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REVIEW

Effect of Dietary Plant Secondary Metabolites on Rumen Fermentation and Microbial Community: A Review

P.S. Banakar, Srobana Sarkar, Bhawna Tyagi, V.V. Vinay, Timmi Chugh, Sachin Kumar, Nitin Tyagi and A.K. Tyagi*

Rumen Biotechnology Lab, Animal Nutrition Division, ICAR- National Dairy Research Institute, Karnal-132001, Haryana, India

ABSTRACT

Plants are a significant source of novel medicinal products and secondary metabolites are a distinctive source of pharmaceutical food additives, flavours and other industrial commodities. These plant secondary metabolites are found to manipulate rumen fermentation process by altering the population of microbial community. Changes in microbial population has further aided in limiting the biohydrogenation process of fatty acids and production of methane. Biohydrgenation of the ingested fatty acids results in saturated fatty acids produced at the cost of unsaturated fatty acids. Animal science advocates are trying various feasible approaches to influence ruminal biohydrogenation processes to achieve ruminant-derived products with reduced saturated fatty acids quantity, which would be of significant value to the wellness of consumers. Dose dependent action of plant secondary metabolites on rumen microbes is not completely investigated. Under the present scenario it would be most apt to explore the potentials of phytochemicals as modulators of ruminal biohydrogenation vis-a-vis most other supplements, such as oil or oil-rich grains.

Keywords: Biohydrogenation, Methane, Plant secondary metabolites, Rumen microbes

INTRODUCTION

At the moment “one health concept” has drawn attention given that, it is an integrative effort of various disciplines working locally, nationally, and globally to attain optimal health for people, animals, and the environment. Scientists, research institutes, food and pharmaceutical industries around the globe are fine tuning their research to identify new sources of natural compounds with fascinating properties that can replace antibiotics or synthetic compounds to improve feed efficiency and promote livestock growth without compromising on the safety of end consumers. Plant secondary metabolites (PSM) such as tannins, saponins and polyphenols are now being extensively studied for their ability to manipulate the rumen fermentation and thereby influencing the performance of animals and the composition of meat and milk products (Wallace, 2007). Manipulation of rumen fermentation depends on the sources and dosage of secondary metabolites in the animal diet. Research reports suggest that at suitable doses, PSM suppresses protozoal populations, increases bacterial and fungal populations, propionate production, microbial yield and decreases methanogenesis to improve the productive performance in ruminants and this change in the fermentation pattern are mainly attributed to their antimicrobial and antioxidant activity that could help to maintain the animal health. Even though ruminant’s diets are rich in poly unsaturated fatty acids (PUFA), their products such as meat, milk and milk products contain mainly saturated fatty acids (SFA) because of the extensive biohydrogenation of ingested PUFA in the rumen.

Modification of the biohydrogenation process plays key role in getting better fatty acid profile in animal products (Durmic *et al.,* 2008; Vasta *et al.,* 2008). This could be achieved either by modifying the rumen environment, changing the competition among different microbial populations during fermentation, augment or reduce specific microbial populations, or target biohydrogenating enzymes through dietary

*Corresponding author: amrishtyagi@1963yahoo.com

manipulation by using feed additives (Khiaosa-Ard *et al.,* 2009).

PLANT SECONDARY METABOLITES AS FEED ADDITIVES

The activity and concentration of phytochemicals in crops is affected by geographic places with varying weather conditions, year-round collection time, sample handling and storage (Bodas *et al.,* 2008). These phytogenic feed additives modify ruminal fatty acid biohydrogenation by exerting antimicrobial effect on biohydrogenating organisms in the rumen (Durmic *et al.,* 2008), accumulating beneficial intermediates (Vasta *et al.,* 2008) and manipulating multiple enzymes engaged in biohydrogenation (Miri *et al.,* 2015), thereby influencing the fatty acid profile of milk and meat. Miri *et al.* (2015) noted that cumin (*Cuminum cyminum*) seed extract (CSE) supplementation increased CLA ruminal concentration by 34.8% and Vaccinic acid (VA) by 11.4%, along with the synchronized rise in CLA and linolenic acid concentration and decline in goat milk stearic acid content. Similar outcomes were recorded in ewes grazing sulla (CT: 2.6% DM) with or without PEG supplementation, where Ruminic acid and VA were smaller, whereas C18:2 n6 and C18:3 n3 were greater than ewes milk receiving sulla + PEG in sulla-fed milk (Cabiddu *et al.,* 2009). However, most research on plant secondary metabolites (PSM) and ruminal bio-hydrogenation were performed through *in vitro* research, while the literature's *in vivo* works are limited.

PSM SUPPLEMETATION ON RUMEN FERMENTATION

PSM and Rumen Biohydrogenation

Recently, phytogenic feed additives have been a major interest of research among ruminant nutritionist. The phytochemicals or PSM are groups of chemical compounds in plants that are not primarily involved in the primary biochemical processes such as plant growth, development and reproduction. These PSM in plants produce a line of defense which ensures survival of the plant structures and reproductive elements by protecting against insect predation. Earlier, these PSM were considered as anti-nutritional factors in animal nutrition because of their antibacterial properties and adverse effects on nutrient utilization. However, recent researches have shown that plant secondary metabolites have potential to modify rumen fermentation favourably, at a relatively low concentration. At appropriate doses, phytobiotics, have suppressed protozoal populations, increased bacterial and fungal populations, propionate production, microbial yield, and decreased methanogenesis to improve the productive performance in ruminants (Hristov *et al.,* 1999; Patra *et al.* 2006; Benchaar *et al.* 2007). Lately, the phytogenic feed additives rich in secondary metabolites are being studied thoroughly with an aim to improve the fatty acid profile in milk and meat by manipulating the process of rumen biohydrogenation.

In vitro incubation of tropical (Jayanegara *et al.,* 2011) and alpine plants (Jayanegara *et al.,* 2012) with additional linseed oil suggest the ability of plant phenolics in modulating fatty acid biohydrogenation by decelerating the process right from the first step. This was indicated by lower disappearance of C18:3 n3 and C18:2 n6 in the incubations of both tropical and alpine plants containing high phenolics in the respective studies. In relation to ruminal biohydrogenation of fatty acids, tannins have been reported to reduce PUFA biohydrogenation, accumulate *trans-11* C18:1, decrease concentration of C18:0 (Jayanegara *et al.,* 2012) and increase the transfer rate of C18:3 n3 from feed to milk (Kalber *et al.,* 2011).

Cabiddu *et al.* (2010) observed a negative relationship between tannins (present in *Vicia sativa* and *Trifolium incarnatum)* and biohydrogenation of C18:3 n-3, appearance of C18:0 and positive correlation of appearance of C18:2 *cis*-9 *trans*-11 *in vitro*. Furthermore, Khiaosa-ard *et al.* (2009) in an *in vitro* study observed a considerable increase of *trans-11* C18:1 at the expense of C18:0 in rumen fluid by adding *Acacia mearnsii* extract (source of CT) at 79 g/kg DM to a grass-clover hay diet supplemented with linseed oil and concluded that tannins inhibited the terminal step of FA biohydrogenation. Also Durmic *et al.* (2008) reported that when extracts from *Acacia iteaphylla* (containing condensed tannins) were incubated *in vitro* with sheep ruminal fluid the production of VA increased while SA decreased.

Addition of *Chrysanthemum coronarium* (@ 5 mg/reaction) and LA to sheep rumen digesta under *in vitro* conditions inhibited ruminal biohydrogenation due to active principle coronaric acid, resulting in increased accumulation of VA with subsequent reduction in stearic acid (Wood *et al.,* 2010). Smilarly, Vasta *et al.* (2009) studied the effects of extracted tannins from carob (*Ceratonia siliqua*), acacia leaves (*Acacia cyanophylla*) and quebracho (*Schinopsis lorentzii*) on *in vitro* rumen biohydrogenation of cow rumen fluid incubated with hay as a substrate. Three concentrations of tannins were added to the substrate (0·0, 0·6 and 1·0 mg/ml RF). In tannin-containing fermenters, vaccenic acid was accumulated (23%) while stearic acid was reduced (16%) and concentration of total conjugated linoleic acid isomers and linoleic acid isomerase (LA-I) activity (nmol CLA produced/min per mg protein) was not affected by tannins.

Lourenco *et al.* (2009) also observed that essential oils rich in monoterpenes (limonene and carvone) resulted in the accumulation of *cis-9, trans-11* CLA in ruminal fluid. Miri *et al.* (2015) reported that dietary supplementation of (*Cuminum cyminum*) seed extract (CSE) in goats (at 1.27% and 2.53% of dry matter intake) increased the ruminal concentration of CLA (C18:2 *cis-9, trans-11*) by 34.8% and VA (C18:1 *trans-11*) by 11.4% in lower supplemented group as compared to CSE free diet. Furthermore, in the same study linoleate isomerase activity (LA-I) of the only stearate forming bacterium, *B. proteoclasticus* was decreased by 53% in the presence of the CSE (4.25 g CSE/ml).

PSM and biohydrogenating microbes

The effect of plant extracts or its secondary metabolites on biohydrogenating bacterial species are variable and it depends on the nature of the plant secondary metabolites. Studies on pure cultures of bacteria showed that *Yucca schidigera* extract (1% v/v) inhibited the growth of *B. fibrisolvens* and *Streptococcus bovis,* while the growth of *Prevotella ruminicola* was increased and the growth of *Selenomonas ruminantium* Z108 was unaffected (Wallace *et al.* 1994). Sivakumaran *et al.* (2004) found that proanthocyanidins fractions from *Dorycnium* with high molecular weight inhibited the growth of *B. fibrisolvens* CF3, while low molecular weight fractions did not interfere with bacterial growth.

Some plant extracts and essential oils from Australian plants have been reported to have a selective inhibitory effect on *B. proteoclasticus* without affecting *Butyrivibrio fibrisolvens,* and some can inhibit the saturation of C18:2 n6, cis-9, trans-11 C18:2 and t11 C18:1 *in vitro* (Durmic *et al.* 2008). Wood *et al.* (2010) observed that addition of *Chrysanthemum coronarium* to pure culture of *B. proteoclasticus* (stearate forming bacteria) inhibited its growth. Furthermore, *C. coronarium* also reduced the rate of LA metabolism. Vasta *et al.* (2010) reported that supplementation of tannins in sheep increased the total protozoa and the relative abundance of *B. fibrisolvens* and decreased the relative abundance of *B. proteoclasticus* in the rumen. Ruminal infusion of garlic oil (GO, 0.8 g/d) in rumen of wethers reduced the relative abundance of *Butyrivibrio proteoclasticus* whereas, GO has no effect on the population of *Butyrivibrio* group of bacteria (Zhu *et al.* 2012). Moreover, defaunation by other compounds also increased some *Butyrivibrio spp.* (Karnati *et al.* 2009). Jafari *et al.* (2016) observed that supplementation of papaya leaves extract (methanolic) at concentrations of 5, 10 and 15 mg/250mg dry matter (DM) reduced the population density of *B. fibrisolvens* as compared to control in buffered rumen fluid after 24h incubation.

PSM and methane production

Manipulation of rumen fermentation is desired to enhance the digestibility of low-quality feed, decrease the production of methane, acetate propionate ratio to enhance animal feed use and decrease greenhouse gas concerns. To accomplish these objectives, the use of plant extracts as feed additives is a favourable choice. Tropical plants or their extracts have a wide variety of secondary metabolites such as saponins, tannins and essential oils (Patra and Saxena, 2009). Plants comprising large quantities of saponins can reduce methane by inhibiting or eliminating protozoa from the rumen (Newbold *et al.,* 1997; Kamra *et al.,* 2000, Goel *et al.* 2008).

Tannins have also been observed to decrease the output of methane, although their efficiency depends on the type and amount of secondary metabolite (Hess *et al.,* 2003; Patra *et al.,* 2008). Plant extracts with elevated flavonoids decreased the production of methane and enhanced microbial biomass owing to enhanced degradability of organic matter (Broudiscou *et al.,* 2002). The reduction in methane is likely due to either a decrease in protozoal numbers and/or methanogenic archaea. Supplementation of saponin, from *Yucca schidigera* plant up to 77 ppm of feed reduced protozoal numbers, and increased bacterial population and organic matter digestion *in vitro* continuous culture systems (Valdez *et al.,* 1986).

Patra *et al.* (2006) assessed the impacts of *Acacia concinna* (Shikakai), seed pulp of *Terminalia chebula* (harad), *Terminalia belerica* (bahera), *Emblica officinalis* (amla) and seed kernel of *Azadirachta indica* (neem seed) extracts in various solvents (ethanol, methanol and water) on methane production and rumen fermentation in *in vitro* gas production tests. Among the extracts, methanolic extract of *T. chebula* suppressed *in vitro* methane production. Total volatile fatty acids (TVFA) were reduced with extracts of *T. chebula* and *A. indica*. Furthermore, *in vitro* dry matter and organic matter degradabilities of feed (g/g) were reduced with all the extracts as compared to the control. A study by Agarwal *et al.* (2008) found a dose-dependent anti-protozoal and anti-methanogenic activity of peppermint oil and eucalyptus oil, with the largest rate of peppermint oil (150 ìl/30 ml of incubation medium) reducing the output of methane by 93 % relative to control.

Methanogenesis inhibition was followed by a significant reduction in feed degradability and production of volatile fatty acids. Incubation of tea saponin (TS) at concentrations of 0, 2, 4, 6 and 8 mg versus 200 mg of corn meal and grass meal (1/1, w / w) in rumen fluid boosted the production of gas and microbial biomass with subsequent decrease in methane, ammonia nitrogen and protozoa. However, concentration of total and individual volatile fatty acids remained unchanged with tea saponins (Hu *et al.,* 2005). Patra *et al.* (2006) conducted an *in vitro* experiment with spices that showed ($P<0.05$) reduction in methane emission by adding ethanol and methanol extracts of fennel, clove and garlic and observed maximum inhibition (83%) with methanol extract of clove. Further, there was an increase in gas production along with total volatile fatty acid with the addition of garlic and clove extracts. Likewise, Kim *et al.,* (2012) studied the effects of plant extracts (wormwood, garlic, onion, ginger, mandarin orange, honeysuckle) on *in vitro* methanogenesis and rumen microbial diversity. Total gas production was the highest for ginger extract, whereas, methane (ml / g DM) was the highest for control, but lowest for garlic extract. With the addition of plant extracts, the concentration of VFAs and pH were not impacted. However, in the garlic and ginger extracts, the ratio of acetate to propionate was lower than that of the control.

Tan *et al.* (2011) performed a test using various proportions of purified condensed tannins (CT) obtained from *Leucaena leucocephala* hybrid-Rendang (LLR) for their impacts on *in vitro* fermentation. For *in vitro* gas production technique, purified CT levels of 0 (control), 10, 15, 20, 25 and 30 mg were used. Total gas (ml / g DM) and VFA concentration (mmol/l) decreased at a decreasing rate with increased CT inclusions. Kim *et al.* (2016) observed that *in vitro* incubation of *Arisaema ringens* extract with buffered rumen liquor reduced methane production by more than 43 %, increased TVFA concentration without a change in ruminal pH and digestibility of dry matter.

CONCLUSIONS

Studies reviewed here show that plant secondary metabolites influence the profile of meat and milk fatty acids through modulation of ruminal biohydrogenation. It would be more appropriate to use phytobiotics as modulators of ruminal biohydrogenation than most other supplements, such as oil or oil-rich grains. Plant secondary metabolites appear to have an anti-methanogenic activity and defaunating properties, favoring lower methane production. Contradictory findings on depression in digestibility of organic matter require further studies to evaluate the ideal amount of supplementation of secondary plant metabolites that enable the production of methane to be mitigated without compromising animal performance.

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