The Journal of Animal & Plant Sciences, 28(2): 2018, Page: 348-364
ISSN: 1018-7081

Review Paper

OPTIONS TO METHANE PRODUCTION ABATEMENT IN RUMINANTS: A REVIEW

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ABSTRACT

The goal of this review was to analyze published data on practices that mitigate enteric methane (CH_4) emissions from ruminants. The current approaches in relation to associated advantages and disadvantages and future options to reduce enteric CH_4 emission are presented. Strategies for reducing ruminant CH_4 output are considered in relationship to rumen ecology, biochemistry, and animal performance. The study is divided into nine sections (Defaunation and inhibition of archaea, Bacteriocins, Methane inhibitors and analogues, Probiotics, Saponins, Tanins, Ionophores, Organic acids, and Lipids). Defaunation or elimination of protozoa to reduce CH_4 emission depending upon diet. Mitigation of CH_4 emission by rumen microbes can be to apply the various chemicals. Some approaches such as immunization and chemical inhibitors directly target against their own rumen methanogens. Organic acids feeding promote propionate production. A reduction in CH_4 production has also been observed with live yeast cells, lactate-utilizing bacteria, and by the selection of plant species that produce secondary metabolites, such as condensed tannins and saponins. Supplements rich in polyunsaturated fat acids such as linoleic acid, and linolenic acid also have a negative effect on CH_4. Based on available results, it appears that dietary supplementation with fat or essential oils are the most promising dietary strategy.

Keywords: Environment, Methane, Ruminant, Additive

INTRODUCTION

Methane emissions in animal husbandry originate from fermentative digestion in animals, natural anaerobic ecosystems, storage of manures, and field application. Within livestock, ruminants (cattle, sheep, and goats) are the primary source of emissions. The actual rate of methane (CH_4) emission is highly dependent on the management strategies implemented on a farm (Kirchgessner *et al.,* 1991; Dämmgen *et al.,* 2012; Broucek, 2015; Van Middelaar *et al.,* 2013).

Integrated research investigating animal, plant, microbe and nutrient level strategies might offer a long term solution of CH_4 production abatement. At the animal level, genetic selection is the area of research with the best chance of finding a solution. Methane emissions can be reduced by the selection of plant species that produce secondary metabolites to reduce methanogenesis, such as saponins, flavonoids and tannins.

Methods of inhibiting methanogens include use of antibiotics, feed additives (fats and oils), nitrate salts, or dicarboxylic acids. The defaunation, vaccination against methanogens, halogenated and chlorinated analogues, bromo-chloromethane, and cyclodextrin are also importat treatments. Methods of enhancing alternative hydrogen (H_2) using microbial species include inoculating with acetogenic species, feeding highly digestible feed components favouring propionate fermentations, and modifying rumen conditions (Cottle *et al.,* 2011).

The sustainability of methane suppressing strategies is an important issue. There is an urgent need for support model that is capable of evaluating the effectiveness of both existing and new technologies for reducing methane emission (Sejian and Naqvi, 2012; Mihina *et al.,* 2012). Despite intensive research in this area, our understanding is far from complete. There have been many reports showing different responses to feed additives such as ionophores, yeast products, fumaric acid, etc., and there is considerable difficulty in applying *in vitro* data to *in vivo* experiments due to the complexity of the ruminal system (Moss, 1994; McGinn *et al.,* 2004; Shibata and Terada, 2010). However, such research will be expensive and long term.

Defaunation and inhibition of archaea: Defaunation and vaccines are mitigation techniques which target the methanogen population of the rumen directly or indirectly, resulting in varying degrees of efficacy (Hook *et al.,* 2010). Strategies are following: a decreasing of the number of the methanogenic archaea (archaebacteria); a reduction of H_2 production; a stimulation of H_2 utilisation beneficial for the animal.

In ruminants, the major portion of the methanogenesis occurs in the the rumen, where feeds including fibrous plant structures are fermented primarily to short-chain volatile fatty acids, carbon dioxide (CO_2), hydrogen, and methane by bacteria, protozoa, fungi and methanogens. The methanogens belong to a separate domain archaea in the kingdom of *Euryarchaeota* and are found in a wide range of other anaerobic environments (Liu and Whitman, 2008; Hook *et al.,* 2010).

Methanogens are very diverse in terms of phylogeny and ecology. They play an important role in the anaerobic food chain, driving anaerobic fermentation through removal of excess H_2 and formate. Most rumen methanogens derive energy for their growth through a series of biochemical reduction of CO_2 with H_2, and some methanogens use acetate and methyl group-containing compounds to produce methane (methanogenesis): $4\ H_2 + CO_2 = CH_4 + 2\ H_2O$

Among 28 genera and 113 species of methanogens known to be present in nature, only seven species have commonly been cultured from the rumen (Janssen and Kirs, 2008; Hook *et al.*, 2010). These are *Methanobacterium formicicum*, *Methanobacterium bryantii*, *Methanobrevibacter ruminantium*, *Methanobrevibacter millerae*, *Methanobrevibacter olleyae*, *Methanomicrobium mobile*, and *Methanoculleus olentangyi*. Analysis of molecular-based studies reveals that the members of family *Methanobacteriaceae* (which includes *Methanobrevibacter* spp., *Methanobacterium* spp., and *Methanosphaera* spp.) are the dominant members (30 % to 99 % of archaea) of the rumen archaea. Members of the order *Methanomicrobiales* (which includes *Methanomicrobium* spp.) are less abundant (0 % to 54 %), and members of the order *Methanosarcinales* (which includes *Methanimicrococcus*) are rare (2 % to 3 %). Usually, CH_4 is produced by two types of methanogens, the slow-growing methanogens that produces CH_4 from acetate (e.g., *Methanosarcina*) and fast growing methanogens that reduce CO_2 with H_2.

Most of the ciliate protozoa living in the rumen, they are not present at birth but normally start establishing in the gastrointestinal tract 3 weeks after birth (Iqbal *et al.,* 2008). Ciliate protozoa are known to affect methanogens in a generic and age manner. There were found to have intracellular bacteria with methanogens (Finlay *et al.,* 1994). The elimination of ciliate protozoa from the rumen has been proposed as a means of increasing the productivity of ruminants (Finlay *et al.,* 1994). The importance of methanogenic bacteria associated with ciliate protozoa was estimated either by removing protozoa from whole rumen fluid or by isolating the protozoa. Methanogenic bacteria associated with rumen ciliates were responsible for between 9 and 25 % of methanogenesis in rumen fluid of sheep (Newbold *et al.,* 1995a).

Defaunation techniques used in research have been reviewed by Hegarty (1999) and Iqbal *et al.* (2008). Defaunation approaches include dietary manipulation (virginiamycin, milk fat); synthetic chemicals including copper sulfate, calcium peroxide, dioctylsodium sulfosuccinate and detergents; and natural compounds like vitamin A, non-protein amino acids and ecdysones, the steroidal hormones which cause skin shedding in insects. Many of the most feed supplements and additives have the ability to reduce protozoal numbers. Some lipids, saponins, tannins and ionophores are toxic to protozoa.

Defaunation effects on mitigated methane production due to (1) reduced fiber digestion, (2) reduced methanogen population associated with protozoa (Machmüller *et al.,* 2003), (3) reduced hydrogen transfer (Finlay *et al.,* 1994), and (4) increased partial pressure of oxygen on the rumen (Eckard *et al.,* 2010).

The removal of protozoa from the rumen has been shown to reduce CH_4 production by up to 50 % (Hegarty, 1999; Martin *et al.,* 2010). Shibata and Terada (2010) described 20 – 40 % reduction in CH_4 production by defaunation. Defaunation reduces methane production by 20 to 50 % depending upon diet (Mathison *et al.,* 1998). Kreuzer *et al.* (1986; cited by Iqbal *et al.,* 2008) reported that defaunation of cattle fed a high concentrate diet decreased methane production by 50 %. CH_4 production was the lowest in defaunated calves and the calves with a mixed protozoan population showed the highest CH_4 production (Shibata and Terada, 2010). Kurihara *et al.* (1997, cited by Shibata and Terada, 2010) wrote that ruminal numbers of protozoa and of cellulolytic bacteria tend to decrease in dry cows fed zinc sulfate and that CH_4 production showed a 60 % reduction. Hegarty (1999) concluded from reviewed data that the defaunation method results in 40 % of methanogenesis in rumen fluid. Machmüller *et al.* (2003) found that effects of myristic acid supplementation inhibited significantly rumen archaea without significantly altering proportions of individual methanogen orders in sheep. This decreased daily CH_4 emission of the animals by 40 % on average. Ciliate protozoa concentration was decreased.

The decrease in CH_4 production of 26 % per kg of dry matter intake (DMI) in protozoa-free lambs was related to a decrease in the proportion of methanogens in the total bacterial population of the whole ruminal content (McAllister and Newbold, 2008; Martin *et al.,* 2010). The study of Morgavi *et al.* (2008) show that the methane emissions decrease of induced by defaunation was stable for to 2 years in sheep. In the absence of protozoa, CH_4 decreased by 20 % in both short- and long-term defaunated animals. Dohme *et al.* (1999) showed that CH_4 production decreased by 61 % in defaunated rumen fluid.

Another method involves defaunation of the rumen to remove syntrophic hydrogen-producing protozoa (Hook *et al.,* 2010; Finn *et al.,* 2015). Methanogenesis is an important liquidating activity for H_2, which is the key element to consider for reducing CH_4 production (Joblin, 1999). The H_2 production and methanogenesis could be altered by increasing the quantity of microbial cells leaving the rumen per unit of carbohydrate consumed. The microbial ecology of the rumen ecosystem is exceedingly complex and the ability

of this system to efficiently convert complex carbohydrates to fermentable sugars is in part due to the effective disposal of H_2 through reduction of CO_2 to methane by methanogens (McAllister and Newbold, 2008). Methanogenic microorganisms remove H_2 produced during fermentation of organic matter in the rumen and hind gut (Cottle *et al*., 2011).

In this respect, acetogenic bacteria normally present in the rumen are of interest because they have the potential to provide an alternative sink for H_2, an essential intermediate in the formation of CH_4. Studies on ruminants fed diets containing concentrates or conserved forages indicate that the rumen contains a diversity of acetogens. In the rumen ecosystem, the ubiquitous protozoa are large producers of this metabolic end product. In addition, a physical association between protozoal cells and methanogens exist in the rumen ecosystem that favours H_2 transfer. The methanogens found both attached and inside ciliate protozoal cells have been estimated to contribute between 9 % and 37 % of the rumen methanogenesis (Finlay *et al*., 1994; Newbold *et al*., 1995a).

Some problems are associated with defaunation. Digestion will be negatively affected if animals are defaunated completely (Machmüller *et al.*, 2003). In addition, the restoration of CH_4 emissions to pre-treatment levels seen for some products has been associated to an adaptation and recovery of protozoal numbers (Martin *et al*., 2010). Other reports indicate that the reduction of methane production by defaunation is only temporary (Iqbal *et al*., 2008). However, in the study of Morgavi *et al*. (2008), the lower CH_4 emission in defaunated animals was maintained for more than 2 years indicating that the changes induced are stable.

Elimination of the rumen protozoa to mitigate methanogenesis is promising, but this option should be carefully evaluated in terms of livestock welfare and performances. In addition, on-farm defaunation techniques are not available up to now. Several feed additives such as ionophores, organic acids and plant extracts have also been assayed (Martin *et al*., 2010; Guerci *et al*., 2013). The absence of protozoa from the rumen can have diverse effects on animals that can be either negative or positive depending on the diet and the type of production targeted (Martin *et al*., 2010).

However, this technique is difficult to apply in the field. Further research is required to identify the feeding conditions that play a role in the effect of defaunation on CH_4 production and to establish practical methods of applying the technique of defaunation. Up to now, however, practical defaunation techniques are not available.

Several biotechnological strategies of immunisation and biological control are currently being explored. The targeted manipulation of the rumen ecosystem provides the best hope for mitigating enteric CH_4 emissions and the biggest challenge. However, vaccines prepared with a different set of methanogen species or tested in other geographical regions did not elicit a positive response. The highly diverse methanogenic community in animals reared under different conditions maybe guilty (Wright *et al*., 2007).

According to Cook *et al*. (2008) experiment, passive immunisation was assayed using antibodies, which were produced in laying hens, against three common methanogens present in the digestive tract of animals. They reported that opinion an ideal treatment for inhibiting methanogenesis would reduce CH_4 production without unfavourably altering ruminal fermentation and VFA profiles, or inhibiting synthesis of microbial protein. One possible future pathway to reduce CH_4 output is to immunize animals against their own methanogens and protozoa. Baker *et al*. (1997; cited by Iqbal *et al*., 2008) have claimed to invoke an immune response to rumen protozoa by administering an immunogenic preparation. This can affect the activity of rumen methanogens as they have a relationship with rumen protozoa.

The development of an antimethanogenic vaccine is also in progress. It is anticipated that vaccination could reduce CH_4 output up very significant. However, the results are still generally inconclusive. Wright *et al*. (2004) found those 8 to 10 weeks after primary immunization, no significant differences in CH_4 production could be measured between the three treatments, although there were trends of an 8 % and 6 % reduction when compared to the control sheep. When CH_4 data were expressed per kg of DMI, sheep immunized with the 3-methanogen mix and the 7-methanogen mix were 4 % and 5 % less, respectively, than the control sheep. Furthermore, authors decided to administer a secondary immunization. As a result, sheep in the 3-methanogen mix group produced 12.8 % less methane than sheep in the control group. Even when the data were corrected for DMI, there was a significant 7.7 % reduction in methane production per kg of DMI between the control and the treatment sheep. Williams *et al*. (2009) described a vaccination regimen which induced a substantial serum antibody response against methanogens in sheep but failed to significantly affect the CH_4 emission by these sheep and the density of methanogens.

Vaccines against rumen archaea may offer mitigation opportunities in the future. However, the CH_4 reduction is to be small and adaptation by ruminal microbes and persistence of the effect is unknown. Much more work is needed to make this technique effective, as there are multiple strains of Archaea in the rumen. If this proves successful, the vaccination would be a valuable tool for CH_4 reductions as it could be applied to a whole ruminant population (Hristov *et al*., 2013a).

This approach has the benefit of using the animal's immune system to produce antibodies against

specific methanogens, instead of chemicals, drugs, or antibiotics that may be potentially harmful to the animal or the environment. Moreover, this reduction in methane was achieved despite targeting probably less than 20 % of the methanogens in the rumen. Whether or not greater efficacy can be achieved using more targeted vaccine formulations, or different feedstuffs, requires elucidation. Up to now, immunisation has not delivered a clear, positive answer in reducing CH_4 emissions by ruminants, highlighting the difficulties of this approach (Martin *et al*., 2010).

The most promising avenues for future research for reducing methanogenesis are the development of new products for reducing protozoal numbers in the rumen and the use of bacterocins or other compounds which specifically target methanogenic bacteria (Mathison *et al*., 1998).

Bacteriocins: Bacteriocins are proteinaceous toxins produced by bacteria to inhibit the growth of bacterial strain (Renuka *et al*., 2013; Guerci *et al*., 2013) which are typically considered to be of narrow spectrum antibiotics. Some bacteriocins (nisin and bovicin) are known to reduce CH_4 production. McAllister and Newbold (2008) reported that bacteriocins could prove effective in directly inhibiting methanogens and redirecting H_2 to other reductive bacteria, such as propionate producers or acetogens.

Nisin, obtained from *Lactobaccilus lactis* ssp. lactis, has been shown to decrease methane production *in vitro* (Martin *et al*., 2010). Although the mechanism is still unclear, nisin reduce rumen methanogenesis by 36 % (Callaway *et al*., 1997). Santoso *et al*. (2004) found decreased methane emission from sheep fed on basal diet supplemented with nisin. However, nisin, in addition to *Yucca schidigera*, had only minor effects on rumen fermentation (Santoso *et al*., 2006).

Bovicin HC5, another bacteriocin produced by *Streptococcus bovis* from the rumen, has been reported to suppress methane production *in vitro* by 50 % (Lee *et al*., 2002). The use of bacteriocins may be prospective for inhibiting methanogen populations in the rumen. According to Lima *et al.* (2009) found *in vitro* studies that HC5 may be useful in limiting CH_4 production in the rumen. Bacteriocins may provide an alternative strategy for decreasing ruminal CH_4 production.

Methane inhibitors and analogues: Methane inhibitors are chemical compounds with inhibitory effects on rumen archaea. The formation of methane by the rumen microbiota is also reduced by the methane analogues in the presence or absence of added substrate. These inhibitors affect one or more of the reactions by which methane is formed from hydrogen and carbon dioxide (Bauchop, 1967; Hristov *et al*., 2015). Control of CH_4 emission by microbes has mainly been focused on application of chemicals that inhibit the growth and activity of methanogens in the rumen (Song *et al*., 2011). Some inhibitors have strong negative effect on methanogenesis. Lanigan (1972) showed five halogenated methane analogues (bromoform, chloroform, iodoform, carbon tetrabromide, and carbon tetrachloride) to inhibit CH_4 creating in the sheep's rumen as well as in rumen fluid *in vitro*. Other authors (Van Nevel and Demeyer, 1996; McCrabb *et al*., 1997; Song *et al*., 2011; Patra, 2014) reported also hydroxylated and chlorinated analogues, cyclodextrin, bromochloromethane, bromoethanesulphonate, chloral hydrate, and bromine analogue of coenzyme M.

Chloroform, as chlorinated methane analogue, depress methanogenesis through inhibition of methyl-CoM reductase but is obviously not suitable for field application. There were found significant reduction in CH_4 production by chloral hydrate, but prolonged feeding led to liver damage (Van Nevel and Demeyer, 1996). Low concentrations of chloroform, carbon tetrachloride, and methylene chloride inhibited the formation of CH_4 by the rumen microbiota in the presence or absence of added substrate (Thiel, 1969). McCrabb *et al*. (1997) conducted trials to determine the effects of dietary supplementation with a complex of bromochloromethane and α-cyclodextrin. Methane production of treated steers was significantly lower than that of control steers over 28 days.

Dumitru *et al*. (2003) found that 4-(ß-D-ribofuranosyl) aminobenzene-5-phosphate (RFA-P) synthase catalyzes the biosynthesis of methanopterin which stop the growth of methane-producing microbes. RFA-P is a key cofactor required for methane formation and for one-carbon transformations in methanogens, but its effects lasted only 3 days in sheep. *In vitro* experimental results indicated that none of these inhibitors affect bacterial metabolism adversely. Supplementation with hydroxylated analogue of methionine tended to decrease CH_4 production further (Patra, 2014).

Bromoethanesulphonate (BES), structural analogue, as the cofactor mercaptoethanesulfonic acid (a bromine analogue of coenzyme M) is also a potent inhibitor of CH_4 emission (Mathison *et al*., 1998). BES involved in methyl group transfer during methanogenesis, is a potentially strong inhibitor of CH_4 production. Lee *et al*. (2009) observed a reduced *in vitro* CH_4 production by supplementing of BES to the culture solution containing timothy or mixed substrate in a dose dependent manner. BES at 5 mM concentration inhibited CH_4 production by more than 95 % compared to the control. Bromochloromethane appeared to be a potent CH_4 inhibitors (Mathison *et al.,* 1998), but its effect on ruminal methanogenesis was transient (Van Nevel and Demeyer, 1996).

Methane inhibitors can reduce CH_4 emissions to zero in the short term but due to microbial adaptation the effects of these compounds are quickly neutralized and feed intake is often depressed (Mathison *et al*., 1998). Basic pathway of CH_4 production in the bovine rumen is the reduction of CO_2 with molecular H_2. In rumen liquid, H_2 is normally converted to CH_4 (Bauchop, 1967). Methods of enhancing H_2 using microbial species include: inoculating with acetogenic species; feeding highly digestible feed components favouring propionate fermentations; and modifying rumen conditions (Cottle *et al*., 2011). Methanogenic microorganisms remove H_2 produced during fermentation of organic matter in the rumen and hind gut. As a consequence, the amount of H_2 produced during fermentation is reduced, resulting in decreased CH_4 production (Hristov *et al*., 2013b; Alstrup *et al*., 2015).

Propionate enhancers are also one of the effective alternatives in methane control. Fumarate and malate are carbon intermediates in the propionate pathway. In this reaction, hydrogen ion (H^+) is needed and therefore, reduction of the dicarboxylic acids may provide an alternative electron sink for H_2 (Morgavi *et al*., 2010; Song *et al*., 2011). Any mitigation strategy that reduces methanogen populations must also include an alternative pathway for H_2 removal from the rumen.

Supplementing nitrate (NO_3) to ruminants has been shown to decrease methane production (Zijderveld van *et al*., 2011). Nitrate has a greater affinity for H_2 than does CO_2 and so, when nitrate is present in the rumen; nitrite and ammonia formation is favoured over methane production. Stoichiometrically, 1 kg NO_3 reduces methane formation by 258.7 g CH_4 (Cottle and Eckard, 2014). The enteric CH_4 was 19.6 % reduced in sheep and 18.2 % in goats fed diet containing 5 % sodium nitrate with sulphur and 0.4% sodium nitrate without sulphur compared to those fed control diet (Arif *et al.,* 2016).

Probiotics: Probiotics are microbial feed additives (the live microorganisms) that influence rumen fermentation and improve health by modulating gut microbiota (Patel *et al*., 2015). The most widely used probiotics are yeast, *Saccharomyces cerevisiae, Lactobacillus sporogenes* and some others. The principle is not yet clear, however, it is assumed that yeast cultures reduce methane production in more manners.

Probiotics increase butyrate or propionate production. The results of Lila *et al*. (2004) showed that a twin strain of *Saccharomyces cerevisiae* live cells stimulated *in vitro* mixed ruminal microorganism fermentation with decreased lactate, and a small decrease of CH_4 and H_2 with hay plus concentrate. The decrease in CH_4 production may be due to the utilization of metabolic H_2 by acetogenic bacteria to produce acetate. Another effect of probioticsis decrease numbers of protozoa. Therefore, the presence of yeast would be beneficial to the rumen microflora. According to Newbold *et al*. (1996), the protozoal population was significantly lower in yeast supplemented. Newbold *et al*. (1995b) and Newbold *et al*. (1998) suggest another possibility, that yeast decreases numbers of rumen ciliate protozoa.

Probiotics increasing microbial synthesis and amplify acetogenesis (Chaucheyras *et al*., 1995; Chaucheyras-Durand *et al*., 2008). The positive effects of a live strain of *Saccharomyces cerevisiae* on H_2 utilization, acetate and CH_4 production by two hydrogenotrophic ruminal microorganisms, an acetogenic bacterial strain and an archaea methanogen, were investigated (Doreau and Jouany, 1998). The addition of yeast cells enhanced by more than 5 times the hydrogenotrophic metabolism of the acetogenic strain and its acetate production. The use of yeasts as ruminant feed additives could help these bacteria to compete (Chaucheyras-Durand *et al*., 2008).

Wallace and Newbold (1993, cited by Iqbal *et al*., 2008) found that probiotics improved productivity by 7–8 % resulting in reduced CH_4 production per unit of product in dairy cows and growing cattle. Mwenya *et al*. (2004) evaluated effects of adding yeast culture, lactic acid bacteria and galacto-oligosaccharides on rumen methanogenesis in sheep. They reported that sheep produced by 10 % less CH_4 when 4 g yeast culture was included per day in a diet (40 % timothy hay, 30 % alfalfa hay, and 30 % concentrate).

McGinn *et al*. (2004) reported that reduction in CH_4 by the yeast was small and not statistically significant in cattle. Study has also shown that CH_4 suppression effects of probiotics are not consistent.

The use of probiotics or the stimulation of rumen microbial populations capable to decrease CH_4 emissions remains a potentially interesting approach. The use of probiotics, i.e. live yeasts, remains a potentially interesting approach, but results have been either unsatisfactory, not conclusive, or have yet to be confirmed *in vivo* (Martin *et al*., 2010).

Saponins: Saponins are glucosides with foaming characteristics that have a direct effect on rumen microbes. They are occurring in many plant species. Saponins are classified based on their carbon skeletons, 11 main classes of saponins were distinguished: dammaranes, tirucallanes, lupanes, hopanes, oleananes, taraxasteranes, ursanes, cycloartanes, lanostanes, cucurbitanes, and steroids (Vincken *et al*., 2007). Teferedegne *et al*. (1999) evaluated 9 different accessions of *Sesbania sesban* for their toxicity to ciliate protozoa. They showed that leaves of *Sesbania sesban* defaunated the rumen of sheep. Saponins decrease protein degradation and favour at the same time microbial protein and biomass synthesis, two processes that result in

reduced availability of H_2 for CH_4 production (Goel *et al*., 2008a; Martin *et al*., 2010).

Research indicates that some saponins might actually be beneficial to rumen digestion. However, saponins are bitter and reduce the palatability of livestock feeds. Some saponins reduce therefore the feed intake and growth rate of nonruminant animals (Das *et al.*, 2012). Results of Goel *et al*. (2008b) indicate that amongst the three plants tested, *Carduus pycnocephalus* has the highest potential reduced CH_4 level by 20–21.5 % on hay or concentrate-hay (68:32) based diets. The saponin containing feeds, fenugreek (*Trigonella foenum-graecum* L.) seeds and *Sesbania seban* did not produce significant reduction in CH_4 production despite decrease in the protozoal numbers by nearly 50 %. Bodas *et al*. (2008) tested 450 plant samples. 35 decreased methane production by more than 15 % versusthose with corresponding control cultures and six species (*C. pycnocephalus*, *P. tremula*, *Prunus avium*, *Q. robur*, *R. nobile* and *S. caprea*). Two plants, *Rheum nobile* and *Carduus pycnocephalus*, consistently decreased methane production without adversely affecting other parameters of the rumen fermentation.

Certain saponin-rich fruits of tropical multipurpose trees could have the potential to reduce methane emission. A comparison of the effects on rumen fermentation of three saponin rich tropical fruits (*Sapindus saponaria*, *Enterolobium cyclocarpum,* and *Pithecellobium saman*) supplemented to forage-based diets was done by Hess *et al*. (2003a). Only *Sapindus saponaria* decreased protozoal count by 54 % and daily CH_4 release by 20 % relative to control diet, but without affecting the methanogen count. Another articleby the same authors (Hess *et al*., 2003b) pointed that fruits of *Sapindus saponaria* had a methane-suppressing potential when were added to a high-fibre diet containing the tropical high-quality legume *Arachis pintoi. Sapindus saponaria* reduced methanogenesis by 11 % on average in grass-alone and legume-supplemented diets. According to Hess *et al.* (2004), supplementation with fruits of *S. saponaria* increased total bacteria count, and decreased total ciliate protozoa count. The results confirmed that it could be a useful supplement in reducing CH_4 emission per animal without affecting N and energy retention of sheep given tropical grass-concentrate diets alone or in combination with a legume.

Pen *et al.* (2006) evaluated effects of increasing concentrations of *Yucca schidigera* and *Quillaja saponaria* extracts on rumen fermentation and CH_4 production of non-lactating Holstein cows fed oat and alfalfa hay. Methane production was strongly inhibited by *Yucca schidigera* but not by *Quillaja saponaria* extract. This suggests that type and origin of saponins alter rumen fermentation differently. An experiment of Holtshausen *et al.* (2009) was conducted to determine whether the addition of saponin-containing *Yucca schidigera* or *Quillaja saponaria* reduces CH_4 production without impairing ruminal fermentation or fiber digestion. The increasing levels of both saponin sources decreased CH_4 concentration in the space of dairy cows. A lower feeding rate of both saponin sources (10 $g.kg^{-1}$ of dry matter) was used *in vivo*. Feeding saponin did not affect milk production, rumen fermentation, or CH_4 production. However, efficiency of milk production was lower for cows fed saponin compared with controls.

The Hu *et al*. (2005) study was conducted to investigate the effect of tea (triterpenoid) saponins on ruminal fermentation *in vitro* and found that tea saponins significantly inhibited the protozoa growth in ruminal fluid. It is suggested that they could modify the rumen fermentation and inhibit the release of CH_4. Using higher doses of tea saponin (8 mg) on 200 mg of substrates in ruminal fluids, authors recorded a 79 % decrease in protozoan counts and 26 % decline in *in vitro* CH_4 release. It has been suggested that antimethanogenic additives could be used as alternatives to antibiotics in ruminant feeds (Bodas *et al*., 2008).

Tanins: Many plants contain tannins. It is assumed that the biological significance of many types of tannin is related to protection against infection, insects, or animal herbivory. Tannins are polyphenolic secondary metabolites of higher plants (Khanbabaee and Ree van, 2001). The two major groups of plant polyphenolic compounds other than lignin are hydrolysable (polyesters of gallic acid and various individual sugars) and condensed tannins (polymers of flavanoids) (McSweeney *et al*., 2001).

Anotherdefinition is that the plant tannins as water soluble phenolic compounds with a molar mass between 300 and 3000, showing the usual phenol reactions and precipitating alkaloids, gelatins and other proteins (Khanbabaee and Ree van, 2001). Condensed tannins contained in the forages have effects on feed digestibility, depending on the quality and biological activity of the tannins (Schofield *et al*., 2001). Forages containing condensed tannins, which can broadly be classed as rumen modifying agents, do reduce emissions (Clark, 2009a; Clark *et al*., 2011). Sejian and Naqvi (2012) demonstrated that *Bergenia crassifolia, Emblica officinalis, Peltiphyllum peltatum, Populus deltoids, Quercus Incana, Rheum Undulatum, Terminalia belerica, Terminalia chebula* and *Vaccinium vitis-idaea* are some other plants containing high tannin contents and have a potential to inhibit *in vitro* as well as *in vivo* CH_4 emission by the rumen microbes. For tannin-containing plants, the antimethanogenic activity has been attributed mainly to the group of condensed tannins. Hydrolysable tannins are considered more toxic to the animal (Field *et al*., 1989).

Introduction of condensed tannins into diets is a likely target to reduce methane production. Tannins

predominant in *Calliandra calothyrus* reduced nutrient degradation and might have affected methanogenesis. Therefore, tannin-rich legumes such as *Calliandra calothyrsus* could be used in combination with good-quality forage species in order to reduce CH_4 emission from rumen fermentation (Hess *et al*., 2003b; Hess *et al*., 2004; Martin *et al*., 2010). Other results of Hess *et al*. (2006) suggest that extracted tannins as well as tannin-rich legumes can be useful in limiting CH_4 emission without major losses in feeding value of the diet while very tannin-rich shrub legumes such as *Calliandra calothyrsus*, despite being also effective in limiting methanogenesis, are restricted in their application due to the simultaneous depression of the feeding value of the diet. Tiemann *et al*. (2008) reported that the inclusion of the tannin-rich plants reduced CH_4 emission per day and per unit of feed and energy intake in growing lambs by up to 24 %.

Woodward *et al.* (2002) investigated the effect of feeding of Sulla (*Hedysarum coronarium*) on CH_4 emission and milk yield in dairy cows. Cows fed sulla produced less CH_4 per kg DMI (19.5 vs. 24.6 g) and per kg milk solid yield (243.3 vs. 327.8 g). Tavendale *et al*. (2005) evaluated inhibitory effects of extractable condensed tannin fractions from *L. pedunculatus* on the common rumen methanogens *Methanobrevibacter ruminantium*. Results indicate that condensed tannin action on methanogenesis can be attributed to indirect effects via reduced hydrogen production (and presumably reduced forage digestibility) and via direct inhibitory effects on methanogens.

Waghorn *et al*. (2002) recorded significant difference in sheep fed diet with *Lotus pedunculatus* (a condensed tannin containing legume) or pasture (11.5 g $CH_4.kg^{-1}$ DMI vs. 25.7 g $CH_4.kg^{-1}$ DMI). Waghorn *et al.* (2002) observed the impact of condensed tannins on rumen methanogenesis to be small but significant; a 16 % reduction. Woodward *et al.* (2001) showed lower daily CH_4 outputs when sheep were fed *Lotus pedunculatus* than ryegrass-based pasture or lucerne (14.5 vs. 20.4 vs. 19.0 g $CH_4.kg^{-1}$ DMI). Friesian dairy cows fed either *Lotus corniculatus* silage had lower total CH_4 outputs than cows fed perennial ryegrass silage (26.90 vs. 35.13 g $CH_4.kg^{-1}$ DMI). The mitigation of CH_4 emissions from animals fed *Lotus* species was due in part to a higher nutritive value relative to pasture but effects of condensed tannins on methanogenesis warrants further investigation. Experiments with feeding of plants or extracts of condensed tannin-containing *Lotus corniculatus*, *Lotus pedunculatus* and *Acacia mearnsii* reduced CH_4 production in small ruminants (sheep, alpaca, goats) by up to 30 % (Pinares-Patiño *et al*., 2003ab; Puchala *et al*., 2005). Angora goats received the condensed tannin-containing forage *Sericea lespedeza* (17.7 %) or a mixture of crabgrass (*Digitaria ischaemum*) and Kentucky 31 tall fescue (*Festuca arundinacea*). Methane emission was significantly lower for *Sericea lespedeza* than for crabgrass with tall fescue (7.4 vs. 10.6 $g.d^{-1}$; 6.9 vs. 16.2 $g.kg^{-1}$ DMI) (Puchala *et al*., 2005).

There are some studies about the effects of plant extracts on CH_4 production both *in vitro* and *in vivo*. However, research indicated that at least some of the measured decrease in CH_4 production is the result of direct effect of condensed tannins in legumes forages on rumen fermentation (Woodward, 2003; Iqbal *et al*., 2008). The extracts of pods of *Acacia concinna*, seed pulp of *Terminalia chebula*, *Terminalia belerica*, *Emblica officinalis* and seed kernel of *Azadirachta indica* were evaluated for their effect on CH_4 production. The results indicate that the plant extracts appear to have a potential to manipulate rumen fermentation. The methanol extract of the seed pulp of *Terminalia chebula* has antimethanogenic activity and extracts of the pods of *Acacia concinna* and *Azadirachta indica* have defaunating properties (Patra *et al*., 2006).

The De Oliveira *et al*. (2007) study evaluated the effect of diets containing sorghum silages with higher and lower tannin concentrations. There was no effect of tannin levels on digestibility and CH_4 emission. The lower ruminal digestibility of neutral detergent fiber in high tannin silage suggests an inhibitory effect of tannins on ruminal fiber digestibility.

Ionophores: Ionophores (monensin and lasalocid) are antimicrobials which have been extensively investigated for their ability to reduce CH_4 production in ruminants. These compounds are also classed as antibiotics and their use is unacceptable in some locations. Their effect on microbes and protozoa was showed. Ionophores are highly lipophilic polyethers that accumulate in cell membranes and catalyze rapid ion movement (Russell and Houlihan, 2003). Ionophores modulate the movement of cations such as sodium, potassium and calcium across cell membranes. These chemicals also reduce acetate production, they cause a direct inhibition on H_2 from rumen fermentation (Russell and Houlihan, 2003; Sejian and Naqvi, 2012). The effects of ionophores on enteric CH_4 production are related to depress of ciliate protozoal populations (Iqbal *et al*., 2008). Ionophores cause a shift in the rumen bacterial population from gram positive to gram negative organisms, with a concurrent shift in fermentation from acetate to propionate (Callaway *et al*., 1997), reaction is associated with a reduction in methanogenesis (Iqbal *et al*., 2008; Sejian and Naqvi, 2012).

According to Guan *et al*. (2006), both monensin and lasalocid decreased in beef total ciliate protozoal populations by 82.5 % in the first 2 wk and by 76.8 % in the first 4 wk after treatment, respectively. Supplementation of monensin in cattle diets can decrease enteric CH_4 emissions by 27 to 30 % for 2 to 4 weeks, depending on energy content of the diet (Guan *et al*.,

2006). Monensin addition to the diet at 24 ppm significantly decreased CH_4 production compared with cows fed diets without monensin (Sauer *et al*., 1998). Monensin supplementation to both faunated and unfaunated goats also reduced CH_4 production, though the degree of reduction in unfaunated animals was smaller than that in faunated animals (Shibata and Terada, 2010). Callaway *et al.* (1997) wrote that maximum CH_4 inhibition by monensin was 18 % *in vitro*. McGinn *et al.* (2004) reported that monensin tended to lower CH_4 emissions per kilogram of DMI by 8.6 % compared with the control in beef cattle. Russell and Strobel (1989) indicated *in vitro* and *in vivo* experiments that monensin decreases CH_4 production by 30 %, but methanogenic bacteria are not particularly sensitive to ionophores. McCaughey *et al.* (1997) observed no benefit from administration of monensin in term of CH_4 production. The energy lost through breathing of CH_4 averaged 4.5 % of gross energy intake.

Beauchemin *et al.* (2008) reported that strategies such as supplementation with ionophores (>24 ppm) are using by North America farmers because there is a high probability that they reduce CH_4 emissions in addition to improving production efficiency. Unfortunately, the inhibitory effects of ionophores on methanogenesis may not persist over time. Omar (2004) found that CH_4 suppression does not persist beyond two weeks (16 days) of ionophore treatment in beef steers. Also Iqbal *et al.* (2008) noted that cattle studies have shown that ionophore-induced suppression of enteric CH_4 production is short lived. Methane emissions of sheep and cattle can be reduced by about 18 % through the use of ionophores in the short term but there are indications that methanogens as well as other members of the microbial population can adapt to their presence (Mathison *et al*., 1998)

Use of ionophores in animal feed has a significant impact on the transfer of antibiotic resistance from animals to man (Russell and Houlihan, 2003). Therefore, ionophores supplement in livestock production is not permitted in EU after the ban on growth promoters in January 2006 (Benchaar and Greathead, 2011).

Organic acids: Organic acids (malate, fumarate, acrylate, and others) have been shown to be the most effective against CH_4 *in vitro* trials (Martin *et al*., 2010). Dicarboxylic organic acids (malate, fumarate) are potential precursors of propionate which stimulate H_2 utilization (Song *et al*., 2011). They can be an alternative sink for metabolic hydrogen and reduce the amount of hydrogen used in CH_4 formation (Lila *et al*., 2004a; McGinn *et al*., 2004; Newbold *et al*., 2005).

Feeding of dicarboxylic organic acids to improve propionate production is other mechanisms for CH_4 inhibition. It was concluded that especially sodium fumarate may be a useful dietary additive for ruminants, because it diverts some H_2 from CH_4 production and because it is able to stimulate proliferation of cellulolytic bacteria and digestion of fibre (Lopez *et al*., 1999). Fumarate and acrylate to be the most effective in *in vitro* trials (Mohammed *et al*., 2004; Newbold *et al*., 2005). The addition of fumarate and malate increased propionate production and reduced CH_4 output. The increasing dietary malic acid led to a decrease of protozoa numbers and total daily CH_4 emissions by 16 % (Foley *et al*., 2009).

Bayaru *et al.* (2001) also demonstrated by *in vivo* studies the potential effects of dicarboxylic acids on CH_4 output. These authors concluded that the dietary fumarate supplementing would have a beneficial effect via decreased methanogenesis, increased propionate production and stimulation of fibre breakdown by rumen microorganisms. The inclusion of dietary additives contained calcium fumarate decreased CH_4 emissions in dairy cows by 10 % (Zijderveld van *et al*., 2011). According to Carro and Ranilla (2003), addition of fumarate did not affect the total gas production in *in vitro* trial. If experiments are done *in vivo*, fumarate could be used as a feed additive for ruminant animals fed high proportions of cereal grains, because it increased pH, acetate and propionate production and it decreased CH_4 production (Carro and Ranilla, 2003).

The opposite of response to organic acids *in vitro*, responses to dietary supplementation *in vivo* were inconclusive and highly variable. McGinn *et al.* (2004) reported that fumaric acid was not effective in reducing CH_4 losses of beef cattle *in vivo*. Newbold *et al.* (2002) reported a dose-dependent response to fumarate in sheep. The effect of fumaric acid - bentonite coupled addition on rumen fermentation efficiency would improve the impact of fumaric acid on rumen fermentation pattern and can be appropriate alternative for antibiotic feed additives in improving ruminants feed efficiency (Abdl-Rahman, 2010). According to Martin (1998), malate was also effective in reducing the drop in ruminal pH normally seen 1 to 2 h after feeding a high-grain diet and improved cattle performance. Therefore, supplementing finishing diets or high-producing dairy cattle diets with malate might be effective in reducing subclinical acidosis.

An exceptional decrease in CH_4 production, up to 75 %, has been shown with 10 % encapsulated fumarate in the diet of lambs without negative effect on animal growth (Wallace *et al*., 2006; cited by Martin *et al*., 2010). Also in the Wood *et al*. (2009) study were encapsulated fumaric acids effective. *In vivo*, growing lambs on a concentrate diet with straw *ad libitum* produced 24.6 $L.d^{-1}$ of CH_4, whereas a 100 $g.kg^{-1}$ addition of fumaric acid encapsulated in partially hydrogenated vegetable oil or encapsulated fumaric acid decreased significantly CH_4 production to 9.6 and 5.8 $L.d^{-1}$, respectively. The 76 % decrease in CH_4 is among the largest reported in the literature to date. In contrast,

encapsulated fumarate had no significant effect in another trial in dairy cows (McCourt *et al*., 2008; cited by Martin *et al*., 2010).

Fumaric acid is a precursor of propionic acid in the fermentation of feed in the rumen and can act as an alternate sink for consumption of hydrogen generated in the rumen. The levels of fumaric acid required to inhibit methanogenesis to a significant extent may cause a drop in pH which might affect feed fermentation adversely. Free fumaric acid (10 % in the ration) and an equivalent amount of encapsulated fumaric acid decreased CH_4 emission to the extent of 49 % and 75 % compared to control sheep without supplementation of fumaric acid (Agrawal and Kamra, 2010, cited by Sejian and Naqvi, 2012).

Rumen additives have shown some success *in vitro* but results from *in vivo* trials with fumaric acid have been disappointing. Fumaric acid caused potentially beneficial changes in ruminal fermentation but no measurable reductions in CH_4 emissions (Foley *et al*., 2009). Fumarate supplementation for an extended period may result in the amplification of small populations of fumarate-reducers (Ungerfeld *et al*., 2007). The inclusion of malate as a feed additive into the diets of ruminants is currently not economically feasible (Martin, 1998). The following constraints are associated with the use of dicarboxylic acids. They are expensive chemicals. They are not suitable for grazing animals as they have to be fed daily. And finally, their efficiency is reduced when concentrates are fed, as evident from *in vitro* trial in which the efficiency was only 4.8 % (Iqbal *et al*., 2008).

Lipids: Generally, lipids cause depressive effect on CH_4 emission by protozoal inhibition, reduction of activity of ruminal methanogens, protozoal numbers, and double bonds in unsaturated fatty acids, and enhanced propionate production (Hristov *et al*., 2013b). Lipid supplementation can reduce methanogenesis without negatively affecting total digestibility and ruminal pH as opposed to concentrates (Hook *et al*., 2010; Sejian *et al*., 2011b). Pinares-Patiño *et al.* (2016) concluded that enhanced dietary lipids contents is an effective means of reducing CH_4 emissions from grazed pasture.

Fat reduces the fermentable substrate and can lower organic matter and fibre degradability. Short-term additives as dietary oils or lipids can be added to reduce CH_4 emissions by decreasing fermentation in the gut and reducing the activity of the micro-organisms in the gut. Fat supplementation can serve as an important energy source in diets of high producing ruminants; however, excessive fat addition will depress fiber degradation in the rumen (Patra, 2014).

The study of Alstrup *et al*. (2015) followed the effect of fat on CH_4 production throughout the lactation, and showed that the mitigation effect of fat on CH_4 production is persistent, and that CH_4 production increases with days in milk. Moate *et al.* (2011) found that for each 10 $g.kg^{-1}$ DM increase in dietary fat concentration were enteric emissions reduced by 0.79 g $CH_4.kg^{-1}$ DMI, or 3.5 %. Martin *et al.* (2010) reported a decrease in CH_4 of 3.8 % with each 1% addition of supplemental fat. A CH_4 decrease of 30 % has been observed when 12 % tallow was added to the diet (Van der Honing *et al*., 1983). Dietary supplementation with fat is the most promising dietary strategy, but the milk production and composition response to supplementary fat is complex and differs among diets. It is also affected by stage of lactation, degree of saturation of the added fat, amount of fat added, and the fat content and composition of the basal diet. For cattle, a 10 g/kg increase in dietary fat decreased CH_4 yield by 1 $g.kg^{-1}$ DMI, but for sheep the decrease was 2.6 $g.kg^{-1}$ DMI (Grainger and Beauchemin, 2011).

Essential oils are naturally occurring plant secondary metabolites, which exhibit antimicrobial activities against gram-negative and gram-positive bacteria, a property that has been attributed to the presence of terpenoid and phenolic compounds (Meale *et al*., 2014). Some essential oils, especially their organosulphur compounds, are capable to affect rumen fermentations and decrease CH_4 production *in vitro* (Busquet *et al*., 2005; Macheboeuf *et al.,* 2006; McAllister and Newbold, 2008). *Allium sativum, Coriandrum sativum, Eucalyptus globules, Foeniculum vulgare, Mentha piperita, Ocimum sanctum, Populus deltoids, Juniper berry, Syzygium aromaticum, Thymus vulgaris, Origanum vulgare, Cinnamonum cassia*, *Rheum rhabarbarum*, *Armoracia rusticana*, and *Rhamnus frangula* are some of the plants which contain high concentration of essential oils and are effective against CH_4 emission and protozoa growth in the rumen (Benchaar and Greathead, 2011). In the Busquet*et al*. (2005) *in vitro* study, treatments of garlic oil, diallyl disulfide, and allyl mercaptan resulted in a decrease in CH_4 production of 73.6, 68.5, and 19.5 %, respectively, compared with the control. However, inhibition of CH_4 production occurred at high doses was associated with a decrease in total volatile fatty acid concentrations and feed digestion (Benchaar and Greathead, 2011). Meale *et al*. (2014) demonstrated *in vivo* experiments that feed ration supplementation with garlic oil (5 $g.d^{-1}$) and juniper berry oil (2 $g.d^{-1}$) had no effects on enteric CH_4 emissions in lactating dairy cows whether expressed as $g.d^{-1}$, $g.kg^{-1}$ DMI, $g.kg^{-1}$ of milk or as $g.kg^{-1}$ DMI.body $weight^{-0.75}$.

The positive effects of fatty acids on CH_4 reduction through their toxicity to methanogens have also been demonstrated (Sejian and Naqvi, 2012). Unsaturated fatty acids serve as electron acceptors during biohydrogenation, causing this depression in CH_4 production. Supplementation of fatty acids to the feed decreases CH_4 production (Martin *et al*., 2008; Iqbal *et*

al., 2008; Brask *et al*., 2013), and it would be one of the dietary strategies to abatement of CH_4 production (Hristov *et al*., 2013a). However, the effect may not last long, it is dependent on fatty acids composition. Medium-chain fatty acids are the more depressive. The study of Beauchemin *et al*. (2009) shows that adding sources of long-chain unsaturated fatty acids to the diet in the form of processed oilseeds can be an effective means of reducing CH_4 emissions.

Saturated acids, lauric and myrstic acid, are particulary toxic to methanogens from bovine ruminal fluid (Sejian and Naqvi, 2012). Myristic acid inhibited significantly rumen archaea without significantly altering proportions of individual methanogen orders (Machmüller *et al.*, 2003). According to Soliva *et al.* (2004), these acids taken alone have similar effects, but a combination between these two acids has a clear synergistic effect leading to a sharp decrease in CH_4. The most effective mixture of lauric and myristic acid, when supplemented to a complete ruminant diet, was the 4:1 rate. It decreased CH_4 release by about 70 % *in vitro*.

Addition of oils to ruminant diets may decrease CH_4 emission by up to 80 % *in vitro* and about 25 % *in vivo* (Machmüller *et al*., 2000; Fievez *et al*., 2003; Singh, 2010).The effects of different oils on ruminal methane fermentation are related to diet composition. Infusion trials of these unsaturated acids (oleic, linoleic and linolenic) showed the greater the depression of CH_4 production (Shibata and Terada, 2010). Linolenic acid has been shown to have a higher effect on CH_4 than linoleic acid *in vitro* (Newbold *et al*., 1996, cited by Martin *et al*., 2010; Jouany *et al*., 2008, cited by Martin *et al*., 2010). In Martin *et al.* (2008) four weeks study they demonstrated that a 5.7 % additivum of lipids from *Linum usitatissimum* seed decreases the quantity of CH_4 emitted daily by dairy cows, with a marked effect of the physical form of *Linum usitatissimum* seed. All the forms of linseed fat (polyunsaturated) acids significantly decreased daily CH_4 emissions but to different extents (−12 % with crude linseed, −38 % with extruded linseed, −64 % with linseed oil) compared with control group. The addition of dietary oil (6 % of DMI) from refined soy oil decreased (40 %) CH_4 output in young bulls (Jordan *et al*., 2006a).

Sunflower seed which is rich in linoleic acid had a similar depressive effect as coconut oil on CH_4 production, and this effect was higher than rapeseed (rich in oleic acid), and especially than linseed (with the amount oflinolenic acid) *in vitro* treatment (Machmüller *et al.,* 1998). Coconut (Cocos nucifera) oil, containing mostsaturatedacids, completely eliminated protozoa from rumen fluid after four to nine days of application, and this period was shorter by more than 30 % on average at the higher level of supply. As compared with the low-fat treatment, coconut oil suppressed CH_4 by 43 % with medium and 57 % with high concentrate level. The maximum CH_4 reduction with sunflower seed and lin seed accounted for about 40 % (Machmüller *et al.,* 1998). McGinn *et al*. (2004) observed a lower acetate concentrations, higher propionate concentrations and lower acetate to propionate ratio when sunflower oil was added to the diets of cattle. They reported a 22 % decrease in CH_4 emissions by addition of sunflower oil to the diet of cattle.

Beauchemin *et al*. (2009) added crushed sunflower seeds, crushed flaxseed, and crushed canola seed to the diets of lactating dairy cows (supplying 3.7 % added fat). All 3 oilseed treatments reduced daily CH_4 production ($g.d^{-1}$) by an average of 13 % compared with a control diet containing calcium salts of palm fat acid (long-chain fatty acid). However, the abatement effect of fat acids supplementation on CH_4 production was not observed in other studies on dairy cows. According to Johnson *et al*. (2002), supplementation of diets with oilseeds did not affect CH_4 emissions but tended to increase the efficiency of milk produced per unit of methane emitted. Cosgrove *et al*. (2008) infused linseed and sunflower oils (3:1) directly into the rumen of 8 month old wether sheep. Sheep tolerated additional oil up to 5 % of DMI, but when 6.2 % was given, feed intakes declined and this treatment was discontinued. Up to 5 % oil infusion did not affect CH_4 production.

Machmüller *et al*. (2000) evaluated on growing lambs diets consisted of maize silage, grass hay and concentrate which was supplemented with the respective lipid source. On average, the five lipid-supplemented diets contained 56 g ether extract per kg dry mater. Coconut oil (medium-chain fatty acids) supplementation reduced significantly CH_4 release per kg live weight by 26 % compared to control, and with the use of rapeseed, sunflower, and linseed seed the relative reduction was 19 %, 27 % and 10 %, respectively. At the study of Kumar *et al.* (2007) eight feeds (mixture of wheat straw and oil seed cakes in 3:1 ratio) were evaluated for CH_4 emission and fermentation pattern with buffalo rumen liquor in an *in vitro* gas production test. The cakes tested were groundnut, soybean, mustard, cotton, karanj, and caster bean. Among the oil cakes, mustard seed cake-based feed produced the minimum CH_4 without affecting other fermentation characteristics adversely.

The efficiency of different oils is also different. Machmüller *et al*. (1998) identified coconut oil as more effective inhibitor than rape seed (canola, rich in oleic acid), sunflower seed (rich in linoleic acid), and linseed oil (rich in both linoleic and linolenic acid). They suggested that coconut oil eliminated protozoa from rumen fluid after four to nine days of application and directly inhibited rumen methanogens, probably by changing their metabolic activity and composition. As compared with the low-fat treatment, coconut oil suppressed methane by 43 % with medium and 57 % with high concentrate level. The methane suppressive effect of

6 % and 9 % total dietary fat was about 40 % and 60 % with coconut oil and about 20 % and 40 % with whole crushed oilseeds rich in polyenoic acids, respectively.

Conclusions: Decreasing CH_4 emissions has become a priority and an integral part of climate control. This review has identified a number of techniques that will result in reduced CH_4 emissions when implemented at a commercial scale. However, the development of more methods is at an early stage of work. Also, the cost of some strategies is likely to be too expensive for commercial husbandry.

More chemicals are used to reduce the formation of CH_4. Direct inhibition of CH_4 generation, vaccination, and probiotics are the promising approaches for future research. But, the use currently available strategies of defaunation are not a permanent solution of ruminant CH_4 due to microbial adaptation. Developing vaccines which stimulate ruminants to produce antibodies against their rumen methanogens may be feasible in principle but the successful development of a vaccine is still a long way off.

Addition of halogenated methane analogues to ruminant diet offers the opportunity to reduce CH_4 production in cattle but rumen microbes can adapt to them. The use of probiotics remains a potentially interesting, but results have been either unsatisfactory, not conclusive, or have yet to be confirmed *in vivo*.

In case of ionophores, many chemical compounds have been tested as additives for ruminants. The monensin has been found to reduce enteric CH_4 emissions. However, the effects are variable and the inhibitory effects on methanogenesis may not persist over time. Also, use of ionophores in livestock production is not permitted in the EU after the ban on growth promoters. Prophylactic effects of miscellaneous compounds should be developed instead of ionosphores.

Most positive reports concern the fat, essential oils, tannins, saponins, and plant extracts. Some essential oils may exert a direct effect on methanogens. Polyunsaturated fatty acids contribute to CH_4 decrease through a toxic effect on cellulolytic bacteria and protozoa. Dietary fat seems a promising nutritional alternative to depress ruminal methanogenesis without affecting other ruminal parameters. Dietary lipids can be effective in reducing CH_4 emissions, but their applicability will depend on effects on feed intake, fiber digestibility, production, and milk composition. This review has shown that addition of fat to the diet can result in a persistent decrease in CH_4 emissions, and not lower animal production. However, the addition of oils to feed may depress dry matter intake and fiber digestibility.

The response to tannin and saponin is highly variable and more research is needed to assess the effectiveness and eventual presence of undesirable residues in animal products.

Possible effects include also plant extracts and improved yeast products. The use of plant extracts is receiving a renewed interest as they are seen as a natural alternative to chemical additives and are well perceived by consumers. However, a limited number of studies have investigated, and it is difficult to provide a comprehensive assessment at this stage about the size of decrease that might be realistically expected *in vivo*.

In summary, this study has shown that CH_4 abatement can be possible. The several interventions for reducing CH_4 emission by ruminants were described. However, there is a need for in vivo investigation of proposed manners. Practical methods of reducing protozoa in the rumen are required.

Acknowledgements: This article was possible through projects APVV of the Slovak Research and Development Agency Bratislava (0632-10 and 15-0060), also by the projects AGB 313011 and CEGEZ 26220120073 supported by the Operational Programme Research and Development funded from the European Regional Development Fund.

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