

#### **CROP & PASTURE SCIENCE**



# Reducing enteric methane of ruminants in Australian grazing systems – a review of the role for temperate legumes and herbs

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### ABSTRACT

In Australia, 71% of agricultural greenhouse gas (GHG) emissions are enteric methane (CH<sub>4</sub>), mostly produced by grazing sheep and cattle. Temperate low CH<sub>4</sub> yielding legumes and herbs can mitigate enteric CH₄ production, but system-level GHG emissions need to be considered. The aims of the study were to: (1) devise a framework to assess GHG reductions when introducing low CH4 yielding species; (2) assess mechanisms of  $CH_4$  reduction in temperate legume and herb species for Australia; (3) use a case study to demonstrate expected changes to system-level GHG emissions with the introduction of low CH<sub>4</sub> yielding legumes; and (4) identify knowledge gaps and research priorities. Results demonstrate lowering emissions intensity (kg CO2-equivalent/kg product) is crucial to mitigate GHG emissions, but livestock productivity is also important. Several pasture species have anti-methanogenic properties, but responses often vary considerably. Of the species investigated biserrula (Biserrula pelecinus) has great potential to reduce enteric  $CH_4$  emissions, but in a case study its emission intensity was similar to subterranean clover (Trifolium subterraneum) but higher than lucerne (Medicago sativa). We conclude that there are temperate legumes and herbs with anti-methanogenic properties, and/or high productivity that could reduce total  $CH_4$ emissions and emissions intensity of ruminant livestock production. There is also great diversity in some plant genotypes that can be exploited, and this will be aided by more detailed understanding of plant secondary compounds associated with CH<sub>4</sub> reduction. This review suggests an opportunity to formulate pasture species mixtures to achieve reduced CH₄ emissions with greater or equal livestock production.

**Keywords:** bioactive plants, grazing systems, greenhouse gas reduction, herbs, legumes, livestock production, methane emissions, temperate pastures.

# Introduction

Australia is a signatory to the Paris Agreement, which aims to limit global warming to no more than 2°C above pre-industrial levels. To meet this goal, there is an imperative to rapidly reduce global greenhouse gas (GHG) emissions. While reductions in GHG emissions are needed from most sectors, limiting global warming to below 2°C cannot be achieved without reducing emissions from the agricultural sector (Reisinger *et al.* 2021). In Australia, agriculture produces about 76.1 Mt carbon dioxide equivalents (CO<sub>2</sub>-e) annually, which is about 15% of the national emissions (Commonwealth of Australia 2022). Approximately 71% of agricultural emissions are methane (CH<sub>4</sub>) emitted from livestock, with enteric CH<sub>4</sub> emissions being the dominant source, equating to 48.2 Mt of CO<sub>2</sub>-e emitted annually (DISER 2021). While there are other emissions sources in agricultural systems [e.g. nitrous oxide (N<sub>2</sub>O) from soil], these sources make significantly lower contributions to the total GHG emissions of the agriculture sector. When the targets for GHG emissions reductions set by most multinational supply chain companies are considered, in conjunction with the fact that around 70% of Australian agricultural product is exported, reducing GHG emissions from agriculture has also become an imperative to maintain market access. The relatively high proportion of GHG emissions that are attributed to livestock means significant research and adoption is needed to reduce GHG emissions from grazing animals to maintain market access.

There is potential to reduce enteric  $CH_4$  through use of several classes of feed additives, including oils, nitrates, phytochemicals, essential oils and methane inhibitors (Almeida *et al.* 2021). While macroalgae, such as red seaweed (*Asparagopsis taxiformis* and *Asparagopsis armata*), and 3-nitrooxypropanol (3-NOP) are considered to have the greatest potential (Black *et al.* 2021) there are also potential limitations. In grazing systems, effective methods for delivery of the required dose and economic feasibility are yet to be determined, while some animal welfare concerns have also been identified for macroalgae (Li *et al.* 2018). These technologies can be implemented in intensive feeding systems, but these barriers must be overcome before they can be used in extensive grazing systems.

In Australia, sheep and beef production is mostly based on grazing, with  $\sim 96\%$  of animals grazed on pasture (MLA 2022). Any feed additives with the potential to reduce enteric CH<sub>4</sub> emissions must be provided to grazing livestock to have an industry-wide impact. Of the 416 million ha of Australian land that is grazed by sheep and cattle, approximately 71 million ha is improved pasture (DAWE 2016), including 23 million ha of sown pasture in the temperate pasture zone (Badgery et al. 2015). While it is difficult to determine the exact numbers of livestock that are supported by sown pastures in the temperate region, there are ~9.4 million cattle or 40% of the herd and 58.4 million sheep or 92% of the flock grazed in this region (MLA 2022), supporting a significant proportion of Australia's red meat and wool production. New solutions to mitigate enteric CH<sub>4</sub> emissions must target these extensive grazing systems.

Pasture species, including a large number of legumes, grasses and herbs, can produce bioactive compounds that reduce enteric CH<sub>4</sub> emissions (Beauchemin et al. 2008; Eckard et al. 2010; Banik et al. 2013a). The main mechanism by which these forages reduce enteric CH<sub>4</sub> is through their expression of specific plant secondary compounds (PSC), however they can also reduce enteric CH<sub>4</sub> emissions intensity, the amount of emissions per unit of product (i.e. meat, milk, wool), by improving the overall nutritional value of pastures. The key anti-methanogenic bioactive compounds in these forages include fats and oils (Moate et al. 2016), phenolic compounds like condensed tannins (CT; Grainger et al. 2009), saponins (Eckard et al. 2010) as well as nitrates and sulfates (Beauchemin et al. 2020). The temperate grazing systems of Australia generally have mixed pastures, containing legumes and grass species, and new options will have to be adapted to these systems.

The anti-methanogenic properties of pasture species have been largely identified through *in vitro* fermentation and pen feeding studies. It is a significant step from these laboratory and controlled environment studies to validating the efficacy of anti-methanogenic pasture species in delivering abatement of GHGs at the farm level. The quality and productivity characteristics of the forages, together with animal interactions and selective grazing, all impact dry matter intake (DMI) of animals thereby affecting enteric CH<sub>4</sub> emissions (Charmley et al. 2016), animal production and ultimately GHG emissions. Furthermore, the agronomic suitability and persistence in a mixed pasture need to be compared to currently recommended species at a regional level. Any changes in plant characteristics, productivity and persistence can flow through to changes to soil properties and influence N<sub>2</sub>O emissions and soil carbon (C) levels. To deliver GHG abatement, animal productivity should be maintained while net emissions are reduced. Farm system-level assessment is needed to determine GHG mitigation potential from introducing these species.

This review: (1) presents a framework to assess legumes and herbs with anti-methanogenic properties, for effectiveness in reducing GHG emissions at a system level when introduced into temperate pasture systems in southern Australia; (2) uses existing literature to short-list potential legume and herb species for temperate pasture systems; (3) provides a case study to demonstrate expected changes to system level emissions via replacement of subterranean clover (*Trifolium subterraneum*) with the introduction of biserrula (*Biserrula pelecinus*), a low methane legume, or lucerne (*Medicago sativa*), a perennial legume with high productivity; and (4) identifies knowledge gaps and research priorities.

# Framework for assessing GHG reduction potential of pasture species

Many forage species have been identified as having antimethanogenic properties, but not all of these will result in reduced GHG emissions for a livestock system. There are a number of other lenses that a species must be assessed through, to ensure GHG abatement occurs. A framework to determine GHG emission reduction potential of pasture species should include: (1) broad screening of pasture species and cultivars, generally screening for plant secondary compounds (PSC) to rank their relative enteric CH<sub>4</sub> reduction potential (once in vivo results or validated in vitro fermentation methods have been used to identify associations with  $CH_4$  reduction); (2) an assessment of their agronomic suitability and productivity for a target region and grazing system; (3) validation of animal production and enteric CH<sub>4</sub> reduction under grazing; and (4) an assessment of changes in all GHGs, and the net GHG balance, at a systems level (Fig. 1). This framework would operate as a funnel with broad, rapid and low-cost screening at the top (assuming the associations between PSC and CH<sub>4</sub> reduction are already



**Fig. 1.** Framework to assess anti-methanogenic potential of pasture species. Generally, the evaluation of species at the top of the funnel has high species throughput, shorter duration and lower cost. Further down the funnel the number of species decreases, the duration can be longer, the cost of evaluation higher and the complexity increases.

established), through to more detailed, time consuming and costly *in vivo* assessments on fewer, more promising species and cultivars at the bottom. A goal is to shorten the time for species to pass through this funnel. Additional opportunities exist to develop even more rapid screening technologies to detect specific PSC associated with  $CH_4$ reduction in pastures once these relationships are established. Potential technologies could include near-infrared spectroscopy, hyperspectral imaging or rapid colorimetric assay approaches. Many species have been proven to have potential for GHG mitigation in temperate grazing systems of Australia at a number of these levels as detailed in following sections.

# Description of compounds and mechanisms of methane suppression

Plant secondary compounds or secondary metabolites of pastures, such as tannins, saponins, and other polyphenolic compounds, have potential to reduce  $CH_4$  emissions in ruminant livestock (Waghorn 2008; Ku-Vera *et al.* 2020; Waters *et al.* 2020; Tava *et al.* 2022). The role of the major PSC are described below.

# Tannins

Tannins are a subset of compounds belonging to the polyphenolic compound group, which is ubiquitous within the plant kingdom. Polyphenolics are plant secondary metabolites that range from simple phenolics right through to high molecular weight polymeric compounds. Tannins are highly polymerised and hydroxylated polyphenol compounds (molecular weight ~500–3000) and they have been historically studied as anti-nutritional factors in relation to animal nutrition (Kumar and Singh 1984) and for bloat prevention (Lees *et al.* 1981; Min *et al.* 2005; Mueller-Harvey 2006). Tannins are a chemically diverse group that comprise two main classes; condensed tannins CT (having a flavan-3-ol base unit and synthesised via the phenylpropanoid pathway) and hydrolysable tannins (HT; characterised as having a gallic acid base unit and synthesised via the shikimate pathway) (Mora *et al.* 2022).

Condensed tannins, also known as polymeric proanthocyanidins, have received the most attention with regards to CH<sub>4</sub> mitigation. Reviews have shown that pastures containing CT can reduce CH<sub>4</sub> production (Aboagye and Beauchemin 2019; Min et al. 2020). However, because CT can form complexes with both proteins and fibre, this can reduce protein digestibility, ruminal fermentation and may reduce the absorption of nutrients, thereby decreasing livestock productivity. Reduced protein availability will lower urine N excretion, and any reduction in fermentation will decrease  $H_2$  production and therefore enteric  $CH_4$ (Min et al. 2020). The potential reduction in livestock productivity of a high CT diet will need to be balanced with the CH<sub>4</sub> reduction goal and other reported benefits of CT including reduced gut parasites, and improved milk production, wool growth, immune responses and reproduction (Waghorn 2008; Min et al. 2020). In addition, CT may affect meat and milk quality as they can inhibit the biohydrogenation of unsaturated fatty acids (Vasta et al. 2019).

Hydrolysable tannins have received little attention with respect to their potential for reducing enteric  $CH_4$ . Historically, HT are known for their potential toxicity to

livestock (McSweenev et al. 1988; Waghorn 2008). However, at low levels HT show some potential for reducing CH<sub>4</sub> emissions via protein-binding (Jayanegara et al. 2015) or directly interacting with rumen microbiota without affecting fibre digestion (Vasta et al. 2019). Jayanegara et al. (2010) found that HT have a more direct effect on either the protozoa (responsible for H<sub>2</sub> production) or the methanogens (responsible for CH<sub>4</sub> production), inhibiting their growth or activity, without reducing fibre fermentation. Similarly, HT may affect meat and milk quality as well but to a lesser degree than CT (Vasta et al. 2019). A greater understanding of the tannin content, particularly HT, and investigation into the range and composition of phenolic compounds in temperate pasture species will help identify opportunities for alternate species and cultivars that have greater CH<sub>4</sub> reduction potential.

#### **Saponins**

Saponins are another class of secondary metabolites synthesised by, and present within, some plant species. Saponins are a heterogeneous subclass of terpenoid compounds with a base structure of either triterpene, steroid, steroidal alkaloid or an acyclic C chain, often occurring as glycosides (Jayanegara *et al.* 2010). Dicotyledonous plant species commonly accumulate triterpenoid-type saponins, whereas plants like Asparagaceae generally synthesise steroidaltype saponins. Grasses often have no saponins, although oats (*Avena sativa*) are an exception, accumulating both steroidal and triterpenoid saponins (Moses *et al.* 2014). Historically, some saponins are known to be toxic, and some saponins can taste bitter thus deterring animal grazing, especially in mixed pastures (Lei *et al.* 2019).

Saponin-rich non-pasture plants, like tea (Camellia sinensis), yucca (Yucca schidigera) and Quillaja saponaria have been shown to decrease CH<sub>4</sub> emissions in vitro (Javanegara et al. 2014). The CH<sub>4</sub> reductions of lucerne and biserrula are thought to be due to their saponin content (Malik and Singhal 2009; Ghamkhar et al. 2018; Kozłowska et al. 2021). Saponins have been measured in all Medicago species studied, but the amount and type of saponin present vary within and between Medicago species (Tava and Pecetti 2012; Szumacher-Strabel et al. 2019) and affect their biological activity (Tava and Pecetti 2012; Kozłowska et al. 2021). Kozłowska et al. (2021) found that two of four saponins extracted from lucerne leaves reduced CH<sub>4</sub> production but the effectiveness of saponins depends on their source, type and concentration. Szumacher-Strabel et al. (2019) reported that total saponin concentration can increase 1.7-4.4 times in ensiled lucerne compared to fresh lucerne, with the magnitude cultivar dependent. Ghamkhar et al. (2018) found 47 metabolites associated with low CH<sub>4</sub> production in biserrula. A species closely related to the genus Biserrula is Astragalus, the largest species in the Fabaceae family, which contains saponins that potentially could lower  $CH_4$  (Ionkova *et al.* 2014). The perennial legume Sulla (*Hedysarum coronarium*) also contains saponins (Tava *et al.* 2022). Knowledge of saponins in pasture species is limited, but the literature suggests that further exploration of saponin content and types is warranted in the search for low  $CH_4$  pastures.

Methane suppression by saponin is thought to be related to inhibition of rumen ciliate protozoa responsible for production of H<sub>2</sub>, as a precursor for methanogens to convert into CH<sub>4</sub>. Thus, CH<sub>4</sub> production decreases as fermentation is shifted in favour of propionate production (Patra *et al.* 2017; Ku-Vera *et al.* 2020). However, despite decreasing protozoal numbers by 40–50%, Jayanegara *et al.* (2010) indicated a weak association between anti-protozoal activity of saponins and methanogenesis. Thus, any bioactive properties and enteric CH<sub>4</sub> mitigating effects will be influenced by the saponin source, the chemical structure and concentration present in feed (Jayanegara *et al.* 2014). Additional bioactive effects associated with saponins include anthelmintic properties *in vitro* (Maestrini *et al.* 2020) and protection against aphids (Goławska *et al.* 2012).

Saponins may contribute to stable foam in the rumen that can cause bloat. However, Majak *et al.* (1980) compared bloat incidence on high saponin and low saponin near-isogenic strains of lucerne and concluded that saponins did not contribute to the occurrence of bloat. Nevertheless, it would be advisable to exercise duty of care and investigate potential bloat risk for pasture species with increased levels of saponins.

### Other polyphenolic compounds

Other classes of phenolic compounds have been largely ignored in livestock research, but they also have the potential to contribute to CH<sub>4</sub> mitigation. The five main groups of non-tannin polyphenols are phenolic acids, flavonoids (e.g. anthocyanins, flavanols, flavanones, flavonols, flavonones, and isoflavones), xanthones, lignans and stilbenes. Banik et al. (2016) found three bioactive fractions from biserrula that reduced CH<sub>4</sub> production by more than 50%, with two of the fractions possibly containing flavonoid glycosides. Jayanegara et al. (2010) demonstrated the ability of phenolic acids (cinnamic, caffeic, p-coumaric and ferulic acids) to decrease CH<sub>4</sub> production significantly during in vitro rumen fermentation. In addition, the molecular structure of phenolic compounds appears to be important, with higher numbers of hydroxyl groups eliciting stronger methane inhibition (Javanegara et al. 2010). In addition, non-tannin phenolic compounds are less likely to decrease the availability of proteins and other nutrients necessary for livestock productivity and may confer additional bioactive effects associated with phenolic compound consumption in humans. For example, non-tannin phenolic compounds can provide antioxidant, antibacterial, antifungal, anti-inflammatory, anti-carcinogenic, anti-diabetic, antihypertensive, antihyperlipidaemic, hepatoprotective, antispasmodic, oestrogenic,

and neuroprotective benefits (Makkar 2003; Siah *et al.* 2012; Durazzo *et al.* 2019).

# Pasture species with CH<sub>4</sub> emission reduction potential

There have been several broad-based assessments of the potential of pasture species to reduce enteric  $CH_4$  production in Australia (e.g. Banik *et al.* 2013*a*) and internationally (e.g. Aboagye and Beauchemin 2019). These assessments have helped to identify species that have the greatest potential to reduce enteric  $CH_4$  in grazing livestock and identify the mechanisms for  $CH_4$  emission reduction. The most promising legume and herb species from a  $CH_4$  reduction and production perspective for temperate environments in Australia are listed in Table 1. The range of  $CH_4$  reduction has been listed based on published data from *in vitro*, pen and grazing studies. The PSC potentially associated with anti-methanogenic properties for each species are also listed; as is the productivity, persistence and general agronomic suitability.

# **Perennial legumes**

The inclusion of perennial legumes in mixed pastures can improve nutritional quality of feed, fix nitrogen, increase ground cover, and reduce variability in pasture production. The two major perennial legumes grown in Australia are lucerne and white clover (*Trifolium repens*) (Nichols *et al.* 2012), but these species are currently not recognised for having anti-methanogenic properties and new low  $CH_4$ cultivars would need to be developed.

#### Lotus

The *Lotus* species have great potential to reduce enteric  $CH_4$  emissions. Across numerous studies a 38% reduction in enteric  $CH_4$  emissions has been reported when compared to common pasture species like perennial ryegrass (*Lolium perenne*), although variability has been considerable (Table 1). Condensed tannins are believed to be the main PSC responsible for reducing  $CH_4$  in *Lotus*. By comparing lotus (*Lotus pedunculatus*) fed to young rams with and without polyethylene glycol to bind with and remove the effects of CT, Waghorn *et al.* (2002) found CT responsible for 16% of the reduction in  $CH_4$  production. This study reported that  $CH_4$  production from lotus (11.5 g/kg DMI) was much lower than that from perennial ryegrass–white clover pasture (25.7 g/kg DMI) indicating other mechanisms are also involved.

Three *Lotus* species have been grown in Australia, namely lotus (*Lotus uliginosus* syn. *L. pedunculatus*), birdsfoot trefoil (*Lotus corniculatus*) and narrowleaf trefoil (*Lotus tenuis*), with *L. uliginosus* by far the most widely grown. Harris *et al.* (1993) reported that 5500 ha of cv. Grasslands Maku (Armstrong 1974) were sown in the coastal regions of eastern Australia for beef and dairy production in 1990, though Blumenthal and McGraw (1999) later reported the combined total area sown to *Lotus* sp. in Australia could be as much as 100 000 ha. However, there are currently no *Lotus* cultivars being grown for certified seed (Australian Seeds Authority Ltd. 2021*a*, 2021*b*), nor are there any eligible for seed certification in Australia (Australian Seeds Authority Ltd. 2021*c*), which is a barrier to increased adoption of *Lotus* sp.

L. uliginosus, L. tenuis and L. corniculatus have proven to be waterlogging tolerant (Real et al. 2008), though only L. tenuis and L. corniculatus tolerate salinity while L. uliginosus tolerates aluminium (Schachtman and Kelman 1991). Drought has proven a significant barrier to lotus persistence (Ayres et al. 2006a). In New South Wales, Grasslands Maku outperformed white clover at temperate sites where summer moisture deficits are relatively short; the reverse was the case in sub-tropical sites (Blumenthal et al. 1999). This is consistent with early reports by Armstrong (1974) that Maku would outperform white clover in soils that are moist, low pH, or low in fertility. Further studies by Ayres et al. (2006b) identified that L. uliginosus was best suited to the high rainfall zone (>1000 mm average annual rainfall (AAR)), whereas L. corniculatus was better suited to the low fertility, low pH regions of 650-1000 mm AAR. This is consistent with the suggestion of Dear et al. (2003) that Lotus sp. have potential on waterlogged and acidic soils and with current advice (NSW DPI 2017). Surveys of growers and agronomists indicated that research was required into establishment, dry matter (DM) production, quality, persistence and seed production (Harris et al. 1993). Barriers to adoption of L. corniculatus in New Zealand were listed by Chapman et al. (1990) as being associated with establishment (appropriate and successful inoculation with rhizobia), and inappropriate grazing management. Under Australian conditions L. corniculatus has not persisted well compared to other adapted species (Haves et al. 2023).

# Sainfoin

Sainfoin (*Onobrychis viciifolia*) reduced enteric CH<sub>4</sub> production by 13% on average, with reductions as high as 48% (Table 1) mainly when compared to ryegrass pasture. When fed to beef cattle sainfoin did not reduce CH<sub>4</sub> emission compared to lucerne after correction for intake (26.1 vs 25.7 g/kg DMI) (Chung *et al.* 2013). Sainfoin accessions collected from different environments varied substantially in terms of *in vitro* CH<sub>4</sub> production, indicating the potential for selection of new low-CH<sub>4</sub>-yielding cultivars (Hatew *et al.* 2015).

Although sainfoin has been successfully grown in north America and northern Europe (Rumball 1982; Dear *et al.* 2003), it has not been a commercial success in Australia since cv. Othello was first introduced in 1980 (Oram 1990; Nichols *et al.* 2012). Evaluations in New Zealand led by Rumball (1982) demonstrated it had a limited role, as it

Functional group	Pasture species/group	Δ CH <sub>4</sub> %, (Range, %)	Potential PSC	Minimum rainfall (mm) <sup>A</sup>	Soil conditions it will tolerate and/or grow well <sup>A</sup>	Production	Persistence	Livestock
Perennial legumes	<i>Lotus</i> spp. (a) Big trefoil (b) Birdsfoot trefoil	- <b>38,</b> (-64 to 2) <sup>1-6</sup>	CT <sup>1, 27</sup>	(a) 1000 (b) 650–750	<ul><li>(a) Acid soils;</li><li>waterlogging</li><li>(b) Moderately acidic soils</li></ul>	**	*0*	(a) Low bloat risk (b) Non-bloating
	Lucerne	- <b>4</b> /+6 <sup>B</sup> (-27 to 20/126) <sup>1, 6-16</sup>	Phen, Sap <sup>27</sup>	375–400	Moderately acid to moderately neutral soils, well-drained	****	****	Bloat risk
	Perennial clovers (a) Red clover (b) White clover	-2, $(-31 \text{ to } 42)^{1, 2, 5, 6, 17-20}$	T, Phen <sup>1, 27</sup>	(a) 700 (b) 750	<ul><li>(a) Well-drained; slightly acid to neutral soils</li><li>(b) Slightly acid to neutral soils</li></ul>	** ***	**	(a) High oestrogen (b) Bloat risk
	Sainfoin	- <b>I3,</b> (-48 to 2) <sup>7-9, 14, 21</sup>	CT, Phen <sup>27</sup>	350	Alkaline, sandy soils	**	**	Non-bloating
	Sulla	<b>−32,</b> (−) <sup>6</sup>	T, Sap <sup>27</sup>	400-800	Neutral to alkaline	***	*	Non-bloating
Annual legumes	Annual clovers (a) Arrowleaf clover (b) Bladder clover (c) Subterranean clover	I, (-28 to 49) <sup>1, 13, 22</sup>	T, Phen <sup>1, 27</sup>	a) 400–500 b) 350–800 c) 375–600	Slightly/moderately acidic to neutral. Bladder and sub-clover prefer well-drained soil	***** *** ****	** ** *****	<ul><li>(a) Bloat risk</li><li>(b) Bloat risk</li><li>(c) Older cultivars</li><li>can have high</li><li>oestrogen</li></ul>
	Biserrula	<b>−77,</b> (−) <sup>1</sup>	Phen <sup>28</sup>	400–525	Acid soils	****	****	Photosensitisation risk
	Burr medic	+ <b>II</b> , (–) <sup>1</sup>	Phen, Sap <sup>27</sup>	300–700	Mildly acidic soil	**	****	No known issues
	Serradella (a) French serradella (b) Yellow serradella	+ <b>7</b> , (2 to 12) <sup>1</sup>	Phen <sup>28</sup>	a) 375–475 b) 400–450	(a) Well-drained; Acid soils; high Al (b) Sandy, acidic well- drained soils. High Al	****	****	No known issues
Perennial herbs	Chicory	-11, (-37 to 2) <sup>2, 6, 22-26</sup>	CT, Phen, Sap <sup>29, 30</sup>	600–750	Acid soil; good fertility	****	***	No known issues
	Plantain	-18, $(-21 \text{ to } -14)^{2, 22}$	CT, Sap <sup>31</sup>	500–650 mm	Low fertility soils	***	***	No known issues

Table I. Temperate pasture species and their potential to reduce methane emissions.

The  $\Delta$  CH<sub>4</sub> production is the percentage change relative to the control, typically perennial ryegrass, of the species of interest using *in vitro*, respiration chambers, sulfur hexafluoride tracer (SF6) or anaerobic digestor on freshly harvested or grazed materials. The average of all studies for each pasture species/grouping is shown in bold. Negative values represent a decrease in CH<sub>4</sub> production relative to the control, typically a grass (83% of all studies); with the predominant grass being perennial ryegrass (61% of all studies). The range of change for each pasture species/grouping is in parenthesis. The plant secondary compounds (PSC) that potentially contribute to the anti-methanogenic properties of the pasture species/grouping; rainfall and soil condition the pastures are suited to; a relative rating of production and persistence (\* poor to \*\*\*\*\* Excellent); and animal health considerations are also indicated.

<sup>1</sup>Banik et al. (2013a); <sup>2</sup>Loza et al. (2021); <sup>3</sup>MacAdam et al. (2022); <sup>4</sup>Pinares-Patiño et al. (2003); <sup>5</sup>Vargas et al. (2018); <sup>6</sup>Waghorn et al. (2020); <sup>7</sup>Amaleviciute-Volunge et al. (2020); <sup>8</sup>Dal Pizzol et al. (2017); <sup>9</sup>Maxin et al. (2020); <sup>10</sup>McCaughey et al. (1999); <sup>11</sup>Medjekal et al. (2018); <sup>12</sup>Melesse et al. (2017); <sup>13</sup>Muir et al. (2020); <sup>14</sup>Niderkorn et al. (2011); <sup>15</sup>Singh et al. (2012); <sup>16</sup>Suybeng et al. (2021); <sup>17</sup>Hammond et al. (2011); <sup>18</sup>Niderkorn et al. (2017); <sup>19</sup>Pavao-Zuckerman et al. (1999); <sup>20</sup>Purcell et al. (2012); <sup>21</sup>Chung et al. (2013); <sup>22</sup>Durmic et al. (2016); <sup>23</sup>Niderkorn et al. (2019); <sup>24</sup>Prusty et al. (2014); <sup>25</sup>Sun et al. (2011); <sup>26</sup>Sun et al. (2012); <sup>27</sup>Tava et al. (2022); <sup>28</sup>Latif et al. (2020); <sup>29</sup>Abbas et al. (2015); <sup>30</sup>Scharenberg et al. (2007); <sup>31</sup>Kara et al. (2018).

<sup>A</sup>Taken from NSW Department of Primary Industries' Pasture Species and varieties (https://www.dpi.nsw.gov.au/agriculture/pastures-and-rangelands).

<sup>B</sup>One study (Medjekal et al. 2016) observed a very high relative increase (126%) in CH<sub>4</sub> production and resulted in average mean CH<sub>4</sub> emission across all studies of +6% (compared to a control). When this single study was excluded, a mean reduction in emissions of 4% was observed. The *median* reduction in emissions including the Medjekal et al. (2016) study was -3%.

CT, condensed tannins; Sap, saponins; T, tannins; Phen, phenolics; Al, aluminium.

failed to persist in damp soils and had limited cool season growth. Hayot Carbonero *et al.* (2011) identified it as being tolerant of cold, drought and low soil fertility. Dear *et al.* (2003) suggested that sainfoin was a potential alternative to lucerne in the well-drained, fine-textured, neutral to alkaline soils in low rainfall mallee areas of Victoria, South Australia and Western Australia. Low productivity and variable establishment were considered issues for adoption (Reed and Flinn 1993; Hayot Carbonero *et al.* 2011; Mora-Ortiz and Smith 2018). Both sainfoin and sulla (*H. coronarium*, mentioned below) can be more productive under a less intensive cutting regime, suggesting that both may lack grazing tolerance, due to their erect growth habit (Reed and Flinn 1993).

The Australian Pastures Genebank previously reported 203 accessions of sainfoin, but in 2022 only 29 accessions were active, and only three available for distribution (APG 2022). The remaining 174 accessions are historic records, with no seed stored. There is a requirement to improve the diversity of this species if its potential is to be fully explored, particularly focusing on material collected from Mediterranean environments, as this material will be most suited to the dry summers and wet winters of southern Australian agricultural regions.

#### Sulla

Cultivars of sulla have been released in Australia (Nichols *et al.* 2012) but few are currently commercially available. Sulla has been reported to have a high level of CT (i.e 6.8%; Waghorn *et al.* 2002), which can reduce  $CH_4$  production by 32% compared to perennial ryegrass and white clover pasture with a similar quality (Table 1). When mixed evenly with lucerne, the  $CH_4$  reduction remained similar, at 26%, highlighting the potential role of mixtures. Sulla also contains saponins (Tava *et al.* 2022) which may also contribute to lower  $CH_4$ .

Sulla is noted for its biennial habit, high biomass, deep taproot and bioactive ingredients (anthelmintic and non-bloating) and production systems have been developed (de Koning *et al.* 2008). Sulla growth can be slow in the first year and first year DM on offer can be increased by sowing with a cover crop that also enhances the summer survival of sulla plants (de Koning *et al.* 2008). Sulla–grass-based pastures in South Australia had higher sheep liveweight gain than grass–subterranean clover pastures and also higher wool growth and less soiling in the breech area (de Koning *et al.* 2010).

#### Lucerne

Lucerne is a widely utilised species in Australia (Nichols *et al.* 2012), sown over an area of 3.2 million ha with the potential for a further 27 million ha (Robertson 2006). Several *in vivo* studies of freshly grown lucerne have reported that it can decrease  $CH_4$  emissions, compared to a range of primarily grass species, but it exhibits considerable

variation in its ability to reduce enteric CH<sub>4</sub> (Table 1). Lucerne contains saponins and this is thought to be responsible for reductions in CH<sub>4</sub>. This is supported by observations that ensiled lucerne has up to 7.3 mg/g DM more saponins than fresh lucerne, which reduced CH<sub>4</sub> production without negatively affecting the basic fermentation parameters (Kozłowska et al. 2020). Several studies have found lucerne hay has reduced CH<sub>4</sub> emission when added to a mixed ration diet (Malik and Singhal 2009, 2016; Kumar et al. 2018); but the lucerne diet was often higher in quality so whether the CH<sub>4</sub> reductions occurred because of this improved quality or a greater saponin content or a combination of both remains unclear. As lucerne is a commonly used species with a well understood agronomy, superior quality attributes leading to high levels of animal performance, available seed and potential to reduce CH<sub>4</sub> emissions compared to other legume pastures, it is expected to play a major role in emissions reduction from livestock systems in the future.

#### **Perennial herbs**

Perennial herbs provide high nutritive feed during late spring and summer (Cranston *et al.* 2015) and in general contain high mineral content (Pirhofer-Walzl *et al.* 2011), including trace elements such as copper (Cu) and selenium (Se) (Hoskin *et al.* 2006). Due to superior animal performance, perennial herbs are being included in pasture mixes as a specialised fodder for finishing lambs and beef cattle (Li and Kemp 2005). In addition, perennial herbs are often rich in secondary phenolic compounds, such as CT, that could potentially reduce  $CH_4$  emissions (Minnée *et al.* 2020; Loza *et al.* 2021).

#### Chicory

Chicory (Cichorium intybus) has the potential to mitigate CH<sub>4</sub> emissions from ruminants as it contains tannins, saponins and other phenolic compounds (Abbas et al. 2015; Table 1). Waghorn et al. (2002) reported that chicory can reduce CH<sub>4</sub> emission of sheep by up to 30% (measured using the sulfur hexafluoride (SF<sub>6</sub>) tracer technique) compared with a mixed ration of perennial ryegrass and white clover. In addition to secondary compounds, the reduction in CH<sub>4</sub> emission from chicory has been associated with high ratios of readily fermentable and structural carbohydrates that increased rumen particle breakdown rate and reduced retention time of particulate matter in the rumen (Barry 1998; Ramirez-Restrepo and Barry 2005). In contrast, Sun et al. (2011) found that there were no differences in CH<sub>4</sub> production between chicory and ryegrass forages (22.8 vs 23.8 g CH<sub>4</sub>/kg DM intake) measured from sheep in an open circuit sheep respiration chamber system.

Nevertheless, chicory has demonstrated great potential to reduce  $CH_4$  emission intensity due to its superior animal performance. For example, Jonker *et al.* (2019) reported

that the milk production from chicory was greater than that from ryegrass–white clover pastures (16.7 vs 15.4 L/day, P < 0.001), but there were no differences in total CH<sub>4</sub> emissions between perennial herbs and ryegrass–white clover pastures from cows over 162 days of lactation, indicating perennial herbs can reduce CH<sub>4</sub> emission intensity compared to traditional ryegrass–white clover pastures.

Chicory is a deep-rooted perennial herb that is adapted to a wide range of climate and soil conditions. Chicory can produce up to 19 t DM/ha.year in favourable conditions (Li and Kemp 2005; Lee *et al.* 2015). The animal performance is superior with liveweight gain up to 290 g/day for lambs and 900 g/day for calves in spring and summer in New Zealand (Li and Kemp 2005; Cranston *et al.* 2015). Including chicory in a pasture mix can increase the voluntary intake, with improved N use efficiency due to its fast particle breakdown, hence improving animal performance (Niderkorn *et al.* 2019).

Grazing management of chicory presents a great challenge to farmers. Chicory can be grazed hard and frequently during its fast-growing period in spring and early summer but grazing in autumn and winter is detrimental to its persistence (Li and Kemp 2005). To maintain a vegetative, high feed quality forage after vernalisation, a more intensive grazing regime is recommended, though the resulting decline in water soluble carbohydrate reserves of roots is likely to compromise its longevity (Mangwe *et al.* 2020). The poor persistence that has been observed is most likely due to improper grazing management. With careful management, such as not grazing during a wet winter, chicory can last for 4–6 years with great productivity under New Zealand conditions (Ramirez-Restrepo and Barry 2005).

Another challenge for the management of chicory is to find a suitable companion species. Due to its high nitrogen (N) demand, legume species are the obvious choice. However, deep-rooted lucerne competes with chicory for water, and aerial seeded annual legumes are a mismatch with the timing of grazing and seed set. Due to its winter-dormancy (Rumball 1986), winter active grasses appear unsuitable as companion species for chicory (Li and Kemp 2005; Cranston *et al.* 2015). Annual legumes with high levels of hard seed, such as biserrula, or legumes that bury seeds underground, such as subterranean clover, are likely to be the most suitable companion species, however they will need further investigation in the field.

#### Plantain

Plantain (*Plantago lanceolata*) is a summer active perennial herb that can maintain high nutritive values during warm summer conditions (Cranston *et al.* 2015) and whilst it has little to no CT, it is rich in other phenolic compounds and saponins (Kara *et al.* 2018; Loza *et al.* 2021) so has potential to reduce CH<sub>4</sub> emissions (Table 1). Cows grazing a mix of plantain, chicory and white clover produced 15% less CH<sub>4</sub> daily, on average, compared with cows grazed on perennial grass-based pastures, most likely due to the content of CT and HT in addition to the improved quality of the diet (Wilson et al. 2020). Pasture productivity can also be high with plantain. Moorhead and Piggot (2009) reported that plantain-based pastures had higher DM production, by 1.8 t DM/ha in summer and 0.9 t DM/ha in autumn, compared to perennial ryegrass-based pastures, but there were no production differences in winter and spring averaged across six sites in Northland, New Zealand. Adding plantain to perennial ryegrass-white clover pastures has potential to increase production levels and to improve DM distribution over time (Moorhead and Piggot 2009). Pure swards of plantain have been shown to yield up to 19 t DM/ha.vear in favourable conditions in New Zealand (Powell et al. 2007; Minneé et al. 2013; Lee et al. 2015). However, yields of 5-9.7 t/ha have been reported in dry environments such as Australia (Reed et al. 2008) and North America (Sanderson et al. 2003). Despite its high organic matter digestibility (80%) when in a vegetative state, growth of lambs was much lower than those grazing chicory but was similar to that for lambs grazing perennial ryegrass (Fraser and Rowarth 1996). Lee et al. (2015) also found that when sown as a pure sward, the crude protein content of plantain can be low (<15%), potentially limiting animal production (Cranston et al. 2015).

Similar to chicory, the poor persistence of plantain is an obstacle for adoption in grazing systems. Plantain requires regular rotational grazing to persist and maintain feed quality, however grazing during winter would reduce the persistence markedly. Labreveux *et al.* (2004) concluded that the plantain cultivars tested may not be appropriate for perennial pastures in north-eastern USA due to very low plant survival. In contrast, Moorhead and Piggot (2009) found that plantain remained the major sward component and produced 1.2 t DM/ha higher yield than perennial ryegrass–white clover pastures in Year 3 across six sites in Northland New Zealand.

#### **Annual legumes**

#### **Biserrula**

Biserrula contains a range of PSC including both phenolics and saponins (Ghamkhar *et al.* 2018; Latif *et al.* 2020) that greatly inhibit the production of CH<sub>4</sub> within *in vitro* studies compared to many other commonly grown forages (Banik *et al.* 2013*a*, 2013*b*, 2019; Vercoe 2016). Pen feeding experiments have also demonstrated reductions in CH<sub>4</sub> emissions on a DMI basis of 42%, 24% and 51% compared with bladder clover (*Trifolium spunosum*), subterranean clover and French serradella (*Ornithopus sativus*), respectively (Hutton *et al.* 2014). Methane production from biserrula was unaffected by growth stage and produced 10.5 mL/g DM on average, compared to 45.5 mL/g DM for subterranean clover *in vitro* (Banik *et al.* 2019).

Biserrula is well adapted to low fertility, acid soils and a wide range of rainfall conditions (325–700 mm) (Loi *et al.* 2010), but currently is not widely grown (Thomas *et al.* 2021).

Biserrula is capable of producing both high quantity (>5 t/ha) and high quality (digestibility of organic dry matter [DOMD] >60% along with crude protein 15-23% and metabolisable energy [ME] >11 MJ/kg DM) with neutral detergent fibre [NDF] values that are comparable or lower than lucerne during the early to mid-growing season (Vercoe 2016; Hackney et al. 2021; McGrath et al. 2021). However, these grazing studies have found that livestock production is less than might be expected based on previously reported forage parameters. Work by McGrath et al. (2021) observed that liveweight gains in sheep after 61 days of grazing a biserrula monoculture were ~6-9 kg lower compared to grazing lucerne monocultures and  $\sim 3$  kg lower than subterranean clover monoculture. Further, Vercoe (2016) observed that average daily gain (ADG g/day) was always lower compared to other annual legumes in monoculture, e.g. subterranean clover and serradella (Ornithopus spp.) but higher rates of ADG were achieved when a 2:1 ratio of annual ryegrass:biserrula was fed. While the reasons for this are not well understood it likely relates to a number of factors. These include: (a) lower palatability (Vercoe 2016); (b) decreased fermentation of biserrula resulting in lower in vitro gas production (Banik et al. 2013a, 2013b) and a subsequent decrease in pasture intake (McGrath et al. 2021); (c) reduced grazing if stock are impacted by a photosensitisation event (Hackney et al. 2007; Kessell et al. 2015) and; (d) development of an aversion to it, in particular in sheep (Revell and Thomas 2004). Aversion to biserrula appears to be more likely to occur when it is the dominant species in the pasture. This aversion may be minimised by ensuring a more heterogenous mixture of species is present in pastures (Swinny et al. 2015; Thomas et al. 2015).

# Serradella

The serradella species are prostrate annual legumes that produce small seeds in woody pods (Nutt et al. 2021). While serradella has not demonstrated reductions in CH<sub>4</sub> emissions in vitro (Table 1), there is potential to use genetic resources to breed lower CH<sub>4</sub> cultivars. Serradella species are suited to deep, acidic sandy soils (Nichols et al. 2007). They are widely adapted and used in ley or phase farming in Western Australia, particularly French serradella and vellow serradella (Ornithopus compressus). In a study of legume persistence across permanent grassland pastures of eastern NSW, Hayes et al. (2023) identified the serradella species as one of the most promising alternative legume species for use in mixed grazed pastures across that extensive region. The relatively lower critical phosphorus requirements of serradella compared with subterranean clover (Bolland and Paynter 1992; Sandral et al. 2019) led Sandral et al. (2019) to postulate that it may be more competitive with cocksfoot (Dactylis glomerata) and phalaris (Phalaris aquatica) in mixed pastures. Lower fertiliser costs would provide an added advantage. Further, suitability mapping of serradella by Hill (1996) identified a large area of NSW suited to serradella. The importance of vernalisation and photoperiod in flowering date stability has recently been studied for adaptability in eastern Australia, to improve the selection of cultivars for target environments (Goward *et al.* 2023). There is also evidence to suggest within-cultivar variability in flowering date and flowering date stability (Haling *et al.* 2022), which can be used to breed better cultivars.

#### Medics

Annual medics have been sown over an estimated area of 24.6 million ha in Australia (Nichols et al. 2012). They contain saponins (Tava and Pecetti 2012) and so may reduce CH<sub>4</sub> emissions. However, Banik et al. (2013a) found no reductions in CH<sub>4</sub> emissions (in vitro) for burr medic (Medicago polymorpha) compared to perennial ryegrass. Studies in annual medics show large variation in total saponin content, saponin type and biological activity of different saponins, which is likely to influence their CH<sub>4</sub> reduction potential. As an example, Pecetti et al. (2010) found the saponin content in spotted medic (Medicago arabica) did not vary through the growing season (24.3-28.3 mg/g DM) but decreased markedly at senescence (10.7 mg/g DM) and had higher saponin levels than those observed in lucerne or barrel medic. Tava and Pecetti (2012) also measured the saponin content of 12 annual medic species and found levels varied from 0.38-1.35% of DM. Lei et al. (2019) screened 201 barrel medic (Medicago truncatula) accessions and found that total saponins in accessions with the lowest concentration were only 60% of those accessions with the highest concentration. Maestrini et al. (2020) reported that crude saponin levels were 2.1%, and 1.7% of DM respectively in burr medic cultivars Angola and Santiago, and all saponins inhibited gastrointestinal nematode eggs in sheep.

Nine species of annual medics have been commercialised in Australia with barrel medic and strand medic (Medicago littoralis) widely grown. Burr medic is considered moderately popular, and the other species are considered special purpose or local use (Nichols et al. 2012). The main use of medics is as lev pastures for neutral and alkaline soils (Nichols et al. 2012). Burr medics are more tolerant of low pH (4.8-5.2 measured in  $CaCl_2$ ) than barrel and strand medics (5.8 measured in  $CaCl_2$ ) (Howie et al. 2007; Nichols et al. 2012) and have greater waterlogging tolerance than other medic species (Francis and Poole 1973). For these reasons, and that they are widely naturalised in Australia, burr medics have the potential to be more widely grown than they currently are. If studies confirm CH<sub>4</sub> abatement from grazing burr medics, then long season cultivars could be developed for high rainfall areas. Spotted medic has performed well on acidic waterlogged soils (Dear et al. 2003) and a breeding program has commenced (Nair et al. 2006) but no cultivars have been released.

#### Subterranean clover

Of the other mainstream pasture species, subterranean clover is of interest because it is the most widely sown annual legume in Australia at 29.3 million ha (Nichols et al. 2012), and when fed to sheep has been found to reduce CH<sub>4</sub> production by 30% compared with feeding ryegrass (Muir et al. 2020). Moreover, while the methanogenic potential of subterranean clover is variable (Table 1), it is also heritable so it can be manipulated by plant breeding (Kaur et al. 2017; Durmic et al. 2022). The other plant traits that contribute to the current success of subterranean clover include its prostate growth making it tolerant to grazing, its ability to bury burrs and protect seed from grazing, a diversity of flowering times to enable it to be grown in many different environments (250-1200 mm of rainfall) and being productive in mixtures (Nichols et al. 2013). There are at least 45 registered cultivars of subterranean clover covering the full environmental gradient that the species can be grown in. Little is known about the range of PSC available in genetic resources. Overall, there is great potential to develop new subterranean clover lines, but the persistence of background populations may make it difficult to determine if a new variety is present, as has been the case with older oestrogenic subterranean clover.

#### Integrated system assessment

#### Farm-scale emissions from low methane legumes

The climate change mitigation potential needs to be considered at a farm-scale when shifting from currently sown legumes to pasture species capable of reducing livestock  $CH_4$  emissions. Farm-scale emissions will be determined not just by enteric  $CH_4$  emissions but also other emissions sources (e.g. N<sub>2</sub>O emissions from soils), including other factors, such as productivity, related to GHG emission intensity. The economic impacts of changing pasture types also need to be considered. Few existing studies have taken this approach to assessing the climate change mitigation potential of replacing currently recommended pasture species with species that have anti-methanogenic properties.

Doran-Browne *et al.* (2015) found that replacing a ryegrass and subterranean clover pasture with *L. corniculatus* decreased enteric CH<sub>4</sub> emissions by up to 19% (up to 5 t CO<sub>2</sub>-e/ha), and reduced emissions intensity by 5–20% for prime lamb production across a range of simulated intakes of *L. corniculatus* (20, 30 and 40% of the diet). Income from productivity gains was 15–30 times higher than from potential emissions trading (at A\$6/t CO<sub>2</sub>-e). Without productivity gains, potential C offset income would not have covered the costs of establishing lotus pasture, though rising C prices may change this. The results from the study highlighted that poor pasture persistence limited profitability (Doran-Browne *et al.* 2015).

As a comparison, Vercoe (2016) studied the effect of increased proportion of biserrula in pastures up to 80%, on a typical sheep property in the 600 mm rainfall zone in Western Australia and found that while biserrula reduced CH<sub>4</sub> it also reduced weight gain of animals. The study found a linear reduction in CH<sub>4</sub> intensity with increasing biserrula from 23 kg CO2-e/kg meat for the base system to 19.7 kg CO<sub>2</sub>-e/kg meat with 80% biserrula or a 0.04 kg  $CO_2$ -e/kg meat reduction for every percentage of increase of biserrula. However, the lower productivity of biserrula meant that farm profit and total emissions per farm also declined with an increasing proportion of biserrula due to a lower stocking rate (Vercoe 2016). Other studies have identified the potential for biserrula to maintain animal production (Hackney et al. 2021) and even lengthen the grazing season as it is more deep-rooted (Hackney et al. 2013), but any reductions in quality will lower production (Harrison et al. 2015; Thomas et al. 2021).

#### Case study

A case study was undertaken to examine how productivity changes (e.g. lamb production) affect total emissions and emissions intensity by introducing biserrula to pasture as an alternative to subterranean clover and lucerne. Data were obtained from a field experiment (McGrath et al. 2021) in which lambs grazed biserrula, subterranean clover and lucerne pastures over a 3-month period and pasture parameters (e.g. DM production, DM digestibility and crude protein content) and animal performance (e.g. liveweight gain, LWG) were recorded. Emissions associated with animal production were calculated on a monthly basis using the equations from the Australian National Greenhouse Gas Inventory (NGGI; Australian Government 2020) and the relevant equations to calculate the emissions were parameterised using data from McGrath et al. (2021). A Global Warming Potential over 100 years (GWP<sub>100</sub>) value of 28 for biogenic CH<sub>4</sub> was used (IPCC 2014). Emissions were calculated on an emissions intensity (kg CO<sub>2</sub>-e/kg LWG) and area (kg  $CO_2$ -e/ha) basis.

Emissions from the relevant sources on a LWG basis are presented in Table 2. Assuming grazing biserrula did not reduce enteric CH<sub>4</sub> emissions, results indicate that emissions intensity for biserrula was 61% greater than for subterranean clover and 71% greater than for lucerne. A 45% reduction in enteric CH<sub>4</sub> emissions was demonstrated by Hutton *et al.* (2014) when biserrula replaced subterranean clover in a 1.2 times maintenance ration. When this 45% reduction in enteric CH<sub>4</sub> emissions is accounted for, this reduced the emissions intensity of biserrula to the equivalent of subterranean clover but it was still 7% greater than for lucerne.

However, if animal production is assumed to match subterranean clover in terms of growth per unit DMI, emissions intensity was reduced by 64% relative to lucerne. **Table 2.** Greenhouse gas emissions intensity (kg  $CO_2$ -e/kg LWG) with breakdown by emissions source for one kg of lamb liveweight production on subterranean clover (Sub), biserrula and lucerne pastures, where (A) biserrula is not assumed to reduce enteric methane emissions, (B) biserrula is assumed to reduce enteric methane emissions by 45% per unit DMI and (C) biserrula is assumed to give 45% emissions reduction, and animal production equivalent to subterranean clover.

	Sub	Biserrula A	Lucerne	Biserrula B	Biserrula C
Enteric methane	3.5	5.6	3.2	3.1	1.9
Indirect N <sub>2</sub> O emissions <sup>A</sup>	0.1	0.2	0.1	0.2	0.1
Manure methane	0.2	0.2	0.1	0.2	0.2
Residue emissions <sup>B</sup>	3.8E-06	7.6E-06	6.3E-06	7.6E6	3.8E-6
Direct nitrous oxide <sup>C</sup>	0.4	0.7	0.5	0.7	0.4
Emissions intensity	4.2	6.7	3.9	4.2	2.6

<sup>A</sup>Emissions associated with the volatilisation of N in manure and subsequent emissions of  $N_2O$ .

 ${}^{B}N_{2}O$  emissions from N released from residues.

<sup>C</sup>N<sub>2</sub>O emissions from N deposited in manure.

Thus, grazing biserrula may have the potential to reduce GHG emissions associated with animal production if issues such as photosensitivity, preferential grazing and reduced fermentation can be ameliorated.

Methane emissions from relevant sources on an area basis are presented in Table 3. Subterranean clover and biserrula pastures had the same stocking rate however subterranean clover pastures gave a higher LWG so, despite the lower emissions intensity of LWG on a subterranean clover pasture, the total emissions for subterranean clover and biserrula pasture were similar on a per hectare basis. Further, despite lamb produced on lucerne pasture having the lowest emissions intensity, the high stocking rate meant that emissions per hectare were more than double that for subterranean clover or biserrula pastures. Assuming a 45% decrease in enteric CH<sub>4</sub> for biserrula resulted in a 36% and 70% reduction in total emissions relative to subterranean clover and lucerne respectively, and where LWG for biserrula was assumed to be the same for subterranean clover, total GHG emissions were reduced by 38 and 71% relative to subterranean clover and lucerne respectively.

This case study demonstrates how the potential for a reduction in the GHG emissions intensity and/or total emissions could be assessed under the proposed framework using calculations that align with the NGGI. For a complete farmscale analysis other emissions sources such as inputs of fuel and fertilisers, and changes to soil organic carbon (SOC) would also need to be included for this temperate forage, as has been demonstrated for Leucaena leucocephala (Harrison et al. 2015). Changing to a pasture that has lower persistence would increase the frequency of re-sowing of pastures, which may result in a decline in SOC due to greater soil disturbance. Reduced persistence leading to shorter pasture phases in a mixed farming (crop/livestock) production system would also likely reduce SOC. Badgery et al. (2014) found that SOC decreased by 0.54 t C/ha.year to 0-30 cm for every year of cropping but increased at 0.78 t C/ha.year for pasture. Chan et al. (2011) found SOC sequestration rates of 0.22, 0.25, and 0.40 t C/ha.year at 0-30 cm for 33%, 50%, and 67% pasture in a cropping rotation, respectively. Changing to a species with lower persistence, where the duration of the cropping phase does not change, will also reduce the farm-scale emissions because fewer animals will be run, producing less enteric CH<sub>4</sub>.

For animal emissions, when we assumed that biserrula reduced enteric  $CH_4$  emissions by 45%, this did not lower the emissions intensity sufficiently so that it was comparable to lucerne. A 50% reduction in enteric  $CH_4$  would be required

**Table 3.** Total GHG emissions (kg  $CO_2$ -e/ha) for lamb production grazing subterranean clover (Sub), biserrula or lucerne pasture. Data from McGrath et al. (2021), where (A) biserrula is not assumed to reduce enteric methane emissions. (B) Biserrula is assumed to reduce enteric methane emissions by 45% per unit DMI or (C) biserrula is assumed to give 45% emissions reduction, and animal production equivalent to subterranean clover.

	Sub	Biserrula A	Lucerne	Biserrula B	Biserrula C
Emissions intensity (kg CO <sub>2</sub> -e/kg LWG)	4.2	6.7	3.9	4.2	2.6
Stocking rate (sheep/ha)	17.7	17.7	32.3	17.7	17.7
LWG (kg)	12.2	7.7	15.3	7.7	12.2
Total	892.3	911.4	1925.8	569.3	554.7

Data from McGrath et al. (2021).

for the emissions intensity of lamb produced on a biserrula pasture to match that of lamb produced on a lucerne pasture. Other in vivo research into effects on enteric CH4 suggested much lower reductions of between 16% and 28% depending on the quality of intake (Vercoe 2016). This suggests that from a systems perspective, biserrula may not have the potential to reduce the emissions intensity of lamb production relative to subterranean clover and lucerne pastures unless the factors that limit livestock production on biserrula are overcome. The GHG emissions of animal production systems, other than enteric CH<sub>4</sub>, are influenced by biophysical characteristics of plants (e.g. root:shoot ratio) and animals (e.g. N retention). For the present study, default values for these equation parameters for pastures, from the NGGI, were used, but these parameters may not be appropriate for the pasture species used in this study. Ideally, these additional parameters will be refined based on future research that estimates the emissions reductions from using low CH<sub>4</sub> yielding species.

Results from this case study highlight an important point in assessing the climate mitigation potential of low CH<sub>4</sub> pasture species - that a reduction in the GHG emissions intensity of animal production does not necessarily lead to a reduction in total emissions at the farm-scale, and can, instead, increase farm-level emissions. However, this could still be part of a larger scale climate change mitigation strategy. Simmons et al. (2020) demonstrated that the farm is not the most appropriate scale to estimate the climate change mitigation potential of management changes because it does not consider the global impacts of feeding and clothing the world population, projected to reach 10 billion people by 2050 (United Nations 2017). Agricultural land is constrained at a global scale so de-intensifying agricultural systems requires more land to produce the same amount of commodities which increases demand for agricultural land, resulting in land clearing (Smith et al. 2019). This is an issue because climate impacts of converting natural land to agricultural land can be greater than the emissions reductions achieved at the farm-scale via de-intensification (Searchinger et al. 2018). Although this kind of 'burden shifting' remains theoretical for agricultural production it has been demonstrated for other land-based commodities such as forestry (Meyfroidt et al. 2010). This means that, based on the analysis presented here, converting a production system to lucerne pastures may have the greatest climate change mitigation potential at a global scale because it uses existing agricultural land more efficiently than either subterranean clover or biserrula, thereby reducing the land required to feed and clothe the global population. Any future applications of the framework need to consider the impacts of increasing demand for agricultural land via de-intensification or sparing agricultural land via intensification.

# **Research gaps and future direction**

This review highlights there are viable temperate legumes and herbs, that have anti-methanogenic properties, are productive, and capable of mitigating enteric  $CH_4$  in grazing systems of temperate Australia. There are many areas for future research and development required before broadscale adoption of these species and accounting for GHG emission reductions can occur. Some of the key areas are highlighted below.

# Understanding variation in CH<sub>4</sub> reduction under grazing

Plant secondary compounds are known to reduce CH<sub>4</sub> emissions through various pathways, however the form of the relationships between various PSC intake and CH<sub>4</sub> emission reduction remain unclear. In vitro experiments indicate that the higher the amount of PSC in a plant the greater the CH<sub>4</sub> emission reduction, but whether there is a threshold at which additional PSC no longer decrease CH<sub>4</sub> emissions or affect digestibility, and therefore DMI, needs to be further explored. Whether in vitro experiments are true indicators of in vivo CH<sub>4</sub> emission measurements can also be confusing; proper validation of any in vitro method must involve rigorous in vivo validation to enable reliable estimation of CH₄ in laboratories. There are also other factors that interact with the actions of PSC to determine in vivo CH<sub>4</sub> reduction, such as plant quality and preferential grazing. Factors such as these would have been responsible for the variability amongst in vivo CH<sub>4</sub> reductions reported by Vercoe (2016) for animals grazing biserrula over various seasons. Understanding how these factors affect CH<sub>4</sub> reductions is essential in being able to predict the potential CH<sub>4</sub> reductions associated with the introduction of a species into a system. Also, any potential animal and human health implications of PSC need to be fully understood (Durmic et al. 2022).

# Same species or new systems?

There is an imperative for rapid action to reduce GHG emissions from agriculture as soon as possible to limit the impacts of climate change. There is a strong case for promoting the adoption of current technologies/pasture species (e.g. biserrula and chicory) that are readily available because they do not require producers to implement new seeding methods and/or invest in new equipment. However, methods need to be developed to accurately account for  $CH_4$  emissions reductions and ensure that adverse GHG or production impacts do not occur. Furthermore, adapting parameters for the NGGI and other accounting frameworks may need to be modified for low  $CH_4$  yielding species.

#### The role of pasture mixtures

Throughout the temperate grazing systems of Australia, pastures are predominantly mixtures of species, generally

containing an annual or perennial legume combined with one or more grass species. There is also increasing interest in including herbs in these pasture mixtures. Many studies have shown that livestock often prefer a 'choice' of pasture species when grazing in order to satisfy a range of forage requirements (quality, quantity, roughage, etc.) (Edwards *et al.* 2008; Soder *et al.* 2009) and that livestock grazing mixes of species are commonly observed to have decreased rates of CH<sub>4</sub> emissions (Wilson *et al.* 2020). In addition, species mixes maximise the productivity of a pasture system by extending out the growing period. Further work is needed to build regionally suitable, diverse pasture mixtures and management that can sustain higher proportions of legumes and herbs.

# Breeding to improve CH<sub>4</sub> reduction

This paper highlights reasonable potential for some species/ cultivars to reduce CH<sub>4</sub>, but there is also great opportunity to develop breeding programs that will encourage the production of new cultivars with increased levels of antimethanogenic activity. As an example, Roldan et al. (2022) increased the CT level in white clover leaf to >2% of DM in two generations of breeding, which in turn reduced in vitro CH<sub>4</sub> production by 19%. The same breeding potential has been identified for subterranean clover (Kaur et al. 2017) and likely exists for lucerne and other widely grown legume species. New breeding programs are required to develop cultivars that have increased anti-methanogenic properties. Even with speed breeding (Pazos-Navarro et al. 2017; Ghosh et al. 2018; Watson et al. 2018) it will be many years before new cultivars come onto the market. As such new cultivars are not likely to be major drivers in the mid-term (5-10 years) and are really part of longer-term solutions (>10 years) to deal with emission reductions.

# Systems analyses

Determining total in vivo GHG reductions for low CH<sub>4</sub> yielding species in a variety of environments is yet to be assessed. The case study in this paper clearly highlights the importance of maintaining high productivity to reduce CH<sub>4</sub> emission intensity, but also found that high productivity increased whole-farm GHG emissions. Meeting the emission reduction targets of government and the supply chain for ruminant products will require a reduction in total GHG emissions but maintaining productivity is important to ensure the most efficient use of existing agricultural land and avoid the need for more land to be brought into agricultural production to meet the demands of a growing global population. The trade-off between total GHG emissions and emission intensity, including the effects on soil C stocks, need to be investigated further and new integrated metrics developed and tested in different environments.

#### Standardised measurements

Standardised and validated protocols are required for the estimation of  $CH_4$  reduction potential. The extent of  $CH_4$  reduction is always assessed relative to some other forage and the use of a standardised comparison pasture species that has low PSC contents, is necessary to quantify differences within and between studies. While perennial ryegrass is often used as a comparative species in temperate pasture regions, it is not grown in lower rainfall grazing systems of temperate Australia. A commonly grown forage within a region would be a more appropriate comparison.

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