

Modifying gut microbiomes in large ruminants: Opportunities in non-intensive husbandry systems



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Implications

- On supplementation of low quality roughages with nitrogen and minerals, high productivity gains are obtained as a result of rumen optimization.
- Better understanding of the microbial diversity, interactions among microorganisms in the rumen, and interactions between rumen microorganisms and the host using nucleic acid-based technologies and DNA sequencing along with other omic technologies during the rumen optimization process will help by developing effective feeding strategies.
- This approach will also help in the development of strategies for obtaining consistent and sustained effects of feed additives such as probiotics and enzymes, many of which currently produce effects that are inconsistent and not long term.

Key words: activators, detoxification of phytotoxins, feed additives, microbial fermentation, non-intensive system, rumen, rumen optimization

Introduction

The microorganisms in the ruminant digestive tracts have a profound influence on the conversion of feeds, especially when they are rich in fiber, into end-products that are used for maintenance and production, and, thereby, affect the transfer of nutrients to animal products as well as to the environment. Currently, more than 50% of ruminant meat and 60% of milk is produced from smallholder ruminant systems (also termed as non-intensive systems or low-input systems), which are mainly fed on poor quality forages (PQFs), including crop residues. This article demonstrates that large gains in animal productivity and a decrease in environmental pollutants in non-intensive systems can be achieved by optimizing the rumen microbial fermentation of ruminants fed PQF diets. Against this backdrop, we suggest that obtaining a better insight into the rumen microbial ecology and diversity of microorganisms in the rumen, during the shift from a poorly functioning rumen that generally occurs in the field when farmers feed a PQF-based diet to a well-functioning rumen achieved

as a result of nutrient supplements, could pave the way for developing other feeding strategies that are needed to enhance the use of both the feed base and other natural resources, particularly in developing countries. These advancements could underpin the development of sustainable feeding strategies required for sustained growth of the livestock sector, especially in the light of the food-fuel-feed competition, which is required to meet the increasing demand of livestock products in the future. Development of these strategies is critical, as a 60–70% increase in consumption of livestock products by 2050 is projected (Makkar, 2016).

Importance of Optimization of Rumen Fermentation

Ruminants have the ability to convert human-inedible PQFs, including crop residues and non-protein nitrogenous (NPN) compounds, into highly nutritious meat and milk, as well as to wool. The continuous rise in human population, urbanization, and income growth are increasing the demand of these products. Therefore, efforts must be made to increase ruminant productivity by using an array of approaches, including manipulating the rumen ecosystem.

In the non-intensive production systems, the PQFs form a major component of the diet and are generally used as a basal diet. When fed alone, these feeds do not favor the microbial growth in the rumen because they are deficient in nitrogen and macro- and micro-minerals such as sulfur, copper, and zinc, among others. Supplementing the PQFs with legumes (Galindo and Marrero, 2005), urea, or urea-molasses multivitamin blocks (UMMB) (Makkar et al., 2008) leads to an increase in microbial populations and a fermentation profile in the rumen that is more favorable for microbial protein synthesis (Wanapat, 2000). It is noteworthy that more than 90% of cellulolytic bacteria require ammonia-N for their proliferation (Currier et al., 2004). As a result, fermentation of crop residues with urea improves fiber digestibility in the rumen (Bakshi et al., 1986, 1987). Microbial shifts in the rumen in most of such studies have been assessed using conventional culture-based techniques (Orpin and Joblin, 1997; Williams and Coleman, 1998; White et al., 1999). However, studies such as those of Tajima et al. (2001) and Zhang et al. (2015) evaluated the diet-dependent shifts in the microbial population of the rumen using real-time PCR and showed that the microbes in the rumen were sensitive to the dietary carbohydrate composition. In these studies, shifts from a hay to a high-grain diet resulted in an increase in the 16S rrs gene copy numbers of *Streptococcus bovis*, *Prevotella bryantii*, and *P. ruminicola*

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and a decline in *Fibrobacter succinogenes* population. The population of *Ehrlichia ruminantium* dropped 14-fold and that of a rumen spirochete, *Treponema bryantii*, decreased by sevenfold, and stabilized by 28 d after the diet change. Using quantitative PCR, Mosoni et al. (2007) showed significant decreases in *F. succinogenes*, *Ruminococcus albus*, and *R. flavefaciens* 16S rrs gene copy numbers in rumen contents of sheep fed a 50:50 ratio concentrate and hay diet compared with a 100% hay diet.

Liu et al. (2015) found that high-grain feeding (hay:grain ratio of 35:65) to rumen-cannulated, castrated male goats (Boer × Yangtze River Delta White) produced a strong shift in bacterial composition and structure compared with feeding a low-grain diet (hay:grain ratio of 100:0). At the genus level, the data revealed that it increased the relative abundance of taxa *Butyrivibrio*, unclassified *Clostridiales*, *Mogibacterium*, unclassified *Anaerolineaceae*, and *Succiniclasticum* and decreased the proportion of unclassified *Ruminococcaceae*, unclassified *Rikenellaceae*, unclassified *Erysipelotrichaceae*, *Howardella*, and unclassified *Neisseriaceae*, confirming the earlier report by Petri et al. (2013). These results demonstrate that microbial ecology in the rumen can be changed substantially by diet manipulation.

Efficiency of Microbial Protein Synthesis

The factors that affect microbial protein synthesis (MPS) include roughage-to-concentrate ratio in the diet, dry matter intake (DMI), rate of passage of digesta in the rumen, the sources and levels of nitrogen and carbohydrate sources, and synchronization of the release of nitrogen and energy (Hoover and Stokes, 1991). There is a strong positive correlation between DMI and microbial growth (Gomes et al., 1994), mainly because of the increased passage rate, resulting in increased passage of dead microbes to the small

intestine (Djouvinov and Todorov, 1994). Higher passage rate reduces digestion in the rumen and a percent of the DMI, but higher intake increases the amount of organic matter availability to the microbes.

Efficiencies of MPS have been reported as approximately 7–14 g/MJ of ME (AFRC, 1992) and 30 g N/kg truly digestible organic matter (TDOM), with values ranging from 10 to 50 g N/kg TDOM (Stern et al., 1994). For optimal MPS, 11–13% crude protein (CP) or ≥ 7.8 g rumen degradable nitrogen (RDN)/MJ ME in the diet are considered sufficient (ARC, 1984). The efficiency of MPS was 13.0, 17.6, and 13.2 g/100 g TDOM for forage, forage concentrate mixed diets, and concentrate-based diets, respectively. Overall, the average efficiency of MPS is 14.8 g/100 g TDOM in the rumen (ARC, 1984). Lebzien and Voigt (1999) reported that MPS may vary between 63 and 313 g/kg fermentable organic matter (FOM) in the rumen, depending on type of the ration fed. This efficiency can differ four to five times between a poorly optimized rumen and a well-optimized rumen (Lebzien and Voigt, 1999) Flachowsky et al. (2006) also observed MPS in the range of 142–171 g/kg FOM in the rumen of lactating dairy cows fed a meadow hay and concentrate mixture with 70:30 to 30:70 ratios. Supplementation with linseed oil at 200 g/day further improved the MPS in both the diet groups. Diets containing a mixture of forages and concentrates increase the efficiency of MPS because of an improved environment for the growth of more diverse microbial species in the rumen. NRC (2001) considered an average of 186 g (75–338 g MPS/kg FOM).

In low to medium milk yielders, MPS in the rumen can meet the protein requirement of animals. The aim always should be to optimize rumen fermentation so that dietary fiber digestion and MPS are maximized (Makkar, 2004). There is an inverse relationship between efficiency of MPS and enteric methane emission (Blummel and Lebzien, 2001).



Lactating crossbred cows fed a diet containing paddy straw and supplements.

Optimization of Rumen Microbial Fermentation for Efficient Utilization of PQFs

Microbial fermentation in the rumen leads to the production of short-chain fatty acids (SCFA), ATPs, H₂, CO₂, CH₄, and NH₃. Methane has a critical role in maintaining the oxidation–reduction potential of the rumen by the removal of hydrogen produced during the fermentation of carbohydrates (Kirchgeßner et al., 1995). Whitford et al. (2001) illustrated that *Methanobrevibacter ruminantium* is the largest group of methanogens in the rumen of lactating dairy cattle fed total mixed ration (TMR), followed by *Methanospaera stadmanae*. However, *Methanobrevibacter* spp. was not identified in grazing cattle (Whitford et al., 2001). Jarvis et al. (2000) isolated *Methanomicrobium mobile* and *Methanobacterium formicum*, followed by an isolate phenotypically similar to *Methanosarcina barkeri* in grazing cattle. A large diversity exists in methanogens in the rumen, and there does not seem to be a direct relationship between total methanogen number and methane production (McSweeney and Mackie, 2012). The amount of methane produced is affected by feed intake (Reynolds et al., 2011), dietary fiber content, level of feeding, roughage-to-concentrate ratio, type of forage, stage of maturity of forage, rate of passage of digesta, efficiency of feed conversion and processing (Bakshi and Wadhwa, 2009), plant secondary metabolites (Bakshi and Wadhwa, 2010, 2012), feed additives (Wadhwa and Bakshi, 2009), phytochemicals (Bakshi and Wadhwa, 2011), and ambient temperature (McAllister et al., 1996). Methane can account for as much as 8–10% of gross energy intake, especially in the non-intensive production systems.

Improving the feed conversion efficiency (FCE) results in more feed nutrients being converted into animal products, associated with low nutrients lost to the environment. The principal environmental pollutants in systems of ruminant production are N and P; carbon as CO₂ and CH₄. A negative curvilinear relationship between methane emissions and FCE was observed in lactating dairy cows (Colman et al., 2011).

Means to Optimize Rumen Microbial Fermentation for Efficient Utilization of PQFs

The optimization of rumen function makes more nutrients available from the PQFs for maintenance and production purposes, as is evident from the increase in digestibility of these low quality roughages under the optimized rumen conditions. This increased digestibility of PQFs increases DMI, resulting in further increases in transfer of nutrients from the PQFs to the animal.

Balancing the Ration with Minerals and Synchronization of Nutrients and Relationship to Microbiota

In addition to synchronization of dietary protein and carbohydrates, trace minerals and vitamins are essential for synthesis of microbial proteins (Sniffen and Robinson, 1987). Feeding either S- (Sniffen and Robinson, 1987), N- (Balcells et al., 1993), or P-deficient diets results in a reduction in MPS (Gunn and Ternouth, 1994).

Sonawane and Arora (1976) showed that *in vitro* MPS was increased together with a reduction in NH₃-N concentration when rumen fluid was incubated with additional Zn as ZnCl₂ or ZnSO₄. The ammoniated rice straw-based ration with or without the minerals supplement (Ca, P, S, and

Zn) fed to Bali cattle improved rumen MPS significantly as compared with the un-supplemented group (222.52 vs. 197.83 g/day). The regression analysis showed that a mineral supplement of 0.19% caused maximum rumen MPS of 223.39 g/day (Mudita et al., 2014). El Ashry et al. (2012) studied the effect of feeding mixed inorganic (sulfates of Mn, Cu and Zn) or chelated minerals (Mn, Cu, and Zn with methionine) on dairy cows from 6 wk before calving and 3 mo after calving.

There was a significant increase in MPS in the group fed chelated minerals compared with the group supplemented with inorganic minerals. Banana flower powder and banana peel are rich in minerals (Bakshi and Wadhwa, 2013) and, when supplemented at 2–4% of DMI, enhanced MPS in cattle and buffalo on a high-concentrate diet (Kang and Wanapat, 2013). The use of a combination of cassava chips and rice bran as slow degraded carbohydrate sources as compared with cassava chips irrespective of the level of cotton seed meal improved digestibility and populations of total viable bacteria and proteolytic bacteria in the rumen of dairy bulls (Wanapat et al., 2013). Feeding balanced rations (TDN, CP, Ca, and P) increased DM and TDN/energy intakes, milk yield and fat (Kannan et al., 2010), net daily income, feed conversion efficiency, reproductive efficiency, and immunity and decreased parasitic load compared with when the unbalanced diets were fed (Wanapat et al., 2012; Garg et al., 2013). There was significant reduction in methane emission from lactating buffaloes (Garg et al., 2013) and cows (Mohini and Singh, 2010) when nutrient digestibilities were improved after balancing their ration for protein, energy, and minerals. Animals on imbalanced rations not only yielded less milk at a higher cost, but also produced more methane per liter of milk (Garg, 2011). Little information is available on changes in rumen microbial ecology in such rumen optimization studies.

Use of Non-protein Nitrogen and Relationship to Microbiota

Incremental incorporation of urea from 0.5 to 2.0% in the rations in which cottonseed cake (undecorticated) and wheat bran was replaced by urea on iso-nitrogen basis; wheat straw was the sole roughage in all the rations at 35%, improving DMI, gain in weight and efficiency of feed utilization in buffalo calves, and did not exert any adverse effects on dressing percentage, gristles percentage, and meat acceptability (Burque et al., 2008; Khan et al., 2015). Garg et al. (2007) showed that urea in the form of slow ammonia release and protected protein supplements to low quality diets was utilized more efficiently for MPS in the rumen. Cherdthong et al. (2011) supplemented slow-release urea (urea-calcium sulfate mixture; U-CaS at 6.7%) to iso-nitrogenous and iso-caloric concentrates containing a high level of cassava chips, coconut meal, palm kernel meal, and rice bran. Rumen-fistulated crossbred (Brahman×native) beef steers were fed concentrate mixture at 5 g/kg of BW daily with rice straw ad libitum. Total viable and cellulolytic bacteria (9.1×10^{11} , and 4.0×10^9 cfu/ml, respectively) and the efficiency of MPS (18.2 g of N/kg of OM truly digested in the rumen) were highest in animals fed the U-CaS concentrate mixture. It was concluded that U-CaS can replace soybean meal or urea in beef cattle diets without adverse effects on rumen fermentation parameters.

Cherdthong et al. (2013) fed feed blocks (rice bran 30%, molasses 42.5%, urea 10.5%, cement 11%, sulfur 1.5%, pre-mix 1.5%, salt 1.0%, and tallow 2%) and rice straw ad libitum with a concentrate mixture at 5 g/kg body weight to Thai native beef cattle. In the experimental feed blocks, UCaS replaced urea up to 18% on a N basis. The results revealed that an inclusion of 18% U-CaS could improve MPS synthesis and total

viable and cellulolytic bacteria predominated in the rumen. Other NPN sources, such as processed (sun dried, ensiled, or deep stacked) poultry litter/poultry droppings, can be incorporated in the concentrate mixture at 30–40% (N basis), depending on the production status of the ruminant (Bakshi et al., 1996; Bakshi and Fontenot, 1998). When wheat/rice/ragi straws and maize stovers/millet stalks were supplemented with UMMB (urea molasses multinutrient blocks), the FCE and productivity improved (Wadhwa and Bakshi, 2014; Choubey et al., 2015). Bull calves fed a basal diet of ad lib paddy straw and 2 kg concentrate mixture with free access to UMMB reduced the CH₄ from cows to the extent of 12–15% (Srivastava and Garg, 2002). In extensive systems based on grazing of ruminants on low quality forages in the rangelands, making available UMMB to animals near the water points have also been shown to increase animal productivity (Makkar et al., 2008). In these studies also, information on rumen microbial ecology is lacking.

Use of Herbal Feed Additives

The herbal feed additives e.g., *Asparagus racemosus*, *Leptidenia reticulata*, *Phellinus linteus*, *Cyathus stercoreus*, *Phialophora hoffmannii*, *Kutaki picorrhiza*, supplemented to the diet either individually or in different combinations (Bakshi and Wadhwa, 2004; Bakshi et al., 2005) increased the rumen microbial numbers (Wanapat et al., 2008) and nutrient digestibility, mainly due to increases in the activities of fibrolytic enzymes (Wadhwa and Bakshi, 2006) and decreased stress to the animals (Hosoda et al., 2006). However, once the herbal feed additives were withdrawn from the diet, no beneficial effect on nutrient utilization or performance

of calves was observed, indicating that for a sustainable effect on animal production, herbal feed additives need to be supplied regularly (Neelam Rani et al., 2006). The optimum level of supplementation in diets may vary depending on the physiological status of the animal. The optimum levels and duration of supplementation of herbs and specific withdrawal periods, therefore, need to be further investigated (Bakshi and Wadhwa, 2012). These effects of herbs are attributed to the presence of a variety of plant secondary metabolites, such as tannins, saponins, and essential oils, among others (Cowan, 1999; Iason, 2005). Dairy cows fed a phytogetic feed additive led to a 6.5% reduction of methane emission as compared with a control (22.2 vs. 23.7 g/L milk) with a simultaneous increase in milk yield by about 1.8 L/day and about a 5% increase in feed conversion efficiency (Anonymous, 2015). Saponins can reduce rumen protozoa populations by binding to cholesterol in the protozoal cell membrane, causing lysis and cell death; and can also shift the carbon from waste products of fermentation such as carbon dioxide and methane to microbial mass (Makkar et al., 1998). The intake of tannins at low levels improved the nutrient utilization (Min et al., 2003), gain in live weight, carcass weight, and dressing percentage, wool production (Montossi et al., 1996), milk production (Wang et al., 1996), reproductive efficiency, and prevention of bloat and reduced the load of internal parasites (Butter et al., 2000). Plants containing condensed tannins, e.g., lotus, can reduce methane considerably (Beauchemin et al., 2008; Grainger et al., 2009). The affinity of condensed tannins for protozoa and methanogens may be particularly important due to the symbiotic role these populations have on methane production (McAllister et al., 1996). Essential oils (EOs) alter growth and metabolism of rumen bacteria because the EOs have antimicrobial activities against both Gram-negative



Buffaloes fed a diet containing low quality roughage and supplements in a non-intensive system.

and Gram-positive bacteria; and they also have fungicidal properties (Calsamiglia et al., 2007). The EOs, due to the hydrophobic nature and affinity for lipids, accumulate in the lipid bilayer of bacterial cell membranes and disrupt their integrity either directly or by denaturing the membrane proteins, causing increased membrane permeability, leading to leakage of cytoplasmic enzymes and metabolites, which affects the trans-membrane ionic gradient. Bacteria can counter balance these effects by using ionic pumps, but a large amount of energy is diverted, resulting in the depletion of the proton-motive force across cell membrane, decreased ATP synthesis slowing microbial growth, and possibly cell death (Burt, 2004; Calsamiglia et al., 2007). The EOs mitigate methane production in the rumen, but most of the studies conducted were *in vitro* (Benchaar et al., 2007). Due to the discrepancies among studies, studies using non-culture-based biotechnological approaches that provide an overall assessment of the composition of microbiota are needed to provide insight into the interaction among various groups of microbes in the rumen as well as microbial shifts responsible for obtaining beneficial effects. These insights are required for the development of feeding strategies using these feed supplements.

Use of Microbial Fermentation Activators

Supplementing cattle diets with fibrolytic enzymes like cellulases and xylanases (Giraldo et al., 2009) can improve voluntary intake and the digestibility of nutrients of PQFs (Beauchemin et al., 2003) and animal productivity (Pinos-Rodríguez et al., 2008). Exogenous enzymes used in ruminant nutrition have been produced by fungi such as *Trichoderma longibrachiantum*, *Aspergillus niger*, *Penicilliumfuniculosum* (Wallace et al., 2001), *Aspergillus oryzae*, as well as *Bacillus* spp. bacteria (Beauchemin et al., 2003). It must, however, be noted that in many studies (Elwakeel et al., 2007; Miller et al., 2008), the use of enzymes has not been found to be effective in improving fiber digestibility, nutrient utilization, and animal productivity.

Among different genera of probiotics (also termed as “direct-fed microbials”), *Lactobacillus*, *Bifidobacteria*, and *Saccharomyces* species are commonly used in both ruminant and non-ruminant species. The beneficial effects of probiotics are manifested by regulation of intestinal microbial homeostasis, stabilizing the gastrointestinal barrier function, secretion of bacteriocin, immunomodulatory effect, reduction of procarcinogenic enzymes, interference in pathogen colonization (Prasad et al., 2014), reduction in the incidence of diarrhea neonatal animals (Agarwal et al., 2002), regulation of rumen pH (Whitley et al., 2009), and decrease in gastrointestinal diseases (Krehbiel et al., 2003). The literature suggests that these probiotics do not provide consistent beneficial effects. This could be attributed to their dependence on diet, host animal genetics, and the environment in which the animal is being reared, among other factors. With the current state of the knowledge, it may be surmised that the probiotics do not seem to have a consistent potential in non-intensive production systems. Again the study of rumen microbial ecology using modern tools could pave the way for developing practical strategies for obtaining benefits from probiotics in low-input, non-intensive production systems.

Managing Physical Nutrition of Animals

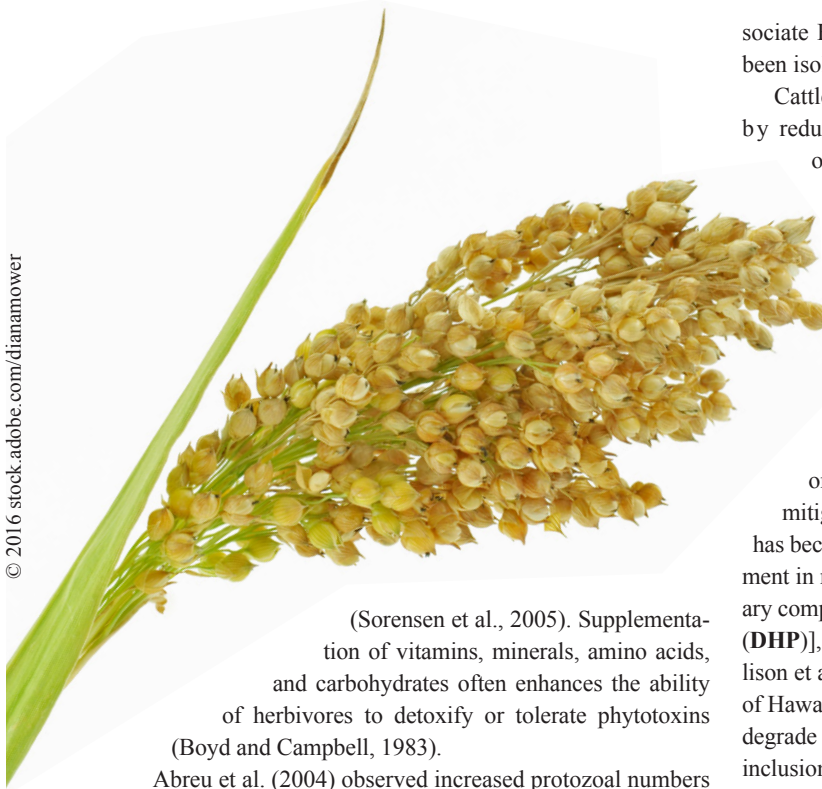
For effective rumen contractions and rumination, sufficient quantity of physically effective fiber (PEF) should be present in the diet. Forages “harvested” at earlier stages of maturity and most of the supplementary feeds contain insufficient amounts of PEF. Under such conditions, both

rate and extent of fiber digestion may be reduced. As these suboptimal conditions persist, rumen contractions may be impaired, resulting in a slower rate of emptying, leading to erratic and, in many cases, lower levels of feed intake and poor rumen function, which ultimately may lead to rumen stasis (Beauchemin, 2007).

It is evident from the above discussion that supplementation strategies can have a large impact on the increase in animal productivity and decrease in environmental pollutants in the non-intensive systems; and this large change is induced through optimization of the rumen function. However, little is known about the role of different groups of microbes, interactions between microbes, and the microbial shifts that need to take place for the rumen of livestock on low quality forages to start working efficiently and how this change can be achieved by supplementation strategies. The understanding of the changes in the rumen microbiome as a result of such shifts is essential. This knowledge could assist in the development of sound feeding strategies based on additives, manipulation of feed ingredients, or manipulation in the proportions of different feed ingredients in a diet to obtain high FCE and a food chain with a maximum transfer of feed nutrients to the animal.

Detoxification of Phytotoxins

In non-intensive production systems in many countries, grazing herbivores are exposed to toxic forages. These forages contain phytotoxins such as tannins, alkaloids, goitrogens, gossypol, saponins, glucosinolates, mimosine, cyanogens, nitrate, and oxalate or a combination of these toxins. The rumen microbiota and its microbiome are considered to contain 100 times more genes than the host animal and provides the ruminant with genetic and metabolic capabilities that the host has not had to evolve on its own, including capabilities to hydrolyze and ferment inaccessible nutrients and toxins (McSweeney and Mackie, 2012). An important reason for the evolution of foregut fermentation is detoxification of phytotoxins by rumen microbes, but in most cases the microorganisms involved have not been isolated and identified. In many instances, the rumen microbiota provides a protective function and effectively modifies or degrades a wide variety of toxic compounds, and adaptation of rumen microbes to overcome the adverse effects of toxic compounds is an important factor to consider. In some cases, the opposite can occur, as toxic metabolites can be produced from innocuous compounds (McSweeney et al., 2003). Utilization of toxins as a source of energy is usually the most important factor driving adaptation of microbial populations to the toxin in the rumen (McSweeney and Mackie, 2012). The ability of the ruminal ecosystem to adapt and increase its capacity to detoxify a plant toxin in response to the amount of toxin consumed is a major factor determining the pathogenesis of plant toxicity in forestomach fermenters (Krause et al., 2005). In many cases, the degradation pathway for a toxin involves a consortium of microorganisms since the enzymes involved may not be present in all microorganisms. Even when a single species of rumen bacteria is capable of degrading a toxin, there are probably several distinct strains of the species present in the rumen, which all contribute to the detoxification (Allison et al., 1992). The size of the population of toxin degrading microorganisms is likely to increase in the naive rumen, which is determined by its ability to derive energy for growth from the normal feed constituents and possibly from toxins, which enable it to compete with other organisms (McSweeney et al., 2005a,b). Some herbivores may regulate intake (both amount and timing) to maintain phytotoxin levels below concentrations that cause acid/base imbalances in the rumen and other negative feedbacks from secondary metabolites



(Sorensen et al., 2005). Supplementation of vitamins, minerals, amino acids, and carbohydrates often enhances the ability of herbivores to detoxify or tolerate phytotoxins (Boyd and Campbell, 1983).

Abreu et al. (2004) observed increased protozoal numbers in sheep receiving the high-saponin fruits of *Sapindus saponaria*, without any effect on their OM digestibility. Patra and Yu (2012) found dose-dependent modulations of ruminal microbial communities by quillaja and yucca saponins; quillaja saponin decreased the abundance of *Ruminococcus flavefaciens* but did not affect *Fibrobacter succinogenes* and *Prevotella*. In contrast, yucca saponin significantly increased the abundance of *R. flavefaciens*, *Prevotella*, and *F. succinogenes* in rumen digesta. Rumen fungi are very sensitive to saponins, e.g., *Neocallimastix frontalis* and *Piromonas rhizinflata* were found to be highly sensitive to yucca saponins (Goel et al., 2008).

Condensed tannins (CT) are the predominant class of tannins in forages, and they do not appear to be degraded by anaerobic microorganisms (Makkar et al., 1995). The effect of tannins on the animal may be either beneficial or harmful, depending on the type of tannin consumed, its chemical structure and molecular weight, the amount ingested, or the animal species involved (Frutos et al., 2004). High levels of tannins in feed are generally regarded as inhibitory to the growth of microorganisms by reducing the availability and digestibility of macronutrients and minerals (Molan et al., 2001), impeding cell wall function (O'Donovan and Brooker, 2001), and interfering with the catalytic activity of extracellular enzymes (Bae et al., 1993) and cell bound enzymes (Makkar et al., 1988). However, plants that are rich in tannins often contain simpler phenolics that appear to be more toxic to bacteria than higher-molecular-weight hydrolyzable tannins (HT) and CT. Molecular ecology studies have confirmed that the Gram-negative Enterobacteriaceae and Bacteroides species predominate in the presence of dietary tannins and that there is a corresponding decrease in the Gram-positive *Clostridium leptum* group (Smith and Mackie, 2004). Calliandra and lotus CT reduce the population of cellulolytic (*Fibrobacter succinogens* and *Ruminococcus flavifaciens*) bacteria in the rumen of sheep, but the proteolytic bacteria and fungi were less affected (McSweeney et al., 1999, 2001). Anaerobic bacteria that dis-

sociate HT-protein complexes but not protein complexed with CT have been isolated from many herbivorous animals (McSweeney et al., 1999).

Cattle can detoxify large quantities of oxalates in their rumen, thereby reducing chances of poisoning. Animals can develop a tolerance for oxalate-containing plants by building up the concentration of *Oxalobacter formigenes*, an oxalate-degrading bacteria, in the rumen (Allison et al., 1985; Hulting and Neff, 2010). If consumed in small amounts over time, with other feed to dilute the concentrations in the rumen, oxalate-accumulating plants cease to be a problem. Livestock should be adapted to oxalate plants over 4 d, incrementally increasing the time allowed to graze the plants, before being left in pastures containing high concentrations of oxalate-accumulating plants.

Due to its high-nutrient content (CP and minerals) (Areghe-ore, 1999), rumen bypass protein supply, and its possible methane mitigation potential (Jayanegara et al., 2011), *Leucaena leucocephala* has become one of the most widely used legumes that is used as a supplement in ruminant feeding practices. However, this feed contains a secondary compound, mimosine and its isomers [3, 4, and 2, 3 dihydroxyperidine (DHP)], which can induce toxicity in ruminants (Ghosh et al., 2007b). Allison et al. (1992) isolated a bacterium *Synergistes jonesii* from the rumen of Hawaii goats and swamp buffaloes (Phesatcha et al., 2013), which can degrade DHP and is resistant to mimosine toxicity. It has been shown that inclusion of *Leucaena leucocephala* leaves up to 30% (Ghosh et al., 2007a) and up to 50% (Peniche-González et al., 2014) in the diet or 3.0 kg/head/day to swamp buffaloes fed rice straw (Phesatcha et al., 2013), improved CP digestibility and efficiency of MPS, and resulted in no observed negative effects of mimosine. Barros-Rodríguez et al. (2014) concluded that in animals that are not adapted to this feed, the intake should not exceed 30% in the diet (on DM basis).

Microbial populations change gradually with prolonged and increasing exposure to toxic substances, and microbial adaptations provide detoxification of plant toxicants such as nitrate, nitrite (Allison and Reddy, 1984), nitropropanoic acid (Anderson et al., 1993), oxalate, prussic acid, sulfate and sulfide, some alkaloids (e.g., mimosine) and, perhaps, even some mycotoxins (Mobashar et al., 2010). New emerging techniques, for example, reverse transcription-PCR (RT-PCR), can be used to monitor the population of the toxin-degrading microbe in the rumen, enabling better understanding of the adaptation process and to develop practical strategies to overcome the toxicity. Microbial detoxification of plants by inoculation of fluoroacetate-degrading bacteria into the rumen was used by Gregg et al. (1998), who inoculated the rumen with a genetically modified strain of *Butyrivibrio fibrisolvens*. This strain contains a gene encoding fluoroacetate dehalogenase from *Moraxella* species, which was efficient at preventing fluoroacetate poisoning in sheep.

Conclusion and Ways Forward

In a non-intensive ruminant husbandry system, in which the poor quality forages constitute the bulk of the dry matter consumed, the rumen function is often impaired by nutrient deficiencies. The desired productivity of the animals and their genetic potential may be achieved by supplementing such diets with the deficient nutrients, which generally include N and minerals, because this enhances the rumen microbial activity and leads to increased efficiency in microbial protein synthesis. To develop effective feeding strategies, it is imperative that the current insights into the

microbial diversity, the interactions among microorganisms in the rumen, and interactions between rumen microorganisms and the host are improved.

Hitherto, knowledge of rumen ecology was primarily obtained using classical culture-based techniques, which only allow the monitoring of less than 20% of the rumen microbial population. Advances in molecular microbial ecology based on 16r RNA gene (rrn) phylogeny now enable a comprehensive identification and quantification of the microbiota. This microbial classification system, together with deep sequencing of DNA from the rumen, has shown the presence of complex communities that have co-evolved with the ruminant host in response to the environmental conditions, such as the composition of diets, temperature, and water availability, among others. These tools have the potential to study the entire microbiome (the predominant genomes) from the complex microbial community in the rumen and to determine community structures as well their function. Gene expression can be sensitively analyzed by amplification of messenger RNA (mRNA) using RT-PCR to produce cDNA as a template for further analysis. Understanding of the rumen microbiome, its metabolic potentials and microbial dynamics, interactions, and shifts in the rumen as a function of nutrients in the diets is expected to play a major role in developing more effective and efficient feeding strategies, especially of ruminants in non-intensive husbandry systems fed poor quality forages. A good model for such studies could be the non-intensive ruminant production systems because of a considerable gain in productivity and nutrient utilization that can be obtained by supplementation of nutrients that are deficient in the low quality forages. Currently, the effects of feed additives such as enzymes and probiotics have been inconsistent. Reasons for this are unclear, but may include lack of knowledge of the changes of rumen as a whole due to these supplementations. Similarly, different strains of a bacterium that detoxify toxins in the rumen could have different detoxification potential, which could be exploited under different conditions. Better understanding of host genetic-microbial interactions and of the development of microbial populations in the rumen after birth could have practical applications. The above stated molecular-based tools have the potential to unravel these complexities, enabling development of more efficient feeding strategies including feed additives that elicit consistent and long-term desired responses. Development of effective immunization-based approaches, for example, against methanogens, or against microbes responsible for urease and proteolysis in the rumen, could also be assisted through these molecular tools. These immunization approaches are expected to have extensive applications in non-intensive livestock production systems.

Literature Cited

Abreu, A., J.E. Carulla, C.E. Lascano, T.E. Diaz, M. Kreuzer, and H.D. Hess. 2004. Effects of Sapindussaponaria fruits on ruminal fermentation and duodenal nitrogen flow of sheep fed a tropical grass diet with and without legume. *J. Anim. Sci.* 82:1392–1400.

AFRC. 1992. Energy and protein requirements of ruminants. CAB International, Wellington, UK.

Agarwal, N., D.N. Kamra, L.C. Chaudhary, L. Agarwal, A. Sahoo, and N.N. Pathak. 2002. Microbial status and rumen enzyme profile of crossbred calves on different microbial feed additives. *Lett. Appl. Microbiol.* 34:329–336. doi:10.1046/j.1472-765X.2002.01092.x.

Allison, M.J., K.A. Dawson, W.R. Mayberry, and J.G. Foss. 1985. Oxalobacter formigenes gen. nov. sp. nov.: Oxalate degrading anaerobes that inhabit gastrointestinal tract. *Arch. Microbiol.* 141:1–7. doi:10.1007/BF00446731.

Allison, M.J., W.R. Mayberry, C.S. McSweeney, and D.A. Stahl. 1992. Synergistes jonesii, gen. sp. nov.: A rumen bacterium that degrades toxic pyridinediols. *Syst. Appl. Microbiol.* 15:522–529. doi:10.1016/S0723-2020(11)80111-6.

Allison, M.J., and C.A. Reddy. 1984. Adaptations of gastrointestinal bacteria in response to changes in dietary oxalate and nitrate. In: C.A. Reddy and M.A. Klug, editors, Current perspectives on microbial ecology. American Society of Microbiology, Washington, DC. p. 248–256.

Anderson, R.C., M.A. Rasmussen, and M.J. Allison. 1993. Metabolism of the plant toxins nitropropionic acid and nitropropanol by ruminal microorganisms. *Appl. Environ. Microbiol.* 59:3056–3061.

Anonymous. 2015. Plant extracts can mitigate methane emissions. <http://www.dairyglobal.net/Articles/General/2015/9/Plant-extracts-can-mitigate-methane-emissions-2692502W/?q=methane§ion=Article&page=1>.

ARC. 1984. The nutrient requirement of ruminant livestock. Agricultural Research Council, CAB, Farham Royal, UK.

Aregheore, E.M. 1999. Nutritive and antinutritive value of some tree legumes used in ruminant livestock nutrition in Pacific island countries. *J. South Pac. Agric.* 6:50–61.

Bae, H.D., T.A. McAllister, J. Yanke, K.J. Cheng, and A.D. Muir. 1993. Effect of condensed tannins on endoglucanase activity and filter paper digestion by *Fibrobacter succinogenes* S85. *Appl. Environ. Microbiol.* 59:2132–2138.

Bakshi, M.P.S., and J.P. Fontenot. 1998. Processing and nutritive evaluation of broiler litter as livestock feed. *Anim. Feed Sci. Technol.* 74:337–345. doi:10.1016/S0377-8401(98)00181-3.

Bakshi, M.P.S., V.K. Gupta, and P.N. Langar. 1986. Fermented straw as a complete basal ration for ruminants. *Agric. Wastes (changed to Biol. Wastes and then to Biores. Technol.)* 16:37–46.

Bakshi, M.P.S., V.K. Gupta, and P.N. Langar. 1987. Effect of moisture level on the chemical composition and nutritive value of fermented straw. *Biol. Wastes (changed to Biores. Technol.)* 21:283–289.

Bakshi, M.P.S., Neelam Rani, M. Wadhwa, and S. Kaushal. 2005. Impact of herbal feed additives on the utilization of nutrients in-vitro. *Indian J. Anim. Nutr.* 22:147–151.

Bakshi, M.P.S., V.M. Wadhawan, M. Wadhwa, and P.N. Langar. 1996. Effect of feeding NPN rich unconventional feedstuffs on the productive performance of buffalo calves. *Bubalus bubalis. J. Buffalo Sci. Tech.* 2:57–62.

Bakshi, M.P.S., and M. Wadhwa. 2004. Effect of feeding herbal feeding herbal feed additives on the productive performance of buffalo calves. *Bubalus bubalis. J. Buff. Sci. Tech.* 10:65–70.

Bakshi, M.P.S., and M. Wadhwa. 2009. Dietary manipulation for mitigation of enteric methane emission. In: Proceedings 6th Asian Buffalo Congress, Lahore, Pakistan. *Pakistan J. Zool. (Environ-SPJZ-2)* 9:887–893.

Bakshi, M.P.S., and M. Wadhwa. 2010. Significance of plant secondary metabolites in relation to methane production by ruminants. In: A.K. Pattanaik, A.K. Verma, and N. Dutta, editors, Animal nutrition: Strategies for environment protection and poverty alleviation. Volume I. Proceedings of VII Biennial Animal Nutrition Association Conference, 17–19 Dec. 2010, Bhubaneswar. p. 25–28.

Bakshi, M.P.S., and M. Wadhwa. 2011. Phytochemicals: Role in enteric methane mitigation and performance of animals. In: V.B. Chaturvedi, N. Dutta, A.K. Verma, P. Singh, and D.N. Kamra, editors, Nutritional interventions for clean and green livestock production. CAFTAN. IVRI, Izatnagar. p. 180–184.

Bakshi, M.P.S., and M. Wadhwa. 2012. Herbal feed additives—role in animal nutrition. In: U.R. Mehra, P. Singh, and A.K. Verma, editors, Animal nutrition—advances and developments. Satish Serial Publishing House, New Delhi. p. 707–733.

Bakshi, M.P.S., and M. Wadhwa. 2013. Nutritional evaluation of cannery and fruit wastes as livestock feed. *Indian J. Anim. Sci.* 83:84–89.

Balcells, J., J.A. Guada, C. Castrillo, and J. Gasa. 1993. Rumen digestion and urinary excretion of purine derivatives in responses to urea supplementation of sodium-treated straw fed to sheep. *Br. J. Nutr.* 69:721–732. doi:10.1079/BJN19930073.

Barros-Rodríguez, M., C.A. Sandoval-Castro, J. Solorio-Sánchez, L.A. Sarmiento-Franco, R. Rojas-Herrera, and A.V. Klieve. 2014. *Leucaena leucocephala* in ruminant nutrition. Invited review. *Trop. Subtrop. Agroecosyst.* 17:173–183.

Beauchemin, K.A. 2007. Ruminal acidosis in dairy cows: Balancing physically effective fiber with starch availability. In: Florida Ruminant Nutrition Symposium, Best Western Gateway Grand, Gainesville, FL. p. 16–27.

Beauchemin, K.A., D. Colombatto, D.P. Morgavi, and W.Z. Yang. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. *J. Anim. Sci.* 81(E. Suppl. 2): E37–E47.

- Beauchemin, K.A., M. Kreuzer, F. O'Mara, and T.A. McAllister. 2008. Nutritional management for enteric methane abatement: A review. *Aust. J. Exp. Agric.* 48:21–27. doi:10.1071/EA07199.
- Benchaar, C., A.V. Chaves, G.R. Fraser, Y. Wang, K.A. Beauchemin, and T.A. McAllister. 2007. Effects of essential oils and their components on *in vitro* rumen microbial fermentation. *Can. J. Anim. Sci.* 87: 413–419.
- Blummel, M., and P. Lebzien. 2001. Predicting ruminal microbial efficiencies of dairy rations by *in vitro* techniques. *Livest. Prod. Sci.* 68:107–117. doi:10.1016/S0301-6226(00)00241-4.
- Boyd, J.N., and T.C. Campbell. 1983. Impacts of nutrition on detoxication. In: J. Calwell and W.B. Jakoby, editors, *Biological basis for detoxication*. Academic Press, New York, N.Y. p. 287–306.
- Burque, A.R., M. Abdullah, M.E. Babar, K. Javed, and H. Nawaz. 2008. Effect of urea feeding on feed intake and performance of male buffalo calves. *J. Anim. Pl. Sci.* 18:1–6.
- Burt, S. 2004. Essential oils: Their antibacterial properties and potential applications in foods—a review. *Int. J. Food Microbiol.* 94:223–253. doi:10.1016/j.ijfoodmicro.2004.03.022.
- Butter, N.L., J.M. Dawson, D. Wakelin, and P.J. Buttery. 2000. Effect of dietary tannin and protein concentration on nematode infection (*Trichostrongylus colubriformis*) in lambs. *J. Agric. Sci.* 134:89–99. doi:10.1017/S0021859699007315.
- Calsamiglia, S., M. Busquet, P.W. Cardozo, L. Castillejos, and A. Ferret. 2007. Invited review: Essential oils as modifiers of rumen microbial fermentation. *J. Dairy Sci.* 90:2580–2595. doi:10.3168/jds.2006-644
- Camboim, E.K.A., A.P. Almedia, M.Z. Tadra-Sfeir, F.G. Junior, P.P. Andrade, C.S. McSweeney, M.A. Melo, and F. Riet-Correa. 2012. Isolation and identification of sodium fluoroacetate degrading bacteria from caprine rumen in Brazil. *The Sci. World J. Article ID 178254*. doi:10.1100/2012/178254.
- Cherdthong, A., M. Wanapat, S. Khantharin, W. Khota, G. Tangmutthapatharakun, K. Phesatcha, S. Foiklang, and S.C. Kang. 2013. Influence of urea calcium mixture in high quality feed block on ruminal fermentation in swamp buffalo. *Buff. Bull.* 32:984–987.
- Cherdthong, A., M. Wanapat, and C. Wachirapakorn. 2011. Influence of urea calcium mixture supplementation on ruminal fermentation characteristics of beef cattle fed on concentrates containing high levels of cassava chips and rice straw. *Anim. Feed Sci. Technol.* 163:43–51. doi:10.1016/j.anifeedsci.2010.10.003.
- Choubey, M., M. Wadhwa, and M.P.S. Bakshi. 2015. Evaluation of urea molasses multi-nutrient blocks containing alternate feed resources in buffaloes. *Buff. Bull.* 34:1–16.
- Colman, D.R., D.E. Beever, R.W. Jolly, and J.K. Drackley. 2011. Gaining from technology for improved dairy cow nutrition: Economic, environmental, and animal health benefits. *J. Anim. Sci.* 27:505–517.
- Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 12:564–582.
- Currier, T.A., D.W. Bohnert, S.J. Falck, C.S. Schauer, and S.J. Bartle. 2004. Daily and alternate-day supplementation of urea or biuret to ruminants consuming low-quality forage: II. Effects on site of digestion and microbial efficiency in steers. *J. Anim. Sci.* 82:1518–1527.
- Djouviov, D.S., and N.A. Todorov. 1994. Influence of dry matter intake and passage rate on microbial protein synthesis in the rumen of sheep and its estimation by cannulation and a non-invasive method. *Anim. Feed Sci. Technol.* 48:289–304. doi:10.1016/0377-8401(94)90179-1.
- El Ashry, G.M., A.A.M. Hassan, and S.M. Soliman. 2012. Effect of feeding a combination of zinc, manganese and copper methionine chelates of early lactation high producing dairy cow. *Food Nutr. Sci.* 3:1084–1091. doi:10.4236/fns.2012.38144.
- Elwakeel, E.A., E.C. Titgemeyer, B.J. Johnson, C.K. Armendariz, and J.E. Shirley. 2007. Fibrolytic enzymes to increase the nutritive value of dairy feedstuffs. *J. Dairy Sci.* 90:5226–5236. doi:10.3168/jds.2007-0305.
- Flachowsky, G., K. Erdmann, P. Lebzien, and L. Hüther. 2006. Investigations on the influence of roughage/concentrate ratio and linseed oil supplementation on rumen fermentation and microbial protein yield in dairy cows. *Slovak J. Anim. Sci.* 39:3–9.
- Frutos, P., G. Hervás, F.J. Giráldez, and A.R. Mantecón. 2004. Review. Tannins and ruminant nutrition. *Span. J. Agric. Res.* 2:191–202. doi:10.5424/sjar/2004022-73.
- Galindo, J., and Y. Marrero. 2005. Manipulación de la fermentación microbiana ruminal. *Rev. Cub.de Cien. Agric.* 39:439–450.
- Garg, M.R. 2011. Methane emission reduction in lactating animals through balanced feeding. In: *Proceedings of Recent Advances in Methane Estimation and Mitigation Strategies in Ruminants*. NIANP, Bangalore, 5–18 Aug.2011. p. 9–13.
- Garg, M.R., P.L. Sherasia, B.M. Bhanderi, S.K. Gulati, and T.W. Scott. 2007. Milk production efficiency improvement in buffaloes through the use of slow ammonia release and protected protein supplement. *Ital. J. Anim. Sci.* 6:1043–1045.
- Garg, M.R., P.L. Sherasia, B.M. Bhanderi, B.T. Phondba, S.K. Shelke, and H.P.S. Makkar. 2013. Effects of feeding nutritionally balanced rations on animal productivity, feed conversion efficiency, feed nitrogen use efficiency, rumen microbial protein supply, parasitic load, immunity and enteric methane emissions of milking animals under field conditions. *Anim. Feed Sci. Technol.* 179:24–35. doi:10.1016/j.anifeedsci.2012.11.005.
- Ghosh, M.K., P.P. Atreja, R. Buragohain, and S. Bandyopadhyay. 2007a. Influence of short-term *Leucaena leucocephala* feeding on milk yield and its composition, thyroid hormones, enzyme activity, and secretion of mimosine and its metabolites in milk of cattle. *J. Agric. Sci.* 145:407–414. doi:10.1017/S0021859607007113.
- Ghosh, M.K., and B. Samiran. 2007b. Mimosine toxicity—a problem of *Leucaena* feeding in ruminants. *Asian J. Anim. Vet. Adv.* 2:63–73. doi:10.3923/ajava.2007.63.73.
- Giraldo, L.A., M.L. Tejido, M.J. Ranilla, S. Ramos, A.R. Mantecon, and M.D. Carro. 2009. Influence of direct-fed exogenous fibrolytic enzymes on ruminal fibrolytic activity in sheep. *Options Mediterr.* 85:297–302.
- Goel, G., H.P.S. Makkar, and K. Becker. 2008. Effects of *Sesbania sesban* and *Carduus pycnocephalus* leaves and fenugreek (*Trigonella foenum-graecum* L.) seeds and their extracts on partitioning of nutrients from roughage- and concentrate-based feeds to methane. *Anim. Feed Sci. Technol.* 147:72–89. doi:10.1016/j.anifeedsci.2007.09.010.
- Gomes, M.J., F.D. Hovell, and X.B. Chen. 1994. The effect of starch supplementation of straw on microbial protein supply in sheep. *Anim. Feed Sci. Technol.* 49:277–286. doi:10.1016/0377-8401(94)90052-3.
- Grainger, C., T. Clarke, M.J. Auldist, K.A. Beauchemin, S.M. McGinn, G.C. Waghorn, and R.J. Eckard. 2009. Mitigation of greenhouse gas emissions from dairy cows fed pasture and grain through supplementation with *Acacia mearnsii* tannins. *Can. J. Anim. Sci.* 89:241–251. doi:10.4141/CJAS08110.
- Gregg, K., B. Hamdorf, K. Henderson, J. Kopečný, and C. Wong. 1998. Genetically modified ruminal bacteria protect sheep from fluoroacetate poisoning. *Appl. Environ. Microbiol.* 64:3496–3498.
- Gunn, K.J., and J.H. Ternouth. 1994. The effect of phosphorus deficiency upon ruminal microbial activity and nitrogen balance in lambs. *Proc. Aust. Soc. Anim. Prod.* 20:445.
- Hoover, W.H., and S.R. Stokes. 1991. Balancing carbohydrates and proteins for optimum rumen microbial yield. *J. Dairy Sci.* 74:3630–3645. doi:10.3168/jds.S0022-0302(91)78553-6.
- Hosoda, K., K. Kuramoto, B. Eruden, T. Nishida, and S. Shioya. 2006. The effects of three herbs as feed supplements on blood metabolites, hormones, antioxidant activity, IgG concentration, and ruminal fermentation in Holstein steers. *Asian-Aust. J. Anim. Sci.* 19:35–41.
- Hulting, A.G., and K. Neff. 2010. Management strategies for dealing with select poisonous plants in Oregon. BEEF 022. Beef Cattle Library, Oregon State University, Corvallis. <http://beefcattle.ans.oregonstate.edu/html/publications>.
- Hundal, J.S., M. Wadhwa, and M.P.S. Bakshi. 2015. Effect of supplementing essential oils on the *in vitro* methane production and digestibility of nutrients from wheat straw used as substrate. *Indian J. Anim. Sci.* 85: (in press).
- Iason, G. 2005. The role of plant secondary metabolites in mammalian herbivory: Ecological perspectives. *Proc. Nutr. Soc.* 64:123–131. doi:10.1079/PNS2004415.
- Jarvis, G.N., C. Strömpl, D.M. Burgess, L.C. Skillman, E.R.B. Moore, and K.N. Joblin. 2000. Isolation and identification of ruminal methanogens from grazing cattle. *Curr. Microbiol.* 40:327–332. doi:10.1007/s002849910065.
- Jayanegara, A., E. Wina, C.R. Soliva, S. Marquardt, M. Kreuzer, and F. Leiber. 2011. Dependence of forage quality and methanogenic potential of tropical plants on their phenolic fractions as determined by principal component analysis. *Anim. Feed Sci. Technol.* 163:231–243. doi:10.1016/j.anifeedsci.2010.11.009.
- Kang, S., and M. Wanapat. 2013. Using plant source as a buffering agent to manipulating rumen fermentation in an *in vitro* gas production system. *Asian-Aust. J. Anim. Sci.* 26:1424–1436.

- Kannan, A., M.R. Garg, and P. Singh. 2010. Effect of ration balancing on methane emission and milk production in lactating animals under field conditions in Rae Bareilly district of Uttar Pradesh. *Indian J. Anim. Nutr.* 27:103–108.
- Khan, M.I., S. Ahmed, A. Rahman, F. Ahmad, A. Khalique, N. Ahmad, Z. Qadir, S. Umar, S. Ullah, and B.E. Azam. 2015. Comparative efficacy of urea and slow-release non-protein nitrogen on performance of Nili-Ravi buffalo calves. *Pak. J. Zool.* 47:1097–1102.
- Kirchgesner, M., W. Windisch, and H.L. Muller. 1995. Nutritional factors for the quantification of methane production. In: W. von Engelhardt, S. Leonhard-Marek, G. Brevis, and D. Giesecke, editors, *Ruminant physiology: Digestion, metabolism, growth and reproduction*. Proceedings 8th International Symposium on Ruminant Physiology. p. 333–348.
- Krause, D.O., W.J.M. Smith, J.D. Brooker, and C.S. McSweeney. 2005. Tolerance mechanisms of streptococci to hydrolysable and condensed tannins. *Anim. Feed Sci. Technol.* 121:59–75. doi:10.1016/j.anifeedsci.2005.02.008.
- Krehbiel, C.R., S.R. Rust, G. Zhang, and S.E. Gilliland. 2003. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. *J. Anim. Sci.* 81:120–132.
- Lebzien, P., and J. Voigt. 1999. Calculation of utilizable crude protein at the duodenum of cattle by two different approaches. *Arch. Anim. Nutr.* 52:1363–1368.
- Liu, J., G. Bian, W. Zhu, and S. Mao. 2015. High-grain feeding causes strong shifts in ruminal epithelial bacterial community and expression of Toll-like receptor genes in goats. *Front. Microbiol.* 6:167.
- Makkar, H.P.S. 2004. Estimation of microbial protein supply in ruminant livestock through quantification of urinary purine derivatives. In: H.P.S. Makkar and X.B. Chen, editors, *Estimation of microbial protein supply in ruminant livestock through quantification of urinary purine derivatives*. Kluwer Academic Press, Dordrecht, The Netherlands. p. 212.
- Makkar, H.P.S. 2016. Smart livestock feeding strategies for harvesting triple gain—the desired outcomes in planet, people and profit dimensions: A developing country perspective. *Anim. Prod. Sci.* (in press).
- Makkar, H.P.S., K. Becker, H.J. Abel, and C. Szegletti. 1995. Degradation of condensed tannins by rumen microbes exposed to quebracho tannins (QT) in rumen simulation technique (RUSITEC) and effects of QT on fermentation processes in the RUSITEC. *J. Sci. Food Agric.* 69:495–500. doi:10.1002/jsfa.2740690414.
- Makkar, H.P.S., M. Sanchez, and A. Speedy. 2008. Feed supplementation blocks—FAO/IAEA Publication 164. FAO, Rome. <http://www.fao.org/docrep/010/a0242e/a0242e00.htm>.
- Makkar, H.P.S., S. Sen, S. Blummel, and K. Becker. 1998. Effect of fractions containing saponins from *Yucca schidigera*, *Quillaja saponaria* and *Accacia auriculoformis* on rumen fermentation. *J. Agric. Food Chem.* 46:4324–4328. doi:10.1021/jf980269q.
- Makkar, H.P.S., B. Singh, and R.K. Dawra. 1988. Effect of tannin-rich leaves of oak on various microbial activities of the rumen. *Br. J. Nutr.* 60:287–296. doi:10.1079/BJN19880100.
- McAllister, T.A., E.K. Okine, G.W. Mathison, and K.-J. Cheng. 1996. Dietary, environmental, and microbiological aspects of methane production in ruminants. *Can. J. Anim. Sci.* 76:231–243. doi:10.4141/cjas96-035.
- McSweeney, C.S., L. Blackall, E. Collins, L.L. Conlan, R.I. Webb, S.E. Denman, and D.O. Krause. 2005a. Enrichment, isolation and characterisation of ruminal bacteria that degrade non-protein amino acids from the tropical legume *Acacia angustissima*. *Anim. Feed Sci. Technol.* 121:191–204. doi:10.1016/j.anifeedsci.2005.02.018.
- McSweeney, C.S., J. Gough, L.L. Conlan, M.P. Hegarty, B. Palmer, and D.O. Krause. 2005b. Nutritive value assessment of the tropical shrub legume *Acacia angustissima*: Anti-nutritional compounds and in-vitro digestibility. *Anim. Feed Sci. Technol.* 121:175–190. doi:10.1016/j.anifeedsci.2005.02.017.
- McSweeney, C.S., and R. Mackie. 2012. Micro-organisms and ruminant digestion: State of knowledge, trends and future prospects. Commission on Genetic Resources for Food and Agriculture. Background Study Paper No. 61.
- McSweeney, C.S., H.P.S. Makkar, and J.D. Reed. 2003. Modification of rumen fermentation for detoxification of harmful plant compounds. In: L. Mannelje, editor, *Proceedings of the 6th International Symposium on the Nutrition of Herbivores*, Merida, Yucatan, Mexico. p. 239–268.
- McSweeney, C.S., B. Palmer, R. Bunch, and D.O. Krause. 1999. Isolation and characterisation of proteolytic ruminal bacteria from sheep and goats fed the tannin-containing shrub legume *Calliandra calothyrsus*. *Appl. Environ. Microbiol.* 65:3075–3083.
- McSweeney, C.S., B. Palmer, D.M. McNeil, and D.O. Krause. 2001. Effect of tropical forage *Calliandra* on microbial protein synthesis and ecology in the rumen. *J. Appl. Microbiol.* 90:78–88. doi:10.1046/j.1365-2672.2001.01220.x.
- Miller, D.R., B.C. Granzin, R. Elliott, and B.W. Norton. 2008. Effects of an exogenous enzyme, Roxazyme® G2 Liquid, on milk production in pasture fed dairy cows. *Anim. Feed Sci. Technol.* 145:194–208. doi:10.1016/j.anifeedsci.2007.05.049.
- Min, B.R., T.N. Barry, G.T. Attwood, and W.C. McNabb. 2003. The effect of condensed tannins on the nutrition of ruminants fed fresh temperate forages: A review. *Anim. Feed Sci. Technol.* 106:3–19. doi:10.1016/S0377-8401(03)00041-5.
- Mobashar, M., J. Hummel, R. Blank, and K.-H. Südekum. 2010. Ochratoxin A in ruminants—a review on its degradation by gut microbes and effects on animals. *Toxins* 2:809–839. doi:10.3390/toxins204809.
- Mohini, M., and G.P. Singh. 2010. Effect of supplementation of urea molasses mineral block (UMMB) on the milk yield and methane production in lactating cattle on different plane of nutrition. *Indian J. Anim. Nutr.* 27:96–102.
- Molan, A.L., G.T. Attwood, B.R. Min, and W.C. McNabb. 2001. The effect of condensed tannins from *Lotus pedunculatus* and *Lotus corniculatus* on the growth of proteolytic rumen bacteria *in vitro* and their possible mode of action. *Can. J. Microbiol.* 47:626–633. doi:10.1139/w01-060.
- Montossi, F.M., J. Hodgson, S.T. Morris, and D.F. Risso. 1996. Effects of the condensed tannins on animal performance in lambs grazing Yorkshire fog (*Holcus lanatus*) and annual ryegrass (*Lolium multiflorum*) dominant swards. *Proc. N.Z. Soc. Anim. Prod.* 56:118–121.
- Mosoni, P., F. Chaucheyras-Durand, C. Béra-Maillet, and E. Forano. 2007. Quantification by real-time PCR of cellulolytic bacteria in the rumen of sheep after supplementation of a forage diet with readily fermentable carbohydrates. Effect of a yeast additive. *J. Appl. Microbiol.* 103:2676–2685. doi:10.1111/j.1365-2672.2007.03517.x.
- Mudita, I.M., I.W. Wirawan, I.G.L.O. Cakra, and I.B.G. Partama. 2014. Optimising rumen function of Bali cattle fed ration based on agriculture by-products with supplementation of multivitamins-minerals. *Int. J. Pure App. Biosci.* 2:36–45.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th revised edn. National Research Council, National Academy of Sciences, Washington, D.C., U.S.A.
- Neelam Rani, M. Wadhwa, S. Kaushal, and M.P.S. Bakshi. 2006. Herbal feed additives and performance of buffalo calves. *Anim. Nutr. Feed Technol.* 6:147–151.
- O'Donovan, L., and J.D. Brooker. 2001. Effect of hydrolysable and condensed tannins on growth, morphology and metabolism of *Streptococcus gallolyticus* (*S. caprinus*) and *Streptococcus bovis*. *Microbiol.* 147:1025–1033. doi:10.1099/00221287-147-4-1025.
- Orpin, C.G., and K.N. Joblin. 1997. The rumen anaerobic fungi. In: P.N. Hobson and C.S. Stewart, editors, *The rumen microbial ecosystem*. 2nd ed. Blackie, London. p. 140–195.
- Patra, A.K., and Z. Yu. 2012. Effects of essential oils on methane production and fermentation by and abundance and diversity of rumen microbial populations. *Appl. Environ. Microbiol.* 78:4271–4280. doi:10.1128/AEM.00309-12.
- Peniche-González, I.N., Z.U. González-López, C.F. Aguilar-Pérez, J.C. Ku-Vera, A.J. Ayala-Burgos, and F.J. Solorio-Sánchez. 2014. Milk production and reproduction of dual purpose cows with a restricted concentrate allowance and access to an association of *Leucaena leucocephala* and *Cynodon nlemfuensis*. *J. Appl. Anim. Res.* 42:345–351. doi:10.1080/09712119.2013.875902.
- Petri, R.M., T. Schwaiger, G.B. Penner, K.A. Beauchemin, R.J. Forster, and J.J. McKinnon. 2013. Changes in the rumen epimural bacterial diversity of beef cattle as affected by diet and induced ruminal acidosis. *Appl. Environ. Microbiol.* 79:3744–3755. doi:10.1128/AEM.03983-12.
- Phesatcha, K., M. Wanapat, and C. McSweeney. 2013. Effect of dried *Leucaena* leaf supplementation on rumen ecology, nutrient digestibility and urinary excretion of 2,3-Dihydroxy Pyridone (2,3-DHP) and 3,4-Dihydroxy Pyridone (3,4-DHP) in swamp buffaloes. *Buff. Bull.* 32(Special Issue 2): 975–979.
- Pinos-Rodríguez, J.M., P.H. Robinson, M.E. Ortega, S.L. Berry, G. Mendoza, and R. Bárcena. 2008. Performance and rumen fermentation of dairy calves supplemented with *Saccharomyces cerevisiae*1077 or *Saccharomyces boulardii*1079. *Anim. Feed Sci. Technol.* 140:223–232. doi:10.1016/j.anifeedsci.2007.08.003.

Prasad, C.S., A.K. Samanta, A.P. Kolte, and A. Dhali. 2014. Role of nutraceuticals in livestock production. In: M.P.S. Bakshi and M. Wadhwa, editors, Recent advances in animal nutrition. Satish Serial Publishers, Delhi. p. 117–142.

Reynolds, C.K., L.A. Compton, and J.A.N. Mills. 2011. Improving the efficiency of energy utilisation in cattle. *Anim. Prod. Sci.* 51:6–12. doi:10.1071/AN10160.

Smith, A.H., and R.I. Mackie. 2004. Effect of condensed tannins on bacterial diversity and metabolic activity in the rat gastrointestinal tract. *Appl. Environ. Microbiol.* 70:1104–1115. doi:10.1128/AEM.70.2.1104-1115.2004.

Sniffen, C.J., and P.H. Robinson. 1987. Symposium: Protein and fiber digestion, passage, and utilization in lactating cows. *J. Dairy Sci.* 70:425–441. doi:10.3168/jds.S0022-0302(87)80027-9.

Sonawane, S.N., and S.P. Arora. 1976. Influence of zinc supplementation on rumen microbial protein synthesis in in-vitro studies. *Indian J. Anim. Sci.* 46:13–18.

Sorensen, J.S., J.D. McLister, and M.D. Dearing. 2005. Plant secondary metabolites compromise the energy budgets of specialist and generalist mammalian herbivores. *Ecology* 86:125–139. doi:10.1890/03-0627.

Srivastava, A.K., and M.R. Garg. 2002. Use of sulfurhexafluoride tracer technique for measurement of methane emission from ruminants. *Indian J. Dairy Sci.* 55:36–39.

Stern, M.D., G.A. Varga, J.H. Clark, J.L. Firkins, J.T. Huber, and D.L. Palmquist. 1994. Evaluation of chemical and physical properties of feeds that affect protein metabolism in the rumen. *J. Dairy Sci.* 77:2762–2786. doi:10.3168/jds.S0022-0302(94)77219-2.

Tajima, K., R.I. Aminov, T. Nagamine, H. Matsui, M. Nakamura, and Y. Benno. 2001. Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. *Appl. Environ. Microbiol.* 67:2766–2774. doi:10.1128/AEM.67.6.2766-2774.2001

Wadhwa, M., and M.P.S. Bakshi. 2006. Herbal feed additives—impact on the rumen environment in buffaloes. *Indian J. Anim. Nutr.* 23:102–109.

Wadhwa, M., and M.P.S. Bakshi. 2009. Animal agriculture and greenhouse gas emission: Mitigation strategies. In: A.K. Pattanaik, A.K. Verma, D.N. Kamra, and K. Sharma, editors, Animal nutrition—preparedness to combat challenges. Proceedings Animal Nutrition Association, World Conference held at NASC Complex, New Delhi, 14–17 Feb. 2009. Vol. I. p. 119–122.

Wadhwa, M., and M.P.S. Bakshi. 2014. Nutritional evaluation of urea molasses multi-nutrient blocks containing agro-industrial wastes in buffaloes. *Indian J. Anim. Sci.* 84:544–548.

Wallace, R.J., S.J. Wallace, N. McKain, V.L. Nsereko, and G.F. Hartnell. 2001. Influence of supplementary fibrolytic enzymes on the fermentation of corn and grass silages by mixed ruminal microorganisms *in vitro*. *J. Anim. Sci.* 79:1905–1916.

Wanapat, M. 2000. Rumen manipulation to increase the efficient use of local feed resources and productivity of ruminants in the tropics. *Asian-Aust. J. Anim. Sci.* 13:59–67.

Wanapat, M., N. Anantasook, P. Rowlinson, R. Pilajun, and P. Gunun. 2013. Effect of carbohydrate sources and levels of cotton seed meal in concentrate on feed intake, nutrient digestibility, rumen fermentation and microbial protein synthesis in young dairy bulls. *Asian-Aust. J. Anim. Sci.* 26:529–536.

Wanapat, M., A. Cherdthong, P. Pakdee, and S. Wanapat. 2008. Manipulation of rumen ecology by dietary lemongrass (*Cymbopogon citratus* Stapf) powder supplementation. *J. Anim. Sci.* 86:3497–3503. doi:10.2527/jas.2008-0885.

Wanapat, M., S. Foiklang, P. Rowlinson, and R. Pilajun. 2012. Effect of carbohydrate sources and cotton seed meal in the concentrate: II. Feed intake, nutrient digestibility, rumen fermentation and microbial protein synthesis in beef cattle. *Trop. Anim. Health Prod.* 44:35–42. doi:10.1007/s11250-011-0014-z.

Wang, Y., G.C. Waghorn, W.C. McNabb, T.N. Barry, M.J. Hedley, and I.D. Shelton. 1996. Effect of condensed tannins in *Lotus corniculatus* upon the digestion of methionine and cysteine in the small intestine of sheep. *J. Agric. Sci.* 127:413–421. doi:10.1017/S0021859600078576.

Whitford, M.F., R.M. Teather, and R.J. Forster. 2001. Phylogenetic analysis of methanogens from the bovine rumen. *BMC Microbiol.* 1:5. doi:10.1186/1471-2180-1-5.

White, B.A., I.K.O. Cann, S.A. Kocherginskaya, R.I. Aminov, L.A. Thill, R.I. Mackie, and R. Onodera. 1999. Molecular analysis of archaea, bacteria and eucarya communities in the rumen: Review. *Asian-Aust. J. Anim. Sci.* 12:129–138.

Whitley, N.C., D. Cazac, B.J. Rude, D. Jackson-O'Brien, and S. Parveen. 2009. Use of a commercial probiotic supplement in meat goats. *J. Anim. Sci.* 87:723–728. doi:10.2527/jas.2008-1031.

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Williams, A.G., and G.S. Coleman. 1998. The rumen protozoa. In: P.N. Hobson and C.S. Stewart, editors, The rumen microbial ecosystem. Blackie Acad. Profess, New York. p. 73–139.

Zhang, X., H. Zhang, Z. Wang, X. Zhang, H. Zou, C. Tan, and Q. Peng. 2015. Effects of dietary carbohydrate composition on rumen fermentation characteristics and microbial population *in vitro*. *Ital. J. Anim. Sci.* 14(3):524–531. doi:10.4081/ijas.2015.3366.