Nitrate and sulphate accumulating shrubs to reduce methane emissions in sheep

Hayley C Norman[1], Matt G. Wilmot[1], Paul Young P, Andrew Toovey[1], Dean Thomas[1], Xixi Li[1], Miranda Macintyre[1], Joshua K. Hendry[1] and Elizabeth Hulm[1]

[1] CSIRO Agriculture Flagship, Private Bag 5, Wembley WA 6913. Email: Hayley.Norman@csiro.au

Abstract
Feeding nitrate and sulphate to ruminants is a proven methane abatement strategy. In extensive systems, provision of these compounds as supplements can be problematic due to issues with individual animals selecting an appropriate dose and risk of toxicity. Several Australian native shrub species that are planted by farmers in the low to medium mixed crop/livestock zone accumulate significant concentrations of nitrate and sulphate. The aim of this project was to explore the potential of these shrubs to offer a safe, profitable, environmentally positive and 'natural' means of reducing methane emissions from sheep grazing poor quality stubbles and pasture resides in autumn. This paper describes results from five on-farm benchmarking studies where we investigated use of *Atriplex nummularia, Enchylaena tomentosa and Rhagodia preissii* in grazing systems. Data concerning nutritive profile of the shrubs and variation in dietary intake, liveweight change, wool production and blood plasma indicators of animal health are presented. Sheep tended to maintain or gain weight during the grazing in autumn and we found little evidence of nitrate toxicity as indicated by blood methaemoglobin. The shrubs had high crude protein, low acid detergent fibre, low to moderate organic matter digestibility and variable concentrations of ash, nitrate and sulphate. Voluntary intake by sheep was variable and influenced by the nutritional value of the shrubs and understorey. We concluded that the shrubs offered an opportunity to increase productivity and reduce methane emissions.

Key words
Old man saltbush, greenhouse gas, carbon, salinity, drought

Introduction
Methane represents up to 12% of gross energy that is lost to the ruminant and contributes to greenhouse gas emissions. Diverting metabolic pathways in the rumen away from methane as the major hydrogen sink has been proposed as a practical approach to reduce methane emissions from ruminants (Leng 2008). Nitrate is a promising hydrogen sink and a rough rule of thumb is that for each addition of 1% potassium nitrate to a diet, methane will be reduced by 10% (Leng 2008). Using respiration chambers, van Zijderveld et al (2010) found that lambs fed a basal diet with 2.6% DM nitrate or 2.6% DM sulphate produced 32% or 16% less CH_4 respectively than lambs fed the same basal diet. If fed both, the reduction in methane was 47%. The use of nitrate in combination with sulphate, could therefore offer an avenue for methane mitigation. Sudden introducing of nitrate in the diet may be detrimental to livestock as nitrite can cause oxidation of haemoglobin in the blood (to methaemoglobin) and nitrate can have a caustic effect on gut.

Several Australian native, drought-hardy, shrubs naturally accumulate high concentrations of nitrate and sulphate, including species of *A. nummularia* (old man saltbush), *R. preissii* (Mallee saltbush), and *E. tomentosa* (ruby saltbush) and *Maireana brevifolia* (bluebush). More than 10 years of research demonstrates that these plants are readily eaten by sheep and cattle without reports of toxicity (Ben Salem et al 2010, Revell et al 2013). It is likely that the high salt levels in the plants (up to 25% DM) and/or secondary plant compounds will restrict shrub intake before a toxic dose of nitrate is ingested. There is *in vitro* evidence that fermentation of M. brevifolia in rumen fluid results in less methane per unit of digestible organic matter than many other forage species (Masters et al 2010).

The aim of this benchmarking study was to investigate the utilisation of these shrubs in current grazing systems with a view to generating parameters for future laboratory and respiration chamber experiments. In autumn 2014, we investigated productivity and health of 5 flocks of sheep grazing these shrubs according to the host farmer's typical practice. The information gathered provides parameters for the design of future laboratory and animal house experiments. We hypothesised that the sheep will voluntarily ingest the shrubs and maintain liveweight, condition during grazing and show no evidence of nitrate toxicity (as evidenced by blood methaemoglobin).

Materials and methods

Table 1 lists the sites and grazing dates. At Tammin, we benchmarked sheep in two adjoining wheat stubbles, where animals in one paddock had access to an adjoining 5.3 ha plot of saltbush. Where the understorey was lacking in quantity or quality, cereal hay was provided to animals *ad lib*.

Table 1 Location of plots, description of feed, stocking rates and animals grazing the benchmarking sites

Location	Feed on offer	Area (ha)	Sheep/ha	Days	Animals
Tammin (WA)	Wheat stubble + saltbush	71	2.4	44	Ewes, early pregnancy
Tammin (WA)	Wheat stubble only	81	2.4	44	Ewes, early pregnancy
Quairading (WA)	4 shrub species + hay	15	3.3	34	Ewes, early pregnancy
Cranbrook (WA)	Saltbush + understorey	1.5	27	44	Weaner wethers
Moulamein (NSW)	Saltbush + hay	2	10	36	Weaner wethers
Barham (NSW)	Saltbush + understorey	50	1	36	Ewes, early pregnancy

Animal management and sampling

Paddocks were stocked with Merino sheep a rate of 1 to 27 sheep/ha, rates were representative of farm practice (with the exception of Barham where the stocking rate was lower). Sheep were removed from the plot after the majority of biomass had been utilised or a maximum of 44 days. For each flock, 20 individuals were randomly selected as 'core' animals and used for monitoring of liveweight, condition score change (Suiter 1994), health and wool growth. Wool samples were collected from 10 cm^2 midside patches at the start and end of grazing. The wool was weighed, scoured, reweighed and clean growth was estimated (Langlands and Wheeler 1968). Dietary selection (C_4 shrub vs C_3 stubble, hay or understorey) was estimated using carbon isotopes within a sample of faeces (Norman et al 2009). Blood samples (10ml) were collected from the jugular of core animals for analysis of blood methaemoglobin and biochemical indicators of animal health. Immediately after collection of blood, 2 ml was poured into a small tube and placed on ice and methaemoglobin was determined using the method of Lacey and Rodnick (2002).

Plant measurement and chemical analysis

For the understorey, above ground forage biomass estimates were collected at the start and end of grazing by calibrated quadrate cuts. At Tammin, grain was removed from the quadrates and weighed separately. Shrub biomass was assessed at the start and end of grazing using the 'Adelaide' technique (Andrew et al 1979). Understorey and shrub samples were dried for 48 hours at 60 °C. Samples were ground in a Cyclotech mill (1mm) and used for laboratory assessment of nutritive value. *In vitro* DMD, adjusted to predict *in vivo* DMD, was determined in duplicate using a modified pepsin-cellulase technique (Norman et al 2010). Duplicate samples of 7 AFIA standards (understorey and hay) or 7 shrub standards (for shrubs) with known *in vivo* DMD were included in each batch to allow raw laboratory values to be adjusted to predict *in vivo* DMD using linear regression (AFIA 2007; Norman et al 2010). Concentrations of NDF and ADF of the shrub material were measured sequentially, according to operating instructions, using an Ankom 200/220 Fibre Analyser (Ankom® Tech. Co., Fairport, NY, USA). Total ash was measured according to the methods of Faichney and White (1983). Total N and C were determined by combustion using a Leco CN628 N Analyser. Anions (nitrate, phosphate, oxalate, sulphate and chloride) were extracted with slight modifications of Cataldi et al. (2003) and analysed by HPLC using suppressed conductivity.

Results

The sheep grazed the paddocks from 34 to 44 days, resulting in a range of 36 to 1188 sheep grazing days/ha. Table 2 summarises the animal production benchmarking data collected. Mean liveweight change ranged from a loss of 48g/day to a gain of 130 g/day. The two most heavily stocked sites were where sheep lost weight. Clean wool growth ranged from 44 to 64 g/sheep.day. At Tammin, where we monitored sheep in stubbles with or without access to saltbush, the sheep with access to the shrubs grew faster in the first 3 weeks of grazing (data not presented) but liveweight change was similar after 44 days. Saltbush as a proportion of the total diet during the last week of grazing ranged from 4.5 % (Moulamein) to 27% (Tammin). At each site there was large differences between the 20 individuals monitored, for example one weaner at Cranbrook had 4% saltbush in his diet whereas another ate 42%. Variation was smallest at Tammin where the mature ewes ate 19 to 36% saltbush. There was little evidence of nitrate toxicity as indicated by blood methaemoglobin. One animal at Tammin consistently had levels of 11% but it was in the stubble only 'control group'. The highest blood methaemoglobin level was 15% for a sheep at Moulamein.

Table 2. Sheep liveweight and condition change, wool growth and blood methaemoglobin.

Location	Liveweight change (g/day)		Condition change (units)		C4 in diet* %		Clean wool (g/day)		Clean wool yield (%)		Methaemoglobin (%, final day)		
	Mean	sem	Mean	sem	Mean	range	Mean	sem	Mean	sem	Mean	sem	Range
Tammin (+ SB)	130	14	0.2	0.01	27.0	19-36	4.7	0.13	56	1.8	0.3	0.25	0-4
Tammin (- SB)	136	15	0.0	0.01			4.4	0.13	53	2.1	1.8	0.75	0-12
Cranbrook	-5	11	0.1	0.01	16.0	4-42	4.5	0.11	58	2.9	0.2	0.18	0-4
Quairading	34	4	0.0	0.01	11.1	6-28					1.3	0.39	0-6
Moulamein	-48	11	0.1	0.00	4.5	1-10	5.6	0.21	47	2.5	2.0	0.55	0-15
Barham	107	15	0.2	0.01	5.0	6-12	6.4	0.18	37	2.0	1.1	0.40	0-4

*Estimated C4 shrub component of diet (saltbush) using C isotopes during the final week of grazing. Data at Quairading is only saltbush intake as the other species were C3.

Across sites, the mean edible dry matter (EDM, leaves and stems <3mm diameter) per shrub ranged from 440 to 690 g, equating to a range of 340 – 602 kg EDM/ha. Understorey varied from < 100 kg/ha to about 3t/ha in the stubble plots at Tammin (Table 3).

Table 3. Biomass on offer at the start of grazing at the benchmarking sites.

Site	Shrub EDM (g/shrub)	Shrub EDM (kg/ha)	Understorey (kg/ha)	Grain (kg/ha)	Total (kg/ha)
Tammin (SB+ Stubble)	602	602	2297	161	3662
Tammin (Stubble)	0	0	2959	245	3204
Cranbrook	341	409	1855		2537
Quairading*	384	250	1109		1743
Moulamein*	720	504	100		1324
Barham	690	415	1000		2105

*Sheep were also offered hay of moderate quality (55-58 % OMD) as per usual farm practice

The nutritional traits of the shrubs at the benchmarking sites are presented in Table 4. *Rhagodia preissii* had the highest OMD with 65% and *Enchylaena tomentosa* the lowest at 51%. OMD of *Atriplex nummularia* ranged from 56 to 63%. Fibre levels were generally low, indicating the shrubs would be a good complement to fibrous senesced pastures and crops. Nitrate levels ranged from 0.12 to 0.97% and sulphate ranged from 0.28 to 0.61%.

Table 4. Nutritional profile of the shrub biomass

Site	Sample	OMD %	NDF %DM	ADF %DM	Hemi %DM	OM %	Nitrate %DM	Sulphate %DM
Quairading	*Rhagodia preissii*	65	21	12	9	87	0.21	0.34
	Enchylaena tomentosa	51	32	19	13	78	0.55	0.47
	Atriplex nummularia	57	24	13	10	70	0.23	0.28
Cranbrook	*Atriplex nummularia*	58	25	14	12	77	0.12	0.43
Barham	*Atriplex nummularia*	56	24	14	10	73	0.22	0.43
Moulamein	*Atriplex nummularia*	62	25	13	12	74	0.97	0.61
Tammin	*Atriplex nummularia*	63	23	13	10	75	0.86	0.27

*Stubbles at Tammin: grain OMD of 92%, wheat leaves OMD of 44% and wheat stems OMD 27%.

Discussion

Across sites, sheep generally maintained liveweight during autumn, a time of year when biomass and nutrients are both lacking and producers are generally providing supplements. At Cranbrook, the farmer achieved 1188 sheep grazing days/ha, with minimal liveweight loss (200g/head over 44 days) without provision of supplements. At all sites, the shrubs were planted on soils that were salt-affected so there few other pasture or cropping options. The producers reported that the shrubs were a valued component of their system. Previous work indicates that addition of the shrubs can quadruple the carrying capacity of saline land in autumn (Bennett et al 2012). Other benefits include improved wool growth and quality, reduced vitamin E deficiency and improved meat quality (Ben Salem et al 2010).

The shrubs did provide a source of dietary nitrate and sulphate that was readily eaten by the sheep without any apparent ill-effects. At Tammin in the final week of grazing, the ewes consumed an average of 2 g nitrate and 0.6 g sulphate per day. The maximum level of consumption during the last week was 3.7 g nitrate and 1.2 g sulphate per day. Based on these numbers, toxicity is unlikely and there should be a modest reduction in methane (< 20%). Associated work is examining differences within and between shrub species in nitrate and sulphate accumulation and the impact of soil nutrients. It is clear some shrub species and genotypes within species accumulate more nitrate and sulphate than others and fertiliser can be used to boost nitrate and sulphate concentrations in leaves. The shrubs also contain other plant secondary compounds such as saponins that have been reported to reduce methane. We are now examining fermentability and methane production in an *in vitro* gas production system with rumen fluid, prior to a respiration chamber experiment to quantify *in vivo* productivity and methane emissions in late 2015.

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