Measurement of antimethanogenic potential and animal productivity of modern cultivars of the tropical legume, Leucaena leucocephala in beef cattle

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Preface

Academic background and personal profile. Ten years ago, I decided to focus my studies on agriculture. For that purpose, I left general education to join the agricultural qualification technique section (TQA: Technique de Qualification Agricole) at the IPEA (Institut Provincial d'Enseignement Agronomique) in La Reid (Theux). Then, I continued my higher education at the HEPL (Haute Ecole de la Province de Liège) still at La Reid where I studied agricultural techniques and management (TGA: Techniques et Gestion Agricole). These studies have revealed a strong passion for subjects related to breeding and more particularly animal nutrition. In the goal of my graduation, I completed a bachelor's thesis on the efficiency of diets for dairy cows. After obtaining my graduation, I continued my studies in Gembloux Agro-Bio Tech (University of Liège) with a master's degree in bioengineering, Agronomic Sciences section. To complete my studies, I attended an internship at the end of which I'm presenting this master's thesis.

Motivation for the choice of the internship. I realised my internship at the Agriculture & Food unit of CSIRO, the Australia's national science research agency, in Townsville (Queensland, Australia). My motivations to choose this research organization abroad were multiples: to discover a different agriculture model, to understand its challenges, to sharpen my knowledge on ruminant nutrition and finally to live an experience in an environment where I could improve my English.

Research project and personal experience. The research project to which I have contributed was entitled "Feeding Leucaena to manage the rumen for maximum beef profit". It involved several experiments, including an indoor methane chamber study. The results presented in this work come from this indoor trial. This research topic corresponded perfectly to my interest, combining breeding, nutrition and environmental preservation. I am delighted with this 6 months experience, during which I put myself in the shoes of a researcher. I have learned scientific rigour, teamwork and the particularities of the research profession. I strongly recommend this internship destination to students who will follow me.

Liège, August 12, 2019

Antoine Stifkens

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I also would like to thanks Anne-Catherine Dalcq from the Statistics, Computing and Modelling Unit of Gembloux Agro-Bio Tech for her precious help in the statistical analysis of the data.

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Abstract

As a contributor to greenhouse gas emissions, it is critical for agriculture sector to reduce its carbon footprint. Knowing that methane from livestock represents 70% of agricultural emissions, developing strategies to mitigate methane production is essential to make the sector more sustainable.

In tropical regions, feeding *Leucaena leucocephala* to beef cattle seems to be a natural and efficient strategy to reduce methane emissions. Moreover, this legume shrub provides high quality feed that boosts animal productivity. The potential for expansion of this legume is massive and greatly supported by the release of new cultivars. However, better knowledges on their methane abatement potential and their animal productivity were needed to accelerate the expansion of this promising crop.

The main objectives of this study were (1) to confirm and quantify the reduction in methane emissions by beef cattle fed with diets containing modern cultivars of Leucaena and (2) to confirm and quantify the animal performance improvement allowed by feeding Leucaena. Four diets were offered to growing steers: they contained 0 (control), 18, 36 or 48% of Leucaena (dry matter basis). The basal diet consisted of *Chloris gayana* hay. Methane emissions were measured in open-circuit respiration chambers and liveweight gains were recorded weekly.

Adding Leucaena in the diet strongly decreased (P<0.001) daily methane emissions by 15.0, 19.7 and 21.6% respectively for 18, 36 and 48% of Leucaena inclusion, according to a quadratic relationship. The tannins and/or mimosine contained in the legume seems to be responsible for this abatement, but this must be clarified by further investigations. Leucaena inclusion in the diet enhanced (P<0.001) animal productivity: the highest daily liveweight gain was 0.46 kg which is, however, below expectations. The poor quality of the basal diet (5.12% of crude protein) is the cause of this low weight gain but also perhaps mimosine toxicity.

The new knowledges acquired by this study should contribute to the expansion of Leucaena-pastures in northern Australia, allowing the beef industry to reduce its carbon footprint while improving its productivity.

Keywords: *Leucaena leucocephala*, methane emissions, beef, animal productivity, Redlands, Wondergraze.

Résumé

En tant que contributeur aux émissions de gaz à effet de serre, il est essentiel que le secteur agricole réduise son empreinte carbone. Sachant que le méthane provenant de l'élevage représente 70 % des émissions agricoles, il est primordial d'élaborer des stratégies pour atténuer la production de méthane afin de rendre ce secteur plus durable.

Dans les régions tropicales, nourrir les bovins viandeux avec du *Leucaena leucocephala* semble être une stratégie naturelle et efficace pour réduire les émissions de méthane. De plus, cet arbuste appartenant à la famille des légumineuses produit du fourrage de haute qualité qui augmente la productivité des animaux. Le potentiel d'expansion de cette légumineuse est énorme et grandement soutenu par l'introduction de nouvelles variétés sur le marché. Cependant, de meilleures connaissances sur leur potentiel de réduction du méthane et leur productivité animale étaient nécessaires pour accélérer l'expansion de cette culture prometteuse.

Les principaux objectifs de cette étude étaient (1) de confirmer et de quantifier la réduction des émissions de méthane des bovins viandeux nourris avec une ration contenant des variétés modernes de Leucaena et (2) de confirmer et quantifier l'amélioration des performances animales permise par l'introduction du Leucaena dans la ration. Quatre rations ont été distribuées aux animaux en croissance : elles contenaient 0 (blanco), 18, 36 ou 48% de Leucaena (sur base de la matière sèche). Le régime de base était composé de foin *Chloris gayana*. Les émissions de méthane ont été quantifiées dans des chambres de respiration en circuit ouvert et la prise de poids était mesurée chaque semaine.

L'ajout de Leucaena à la ration a fortement diminué (P<0,001) les émissions quotidiennes de méthane par 15,0, 19,7 et 21,6% respectivement pour 18, 36 et 48% d'inclusion de Leucaena, selon une relation quadratique. Les tanins et/ou la mimosine présents dans la légumineuse semblent être responsables de cette réduction, mais cela devrait être clarifié par des recherches supplémentaires. L'inclusion de Leucaena dans la ration a amélioré (P<0,001) la productivité animale : le gain de poids vif quotidien le plus élevé a été de 0,46 kg, ce qui est toutefois inférieur aux attentes. La mauvaise qualité de la ration de base (5,12% de protéines brutes) est à l'origine de cette faible prise de poids mais également une probable intoxication à la mimosine.

Les nouvelles connaissances acquises par cette étude devraient contribuer à l'expansion des pâturages de Leucaena dans le nord de l'Australie, permettant à l'élevage bovin de réduire son empreinte carbone tout en améliorant sa productivité.

Mots-clés : *Leucaena leucocephala*, émissions de méthane, bovins viandeux, productivité animale, Redlands, Wondergraze.

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List of Abbreviations

ADF	Acid Detergent Fiber
AE	Animal Equivalent
AI	Artificial Insemination
ATP	Adenosine Triphosphate
BL	Baseline
BW	Bodyweight
C	Carbon
CFC	Chlorofluorocarbon
CP	Crude Protein
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CT	Condensed tannin
DHP	Dihydroxy Pyridine
DMD	Dry Matter Digestibility
DMI	Dry Matter Intake
FADH	Flavin Adenine Dinucleotide
GDP	Gross Domestic Product
GHG	Greenhouse Gas
GWP	Global Warming Potential
HT	Hydrolysable Tannin
LCA	Life Cycle Assessment
LCFA	Long Chain Fatty Acid
LU	Livestock Unit
LW	Liveweight
LWG	Liveweight gain
N	Nitrogen
NAD	Nicotinamide Adenine Dinucleotide
NDF	Neutral Detergent Fiber
OM	Organic Matter
PEG	Polyethylene Glycol
Pi	inorganic Phosphate ion
PUFA	Polyunsaturated Fatty Acid
RFI	Residual Feed Intake
RGH	Rhodes Grass Hay
RL	Redlands
VFA	Volatile Fatty Acid
WG	Wondergraze

Chapter 1. Introduction

The dramatic accumulation of greenhouse gases into the atmosphere is the most worrying environmental problem for our future. As a contributor to these emissions, it is critical for the agriculture sector to reduce its carbon footprint. In Australia, the first greenhouse gas emitted by agriculture is methane from livestock, accounting for 70% of agricultural emissions and 8% of total emissions. Developing strategies to mitigate methane production is essential to make livestock sector more sustainable.

In northern Australia, beef cattle spend 85 to 90% of their lifespan in pastures. Thus, any pasture-based mitigation strategy has massive potential to reduce methane emissions. Leucaena-pasture is one of these strategies well-adapted for grazing systems in tropical conditions. Recent studies demonstrate that Leucaena reduces methane emissions by 18% when fed at 44% of the diet (Kennedy et al., 2012). Moreover, this nutritious legume shrub offers a rare opportunity to increase productivity and profitability of beef production by reducing the adverse effect of the dry period on forage quality and availability (Bowen et al., 2018).

Today, ~200,000 ha of Leucaena-grass pasture have been sown, while approximately 13.5 M ha are suitable for this crop in Queensland (Shelton et al., 2007). The potential for expansion is therefore considerable and is greatly supported by the release of new cultivars that offer major advantages over old cultivars, being resistant to pest and disease and higher yielding. However, it is necessary to address knowledge gaps about animal productivity and methane abatement potential related with these new cultivars to accelerate their uptake by beef producers. And that is exactly what this study is about.

This master thesis has a similar structure as a scientific paper, namely: literature review, objectives, materials and methods, results, discussion and conclusions. The literature review, subdivided into 4 parts, tries to provide the necessary background to understand the problematic. The first part outlines the process of methane production by ruminants, the second one presents the existing strategies to reduces methane emissions from ruminants, then, the third part is devoted to the description of the Australian beef industry and finally, the last one refers to the tropical legume Leucaena.

Chapter 2. Literature review

2.1. Methanogenesis by ruminants

Methane emissions by ruminants tarnish the image of the livestock sector. However, these releases are a consequence of a valuable process: the conversion of fibrous biomass into high quality energy and protein to feed humans through microbial fermentation (Immig, 1996). To understand the problem, this section is dedicated to the effect of methane on global warming, the production process by ruminants and finally the factors that influence methane production.

2.1.1. Methane: a potent greenhouse gas

The gases composing the atmosphere are transparent to incoming visible solar radiation and partially opaque to outgoing long-wave radiation emitted from Earth's surface. As a result, the heat of the sun is trapped in the atmosphere: this process is called the greenhouse effect. It is a natural warming and essential to make our planet habitable. Indeed, without this phenomenon, the average temperature of the Earth would be 33°C lower and would reach -18°C. The main greenhouse gases (GHG) include water vapour (H_2O), carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), tropospheric ozone (O_3) and chlorofluorocarbons (CFCs; Schiffer et al., 1991; Allaby, 2002). The problem we now face is that the greenhouse gas concentration is increasing owing human activities. As a result, more radiation is trapped which leads to global warming (Milich, 1999).

Impact of methane on global warming

Table 1 | Lifetime and global warming potential (GWP) of 4 important GHG (IPCC, 2014)

GHG	Lifetime (years)	GWP - 100 years
CO_2	5 - 200	1
CH_4	12.4	28
NO_2	121	265
CF_4	50,000	6630

Methane is the second most important anthropogenic greenhouse gas after carbon dioxide in its contribution to global warming. Its 100 years - global warming potential (GWP) is 28 times more important than carbon dioxide (IPCC, 2014). However, its lifetime is shorter (Table 1) and its

concentration in the atmosphere is 200 times lower than carbon dioxide. Despite that, methane is responsible for 16% (Karakurt et al., 2012) to 20% (Yusuf et al., 2012) of the global warming.

The methane concentration in the atmosphere has been relatively stable and has never exceeded 800 ppb over the last 800,000 years until industrialization. It began to rise in the 19th century (Figure 1), along with carbon dioxide and nitrous oxide, and reached 1868 ppb in 2018 (Yusuf et al., 2012). This dramatic increase, correlated with human population growth (Milich, 1999), must be stopped to hold the global temperature rise below 2°C before the end of the century (Gerber, Steinfeld, et al., 2013).

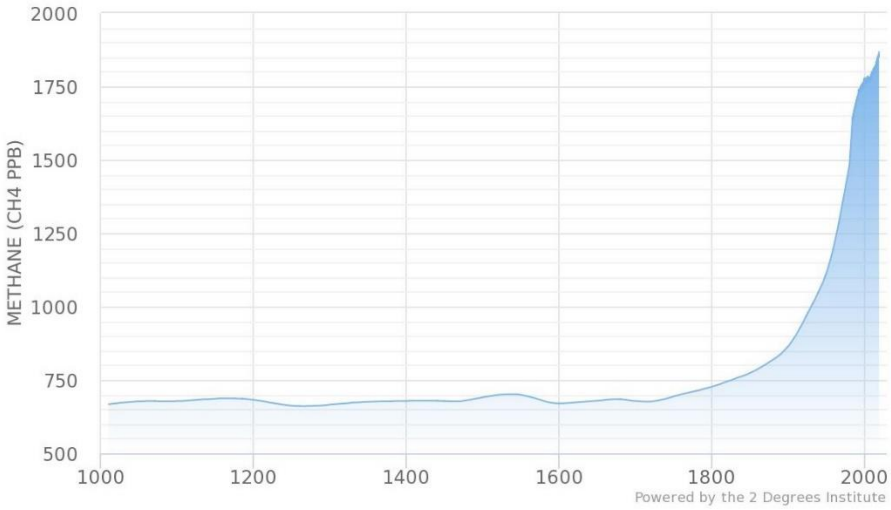

Figure 1 | Trend in methane (CH_4) concentration in part per billion (ppb) since the year 1000 until 2018 (2 Degrees Institute 2019 - WebLink)

Livestock contribution

Methane is produced through anaerobic decomposition of organic matter by methanogen microorganisms, belonging to the *Archea* domain. It is released from both natural (40%) and anthropogenic (60%) sources (Karakurt et al., 2012). The main sources and their contribution are listed in the Table 2.

Enteric fermentation represents ~55% of agricultural emissions and agriculture contributes to ~50% of anthropogenic emissions (Table 2). Methane release from enteric fermentation represents therefore 28% of all anthropogenic emissions and 16-17% of total emissions (natural and anthropogenic). On an Australian-wide scale, 8% of all national emissions and 70% of

agricultural emissions come from enteric methane produced by cattle and sheep (Hegarty et al., 1999; Beauchemin et al., 2008; Yusuf et al., 2012).

Table 2 | Main sources of anthropogenic methane and their contribution (from Karakurt et al., 2012; Yusuf et al., 2012)

Natural emissions (40%)	Anthropogenic emissions (60%)			
	Agriculture (50-53%)	Waste (19%)	Energy (28%)	Industry (0.1%)
Wetlands Termites Wildfires Grasslands Coal beds Lakes	**Enteric fermentation (53-60%)** Rice cultivation (18%) Manure management (11%) Others (18%)	Landfills Wastewater Waste combustion	Coal mining Oil and gas drilling and processing Biomass combustion	-

These fermentation takes place in the rumen of ruminants during the normal processes of digestion which is detailed in the following section (2.1.2). The concerned ruminants are cattle, buffaloes, sheep, goats and camels. Among them, cattle represents 70% of the methane emissions because of their large size, energy intake and numbers (Milich, 1999).

Enteric methane release from dairy cattle and beef represents respectively 46.5% and 42.6% of global emissions of GHG by these sectors (Gerber, Steinfeld, et al., 2013). Therefore, this source seems to be a good candidate for investigating and applying mitigation strategies.

2.1.2. Production process by ruminants

A major advantage of ruminants is the transformation of fibrous biomass, which cannot be used in human nutrition, into high-quality energy and protein sources. However, a byproduct of this process is methane released by eructation (Immig, 1996). This section is dedicated to understanding this process.

Overview

In the rumen, methane is produced by methanogens mainly from CO_2 and H_2. These two metabolites are end-products of carbohydrate fermentation by bacteria, protozoa and fungi. Methanogenesis is essential to avoid the negative feedback of H_2 on fermentation and to maintain the reductive potential of the rumen environment.

Carbohydrates fermentation

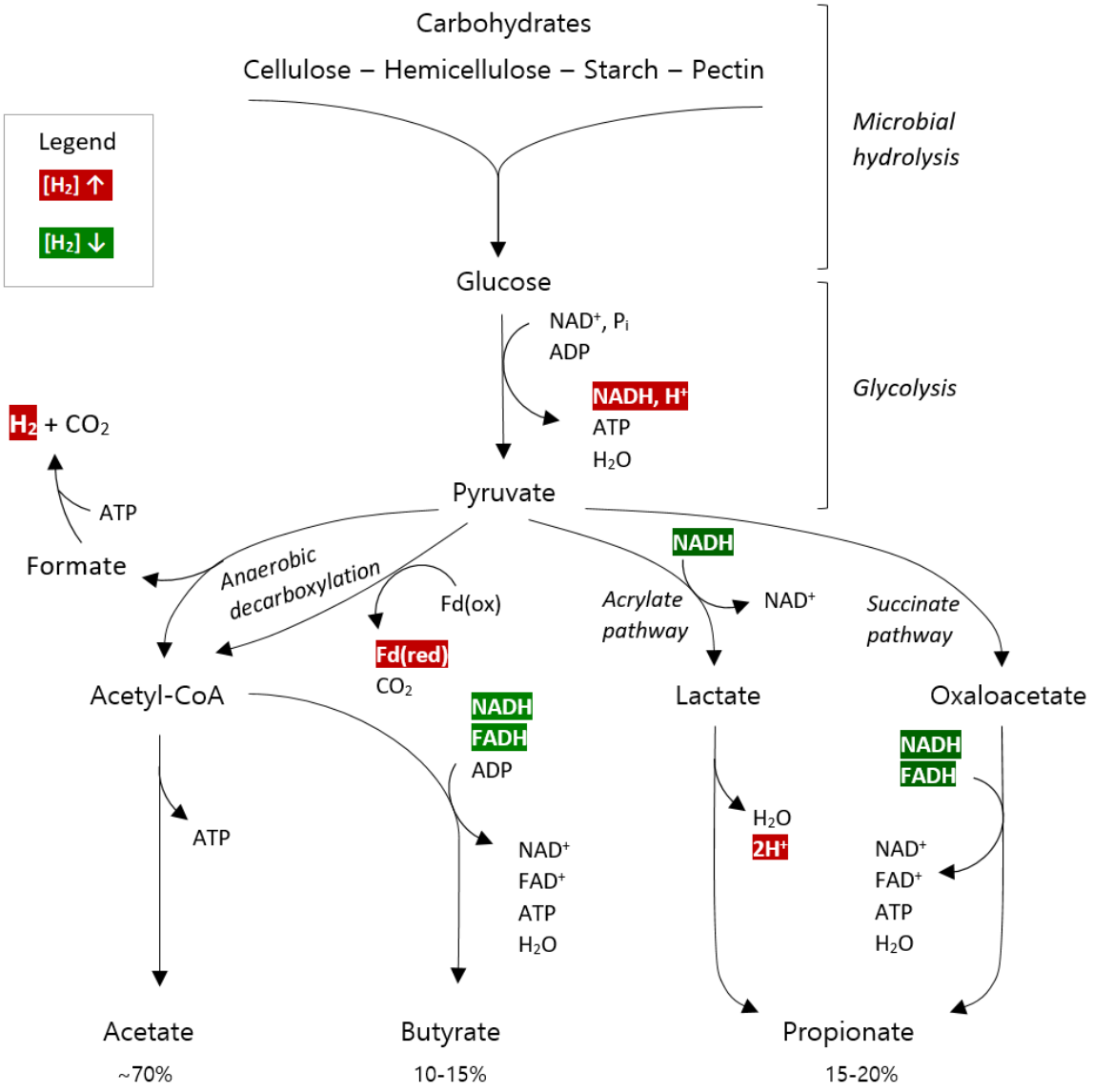

Figure 2 | Principal anaerobic metabolic pathway that generates or utilises hydrogen in the rumen during carbohydrates fermentation (from Immig, 1996; Hegarty, 1999; Morgavi et al., 2010)

The first step of carbohydrate digestion is microbial hydrolysis. The polysaccharides are broken down into simple five and six-carbon monomers as glucose by extracellular enzymes (Immig, 1996). Then, glycolysis occurs in the rumen microbes and leads to the oxidation of glucose to pyruvate with the generation of nicotinamide adenine dinucleotide (NADH), adenosine triphosphate (ATP) and water (H_2O; Figure 2).

Because NADH accumulation makes enzymes less active, it must be reoxidised to avoid the feedback inhibition on fermentation. In aerobic condition, oxygen (O_2) is the final acceptor of H_2 and electrons to form H_2O. But the rumen is an anaerobic environment, so this path cannot be used (Cottle et al., 2011). The main mechanism employed by rumen microbes to regenerate NAD^+ is by the enzyme NADH ferredoxin oxidoreductase coupled to an hydrogenase (Hegarty et al., 1999).

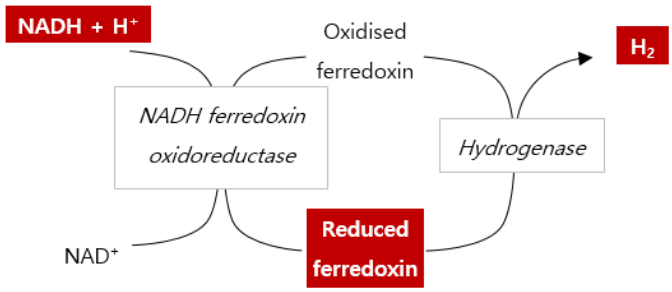

Figure 3 | Mechanisms of regenerating NAD^+ and oxidised ferredoxin (Hegarty et al., 1999)

The end product of glycolysis, pyruvate, is fermented by the microbes for their own energy needs. During this process, volatiles fatty acids (VFA) are produced as by-product, but represent an essential energy substrate for the host animal. As shown in the Figure 2, the 3 major VFAs are acetate, propionate and butyrate, respectively from the fermentation of cellulose, starch and sugars in the following proportions: 70%, 15-20% and 10-15% (Immig, 1996).

The synthesis of acetate and butyrate requires Acetyl CoA, which comes from anaerobic decarboxylation of pyruvate. This step takes place by 2 reactions catabolized by 2 different enzymes: pyruvate oxidoreductases and pyruvate formate lyase. The first reaction leads to the reduction of ferredoxin, which must be reoxidized, resulting in H_2 release (Figure 3). The second step generates formate, which is rapidly degraded to H_2 and CO_2. These two steps, which allow the production of acetate and butyrate, lead to the major release of CO_2 and H_2. In contrast, propionate synthesis helps to reduce H_2 concentration by using NADH, precluding its H_2 emitting reoxidation by the coupled enzymes (Figure 3; Hegarty, 1999).

Although the synthesis of propionate and butyrate require reduced cofactor (NADH, FADH), the global balance is in favor of their accumulation in the rumen. They are reoxidized trough the couple of enzymes NADH ferredoxin oxidoreductase /hydrogenase what release H_2 (Figure 3). The activity of NADH ferredoxin oxidoreductase is suppressed at high H_2 concentration, thus it is essential to proceed with the disposal of this gas.

Disposal of dihydrogen

H_2 disposal is essential to ensure continuity of reoxidation of NADH to NAD^+, and therefore to allow carbohydrate fermentation, ATP production and microbial growth (Cottle et al., 2011). The main mechanisms allowing direct H_2 disposal – or NADH consumption avoiding H_2 liberation – are shown and explained hereafter.

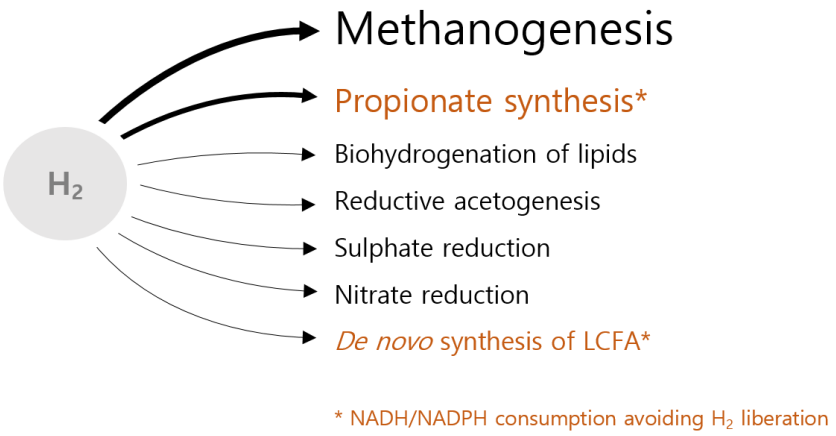

Figure 4 | Main mechanisms allowing direct H_2 disposal or reduced cofactor consumption avoiding H_2 liberation in the rumen ecosystem

- Methanogenesis

Methanogenesis is achieved by Archaea methanogens that oxidise H_2 as an energy source while producing CH_4 (Cottle et al. 2011). This mechanisms is the largest H_2 sink in the rumen: it allows the removal of 48% to 80% of produced H_2 (Morvan et al., 1996; Guyader, 2015). It avoids the feedback inhibition from H_2 but constitutes a considerable loss of energy: about 6-10% of the gross energy ingested is lost in the form of methane (Immig, 1996).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

The number of methanogens is correlated with those of cellulolytic microorganisms and with protozoa, implying that fiber-rich diets are more methanogenic (Morvan et al., 1996). From this statement, Demeyer and van Nevel (1979) proposed to calculate the methane production from the fermentation end-products (acetate, butyrate and propionate), based on stoichiometric equation. *In vitro* studies that used this equation found hydrogen recoveries ranging from 61 to 89% (part of the hydrogen present in methane), which indicates that alternative hydrogen sinks exist (Immig, 1996), as detailed hereafter.

- Propionate synthesis

As seen before, fermentation of carbohydrates leads to 3 major VFAs, according the reactions summarized hereafter (Table 3).

Table 3 | Fermentative reactions leading to the production of volatiles fatty acids (VFAs) from glucose, and their contribution to the hydrogen (H_2) pool (from Olijhoek and Lund 2017)

VFA	Reactions				H_2 contribution
Acetate	$C_6H_{12}O_6 + 2\ H_2O$	→	$2\ CH_3COOH$	$+ 2\ CO_2 + \mathbf{4\ H_2}$	source (+4)
Propionate	$C_6H_{12}O_6 + \mathbf{2\ H_2}$	→	$2\ CH_3CH_2COOH$	$+ 2\ H_2O$	sink (-2)
Butyrate	$C_6H_{12}O_6$	→	$CH_3CH_2CH_2COOH$	$+ 2\ CO_2 + \mathbf{2\ H_2}$	source (+2)

Propionate synthesis uses H_2 while acetate and butyrate formation releases it. Propionate formation would be responsible for 19-33% of the H_2-disposal (Guyader, 2015). It is synthetized by two main pathways (Figure 2): the acrylate pathway and the succinate pathway. Both contain a stage where reduced cofactors are oxidized, preventing the reduction of those electrons to H_2. There is therefore a competition for H_2 between propionic synthesis and methanogenesis (Hegarty, 1999).

- Biohydrogenation of lipids

Biohydrogenation of polyunsaturated fatty acids (PUFA) consists of saturation of double bonds within the C-backbone with hydrogen. This process is a H_2 sink but contributes to only 1-2% of its disposal (Gerber, Henderson, et al., 2013).

However, in their review, Cottle et al. (2011) indicates that the introduction of lipids to the diet reduces methane production significantly. The anti-methanogenic role of lipids is not really ascribable to the use of H_2 for biohydrogenation but rather to the depressive effect on bacteria and protozoa which can lead to lower digestibility and DMI (dry matter intake).

- Reductive acetogenesis

Acetogenic bacteria are a microbial group that may also contribute to the H_2 disposal: they use it to reduce CO_2 and form acetate as shown in this reaction:

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$$

An advantage of this H_2 utilization is that acetate is an energy source for ruminants, while methane is lost in the atmosphere (Morvan et al., 1996; Hegarty, 1999). Unfortunately, reductive

acetogenesis represents a negligible contribution to H_2 disposal in the rumen because (1) acetogenic bacteria are able to use other source of energy than H_2 oxidation (Morgavi et al., 2010) and (2) they are less efficient at this process than methanogens (Moss et al., 2000). However, this metabolic pathway competing with methanogenesis has the merit of being investigated because of the beneficial production of acetate instead of CH_4 (Immig, 1996).

- Sulphate reduction

Sulphate-reducing bacteria also contribute to the H_2 disposal by using it to reduce sulphates (SO_4) and produce sulphides (H_2S; Morvan et al., 1996). However, H_2S has potential toxic effects in the host animal by damaging the colonic epithelium (Hegarty, 1999).

Sulphate reduction occurs mainly in the hindgut, where it competes with methanogens. This competition does not seem to occur in the rumen due to the low availability of sulphate, while this electron acceptor is released in the hindgut by the bacteria from mucins (Immig, 1996).

- Nitrate reduction

The reduction of nitrate by bacteria to ammonia can be an alternative H_2 sink. This reaction occurs in 2 steps. The first one is the reduction of nitrate (NO_3) to nitrite (NO_2), followed by the reduction of nitrite to ammonia (NH_3), as shown hereafter:

$$NO_3^{--} + H_2 + 2H^+ \rightarrow NO_2^- + 2H_2O$$
$$NO_2 + 3H_2 \rightarrow NH_3 + O_2$$

The advantage of this reaction is that it provides ammonia which is a beneficial product for the host animal. Indeed, this fermentable nitrogen may be used for protein synthesis, which is particularly interesting if crude protein (CP) is lacking in the diet (Cottle et al., 2011). However, the intermediate product, nitrite, can accumulate in the rumen, because the first reaction may be faster than the second (Hegarty, 1999). When absorbed in the bloodstream, nitrite converts haemoglobin to methemoglobin which reduces the oxygen-carrying capacity of the blood (Chuan Wang et al., 1961).

- Microbial synthesis of long chain fatty acids

Rumen microbes are made of protein, polysaccharides, lipids and nucleic acids. All these molecules contain hydrogen. The proliferation of microbes in the rumen therefore implies a consumption of H_2 (Guyader, 2015). Although lipids represents only ~12% of microbial biomass, *de novo* synthesis of long chain fatty acids (LCFA) is an extremely H_2-consuming process. It

require NADPH (another reduced cofactor) during the elongation step, precluding the H_2 release owing its reoxidation (Hegarty, 1999). However, microbial synthesis seems to be a small contributor to H_2 disposal in the rumen and is addressed in few studies.

2.1.3. Factors that influence methane production

Based on the elements seen in the section 2.1.2, we can conclude that methanogenesis depends on H_2 production in the rumen and is competing with alternative but still rather marginal H_2-sinks. Dihydrogen production, and therefore methane production, is determined by two main factors: feed intake and diet composition (Shibata et al., 2010).

Feed intake

Methane production is highly correlated with the DMI (DMI; Shibata et al., 2010). For this reason, DMI is used as an indicator to predict large-scale methane emissions. Recently, Charmley et al. (2016) calculated that each kg of DMI generates on average 20.7 g of methane for forage-based diets (forage content of >70%).

Nevertheless, if we consider the yield of methane (i.e. CH_4 per kg DMI), a high level of intake can reduce the ruminal residence time of feeds in the rumen hence induce lower ruminal fermentation and CH_4 emission per kg of DMI (Moss et al., 2000). Hence, feed intake does not explain all the variations of CH_4 emissions, the diet composition plays an important role.

Diet composition

As shown in Figure 2 and Table 3, the VFA profile directly influences the amount of H_2 produced. Acetate production leads to an increase in H_2 concentration, whereas propionate production leads to its decrease.

The acetate: propionate ratio is the result of fermentation pathways used by bacteria, which are driven by diet composition, and more precisely by the types of carbohydrate involved. High-fiber diets stimulate cellulolytic bacteria and protozoa and therefore acetate production. Conversely, starchy diets stimulate amylolytic bacteria, leading to propionate production (Kumar et al., 2009).

The shift in fermentation patterns is related to a change in ruminal pH, which in turn affects methanogenesis. Indeed, methanogens have an optimal pH for methane production about 7 to 7.2 and fiber-rich diets often result in a rumen pH close to these values (Kumar et al., 2009).

Conversely, starchy diets lead to a reduction in ruminal pH and through this, inhibit the development of methanogens (Hook et al., 2010).

Aside from carbohydrates, other components of the diet may have effects on methanogenesis through the activation of alternative H_2-sinks or the inhibition of *Archaea*. These aspects will be discussed in the section 2.2.2 as they can be used as strategies to reduce methane emissions.

2.2. Strategies to reduce methane emissions from ruminants

Table 4 | Summary of the strategies to reduce methane emission, mechanism of abatement and consideration to take into account (adapted from Hook et al., 2010)

Strategies	Mechanism of CH_4 abatement	Considerations to take into account
MANAGEMENT STRATEGIES		
Reproduction management	Herd size ↓ Replacement rate ↓ ➔ less animals needed	Reproductive techniques are not always available
Genetic improvement	Feed efficiency ↑ Animal productivity ↑	Low genetic progress in extensive systems
NUTRITIONAL STRATEGIES		
Starchy diet	Propionate ↑ ➔ H_2 consumption ↑ Acetate ↓ ➔ H_2 production ↓ pH ↓ ➔ fibrolytic community and methanogens ↓	Production of starchy concentrate emits more GHG than fodder; acidosis risk; competition with human nutrition
Electron acceptors (nitrate)	Stimulation of H_2-sink ➔ H_2 ↓	Nitrite toxicity
Plant compounds		
Essential oil	Antimicrobial activity	More research needed
Saponins	Inhibition of protozoa ➔ H_2 ↓	Animal productivity lowered
Tannins	Feed digestibility ↓ methanogens ↓	Toxicity; affect digestibility; protection of protein
Lipids	Inhibition of protozoa, DMI ↓ ➔ H_2 ↓ methanogens ↓	Intake and animal performance reduced; favor high-oil by-products
Macroalgae	Antibacterial secondary metabolites (tannins, bromoform) inhibit methanogens Propionate ↑ ➔ H_2 consumption ↑	Need more investigation Difficulty of supply
Monensin	Inhibit fibrolytic community ➔ H_2 ↓ propionate ↑ ➔ H_2 consumption ↑	Unpopular option Banned in the EU
Defaunation	Removal of protozoa ➔ methanogens ↓ and H_2 ↓	Toxicity
Vaccines	Immune response against methanogens	Not yet efficient; strains change with diet and location

Many authors have reviewed the strategies to reduce methane emissions from ruminants (Kumar et al., 2009; Hook et al., 2010; Shibata et al., 2010; Cottle et al., 2011; Gerber, Henderson, et al., 2013) and new ones are constantly being developed. Mitigation strategies can be classified into 2 groups: management strategies allowing an improvement in animal productivity and nutritional strategies impacting enteric fermentation. They are summarized in Table 4 and then detailed in the following subsection.

2.2.1. Management strategies: increasing animal productivity

The increase in animal productivity is the most promising and cost effective option to reduce methane emissions per kg of commodity produced (Shibata et al., 2010; Gerber, Steinfeld, et al., 2013). To understand this concept, we need to consider emissions relative to feed intake (DMI), methane yield or product output (meat, milk, wool), methane intensity, rather than absolute emissions per animal. When productivity increases, the relative CH_4 emissions decrease because fewer animals are needed to provide the same product output (Cottle et al., 2011). Possible management strategies for increasing animal productivity are discussed in this section.

Reproductive management

Improving fertility helps to reduce CH_4 emissions from beef production systems because it allows breeders to reduce herd size and replacement rates. Examples of strategies from the FAO review related to the reproductive management are listed and briefly explained below (Gerber, Henderson, et al., 2013):

- choice of adapted breeds: adapted animals will be less subject to environmental stressors that affects fertility (e.g. heat stress can cause embryo loss);
- mating choice avoiding inbreeding: using pure-breeding must be performed by making sure to minimize inbreeding-induced reduction in fertility;
- allow early puberty and parturition by adequate nutritional status: this aims to reduce the unproductive but CH_4-producing period of the animal's life;
- maintain a low yearly calving interval by ensuring fertilization within 85 days after parturition: early weaning helps to achieve this goal;
- enhance periparturition care and health by monitoring the metabolic status and performing a pregnancy diagnosis on time: postpartum disease results in poor fertility or even anticipated culling, which have a significant impact on emissions from the system;

- use reproductive technologies such as artificial insemination (AI), genomics, embryo transfer, gender-selected semen and estrus synchronization allows faster genetic progress of offspring.

Genetics

Genetic selection can change breeds to more efficient and productive animals, and therefore can help to reduce CH_4 emissions per unit of product. Two obvious examples are the Holstein and the Belgian Blue, breeds that have made spectacular genetic progress. However, animal productivity does not only depends on milk or meat yields but also fertility, longevity and disease resistance. Consequently, selection of animals must also consider these traits to achieve higher productivity sustainable over time (Moss et al., 2000).

Aside traits related to milk and meat yield, residual feed intake (RFI) can be used to reduce indirectly CH_4 emissions. RFI is the difference between feed intake and feed requirements for maintenance and production. It measures the efficiency of the animals. Selection for low RFI, i.e. for animals that eat less than expected and consequently are more efficient animals, results in a reduction of daily methane production (Hegarty et al., 2007).

Although high variability in methane emissions between animals has been reported, direct selection for low CH_4-emitters is difficult to apply because traits related to methane emission have low heritability, they are difficult to measure and they are unfavorably correlated with production traits (Cottle et al., 2011).

The use of genetic selection to reduce methane emissions by improving animal productivity implies rapid genetic progress, which is enabled by using reproductive technologies. However, these technologies are not available worldwide, this limits the genetic-based strategies to reduce methane emissions.

2.2.2. Nutritional strategies: promote alternative H_2-sinks or inhibits *Archaea*

Nutritional strategies allowing an abatement of methane emissions act by promoting H_2-sinks, inhibiting *Archea* or through a combination of both. They are discussed in the following sections and their mechanism explained. A distinction has been made between rumen manipulation through feeding management and through artificial methods.

Rumen manipulation through feeding management

- Starchy diets to stimulate propionate production

As discussed earlier, inclusion of starch results in shifting the rumen microbes towards propionic bacteria. They ferment pyruvate to propionate by oxidizing reduced cofactors, preventing the production of H_2. Furthermore, the activity of these bacteria leads to lower ruminal pH which makes less favorable conditions for methanogens. Therefore, starchy diets act on both modes of action of methane mitigation: alternative H_2 sink and antimethanogens.

[*What about the GHGs emitted by grain production?*

The production of grain emits much more GHGs than growing fodder, because N_2O and CO_2 emissions are greater (Gerber, Henderson, et al., 2013). On the other side, feeding grain decreases CH_4 production and above all increases productivity, resulting in a reduction of total emissions over the lifetime of an animal. Hence most of the life cycle assessment (LCA) shown that feedlots are generally beneficial from a GHG point of view compared to grass-finishing systems, despite grassland C sequestration. That is how surprisingly, feedlot development over the past 3 decades in Australia is one major contributing factor to the decline in GHG emissions from the beef industry (Wiedemann et al., 2017).

However, depending on the type of grazing system, pasture-finished beef production may emits less GHGs than those raised in feedlots, or even sequester C. As an example, grazed savanna woodlands in Queensland have a positive C balance due to the sequestration in woody vegetation and a low stocking rate of >4 ha/AE (animal equivalent = 450 kg steer) (Bray et al., 2014). Another study to assess the total C balance of a grazed grassland located in Wallonia (southern Belgium) reported that intensively managed (2 LU/ha; livestock unit = 600 kg steer) old pasture (>100 years) is a relatively stable C sink (Gourlez de la Motte et al., 2016). Thus, under certain conditions, both intensive and extensive managed grassland can offset GHGs emissions from cattle through C sequestration. Furthermore, we must remember that pastures contribute to biodiversity and landscape function, minimize water run-off by preventing soil erosion and utilize less pesticides than arable systems.]

We must also consider that excess of fermentable carbohydrates may cause the ruminal pH to drop leading to acidosis. This pathology disrupts the rumen microbes and can damage ruminal and intestinal walls, decrease blood pH, cause dehydration and even death (Hook et al., 2010; Gerber, Henderson, et al., 2013). In addition to that, fed with cereals, ruminants become competitors with human for food. Thereby this practice is not viable in some countries where food security is precarious or for low-cost extensive systems (Gerber, Steinfeld, et al., 2013).

- Electron acceptors to stimulate alternative H_2 sinks

Electron acceptors include nitrate and sulphate as seen before (see page 9), but also malate, fumarate, succinate, nitropropanol, nitroethane, nitroethanol, sodium laurate or lauricidin (Cottle et al., 2011). Their inclusion in the rumen leads to H_2 consumption and reduces methanogenesis. Among them, nitrate appears to be the most efficient H_2-sink (Gerber, Henderson, et al., 2013). As a reminder, its advantage is ammonia formation, a useful product for ruminants on low-N diets. The disadvantage is the possible nitrite accumulation which leads to reduced oxygen-carrying capacity of the blood (see page 9 Nitrate reduction).

Van Zijderveld et al. (2010) investigated the potential for reducing methane production through nitrate supplementation in sheep. The addition of 2.6% nitrate to the diet reduced the CH_4 production by 32%. This is because the reduction of nitrate is thermodynamically more favourable than reduction of CO_2 to CH_4. In other words, when nitrate is present in the rumen, ammonia formation is favoured over methanogenesis (Hegarty, 1999; Morgavi et al., 2010; Van Zijderveld et al., 2010). During this study, the authors have monitored the conversion rate of haemoglobin to methemoglobin in order to see if animals were suffering from nitrite intoxication. The highest value was about 7% of methemoglobin when ruminants can tolerate up to 30-40% (Van Zijderveld et al., 2010; Olijhoek et al., 2017).

Consequently, nitrate reduction seems to be H_2-sink which competes efficiently methanogenesis and is particularly interesting when diets are deficient in CP, but carries the risk of nitrite poisoning. This abatement method is particularly suitable for producers who use urea as a nitrogen source. The urea can be substituted by nitrate.

- Plant secondary compounds

Secondary compounds are chemicals that are not directly involved in the process of plant growth but are associated with grazing avoidance mechanisms. Among them, essential oils, saponins and tannins may reduce methane production by ruminants (Gutteridge and Shelton 1998).

Some essential oils exert an antimicrobial effect on gram-positive bacteria, that can reduce the H_2 production in the rumen. However, essential oils were studied only *in vitro* and antimethanogenic effects were not clearly reported (Gerber, Henderson, et al., 2013).

Saponins are natural detergents forming complex with sterols in protozoal cell membranes, resulting in their death. The suppression of protozoa leads to a lower production of acetate and

thereby of H_2 and inhibits the methanogens. But, saponins inclusions have a downside because they decrease organic matter digestibility and animal productivity (Goel et al., 2012).

Finally, among the secondary compounds, there are tannins and their inclusion in diets offer mitigation strategies. Tannins are secondary compounds used by plants where they play a role in protection from predators such as bacteria, insects, fungi and grazing animals. They are classified in two groups: hydrolysable (HTs) and condensed tannins (CTs). They act both as anti-nutritional agent by binding dietary protein, polymers and minerals in aqueous solution. However, they may have beneficial effects depending on their concentration and their nature (Gutteridge et al., 1998; Goel et al., 2012). By binding the macromolecule, the tannins form complexes that inhibit methane as shown in the Figure 5.

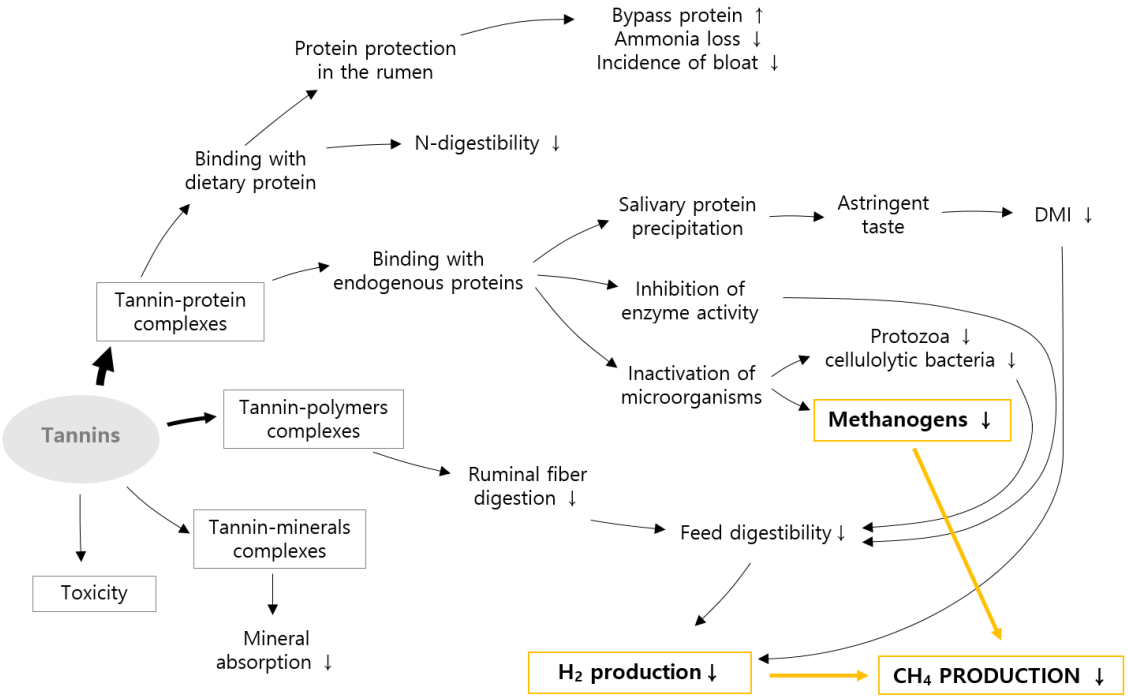

Figure 5 | Simplified schema of tannins interactions and their mode of action on methanogenesis. DMI, dry matter intake; H_2, dihydrogen; CH_4, methane; N, nitrogen (from Kumar et al., 1995; McSweeney et al., 2001; Goel et al., 2012)

Tannins lower methane production by directly inhibiting methanogens and/or indirectly by reducing both feed degradation in the rumen and feed intake. The effects of tannins vary according to their structure, molecular weight and concentration. The challenge is to find those who are specific in decreasing methanogenesis without decreasing the feed digestibility and consumption. Goel and Makkar (2012) reported that HTs tend to be more specific at this point than CTs: they act only by suppressing methanogens while CTs act more on reduction of fiber digestion. Further, tannins have other advantages by increasing rumen bypass protein, reducing

ammonia loss and avoiding bloat in the case of soluble N-rich diets (Goel et al., 2012; Gerber, Henderson, et al., 2013).

Tannins can be fed as supplements (e.g. chestnut tannin, grape marc) or as tannin-rich plants. Numerous tropical legumes are particularly tannin-rich and their antimethanogenic effects are reported. The interest in legumes is all the more important as their protein content is high, which has direct positive nutritional and animal health benefits for the animal (Gerber, Henderson, et al., 2013).

- Lipid inclusion

In their review, Cottle et al. (2011) reported that lipid inclusion lowered methane production by 3.5% to 5.6% for each percent of added lipid. Biohydrogenation of LCFA is an alternative H_2-sink, but its H_2-disposal contribution is minor. Methane abatement is mainly due to the suppressive effect on bacteria and protozoa which can lead to lower fiber digestibility and voluntary intake if lipid comprises over 5% of the diet (Hook et al., 2010).

This abatement strategy has the disadvantage of reducing DMI and animal performance and is in addition not always cost-effective. However, it should become more interesting by using high-oil by-products as cheap source of lipids (Gerber, Henderson, et al., 2013). It has been reported that feeding grape marc, a high-oil by-product of winemaking, is effective in reducing methane emissions by about 10 to 20 %. However, grape marc also contains tannins, which seem to be responsible for the half of this methane abatement (Moate et al., 2014).

- Macroalgae as antimethanogenic feed additive

The antimethanogenic effects of macroalgae have recently been reported and seems to have no negative impact on digestibility and animal productivity. The CH_4 abatement is due to the presence of antibacterial secondary metabolites as tannins and bromoform, which affect growth of methanogens. This implies a shift of rumen fermentation towards propionate formation. A low dose (2-5% of substrate OM) of dried and grounded *Asparagopsis taxiformis* – a red macroalgae – lead to a virtual elimination of CH_4 *in vitro* (Kinley et al., 2016). This potent antimethanogenic properties has been confirmed *in vivo* with sheep, where low inclusion of *Asparagopsis* (~0.42 g/kg LW) demonstrated a reduction of CH_4 emissions by 50-80% over a 72-day feeding period (Li et al., 2018). Further works are needed to define the effect of this algae on feed intake, digestibility, animal productivity and animal health. Moreover, production and processing must be investigated to make this practice usable at large scale.

Artificial rumen manipulation

- Monensin

Monensin is an ionophore antibiotic isolated from a bacteria of the genus Streptomyces. Originally it was use to increase feed efficiency and prevent ketosis in dairy cows. Furthermore, it has been shown to have an antimethanogenic effect by inhibiting protozoa and cellulolytic bacteria, causing a shift in VFA pattern towards propionate instead of acetate. This change lowers the H_2 supply in the rumen, and consequently the methane production by *Archea* (Kumar et al., 2009; Hook et al., 2010).

The use of antibiotics as feed additive is unpopular with consumers and could be barriers to its adoption. Moreover, it has been banned in the European Union since 2006 although it can still be used if prescribed by a veterinarian (EC 1831/2003).

- Defaunation

Defaunation consists in the removal of protozoa from the rumen by using chemicals and physical techniques. Protozoa generate large amounts of H_2 through formation of acetate and butyrate which leads to a symbiotic relationship with methanogens. Therefore removal of protozoa results in CH_4 abatement by decreasing H_2 production, activating the H_2-consuming propionate formation and reducing the methanogen population (Morgavi et al. 2010).

This method has never been used routinely because of the toxicity of the defaunating agents (e.g. bromochloromethane, 2-bromoethanesulfonic acid) to the ruminant. Further, although protozoa are not essential to the ruminant, they contribute to feed degradation, especially of the structural carbohydrates (Moss et al., 2000; Morgavi et al., 2010).

- Vaccines

Vaccines can be used to trigger antibody production by the immune system of host animal against *Archea*. In theory, antibodies could continuously reach the rumen with saliva and specifically target methanogen strains. Current research on this method reported only 5 to 10% of CH_4 abatement, probably because not all methanogens strains are yet clearly identified (Gerber, Henderson, et al., 2013). Moreover, methanogens can differ according to the diet or the geographical location which complicates vaccine development. The use of vaccines as strategy for CH_4 reduction still need more investigation to be ready for practical application (Hook et al., 2010).

2.3. The Australian beef industry

2.3.1. Context

Australian agriculture contributes to 3% of gross domestic product (GDP) and directly employs 250,700 full-time equivalent workers. Moreover, it provides indirectly over 1.6 million jobs throughout the agricultural supply chain. Seventy percent of the agricultural production is exported, which represents 21% of total merchandise exports and makes of Australia a significant player in the global market of agriculture raw materials (ABARES, 2018; ABS, 2018).

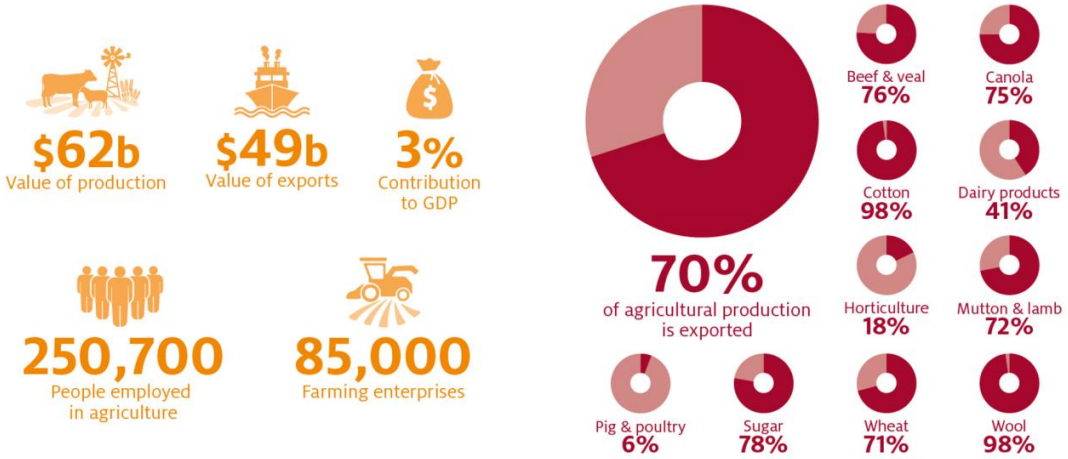

Figure 6 | Agriculture commodities statistics: contribution of agriculture to the Australian economy in 2016-17 (on left) and share of agricultural production exported 3-year average 2014-15 to 2016-17 (on right) (ABARES, 2018)

In 2018, the gross value of cattle and veal production was AUD$11.5 billion and accounted for 20% of the total farm value (ABARES Agricultural commodities March 2019). Further the beef industry accounts for 55% of farms with agriculture activity (NFF, 2017). According to its gross value, cattle and calves is the first agricultural commodities produced in Australia (Table 5 | Top three of agricultural commodities produced in Australia, ranked by gross value in 2018 Table 5).

Table 5 | Top three of agricultural commodities produced in Australia, ranked by gross value in 2018 (ABARES, 2018)

Agricultural commodities	Gross value in 2018 (AUD$)
Cattle and calves	11.5 billion
Wheat	5.1 billion
Milk	4.1 billion

2.3.2. Beef production and markets

The Australian cattle herd was 26.2 million heads in 2017, of which 90% were beef cattle and 10% dairy cattle (ABS, 2018). Queensland alone accounts for almost the half of the national herd (Figure 7 | Australian cattle and breakdown by State (on left) and Australian beef export by location (on right) (MLA, 2018)Figure 7). Beef cattle farmers produce roughly 2.5 million tonnes carcasses weight of beef and veal each year, which represent 3% of world's production (NFF, 2017).

Because 76% of the production is exported, Australia was the third largest beef exporter in 2017, behind India and Brazil. Exports occurred to over 78 countries as live or processed beef. Live beef exports represented ~960,000 heads and are stimulated by a growing feedlot industry in South-East Asia, particularly in Indonesia and Philippines (Martin et al., 2013). However, processed beef represents the major beef export, equivalent to $8 billion or 86% of the exported beef value (ABS). The three major destinations are Japan (28.5%), United States (21.8%) and Korea (14.5%; MLA, 2018).

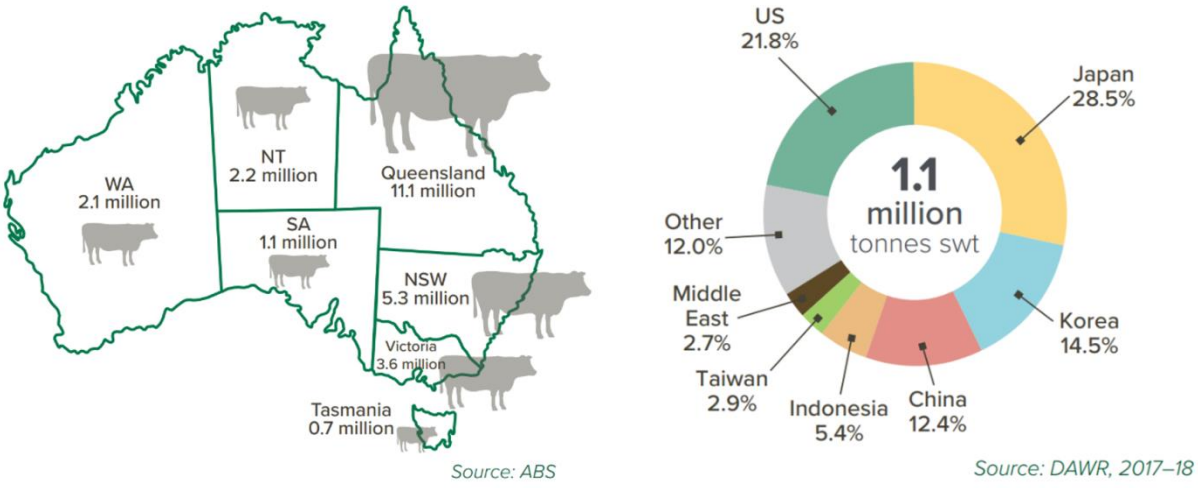

Figure 7 | Australian cattle and breakdown by State (on left) and Australian beef export by location (on right) (MLA, 2018)

2.3.3. Northern vs southern beef industry

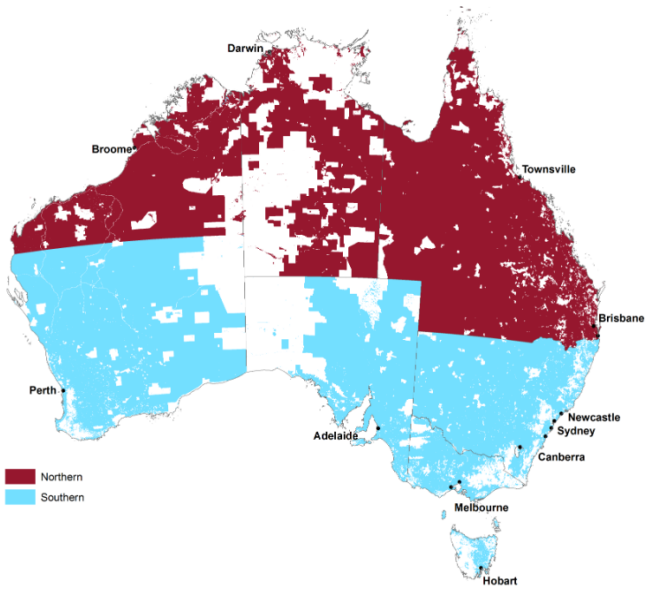

Figure 8 | Map of Australian beef cattle industry regions (Martin et al., 2013)

The Australian beef industry is generally divided into 2 regions: the northern and the southern region (Figure 8). Northern beef producers use mainly *Bos indicus* breeds such as Brahman and Santa Gertrudis which are better suited to tropical conditions. Whereas Southern farmers use *Bos taurus* breeds such as Angus, Hereford and Charolais. Because meat from *Bos indicus* is of lower quality, the northern industry targets mainly the export market while the southern beef is predominantly sold into high value domestic market. Hereafter are the major markets according to their regions (Martin et al., 2013).

Table 6 | Major beef market according to their regions (from Martin et al. 2013)

Regions	Major markets
Upper Northern Territory, Northern Western Australia	Live export market
Queensland	Beef export market
Southern states	Domestic beef market (~50%), beef export market (~50%)

Northern and southern beef industries differ in markets access, but also in their climate, pasture, production system and industry infrastructure. The following section are mainly focused on the northern region.

2.3.4. Northern climate

Northern Australia is divided into three climate zones: tropical in the far north, subtropical along the East coast and hot arid inland (Figure 9). Rainfall is dominated by monsoon systems with distinct wet and dry seasons. Wet season occurs from November to April, followed by the dry season. The season intensity differs from one region to another depending on latitude, topography and distance from the coast.

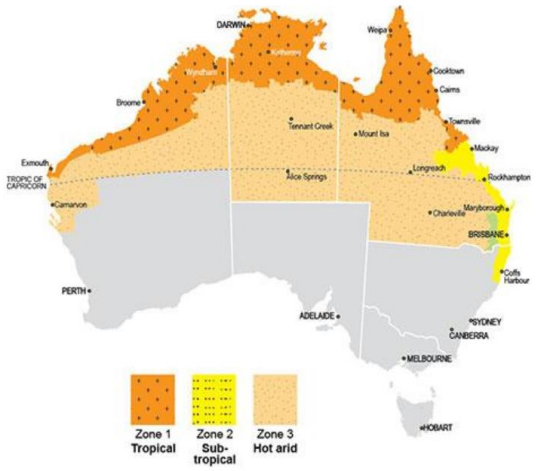

Figure 9 | Map of climate zones in northern Australia (Bureau of Metrology 2001)

As an example, rainfall in Townsville and Cairns were respectively 1128 and 1987 mm on average over the last 78 years (BOM, 2019). The climate graph of Townsville is shown in Figure 10 to give an idea of monthly changes in temperature and precipitation in the tropical region.

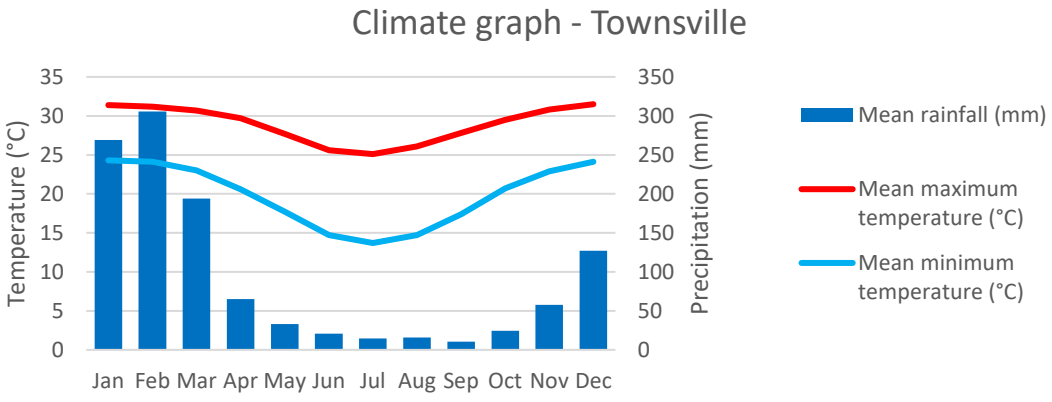

Figure 10 | Climate graph of Townsville based on 78-years average records (1940 - 2018; BOM, 2019)

2.3.5. Northern production system

Northern beef farms collectively account for half of the national herd but occupy 75% of farming land dedicated to beef in Australia. Consequently, they are more extensive than their southern neighbors.

Farm size

The average size of beef cattle producing farms in the northern region was 1580 heads and 23,436 hectares in 2014. Farm with fewer than 100 head represent only 2% of the national beef cattle and by the way are generally not included in the statistics of the Australian Agricultural and grazing Industries Survey (AAGIS).

Table 7 | Average herd size and area operated by northern and southern beef farms in 2014 (ABARES, 2018)

Characteristics of farm		Northern	Southern
Average herd size (head)		1580	431
Distribution	100 – 400	39%	73%
	400 – 1600	38%	23%
	1600 – 5400	18%	3%
	>5400 head	5%	1%
Average area operated (ha)		23,436	5,561

Feeding

Beef cattle are predominately raised on pasture for the majority of their life. About 40% enter feedlots where they are fed with grain-based diets to be finished over 50-120 days, i.e. 10-15% of their lifespan. This second feeding process stage aims to ensure rapid weight gain and therefore meet the market requirements faster (ALFA, 2019).

Queensland is the largest producer of grain finished cattle, accounting for 58.4% of Australian lotfed cattle turn-off (MLA, 2018). Feedlots are mainly located in the south-east of this state, close to inputs (grain, water, feeder cattle) and processing facilities. This industry has increased significantly over the past 25 years due to the ability to consistently meet market requirement irrespective of seasons or drought (ALFA, 2019).

However, Australian beef cattle are still mainly grass-fed. Pasture improvement to ensure greater stability of supply over the seasons and to increase nutritional value is one of the levers allowing higher productivity, and therefore lower methane intensity (emission per unit animal product). Introducing legumes in pasture helps to achieve this goal.

Pasture – role of legumes

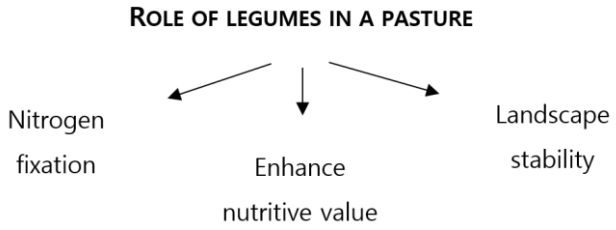

Figure 11 | Main roles of legumes in a pasture (from Humphreys, 1995)

Legumes have the ability to fix atmospheric nitrogen (N_2) through symbiosis with Rhizobia bacteria. This symbiotic relationship allows a large supply of nitrogen in the form of ammonia (NH_3) to the host plant, making them rich in protein, as well as providing N for grasses in the sward. Biological nitrogen fixation in legumes is inexpensive and environmentally clean and avoids the consumption of fossil fuel-consuming fertilizer. This is particularly important in northern Australia where it is cost-prohibitive to use inorganic fertilizers. Further, legume ground cover ensures landscape stability by reducing soil erosion and runoff (Humphreys, 1995).

2.4. Leucaena: an efficient legume to reduce methane emission in tropical regions

2.4.1. Introduction

Leucaena leucocephala is a tropical legume shrub originating from Mexico and Central America. It can be used for many purposes such as fodder, human consumption, firewood, erosion control, shading, windbreaking. This versatility led to its spread and naturalization throughout the tropics. This is how Leucaena was introduced into Australia in the late 19th century (Shelton et al., 1998).

This legume is now listed as a weed in 25 countries including Australia. In Queensland, two distinct subspecies cohabit: *Leucaena leucocephala ssp. Leucocephala* and *Leucaena leucocephala ssp. glabrata.* The first one was naturalized in 1920 and is now considered as a weedy shrub. It is an aggressive colonizer of roadsides, disturbed sites and creeks. The second subspecies was developed in Queensland by CSIRO in 1960s in order to supply highly nutritious forage for cattle and continues to be improved in terms of yield, establishment, disease and reduced weed potential (Shelton et al., 1998; Walton, 2003).

Botanical characteristics

Leucaena leucocephala (Lam.) de Wit belongs to the Mimosea tribe of the Mimosoidea subfamily of the Fabaceae family. It is a legume able to fix the atmospheric nitrogen, through nodulation with *Rhizobia*. It is a perennial, thornless long-lived shrub or small tree, 7 – 18 m tall. The leaves are bipinnate, 10 – 25 cm long and arranged alternately along the stem. Each leaf has 6-8 pairs of pinnae bearing 11 – 23 pairs of leaflets 8 – 16 mm long (Shelton et al., 1998; Walton, 2003).

Establishment in Australia

Leucaena-grass pastures are predominately located in Queensland, where the ideal growing conditions are met: warm temperature, rainfall between 650 and 3,000 mm and soil types of moderate-high fertility (Shelton et al., 1998). Shelton and Dalzell (2007) estimates that there are approximately 13.5 M ha suitable for planting Leucaena in Queensland, which represents 9.2% of grazing area in the state (Department of agriculture and fisheries 2018). Although the rate of adoption of Leucaena is raising rapidly, the latest estimates report that ~200,000 ha are sown with this plant, thus only 1.5% of the potential development (Beutel et al., 2018).

The main reasons explaining the untapped potential of Leucaena-grass pastures are slow establishment, psyllid insect sensitivity and toxicity for cattle (Shelton et al., 1998). These obstacles limiting the expansion of Leucaena plantings and the way they can be tackled are discussed further in the following sections.

2.4.2. Composition of Leucaena

Like other legumes, a major asset of Leucaena is its high protein content (24.4%; Table 8). Its nutritional values are similar with that of alfalfa and higher that Rhodes grass (*Chloris gayana*), which is a grass species widely used in northern Australian pastures (Shelton et al., 1998). Table 8 shows the comparative chemical composition of these 3 species.

Table 8 | Comparative chemical composition of *Leucaena leucocephala*, *Chloris gayana* and *Medicago sativa*. NDF, neutral detergent fiber. (from Gutteridge et al., 1998; Mlay et al., 2006; Vandermeulen et al., 2018)

	C. gayana Rhodes grass	*L. leucocephala* Leucaena leaf	*M. sativa* Alfalfa leaf
Crude protein (%)	5.5 – 13.5 [a,c]	24.4 [a]	26.9 [b]
Ether extract (%)	0.9 [a]	3.4 [a]	–
Ash (%)	6.9 [a]	13.4 [a]	16.6 [b]
Organic matter (%)	93.1 [a]	86.6 [a]	83.4 [b]
NDF (%)	71.4 [a]	31.5 [a]	31.4 [b]
Gross energy (KJ/g)	16.6 [a]	17.2 [a]	18.5 [b]
Tannins (g/kg)	1.68 [c]	101.5 [a]	0.13 [b]

Reference: [a] Mlay et al. (2006), [b] Gutteridge and Shelton (1998), [c] Vandermeulen and al. (2018)

Leucaena has a particularly high tannin content (~10%) compared to Rhodes grass and Alfalfa (*Medicago sativa*) (<0.2%). These molecules bind with dietary and endogenous protein leading to increased protein bypass, reduction of feed digestibility and methane abatement (see Figure 5 page 17). This last effect is of specific interest to this review and will be discussed in detail in section 2.4.6.

Toxicity and solution

Leucaena contains significant amounts (4-12 %) of mimosine, especially in the edible parts for the ruminants: tips of actively growing shoots, young leaves and young pods. It is a toxic amino acid that disturbs cell division leading to alopecia (hair loss) and sometimes damaging internal organs. Fortunately, toxicity due to mimosine is rare because it is quickly converted into 3,4-dihydroxy pyridine (3,4-DHP) by the enzymes present in the shrub and the rumen bacteria. However, this

byproduct is also toxic and can cause alopecia, salivation, low fertility, depressive appetite, lesions, enlarged thyroid glands and sometimes mortality (Jones et al., 1984; Jones, 1994).

To overcome DHP toxicity, *Synergistes jonesii* was introduced into Australian ruminants in 1981. This ruminal bacterium can degrade 3,4-DHP to 2,3-DHP and 2,3-DHP to harmless byproduct, resulting in the complete detoxification of mimosine. It has been commercialized since 1995 as an *in-vitro* mixed-culture inoculum for producers using Leucaena-grass pasture (Jones, 1994; Dalzell et al., 2012).

Dalzell and coworkers (2012) studied the prevalence of mimosine and DHP toxicity in cattle grazing Leucaena pastures in Queensland. As expected, they did not find mimosine toxicity but almost half of the animals (48%) appeared to be exposed to subclinical DHP toxicity (urinary DHP concentration >100 µg/mL). The authors noted the importance of protecting animals from Leucaena toxicity, by scrupulously following the recommended procedures (Dalzell et al., 2012). Ideally, 10% of the herd grazing Leucaena should be inoculated directly with the *in vitro*–produced *Synergistes jonesii* culture on an annual basis. The DHP-degrading bacteria appears to be naturally spread to the non-inoculated animals via the feaces in the form of dust (Jones et al. 2009).

2.4.3. Benefits of Leucaena pastures

Shelton and Dalzell (2007) reviewed the benefits of Leucaena pasture and classified them in 3 groups: production, economic and environmental. There are discussed in the following subsections.

Production benefits

Leucaena has deep and well developed taproot and rapid growth, in symbiosis with nitrogen-fixing bacteria. As a result, it produces a large quantity of highly nutritious and digestible forage: 3 to 30 t of DM/ha/yr irrespective of drought (Shelton et al., 1998). Moreover, Leucaena can survive and remain productive for more than 30 years under regular grazing. This productivity leads to rapid animal growth rate (250 – 300 kg LW/yr) and an increased carrying capacity (1.5 ha/steer) resulting in animal production up to 4 times higher per area compared to grass pasture. Furthermore, Leucaena pasture in good growing conditions allows cattle to meet all export weight-for-age and carcass quality requirements without the need to finish cattle with grain-based rations in feedlots (Shelton et al., 2007).

Economic benefits

Leucaena can grow without using urea supplementation, synthetic fertilizers or pesticides. Therefore, it makes possible to finish cattle in compliance with organic farming standards. Leucaena-fed steers can be valued at a better price in the organic market. In addition, animal welfare is improved and environmental impact lowered compared with feedlots, making meat more ethical according to consumers expectations. Furthermore, the continuous feed supply from Leucaena allows producers to keep their cattle in good condition during the dry season and sell at a more convenient time, when the availability of finished cattle for abattoirs is limited, resulting in higher price. Moreover, planting Leucaena doubles the value of the land compared to buffel grass (*Pennisetum ciliare*) pastures (Shelton et al., 2007).

In their economic study, Bowen et al. (2018) compared the profitability of six forage types and ranked Leucaena-grass pastures first, with a gross margin of $181/ha (Table 9). This greater profitability is the result of relatively low forage cost, compared with annual forage crops, combined with high productivity.

Table 9 | Profitability, expressed as gross margin, of six forage options for beef production in the subtropics and northern Australia (from Bowen et al., 2018)

Forages options	Average gross margin ($/ha)
Leucaena (*Leucaena leucocephala* spp*. Glabrata*) -grass pastures	181
Butterfly pea (*Clitoria ternatea*) -grass pastures	140
Oats (*Avena sativa*)	102
Perennial grass (C_4 species)	96
Sorghum (*Sorghum* spp.)	24
Lablab (*Lablab purpureus*)	18

Finally, the C sequestration in stems and roots and the antimethanogenic potential of Leucaena may be eligible for carbon credits under national or international abatement schemes. This means that farmers will be able to earn extra income in form of carbon credits by using Leucaena (Shelton et al., 2007; Taylor et al., 2016).

Environmental benefits

By fixing atmospheric nitrogen, Leucaena enhances soil fertility and promotes grass growth and strong ground cover, resulting in soil protection against erosion and germination of weeds (Shelton et al., 2007). When grown in catchments draining into the Great Barrier reef Lagoon, reducing erosion is all the more important knowing that it is – among others – responsible for

loading sediments and nutrients into the ocean which affects the health of the Great Barrier Reef (Star et al. 2011).

With its deep roots, Leucaena reduces deep drainage of water and therefore controls dryland salinity. Salinization processes have grown because of land clearing for pastures and crops that occurred in the past. Since Leucaena replaces the original native woodland vegetation in its use of water, its adoption restores the hydrological balance of catchments which prevents salinity problems (Shelton et al., 2007).

Leucaena contributes to reducing GHG emissions from the beef industry by storing C in its woody branches and roots, increasing the surface soil organic C and reducing CH_4 emissions by cattle (Shelton et al., 2007). Additional storage of carbon in the soil of Leucaena-pasture ≤20 years old offsets the CH_4 and N_2O emitted by cattle grazing these pastures, making the GHG balance of Leucaena-fed beef positive (Radrizzani et al., 2011). The CH_4 mitigation benefit will be discussed further in section 2.4.6.

2.4.4. Possible constraints

Leucaena pastures also have weaknesses: the main ones are sensitivity to psyllid and potential adverse environmental impacts in case of mismanagement. There are discussed hereafter.

Sensitivity to psyllid

One major obstacle to the expansion of Leucaena into humid (>800 mm annual rainfall) coastal areas of Queensland is psyllid known as *Heteropsylla cubana*. The psyllids are aphid-like insects, 2 mm in length, winged and light green adapted to feeding on the young growing shoots of Leucaena. Infestations can cause yields fall by up to 79%. The best strategy to control this pest is the development of psyllid-resistant cultivars (Shelton et al., 1998). A new psyllid resistant variety, Redlands, was released in 2017 for commercial plantings, allowing producers to overcome the problem.

Weed potential

Leucaena produces large amounts of seeds that can be moved by water and livestock. As a result, it can invade neighboring properties in case of mismanagement. The Leucaena Network has developed a Code of Practice to minimize this weed risk. However, it must be remembered that most current weed infestations are not due to current agricultural practices but to the historical introduction and use as an ornamental tree and for slope stabilization (Shelton et al., 2007).

Soil acidification

Like other legume-based pastures, Leucaena pastures accelerate acidification through biological nitrogen fixation, which requires base cations uptake, and increase in soil organic matter, resulting in higher release of carbonic and carboxylic acids. Acidification can degrade soil chemical properties, soil fertility and thus suppress vegetation growth. To counter this process, it is recommended to spread lime regularly, especially considering that Leucaena prefers soil with neutral to alkaline pH (Shelton et al., 2007).

2.4.5. Leucaena cultivars

In Australia, five cultivars of Leucaena are available for sowing by producers. Their characteristics are described in the following table.

Table 10 | Characteristics of the five Leucaena cultivars available in Australia

Cultivars	Year of release	Brief description	Psyllid resistance	Seed production
Peru	1962	Shrubby growth with good basal branching but superseded by new cultivars.	Very susceptible	Very high
Cunningham	1976	Shrubby growth habit, highly productive but very susceptible to frost.	Very susceptible	Very high
Tarramba	1994	Taller more tree-like growth habit (require specific management to promote basal branching). Cold tolerant.	Tolerant	High
Wondergraze	2011	More branched shrub, cold tolerant.	Tolerant	High
Redlands	2017	Suitable for planting in locations with high psyllid incidence.	Highly tolerant	Moderate

Globally, the new cultivars show higher tolerance to psyllid and frost and are less prolific seeders. Consequently, they make it possible to overcome the constraints to the expansion of Leucaena-grass pastures (sensitivity to psyllid and weed potential). However, little is known about their productivity due to their recent release.

2.4.6. Leucaena and methanogenesis

Antimethanogenic properties of Leucaena have been recently confirmed *in vivo* with cattle and sheep (Kennedy et al., 2012; Soltan et al., 2013; Archimède et al., 2016). In a limited indoor study (only 3 steers per diet), Kennedy and Charmley (2012) reported 18% methane abatement when Leucaena was fed at 44% of the diet. In another experiment, sheep were fed 35% Leucaena and a 14.1% drop in methane emissions was measured (Soltan et al., 2013).

The main antimethanogenic compounds of Leucaena are considered to be the tannins but results are still unclear and vary between experiments. Their effects have been presented in Figure 5. Condensed tannins are generally suggested as being responsible for methane abatement in Leucaena, but hydrolysable tannins have been also implicated (Goel et al., 2012).

Tannins are not the only antimethanogenic compound in Leucaena. Soltan et al. (2013) observed a decline in CH_4 production despite their inactivation by polyethylene glycol (PEG). They hypothesized that the mimosine could affect methanogenesis, knowing that it exhibits antibacterial and antifungal activities. An experiment has confirmed this hypothesis and concluded that mimosine seems to stimulate acetogenesis as an alternative H_2 sink, that would lead to reduced methanogenesis (Soltan et al., 2017).

In addition to reducing CH_4 emissions per kg of DMI through the effects of tannins and/or mimosine, Leucaena's high nutritive value enhances animal productivity, resulting in lower methane intensity (emission per unit animal product). Thereby, Leucaena fed beef emits 27% less CH_4 per kg liveweight (LW) compared to grass-fed beef (Harrison et al., 2015).

2.5. Synthesis of the literature review

The highlights of this literature review are summarized in the following key points:

- methane is a potent GHG which represents 16 to 20% of global warming;
- enteric fermentation by ruminants is the main source of anthropogenic methane emissions, accounting for 28% worldwide;
- in Australia, about 10% of all national emissions and 70% of agricultural emissions come from enteric methane produced by cattle and sheep;
- enteric methane released into the atmosphere also constitutes a loss of 6 to 10% of the energy contained in the feed;
- methanogenesis contributes to the digestive performance of ruminants by removing dihydrogen. Accumulation of hydrogen has a negative feedback on the reductive potential of the rumen in the case of accumulation;
- there are a wide range of strategies to reduce methane emissions from ruminants, but many of them are counterproductive (i.e. result in the under-utilisation of low-cost fibrous feed resources, depress dry matter intake, have toxic effect) and/or use artificial additives that are not well accepted by consumers (i.e. Monensin, defaunating agent);
- the tropical legume shrub *Leucaena leucocephala* seems to be a well-adapted, natural and efficient strategy to reduce methane emission by beef cattle in northern Australia;
- Leucaena increases productivity and profitability and has recently shown that it reduces methanogenesis;
- the antimethanogenic properties of Leucaena seems to be due to the presence of tannins and/or the toxic alkaloid mimosine in their edible parts;
- the potential of development of Leucaena-pastures in Queensland is enormous knowing that it has only been sown on 1.5% of the appropriate land and new cultivars has been released, making possible to overcome the constraints of the expansion of the crop.

Chapter 3. Objectives of this study

The specific objectives of this study are, in order of importance, to:

I. confirm and quantify the reduction in methane emissions by beef cattle fed with diets containing Leucaena;
II. confirm and quantify the animal performance improvement allowed by feeding Leucaena;
III. compare methane abatement and animal performance of the new psyllid resistant cultivar "Redlands" with the standard modern cultivar "Wondergraze";
IV. measure the yield in edible parts of Leucaena from a paddock sown for 3 years.

On a more global scale, this study addresses knowledge gaps on the crop of Leucaena and its use to feed cattle, especially with the new cultivars. This new knowledge will contribute to the expansion of Leucaena-pastures in northern Australia, allowing the beef industry to reduce its carbon footprint while improving its productivity.

Chapter 4. Materials and methods

The experiment was conducted at CSIRO's Lansdown Research Station (19°39′S, 146°50′E) located 45 km south of Townsville, north-east Australia, from March 18th until June 28th of 2019. The experimental protocol complied with the Australian code for the care and use of animals for scientific purposes (8th Edition 2013) and was approved by the CSIRO Queensland Animal Ethics Committee (AEC Number: 2019-02).

4.1. Experimental design and animals

Sixteen Droughtmaster (*Bos Taurus*) steers with an initial LW of 428 ± 25 kg were used for the trial. After four months of grazing, they were housed in individual covered pens (3 × 4 m) and allocated to four groups, based on methane emissions, recorded in a previous experiment. Within each group animals were nominally assigned, at random, to one of four treatments; 0, 18, 36 and 48% inclusion of Leucaena in the diet.

Prior to introducing treatments, the first four weeks were used to familiarize the cattle with handling, entry and exit to the methane chambers and for adaptation to the Rhodes grass (*Chloris gayana*) hay (RGH). During this period, individual methane emissions were measured in open-circuit respiration chambers, creating the baseline (BL), i.e. the methane production of each animal fed with 100% RGH. There then followed, a two-week period (P0) to gradually introduce Leucaena to the diet until the desired inclusion levels had been reached. The two newest cultivars of Leucaena were used in this trial (Redlands and Wondergraze). After the Leucaena adaptation, four periods of two weeks (P1 to P4) followed, during which individual methane emissions were measured in open-circuit respiration chambers. Table 11 summarizes the feeding plan for steers with the different periods.

Table 11 | Feeding planning of steers specifying the Leucaena inclusion and the cultivar fed for each period. The basal diet was composed of Rhodes grass hay. DM, dry matter; RL, Redlands (in green); WG, Wondergraze (in yellow)

Animal	Group	Periods					
		BL	P0	P1	P2	P3	P4
		Leucaena inclusion (% of diet on DM basis) $_{cultivar\ fed}$					
67	1	0	0	0	0	0	0
30	1	0	0 → 18 $_{RL}$	18 $_{RL}$	18 $_{RL}$	18 $_{WG}$	18 $_{WG}$
19	1	0	0 → 36 $_{RL}$	36 $_{RL}$	36 $_{RL}$	36 $_{WG}$	36 $_{WG}$
57	1	0	0 → 48 $_{WG}$	48 $_{WG}$	48 $_{WG}$	48 $_{RL}$	48 $_{RL}$
22	2	0	0	0	0	0	0
48	2	0	0 → 18 $_{WG}$	18 $_{WG}$	18 $_{WG}$	18 $_{RL}$	18 $_{RL}$
28	2	0	0 → 36 $_{WG}$	36 $_{WG}$	36 $_{WG}$	36 $_{RL}$	36 $_{RL}$
32	2	0	0 → 48 $_{RL}$	48 $_{RL}$	48 $_{RL}$	48 $_{WG}$	48 $_{WG}$
26	3	0	0	0	0	0	0
27	3	0	0 → 18 $_{RL}$	18 $_{RL}$	18 $_{RL}$	18 $_{WG}$	18 $_{WG}$
18	3	0	0 → 36 $_{RL}$	36 $_{RL}$	36 $_{RL}$	36 $_{WG}$	36 $_{WG}$
1	3	0	0 → 48 $_{WG}$	48 $_{WG}$	48 $_{WG}$	48 $_{RL}$	48 $_{RL}$
9	4	0	0	0	0	0	0
65	4	0	0 → 18 $_{WG}$	18 $_{WG}$	18 $_{WG}$	18 $_{RL}$	18 $_{RL}$
40	4	0	0 → 36 $_{WG}$	36 $_{WG}$	36 $_{WG}$	36 $_{RL}$	36 $_{RL}$
29	4	0	0 → 48 $_{RL}$	48 $_{RL}$	48 $_{RL}$	48 $_{WG}$	48 $_{WG}$

4.2. Diets and feeding management

Steers had ad libitum access to water and diet. The basal diet consisted of RGH purchased from a neighboring farm. It was chopped to provide particle length of 5-10 cm to facilitate mixing with Leucaena. The Leucaena was hand harvested and chopped in a mulcher three days a week (on Mondays, Wednesdays and Fridays) and stored in a cold room until feeding. A mineral vitamin supplement was provided in the form of a lick block throughout the trial (see appendix 1 for composition).

Just prior feeding, the hay and fresh Leucaena were weighed out for each steer and mixed in wheeled feed bins according to the four inclusion levels: 0 (control), 18, 36 and 48 %, on a DM basis. The bins were located in the pens or the respiration chambers depending where the corresponding animals were. The diets were distributed between 9:00 AM and 10:00 AM.

Figure 12 | Photo of four individual wheeled bins used to offer diets to steers

To avoid mimosine and DHP toxicity, steers were inoculated using a drenching gun with 500 mL *Synergistes jonesii* mixed culture ~10 days after commencement of Leucaena feeding (on April 24th for group 1 & 2 and on May 1st for Group 3 & 4). Animals fed with Redlands were dosed with Redlands adapted culture and those fed with Wondergraze were dosed with Wondergraze adapted culture. When changing varieties, between P2 and P3, steers were reinoculated with the specific culture (on May 22th for group 1 & 2 and on June 5th for Group 3 & 4). The inoculums were provided by the Queensland Department of Agriculture and Fisheries (QDAF, Brisbane).

4.3. Leucaena plantation

Both cultivars of Leucaena (Redlands and Wondergraze) were established at Lansdown Research Station in March/April 2017. The tropical legumes were sown in an existing grass paddock of 20 ha in twin rows spaced 12 meters apart. In March 2018, additional seeding intervention was undertaken by establishment of seedlings in pots under shade and transplanting in the paddock to fill the gaps and ensure sufficient biomass.

The paddock was grazed for three weeks in April/May 2018 to remove excess biomass and the Leucaena rows slashed to 30 cm above the ground level in July 2018 to promote branching. Then, based on a soil report which identified a phosphorus deficiency (7 ppm; see appendix 2 for soil analysis), the paddock was fertilized in September 2018 with 250 kg/ha of single superphosphate and 150 kg/ha of muriate of potash. Following these operations and favored by an exceptional rainy season (~1500 mm in January and February 2019), the Leucaena plantation reached sufficient biomass to initiate the experiment in March 2019. Grasses in the sward were mowed in April 2019 to make the rows more accessible for harvesting.

4.3.1. Harvesting method

Leucaena was hand harvested as stems cut at 1 m above ground level. Stems were transferred to the feed shed and green material (leaves and green stems <5 mm diameter) removed by hand and shredded using a garden chopper. Thicker stems and ripe pods were discarded. The length of harvested rows of Leucaena was measured using a measuring wheel whenever Leucaena was harvested. The corresponding amount of edible material was recorded, the dry matter calculated and yield of edible Leucaena was expressed per linear m and per hectare.

Figure 13 | Harvesting of Leucaena stems in the paddock

4.4. Sampling, measurement and chemical analysis

4.4.1. Feed intake

The daily ration was offered in individual wheeled bins secured in individual pens, which guaranteed total control of the intake. Feed intake was measured for the 16 steers throughout the trial by calculating the difference between feed offered and feed removed 23 hours later.

4.4.2. Live weight

All animals were weighed using a cattle weighing scale every Wednesday throughout the trial, prior to feeding.

4.4.3. Measurement of gas emissions

Four open-circuit respiration chambers were used to determine individual CH_4, and H_2 emissions. These chambers had an internal volume of 23.04 m^2 (4 × 2.4 × 2.4 m) and were constructed of a steel frame over which 4.5 mm clear polycarbonate was attached providing full visibility for each animal. A modified squeeze crush defined a confinement area in each chamber and the floor was made of plastic grids allowing feaces and urine to flow into a container located below. Chambers were maintained at 2.0°C below ambient air temperature and under slight negative pressure (-10 Pa). Air was drawn from outside the building at a rate of 3000 L/min.

Air samples from each chambers and from two outside air ports were collected for 180 s. They were filtered, dehumidified, dried and refrigerated before analysis. The composition of air samples was determined by infrared for CH_4 (Servomex 4100 Servomex Group Ltd., Crowborough, United Kingdom) and by gas chromatography for H_2 (Servomex Chroma, Servomex Group Ltd., Crowborough, UK). A full description of the components and functioning of these chambers is provided by Charmley et al. (2016).

The doors of the chambers being opened for 1 h for feeding and cleaning, measurements were taken over 23 h and extrapolated to a 24 h production. Daily CH_4 and H_2 production (g) were calculated by averaging the last 90 s of each sampling period.

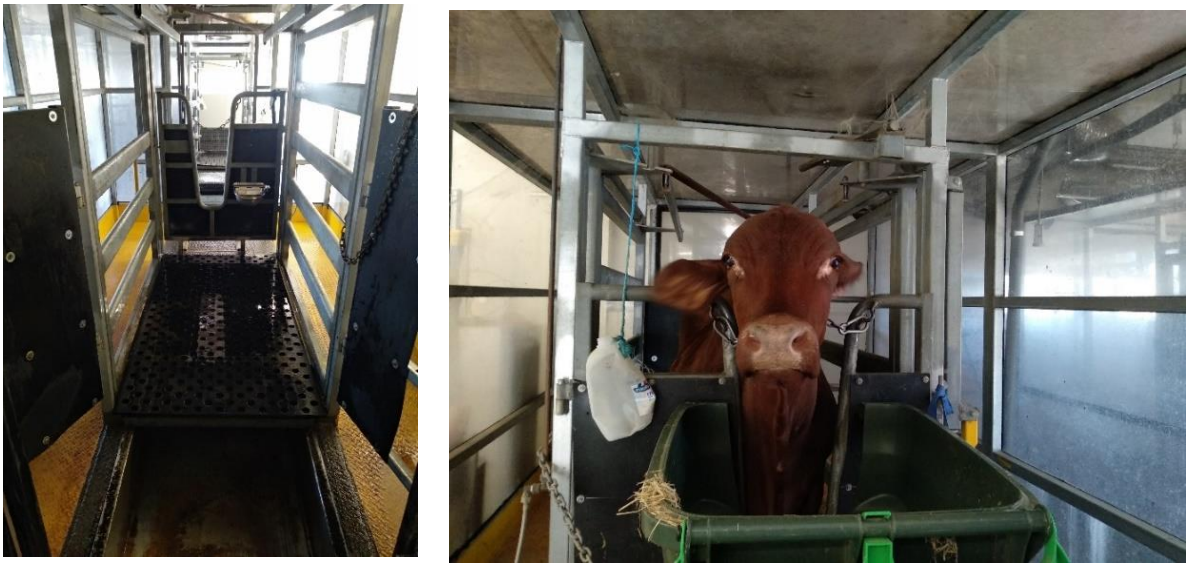

Figure 14 | Photo of a methane chamber, taken from the back door, through which the steers enter (on left) and from the front door by which animals were fed and come out (on right)

For the baseline and each 2-week periods (P1–P4), animals were held in individual pens from day 1 to 11, transferred to open circuit respiration chambers on day 12 and returned to individual

pens on day 14. Thus, methane emissions were collected over two consecutive days, as shown in Figure 15.

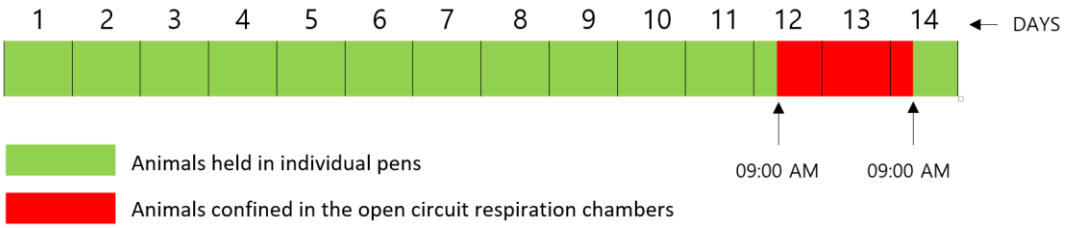

Figure 15 | Organization of periods and confinement time in open circuit respiration chamber

4.4.4. Feed sampling and analysis

Feed sampling occurred according to the protocol shown in the Figure 16. It was repeated each time when four steers went in the chambers, i.e. 20 times. Samples of fresh feed offered (hay and Leucaena) were collected the day before entering the chambers (D11) and during the two days of animal confinement (D12 & D13). These samples were pooled for the 4 steers in the same group over the 3 days. Ten percent of the daily refusals were collected, and the samples pooled by animal.

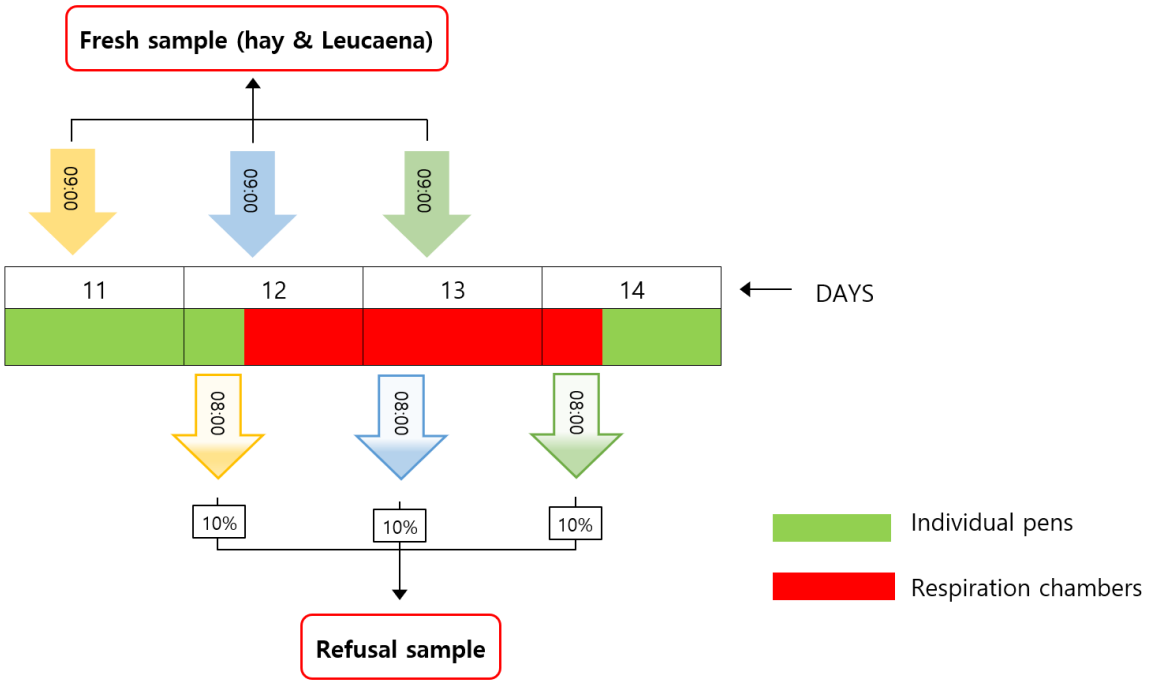

Figure 16 | Sampling protocol for fresh feed and refusal

Feed samples were dried in oven at 65ºC, ground to 1 mm and packed in plastic bags before being sent to CSIRO laboratories in Perth for analysis. ADF, NDF and nitrogen contents and dry

matter digestibility (DMD) of feed samples were predicted by near infrared spectroscopy. The calibration database was large about 1400 samples composed of tropical forages collected in Queensland. All samples (Leucaena, hay, mixture of the both and refusals) were predicted with the same prediction equations, whose characteristics are presented in the Table 12.

Table 12 | Characteristics of forage prediction equations used to determine ADF, NDF, nitrogen and DMD of forages offered in this trial

Constituent	Wavelengths	Scatter correction	SEC	RSQ	SECV	1-VR
ADF	700 - 2499	SNV and detrend	1.47	0.976	1.71	0.967
NDF	700 - 2499	none	2.61	0.964	2.77	0.960
Nitrogen	1106 - 2491	SNV and detrend	0.08	0.988	0.08	0.986
DMD	700 - 2499	SNV and detrend	0.025	0.956	0.026	0.951

ADF, acid detergent fiber. NDF, neutral detergent fiber. DMD, dry matter digestibility. SEC, standard error of deviation. SECV, standard error of cross validation. RSQ, coefficient of determination. 1-VR, (one minus variance ratio) describe the variance explained during cross validation process.

Validation work was undertaken for the fiber fraction (ADF, NDF) and for the DMD. For that, eight feed samples (~10% of the samples) were analysed by wet chemistry. The ADF and NDF contents were determined by the Ankom method (Ankom Tech. Co., Fairport, NY, USA) and the DMD by the in house pepsin cellulase method corrected for in vivo digestibility for cattle (Klein et al., 1993). R^2 of 0.975, 0.935, 0.941 were found respectively for NDF, ADF and DMD at the end of this validation work. Ash content of feed samples was measured by combustion at 550°C, then OM content was deducted (100-ash). Crude protein content was calculated by multiplying nitrogen by 6.25 and hemicellulose was obtained by subtracting the ADF content from the NDF content.

4.4.5. Ruminal pH

Ruminal pH was measured 3 h after feeding, on day 14, following the release of the steers from the methane chambers. The animals were restrained in a commercial cattle crush while the operator inserted an oral stomach tube. The rumen fluid was sucked up with a hand pump attached to the suction tube. The pH was determined immediately using a digital pH meter.

4.5. Calculations

CH_4 and H_2 production was calculated by averaging the two days of measurement. In a unique case (out of 80), gas production was based on a single measurement due to a low DMI (less than 20% compared to previous days), which led to a biased result. CH_4 and H_2 production was

expressed in grams per day, per kg of DMI, per kg of OM and as a percent of deviation from the baseline. The baseline being the gas production when the same animals were fed with 100% of RGH.

The calculation of the nutrients ingested was based on the amount of feed offered and its chemical composition, and on the amount of feed refused ant its chemical composition. Daily liveweight gains were calculated by regression. In order to compare Redlands and Wondergraze, animal productivity, DMI, gas emissions and ruminal pH were averaging by removing the controls from the dataset, and regardless the Leucaena inclusion levels.

4.6. Statistical analysis

All statistics analyses were performed using the software RStudio (version 3.5.1). The individual steer was the experimental unit for DMI, gas (CH_4 and H_2) emissions, liveweight gain and ruminal pH. The influence of the fixed factors (dose of Leucaena in the diet and cultivar fed) on gas emissions, DMI and ruminal pH was analyzed using a mixed model procedure through the lmer function of the lme4 package. A first analysis was carried out on the complete dataset excluding the controls to study the influence of the cultivar on DMI, gas emissions and ruminal pH. The model used was as follows: ~ dose + cultivar + dose*cultivar + (cultivar|animal) + (1|period). Since the cultivar fed had no influence and that no interaction was detected, this factor was not considered in the final model, taking into account the complete dataset and only the "dose" factor: ~ dose + (1|animal) + (1|period). To refine the analysis, multiple comparisons were conducted using the SNK.test function of the agricolae package. Then, the shape of relationship that linked the "dose" to the variables of interest were investigated with the lm function.

The effect of confining steers in the methane chambers on DMI (kg/d and g/kg BW) was assessed using a Student's t-test. The influence of "dose", "cultivar" and their interaction on nutrient ingested by the steers was studied using a two-way ANOVA. Then, multiple comparisons were carried out to identify the groups of means non significantly different. Finally, taking into account the complete dataset excluding the controls and regardless the levels of Leucaena in the diets, the influence of the cultivars was still investigated for productivity (yield in term of DM/m of row and animal productivity in term of daily LWG), DMI, gas emissions, ruminal pH and nutritional values, using a one-way ANOVA. Effects were declared significant at $P \leq 0.05$, P-values between 0.05 and 0.1 were reported and no significance (ns) was mentioned at $P > 0.1$.

Chapter 5. Results

5.1. Feed intake and chemical composition

Feed intakes measured throughout the entire trial (i.e. not only when animals were confined in the chambers) reported in Table 13 show that the proportion of Leucaena present in the diet had a significant influence on dry matter intake ($P<0.001$). Steers fed 36% of Leucaena had the highest intake, followed by animals fed 48%, then 18% and finally 0%. The DMI per kg of BW followed the same trend although there was no difference between 18 and 48% Leucaena inclusion. The cultivar had no effect on DMI and DMI per kg of BW ($P>0.1$). The confinement of the steers had an influence on intake. On average, it was reduced by 0.37 kg/d when animals were confined in the respiration chambers.

Table 13 | Dry matter intake (mean±sem) by steers throughout the 56 days of the trial by dose of Leucaena in the diet, cultivar and location of the animals

Factor		DMI[a] kg/d	DMI g/kg BW[b]
Dose of Leucaena in the diet (%)			
	0	5.18 ±0.86d	13.17 ±1.96c
	18	6.85 ±0.84c	16.41 ±1.91b
	36	7.92 ±0.87a	17.49 ±1.50a
	48	7.44 ±0.95b	16.75 ±1.83b
	P	***	***
Cultivar			
	Redlands	7.42 ±0.99	16.93 ±1.85
	Wondergraze	7.39 ±0.99	16.84 ±1.78
	P	ns	ns
Location			
	Pens	6.90 ±1.50	16.09 ±2.73
	Chambers	6.53 ±1.33	15.18 ±2.38
	P	**	***

[a] DMI, dry matter intake. [b] BW, body weight.

The average chemical composition of RGH and both Leucaena cultivars displayed in Table 14 indicate that forage quality was consistent over time as evidenced by the low standard error of the mean. The two cultivars, Redlands and Wondergraze, did not differ in terms of DM, OM, DMD, NDF and ADF while Redlands had ($P<0.05$) higher nitrogen, CP and hemicellulose contents.

Table 14 | Chemical composition (mean±sem) of Rhodes grass (*Chloris gayana*) hay and both cultivars of Leucaena (Redlands and Wondergraze)

	Chloris gayana [a]	*Leucaena leucocephala*		
		Redlands	Wondegraze	*P*
N (number of samples)	12	18	18	-
DM[b] (%)	86.48± 0.01	35.99± 0.03	34.72± 0.03	ns
Ash (% DM)	7.00± 0.29	7.50± 0.51	7.32± 0.73	ns
OM[c] (% DM)	93.00± 0.29	92.50± 0.51	92.68± 0.73	ns
Nitrogen (% DM)	0.82 ±0.06	2.46 ±0.29	2.27 ±0.24	*
CP[d] (% DM)	5.12 ±0.39	15.36 ±1.84	14.17 ±1.52	*
DMD[e] (%)	41.61 ±1.47	61.34 ±4.42	61.88 ±3.14	ns
NDF[f] (% DM)	73.09 ±1.18	46.14 ±2.07	45.05 ±2.00	ns
ADF[g] (% DM)	47.59 ±1.56	32.50 ±3.33	33.83 ±2.85	ns
Hemicellulose (% DM)	25.50 ±1.45	13.64 ±3.03	11.22 ±2.40	*

[a] Rhodes grass (*Chloris gayana*) hay not included in the statistical analysis. [b] DM, dry matter. [c] OM, organic matter. [d] CP, crude protein. [e] DMD, dry-matter digestibility. [f] NDF, neutral detergent fiber. [g] ADF, acid detergent fiber.

Table 15 | Nutrient intake per day when steers were confined in the chambers according to Leucaena inclusion level in the diet (basal diet composed of Rhodes grass hay) and cultivar fed

Treatment	DMI[a] (kg/d)	OM[b] (kg/d)	Ash (g/d)	N[c] (g/d)	CP[d] (g/d)	NDF[e] (kg/d)	ADF[f] (kg/d)	Hemi.[g] (kg/d)
Dose (%)								
0	4.79c	4.45c	341c	41c	257c	3.48c	2.25c	1.23c
18	6.20b	5.75b	448b	76b	474b	4.13b	2.70b	1.43b
36	7.86a	7.29a	570a	115a	721a	4.87a	3.26a	1.61a
48	7.31a	6.78a	535a	119a	745a	4.33b	2.94b	1.39bc
Cultivar								
Redlands	7.11	6.59	523	106	660	4.46	2.96	1.50
Wondergraze	7.14	6.63	513	101	634	4.43	2.98	1.45
SEM	0.18	0.17	13.38	4.28	26.77	0.10	0.07	0.03
P								
Dose	***	***	***	***	***	***	***	**
Cultivar	ns	ns	ns	ns	ns	ns	ns	0.07
Dose×Cultivar	ns	ns	ns	ns	ns	ns	ns	ns

[a] DMI, dry-metter intake. [b] N, nitrogen. CP[d], crude protein. NDF[e], neutral detergent fiber. ADF[f], acid detergent fiber. Hemi.[g], hemicellulose.

As shown in Table 15, the inclusion of Leucaena in the diet had a significant influence on nutrients ingested. Steers fed with 36 and 48% of Leucaena ingested the largest amount of OM and CP, followed by those fed with 18%, control steers exhibited the lowest intake of OM and CP.

Regarding fiber fractions (NDF, ADF, hemicellulose), their intake was highest for animals at 36% Leucaena, intermediate for those fed 18 and 48% Leucaena and least for control animals. Consistently with the other intake parameters and chemical composition, the cultivar had no impact on nutrient intake (P>0.05).

5.2. Gas emissions, ruminal pH and liveweight gain

The proportion of Leucaena in the diet had a significant influence on liveweight gain (Table 16): steers fed the Leucaena-free diet lost weight while animals fed Leucaena gained weight. However, no difference was found between the three levels of Leucaena inclusion in the diet. Methane emissions were significantly influenced by the dose of Leucaena. Expressed in g/kg of DMI, the lowest methane emissions were measured in steers at 48 and 36% Leucaena. Methane emissions were intermediary for steers fed 18% Leucaena and highest for control animals. Dihydrogen was significantly influenced by the dose of Leucaena when expressed in g/d. However, no dose effect was detected when expressed in g/kg DMI or deviation from baseline, i.e. compared with H_2 emission by the same animals fed with 100% Rhodes grass (*Chloris Gayana*) hay. Ruminal pH was influenced by the dose (P<0.05), being higher when Leucaena was present in the diet.

Table 16 | Liveweight gain, gas emissions and ruminal pH according to the level of Leucaena in the diet

	Leucaena inclusion in the diet[a] (%)				SEM[f]	*P*
	0	18	36	48		
LW[b] gain (kg/d)	-0.49b	0.14a	0.46a	0.42a	0.103	***
DMI[c]	4.79c	6.20b	7.86a	7.31a	0.18	***
Methane						
g/day	94c	111b	126a	114b	1.94	**
g/kg DMI	19.95a	18.14b	16.15c	15.84c	0.28	***
% dev[d]	+2.4a	-15.0b	-19.7b	-21.6b	0.02	***
g/kg OM[e]	21.48a	19.55b	17.42c	17.09c	0.30	***
Dihydrogen						
g/day	0.24b	0.19b	0.33ab	0.46a	0.03	***
g/kg DMI	0.0498	0.0313	0.0420	0.0640	0.0042	NS
% dev	+25.6	-81.51	+5.4	-53.7	20	NS
Ruminal pH	6.69b	6.97a	6.96a	6.86a	0.03	*

[a] The basal diet is composed of Rhodes grass (*Chloris Gayana*) hay. [b] LW, liveweight. [c] DMI, dry matter intake. [d] deviation from baseline (the baseline is the gas emission by the same animals fed with 100% Rhodes grass (*Chloris Gayana*) hay). [e] OM, organic matter. [f] SEM, standard error of mean.

Table 17 | Results of single regression analysis between the level of Leucaena in the diet (in %) with dry matter intake (kg/d) and methane emissions

Predicted variables	Linear relationship						Quadratic relationship							
	Intercept		β (dose)		r.s.d.[b]	R^2	Intercept		β_1 (dose^2)		β_2 (dose)		r.s.d.	R^2
	Estimate (SE[a])	*p*	Estimate (SE)	*p*			Estimate (SE)	*p*	Estimate (SE)	*p*	Estimate (SE)	*p*		
DMI (kg/d)	5.03 (0.2)	<.001	0.059 (0.007)	<.001	0.95	0.57	4.69 (0.22)	<.001	-0.0015 (0.0004)	<.001	0.129 (0.022)	<.001	0.88	0.63
Methane														
g/kg DMI[c]	19.81 (0.33)	<.001	-0.090 (0.01)	<.001	1.53	0.54	20.02 (0.38)	<.001	0.0009 (0.0007)	ns	-0.13 (0.04)	0.0011	1.53	0.55
g/kg OM[d]	21.33	<.001	-0.096 (0.01)	<.001	1.65	0.54	21.56 (0.41)	<.001	0.0009 (0.0008)	ns	-0.14 (0.04)	0.012	1.65	0.54
Dev[e] %	-0.011 (0.02)	ns	-0.0048 (0.0006)	<.001	0.09	0.51	0.002 (0.002)	ns	1.35e-04 (4.18e-05)	0.002	-1.13e-02 (2.07e-03)	<.001	0.08	0.58

[a] SE, Standard error. [b] r.s.d., residual standard deviation. [c] DMI, dry matter intake. [d] OM, organic matter. [e] deviation from baseline (the baseline is the gas emission by the same animals fed with 100% Rhodes grass (*Chloris Gayana*) hay).

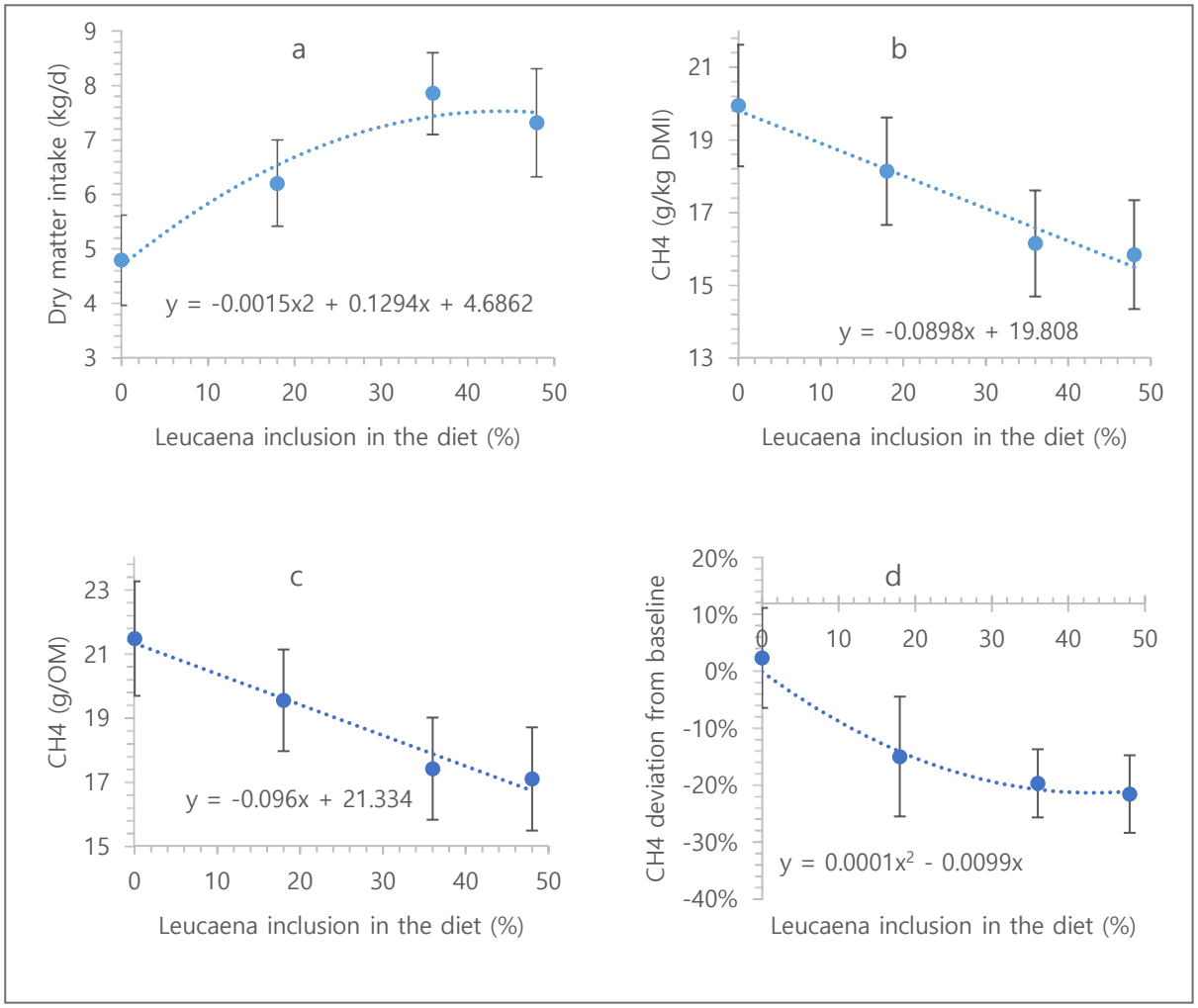

Figure 17 | Changes in dry matter intake (a), methane (CH_4) emission relative to dry matter intake (b), methane emissions relative to organic matter ingested (c) and methane deviation from baseline (c) according the dose of Leucaena present in the diet up to a maximum of 48%. The baseline is the gas emission by the same animals fed with 100% of Rhodes grass (*Chloris Gayana*) hay. For graph d, line was constrained to pass through the origin as the intercept was not significant (see Table 17).

Table 17 presents the results for the regression between methane emissions (g/kg DMI, g/kg OM and deviation from baseline) and DMI (kg/d) according the predictive variable dose of Leucaena. The relationship between DMI and the dose of Leucaena in the diet was identified as quadratic (Figure 17a). A significant regression equation was found (p<.001), with an R^2 of 0.634. The relationship between methane in g/kg DMI and the dose of Leucaena in the diet was identified as linear (Figure 17b). A significant regression equation was found (p<.001), with an R^2 of 0.57. The relationship between methane deviation from baseline and the dose of Leucaena in the diet was identified as quadratic (Figure 17d). A significant regression equation was found (p<.001), with an R^2 of 0.58.

5.3. Comparison of Redlands and Wondergraze

Table 18 | Comparison of Redlands and Wondergraze in term of productivity, dry matter intake, gas emissions, ruminal pH. Means were calculated from the complete dataset excluding the controls (0%) for both cultivars. They therefore include the three levels of Leucaena inclusion (18, 36 and 48%)

	Redlands	Wondergraze	SEM	*P*
Productivity				
Crop (kg DM/m of row[a])	0.51	0.43	0.037	ns
Crop (t DM/ha)	0.46	0.39	0.033	ns
LW gain[b] (kg/d)	0.30	0.30	0.057	ns
DMI (kg/d)	7.11	7.14	0.156	ns
Methane emissions				
g/d	115	119	1.640	ns
g/kg DMI	16.41	17.01	0.257	ns
% dev	-20.01	-17.41	0.012	ns
g/kg OM	17.72	18.32	0.276	ns
Dihydrogen emissions				
g/d	0.267	0.387	0.034	0.08
g/kg DMI	0.0377	0.0537	0.005	0.08
% dev	-42.17	-44.34	0.242	ns
Ruminal pH	6.95	6.91	0.029	ns

[a] One hectare of Leucaena pasture with rows spaced 12 meters has 883 linear meters of rows

[b] Liveweight gain calculated by regression

As shown in Table 18, no significant differences ($P > 0.05$) have been identified between Redlands and Wondergraze in term of productivity, intake, CH_4 and H_2 emissions and ruminal pH.

Chapter 6. Discussion

6.1. Forage quality

The Rhodes grass (*Chloris gayana*) hay used was representative to those commonly grazed across northern beef systems. According to Perry et al. (2016), who suggest values to characterize a RGH of low or high quality, the hay used in the trial may be considered of low quality (see Table 14).

The crude protein content of Leucaena (~15%) is lower than those found in the literature: Mlay et al. (2006) reported ~24% and Shelton and Dalzell (2007) 20% of CP. This difference could be explained by the harvesting method of the plant material: in this trial, the collected parts of the shrub included leaves, green pods and stems smaller than 0.5 cm in diameter. Whereas the values reported by these authors correspond to leaves alone. The inclusion of stems had diluted the nutritional values of leaves and leads to a higher fiber content. However, this harvesting method appears to be more representative of the forage typically consumed by grazing animals.

6.2. Feed intake

The inclusion of Leucaena in the diet clearly stimulated the ingestion of dry matter according a quadratic relationship (Table 17; Figure 17a). This can be explained by the lower fiber content and the higher digestibility (DMD) of diets containing Leucaena which involve an accelerated rate of passage of forages through the rumen: the forages stay in the rumen for less time, leaving space for a new meal more quickly.

Another possible explanation could be the higher crude protein content of diets with Leucaena, which stimulates the microbial growth and accelerates the digestion. However, the tannins present in Leucaena should bind with proteins and protect them from ruminal fermentation. Due to the formation of the tannin-proteins complex, the ruminal bacteria possibly do not benefit from this additional supply of nitrogen compounds provided by Leucaena.

The increased forage intake of diets with Leucaena can also be explained by the high palatability of the legume. However, it is known that the tannins may reduce intake of legume by decreasing palatability or negatively affecting digestion. Nevertheless, it appears that Leucaena remains a

highly palatable forage despite the presence of tannins. Indeed, Faint et al. (1998) did not find any relationship between palatability and condensed tannins content when cattle grazed Leucaena pastures.

6.3. Methane measurement

Methane emissions were measured in individual open circuit chambers which are considered as a reference method due its accuracy and repeatability (Llonch et al., 2016; Olijhoek et al., 2017). But this method still has an inconvenient feature. It involves the confinement of the animal in a restrained space without direct contact with their herdmates, which can generate stress and alter feeding behavior and intake. Llonch et al. (2016) assessed the impact of confinement within chambers on feed intake and methane emissions and studied the relationship between stress and changes in feed intake during confinement. They found a 14.9% reduction in forage intake (when considering DMI/kg BW) when animals were confined in the chambers compared to when they were housed into individual pens. The DMI reduction was higher in more stressed animals. The authors concluded that the stress associated to the confinement reduces the DMI resulting in an increased methane emissions (g CH_4/kg DMI) in fibrous diets. In this trial, a 5.7% reduction in DMI (g/kg BW) was measured during the confinement in the chambers. The animals were probably less stressed than those used by Llonch et al. (2016) due to the following reasons: (1) a familiarization period of the cattle with the handling procedures has been carried out and (2) the majority of the steers were involved in a previous study also using the chambers. The stress exposure was minimized resulting to reliable measurements.

6.4. Methane abatement potential of Leucaena

Methane yield per kg DMI from the hay was close to values described in the literature: steers fed with the control diet (Rhodes grass hay only) produced 19.95 g CH_4/kg DMI while Kennedy and Charmley (2012) reported 19.6 g CH_4/kg DMI and Charmley et al. (2016) 20.7 g CH_4/kg DMI. The inclusion of Leucaena in the diet strongly reduced methane production by steers. There was a single quadratic relationship (Figure 17d) between the deviation in methane production from the baseline (%) and the Leucaena inclusion in the diet (considering a range from 0 to 48% of Leucaena in the diet). Steers fed 48% of Leucaena showed a drop of 21.6% in methane yield, which confirm the findings of Kennedy and Charmley (2012). In their limited indoor study (measurements on 3 steers), they reported 18% methane abatement when Leucaena was fed at 44% of the diet.

A variety of mechanisms can be responsible for this methane abatement but are difficult to clearly identify on the basis of available observations. Knowing that diets with Leucaena contain less fiber, more soluble sugars and crude proteins (including a portion of soluble proteins) and that the particles size is reduced, it would be justified to expect a shift of the ruminal fermentation towards propionate formation. This modification, coupled with a decrease in ruminal pH would explain a part of the methane abatement. However, the ruminal pH was slightly higher with Leucaena, which refute this hypothesis.

Tannins present in the tropical legume could explain its antimethanogenic properties. Several authors reported a linear relationship between condensed tannins and CH_4 reduction *in vitro* (Rira et al., 2015; Vandermeulen et al., 2018) and *in vivo* (Archimède et al., 2016). Tannins lower methane production by directly inhibiting methanogens and/or indirectly by reducing both feed degradation in the rumen and feed intake. The direct inhibition of methanogens seems to be applicable in this trial (given that the DMI is not reduced) but investigation[1] on *Archea* population in the rumen are needed to understand the mode of action of tannins from Leucaena.

Mimosine could also explain partially the methane abatement. Indeed, Soltan et al. (2013) identified this alkaloid as an another antimethanogenic compound in Leucaena. They reported that mimosine seems to stimulate acetogenesis as an alternative H_2 sink, that would lead to reduced methanogenesis. This mode of action is difficult to confirm with the data from this trial as H_2 production increased with the level of Leucaena when the control diet is omitted (Table 16).

6.5. Animal productivity

Steers fed with Leucaena ingested higher amounts of forage with superior nutritional value (Table 15). As a result, animal productivity increased when diets contained Leucaena up to 36% of inclusion (Table 16). Bowen et al. (2010) reported that Leucaena-grass pasture can achieve a daily gain of 0.9 kg of LW/head. In this trial, the highest daily gain was found with 36% of Leucaena and reached 0.46 kg/head/day. This lower productivity is attributable to the poor CP content of RGH (only 5.12%). Shelton and Dalzell (2007) stated that 12-13% of CP in the diet is required to achieve a target of 300 kg LWG/hd/yr. However, this protein supply was not reached in this trial: the CP content of ingested diets was 5.3, 7.6, 9.1 and 10.3% respectively for 0, 18, 36 and 48% of Leucaena inclusion (values calculated from Table 15).

[1] Rumen samples were collected during the trial for genetic studies of microbial diversity and microbiome analysis, but the results were not yet available to include them into this work.

Another explanation for this lower than expected productivity could be mimosine toxicity, or more precisely DHP intoxication which is a byproduct of its degradation. This hypothesis is based on the slight decrease in daily intake and LWG from 36 to 48% of Leucaena. This seems to be in line with the statement of Dalzell et al. (2012) who reported that clinical Leucaena toxicity symptoms can occur in cattle when the legume comprises over 30% of dietary DM intake. However, precautions have been taken by inoculating steers with *Synergistes jonesii* culture, which was intended to detoxify mimosine and its byproducts. But it is possible that bacterial strains may not be fully adapted to the new Leucaena cultivars (Redlands and Wondergraze) used in the trial. The effectiveness of these inocula should be assessed by quantifying[2] mimosine metabolites in urine and rumen fluid. These analysis will make it possible to accept or reject the hypothesis of toxicity, which would explain why productivity did not increase beyond 36% of Leucaena inclusion.

6.6. Forage biomass

The edible biomass of Leucaena forage was 0.45 and 0.38 t DM/ha for Redlands and Wondergraze. This was apparently the first time the yield in edible Leucaena had been measured at the scale of an entire paddock (20 ha). Shelton et al. (1998) previously reported 3 to 30 t DM/ha/yr but it seems to correspond to the total biomass (wood included), and not only to the edible part of the legume. Bowen et al. (2016) assessed the yield in edible parts by harvesting 8 m of rows according to a similar procedure to that used in this trial and they found 0.4 t DM/ha. Despite the short distance harvested, they obtained an estimate very close to the yield measured in this trial. It seems important to remember here that the spacing between the rows was 12m, and that the paddock also produced approximately 4 t/ha tropical grass hay.

6.7. Comparison of Wondergraze and Redlands

In term of productivity, gas emissions and ruminal pH, these two modern cultivars were very similar. Redlands's primary benefit is its very high tolerance to psyllids. However, no psyllid incidence was detected in either cultivar, so this feature could not be proven during this trial. Although no differences were observed in term of yield (t DM/ha) between the two cultivars, based on the feedback of the staff who harvested Leucaena, it was observed that the ratio edible forage/wood was higher for Redlands. Indeed, Wondergraze seems to produce more wood for

[2] Rumen fluid and urine were collected during the trial for measurement of mimosine metabolites, but the results were not yet available to include them into this work.

the same amount of forage, resulting in taller shrub and less easily accessible for cattle. Furthermore, Wondergraze produced more pods, and consequently more seeds than Redlands.

6.8. Extrapolation to grazing systems

Four diets were offered to steers: they contained 0, 18, 36 or 48% of Leucaena (the basal diet being composed of RGH). These levels were chosen to study the relationship between the inclusion of the tropical legume and CH_4 emissions over a relatively wide range. The expectation was to observe a quadratic relationship showing a plateau in response to Leucaena inclusion level. This kind of response was effectively reported for CH_4 emissions (expressed in deviation from baseline; Figure 17d) and DMI (Figure 17a). It can therefore be assumed that increasing the dose of Leucaena beyond 48% in the diet will not induce any further change in the DMI or methane emissions.

In the paddock used in this trial, the yield of RGH was estimated at 4.25 t DM/ha while the yield in edible Leucaena was 0.45 and 0.38 t DM/ha for Redlands and Wondergraze. Therefore, the proportion of edible Leucaena was 9.6% and 8.1% for Redlands and Wondergraze of total crop biomass. Bowen et al. (2016) also calculated this proportion and found 10.5% of edible Leucaena forage in the paddock. It is then justified to wonder if the proportions chosen in this experiment (up to 48% of DMI) are relevant when we compare what a Leucaena-grass pasture can provide (~10% of legume).

But, knowing that Leucaena is much more palatable than tropical grasses and that it is never soiled by faeces and urine, we can consider that that all of edible legume forage will be consumed while part of grass pasture will be wasted. It should be noted that utilization rates (% of total standing biomass at the end of the wet season removed by the start of the following season) should not exceed 30% (Ash et al., 2011). As a result, we can reasonably assume that Leucaena will represent a proportion of the diet ingested close to or even higher than 18%. This assumption is confirmed by Bowen et al. (2016) who calculated the proportion of C_3 (legume) forage biomass ingested by animals from analysis of faeces. They estimated the proportion of legume at 51% in the diet while the Leucaena-grass pasture contained only 10% on Leucaena. Graham et al. (2013) also reported proportion of Leucaena in the diet from 14 to 74% in eight different property in southern Queensland, which confirm that biomass available in the pasture do not correspond to biomass ingested.

Chapter 7. Conclusions and the future

In tropical regions, feeding Leucaena to beef cattle is a promising practice that increases productivity and reduces methane emissions. The potential of this legume is massive and greatly supported by the release of new cultivars being resistant to pest and disease but also being higher yielding (e.g. Wondergraze and Redlands). However, a better knowledge of these cultivars relating to methane abatement potential and animal productivity were needed to accelerate their commercial uptake and the expansion of this valuable crop. The objective of this study was to fill in these knowledge gaps and the main findings were as follows:

I. Leucaena inclusion in the diet significantly reduced methane emissions; for the three levels of inclusion investigated (18, 36, 48% of Leucaena in the diet), methane emissions were reduced by 15.0, 19.7 and 21.6% respectively, according a to quadratic relationship;
II. Leucaena inclusion in the diet significantly enhanced animal productivity; the highest daily liveweight gain was 0.46 kg which is, however, below expectations; the poor quality of the basal diet (Rhodes grass hay) is the cause of this low weight gain but also perhaps mimosine toxicity;
III. the new cultivars Redlands and Wondergraze have shown similar results in terms of methane abatement and animal performance; however, Redlands had a higher ratio leaf/wood and was a less prolific seeder;
IV. the yield in edible parts of Leucaena from the paddock sown for 3 years was 0.45 and 0.38 t DM/ha for Redlands and Wondergraze; this production was equivalent to ~10% of the edible biomass available in the paddock.

This study has provided a better knowledge of the new cultivars and will contribute to the expansion of Leucaena-pastures in northern Australia, allowing the beef industry to reduce its carbon footprint while improving its productivity. However, some questions remain outstanding and can be addressed in the future. The effectiveness of the *Synergistes jonesii* inoculum to detoxify the mimosine of the new cultivars should be assessed to ensure they do not present any risk of toxicity. Another interesting research would be to investigate the mode of action and causative agents (e.g. tannins, mimosine) of Leucaena. A better understanding of these mechanisms would make it possible to select cultivars for high antimethanogenic properties in addition to desirable nutritive characteristics.

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Appendix

Appendix 1: Composition of the mineral vitamin supplement provided throughout the trial

TRACE ELEMENT

With Copper & Cobalt

OLSSON'S

20, 40 & 100kg

A supplement used for ruminants to correct deficiencies in trace elements.

TYPICAL ANALYSIS

Molasses	5%
Salt (NaCl)	Max. 45%
MACRO INGREDIENTS	
Calcium (Ca)	14.5%
Phosphorus (P)	0.6%
Sulphur (S)	0.8%
Magnesium (Mg)	0.02%
MICRO INGREDIENTS	
Copper (Cu)	1000mg/kg
Cobalt (Co)	65mg/kg
Ferrous Iron (Fe++)	1350mg/kg
Zinc (Zn)	300mg/kg
Iodine (I)	1500mg/kg
Selenium (Se)	26mg/kg
Iron (Fe)	650mg/kg

FOR ANIMAL TREATMENT ONLY.

THIS PRODUCT DOES NOT CONTAIN RESTRICTED ANIMAL MATERIAL.

Olsson's Trace Element is a macro mineral supplement designed for a broad range of mineral deficiencies.

The effect of balancing these essential elements is the efficient breakdown of feed consumed by the animal. A deficiency in trace elements will have a varied effect on an animal. Deficiencies in copper, iron, zinc, manganese, cobalt, iodine, & selenium can reduce the animal's growth and reproduction rates, and result in less efficient feed conversion and depressed immunity. Balancing these elements will improve general production of the herd.

Trace Element with Copper & Cobalt can be used year round and fed on an ad lib basis.

DIRECTIONS FOR USE

Feeding Instructions:
Use in accordance with the Olsson Indicator system.

Sheep/Goats: 5-10g per head per day

Cattle: 50-100g per head per day

Place near water troughs, dams and stock camps. Place out sufficient blocks to avoid overcrowding of stock. Replace blocks immediately when consumed.

Intake is recommendation only. Higher intakes are normally the result of mineral deficiencies.

Avoid contact with skin and eyes.

Storage Instructions:
Store out of direct sunlight and under cover. Edible carton and packaging.

OLSSON'S HEAD OFFICE SYDNEY 19-25 Nelson Road, Yennora NSW 2161 • **HOTLINE** 1800 804 096 • **www.olssons.com.au**

Appendix 2: Soil analysis of the Leucaena paddock

	Mean of 10 analysis
pH (1:5 Water)	5.90
pH (1:5 CaCl2)	4.64
Electrical Conductivity	0.03
EC Saturation Index	0.19
Chloride	16.00
Nitrate Nitrogen (ppm)	2.01
Ammonium Nitrogen (ppm)	1.79
Phosphorus - Colwell (ppm)	7.17
Phosphorus Buffer Index - Colwell (ppm)	65.40
Calcium (Amm. Acet.) (ppm)	2.90
Potassium (Amm. Acet.) (ppm)	0.17
Magnesium (Amm. Acet.) (ppm)	1.69
Sodium (Amm. Acet.) (ppm)	0.16
Calcium/Magnesium Ratio (Amm. Acet.) (ppm)	1.71
Aluminium (KCl) (ppm)	0.23
Cation Exchange Capacity - incl. Al (Amm. Acet.) (ppm)	5.07
Sodium % of Cations - incl. Al (Amm. Acet.) (ppm)	3.38
Aluminium (KCl) % of Cations	6.28
Copper (DTPA) (mg/kg) (ppm)	1.08
Iron (DTPA) (ppm)	98.20
Manganese (DTPA) (ppm)	32.90
Zinc (DTPA) (ppm)	0.89
Boron (ppm)	0.37
Sulphur (MCP) (ppm)	7.17
Organic Carbon %	0.63
Phosphorus - BSES (ppm)	9.16
Organic Matter %	1.09
Available Potassium (Amm. Acet.) (ppm)	67.50

Appendix 3: Photos of the experiment

CSIRO's Lansdown Research Station

Individual covered pens

Droughtmaster (*Bos Taurus*) steers in its individual pens

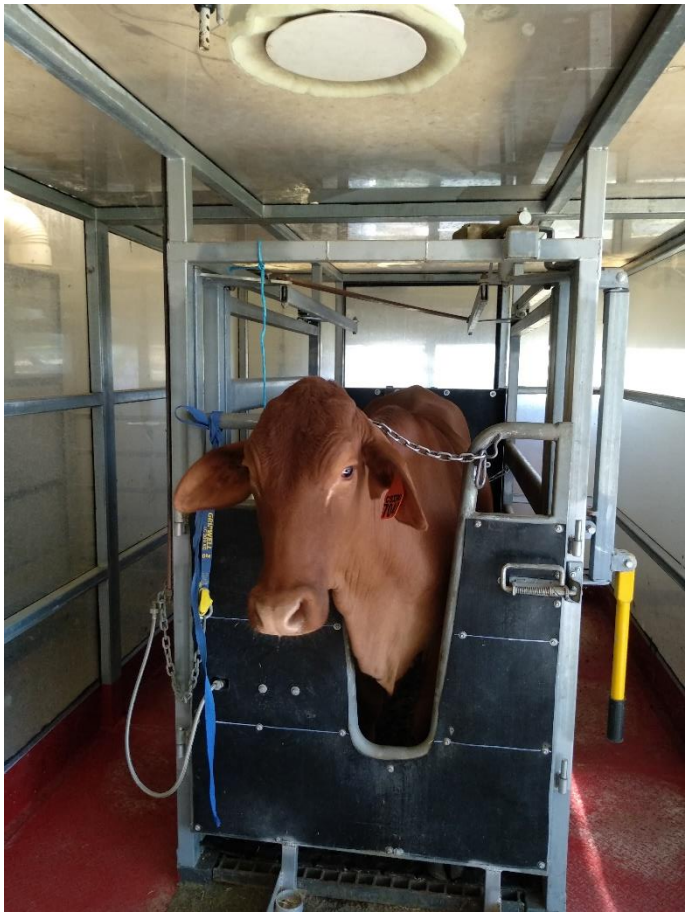
Leucaena rows just before start of experiment (Photo: Ed Charmley)

Leucaena stems being collected from the paddock (Photo: Ed Charmley)

Stripping edible material from Leucaena stems (Photo: Ed Charmley)

Garden chopper used to chop up the stripped edible material (Photo: Ed Charmley)

Leucaena before (on left) and after (on right) chopping (Photos: Ed Charmley)



Steer housed in methane chamber waiting for its feed