Managing livestock to reduce methane emissions

Assessment of strategies for abatement of enteric methane

A.K. Almeida and R.S. Hegarty

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1. A meta-analysis of the efficacy and productivity consequences of mitigation strategies for livestock methane emissions

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Introduction

Recognizing the urgent need to address climate change, nations have agreed to reduce greenhouse gas (GHG) output, aiming for net zero emissions by the second half of the century (UNFCCC, 2015). Livestock enteric methane (CH_4) contributes to 11.6% of global GHG emissions from anthropogenic activities (Ripple *et al.*, 2014), and efforts are being made to identify and encourage actions to reduce these emissions (GRA 2020).

Enteric methane (CH_4) emission represents a natural energy waste by the ruminant fermentation process, and it is the main source of GHG in agriculture. Enteric CH_4 accounts for 43% of the GHG emissions from livestock, globally (Herrero *et al.*, 2016). In Australia 73% of the GHG emissions from the agriculture sector are CH_4 (***Figure 1***). As CH_4 has a relatively brief lifetime in the atmosphere (i.e., from 8.4 to 12 years, Ehhalt et al., 2001), mitigating CH_4 may represent a substantial pathway to achieve climate stabilisation targets. In NSW, CH_4 emissions are mainly due to the 25.2 million head of sheep and 4.73 million head of *cattle* (MLA, 2019). There are opportunities to reduce CH_4 emissions while improving ruminant efficiency from a metabolic to a farming system level. It is a healthy reminder from Capper et al., (2009) that speaking of the change in the US dairy industry since 1944 says "Modern dairy practices require considerably fewer resources than dairying in 1944 with 21% of animals, 23% of feedstuffs, 35% of the water, and only 10% of the land required to produce the same 1 billion kg of milk. Waste outputs were similarly reduced, with modern dairy systems producing 24% of the manure, 43% of CH_4, and 56% of N_2O per billion kg of milk compared with equivalent milk from historical dairying."

The developing suite of mitigation options for enteric methane have been regularly reviewed (Martin et al., 2010; Cottle et al., 2011; Hristov et al., 2013; Grossi et al., 2019). Progress towards carbon neutrality for the red meat sector in Australia may involve nutritional strategies, as well as whole farm systematic approaches (e.g., vaccines, improving reproductive rate, stock number and productivity, pasture management, genetics, among others). The nutritional approach, more specifically, rumen manipulation, encompasses a wide range of possibilities (e.g., oils, algae, nitrate, ionophores, protozoa population control, bacteriocins, phytochemicals, 3-nitrooxypropanol, acetogens, organic acids, among others). In this meta-analysis, we focus on strategies that are likely to be adopted in NSW and contribute to carbon neutrality by 2050. Therefore, the approach of this assessment is to use CH_4 mitigation data published since 2000 to quantify the technical potential of strategies for CH_4 mitigation by ruminants (cattle and sheep), as well as quantifying the co-benefits and identifying barriers to implementation. The ultimate purpose of this assessment is to identify the CH_4 mitigation potential of system-based and nutritional strategies that may be adopted regionally to inform the development of efficient policies to support CH_4 abatement.

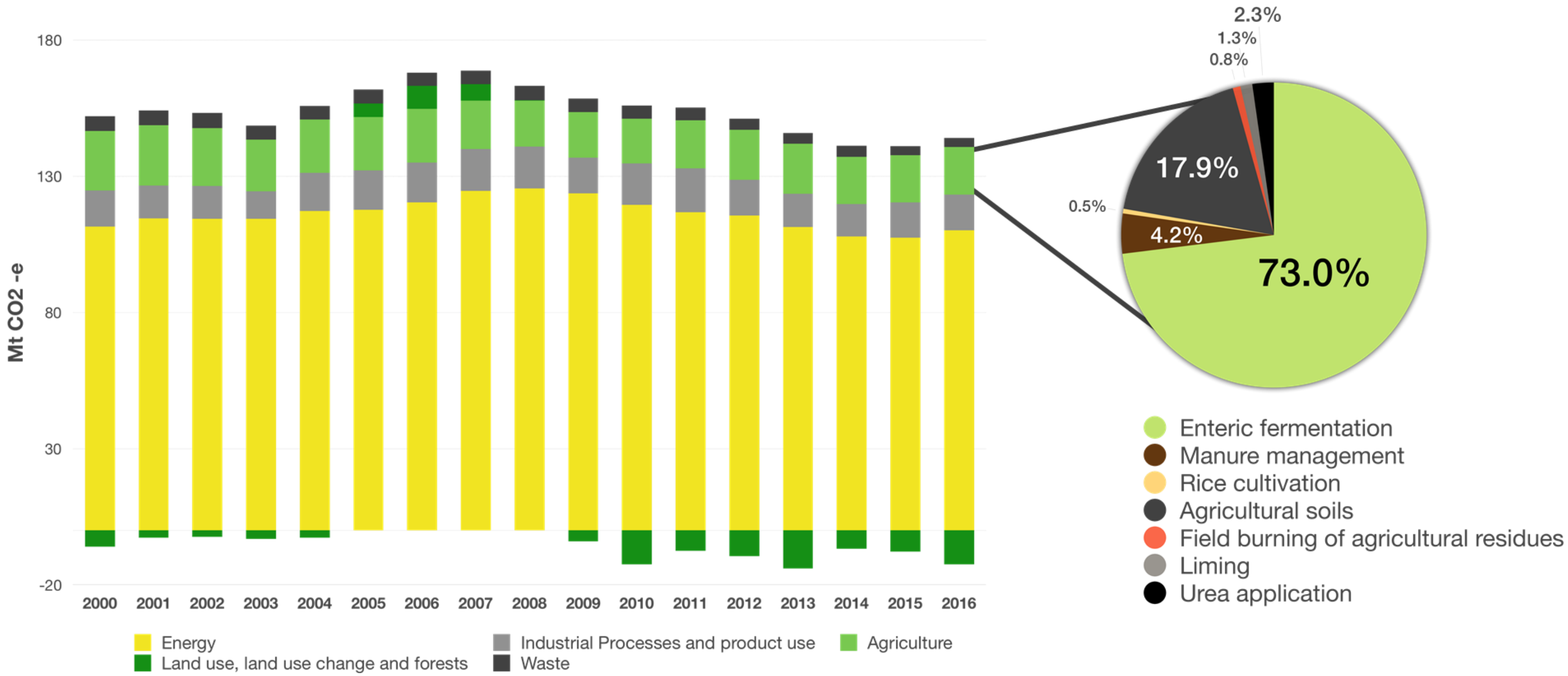

Figure 1 Sources of GHG emissions (Mt CO2-e) in NSW emphasizing the agriculture sector contribution in 2016 (Source: State and Territory Greenhouse Gas Inventories; DEE, 2016).

Material and methods

Published literature screening

A database was created with publications from 2000 to 2020, using only reports of in vivo trials that measured ruminant enteric CH_4 emissions. Studies included addressed the effects of nutritional (oils, algae, nitrate, ionophores, protozoa population control, bacteriocins, phytochemicals, 3-nitrooxypropanol, acetogens, organic acids) as well as system-based abatement measures (vaccines, improving reproductive rate, stock number and productivity, pasture management, genetics). Keywords used to identify papers were as follows: “ruminant”, “enteric”, “methane emission”, and one of the potential strategies. Pertinent literature cited in each considered article was also screened for inclusion. All data were from articles published in indexed journals identified through searches conducted using the Google Scholar search engine (https://scholar.google.com) from 18th of August 2019 to 26th of April 2020. Data were entered in an Excel spreadsheet in a systematic fashion in which each row represented a treatment and each column represented an exploratory variable (Sauvant et al., 2008). All publications used in the meta-analysis are listed in Section 3 and a summary of the data is presented in ***Table 1***. Data reported in divergent units of measure were transformed to matching units. When a study did not report all needed results and it was possible to calculate from the reported data, appropriate calculations were performed from the reported data.

The investigated factors were body weight (BW), dry matter (DM) intake, average daily growth (ADG) (g/d), milk production (kg/d) diet chemical composition (crude protein, CP; neutral detergent fiber, NDF; fat; and non-fiber carbohydrate, NFC), and digestibility of nutrients (DM, CP, fat and NDF). Methane emissions were reported as methane production rate (MPR: g CH_4/head/d), methane yield (MY: g CH_4/kg DMI) and methane emission intensity (MI: g CH_4/kg animal product, typically milk or liveweight gain).

Inclusion criteria

For inclusion in the meta-analysis, studies were required to have MY (g/kg DMI) measured, as well as data reported on composition of diets and intake. Besides these, other variables required in the dataset were digestibility, animal performance and rumen fermentation indicators (pH, molar proportion of volatile fatty acids, and protozoa count), as previously described. Overall, 108 publications met these requirements and were included in the analysis (***Table 1***).

Statistical Analysis

The meta-analysis was performed using the MIXED procedure of SAS (version 9.4, SAS/STAT, SAS Institute Inc., Cary, NC), considering study as a random effect. Furthermore, to account for variations in precision across studies, the inverse of the squared standard error of the mean (SEM) (Wang and Bushman, 1999) of MY was used as a factor in the WEIGHT statement of the model (St-Pierre, 2001). The slopes and intercepts by study were included as random effects, specifying an unstructured variance-covariance matrix for the intercepts and slopes. (St-Pierre, 2001). When pertinent, covariates were included in the model based on previous literature, as listed in results section. In this sense, if the covariate was found non-significant, it was then removed from the model if $P > 0.05$ (St-Pierre, 2001).

Table 1 Description of the database used in the meta-analysis of the effect of different strategies for enteric methane abatement.

Study code	Source	Animal	Strategy	CH_4 Method	n
1	Alemu et al. (2019)	sheep	phytochemicals, NO3	GreenFeed	22
2	Beauchemin et al. (2006)	beef	phytochemicals, oil, organic acid	chamber	8
3	Beauchemin et al. (2007)	beef	oil	chamber	4
4	Beauchemin et al. (2009)	dairy	oil, protozoa control	chamber	4
5	Benchaar (2016)	dairy	Phytochemicals, ionophores	SF6	8
6	Benchaar et al. (2015)	dairy	oil, protozoa control	chamber	6
7	Bird et al. (2008)	sheep	Protozoa control	chamber	7
8	Caetano et al. (2019)	beef	Phytochemicals	GreenFeed	10
9	Carulla et al. (2005)	sheep	Phytochemicals, protozoa control	chamber	6
10	Carvalho et al. (2016)	beef	oil	SF6	9
11	Chung et al. (2013)	beef	Phytochemicals, protozoa control	chamber	8
12	Cooprider et al. (2011)	beef	Ionophores	chamber	4
13	Cosgrove et al. (2008)	sheep	oil	SF6	2
14	Ding et al. (2012)	sheep	oil	other	3
15	Duthie et al. (2018)	beef	NO3	chamber	18
16	El-Zaiat et al. (2014)	sheep	phytochemicals, NO3	chamber	6
17	Fiorentini et al. (2014)	beef	oil, protozoa control	SF6	9
18	Grainger et al. (2008)	dairy	oil	SF6	6
19	Grainger et al. (2008b)	dairy	Ionophores	chamber/SF6	15
20	Grainger et al. (2009)	dairy	Phytochemicals	SF6	10
21	Grainger et al. (2010)	dairy	Ionophores	chamber/SF6	10/15
22	Granja-Salcedo et al. (2019)	beef	NO3	SF6	10
23	Guyader et al. (2015a)	dairy	phytochemicals, NO3	chamber	4
24	Guyader et al. (2015b)	dairy	oil, NO3, protozoa control	chamber	4
25	Guyader et al. (2016)	dairy	oil, NO3	chamber	8

26	Haisan et al. (2014)	dairy	3-NOP	SF6	5
27	Haisan et al. (2017)	dairy	3-NOP	SF6	6
28	Hegarty et al. (2008)	sheep	Protozoa control	chamber	6
29	Hess et al. (2016)	sheep	Phytochemicals, protozoa control	chamber	6
30	Hollmann et al. (2012)	dairy	oil	chamber	6
31	Holtshausen et al. (2009)	dairy	Phytochemicals, protozoa control	chamber/SF6	4
32	Hosoda et al. (2005)	dairy	Phytochemicals	chamber	4
33	Hristov et al. (2013)	dairy	Phytochemicals	SF6	8
34	Hristov et al. (2015)	dairy	3-NOP	GreenFeed	12
35	Hulshof et al. (2012)	beef	NO3	SF6	8
36	Hünerberg et al. (2013a)	beef	oil	chamber	8
37	Hünerberg et al. (2013b)	beef	oil	chamber	4
38	Johnson et al. (2002)	dairy	oil	SF6	4
39	Jordan et al. (2006a)	beef	oil	chamber	10
40	Jordan et al. (2006b)	beef	oil	SF6	12
41	Jordan et al. (2007)	beef	oil	SF6	4
42	Jose Neto et al. (2019)	beef	oil	SF6	9
43	Kim et al. (2019)	beef	3-NOP	GreenFeed	9
44	Kinley et al. (2020)	beef	Seaweed	chamber	5
45	Klevenhusen et al. (2011)	sheep	Phytochemicals, protozoa control	chamber	6
46	Lee et al. (2015)	beef	NO3	chamber	8
47	Lee et al. (2017a)	beef	NO3	chamber	7
48	Lee et al. (2017b)	beef	NO3	chamber	7
49	Li et al. (2012)	sheep	NO3	chamber	5
50	Li et al. (2013)	sheep	NO3	chamber	6
51	Li et al. (2018)	sheep	Seaweed	chamber	6
52	Liu et al. (2011)	sheep	phytochemicals, oil	chamber	8
53	Lopes et al. (2016)	dairy	3-NOP	GreenFeed	6
54	Ma et al. (2015)	sheep	Phytochemicals	chamber	6

55	Ma et al. (2017)	sheep	Phytochemicals	chamber	6
56	Machmüller et al. (2000)	sheep	oil, protozoa control	chamber	3
57	Machmüller et al. (2001)	sheep	oil, protozoa control	chamber	3
58	Machmüller et al. (2003)	sheep	oil, protozoa control	chamber	3
59	Malik et al. (2017)	sheep	Phytochemicals, protozoa control	SF6	10
60	Mao et al. (2010)	sheep	phytochemicals, oil	chamber	8
61	Martin et al. (2008)	dairy	oil	SF6	8
62	Martin et al. (2016)	dairy	oil	SF6	4
63	Martinez-Fernandez et al. (2018)	beef	3-NOP	chamber	4
64	McGinn et al. (2004)	beef	oil, organic acid, ionophores	chamber	8
65	McGinn et al. (2009)	beef	oil	SF6	30
66	Melgar et al. (2020)	dairy	NO3	GreenFeed	24
67	Moate et al. (2011)	dairy	oil	chamber	4
68	Moate et al. (2014)	dairy	Phytochemicals, protozoa control	SF6	10
69	Mohammed et al. (2004)	dairy	Phytochemicals, protozoa control	chamber	4
70	Moreira et al. (2013)	sheep	Phytochemicals	SF6	3
71	Mwenya et al. (2005)	dairy	Ionophores	other	4
72	Newbold et al. (2014)	beef	NO3	chamber	6
73	Nguyen and Hegarty (2017)	beef	oil, protozoa control	chamber	6
74	Nolan et al. (2010)	sheep	NO3	chamber	4
75	Norris et al. (2020)	beef	Phytochemicals, protozoa control	chamber	8
76	Odongo et al. (2007)	dairy	oil	chamber	6
77	Odongo et al. (2007b)	dairy	Ionophores	other	12
78	Olijhoek et al. (2016)	dairy	NO3	chamber	4
79	Oliveira et al. (2007)	beef	Phytochemicals	SF6	8
80	Patra et al. (2011)	sheep	Phytochemicals	chamber	4
81	Pen et al. (2007)	sheep	Phytochemicals	chamber	4

82	Rebelo et al. (2009)	beef	NO3	SF6	10
83	Reynolds et al. (2014)	dairy	3-NOP	GreenFeed	6
84	Romero-Perez *et al.* (2014)	beef	3-NOP	chamber	8
85	Romero-Perez et al. (2015)	beef	3-NOP	chamber	8
86	Roque et al. (2019)	dairy	Seaweed	GreenFeed	12
87	Rossi et al. (2017)	beef	oil	SF6	7
88	Santoso et al. (2004)	sheep	Phytochemicals	chamber	4
89	Silva et al. (2018)	beef	oil	SF6	6
90	Soltan et al. (2013)	sheep	Phytochemicals	chamber	6
91	Staerfl et al. (2012)	beef	Phytochemicals, protozoa control	chamber	6
92	Sun et al., (2017)	beef	NO3	chamber	4
93	Tiemann et al. (2008)	sheep	Phytochemicals	chamber	6
94	Troy et al. (2015)	beef	oil, NO3	chamber	6
95	Van Wesemael et al. (2019)	dairy	3-NOP	GreenFeed	10
96	van Zijderveld et al. (2010)	sheep	NO3	chamber	5
97	van Zijderveld et al. (2011a)	dairy	oil, Phytochemicals	chamber	10
98	van Zijderveld et al. (2011b)	dairy	NO3	chamber	5
99	Velazco et al. (2014)	beef	NO3	GreenFeed	10
100	Veneman *et al.* (2015)	dairy	oil, NO3	chamber	6
101	Villar et al. (2019)	beef	oil, NO3	chamber	4
102	Vyas et al. (2016)	beef	3-NOP	chamber	5
103	Vyas et al. (2018a)	beef	3-NOP	chamber	5
104	Vyas et al. (2018b)	beef	3-NOP, ionophores	chamber	5
105	Waghorn et al., (2008)	dairy	Ionophores	SF6	16
106	Wang et al. (2009)	sheep	Phytochemicals	chamber	4
107	Yang et al. (2017)	beef	Phytochemicals, protozoa control	chamber	4
108	Zhou et al. (2011)	sheep	Phytochemicals, protozoa control	chamber	3

Differences between means were determined using the P-DIFF option of the LSMEANS statement, which is based on Fisher's F-protected least significant difference test. Significant difference was declared at $P < 0.05$.

Results and discussion

Reducing enteric methane emissions by managing nutritional processes

Nutritional management can alter DMP and MY by multiple means that affect residual hydrogen availability in the rumen or directly target methanogens. These means include:

- Quantity of fermentable material ingested (general positive relationship)
- Rate of digesta passage through the reticulorumen (negative relationship with methane yield)
- Proportions of starch and fibre in ingested feed
- General suppression of fermentation or suppression of fibre fermentation (eg grain-feeding causing low pH; oil coating fibre and microbes)
- Unique steps in methanogenic biochemistry (vaccine, 3NOP, macroalgae)

While mitigation efficacy of these strategies have been reported and often combined in broad-ranging reviews (Martin et al., 2010, Cottle et al., 2011, Asizua et al., 2014, Patra, 2016, Grossi et al., 2019), the effect of feed management on animal productivity is less well assessed. An appraisal of abatement mechanisms and quantitative potential enteric methane abatement of each considered dietary strategy, its risks and barriers to implementation in NSW, as well as potential co-benefits are provided below.

Oils

Among the several dietary strategies specifically developed to mitigate enteric CH_4 production, oil inclusion in the diet is the one with most papers published in the last 20 years, that were included in the meta-analysis (n = 35; see Table 1). Oils (i.e., polyunsaturated fatty acids and the medium-chain saturated fatty acids) have been previously recognised to suppress CH_4 production in ruminants (Blaxter and Czerkawski, 1966). For instance, adding oil to the ruminant diet reduces H_2 producers (i.e., protozoa; Mao et al., 2010, Guyader et al., 2015), as well as methanogen populations (Mao et al., 2010), and may act as a [H] acceptor through fatty acid biohydrogenation, although small (Ungerfeld, 2015). Not surprisingly, among the papers included, only two (Johnson *et al.*, 2002, Silva *et al.*, 2018) out of 35 showed that adding oil to the diet of dairy cattle, beef cattle, or sheep did not affect enteric CH_4 production.

Our results revealed that the MY potential mitigation ranges from 12 to 20% (95% CI of the mean effect size; mean reduction of 15%). In this regard, for every increase of 1% of oil (10 g/kg DM) supplying from 2.85 to 6.20% - 95% CI of oil inclusion, the MY was reduced by 1.02 ± 0.113 g CH_4/kg DMI or 4.37% ($P < 0.01$; RMSE = 4.56). Previous meta-analysis (i.e., 17 studies) examined the reduction in MY in response to oil in the diet and reported that for each 1% oil added to the diet MY were reduced by 5.6% (Beauchemin *et al.*, 2007).The extent of the CH_4 mitigation by dietary oil may vary with basal diet, as negative effects have been reported on DMI, fibre digestion, as well as animal performance, from adding oil to ruminants fed high-fiber diets (Beauchemin and McGinn, 2006, Hollmann *et al.*, 2012, Troy *et al.*, 2015). Oil can be added to a low-fibre diet without impairing fibre

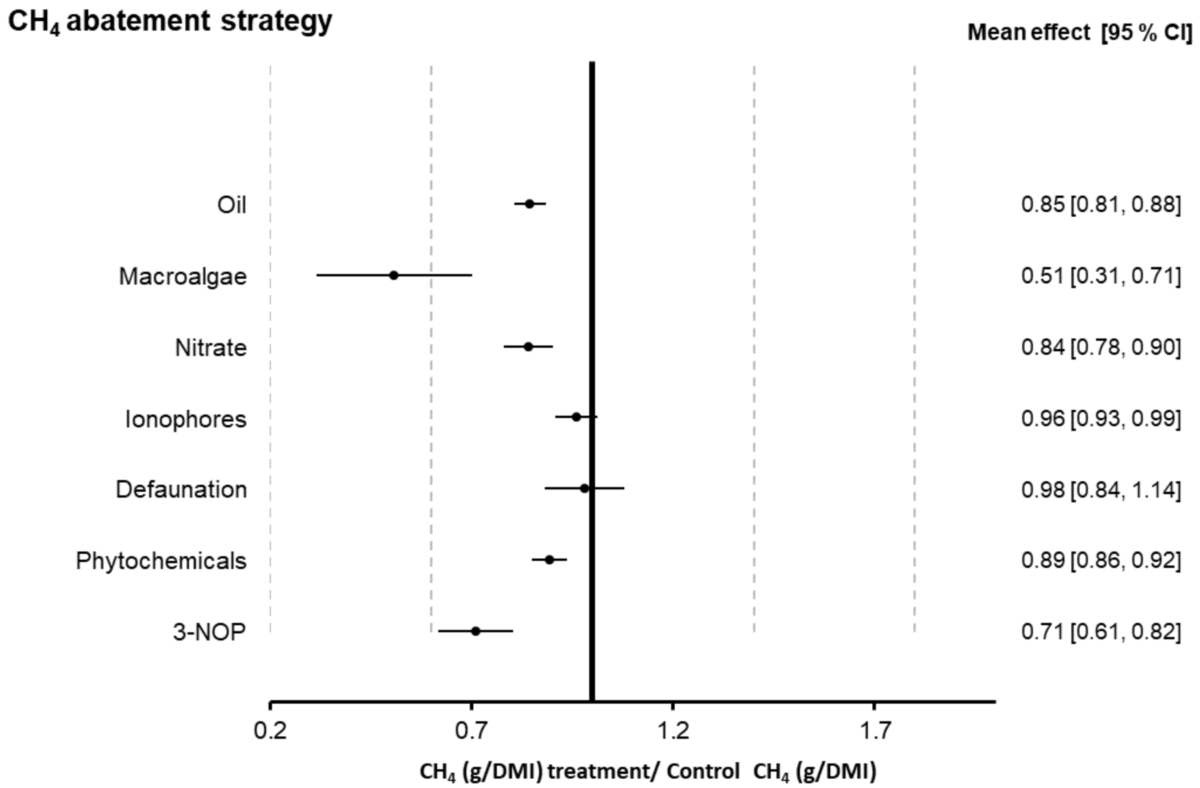

Figure 2 Forest plot depicting the standardized mean effect of the estimated ratio of methane yields for mitigation strategy vs control emissions (mean methane emission in treatment with mitigation strategy divided by mean methane emission in control) and the 95% confidence interval (95% CI). Values below 1.0 indicate that the mitigation strategy yields a reduction in methane emissions. Source: Almeida et al., 2021.

digestibility, but not to high-fibre diets (Machmüller *et al.*, 2001, Machmuller *et al.*, 2003, Benchaar *et al.*, 2015). Therefore, there is a risk that these adverse effects on fibre digestibility could restrict the use of oils as a mitigation strategy for grazing livestock. Oil addition within the range of the present meta-analysis reduced DMI by 1.24 to 6.17% (***Figure 3a***), and reduced NDF digestibility by 6.30% to 13.0% (95% CI of NDF content from 29.8 to 42.4%; ***Figure 3b***). No effect on growth rate could be detected using the present database, whereas oil addition decreased milk production by 1.17 to 13.7% (95% CI). Supplementation with unsaturated fatty acid rich-oils may influence the biohydrogenation, yielding the production of trans-10 18:1 fatty acid, which may result in a higher level of anti-lypogenic CLA (trans-10, cis 12) production in the mammary gland (Griinari and Bauman 1999; Odongo *et al.*, 2007), and therefore may cause milk depletion (Baumgard *et al.*, 2002). The overall reduction in MI (g CH_4/kg of milk or weight gain) ranged from 14.4 to 21.5% ($P<0.01$; ***Figure 3c***). Moreover, the practicality of oil supplementation in the diet in a farm setting should be evaluated considering its benefits in methane mitigation, as well as animal performance and cost of feeding in each scenario. Oil as a mitigation strategy can readily be applied to feedlot and dairy systems. The main barrier in grazing systems is the fibre digestibility reduction.

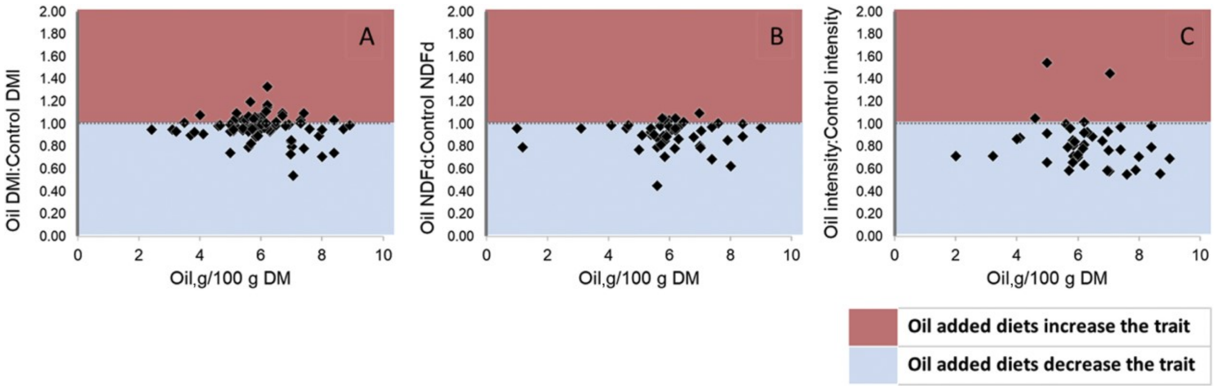

Figure 3 Mean effect of the estimated ratio of diets containing oil and control diets in DMI (a), NDF digestibility (b) and CH_4 intensity (g CH_4/kg animal product) (c). Source: Almeida et al., 2021.

Seaweeds

Seaweeds are macroalgae, being complex and diverse multicellular organisms that can grow in both marine and fresh water environments (van der Spiegel *et al.*, 2013). The term “seaweed” has no taxonomic importance but is commonly used to refer to the marine algae (Makkar *et al.*, 2016). Based on the pigment involved in their photosynthetic process, seaweeds can be categorised as red algae (*Rhodophyceae*), brown algae (*Phaeophyceae*), and green algae (*Chlorophyceae*) (Chapman and Chapman, 1980). There are more than 13,000 species of macroalgae (Huisman *et al.*, 1998) and some species of macroalgae have recently been proposed as a novel ingredient in ruminant diets (van der Spiegel *et al.*, 2013, Halmemies-Beauchet-Filleau *et al.*, 2018). Seaweeds vary in chemical composition (Machado et al., 2014, Makkar et al., 2016), digestibility (i.e., 15 to 94% as reviewed by Makkar et al., 2016) and show a wide diversity and content of secondary metabolites (Carroll *et al.*, 2019), including those by which CH_4 mitigation is achieved (Dubois *et al.*, 2013, Machado *et al.*, 2014, Kinley and Fredeen, 2015).

Previous studies have identified that the red macroalgae *Asparagopsis taxiformis* has a high efficacy in CH_4 abatement *in vitro* (Kinley and Fredeen, 2015, Machado et al., 2016, Machado et al., 2018) and *in vivo* (Li *et al.*, 2018) due to its high content of bromoform, a halogenated CH_4 analogue (Lanigan, 1972). Halogenated CH_4 analogues inhibit the enzymatic activity (i.e., methyltransferase enzyme) by reacting with the reduced vitamin B12 cofactor required in one of the final steps of CH_4 formation, decreasing the cobamide-dependent pathway (Wood *et al.*, 1968).

In vivo animal trials testing seaweeds as a mitigation option are only recently published. One study in sheep (Li et al., 2018), one in dairy cattle (Roque *et al.*, 2019) and one in feedlot cattle (Kinley *et al.*, 2020) are available, showing a dose dependent MY reduction from 30.0% up to 69.0% (95% CI; $P < 0.01$; $n = 3$; mean reduction of 49.0%), when using *Asparagopsis* inclusion from 0.5% to 3.0%. Solving the equation of the relationship between MY and *A. taxiformis* intake generated by Li et al., (2018) revealed reduction of 3.50 g CH_4/kg DMI (i.e., or 23.3% CH_4 mitigation) for every gram of *A. taxiformis* intake, without decreasing DMI or affecting blood chemistry and pathology, but with some effect on fermentation (Li *et al.*, 2018). Roque *et al.*, 2019 reported 38% DMI reduction and reduced milk production when *Asparagopsis* was fed to dairy cows at 1% of dry matter.

A previous study reported moderate potential of seaweed to be market-ready as a ruminant feed within 2 to 3 years (Halmemies-Beauchet-Filleau et al., 2018) and preparations are well underway for marketing of a commercial product in Australia (CSIRO 2020;

https://research.csiro.au/futurefeed/). The biochemical profile varies between species of seaweeds (Machado et al., 2014, Carroll et al., 2019), and it seems that Asparagopsis is the most effective macroalgae for CH4 mitigation. However, the effect of feeding macroalgae to ruminants on diet digestibility, animal performance and health, together with CH4 abatement are yet to be extensively addressed using in vivo trials. Moreover, the mitigation effect of seaweed appears to vary among its species in vitro, and is influenced by basal diet (Machado et al., 2014, Machado et al., 2016, Maia et al., 2016), thus future in vivo studies should focus on the influence of basal diet on seaweed mitigation effect. The animal performance response in a pasture-based setting is yet to be defined, and lifecycle analyses to address constraints linked with seaweed production itself must be considered. Among caveats to tackle before the adoption of seaweed as a mitigation strategy is the fact that high harvesting rates in the wild may disrupt the equilibrium of coastal ecosystems (Makkar et al., 2016), also cultivation of seaweeds may increase bromoform production, which may damage to the atmospheric ozone layer (Carpenter and Liss, 2000, Quack and Wallace, 2003).

Nitrate

The mechanism by which NO_3^- may lower ruminal methane production is through competition with methanogenesis for reducing equivalents. Because NO_3^- has a higher affinity for H_2 than does CO_2 in the rumen (Jones, 1972, Latham *et al.*, 2016), CH_4 production is reduced when feeding NO_3^- to ruminants. In the rumen, NO_3^- is initially reduced to NO_2^- (nitrite) and then to NH_3, decreasing the availability of H_2 for methanogens (Lewis, 1951, Nolan *et al.*, 2016).

The current meta-analysis revealed that NO_3^- supplementation decreased MY by 15.7% compared with a control diet (16.1±0.855 g vs. 19.1±0.853 CH_4/kg DMI; P<0.01; n = 25) in ruminants. Dietary NO_3^- inclusion from 17.2 to 22.1 g/kg DM (95% CI) led to MY reduction of from 10.0 to 22.1% (95% CI; P <0.05; n = 25). Unlike oils, NO_3^- supplementation does not impair fibre digestibility (i.e., NDF digestibility; P=0.86; n = 12; ***Figure 4b***), or DMI (P=0.86; n = 25; ***Figure 4a***), which is a beneficial outcome in grazing systems. Moreover, dietary NO_3^- supplies non-protein nitrogen to the rumen biota, reducing the need for other dietary nonprotein nitrogen sources (Hulshof et al. 2012; Li et al. 2012).The overall reduction in CH_4 intensity (g CH_4/kg of milk or weight gain) from NO_3^- inclusion ranged from 10.7 to 18.7% (P<0.01; n = 11 ***Figure 4c***).

Since 2015, the Australian Emissions Reduction Fund has included a method through which carbon credits can be generated for feeding of NO_3^- to grazing ruminants (DoE, 2015). The major limitation of feeding NO_3^- to ruminants is the possibility of accumulation and absorption of intermediates of NO_3^- reduction (i.e., NO_2^- into the bloodstream). Besides being a precursor to carcinogenic compounds, NO_2^- can impair the capacity of blood to transport oxygen to an animal's tissues due to methaemoglobinaemia (Lewis, 1951, Sindelar and Milkowski, 2012, Bedale *et al.*, 2016). However, in most studies used in the present analysis, blood methaemoglobin concentrations in nitrate-supplemented animals were higher than non-supplemented ones (Velazco *et al.*, 2014, Guyader *et al.*, 2016, Rebelo *et al.*, 2019). None of the included studies that measured blood methaemoglobin levels observed clinical symptoms of methaemoglobinaemia (i.e., cyanosis and hypoxia that may arise at methaemoglobin > 20% of total haemoglobin (Mensinga *et al.*, 2003)).

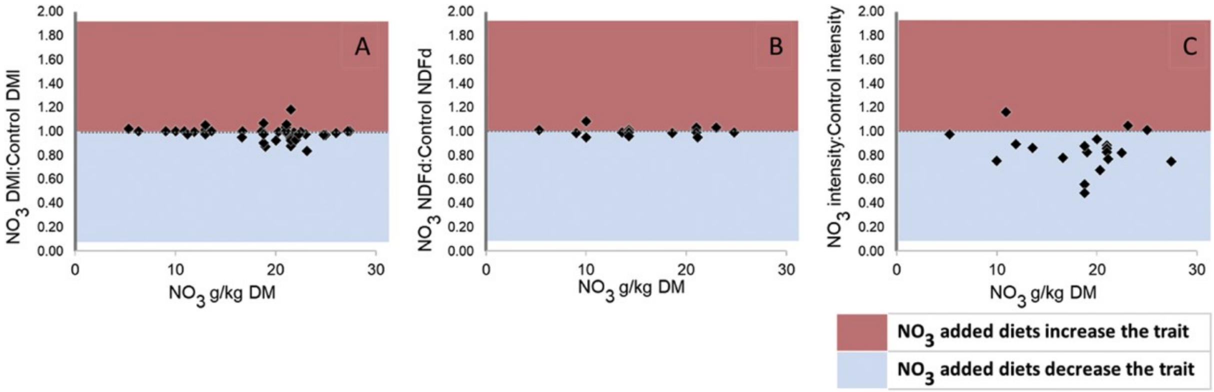

Figure 4 Forest plot depicting the standardized mean effect of the estimated ratio of diets containing nitrate (NO3) and control diets in DMI (a), NDF digestibility (b) and CH_4 intensity (g/kg animal product) (c). Source: Almeida et al., 2021.

In this regard, management and nutritional strategies to reduce the risk of NO_2^- poisoning may be used, including adapting animals to NO_3^- (i.e., microbial acclimation; (Lee and Beauchemin, 2014, Nolan *et al.*, 2016), slowing the rate of NO_3^- reduction reaction in the rumen (e.g., by encapsulated in lipid; de Raphélis-Soissan et al., 2017), as well as combining different mitigation strategies (e.g., NO_3^- + oil; Nolan *et al.*, 2016, Lee *et al.*, 2017, Villar *et al.*, 2019), allowing the reduction of NO_3^- level in the diet. Evaluating the combined effect of using nitrate and an oil source for CH_4 mitigation, the change in MY varied from 38.6% MY reduction up to 3.5% MY increase; (95%CI; $P < 0.05$; $n = 4$). Although these results were based on only four papers that evaluated the interaction of oil and NO_3^-, it seems that the combination of NO_3^- supplementation with oil would reduce the likelihood of nitrate poisoning.

Ionophores

Ionophores are compounds of diverse chemical structures that are able to anchor to the lipid bilayer of cell membranes of organisms and translocate protons (H+) and metal ions (futile ion flux) through the membrane leading to eventual death of the microbial cell (i.e., gram+ bacteria and protozoa) (Russell and Strobel, 1989, Chow *et al.*, 1994). Typically, this shifts the microbial population toward gram- bacteria that are least sensitive to ionophores, at the expense of H^+-, ammonia-, and lactate-producing organisms, resulting in higher propionate production, less CH_4, greater protein availability and higher ruminal pH (Russell and Houlihan, 2003). In Australia, very few beef feedlots would operate without an ionophore (monensin or lasaocid) though several non-ionophores can be used (eg. Bambermycin or virginiamycin).

Several ionophores are registered and approved for use as feed additives (e.g., monensin, lasalocid, narasin, laidlomycin), but this varies between countries, with laidlomycin not available in Australia for example. The primary reason for modifier inclusion is to reduce the risk of lactic acidosis and associated with this is a substantial improvement in animal health and performance and so feed-yard economics. Monensin, the most widely used ionophore in ruminant nutrition, is produced by *Streptomyces spp*. Over time, rumen microbes adapt, reducing the ionophore response, including the CH_4 mitigation (Callaway *et al.*, 2003). Rotating ionophores and antibiotics (daily, weekly or biweekly) may improve the effectiveness of ionophores on feed efficiency over time (Guan et al., 2006, Crossland et al., 2017). As ruminal bacteria may become resistant to ionophores, one may argue that ionophore resistance poses a public health threat, however genes linked to ionophore resistance in ruminal bacteria have not yet been identified, indicating that is not likely that ionophore resistance can spread across microorganisms (Russell and Houlihan, 2003).

The present study revealed that including ionophores (monesin was used in the majority of studies) in cattle diets reduced MY by only 4% (95%CI from 0.5 to 7.4%; P = 0.05; n = 10; ***Figure 2***). Moreover, the use of ionophores as a mitigation strategy is limited to the period prior microbiome adaptation. Ionophores have been commonly used as a performance enhancer for four decades. The current study showed only a modest MI reduction (g/CH_4/kg ADG: 95%CI from 0 to 14.7%; P = 0.04; n = 5). Ionophores affect ammonia production, changing the fermentation dynamics towards improving energetics and N use in the rumen, as well as acidosis control. Ionophores are used mainly in a feedlot setting and dairy herds (i.e., cattle fed high grain diets), and CH_4 mitigation appear to be only a small co-benefit of the use of ionophores.

Protozoa population control

Some methanogens in the rumen exist as endo- and ecto-symbionts with ciliate protozoa (Finlay *et al.*, 1994, Tokura *et al.*, 1997) and such symbionts may account for up to 37% of the rumen methanogens (Finlay et al. 1994). Although protozoa are a significant proportion of the biomass in the rumen ecosystem, they are not essential (Williams and Coleman, 1992; Newbold et al., 2015). On the contrary, some co-benefits of rumen defaunation (removing protozoa) have been reported, such as increases in growth rate and live weight gain of ruminants (Eugène et al, 2004; Newbold et al., 2015) especially when the feed is deficient in protein relative to energy content. In addition, rumen protozoa are significant H_2 producers, which due to their preferred production of acetate and butyrate synthesis rather than propionate (Williams and Coleman 1992).

In brief, one may use physical and chemicals techniques to achieve defaunation of the rumen: the most commonly used technique is the isolation of animals from their mothers at birth, followed by use of detergents and other chemicals (e.g., sodium lauryl sulfate, alkanes, synperonic NP9, calcium peroxide, copper sulfate), as well as emptying and washing the rumen (Hegarty et al., 2008; Newbold et al., 2015). Moreover, some feed additives used to mitigate CH_4 or as efficiency enhancers may control protozoan population: ionophores, oil, and NO_3 supplementation. It is noteworthy that none of the available techniques are considered practical and/or efficient for commercial application to the date.

When a protozoa population-controlling additive was used, the protozoa population reduced by 23% (95% CI = 12 – 35%; P < 0.01; n = 22) but rumen MY diminished by only 2% on average (95% CI = -0.16 – +14.0 %; P = 0.03; n = 22; ***Figure 2***), noting variation inherent to diet. The reduction in MY may be due to a reduced methanogen population, an altered pattern of volatile fatty acid production and hydrogen availability; and dry matter digestion in the rumen. Our results did not show reduction in DM digestibility (P = 0.91) or NDF digestibility (P = 0.87). The decline in methanogenesis associated with removal of protozoa is greatest on high concentrate diets and this is in keeping with protozoa being relatively more important sources of hydrogen on starchy diets, as many starch-fermenting bacteria do not produce H_2.

Phytochemicals

For the purpose of the present study, a wide array of heterogeneous plant secondary compounds with CH_4 mitigation effects were grouped in phytochemicals (Patra and Saxena, 2010) including tannin-rich feeds, essential oils, and saponins but excluding macroalgal bromoform. When using phytochemicals as feed additives, one should pay close attention to the dose and quality, as they may possess anti-nutritional characteristics at higher levels. The aim is to find the equilibrium between the beneficial CH_4 abatement and optimum nutrient utilisation. This balance is particularly complex to attain as the composition and quantity of phytochemicals varies widely within natural sources (e.g., legumes), even when fed as extracts. More than 200,000 plant secondary compounds have been identified (Hartmann, 2007), and some have anti-methanogenic proprieties.

Saponins are high molecular-weight glycosides that occur in a variety of plants, with triterpene saponins (i.e., saccharide chain units linked to a triterpene) more abundant in nature than steroidal saponins (Hostettmann and Marston, 2005). The CH_4-suppressing traits of saponin-rich plants are related to the inhibition of rumen ciliate protozoa, which may enhance efficiency of synthesis of microbial protein (Patra and Saxena, 2009). Similar beneficial effects on N and energy ruminal metabolism have been observed when feeding tannins to ruminants (Norris et al., 2020). Tannins are high molecular weight polyphenolic compounds soluble in water and have capacity to interact with proteins (and carbohydrates) due to the presence of a large number of phenolic hydroxyl groups forming complexes (Patra and Saxena, 2010). They exist as hydrolysable tannins (HT) and condensed tannins (CT); both have antimethanogenic effects, however CT are usually used as feed additive as HT represents high risk of toxicity to the animal (Field and Lettinga, 1987, McSweeney *et al.*, 2001).

Essential oils

Essential oils are not based on long chain fatty acids but are bio-active molecules with antimicrobial properties that can directly inhibit methanogens and hydrogen-producing microorganisms. They include garlic oil, thymol, cinnamaldehyde, peppermint, menthol and eucalyptus oils, as well as commercial blends. The type of essential oil will define the effect on CH_4 production. Moreover, it is important to consider the potential anti-nutritional effect of essential oils and also the adaptation of rumen microbes to essential oils, the change in flavour of animal products due to presence of residues in meat and milk, as well as acceptability by the animals, which could affect DMI (Rae, 1999, Calsamiglia *et al.*, 2007). In this regard, the present meta-analysis indicated no effect of phytochemical inclusion on DMI of dairy, beef cattle, and sheep (mean effect of 1.00 ± 0.00386; 95% CI 0.992 – 1.01; P = 0.81; n = 33; ***Figure 5***).

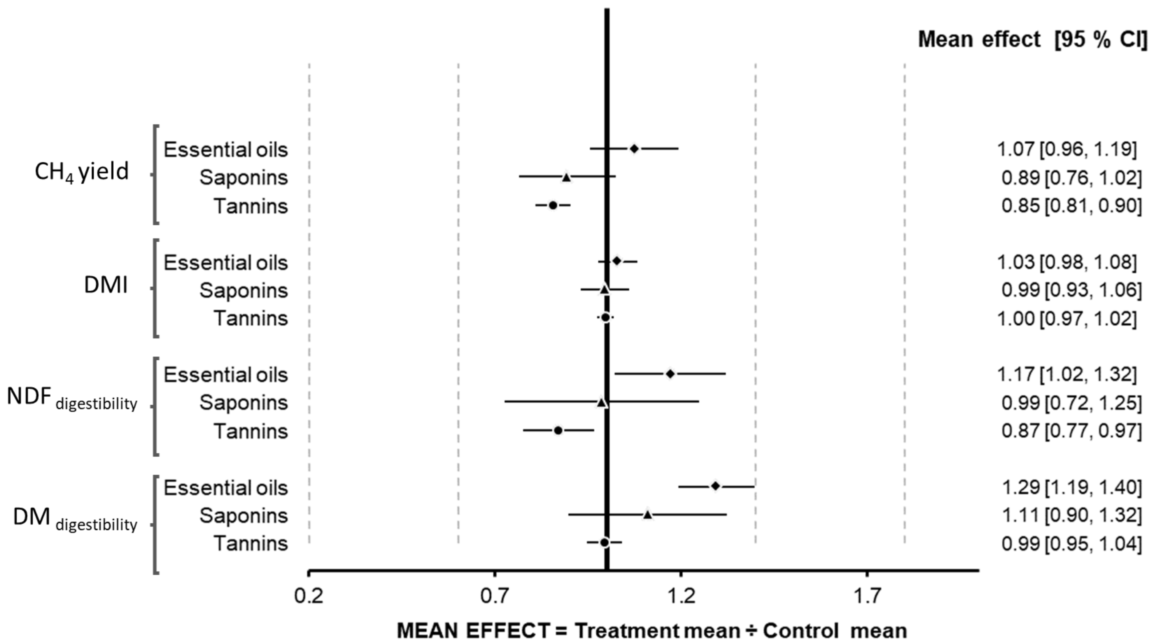

Figure 5 Mean effect of the estimated ratio of diets essential oils (diamond), saponins (triangle), and tannins (circle) and control diets in methane yield, DMI, NDF digestibility and DM digestibility. Source: Almeida et al., 2021.

Among studies included in the present analysis, 24% trialled saponins, 50% used tannins and 21% fed essential oils, while 5% of these studies examined other phytochemicals (e.g., flavonoids). The estimated mean reduction on MY through phytochemical supplementation was 10% compared with the control diet (16.7 ± 1.11 g vs. 18.6±1.12 CH_4/kg DMI; P<0.01; n = 33), with tannins and saponins having greatest effect (***Figure 5***). The observed mean reduction in MY due to phytochemical supplementation ranged from 8 to 14 % (***Figure 2***; 95% CI; P<0.01; n = 33). Additionally, phytochemical inclusion in the diet of ruminants affected fibre digestibility (mean reduction in NDF digestibility of 4.69%; 95% CI 0.86 – 8.56; P = 0.02; n = 21), without affecting total tract DM digestibility (mean effect 95% CI 0.97 – 1.01; P = 0.97; n = 21). Overall, phytochemical supplementation tended to reduce CH_4 intensity in ruminant animals (mean effect 0.922 ± 0.0351; 95% CI 0.83 – 1.00; P = 0.08; n = 21).

Notably the dietary inclusion of phytochemicals does not identify a simple recommendation due to high variation in concentration and type of compound, and spatial and temporal inconsistency.

3-nitrooxypropanol (3-NOP)

The commercially developed compound 3-NOP provides a novel and promising feed additive used to mitigate CH_4.It is a structural analogue of the nickel enzyme methyl CoM reductase produced by the methanogenic archaea, thus it inhibits the last step of CH_4 formation in the rumen (Duin et al., 2016). Previous, studies have shown that 3-NOP is a potent CH_4 suppressant, effective in a wide range of diet types, exhibiting no DMI nor digestibility impairment in beef or dairy cattle (Romero-

Perez et al., 2014, Haisan et al., 2017, Jayanegara et al., 2017). Research has found that 3-NOP is metabolised rapidly, not building up in the mammal's bloodstream (Thiel et al., 2019a). Moreover, 3-NOP and its metabolites were not found to have mutagenic or genotoxic potential (Thiel et al., 2019b). Thus, 3-NOP does not seem to represent a food security threat or risk to animal health.

Reviewing previous studies, the in-feed doses of 3-NOP fed to ruminants ranged from 40 to 340 mg 3-NOP/kg DM (64.2 to 122 mg 3-NOP/kg DM; 95% CI) and responses are highly dose dependent. From our meta-analysis, 3-NOP supplementation decreased ruminant methane emission by 23.3% compared with a control diet (15.1 ± 0.995 g vs. 19.7±1.11 CH_4/kg DMI; P<0.01; n = 14). The mean reduction in the MY ranged from 18 to 39 % (95% CI; P < 0.01; n = 14; ***Figure 2***). All individual studies used in the present meta-analysis underscored the efficacy of 3-NOP in lowering enteric CH_4 emissions (Table 1).

Previously, CH_4 abatement achieved with dietary 3-NOP in ruminants was associated with a decrease in DMI (Romero-Perez et al., 2014; Vyas et al., 2016) and this was borne out in the current analysis where 3-NOP supplementation reduced DMI up to 4.5 % (P = 0.02; ***Figure 6a***; n = 14). In the present study, the 3-NOP supplementation did not alter fibre digestibility (i.e., NDF; P = 0.25; ***Figure 6b***; n = 5). Moreover, the reduction in MY with 3-NOP ranged from 6.5 to 38% in dairy cattle (mean of 22.2%; P < 0.01; n = 7) and from 1.5 up to 59% in beef cattle (mean of 30.0%; P < 0.01; n = 7). In contrast, previous studies suggested stronger antimethanogenic effects of 3-NOP in dairy cattle than in beef cattle (Dijkstra et al., 2018), and also that 3-NOP has a greater CH_4 suppressing effect in high-forage than high-grain feedlot diets (Kim et al., 2019). It seems that the antimethanogenic effectiveness of 3-NOP differs between ruminant species; one may expect a larger variation within the grazing beef compared to dairy production systems.

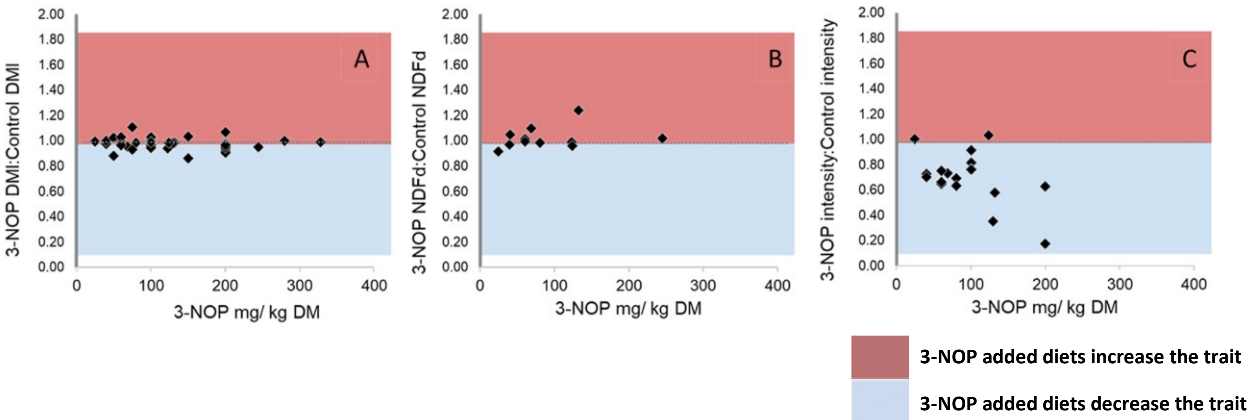

Figure 6 Mean effect of the estimated ratio of diets containing 3-nitrooxypropanol (3-NOP) and control diets in DMI (a), NDF digestibility (b) and CH_4 intensity (c). Source: Almeida et al., 2021.

The overall reduction in CH_4 intensity (g CH_4/kg of milk or weight gain) from 3-NOP ranged from 13.2 to 39.9% (P<0.01; ***Figure 6c***). Thus, 3-NOP may offer a reliable and effective strategy for CH_4 abatement in beef, sheep and dairy cattle, yet as a relatively novel feed additive, there may be resistance to adoption, particularly as the magnitude of the mitigation appears to differ between ruminant species. Optimal doses of 3-NOP to be used are yet to be defined to enable registration as a permitted feed additive, enabling the use of 3-NOP in the meat, wool and dairy industries in Australia.

Other feed additives with CH_4 mitigation effects

Among the reviewed CH_4 reduction strategies, it was found that comprehensive data regarding the use of bacteriocins (for review: Garsa et al., 2019), organic acids and prebiotics (e.g., acetogens, yeasts) (Martin et al., 2010, Patra, 2016) is too sparse to support adoption in the next 10 years. Moreover, these technologies generally yield modest results in CH_4 abatement, thus they were not suitable to be included in the present meta-analysis.

2. Farm management practices as a route to enteric methane mitigation

On-farm management approaches to mitigate CH_4 emissions may also be used. Usually they involve management decisions and interventions targeted to increase productivity and/or minimise consumption of feed by non-productive animals. In this regard, breeding strategies, reproductive rate, stock number and productivity, pasture management, as well as vaccination, may contribute (in some cases additively) as CH_4 reduction strategies for the enterprise. Unlike most of the feed additive strategies previously described in the section 1 meta-analysis, the whole farm system options may be readily applied to grazing animals as well as feedlot and dairy cattle.

Modifications of the on-farm production system itself are particularly desirable in reducing livestock emissions, because in general they use known and available technologies; do not require new chemical products; and are associated with increased animal productivity. These changes include intensification made possible through improved feedbase (Pinares-Patiño et al., 2009), improved reproductive performance (Alcock and Hegarty 2011) and/or improved metabolic efficiency of the animal, that could be achieved by breeding (Okine et al., 2006) or improved health (Eckard et al. 2010). All these strategies rely upon decreasing the proportion of feed consumed (and therefore the quantity of methane produced) by the breeding females in the flock or herd. By either reducing daily emissions from this group or increasing the commercial product they yield, the emission/unit product is reduced. Consequently, they provide significant flexibility for the land manager to rearrange his land allocation and stock numbers around personal priorities for emission and productivity targets (Alcock and Hegarty 2011).

The impact of changing the system's feed or animal management on enterprise emissions is wholly dependent upon what the manager chooses to do with livestock numbers and area under grazing. All that these management strategies provide to a livestock manager is opportunity; opportunity to choose the balance of emission change or productivity change they want. Alcock and Hegarty (2011) explored this for lamb producers at Cowra in NSW and found most of the management technologies considered reduced emissions intensity by 10% - 20% comparing the worst with the best practices. However, if breeding stock are managed to be more productive (e.g., by superior nutrition leading to greater product/breeder) the farmer is faced with the challenge of whether to:

- increase stock numbers to gain extra product (for the same or more emissions).
- reduce stock number to maintain previous product output with less enterprise emissions and so make land available for other uses (eg. Tree planting, conservation zone)

The means by which animal and feed management can be changed to provide this opportunity are described in the sections below.

Animal management

There are multiple aspects of managing the animals in the grazing system that can affect emission intensity and total emission from the enterprise. These include decisions around reproduction, age at first joining, lifetime in the herd as well as health management and selection of the genotype of the animals being bred. These changes are considered individually and then the impact of changing reproductive rate (as a sum of these strategies) in NSW livestock by 5-10% is assessed using simulations to verify the sensitivity of the current methodology used to calculate methane emissions for the National Greenhouse Gas Inventory.

Age at first joining

Animals intended for breeding are not contributing economically until they deliver their first progeny, but consume feed and generate GHG emissions throughout their life. So one way to increase efficiency in breeding is to reduce the time before animals breed. This has substantial impacts in reducing the emission intensity of milk (Christie et al., 2016) and of lamb, as well as increasing profitability (Alcock et al., 2015; Tocker et al., 2020). Similarly, in beef production, joining at a younger age was shown to be economically preferable to joining at later ages, irrespective of the culling for age rule (Nunez-Dominguez et al., 1991). However a caveat remains that unless replacement females are at a suitable weight to achieve successful early joining, then joining underweight young animals will be disadvantageous to the economic yield of the flock (Farrell et al., 2019) and it would be expected that the same adverse effect would diminish emission intensity gains due to the supplementary feeding of lighter weight dry females that either fail to get pregnant or fail to rebreed due to weight and condition.

Lifetime in the herd or flock

Culling for age is a standard management practice across most NSW beef and sheep properties. Optimum age structure in a flock or herd can be readily estimated for optimised genetic gain (eg. Turner et al., 1968). However, there is increasing awareness that culling for age is a simplistic and far from optimum approach to manage flock structure as it fails to capitalize on the opportunity of retaining high performing individuals in the herd/flock (Hatcher et al., 2018; Richards et al., 2018). The implications of retaining the more-productive females in the flock/herd is two-fold for emissions; (i) The number of non-productive females being reared to mating age is reduced, resulting in less methane produced by animals not contributing to marketable livestock and (ii) The non-productive (but methane producing) rearing period of replacements becomes a lower proportion of the average lifetime of breeders in the herd/flock, so the emission intensity over the lifetime is reduced. The same principles apply in dairy where extended lactations (within a year) reduce emissions intensity of milk production (Lehmann et al., 2014). Doran-Browne et al. (2015) found the economic impact of extending productive life was greater in the dairy herd than in wool growing enterprises.

Fecundity management

Consistent with reducing emission intensity, increasing the reproductive output can be achieved by nutritional and animal management/culling as described above, but can also be modified by targeted interventions to ovulation. Specifically, this includes ovulation stimulation by a nutritional pulse (flushing; Banchero et al., 2021), and breeding for improved ovulation (Fogarty, 2009). An injection providing active immunization of sheep against androstenedione increased ovulation rate and was commercially released (Fecundin; Coopers Animal Health Australia) but is no longer available.

Harrison et al., (2014) showed that increasing fecundity, reduced emission intensity from 9.3 to 7.3 tCO_2-e/t clean fleece weight plus liveweight, in a sheep breeding enterprise. There is scope for strategic supplements to be used by breeding flocks in NSW to increase reproductive output and consequently change herd/flock structure and reduce emission intensity.

Improved health management

Health management has a larger role in dairy than in beef or sheep enterprises and the role of health management as an emission reduction strategy has been reviewed by Knapp et al. (2014). The principal period of infectious and metabolic disease is in the first 60 days post-calving and disease in this period is likely to multiply its impact by not only poor milk production but poor reproduction. A 5% reduction in culling for disease and associated rise in milk yield/cow was estimated to reduce the emission intensity of milk by 8-12% (Knapp et al., 2014).

In sheep, culling for age at a younger age is undertaken, in part, to avoid the rise in dental health problems as sheep change from “full mouth” with all teeth, through to broken then gummy as teeth fall out with age. However, moving to a younger average age for such health reasons lowers flock economic performance (Farrell et al., 2019). As previously identified, all health management strategies that improve productivity per animal will reduce emission intensity (Hegarty et al., 2010).

The shorter lifetimes for beef cattle compared with dairy cattle and wool sheep mean that health management has lesser importance as an emissions reduction strategy in beef. Nevertheless, maintaining herd health through good husbandry including disease prevention practices is important to achieving target growth rates and reproductive performance.

Genetics

Methane yield (MY) has been found to have a low heritability (0.1 – 0.2) in both sheep and cattle but no undesirable correlation with economic (growth, reproduction or commercial) traits (Herd et al., 2019). The converse implication of these low phenotypic and genotypic correlations between methane yield and productivity traits is that there seems little scope for indirectly reducing MY by selection for correlated traits. New Zealand has increasingly used direct measurement of GHG emissions from sheep in portable accumulation chambers to underpin direct genetic improvement in MY, and are looking to inform selection through breeding values (Jonker et al., 2018; Ludemann et al., 2012) into a highly regulated seedstock supply chain for rams in that country. In Australia while heritability of MY has also been shown for sheep (Robinson *et al*., 2014) and cattle (Donoghue et al., 2015), the principal focus has been on implementing breeding values for beef in BREEDPLAN, Australia's mechanism for providing Estimated breeding values (EBV) to cattle breeders. These mechanisms are embedded in BREEDPLAN for the Angus breed but have not as yet been activated. The key criticism of genetic improvement is the slow progress and that applying pressure on one trait reduces pressure able to be applied on other traits. Despite this, the certainty of progress and the readily-adopted mechanism for implementation make it a desirable approach, which could deliver slow but steady improvement (Fennessy et al., 2019). While recognizing that the rumen and faecal biomes are correlated with methane production (Andrade et al., 2020) and that buccal biomes have also been assessed as potential selection tools for breeding for low MY, they are unlikely to develop as stand-alone chemical markers in a genomic empowered world.

The ability to make genetic gain using genomic markers and genomic breeding values (Jonker et al., 2018) will make breeding a far more attractive strategy in coming decades. In this regard, previous studies indicated that MY of beef cattle may be reduced by 1.45% annually if genetic selection is focused on CH_4 (Fennessy et al., 2019). All of the above changes can improve the reproductive performance, and the implications of this for NSW emissions are simulated below.

Simulations of NSW impacts of improved reproductive performance

The state inventory shows 91% of enteric methane is sourced from sheep and grazing cattle (i.e., 92% of ruminant population; ***Figure 7***). Moreover, cows (15,868,299 head) and ewes kept for reproduction purposes (42,121,348 head) comprise 62% of the total ruminant population in NSW (***Figure 8***), and contribute 60% of the state's enteric methane emissions.

Simulations were run to assess the effect of an increase in reproductive rate on emissions and emission intensity. Baseline values of average NSW beef cow (85%) and sheep (125%) fertility were assumed. Simulations examined the emission impacts of improving reproductive rates by 5 and 10%. The effects of raising the reproductive rate of breeders on NSW emissions, based on the state ruminant population (ABS, 2018), were modelled considering adoption rates of 50, 60, 70 & 80%.

The method used in the national and state inventory to estimate enteric methane emissions differs between sheep and cattle. Sheep emissions are based on calculations that include liveweight, growth and dry matter digestibility (DMD) of feed to calculate dry matter intake (DMI), while beef emissions rely on animal liveweight and growth to calculate DMI. There is an assumed relationship between dry matter intake (DMI) and methane (CH_4) production of 20.7 g CH_4/kg DMI (Charmley et al., 2015) for beef cattle, whereas for sheep the linear equation proposed by Howden et al. (1996) is adopted: CH_4 (kg) = DMI × 0.0188 + 0.00158.

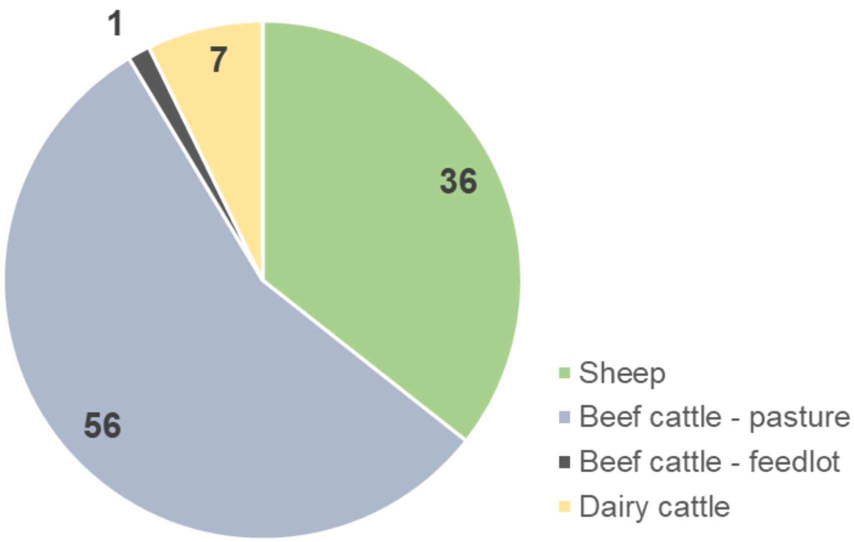

Figure 7 Contribution of each ruminant species and category (%) to the total enteric methane production in New South Wales (NSW). Using the National inventory report methodology and total number of ruminants in NSW (Source: AGEIS, ABS, from MLA, 2020), the total methane emission of NSW was estimated as 527 M tonnes of CH_4 per year.

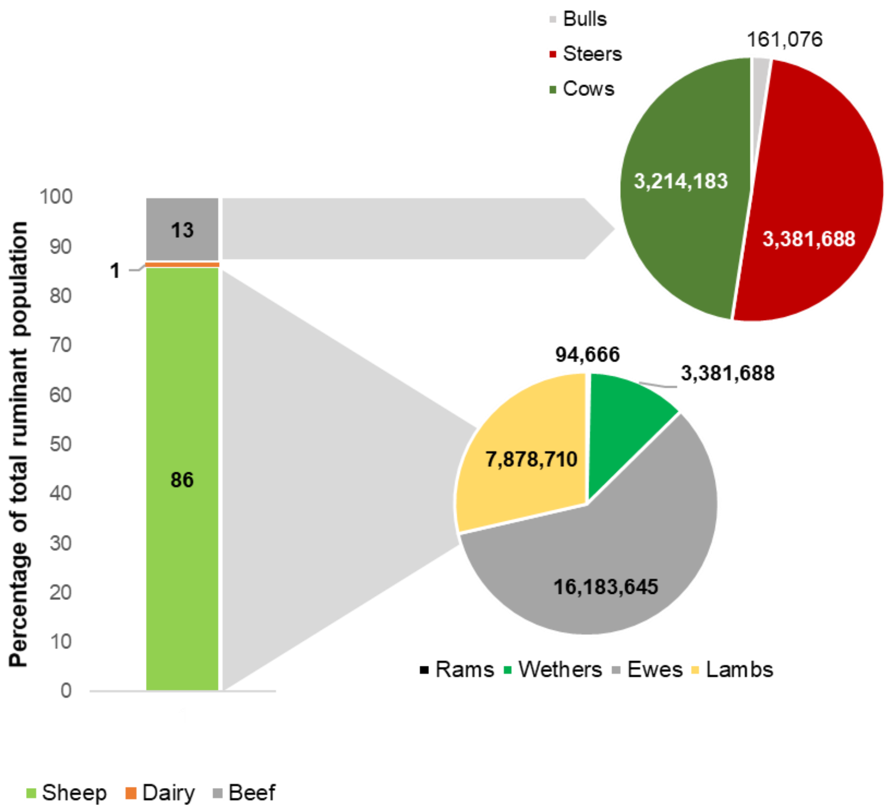

Figure 8 Ruminant population by species (vertical bar) and categories (pie chart) in New South Wales (NSW). The total number of ruminants in NSW is 31,031,785 head (Source: AGEIS, ABS, from MLA, 2020), from which 86% are sheep, 13% are beef cattle and only 1% are dairy cattle.

Sensitivity analysis

Considering that females for breeding purposes, between sheep (12,982,446 head) and beef cows (2,617,012 head), are more than half of the ruminant population and emit 60% of the enteric methane, targeting improved fertility in breeding females is a real opportunity to reduce methane emission in NSW. The simulations were run reflecting a constant output of saleable animals, meaning that as reproductive rate increased, the number of breeding females in the state decreased.

Specifically, improving fertility by 5% is predicted to result in a reduction in current emissions by 1.56, 1.87, 2.18 and 2.50% for 50, 60, 70 and 80% adoption, respectively (***Figure 9***). Similarly, by improving fertility by 10%, the expected abatement in methane emissions would be 2.97, 3.56, 4.16, and 4.75% for 50, 60, 70 and 80% adoption, respectively (***Figure 9***).

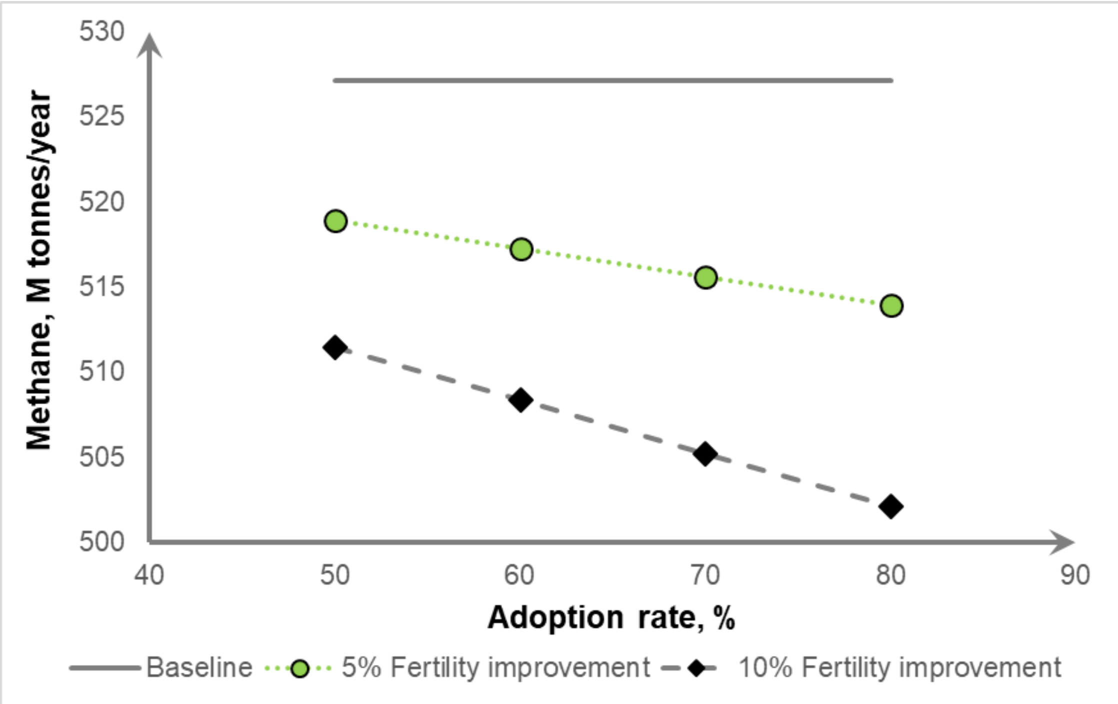

Figure 9 Sensitivity of total methane production per year in New South Wales (NSW) to 5% and 10% fertility improvement in sheep and beef cattle compared to the baseline methane production.

In this simulation, the main driver in methane abatement (M tonnes/year) was the reduction in breeding females while maintaining the same output. Using a different approach, a modelling study of cattle properties in dry inland regions (Cullen et al., 2016) found that improved reproduction gave a 22-28% decline in GHG emissions intensity (t CO_2-e t^{-1} liveweight sold), largely due to higher weaning rates, but would likely increase total methane production due to increase in number of animals. This improved profitability by $38-62 000 due to more liveweight sold for the same breeder numbers.

Methanogen vaccines

Vaccination is a standard animal management practice in NSW and may offer a management tool that reduces emissions without requiring animal or feed-base management change. Vaccines have the potential to modify the ruminal microbiome targeting methane-producing microbes in the rumen through a non-nutritional mechanism. The vaccine would induce a serum antibody response against methanogenic microbes, resulting in CH_4 abatement (Williams *et al.*, 2009). These antibodies flow via saliva into the digestive tract where the methanogens are inhibited (Wright *et al.*, 2004). To date, vaccination has not provided any effective and clear response in modifying CH_4 emissions by ruminants. Given the inherent diversity within the rumen, and amongst methanogens across the globe, the use of vaccines to mitigate CH_4 in ruminants faces challenges. Early *in vivo* studies showed variation from 7% reduction of CH_4 up to 20% increase in CH_4 (Wright *et al.*, 2004, Williams *et al.*, 2009). If an effective vaccine is developed, the vaccine may be universally applied to flocks and herds. The New Zealand-based team that took over vaccine research from CSIRO has released data showing immunoglobulin development in sheep (Subharat et al., 2016), but has not published evidence of consistent mitigation being achieve *in vivo*.

Feedbase management

Improving quality of available forage

The key consequences of improved pasture quality occur through animal productivity impacts such as faster weight gain and/or wool growth, shorter lifespan for slaughter animals, higher breeder body condition score so reduced age to joining, so reduced need for replacement animals and perhaps longer persistence in the herd/flock.

Thus, improving nutrition supports the implementation of the animal management strategies described above.

Very simplistically and without considering known flow-on effects of improved digestibility on growth (as the national inventory largely does not permit this), the effect of increased DMD for sheep in NSW was simulated using the National Inventory Report (https://www.industry.gov.au/data-and-publications/national-greenhouse-accounts-2019/national-inventory-report-2019) framework. In the inventory, seasonal values for DMD are assumed to be 75, 61, 64, and 72% for spring, summer, autumn and winter, respectively for sheep only. Simulating a 5% increase in the average digestibility of NSW pasture in each season, the estimated methane output from the sheep industry would increase by 6% (considering 100% adoption rate), as a consequence of increase in intake by grazing sheep. However, this simulation does not include DMD effects on average daily weight gain in sheep, so does not reflect the reality of the biological system where pasture and animals are interdependent.

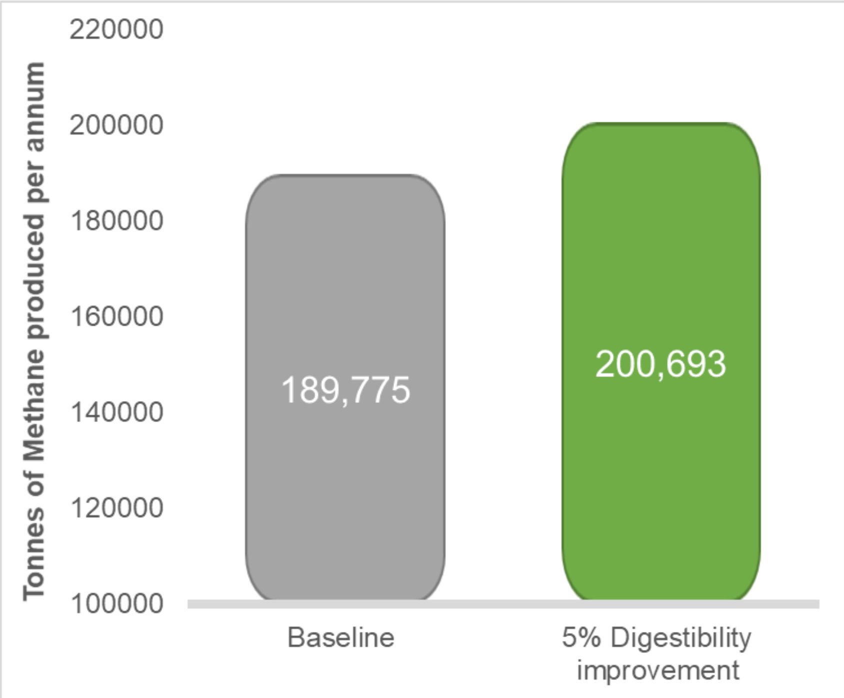

Figure 10 Sensitivity of total methane production per year in New South Wales to a 5% improvement in dry matter digestibility of pastures across all seasons, based on the National Inventory Report calculations, compared to the baseline methane production (https://www.industry.gov.au/data-and-publications/national-greenhouse-accounts-2019/national-inventory-report-2019).

In practice, however, improved DMD may not lift feed intake, so the realized impact is likely to be no change in total methane produced but likely to be accompanied by an increase in growth, thus the carbon footprint of the meat produced would decrease. While this is not apparent in the national Inventory, reflecting its insensitivity, these system-effects on emissions intensity are the basis for most progress in reducing the methane emissions from ruminant production. Therefore, the responsiveness of the set of equations used in the framework must be improved before the producer could benefit from carbon credit income from improving pasture digestibility. There must be a coming together of feedbase change and animal number or management change, for a grazing system to maintain some sort of equilibrium and thus to be sustainable. This coming together is exemplified in the push to intensive animal agriculture and is discussed below.

Intensification of livestock systems

The impact of intensification on enteric emissions has been regularly displayed in the decline in milk emission intensity that has been reported globally (Niu *et al.*, 2018, Naranjo *et al.*, 2020). Life cycle assessment and whole-farm modelling has indicated the potential to reduce emissions intensity of pasture-fed sheep and beef production through on- or off-farm feedlot finishing or supplementary grain feeding (White et al., 2010; Harrison et al., 2014; Taylor and Eckard, 2016; Wiedemann et al., 2017). As with improved reproductive performance, intensifying production creates opportunity to raise more stock on the same land area or keep the same number of animals but on a reduced area of land, while keeping product output unchanged. With "systems thinking" this gives the land manager the opportunity to take some land out of grazing should they wish, perhaps for reforestation; this choice will depend on the balance of environmental and economic priorities for the livestock manager. While the long-term impact of this has been modelled for sheep enterprises in NSW (Alcock et al., 2015a; Doran-Browne et al., 2016), further system modelling on the merit of land use change associated with intensity, animal efficiency and reproductive performance is required especially for beef.

What is clear is that managing the animals through grazing, genetic selection, culling and early breeding decisions, together with managing the pasture feedbase in its composition, quality and potentially methane suppressing swards (see also phytochemicals section, above), supplementary feeding and feedlot finishing have great potential to mitigate emissions both in absolute terms (kg CH_4/d) and in emission intensity of the livestock products. The enterprise –wide outcome is subject to matching decisions on total livestock numbers and land area used. The efficacy of combinations of subsets of this suite of decisions is evident in assessment in the dairy industry (Beukes et al., 2010), sheep industry (Alcock et al., 2015), and in explaining differences in greenhouse outputs and profitability of diverse beef enterprises (Harrison et al., 2016). These positive abatement responses to animal and feed management are seen across globally-diverse feeding/grazing systems (Gerssen-Gondelach et al., 2017), even when the assessment is made on a life cycle basis. This gives confidence that a set of mitigation strategies can deliver consistent mitigation outcomes in grazing systems across NSW.

Including methane suppressing forages in grazing systems

Just as anti-methanogen vaccines offer a methane-specific animal option, so anti-methanogenic forages offer a methane-specific plant option for enteric methane mitigation.

No matter what grazing-based production system is in use, there is scope to change not only the general nutritional value of the forage, but to introduce specific methane suppressing forages. As described in the phytochemicals section, plants that have elevated levels of condensed tannins offer the primary forage-based possibility of reducing enteric methane production while maintaining or increasing animal performance. These include *Lotus spp.,* (Banik et al., 2013), *Desmanthus* (Suybeng et al., 2019) and the more tropically-adapted *Leucaena* and *Gliricidia* browses which are all at commercial or near commercial stages of availability. Desmanthus is suited to the warm non-frosting regions of NSW. Similarly, the tree legume *Leucaena leucocephala* is also suited to these areas and can deliver commercially significant methane mitigation (Tomkins et al., 2019) but is not currently recommended in NSW due to its propensity to become a weed. While other forages have shown promise (eg *Biserrula pelecinus*, *Eremophila glabra;* Durmic et al., 2021) they are not close to commercial release.

One of the key advantages of leguminous methane-active forages is the additional dietary protein they supply which can be seasonally important in drier regions such as NSW's north and north-west. Thus, the advantage of these methane inhibitory forages is they not only suppress emissions chemically, but can affect total diet digestibility and dry matter intake and therefore have a flow-on catalytic role in stimulating animal liveweight gain and thereby moving the system to both lower emissions and higher productivity.

Conclusion

The meta-analysis assessed the available CH_4 mitigation strategies in ruminant production systems. In this regard, seaweed, 3-NOP, oil and NO_3^- , as feed additives, are among the most promising technical options for direct abatement of livestock CH_4 emissions from ruminant production in the next 10 to 20 years.

However, whole-farm strategies that combine changes to on-farm management of livestock and improved feed base provide multiple and interacting opportunities to reduce total enteric emissions, emission intensity, and either increase production or reduce the land area used and number of stock required, enabling mitigation at regional and global scales. Further investigation is required of combinations of different strategies for CH_4 mitigation using a systemic approach, to inform policy recommendations.

3. References for Section1 meta-analysis

Alemu AW, Romero-Perez A, Araujo RC and Beauchemin KA 2019. Effect of Encapsulated Nitrate and Microencapsulated Blend of Essential Oils on Growth Performance and Methane Emissions from Beef Steers Fed Backgrounding Diets. Animals (Basel) 9.

Beauchemin KA and McGinn SM 2006. Methane emissions from beef cattle: Effects of fumaric acid, essential oil, and canola oil1. Journal of animal science 84, 1489-1496.

Beauchemin KA, McGinn SM and Petit HV 2007. Methane abatement strategies for cattle: Lipid supplementation of diets. Canadian Journal of Animal Science 87, 431-440.

Beauchemin KA, McGinn SM, Benchaar C and Holtshausen L 2009. Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: effects on methane production, rumen fermentation, and milk production. J Dairy Sci 92, 2118-2127.

Benchaar C 2016. Diet supplementation with cinnamon oil, cinnamaldehyde, or monensin does not reduce enteric methane production of dairy cows. Animal 10, 418-425.

Benchaar C, Hassanat F, Martineau R and Gervais R 2015. Linseed oil supplementation to dairy cows fed diets based on red clover silage or corn silage: Effects on methane production, rumen fermentation, nutrient digestibility, N balance, and milk production. J Dairy Sci 98, 7993-8008.

Bird SH, Hegarty RS and Woodgate R 2008. Persistence of defaunation effects on digestion and methane production in ewes. Australian Journal of Experimental Agriculture 48.

Caetano M, Wilkes MJ, Pitchford WS, Lee SJ and Hynd PI 2019. Effect of ensiled crimped grape marc on energy intake, performance and gas emissions of beef cattle. Animal Feed Science and Technology 247, 166-172.

Carulla J, Kreuzer M, Machmüller A and Hess H 2005. Supplementation of Acacia mearnsii tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Australian Journal of Agricultural Research 56, 961-970.

Carvalho IPCd, Fiorentini G, Berndt A, Castagnino PdS, Messana JD, Frighetto RTS, Reis RA and Berchielli TT 2016. Performance and methane emissions of Nellore steers grazing tropical pasture supplemented with lipid sources. Revista Brasileira de Zootecnia 45, 760-767.

Chung Y-H, Mc Geough E, Acharya S, McAllister T, McGinn S, Harstad O and Beauchemin K 2013. Enteric methane emission, diet digestibility, and nitrogen excretion from beef heifers fed sainfoin or alfalfa. Journal of animal science 91, 4861-4874.

Cooprider KL, Mitloehner FM, Famula TR, Kebreab E, Zhao Y and Van Eenennaam AL 2011. Feedlot efficiency implications on greenhouse gas emissions and sustainability. J Anim Sci 89, 2643-2656.

Cosgrove GP, Waghorn GC, Anderson CB, Peters JS, Smith A, Molano G and Deighton M 2008. The effect of oils fed to sheep on methane production and digestion of ryegrass pasture. Australian Journal of Experimental Agriculture 48.

de Oliveira SG, Berchielli TT, Pedreira MdS, Primavesi O, Frighetto R and Lima MA 2007. Effect of tannin levels in sorghum silage and concentrate supplementation on apparent digestibility and methane emission in beef cattle. Animal Feed Science and Technology 135, 236-248.

Ding X, Long R, Zhang Q, Huang X, Guo X and Mi J 2012. Reducing methane emissions and the methanogen population in the rumen of Tibetan sheep by dietary supplementation with coconut oil. Trop Anim Health Prod 44, 1541-1545.

Duthie CA, Troy SM, Hyslop JJ, Ross DW, Roehe R and Rooke JA 2018. The effect of dietary addition of nitrate or increase in lipid concentrations, alone or in combination, on performance and methane emissions of beef cattle. Animal 12, 280-287.

El-Zaiat H, Araujo R, Soltan YA, Morsy AS, Louvandini H, Pires A, Patino HO, Corrêa PS and Abdalla AL 2014. Encapsulated nitrate and cashew nut shell liquid on blood and rumen constituents, methane emission, and growth performance of lambs. Journal of animal science 92, 2214-2224.

Fiorentini G, Carvalho IPC, Messana JD, Castagnino PS, Berndt A, Canesin RC, Frighetto RTS and Berchielli TT 2014. Effect of lipid sources with different fatty acid profiles on the intake, performance, and methane emissions of feedlot Nellore steers1. Journal of animal science 92, 1613-1620.

Grainger C, Williams R, Eckard RJ and Hannah MC 2010. A high dose of monensin does not reduce methane emissions of dairy cows offered pasture supplemented with grain. J Dairy Sci 93, 5300-5308.

Grainger C, Clarke T, Beauchemin KA, McGinn SM and Eckard RJ 2008a. Supplementation with whole cottonseed reduces methane emissions and can profitably increase milk production of dairy cows offered a forage and cereal grain diet. Australian Journal of Experimental Agriculture 48.

Grainger C, Clarke T, Auldist M, Beauchemin K, McGinn S, Waghorn G and Eckard RJ 2009. Potential use of Acacia mearnsii condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. Canadian Journal of Animal Science 89, 241-251.

Grainger C, Auldist MJ, Clarke T, Beauchemin KA, McGinn SM, Hannah MC, Eckard RJ and Lowe LB 2008b. Use of monensin controlled-release capsules to reduce methane emissions and improve milk production of dairy cows offered pasture supplemented with grain. J Dairy Sci 91, 1159-1165.

Granja-Salcedo YT, Fernandes RM, de Araujo RC, Kishi LT, Berchielli TT, de Resende FD, Berndt A and Siqueira GR 2019. Long-Term Encapsulated Nitrate Supplementation Modulates Rumen Microbial Diversity and Rumen Fermentation to Reduce Methane Emission in Grazing Steers. Front Microbiol 10, 614.

Guyader J, Doreau M, Morgavi DP, Gerard C, Loncke C and Martin C 2016. Long-term effect of linseed plus nitrate fed to dairy cows on enteric methane emission and nitrate and nitrite residuals in milk. Animal 10, 1173-1181.

Guyader J, Eugène M, Doreau M, Morgavi DP, Gérard C, Loncke C and Martin C 2015a. Nitrate but not tea saponin feed additives decreased enteric methane emissions in nonlactating cows1. Journal of animal science 93, 5367-5377.

Guyader J, Eugène M, Meunier B, Doreau M, Morgavi DP, Silberberg M, Rochette Y, Gerard C, Loncke C and Martin C 2015b. Additive methane-mitigating effect between linseed oil and nitrate fed to cattle1. Journal of animal science 93, 3564-3577.

Haisan J, Sun Y, Guan L, Beauchemin K, Iwaasa A, Duval S, Barreda D and Oba M 2014. The effects of feeding 3-nitrooxypropanol on methane emissions and productivity of Holstein cows in mid lactation. Journal of dairy science 97, 3110-3119.

Haisan J, Sun Y, Guan L, Beauchemin KA, Iwaasa A, Duval S, Kindermann M, Barreda DR and Oba M 2017. The effects of feeding 3-nitrooxypropanol at two doses on milk production, rumen fermentation, plasma metabolites, nutrient digestibility, and methane emissions in lactating Holstein cows. Animal Production Science 57, 282-289.

Hegarty RS, Bird SH, Vanselow BA and Woodgate R 2008. Effects of the absence of protozoa from birth or from weaning on the growth and methane production of lambs. Br J Nutr 100, 1220-1227.

Hess HD, Beuret RA, Lötscher M, Hindrichsen IK, Machmüller A, Carulla JE, Lascano CE and Kreuzer M 2016. Ruminal fermentation, methanogenesis and nitrogen utilization of sheep receiving tropical grass hay-concentrate diets offered with Sapindus saponaria fruits and Cratylia argentea foliage. Animal Science 79, 177-189.

Hollmann M, Powers WJ, Fogiel AC, Liesman JS, Bello NM and Beede DK 2012. Enteric methane emissions and lactational performance of Holstein cows fed different concentrations of coconut oil. J Dairy Sci 95, 2602-2615.

Holtshausen L, Chaves AV, Beauchemin KA, McGinn SM, McAllister TA, Odongo NE, Cheeke PR and Benchaar C 2009. Feeding saponin-containing Yucca schidigera and Quillaja saponaria to decrease enteric methane production in dairy cows. J Dairy Sci 92, 2809-2821.

Hosoda K, Nishida T, Park W-Y and Eruden B 2005. Influence of Mentha× piperita L.(peppermint) supplementation on nutrient digestibility and energy metabolism in lactating dairy cows. Asian-Australasian journal of animal sciences 18, 1721-1726.

Hristov AN, Lee C, Cassidy T, Heyler K, Tekippe J, Varga G, Corl B and Brandt R 2013. Effect of Origanum vulgare L. leaves on rumen fermentation, production, and milk fatty acid composition in lactating dairy cows. Journal of dairy science 96, 1189-1202.

Hristov AN, Oh J, Giallongo F, Frederick TW, Harper MT, Weeks HL, Branco AF, Moate PJ, Deighton MH and Williams SRO 2015. An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. Proceedings of the National Academy of Sciences 112, 10663-10668.

Hulshof R, Berndt A, Gerrits W, Dijkstra J, Van Zijderveld S, Newbold J and Perdok H 2012. Dietary nitrate supplementation reduces methane emission in beef cattle fed sugarcane-based diets. Journal of animal science 90, 2317-2323.

Hünerberg M, McGinn SM, Beauchemin KA, Okine EK, Harstad OM and McAllister TA 2013a. Effect of dried distillers grains plus solubles on enteric methane emissions and nitrogen excretion from growing beef cattle1. Journal of animal science 91, 2846-2857.

Hünerberg M, McGinn SM, Beauchemin KA, Okine EK, Harstad OM and McAllister TA 2013b. Effect of dried distillers' grains with solubles on enteric methane emissions and nitrogen excretion from finishing beef cattle. Canadian Journal of Animal Science 93, 373-385.

Johnson KA, Kincaid RL, Westberg HH, Gaskins CT, Lamb BK and Cronrath JD 2002. The Effect of Oilseeds in Diets of Lactating Cows on Milk Production and Methane Emissions. Journal of dairy science 85, 1509-1515.

Jordan E, Lovett DK, Hawkins M, Callan JJ and O'Mara FP 2007. The effect of varying levels of coconut oil on intake, digestibility and methane output from continental cross beef heifers. Animal Science 82, 859-865.

Jordan E, Kenny D, Hawkins M, Malone R, Lovett DK and O'Mara FP 2006a. Effect of refined soy oil or whole soybeans on intake, methane output, and performance of young bulls. J Anim Sci 84, 2418-2425.

Jordan E, Lovett DK, Monahan FJ, Callan J, Flynn B and O'Mara FP 2006b. Effect of refined coconut oil or copra meal on methane output and on intake and performance of beef heifers1. Journal of animal science 84, 162-170.

Jose Neto A, Messana JD, Rossi LG, Carvalho IPC and Berchielli TT 2019. Methane emissions from Nellore bulls on pasture fed two levels of starch-based supplement with or without a source of oil. Animal Production Science 59.

Kim S-H, Lee C, Pechtl HA, Hettick JM, Campler MR, Pairis-Garcia MD, Beauchemin KA, Celi P and Duval SM 2019. Effects of 3-nitrooxypropanol on enteric methane production, rumen fermentation, and feeding behavior in beef cattle fed a high-forage or high-grain diet. Journal of animal science 97, 2687-2699.

Kinley RD, Martinez-Fernandez G, Matthews MK, de Nys R, Magnusson M and Tomkins NW 2020. Mitigating the carbon footprint and improving productivity of ruminant livestock agriculture using a red seaweed. Journal of Cleaner Production 259.

Klevenhusen F, Zeitz JO, Duval S, Kreuzer M and Soliva CR 2011. Garlic oil and its principal component diallyl disulfide fail to mitigate methane, but improve digestibility in sheep. Animal Feed Science and Technology 166-167, 356-363.

Lee C, Araujo RC, Koenig KM and Beauchemin KA 2015. Effects of encapsulated nitrate on enteric methane production and nitrogen and energy utilization in beef heifers1,2. Journal of animal science 93, 2391-2404.

Lee C, Araujo RC, Koenig KM and Beauchemin KA 2017a. Effects of encapsulated nitrate on growth performance, carcass characteristics, nitrate residues in tissues, and enteric methane emissions in beef steers: Finishing phase. Journal of animal science 95.

Lee C, Araujo RC, Koenig KM and Beauchemin KA 2017b. Effects of encapsulated nitrate on growth performance, nitrate toxicity, and enteric methane emissions in beef steers: Backgrounding phase. Journal of animal science 95.

Li L, Davis J, Nolan J and Hegarty R 2012. An initial investigation on rumen fermentation pattern and methane emission of sheep offered diets containing urea or nitrate as the nitrogen source. Animal Production Science 52, 653-658.

Li L, Silveira CI, Nolan JV, Godwin IR, Leng RA and Hegarty RS 2013. Effect of added dietary nitrate and elemental sulfur on wool growth and methane emission of Merino lambs. Animal Production Science 53.

Li X, Norman HC, Kinley RD, Laurence M, Wilmot M, Bender H, de Nys R and Tomkins N 2018. Asparagopsis taxiformis decreases enteric methane production from sheep. Animal Production Science 58, 681-688.

Li, X., Durmic, Z., Liu, S., McSweeney, C.S. and Vercoe, P.E., 2014. Eremophila glabra reduces methane production and methanogen populations when fermented in a rusitec. *Anaerobe*, *29*, pp.100-107

Liu H, Vaddella V and Zhou D 2011. Effects of chestnut tannins and coconut oil on growth performance, methane emission, ruminal fermentation, and microbial populations in sheep. J Dairy Sci 94, 6069-6077.

Lopes J, de Matos L, Harper M, Giallongo F, Oh J, Gruen D, Ono S, Kindermann M, Duval S and Hristov AN 2016. Effect of 3-nitrooxypropanol on methane and hydrogen emissions, methane isotopic signature, and ruminal fermentation in dairy cows. Journal of dairy science 99, 5335-5344.

Ma T, Chen DD, Tu Y, Zhang NF, Si BW and Diao QY 2017. Dietary supplementation with mulberry leaf flavonoids inhibits methanogenesis in sheep. Anim Sci J 88, 72-78.

Ma T, Chen DD, Tu Y, Zhang NF, Si BW, Deng KD and Diao QY 2015. Effect of dietary supplementation with resveratrol on nutrient digestibility, methanogenesis and ruminal microbial flora in sheep. J Anim Physiol Anim Nutr (Berl) 99, 676-683.

Machmüller A, Ossowski DA and Kreuzer M 2000. Comparative evaluation of the effects of coconut oil, oilseeds and crystalline fat on methane release, digestion and energy balance in lambs. Animal Feed Science and Technology 85, 41-60.

Machmüller A, Soliva CR and Kreuzer M 2003. Effect of coconut oil and defaunation treatment on methanogenesis in sheep. Reproduction Nutrition Development 43, 41-55.

Machmüller A, Dohme F, Soliva CR, Wanner M and Kreuzer M 2001. Diet composition affects the level of ruminal methane suppression by medium-chain fatty acids. Australian Journal of Agricultural Research 52, 713-722.

Malik PK, Kolte AP, Baruah L, Saravanan M, Bakshi B and Bhatta R 2017. Enteric methane mitigation in sheep through leaves of selected tanniniferous tropical tree species. Livestock Science 200, 29-34.

Mao H-L, Wang J-K, Zhou Y-Y and Liu J-X 2010. Effects of addition of tea saponins and soybean oil on methane production, fermentation and microbial population in the rumen of growing lambs. Livestock Science 129, 56-62.

Martin C, Rouel J, Jouany JP, Doreau M and Chilliard Y 2008. Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. J Anim Sci 86, 2642-2650.

Martin C, Ferlay A, Mosoni P, Rochette Y, Chilliard Y and Doreau M 2016. Increasing linseed supply in dairy cow diets based on hay or corn silage: Effect on enteric methane emission, rumen microbial fermentation, and digestion. J Dairy Sci 99, 3445-3456.

Martinez-Fernandez G, Duval S, Kindermann M, Schirra HJ, Denman SE and McSweeney CS 2018. 3-NOP vs. halogenated compound: Methane production, ruminal fermentation and microbial community response in forage fed cattle. Frontiers in Microbiology 9, 1582.

McGinn SM, Beauchemin KA, Coates T and Colombatto D 2004. Methane emissions from beef cattle: Effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid1. Journal of animal science 82, 3346-3356.

McGinn SM, Chung YH, Beauchemin KA, Iwaasa AD and Grainger C 2009. Use of corn distillers' dried grains to reduce enteric methane loss from beef cattle. Canadian Journal of Animal Science 89, 409-413.

Melgar A, Harper M, Oh J, Giallongo F, Young M, Ott T, Duval S and Hristov A 2020. Effects of 3-nitrooxypropanol on rumen fermentation, lactational performance, and resumption of ovarian cyclicity in dairy cows. Journal of dairy science 103, 410-432.

Moate PJ, Williams SRO, Grainger C, Hannah MC, Ponnampalam EN and Eckard RJ 2011. Influence of cold-pressed canola, brewers grains and hominy meal as dietary supplements suitable for reducing enteric methane emissions from lactating dairy cows. Animal Feed Science and Technology 166-167, 254-264.

Moate PJ, Williams SR, Torok VA, Hannah MC, Ribaux BE, Tavendale MH, Eckard RJ, Jacobs JL, Auldist MJ and Wales WJ 2014. Grape marc reduces methane emissions when fed to dairy cows. J Dairy Sci 97, 5073-5087.

Mohammed N, Ajisaka N, Lila Z, Hara K, Mikuni K, Hara K, Kanda S and Itabashi H 2004. Effect of Japanese horseradish oil on methane production and ruminal fermentation in vitro and in steers. Journal of animal science 82, 1839-1846.

Moreira GD, Lima Pde M, Borges BO, Primavesi O, Longo C, McManus C, Abdalla A and Louvandini H 2013. Tropical tanniniferous legumes used as an option to mitigate sheep enteric methane emission. Trop Anim Health Prod 45, 879-882.

Mwenya B, Sar C, Santoso B, Kobayashi T, Morikawa R, Takaura K, Umetsu K, Kogawa S, Kimura K, Mizukoshi H and Takahashi J 2005. Comparing the effects of β1-4 galacto-oligosaccharides and l-cysteine to monensin on energy and nitrogen utilization in steers fed a very high concentrate diet. Animal Feed Science and Technology 118, 19-30.

Newbold, C.J., De La Fuente, G., Belanche, A., Ramos-Morales, E. and McEwan, N.R., 2015. The role of ciliate protozoa in the rumen. *Frontiers in microbiology*, *6*, p.1313.

Newbold J, Van Zijderveld S, Hulshof R, Fokkink W, Leng R, Terencio P, Powers W, Van Adrichem P, Paton N and Perdok H 2014. The effect of incremental levels of dietary nitrate on methane emissions in Holstein steers and performance in Nelore bulls. Journal of animal science 92, 5032-5040.

Nguyen SH and Hegarty RS 2017. Effects of defaunation and dietary coconut oil distillate on fermentation, digesta kinetics and methane production of Brahman heifers. J Anim Physiol Anim Nutr (Berl) 101, 984-993.

Nolan JV, Hegarty R, Hegarty J, Godwin I and Woodgate R 2010. Effects of dietary nitrate on fermentation, methane production and digesta kinetics in sheep. Animal Production Science 50, 801-806.

Norris AB, Crossland WL, Tedeschi LO, Foster JL, Muir JP, Pinchak WE and Fonseca MA 2020. Inclusion of quebracho tannin extract in a high-roughage cattle diet alters digestibility, nitrogen balance, and energy partitioning. J Anim Sci 98.

Odongo NE, Or-Rashid MM, Kebreab E, France J and McBride BW 2007a. Effect of supplementing myristic acid in dairy cow rations on ruminal methanogenesis and fatty acid profile in milk. J Dairy Sci 90, 1851-1858.

Odongo NE, Bagg R, Vessie G, Dick P, Or-Rashid MM, Hook SE, Gray JT, Kebreab E, France J and McBride BW 2007b. Long-term effects of feeding monensin on methane production in lactating dairy cows. J Dairy Sci 90, 1781-1788.

Olijhoek DW, Hellwing ALF, Brask M, Weisbjerg MR, Hojberg O, Larsen MK, Dijkstra J, Erlandsen EJ and Lund P 2016. Effect of dietary nitrate level on enteric methane production, hydrogen emission, rumen fermentation, and nutrient digestibility in dairy cows. J Dairy Sci 99, 6191-6205.

Patra AK, Kamra DN, Bhar R, Kumar R and Agarwal N 2011. Effect of Terminalia chebula and Allium sativum on in vivo methane emission by sheep. J Anim Physiol Anim Nutr (Berl) 95, 187-191.

Pen B, Takaura K, Yamaguchi S, Asa R and Takahashi J 2007. Effects of Yucca schidigera and Quillaja saponaria with or without β 1–4 galacto-oligosaccharides on ruminal fermentation, methane production and nitrogen utilization in sheep. Animal Feed Science and Technology 138, 75-88.

Rebelo LR, Luna IC, Messana JD, Araujo RC, Simioni TA, Granja-Salcedo YT, Vito ES, Lee C, Teixeira IAMA, Rooke JA and Berchielli TT 2019. Effect of replacing soybean meal with urea or encapsulated nitrate with or without elemental sulfur on nitrogen digestion and methane emissions in feedlot cattle. Animal Feed Science and Technology 257.

Reynolds CK, Humphries DJ, Kirton P, Kindermann M, Duval S and Steinberg W 2014. Effects of 3-nitrooxypropanol on methane emission, digestion, and energy and nitrogen balance of lactating dairy cows. Journal of dairy science 97, 3777-3789.

Romero-Perez A, Okine E, McGinn S, Guan L, Oba M, Duval S, Kindermann M and Beauchemin K 2014. The potential of 3-nitrooxypropanol to lower enteric methane emissions from beef cattle. Journal of animal science 92, 4682-4693.

Romero-Perez A, Okine E, McGinn S, Guan L, Oba M, Duval S, Kindermann M and Beauchemin K 2015. Sustained reduction in methane production from long-term addition of 3-nitrooxypropanol to a beef cattle diet. Journal of animal science 93, 1780-1791.

Roque BM, Salwen JK, Kinley R and Kebreab E 2019. Inclusion of Asparagopsis armata in lactating dairy cows' diet reduces enteric methane emission by over 50 percent. Journal of Cleaner Production 234, 132-138.

Rossi LG, Fiorentini G, Vieira BR, Neto AJ, Messana JD, Malheiros EB and Berchielli TT 2017. Effect of ground soybean and starch on intake, digestibility, performance, and methane production of Nellore bulls. Animal Feed Science and Technology 226, 39-47.

Santoso B, Mwenya B, Sar C, Gamo Y, Kobayashi T, Morikawa R, Kimura K, Mizukoshi H and Takahashi J 2004. Effects of supplementing galacto-oligosaccharides, Yucca schidigera or nisin on rumen methanogenesis, nitrogen and energy metabolism in sheep. Livestock Production Science 91, 209-217.

Silva RA, Fiorentini G, Messana JD, Lage JF, Castagnino PS, San Vito E, Carvalho IPC and Berchielli TT 2018. Effects of different forms of soybean lipids on enteric methane emission, performance and meat quality of feedlot Nellore. The Journal of Agricultural Science 156, 427-436.

Soltan YA, Morsy AS, Sallam SM, Lucas RC, Louvandini H, Kreuzer M and Abdalla AL 2013. Contribution of condensed tannins and mimosine to the methane mitigation caused by feeding Leucaena leucocephala. Arch Anim Nutr 67, 169-184.

Staerfl SM, Zeitz JO, Kreuzer M and Soliva CR 2012. Methane conversion rate of bulls fattened on grass or maize silage as compared with the IPCC default values, and the long-term methane mitigation efficiency of adding acacia tannin, garlic, maca and lupine. Agriculture, Ecosystems & Environment 148, 111-120.

Sun YK, Yan XG, Ban ZB, Yang HM, Hegarty RS and Zhao YM 2017. The effect of cysteamine hydrochloride and nitrate supplementation on in-vitro and in-vivo methane production and productivity of cattle. Animal Feed Science and Technology 232, 49-56.

Tiemann TT, Lascano CE, Wettstein HR, Mayer AC, Kreuzer M and Hess HD 2008. Effect of the tropical tannin-rich shrub legumes Calliandra calothyrsus and Flemingia macrophylla on methane emission and nitrogen and energy balance in growing lambs. Animal 2, 790-799.

Troy SM, Duthie C-A, Hyslop JJ, Roehe R, Ross DW, Wallace RJ, Waterhouse A and Rooke JA 2015. Effectiveness of nitrate addition and increased oil content as methane mitigation strategies for beef cattle fed two contrasting basal diets1. Journal of animal science 93, 1815-1823.

Van Wesemael D, Vandaele L, Ampe B, Cattrysse H, Duval S, Kindermann M, Fievez V, De Campeneere S and Peiren N 2019. Reducing enteric methane emissions from dairy cattle: Two ways to supplement 3-nitrooxypropanol. Journal of dairy science 102, 1780-1787.

van Zijderveld SM, Dijkstra J, Perdok HB, Newbold JR and Gerrits WJ 2011a. Dietary inclusion of diallyl disulfide, yucca powder, calcium fumarate, an extruded linseed product, or medium-chain fatty acids does not affect methane production in lactating dairy cows. J Dairy Sci 94, 3094-3104.

van Zijderveld SM, Gerrits WJ, Dijkstra J, Newbold JR, Hulshof RB and Perdok HB 2011b. Persistency of methane mitigation by dietary nitrate supplementation in dairy cows. J Dairy Sci 94, 4028-4038.

van Zijderveld SM, Gerrits WJ, Apajalahti JA, Newbold JR, Dijkstra J, Leng RA and Perdok HB 2010. Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. J Dairy Sci 93, 5856-5866.

Velazco JI, Cottle DJ and Hegarty RS 2014. Methane emissions and feeding behaviour of feedlot cattle supplemented with nitrate or urea. Animal Production Science 54.

Veneman JB, Muetzel S, Hart KJ, Faulkner CL, Moorby JM, Perdok HB and Newbold CJ 2015. Does Dietary Mitigation of Enteric Methane Production Affect Rumen Function and Animal Productivity in Dairy Cows? PLoS One 10, e0140282.

Villar ML, Hegarty RS, Nolan JV, Godwin IR and McPhee M 2019. The effect of dietary nitrate and canola oil alone or in combination on fermentation, digesta kinetics and methane emissions from cattle. Animal Feed Science and Technology.

Vyas D, McGinn S, Duval S, Kindermann M and Beauchemin K 2016. Effects of sustained reduction of enteric methane emissions with dietary supplementation of 3-nitrooxypropanol on growth performance of growing and finishing beef cattle. Journal of animal science 94, 2024-2034.

Vyas D, McGinn S, Duval S, Kindermann M and Beauchemin K 2018a. Optimal dose of 3-nitrooxypropanol for decreasing enteric methane emissions from beef cattle fed high-forage and high-grain diets. Animal Production Science 58, 1049-1055.

Vyas D, Alemu AW, McGinn SM, Duval SM, Kindermann M and Beauchemin KA 2018b. The combined effects of supplementing monensin and 3-nitrooxypropanol on methane emissions, growth rate, and feed conversion efficiency in beef cattle fed high-forage and high-grain diets. Journal of animal science 96, 2923-2938.

Waghorn GC, Clark H, Taufa V and Cavanagh A 2008. Monensin controlled-release capsules for methane mitigation in pasture-fed dairy cows. Australian Journal of Experimental Agriculture 48.

Wang CJ, Wang SP and Zhou H 2009. Influences of flavomycin, ropadiar, and saponin on nutrient digestibility, rumen fermentation, and methane emission from sheep. Animal Feed Science and Technology 148, 157-166.

Yang K, Wei C, Zhao GY, Xu ZW and Lin SX 2017. Effects of dietary supplementing tannic acid in the ration of beef cattle on rumen fermentation, methane emission, microbial flora and nutrient digestibility. J Anim Physiol Anim Nutr (Berl) 101, 302-310.

Zhou YY, Mao HL, Jiang F, Wang JK, Liu JX and McSweeney CS 2011. Inhibition of rumen methanogenesis by tea saponins with reference to fermentation pattern and microbial communities in Hu sheep. Animal Feed Science and Technology 166-167, 93-100.

4. References for Sections 1 and 2 excluding meta-analysis data sources

Alcock, D.J., Harrison, M.T., Rawnsley, R.P. and Eckard, R.J., 2015. Can animal genetics and flock management be used to reduce greenhouse gas emissions but also maintain productivity of wool-producing enterprises?. Agricultural Systems, 132, pp.25-34.

Alcock, D.J. and Hegarty, R.S., 2011. Potential effects of animal management and genetic improvement on enteric methane emissions, emissions intensity and productivity of sheep enterprises at Cowra, Australia. Animal Feed Science and Technology, 166, pp.749-760.

Almeida, A.K., Hegarty, R.S. and Cowie, A., 2021. Meta-analysis quantifying the potential of dietary additives and rumen modifiers for methane mitigation in ruminant production systems. Animal Nutrition. (In press)

Andrade, B.G., Afli, H., Bressani, F.A., Cuadrat, R.R., de Oliveira, P.S., Mourão, G.B., Coutinho, L.L., Reecy, J.M., Koltes, J.E., de Souza, M.M. and Neto, A.Z., 2020. Fecal and Ruminal Microbiome Components Associated With Methane Emission in Beef Cattle. https://www.researchsquare.com/article/rs-86658/latest.pdf viewed 13/05/21

Asizua D, Mpairwe D, Kabi F, Mutetikka D, Kamatara K, Hvelplund T, Weisbjerg MR, Mugasi S and Madsen J 2014. Growth performance, carcass and non-carcass characteristics of Mubende and Mubende× Boer crossbred goats under different feeding regimes. Livestock Science 169, 63-70.

Banchero, G.E., Stefanova, K., Lindsay, D.R., Quintans, G., Baldi, F., Milton, J.T.B. and Martin, G.B., 2021. Ovulation and ovulation rate in ewes under grazing conditions: factors affecting the response to short-term supplementation. Animal, 15(2), p.100100.

Banik, B.K., Durmic, Z., Erskine, W., Ghamkhar, K. and Revell, C., 2013. In vitro ruminal fermentation characteristics and methane production differ in selected key pasture species in Australia. Crop and Pasture Science, 64(9), pp.935-942.

Baumgard LH, Matitashvili E, Corl BA, Dwyer DA and Bauman DE 2002. trans-10, cis-12 Conjugated Linoleic Acid Decreases Lipogenic Rates and Expression of Genes Involved in Milk Lipid Synthesis in Dairy Cows1. Journal of dairy science 85, 2155-2163.

Beauchemin KA and McGinn SM 2006. Methane emissions from beef cattle: Effects of fumaric acid, essential oil, and canola oil1. Journal of animal science 84, 1489-1496.

Bedale W, Sindelar JJ and Milkowski AL 2016. Dietary nitrate and nitrite: Benefits, risks, and evolving perceptions. Meat science 120, 85-92.

Benchaar C, Hassanat F, Martineau R and Gervais R 2015. Linseed oil supplementation to dairy cows fed diets based on red clover silage or corn silage: Effects on methane production, rumen fermentation, nutrient digestibility, N balance, and milk production. J Dairy Sci 98, 7993-8008.

Beukes, P.C., Gregorini, P., Romera, A.J., Levy, G. and Waghorn, G.C., 2010. Improving production efficiency as a strategy to mitigate greenhouse gas emissions on pastoral dairy farms in New Zealand. *Agriculture, Ecosystems & Environment*, *136*(3-4), pp.358-365.

Blaxter KL and Czerkawski J 1966. Modification of the methane production of the sheep by supplementation of ITS diet. Journal of the Science of Food and Agriculture 17, 417-421.

Browne NA, Eckard RJ, Behrendt R and Kingwell RS 2011. A comparative analysis of on-farm greenhouse gas emissions from agricultural enterprises in south eastern Australia. Animal Feed Science and Technology 166-167, 641-652.

Callaway T, Edrington T, Rychlik J, Genovese K, Poole T, Jung YS, Bischoff K, Anderson R and Nisbet DJ 2003. Ionophores: their use as ruminant growth promotants and impact on food safety.

Calsamiglia S, Busquet M, Cardozo PW, Castillejos L and Ferret A 2007. Invited review: Essential oils as modifiers of rumen microbial fermentation. J Dairy Sci 90, 2580-2595.

Carpenter L and Liss P 2000. On temperate sources of bromoform and other reactive organic bromine gases. Journal of Geophysical Research: Atmospheres 105, 20539-20547.

Carroll AR, Copp BR, Davis RA, Keyzers RA and Prinsep MR 2019. Marine natural products. Natural product reports.

Charmley, E.S.R.O., Williams, S.R.O., Moate, P.J., Hegarty, R.S., Herd, R.M., Oddy, V.H., Reyenga, P., Staunton, K.M., Anderson, A. and Hannah, M.C., 2015. A universal equation to predict methane production of forage-fed cattle in Australia. Animal Production Science, 56(3), pp.169-180.

Chow JM, Van Kessel JAS and Russell JB 1994. Binding of radiolabeled monensin and lasalocid to ruminal microorganisms and feed. Journal of animal science 72, 1630-1635.

Christie, K.M., Harrison, M.T., Trevaskis, L.M., Rawnsley, R.P. and Eckard, R.J., 2016. Modelling enteric methane abatement from earlier mating of dairy heifers in subtropical Australia by improving diet quality. *Animal Production Science*, *56*(3), pp.565-573.

Capper, J.L., Cady, R.A. and Bauman, D.E., 2009. The environmental impact of dairy production: 1944 compared with 2007. Journal of animal science, 87(6), pp.2160-2167.

Cottle DJ, Nolan JV and Wiedemann SG 2011. Ruminant enteric methane mitigation: a review. Animal Production Science 51, 491-514.

Crossland WL, Tedeschi LO, Callaway TR, Miller MD, Smith WB and Cravey M 2017. Effects of rotating antibiotic and ionophore feed additives on volatile fatty acid production, potential for methane production, and microbial populations of steers consuming a moderate-forage diet. J Anim Sci 95, 4554-4567.

Cullen, B.R., Eckard, R.J., Timms, M. and Phelps, D.G., 2016. The effect of earlier mating and improving fertility on greenhouse gas emissions intensity of beef production in northern Australian herds. The Rangeland Journal, 38(3), pp.283-290.

DEE (2016) 'State and Territory Greenhouse Gas Inventories 2016.' (Department of the Environment and Energy: Canberra) Available at http://www. environment.gov.au [Verified 22 October 2019].

de Raphelis-Soissan, V., Nolan, J.V., Newbold, J.R., Godwin, I.R. and Hegarty, R.S., 2016. Can adaptation to nitrate supplementation and provision of fermentable energy reduce nitrite accumulation in rumen contents in vitro? Animal Production Science, 56(3), pp.605-612.

Dijkstra J, Bannink A, France J, Kebreab E and van Gastelen S 2018. Short communication: Antimethanogenic effects of 3-nitrooxypropanol depend on supplementation dose, dietary fiber content, and cattle type. J Dairy Sci 101, 9041-9047.

Donoghue, K.A., Bird-Gardiner, T.L., Arthur, P.F., Herd, R.M. and Hegarty, R.F., 2015, September. Genetic parameters for methane production and relationships with production traits in Australian beef cattle. In Proceedings of the Association for the Advancement of Animal Breeding and Genetics (Vol. 21, pp. 114-117).

Doran-Browne N, Behrendt R, Kingwell R and Eckard R 2015. Modelling the potential of birdsfoot trefoil (Lotus corniculatus) to reduce methane emissions and increase production on wool and prime lamb farm enterprises. Animal Production Science, 55(9), 1097-1105.

Doran-Browne NA, Ive J, Graham P and Eckard RJ 2016. Carbon-neutral wool farming in south-eastern Australia. Animal Production Science, 56(3), 417-422.

Dubois B, Tomkins NW, Kinley RD, Bai M, Seymour S, Paul NA and de Nys R 2013. Effect of tropical algae as additives on rumen in vitro gas production and fermentation characteristics. American Journal of Plant Sciences 4, 34-43.

Duin, E.C., Wagner, T., Shima, S., Prakash, D., Cronin, B., Yáñez-Ruiz, D.R., Duval, S., Rümbeli, R., Stemmler, R.T., Thauer, R.K. and Kindermann, M., 2016. Mode of action uncovered for the specific reduction of methane emissions from ruminants by the small molecule 3-nitrooxypropanol. Proceedings of the National Academy of Sciences, 113(22), pp.6172-6177.

Durmic, Z., Black, J., Martin, G. and Vercoe, P., 2021. Harnessing plant bioactivity for enteric methane mitigation in Australia. Animal Production Science.

Ehhalt, D., Prather, M., Dentener, F., Derwent, R., Dlugokencky, E., Holland, E., Isaksen, I., Katima, J., Kirchhoff, V., Matson, P. and Midgley, P., 2001. Atmospheric chemistry and greenhouse gases. J.T. Houghton, Y. Ding, D.J. Griggs, M. Noguer, P.J. van der Linden, X. Dai, et al. (Eds.), Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the IPCC. Cambridge, United Kingdom, Cambridge University Press, New York (2001), pp. 239-287.

Farrell, L.J., Tozer, P.R., Kenyon, P.R., Ramilan, T. and Cranston, L.M., 2019. The effect of ewe wastage in New Zealand sheep and beef farms on flock productivity and farm profitability. Agricultural Systems, 174, pp.125-132.

Fennessy, P.F., Byrne, T.J., Proctor, L.E. and Amer, P.R., 2019. The potential impact of breeding strategies to reduce methane output from beef cattle. Animal Production Science, 59(9), pp.1598-1610.

Field JA and Lettinga G 1987. The methanogenic toxicity and anaerobic degradability of a hydrolyzable tannin. Water Research 21, 367-374.

Finlay BJ, Esteban G, Clarke KJ, Williams AG, Embley TM and Hirt RP 1994. Some rumen ciliates have endosymbiotic methanogens. FEMS microbiology letters 117, 157-161.

Fogarty, N.M., 2009. A review of the effects of the Booroola gene (FecB) on sheep production. Small Ruminant Research, 85(2-3), pp.75-84

Garsa, A.K., Choudhury, P.K., Puniya, A.K., Dhewa, T., Malik, R.K. and Tomar, S.K., 2019. Bovicins: the bacteriocins of streptococci and their potential in methane mitigation. Probiotics and antimicrobial proteins, 11(4), pp.1403-1413.

Gerssen-Gondelach, S.J., Lauwerijssen, R.B., Havlík, P., Herrero, M., Valin, H., Faaij, A.P. and Wicke, B., 2017. Intensification pathways for beef and dairy cattle production systems: Impacts on GHG emissions, land occupation and land use change. Agriculture, Ecosystems & Environment, *240*, pp.135-147.

GRA 2020 Global Research Alliance on Agricultural Greenhouse Gases https://globalresearchalliance.org/research/livestock/

Griinari, J.M. and Bauman, D.E., 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. Advances in conjugated linoleic acid research, 1(1), pp.180-200.

Grossi G, Goglio P, Vitali A and Williams AG 2019. Livestock and climate change: impact of livestock on climate and mitigation strategies. Animal Frontiers 9, 69-76.

Guan H, Wittenberg KM, Ominski KH and Krause DO 2006. Efficacy of ionophores in cattle diets for mitigation of enteric methane. J Anim Sci 84, 1896-1906.

Guyader J, Doreau M, Morgavi DP, Gerard C, Loncke C and Martin C 2016. Long-term effect of linseed plus nitrate fed to dairy cows on enteric methane emission and nitrate and nitrite residuals in milk. Animal 10, 1173-1181.

Haisan J, Sun Y, Guan L, Beauchemin KA, Iwaasa A, Duval S, Kindermann M, Barreda DR and Oba M 2017. The effects of feeding 3-nitrooxypropanol at two doses on milk production, rumen fermentation, plasma metabolites, nutrient digestibility, and methane emissions in lactating Holstein cows. Animal Production Science 57, 282-289.

Halmemies-Beauchet-Filleau A, Rinne M, Lamminen M, Mapato C, Ampapon T, Wanapat M and Vanhatalo A 2018. Alternative and novel feeds for ruminants: nutritive value, product quality and environmental aspects. Animal 12, s295-s309.

Harrison, M.T., Jackson, T., Cullen, B.R., Rawnsley, R.P., Ho, C., Cummins, L. and Eckard, R.J., 2014. Increasing ewe genetic fecundity improves whole-farm production and reduces greenhouse gas emissions intensities: 1. Sheep production and emissions intensities. Agricultural Systems, *131*, pp.23-33.

Harrison MT, Christie KM, Rawnsley RP and Eckard RJ, 2014. Modelling pasture management and livestock genotype interventions to improve whole-farm productivity and reduce greenhouse gas emissions intensities. Animal Production Science, 54(12), 2018-2028.

Harrison, M.T., Cullen, B.R., Tomkins, N.W., McSweeney, C., Cohn, P. and Eckard, R.J., 2016. The concordance between greenhouse gas emissions, livestock production and profitability of extensive beef farming systems. Animal Production Science, 56(3), pp.370-384.

Hatcher, S., Dominik, S., Richards, J.S., Young, J., Smith, J., Tearle, R., Brien, F.D. and Hermann, N., 2018. Ewe culling and retention strategies to increase reproductive rates in Merino sheep. Animal Production Science, 58(8), pp.1545-1551.

Hartmann T 2007. From waste products to ecochemicals: fifty years research of plant secondary metabolism. Phytochemistry 68, 2831-2846.

Hegarty, R.S., Alcock, D., Robinson, D.L., Goopy, J.P. and Vercoe, P.E., 2010. Nutritional and flock management options to reduce methane output and methane per unit product from sheep enterprises. Animal Production Science, 50(12), pp.1026-1033.

Herd, R.M., Velazco, J.I., Smith, H., Arthur, P.F., Hine, B., Oddy, H., Dobos, R.C. and Hegarty, R.S., 2019. Genetic variation in residual feed intake is associated with body composition, behavior, rumen, heat production, hematology, and immune competence traits in Angus cattle. Journal of Animal Science, 97(5), pp.2202-2219.

Herrero M, Henderson B, Havlík P, Thornton PK, Conant RT, Smith P, Wirsenius S, Hristov AN, Gerber P, Gill M, Butterbach-Bahl K, Valin H, Garnett T and Stehfest E 2016. Greenhouse gas mitigation potentials in the livestock sector. Nature Climate Change 6, 452-461.

Hollmann M, Powers WJ, Fogiel AC, Liesman JS, Bello NM and Beede DK 2012. Enteric methane emissions and lactational performance of Holstein cows fed different concentrations of coconut oil. J Dairy Sci 95, 2602-2615.

Hostettmann K and Marston A 2005. Saponins. Cambridge University Press.

Howden, S.M., White, D.H. and Bowman, P.J., 1996. Managing sheep grazing systems in southern Australia to minimise greenhouse gas emissions: adaptation of an existing simulation model. Ecological Modelling, 86(2-3), pp.201-206.

Huisman JM, Cowan RA and Entwisle TJ 1998. Biodiversity of Australian Marine Macroalgae — A Progress Report. In Botanica Marina, p. 89.

Jayanegara A, Sarwono KA, Kondo M, Matsui H, Ridla M, Laconi EB and Nahrowi 2017. Use of 3-nitrooxypropanol as feed additive for mitigating enteric methane emissions from ruminants: a meta-analysis. Italian Journal of Animal Science 17, 650-656.

Johnson KA, Kincaid RL, Westberg HH, Gaskins CT, Lamb BK and Cronrath JD 2002. The Effect of Oilseeds in Diets of Lactating Cows on Milk Production and Methane Emissions. Journal of dairy science 85, 1509-1515.

Jonker A, Hickey SM, Rowe SJ, Janssen PH, Shackell GH, Elmes S, Bain WE, Wing J, Greer GJ, Bryson B and MacLean S 2018. Genetic parameters of methane emissions determined using portable accumulation chambers in lambs and ewes grazing pasture and genetic correlations with emissions determined in respiration chambers. Journal of Animal Science, 96(8), 3031-3042.

Jones GA 1972. Dissimilatory metabolism of nitrate by the rumen microbiota. Canadian Journal of Microbiology 18, 1783-1787.

Kim S-H, Lee C, Pechtl HA, Hettick JM, Campler MR, Pairis-Garcia MD, Beauchemin KA, Celi P and Duval SM 2019. Effects of 3-nitrooxypropanol on enteric methane production, rumen fermentation, and feeding behavior in beef cattle fed a high-forage or high-grain diet. Journal of animal science 97, 2687-2699.

Kinley R and Fredeen A 2015. In vitro evaluation of feeding North Atlantic stormtoss seaweeds on ruminal digestion. Journal of Applied Phycology 27, 2387-2393.

Kinley RD, Martinez-Fernandez G, Matthews MK, de Nys R, Magnusson M and Tomkins NW 2020. Mitigating the carbon footprint and improving productivity of ruminant livestock agriculture using a red seaweed. Journal of Cleaner Production 259.

Knapp, J.R., Laur, G.L., Vadas, P.A., Weiss, W.P. and Tricarico, J.M., 2014. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. Journal of dairy science, 97(6), pp.3231-3261.

Koneswaran G and Nierenberg D 2008. Global farm animal production and global warming: impacting and mitigating climate change. Environ Health Perspect 116, 578-582.

Lanigan G 1972. Metabolism of pyrrolizidine alkaloids in the ovine rumen. IV. Effects of chloral hydrate and halogenated methanes on rumen methanogenesis and alkaloid metabolism in fistulated sheep. Australian Journal of Agricultural Research 23, 1085-1091.

Latham EA, Anderson RC, Pinchak WE and Nisbet DJ 2016. Insights on Alterations to the Rumen Ecosystem by Nitrate and Nitrocompounds. Frontiers in Microbiology 7.

Lee C and Beauchemin KA 2014. A review of feeding supplementary nitrate to ruminant animals: nitrate toxicity, methane emissions, and production performance. Canadian Journal of Animal Science 94, 557-570.

Lee C, Araujo R, Koenig K and Beauchemin K 2017. Effects of encapsulated nitrate on growth performance, carcass characteristics, nitrate residues in tissues, and enteric methane emissions in beef steers: finishing phase. Journal of animal science 95, 3712-3726.

Lehmann, J.O., Mogensen, L. and Kristensen, T., 2014. Extended lactations may improve cow health, productivity and reduce greenhouse gas emissions from organic dairy production. Organic agriculture, 4(4), pp.295-299.

Lewis D 1951. The metabolism of nitrate and nitrite in the sheep; the reduction of nitrate in the rumen of the sheep. The Biochemical journal 48, 175-180.

Li X, Norman HC, Kinley RD, Laurence M, Wilmot M, Bender H, de Nys R and Tomkins N 2018. Asparagopsis taxiformis decreases enteric methane production from sheep. Animal Production Science 58, 681-688.

Ludemann CI, Byrne TJ, Sise JA and Amer PR 2012. Selection indices offer potential for New Zealand sheep farmers to reduce greenhouse gas emissions per unit of product. International Journal of Agricultural Management, 1(4), 29-40.

Machado L, Magnusson M, Paul NA, de Nys R and Tomkins N 2014. Effects of marine and freshwater macroalgae on in vitro total gas and methane production. PLoS One 9, e85289.

Machado L, Magnusson M, Paul NA, Kinley R, de Nys R and Tomkins N 2016. Dose-response effects of Asparagopsis taxiformis and Oedogonium sp. on in vitro fermentation and methane production. Journal of Applied Phycology 28, 1443-1452.

Machmüller A, Dohme F, Soliva CR, Wanner M and Kreuzer M 2001. Diet composition affects the level of ruminal methane suppression by medium-chain fatty acids. Australian Journal of Agricultural Research 52, 713-722.

Machmuller A, Machmuller A, Soliva CR and Kreuzer M 2003. Methane-suppressing effect of myristic acid in sheep as affected by dietary calcium and forage proportion. Br J Nutr 90, 529-540.

Maia MR, Fonseca AJ, Oliveira HM, Mendonça C and Cabrita AR 2016. The potential role of seaweeds in the natural manipulation of rumen fermentation and methane production. Scientific reports 6, 32321.

Makkar HPS, Tran G, Heuzé V, Giger-Reverdin S, Lessire M, Lebas F and Ankers P 2016. Seaweeds for livestock diets: A review. Animal Feed Science and Technology 212, 1-17.

Martin C, Morgavi DP and Doreau M 2010. Methane mitigation in ruminants: from microbe to the farm scale. Animal 4, 351-365.

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

Mensinga TT, Speijers GJA and Meulenbelt J 2003. 17Health Implications of Exposure to Environmental Nitrogenous Compounds. Toxicological Reviews 22, 41-51.

Naranjo A, Johnson A, Rossow H and Kebreab E 2020. Greenhouse gas, water, and land footprint per unit of production of the California dairy industry over 50 years. J Dairy Sci 103, 3760-3773.

Niu M, Kebreab E, Hristov AN, Oh J, Arndt C, Bannink A, Bayat AR, Brito AF, Boland T, Casper D, Crompton LA, Dijkstra J, Eugene MA, Garnsworthy PC, Haque MN, Hellwing ALF, Huhtanen P, Kreuzer M, Kuhla B, Lund P, Madsen J, Martin C, McClelland SC, McGee M, Moate PJ, Muetzel S, Munoz C, O'Kiely P, Peiren N, Reynolds CK, Schwarm A, Shingfield KJ, Storlien TM, Weisbjerg MR, Yanez-Ruiz DR and Yu Z 2018. Prediction of enteric methane production, yield, and intensity in dairy cattle using an intercontinental database. Glob Chang Biol 24, 3368-3389.

Nolan JV, Godwin IR, de Raphélis-Soissan V and Hegarty RS 2016. Managing the rumen to limit the incidence and severity of nitrite poisoning in nitrate-supplemented ruminants. Animal Production Science 56, 1317-1329.

Norris AB, Crossland WL, Tedeschi LO, Foster JL, Muir JP, Pinchak WE and Fonseca MA 2020. Inclusion of quebracho tannin extract in a high-roughage cattle diet alters digestibility, nitrogen balance, and energy partitioning. J Anim Sci 98.

Nunez-Dominguez, R., Cundiff, L.V., Dickerson, G.E., Gregory, K.E. and Koch, R.M., 1991. Lifetime production of beef heifers calving first at two vs three years of age. Journal of Animal Science, 69(9), pp.3467-3479.

Odongo NE, Or-Rashid MM, Kebreab E, France J and McBride BW 2007. Effect of supplementing myristic acid in dairy cow rations on ruminal methanogenesis and fatty acid profile in milk. J Dairy Sci 90, 1851-1858.

Patra AK 2016. Recent Advances in Measurement and Dietary Mitigation of Enteric Methane Emissions in Ruminants. Front Vet Sci 3, 39.

Patra AK and Saxena J 2009. The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production. Nutr Res Rev 22, 204-219.

Patra AK and Saxena J 2010. A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. Phytochemistry 71, 1198-1222.

Pinares-Patiño, C.S., Waghorn, G.C., Hegarty, R.S. and Hoskin, S.O., 2009. Effects of intensification of pastoral farming on greenhouse gas emissions in New Zealand. New Zealand veterinary journal, 57(5), pp.252-261.

Quack B and Wallace DWR 2003. Air-sea flux of bromoform: Controls, rates, and implications. Global Biogeochemical Cycles 17.

Rae HA 1999. Onion toxicosis in a herd of beef cows. The Canadian Veterinary Journal 40, 55.

Rebelo LR, Luna IC, Messana JD, Araujo RC, Simioni TA, Granja-Salcedo YT, Vito ES, Lee C, Teixeira IAMA, Rooke JA and Berchielli TT 2019. Effect of replacing soybean meal with urea or encapsulated nitrate with or without elemental sulfur on nitrogen digestion and methane emissions in feedlot cattle. Animal Feed Science and Technology 257.

Richards, J.S., Sladek, M.A. and Lee, G.J., 2018. Cumulative reproductive performance effect on overall lifetime productivity in Merino sheep. Animal Production Science, 58(8), pp.1470-1480.

Ripple WJ, Smith P, Haberl H, Montzka SA, McAlpine C and Boucher DH 2014. Ruminants, climate change and climate policy. Nature Climate Change 4, 2-5.

Robinson, D.L., Goopy, J.P., Donaldson, A.J., Woodgate, R.T., Oddy, V.H. and Hegarty, R.S., 2014. Sire and liveweight affect feed intake and methane emissions of sheep confined in respiration chambers. Animal, 8(12), pp.1935-1944.

Romero-Perez A, Okine E, McGinn S, Guan L, Oba M, Duval S, Kindermann M and Beauchemin K 2014. The potential of 3-nitrooxypropanol to lower enteric methane emissions from beef cattle. Journal of animal science 92, 4682-4693.

Roque BM, Salwen JK, Kinley R and Kebreab E 2019. Inclusion of Asparagopsis armata in lactating dairy cows' diet reduces enteric methane emission by over 50 percent. Journal of Cleaner Production 234, 132-138.

Russell JB and Houlihan AJ 2003. Ionophore resistance of ruminal bacteria and its potential impact on human health. FEMS microbiology reviews 27, 65-74.

Russell JB and Strobel H 1989. Effect of ionophores on ruminal fermentation. Applied and environmental microbiology 55, 1.

Sauvant D, Schmidely P, Daudin J J, St-Pierre, N R. (2008). Meta-analyses of experimental data in animal nutrition. Animal, 2:1203–1214.

Scaramuzzi, R.J., Campbell, B.K., Cognie, Y. and Downing, J.A., 1987. Increasing prolificacy of ewes by means of gonadotrophin therapy and treatment with Fecundin. In “New Techniques in Sheep Production” (pp. 47-56). Butterworth-Heinemann.

Silva RA, Fiorentini G, Messana JD, Lage JF, Castagnino PS, San Vito E, Carvalho IPC and Berchielli TT 2018. Effects of different forms of soybean lipids on enteric methane emission, performance and meat quality of feedlot Nellore. The Journal of Agricultural Science 156, 427-436.

Sindelar JJ and Milkowski AL 2012. Human safety controversies surrounding nitrate and nitrite in the diet. Nitric oxide 26, 259-266.

St-Pierre, NR. 2001. Integrating quantitative findings from multiple studies using mixed model methodology. Journal of Dairy Science 84:741–755.

Subharat S, Shu D, Zheng T, Buddle BM, Kaneko K, Hook S, and Wedlock DN . 2016. Vaccination of sheep with a methanogen protein provides insight into levels of antibody in saliva needed to target ruminal methanogens. PLoS One, 11, e0159861.

Suybeng, B., Charmley, E., Gardiner, C.P., Malau-Aduli, B.S. and Malau-Aduli, A.E., 2019. Methane Emissions and the Use of Desmanthus in Beef Cattle Production in Northern Australia. Animals, 9(8), p.542.

Taylor C and Eckard, R 2016. Comparative analysis of greenhouse gas emissions from three beef cattle herds in a corporate farming enterprise. Animal Production Science, 56(3), 482-494.

Thiel A, Rumbeli R, Mair P, Yeman H and Beilstein P 2019a. 3-NOP: ADME studies in rats and ruminating animals. Food Chem Toxicol 125, 528-539.

Thiel A, Schoenmakers ACM, Verbaan IAJ, Chenal E, Etheve S and Beilstein P 2019b. 3-NOP: Mutagenicity and genotoxicity assessment. Food Chem Toxicol 123, 566-573.

Tocker, J., Behrendt, R., Raeside, M. and Malcolm, B., 2020. The impact of ewe lamb mating and different feeding strategies over summer–autumn on profit and risk: a case study in south-west Victoria. *Animal Production Science*.

Tokura M, Ushida K, Miyazaki K and Kojima Y 1997. Methanogens associated with rumen ciliates. FEMS Microbiology Ecology 22, 137-143.

Tomkins, N., Harrison, M., McSweeney, C.S., Denman, S., Charmley, E., Lambrides, C.J. and Dalal, R., 2019. Greenhouse gas implications of leucaena-based pastures. Can we develop an emissions reduction methodology for the beef industry?. Tropical Grasslands-Forrajes Tropicales, 7(4), pp.267-272.

Troy SM, Duthie C-A, Hyslop JJ, Roehe R, Ross DW, Wallace RJ, Waterhouse A and Rooke JA 2015. Effectiveness of nitrate addition and increased oil content as methane mitigation strategies for beef cattle fed two contrasting basal diets1. Journal of animal science 93, 1815-1823.

Turner, H.N., Brown, G.H., Ford, G.H., 1968. The influence of age structure on total productivity in breeding flocks of Merino sheep. I. Flocks with a fixed number of breeding ewes, producing their own replacements. Aust. J. Agric. Res. 19 (3), 443.

UNFCCC (2015b) 'Adoption of the Paris Agreement. United Nations/framework convention on climate change, 21st conference of the Parties FCCC/CP/2015/L.9/Rev.1.' Available at https://unfccc.int/resource/docs/2015/cop21/eng/l09r01.pdf [Verified 18 February 2020]

Ungerfeld EM 2015. Shifts in metabolic hydrogen sinks in the methanogenesis-inhibited ruminal fermentation: a meta-analysis. Front Microbiol 6, 37.

van der Spiegel M, Noordam MY and van der Fels-Klerx HJ 2013. Safety of Novel Protein Sources (Insects, Microalgae, Seaweed, Duckweed, and Rapeseed) and Legislative Aspects for Their Application in Food and Feed Production. Comprehensive Reviews in Food Science and Food Safety 12, 662-678.

Velazco JI, Cottle DJ and Hegarty RS 2014. Methane emissions and feeding behaviour of feedlot cattle supplemented with nitrate or urea. Animal Production Science 54.

Villar ML, Hegarty RS, Nolan JV, Godwin IR and McPhee M 2019. The effect of dietary nitrate and canola oil alone or in combination on fermentation, digesta kinetics and methane emissions from cattle. Animal Feed Science and Technology.

Wang, M.C. and Bushman, B.J., 1999. Integrating results through meta-analytic review using SAS software. SAS institute.

Wiedemann S, Davis R, McGahan E, Murphy C and Redding M 2017. Resource use and greenhouse gas emissions from grain-finishing beef cattle in seven Australian feedlots: a life cycle assessment. Animal Production Science, 57(6), 1149-1162.

White TA, Snow VO and King WM 2010. Intensification of New Zealand beef farming systems. Agricultural Systems, 103(1), 21-35.

Williams, A.G. and Coleman, G.S., 1992. Role of protozoa in the rumen. In The Rumen Protozoa (pp. 317-347). Springer, New York, NY.

Williams YJ, Popovski S, Rea SM, Skillman LC, Toovey AF, Northwood KS and Wright AD 2009. A vaccine against rumen methanogens can alter the composition of archaeal populations. Appl Environ Microbiol 75, 1860-1866.

Wood JM, Kennedy FS and Wolfe RS 1968. Reaction of multihalogenated hydrocarbons with free and bound reduced vitamin B12. Biochemistry 7, 1707-1713.

Wright AD, Kennedy P, O'Neill CJ, Toovey AF, Popovski S, Rea SM, Pimm CL and Klein L 2004. Reducing methane emissions in sheep by immunization against rumen methanogens. Vaccine 22, 3976-3985.

5. Estimated feasible abatement potential

Table 2 This table summarizes the practicality, availability, risks, barriers and co-impacts that are likely to influence adoption, and therefore the realization of the technical potential identified through the meta-analysis (Part A). An estimate of feasible abatement potential per unit dry matter intake is provided, based on consultant's assessment, for different livestock production systems

Abatement strategy	Abatement mechanism	Ease of implementation	Risks and barriers	Co-benefits/disbenefits	**Abatement potential** (% reduction per unit DMI)	Estimate of feasible abatement in 2030: % Effectiveness[1] / % Adoption[2]	Confidence for 2030
Dietary modification:							
Macroalgae (*Asparag-opsis* seaweed)	Bromoform inhibits methano-genesis	Theoretically simple: Easy to supply in ration to lot-fed and supplemented cattle. Availability: Unavailable. Requires scaling up seaweed production and development of supply chain. Alternatively, effort also underway to produce active ingredient using biosynthesis.	Risk: *Industrial*: ozone depleting, volatile, potentially carcinogenic, hydrocarbon *Animal*: high risk of failure: Few in vivo studies – not yet proven Early stages of technology development w.r.t production of bromoform. Potential for adverse impact on animal health. Barriers: availability, cost of growth and drying. Unknown volatility.	-ve Halogenated ozone-depleting substance Potentially carcinogenic. Stability in dried form unknown. Seaweed production could impact water quality. Land required for algae production. Biosynthesis alternative also requires land for sugar production.	30-69 Suitability: intensive systems	50% Ext cattle[3] 5% Int cattle[4] 5% Feedlot 60% Dairy 60% Sheep 5%	M

[1] Reduction in enteric methane emissions per unit DMI in the year 2030 compared with 2020
[2] Assuming a market incentive that removes cost barriers, and regulatory barriers removed
[3] Extensive grazing - rangelands
[4] More intensive grazing systems, generally on improved pastures

		Will not require APVMA registration as making no label claim.					
3-NOP	Inhibits methanogenesis by inactivating the enzyme methyl coenzyme M reductase	Potentially simple: lickblock or feed additive No impact on feed intake and fibre digestibility at mod levels. Moderate cost Availability: Commercialised Undergoing registration in Europe Registration required for Australian use. Slow-release forms being tested.	Barriers: Not registered Requires frequent ingestion Risk: *Industrial* – none known *Animal* – excess (>200 mg/kg DM) can lower DMI *Product* No known residue problems Cost: what market bears	+ve Enhanced productivity (3% gain recorded) -ve No known residue problems	18-39 Suitability: intensive systems Adoption if slow release available: Ext cattle 30% Int cattle 30% Feedlot 70% Dairy 70% Sheep 10%	29% Ext cattle 0% Int cattle 2% Feedlot 60% Dairy 60% Sheep 0%	H
Nitrate	Alternative hydrogen sink	Simple: provide in lick block. No impact on feed intake and fibre digestibility. Current ERF method for grazing cattle in lick-blocks.	*Industrial risk* – holding potentially explosive nitrates *Livestock*: Risk of death at high rate (Risk controlled using lick or slow release forms.) Some adverse dairy experience *Product*: Low risk of nitrate and nitrite in milk	+ve Can enhance wool production? -ve Nitrate production is GHG-intensive process	10-22 Suitability: all grazing cattle and sheep; feedlots, dairy	16% Adoption: Ext cattle 30% Int cattle 10% Feedlot 80% Dairy 40%	H

		Availability: Commercially available	Barrier: cost			Sheep 20%	
Oils	Suppression of ciliate protozoa and archaea, biohydrogenation of free unsaturated fatty acids, reduction in organic matter fermentation, replacing fermentable carbohydrates	Simple: Easy to supply in ration to lot-fed and supplemented cattle. Commercially available Grazing animals. No. Only in oilseed like whole cotton seed Availability: unrestricted	Industrial: None Livestock: 7% limit in diet No serious risk but can reduce DMI and fibre digestibility, so may not be suitable for animals on high-fibre diet (grazing lower quality pastures) *Product:* may be additional opportunity to alter ω–3: *ω*–6 positively Barriers: cost & unsuitability for grazing	+ve Opportunity to improve *ω–3* fatty acids for meat quality. Can lift energy intake & so performance -ve Land required to produce oil crops.	12-20 Suitability: intensive systems	15% Ext cattle 0% Int cattle 5% Feedlot 80% Dairy 40% Sheep 5%	H
Phyto-chemicals feed additive Eg grape marc, tannins saponins Forage: eg Leucaena	Antimicrobial action of tannins	Moderate complexity: Additive: not readily available in formulation for easy delivery Availability: limited, inconsistent spatially and temporally	Barrier: cost, Not APVMA approved productivity change practicality, Legumes: Cost to establish and manage forage spp Barrier: Leucaena not approved for planting in NSW (weed risk). Tagasaste possible alternative	+ve Legumes: Provides additional protein source in dry season to lift intake and performance -ve Tannins can reduce protein availability and diet acceptability Legumes: Weed risk on escape	8-14%	10% Feed additive: Ext cattle 0% Int cattle 5% Feedlot 10% Dairy 20% Sheep 5% Forage: Ext cattle 20% Int cattle 10% Feedlot 0%	L to M

						Dairy 10% Sheep 5%	
Ionophores	Inhibit ciliate protozoa	Simple in feedlot, dairy (add to ration/supplement) Moderate complexity for grazing animals: capsules? Availability: Commercially available (Monensin, Salinomycin, Lasalocid)	Barrier: Antibiotic rumen modifier Low efficacy cost	+ve Enhanced productivity Lower risk of acidosis -ve Perceived antimicrobial stewardship conflict with use	0.5-7.4% Short-term effect only Suitability: feedlots, dairy. Feedlot 90% Dairy 70%	4% Feedlot 90% Dairy 70% Ext cattle 0% Int cattle 0%	H
Protozoa control	Eliminate or long term suppression of protozoa	No commercial protozoa control strategy available	Risks: variable accordingly methodology Barriers: Lack of practical technology	+ve Increase protein flow so increase productivity I tropics and increases wool growth -ve May be a compound affecting all eukaryotic cells (including animal gut & tissue)	-0.6-+14% Suitability: feedlots, dairy. If available: Ext cattle 60% Int cattle 20% Feedlot 10% Dairy 60% Sheep 40%	2%	M
Non-dietary strategies							
Vaccines	Inhibits rumen Archaea by salivary immunoglobulins	Simple delivery Low cost Availability: unavailable	Risk: Principle of vaccine for rumen microbe may be faulty.		Effectiveness: 5-20%[5] Suitability: All stock	20%	L by 2030 M by 2050

[5] MLA 2018

			Barriers: Unproven, Unavailable			Dairy & feedlot: 80% beef & sheep: 20%	
Breeding	Select for reduced methane yield	Slow to deliver results, but easy uptake, permanent cumulative benefit.	Risk: low Selection may compromise other economic traits Barriers Economic value on CH_4	+ve Increased profitability Enhanced growth rate: Reduced CH_4 is correlated with faster growth	1.45% reduction per year[6] 2030: 3-7% Suitability: All stock Adoption: 2030 Dairy 0-10% beef 0-4% sheep 0-20% 2050: 15% abatement Adoption: dairy 50-90% beef/sheep 30-50%[7]	5% Dairy: 80% beef & sheep: 20%	H
Herd manage-ment	Improved emissions intensity: Cull unproductive animals, supplementary feeding, grazing management, to increase product	Simple: Known nutritional and animal management strategies	Risk: Low Barriers: none significant (consistent with best practice)	+ve Increased profitability -ve May lead to more productive animals so	Effectiveness: variable Suitability: All stock but mostly grazed cattle and sheep	Dairy: 5% Adoption: 80% Beef: 15% Adoption: 80% Sheep: 10%	H

[6] Fennessy et al, 2019
[7] Reisinger et al., 2018

	yield per unit feed consumed			higher total emissions in sheep and beef enterprises despite lower emissions intensity	Ext cattle 30% Int cattle 10% Feedlot 20% Dairy 10% Sheep 20%	Adoption: 50%	
Pasture management	Improved emissions intensity: Lower CH_4 per unit feed and faster growth rate with higher quality diet: Encourage higher quality pasture species through grazing management, pasture improvement	More nutritional pastures lead to a lower % of consumed energy going to maintenance, so product per unit CH_4 increased	Risk: low risk for intensity effect on back of productivity rise but high uncertainty in methane yield effect Barriers: cost (limited abatement relative to cost) Emission mitigation varies throughout the year with seasonal variation in pasture quality	+ve Increased SOC, higher returns, more resilient farming system -ve Higher fertiliser use may cause eutrophication, N_2O emissions	Effectiveness: 0.4 - 4.2% Applicability: Grazed cattle and sheep	2% Ext cattle 10% Int cattle 10% Feedlot 0% Dairy 10% Sheep 20%	M

References (Table 2)

Fennessy, P.F., Byrne, T.J., Proctor, L.E. and Amer, P.R., 2019. The potential impact of breeding strategies to reduce methane output from beef cattle. Animal Production Science, 59(9), pp.1598-1610.

MLA 2018 Greenhouse Gas mitigation potential of the Australian red meat production and processing sectors https://www.mla.com.au/research-and-development/search-rd-reports/final-report-details/Greenhouse-gas-mitigation-potential-of-the-Australian-red-meat-production-and-processing-sectors/3726

Reisinger, A, Clark, H, Abercrombie, R, Aspin, M, Ettema, P, Harris, M, Hoggard, A, Newman, M, Sneath, G (2018) Future options to reduce biological GHG emissions on-farm: critical assumptions and national scale impact. A report to the Biological Emissions Reference Group. Available at https://www.mpi.govt.nz/dmsdocument/32128-bergreport-future-options-final-dec-2018 [Verified 6 December 2018]

6. Kangaroo Farming: A role in achieving carbon neutrality in the red meat industry of NSW by 2050?

Roger Hegarty and Amelia Almeida

Industry situation

The prospect of reducing Australia's GHG emission by replacing ruminants with kangaroos has been suggested (Garnaut 2008) and the broader environmental arguments for replacement of sheep with kangaroos in rangelands have been promoted (Grigg 2002). A "NSW Kangaroo Taskforce" has recently been formed to try to better understand the interplay of kangaroos and ruminants in co-grazing and to advance the role and utilisation of kangaroos. There are approximately 40 million kangaroos in Australia (Wilson & Edwards 2019). Four macropod species form the core of Australia's (and the NSW) kangaroo meat and skins industry, being Red, Eastern greys, Western greys and Wallaroos (all referred to as 'kangaroos' hereafter). All states involved have a kangaroo management or harvest plan. Nowhere are these native animals domesticated and 'farmed' but the industry relies on wild harvest (animals shot on-farm) and transfer of the carcass to an accredited abattoir for processing for pet food or human consumption.

Kangaroos in NSW are protected under the Biodiversity Conservation Act 2016 but can be harvested under license. The kangaroo harvest has two components, being those shot by professional shooters under a commercial quota ('commercial harvest') and those culled by land owners and non-professionals on non-commercial permits granted for damage mitigation (eg reducing high kangaroo numbers during drought). This is called 'non-commercial' culling but these carcases are disposed of on-farm and not commercially sold. There is currently little market (and so no value to the shooter) for kangaroo skins due to global pressure against their use by animal rights lobbyists.

The maximum commercial harvest is set on a state basis as a function of the estimated kangaroo species populations, being on average 14.6% of population estimates for NSW in 2018 (OEH 2018). The smaller non-commercial culling is based on perceived overpopulation relative to feed availability and demand by domesticated livestock. It is important to recognise that generally commercial harvest quotas have not been met in recent years and in 2018 the harvest taken was only 3-4% of population despite the quota being 14.6% of population.

The place of kangaroos in grazing systems, especially in the rangelands, has been often considered, including by Wilson (ANU), Hacker (formerly NSW DPI) and Grigg (UQ), some of Australia's foremost experts in kangaroo ecology and management. This synopsis report largely draws on the publications of these researchers.

At a value paid to the shooter of AU\$0.60/kg and at an average carcass weight of 23kg. a carcass is worth \$13.80 (2018 value) or national on-farm value of \$550 Million. This is less than 1/7th of the value of sheep production and less than 1/10th of the value of grazing beef nationally (Wilson & Edwards 2019).

Forging a future for kangaroo harvesting in NSW

With growing interest in regenerative agriculture that improves the environmental quality of land through its agricultural use, there is interest in rethinking kangaroo management to: (1) improve the economic viability of grazing enterprises; (2) deliver a suite of environmental benefits on-farm, and (3) provide a meat of unique nutritional attributes. Achieving such changes is an end-goal for the NSW Kangaroo Taskforce as they look to more effectively integrate the kangaroo harvest industry in a sustainable alliance with the ruminant grazing sector (Cooney et al., 2009; McLeod and Hacker 2020) for on-farm environmental and economic advantage.

What could kangaroo management look like in the future?

Farming of kangaroos in intensively managed small areas has been considered (Shepherd 1983) but largely discarded due to management challenges of these undomesticated species. In considering whether domestication is practical, while recognising the many 'tame' hand-reared joeys that are kept, the experience with the fox is that more than 30 years of selective breeding have not yet produced a truly domesticated fox (Kukekova et al., 2018). It is anticipated that wild harvest will continue to be the basis of Australia's kangaroo industry and there are efforts to engage landowners more in this by sharing in the financial rewards with the shooter (Grigg 2002; Cooney et al., 2009).

The remainder of this report considers the strengths weaknesses, opportunities and threats to the NSW kangaroo harvesting industry becoming a major managed industry in rural NSW and its possible role in mitigation of NSW GHG emissions.

Industry Strengths

What are the attributes of a wild-harvest based kangaroo industry that will underpin its growth?

A naturally occurring and resilient animal

NSW had an estimated 12.8 million kangaroos in 2018 (OEH 2019) on public and private lands, but all kangaroos are the property of the Crown and protected under the Biodiversity Conservation Act (2016). While the populations of commercial species are thought to have risen with agricultural development including land-clearing and water supply, they are native to the regions so climatically adapted animals. Their physiology, low energy and water requirements (relative to ruminants) (Grigg 2002) are advantageous over ruminants in harsh season but in the recent 2017-2020 drought large numbers of kangaroos have died (ABC 2019) as in previous droughts (***Figure 11***), so being native is not a guarantee of productivity in all seasons.

Reproductive capability in harsh environments

While not unique among mammals, kangaroos have the ability to support more than one young at the same time, in different stages of development by means of embryonic diapause (Renfree 1979). As a result, from a single mature red or grey kangaroo, in 40 months 10 or 6 young can be produced (Shepherd 1983). This allows for sustained productivity in good seasons and a quick return to reproductive development after poor seasons (droughts).

Hard footed v soft-footed

While ungulates breaking up the soil with cloven hooves has been argued to be very desirable on the African plains and a key point in the Savory method of grazing, disturbing the soil in NSW's non-arable regions is not desirable due to wind erosion and opening up the soil to weed germination. While kangaroos unquestionably make paths though fences, their long footpads are generally thought to be soft on the soil surface as they spread the weight, unlike ruminants whose weight is carried on 4 small pairs of hard hooves. There is some evidence that ground pressures are lower from the kangaroo foot than from sheep but more work is required. (Grigg 2002; Norris & Andrews 2010). The advantage for kangaroos in this is that they are less destructive of the soil surface, litter layer and young or delicate ground covers.

Lower maintenance energy cost

While all life has a similar maintenance energy requirement of approximately 0.3 MJ per kilogram of metabolic body weight (kg Liveweight$^{0.75}$), there are between-species differences and kangaroos appear to have a lower energy requirement than do ruminants as a generalisation (Grigg 2002; Wilson 2018). Values of 0.7 and 0.5 have previously been used to reflect the lower field metabolic rate of kangaroos relative to sheep, but Munn et al (2009) found in red kangaroos an experimental value of 0.35. This may well be part of their adaption to rangeland living but it will also contribute to a lower energy cost in meat production and thus a lower emission intensity for kangaroo meat (g CH_4/kg meat). Further direct or indirect calorimetry is needed to broaden the base of understanding of energy requirements and intake of commercial macropods.

It must be remembered that kangaroos exhibit sexual dimorphism in body weight (Spiegel and Greenwood 2019), with the average male and female kangaroos harvested in NSW in 2017 weighing 30.0 and 19.4 kg respectively (OEH 2018). While 95% of harvested animals were males, the wild population must if anything have at least an equal or higher ratio of females to males, so there is half the kangaroo population that less desirable for harvest and meat production by virtue of inadequate body weight. In effective the female portion of the mob is an overhead that increases the feed required and methane output for every kangaroo commercially slaughtered. There is however no law precluding harvesting of females but strict procedures for dealing with dependent young are laid out in the code. For sheep in contrast, every domesticated ruminant can be slaughtered for meat.

Efficient Meat Production

Wilson (2018) calculated based on the feed intake of a kangaroo being 0.5 DSE (1.0 DSE being feed intake of a non-pregnant sheep), compared to 8 DSE for a growing steer, that for the same quantity of feed consumed, kangaroos will produce 210 kg of useable meat compared to 180kg from a steer. This again is a very crude approximation and would need stronger replication and allowance for energy cost of maintaining breeders (ruminant and kangaroo) before such claims can be made with confidence, but it indicates that conversion of feed to meat is comparable for kangaroos and ruminants. The point raised in the previous section on the large number of females needing to eat but contributing less to the commercial carcass also needs to be considered in estimating the efficiency of every kilogram of meat harvested. On the positive note, there is extensive value adding in the kangaroo carcass processing. Mr Doug Jobson (Macromeats) estimated that the company would only put to waste (render), 1000 kg from the 150,000 kg of kangaroo carcasses processed each week.

Meat is low in total fat and cholesterol, high in Zinc and unsaturated fat

The properties of wild-harvested kangaroo meat have recently been reviewed (Spiegel and Greenwood 2019). On the positive side it has a low total fat content of approximately 2% and the fats contain a higher proportion of unsaturated fatty acids than in ruminants, which is desirable. Further, kangaroo meat has been found to have levels of the desirable conjugated linoleic acid (CLA; Lehnen et al., 2015) and its precursor *trans* vaccenic acid that are more than 3 x that of lambs higher (Engelke et al., 2004). The protein content (even when expressed as a percentage in fat-trimmed lean) is marginally higher than in ruminants (Ford and Fogerty 1982; cited by Wilson and Edwards 2019).

Minimal on-farm infrastructure requirement

Sheep in NSW's western division require little treatment for internal parasites but are seasonally challenged by blowfly. Consequently, they require routine mustering yarding, crutching and shearing, though the movement to shedding sheep-breeds is reducing this. Such management practices are not required for wild harvest of kangaroos so the operating costs in terms of labour and infrastructure are likely to be lower than for grazing domestic stock. This may well enable kangaroo-based enterprises to still run profitably despite a lower income than sheep properties. Further, as the animals arrive at the abattoir gutted (+/- pluck), there is less functionality and less waste disposal required at the commercial abattoir. However, chilling capability on farm, in storage and in transit however are likely to need further development if the scale of enterprise increases. The human resource also needs consideration as the number of commercial shooters licensed to harvest kangaroos is diminishing in the past decade (Wilson and Edwards 2019) and this impacts on the ability of the industry to operate at scale.

Greater biodiversity

Kangaroos are already endemic in the areas likely to be used for their increased production, so increasing their numbers is not increasing biodiversity or protecting these non-endangered species. While no data was found on associated changes in wildlife diversity, kangaroos have been found to be less destructive to an array of seedlings during germination and recruitment than were sheep (Tiver & Andrew 1997).

In broader biodiversity metrics however, Eastern grey Kangaroos (EGKs) have been found to have negative effects on a range of other environmental species as summarised in Table 3 below, copied directly from a compilation of 8 published studies (ACTEPA 2015). These studies presented negative effects of kangaroos grazing on vegetation, beetles and ground dwelling lizards as well as some birds, so the argument for kangaroos increasing diversity is not a convincing one.

Reduced enteric methane emissions compared to ruminants

Australia's Climate Change Commissioner and high-profile scientist and climate advocate, Dr. Tim Flannery has been advocating kangaroos should have a full functional role in Australia's meat industry (Flannery 2002). The use of kangaroos to produce low methane red meat has been modelled for the rangelands (Wilson and Edwards 2008) and showed some economic feasibility relative to ruminants. At the current time the carbon price (Australian Carbon Credit Unit: ACCU) is below that modelled and the cattle and sheep prices are much higher while kangaroo prices remain much the same ($1/kg), greatly detracting from the economic feasibility of changing the animal balance in favour of kangaroos. While kangaroos have sometimes been found to produce no foregut methane (Kempton et al., 1976), recent reviews of a broader range of herbivores have shown a less discrete difference in enteric emissions between ruminant and non-ruminant species (Klieve and Ouwerkerk 2007; Clauss et al., 2020; de la Fuente et al 2019). A statistically robust meta-analysis has not been conducted, but a loss of <4% of gross energy intake as enteric methane from macropods has been consistent, in difference to a default methane yield of 7% for ruminants. Building on the work of Klieve and Ouwerkerk (2007) and on many years of comparative digestive studies, Clauss et al (2020) have recently reassessed numeric enteric methane emission levels from a range of herbivores of diverse body size and gut configurations and have concluded that rather than a sharp dichotomy between ruminants and non-ruminant herbivores, methane emission share a relatively reliable continuum across species. So there is a solid basis for anticipating a lower methane/unit feed intake by kangaroos than the ruminants they co-graze with. Some of the research studies are summarized in Table 4. The studies reported do not include incubation of rumen digesta (Dellow et al., 1988) or of faecal material (Hackstein and Van Alen 1996).

Table 3 Summary of research on the effects of kangaroo grazing on biodiversity, based on field work in the ACT and published or in review since the publication of the ACT Kangaroo Management Plan in 2010. Copied from ACTEPA 2015.

Title of study	Year	Primary Author	Studied Taxon	Negative effect of high density of EGKs?	Recommended kangaroo density (EGK/ha)
Biomass and floristic patterns in the ground layer vegetation of box-gum grassy eucalypt woodland in Goorooyarroo and Mulligans Flat Nature Reserves, Australian Capital Territory	2010	Sue McIntyre	Ground-layer plants	Yes	-
Experimental reduction of native vertebrate grazing and addition of logs benefit beetle diversity at multiple scales	2011	Philip Barton	Beetles	Yes	0.4
Back to the brink – population decline of the endangered grassland earless dragon (*Tympanocryptis pinguicolla*) following its rediscovery	2012	Wendy Dimond	The grassland earless dragon	Yes	-
Bringing forward the benefits of coarse woody debris in ecosystem recovery under different levels of grazing and vegetation density	2013	Adrian Manning	Reptiles	Yes	0.4
Eaten Out of House and Home: Impacts of Grazing on Ground-Dwelling Reptiles in Australian Grasslands and Grassy Woodlands	2014	Brett Howland	Grass and Reptiles	Yes	< 0.5
Restoration of eucalypt grassy woodland: effects of experimental interventions on ground-layer vegetation	2015	Sue McIntyre	Ground-layer plants	Yes	
Habitat preferences of the threatened striped legless lizard: implications for the management of grazing in grasslands.	2015	Brett Howland	Striped legless lizards	Yes	< 1.2
Birds of a feather flock together: using trait-groups to understand the effect of macropod grazing on bird communities in grassy habitats	In review	Brett Howland	Birds	Some yes Some no	varied

Table 4 Methane emission from macropods (summary of available whole animal literature)

Species	*LW (kg)*	*GEI*	*% GEI*	*gCH_4/l/d*	*g/kg DMI*	*Reference*
M. Fuliginosus	*21.7*	*4.517*	*2.65*	*3.05*	*12.68*	*Vendl et al.,2015*
M. Fuliginosus	*21.8*	*7.67*	*1.60*	*3.09*	*7.54*	*Vendl et al.,2015*
M. rufus	*17.3*	*5.292*	*2.70*	*2.98*	*12.87*	*Vendl et al.,2015*
M. rufus	*17.7*	*7.238*	*1.63*	*2.6*	*2.70*	*Vendl et al.,2015*
M.rufogriseus(RNW)	*17.0*			*2.23*		*Madsen & Bertelsen 2012*
M.rufogriseus(RNW)	*14.0*			*1.12*		*Madsen & Bertelsen 2012*
M.rufogriseus(RNW)	*13.5*			*1.49*		*Madsen & Bertelsen 2012*
M.rufogriseus(RNW)	*17.5*			*1.42*		*Madsen & Bertelsen 2012*
M.rufogriseus(RNW)	*17.5*			*2.53*		*Madsen & Bertelsen 2012*
M.rufogriseus(RNW)	*14.0*			*1.47*		*Madsen & Bertelsen 2012*
M.rufogriseus(RNW)	*13.5*			*2.44*		*Madsen & Bertelsen 2012*
M.rufogriseus(RNW)	*18.5*			*1.03*		*Madsen & Bertelsen 2012*
M.rufogriseus(RNW)	*20*			*1.99*		*Madsen & Bertelsen 2012*
M.rufogriseus(RNW)	*16.6*			*1.39*		*Madsen & Bertelsen 2012*
M.rufogriseus(RNW)	*14*			*1.79*		*Madsen & Bertelsen 2012*
M.rufogriseus(RNW)	*16*			*1.93*		*Madsen & Bertelsen 2012*
M. giganteus	*13.91*			*0.48*	*0.723*	*Kempton et al 1976*
M. eugenii	*4.4*	*10.4*		*1.098*		*Von Englehardt et al 1978*
M. eugenii	*12*	*6.5*		*1.872*		*Von Englehardt et al 1978*
M. eugenii	*10.8*	*11*		*2.851*		*Von Englehardt et al 1978*
M. eugenii	*9.1*	*1.4*		*3.057*		*Von Englehardt et al 1978*

M. fuliginous = western grey kangaroo; M. rufus = red kangaroo; M rufogriseus = red necked wallaby M. eugenii = Tammar wallaby

Industry Weaknesses

Hygiene

Because carcases are field dressed (gut/head/tail removed but skin retained) in remote locations and not refrigerated immediately post-slaughter, contamination of carcasses with dust and bacteria has been a major basis for industry criticism (eg AWPC 2015). There were numerous accounts of Escherichia coli detection on the majority of kangaroo samples tested (Anderson et al., 1964), but requirements on hygiene for field dressing and chilling have improved since these studies and Eglezos et al., 2007 reported approximately 14% of carcasses tested positive for E. coli and less than 1% for Salmonella, which they considered comparable with carcass contamination rates for beef in Australia.

There is no doubt that food safety is a pivotal point in quality assurance for the kangaroo harvest industry and moves to better cover and chill carcasses is likely to be required for industry expansion to be sustainable without risking markets.

Concurrent reduction in ruminant numbers

Just as sheep and cattle do not voluntarily consume identical diets in a grazing environment (Animut and Goetsch 2008), so kangaroos and ruminants do not voluntarily choose an identical diet. Edwards et al (1995) reported 52-73% overlap in diet selected by sheep and by red kangaroos in the rangelands and the diet preference of sheep changed when no kangaroos were present. There are many other studies of diet overlap between macropods and ruminants, giving clear evidence there is diet overlap, meaning kangaroos use some food that would otherwise be eaten by livestock. Consequently, and for emission reduction to be achieved, livestock populations must be reduced in association with an increased kangaroo population. This would require a major mindset change and a strong economic motivation to replace sheep with kangaroos. Nutritionally two kangaroos can be roughly considered to have the energy requirements of one sheep (Grigg 2002), and with 50% diet overlap this could be reduced to a 1:1 replacement of sheep with kangaroos. The removal of ruminants is still fundamental to achieve environmental benefits from increased kangaroo populations. The resistance of graziers to make the substitution is a key weakness in the strategy to reduce NSW greenhouse gas emissions by substituting kangaroos for sheep.

Seasonal drought population fluctuations

Being native does not make kangaroos immune to starvation and seasonal adversity. The kangaroo population, like the livestock population, will have to be managed in accordance with seasonal feed resources. While they are able to readily respond to good seasons, they, like livestock, die rapidly in sustained drought (***Figure 11***).

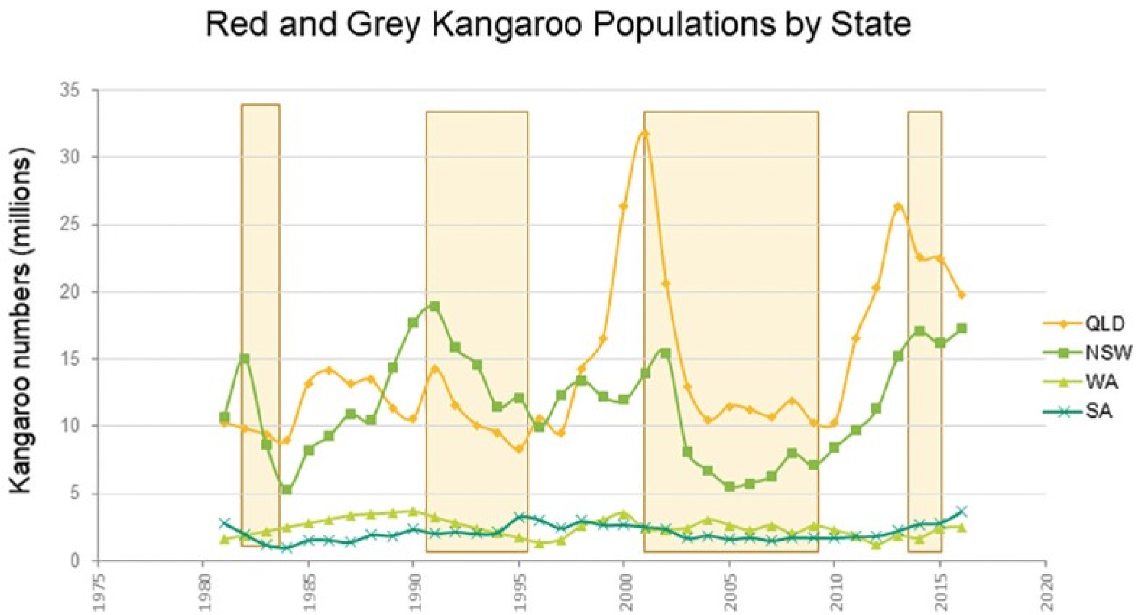

Figure 11 Kangaroo numbers over time by state, reflecting the decline in kangaroo numbers that occurs during drought periods (shaded areas). Reproduced with permission from Wilson and Edwards 2019.

Fencing and Exclusion fencing

Kangaroos are not retained by standard livestock fences and even most property boundary fences can be penetrated if not jumped over by kangaroos (Kondinin 2016). 'Roo holes' are often started by feral pigs but readily expanded and routinely used by kangaroos to traverse property boundaries. In recent years, government support for 'Exclusion fences' had been used by some graziers and groups of graziers to fence out kangaroos as well as dogs and pigs, and in so doing, protect sensitive habitat (Wilson and Edwards 2019). The current enthusiasm for exclusion fencing principally for dog control is strongly dependent upon government subsidy for costs ($7000-$8000/km installed) but may influence the distribution for kangaroos in coming decades. In southern states, processors accept female kangaroos for meat processing.

Animal welfare & public perception

Kangaroo commerce has a strong battery of opponents attacking both the operation of a kangaroo meat industry (eg AWPC 2015) and skin industry (PETA 2020). Currently more than 90% of kangaroos harvested by professional shooters are male (OEH 2018) in NSW and females (does) are not generally shot by professional shooters. It is likely that if the kangaroo population was expanded to grow the industry, females would also need to be taken in to the commercial chain and this will bring an increased level of protests since mature females are typically pregnant and their growth and final body weight is lighter than for males.

Since kangaroos are owned by the crown and not the landowner on whose property they live, there are more opportunities for legal restriction of kangaroo harvesting than for domestic livestock production. The licensing system for shooters (harvesters) and standards for harvesting vehicles, the quota on harvest and the release of permits ('tags') for non-commercial on-farm control all provide opportunities for regulation. Similarly, the export of kangaroo products can also be regulated. So this exposure of the entire kangaroo processing change to multiple sites of regulation is a weakness to the industry, but many of the challenges are shared by the 'competing' livestock processing industries.

Industry Opportunities

Agriculture needs to work with the environment not against it

The extreme drought leading up to 2020 has challenged the sustainability of traditional agriculture in Australia. Increasingly the vision is to move to agriculture that enriches the environment rather than degrades it. In its simplest sense this can be called "regenerative agriculture" and seems likely to build the foundation of sustainability previously developed by Landcare, into something beyond maintenance, a new way of enrichment of the farming/grazing system. This enthusiasm is consistent with a reduction in domestic livestock and a replacement with native livestock that may offer lower GHG emissions and less physical damage to the environment and less human intervention into the landscape.

Carbon credit value from Emission Reduction Fund methodology

A shifting in the balance from planned exclusive ruminant grazing to planned co-grazing of livestock with kangaroos may well be eligible for development into a mitigation method to be approved and utilised in ERF projects and thereby generate claimable ACCUs with the nation's Clean Energy Regulator. This would need to be tested, in particular, passing the additionality test.

Industry Threats

A very broad assessment of physical threats to the kangaroo harvest industry has been included in the Agriculture Victoria (2020) plan and points from this are summarised below. References for each variable are provided in the Victorian Kangaroo Harvest Management Plan (Agriculture Victoria 2020).

- Climate change
- Animal disease
- Drought and floods
- Habitat loss and modification
- Genetic impact of removing large males
- Predation (human, dingo)
- Vehicle collisions

This list fails to address the social pressures that have been the primary threat to the industry in recent decades and will foreseeably remain so in the future. While tests have shown consumers in Australia consider kangaroo meat to typically have 'everyday eating-quality' (See Spiegel and Greenwood 2020 for review), it is not the eating quality but the social acceptability of the meat that may curtail its development. While RSCPA have been positively engaged in the development of the kangaroo industry, most researchers have highlighted the need to include all relevant stakeholders in the discussions to develop the industry (Cooney et al., 2009; Wilson 2018, Wilson and Edwards 2019; McLeod and Hacker 2020). This gives the greatest opportunity to accommodate welfare and social concern issues in the management protocols being developed.

Implications for NSW Livestock and Agricultural Emissions

There have been descriptions of how kangaroos could take their place as a 3rd commercial animal in Australia's grazing systems (eg. Cooney et al., 2009; Wilson and Edwards 2019; McLeod and Hacker 2020) and how substituting kangaroos for sheep can reduce agricultural emissions the impacts that this could make on agriculture's emissions profile (Wilson and Edwards 2008). The emission rate of Vendl (2015) has provided an improved emission basis for kangaroos over that previously used. In 2017 NSW had 28.5M sheep and 6.81M beef cattle producing 5,200 and 8,500 Gg of enteric methane (CO_2 equivalents Table 5). The corresponding NSW kangaroo population of 14.4M animals was estimated to be releasing 403 Gg of enteric methane (Table 5).

Table 5 Population and emission estimates for kangaroos and livestock in NSW in 2017

	Population estimate 2017	Average live-weight /kangaroo# (kg)	Total methane production (Gg CO2e/year)
NSW Kangaroos (2017)~	14,403,862	28.5	403$
NSW Cattle (all, 2017)*	6,807,902		8,482
NSW Sheep (2017)*	28,476,308		5,176
National Livestock Enteric CH_4*~			51,543

~: Note Enteric emission from kangaroos are not included in the national inventory as the inventory covers only emissions related to the activity of man not natural ecosystems.

#: Assuming living population consists of 1/4 mature males at average carcass weight of 30kg, 1/4 mature females at average carcass weight of 19.4kg (carcass weights are average as harvested in 2017 in NSW; OEH 2018). Assuming an average dressing percentage of 65% (Hopwood 2008) this gives average live-weights of 54 and 30kg respectively. Of the remaining 50% of the population it is assumed that ½ are immature males at half-mature weight (27kg) and ½ are immature females at half-mature weight (15 kg).

$: Daily enteric methane emissions from kangaroos were calculated as the product of average live weight of NSW kangaroos by population by days/year and the average body mass specific methane volume of Vendl et al., (2015) of 0.150 L CH_4/kg Bodyweight/d

*: State and national livestock numbers and emission for 2017 were taken from the National Greenhouse gas inventory for 2017. https://ageis.climatechange.gov.au/ (AGEIS 2020)

There is a biological possibility for kangaroos to be substituted for grazing ruminants in NSW, as a 'sheep replacement therapy' for the grazing landscape (Grigg 2002). The practicality of this substitution and the scale to which it will occur will depend on the balance of components addressed in the above SWOT analysis:

(1) A multisector approach to industry development. While several models have been presented, the coinciding of the McLeod and Hacker (2020) "active adaptive management" approach and

the formation of the NSW Kangaroo Taskforce provides a positive strategy and multi-party steering group to innovate in an era when the agriculture sector is looking to change practice to enrich instead of deplete the landscape.

(2) Economics of generating saleable product from harvested animals. At present shooters receive a long-term high price of $1- $1.10/kg for kangaroo carcasses due to drought shortages. Currently mutton carcasses approximately 6 times as much/ kilogram ($6.55/kg over the hooks in NSW; MLA 2020). Due to global market closure, kangaroo skins that were once a co-product are now a by-product of the meat industry, with only selected skins being tanned. Preferentially (for carcass weight and welfare reasons) male kangaroos are primarily harvested. Skin value does not add a specific return to the shooter. In contrast, every sheep is equally able to go to market and provide meat and skin value.

(3) Manufacturer and consumer perception of kangaroo-sourced materials. This is a key vulnerability of kangaroo processing, exemplified by the minimal processing of kangaroo skins that currently occurs. The reality is that both the meat and the skins have attributes that make them highly marketable on their physical attributes but animal activists have run effective campaigns based on both animal cruelty and food hygiene do prevent kangaroo products being manufactured.

(4) Lower enteric emissions. While kangaroos do produce enteric methane, it is clear they produce approximately 73% less methane/kg liveweight/d than do ruminants. The simple ramifications of this are that the methane emission associated with for every kilogram of sheep body weight will drop by 73% if that kilogram of sheep is replaced with 1 kilogram of kangaroo. Herd modelling of the reproductive and productive lifetime of sheep and of kangaroos is needed to estimate the relative emission/unit meat or emission/$ product through species change. There may be scope for developing managed co-grazing as an ERF methodology.

7. References for Section 6

ABC (2019). Kangaroo harvest halted in western Queensland as millions starve in drought. https://www.abc.net.au/news/2019-11-05/harvest-cancelled-while-millions-of-kangaroos-starve-in-drought/11669190 Downloaded 22/04/2020

ACTEPA 2015. ACT conservation research: the effects of kangaroo grazing on biodiversity. https://www.environment.act.gov.au/__data/assets/pdf_file/0007/902446/Effects-of-kangaroo-grazing-and-biodiversity.pdf

AGEIS (2020). Australian Greenhouse Emissions Information System, Department of Industry, Science, Energy and Resources. https://ageis.climatechange.gov.au/NGGITrend.aspx Accessed 23/04/2020.

Agriculture Victoria (2020). Victorian Kangaroo Harvest Management Plan 2020 . http://agriculture.vic.gov.au/__data/assets/pdf_file/0011/495029/Kangaroo-harvest-management-plan-2020.pdf?v=2

Anderson, K., Crowder, E.F. and Woodruff, P., 1964. The Isolation of Salmonellae from Kangaroo Meat sold as Pet Food. *Medical Journal of Australia*, *2*(17), pp.668-9.

Animut, G. and Goetsch, A.L., 2008. Co-grazing of sheep and goats: benefits and constraints. *Small Ruminant Research*, *77*(2-3), pp.127-145.

AWPC 2015. Kangaroo meat can kill you and your pets. https://awpc.org.au/kangaroo-meat-can-kill-you-and-your-pets/ Downloaded 23/04/2020.

Madsen, J. and Bertelsen, M.F., 2012. Methane production by red-necked wallabies (Macropus rufogriseus). *Journal of Animal Science*, *90*(4), pp.1364-1370.

Clauss, M., Dittmann, M.T., Vendl, C., Hagen, K.B., Frei, S., Ortmann, S., Müller, D.W., Hammer, S., Munn, A.J., Schwarm, A. and Kreuzer, M., 2020. Comparative methane production in mammalian herbivores. *Animal*, *14*(S1), pp.s113-s123.

Cooney, R., Baumber, A., Ampt, P. and Wilson, G., 2009. Sharing Skippy: how can landholders be involved in kangaroo production in Australia?. *The Rangeland Journal*, *31*(3), pp.283-292.

de la Fuente, G., Yañez-Ruiz, D.R., Seradj, A.R., Balcells, J. and Belanche, A., 2019. Methanogenesis in animals with foregut and hindgut fermentation: a review. *Animal Production Science*, *59*(12), pp.2109-2122.

Dellow, D.W., Hume, I.D., Clarke, R.T.J. and Bauchop, T., 1988. Microbial Activity in the Forestomach of Free-Living Macropodid Marsupials-Comparisons With Laboratory Studies. *Australian Journal of Zoology*, *36*(4), pp.383-395.

Edwards, G.P., Dawson, T.J. and Croft, D.B., 1995. The dietary overlap between red kangaroos (Macropus rufus) and sheep (Ovis aries) in the arid rangelands of Australia. *Australian Journal of Ecology*, *20*(2), pp.324-334.

Eglezos, S., Huang, B. and Stuttard, E., 2007. A survey of the microbiological quality of kangaroo carcasses processed for human consumption in two processing plants in Queensland, Australia. *Journal of food protection*, *70*(5), pp.1249-1251.

Von Engelhardt, W., Wolter, S., Lawrenz, H. and Hemsley, J.A., 1978. Production of methane in two non-ruminant herbivores. *Comparative Biochemistry and Physiology Part A: Physiology*, 60(3), pp.309-311.

Engelke, C.F., Siebert, B.D., Gregg, K., Wright, A.D.G. and Vercoe, P.E., 2004. Kangaroo adipose tissue has higher concentrations of cis 9, trans 11-conjugated linoleic acid than lamb adipose tissue. *Journal of Animal and Feed Sciences*, *13*, pp.689-692.

Flannery, T., 2002. The future eaters: an ecological history of the Australasian lands and people. Grove Press.

Ford, G.L.; Fogerty, A.C. 1982. Fatty acids of kangaroo and wallaby meat. CSIRO Food Research Quarterly. 42(3/4): 57-60 12 refs. http://hdl.handle.net/102.100.100/288144?index=1

Garnaut, R., 2008. The Garnaut climate change review. *Cambridge, Cambridge*.

Grigg, G.C., 2002. Conservation benefit from harvesting kangaroos: status report at the start of a new millennium-a paper to stimulate discussion and research. 53-76.

Hackstein, J.H. and van Alen, T.A., 1996. Fecal methanogens and vertebrate evolution. *Evolution*, *50*(2), pp.559-572.

Kempton, T.J., Murray, R.M. and Leng, R.A., 1976. Methane production and digestibility measurements in the grey kangaroo and sheep. *Australian Journal of Biological Sciences*, *29*(3), pp.209-214.

Klieve, A.V. and D. Ouwerkerk. 2007. Comparative greenhouse gas emissions from herbivores, p. 487 – 500. *In* Proceedings of the 7th International Symposium on the Nutrition of Herbivores (Beijing, China). Q.X. Meng, L.P. Ren and Z.J. Cao (ed.) *China Agricultural University Press, Beijing.*

Kondinin 2016. Research Report: Exclusion fencing: fighting ferals. https://www.farmingahead.com.au/digital_assets/c3b39f7a-63f0-415b-832c-ea4d0b070e2b/Research-Report-72-Exclusion_Fencing-LR_v2.pdf Accessed 23/04/2020

Kukekova, A.V., Johnson, J.L., Xiang, X., Feng, S., Liu, S., Rando, H.M., Kharlamova, A.V., Herbeck, Y., Serdyukova, N.A., Xiong, Z. and Beklemischeva, V., 2018. Red fox genome assembly identifies genomic regions associated with tame and aggressive behaviours. *Nature ecology & evolution*, *2*(9), pp.1479-1491.

Lehnen, T.E., da Silva, M.R., Camacho, A., Marcadenti, A. and Lehnen, A.M., 2015. A review on effects of conjugated linoleic fatty acid (CLA) upon body composition and energetic metabolism. *Journal of the International Society of Sports Nutrition*, *12*(1), p.36.

McLeod, S.R. and Hacker, R.B., 2020. Balancing stakeholder interests in kangaroo management–historical perspectives and future prospects. *The Rangeland Journal*, *41*(6), pp.567-579.

MLA 2020. Meat & Livestock Australia - weekly market statistics. https://www.mla.com.au/CachedNLRSReports/Meat-and-Livestock-Weekly-Stats-22-Apr-2020.PDF?id=637233374367037513 accessed 24/04/2020.

Munn, A.J., Dawson, T.J., McLeod, S.R., Croft, D.B., Thompson, M.B. and Dickman, C.R., 2009. Field metabolic rate and water turnover of red kangaroos and sheep in an arid rangeland: an empirically derived dry-sheep-equivalent for kangaroos. *Australian Journal of Zoology*, *57*(1), pp.23-28.

Norris, D. and Andrews, P., 2010. Re-coupling the carbon and water cycles by Natural Sequence Farming. *International journal of Water*, *5*(4), pp.386-395.

OEH 2018. 2017 Annual Report: New South Wales Commercial Kangaroo Harvest Management Plan 2017–21. https://www.environment.nsw.gov.au/-/media/OEH/Corporate-Site/Documents/Animals-and-plants/Wildlife-management/Kangaroo-management/kangaroo-management-program-2017-annual-report-180095.pdf accessed 24/04/2020

OEH 2019. 2019 Quota Report New South Wales Commercial Kangaroo Harvest Management Plan 2017–2021 https://www.environment.nsw.gov.au/-/media/OEH/Corporate-Site/Documents/Animals-and-plants/Wildlife-management/Kangaroo-management/commercial-kangaroo-harvest-management-plan-2017-2021-quota-report-180591.pdf accessed 27/04/2020

PETA 2020. Paul Smith confirms kangaroo-skin ban. https://www.peta.org.au/news/paul-smith-kangaroo-skin-ban/ Downloaded 23/04/2020

Renfree MB 1979. Initiation of development of diapausing embryo by mammary denervation during lactation in a marsupial. *Nature* 278 549–551

Shepherd N.C. 1983. The feasibility of farming kangaroos. Australian Rangeland Journal 5, pp.35-44.

Spiegel, N.B. and Greenwood, P.L., 2019. Meat production from wild kangaroo: The species, industry, carcass characteristics and meat quality traits. In *More than beef, pork and chicken–the production, processing, and quality traits of other sources of meat for human diet* (pp. 347-383). Springer, Cham.

Tiver, F. and Andrew, M.H., 1997. Relative effects of herbivory by sheep, rabbits, goats and kangaroos on recruitment and regeneration of shrubs and trees in eastern South Australia. *Journal of Applied Ecology*, pp.903-914.

Vendl, C., Clauss, M., Stewart, M., Leggett, K., Hummel, J., Kreuzer, M. and Munn, A., 2015. Decreasing methane yield with increasing food intake keeps daily methane emissions constant in two foregut fermenting marsupials, the western grey kangaroo and red kangaroo. *Journal of Experimental Biology*, *218*(21), pp.3425-3434.

Wilson, G., 2018. Co-production of livestock and kangaroos: a review of impediments and opportunities to collaborative regional management of wildlife resources. In *Conservation through Sustainable Use of Wildlife Conference* (Vol. 30).

Wilson, G.R. and Edwards, M.J., 2008. Native wildlife on rangelands to minimize methane and produce lower-emission meat: kangaroos versus livestock. *Conservation Letters*, *1*(3), pp.119-128.

Wilson, G.R. and Edwards, M., 2019. Professional kangaroo population control leads to better animal welfare, conservation outcomes and avoids waste. *Australian Zoologist*, *40*(1), pp.181-202.

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