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Identifying non-agricultural and agricultural plant species with anti-methanogenic properties

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Introduction

This project addressed an urgent need of the livestock industries for cost-effective methods of reducing methane emissions by ruminants grazing pasture. The predominance of pasture in the diet of ruminants in NZ presents some specific challenges for mitigation of methane production. A forage-based solution, while elusive to date, could provide a simple, cost-effective solution for a significant proportion of ruminant livestock in New Zealand.

A comparatively limited number of mainstream pasture species have been tested in NZ including ryegrasses, commonly used legumes such as white and red clovers, less commonly used legumes sulla, lotus, lucerne and Caucasian clover, and herbs such as plantain and chicory. There is a much broader range of species that are used for feeding ruminants that have not yet been tested, and a very wide range of plants that ruminants eat under extensive natural grazing situations.

There is considerable evidence of suppression of methanogenesis by nutritional modification, supporting the proposition of broadening the search for effective plants and for plant traits which could then be mimicked in mainstream pasture species. While only providing 'proof-of-concept' evidence for NZ conditions, rather than offering a practical cost-effective solution, concentrate (grain-rich) diets reduce methane production compared with forage diets because of the different fermentation characteristics of starch and fibre (Blaxter and Clapperton, 1965). More recent studies show a direct association between the proportion of forage in a cow's diet and the production of methane (kg CH4/kg milk produced) (Johnson et al. 2002; Lovett et al. 2006). Paradoxically, the increased use of grain for feeding ruminants has associated greenhouse gas emissions during the production of grain, partially offsetting the lower enteric methane emissions.

Dietary additives such as plant oils generally suppress methanogenesis. Beauchemin et al. 2008 in a review of 17 studies evaluating addition of both oil extracts and oil seeds showed that methane production decreased by nearly 6% for each 1% of oil added to the diet (r^2 = 0.67). The mechanism of suppression (direct suppression of methanogens, suppression of organic matter fermentation, hydrogenation of fatty acids) and the effectiveness differ depending on characteristics of the oil such as fatty acid profile, form of administration and basal diet. Thus, it is to be expected that not all oil-containing plants will give effective

suppression of methanogenesis without adverse effects on intake and fibre digestibility, making careful screening of a range of plants necessary.

Antimicrobials, such as the ionophore monensin, are another potential mitigant. Monensin can suppress methane production by 3-8% (Sauer et al. 1998; McGinn et al. 2004; Van Vugt et al. 2005; Odongo et al. 2007). This effect is by decreasing the rumen acetate:propionate ratio and decreasing the population of rumen protozoa. However, inhibitory effects may be short-lived (e.g. < 2 months) as rumen microbial populations can adapt and methane production increase to pre-treatment levels (Johnson and Johnson, 1995), although other studies show effects may persist for 6 months (e.g. Odongo et al. 2007). While this indicates a mechanism of reduction, there is public resistance to the use of synthetic antimicrobials as feed additives, and it could be used as a non-tarriff trade barrier. However, if similar mechanisms can be mimicked by naturally occurring plant compounds it would circumvent some of these issues.

Plant secondary compounds such as tannins and saponins decrease methane production. There is wide variation in both classes of secondary compounds and, as with plant oils, the suppression of methanogenesis can be very specific to the type and composition of secondary compounds (Hess et al. 2003; Beauchemin et al. 2007). However, the very diverse range of plants containing these secondary compounds suggesting wide scope for pursuing mitigants among such plants. Several legume species (e.g. *Lotus pedunculatus*, *Lotus corniculatus*) contain condensed tannins and have been shown to suppress methane production (e.g. Pinares-Patino et al. 2003; Waghorn et al. 2003; Woodward et al. 2004), and while they are often agronomically difficult to use in intensive ruminant production systems, they have an advantage over other tannin-containing plants because of high nutritive value and often high palatability (provided tannin concentration is not greater than approximately 4% of DM, in which case dry matter intake may be suppressed). There is scope for parallel improvement of their agronomic characteristics for more efficient and widespread use of these secondary compounds.

There are other plant secondary-compounds of putative value for gut function that are being researched for their human health prebiotic effects (selective stimulation of growth of desirable bacteria). Some of these secondary compounds have demonstrated ruminant methane mitigation properties. A recent review (Calsamiglia et al. 2007) outlines 12 commonly used species of herb where the active compounds with antimicrobial and

antimethanogenic activity have been identified, and the mode of action described. Typically they increase propionate production, decrease acetate or methane production and also modify protein degradation. For example, four component compounds of garlic oil have suppressed methane production using an *in vitro* batch-culture fermentation (Busquet et al. 2005), and subsequent studies suggests this to be a result of direct inhibition of rumen methanogens (methane producing bacteria) (see McAllister and Newbold, 2008). Analogous compounds with antifungal and antibacterial properties are known to exist in onion (*Allium cepa*). Some brassica species (*Brassica oleracea*) contain compounds (glucosinolates) with activity against some gut bacteria. Similarly, manuka honey is known to possess antimicrobial activity. Within this grouping of plant species we will include examples of those used in traditional Maori medicine for their gut health and skin healing properties. For example, Koromiko (*Hebe salicifolia*) has known beneficial effects against diarrhoea and dysentery making it an obvious candidate for further study, although it is not known if the specific bioactive compounds it contains have beneficial effects on gut health directly or if it acts as a prebiotic. Other species with noted benefits that warrant screening include leaves and rhizomes of harakeke (*Phorium tenax*; flax) and roots of Rarauhe (*Pteridium aquilinum*; bracken fern – will be checked for potential toxicity before any animal feeding tests are conducted). The use of plant-derived compounds to selectively alter microbial populations may be useful to promote useful bacterial populations to encourage the utilization of hydrogen by metabolic pathways other than methanogenesis e.g. stimulate propionate producing microorganisms that compete with methanogens for hydrogen, or those that produce acetate via reductive acetogenesis (see Attwood and McSweeny, 2008).

Legume diets were generally considered to result in lower emissions per unit of dry matter intake (or per unit of animal product) than do grass diets. While more recent studies with white clover that have been completed since this project started do not to support this generalisation, a range were included in this study because of the diversity in types of legume. The effects on methane emissions were often attributed to lower fibre content, faster rate of passage through the rumen because of the faster degradation rate, and in some cases higher dry matter intake of legumes compared with grasses. However morphological characteristics such as low leaf:stem ratio and advancing stage of maturity (stem formation, secondary thickening) tend to result in increasing methane production. Legumes such as white clover that do not form true stems and maintain a high proportion of leaf in the grazed herbage indicate desirable morphological characteristics. There is a range of legume species with these general characteristics and recent advances in the ability to hybridise among different

species of *Trifolium* provides scope for both agronomic improvement while also maintaining the morphological characteristics associated with low production of methane or enhancing specific characteristics that suppress methane production. One particular species of *Trifolium* had shown promise in a previous in vitro assay, and this was included for further investigation.

This discovery and proof-of-concept project was based on assessing the potential for plant species to reduce methane production, using lab-based screening assays. The aim was to screen a wide range of plant species that were categorised in a structured way to maximise the chances of identifying species with the bioactive compounds. This structured grouping was based on several criteria such as compounds known to suppress methane production, currently-used plants that have not yet been assessed, and more speculatively a grouping of plants that influence gut microbial ecology. However, it was sufficiently broad to include plants with no currently known potential for ruminant feeding so as to give the highest possible opportunity for identifying novel traits/bioactive compounds. While there was possibility for species that are currently used to possess traits of interest, given the screening that has taken place among the mainstream species, it was considered more likely that traits would be identified in species that are either not widely used or, unlikely to be used because of limitations in their agronomic or nutritional value.

Material and Methods

PLANT COLLECTION

Five, broad classifications of plant species were initially created. These consisted of agriculturally relevant forage species for which there was no existing information, grouped as legumes (including tannin-containing legumes), grasses and other species including brassicas, indigenous plants traditionally used by local Maori for their anti-microbial or healing properties,plants species containing oils, essential oils or other secondary compounds such as saponins, and a further speculative group of plant species having curiosity value rather than belonging to any particular classification system or having any prior known properties. Where the focus was on particular plant constituents (e.g. oils, saponins, tannins) reference sources were used to identify appropriate, readily available example species.

The selection of candidate species of indigenous plants and their collection was subcontracted to local Rangitaane iwi, so as to draw on their knowledge of the species and plant parts used in traditional Maori therapy, and the timing and location of harvest for optimum effect. Native plant species list where plants were known to have beneficial antibiotic/antimicrobial properties and used internally (majority used both externally and internally) by Maori to relieve stomach disorders, or to cure septic wounds.

These plants were chosen for initial investigation because they act alone i.e. effects not masked by combinations with others in the medicinal preparations. The criteria for the choice was designed to limit any special preparations while retaining the closest match to the desired microbial suppressive effects. There are some from the list below that require some preparation before use to extract the rongoā compounds. However, these preparations were similar to a digestive process intended to release fluids or soften the plant material prior to their use. Tanenuiarangi Manawatu Incorporated advised on any special preparation needed to release medicinal properties.

The list of more speculative plants was drawn up in a less systematic way, based largely on availability and uniqueness in characteristics. Wherever possible samples of each plant species were collected from local sources. This included on-farm (e.g. brassicas), plant nursery sources at AgResearch or local garden centres, and roadsides or wasteland areas for some of the species that could be classfied as weeds. Where there was no readily available material from local sources, seed was obtained and plants cultivated under nursery conditions at AgResearch, or material requested from sources in other regions. Samples of the species were obtained from local parks and reserves. Depending on the time of year and stage of development of the species being sampled, plants were often separated into different plant components such as leaves, stems, flowers or fruit, and these components were assayed individually. All material was frozen as soon after harvest as practical and then freeze-dried and ground to pass a 1mm sieve. Subsamples were then taken from this for submission for *in vitro* assay.

IN VITRO SCREENING

The *in vitro* screening process was carried out in a fully automated rumen batch culture system that measures total gas production as well as methane and hydrogen concentration (Muetzel et al., 2011).

The freeze dried plant samples were incubated at 10 mg/ml of medium or were added dissolved in dimethylsulfoxide (DMSO) to 600 mg of the control ryegrass. A leak test and a pressure calibration were carried out using bottles filled with water before and after the incubation. Before incubation the bottles were randomised, placed into the incubation rack and pre-warmed in the incubator. The buffer used is described by (Mould et al., 2005) and contains 6.0 mM Na_2HPO_4, 9.6 mM KH_2PO_4, 0.5 mM $MgCl_2$, 64.5 mM $NaHCO_3$ and 17.8 mM NH_4HCO_3. A total of 2.4 L of buffer was prepared, placed in a 39 °C water bath and gassed with CO_2 for at least 30 min before the reducing agent (NaOH 2.5 mM and Cystein-HCl 2.5 mM) was added just prior to collection of rumen fluid. Before rumen fluid collection, calibration gases were injected to the GC.

Ruminally fistulated non-lactating cows were fed a maintenance ration of pasture and were brought in to the cattle yards for rumen fluid collection. Rumen fluid was collected from one cow via a large fistula by manually removing digesta from various parts of the rumen and squeezing the contents into a pre-warmed thermos flask (750 ml). The thermos bottles were filled to the top to avoid oxygen contamination during the transport to the laboratory. In the lab the rumen fluid was filtered through one layer of cheese cloth and 600 ml of the filtered rumen fluid was immediately transferred into the reduced buffer. The medium was then bubbled for another 15 min with CO_2 to equilibrate the solution before being dispensed in 60 ml aliquots into the incubation bottles. The pre-warmed incubation bottles were flushed with CO_2 before the medium was dispensed. The bottles were then capped with a butyl rubber stopper, placed in the incubator and connected to the gas system by stabbing the 23 g needle through the butyl stopper.

The pressure in the bottles was measured every minute via a pressure sensor connected to a 2 way valve and this was converted to a volume of gas using a separate calibration curve for each of the 32 pressure sensors. If the accumulated volume of gas exceeds 6 ml, the volume needed to flush the gas lines 5 times, the valve opens and the gas sample is flushed through a sampling loop of the gas chromatograph. After 15 seconds the valves closes and the sampling loop is switched automatically in line with the GC column for the measurement of methane and hydrogen concentrations. The gas composition was determined using a GC 2010 gas chromatograph (Shimazu, Kyoto, Japan) fitted with a column and a TCD and a FID detector to quantify hydrogen and methane simultaneously. The gases were separated in a HP-Molsieve column (30 m length, .53 mm ID, 25 µM film) using a isothermic run at 85 °C. N_2

was used as the carrier gas at a rate of 13 ml/min. The temperature of the FID and TCD detector was 250 and 105 °C respectively. The samples were injected into the GC as a single chromatogram lasting 25 h, allowing a sample to be injected every 60 s. To ensure proper data acquisition of the GC, a standard sample (Control) was injected every hour into the system to account for any drift or decrease in response over the whole incubation period. Incubation usually last for 48 h after which a sample for short chain fatty acid analysis was taken from each bottle (a 0 h sample was taken at the start of the incubation from the medium). At the end of the incubation another set of gas standards were injected automatically into the system.

Gas production (GP) data were calculated as the cumulative gas production and are reported as ml of gas produced per g of substrate incubated. Methane and hydrogen production were also calculated as cumulative production taking into account the residual gas concentration after each purging event. Data for methane and hydrogen are reported either as production (ml/g DM) or relative to total gas production (% GP).

SAMPLE PREPARATION AND ANALYSIS

Samples (1.8ml) of the buffered rumen fluid at the start of the incubation and the contents of the bottle after 48 hours incubation were taken for analysis of short chain fatty acids. Samples were centrifuged (21 000 g, 10 min, 4 °C) and 900 µl of the supernatant was transferred into 100 µl of internal standard solution (19 mM ethylbutyrate in 20% (v/v) phosphoric acid). Samples were frozen for at least 16 h, centrifuged again as above and 800 µl of the supernatant was transferred into a 2 ml gas chromatography vial and crimped immediately. SCFA were determined in a Hewlett-Packard (HP) 6890 series GC with an auto-injector and flame ionization detector. The column was a Zebron ZB FFAP, the detector temperature was held at 240°C and the oven temperature was increased from 85 to 180°C at 10°C/min and held for 10 min. The carrier gas was helium at 5.5 ml/min. SCFA were quantified from a standard curve of known concentrations of individual fatty acids.

SCFA production was calculated by subtracting the 0 hour medium concentration from the final concentrations and converting into mmoles per g of substrate incubated. Individual SCFA are reported as a proportion of the total amount of SCFA.

Two sample extraction techniques were employed. The first method employed steam distillation extraction to yield an ethereal extract amenable for analysis by gas chromatography mass spectrometry for characterising and quantifying the samples volatile essential oil components. The ethereal extract was also amenable for evaluation of its *in vitro* activity. The second method employed soxhlet solvent extraction to yield a plant residue and extract amenable for *in vitro* evaluation of activity.

Sample steam distillation extraction was performed as follows. Samples (1 g) were added to saturated sodium chloride (50 ml) in a 150-ml round bottom flask and heated to reflux on one side of a Likens Nickerson steam distillation apparatus. Ether (25 ml; t-butyl methyl ether) was added to a 100-ml round bottom flask and heated to reflux on the other side of the Likens Nickerson steam distillation apparatus and the extraction was performed for 2 hours. The resulting ethereal extract was analysed by a gas chromatograph (Shimadzu GC17a) equipped with a wax column (ZB wax; 30m x 0.25mm ID x 0.25 um film thickness) and mass spectrometry (Shimadzu QP5050a). Compounds were initially identified from mass spectral database libraries and subsequently confirmed from analysis of authentic compounds. For *in vitro* activity assays the ethereal extract was reduced to dryness by rotary evaporation and redissolved in DMSO.

Sample soxhlet extractions were performed as follows. Samples (5 g) were weighed into cellulose soxhlet thimbles (50 mm x10 mm) and added to a soxhlet extraction apparatus. Hexane was chosen as the extraction solvent to prevent extraction of the soluble sugars or water soluble components and the extraction was performed for 2 hours. For *in vitro* activity assays residual hexane was removed from the extracted plant material (residue) by vacuum and the hexane extract was reduced to dryness by rotary evaporation and redissolved in DMSO.

DATA ANALYISIS

All samples were incubated in duplicate bottles and the incubation was repeated with rumen fluid from another donor animal. In each incubation run a negative and a positive control were incubated along with the test plants. The negative control was a ryegrass sample and the positive control was the same ryegrass where 60 µl of a 30 mM solution of bromoethane sulfonate (BES) was added. Because of the natural variability of *in vitro* incubations the results for all test plants within an incubation run were normalised for the negative and the

positive controls. To compare the effects of the plants on methane emissions the SCFA production data and the proportion of methane during early (8 h) and late (24 h) fermentation were used. For SCFA, the negative control was assumed to be 100 %, whereas for methane the difference in methane emissions of the negative and positive control was assumed to be 100%. In addition the proportion of propionate hydrogen formed served as indicators for sugar fermentation or a methanogen inhibitor respectively.

Results and Discussion

The plants tested are shown in Table A1 in the annex. Various groupings of plants, covering edible and non-edible species, and indigenous and exotic species were screened in this programme. Most plant species fitted within a broad classification into five main groups. These groups were brassicas, grasses, legumes, plant species with particular secondary compounds that had been shown in other studies to confer anti-methanogenic properties, and indigenous plants with properties used in traditional Maori medicine for a variety of therapeutic uses. A fifth group included a diverse range of plant species with many different factors supporting their inclusion for assay. Some were chosen where there is residue available from existing processing industries, the example here being hops where vine after stripping the cones, and residues are extracting resins could be available from processing facilities for livestock feeding. Others species were selected on the basis of known use as herbal remedies for various ailments, outside of traditional Maori medicine, such as treating stomach ailments or providing antimicrobial properties. Yet other candidates were plant species or plant parts sometimes consumed by livestock, and/or where there could be harvesting and/or processing based on availability e.g. seaweeds, pine needles. Not all were edible plants, and some are considered as poisonous to livestock. Extending the selection beyond just forage species was considered an important aspect of this project, to allow for the identification of possible secondary or other compounds in plant that would provide inhibitory characteristics. A true inhibitor is considered the most desirable way for reducing methanogenesis because it could be effective at low proportions in the diet. Alternative pathways for reducing methane production, such as increasing the formation of propionate to act as a sink for hydrogen, require comparatively larger quantities of the active constituent to achieve similar levels of suppression.

SCFA concentration was used to assess the effect of the tested plants on overall fermentation. Total SCFA production of the test plants varied from 20% higher than the control (ryegrass) to only 15% of the control samples. Differences in methane emissions as a percentage of total gas production were evaluated after 8 and 24 hours of incubation. Expressing the data relative to total gas production is a way to the determine if there is a specific effect on methane emissions rather than a decrease in overall methane production associated with a decrease in overall fermentation. Early in the incubation the effects on methane emissions were generally higher, ranging from around 85% inhibition to 110% increase in methane emissions. The range was similar for the 24 h methane emissions but the average effect was smaller at later stages of fermentation. Methane emissions were lower than the positive control for only a few plants including garlic (*Allium sativum*), horopito (*Pseudowintera colorata*), osage orange (*Maclura pomifera*), oregano (*Origanum vulgare*) and cauliflower (*Brassica oleracea*). Within these plants oregano and horopito decreased SCFA production compared to the ryegrass control by 34% and 86%, respectively. The negative effect of horopito on total fermentation makes it an unlikely candidate for further investigation. Horse apple and garlic both increased the proportion of propionate significantly but the summation of hydrogen being diverted into propionate and that released as molecular hydrogen explained less than 50% of the hydrogen produced when calculated from the fermentation equations of (Wolin, 1960). Only garlic and oregano showed a significant release of molecular hydrogen, which is an indicator of direct methanogen inhibition. The decrease of methanogenesis observed for cauliflower was surprising, since there was no hydrogen released, as would be expected if the plant contained an inhibitor of methanogenesis, nor was the fermentation profile changed towards greater production of propionate, a hydrogen sink. However, a nitrate analysis revealed that the sample contained 9580 mg/kg DM of nitrate which is a potent hydrogen sink (Van Zijderveld et al., 2011; Sakthivel et al., 2012). Garlic which showed both an increase in molecular hydrogen production and a shift towards propionate production showed the highest methane reduction potential observed so far. However, garlic and its effect on ruminal methane emissions has been studied for a long time and is well documented *in vitro* (Hart et al., 2008; Staerfl et al., 2010) but attempts to reduce methane emissions in sheep or cattle have not been successful (Klevenhusen et al., 2011; Patra et al., 2011; Staerfl et al., 2012). Similarly, oregano as a methane mitigation tool has been studied intensively (Wang et al., 2009; Tekippe et al., 2011) and for these reasons garlic and oregano were not examined further.

Bee balm (*Monarda fistulosa*) showed a similar profile in fermentation to that of oregano and lavender, both characterized a relatively high proportion of hydrogen produced, indicating the presence of an inhibitor. Classical approaches for identifying a sample's bioactive constituents employ sample extraction and separation of extract's constituents by their chemical properties in combination with a bioassay to inform where activity resides. Given the fermentation response of bee balm was comparable to that of oregano and lavender and previous studies identified the antimicrobial activity was associated with the volatile essential oil component, initial approaches for identifying bee balm's activity focused on extraction of the volatile essential oil component. The volatiles of this plant were extracted by steam distillation and analysed by gas chromatography mass spectrometry. Carvacrol, (60 %), thymoquinone (15 %) and p-cymene (15 %) were identified to be the major components present and various terpene minor components (10 %) were also identified. The essential oil extract comprised of 1 % of the bee balm's dry weight. A parallel steam distillation of oregano confirmed carvacrol and thymol were the major components of oregano's essential oil. In order to establish whether carvacrol was the inhibitory substance responsible for the increased emission of hydrogen, the steam distillation extract and a hexane soxhlet extract of bee balm were incubated *in vitro* in proportions found in the bee balm itself. In addition, the residue from the hexane extraction was also incubated.

During the initial screening the gas production of the sample was comparatively low and the proportion of methane production was only about a third of the control values (Table 3). During the re-screening the gas production increased by 20% and the proportion of methane by around 50% compared to the initial screening. Hydrogen emissions however were increased by 50%. The material that was extracted and incubated was from a separate subsample and did not show any activity against methane emissions *in vitro*. However for comparison with the extracts DMSO was added to the bee balm sample and DMSO could have been converted to dimethyl sulfide, using one mole of hydrogen for every mole of DMSO converted. This may be the reason why no hydrogen was observed from the incubation of the bee balm in the presence of DMSO since the bee balm itself does not contain a very strong inhibitor compared to garlic. However, the fact that no reduction of methane was observed over the screening results even in the presence of DMSO, makes it questionable whether the second batch of bee balm contained any activity against methanogens. This could explain the lack of an effect of the steam distillation and the hexane extract. However the steam distillation extract contained 60% carvacrol and at the same

concentration carvacrol did not show any activity against methanogens even when considering that hydrogen might be consumed by the reduction of DMSO.

From the results obtained we infer that they apply to the species as a whole. However, we are necessarily limited to screening what we consider was sample material representative of that species. There is variability in animal rumen microbial communities and variability within plant species that could potentially influence the results obtained. The rumen microbial community differs between animals even when they are managed under the same conditions in terms of diet. This effect relating to differences between animals was apparent in the results for bee balm. Similarly plants vary in the chemical composition in relation to time of year, stage of growth, cultivar and even between individual plants within a species. This was apparent for one of the *Trifolium* species that initially had shown promising effects that could not be repeated with subsequent batches of material. One approach to maximising the chances of detecting bioactivity that might be present in a plant species was to separate the harvested plant into component species, as for example is done for traditional uses of indigenous plants. This would reduce the chances of bioactive constituents residing in just one part of the plant from being diluted by the presence of other non-bioactive plant parts.

So far the results of the bee balm are equivocal and pursuing this line further would be challenging because of the variability of results between assays and between different batches of the plant material. Identifying the reasons for this variability, while a desirable goal, would be difficult with the research tools and methodologies currently available.

Conclusions

Out of 220 plant species or plant parts screened, 10 samples decreased the specific methane emissions by more than 50% and 4 out of these 10 had no negative effect on overall fermentation. However, most of the observed effects could be attributed to substrates that shift the fermentation pathways into propionate, or hydrogen sinks such as nitrate. Two species of plants (garlic and oregano) showed a true methanogenic inhibitor effect but for both of them the effect on methane emissions has been described, and in the case of garlic the active components are known. However, the programme has shown that the methods used can identify natural plants with specific inhibitors of methane and identify false-positive samples where the decrease in methane is driven either by carbohydrates directing fermentation towards propionate formation or hydrogen sinks like nitrates. Importantly, there

is variation among plant species in their effects on methane emissions, and this should be explored further.

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Table 1: Indigenous plant species selected for screening and their Maori name, the plant part used and the traditional purpose of use.

Species name	Maori name	Plant part	Traditional use
Angiosperm trees			
Alectryon excelsus	titoki	oil	
Beilschmiedia tawa	tawa	innerbark	flatulence
Elaeaocarpus dentatus	hinau	bark	diarrhoea
Kunzea ericoides	kanuka	bark (boiled)	diarrhoea
Leptospermum scoparium	manuka	tea-tree	
Melicytus ramiflorus	mahoe	berry	antiseptic
Metrosideros excelsa	pohutukawa	bark	stomach complaints
Pennantia corymbosa	kaikomako		
Gymnosperms			
Agathis australis	kauri	sap; bark/cones	antiseptic
Podocarpus totara	totara	bark leaves	antiseptic
Ferns and fern allies			
Adiantum spp	maidenhair	whole plant	antiseptic
Cyathea dealbata	ponga	pith	antiseptic
Shrubs			
Geniostoma rup ligustri	hangehange	leaves	stomach complaints
Hebe stricta	koromiko	stem tips	diarrhoea
Leucopogon fasciculatus	mingimingi	leaves	antiseptic
Macropiper excelsum	kawakawa	whole plant	stomach complaints
Myoporum laetum	ngaio	leaves	antiseptic
Pseudowintera colorata	horopito	whole plant	general
Monocots			
Typha orientalis	raupo	whole plant	antiseptic
Cortideria spp	toitoi	leaf base	stomach complaints
Phormium tenax	harakeke	flax leaf gum	diarrhoea

Table 2 Description of all plant species tested, according to common name, botanical name, and the plant part(s) assayed.

ID	Common name	Botanical name	Plant part
1	turnips	*Brassica rapa* Var. Pasja	whole plant
2	turnips	*Brassica rapa* Var. Appin	top/ leaf
3	turnips	*Brassica rapa* Var. Appin	bulb
4	rape (winter)	*Brassica napus* Var. Goliath	leaf
5	rape (winter)	*Brassica napus* Var. Goliath	stem top
6	rape (winter)	*Brassica napus* Var. Goliath	stem base
7	rape (winter)	*Brassica napus* Var. Goliath	whole plant
8	rape (summer)	*Brassica napus* Var. Titan	whole plant
9	kale	*Brassica oleracea* Var. Kestrel	leaf
10	kale	*Brassica oleracea* Var. Kestrel	upper stem
11	kale	*Brassica oleracea* Var. Kestrel	lower stem
12	kale	*Brassica oleracea* Var. Kestrel	top/leaf
13	kale	*Brassica oleracea* Var. Kestrel	stem base
14	swedes	*Brassica napus* Var. Aparima Gold	top/leaf
15	swedes	*Brassica napus* Var. Aparima Gold	bulb
16	swedes	*Brassica napus* Var. Dominion Swede	top/ leaf
17	swedes	*Brassica napus* Var. Dominion Swede	bulb
18	fodder beet (mangels)	*Beta vulgaris* Var. Collosse	top/leaf
19	fodder beet (mangels)	*Beta vulgaris* Var. Collosse	bulb
20	fodder beet	*Beta vulgaris* Var. Collosse	bulb
22	broccoli	*Brassica oleracea*	leaf
23	broccoli	*Brassica oleracea*	head
24	broccoli	*Brassica oleracea*	leaf
25	broccoli	*Brassica oleracea*	stem
26	cabbage	*Brassica oleracea*	whole
27	onion	*Allium cepa*	bulb
28	cauliflower	*Brassica oleracea*	head
29	cauliflower	*Brassica oleracea*	leaf/midrib
30	garlic	*Allium sativum*	bulb
31	beetroot	*Beta vulgaris*	leaf
32	beetroot	*Beta vulgaris*	bulb
33	brussel sprouts	*Brassica oleracea*	leaf
35	chinese cabbage	*Brassica rapa* Var. Chinensis	leaf
36	kohlrabi	*Brassica oleracea*	leaf
38	bake beans + tomato paste	*Phaseolus vulgaris*	seed/fruit(cooked)
39	bake beans only	*Phaseolus vulgaris*	seeds(cooked)
40	rhubarb	*Rheum rhabarbarum*	stalks
41	rhubarb	*Rheum rhabarbarum*	leaf
51	kawakawa	*Macropiper excelsum*	leaf
52	koromiko	*Hebe stricta*	leaf&stem
53	hangehange	*Geniostoma ligustrifolium*	leaf
54	totara	*Podocarpus totara*	leaf

ID	Common name	Botanical name	Plant part
55	raupo	*Typha orientalis*	leaf
57	kanuka	*Kunzea ericoides*	leaf&stem
58	mahoe	*Melicytus ramiflorus*	fruit
59	ngaio	*Myoporum laetum*	leaf
60	kauri	*Agathis australis*	stem&fruit
61	pepper wood/red horopito	*Pseudowintera colorata*	leaf&stem
63	ponga	*Cyathea dealbata*	pith
65	titoki	*Alectryon excelsus*	leaf
68	maiden hair fern	*Adiantum*	frond
74	poroporo	*Solanum aviculare*	fruit
75	poroporo	*Solanum aviculare*	leaf
101	agapanthus	*Agapanthus africanus*	whole plant
102	foxglove	*Digitalis purpurea*	leaf
103	sheeps burnett	*Sanguisorba minor*	leaf/stem
104	chicory -red	*Cichorium intybus*	leaf/stem
105	chicory -red	*Cichorium intybus*	root
106	giant creeping buttercup	*Ranunculus*	leaf/stem
107	fennel	*Foeniculum vulgare*	leaf/flower
108	fennel	*Foeniculum vulgare*	stalk
109	saltbush	*Atriplex nummularia*	leaf/stem
110	safflower	*Carthamus tinctorius*	flower head
111	hops	*Humulus lupulus* Var. Pacific Jade	hop pellets
112	hops	*Humulus lupulus* Var. NZ Hallertau Aroma	hop pellets
113	hops	*Humulus lupulus* Var. Pacific Jade	hop cones
114	hops	*Humulus lupulus* Var. NZ Hallertau Aroma	hop cones
115	hops	*Humulus lupulus* Hop marc #1	milled
116	hops	*Humulus lupulus* Hop marc #2	milled
117	tobacco	*Nicotiana tabacum*	flowers
118	tobacco	*Nicotiana tabacum*	young leaf
119	tobacco	*Nicotiana tabacum*	older leaf
120	tobacco	*Nicotiana tabacum*	stalks
121	chestnut	*Aesculus hippocastanum*	fruit
122	acorn	*Quercus robur*	fruit
123	pine needles	*Pinus radiata*	tip growth
124	chamomile	*Matricaria matricarioides*	whole plant
125	gorse	*Ulex europaeus*	end growth
126	willow	*Salix matsudana*	young growth
127	goats rue	*Galega officinalis*	whole plant
128	ragged robin	*Lychnis flos-cuculi*	leaf
129	mint	*Mentha spicata*	leaf/stem
130	plantain	*Plantago lanceolata*	leaf
131	plantain	*Plantago lanceolata*	stem+seedhead
132	nightshade	*Solanum nigrum*	young
133	grape leaf	*Vitis vinifera*	leaf
134	oxalis species	*Oxalis*	leaf

ID	Common name	Botanical name	Plant part
135	paperbark	*Melaleuca linariifolia*	leaf/stem
136	hop leaf	*Humulus lupulus* Var. Rakua	fresh
137	hop flowers	*Humulus lupulus* Var. Rakua	fresh
138	hop vine	*Humulus lupulus* Var. Rakua	fresh
139	broad leaf dock	*Rumex obtusifolius*	fresh leaf
140	tea tree	*Melaleuca alternifolia*	leaf&stem
141	oolong tea	*Camellia sinensis*	leaf
142	lemongrass	*Cymbopogon citratus*	leaf
143	plantago sp	*Plantago*	leaf
144	sage	*Salvia officinalis*	leaf
145	lavander	*Lavandula spica*	leaf/stem
146	lavander	*Lavandula spica*	flowers
147	osage orange	*Maclura pomifera*	fruit
148	safflower	*Carthamus tinctorius*	leaf/stem/flower
151	sulla	*Hedysarium coronarium*	whole plant
152	birdsfoot trefoil	*Lotus corniculatus* Var. Goldie	whole plant
153	lotus major	*Lotus pedunculatus* Var. Sunrise	whole plant
154	sainfoin 4522	*Onobrycus viciifolia*	whole plant
155	Dorycnium hirsutum	*Dorycnium hirsutum*	whole plant
156	Dorycnium pentaphyllum	*Dorycnium pentaphyllum*	whole plant
157	Acacia saligna	*Acacia saligna*	leaf
158	Acacia saligna	*Acacia saligna*	stem
159	Trifolium ambiguum	*Trifolium ambiguum*	whole plant
160	crimson clover	*Trifolium incarnatum*	whole plant
161	crimson clover	*Trifolium incarnatum*	whole plant
164	sulla	*Hedysarum coronarium*	whole plant
165	lotus major	*Lotus pedunculatus*	whole plant
166	crown vetch	*Coronaria varia*	whole plant
167	ball clover	*Trifolium nigrescens* Var. NMD	whole plant
168	ball clover	*Trifolium nigrescens* Var. NPD	whole plant
169	ball clover	*Trifolium nigrescens* Var. NND	whole plant
170	ball clover	*Trifolium nigrescens*	whole plant
171	Chamaecytisus palmensis	*Chamaecytisus palmensis*	leaf
172	yellow-flowered lupin	*Lupinus arboreus*	leaf
173	sweet clover	*Melilotus alba*	whole plant
174	ball clover	*Trifolium nigrescens* Var. NMD	whole plant
175	ball clover	*Trifolium nigrescens* Var. NPD	whole plant
176	ball clover	*Trifolium nigrescens* Var. NND	whole plant
177	Dorycnium pentaphyllum	*Dorycnium pentaphyllum*	whole plant
178	Lathyrus latifolius	*Lathyrus latifolius*	whole plant
179	caucasian clover	*Caucasian clover*	whole plant
181	ball clover	*Trifolium nigrescens* Var. NPD	whole plant
183	haresfoot clover	*Trifolium arvense*	whole plant
184	haresfoot clover	*Trifolium arvense*	whole plant
185	low hop clover	*Trifolium campestre*	whole plant

ID	Common name	Botanical name	Plant part
189	suckling clover	*Trifolium dubium*	whole plant
190	alsike clover	*Trifolium hybridum*	whole plant
191	bigflower clover	*Trifolium michelianum*	whole plant
201	yorkshire fog	*Holcus lanatus*	lamina
202	tall fescue	*Festuca arundinacea*	lamina
203	cocksfoot	*Dactylis glomerata* Var. Vision	lamina
204	prairie grass	*Bromus unioloides*	lamina
205	timothy	*Phleum pratense*	lamina
206	browntop	*Agrostis stolonifera* Var. Muster	lamina
207	harding grass	*Phalaris tuberosa*	lamina
208	Alaska brome grass	*Bromus sitchensis*	lamina
210	sorghum	*Sorghum bicolor*	lamina
251	laughing gym (mushroom)	*Gymnopilus junonius*	top/stem
252	shining gum	*Eucalyptus nitens*	leaf
253	giant yucca	*Yucca gigantea*	leaf
254	fence aloe	*Aleo tenuior* Var. Rubriflora	leaf
255	soap aloe	*Aleo saponoria*	leaf
256	peppercorns	*Piper nigrum*	seeds
257	brown alga (seaweed# 1)	*Marginariella boryana*	whole plant
259	strap kelp (seaweed #3)	*Lessonia variegata*	whole plant
260	sea lettuce (seaweed #4)	*Ulva*	whole plant
261	giant kelp (seaweed #5)	*Macrocystis pyrifera*	whole plant
262	bull kelp (seaweed #6)	*Durvillaea antarctica*	whole plant
263	mallow	*Malva sylvestris*	leaf
264	mallow	*Malva sylvestris*	stem
267	horse chestnut	*Aesculus hippocastanum*	leaflets
268	horse chestnut	*Aesculus hippocastanum*	stalk from leaves
271	horse chestnut	*Aesculus hippocastanum*	seed coat/husk
272	lawn chamomile	*Matricaria recutita*	leaf
274	mock orange	*Philadelphus virginalis*	leaf
275	mock orange	*Philadelphus virginalis*	stalk from leaves
277	white campion	*Silene latifolia*	leaf
278	spineless yucca	*Yucca elephantipies*	leaf
279	spineless yucca	*Yucca elephantipies*	stem
283	bay laurel	*Laurus nobilis*	leaf
284	bay laurel	*Laurus nobilis*	branch/stalk
285	basil	*Ocimum basilicum*	leaf
286	sage	*Salvia officinalis*	leaf
288	rosemary	*Rosmarinus officinalis*	leaf, young stalks
289	rosemary	*Rosmarinus officinalis*	branch/stalk
291	mint	*Mentha*	leaf
292	mint	*Mentha*	leaf
295	German chamomile	*Matricaria chamomilla* Var Recutiata	leaf
298	oregano	*Origanum vulgare*	leaf
301	angelica	*Angelica archangelica*	leaf

ID	Common name	Botanical name	Plant part
302	dill	*Anethum graveolens*	leaf
303	celery	*Apium graveolens*	leaf
304	traggon	*Artemisai dracenculus*	leaf
305	wormwood	*Artemisia absinthium*	leaf
306	caraway	*Carum carvi*	leaf
309	bee balm	*Mondarda fistulosa*	leaf
310	parsley	*Petroselinum crispum*	leaf
315	sweet pepper, capsicum	*Capsicum annuum*	leaf
316	sweet pepper, capsicum	*Capsicum annuum*	fruit
320	blueberry	*Vaccinium sp*	leaf
321	blueberry	*Vaccinium sp*	stalk/branch
328	annual nettle	*Urtica urens*	whole
329	perennial nettle	*Urtica dioica*	whole
330	tree nettle	*Urtica ferox*	whole
331	onion weed	*Nothoscordum borbonicum*	whole
332	fly agaric	*Amanita australis* (fungi)	top/stem
336	manuka tea	*Leptospermum scoparium*	leaf
338	manuka oil	*Leptospermum scoparium*	liquid
339	hop extract CO_2	*Humulus lupulus*	paste
340	hop extract CO_2	*Humulus lupulus*	paste
341	giant yucca	*Yucca elephantipies*	fl. Stem
342	hemlock	*Conium maculatum*	leaf/stem
344	elderberry	*Sambucus nigra*	leaf/stem
347	gingko	*Ginkgo biloba*	leaf
348	field bindweed	*Convolvulus arvensis*	leaf/stem
349	spider wort	*Tradescantia pallida*	leaf/stem
350	Japanese honeysuckle	*Lonicera japonica*	leaf/stem/flw
351	forage kochia	*Kochia prostrata*	leaf/stem
352	white mulberry	*Morus alba*	leaf
353	black mulberry	*Morus nigra*	leaf/berries
NMF	ball clover	*Trifolium nigrescens*	flower
NML	ball clover	*Trifolium nigrescens*	leaf
NMP	ball clover	*Trifolium nigrescens*	petiole
NNF	ball clover	*Trifolium nigrescens*	flower
NNL	ball clover	*Trifolium nigrescens*	leaf
NNP	ball clover	*Trifolium nigrescens*	petiole
NPF	ball clover	*Trifolium nigrescens*	flower
NPL	ball clover	*Trifolium nigrescens*	leaf
NPP	ball clover	*Trifolium nigrescens*	petiole
WCF	white clover	*Trifolium repens*	flower
WCL	white clover	*Trifolium repens*	leaf
WCP	white clover	*Trifolium repens*	petiole

Table 3 Ranking of plant species and plant parts on specific methane reduction potential (CH_4 as a proportion of total gas production; positive numbers indicate the extent of suppression compared with the negative control and negative numbers indicate an increase in methane emission compared with the positive control; within the column the shading grades from dark green for highest levels of suppression to red for highest levels of increase in methane emission), the effect on overall fermentation determined from the production of short chain fatty acids (SCFA; negative numbers indicate suppression of fermentation and positive numbers indicate an increase in fermentation relative to the negative control; within the column shading ranging from dark green indicating highest levels of enhanced fermentation to red indicating highest levels of suppressed fermentation) and categorisation on whether the decrease in methane is associated with propiogenic pathways (C3) which act as a hydrogen sink, or an inhibitory effect towards methanogens (H2).

ID	Common name	Plant part	CH4	SCFA	C3	H2
30	garlic	bulb	89%	9%	strong	strong
61	pepper wood/red horopito	leaf&stem	87%	-86%	weak	--
147	osage orange	fruit	82%	-9%	strong	--
298	oregano	leaf	78%	-34%	--	weak
29	cauliflower	leaf/midrib	74%	0%	--	--
137	hop flowers	fresh	60%	-66%	weak	--
339	hop extract CO2	paste	59%	-47%	strong	--
74	poroporo	fruit	58%	-34%	--	--
144	sage	leaf	56%	-24%	--	--
35	chinese cabbage	leaf	55%	-3%	--	--
340	hop extract CO2	paste	49%	-35%	weak	--
251	laughing gym (mushroom)	top/stem	46%	-14%	strong	--
316	sweet pepper, capsicum	fruit	45%	4%	weak	--
341	spineless yucca	flower&stem	44%	4%	strong	--
344	elderberry	leaf&stem	44%	-15%	--	--
146	lavander	flowers	43%	-37%	--	weak
114	hops	hop cones	43%	-67%	--	--
19	fodder beet (mangels)	bulb	42%	1%	weak	--
31	beetroot	leaf	40%	-32%	--	--
20	fodder beet	bulb	40%	7%	weak	--
309	bee balm	leaf	40%	-19%	--	--
145	lavander	leaf&stem	39%	-42%	--	weak
75	poroporo	leaf	38%	-33%	--	--
143	plantago sp	leaf	38%	-44%	--	--
111	hops	hop pellets	37%	-81%	strong	--
320	blueberry	leaf	37%	-56%	--	--
210	sorghum	lamina	34%	-15%	--	--
271	horse chestnut	seed coat/husk	33%	-48%	--	--
288	rosemary	leaf, young stalks	32%	-60%	strong	--
32	beetroot	bulb	32%	11%	--	--
306	caraway	leaf	32%	-6%	--	--
101	agapanthus	whole plant	31%	-4%	weak	--
191	bigflower clover	whole plant	29%	-13%	--	weak
112	hops	hop pellets	29%	-72%	strong	--
17	swedes	bulb	29%	10%	--	--

ID	Common name	Plant part	CH4	SCFA	C3	H2
253	giant yucca	leaf	29%	-16%	weak	--
27	onion	bulb	28%	3%	--	--
301	angelica	leaf	27%	-3%	--	weak
NNF	ball clover	flower	27%	6%	--	--
NMF	ball clover	flower	25%	-4%	--	--
181	ball clover	whole plant	24%	-3%	--	--
166	crown vetch	whole plant	24%	-8%	--	--
336	manuka tea	leaf	24%	-23%	--	--
159	*Trifolium ambiguum*	whole plant	23%	-12%	--	--
157	*Acacia saligna*	leaf	23%	-50%	--	--
22	broccoli	leaf	22%	4%	--	--
148	safflower	leaf/stem/flower	22%	-8%	--	--
NMP	ball clover	petiole	21%	1%	--	--
350	Japanese honeysuckle	leaf/stem/flower	21%	-23%	--	--
264	mallow	stem	20%	-20%	--	--
315	sweet pepper, capsicum	leaf	20%	-22%	--	--
328	annual nettle	whole	20%	-26%	--	--
121	chestnut	fruit	20%	-27%	--	--
15	swedes	bulb	19%	1%	--	--
102	foxglove	leaf	19%	-28%	--	--
113	hops	hop cones	18%	-78%	weak	--
338	manuka oil	liquid	18%	-13%	--	--
123	pine needles	tip growth	17%	-64%	--	--
40	rhubarb	stalks	17%	-6%	--	--
63	ponga	pith	17%	-28%	--	--
252	shining gum	leaf	16%	-67%	--	--
133	grape leaf	leaf	16%	-42%	--	--
3	turnips	bulb	16%	4%	--	--
347	gingko	leaf	16%	-19%	--	--
25	broccoli	stem	15%	10%	--	--
286	sage	leaf	15%	-33%	--	--
304	traggon	leaf	15%	-20%	--	--
24	broccoli	leaf	15%	6%	--	--
272	lawn chamomile	leaf	15%	-16%	--	--
28	cauliflower	head	14%	15%	--	--
305	wormwood	leaf	13%	-14%	--	weak
279	spineless yucca	stem	13%	-8%	weak	--
179	caucasian clover	whole plant	13%	2%	--	--
7	rape (winter)	whole plant	13%	6%	--	--
26	cabbage	whole	13%	14%	--	--
NPF	ball clover	flower	13%	-1%	--	--
116	hops	milled	12%	-37%	--	--
11	kale	lower stem	12%	-2%	--	--
9	kale	leaf	12%	7%	--	--
267	horse chestnut	leaflets	11%	-23%	--	--
295	German chamomile	leaf	11%	-7%	--	weak
291	mint	leaf	11%	-18%	--	--

ID	Common name	Plant part	CH4	SCFA	C3	H2
139	broad leaf dock	fresh leaf	10%	-14%	--	--
115	hops	milled	10%	-38%	--	--
117	tobacco	flowers	10%	-28%	--	--
353	black mulberry	leaf/berries	10%	3%	--	--
NML	ball clover	leaf	10%	8%	--	--
132	nightshade	young	10%	-12%	--	--
59	ngaio	leaf	10%	-17%	--	--
12	kale	top/leaf	9%	-1%	--	--
165	lotus major	whole plant	9%	-40%	--	--
171	*Chamaecytisus palmensis*	leaf	9%	-6%	--	--
23	broccoli	head	9%	13%	--	--
4	rape (winter)	leaf	8%	8%	--	--
2	turnips	top/ leaf	8%	4%	--	--
254	fence aloe	leaf	8%	-10%	--	--
103	sheeps burnett	leaf/stem	7%	-18%	--	--
53	hangehange	leaf	7%	-16%	--	--
18	fodder beet (mangels)	top/leaf	7%	-12%	--	--
255	soap aloe	leaf	7%	7%	--	--
292	mint	leaf	7%	-22%	--	--
190	alsike clover	whole plant	7%	-11%	--	--
170	ball clover	whole plant	6%	3%	--	--
174	ball clover	whole plant	6%	-13%	--	--
6	rape (winter)	stem base	6%	-14%	--	--
104	chicory -red	leaf&stem	6%	-5%	--	--
185	low hop clover	whole plant	6%	-4%	--	--
310	parsley	leaf	6%	5%	--	--
303	celery	leaf	5%	3%	--	--
13	kale	stem base	4%	-3%	--	--
5	rape (winter)	stem top	4%	2%	--	--
107	fennel	leaf/flower	4%	-4%	--	--
342	hemlock	leaf/stem	4%	11%	--	--
16	swedes	top/leaf	4%	-11%	--	--
41	rhubarb	leaf	4%	-11%	--	--
NPP	ball clover	petiole	4%	8%	--	--
52	koromiko	leaf&stem	4%	-25%	--	--
277	white campion	leaf	4%	-17%	--	--
141	oolong tea	leaf	4%	-14%	--	--
352	white mulberry	leaf	3%	6%	--	--
268	horse chestnut	stalk from leaves	3%	-24%	--	--
349	spider wort	leaf&stem	3%	-12%	--	--
134	Oxalis species	leaf	3%	-29%	--	--
NNP	ball clover	petiole	2%	18%	--	--
NPL	ball clover	leaf	2%	7%	--	--
278	spineless yucca	leaf	2%	-13%	weak	--
184	haresfoot clover	whole plant	2%	-22%	--	--
302	dill	leaf	1%	1%	--	--
129	mint	leaf&stem	1%	-14%	--	--

ID	Common name	Plant part	CH4	SCFA	C3	H2
119	tobacco	older leaf	1%	4%	--	--
33	brussel sprouts	leaf	1%	16%	--	--
164	sulla	whole plant	1%	-11%	--	--
201	yorkshirefog	lamina	1%	-7%	--	--
WCL	white clover	leaf	-1%	1%	--	--
329	perennial nettle	whole	-2%	-22%	--	--
36	kohlrabi	leaf	-2%	4%	--	--
WCP	white clover	petiole	-2%	6%	--	--
189	suckling clover	whole plant	-2%	-4%	--	--
8	rape (summer)	whole plant	-2%	5%	--	--
126	willow	young growth	-2%	-11%	--	--
202	tall fescue	lamina	-2%	-1%	--	--
348	field bindweed	leaf&stem	-2%	-11%	--	--
167	ball clover	whole plant	-2%	1%	--	--
330	tree nettle	whole	-3%	-21%	--	--
NNL	ball clover	leaf	-3%	8%	--	--
206	browntop	lamina	-3%	-9%	--	--
152	birdsfoot trefoil	whole plant	-3%	-29%	--	--
169	ball clover	whole plant	-4%	1%	--	--
204	prairie grass	lamina	-4%	5%	--	--
58	mahoe	fruit	-4%	-15%	--	--
156	*Dorycnium pentaphyllum*	whole plant	-4%	-39%	--	--
153	lotus major	whole plant	-4%	-12%	--	--
331	onion weed	whole	-4%	-9%	--	--
151	sulla	whole plant	-4%	-3%	--	--
60	kauri	stem&fruit	-4%	-72%	--	--
10	kale	upper stem	-5%	8%	--	--
130	plantain	leaf	-5%	-24%	--	--
205	timothy	lamina	-6%	7%	--	--
1	turnips	whole plant	-6%	5%	--	--
14	swedes	top/leaf	-6%	7%	--	--
274	mock orange	leaf	-6%	-15%	--	--
38	bake beans + tomato paste	seed/fruit(cooked)	-6%	7%	--	--
106	giant creeping buttercup	leaf&stem	-7%	-5%	--	--
155	*Dorycnium hirsutum*	whole plant	-7%	-34%	--	--
154	sainfoin 4522	whole plant	-7%	-9%	--	--
203	cocksfoot	lamina	-8%	-6%	--	--
168	ball clover	whole plant	-8%	1%	--	--
172	yellow-flowered lupin	leaf	-8%	-11%	--	--
262	bull kelp	whole plant	-8%	-37%	--	--
321	blueberry	stalk/branch	-9%	-70%	--	--
158	Acacia saligna	stem	-9%	8%	--	--
285	basil	leaf	-9%	-17%	--	--
105	chicory -red	root	-10%	-6%	--	--
283	bay laurel	leaf	-11%	-42%	--	--
WCF	white clover	flower	-11%	-17%	--	--
160	crimson clover	whole plant	-13%	3%	--	--

ID	Common name	Plant part	CH4	SCFA	C3	H2
118	tobacco	young leaf	-13%	7%	--	--
284	bay laurel	branch/stalk	-13%	-36%	--	--
207	harding grass	lamina	-14%	-18%	--	--
124	chamomile	whole plant	-14%	-24%	--	--
108	fennel	stalk	-14%	-20%	--	--
136	hop leaf	fresh	-15%	-15%	--	--
259	strap kelp	whole plant	-15%	-68%	--	--
183	haresfoot clover	whole plant	-15%	-36%	--	--
175	ball clover	whole plant	-16%	-20%	--	--
39	bake beans only	seeds(cooked)	-17%	7%	--	--
65	titoki	leaf	-18%	-56%	--	--
260	sea lettuce	whole plant	-20%	-61%	--	--
351	forage kochia	leaf&stem	-21%	-18%	--	--
161	crimson clover	whole plant	-21%	-18%	--	--
122	acorn	fruit	-21%	-24%	weak	--
142	lemongrass	leaf	-21%	-4%	--	--
263	mallow	leaf	-21%	-13%	--	--
55	raupo	leaf	-22%	-30%	--	--
51	kawakawa	leaf	-22%	-30%	--	--
131	plantain	stem+seedhead	-22%	-29%	--	--
54	totara	leaf	-23%	-49%	--	--
128	ragged robin	leaf	-23%	-16%	--	--
208	Alaska brome grass	lamina	-23%	-13%	--	--
138	hop vine	fresh	-24%	-31%	--	--
275	mock orange	stalk from leaves	-24%	-47%	--	--
120	tobacco	stalks	-26%	-33%	--	--
176	ball clover	whole plant	-28%	-21%	--	--
125	gorse	end growth	-28%	-16%	--	--
256	peppercorns	seeds	-29%	-28%	--	--
257	brown alga	whole plant	-32%	-70%	strong	--
289	rosemary	branch/stalk	-32%	-69%	--	--
127	goats rue	whole plant	-32%	-8%	--	--
173	sweet clover	whole plant	-34%	-12%	--	--
177	*Dorycnium pentaphyllum*	whole plant	-35%	-56%	--	--
140	tea tree	leaf&stem	-37%	-73%	--	--
261	giant kelp	whole plant	-39%	-46%	--	--
135	paperbark	leaf/stem	-40%	-72%	--	--
68	maiden hair fern	frond	-43%	-53%	--	--
110	safflower	flower head	-45%	-38%	--	--
178	Lathyrus latifolius	whole plant	-46%	-27%	--	--
57	kanuka	leaf&stem	-47%	-81%	--	--
109	saltbush	leaf/stem	-51%	-44%	--	--
332	fly agaric	top/stem	-95%	-42%	--	--

Table 4 Gas production (GP), methane (CH_4) and hydrogen (H_2) emissions from bee balm (ID 309), the steam distillation extract (SD_E) the hexane extract (HX_E) extract, the residue (HX_R) and carvacrol after 8 h of *in vitro* incubation, compared with plant species 309 and ryegrass dissolved in dimethyl sulfoxide (DMSO).

Series	Substrate	Additive	GP [ml/g]	CH_4 [%GP]	H_2 [%GP]
Screening	309	--	82	3.6	1.0
Re- screening	309	--	108	7.6	1.5
Extraction *	309	DMSO	169	8.7	0.2
	Ryegrass	DMSO	149	9.1	0.3
	Ryegrass	Carvacrol	103	8.7	0.5
	Ryegrass	309 SD_E	157	10.8	0.2
	Ryegrass	309 HX_E	160	10.2	0.1
	309 HX_R	--	180	10.0	0.3

* extracts were dissolved in DMSO and added on to ryegrass.