Animal (2013), **7:s2**, pp 253–265 © The Animal Consortium 2013
doi:10.1017/S1751731113000852

animal

Plant components with specific activities against rumen methanogens

A. Cieslak[1†], M. Szumacher-Strabel[1], A. Stochmal[2] and W. Oleszek[2]

[1]*Department of Animal Nutrition and Feed Management, Poznan University of Life Sciences, Wolynska 33, 60-637 Poznan, Poland;* [2]*Institute of Soil Science and Plant Cultivation, State Research Institute, Department of Biochemistry and Crop Quality, Czartoryskich 8, 24-100 Pulawy, Poland*

(Received 18 January 2013; Accepted 14 April 2013)

A wide range of plant bioactive components (phytochemicals) have been identified as having potential to modulate the processes of fermentation in the rumen. The use of plants or plant extracts as natural feed additives has become a subject of interest 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 reduction potential, there have not yet been major breakthroughs that could be applied in practice. A key tenet of this paper is the need for studies on the influence of plant components on methane production to be performed with standardized samples. Where there are consistent effects, the literature suggests that saponins mitigate methanogenesis mainly by reducing the number of protozoa, condensed tannins both by reducing the number of protozoa and by a direct toxic effect on methanogens, whereas essential oils act mostly by a direct toxic effect on methanogens. However, because the rumen is a complex ecosystem, analysis of the influence of plant components on the populations of methanogens should take into account not only the total population of methanogens but also individual orders or species. Although a number of plants and plant extracts have shown potential in studies in vitro*, these effects must be confirmed* in vivo*.*

Keywords: ruminants, methane, tannins, saponins, essential oils

Implications

This review demonstrates that plant phytochemicals can have important effects on rumen methanogens, either by affecting methanogens directly and/or indirectly by affecting rumen protozoa. Modulation of the rumen microbiota, including methanogens and protozoa, with plant extracts such as saponins, tannins and essential oils has implications in improvement of animal nutrition through decreased dietary energy loss, and in limitation of the negative impact on the environment through mitigation of methane production.

Characteristics of the rumen ecosystem in relation to methanogenesis

The rumen ecosystem is one of the richest microbial environments, inhabited by numerous microorganism species including roughly 10^{11} bacterial cells/ml of rumen fluid, roughly 10^6 protozoal cells/ml of rumen fluid, roughly 10^3 fungal cells/ml of rumen fluid and roughly 10^9 methanogen cells/ml of rumen fluid. Only ~10% of the microbial population of this ecosystem has been identified and described (Pers-Kamczyc *et al.*, 2011), mainly due to the difficulty with maintaining them in *in vitro* culture. According to the literature data, the population of methanogens is very important to rumen functioning. Together with bacteria and fungi, methanogens are the earliest colonizers of the rumen. *Archaea* can be found in the lamb rumen as soon as 30 h after birth (Morvan *et al.*, 1994). The population of these microorganisms in lambs in the 1st week of life was 10^4 cells/g of the rumen contents, whereas in the 3rd week of life it was 10^8 to 10^9 cells/g (Skillman *et al.*, 2004). It is estimated that the methanogen population accounts for ~3% to 5% of rumen microbial biomass. So far 113 species of methanogen have been described, and although there are many more in other ecosystems, only a few have been described in the rumen (Janssen and Kirs, 2008).

Methanogens appearing first in the rumen are of the genus *Methanobrevibacter* (Skillman *et al.*, 2004). In the case of *Methanobacterium*, representatives of the family colonize the rumen environment rapidly, but, in contrast to *Methanobrevibacter*, they disappear rapidly, that is, on the 12 to 19th day after birth (Zhu *et al.*, 2007). According to some literature data, predominant methanogens in the rumen ecosystem of farm animals include microorganisms of the genus *Methanobrevibacter* and *Methanosaricina*, and especially the following species: *Methanobacterium formicicum*, *Methanobrevibacter ruminantium*, *Methanosaricina barkeri*, *Methanosaricina mazei*

† E-mail: adamck@jay.up.poznan.pl

and *Methanomicrobium mobile* (Stewart *et al*., 1997; St-Pierre and Wright, 2012). Other data demonstrated predominance of only *M. ruminantium* (Leahy *et al*., 2010), whereas other data indicated the order *Methanobacteriales* as predominant in the rumen (Jarvis *et al*., 2000). However, the literature is still expanding and newer methanogens are being identified (Wright *et al*., 2008; Zhou *et al*., 2009; King *et al*., 2011; Lee *et al*., 2013).

There are also differences in the methanogen populations associated with rumen solid- and liquid phases. *M. mobile*, *Methanobacterium aarhusense* and *Methanosphaera stadtmanii* are species occurring only as free-living forms in rumen fluid, which account for a small percentage of the general *Archaea* population (Zhu *et al*., 2007). *Archaea* associated with solid phases may be a much larger part of the total population of rumen methanogens (Tajima *et al*., 2001). Zhu *et al.* (2007) named *Methanobrevibacter* spp., *Methanosphaera* spp. and unidentified methanogens as species characteristic for the solid fraction. However, Shin *et al.* (2004) showed that the most numerous group of *Archaea* inhabiting solid particles are the families *Methanomicrobiaceae* and *Methanobacteriaceae*. In the group of microorganisms related to the rumen wall, predominant methanogens include *Methanobrevibacter* spp. and *Methanosphaera* spp. (Zhu *et al*., 2007). A considerable part of the population of methanogens in the rumen are of unknown taxonomy and properties.

Previous research demonstrated that quantitative as well as qualitative variability of microorganisms (including methanogens) in the rumen depends on many factors, such as: animal species, geographical location, feed and also on the use of feed additives that affect rumen fermentation (Szumacher-Strabel *et al*., 2009; Hook *et al*., 2010; Huang *et al*., 2012; Popova *et al*., 2012; Kumar *et al*., 2013).

Analysis of the process of methanogenesis in the rumen requires understanding of interactions between hydrogen producers (bacteria, protozoa, fungi) and consumers (methanogens) in the rumen ecosystem in terms of the possibility of reducing the negative effects (both environmental and economic) of methane production in the rumen. Therefore, growth and development of *Achaea* directly depends on the population of microorganisms, for example, those utilizing cellulose, which leads to an increase in the concentration of hydrogen (substrate for the process of methanogenesis), a by-product of crude fiber hydrolysis (Morvan *et al*., 1996; Wang *et al*., 2011). Syntropic interaction was demonstrated between rumen bacteria and methanogens by Wolin *et al.* (1997). Bacteria provide a substrate for methanogenesis in the form of hydrogen and formic acid, whereas methanogens decrease the concentration of bacterial metabolism products (i.e. in the process of horizontal hydrogen transfer). High amounts of bacterial metabolism products may be harmful to other microorganisms inhabiting the rumen environment. Maintaining low (10^{-6} to 10^{-7} mole/dm^3) concentration of hydrogen favorably influences the development of particular groups of microorganisms that are important for a range of rumen processes (Wolin and Miller, 1988; Ushida and Jouany, 1996). Additionally, the hydrogen concentration in the rumen affects effectiveness of the process of methanogenesis. Acidification to pH 5.5 causes loss of the ability to bind H^+ by *Archaea*, leading to further acidification of the rumen and a reduction in the methanogen population (Russell *et al*., 1988; Van Kessel and Russell, 1996).

Rumen archaea use large amounts of hydrogen for methane production (Hungate, 1967). The simplest reaction eliminating hydrogen from the rumen of ruminants is a combination of H_2 and CO_2 according to the following equation: $CO_2 + 4H_2 = CH_4 + 2H_2O$ (Whitmann *et al*., 1992; Morgavi *et al*., 2010). The theory of hydrogen utilization was confirmed in the study of Demeyer and De Graeve (1991), where hydrogen addition to rumen fluid caused an increase in methane production by 94% with little change in the production of volatile fatty acids (VFA). Methane may also come from other rumen reactions, including the reduction of formic acid, methanol, methylamine, diethylamine, triethylamine (Hungate *et al*., 1970). It is likely that the scale of methane production in the above processes is modest (Wolin *et al*., 1997).

The process of methanogenesis, and thereby the number of methanogens in the rumen has been directly correlated with the protozoal population (Newbold *et al*., 1995). Some *in vitro and in vivo* studies demonstrated that the lack of the protozoal population in the rumen ecosystem has a significant effect on both the population of methanogens and the level of methane production (Cieslak *et al*., 2009a; Morgavi *et al*., 2012). The research also showed that sheep maintained without protozoa for more than 2 years have reduced methanogenesis in comparison with sheep kept without protozoa for only 2 months (Morgavi *et al*., 2012). Similarly, earlier studies also demonstrated that short-term (up to 3 months) defaunation of the sheep rumen causes reduction of methane emission in comparison with the rumen of sheep inhabited by all groups of microorganisms and after long-term defaunation for ca. 1 year (Ranilla *et al*., 2004). The results from other study emphasize the need to consider the different methanogen communities (free-living *v.* protozoa-associated methanogen) when developing strategies for mitigating methane emissions in ruminants (Tymensen *et al*., 2012). Further, it is important to recognize that different archaeal phylotypes are associated with specific groups of protozoa (Ohene-Adjei *et al*., 2007). A number of experiments have shown that the methanogens living in close symbiosis with rumen protozoa include: *M. formicicum*, *M. ruminantium*, *M. barkeri*, *M. mazei* and *M. mobile* (Stewart *et al*., 1997). Regensbogenova *et al.* (2004) demonstrated that *M. mobile* occurs mainly in single-species cultures of *Metadinium medium*, *Entodinium furca monolobum* and *Diplodinium dentatum*, while Kamra (2005) named four species of rumen ciliates of the group *Entodiniomorpha*, which are in close symbiosis with the methanogens: *Entodinium elonginucleatum*, *Entodinium bursa*, *Eudiplodinium maggii* and *Eremoplastron bovis*. Other researchers (Finlay *et al*., 1994; Kisidayova *et al*., 2000; Cieslak *et al*., 2006) added to the list *Dasytricha ruminantium*, *Entodinium caudatum*, *E. f. monolobum*,

D. dentatum, *Eremoplastron dilobum*, *Epidinium ecaudatum* and *Ophryoscolex caudatus*. It is presumed that the species preferences of the methanogens result from differences in the protozoal cell surface (Vogels *et al.*, 1980). However, literature data are ambiguous mainly because of the difficulty of culturing methanogens *in vitro*.

This review focuses on the effects of plant components on rumen methanogens and an important first step in understanding responses, and variation in responses, is to consider the structure, biochemistry and chemical analysis of these compounds.

Standardization of plant material and extract for biological activity studies

Saponin sources

One of the most commonly researched plant materials for anti-methanogenic activity is *Yucca schidigera*. It was approved by Food and Drug Administration as a food and feed additive with the 'GRAS' label (Generally Recognized as Safe). Two different product of yucca are available on market, that is, yucca powder (YP) and yucca extract (YE). *Y. schidigera* contains as much as 10% of steroidal saponins on a dry matter (DM) basis, which makes this plant one of the richest commercial source of saponins. YP, which is just powdered yucca log also contains 10% saponins. YE is prepared by squeezing the juice present in yucca logs, followed by the condensation to produce a syrup, and may contain 15% to 19% of steroidal saponins. Acid-hydrolyzed fractions of these saponins contain both furostanol and spirostanol aglycones. These include sarsapogenin, markogenin, smilagenin, samogenin, gitogenin and neogitogenin (Kaneda *et al.*, 1987). In the plant, these can be found in a multi-component mixture of glycosides (Tanaka *et al.*, 2000; Oleszek *et al.*, 2001, Kowalczyk *et al.*, 2011). They can be found both as monodesmosides with one sugar chain attached at the 3-O- and bidesmosides with two sugar chains at the 3-O- and 26-O- positions. Tanaka *et al.* (2000) identified as many as 13 structurally different monodesmosidic saponins, giving them trivial names from YS-I to YS-XIII.

In the work of Oleszek *et al.* (2001) eight individual saponins were isolated and identified out of which five were known spirostanol and three new furostanol structures. However, monodesmosides made up about 93% of the total saponins present. Recent LC-MS analysis showed that there are substantial qualitative and quantitative differences in the concentration of monodesmoside and bidesmoside saponin components between YP and YE; YE contains predominantly monodesmosides, whereas YP has higher amount of bidesmosidic forms. The bidesmosides in YP result from the presence of yucca bark (Kowalczyk *et al.*, 2011).

The effects of *Y. schidigera* have been historically attributed to its saponin content. However, experiments performed with rats showed effects of both saponin-containing and non-saponin-containing fractions (Duffy *et al.*, 2001). Several mechanisms have been proposed for the mode of action of *Y. schidigera* extract, but none has been conclusively proven.

The above discussion shows the difficulty of standardizing batches of yucca product, and also the potential for large variation between batches. Typical quality control procedures for the yucca products involve acid hydrolysis of the butanol phase followed by GC-FID analysis of resulting sapogenins. No information is given on glycosidic composition. In fact, glycosidic composition rather than aglycone content determines the biological activity of these compounds.

Additional difficulties in interpretation of experimental data when YE is used relate to the composition of the rest of the product. Even if concentration of steroidal saponins is 20% of the sample, the other 80% still remains unknown. A high proportion of YE is made up of polysaccharides of unknown composition and their effects on extract activity is not known.

Similar problems arise with other saponin sources, for example, quillaya saponins, the second largest commercial source of triterpene saponins. The term quillaya saponins covers the multicomponent mixture of individual glycosides of high polarity and complex structural diversity. Most of the published work on this plant refers to one of the best known component (QS-21) of acylated bidesmosidic triterpene saponin. No analytical protocols are available to standardize these saponins and not much is known about their stability under different extraction/purification conditions. Usually acylation is very sensitive to temperature, pH or other extraction/purification treatments. Thus, the development of analytical protocols for standardization of quillaya saponins is essential.

Tannins and proanthocyanidins

Tannins are a complex mixtures of individual compounds having molecular weights ranging from 500 to over 3000 (gallic acid esters) and up to 20 000 (proanthocyanidins). Their standardization before *in vitro* or *in vivo* trials represents a unique analytical problem. They are usually standardized using a spectrophotometric technique with Folin–Ciocalteu reagent, with results expressed as gallic acid equivalents. In fact this method is not specific, measuring total reducing capacity. There is a need for more specific analytical approaches, such as HPLC for procyanidins and GCMS for proanthocyanidins.

Essential oils

Widely occurring in plants and animals, the essential oils may consist of volatile constituents of terpenoid or non-terpenoid origin. Under this group, hundreds of large or small molecules can be present, consisting of hydrocarbons and their oxygenated derivatives. Some of these compounds may additionally contain nitrogen or sulfur. They may exist in the form of alcohols, acids, esters, epoxides, aldehydes, ketones, amines and sulfides but in some cases halogenated compounds are also found. The composition of essential oils is usually characteristic for the particular plant species and responsible for its fragrance. Each of the compounds may have different effects on organisms causing damage to the plant tissue (e.g. herbivorous insects or microorganisms).

As with other groups of secondary metabolites, the level and composition of essential oils change depending on the variety, environmental factors, time of harvest, etc. A further source of variation in commercially available essential oils is differences in the technology used for their isolation, as well as storage. The most effective analytical method for essential oils remains GS-MS. More advanced techniques, such as GC/FT-IR and NMR, are required for identification of new components of essential oils.

Effect of plant components on rumen methanogens

Plant components (e.g. saponins, tannins, essentials oils) may affect methanogenesis by inhibiting growth, development and activity of the population of methanogens both indirectly (by reducing the number of protozoa associated with methanogens) and directly, by affecting methanogens. Moreover, plant components may also case a shift toward propionate production, which affects methanogenesis through reduced competition for hydrogen.

Saponins

Although saponins have reduced the amount of enteric methane production by up to 50% in some studies (Szumacher-Strabel and Cieslak, 2010; Patra and Saxena, 2010; Bodas *et al*., 2012), these effects need to be confirmed in more *in vivo* studies. There is some ambiguity in the literature concerning the mechanism of saponins action to reduce methanogens and methanogenesis. According to Guo *et al.* (2008), mitigation of methanogenesis using tea saponins results from decreased activity of the *mcrA* gene (an indicator of the methanogenic activity of the methanogen population), without changing the total methanogen numbers. This effect was noted when using a mixed rumen culture for *in vitro* studies, but not when pure cultures of *M. ruminantium* were tested. Other researchers used 3 g/day of tea saponins in sheep diets and concluded that there was no effect on the populations of methanogens (Mao *et al*., 2010; Zhou *et al*., 2011). Earlier *in vitro* research had suggested mitigation of the process of methanogenesis without reduction in the number of methanogens with the use of saponins from *Sapindus saponaria* or tea saponins (Hess *et al*., 2003; Hu *et al*., 2005). It seems likely that the reduction in methanogenesis was related to a reduction in *Archaea* associated with protozoa as a result of partial defaunation. A reduction in the protozoal population decreases the amount of hydrogen available in the rumen for the process of methanogenesis (Szumacher-Strabel and Cieslak, 2010).

Saponins, due to their structure (hydrophobic sapogenin, i.e. aglycone, and a hydrophilic sugar part – glycone, which may comprise glucose, arabinose, xylose, galactose) can interact with cholesterol present in eukaryotic cell membranes and thereby cause destruction of this cell type (Cheeke, 1996; Wina *et al*., 2005). This may explain also a lack of direct saponin effect on methanogen cells. Wina *et al.* (2005) found that lower doses (1 mg/ml) of methanol extract of *Sapindus rarak* containing saponins, used in *in vitro* research, did not reduce the concentration of methanogen RNA. The reduction was observed only for higher concentrations (4 mg/ml). Similar conclusions were drawn by other authors (Staerfl *et al*., 2010; Wang *et al*., 2011; Zmora *et al*., 2012b and 2012c). Moreover, Bodas *et al.* (2012) demonstrated that low saponin concentrations indirectly influence methane production in the rumen by reducing the number of protozoa, whereas higher saponin concentrations have direct negative effect on methanogens.

As the rumen is a highly dynamic ecosystem, examination of the influence of phytofactors on microorganisms involved in the process of methanogenesis should take into account factors that can deactivate biological properties of saponins, including degradation, hydrolysis, deglycosylation, detoxication of saponins (Miles *et al*., 1992; Makkar and Becker 1997; Odenyo *et al*., 1997; Wang *et al*., 1998; Teferedegne *et al*., 1999). According to other authors, the duration of saponin administration and the ratio of forage to concentrate may have a significant influence on their effect (Newbold *et al*., 1997, Teferedegne *et al*., 1999; Goel *et al*., 2008).

Tannins

Research on the effects of tannins on the populations of methanogens has been carried out both under *in vitro* and *in vivo* conditions, however, results were not always unequivocal (Tavendale *et al*., 2005; Bhatta *et al*., 2009; Szumacher-Strabel *et al*., 2011; Cieslak *et al*., 2012). The range of mitigation of methane production by using tannins is quite broad, from 2% to 58% in comparison with analyzed control groups (Patra and Saxena, 2010a; Bodas *et al*., 2012), and factors responsible for the mitigation are various, for example, tannin type, plant source. Tavendale *et al.* (2005) suggested that inhibition of methanogen growth is due to the bacteriostatic and bactericidal effects of condensed tannins (CT). The study demonstrated deactivation of methanogens (*M. ruminantium*), linked to reduction of produced methane (Tavendale *et al*., 2005). Similar conclusions were drawn by Pellikaan *et al.* (2011).

Other researchers analyzed six commercial sources of tannins containing hydrolysable tannins (HT) or HT and CT, and showed greater potential for mitigation of methanogenesis after using a mixture of HT and CT (Bhatta *et al*., 2009). The mixture of HT and CT suppresses methanogenesis by reducing the methanogenic populations in the rumen either directly or by reducing the protozoal population, thereby reducing methanogens symbiotically associated with the protozoal population (Bhatta *et al*., 2009). However, Goel and Makkar (2012) showed that HT led to a larger reduction in the population of methanogens or microorganisms providing them with H_2 than CT. Other authors noted not only a direct tannin effect on methanogens but also a direct influence on protozoa associated with them (Patra and Saxena, 2009). Research with dairy cattle showed that addition of CTs from *Vaccinium vitis idaea* at 2 g/kg dietary DM caused mitigation of methanogenesis mainly resulting from a reduction in protozoal numbers without a negative effect on the digestibility of organic matter and VFA production (Cieslak *et al*., 2012).

CT extracted from *Leucaena leucocephala* caused a linear reduction in total methanogens (up to 99%) and total protozoa (up to 83%) with increasing levels (from 20 to 60 mg/g DM of substrate) of CT in an *in vitro* study (Tan *et al.*, 2011). However, the reduction in protozoal population was not always proportionally related to the decrease in methanogen population. Another study also confirmed that tannin effects on the protozoal population are varied, probably because some tannins have a direct effect on methanogens, which are not associated with protozoa (Bhatta *et al.*, 2012). Other researchers have demonstrated a decline in the methanogen population associated with protozoa, for example, species belonging to *Methanobacteriaceae*, and simultaneous increase in the number of free-living *Methanobacteriales*, after inhibition of protozoa (Goel and Makkar, 2012). A reduction in the number of protozoa is not always accompanied by a reduction in the number of methanogens. Limitation of the population of one methanogen may cause an increase in the populations of the others. As phytofactors may increase populations of some microorganisms by decreasing others, the analysis of rumen microorganisms should include also the quantitative and qualitative analysis of other organisms in the rumen (Zmora *et al.*, 2012a).

Interpretation of results with extracts containing one bioactive phytofactor, even in a predominant concentration, is difficult because of the complexity of structures, as well as the possibility of interactions between individual bioactive extract components or bioactive extract components and feed components. Soltan *et al.* (2012) also suggested that the potential methanogenic properties of feed containing tannins may be related not only to the tannin content, but also to other factors. Another cause of lack of the effect of feed additives containing tannins on the number of methanogens in rumen fluid under *in vitro* conditions may be the use of too low concentrations of tannins in the supplement (Szumacher-Strabel *et al.*, 2011). Jayanegara *et al.* (2012) stressed that methane declined when dietary tannins increased, however, when the amount of tannin (in the batch culture system) is too high (more than 100 g tannin/kg DM) the accuracy of estimates of the impact of tannins on methanogenesis decreased.

Evaluation of the effects of tannins on rumen methanogenesis should also include other responses whose modulation may indirectly cause mitigation. For example, the use of extracts or feed containing considerable amount of tannins may limit feed intake or reduces digestibility of organic matter, and therefore decreases the amount of methane produced (e.g. Carulla *et al.*, 2005).

There is scarce information about the direct effect of tannins on methanogens. Smith *et al.* (2005) demonstrated a number of mechanisms improving tolerance of bacteria to the unfavorable environment produced by tannins. These include modification of the cell membrane, secretion of a protective exo-polysaccharide layer around the cells, and degradation/modification of tannins.

Essential oils

Essential oils are mixtures of terpenoids, low-molecular-weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters, and/or lactones (Dorman and Deans, 2000), and are usually extracted from plant material by water or aqueous alcohol steam distillation. Antimicrobial effects of essential oils are manifested in their high affinity for microbial cell membranes (Jouany and Morgavi, 2007). However, the effect of individual essential oils depends on their structure, which results from chemical composition and type of functional group, for example, terpenoids or phenols. They cause disturbances of ion transport (electrons) through the cell membrane, mitigate protein translocation, phosphorylation and enzyme-dependent reactions taking place in the membrane (Jouany and Morgavi, 2007). Essential oils affect individual groups of microorganisms differently because of differences in the structure of the cell membrane, for example, between bacteria and methanogens.

In some studies, essential oils stimulated some protozoa species, for example, *Isotricha* spp. or *Dasytricha* spp. and this led to an increase in associated *Methanobrevibacter smithii*. However, in many studies authors showed decreased rumen methane production in response to essential oil supplements, without alterating protozoa population (Evans and Martin, 2000; Busquet *et al.*, 2005). The direct influence of essential oils on methanogen cells may be related to the structure and properties of the oil used or secondary plant metabolites contained therein. In an experiment carried out by Busquet *et al.* (2005), garlic oil (*Allium sativum*) was used as the supplement. In contrast to the essential oils that are active only against Gram-positive bacteria, garlic oil is active against Gram-negative and Gram-positive bacteria, fungi, viruses and parasites, and the main mechanism of action is related to the ability to react with –SH groups (O'Ghara *et al.*, 2000). Similar results of a decrease in total methanogens were obtained by Kongmun *et al.* (2011) after using the addition of 7% coconut oil with 100 g/day of garlic powder in rumen fistulated swamp buffalo bulls. In their study, there was no relationship between the protozoal population and the methanogen population, probably because methanogens are found in a wide range of environments in the rumen: (i) free in the rumen fluid; (ii) attached to particulate material and rumen protozoa and (iii) attached to the rumen epithelium (Janssen and Kirs, 2008). According to Goel *et al.* (2009), decreases in methanogen populations can be correlated with decreased bacteria populations, for example *Ruminococci* that produce significant amounts of hydrogen. However, these effects may depend on the concentration of the specific treatment factor used and, according to McIntosh *et al.* (2003), ruminal methanogens seem to be affected only at high concentrations of essential oils. In their experiment, EO blend (containing thymol, eugenol, vanillin and limonene) of the concentration up to 160 ppm did not alter the *M. smithii* population, which was inhibited only at a concentration of 1000 ppm.

Essential oils can modulate either the rumen methanogen population or methanogen activity and they do not always influence the total number of methanogens inhabiting the rumen, instead affecting the distribution among methanogen species. This thesis is confirmed by the results of Ohene-Adjei *et al.* (2008), where an increase in the diversity

Table 1 *Effects of saponin sources on methane production, methanogen and protozoa populations in the rumen*

Reference	Saponin sources	Diet/substrate	Test system/dosage	Methane	Methanogen	Protozoa
Narvaez *et al.* (2013)	*Yucca schidigera* extract (153 mg smilagenin equivalent per g DM of steroidal sapogenins)	Total mixed ration (forage/concentrate total mixed ration barley silage–barley grain) 65 : 35	Serum bottle/650 μg per ml	−15%	−52%	NA
Li and Powers (2012)	Yucca saponin (8.5% saponin) (YS)	Total mixed ration (C) (concentrate/forage) 54 : 46	Holstein steers/		NA	NA
	Quillaja saponin (3.6% saponin) (QS)		Exp1/			
			C + 0.64% YS	−15%		
	Tea saponin (21.6% saponin) (TS)		C + 1.5% QS	−9%		
			Exp2/			
			C + 0.25 TS	NE		
			Exp3/			
			C + 1.5 QS	+5%		
			C + 0.5 TS	−18%		
			C + 1.5% YS	NE		
Zhou *et al.* (2011)	Tea saponin (60% triterpenoid saponins)	Chinese wild rye/concentrate 60 : 40	Refaunated or defaunated sheep/ 3 g per day	−11% refaunated, −18% defaunated	NE refaunated, NE defaunated	−43% refaunated, −100% defaunated
Wang *et al.* (2011)	Gynosaponins powder (98% gynosaponins)	Rice straw 100	Batch culture/100 and 200 mg/l	−73%, −89%	−42%, −73%	NA
Mao *et al.* (2010)	Tea saponins (60% saponins)	Wild rye/concentrate 60 : 40	Growing lambs/3 g per day	−27%	NE	−41%
Holtshausen *et al.* (2009)	*Yucca schidigera* (6% saponin)	Barley silage/concentrate 51 : 49	Batch culture/15, 30 and 45 mg/g substrate	−8%, −15%, −26%	NA	NA
	Quillaja saponaria (6% saponin)	Barley silage/concentrate 51 : 49	Dairy cows/10 g per kg of DM	NE, −11%, −12%		
	Yucca schidigera (6% saponin)			NE		
	Quillaja saponaria (6% saponin)			NE		
Wang *et al.* (2009)	*Yucca schidigera* extract (Desert King International Product, United States of America)	Hay/concentrate 75 : 35	Sheep/170 mg per day	−15%	NA	NA
Guo *et al.* (2008)	Tea saponin (60% triterpenoid saponin)	Grass meal/corn meal 50 : 50	Serum bottle/0.4 mg per ml	−8%	NE	−50%
Pen *et al.* (2008)	*Quillaja saponaria* extract (QSE) (5% to 7% saponins)	Oat hay/concentrate 50 : 50	Continuous culture fermentation vessels/ 2 and 4 ml(QSE)/l	NE	NA	−62%, −75%
	Yucca schidigera extract (YSE) (8% to 10% saponins)		Continuous culture fermentation vessels/ QSE : YSE, 2 : 0 ml/l, 2 : 2 ml/l, and 2 : 4 ml/l	−14%, −16%, NE		−50%, −75%, −75%
Goel *et al.* (2008)	*Sesbania sesban* leaves extract (63.5% saponins)	Hay/concentrate 50 : 50	HGT/10.9 and 21.2 mg	NE	−78%	−14%, −36%
	Knautia arvensis leaves extract (82.4% saponins)		HGT/3.88 and 7.76 mg		−21%	−14%, −25%
	Trigonella foenum-graecum seeds extract (34.5% saponins)		HGT/5.62 and 11.54 mg		−22%	−15%, −39%
Pen *et al.* (2007)	*Yucca schidigera* extract	Italian ryegrass hay/concentrate 60 : 40	Sheep/1.31 to 1.64 g saponin/day	NE	NA	NE
	Quillaja saponaria		Sheep/0.8 to 1.3 g saponin/day			
Pen *et al.* (2006)	*Yucca schidigera* Extract (80–100 g/kg saponins)	Oat hay/concentrate 50 : 50	Serum bottle/2, 4 and 6 ml/l	−17%, −29%, −42%	NA	−29%, −55%, −56%
	Quillaja saponaria (50–70 g/kg saponins)			NE		−34%, −41%, −40%
Hu *et al.* (2005)	Tea saponins (60% saponins)	Grass meal/corn meal 50 : 50	HGT/0.2 and 0.4 mg/ml	−13%, −16%	NA	−13%, −16%

DM = dry matter; HGT = hohenheim gas test system; NE = no effect; NA = not analyzed; –decrease; +increase.

Table 2 *Effects of tannin sources on methane production, methanogen and protozoa populations in the rumen*

Reference	Tannin sources	Diet/substrate	Test system/dosage	Methane	Methanogen	Protozoa
Hassanat and Benchaar (2013)	*Acacia mearnsii* extract (82% CT)	Total mixed ration (forage/concentrate) 65 : 35	Serum bottle/4, 10, 20, 30 and 40 mg	NE, −12%, −21%, −32%, −38%	NA	NA
	Schinopsis balansae extract (90.4% CT)			NE, NE, −23%, −34%, −40%		
	Castanea sativa extract (5.7% CT and 75.5% HT)			NE, −13%, −23%, −31%, −40%		
	Quercus aegilops extract 8.0% CT and 71.2% HT)			NE, −11%, −19%, −26%, −36%		
Soltan *et al.* (2012)	*Acasia saligna* leaves (6.3% CT)	*Acasia saligna* 100	Serum bottle/500 mg	−38%	NA	−9%
	Laucaena leucocephala leaves (4.6% CT)	Laucaena leucocephala 100		−36%		−23%
	Prosopis juliflora leaves (0.04% CT)	Prosopis juliflora 100		NE		34%
	Atriplex halimus leaves (0.02% CT)	*Atriplex halimus* 100		NE		10%
Niderkorn *et al.* (2011)	*Onobrychis viciifolia* sainfoin (1.52% CT)	*Onobrychis viciifolia*/600 mg	Serum bottle/600 mg/3.5 h	NE	NA	NA
	Cocksfoot/sainfoin 50 : 50	Cocksfoot plus sainfoin/600 mg		NE		
	Ryegrass/sainfoin 50 : 50	Ryegrass plus sainfoin/600 mg		NE		
	Onobrychis viciifolia sainfoin (1.52% CT)	*Onobrychis viciifolia*/600 mg	Serum bottle/600 mg/24 h	NE		
	Cocksfoot/sainfoin 50 : 50	Cocksfoot plus sainfoin/600 mg		+8%		
	Ryegrass/sainfoin 50 : 50	Ryegrass plus sainfoin/600 mg		NE		
Tan *et al.* (2011)	*Leucaena leucocephala* extracts (100% CT)	Guinea grass 100	HGT/10, 15, 20, 25 and 30 mg	−33%, −47%, −57%, −59%, −63%	−25%, NE, −99%, −94%, −95%	−86%, −83%, −62%, −55%, −55%
Hariadi and Santoso (2010)	*Acacia mangium* (5.4% tannins)	Elephant grass/ *Accacia mangium* 80:20	Glass syringes/60 mg	−29%	NA	NE
	Biophytum petersianum (4.3% tannins)	Elephant grass/*Biophytum petersianum* 80 : 20		−25%		NE
	Psidium guajava (3.5% tannins)	Elephant grass/*Psidium guajava* 80 : 20		−18%		NE
	Phaleria papuana (3.1% tannins)	Elephant grass/*Phaleria papuana* 80:20		NE		−31%
	Persea americana (2.4% tannins)	Elephant grass/*Persea americana* 80 : 20		NE		NE
	Sesbania grandiflora (1.9% tannins)	Elephant grass/*Sesbania grandiflora* 80 : 20		NE		−55%
Grainger *et al.* (2009)	*Acacia mearnsii* extracts (603 g CT/kg DM)	Grazing ryegrass/cracked triticale grain 89 : 11	Dairy cows/0.9 or 1.8% DMI	−14%, −29%	NA	NA
Bhatta *et al.* (2009)	Mimosa tannins (7.78% HT plus 1.5% CT)	Timothy hay/concentrate 65 : 35	HGT/5, 10, 15, 20 and 25% tannin-containing samples of the basal diet DMI	−11%, −14%, −27%, −32%, −41%	NE	NE, −27%, +26%, −15%, −9%
	Quebracho tannin (7.62% HT plus 3.67% CT)			NE, −31%, −37%, −45%, −45%	−20%, −27%, −27%, −27%, −35%	−4%, −16%, −32%, −28%, −55%
	Quebracho tannin (3.94% HT plus 1.33% CT)			−13%, −23%, −26%, −31%, −38%	NE, −34%, −26%, −22%, −30%	−8%, −20%, −34%, −33%, −34%
Ramirez-Restrepo *et al.* (2010)	*Salix* spp. (34% CT)	Ryegrass/white clover	Sheep (5th or 11th weeks)/grazed willow (*Salix* spp.) fodder blocks 12 g CT/kg DMI	−19% (5th week) NE (11th week)	NA	NA
Animut *et al.* (2008a)	*Lespedeza striata* forage (151 g CT/kg DM)	*Lespedeza striata*/ *Sorghum bicolor* (33 : 67, 67 : 33, 100)	Goats/200, 447 and 613 g/day	−33%, −47%, −58%	NA	−42%, −56%, −69%
Animut *et al.* (2008b)	*Lespedeza striata* forage (140 g CT/kg DM)	*Lespedeza striata*/*Lespedeza cuneata* 100 : 0, 50 : 50, 0 : 100	Goats/720, 719 and 745 g/day	−49%, −54%, −51%	NA	−34%, −22%, −51%
	Lespedeza cuneata forage (151 g CT/kg DM)					
Beauchemin *et al.* (2007)	Quebracho tannins (91% CT)	Barley silage/concentrate 70 : 30	Beef cattle/1 or 2% of DMI	NE	NA	NA
Zeleke *et al.* (2006)	*Acacia angustissima* 459	*Brachiaria humidicola* grass/*Acacia angustissima 20 : 80*	Rusitec/2.8 g DM/day	−12%	NA	NA
	Sesbania sesban 10865	Brachiaria humidicola grass/*Sesbania sesban* 20 : 80		−37%		

CT = condensed tannins; HT = hydrolysable tannins; DM = dry matter; DMI = dry matter intake; HGT = hohenheim gas test system; NE = no effect; NA = not analyzed; –decrease; +increase.

Table 3 *Effects of essential oil sources on methane production, methanogen and protozoa populations in the rumen*

Reference	Essential oil source	Diet/substrate	Test system/dosage	Methane	Methanogens	Protozoa
Lin *et al.* (2012a)	Combination of essential oil (809 g/kg eugenol in thyme oil; 837 g/kg carvacrol in oregano oil; 855 g/kg cinnamaldehyde in cinnamon oil; 801 g/kg limonene in lemon oil) plus monosodium fumarate	Ground maize/ground *Leymus chinensis* hay 50 : 50	Serum bottles/500 mg/l of essential oil plus 0, 5, 10 and 15 mM monosodium fumarate	−51%, −63%, −80%, −56%	−38%, −48%, −41%, −48%	−95%, −95%, −94%, −94%
Lin *et al.* (2012b)	Combination of essential oil (eugenol, carvacrol, citral, cinnamaldehyde; purity >99%) plus monosodium fumarate	Ground corn kernels/ ground *Leymus chinensis* hay 50 : 50	IVGPS (24 h)/200 mg/l essential oil plus 0, 5, 10 and 15 mM of monosodium fumarate	−31%, −76%, −84%, −65%	−23%, −16%, −34%, −16%	−88%, −85%, −88%, −82%
Manh *et al.* (2012)	Eucalyptus leaf meal powder	Concentrate 0.5% of BW/rice straw ad libitum	Dairy cows/100 and 200 g/day)	−16%, −26%	NA	NE, −22%
Chaves *et al.* (2012)	Cinnamon leaf (eugenol; 0.76 v/v)	Barley silage	Serum vials (6, 12 and 24 h)/37.5, 75 and 120 mg/kg silage DM	6 h: −47%, −29%, NE; 12 h: NE; 24 h: NE	NA	NA
	Oregano (carvacrol, thymol; >0.6 v/v)			6 h: NE, −24%, −24%; 12 h: NE24 h: NE		
	Sweet orange (limonene; >0.95 v/v)			6 h: NE; 12 h: NE; 24 h: NE		
Patra and Yu (2012)	Clove oil	Ground alfalfa hay/concentrate 50 : 50	Serum bottles/0.25, 0.50 and 1.0 g/l Fermentation medium	−11%, −17%, −34%	−6%, −3%, −12%	+2%, −6%, −27%
	Eucalyptus oil			−26%, −8%, −17%	−1%, −0.4%, −6%	+1%, −2%, −7%
	Garlic oil			−22%, −28%, −42%	−8%, −14%, −16%	−0.2%, +3%, −7%
	Origanum oil			−12%, −38%, −86%	−15%, −20%, −38%	−11%, −32%, −35%
	Peppermint oil			−8%, −20%, −25%	−15%, −20%, −20%	−1%, −11%, −33%
Sallam *et al.* (2011)	*Achillea santolina* (16-dimethyl 15-cyclooactdaiene; 60.5%)	Concentrate/forage 50 : 50	IVGPS (24h)/25, 50 and 75 μl/75 ml rumen fluid	+37%, +56%, −30%	NA	−9%, −5%, −45%
	Artemisia judaica (piperitone and camphor; 49.1% and 34.5%)			+46%, +43%, −4%		NE
	Mentha microphylla (piperitone oxide and *cis*-piperitone oxide; 46.7% and 28%)			−92%, −100%, −100%		−12%, −21%, −49%
	Schinus terebinthifolius (γ-muurolene and α-thujene; 45.3% and 16.0%)			NE		NE, −21%, −41%
Araujo *et al.* (2011)	Carvacrol (2-methyl-5-isopropyl-1-phenol) Eugenol (2-methoxy-4-(2-propenyl)-phenol) 1,8-cineol (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane)	Concentrate/forage 80 : 20	Serum bottles/50 mg/l	−95%, −67%, −13%	NA	NA
Patra *et al.* (2010)	*Foeniculum vulgare* seed extracts (ethanol and methanol)	Wheat straw/concentrate 50 : 50	HGT (24 h)/ethanol and methanol extracts of 0.5 ml/30 ml	−39%, −71%	NA	−41%, −24%
	Syzygium aromaticum flower bud extracts (ethanol and methanol)			−47%, −86%		−43%, −48%
Sallam and Abdelgaleil (2010)	Citrus essential oil (DL-limonene and γ-terpinene; 83.9% and 10.75%)	Roughage/concentrate 50 : 50	SAGPT (24 h)/25, 50 and 75 μl/75 ml rumen fluid	NE, −16%, −33%	NA	NE, −35%, −35%
	Limonene (100%)			+14%, −20%, −10%	NA	−47%, −48%, −40%
Wang *et al.* (2009)	Oregano extract *Ropadiar*	Hay/concentrate 75 : 25	Sheep/250 mg per day	−12%	NA	NA
Sallam *et al.* (2009)	*Eucalyptus oil* eucalyptol (1,8-cineole)	Roughage/concentrate 50 : 50	IVGPS (24 h)/25, 50, 100 and 150 μl/75 ml	−26.5%, −47%, −77%, −85%	NA	−23%, −36%, −59%, −64%
Agarwal *et al.* (2009)	Peppermint oils–*Mentha piperita*	Wheat straw/concentrate mixture 50 : 50	HGT (24 h)/0.33, 1.0 and 2.0 μl/ml	NA	+105%, −82%, −80%	−30%, −78%, −88%
Soliva *et al.* (2008)	Pine oil–*Pinus mugos* (monoterpene hydrocarbons α-pinen, β-pinen, limonellen, ω3-caren, β-phellandren with proportions of 22%, 13%, 12%, 25% and 14%)	Mixed forage/concentrate 50 : 50	Rusitec(10 days)/0.008 g DM/day	NE	NA	−8%

Table 3 *Continued*

Reference	Essential oil source	Diet/substrate	Test system/dosage	Methane	Methanogens	Protozoa
Macheboeuf *et al.* (2008)	Carvacrol (2-methyl-5-isopropyl-1-phenol; >980 g/kg)	Corn/soybean/hay 45 : 30 : 25	Batch (16 h)/1.5, 2, 3 and 5 mM	−13%, −32%, −85%, −98%	NA	NA
	Cinnamaldehyde ((*E*)-3-phenyl-2-propenal; >980 g/kg)		Batch (16 h)/1, 2, 3 and 5 mM	NE, −13%, −19%, −94%		
	Thymol (5- methyl-2-isopropyll-phenol; >980 g/kg)		Batch (16 h)/1, 2, 3 and 6 mM	NE, −32%, −84%, −99%		
	Anethum graveolens (400 g/kg carvone, 320 g/kg limonene)		Batch (16 h)/2.5, 5, 10 and 25 mM	NE, NE, −47%, −76%		
	Cinnamomum verum (790 g/kg cinnamaldehyde)		Batch (16 h)/1, 3, 5 and 10 mM	NE, −26%, −98%, −98%		
	Thymus vulgaris (470 g/kg thymol, 200 g/kg terpinene, 200 g/kg p-cymene)		Batch (16 h)/0.5, 1, 2 and 3 mM	NE, NE, −62%, −94%		
	Origanum vulgare (890 g/kg carvacrol, 50 g/kg thymol)		Batch (16 h)/1, 2, 3 and 5 mM	NE, NE, −63%, −97%		
	Origanum vulgare thymol chemotype (210 g/kg carvacrol, 350 g/kg thymol)		Batch (16 h)/0.5, 1, 2 and 3 mM	NE, NE, −60%, −95%		

HGT = hohenheim gas test, IVGPS = *in vitro* gas production system; DM = dry matter; SAGPT = semi-automatic gas production technique; NE = no effect; NA = not analyzed; − decrease; + increase.

of methanogenic *Archaea* (*Methanosphaera stadtmanae*, *M. smithii* and some uncultured groups) was observed in response to treatment with cinnamaldehyde, garlic and juniper berry oil, without alteration of the total rumen methanogenic capacity.

Therefore, inhibition of the process of methane production in the rumen is not always accompanied by changes in the diversity of methanogens or a decrease in their population. Twofold increase in the number of rumen methanogens resulting from using the addition of 0.33 μl peppermint oil per ml of incubation medium *in vitro* was followed by 20% decrease in methane production, while using higher concentrations of peppermint oil (1 or 2 μl/ml) decreased methanogen populations (on average by 82%) and methanogenesis by 61% (Agarwal *et al.*, 2009). A similar effect was observed in an *in vitro* experiment where limonene, the main component of fir oil (*Abies alba*), at 40 or 400 mg/l (Cieslak *et al.*, 2009b) was used. The addition of limonene in greater amounts caused reduction of the population of methanogens (on average by 25%) with simultaneous mitigation of the process of methanogenesis (on average by 28%; Cieslak *et al.*, 2009b). The lower level had no effect on methanogen numbers or methane production. This confirmed earlier observations that medium and high concentrations of essential oils can affect the number of methanogens (Cieslak *et al.*, 2009b). The authors also do not exclude that the addition of limonene, indirectly reducing the number of microorganisms providing the substrate for methanogens in the form of H_2 and CO_2, can reduce the amount of methane produced in the rumen.

The possible mechanism of action of essential oils on methane production in the rumen may be the result of direct inhibition of methanogens (Calsamiglia *et al.*, 2007). Essential oils may affect the unique methanogen cell membrane structure (isoprenoid unit) that leads to cell destruction. Studies with essential oils have not considered the possibility that methanogen species may change in response to treatments as an adaptive response. This would complicate targeted efforts to minimize the methane production (Ohene-Adjei *et al.*, 2008). In addition to studies of effects of essential oils on methanogens, a number of other studies have made indirect observations based on measurements of VFA production and VFA proportions (Szumacher-Strabel and Cieslak, 2010; Vasta and Bessa, 2012).

Several studies have investigated effects of saponins, tannins and essential oils on rumen fermentation, including methane production, but only a few have specifically determined their effects on methanogen population. The most recent results are presented in Table 1 for saponins, Table 2 for tannins and Table 3 for essential oils.

Conclusions

Studies of the influence of plant bioactive components (phytochemicals) on methane control in ruminants should be performed using as far as possible standardized samples. Natural products like saponins, tannins or essential oils occur

in the plant material as multicomponent mixtures. The composition of the mixture and quantitative relations of individual compounds may change under different circumstances. The plant variety, time of harvest, climate, water availability or even sample treatment after plant harvest may influence qualitative/quantitative relations of the sample.

We can generally conclude that the saponins mitigate methanogenesis mainly by reducing the number of protozoa; CTs act both by reducing the number of protozoa and by a direct toxic effect on methanogens, whereas essential oils act mostly by a direct toxic effect on methanogens. However, because the rumen is the complex ecosystem, analysis of influence of plant components on the populations of methanogens should take into account not only the total population of methanogens but also individual orders or species. Most of tested plant and plant components have presented their antimicrobial activity in *in vitro* research when tested at high doses. The limited scientific information available from long-term *in vivo* trials suggested that benefits associated with bioactive components *in vitro* are not always obtained *in vivo* or are diminished over time due, for example, microbial adaptation. Hence, there is an urgent need to establish a clear definition of the optimal active dose of plant components that can be used as additives for ruminants.

Acknowledgements

The following manuscript was supported in part by the Polish Ministry of Science and Higher Education, Grant no. N N311 476339.

This paper was published as part of a supplement to *animal*, publication of which was supported by the Greenhouse Gases & Animal Agriculture Conference 2013. The papers included in this supplement were invited by the Guest Editors and have undergone the standard journal formal review process. They may be cited. The Guest Editors appointed to this supplement are R. J. Dewhurst, D. R. Chadwick, E. Charmley, N. M. Holden, D. A. Kenny, G. Lanigan, D. Moran, C. J. Newbold, P. O'Kiely, and T. Yan. The Guest Editors declare no conflict of interest.

References

Agarwal N, Shekhar C, Kumar R, Chaudhary LC and Kamra DN 2009. Effect of peppermint (*Mentha piperita*) oil on *in vitro* methanogenesis and fermentation of feed with buffalo rumen liquor. Animal Feed Science and Technology 148, 321–327.

Animut G, Goetsch AL, Puchala R, Patra AK, Sahlu T, Varel VH and Wells J 2008a. Methane emission by goats consuming diets with different levels of condensed tannins from lespedeza. Animal Feed Science and Technology 144, 212–227.

Animut G, Goetsch AL, Puchala R, Patra AK, Sahlu T, Varel VH and Wells J 2008b. Methane emission by goats consuming different sources of condensed tannins. Animal Feed Science and Technology 144, 228–241.

Araujo RC, Pires AV, Mourão GB, Abdallab AL and Sallam SMA 2011. Use of blanks to determine *in vitro* net gas and methane production when using rumen fermentation modifiers. Animal Feed Science and Technology 166–167, 155–162.

Beauchemin KA, McGinn SM, Martinez TF and McAllister TA 2007. Use of condensed tannin extract from quebracho trees to reduce methane emissions. Journal of Animal Science 85, 1990–1996.

Bhatta R, Baruah L, Saravanan M, Suresh KP and Sampath KT 2012. Effect of medicinal and aromatic plants on rumen fermentation, protozoa population and methanogenesis *in vitro*. Journal of Animal Physiology and Animal Nutrition, doi: 10.1111/j.1439-0396.2012.01285.x.

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

Bodas RN, Prieto NR, García-González RS, Andrés S, Giráldez FJ and López S 2012. Manipulation of rumen fermentation and methane production with plant secondary metabolites. Animal Feed Science and Technology 176, 78–93.

Busquet M, Calsamiglia S, Ferret A, Carro MD and Kamel C 2005. Effect of garlic oil and four of its compounds on rumen microbial fermentation. Journal of Dairy Science 88, 4393–4404.

Calsamiglia S, Busquet M, Cardozo PW, Castillejos L and Ferret A 2007. Invited review: essential oils as modifiers of rumen microbial fermentation. Journal of Dairy Science 90, 2580–2595.

Carulla JE, Kreuzer M, Machmueller A and Hess HD 2005. Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage fed sheep. Australian Journal of Agricultural Research 56, 961–970.

Chaves AV, Baah J, Wang Y, McAllister TA and Benchaar Ch 2012. Effects of cinnamon leaf, oregano and sweet orange essential oils on fermentation and aerobic stability of barley silage. Journal of the Science of Food and Agriculture 92, 906–915.

Cheeke PR 1996. Biological effects of feed and forage saponins and their impact on animal production. Advanced Experimental Medicine Biology 405, 377–385.

Cieslak A, Váradyová Z, Kišidayová S and Szumacher-Strabel M 2009a. The effects of linoleic acid on the fermentation parameters, population density, and fatty-acid profile of two rumen ciliate cultures, *Entodinium caudatum* and *Diploplastron affine*. Acta Protozoologica 48, 51–61.

Cieslak A, Zmora P, Nowakowska A and Szumacher-Strabel M 2009b. Limonene affect rumen methanogenesis inhibiting the methanogens population. Bioactive plant compounds – structural and applicative aspects. Acta Biochimica Polonica 56, 59–61.

Cieslak A, Zmora P, Pers-Kamczyc E and Szumacher-Strabel M 2012. Effects of tannins source (*Vaccinium vitis idaea* L.) on rumen microbial fermentation *in vivo*. Animal Feed Sciences and Technology 176, 102–106.

Cieslak A, Miltko R, Belzecki G, Szumacher-Strabel M, Potkanki A, Kwiatkowska E and Michalowski T 2006. Effect of vegetable oils on the methane concentration and population density of the rumen ciliate, *Eremoplastron dilobum*, grown *in vitro*. Journal of Animal and Feed Sciences 15, 15–18.

Demeyer DI and De Graeve K 1991. Differences in stoichiometry between rumen and hindgut fermentation. Journal of Animal Physiology and Animal Nutrition 22, 50–61.

Dorman HJ and Deans SG 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology 88, 308–316.

Duffy CF, Killeen GF, Connolly CA and Power RF 2001. Effects of dietary supplementation with *Yucca schidigera* Roezl ex Ortgies and its saponin and non-saponin fractions on rat metabolism. Journal of Agricultural Food Chemistry 49, 3408–3413.

Evans JD and Martin SA 2000. Effects of thymol on ruminal micro-organisms. Current Microbiology 41, 336–340.

Finlay BJ, Esteban G, Clarke KJ, Williams AG, Embley TM and Hirt RR 1994. Some rumen ciliates have endosymbiotic methanogens. FEMS Microbiology Letters 117, 157–161.

Goel G and Makkar HPS 2012. Methane mitigation from ruminants using tannins and saponins. Tropical Animal Health Production 44, 729–739.

Goel G, Makkar HPS and Becker K 2008. Changes in microbial population, methanogenesis and rumen fermentation in response to saponin rich fractions of different plant materials. Journal of Applied Microbiology 105, 770–777.

Goel G, Makkar HPS and Becker K 2009. Inhibition of methanogens by bromochloromethane: effects on microbial communities and rumen fermentation using batch and continuous fermentations. The British Journal of Nutrition 101, 1484–1492.

Grainger C, Clarke T, Auldist MJ, Beauchemin KA, McGinn SM, Waghorn GC and Eckard RJ 2009. Potential use of *Acacia mearnsii* condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. Canadian Journal of Animal Science 89, 241–251.

Guo YQ, Liu JX, Lu Y, Zhu WY, Denman SE and McSweeney CS 2008. Effect of tea saponin on methanogenesis, microbial community structure and expression of mcrA gene, in cultures of rumen microorganisms. Letters Applied Microbiology 47, 421–426.

Hariadi BT and Santoso B 2010. Evaluation of tropical plants containing tannin on *in vitro* methanogenesis and fermentation parameters using rumen fluid. Journal of the Science of Food and Agriculture 90, 456–461.

Hassanat F and 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*. Journal of Science of Food and Agriculture 93, 332–339.

Hess HD, Kreuzer M, Diaz TE, Lascano CE, Carulla JE, Soliva CR and Machmüller A 2003. Saponin rich tropical fruits affect fermentation and methanogenesis in faunated and defaunated rumen fluid. Animal Feed Science and Technology 109, 79–94.

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

Hook SE, Wright AD and McBride BW 2010. Methanogens: methane producers of the rumen and mitigation strategies. Archaea, Article ID: 945785, doi: 10.1155/2010/945785.

Hu WL, Liu JX, Ye JA, Wu YM and Guo YQ 2005. Effect of tea saponin on rumen fermentation *in vitro*. Animal Feed Science and Technology 120, 333–339.

Huang XD, Tan HY, Long R, Liang JB and Wright AD 2012. Comparison of methanogen diversity of yak (*Bos grunniens*) and cattle (*Bos taurus*) from the Qinghai-Tibetan plateau, China. BMC Microbiology 12, 237–247.

Hungate RE 1967. Hydrogen as an intermediate in the rumen fermentation. Archives of Microbiology 40, 952–958.

Hungate RE, Smith W and Bauchop T 1970. Formate as an intermediate in the rumen fermentation. Journal of Bacteriology 102, 389–397.

Janssen PH and Kirs M 2008. Structure of the archaeal community of the rumen. Applied and Environmental Microbiology 74, 3619–3625.

Jarvis GN, Strompl C, Burgess DM, Skillman LC, Moore ER and Joblin KN 2000. Isolation and identification of ruminal methanogens from grazing cattle. Current Microbiology 40, 327–332.

Jayanegara A, Leiber F and Kreuzer M 2012. Meta-analysis of the relationship between dietary tannin level and methane formation in ruminants from *in vivo* and *in vitro* experiments. Journal of Animal Physiology and Animal Nutrition 96, 365–375.

Jouany JP and Morgavi DP 2007. Use of 'natural' products as alternatives to antibiotic feed additives in ruminant production. Animal 1, 1443–1466.

Kamra DN 2005. Rumen microbial ecosystem. Current Science 89, 124–132.

Kaneda N, Nakanishi H and Staba J 1987. Steroidal constituents of *Yucca schidigera* plants and tissue cultures. Phytochemistry 26, 1425–1429.

King EE, Smith RP, St-Pierre B and Wright AD 2011. Differences in the rumen methanogen populations of lactating Jersey and Holstein dairy cows under the same diet regimen. Applied and Environmental Microbiology 77, 5682–5687.

Kisidayova S, Varyadova Z, Zelenak I and Siroka P 2000. Methanogenesis in rumen ciliate cultures of *Entodinium caudatum* and *Epidinium ecaudatum* after long-term cultivation in a chemically defined medium. Folia Microbiologica 45, 269–274.

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

Kowalczyk M, Pecio L, Stochmal A and Oleszek W 2011. Qualitative and quantitative analysis of steroidal saponins in crude extract and bark powder of *Yucca schidigera* Roezl. Journal of Agricultural Food Chemistry 59, 8058–8064.

Kumar S, Dagar SS, Puniya AK and Upadhyay RC 2013. Changes in methane emission, rumen fermentation in response to diet and microbial interactions. Research in Veterinary Science 94, 263–268.

Leahy SC, Kelly WJ, Altermann E, Ronimus RS, Yeoman CJ, Pacheco DM, Li D, Kong Z, McTavish S, Sang C, Lambie SC, Janssen PH, Dey D and Attwood GT 2010. The genome sequence of the rumen methanogen *Methanobrevibacter ruminantium* reveals new possibilities for controlling ruminant methane emissions. PLoS One 5, e8926. doi:10.1371/journal.pone.0008926.

Lee J-H, Rhee M-S, Kumar S, Lee G-H, Chang D-H, Kim D-S, Choi S-H, Lee D-W, Yoon M-H and Kim B-C 2013. Genome sequence of Methanobrevibacter sp. strain JH1, isolated from rumen of Korean native cattle. Genome Announcements 1, e00002-13. doi:10.1128/genomeA.00002-13.

Li W and Powers W 2012. Effects of saponin extracts on air emissions from steers. Journal of Animal Science 90, 4001–4013.

Lin B, Lu Y, Wang JH, Liang Q and Liu JX 2012a. The effects of combined essential oils along with fumarate on rumen fermentation and methane production *in vitro*. Journal of Animal and Feed Sciences 21, 198–210.

Lin B, Wang JH, Lu Y, Liang Q and Liu JX 2012b. *In vitro* rumen fermentation and methane production are influenced by active components of essential oils combined with fumarate. Journal of Animal Physiology and Animal Nutrition 97, 1–9.

Macheboeuf D, Morgavi DP, Papon Y, Mousset JL and Arturo-Schaan M 2008. Dose–response effects of essential oils on *in vitro* fermentation activity of the rumen microbial population. Animal Feed Science and Technology 145, 335–350.

Makkar HPS and Becker K 1997. Degradation of *Quillaja saponin*s by mixed culture of rumen microbes. Letters Applied Microbiology 25, 243–245.

Manh NS, Wanapat M, Uriyapongson S, Khejornsart P and Chanthakhoun V 2012. Effect of eucalyptus (Camaldulensis) leaf meal powder on rumen fermentation characteristics in cattle fed on rice straw. African Journal of Agricultural Research 7, 1997–2003.

Mao HL, Wang JK, Zhou YY and 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. Livestock Science 129, 56–62.

McIntosh FM, Williams P, Losa R, Wallace RJ, Beever DA and Newbold CJ 2003. Effects of essential oils on ruminal microorganism and their protein metabolism. Applied and Environmental Microbiology 69, 5011–5014.

Miles CO, Wilkins AL, Munday SC, Holland PT, Smith BL, Lancaster MJ and Embling PP 1992. Identification of the calcium salt of epismilagenin beta-D-glucuronide in the bile crystals of sheep affected by *Panicum-dichotomiflorum* and *Panicum-schinzii* toxicoses. Journal of Agricultural Food Chemistry 40, 1606–1609.

Morgavi DP, Forano E, Martin C and Newbold CJ 2010. Microbial ecosystem and methanogenesis in ruminants. Animal 4, 1024–1036.

Morgavi DP, Martin C, Jouany JP and Ranilla MJ 2012. Rumen protozoa and methanogenesis: not a simple cause–effect relationship. The British Journal of Nutrition 107, 388–397.

Morvan B, Bonnemoy F, Fonty G and Gouet P 1996. Quantitative determination of H_2-utilizing acetogenic and sulfate-reducing bacteria and methanogenic archaea from digestive track of different mammals. Current Microbiology 32, 129–133.

Morvan B, Dore J, Rieulesme F, Foucat L, Fonty G and Gouet P 1994. Establishment of hydrogen-utilizing bacteria in the rumen of the newborn lamb. FEMS Microbiology Letters 117, 249–256.

Narvaez N, Wang Y and 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*. Journal of Science of Food and Agriculture, doi: 10.1002/jsfa.6068.

Newbold CJ, Lassalas B and Jouany JP 1995. The importance of methanogens associated with ciliate protozoa in ruminal methane production *in vitro*. Letters in Applied Microbiology 21, 230–234.

Newbold CJ, ElHassan SM, Wang J, Ortega ME and Wallace RJ 1997. Influence of foliage from African multipurpose trees on activity of rumen protozoa and bacteria. The British Journal of Nutrition 78, 237–249.

Niderkorn V, Baumont R, Le Morvan A and Macheboeuf D 2011. Occurrence of associative effects between grasses and legumes in binary mixtures on *in vitro* rumen fermentation characteristics. Journal of Animal Science 89, 1138–1145.

O'Ghara EA, Hill DJ and Maslin DJ 2000. Activities of garlic oil, garlic powder, and their diallyl constituents against Helicobacter pylori. Applied and Environmental Microbiology 66, 2269–2273.

Odenyo AA, Osuji PO and Karanfil O 1997. Effect of multipurpose tree (MPT) supplements on ruminal ciliate protozoa. Animal Feed Science and Technology 67, 169–180.

Ohene-Adjei S, Teather RM, Ivan M and Forster RJ 2007. Postinoculation protozoan establishment and association patterns of methanogenic Archaea in the ovine rumen. Applied and Environmental Microbiology 73, 4609–4618.

Ohene-Adjei S, Chaves AV, McAllister TA, Benchaar TA, Teather C and Forster RJ 2008. Evidence of increased diversity of methanogenic archea with plant extract supplementation. Microbial Ecology 56, 234–242.

Oleszek W, Sitek M, Stochmal A, Piacente S, Pizza C and Cheeke P 2001. Steroidal saponins of *Yucca schidigera* Roezl. Journal of Agricultural Food Chemistry 49, 4392–4396.

Patra AK and Saxena J 2009. Dietary phytochemicals as rumen modifiers: a review of the effects on microbial populations. Antonie van Leeuwenhoek 96, 369–375.

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

Patra AK and Yu Z 2012. Effects of essential oils on methane production and fermentation by, and abundance and diversity of, rumen microbial populations. Applied and Environmental Microbiology 78, 4271–4280.

Patra AK, Kamra DN and Agarwal N 2010. Effects of extracts of spices on rumen methanogenesis, enzyme activities and fermentation of feeds *in vitro*. Journal of the Science of Food and Agriculture 90, 511–520.

Pellikaan WF, Stringano E, Leenaars J, Bongers DJGM, van Laar-van Schuppen S, Plant J and Mueller-Harvey I 2011. Evaluating effects of tannins on extent and rate of *in vitro* gas and CH4 production using an automated pressure evaluation system (APES). Animal Feed Science and Technology 166–167, 377–390.

Pen B, Sar C, Mwenya B and Takahashi J 2008. Effects of *Quillaja saponaria* extract alone or in combination with *Yucca schidigera* extract on ruminal fermentation and methanogenesis *in vitro*. Animal Science Journal 79, 193–199.

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

Pen B, Sar C, Mwenya B, Kuwaki K, Morikawa R and Takahashi J 2006. Effects of *Yucca schidigera* and *Quillaja saponaria* extracts on *in vitro* ruminal fermentation and methane emission. Animal Feed Science and Technology 129, 175–186.

Pers-Kamczyc E, Zmora P, Cieslak A and Szumacher-Strabel M 2011. Development of nucleic acid based techniques and possibilities of their application to rumen microbial ecology research. Journal of Animal and Feed Sciences 20, 315–337.

Popova M, Morgavi DP and Martin C 2012. Methanogens and methanogenesis in the rumen and cecum of lambs fed two different high-concentrate diets. Applied and Environmental Microbiology doi:10.1128/AEM.03115-12.

Ramirez-Restrepo CA, Barry TN, Marriner A, Lopez-Villalobos N, McWilliam EL, Lassey KR and Clark H 2010. Effects of grazing willow fodder blocks upon methane production and blood composition in young sheep. Animal Feed Science and Technology 155, 33–43.

Ranilla MJ, Morgavi DP and Jouany JP 2004. Effect of time after defaunation on methane production *in vitro*. Reproduction Nutrition Development 44, S35–S36.

Regensbogenova M, Kisidayova S, Michalowski T, Javorsky P, Moon-Van Der Staay GWM, Moon-Van Der Staay SY, Hackstein JHP, McEwan NR, Jouany JP, Newbold JC and Pristas P 2004. Rapid identification of rumen protozoa by restriction analysis of amplified 18S rRNA gene. Acta Protozoologica 43, 219–224.

Russell MJ, Hall AJ, Cairns-Smith AG and Braterman PS 1988. Submarine hot springs and the origin of life. Nature 336, 117–117.

Sallam SMA and Abdelgaleil SAM 2010. Effect of different levels of citrus essential oil and its active components on rumen microbial fermentation and methane emission *in vitro*. Cuban Journal of Agriculture Science 44, 367–371.

Sallam SMA, Bueno ICS, Brigide P, Godoy PB, Vitti DMSS and Abdalla AL 2009. Efficacy of eucalyptus oil on *in vitro* rumen fermentation and methane production. Options Mediterraneennes 85, 267–272.

Sallam SMA, Abdelgaleil SAM, Bueno ICDS, Nasser MEA, Araujo R and Abdalla AL 2011. Effect of some essential oils on *in vitro* methane emission. Archives of Animal Nutrition 65, 203–214.

Shin EC, Choi BR, Lim WJ, Hong SY, An CL, Cho KM, Kim YK, An JM, Kang JM, Lee SS, Kim H and Yun HD 2004. Phylogenetic analysis of archaea in three fractions of cow rumen based on the 16S rDNA sequence. Anaerobe 10, 313–319.

Skillman LC, Evans PN, Naylor GE, Morvan B, Jarvis GN and Joblin KN 2004. 16S ribosomal DNA-directed PCR primers for ruminal methanogens and identification of methanogens colonising young lambs. Anaerobe 10, 277–285.

Smith AH, Zoetendal EG and Mackie RI 2005. Bacterial mechanisms to overcome inhibitory effects of dietary tannins. Microbial Ecology 50, 197–205.

Soliva CR, Widmer S and Kreuzer M 2008. Ruminal fermentation of mixed diets supplemented with St. John's Wort (*Hypericum perforatum*) flowers and pine (*Pinus mugo*) oil or mixtures containing these preparations. Journal of Animal and Feed Sciences 17, 352–362.

Soltan YA, Morsy AS, Sallam SMA, Louvandini H and Abdalla AL 2012. Comparative *in vitro* evaluation of forage legumes (prosopis, acacia, atriplex, and leucaena) on ruminal fermentation and methanogenesis. Journal of Animal and Feed Sciences 21, 759–772.

Staerfl SM, Kreuzer M and Soliva CR 2010. *In vitro* screening of unconventional feeds and various natural supplements for their ruminal methane mitigation potential when included in a maize-silage based diet. Journal of Animal and Feed Sciences 19, 651–664.

Stewart CS, Flint HJ and Bryant MP 1997. The rumen bacteria. In The rumen microbial ecosystem (ed. PN Hobson and CS Stewart), pp. 10–72. Blackie Academic and Professional, London, UK.

St-Pierre B and Wright AD 2012. Diversity of gut methanogens in herbivorous animals. Animal 7 (suppl. 1), 49–56.

Szumacher-Strabel M and Cieslak A 2010. Potential of phytofactors to mitigate rumen ammonia and methane production. Journal of Animal and Feed Sciences 19, 319–337.

Szumacher-Strabel M, Cieslak A and Nowakowska A 2009. Effect of oils rich in linoleic acid on *in vitro* rumen fermentation parameters of sheep, goats and dairy cows. Journal of Animal and Feed Sciences 18, 440–452.

Szumacher-Strabel M, Zmora P, Roj E, Stochmal A, Pers-Kamczyc E, Urbanczyk A, Oleszek W, Lechniak D and Cieslak A 2011. *In vitro* screening of the wild dog rose (*Rosa canina*) potential to mitigate rumen methane production. Journal of Animal and Feed Sciences 20, 285–299.

Tajima K, Nagamine T, Matsui H, Nakamura M and Animov RI 2001. Phylogenetic analysis of archaeal 16S rRNA libraries from the rumen suggests the existence of a novel group of archaea not associated with known methanogens. FEMS Microbiology Letters 200, 67–72.

Tan HY, Sieo CC, Abdullah N, Liang JB, Huang XD and Ho YW 2011. Effects of condensed tannins from Leucaena on methane production, rumen fermentation and populations of methanogens and protozoa *in vitro*. Animal Feed Science and Technology 169, 185–193.

Tanaka O, Ikeda T, Ohtani K, Kasai R and Yamasaki K 2000. Antiyeast steroidal saponins from *Yucca schidigera* (Mohave Yucca), a new anti-food-deteriorating agent. Journal of Natural Products 63, 332–338.

Tavendale MH, Meagher LP, Pacheco D, Walker N, Attwood GT and Sivakumaran S 2005. Methane production from *in vitro* rumen incubations with *Lotus pedunculatus* and *Medicago sativa*, and effects of extractable condensed tannin fractions on methanogenesis. Animal Feed Science and Technology 123–124, 403–419.

Teferedegne B, McIntosh F, Osuji PO, Odenyo A, Wallace RJ and Newbold CJ 1999. Influence of foliage from different accessions of the sub-tropical leguminous tree, *Sesbania sesban*, on ruminal protozoa in Ethiopian and Scottish sheep. Animal Feed Science and Technology 78, 11–20.

Tymensen LD, Beauchemin KA and McAllister TA 2012. Structures of free-living and protozoa-associated methanogen communities in the bovine rumen differ according to comparative analysis of 16S rRNA and mcrA genes. Microbiology 158, 1808–1817.

Ushida K and Jouany JP 1996. Methane production associated with rumen-ciliated protozoa and its effect on protozoan activity. Letters in Applied Microbiology 23, 129–132.

Van Kessel JS and Russell JB 1996. The effect of pH on ruminal methanogenesis. FEMS Microbiology Ecology 20, 205–210.

Vasta V and Bessa RJB 2012. Manipulating ruminal biohydrogenation by the use of plants bioactive compounds. In Dietary phytochemicals and microbes (ed. AK Patra), pp. 263–284. Springer, London, UK.

Vogels GD, Hoppe WF and Stumm CK 1980. Association of methanogenic bacteria with rumen ciliates. Applied and Environmental Microbiology 47, 219–221.

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

Wang XF, Mao SY, Liu JH, Zhang LL, Cheng YF, Jin W and Zhu WY 2011. Effect of the gynosaponin on methane production and microbe numbers in a fungus-methanogen co-culture. Journal of Animal and Feed Sciences 20, 272–284.

Wang Y, McAllister TA, Newbold CJ, Rode LM, Cheeke PR and Cheng KJ 1998. Effects of *Yucca schidigera* extract on fermentation and degradation of steroidal saponins in the rumen simulation technique (RUSITEC). Animal Feed Science and Technology 74, 143–153.

Whitmann WB, Bowen TL and Boone DR 1992. The methanogenic bacteria. In The prokaryotes, 2nd ed. A handbook on the biology of bacteria: ecophysiology, isolation, identification, applications (ed. A Balows), pp. 719–767. Springer-Verlag, New York, USA.

Wina E, Muetzel S, Hoffmann E, Makkar HPS and Becker K 2005. Saponins containing methanol extract of *Sapindus rarak* affect microbial fermentation, microbial activity and microbial community structure *in vitro*. Animal Feed Science and Technology 121, 159–174.

Wolin MJ and Miller TL 1988. Microbe–microbe interactions. In The rumen microbial ecosystem (ed. PN Hobson), pp. 343–359. Elsevier Science Publishers, New York, USA.

Wolin MJ, Miller C and Stewart CJ 1997. Microbe–microbe interactions. In The rumen microbial ecosystem (ed. PN Hobson and CS Stewart), pp. 467–491. London Blackie Academic and Professional, UK.

Wright ADG, Ma X and Obispo NE 2008. Methanobrevibacter phylotypes are the dominant methanogens in sheep from Venezuela. Microbial Ecology 56, 390–394.

Zeleke AB, Clement C, Hess HD, Kreuzer M and Soliva CR 2006. Effect of foliage from multi-purpose trees and a leguminous crop residue on *in vitro* methanogenesis and ruminal N use. International Congress Series 1293, 168–171.

Zhou M, Hernandez-Sanabria E and Guan LL 2009. Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. Applied and Environmental Microbiology 75, 6524–6533.

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

Zhu WY, Mao SY, Liu JX, Cheng YF, Iqbal MF and Wang JK 2007. Diversity of methanogens and their interactions with other microorganisms in methanogenesis in the rumen. The Proceedings of the VII International Symposium on the Nutrition of Herbivores (ed. QX Meng), pp. 17–22. China Agricultural University Press, Beijing, China.

Zmora P, Cieslak A, Pers-Kamczyc E, Nowak A, Szczechowiak J and Szumacher-Strabel M 2012b. Effects of *Mentha piperita* L. on *in vitro* rumen methanogenesis and fermentation. Acta Agriculturae Scandinavica, Section A – Animal Science 62, 46–52.

Zmora P, Cieslak A, Pers-Kamczyc E and Szumacher-Strabel M 2012c. The *in vitro* study on the effect of *Salvia officinalis* L. on rumen fermentation. Journal of Animal and Feed Sciences 21, 613–623.

Zmora P, Cieslak A, Jedrejek D, Stochmal A, Pers-Kamczyc E, Oleszek W, Nowak A, Szczechowiak J, Lechniak D and Szumacher-Strabel M 2012a. Preliminary *in vitro* study on the effect of xanthohumol on rumen methanogenesis. Archives of Animal Nutrition 66, 66–71.