts in CT-containing legumes (i.e. < 50 g CT/kg DM) did not (Wang *et al.* 1996; Carulla *et al.* 2005) even as the plant matured (Ramírez-Restrepo *et al.* 2006). Consequently, it is reasonable to assume that the CT concentration in *Desmanthus* spp. could enhance rumen metabolism and systemic dynamic physiology (Tedeschi *et al.* 2014; Tedeschi and Fox, 2016). Tannin chemistry in terms of molecular size and monomer composition is likely to influence the strength of interaction of tannins with dietary proteins and fibre, and thus forage quality (Barahona *et al.* 2003). For example, the astringency (i.e. ability to bind protein) of *C.*

calothyrsus seemed to be related to the tannin structure (Lascano *et al.* 2003; Stewart *et al.* 2000). However, further research is required to elucidate the magnitude of significant molecular interactions and the systemic exposure to analogous physiologic mechanisms.

Overall, although in our study *Desmanthus* spp reduced *in vitro* CH_4 production and VFA concentrations compared to the control grass, the potential sustainable impact of *Desmanthus* spp. on tropical farming systems should be determined by subsequent pastoral experimentation.

6. Conclusion

Differences in fermentative traits between the *Desmanthus* cultivars along the seasons may provide opportunities to minimise the environmental footprint of pastoral systems in northern Australia. However, the degree of further collaboration between the academia, primary producers and agricultural industries will make a major difference to confront the challenges imposed by climate variability and the imperative need to cope with the growing food security vulnerability.

7. References

Ammar H, López S, González JS, Ranilla MJ (2004) Tannin levels in the foliage of some Spanish shrub species at different stages of development. In 'Options Méditerranéennes: Série A. Séminaires Méditerranéens No. 59. Nutrition and feeding strategies of sheep and goats under harsh climates'. (Eds H Ben Salem, A Nefzaoui , P Morand-Fehr) pp. 159–163. (CIHEAM: Zaragoza)

Animut G., Puchala R, Goetsch AL, Patra AK, Sahlu T, Varel VH, Wells J (2008) Methane emission by goats consuming diets with different levels of condensed tannins from lespedeza. *Animal Feed Science and Technology* **144**, 212–227. doi:10.1016/j.anifeedsci.2007.10.014

AOAC (1995) 'Official Methods of Analysis of AOAC INTERNATIONAL, 16th ed.' (Association of Official Analytical Chemists: Arlington)

Asquith TN, Butler LG (1985) Use of dye-labeled protein as spectrophotometric assay for protein precipitants such as tannin. *Journal of Chemical Ecology* **11**, 1535–1544.

Barahona R, Lascano CE, Narvaez N, Owen E, Morris P, Theodorou MK (2003) *In vitro* degradability of mature and immature leaves of tropical forage legumes differing in condensed tannin and non-starch polysaccharide content and composition. *Journal of the Science of Food and Agriculture* **83**, 1256–1266. doi:10.1002/jsfa.1534.

Barry TN, Duncan SJ (1984) The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. *British Journal of Nutrition* **51**, 485–491.

Bhatta R, Shinde AK, Vaithiyanathan S, Sankhyan SK, Verma D (2002) Effect of polyethylene glycol-6000 on nutrient intake, digestion and growth of kids browsing *Prosopis cineraria*. *Animal Feed Science and Technology* **101**, 45–54.

Bhatta R, Uyeno Y, Tajima K, Takenaka A, Yabumoto Y, Nonaka I, Enishi O, Kurihara M (2009) Difference in the nature of tannins on *in vitro* ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. *Journal of Dairy Science* **92**, 5512–5522. doi:10.3168/jds.2008-1441

Bergman EN (1990) Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews* **70**, 567–590.

Carulla J, Kreuzer M, Machmüller A, Hess H (2005) Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. *Crop and Pasture Science* **56**, 961–970.

Cook B, Pengelly BC, Brown S, Donnelly JB, Eagles D, Franco A, Hanson J, Mullen BF, Partridge I, Peters M, Schultze-Kraft R (2005) Tropical Forages: an interactive selection tool. [CD-ROM], CSIRO, DPI&F(Qld), CIAT and ILRI, Brisbane, Australia [Online]. Available at http://www.tropicalforages.info/ (Verified 22 March 2016)

Cox K, Gardiner C (2013) Pasture legumes in Queensland–a new wave? In 'Proceedings of the Northern Beef Research Update Conference'. pp. 15-20. (Queensland North Australia Beef Research Council: Gympie, Qld)

Dalzell SA, Shelton HM, Mullen BF, Larsen PH, McLaughlin KG (2006) 'Leucaena: a guide to establishment and management.' (Meat & Livestock Australia Ltd.: Sydney, NSW)

Department of Primary Industries Queensland (1988) ´Native pastures in Queensland. The resources and their management.´ (Department of Primary Industries, Queensland Government: Brisbane)

Department of Primary Industries Queensland (1999) ´Managing grazing in northern Australia.´ (Department of Primary Industries, Queensland Government: Brisbane)

Gardiner CP, Swan SJ (2008) Abandoned pasture legumes offer potential economic and environmental benefits in semiarid clay soil rangelands. In: 'A climate of change in the rangelands. Australian Rangeland Society, 15th Biennial Conference Proceedings', 28 September - 2 October 2008, Charters Towers, Australia (Australian Rangeland Society: Mount Lawley, WA)

Gardiner C, Parker AJ (2012) Steer liveweight gains on Progardes™ *Desmanthus*/buffel pastures in Queensland. In 'Proceedings of Second Joint Conference of the New Zealand Society of Animal Production and the Australian Society of Animal Production', Lincoln University, Christchurch, New Zealand, 2–5 July 2012. pp. 1. (Lincoln University: Christchurch)

Gardiner C, Kempe N, Hannah I, McDonald J (2013) PROGARDES ™: a legume for tropical/subtropical semi-arid clay soils. *Tropical Grasslands-Forrajes Tropicales* **1**, 78–80.

Gardiner CP (2016) Developing and commercializing new pasture legumes for clay soils in the semi-arid rangelands of northern Australia: The new *Desmanthus* cultivars JCU 1-5 and the Progardes story. In 'Tropical forage legumes. Harnessing the potential of *Desmanthus* and other genera for heavy clay soils'. (Eds JR Lazier, N Ahmad) pp. 283-304. (CABI: Boston)

Goering HK, Van Soest PJ (1970) 'Forage fiber analyses (apparatus, reagents, procedures, and some applications) Agriculture Handbook No. 379.' (ARS-USDA: Washington DC)

Gonzalez-V. EA, Hussey MA, Ortega-S. JA (2005) Nutritive value of *Desmanthus* associated with Kleingrass during the establishment year. *Rangeland Ecology & Management* **58**, 308–314.

Grainger C, Clarke T, Auldist MJ, Beauchemin KA, McGinn SM, Waghorn GC, Eckard RJ (2009) Potential use of *Acacia mearnsii* condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. *Canadian Journal of Animal Science* **89**, 241–251.

Hess HD, Tiemann TT, Noto F, Carulla JE, Kreuzer M (2006) Strategic use of tannins as means to limit methane emission from ruminant livestock. *International Congress Series* **1293**, 164–167. doi:10.1016/j.ics.2006.01.010

Hill JO, Coates DB, Whitbread AM, Clem RL, Robertson MJ, Pengelly BC (2009) Seasonal changes in pasture quality and diet selection and their relationship with liveweight gain of steers grazing tropical grass and grass–legume pastures in northern Australia. *Animal Production Science* **49**, 983–993. doi:10.1071/EA06331

Jayanegara A, Goel G, Makkar HPS, Becker K (2010) Reduction in methane emissions from ruminants by plant secondary metabolites: Effects of Polyphenols and Saponins. In 'Sustainable Improvement of Animal Production and Health'. (Eds NE Odongo, M Garcia, GJ Viljoen) pp. 151–157. (FAO: Rome)

Jayanegara A, Goel G, Makkar HPS, Becker K (2015) Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population *in vitro*. *Animal Feed Science and Technology* **209**, 60–68. doi:10.1016/j.anifeedsci.2015.08.002

Jones RM, Brandon NJ (1998) Persistence and productivity of eight accessions of *Desmanthus virgatus* under a range of grazing pressures in subtropical Queensland. *Tropical Grasslands* **32**, 145–152.

Jones WT, Mangan JL (1977) Complexes of the condensed tannins of sainfoin (*Onobrychis viciifolia* Scop.) with fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. *Journal of the Science of Food and Agriculture* **28**, 126–136.

Kamalak A, Filho JMP, Canbolat O, Gurbuz Y, Ozay O, Ozkan CO (2004) Chemical composition and its relationship to *in vitro* dry matter digestibility of several tannin-containing trees and shrub leaves. *Livestock Research for Rural Development* **16**, 1–5.

Kanani J, Lukefahr SD, Stanko RL (2006) Evaluation of tropical forage legumes (*Medicago sativa*, *Dolichos lablab*, *Leucaena leucocephala* and *Desmanthus bicornutus*) for growing goats. *Small Ruminant Research* **65**, 1–7. doi:10.1016/j.smallrumres.2005.04.028

Kinley RD, de Nys R, Vucko MJ, Machado L, Tomkins NW (2016) The red macroalgae *Asparagopsis taxiformis* is a potent natural antimethanogenic that reduces methane production during *in vitro* fermentation with rumen fluid. *Animal Production Science* **56**, 282–289. doi:10.1071/AN15576

Lascano C, Avila P, Stewart J (2003) Intake, digestibility and nitrogen utilization by sheep fed with provenances of *Calliandra calothyrsus* Meissner with different tannin structure. *Archivos Latinoamericanos de Producción Animal* **11**, 21–28.

Loch DS (2015) *Desmanthus* varieties JCU1 -5 descriptors. Plant Varieties Journal 28, 2. pp. 59–63 and 159–192. Available at https://www.ipaustralia.gov.au/sites/g/files/net856/f/journals/pvj_vol_28_2.pdf (Verified 18 September 2016)

Makkar HP (2003) 'Quantification of tannins in tree and shrub foliage: a laboratory manual.' (Kluwer Academics Publishers: Dordrecht, The Netherlands)

Manteca X, Villalba JJ, Atwood SB, Dziba L, Provenza FD (2008) Is dietary choice important to animal welfare? *Journal of Veterinary Behavior: Clinical Applications and Research* **3**, 229–239. doi:10.1016/j.jveb.2008.05.005

McLeod MN (1974) Plant tannins - Their role in forage quality. *Nutrition Abstracts & Reviews* **44**, 803–815.

McSweeney CS, Odenyo A, Krause DO (2002) Rumen microbial responses to antinutritive factors in fodder trees and shrub legumes. *Journal of Applied Animal Research* **21**, 181–205. doi:10.1080/09712119.2002.9706369

McSweeney CS, Palmer B, McNeill DM, Krause DO (2001) Microbial interactions with tannins: nutritional consequences for ruminants. *Animal Feed Science and Technology* **91**, 83–93.

Min BR, Attwood GT, McNabb WC, Molan AL, Barry TN (2005) The effect of condensed tannins from *Lotus corniculatus* on the proteolytic activities and growth of rumen bacteria. *Animal Feed Science and Technology* **121**, 45–58. doi:10.1016/j.anifeedsci.2005.02.007

NHMRC (2013) 'Australian code for the care and use of animals for scientific purposes.' 8th ed. (National Health and Medical Research Council: Canberra)

Pengelly BC, Conway MJ (2000) Pastures on cropping soils: which tropical pasture legume to use? *Tropical Grasslands* **34**, 162–168.

Porter LJ, Hrstich LN, Chan BG (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* **25**, 223–230.

Provenza FD, Villalba JJ (2010) The role of natural plant products in modulating the immune system: An adaptable approach for combating disease in grazing animals. *Small Ruminant Research* **89**, 131–139. doi:10.1016/j.smallrumres.2009.12.035

Provenza FD, Villalba JJ, Haskell J, MacAdam JW, Griggs TC, Wiedmeier RD (2007) The value to herbivores of plant physical and chemical diversity in time and space. *Crop Science* **47**, 382–398. doi:10.2135/cropsci2006.02.0083

Ramírez-Restrepo CA, Barry TN, López-Villalobos (2006) Organic matter digestibility of condensed tannin-containing *Lotus corniculatus* and its prediction *in vitro* using cellulase/hemicellulose enzymes. *Animal Feed Science and Technology* **125**, 61–71.

Ramírez-Restrepo CA, Barry TN, Marriner A, López-Villalobos N, McWilliam EL, Lassey KR, Clark H (2010) Effects of grazing willow fodder blocks upon methane production and blood composition in young sheep. *Animal Feed Science and Technology* **155**, 33–43. doi:10.1016/j.anifeedsci.2009.10.003

Ramírez-Restrepo CA, O'Neill CJ, López-Villalobos N, Padmanabha J, McSweeney C (2014) Tropical cattle methane emissions: the role of natural statins supplementation. *Animal Production Science* **54**, 1294–1299. doi:10.1071/AN14246

Ramírez-Restrepo CA, Charmley E (2015) An integrated mitigation potential framework to assist sustainable extensive beef production in the tropics. In ´Grassland: A Global Resource Perspective´. (Eds PK Ghosh, SK Mahanta, JB Singh, PS Pathak) pp 417-436. (Range Management Society of India: Jhansi)

Rangel JH de A, Gardiner CP (2009) Stimulation of wool growth by *Desmanthus* spp. as a supplement in a Mitchell grass hay diet. *Tropical Grasslands* **43**, 106–111.

Shelton HM, Lowry JB, Gutteridge RC, Bray RA, Wildin JH (1991) Sustaining productive pastures in the tropics. 7. Tree and shrub legumes in improved pastures. *Tropical Grasslands* **25**, 119–128.

Singh B, Sahoo A, Sharma R, Bhat TK (2005) Effect of polethylene glycol on gas production parameters and nitrogen disappearance of some tree forages. *Animal Feed Science and Technology* **123–124**, 351–364. doi:10.1016/j.anifeedsci.2005.04.033

Singh S, Kushwaha BP, Nag SK, Mishra AK, Bhattacharya S, Gupta PK, Singh A (2011) *In vitro* methane emission from Indian dry roughages in relation to chemical composition. *Current Science* **101**, 57-65.

Stewart JL, Mould F, Mueller-Harvey I (2000) The effect of drying treatment on the fodder quality and tannin content of two provenances of *Calliandra calothyrsus* Meissner. *Journal of the Science of Food and Agriculture* **80**, 1461–1468.

Tan HY, Sieo CC, Abdullah N, Liang JB, Huang XD, Ho YW (2011) Effects of condensed tannins from *Leucaena* on methane production, rumen fermentation and populations of methanogens and protozoa *in vitro*. *Animal Feed Science and Technology* **169**, 185–193.

Tavendale MH, Meagher LP, Pacheco D, Walker N, Attwood GT, Sivakumaran S (2005) Methane production from *in vitro* rumen incubations with *Lotus pedunculatus* and *Medicago sativa*, and effects of extractable condensed tannin fractions on methanogenesis. *Animal Feed Science and Technology* **123–124**, 403–419. doi:10.1016/j.anifeedsci.2005.04.037

Tedeschi LO, Ramírez-Restrepo CA, Muir JP (2014) Developing a conceptual model of possible benefits of condensed tannins for ruminant production. *Animal* **8,** 1095-1105. doi:10.1017/S1751731114000974

Tedeschi Lo, Fox DG (2016) ´The ruminant nutrition system: An applied model for predicting nutrient requirements and feed utilization in ruminants.´ (XanEdu Publishing Inc: Acton)

Tiemann TT, Lascano CE, Kreuzer M, Hess HD (2008) The ruminal degradability of fibre explains part of the low nutritional value and reduced methanogenesis in highly tanniniferous tropical legumes. *Journal of the Science of Food and Agriculture* **88**, 1794–1803. doi:10.1002/jsfa.3282.

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583–3597.

Vera RR, Seré C (1985) Evaluation of tropical pasture species with a farming system perspective. In 'Proceedings XV International Grassland Congress'. Aug. 1985, Kyoto, Japan, pp. 1187–1188. (Science Council of Japan/Japanese Society of Grassland Science: Nishi-nasuno)

Waghorn GC, Shelton ID, McNabb WC (1994) Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 1. Non-nitrogenous aspects. *Journal of Agricultural Science (Cambridge)* **123**, 99–107.

Wang Y, Douglas GB, Waghorn GC, Barry TN, Foote AG, Purchas RW (1996) Effect of condensed tannins upon the performance of lambs grazing *Lotus corniculatus* and lucerne (*Medicago sativa*). *Journal of Agricultural Science (Cambridge)* **126**, 87–98.

CHAPTER VII

General discussion and perspectives

The research undertaken in this thesis demonstrated that shrubs and trees influence the behaviour of heifers grazing in a temperate silvopastoral system and the *in vitro* fermentation in the rumen. The different sections of the thesis aimed at determining (i) how are shrub and tree species integrated in ruminant production systems; (ii) which temperate woody forage species are consumed by browsing cattle; (iii) once consumed, how will woody forage species modulate rumen fermentation; (iv) what are the effects of secondary compounds of woody forage on the rumen protein metabolism; and (v) how will tropical browse species influence rumen fermentation including methane production. The graphical abstract (Figure 3) illustrates the different issues related to silvopastoral systems that are relevant to study; topics surrounded with a green rectangle represent what was achieved in this work.

1. Use of shrubs and trees in silvopastoral systems

The first section of this work concerned the use of shrubs and trees in temperate and tropical ruminant production systems (Chapter 2 and Figure 3). This issue was addressed also in Chapters 4 and 5 and in Chapter 6 as grassland-based systems in Belgium and Australia are very different, notably regarding herd size and pasture management including stocking density. In Wallonia, dairy and beef cattle farms include on average around 40 dairy cows and 35 suckler cows respectively (SPW 2016) while in coastal and central Queensland beef cattle herds have been reported to carry on average between 500 to 2000 heads of cattle (McIvor 2005). Systems with grazing cattle in Wallonia are rather intensive (Hautier *et al.* 2014), while the northern Australian systems are more extensive (Lefroy *et al.* 1992; Cox and Gardiner 2013; Ramírez-Restrepo and Charmely 2015). In this work, woody forage was integrated within a hedgerow in the experimental trial of Chapters 4 and 5, while *Desmanthus* species studied in Chapter 6 are shrubby legumes that are usually sown to improve pastures (Gardiner *et al.* 2013). In both of these temperate and tropical systems, shrubs and trees were available to grazing cattle by direct browsing. However, other ways of integrating the woody fodder in ruminants' diet do exist as it was illustrated in Chapter 2 with cut-and-carry systems and the preservation of forage. Those types of silvopastoral systems need also to be investigated to better understand how to manage properly the woody forage component. Furthermore, as the primary purpose of woody forage within production systems in this work is to feed livestock, the biomass production is thus essential. Biomass yield varies between species (Clem and Hall 1994; Liagre 2006; Mullen and Gutteridge 2002) as it is illustrated in Table 2 with the studied species such as *Fraxinus excelsior* or *Desmanthus virgatus.*

Productivity of shrubs and trees has not been investigated in this work but should be evaluated with both above-mentioned temperate and tropical species (Figure 3). Relying on woody fodder as plain forage is occasional under the climate conditions of Belgium, while this practice is prevalent notably during the seasonally dry periods (April-October) in northern Australia. Therefore the productivity of woody plant is an important point to investigate in the future.

The implementation of silvopastoral systems requires to determine the most appropriate woody species according to the system conditions. Where the woody fodder is directly consumed by the animal as the ones studied in this work (i.e. hedge and sown tropical shrubby legumes), the ability to stand browsing is crucial to avoid overbrowsing. Planting shrubs and trees with similar palatability might prevent this problem (Papachristou and Papanastasis 1994). For example, *F. excelsior* seemed more sensitive to browsing by sheep than *Acer pseudoplatanus*, which might be explained notably by a difference of palatability between species (Eason *et al.* 1996). Beyond taste other natural browsing deterrents might also avoid overbrowsing as the presence of spines in *Crataegus monogyna*. Furthermore, the ecological territory is an important criterion as not all species will adapt properly within the system. For example, *Acer campestre* and *F. excelsior* are not suitable for acidic soils (Rameau *et al.* 1989; Cornier *et al.* 2011), which requires studying the climatic and edaphic conditions of the region in which a species might be planted. This will greatly determine the opportunity of each shrub and tree species to establish, as it was noticed that *Sambucus nigra* did not persist well after plantation on the study pasture of the Centre des Technologies Agronomiques (CTA, Strée, Belgium; Marche C., personal communication). In northern Australia, the *Desmanthus* cultivars studied in this work have been selected notably because of their persistence in the conditions of this region (Gardiner and Swan, 2008). Before planting, there is a need to check if the species is invasive or not. For example, *C. sanguinea* has been reported for its invasiveness (Rameau *et al.* 1989), and *Robinia pseudoacacia* that was present in the hedge but not consumed by heifers is also considered as an invasive species in Belgium (Halford *et al.* 2011). Once established, diseases might develop which is obviously indesired. Among the studied temperate species, ash (*F. excelsior*) dieback is caused by the fungus *Chalara fraxinea* (Delhaye *et al.* 2010). No disease has been reported for *Desmanthus* spp. (Cook *et al.* 2005), which is interesting since, as woody forage candidate plant, it comes in competition with *Leucaena leucocephala* which causes productivity loss or even death if the mimosine toxicity is not properly dealt with (Hegarty *et al.* 1964; Jones *et al.* 1976).

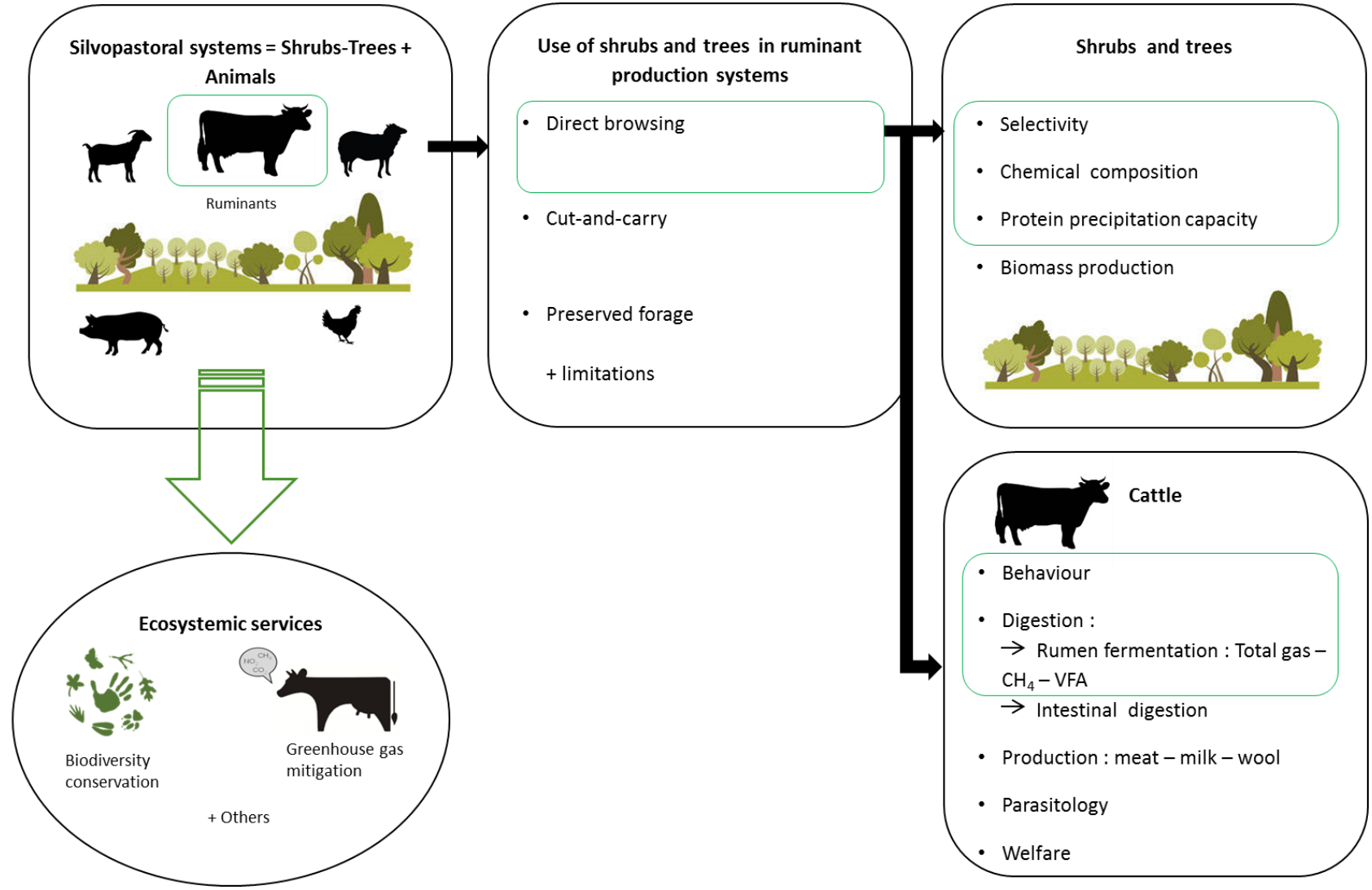

Figure 3. Graphical abstract aiming at describing several main components of silvopastoral systems and what was achieved during the thesis.

2. Heifers behaviour and selectivity towards woody plants

In Chapter 4, the study based on the behaviour and selectivity of dairy heifers grazing in a pasture fenced with shrubs and trees in a hedge demonstrated that having access to woody plants did influence heifers' behaviour (Figure 3). In a similar study presented in Chapter 5, the heifers' feeding behaviour that was recorded only during spring (May 2012) was also impacted by the presence of the hedge. However, results presented in each trial differed in several points. Firstly, heifers browsed shrub and tree forage over the entire grazing season (i.e. in spring (May), early summer (July) and late summer (September)) in the experiment of Chapter 4, while the browsing activity has not been observed continually during the study presented in Chapter 5 (i.e. one week out of the four of this experiment). Secondly, the browsing behaviour recorded in the first study was higher when pre-grazing pasture biomass was lower, while the opposite resulted from the second experiment. Therefore the first study confirmed that woody forage may supplement low-quality fodder throughout the season by providing high quality biomass (Leng 1992; Hirata *et al.* 2008). The second study showed that cattle rely more on shrub and tree forage when the pasture biomass availability is lower, which was usually observed during dry periods (Katjiua and Ward 2006; Liagre 2006). However, the pasture biomass available for grazing was assessed but the woody forage biomass was not in both trials. This is particularly relevant when studying the behaviour in relation to the forage biomass and nutritive value, because in summer (Chapter 4) the availability of biomass from the hedge might have influenced the time spent browsing. Indeed, heifers browsed the hedge in spring and it might have not grown sufficiently between spring and summer to supply enough forage to maintain similar level of browsing. The pasture and hedge biomass availability should then be evaluated in future trials. Thirdly, the rumination activity was influenced by the presence of the hedge in the first study while it was not in the second. This might be explained by the lower consumption of woody foliage during the second experiment; pasture biomass was then the main source of forage in the diet of both groups which has as a consequence not impacted the rumination time.

Furthermore, the study in Chapter 4 demonstrated that heifers consumed mainly 4 shrub and tree species from the hedge: *Carpinus betulus, Cornus sanguinea, Corylus avellana* and *Crataegus monogyna*. Except for *C. sanguinea*, the same species was selected by heifers in the second trial. The heifers' behaviour and selectivity towards woody species have been related to nutritive value measured by NIRS according to the season in Chapter 4. As mentioned before, in spring, heifers browsed longer while the availability of pasture biomass

was the highest. One explanation was found in the comparison of nutritive value between woody plants and the pasture which showed that the woody forage in spring was on average more digestible than the pasture with similar CP contents. However, both studies measured the selectivity for each species using the time spent browsing the species but not its relative biomass in the diet. This is an important parameter to measure in future experiments to determine the real amount of forage ingested by cattle.

3. Nutritive value and *in vitro* fermentation profile including methane production of woody species

When comparing the nutritive value in terms of chemical composition and *in vitro* rumen fermentation profile evaluated in Chapter 5, the four most ingested species were not among the most interesting species. However, *C. avellana* and *C. monogyna* were among the three CT-rich species (Table 12), and had also a high protein precipitation capacity (PPC) with *C. avellana* displaying the highest potential to precipitate proteins in ruminal conditions (Table 12). As it is known that CP are highly soluble early in the grazing season (Abdalla *et al.* 1988), the capacity of both species to protect proteins from degradation in the rumen and then possibly increase the protein available for the animal (Lascano *et al.* 2003) might be one driver for heifers to ingest particularly these species in spring. Furthermore, CT is known to prevent bloat induced notably by immature clover forage (Lees 1992). Bloat prevention might be another driver that induced the ingestion of CT-containing plants, and especially highly CT-rich species as *C. avellana* and *C. monogyna* (Table 12). However, though both species seemed promising in terms of protein precipitation in ruminal conditions and might avoid bloat, their CT concentration was over 50 g/kg depigmented sample which has been reported to be the limit to avoid impaired effects notably on voluntary feed intake (Barry and Duncan 1984; Wang *et al.* 1996; Carulla *et al.* 2005). Further *in vitro* and *in vivo* trials are therefore needed to support our hypotheses that the ingestion of shrub and tree forage when pasture biomass is high is driven by nutritional aspects and to ensure proper rumen function such as bloat prevention, without negative effects on intake and animal productivity.

Fraxinus excelsior seemed the most promising species when taking into account both chemical composition and *in vitro* rumen fermentation profile. The high nutritive value of this species has previously been reported and might explain the considerable incorporation of this species in ruminants' diet for years in various systems (Thiébault 2005; Liagre 2006; Jayanegara *et al.* 2011). However, the above-mentioned disease that strikes *F. excelsior* could limit its integration within silvopastoral systems as the persistence of this species would not

be ensured (Figure 3). The CP values of *F. excelsior* and *C. avellana* that were collected in spring (Table 9) are in the range of the results from the literature compiled by Luske and van Eekeren (2014), but higher than those of the study of Emile *et al.* (2016). It might be explained because in the latter study they collected samples in summer while our experiment took place in spring.

The comparison of the chemical composition of temperate woody forage obtained by NIRS in Chapter 4 (samples collected in May 2013; Table 5) to that of Chapter 5 measured using chemical analyses (samples collected in May 2012; Table 9) showed high differences for some species. Leaves of *Sambucus nigra* collected in May 2013 contained less than half of the amount of crude protein (CP) measured in the samples collected the year before (152 *versus* 320 g CP/kg DM). These differences might depend on the year of plant collection; for example, if the weather is different from one year to another, it might influence the plant chemical composition (Van Soest 1994). Furthermore, as the database used for the prediction was composed of Mediterranean woody species (Meuret *et al.* 1993; Agreil *et al.* 2005) and not the temperate species that we studied, the predicted values might also have slightly deviated from the results obtained with laboratory analyses, as it was discussed in a similar way in Chapter 4 with FNIRS. Although in this example the forage has been collected at similar stage of growth over two consecutive years, shrubs and trees need to be managed considering the evolution of the biomass and chemical composition with time. Indeed, it is determinant to decide if periodic maintenance of the hedge is necessary throughout the grazing season or if we can let plants grow. On the one hand, the first case requires more labour for farmers, while on the other hand the latter may induce a decline of nutritive value. If the hedge is initially pruned and kept at a pre-determined browsing height, maintenance could be performed mostly by the animals themselves; in Chapter 4, it was measured that heifers could reach leaves up to 2.1 m, and thus shrubs and trees should be pruned to keep them under this height. This can be achieved if the browsing pressure is managed adequately to allow an appropriate balance between overbrowsing and excessive woody plant growth. This needs investigation because each system (i.e. type of animal (cattle *vs* small ruminants), plantation design (hedge *vs* isolated trees), plant type (trees *vs* shrubs) and species, etc.) will have its own specificities.

As regards CH_4 production, the four most ingested species in Chapter 4 are among the forage with the greatest anti-methanogenic potential (Table 10); *C. betulus* and *C. sanguinea* displayed the lowest CH_4 production followed then by *C. avellana* and *C. monogyna*. It

seemed that the species which produced less CH_4 presented lower rumen fermentation (Table 10), and this was observed with the temperate species *Castanea sativa* for which the lower *in vitro* CH_4 production was accompanied by impaired rumen fermentation (Jayanegara *et al.* 2011). Overall, CH_4 produced by the *in vitro* fermentation of the tropical *Desmanthus* was lower (in the range 5.94-14.6 ml/g DM after 24 h of incubation (data not shown)) than that of temperate shrub and tree species (14.0-38.5 ml/g DM at 24 h; Table 10). Reduced enteric CH_4 production can result from a reduction in rumen fermentation rate as mentioned above or from an alteration of the production of VFA's (Bell and Eckard 2012). The first explanation supported our observation because total gas and *in vitro* organic matter digestibility of *Desmanthus* species (Table 17) was lower compared to temperate species of May (Table 5 and Table 10). However, it is important to keep in mind that the implementation of these two *in vitro* studies was different as the ruminal fluid came from different animals (Brahman Zebu steers (*Bos indicus*) *vs* dry Red-Pied cows (*Bos taurus*)) and feeding systems (grazing *vs* housed). These breeds were the most relevant to use respectively, because the behaviour and selectivity study was performed with dairy cattle (Chapter 5), and *Desmanthus* species are expected to be fed to grazing Zebus. Methane production by temperate species was only measured during one season (i.e. species collected in May) and the evolution of plant maturity over seasons (Feeny 1970; Riipi *et al.* 2002) might influence rumen fermentation and thus digestibility and gas production including CH_4. In both studies about CH_4 production by temperate (Chapter 5) and tropical (Chapter 6) shrub and tree forage, we focused on the *in vitro* CH_4 produced by each forage but this should be consider in the whole farm greenhouse gas budget in order to examine long-term sustainability of reductions in CH_4 production (Beauchemin *et al.* 2008). In Wallonia, trials achieved to determine the CH_4 (Dumortier *et al.* 2017) and carbon balance (Gourlez de la Motte *et al.* 2016) of an intensively grazed pasture have been performed at the paddock level. On the long-run, it would be relevant to complete such research on silvopastoral systems as the one studied in this work. In Australia, measurements at pasture level have not been performed with the newly-developed *Desmanthus* cultivars but research with *L. leucocephala* (McSweeney and Tomkins 2015) could be applied to these legumes. However, measurements of methane emissions on silvopatstoral systems require an accurate methodology that provides individual animal measurements, issue that is critical with new forages for evalution under greenhouse gas research.

4. Other services of shrubs and trees within silvopastoral systems

Although the investigation of this work was primarily based on shrubs trees as forage resource for livestock, silvopastoral systems are expected to lead to ecosystemic services and environmental benefits if there are managed properly (Kaur *et al.* 2002; Jose 2009; Pulido-Santacruz and Renjifo 2011). This is illustrated in Figure 3 with several examples of possible benefits and it was also briefly described in the introduction and in Chapter 2. Indeed in Wallonia, and more generally in Europe, the re-establishment of woody perennials into the agricultural landscapes results primarily from environmental concerns (Nerlich *et al.* 2013; Luske and van Eekeren 2014). Shrubs and trees can contribute to biodiversity conservation (Liagre 2006; Pulido-Santacruz and Renjifo 2011), erosion control (Liagre 2006) and carbon storage (Montagnini and Nair 2004). Woody species have also been demonstrated to lower methane emission which is notably associated to the plant CT content (Ramírez-Restrepo et al. 2010). This was also observed in our *in vitro* experiment since negative correlations between CT and CH_4 production by the tropical *Desmanthus* cultivars (Chapter 6) have been measured. Furthermore, the PPC of some temperate species, such as *C. avellana* and *C. monogyna*, could be interesting to balance rumen by reducing N degradation in the rumen, and then urine excretion and N_2O release in the environment. However, this potential benefit needs to be further study to confirm it.

Besides the environmental benefits, the implementation of woody plants on grasslands raised also the interest of farmers in matters such as the anthelmintic potential of woody foliage. Indeed it is known that gastrointestinal parasites are responsible to major production loss (Vlassoff and McKenna 1994). Over the last decades, parasitic infestation has been controlled mainly with synthetic anthelmintic drugs (Williams 1997) but this has raised public concern regarding the use of chemical compounds in farming systems and the uncertainty of residual particles in both human food products (Waller and Thamsborg 2004) and the environment (McKellar 1997). These concerns have led to the interest in organic and sustainable agriculture (Waller and Thamsborg 2004) while systematic preventive deworming of cattle is commonly practiced by farmers without examining the infestation level of the herd (Marche C., personal communication). Therefore, production systems that are based on natural and efficient bio-active compounds present in shrub and tree foliage might play a significant role in controlling parasites infestation. The primary stage of such research was initiated in a study (Vandermeulen 2012) which demonstrated that the access to temperate shrubs and trees (Table 11) decreased significantly the number of eggs of *Trichostrongylus*

spp. in experimental heifers faeces compared to the animals without access to the hedge (0 eggs/faeces *versus* 50 eggs/g faeces respectively). However there was no significant reduction of *Coccidia* spp. although the number of eggs was lower in faeces of the group with access to the hedge (383 eggs/g faeces for experimental *versus* 600 for heifers of the control group). Anthelmintic properties of secondary compounds such as CT have been reported (Ramírez-Restrepo *et al.* 2004, 2005; Mupeyo *et al.* 2011). Though the preliminary study did not assess the content in secondary compound including CT (Vandermeulen 2012), it was evaluated in Chapter 5 (Table 12) and showed that CT in woody foliage was in the range 1.5-75.6 g/kg depigmented sample in May. During this season, the CT values were low except for *C. avellana* (58.2 g/kg depigmented sample) and *C. monogyna* (75.6 g/kg depigmented sample) that were 2 out of the 3 selected species as determined in Chapter 5. Therefore, as CT-rich species have been ingested by heifers with access to the hedge, it is reasonable to hypothesize that the reduction in *Trichostrongylus* spp. might notably be explained by the presence of CT in the browed species. However, this needs to be confirmed by *in vivo* trials (Figure 3).

As expressed earlier, condensed tannins have also been demonstrated to reduce CH_4 emission from ruminants (Animut *et al.* 2008; Ramírez-Restrepo *et al.* 2010). The potential reduction effect of CT on CH_4 by *Desmanthus* species have been discussed in Chapter 6. Furthermore, we also measured significant negative correlations between CH_4 and HT in the tropical legume cultivars. The HT would have less detrimental impact on digestibility than CT (Jayanegara *et al.* 2010), but this form of tannin has less extensively studied (Bhatta *et al.* 2009; Jayanegara *et al.* 2015) due to higher risk of toxicity (Goel and Makkar 2012). According to the results of Leblois (2012) and Chapter 5, HT concentration in some temperate species in July reached 131 g/kg in *C. sanguinea*, 128 g/kg in *C. betulus*, 87 g/kg in *Acer campestre* and 16 g/kg in *F. excelsior*, while no HT was detected in *C. avellana* that only contained CT. *Cornus sanguinea* and *C. betulus* contained much higher HT than CT, and they also had low *in vitro* CH_4 production (Table 10). Though HT concentration was measured in samples collected in July while CH_4 in May, this secondary compound may play a role in reducing methanogenesis also for temperate species, but this should be more strictly confirmed in future experiments. In this work, we evaluated and discussed a type of polyphenols, mainly CT and HT, while other secondary compounds should also be studied as saponins (Jayanegara *et al.* 2010).

Trees and shrubs on pasture can bring shade to cattle in summer (Liagre 2006) and reduce heat stress which induces thermal discomfort (Van laer *et al.* 2015a) and depresses

milk production in dairy cattle (Van laer *et al.* 2015b). This benefit is observed even in temperate summer as known in Belgium (Van laer *et al.* 2015a) and this is supported by our findings in Chapter 4 as the group of heifers with access to the hedge rested longer under the trees' shade in summer, when the temperature was higher, than the control group. The hedges can also protect animals against wind and raise the soil and air temperature which is appreciated by livestock in winter (Liagre 2006). However, we did not set up our study during this time of the year but the investigation of the influence of shrubs and trees in a hedge on the microclimate should be considered (Hawkes and Wedderburn 1994).

As it was discussed in Chapter 2, the influence of temperate shrubs and trees on animal productivity in terms of milk, meat and/or wool production is crucial. Research about the positive effect of browsing *Desmanthus* has been performed on wool growth and weight loss of sheep (Rangel and Gardiner 2009) and on steers live weight gain (Gardiner and Parker 2012) but further studies are required to confirm these positive outcomes (Figure 3). As expressed earlier, the productivity of both temperate and tropical woody forage needs to be investaged to determine the importance of this component within the whole systems and further to relate it to animal performance. In temperate regions, as the renewed interest of using shrubs and trees to feed ruminants is recent (Bestman *et al.* 2014; Luske and van Eekeren 2014; Smith *et al.* 2014), little is known about this practice and the current knowledge is mainly relying on what was done in the past (Thiébault 2005; Liagre, 2006).

It can be concluded that temperate and tropical silvopastoral systems including shrubs and trees as fodder for ruminants offer opportunities to diversify farming systems. The browsing of shrubs and trees by cattle seems to be induced by several drivers according to the season such as the nutritive value of woody plants compared to that of pasture, the appropriate function of the rumen, and possibly a need for self-medication. Some woody forage appears promising in terms of nutritive value, as well as in terms of potential to modulate the rumen fermentation and further the protein digestion, or reduced methanogenesis. Future investigations should be encouraged to provide answers on topics which remain little explored and would help to integrate properly shrubs and trees in ruminant production systems.

5. References

Abdalla HO, Fox DG, Seaney RR (1988) Variation in Protein and Fiber Fractions in Pasture During the Grazing Season. *Journal of Animal Science* **66**, 2663–2667. doi:10.2527/jas1988.66102663x

Agreil C, Fritz H, Meuret M (2005) Maintenance of daily intake through bite mass diversity adjustment in sheep grazing on heterogeneous and variable vegetation. *Applied Animal Behaviour Science* **91**, 35–56. doi:10.1016/j.applanim.2004.08.029

Animut G, Puchala R, Goetsch AL, Patra AK, Sahlu T, Varel VH, Wells J (2008) Methane emission by goats consuming diets with different levels of condensed tannins from lespedeza. *Animal Feed Science and Technology* **144**, 212–227. doi:10.1016/j.anifeedsci.2007.10.014

Barry TN, Duncan SJ (1984) The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. *British Journal of Nutrition* **51**, 485–491.

Beauchemin KA, Kreuzer M, O'Mara F, McAllister TA (2008) Nutritional management for enteric methane abatement: a review. *Australian Journal of Experimental Agriculture* **48**, 21–27.

Bell M, Eckard RJ (2012) Reducing Enteric Methane Losses from Ruminant Livestock – Its Measurement, Prediction and the Influence of Diet. In 'Livestock Science' (Ed. K Javed) pp. 135–150. (InTech: Rijeka)

Bestman M, van Eekeren N, Luske B, Vonk M, Anssems E, Boosten M, van Bree M (2014) Introducing trees in dairy and poultry farms. Experiences dairy and poultry farmers' networks in The Netherlands. In '18th IFOAM Organic World Congress 2014', Istanbul, Turkey, 13-15 October 2014 [Online]. Available at http://orgprints.org/23894/9/23894.pdf (verified 22 March 2016).

Bhatta R, Uyeno Y, Tajima K, Takenaka A, Yabumoto Y, Nonaka I, Enishi O, Kurihara M (2009) Difference in the nature of tannins on *in vitro* ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. *Journal of Dairy Science* **92**, 5512–5522. doi:10.3168/jds.2008-1441

Carulla J, Kreuzer M, Machmüller A, Hess H (2005) Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. *Crop and Pasture Science* **56**, 961–970.

Clem RL, Hall TJ (1994) Persistence and productivity of tropical pasture legumes on three cracking clay soils (Vertisols) in north-eastern Queensland. *Animal Production Science* **34**, 161–171.

Cook B, Pengelly BC, Brown S, Donnelly JB, Eagles D, Franco A, Hanson J, Mullen BF, Partridge I, Peters M, Schultze-Kraft R (2005) Tropical Forages: an interactive selection tool. [CD-ROM], CSIRO, DPI&F(Qld), CIAT and ILRI, Brisbane, Australia [Online]. Available at http://www.tropicalforages.info/ (verified 22 March 2016)

Cornier T, Toussaint B, Duhamel F, Blondel C, Henry E, Mora F (2011) 'Guide pour l'utilisation d'arbres et d'arbustes pour la végétalisation à vocation écologique et paysagère en Région Nord-Pas de Calais' Centre régional de phytosociologie / Conservatoire botanique national de Bailleul, pour le Conseil régional Nord-Pas de Calais et la DREAL Nord-Pas de Calais, 48 p. Bailleul.

Cox K, Gardiner C (2013) Pasture legumes in Queensland–a new wave? In 'Proceedings of the Northern Beef Research Update Conference'. pp. 15-20. (Queensland North Australia Beef Research Council: Gympie, Qld)

Delhaye N, Helson M, Chandelier A (2010) La chalarose du frêne: premiers foyers en Wallonie. *Forêt Wallonne* **108**, 49–56.

Dumortier P, Aubinet M, Beckers Y, Chopin H, Debacq A, Gourlez de la Motte L, Jérôme E, Wilmus F, Heinesch B (2017) Methane balance of an intensively grazed pasture and estimation of the enteric methane emissions from cattle. *Agricultural and Forest Meteorology* **232**, 527–535. doi:10.1016/j.agrformet.2016.09.010

Eason WR, Gill EK, Roberts JE (1996) Evaluation of anti-sheep tree-stem-protection products in silvopastoral agroforestry. *Agroforestry Systems* **34**, 259–264.

Emile JC, Delagarde R, Barre P, Novak S (2016) Nutritive value and degradability of leaves from temperate woody resources for feeding ruminants in summer. In 'Book of Abstract of The 3rd European Agroforestry Conference, Celebrating 20 years of Agroforestry research in Europe'. (Ed. M Gosme) pp. 372–375. (Montpellier, France, 23–25 May 2016.

Feeny P (1970) Seasonal Changes in Oak Leaf Tannins and Nutrients as a Cause of Spring Feeding by Winter Moth Caterpillars. Ecology 51:565-581. doi:10.2307/1934037

Gardiner CP, Swan SJ (2008) Abandoned pasture legumes offer potential economic and environmental benefits in semiarid clay soil rangelands. In: 'Proceedings of the 15th Biennial Conference of the Australian Rangeland Society. A climate of change in the rangelands'. 4 pp. (Australian Rangeland Society: Mount Lawley, WA).

Gardiner C, Parker AJ (2012) Steer liveweight gains on Progardes desmanthus/buffel pastures in Queensland. In 'Proceedings of Second Joint Conference of the New Zealand Society of Animal Production and the Australian Society of Animal Production'. pp. 1. (Lincoln University: Christchurch).

Gardiner C, Kempe N, Hannah I, McDonald J (2013) PROGARDES TM: a legume for tropical/subtropical semi-arid clay soils. *Tropical Grasslands-Forrajes Tropicales* **1**, 78–80.

Goel G, Makkar HPS (2012) Methane mitigation from ruminants using tannins and saponins. *Tropical Animal Health and Production* **44**, 729–739. doi:10.1007/s11250-011-9966-2

Gourlez de la Motte L, Jérôme E, Mamadou O, Beckers Y, Bodson B, Heinesch B, Aubinet M (2016) Carbon balance of an intensively grazed permanent grassland in southern Belgium. *Agricultural and Forest Meteorology* **228–229**, 370–383. doi:10.1016/j.agrformet.2016.06.009

Halford M, Heemers L, Mathys C, Vanderhoeven S, Mahy G (2011) Socio-economic survey on invasive ornamental plants in Belgium. AlterIAS project, Final report February 2011. (Biodiversity & Landscape Unit, University of Liège-Gembloux Agro-Bio Tech: Gembloux)

Hautier L, Campion M, Ninane M, Knoden D, Luxen P, Burny P, Beckers Y, Dufrêne M, Stilmant D (2014) L'élevage des ruminants, un élément clé de notre territoire. In '19ème Carrefour des Productions Animales. La viande bovine remise en question : de sa production à sa consommation'. pp. 30–44 (Centre Wallon de Recherches Agronomiques & Gembloux Agro-Boi Tech : Gembloux)

Hawkes MF, Wedderburn ME (1994) Microclimate changes under pinus radiata agroforestry regimes in New Zealand. *Agricultural and Forest Meteorology* **71**, 133–145.

Hegarty MP, Schinckel PG, Court RD (1964) Reaction of sheep to the consumption of *Leucaena glauca* Benth. and to its toxic principle mimosine. *Australian Journal of Agricultural Research* **15**, 153–167.

Hirata M, Hasegawa N, Takahashi T, Chowdappa R, Ogura S, Nogami K, Sonoda T (2008) Grazing behaviour, diet selection and feed intake of cattle in a young tree plantation in southern Kyushu, Japan. *Tropical Grasslands* **42**, 170–180.

Jayanegara A, Goel G, Makkar HPS, Becker K (2010) Reduction in methane emissions from ruminants by plant secondary metabolites: Effects of Polyphenols and Saponins. In 'Sustainable Improvement of Animal Production and Health'. (Eds NE Odongo, M Garcia, GJ Viljoen) pp. 151–157. (FAO: Rome)

Jayanegara A, Marquardt S, Kreuzer M, Leiber F (2011) Nutrient and energy content, *in vitro* ruminal fermentation characteristics and methanogenic potential of alpine forage plant species during early summer. *Journal of the Science of Food and Agriculture* **91**, 1863–1870. doi:10.1002/jsfa.4398

Jayanegara A, Goel G, Makkar HPS, Becker K (2015) Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population *in vitro*. *Animal Feed Science and Technology* **209**, 60–68. doi:10.1016/j.anifeedsci.2015.08.002

Jones RJ, Blunt CG, Holmes HG (1976) Enlarged thyroid glands in cattle grazing leucaena pastures. *Tropical Grasslands* **10**, 113–116.

Jose S (2009) Agroforestry for ecosystem services and environmental benefits: an overview. *Agroforestry Systems* **76**, 1–10. doi:10.1007/s10457-009-9229-7

Katjiua M, Ward D (2006) Cattle diet selection during the hot-dry season in a semi-arid region of Namibia. *African Journal of Range & Forage Science* **23**, 59–67. doi:10.2989/10220110609485887

Kaur B, Gupta SR, Singh G (2002) Carbon storage and nitrogen cycling in silvopastoral systems on a sodic in northwestern India. *Agroforestry Systems* **54**, 21–29.

Lascano C, Avila P, Stewart J (2003) Intake, digestibility and nitrogen utilization by sheep fed with provenances of *Calliandra calothyrsus* Meissner with different tannin structure. *Archivos Latinoamericanos de Producción Animal* **11**, 21–28.

Leblois J (2012) 'Impact du broutage de fourrages ligneux tempérés sur les fermentations ruminales et le métabolisme protéique chez les bovins'. Master's Thesis, University of Liège-Gembloux Agro-Bio Tech, Belgium.

Lefroy EC, Dann PR, Wildin JH, Wesley-Smith RN, McGowan AA (1992) Trees and shrubs as sources of fodder in Australia. *Agroforestry Systems* **20**, 117–139.

Leng RA (1992) 'Tree foliage in ruminant nutrition.' (FAO: Rome)

Lees GL (1992) Condensed tannins in some forage legumes: Their role in the prevention of ruminant pasture bloat. In 'Plant Polyphenols. Synthesis, Properties, Significance'. (Eds RW Hemingway, PE Laks) pp. 915–934. (Prenum Press: New York)

Liagre F (2006) 'Les haies rurales.' (France Agricole Editions: Paris)

Luske B, van Eekeren N (2014) Renewed interest for silvopastoral systems in Europe – an inventory of the feeding value of fodder trees. In 'Proceedings of the 4th ISOFAR Scientific Conference, 'Building Organic Bridges'' (Eds G Rahmann, U Aksoy) pp. 811–814. (Thuenen: Braunschweig)

McIvor JG (2005) Chapter 9. Australian Grasslands [Online]. *In* 'Grasslands of the World'. (Eds. JM Suttie, SG Reynolds, C Batello) Plant Production and Protection Series No. 34 (FAO: Rome). Available at http://www.fao.org/docrep/008/y8344e/y8344e0g.htm#bm16 (verified 30 January 2017)

McKellar QA (1997) Ecotoxicology and residues of anthelmintic compounds. *Veterinary Parasitology* 7g2, 413–435.

McSweeney CS, Tomkins N (2015) Impacts of Leucaena plantations on greenhouse gas emissions in northern Australian cattle production systems. Project B.CCH.6510 Final Report (Meat & Livestock Australia Limited: Sydney)

Meuret M, Dardenne P, Biston R, Poty O (1993) The use of NIR in predicting nutritive value of Mediterranean tree and shrub foliage. *Journal of Near Infrared Spectrometry* **22**, 45–54.

Montagnini F, Nair PKR (2004) Carbon sequestration: an underexploited environmental benefit of agroforestry systems. *Agroforestry Systems* **61**, 281–295.

Mullen BF, Gutteridge RC (2002) Wood and biomass production of Leucaena in subtropical Australia. *Agroforestry Systems* **55**, 195–205.

Mupeyo B, Barry TN, Pomroy WE, Ramírez-Restrepo CA, López-Villalobos N, Pernthaner A (2011) Effects of feeding willow (*Salix* spp.) upon death of established parasites and parasite fecundity. *Animal Feed Science and Technology* **164**, 8–20. doi:10.1016/j.anifeedsci.2010.11.015

Nerlich K, Graeff-Hönninger S, Claupein W (2013) Agroforestry in Europe: a review of the disappearance of traditional systems and development of modern agroforestry practices, with emphasis on experiences in Germany. *Agroforestry Systems* **87**, 475–492. doi:10.1007/s10457-012-9560-2

Papachristou TG, Papanastasis VP (1994) Forage value of Mediterranean deciduous woody fodder species and its implication to management of silvo-pastoral systems for goats. *Agroforestry Systems* **27**, 269–282.

Pulido-Santacruz P, Renjifo LM (2011) Live fences as tools for biodiversity conservation: a study case with birds and plants. *Agroforestry Systems* **81**, 15–30. doi:10.1007/s10457-010-9331-x

Rameau JC, Mansion D, Dumé G (1989) Flore forestière française. Guide écologique illustré. Tome 1, Plaines et collines. (Institut pour le développement forestier : Paris)

Ramírez-Restrepo CA, Barry TN, López-Villalobos N, Kemp PD, McNabb WC (2004) Use of *Lotus corniculatus* containing condensed tannins to increase lamb and wool production under commercial dryland farming conditions without the use of anthelmintics. *Animal Feed Science and Technology* **117**, 85–105. doi:10.1016/j.anifeedsci.2004.05.005

Ramírez-Restrepo CA, Barry TN, Pomroy WE, López-Villalobos N, McNabb WC, Kemp PD (2005) Use of *Lotus corniculatus* containing condensed tannins to increase summer lamb growth under commercial dryland farming conditions with minimal anthelmintic drench input. *Animal Feed Science and Technology* **122**, 197–217. doi:10.1016/j.anifeedsci.2005.03.009

Ramírez-Restrepo CA, Barry TN, Marriner A, López-Villalobos N, McWilliam EL, Lassey KR, Clark H (2010) Effects of grazing willow fodder blocks upon methane production and blood composition in young sheep. *Animal Feed Science and Technology* **155**, 33–43. doi:10.1016/j.anifeedsci.2009.10.003

Ramírez-Restrepo CA, Charmley E (2015) An integrated mitigation potential framework to assist sustainable extensive beef production in the tropics. In 'Grassland: A Global Resource Perspective'. (Eds PK Ghosh, SK Mahanta, JB Singh, PS Pathak) pp. 417-436 (Range Management Society of India: Jhansi)

Rangel JH de A, Gardiner CP (2009) Stimulation of wool growth by *Desmanthus* spp. as a supplement in a Mitchell grass hay diet. *Tropical Grasslands* **43**, 106–111.

Riipi M, Ossipov V, Lempa K, Haukioja E, Koricheva J, Ossipova S, Pihlaja K (2002) Seasonal changes in birch leaf chemistry: are there trade-offs between leaf growth and accumulation of phenolics? Oecologia **130**,380–390. doi:10.1007/s00442-001-0826-z

Smith J, Kuoppala K, Yáñez-Ruiz D, Leach K, Rinne M (2014) Nutritional and fermentation quality of ensiled willow from an integrated feed and bioenergy agroforestry system in UK. In 'Maataloustieteen Päivät 2014, 8-9.1. 2014 Viikki, Helsinki, Suomen Maataloustieteellisen Seuran julkaisuja No. 30'. (Eds M Hakojärvi, N Schulman). 9 p.

SPW (2016) L'agriculture wallonne en chiffres [Online]. Available at http://agriculture.wallonie.be/apps/spip_wolwin/IMG/pdf/Complet_FR_2014-2.pdf (Verified 15 December 2016).

Thiébault S (2005) L'apport du fourrage d'arbre dans l'élevage depuis le Néolithique. *Anthropozoologica* **40**, 95–108.

Vandermeulen S (2012) 'Intérêt du broutage des haies et bandes boisées pour les bovins sur parcours en Wallonie.' Master's Thesis, University of Liège-Gembloux Agro-Bio Tech, Belgium.

Van laer E, Moons CPH, Ampe B, Sonck B, Vandaele L, De Campeneere S, Tuyttens FAM (2015a) Effect of summer conditions and shade on behavioural indicators of thermal discomfort in Holstein dairy and Belgian Blue beef cattle on pasture. *Animal* **9**, 1536–1546. doi:10.1017/S1751731115000804

Van laer E, Tuyttens FAM, Ampe B, Sonck B, Moons CPH, Vandaele L (2015) Effect of summer conditions and shade on the production and metabolism of Holstein dairy cows on pasture in temperate climate. *Animal* **9**, 1547–1558. doi:10.1017/S1751731115000816

Van Soest PJ (1994) 'Nutritional ecology of the ruminant.' (Cornell University Press: Ithaca)

Vlassoff A, McKenna PB (1994) Nematode parasites of economic importance in sheep in New Zealand. *New Zealand Journal of Zoology* **21**, 1–8. doi:10.1080/03014223.1994.9517971

Waller PJ, Thamsborg SM (2004) Nematode control in "green" ruminant production systems. *Trends in Parasitology* **20**, 493–497. doi:10.1016/j.pt.2004.07.012

Wang Y, Douglas GB, Waghorn GC, Barry TN, Foote AG, Purchas RW (1996) Effect of condensed tannins upon the performance of lambs grazing *Lotus corniculatus* and lucerne (*Medicago sativa*). *The Journal of Agricultural Science* **126**, 87–98.

Williams JC (1997) Anthelmintic treatment strategies: current status and future. *Veterinary Parasitology* **72**, 461–477.

Author's publications related to this thesis

1. Articles

Three articles accepted/published (peer-rewiewed):

Vandermeulen S, Pascal M, Marche C, Pilet A, Beckers Y, Bindelle J (2013) Hedges and woody strips browsing by cattle on pasture in Wallonia, Belgium. In: 'Revitalising Grasslands to Sustain Our Communities: Proceedings 22nd International Grassland Congress.' (Eds DL Michalk, GD Millar, WB Badgery, KM Broadfoot), pp. 1011-1012 (New South Wales Department of Primary Industry: Orange).

Vandermeulen S, Decruyenaere V, Ramírez-Restrepo CA, Bindelle J (2014) Compatibility of using TiO_2 and the faecal near-infrared reflectance spectrometry for estimation of cattle intake. *Grassland Science in Europe*, **19**, 625-627.

Vandermeulen S, Ramírez-Restrepo CA, Marche C, Decruyenaere V, Beckers Y, Bindelle J (In press). Behaviour and browse species selectivity of heifers grazing in a temperate silvopastoral system. *Agroforestry Systems*, 1-12.

Two articles submitted to peer-reviewed journal and presently under "minor revision":

Vandermeulen S, Ramírez-Restrepo CA, Beckers Y, Claessens H, Bindelle J. Agroforestry for ruminants: a review of trees and shrubs as fodder in silvopastoral temperate and tropical production systems. *Animal Production Science*.

Vandermeulen S, Singh S, Ramírez-Restrepo CA, Kinley R, Gardiner CP, Holtum J, Hannah I, Bindelle J. *In vitro* assessment of ruminal fermentation, digestibility and methane production of three species of *Desmanthus* for application in northern Australian grazing systems. *Crop and Pasture Science.*

2. Conference

Vandermeulen S*, Ramírez-Restrepo CA, Beckers Y, Claessens H, Bindelle J (2016) Review of shrubs and trees in intensive ruminant systems in temperate areas. Presented at *The 1st International Global Farm Platform Conference: Steps to Sustainable Livestock*, Bristol, United Kingdom, 12 – 15 January 2016.

3. Posters

Vandermeulen S*, Pascal M, Marche C, Pilet A, Beckers Y, Bindelle J (2013) Hedges and woody strips browsing by cattle on pasture in Wallonia, Belgium. Presented at *The 22nd International Grassland Congress*, Sydney, Australia, 15 - 19 September 2013.

Vandermeulen S, Decruyenaere V, Ramírez-Restrepo CA, Bindelle J* (2014) Compatibility of using TiO_2 and the faecal near-infrared reflectance spectrometry for estimation of cattle intake. Presented at *The 25th General Meeting of the European Grassland Federation*, Aberystwyth, Wales, U.K., 7 - 11 September 2014.

Vandermeulen S*, Yando E, Marche C, Ramírez-Restrepo CA, Bindelle J (2014) Is the behavior of grazing cattle influenced by the presence of a hedge in the pasture? Presented at *The 19th National Symposium on Applied Biological Sciences*, Gembloux, Belgium, 7 February 2014.

Vandermeulen S, Ramírez-Restrepo CA, Singh S, Kinley R, Gardiner CP, Holtum J, Bindelle J, Hannah I* (2015) Intra-cultivar potential of *Desmanthus* spp. as a greenhouse gas mitigation strategy for tropical livestock pastoral systems. Presented at *The Tropical Agriculture Conference 2015, Meeting the Productivity Challenge in the Tropics*, Brisbane, Australia, 16 – 18 November 2015.

Vandermeulen S, Leblois J *, Ramírez-Restrepo CA, Beckers Y, Lognay G, Bindelle J (2016) *In vitro* evaluation of protein precipitation capacity of temperate browse species. Presented at *21th National Symposium on Applied Biological Sciences*, Antwerpen, Belgium, 5 February 2016.

*Speaker