Ann. Anim. Sci., Vol. 19, No. 1 (2019) 3–29 *DOI: 10.2478/aoas-2018-0037*

sciendo

MANIPULATION OF RUMEN FERMENTATION AND METHANE GAS PRODUCTION BY PLANT SECONDARY METABOLITES (SAPONIN, TANNIN AND ESSENTIAL OIL) – A REVIEW OF TEN-YEAR STUDIES

Saied Jafari[1], Mahdi Ebrahimi[1,2♦], Yong M. Goh[1, 3], Mohamed A. Rajion[1], Mohamed F. Jahromi[4], Wisam S. Al-Jumaili[1, 5]

[1]Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2]Department of Plant Sciences & Biotechnology, Faculty of Life Sciences & Biotechnology, Shahid Beheshti University, Tehran, Iran
[3]Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[4]Agriculture Biotechnology Research Institute of Iran (ABRII), East and North-East Branch, P.O.B. 91735 844, Mashhad, Iran
[5]Department of Animal Resources, College of Agriculture, University of Al-Qassim Green, Babylon, 51013 Al-Qassim, Iraq
♦Corresponding author: mehdiebrahimii@gmail.com

Abstract

A wide range of plant secondary metabolites (PSM) have been shown to have the potential to modulate the fermentation process in the rumen. The use of plants and plant extracts as natural feed additives has become an interesting topic not only among nutritionists but also other scientists. Although a large number of phytochemicals (e.g. saponins, tannins and essential oils) have recently been investigated for their methane (CH_4) reduction potential, there have not yet been major breakthroughs that could be applied in practice. However, the effectiveness of these PSM depends on the source, type and the level of their presence in plant products. The aim of the present review was to assess ruminal CH_4 emission through a comparison of integrating related studies from published papers, which described various levels of different PSM sources being added to ruminant feed. Apart from CH_4, other related rumen fermentation parameters were also included in this review.

Key words: rumen, methane, fermentation, plant secondary metabolite

Methanogenesis is one of the important means to remove H_2 (produced as a result of the carbohydrate decomposition) from the rumen (Mihaela et al., 2014). The microorganisms that produce methane (CH_4) as the end product of their respiration are called methanogens and the process through which methanogens produce CH_4 is called methanogenesis.

Methanogenesis is energy consuming and accounts for almost 20% of gross energy intake of the animals (Bhatta et al., 2012). This energy is eventually wasted in

the form of CH_4 (Johnson and Johnson, 1995). Beside energy spoilage through the CH_4 formation, it is 23 times higher than CO_2 in trapping the atmospheric heat and it is considered as a greenhouse gas (GHG) which plays a pivotal role in the global warming with negative consequences on the worldwide environment (Bodas et al., 2012). In the ruminant, methanogenesis occurs both in the rumen and in the hindgut, but the majority of the CH_4 originates from the rumen in which CH_4 takes up nearly 90% of the total CH_4 production of ruminants (Kumar et al., 2013); therefore, plenty of research is needed to find a suitable feed alternative to mitigate rumen CH_4 production for better and greener environment, and eventually, better livestock production as well.

Dietary strategies to reduce rumen methanogenesis

Production of CH_4 is an intrinsic process of ruminal fermentation as mentioned earlier and suppressing or abating its formation is a big challenge. Many types of CH_4 inhibitors have been repeatedly experimented to mitigate the production of enteric CH_4 (Patra, 2014).

However, most of them have shown negative effects on rumen fermentation characteristics when added at high doses to achieve effective CH_4 inhibition (Patra and Yu, 2013). In addition, some of these inhibitors are toxic to animals (Patra, 2012). Meanwhile, contemporary consumer demands orient towards the use of phytochemicals which are natural products to alter rumen fermentation. Plants produce a diverse array of plant secondary metabolites (PSM), which are not biologically involved in primary biochemical processes such as plant growth, development and reproduction (Bhatta et al., 2015). Besides, more than 200,000 defined PSM structures have been identified (Hartmann, 2007). The majority of PSM can generally be classified into three groups; saponins, tannins and essential oils (EO).

Saponins are a class of PSM that possess a great complexity in their structures as well as their biological activities (Jayanegara et al., 2014). Basically, chemical structure of saponins consists of a sugar moiety (e.g. glucose, galactose, glucuronic acid) which is linked to a hydrophobic aglycone or sapogenin (Francis et al., 2002). Accordingly, the biological activity of saponins depends on the nature, number and sequence of the sugars in the structures (Chwalek et al., 2006).

Tannins are complex mixtures of individual compounds having molecular weights ranging from 500 to over 3000 (gallic acid esters) and up to 20000 (proanthocyanidins) which are usually subdivided into two groups based on the chemical structure: hydrolyzed tannin (HT) and condensed tannin (CT) (Bhatta et al., 2009).

Widely occurring in plants and animals, EO may consist of volatile constituents of terpenoid or non-terpenoid origin (Cieslak et al., 2013). Under this group, hundreds of large or small molecules can be present, consisting of hydrocarbons and their oxygenated derivatives. The composition of EO is usually characteristic for the particular plant species and responsible for its fragrance (Cieslak et al., 2013).

The effectiveness of PSM (e.g. saponins, tannins and EO) has been screened in *in vitro* and *in vivo* studies in the last few decades. Our purpose for the current review is to provide deeper insights into the use of PSM to reduce CH_4 emission from ruminants and consequently to reduce the impact of global warming.

Effect of saponins on rumen methanogenesis and fermentation characteristics

Review of recent studies about the effects of saponin sources on rumen CH_4 and fermentation parameters are shown in Tables 1 and 2, respectively. Saponins or saponin-like substances have been reported to suppress CH_4 production and to modulate rumen fermentation patterns (Patra and Yu, 2012, 2013, 2014 a, b and 2015). In a series of *in vitro* studies by us, the addition of papaya leaf (a saponin-rich source), methanolic extract of papaya leaf and different solvent extracts of papaya leaf reduced the CH_4 production by 37, 34 and 30% as compared to control group, respectively (Jafari et al., 2016 a, b and c). As expected, reduced CH_4 production in our study was accompanied by reduced acetic/propionate ratio providing the sink for metabolic H_2 during rumen fermentation. Consistently, Wanapat et al. (2014) reported the reduction of CH_4 by the inclusion of mangosteen peel powder (saponin-rich fruit) without a negative effect on dry matter intake, ruminal pH, total volatile fatty acid and NH_3N concentration while increasing propionic concentration and decreasing acetic-propionic ratio in swamp buffaloes. Cieslak et al. (2013) reported that there was some ambiguity in the literature concerning the mechanism of action of saponins to reduce methanogens and methanogenesis. According to Guo et al. (2008), mitigation of methanogenesis using tea saponin resulted from decreased activity of the mcrA gene (an indicator of the methanogenic activity of the methanogen population), without changing the total methanogen numbers. However, 3 g/day of tea saponins in sheep diets had no effect on the populations of methanogens (Mao et al., 2010; Zhou et al., 2011). Earlier *in vitro* research had suggested mitigation of methanogenesis without a reduction in the number of methanogens with the use of saponins from *Sapindus saponaria* or tea saponins (Hu et al., 2005). However, Jayanegara et al. (2014) reported that saponins decreased CH_4 emission due to a lower relative abundance of methanogen population in the presence of the respective substances in the rumen. A combination of nitrate and quillaja saponin (saponin source) was shown to reduce CH_4 production by almost 60% using an *in vitro* model of rumen cultures (Patra and Yu, 2013). Patra and Yu (2013) proposed that quillaja saponin functioned as an inhibitor to rumen protozoa, decreased H_2 production by protozoa and protozoa-associated methanogens. Patra and Yu (2014 a, b) reported that CH_4 production was the lowest (45.7% depression) when combined with other CH_4 inhibitors (e.g. sulfate and nitrate).

Some authors have reported an insignificant effect of a saponin-rich source on *in vivo* CH_4 emissions of ruminants (Li and Powers, 2012). In contrast, some studies observed a CH_4 reduction *in vivo* on the addition of saponin-rich sources into basal diets (Wang et al., 2011; Zhou et al., 2011). Thus, like in the *in vitro* studies, the effects of saponins on *in vivo* CH_4 emission from ruminants have produced contrasting results. According to some studies (e.g. Bodas et al., 2012), the duration of saponin administration and the ratio of forage to concentrate may have a significant influence on their effectiveness. Nonetheless, in addition to suppressing CH_4 production, the use of saponins may also confer nutritional benefits as they might increase microbial protein synthesis due to inhibition of protozoa, and might increase the fiber-degrading bacteria and fungi in the rumen, which is beneficial for utilizing in low-quality-based diets (Rira et al., 2015).

Table 1. Effects of saponin sources on methane (CH_4) production in rumen

Reference	Saponin source	Diet/substrate	Test system/dosage	CH_4 reduction
Bharathidhasan et al. (2013)	Purified saponin (1.55, 3.10, 4.65 and 6.20 mg/30 mL rumen inoculum)	Hybrid Cumbu Napier grass	*In vitro* (gas production using syringe)	14.04%, 21.90%, 34.30% and 37.60%
Guyader et al. (2015)	Tea saponin (68% tea saponin)	50% grass hay + 50% concentrates	*In vivo* (non lactating Holstein cows) 0.5% tea saponin + 2.3% nitrate	28%
Jafari et al. (2016 a)	Papaya leaf (7.5, 12.5 and 25% of diet)	Concentrate + alfalfa (50:50)	*In vitro* (rumen fluid from crossbred goat)	(17%, 34% and 37%)
Jafari et al. (2016 b)	Papaya leaf methanol extract (PLE; 5, 10 and 15 mg of PLE/0.25 g DM)	Concentrate + alfalfa (50:50)	*In vitro* (rumen fluid from crossbred goat)	(ns, ns and 34%)
Jafari et al. (2016 c)	Papaya leaf solvent fractions (PLF, 15 mg of PLF/0.25 g DM)	Concentrate + alfalfa (50:50)	*In vitro* (rumen fluid from crossbred goat)	(25%, 29%, ns, 25% and ns)
Li and Powers (2012)	Yucca saponin (8.5% saponin)	Total mixed ration (forage/concentrate)	*In vivo*	NA
Narvaez et al. (2013)	*Yucca schidigera*	Forage/concentrate (65:35)	Serum bottle/650 µg per mL	15%
Patra et al. (2012)	*Yucca schidigera* (0.2, 0.4 and 0.6 g/L of culture)	Concentrate + alfalfa (50:50)	*In vitro* (serum bottle)	27.4%, 24.8% and 26.0%
Patra and Yu (2013)	A. Quillaja saponin (0.6 g/L), B. Quillaja saponin (1.2 g/L), C. Quillaja saponin (1.2 g/L) + propionic acid (8 mM) + nitrate (10 mM)	Corn silage (45%) + alfalfa hay (10%) + dairy protein product (20%) + concentrate mixture (25%).	*In vitro* (batch fermentation)	A. 11% B. 24% C. 85%

Patra and Yu (2014)	A. Quillaja saponin (0.6 g/L) B. Quillaja saponin (0.6 g/L) + nitrate (5 mM), and sulfate (5 mM).	Corn silage (45%) + alfalfa hay (10%)+ dairy protein product (20%) + concentrate mixture (25%).	*In vitro* (batch fermentation)	A. 8% B. 47%
Patra and Yu (2015)	A. Quillaja saponin B. Saponin + garlic C. Saponin + nitrate D. Saponin + garlic + nitrate	Concentrate + alfalfa (70:30)	*In vitro* (alfalfa + concentrate, 70:30)	A. 36% B. 45% C. 55% D. 70%
Rira et al. (2015)	*Yucca schidigera* (4.4% saponin)	Dates by-products + the vetch-oat	*In vitro* (syringe) 8 mg/mL of saponins	60%
Wanapat et al. (2014)	Mangosteen peel powder (10.9% saponin)	Concentrate + rice straw	*In vivo* (swamp buffaloes) 100 (g/head/day)	7%

DM – dry matter; NA – not applicable; ns – not significant. Solvents were hexane, chloroform, ethyl acetate, butanol and water, respectively.

Table 2. Effects of saponin sources on fermentation parameters in rumen

Reference	Saponin source	pH	TVFA	Acetic/propionic	NH_3N
Feng et al. (2012)	Gross saponin of *Tribulus terrestris*	A. 6.79 ns B. 6.80 ns C. 6.85 ns D. 6.84 ns	A. 63.89 mmol/L B. 62.17 C. 60.98 D. 60.55	A. 2.88 ns B. 2.77 ns C. 2.64 (–9%) D. 2.59 (–11%)	A. 14.82 mg/dL B. 14.56 C. 13.36 (–15%) D. 12.85 (–18%)
Jafari et al. (2016 a)	Papaya leaf (7.5, 12.5 and 25% of diet)	(ns, 7.35, ns)	(90.01 mM, ns, ns)	(ns, ns, 1.80)	(23.05, 23.44, 22.55 mg/dL)
Jafari et al. (2016 b)	Papaya leaf methanol extract (PLE; 5, 10 and 15 mg of PLE/0.25 g DM)	(ns, ns, ns)	(ns, ns, ns)	(ns, 2.02, 1.93)	(ns, 18.91, 19.56 mg/dL)
Jafari et al. (2016 c)	Papaya leaf solvent fractions (PLF, 15 mg of PLF/0.25 g DM)	(ns, 7.46, ns, ns, ns)	(ns, 7.46 mM, ns, ns, ns)	(1.88,1.83, ns, 1.69, 1.70)	(ns, 13.72 mg/dL, 13.63 mg/dL, ns, ns)
Mao et al. (2010)	Tea saponin (60% saponin)	6.8 (–1.5%)	26 mmol/L	5.0 ns	143 mg/L
Narvaez et al. (2013)	*Yucca schidigera*	NA	118.20 mmol/L (+8%)	1.4 (–55%)	26.80 mmol/L (–30%)
Patra et al. (2012)	Yucca saponin (0.2, 0.4 and 0.6 g/L)	5.572, 5.54, 5.58	A. 131 Mm B. 137 C. 140	A. 3.66 B. 3.71 C. 3.74	A. 15.19 mmol/L B. 18.12 C. 18.68
Patra and Yu (2013)	A. Quillaja saponin (0.6 g/L) B. Quillaja saponin (1.2 g/L) C. Quillaja saponin (1.2 g/L) + propinoic acid (8 mM) + nitrate (10 mM)	A. 5.59 (–2%) B. 5.59 (–2%) C. 5.89 (+3%)	A. 108.9 mM (+15%) B. 109.8 (+17%) C. 90.6 ns	A. 2.88 (–17%) B. 2.82 (–18%) C. 2.87 (–17%)	A. 27.01 B. 25.8 C. 23.3 (–8%) mM
Patra and Yu (2014)	A. Quillaja saponin (0.6 g/l) B. Quillaja saponin (0.6 g/L) + nitrate (5 mM), and sulfate (5 mM).	A. 6.42 B. 6.58	A. 92.8 B. 98.3	A. 2.28 (–9%) B. 2.26 (–10%)	A. 15.7 mM B. 18.6

Patra and Yu (2015)	A. Quillaja saponin B. Saponin + garlic C. Saponin + nitrate D. Saponin + garlic + nitrate	A. 5.84 ns B. 5.95 ns C. 6.01 ns D. 6.05 ns	A. 122.8 ns mM B. 128.3 (+8%) C. 133.8 (+6%) D. 126.2 ns	A. 1.99 (–19%) B. 2.11 (–14%) C. 2.23 (–9%) D. 2.08 (–15%)	NA
Wanapat et al. (2014)	Mangosteen peel powder (MSP)	6.6 ns	100.1 ns	3.2 (–18%)	11.6ns
Wang et al. (2011)	Gynosaponin powder (98% gynosaponins)	NA	18.38 mM (–38%), 12.90 (–56%)	NA	NA
Zhou et al.(2011)	Tea saponin 60% (triterpenoid saponin)	A. 6.48 ns B. 6.36 ns	A. 61.5 ns mmol/L B. 60.9 ns	A. 3.02 (–13%) B. 2.43 (–30%)	A. 10.5 mg/dL (–3%) B. 8.0 (–34%)

Solvents were hexane, chloroform, ethyl acetate, butanol, and water; TVFA, total volatile fatty acid; NA, not applicable; ns – not significant; (–) decrease and (+) increase as compared to the control group.

Table 3. Effects of tannin sources on methane (CH_4) production in rumen

Reference	Tannin source	Diet/substrate	Test system/dosage	Methane reduction
1	2	3	4	5
Anantasook et al. (2013)	Rain tree pod meal (60 g/kg of total DM intake)	Total mixed ration (concentrate + rice straw treated with urea) at 25 g/kg BW	*In vivo* (growing steer) 40:60 (roughage: concentrate)	10%
Bhatta et al. (2014)	A. *Autocarpus integrifolis* B. *Azardirachta indica* *C. Ficus bengalensis*	Roughage + concentrate (40:60)	*In vitro* (from 2.5 to 30% of total mixed ration, DM Basis)	A. 12–33 (%) B. 24–61 (%) C. 15–46 (%)
Bhatta et al. (2012)	A. *Autocarpus integrifolia* leaf (186 g/kg DM) of CT B. *Ficus religiosa* leaf (13.5 g/kg DM) of HT C. *Jatropha curcus* (5.6 g/kg DM) of HT D. *Sesbania grandiflora* (13.1 g/kg DM) of HT	*Elusine coracana* straw and commercial concentrate mixture in 1:1 ratio.	*In vitro* (gas production) 200 mg sample/30 mL buffered rumen inoculum	A. 4.73 (mL/total gas reduction) B. 3.58 (mL/total gas reduction) C. 3.43 (mL/total gas reduction) D. 2.02 (mL/total gas reduction)
Bhatta et al. (2012)	A. *Clerodendrum inerme* (23.7 g/kg DM) of HT B. *Gymnema sylvestre* (23.9 g/kg DM) of HT C. *Sapindus laurifolia* (82 g/kg DM) of CT	*Elusine coracana* straw and commercial concentrate mixture in 1:1 ratio.	*In vitro* (gas production) 200 mg sample/ 30 mL buffered rumen inoculum	A. 7.9% B. 9.9% C. 12.7%
Bueno et al. (2015)	Acacia (*Acacia molissima*) tannin extract	Forages (600–800 g/kg) and concentrates (200–400 g/kg)	*In vitro* (gas production test) (50 g of leucocyanidin (CT)/kg of DM)	A. Goat (13%) B. Sheep (23%) C. Buffalo (22%) D. Cattle (9%)
Hassanat and Benchaar (2013)	A. *Acacia mearnsil* extract (82% CT) B. *Schinopsis balansae* extract (90.4% CT) C. *Castanea sativa* extract	Total mixed ration (forage / concentrate)	*In vitro* (serum bottle) 10, 20, 30 and 40 mg	A. 12%, 21%, 32% and 38% B. NE, 23%, 34% and 40% C. 13%, 23%, 31% and 40%

	(5.7% CT and 75.5% HT) D. *Quercus aegilops* extract (8.0% CT and 71.2% HT)			D. 11%, 19%, 26% and 36%
Hatew et al. (2015)	Sainfoin (*Onobrychis viciifolia*) accessions: A. Rees 'A' B. CPI63763 C. Cotswold Common D. CPI63767	250 mg lucerne (tannin free) / 30 ml of inoculum	*In vitro* (fermentation bottle) 120 g CT/kg of substrate DM	A. 30% B. 45% C. 30% D. 48%
Jayanegara et al. (2015)	A. Chestnut (1 mg/mL) B. Sumac (1 mg/mL)	380 mg of concentrate + hay (30:70) / 30 mL of inoculum	*In vitro* (glass syringe)	6% and 7%
Jayanegara et al. (2015)	A. Chestnut B. Sumac C. Mimosa D. Quebracho	380 mg (concentrate + hay (30:70) / 30 mL of inoculum	*In vitro* (glass syringe) 1mg of purified tannin/mL of inoculum	A. 23% B. 20% C. 23% D. 27%
Jayanegara et al. (2011)	A. *Trigonella foenumgraecum* leaf B. *Sesbania sesban* leaf	Hay: concentrate (50:50)	*In vitro* (gas production) 380 mg/40 mL incubation fluid	2. 20%
Jayanegara et al. (2010)	A. purified chestnut B. sumac tannins	Hay: concentrate (70:30)	*In vitro* (gas production) 1 mg/mL	A. 6.5% B. 7.2%
Naumann et al. (2015)	A. Panicled-tick clover (PTC) B. Sericea lespedeza (SL)	Corn : alfalfa	A. 45% replacement of alfalfa with PTC B. 45% replacement of alfalfa with SL	A. 65% B. 24.4%
Pinski et al. (2015)	Quebracho condensed tannin extract (75–77% QCT)	Corn : alfalfa	*In vitro 25*, 50, 75 g/kg (DM basis)	ns, ns, ns
Rira et al. (2015)	*Acacia cyanophylla* (CT 63%)	dates by products and the vetch-oat	*In vitro* (syringe) 30% and 60%	56.25% and 36.50%

Table 3 – contd.

1	2	3	4	5
Soltan et al. (2013)	*Leucaena*		A. *In vitro* B. *In vivo*	A. 41.4 mL/g truly degraded organic matter B. 47.4 (–14%) l/kg digestible organic matter
Soltan et al. (2012)	A. *Acacia saligna* leaves (6.3% CT) B. *Laucaena leucocephala* leaves (4.6% CT) C. *Prosopis juliflora* leaves (0.04% CT) D. *Atriplex halimus* leaves (0.02% CT)	A. *Acasia saligna* B. *Laucaena leucocephala* C. *Prosopis juliflora* D. *Atriplex halimus*	*In vitro* (serum bottle)/500 mg	A. 38% B. 36% C. NE D. NE
Tan et al. (2011)	*Leucaena leucocephala* extracts (100% CT)	Guinea grass 100	*In vitro* (Hohenheim gas test)/10, 15, 20, 25 and 30 mg	–33%, –47%, –57%, –59% and –63%
Wanapat et al. (2014)	Mangosteen peel powder		*In vivo* (swamp buffaloes) 100 (g/head/day)	7.00%
Wischer et al. (2013)	A. chestnut *(Castanea sativa)* B. valonea *(Quercus valonea)*	Grass silage (100%)	*In vitro* (rusitec) 1.5 g of tannin source	A. 63% B. 34%

DM – dry matter; HGT – Hohenheim gas test system; NA – not applicable; NE – no effect; ns – not significant; – decrease; + increase compared to control.

Table 4. Effects of tannin sources on fermentation parameters in rumen

Reference	Tannin source	pH	TVFA	Acetic/propionic ratio	NH_3N
1	2	3	4	5	6
Anantasook et al. (2013)	Rain tree pod meal (60 g/kg of total DM intake) A. R:C ratio (60:40) B. R:C (40:60)	A. 6.2 (–1%) B. 6.3 (+1%)	A. 111.9 mM (+2%) B. 117.1mM (+4%)	A. –19% B. –17%	A. 15.2 mg/dL (+5%) B. 15 mg/dL (+1%)
Bhatta et al. (2015)	Acacia *(Acacia molissima)* tannin extract		A. 15.7-16.5 B. 12.7 (7%) – 10.6 (23%) C. 13.7 (6%) – 11.3 (22%)		A. 48.5 (13%) – 35.7 (36%) B. 41.5 (20%) – 31.5 (39%) C. 51.8 (7%) – 41.6 (25%)
Bhatta et al. (2012)	A. *Autocarpus integrifolia* leaf (186 g/kg DM) of CT B. *Ficus religiosa* leaf (13.5 g/kg DM) of HT C. *Jatropha curcus* (5.6 g/kg DM) of HT		A. 12.6 mmol/dL B. 10.4 C. 10.44. 13.5		A. 6.3 mg/dL B. 18.2 C. 19.6
Bhatta et al. (2012)	A. *Clerodendrum inerme* (23.7 g/kg DM) of HT B. *Gymnema sylvestre* (23.9 g/kg DM) of HT *C. Sapindus laurifolia* (82 g/kg DM) of CT		A. 13.1 mM/DI B. 4.22 C. 10.3		A. 7.70 mg/dL B. 16.5 C. 7
Ebrahimi et al. (2015)	Oil palm frond (tannin source) 25% and 50% in diet	5.8 ns and 5.9	96.36 ns and 96.47 ns		-

Table 4 – contd.

1	2	3	4	5	6
Hassanat and Benchaar (2013)	A. *Acacia mearnsii* extract (82% CT) B. *Schinopsis balansae* extract (90.4% quebracho CT) 200 g/kg C. *Castanea sativa* extract (5.7% CT and 75.5% chestnut HT) 200 g/kg D. *Quercus aegilops* extract 8.0% CT and 71.2% HT) 200 g/kg	A. 6.50 (+2%) B. 6.54 (+2.5%) D. 6.45 (+1%)	A. 111.1 mmol/L (–15%) B. 107.2 mmol/L (–18%) C. 113.6 mmol/L (–13%) D. 113.1 mmol/L (–13%)	A. 2.96 (–9%) B. 2.89 (–11%) D. 3.46 (+6%)	A. 4.03 mmol/L (–62%) B. 3.63 mmol/L (–66%) C. 3.48 mmol/L (–67%) D. 4.86 mmol/L (–55%)
Hatew et al. (2015)	Sainfoin (*Onobrychis viciifolia*) (120 g/kg of substrate DM) accessions: A. Rees 'A' B. CPI63763 C. Cotswold Common D. CPI63767		A. –3% B. –3% C. –6% D. –14%	A. –32% B. –43% C. –30% D. –52%	
Jayanegara et al. (2015)	A. Chestnut (1 mg/mL) B. Sumac (1 mg/mL) C. Mimosa (1 mg/mL) D. Quebracho (1 mg/mL)		A. –16% B. –10% C. –15% D. –15%	A. –11% B. –9% C. –10% D. –11%	
Jayanegara et al. (2015)	A. Chestnut (1 mg/mL) B. Sumach (1 mg/mL) C. Mimosa (1 mg/mL) D. Quebracho (1 mg/mL)		A. –7% B. –3% C. –11% D. –15%	A. –12% B. –8% C. –5% D. –12%	
Pinski et al. (2015)	Quebracho condensed tannin extract (QCT)	6.09, 6.15 ns, 6.16 ns	77.86 mM, 101.50, 94.57	1.18 (–24%), 2.00 (+28%), 1.80	24.39 mg/dL (–3%), 21.90 (–13%), 22.35 (–11%)

Table 4 – contd.

1	2	3	4	5	6
Rira et al. (2015)	*Acacia cyanophylla* (CT 63%)		–42%		
Soltan et al. (2012)	A. *Acasia saligna* leaves (6.3% CT) B. *Laucaena leucocephala* leaves (4.6% CT) C. *Prosopis juliflora* leaves (0.04% CT) D. *Atriplex halimus* leaves (0.02% CT)	A. 6.95 ns B. 6.94 ns C. 6.98 ns D. 6.87 ns	A. 65.96 mmol /L B. 65.72 C. 68.37 D. 62.93	A. 4.21 (+4%) B. 4.30 (+7%) C. 3.79 (+5%) D. 3.70 (+7%)	A. 24.5 mg/100 mL B. 27.4 (+10%) C. 30.9 (+25%) D. 26.4 (+6%)
Tan et al. (2011)	*Leucaena leucocephala* extracts (100% CT)	A. 7.14 ns B. 7.14 ns C. 7.14 ns D. 7.13 ns E. 7.13 ns	A. 47.6 mmol/L (–17%) B. 46.2 (–19%) C. 47.8 (–16%) D. 44.4 (–22%) E. 46.7 (–18%)	A. 3.66 (–1%) B. 3.71 (–3%) C. 3.70 (–2%) D. 3.87 (–7%) E. 3.80 (–5%)	
Wanapat et al. (2014)	Mangosteen peel powder (MSP)	6.6 ns	100.1 ns	3.2 (–18%)	11.6 ns
Wischer et al. (2013)	A. chestnut (*Castanea sativa*) TT <76% B. valonea (*Quercus valonea*), TT>67%		A. 31 (mmol/day) (–16%) B. 34 (–8%)	A. 1.17 (–6%) B. 1.26 (+1%)	A. 3.7 mmol/day (–16%) B. 4 mmol/day (–9%)

DM – dry matter; NA – not applicable, NE – no effect; ns – not significant; TVFA, total volatile fatty acid ; – decrease; + increase compared to control.

Table 5. Effects of essential oils on methane (CH_4) production in rumen

Reference	EO source	Diet/substrate	Test system/dosage	Methane reduction
1	2	3	4	5
Castro-Montoya et al. (2015)	200 g/kg (m/m) of coriander oil + geranyl acetate + eugenol		*In vivo* A. Dairy cattle B. Beef cattle	A. 14% B. 20%
Castro-Montoya et al. (2015)	Agolin Ruminant (blend of EO)	A. Concentrate + maize silage (50:50) B. Concentrate + maize silage + Grass silage (30:35:35)	*In vitro* A. (batch incubation) 30 ppm (m/v) B. Gas production (24 h) 30 ppm (m/v)	A. NE B. 17%
Cobellis et al. (2015)	A. Oregano B. Rosemary	Alfalfa hay + corn meal (1:1)	*In vitro* (0.5, 1.0, 1.5, 2.0 g/L)	A. 8.66 (mL), 4.18 (54%), 2.57 (72%), 2.71 (70%) B. 9.36, 9.12, 8.66, 8.43 (8%)
Durmic et al. (2014)	*Agonis fragrans, Eucalyptus plenissima, Eucalyptus staigeriana, Leptospermumpettersoni, Melaleuca alternifolia, Melaleuca ericifolia, Melaleuca teretifolia, Santalumspicatum*	Commercial pellet (barley + oats + wheat + lupin + straw + mill mix + mineral)	*In vitro* (batch fermentation) (25 µL/100 mg DM)	43%, 35%, 71%, 70%, 32%, 75%,75%, 45%
Jahani-Azizabadi et al. (2014)	A. coriander seed essential oils B. oregano, cinnamon C. caraway D. cumin E. cinnamon F. pistachio hull G. thyme	Alfalfa hay: concentrate (50:50)	*In vitro* (RUSITEC) 35, 70, 140, and 280 µL/L of the total culture medium.	A. (ns, ns, 16%, 21%) B. (ns, ns, ns, 32%) C. (17%, 22% , ns, ns) D. (ns, ns, ns, ns) E. (ns, ns, ns, 13%) F. (14%, ns, 21%, 17%) G. (ns, ns, 13%, 44%)
Kongman et al. (2010)	Garlic powder + coconut oil	Roughage: concentrate 60:40	*In vitro* (gas production) (8:4 mg, 4:8, 0:16)	18%, 9%, 15%

Kongman et al. (2011)	Coconut oil (CO) + garlic powder (GP)	Concentrate (0.5% of BW) + rice straw	*In vivo* (swamp buffaloes)	A. 26.6 ns mmol/L B. 25.0 (9%)
Lin et al. (2012 a)	Thyme oil (eugenol) + oregano oil (carvacrol) + cinnamon oil (cinnamaldehyde) + lemon oil (limonene) A. (1:2:3:4), B. (2:1:4:3), C. (3:4:1:2), D. (4:3:2:1), E. (1:1:1:1)	Ground maize/ground *Leymus chinensis* hay	*In vitro* (serum bottles) 50, 200 and 500 mg/L of medium	A. 0.83 (mmol), 0.81, 0.41 B. 0.85, 0.80, 0.42 C. 0.90, 0.76, 0.37 D. 0.93, 0.77, 0.38 E. 0.87, 0.79, 0.37
Lin et al. (2012 b)	(Thyme oil, eugenol) + oregano oil, carvacrol + cinnamon oil, cinnamaldehyde + lemon oil, limonene (1:1:1:1) + Monosodium fumarate (0, 5, 10 and 15 mmol/L)	Ground corn kernels/ground *Leymus chinensis* hay 50:50	*In vitro* (syringe) EO + 0, 5, 10 and 15 mM/L monosodium fumarate)	31%, 76%, 84% and 65%
Manh et al. (2012)	Eucalyptus leaf meal powder	Concentrate 0.5% of BW/rice straw *ad libitum*	*In vivo* (dairy cows) 100 and 200 g/day)	16%, 26%
Mateos et al. (2013)	A. Garlic oil (0.65g diallyl disulfide + 0.15 g diallyl trisulfide + 0.10 g allicin/g of oil) B. Cinamaldehyde (99% purity)	Alfalfa + concentrate (50:50)	*In vitro* (gas production) 0.2, 0.6, 1.8, 5.4 (g/kg of substrate)	A. 0.53 ns mmol, 0.47 (13%), 0.32 (40%), 0.20 (63%) B. 0.54 ns mmol, 0.51 ns, 0.49 ns, 0.37 (31%)
Mateos et al. (2015)	A. Garlic oil (0.65g diallyl disulfide + 0.15 g diallyl trisulfide + 0.10 g allicin/g of oil) B. Cinamaldehyde (99% purity)	Barley straw + concentrate (15:85)	*In vitro* (gas production) 0.2, 0.6, 1.8, 5.4 (g/kg of substrate)	A. 10%, 10%, 25%, 61% B. 76%
Meale et al. (2014)	A. Garlic oil (GO) B. Juniper berry oil (JBO)	Forage : concentrate 60:40	*In vivo* (lactating dairy cows) A. GO (5 g/day) B. JBO (2 g/day)	A. NE B. NE

Table 5 – contd.

1	2	3	4	5
Patra and Yu (2012)	A. Clove oil B. Eucalyptus oil C. Garlic oil D. Origanum oil E. Peppermint oil	Ground alfalfa hay/concentrate	*In vitro* (serum bottles/0.25, 0.50 and 1.0 g/L fermentation medium)	A.11%, 17%, 34% B.26%, 8%, 17% C.22%, 28%, 42% D.12%, 38%, 86% E. 8%, 20%, 25%)
Patra et al. (2010)	A. *Foeniculum vulgare* seed extracts (ethanol and methanol) B. *Syzygium aromaticum* flower bud extracts (ethanol and methanol)	Wheat straw/concentrate 50:50	HGT (24 h)/ethanol and methanol extracts of 0.5 mL/30m	A. 39%, 71% B. 47%, 86%
Pinski et al. (2015)	cinnamon oil		*In vitro* (125, 250, 500 mg/L)	13%, 18%, 37%
Rira et al. (2015)	A. *Juniperus phoenicea* B. *Mentha pulegium*	Hay: concentrate (1:1)	*In vitro* (syringe) 30% and 60%	A. 56.25% B. 36.50%
Thao et al. (2015)	*Eucalyptus (E. camaldulensis)* leaf (> 1% EO <2%)	Total mixed ration (TMR)	*In vivo* (40, 80 and 120 g/head/day)	8.5%, 14%, 12%
Tomkins et al. (2015)	CRINA (Blend of EO)	Rhodes grass *(C. gayana)* hay *(ad libitum)*	*In vivo* (Brahman steers) 1 and 2 g/d	79.8 ns (g/d) and 74.5 ns
Verma et al. (2012)	25 g garlic bulb + 1 mL peppermint oil	50% wheat straw + 50% concentrate	*In vivo* (buffaloes)	136.04 (13%)

DM – dry matter; HGT – Hohenheim gas test system; NA – not applicable; NE – no effect; ns – not significant;(–), decrease and (+) increase compared to control.

Table 6. Effects of essential oils on fermentation parameters in rumen

Reference	EO source	Ph	TVFA	Acetic/propionic acid ratio	NH_3N
1	2	3	4	5	6
Cobellis et al. (2015)	A. Oregano B. Rosemary	NA	A. 75.34 ns Mm, 34.72 (–57%), 31.92 (–60%), 27.70 (–66%) B. 76.67 ns, 80.53 ns, 95.06 ns, 92.91 ns	A. 4.28 ns, 4.53 ns, 4.42 ns, 4.09 ns B. 4.05 ns, 3.58 (–10%), 3.96 ns, 4.82 (+20%)	NA
Jahani-Azizabadi et al. (2014)	A. coriander seed essential oils B. oregano, cinnamon C. caraway D. cumin E. cinnamon F. pistachio hull G. thyme	A. 6.34 B. 6.34 C. 6.29 D. 6.28 E. 6.31 F. 6.34 G. 6.31	NA	NA	A. 41.2 mg/dL B. 41.05 C. 41.02 D. 43.97 E. 37.55 F. 42.55 G. 32.72
Kongmun et al. (2011)	A. 7% coconut oil + 50 g/day garlic powder B. 7% coconut oil + 100 g/day garlic powder.	A. 6.9 ns B. 6.8 ns	A. 93.3 mM (–6%) B. 89.9 (-9%)	A. 2.7 (–6%) B. 2.4 (-17%)	A. 9.3 ns mg/L B. 9.9 ns
Kongmun et al. (2010)	Coconut oil and garlic powder (*A. sativa*) ratio		167.6 mM (–7%), 178.5 ns, 160.9 (–11%)	1.7(–10%), 1.8 ns, 1.6 (–15%)	24.1 mg/dL, 23.4 ns, 16.9 (–5%)
Manh et al. (2012)	Eucalyptus leaf meal powder	6.7 ns, 6.7 ns	103 mmol/L (–14%), 92.8 (–23%)	3.2 ns, 3.0 (–9%)	10.6 (–28%) mg/dL, 10.0 (–32%)
Mateos et al. (2013)	A. Garlic oil (0.65g diallyl disulfide + 0.15 g diallyl trisulfide + 0.10 g allicin/g of oil) B. Cinamaldehyde (99% purity)	A. 6.66 ns, 6.64 ns, 6.67 ns, 6.65 ns) B. 6.62 ns, 6.65 ns, 6.65 ns, 6.64 ns)	A. 2.07 mmol, 2.08, 1.99, 1.93 B. 2.09, 2.09, 2.12, –9%	A. 2.87, 2.62 (–10%), 2.23 (–23%), 2.05 (–30%) B. 2.86, 2.88, +7%, –9%	A. 228 ns (mg/L), 231 ns, 243 ns, 230 ns B. 225 ns, 199 (–14%), 188 (–19%), 250 (+7%)

Table 6 – contd.

1	2	3	4	5	6
Patra and Yu (2012)	A. Clove oil B. Eucalyptus oil C. Garlic oil D. Origanum oil E. Peppermint oil	A. 5.49, 5.52, 5.56 B. 5.46, 5.48, 5.52 C. 5.49, 5.51, 5.54 D. 5.51, 5.58, +10% E. 5.49, 5.53, 5.58	A. 97.3 mM, 101, 91.0 B. +6%, +8%, +7% C. 106.3, 100.8, 101.3 D. 98.3, 90, 61(–38%) E. 103.4, 105.7, 103.3	A. 2.24, 2.28, 2.47 B. 2.19, 2.18, 2.14 C. 2.09, 2.10,-11% D. 2.22, 3.19 (+43%), 2.85 E. 2.09 (–6%), 2.22, 2.60 (+17%)	A. 31.3 mM, 23.3, –18% B. –29%, 27.9, 27.1 C. 27.8, 26.2, 27.7 D. 27.7, 24.7 (–13%), 18.6 (–34%) E. 10.8 (–61%), 23.8 (–16%), 23.7 (–16%)
Pinski et al. (2015)	Cinnamon oil (CNO)	6.15 ns, 6.19 ns, 6.27 (+1.7%)	76.70 ns (mM), 77.64 ns, 62.89 (–25%)	1.25 ns, 1.34 ns, 1.50 ns	27.28 ns (mg/dL), 26.86 ns, 26.18 (–2.5%)
Tekippe et al. (2012)	A. *Ambrosia artemisiifolia* B. *Artemisia annua* C. *Asimina triloba* D. *Oplopanax horridus* E. *Oplopanax horridus* F. *Heracleum maximum* G. *Origanum vulgare*	NA	NA	A. 2.04 ns B. 2.00 ns C. 1.83 (–7%) D. 1.92 (–3%) E. 1.96 ns F. 2.03 ns G. 2.02 ns	NA
Tekippe et al. (2013)	A. *Artemisia annua* B. *Artemisia afra Jacq.* C. *Artemisia annua* D. *Oplopanax horridus* E. *Origanum majorana* F. *Rhus typhina* G. *Spilanthes acmella*	NA	NA	A. 2.61 ns B. 2.52 ns C. 2.63 ns D. 2.45 ns E. 2.42 ns F. 2.44 ns G. 2.46 ns	NA
Tekippe et al. (2011)	*Origanum vulgare L.* leaf	6.1 ns	3.0 ns mM	2.86 ns	5.5 mM (+18%)

Table 6 – contd.

1	2	3	4	5	6
Thao et al. (2015)	Eucalyptus (*E. camaldulensis*) leaf (> 1% EO <2%) 40, 80 and 120 g/head/day	6.5 ns, 6.4 ns, 6.5 ns	104.6 ns mM/L, 106.6 (+5.5%), 108.5 (+4%)	(3.00 ns, –19%, 2.59) (–30%), 2.73(–27%)	10.3 ns mg/dL, 8.5 ns, 7.8 (–34%)
Tomkins et al. (2015)	CRINA (blend of EO)	6.8 ns, 6.9 ns	51.1 ns Mm, 56.5 ns	5.3 ns, 5.3 ns	-
Zmora et al. (2013)	*Mentha piperita L.* leaf (1.2–3.9% v/w of EO)	6.81 ns, 6.81 ns	38.95 ns mmol, 43.50 ns	3.99 ns, 3.82 ns	14.13 (–12%) mmol, 14.99 (–7%)

NA – not applicable; NE – no effect; ns – not significant; TVFA – total volatile fatty acids; (–), decrease and (+) increase compared to control.

Effect of tannins on rumen methanogenesis and fermentation characteristics

Review of recent studies about the effects of tannin sources on rumen CH_4 and fermentation parameters are shown in Tables 3 and 4, respectively. Tannins as a class of PSM could be divided into two groups based on chemical structure, which are hydrolysable tannins (HT) and condensed tannins (CT) (Goel and Makkar, 2012). Many factors like tannin type and plant source may influence results derived from the effect of tannin on methanogenesis (Goel and Makkar, 2012). Bouchard et al. (2013) indicated that beneficial effects of CT on enteric CH_4 formation typically occur at dietary concentrations between 20 and 40 g CT/ kg DM. However, Bueno et al. (2015) showed the lack of tannin effect on CH_4 emissions in their studies. In spite of the fact that anti-methanogenic activity of phenolic compounds has been consistently demonstrated in several *in vitro* and *in vivo* studies, their effectiveness usually depends on the species of microorganisms and the concentration, type or source of CT (Patra and Saxena, 2011). In contrast to the former study, Bhatta et al. (2013) and Anantasook et al. (2014) reported that the ruminal CH_4 concentration was reduced by tannin addition. Inoculum from different domesticated ruminant species has unequal rumen fermentation and degradability as a result of differing microbial diversity due to their respective feeding strategies behavior. Rumen fluid from bovines emits more CH_4 than from small ruminants, when measured on a degraded organic matter basis. Bhatta et al. (2015) introduced PSM such as tannins as rumen modifiers because these compounds are natural products, which are generally accepted as environmentally safe and friendly in food production systems. It should be noted that not all types of tannins produce beneficial nutritional and environmental responses (Bhatta et al., 2014). Generally, tannins with low molecular weight showed greater inhibitory effects on rumen microbes, because of their higher protein-precipitating capacities than high molecular weight polymeric tannins (Bhatta et al., 2014).

Recently, it was also confirmed that samples containing both HT plus CT were more effective in reducing *in vitro* CH_4 production than those containing only HT (Bhatta et al., 2012). Moreover, earlier study had shown that phenolic fractions present in tannin extracts were more effective than leaves containing tannins (Bhatta et al., 2009). Bhatta et al. (2014) declared that tannins can directly suppress methanogenesis by affecting rumen archaea and not by defaunation (removal of protozoa) per se. Protozoa can synergistically provide H_2 as a source of electrons to the methanogens, and hence, antiprotozoal effects of tannins would be expected to decrease CH_4 production by methanogens attached to protozoa. The effects of HT and CT may be different on ruminal ciliated protozoa, with HT generally being less inhibitory against protozoa than CT (Sliwinski et al., 2002). Pinski et al. (2015) concluded that addition of CT at concentration less than 50 g/kg of DM did not adversely affect ruminal fermentation parameters. Beauchemin and McGinn (2007) also did not observe any reduction in CH_4 production by feeding quebracho tannin extract up to 2% (1.8% CT) of the dietary DM. However, a meta-analysis study by Jayanegara et al. (2012) concluded that increasing tannin concentrations (up to 177 g/kg) reduced CH_4 production *in vitro* and *in vivo*. This discrepancy may be related to several factors such as supplement source, dose level, diet composition and the period of adaptation to the product. It is known that tannins reduce degradation of dietary protein in the

rumen by forming protein–tannin complexes or the inhibition of the activities of the protease enzyme by tannin (Hatew et al., 2015). In an *in vitro* gas production test, supplementation of *Acacia cyanophylla* (containing 63% of CT) at 60% and 30% resulted in 37.5% and 56.25% lower CH_4, respectively (Rira et al., 2015). The results were attributed to the high CT content in *Acacia cyanophylla* which had been reported to be toxic for rumen microbial population, especially ciliate protozoa, fiber degrading microbes and methanogens (Kamra et al., 2006). In addition, the inhibition of CH_4 production was accompanied by alteration in total volatile fatty acid profile and the acetate/propionate ratio through an increase in the concentration of propionate with *Acacia cyanophylla* supplementation. Jayanegara et al. (2015) reported that all the purified tannins (chestnut, sumac, mimosa and quebracho) at concentration of 1 mg/mL of rumen liquid were able to decrease ruminal CH_4 emissions *in vitro*, and confirmed their previous results obtained about the inhibitory effect of tannins on rumen methanogenesis (Jayanegara et al., 2011, 2012 and 2013). Moreover, a meta-analysis study concluded that increasing tannin concentration (0 to 177 g/kg) reduced CH_4 production *in vitro* and *in vivo* (Jayanegara et al., 2011).

Jayanegara et al. (2015) proposed two inhibitory mechanisms of tannins on CH_4 emission from ruminants; (1) through reduction in fibre digestion, which decreases H_2 production, and (2) through inhibition of the growth of methanogens. Tan et al. (2011) reported that CT at a relatively low level of 15 mg/500 mg DM of CT, reduced CH_4 production, decreased methanogen and protozoal populations and reduced nitrogen disappearance with only 7% reduction in dry matter digestibility. An *in vitro* study showed that 50 g/kg dietary HT from chestnut or CT from acacia reduced CH_4 production and ruminal protein degradation, but with a slight negative impact on total VFA concentration (Hassanat and Benchaar, 2013). Hassanat and Benchaar (2013) also reported up to the 40% reduction of CH_4 production compared with control when the substrate was incubated with CT at $\geq$100 g/kg with minimum detrimental effects on the efficiency of ruminal fermentation. They concluded that tannin sources could affect rumen methanogenesis without affecting other fermentation parameters and their impacts on rumen fermentation varies according to their type, source and concentration.

Effect of essential oils on rumen methanogenesis and fermentation characteristics

Review of recent studies about the effects of EO sources on rumen CH_4 and fermentation parameters are shown in Table 5 and 6, respectively. EO consist of volatile constituents of terpenoid or non-terpenoid origin (Rira et al., 2015). Under this group, hundreds of large or small molecules can be present, consisting of hydrocarbons and their oxygenated derivatives. EO are known for their antimicrobial activity and are commonly used for the treatment of microbial infections (Rira et al., 2015). Conflicting results have been reported on the effects of EO on rumen methanogenesis. Rumen CH_4 production reduction has been observed in response to EO supplements. Tekippe et al. (2012) screened among a collection of 100 EO and plants which were naturalized to, or successfully grown in North America and identified that three EO from *Anethum graveolens* (dill weed), *Lavandula latifolia*,

and *Ocimum basilicum* and one plant sample *(Origanum vulgare)* with a potential for reducing CH_4 production *in vitro*. Concentration of NH_3N was also very low at the end of the incubation for both EO and plant samples. Castro-Montoya et al. (2015) reported that the blend of EO tended to decrease daily CH_4 emissions from dairy and the decrease was sustained for the six weeks of supplementation. In previous studies with other EO sources, *in vitro* CH_4 inhibition was achieved only at extremely high concentrations; for example, Evans and Martin (2000) found that after 24 h thymol strongly inhibited *in vitro* CH_4 production when added at a concentration of 400 ppm, but production of acetate and propionate strongly decreased. When thymol was incubated at a concentration of 200 ppm or lower, there were no effects on CH_4, acetate and propionate production. Tomkins et al. (2015) showed that administration of daily CRINA (commercially made with blend of EO) into the rumen had a significant effect on rumen fermentation and decreased enteric methanogenesis when used at rates of 1 or 2 g/d. Similarly, Busquet et al. (2005) found that garlic oil and diallyl disulfide decreased *in vitro* CH_4 production and total VFA production when applied at a concentration of 300 ppm. However, lower concentrations (30 ppm) of both EO showed no negative effect on fermentation parameters.

Cobellis et al. (2015) concluded that the effects of EO are due to their antimicrobial activity against ruminal microorganisms such as methanogenic archaea and hyper-ammonia-producing bacteria. However, EO also showed adverse effects on fiber digestion. The effect of EO in reducing *in vitro* CH_4 production through a direct inhibition of methanogenic archaea and/or an indirect depression of some microbial metabolic processes involved in methanogenesis has been well documented (Rira et al., 2015). Patra and Yu (2012) found a decrease in the abundance of rumen archaea and protozoa by all the tested EO (clove, eucalyptus, garlic, oregano and peppermint) but also in that of cellulolytic bacteria. Total VFA concentration was also markedly reduced by oregano EO doses. In a similar study, Cardozo et al. (2004) found that the effects of some EO on rumen VFA profiles were more pronounced at low rumen pH. They also suggested that pH is able to affect dissociated or undissociated status of EO molecules. The results of the proposed investigation showed that oregano EO, at the highest concentration, was a potent inhibitor of ruminal CH_4 and NH_3N production mostly due to the antimicrobial properties of carvacrol, its major compound. Pinski et al. (2015) showed that except for cinnamon oil, EO tested in the study had no effect on culture CH_4 production. However, previous *in vitro* (Sallam et al., 2009) and *in vivo* (Manh et al., 2012) experiments have reported that eucalyptus oil decreases CH_4 production. These inconsistencies might be related to differences in supplement source, dose level and diet composition.

Conclusion and future directions

A conclusion from the current review is that the effects of saponin, tannin and EO on ruminal fermentation are desirable if they improve fermentation characteristics such as increase VFA concentration, decrease NH_3N concentration and decrease CH_4 production. However, a reduction in VFA production as a result of plant additive supplementation, even if accompanied by reductions in CH_4 production, would generally be viewed to be nutritionally unfavorable. Moreover, the literature suggests

that saponins mitigate methanogenesis mainly by reducing the number of protozoa, tannins especially condensed tannin both by reducing the number of protozoa and by a direct toxic effect on methanogens, whereas EO act mostly by a direct toxic effect on methanogens. Although a large number of phytochemicals (e.g. saponins, tannins and essential oils) have been investigated for their CH_4 reduction potential, there have not yet been major breakthroughs that could be applied in practice. Therefore, the future challenge will be to identify cost-effective PSM components which favorably alter ruminal fermentation by decreasing CH_4 production without reducing total VFA concentrations.

Acknowledgments

The authors are thankful to the Faculty of Veterinary Medicine, Universiti Putra Malaysia.

References

Anantasook N., Wanapat M., Cherdthong A. (2014). Manipulation of ruminal fermentation and methane production by supplementation of rain tree pod meal containing tannins and saponins in growing dairy steers. J. Anim. Physiol. Anim. Nutri., 98: 50–55.

Beauchemin K.A., McGinn S. (2007). Methane emissions from beef cattle: effects of fumaric acid, essential oil, and canola oil. J. Anim. Sci., 84: 1489.

Bharathidhasan A., Viswanathan K., Balakrishnan V., Valli C., Ramesh S., Senthilkumar S.M.R. (2013). Effects of Purified Saponin on Rumen Methanogenesis and Rumen Fermentation Characteristics Studied Using In Vitro Gas Production Technique. Inter. J. Vet. Sci., 2: 44–49.

Bhatta R., Uyeno Y., Tajima K., Takenaka A., Yabumoto Y., Nonaka I., Enishi O., Kurihara M. (2009). Difference in the nature of tannins on *in vitro* ruminal methane and volatile fatty acid production, and methanogenic archaea and protozoal populations. J. Dairy Sci., 92: 5512–5522.

Bhatta R., Saravanan M., Baruah L., Sampath K. (2012). Nutrient content, *in vitro* ruminal fermentation characteristics and methane reduction potential of tropical tannin-containing leaves. J. Sci. Food Agri., 92: 2929–2935.

Bhatta R., Saravanan M., Baruah L., Sampath K.T., Prasad C.S. (2013 a). Effect of plant secondary compounds on *in vitro* methane, ammonia production and ruminal protozoa population. J. Appl. Microbiol., 115: 455–465.

Bhatta R., Baruah L., Saravanan M., Suresh K.P., Sampath K.T. (2013 b). Effect of medicinal and aromatic plants on rumen fermentation, protozoa population and methanogenesis *in vitro*. J. Anim. Physiol. Anim. Nutri., 97: 446–456.

Bhatta R., Saravanan M., Baruah L., Prasad C.S. (2015). Effects of graded levels of tannin-containing tropical tree leaves on *in vitro* rumen fermentation, total protozoa and methane production. J. Appl. Microbiol., 118: 557–564.

Bodas R., Prietoa N., García-González R., Andrésa S., Giráldeza F.J., López S. (2012). Manipulation of rumen fermentation and methane production with plant secondary metabolites. Anim. Feed Sci. Technol., 176: 78 –93.

Bouchard K., Wittenberg K.M., Legesse G., Krause D.O., Khafipour E., Buckley K.E., Ominski K.H. (2013). Comparison of feed intake, body weight gain, enteric methane emission and relative abundance of rumen microbes in steers fed sainfoin and lucerne silages under western Canadian conditions. Grass Forage Sci., 70: 116–129.

Bueno C., Brandi R.A., Franzolina A., Benete G., Fagundes G.M., Abdalla A.L., Louvandini H., Muir J.P. (2015). *In vitro* methane production and tolerance to condensed tannins in five ruminant species. Anim. Feed Sci. Technol., 205: 1–9.

Busquet M., Calsamiglia S., Ferret A., Kamel C. (2006). Plant extracts affect *in vitro* rumen microbial fermentation. J. Dairy Sci., 89: 761–771.

Cardozo P.W., Calsamiglia S., Ferret A., Kamel C. (2004). Effects of natural plant extracts on ruminal protein degradation and fermentation profiles in continuous culture. J. Anim. Sci., 82: 3230–3236.

Castro-Montoya J., Peiren N., Cone J.W., Zweife B., Fievez V., De Campeneere S. (2015). *In vivo* and *in vitro* effects of a blend of essential oils on rumen methane mitigation. Livest. Sci., 180: 134–142.

Chwalek M., Lalun N., Bobichon H., Ple K., Voutquenne-Nazabadioko L. (2006). Structure-activity relationships of some hederagenin diglycosides: Haemolysis, cytotoxicity and apoptosis induction. Biochim. Biophys. Acta, 1760: 1418–1427.

Cieslak A., Szumacher-Strabel S., Oleszek W. (2013). Plant components with specific activities against rumen methanogens. Anim., 7: 253–265.

Cobellis G., Petrozzi A., Forte C., Acuti G., Orrù M., Marcotullio M.C. (2015). Evaluation of the effects of mitigation on methane and ammonia production by using *Origanum vulgare* L. and *Rosmarinus officinalis* L. essential oils on *in vitro* rumen fermentation systems. Sustainability,7:12856–12869.

Durmic Z., Moate P.J., Eckard R., Revell D.K., Williams R., Vercoe P.E. (2014). In vitro screening of selected feed additives, plant essential oils and plant extracts for rumen methane mitigation. J. Sci. Food Agr., 94: 1191–1196.

Ebrahimi M., Rajion M.A., Meng G.Y., Shokryzadan P., Sazili A.Q., Jahromi M.F. (2015). Feeding Oil Palm (Elaeis Guineensis, Jacq.) Fronds Alters Rumen Protozoal Population and Ruminal Fermentation Pattern in Goats. J. Anim. Sci., 14, article 3877, https://doi.org/10.4081/ijas.2015.3877.

Evans J.D., Martin S.A. (2000). Effects of thymol on ruminal microorganisms. Curr. Microbiol., 41: 336–340.

Feng Z.H., Cao Y.F., Gao Y.X., Li Q.F., Li J.G. (2012). Effect of Gross Saponin of Tribulus terrestris on Ruminal Fermentation and Methane Production in vitro. J. Anim. Vet. Ad., 11: 2121–2125.

Francis G., Kerem Z., Makkar H.P.S., Becker K. (2002). The biological action of saponins in animal systems: a review. Br. J. Nutr., 88: 587–605.

Goel G., Makkar H.P.S. (2012). Methane mitigation from ruminants using tannins and saponins. Trop. Anim. Health Prod., 4: 729–739.

Guo Y.Q., Liu J.X., Lu Y., Zhu W.Y., Denman S.E., McSweeney C.S. (2008). Effect of tea saponin on methanogenesis, microbial community structure and expression of mcrA gene, in cultures of rumen microorganisms. Lett. Appl. Microbiol., 47: 421–426.

Hartmann T. (2007). From waste products to ecochemicals: fifty years research of plant secondary metabolism. Phytochem., 68: 2831–2846.

Hassanat F., Benchaar C. (2013). Assessment of the effect of condensed (acacia and quebracho) and hydrolysable (chestnut and valonea) tannins on rumen fermentation and methane production *in vitro*. J. Sci. Food Agri., 93: 332–339.

Hatew B., Stringano E., Harvey M., Hendriks W.H., Hayot C., Smith C., Pellikaan W. (2015). Impact of variation in structure of condensed tannins from sainfoin *(Onobrychis viciifolia)* on *in vitro* ruminal methane production and fermentation characteristics. J. Anim. Physiol. Anim. Nutri., DOI: 10.1111/jpn.12336.

Hu W.L., Liu J.X., Ye J.A., Wu Y.M., Guo Y.Q. (2005). Effect of tea saponin on rumen fermentation *in vitro*. Anim. Feed Sci. Technol., 120: 333–339.

Jafari S., Goh Y.M., Rajion M.A., Jahromi M.F., Ebrahimi M. (2016 a). Ruminal methanogenesis and biohydrogenation reduction potential of papaya *(Carica papaya)* leaf: an *in vitro* study. It. J. Anim. Sci., 15: 157–165.

Jafari S., Goh Y.M., Rajion M.A., Jahromi M.F., Ebrahimi M. (2016 b). Manipulation of rumen microbial fermentation by polyphenol rich solvent fractions from papaya leaf to reduce green-house gas methane and biohydrogenation of C18 PUFA. J. Agri. Food Chem., DOI: 10.1021/acs.jafc.6b00846.

Jafari S., Goh Y.M., Rajion M.A., Jahromi M.F., Ebrahimi M. (2016 c). Papaya *(Carica papaya)* leaf methanolic extract modulates *in vitro* rumen methanogenesis and rumen biohydrogenation. J. Anim. Sci., doi:10.1111/asj.12634.

Jahani-Azizabadi H., Danesh Mesgaran M., Vakili A.R., Rezayazdi K. (2014). Effect of some plant essential oils on in vitro ruminal methane production and on fermentation characteristics of a mid-forage diet. J. Agr. Sci. Technol., 16: 1543–1554.

Jayanegara A., Goel G., Makkar H.P.S., Becker K. (2010). Reduction in Methane Emissions from Ruminants by Plant Secondary Metabolites: Effects of Polyphenols and Saponins. Food Agri Org UN: 151–157.

Jayanegara A., Kreuzer M., Wina E., Leiber E. (2011). Significance of phenolic compounds in tropical forages for the ruminal bypass of polyunsaturated fatty acids and the appearance of biohydrogenation intermediates as examined *in vitro*. Anim. Prod. Sci., 51: 1127–1136.

Jayanegara A., Kreuzer M., Leiber F. (2012). Ruminal disappearance of polyunsaturated fatty acids and appearance of biohydrogenation products when incubating linseed oil with alpine forage plant species in vitro. Livest. Sci., 147: 104–112.

Jayanegara A., Ikhsan T., Toharmat T. (2013). Assessment of methane estimation from volatile fatty acid stoichiometry in the rumen *in vitro*. J. Indo. Trop. Anim. Agri., 38: 103–108.

Jayanegara A., Wina E., Takahashi J. (2014). Meta-analysis on methane mitigating properties of saponin-rich sources in the rumen *in vitro*: influence of addition levels and plant sources. Asian-Australas. J. Anim. Sci., 27: 1426–1435.

Jayanegara A., Goel G., Makkar P.S.H., Becker K. (2015). Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population in vitro. Anim. Feed Sci. Technol., 209: 60–68.

Johnson K.A., Johnson D.E. (1995). Methane emissions from cattle. J. Anim. Sci., 73: 2483–2492.

Kamra D.N., Agarwal N., Chaudhary L.C. (2006). Inhibition of ruminal methanogenesis by tropical plants containing secondary compounds. Intl. Congress Ser., 1293: 156–163.

Kongmun P., Wanapat M., Pakdee P., Navanukraw C. (2010). Effect of coconut oil and garlic powder on in vitro fermentation using gas production technique. Livest. Sci., 127: 38–44.

Kongmun P., Wanapat M., Pakdee P., Navanukraw C., Yu Z. (2011). Manipulation of rumen fermentation and ecology of swamp buffalo by coconut oil and garlic powder supplementation. Livest. Sci., 135: 84–92.

Kumar S., Choudhury P.K., Carro M.D., Griffith G.W., Dagar S.S., Puniya M., Calabro S., Ravella S.R., Dhewa T., Upadhyay R.C., Sirohi S.K., Kundu S.S., Wanapat M., Puniya A.K. (2013). New aspects and strategies for methane mitigation from ruminants. Appl. Microbiol. Biotechnol., DOI 10.1007/s00253-013-5365-0.

Li W., Powers W. (2012). Effects of saponin extracts on air emissions from steers. J. Anim. Sci., 90: 4001–4013.

Lin B., Lu Y., Wang J.H., Liang Q., Liu J.X. (2012). Effects of combined essential oils along with fumarate on rumen fermentation and methane production in vitro. J. Anim. Feed Sci., 21: 198–210.

Lin B., Wang JH., Lu Y., Liang Q., Liu JX. (2013). In vitro rumen fermentation and methane production are influenced by active components of essential oils combined with fumarate. J. Anim. Physiol. Anim. Nutr., 97:1–9.

Manh N.S., Wanapat M., Uriyapongson S., Khejornsart P., Chanthakhoun V. (2012). Effect of eucalyptus (Camaldulensis) leaf meal powder on rumen fermentation characteristics in cattle fed on rice straw. Afri. J. Agri. Res., 7: 1997–2003.

Mao H.L., Wang J.K., Zhou Y.Y., Liu JX. (2010). Effects of addition of tea saponins and soyabean oil on methane production, fermentation and microbial population in the rumen of growing lambs. Livest. Sci., 129: 56–62.

Mateos J., Ranilla M.J., Tejido M.L., Saro C., Kamel C., Carro M.D. (2013). The influence of diet type (dairy versus intensive fattening) on the effectiveness of garlic oil and cinnamaldehyde to manipulate in vitro ruminal fermentation and methane production. Anim. Prod. Sci., 53: 299–307.

Meale S.J., Chaves A.V., McAllister T.A., Iwaasa A.D., Yang W.Z., Benchaar C. (2014). Including essential oils in lactating dairy cow diets: effects on methane emissions. Anim. Prod. Sci., 54: 1215–1218.

Mihaela G., Criste A., Cocan D., Constantinescu R., Raducu C., Miresan V.

(2014). Methane production in the rumen and its influence on global warming. Pro-Envir.,7: 64–70.

Narvaez N., Wang Y., McAllister T. (2013). Effects of extracts of Humulus lupulus (hops) and Yucca schidigera applied alone or in combination with monensin on rumen fermentation and microbial populations in vitro. J. Sci. Food Agr., 93: 2517–2522.

Naumann H.D., Lambert B.D., Armstrong S.A., Fonseca M. A., Tedeschi L.O., Muir J.P. (2015). Effect of replacing alfalfa with panicled-tick clover or sericea lespedeza in corn-alfalfa-based substrates on in vitro ruminal methane production. J. Dairy Sci., 98: 3980–3987.

Patra A.K. (2012). Enteric methane mitigation technologies for ruminant livestock: a synthesis of current research and future directions. Envir. Monitor. Assess., 184: 1929–1952.

Patra A.K. (2014). A meta-analysis of the effect of dietary fat on enteric methane production, digestibility and rumen fermentation in sheep, and a comparison of these responses between cattle and sheep. Livest. Sci., 162: 97–103.

Patra M., Saxena J. (2011). Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. J. Food Agri., 91: 24–37.

Patra A.K., Yu Z. (2012). Effects of essential oils on methane production and fermentation by, and abundance and diversity of, rumen microbial populations. Appl. Environ. Microbiol., 78: 4271–4280.

Patra A.K., Yu Z. (2013). Effective reduction of enteric methane production by a combination of nitrate and saponin without adverse effect on feed degradability, fermentation, or bacterial and archaeal communities of the rumen. Bioresour. Technol., 148: 352–360.

Patra A.K., Yu G. (2014 a). Combinations of nitrate, saponin, and sulfate additively reduce methane production by rumen cultures *in vitro* while not adversely affecting feed digestion, fermentation or microbial communities. Bioresour. Technol., 155: 129–135.

Patra A.K., Yu Z. (2014 b). Effects of vanillin, quillaja saponin, and essential oils on in vitro fermentation and protein degrading microorganisms of the rumen. Appl. Microbiol. Biotechnol., 98: 897–905.

Patra A.K., Yu Z. (2015). Effects of Adaptation of In vitro Rumen Culture to Garlic Oil, Nitrate, and Saponin and Their Combinations on Methanogenesis, Fermentation, and Abundances and Diversity of Microbial Populations. Front Microbiol., 6: 14–34.

Patra A.K., Kamra D.N., Agarwal N. (2010). Effects of extracts of spices on rumen methanogenesis, enzyme activities and fermentation of feeds in vitro. J. Sci. Food Agr., 90: 511–520.

Patra A.K., Stiverson J., Yu Z. (2012). Effects of quillaja and yucca saponins on communities and select populations of ruminal bacteria and archaea, and fermentation *in vitro*. J. Appl. Microbiol., 113: 1329–1340.

Pinski B., Günal M., AbuGhazaleh AA. (2015). The effects of essential oil and condensed tannin on fermentation. Anim. Prod. Sci., http://dx.doi.org/10.1071/AN15069.

Rira M., Chentli A., Boufener S., Boussebou H. (2015). Effects of plants containing secondary metabolites on ruminal methanogenesis of sheep *in vitro*. Energy Procedia., 74: 15–24.

Sallam S.M.A., Bueno I.C.S., Brigide P., Godoy P.B., Vitti D.M.S.S., Abdalla A.L. (2009). Efficacy of eucalyptus oil on *in vitro* rumen fermentation and methane production. Options Mediterraneennes., 85: 267–272.

Sliwinski B.J., Carla R.S., Machmuller A., Kreuzer M. (2002). Efficacy of plant extracts rich in secondary constituents to modify rumen fermentation. Anim. Feed Sci. Technol., 101: 101–114.

Soltan Y.A., Morsy A.S., Sallam S.M.A., Louvandini H., Abdalla A.L. (2012). Comparative in vitro evaluation of forage legumes (Prosopis, Acacia, Atriplex, and Leucaena) on ruminal fermentation and methanogenesis. J. Anim. Feed Sci., 21: 759–772.

Soltan Y.A., Morsy A.S., Sallam S.M.A., Lucas R.C., Louvandini H., Kreuzer M., Abdalla A.L. (2013). Contribution of condensed tannins and mimosine to the methane mitigation caused by feeding Leucaena leucocephala. Arch. Anim. Nutr., 67: 169–184.

Tan H.Y., Sieo C.C., Abdullah N., Liang J.B., Huang X.D., Ho YW. (2011). Effects of condensed tannins from *Leucaena* on methane production, rumen fermentation and populations of methanogens and protozoa *in vitro*. Anim. Feed Sci. Technol., 169: 185–193.

Tekippe J.A., Hristov A.N., Heyler K.S., Cassidy T.W., Zheljazkov V.D., Ferreira J.F.S., Karnati S.K., Varga G.A. (2011). Rumen fermentation and production effects of Origanum vulgare L. in lactating dairy cows. J. Dairy Sci., 94: 5065–5079.

Tekippe J.A., Hristov A.N., Heyler K.S., Zheljazkov V.D., Ferreira J.F.S., Cantrell C.L., Varga G.A. (2012). Effects of plants and essential oils on ruminal in vitro batch culture methane production and fermentation. Can. J. Anim. Sci., 92: 395-408.

Tekippe J.A., Tacoma R., Hristov A.N., Lee C., O.H.J., Heyler K.S., Cassidy T.W., Varga G.A., Bravo D. (2013). Effect of essential oils on ruminal fermentation and lactation performance of dairy cows. J. Dairy Sci., 96: 7892–7903.

Thao N.T., Wanapat M., Kang S., Cherdthong A. (2015). Effects of Supplementation of Eucalyptus (E. Camaldulensis) Leaf Meal on Feed Intake and Rumen Fermentation Efficiency in Swamp Buffaloes. Asian-Australas. J. Anim. Sci., 28: 951–957.

Tomkins N.W., Denman S.E., Pilajun R., Wanapat M., McSweeney C.S., Elliott R. (2015). Manipulating rumen fermentation and methanogenesis using an essential oil and monensin in beef cattle fed a tropical grass hay. Anim. Feed Sci. Technol., 200: 25–34.

Verma V., Chaudhary L.C., Agarwal N., Bhar R., Kamra D.N. (2012). Effect of Feeding Mixture of Garlic Bulb and Peppermint Oil on Methane Emission, Rumen Fermentation and Microbial Profile in Buffaloes. Anim. Nutr. Feed Technol., 12: 157–164.

Wanapat M., Chanthakhoun V., Phesatcha K., Kang S. (2014). Influence of mangosteen peel powder as a source of plant secondary compounds on rumen microorganisms, volatile fatty acids, methane and microbial protein synthesis in swamp buffaloes. Livest. Sci., 162: 126–133.

Wang X.F., Mao S.Y., Liu J.H., Zhang L.L., Cheng Y.F., Wand J., Zhu WY. (2011). Effect of the gynosaponin on methane production and microbe numbers in a fungus methanogen coculture. J. Anim. Feed Sci., 20: 272–284.

Wischer G., Boguhn J., Steinga H., Schollenberger M., Rodehutscord M. (2013). Effects of different tannin-rich extracts and rapeseed tannin monomers on methane formation and microbial protein synthesis in vitro. Animal, 7: 1796–1805.

Zhou C.S., Xiao W.J., Tan Z.L., Salem A.Z.M., Geng M.M., Tang S.X., Wang M., Han X.F., Kang JH. (2012). Effects of dietary supplementation of tea saponins (*Ilex kudingcha C.J.Tseng*) on ruminal fermentation, digestibility and plasma antioxidant parameters in goats. Anim. Feed Sci. Technol., 176: 163–169.

Zhou Y.Y., Mao H.L., Jiang F., Wang J.K., Liu J.X., McSweeney C.S. (2011). Inhibition of rumen methanogenesis by tea saponins with reference to fermentation pattern and microbial communities in Hu sheep. Anim. Feed Sci. Technol., 166–167: 93–100.

Zmora P., Cieslak A., Pers-Kamczyc E., Nowak A., Szczechowiak J., Szumacher-Strabel M. (2013). Effect of Mentha piperita L. on in vitro rumen methanogenesis and fermentation. Acta Agr., 62: 46–52.

Received: 8 III 2018
Accepted: 27 VII 2018