THE USE OF PLANT BIOACTIVE COMPOUNDS TO MITIGATE ENTERIC METHANE IN RUMINANTS AND ITS APPLICATION IN INDONESIA

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ABSTRACT

Worldwide, increasing greenhouse gas (GHG) emissions have become a major concern as they are now considered to be the cause of global warming. Several strategies have been planned and taken by different countries including Indonesia to mitigate this situation. Agriculture is considered to be one of major contributors to GHG, especially methane coming from ruminant digestive processes. More than 85% of the methane produced by ruminants comes from enteric fermentation. Several options have been proposed to lower this enteric methane production. This paper describes a review on diet manipulation using feed additives, especially plant bioactive compounds, to mitigate the GHG emission from ruminant livestock. Plant bioactive compounds have been found with various chemical structures. Some of them such as saponin, tannin, essential oils, organosulphur compounds, have been reported to have ability to reduce enteric methane production. Indonesia has many plant resources that have potential as methane reducing agents. *Sapindus rarak* fruit especially its methanol extract contain saponins which reduce the activity of methanogens in the rumen *in vitro,* hence reduce methane production (11%). Feeding *S. rarak* to sheep increased daily weight gain but not that of local cattle. Shrub legumes such as *Calliandra calothyrsus* and *Leucaena leucocephala* contain tannins which can reduce methanogenesis (3 – 21% methane reduction). Besides tannin, these shrub legumes are a good source of protein. Feeding shrub legumes can be beneficial as a protein source and a methane reducer. Other sources of methane reducing agents have been tested in other countries and some can be applied for Indonesian situation. The strategy to reduce methane by plant bioactive compounds should be developed to be simple and relatively cheap so it will benefit the local farmers. Extraction of these compounds may be expensive, therefore, costs should be considered carefully when proposing to use the extract as a feed additive for livestock raised by small farmers. It is recommended to develop a feeding system for ruminant using tannin containing plants/ legumes, saponin containing plants or other plants.

Key words: Bioactive compounds, methane, ruminant

ABSTRAK

PEMANFAATAN SENYAWA BIOAKTIF TANAMAN UNTUK MENEKAN PRODUKSI GAS METANA PENCERNAAN DALAM RUMINAN DAN APLIKASINYA DI INDONESIA

Di seluruh dunia, meningkatnya emisi gas rumah kaca (GRK) telah menjadi topik perhatian utama karena gas ini dianggap sebagai penyebab pemanasan global. Beberapa strategi telah direncanakan atau dipilih oleh beberapa negara termasuk Indonesia untuk mengurangi pengaruh pemanasan global. Pertanian merupakan kontributor utama GRK, terutama gas metana yang berasal dari proses pencernaan ruminansia. Lebih dari 85% metana yang diproduksi oleh ternak ruminansia berasal dari fermentasi pencernaan. Beberapa usaha telah diajukan atau dilakukan untuk menekan produksi gas metana pencernaan. Makalah ini menelaah manupilasi pakan dengan pakan aditif terutama senyawa bioaktif yang telah ditemukan dalam bentuk senyawa kimia yang bermacam-macam. Beberapa dari mereka seperti saponin, tanin, lemak esensial, senyawa organosulfur, dan sebagainya, telah dilaporkan mempunyai kemampuan untuk mengurangi produksi gas metana pencernaan. Indonesia mempunyai banyak sumber tanaman yang potensial sebagai agen penurun gas metana. Buah *Sapindus rarak* terutama ekstrak metanol mengandung saponin yang dapat menekan aktivitas bakteri metanogen di dalam rumen secara *in vitro* sehingga menekan produksi gas metana (11%). Pemberian *S. rarak* meningkatkan pertambahan bobot badan harian domba tetapi hal ini tidak terjadi pada sapi. Leguminosa herba seperti *Calliandra calothyrsus* dan *Leucaena leucocephala* yang mengandung senyawa tanin dapat menekan proses metanogenesis (produksi metana ditekan sebesar 3 – 21%). Selain tanin, leguminosa herba juga sebagai sumber protein pakan yang baik. Pemberian leguminosa herba memberi pengaruh menguntungkan sebagai sumber protein dan penekan gas metana. Beberapa agen penekan metana lain telah diujicobakan di negara lain dan beberapa dapat diaplikasikan untuk kondisi Indonesia. Strategi untuk menekan gas metana dengan senyawa bioaktif tanaman harus dikembangkan secara mudah dan murah sehingga bermanfaat bagi peternak lokal. Ekstraksi senyawa-senyawa ini mungkin akan mahal sehingga harus dipertimbangkan bila penggunaan ekstrak tanaman ini akan direkomendasikan kepada peternak kecil. Direkomendasikan untuk mengembangkan sistem pemberian pakan untuk ruminansia menggunakan tanaman yang mengandung senyawa tanin, saponin atau tanaman lain untuk menekan gas metana.

Kata kunci: Senyawa bioaktif, metana, ruminansia

INTRODUCTION

Agriculture is Indonesia's third-highest emitting sector, behind Land Use, Land-Use Change and Forestry (LULUCF) and peat, with emissions of 132 million tons (Mt) CO_2 in 2005 (based on land use at the time), which is 13.6% of the National GHG emission. Emission from this sector is expected to grow by 25 percent to 164 Mt CO_2 in 2030 (NCCC, 2010). Agricultural carbon emissions are mostly not carbon dioxide, but other GHGs like methane and nitrogen oxide. Agriculture in Indonesia is responsible for about 69% of total methane emissions. Seventy one percent of methane emission in agriculture sector comes from rice field, whereas livestock contributed for 16.5% of methane emission from agriculture sector (SUGANDHY *et al.*, 1999). Emissions from livestock are expected to rise from 25 Mt of CO_2 to 39 Mt of CO_2 in 2030, being responsible for more than 50 percent of additional emissions (NCCC, 2010).

Several strategies have been planned to reduce the emission from agriculture including livestock. The Indonesian government plans to abate 105 Mt CO_2 by 2030 from the agriculture sector through improving water management in rice cultivation, the restoration of degraded land and feed supplementation or feeding strategies for livestock. To put the strategy into action, a consortium of research and development in agriculture on climate change issue has been developed in 2008 with members from more than 10 institutions. An agricultural model system with efficient farming and low methane emission was established in Subang, West Java.

The appropriate strategy or methods for mitigation may be differ for each country/region, this depends on the situation and culture of farmers, farm size and management, livestock species, climate of the region, etc.

Recently, several research programmes on the utilization of plant secondary compounds to suppress enteric methane production have been conducted and this paper reviews some of plant secondary compounds which have potential as methane reducing agents.

PLANT BIOACTIVE COMPOUNDS ON METHANOGENESIS

Plant bioactive (PB) or plant secondary compounds are chemicals synthesized in plants but are not involve in the primary biochemical processes of plant growth. They can act as protective agents against predator. Thousands PB have been discovered and classified as saponins, tannins, essential oils, alkaloids, protein inhibitors, non-protein amino acids, lipids, metal-binding substances, phenolic compounds, sesquiterpene lactone, organic acids, pigments and others. Several PB have been reported to have negative effects to animals, however, some of them may have potential to improve animal production and also suppress methane production. Recent reviews on the use of plant bioactive compounds to reduce methanogenesis are available (ROCHFORT *et al.*, 2008; PATRA and SAXENA 2010; PATRA 2010, JAYANEGARA *et al.*, 2011).

A European Union collaborative project (RUMEN UP project) screened 450 plant species found in Europe for anti methanogenic activity when added to ruminant feeds. This resulted in 35 plants which decreased methane production *in vitro* by more than 15% and 6 plants by more than 25%. Only two plants, *Rheum nobile* and *Carduus pycnocephalus* consistently decreased methane production without adversely affecting other parameters of the rumen fermentation (BODAS *et al.*, 2008). The Australian ENRICH project evaluated 128 Australian native shrubs and found that more than 25% of those plants caused methane reduction *in vitro* in the range of 25 – 90% (DURMIC, unpublished report) and further work is still going on. Those plants that have been evaluated in Europe or Australia must contain various bioactive compounds.

Saponins

Saponins are structurally diverse family of plant bioactive compounds. In general, they consist of an aglycone attached to one or more sugar moieties. They are classified as triterpene saponins and steroid saponins based on their aglycones which can be in the form of triterpene (30 C-atoms) or steroid (27 C-atoms), respectively. The most prominent property of saponin is its ability to form very stable foam as a consequence of its surfactant ability. The polar group of the sugar moiety and the non polar group of the aglycone enable saponin to lower the surface tension. Table 1 shows the effect of saponin extract or saponin containing plants on methanogenesis or methanogen either *in vitro* or *in vivo*. Most of the *in vitro* results show that saponin reduced methane production while the effect of saponin on *in vivo* results varied.

Studies on the saponin effect on methanogenesis in Indonesia is still limited. The source of saponin is mostly the *Sapindus rarak* fruit's pericarp. The fruit's

Table 1. Effect of saponin extracts on *in vitro* and *in vivo* methanogenesis or methanogens and other parameters

No	Sources of saponin	Concentration	*In vitro* system	Feed	Effect on methane or methanogens	Other effect	References
1	*Camellia sinensis* (tea) saponin	0.4 mg/ml buffered rumen liquor	bottle - gas test-rumen sheep fed alfalfa hay : concentrate (60 : 40)	corn meal and grass meal (750 mg; 50 : 0, w/w)	depressed : methane production (8%)	depressed : protozoal relative abundance (51%), fungal relative abundance (79%)	GUO *et al.* (2008)
					expression of mcrA gene (76%)	increased: molar proportion of propionate (11%)	
						F. succinogenes (41%)	
						no effect on total VFA production, methanogens, *R. flavefaciens*	
2	*Knautia arvensis* leaves, 80% methanol extract	0, 3.88 and 7.76 mg/40 ml buffered RL	gas test - conc : hay fed Holstein cattle	concentrate : hay (1 : 1)	reduced methane production (5.50 – 6.43%)	reduced protozoal numbers (14 – 25%).	GOEL *et al.* (2008a; b)
						increased : fibrobacter (24%), ruminococcus (18%)	
						no effect on total VFA, propinoate. methanogens (21%), fungi (30%)	
3	*Quillaja saponaria* extract contained 50 – 70 g/kg saponins	2, 4 and 6 ml/l buffered RL	continous - dairy cow rumen liquor	oat and alfalfa hay (1 : 1,w/w)	no effect on methane production	decreased : protozoal numbers (34 – 40%), ammonia production (21 – 20%)	PEN *et al.* (2006)
						increased : molar proportion of propionate (15 – 19%)	
						No effect on total VFA production	
4	*Trigonella foenum-graecum* seed, 80% methanol extract	0, 5.62 and 11.54 mg/40ml buffered RL	gas test-conc : hay fed Holstein cattle	Concentrate : hay (1 : 1)	depressed : methanogens (22%)	decreased : protozoal numbers (15 – 39%), fungi (65%)	GOEL *et al.* (2008a; b)
					decreased methane production (1.82 – 1.97%)	increased : fibrobacter (42%), ruminococcus (40%)	
						no effect on total VFA, propionate	

Table 1. Effect of saponin extracts on *in vitro* and *in vivo* methanogenesis or methanogens and other parameters (continued)

No	Sources of saponin	Concentration	*In vitro* system	Feed	Effect on methane or methanogens	Other effect	References
5	*Yucca schidigera* whole plant powder (6% saponin)	15, 30, 45 mg/g substrate	batch culture - Holstein cow rumen liquor	51 : 49 forage (barley silage) : concentrate	depressed: methane production (8 – 26%)	depressed : total gas (2 – 4%), ammonia (81 – 100%) Increased molar proportion of propionate (10 – 36%)	HOLTSHAUSEN *et al*. (2009)
6	*Camellia sinensis (*tea) saponin extract	3 g/day	Huzhou lamb	concentrate : chinese rye (40 : 60)	reduced: methane production (28%) no effect on methanogens	reduced : protozoal numbers (41%), ammonia production (15%), *R. flavefaciens* (51%) enhanced : total VFA (13%), MCP (22%) no effect on propionate, fungi, *F. succinogenes*	MAO *et al*. (2010)
7	*Quillaja saponaria* whole plant powder (3% saponin)	10 g/kg DM	Holstein cow	51 : 49 forage (barley silage) : concentrate	no effect on methane production	no effect on protozoal numbers, ammonia, total VFA, propionate	HOLTSHAUSEN *et al*. (2009)
8	*S. rarak* fruits pericarps, methanol extract	0.24, 0.48, 0.72 g/kg BM - daily for 100 days = 4,8, 12 g/day	Thin tail sheep	elephant grass : wheat bran (65 : 35 w/w)	no effect on methanogens	reduced: protozoal numbers (34 – 80%), ammonia production (12 – 18%) increased propionate (14 – 19%) No effect on total VFA production, fungi, MCP	WINA (2005)
9	*Yucca schidigera* whole plant powder (6% saponin)	10 g/kg DM	Holstein cow	51 : 49 forage (barley silage) : concentrate	no effect on methane production	no effect on protozoal numbers, ammonia, total VFA, propionate	HOLTSHAUSEN *et al*. (2009)

pericarp powder, its crude extract and the whole fruit suppressed *in vitro* methane production by 21%, 31% (THALIB, 2004) and 10%, respectively (SUHARTI, 2010). However, there has no report on the effect of *S. rarak* saponin on *in vivo* methane production yet although *S. rarak* saponin significantly reduced protozoal population even after 100 days (WINA *et al.*, 2005). Feeding *S. rarak* to sheep increased daily weight gain (WINA, 2005; THALIB and WIDIAWATI, 2008; THALIB *et al.*, 2010) but not that of local cattle (ASTUTI *et al.*, 2009).

Similar effect on reduced protozoa was obtained by adding methanol extract of *Sapindus trifoliatus*, *Albizia saponaria* bark, *Malvavicus arboreus* leaves, *Enterolobium cyclocarpum* bark or leaves to *in vitro* fermentation (WINA, unpublished). These extracts most probably have the ability to reduce methane or methanogens.

Saponin reduced protozoal population and also some methanogens that were associated with protozoa, although the effect on methanogens did not always correlate with the effect on protozoa (JAYANEGARA *et al.*, 2010). It was expected that reduced methanogens would also reduce methane emission. In a study with extracts of *S. sesban*, Fenugreek and Knautia showed that these extracts decreased protozoal numbers and methanogen populations but surprisingly did not decrease methane production (GOEL *et al.*, 2008). There was a weak relationship between methanogenesis and methanogens in the rumen.

The formation of methane in the rumen requires the involvement of several microbes and enzymes. It was reported that the activity of the methyl coenzyme-M reductase (*mcr*A) gene of *Methanobrevibacter ruminantium* was reduced by tea saponin (GUO *et al.*, 2008), indicating that the rate of methanogenesis and not the numbers of methanogen was inhibited. It is likely that H_2 availability rather than the number of methanogens controlled the methanogenesis.

Increasing H_2 utilization by organisms other than methanogens would also reduce methane production. THALIB and WIDIAWATI (2008) suggested a combination of *Acetoanaerobium noterae*, an acetogenic bacteria with *S. rarak* saponin extract to be fed to sheep and its rumen liquor reduced methane production in the batch culture system by 20%. Furthermore, the above combination was enriched with saponin containing legumes and when fed to small ruminants, their rumen liquor had higher ability to decrease methane production (THALIB, unpublished).

A combination of feeding saponins from Sapindus (soapberry) fruit with condensed tannins from mangosteen peel (4% of total diet) to Friesian Holstein steers significantly depressed methane production and also the percentage of methanogens in total ruminal DNA (POUNGCHOMPU *et al.*, 2009). Other combination between bioactive compounds with probiotic has also been suggested. However, the exact mechanism to reduce methane is not clear yet and requires further studies.

Tannins

Tannins are polyphenolic compounds with diversed structures which are abundantly found in plants. They are classified into two major groups; i.e hydrolysable tannins which contain gallic acid or ellagic acid esterfied with polyols (generally d-glucose, shikimic acid, quinic acid) as a central core and condensed tannins. The term of “condensed” is confusing as hydrolysable tannin can also undergo condensation reaction. Condensed tannins are proanthocyanidins, which produced red anthocyanidins upon heating in acidic alcohol solution (REED, 1995). In fact, this classification is rather confusing as more mixtures of both have been found, such as catechin gallates and gallic acid esters of proanthocyanidin. The most important aspect of these tannins is their ability to form strong complexes with proteins from plants, rumen microbes or enzymes.

Table 2 show the effect of tannin extracts or tannin containing plants on methane production or methanogens both *in vitro* and *in vivo*. The effect of tannin on methane production *in vitro* (maximum inhibition 72%) was less than the effect of saponins on methane production (max inhibition 96%). The effect also depends on the structure of tannins. The non-tannin phenolics contribute to reducing methane production and those with higher numbers of hydroxyl groups have higher methane reducing effects. The hydrolysable tannins are more effective in decreasing *in vitro* CH_4 production than the condensed tannins (JAYANEGARA *et al.*, 2010). However, this *in vitro* result should be further evaluated *in vivo* since hydrolysable tannin is easily degraded in the rumen.

Addition of tannin to the rumen will depress fibre digestion in the rumen and inhibit protozoa population to a certain extent. Therefore, the inhibition of methanogenesis was probably the result of suppression of fibre degradation as indirect effect of condensed tannin. The inhibition of fibre degradation will shift Short Chain Fatty Acid composition to less acetate, less hydrogen sink, hence less CH_4 formation. HARIADI and SANTOSO (2010) found that there was a negative correlation between total tannin content and methane production at 48h of incubation (r = -0.76). The direct effect of hydrolysable tannins on methanogens, however, cannot be excluded (HESS *et al.*, 2006; JAYANEGARA *et al.*, 2010). The study using pure cultures (TAVENDALE *et al.*, 2005) showed that tannin reduced methane production by reducing methanogenic bacteria.

Table 2. Effect of tannin containing plants/extracts on *in vitro* and *in* vivo methanogenesis or methanogens and other parameters

No	Tannin	Concentration	*In vitro* system/ animal	Feed	Effect on methane or methanogens	Other effects	References
1	Phenolic acids (Cinnamic, phenylacetic acid, caffeic, p-coumaric, ferulic acids)	2 and 5 mM	gas syringe	hay	decreased methane (0.2 – 6.3%)	no effect on total gas and OM digestibility	JAYANEGARA (2009)
2	*Castanea sative* Mill (Chesnut) and *Rhus typhina* (sumach) tannin	0,5; 0.75; 1.0 mg/ml	gas syringe	hay : conc (70 : 30)	decreased methane 6.5 and 7.2% at 1mg/ml inclusion	no effect on OM digestibility	JAYANEGARA *et al*. (2010)
3	*Acacia mearnsii* (Mimosa) and *Schinopsis quebracho* (quebracho) tannin	0,5; 0.75; 1.0 mg/ml	gas syringe	hay : conc (70 : 30)	no effect on methane	decreased total gas and OM digestibility	JAYANEGARA *et al*. (2010)
4	*Acacia mearnsii* black wattle (mimosa); 700 g/kg DM CT; *Castanea sative* Mill (chestnut); 800 g/kg DM HT	14.9 mg/kg DM	crossbred steer	corn : cotton seed meal : sorghum hay (641 : 100 : 71)	no effect on methane emission	no effect on nutrient intake, digestibility, ADG	KRUEGER *et al*. (2010)
5	*Calliandra calothyrsus* leaves	175 g/kg DM	SwissWhite Hill Sheep	*Brachiaria brizantha* : Vigna unguiculata (550 : 450g/kg), Vigna : *C. calothyrsus* (2 : 1, 1 : 2)	reduced methane release daily 3% and 21% (for Vigna : Cal = 2 : 1 and 1 : 2)	OM, CP, NDF, ADF digestibility reduced. Reduced N retention	TIEMANN *et al*. (2008)
6	*Flemingia macrophylla* leaves	111g/kgDM	SwissWhite Hill Sheep	*Brachiaria brizantha* : *Vigna unguiculata* (550 : 450g/kg), Vigna : *F.macrophylla* (2 : 1, 1 : 2)	reduced methane release 3% and 17% (for Vigna : F.macro = 2 : 1 and 1 : 2)	OM, CP, NDF, ADF digestibility reduced. Reduced energy retention	TIEMANN *et al*. (2008)
8	*Mimosa pigra*	0, 25, 50, 75% replaced basal diet	goat	*Muntingia calabura* as basal diet	decreased methane 42% at 50% inclusion	increased DM intake, daily gain, N retention	KONGVONGXAY *et al.* (2011)

Tree legumes which are found abundantly in tropical countries like Indonesia have been reported to reduce methane. TIEMANN *et al.* (2008) reported that feeding the tannin containing legumes, *Calliandra calothyrsus* or *Flemingia macrophylla* to sheep reduced methane production. The Indonesian consortium on climate change has promoted a feeding system using *Leucaena* spp. as a source of protein and also as a methane reducing feed to be applied by small farmers CSIRO Australia also tries to promote Leucaena as an antimethanogenic feed. The substitution of Rhodesgrass with Leucaena reduced methane in a dose dependent level (CHARMLEY, 2009). However, the condensed tannin content in Leucaena varies from 0-170 mg/g suggesting that the ability to reduce methane may also vary. Not only the level of tannin but the different structures of tannin in different tree legumes may also affect its nutritional value (TIEMANN *et al.*, 2010) and ability to reduce methane emission (TIEMANN *et al.*, 2008).

Besides tannin, these shrub legumes are good sources of protein. Several feeding trials to utilize *Calliandra calothyrsus* as supplemented feed showed that this tannin containing legume improved animal performances when it fed fresh (WINA and TANGENDJAJA, 2006). Therefore, shrub legumes can be beneficial as a protein source and also methane reducer.

Some farmers have been using local adapted legumes, at about 30% of the diet to feed their livestock. However, the tannin level in this type of diet was less than 2% (20g/kg DM), where probably a minor effect of tannin might be shown. JAYANEGARA *et al.* (2011) concluded in their meta analysis study of the effect of tannin that the reliable and distinguishable effect of tannins, that is reducing methane without adverse effect on animal performance can be expected only from levels > 20 g/kg DM. More studies on tannin containing legumes in different composition of feed and their effect on animal performance and methane emission should be done.

Other plant bioactives beside tannins and saponins

Table 3 shows the use of other plant bioactives besides tannins and saponin as methane reducing agent. One of them is essential oils. Essential oils are volatile oils extracted by steam distillation or solvent extraction from different parts of plants. The type and composition of essential oils vary among plants and also different parts of the same plant. Essential oils have been long known to have antibacterial properties (BENCHAAR *et al.*, 2008). Recently, studies on the use of essential oils to improve rumen fermentation and reduce methane production in the rumen have become an interest. PATRA *et al.* (2006) examined several plant spices and found only the methanol and ethanol extracts of *Foeniculum vulgare* and *Syzgium aromatic*um that contain essential oils showed the ability to reduce methane.

There are several possible explanations on the mode of action of essential oils reducing methane production, but the exact mechanism of essential oils affecting methanogenesis requires further studies. First, essential oils depressed methane production by reducing protozoal population and methanogens associated protozoa population. Unlike tannin or saponin, the result of meta analysis from 36 published studies showed that each 1% suppression of protozoal numbers accounted for about 0.45% inhibition of methane by essential oils compared to 0.17, 0.29% inhibition by saponins, tannins, respectively (PATRA, 2010). Second, cinnamaldehyde (essential oil of cinnamon) supplementation inhibited *M. ruminantium*, but increased *Methanosphaera stadtmanae* and *M. smithii* and some uncultured groups. The alteration of methanogen community or reduction in the activity of methanogens may decrease methanogenesis (OHENE-ADJEI *et al.*, 2008).

Commercial product containing mixture of essential oils is available in the market. One of them is a mixture of thymol, eugenol, vanillin and limonene (BENCHAAR *et al.*, 2006). This product at 2 g/day improved feed efficiency of beef cattle fed a silage base diet. The methane emission, however, was not measured. BEAUCHEMIN and MCGINN (2006) reported no effect of essential oil mixture fed to beef cattle (1g/day) on methane emission. Adaptation of ruminal microbes to essential oil may cause no effect of this compound on rumen parameters.

The extraction of essential oil from plants or spices is usually done by steam distillation, but the yield is very low and mainly used for human consumption. Competing with human consumption, the use of spices and essential oils may not be feasible for ruminant.

Other compounds that received some interest on reducing methanogenesis are organosulphur compounds in garlic extract (BUSQUET *et al*., 2005). The four major of these organosulphur compounds are diallyl sulphide, diallyl disulphide, allyl mercaptan, and allicin. It was reported that garlic oil and its component diallyl disulphide (300 mg/l of ruminal fluid) reduced methane production by 74 and 69%, respectively, in batch culture system without altering digestibility (BUSQUET *et al*., 2005). When combination of garlic (100g/day) and coconut oil (7%) was given to swamp buffalo, it reduced methane production without affecting total methanogens or its diversity (KONGMUN *et al.*, 2011).

Cashew Nut Shell Liquid (CNSL) is obtained from the shell of cashew nut. Indonesia produces a lot of cashew nuts and the shell which are mainly thrown

Table 3. Effect of other bioactive compounds on *in vitro* methanogenesis or methanogens and other parameters

No	Other bioactive compounds	Concentration	*In vitro* system	Feed	Effect on methane or methanogens	Other effects	References
1	*Allium sativum* (garlic), ethanol, methanol extracts	0.5 ml (20 g/100ml)	gas syringe	wheat straw : concentrate (1 : 1)	reduced methane 27, 68% by ethanol, methanol extracts	increased protozoal numbers, decreased acetate/propionate, no effect on total gas	PATRA *et al*. (2006)
2	Allicin and dially disulfide	0.5, 5, 10 mg/l	bottle, sheep rumen liquor	alfalfa : concentrate (1 : 1)	no effect on total methane	decreased H recovery	KAMEL *et al.* (2008)
3	*Foeniculum vulgare* (fennel), ethanol, methanol extracts	0.5 ml (20 g/100 ml)	gas syringe	wheat straw : concentrate (1 : 1)	reduced methane 35, 83% by ethanol, methanol extracts	decreased acetate/propionate, reduced total gas	PATRA *et al*. (2006)
4	*Syzygium aromaticum* (clove), ethanol, methanol extracts	0.5 ml (20 g/100 ml)	gas syringe	wheat straw : concentrate (1 : 1)	reduced methane 53, 64% by ethanol, methanol extracts	DMD and protozoal numbers decreased, reduced total gas	PATRA *et al*. (2006)
5	Cashew nut shell liquid (CNSL)	500 mg/l	test tube	hay : concentrate (90 : 10, 70 : 30, 50 : 50, 30 : 70 and 10 : 90)	reduced methane 45.26 – 65.62%	reduced total gas 29.54 – 39.70%; increased propionate 24 – 40%	

away as the waste. This liquid contains anacardic acid, cardanol and cardol, that are salicylic acid derivatives with an alkyl group. Indonesian people believe that the cashew nut shell is very toxic and the oil from the shell will cause skin irritation. The use of CNSL as methane reducing agent has been patented recently by KOBAYASHI *et al.* (2010). CNSL is considered to be a strong potential candidate for a feed additive. At the level of 200 µg/ml CNSL included in the *Rusitec* fermentation, less methane production was observed. The highest inhibition of methane production (70.1%) was observed with a 70:30 concentrate to hay substrate mixture (WATANABE *et al*., 2010). This also requires *in vivo* experiment to show the biological activity of CNSL on methanogenesis or methanogens.

There are many more bioactive compounds in Indonesia that may have potential of reducing methane or methanogens, however, many have not been explored yet.

CONCLUSION

The inhibition of enteric CH4 emission in ruminant is possible through the use of plant bioactive compounds, however, long term effects of these plant bioactive compounds to reduce methanogens or methanogenesis have never been reported. Therefore, long term *in vivo* experiments should be done to evaluate whether the use of plant bioactive compounds can give a persistent effect to mitigate methane emission. Screening and selection on various potential local plants containing secondary compounds that inhibit methanogenesis without adversely affecting feed utilization should be done and when combined with other feed ingredients require further evaluation, perhaps in a collaborative project.

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