



# Influence of Feed and Feeding Strategies on Rumen Microbiome: A Review

Valli Chinnamani<sup>1</sup>, R. Murugeswari<sup>2</sup>, G. Srinivasan<sup>3</sup>, A. Ruba Nanthini<sup>2</sup>

10.18805/IJAR.B-5307

## ABSTRACT

The presence of complex microorganisms in the rumen of ruminants are able to process the lignocellulose from low quality feed and fodder into volatile fatty acids, convert non-protein nitrogen into microbial protein and synthesize B vitamins, thus, ruminants are able to produce food of animal origin without competition for feed with non-ruminants and man. On the other hand, they contribute 14 per cent of anthropogenic methane, released annually into the atmosphere. Manipulating the rumen microbiome is considered as an important approach to reduce environmental impact, while increasing production efficiency in ruminants. A systematic literature search was undertaken and relevant research and review articles were downloaded, classified and salient points extracted to prepare the narrative review on "Influence of Feed and Feeding strategies on Rumen microbiome". The microbial community in the rumen is the most diverse composed of bacteria, archaea, protozoa, fungi and uncharacterized virome. Differences in ruminal microbial community compositions are predominantly attributable to diet. Dietary manipulation offers a viable solution to alter the rumen microbiome so that the present-day challenges faced by the ruminant livestock industry is addressed.

**Key words:** Feed effect, Feed efficiency, Methane mitigation, Rumen microbes.

The organization for economic cooperation and development (OECD) - FAO, Agricultural Outlook 2023-2032, forecasts a continued growth in the demand and production of meat and dairy in the decade ahead (FAO, 2023) World milk production is projected to grow at 1.6 % per annum, faster than most other main agricultural commodities, the per capita consumption of fresh dairy products is also projected to increase by 1.0% per annum over the coming decade, faster than over the past ten years, driven by higher per-capita income growth (FAO, 2019). Global demand for meat is also growing and over the past 50 years, meat production has more than tripled, the world now produces more than 340 million tonnes of meat per year. Ruminants contribute substantially to the production of milk, meat, provide products such as skins, bones, dung, heating material and are draft animals (Flachowsky *et al.*, 2013). The presence of complex microorganisms in the rumen of ruminants (Hungate, 1966) are able to process the lignocellulose from low quality feed and fodder into volatile fatty acids, convert non-protein nitrogen into microbial protein and synthesize B vitamins, thus are able to produce food of animal origin without competition for feed with non-ruminants and man (Flachowsky *et al.*, 2017). However, ruminant livestock production is responsible for approximately 14% of anthropogenic methane, released annually into the atmosphere (Gerber *et al.*, 2013) which, is not only a major problem to the environment, but also causes a loss of 2 to 8% of the feed energy as methane (IPCC, 2006). Improvements in the production efficiency of ruminants between the period 2000 to 2018 has led to decline in methane intensity of meat and milk from ruminants in most regions of the world, despite this decrease in

<sup>1</sup>Faculty of Basic Sciences, Madras Veterinary College Campus, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 051, Tamil Nadu, India.

<sup>2</sup>Institute of Animal Nutrition, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 051, Tamil Nadu, India.

<sup>3</sup>Pulikulam Cattle Research Station, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 051, Tamil Nadu, India.

**Corresponding Author:** Valli Chinnamani, Faculty of Basic Sciences, Madras Veterinary College Campus, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 051, Tamil Nadu, India. Email: valliviba@yahoo.co.in

**How to cite this article:** Valli, C., Murugeswari, R., Srinivasan, G. and Nanthini, A.R. (2024). Influence of Feed and Feeding strategies on Rumen Microbiome: A Review. Indian Journal of Animal Research. 58(9): 1444-1451. doi: 10.18805/IJAR.B-5307.

**Submitted:** 19-01-2024 **Accepted:** 08-04-2024 **Online:** 20-08-2024

methane intensity, total methane emitted globally by ruminants increased during the same period (Chang *et al.*, 2021). Hence, avenues are being sought to decrease the emissions of greenhouse gases from ruminants while simultaneously increasing production to meet the rising demand for animal products (Ungerfeld *et al.*, 2022). Improvement of rumen efficiency can be achieved by manipulating animal diet, improving host microbial interactions and plant microbial interactions to maximise productivity whilst reducing environmental costs (Huws *et al.*, 2018). Microbiota manipulation has been attempted to improve energy harvesting, reduce methane emission, to prevent and treat ruminal diseases (Malmuthuge and Guan, 2017; McCann *et al.*, 2016). It is in this context that this narrative review was conceptualized and written.

## Rumen microbiome

The microbial community in the rumen is the most diverse gut ecosystems of the animal kingdom (Weimer, 2015). It is composed of bacteria ( $10^{10}$  to  $10^{11}$  organisms/ml) archaea ( $10^8$  to  $10^9$  organisms/ml), protozoa ( $10^5$  to  $10^6$  organisms/ml), fungi ( $10^3$  to  $10^4$  organisms/ml) and uncharacterized virome (Newbold and Ramos, 2020). The Hungate 1000 project, has produced 501 genomes (480 bacteria and 21 archaea) from rumen microbes (Seshadri *et al.*, 2018). Members of the genera *Fibrobacter* and *Ruminococcus* are predominant fiber-digesting bacteria in the rumen, specifically the species *Fibrobacter succinogenes*, *Ruminococcus flavefaciens* and *Ruminococcus albus* (Koike and Kobayashi, 2001). Ruminants harbour distinct protozoal populations from birth, the most common protozoa in the rumen are ciliates, with a few flagellate species (Williams and Coleman, 1992). Predominant protozoal genera in the rumen are Entodinium, Epidinium, Metadinium, Diploplastron, Polyplastron and Diplodinium (Lima *et al.*, 2015). Anaerobic fungi represent 10 to 20% of the rumen microbiome and they are thought to be crucial fibre degraders (Krause *et al.*, 2013). There are 5 major fungal phyla, which include 55 fungal genera, the predominant genera are Ascomycota (27%), Basidiomycota (3%) and Neocallimastigomycota (1%) (Kumar *et al.*, 2015). However, Belanche *et al.* (2019) reported that the majority of the fungal species were anaerobic and belonged to Neocallimastigomycota. The most abundant archaeal phylum in the rumen was Euryarchaeota (99%), ten archaeal genera were detected in the rumen and the most abundant genus was Methanobrevibacter (91%) (Zhou *et al.*, 2017). Ruminal methanogens have been broadly clustered into 3 distinct genera *Methanobrevibacter*, *Methanosphaera* and *Methanomassiliicoccales* in dairy cows (Zhu *et al.*, 2017; Pitta *et al.*, 2021).

## Effect of feed type on rumen microbiome

A global comparison study of the rumen microbiome concluded that, a common core of bacteria and archaea dominated in nearly all samples, differences in microbial community compositions were predominantly attributable to diet (Henderson *et al.*, 2015). Calves fed with milk and concentrate are reported to have a higher relative abundance of methanogens and bacteria known to degrade readily fermentable carbohydrates than milk only fed calves (Dias *et al.*, 2017). Firmicutes and Bacteroidota were the two major bacterial phyla detected in preweaning calves (Li *et al.*, 2019; Dias *et al.*, 2018), Firmicutes was dominant in high-grain diets, while Bacteroidota was dominant in hay diets (Faniyi *et al.*, 2019). Calf starter fed to calves from the seventh day onward led to the emergence of *Prevotella* as the predominant rumen bacterium by fourteenth day, *Megasphaera* species increased between 7 and 14 days and *Butyrivibrio*, *Succinivibrio*, *Catenibacterium* increased after 14<sup>th</sup> day with decline in *Streptococcus* and *Lactobacillus*. Archaeal species increased by 28<sup>th</sup> day (O'Hara *et al.*,

2020). Calves fed milk replacer at 0.749 kg/d had a higher proportion of ruminal fibrolytic bacteria (*B. fibrisolvens* and *Clostridium* cluster IV) than calves fed 1.498 kg of milk replacer/d for 5 weeks (Hao *et al.*, 2021).

High forage diets, in comparison to high-concentrate diets, promoted higher bacterial diversity and abundance of Bacteroides, Fibrobacter and Ruminococcus (Wang *et al.*, 2019a). On feeding hay, higher relative abundances of *Prevotella*, *Eubacterium*, *Oscillibacter* and *Succinivibrio* and lower relative abundances of *Ruminococcus*, *Clostridium* and *Olsenella* were identified compared with the feeding of high concentrate (Kim *et al.*, 2018). High concentrate diets negatively affected protozoal growth (Hristov *et al.*, 2001). Large increases in grain feeding increased the abundances of *Selenomonas ruminantium*, *Streptococcus bovis* and *Prevotella bryantii* while decreasing the abundances of *Butyrivibrio fibrisolvens* and *Fibrobacter succinogenes* in rumen digesta (Fernando *et al.*, 2010). During subacute rumen acidosis induced by high grain diet or sudden change from high forages to a grain based diet, a decline in the population of Bacteroidetes occurred (Cardo, 2015). The relative abundances of the phyla Bacteroidetes, Fibrobacteres, Verrucomicrobia and Proteobacteria were higher in low SARA risk lambs, Firmicutes, Tenericutes and Actinobacteria were higher in the high SARA risk lambs (Li *et al.*, 2017). High concentrate diet in goats can induce ruminal microbiota dysbiosis, phylum-wide shift in the *Cyanobacteria* and *Verrucomicrobia* (Hua *et al.*, 2017). Rumen bacterial composition of goats fed with high grain diet had relative abundance of *Actinobacteria* and lower relative abundance of *Tenericutes*, *Verrucomicrobia*, *Proteobacteria* and *Fibrobacteres*, no significant differences were observed in relative abundance of *Bacteroidetes* and *Firmicutes* between hay and high grain fed goats (Zhang *et al.*, 2018). High proportions of rapidly fermented carbohydrates and low ruminal pH resulted in rapid proliferation of *Streptococcus bovis* and *Lactobacillus* sp. (McCann *et al.*, 2016). Dairy goats fed high levels of rumen degradable starch, promoted lower levels of genes encoding for enzymes involved in cellulose degradation and starch branching which were mostly present in Prevotellaceae, Ruminococcaceae and Bacteroidaceae (Shen *et al.*, 2020). High concentrate diets increased the relative abundance of *Eubacterium* spp., *Clostridium* spp., *Ruminococcus* spp., *Lactobacillus*, *Bifidobacterium* spp. and decreased the relative abundance of *Prevotella* spp., *Fibrobacter succinogenes* and *Bacteroides* spp (Zhang *et al.*, 2020a).

In Sunit sheep that were only grazed or adopted to barn feeding with grazing the concentrations of propionic acid were negatively correlated to the abundance of *Bacteroides* and positively correlated to *Ruminococcus* (Wang *et al.*, 2018). Belanche *et al.* (2019) reported that the adaptation process from non-grazing to grazing diet led to an increase in the microbial concentration, diversity, microbial network complexity and resulted in the abundance

of key microbes *Ruminococcus*, *Butyrivibrio*, *Orpinomyces*, *Prevotella*, *Entodiniinae*, *Streptococcus*, *Selenomonas* and *Methanomassiliicoccaceae*.

### Feed energy and protein levels on rumen microbiome

There was an increase in the number of Bacteroidetes within the rumen in buffalo fed high levels of hay, which decreased the dietary energy concentration (Pitta *et al.*, 2014a). Relative abundance of Firmicutes, Lachnospiraceae, Veillonellaceae, Saccharofermentants Erysipelotrichaceae and Butyrivibrio were reported in high feed intake, Mao *et al.* (2012) indicated that the abundance of Prevotella was highly correlated with the content of crude protein. Protozoa and fungi count remained unchanged with increasing levels of crude protein, but archaea counts increased (Chanthakhoun *et al.*, 2012). Increasing crude protein raised proteolytic bacteria abundance (*Butyrivibrio fibrisolvens* and *Prevotella ruminicola*) in crossbred beef steers fed total mixed ration (Wang *et al.*, 2017). A diet with 5.3% ruminally degradable protein resulted in a higher bacterial nitrogen flow 415 g/d as opposed to 365 g/d when 4.8% ruminally degradable protein was fed (Volden *et al.*, 1999). Reducing 30% lysine via rumen protected lysine significantly decreased relative abundance of Firmicutes (Kong *et al.*, 2020). Low energy diet increased the relative abundance of phyla of Fibrobacteres whereas, at the genus level, it increased the relative abundance of Butyrivibrio and Prevotellaceae (Li *et al.*, 2020). Srinivasan *et al.* (2023) reported that Methanogen belonging to the order Methanomassiliicoccales was not present in the rumen liquor of Pulikulam cattle fed high energy diet.

### Feed processing on rumen microbiome

Pelleting high grain TMR altered the rumen fermentation and amplified the ruminal microbiome, Fibrobacteres was significantly decreased in the high grain TMR pelleted group, pelleting resulted in a decrease in the percentage of unclassified BS11 gut group and Succiniclasticum, while it led to an increase in the abundance of simple sugar fermenters like Megasphaera, Olsenella, Dialister and Sharpea (Trabi *et al.*, 2019). Chopping roughage increased ruminal bacteria papillibacter and *Eubacterium hallii* which are involved in butyrate production and also increased synergistetes and mogibacterium, which are involved in bacterial colonization (Wang *et al.*, 2020). In yearling sheep, feeding pelleted hay led to increase in bacterial richness and was positively associated with several succiniclasticum, prevotella and uncultured taxa in the ruminococcaceae and rickenellaceae families and bacteroidales order (Ishaq *et al.*, 2019b). The relative abundance of prevotella was significantly increased while the relative abundance of succinivibrionaceae was decreased in cattle fed whole-plant corn silage compared with those fed corn straw silage (Cui *et al.*, 2022).

### Fat or fatty acid supplementation on rumen microbes

Vegetable oil supplementation reduced rumen protozoan population in ruminants and also resulted in a negative effect on cellulolytic bacteria causing inhibitory effects of the fibre digestion (Ibrahim *et al.*, 2021). Ruminal microorganisms belonging to the genera fibrobacter, ruminococcus, butyrivibrio and prevotella can be very sensitive to fat (Huws *et al.*, 2014). Soybean oil and linseed oil supplementation potentially decreased the populations of *Butyrivibrio fibrisolvens*, *Ruminococcus albus* and *F. succinogenes* and decreased total volatile fatty acid production in the rumen (Yang *et al.*, 2009). Studies have shown that linseed and linseed oil affect the bacteria communities in the rumen, especially the cellulolytic group (Enjalbert *et al.*, 2017).

Dietary PUFA can influence the composition of the rumen microbiota (Fievez *et al.*, 2007). Oil rich supplement containing C18:2n-6 and C18:3n-3 inhibited the growth of fibrolytic fungi, Fibrobacter succinogenes and *Ruminococcus flavefaciens* in the rumen and suppressed the methanogenesis (Zhang *et al.*, 2008). Hristov *et al.* (2013) reported that both C18:2n-6 and C18:3n-3 were effective to decrease rumen ciliate. Coconut oil, which has a high MCFA content, is more toxic to protozoa and fibrolytic bacteria, moreover, it selectively inhibits certain ruminal archaea populations (Patra and Yu, 2014). Supplementation of MCFA at 0.063 per cent dietary DM may not affect overall animal performance or total tract nutrient digestibility but decrease the daily range of pH and the bacterial richness in the rumen (Burdick *et al.*, 2022). Ca soap supplementation increased protozoa population in cull ewes (Bhat *et al.*, 2013). The supplementation of rumen protected fat increased the population of *F. succinogenes* and *R. albus* significantly as compared to the diet without rumen protected fat while the population of *R. flavefaciens* and total cellulolytic bacteria was found significantly lowest in the diet Ca soap (Behan *et al.*, 2019).

### Mineral and vitamin supplementation on rumen microbes

Trace mineral supply from rumen sustained release boluses influenced rumen microbiota of lactating yaks, relative abundances of planctomycetes tended to be higher and the erysipelotrichaceae and *Tenericutes bacterium* tended to be lower in mineral supplementation (Zhao *et al.*, 2022). The population of *Dasytricha ruminantium*, *Ophryoscolex caudatus*, *Polyplastron multivesiculatum* and *Diploplastron affine* were significantly higher in sheep supplemented with organic or inorganic Se than in animals given the basal diet alone (Mihalikova *et al.*, 2005). Dietary Se increased total bacteria, total anaerobic fungi, total protozoa, *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Butyrivibrio fibrisolvens* (Zhang *et al.*, 2020b; Liu *et al.*, 2019; Zhang *et al.*, 2020c). Dietary supplementation of alkaline mineral complex buffer, in Holstein Friesian cows in transition period changed the composition and structure of rumen

microorganisms it considerably increased the abundance of Firmicutes Ruminococcaceae and Christensenellaceae considerably 14 days before the expected delivery date (Guo *et al.*, 2023). Total rumen bacterial evenness and diversity in rams were reduced by supplementation with a Zn AA complex, but not in rams supplemented with an equal concentration of ZnSO<sub>4</sub>, likely due to differences in bioavailability between organic and inorganically sourced supplement formulations (Ishaq *et al.*, 2019a). Supplementing with Na<sub>2</sub>SO<sub>4</sub> increased the diversity and the richness of rumen microbiota and the relative abundances of the phylum Firmicutes and genera Ruminococcus 2, Rikenellaceae RC9 gut group and Desulfovibrio, whereas it decreased the relative abundances of the phylum Bacteroidetes and genera Prevotella 1, Prevotellaceae UCG-001 and Treponema 2 (Zhao *et al.*, 2022). Major cellulolytic bacteria like *Ruminococcus albus*, *Ruminococcus flavefaciens* and anaerobic fungi in the rumen Neocallimastix require thiamine for optimal proliferation (Kandathil and Bandla, 2019). Activity of cellulase and populations of total bacteria, protozoa, fungi, dominant cellulolytic bacteria, *Prevotella ruminicola* and *Ruminobacter amylophilus* increased linearly with increase in levels of riboflavin supplementation in Holstein bulls (Wu *et al.*, 2021).

### Phytobiotics on rumen microbiome

Herbal extracts from different sources may have stimulatory or inhibitory effects on fibrolytic bacteria due to their non volatile compounds (Khovidhunkit *et al.*, 2000). Essential oils have inhibitory effects on gram-positive bacteria due to main bioactive compounds (Cristani *et al.*, 2007; Khorrami *et al.*, 2015). A study by Patra and Yu (2014) observed that protozoa population decreased, but increased abundances of *Ruminococcus flavefaciens*, *Prevotella bryantii*, *Butyrivibrio fibrisolvens*, *Prevotella ruminicola*, *Clostridium aminophilum* and *Ruminobacter amylophilus* with increasing doses of vanillin. Calves receiving 4 mL/d of phytobiotic-rich herbal extract (Immunofin, IMPE) had a greater abundance of total bacteria, *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* (Jahani-Azizabadi *et al.*, 2022). Saponins not only have adverse effects on rumen protozoa but also affect specific bacteria and fungi (Wang *et al.*, 2019b). Supplementation with low levels (10 g/cattle per day) of tea saponin could significantly increase total VFAs and the relative abundances of a variety of microbiota including Proteobacteria, Actinobacteria, Saccharomyces and Aspergillus (Qu *et al.*, 2023).

Supplementations of rain tree pod meal (0 and 60 g/kg of total DM intake), containing crude tannins and saponins at 84 and 143 g/kg of DM, did not affect total bacterial, *Ruminococcus albus* and viable proteolytic bacteria, however the numbers of fungi, cellulolytic bacteria, *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* were higher while amylolytic bacteria was lower and the population of *Fibrobacter succinogenes*, was found to be higher (Anantasook *et al.*, 2013). An increased abundance of Prevotellaceae but decreased abundances

of Ruminococcaceae and Lachnospiraceae were observed in young growing cattle after they were fed saponins (Wang *et al.*, 2019b).

The inclusion of chestnut tannins led to the enrichment of the genera Anaerovibrio, Bibersteinia, Escherichia, Pseudobutyrvibrio and Streptococcus (Mannelli *et al.*, 2019). The abundance of *Ruminococcus flavefaciens*, methanogenic archaea and protozoa populations were reduced, whereas total ruminal bacteria were enhanced in the presence of condensed tannins (Fagundes *et al.*, 2020). The relative abundances of Methanobrevibacter and total methanogens decreased following supplementation with red seaweed extracts, which was consistent with the decreased CH<sub>4</sub> production (Choi *et al.*, 2022).

### Feed efficiency and rumen microbial diversity

A lower abundance of active bacterial families of Lachnospiraceae, Lactobacillaceae and Veillonellaceae have been reported in animals with high feed efficiency (Li and Guan, 2017). Lower abundance of archaeal species of *Methanobrevibacter smithii* were present in the rumen contents of animals with higher feed efficiency (Carberry *et al.*, 2014). In high-efficiency animals, Selenomonas and members of the Succinivibrionaceae family positively interacted with each other (Xue *et al.*, 2022).

### CONCLUSION

The rumen microbial community is affected by a great number of internal and external factors, such as host, physiological status, diet and environment (Pitta *et al.*, 2014b). The fundamental knowledge on rumen microbes will lead to the development of advanced methods to manipulate the rumen microbiome and thereby improve the production efficiency of ruminants (Wang *et al.*, 2023). Dietary manipulation offers a viable solution to alter the rumen microbiome so that the present-day challenges faced by the ruminant livestock industry is addressed (Stanton *et al.*, 2019). Recent advances in high-throughput sequencing and bioinformatic analyses have helped to reveal how the composition of the rumen microbiome varies significantly with changes in diet. These efforts are beginning to explain how shifts in the microbiome affect feed efficiency. Efforts must now be made to understand how this rumen microbial community interacts with the physiological function of the rumen and the host, ultimately influencing both the health and productivity of the animal.

### Conflict of interest

All authors declare that they have no conflict of interest.

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