



Rumen Microbiota and Nutrient Metabolism: A Review

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ABSTRACT

The rumen consists of a complex ecosystem where nutrients consumed by ruminants are digested by fermentation process, which is executed by diverse microorganisms such as bacteria, protozoa, fungi, bacteriophage and oscillospira. A symbiotic relationship is found among different groups of microorganisms due to the diverse nature of these microbial species and their adaptability and interactions. The ruminant provides necessary environment for the establishment of such microorganisms, while the microorganisms obtain energy from the host animal through microbial fermentation end products. The rumen microbial ecosystem fulfills several functions like fibrolytic, lipolytic and proteolytic functions and produce metabolites including volatile fatty acids (VFA), biohydrogenated lipids, microbial protein, methane etc. The purpose of this review is to contribute a better understanding of the fermentation processes that are taking place in the rumen and to provide information that can be applied for the development of new nutritional strategies to improve the digestion process for achieving maximum production.

Key words: Biohydrogenated lipid, Methane, Microbial protein, Rumen microbes, Rumen, Symbiosis, VFA.

Animal husbandry is an important link during the exchange between humans and nature. The development of animal husbandry not only provides food for humans but also contribute to the agricultural economy and makes an important contribution to the economy. Ruminant animal has complex stomach that is divided into four chamber *i.e.* rumen, reticulum, omasum and abomasum. The rumen is described as a “black box” due to presence of various microbes and the ruminal microbiota is regarded as a new organ consisting of trillions of microbes. The rumen microbiota is responsible for the successful conversion of low-quality feedstuffs into energy for host (Seymour *et al.*, 2005).

Rumen microbiota contains several kingdoms including Bacteria, Bacteriophages, Protozoa, Fungi and Oscillospira. The rumen microbial ecosystem fulfills several functions like fibrolytic, lipolytic and proteolytic functions and produce metabolites including volatile fatty acids (VFA), biohydrogenated lipids, microbial protein etc. These metabolites are either absorbed across the rumen epithelium or in the lower gastrointestinal tract and then enter into the blood-stream to be available to the host.

Ruminants have an ability to convert the low quality fibrous feed materials into valuable products such as meat, milk and fibers, which are useful to humans. It has been reported that ruminant livestock farming has the potential to minimize the use of human edible feedstuffs by utilizing available forage resources within a given system (Eisler *et al.*, 2014).

Rumen

The rumen is the largest compartment of ruminal stomach and is the first chamber in the alimentary canal of ruminant animals. The capacity of an adult dairy cow's rumen is about 150 to 200 liters. It is one of the most dense microbial habitats in the world. Microscopic organisms called rumen

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microbes break down (or digest) the ingested feed by a fermentation process. The rumen is the major site of fermentation in ruminant animals.

Physicochemical properties of the rumen

- The normal rumen temperature is in the range of 39 to 39.5°C (Wahrmund *et al.*, 2012) and immediately after eating it may increase up to 41°C as the fermentation process generates heat (Brod *et al.*, 1982).
- The pH of rumen usually remains in the range of 5.5 to 7.0 (Krause and Oetzel, 2006). The pH depends on the production of saliva, generation and absorption of short-chain fatty acids, the type and level of feed intake and the exchange of bicarbonates and phosphates through the ruminal epithelium (Aschenbach *et al.*, 2011).
- The osmotic pressure in the rumen depends on the presence of ions and molecules, which generate a gas tension. The ruminal fluid's osmolality is approximately 250 mOsm/kg (Lodemann and Martens, 2006).

- In ruminant digestive system, saliva is main component acting as buffering agent. Normal salivary buffering capacity of saliva is 125 meq/l bicarbonate and pH is 8.4. So saliva will help to maintain ruminal environment in balance.
- The normal ruminal redox potential remains in the range of -130 to -200 mV.

Ruminal microbiota

The ruminal ecosystem consists of a wide diversity of microorganisms that are in a symbiotic relationship in a strict anaerobic environment (Ozutsumi *et al.*, 2005). It consist bacteria (10^{10} to 10^{11} organisms/ml), bacteriophage (10^8 to 10^9 organisms/ml), protozoa (10^5 to 10^6 organisms/ml), fungi (10^3 to 10^4 organisms/ml), oscillospira (10^4 organisms/ml) and uncharacterized virome.

Establishment of rumen microflora

The rumen in adult ruminants harbors a dense and diverse microbiota, whereas the rumen in newborn calves and lambs has a rather simple microbiota. The rumen is inoculated with different type of microorganism during lactation, diet ingestion and contact with the environments so that it is gradually colonized by a large number of diverse microbes that affect the epithelial cell function and gut-associated lymphoid tissue development (Jami *et al.*, 2013).

Ruminal microbes and nutrient metabolism

The microbes that reside in the rumen influence the host metabolism by degrading the dietary materials, though these microbes are not considered as one of the specific tissues of the host. This microbiota participates in the digestion of the diet by their own secreted enzymes. It has been demonstrated that the rumen microbiome plays a critical role in feed efficiency, milk yield and milk components in dairy cows (Scharen *et al.*, 2018).

Ruminal bacteria

The rumen contains a variety of bacterial genera, which constitute the majority of the microorganisms that live in anaerobic environment (Pitta *et al.*, 2010).

Cellulose degrading bacteria

The ruminant diet is mainly plant based and cellulose is the main component of the cell wall of these plants. So, cellulolytic ruminal microorganisms play an important role in animal nourishment (Russell *et al.*, 2009). When cellulose enter into rumen, cellulose degrading bacteria (Table 1) produce extracellular cellulase enzymes (Weimer, 2015) to break β (1-4)-glycosidic bonds of the cellulose and produce monosaccharide glucose, which is then fermented to

pyruvate primarily by the Embden Mayerhoff (EMP) and pentose phosphate pathway. Pyruvate is then convert to VFAs. These VFAs are directly absorbed from the rumen by simple diffusion.

The ability of cellulolytic bacteria to degrade cellulose depends mainly on the type of forage, crop maturity and the members of the bacterial communities (Fondevila and Dehority, 1996). The establishment of this bacterial group can be affected by the presence of certain types of lipids in the diet. For example, medium chain fatty acids are often toxic to cellulolytic bacteria, reducing the digestibility of the fiber.

Amylolytic bacteria

Starch is an important component of the diet of cattle and high milk producing cows which are fed with concentrates containing major proportions of grains. Starch is an easily fermentable energy source for ruminants. When starch undergoes ruminal fermentation in rumen, amylolytic bacteria (Table 2) produce maltase enzyme that convert starch into disachharide maltose and further into monosaccharide glucose. Glucose converted into pyruvate primarily by the EMP and pentose phosphate pathway and subsequently into (volatile fatty acids) VFAs. When feed contains large amounts of starch, the production of total VFAs per kilogram feed is greater than when feed contains large amount of fiber is fed. At the same time, the amount of propionic acid relative to amount of other acids, also increases when the diet rich in starch is fed.

On normal ruminant diet, amylolytic bacterial population remains in the range of 10^4 - 10^7 cells/grams but, its population may be greater than 10^{11} /grams in ruminal contents, when the concentration of fermentable sugar increases in the diet (Nagaraja and Titgemeyer, 2007). Furthermore, *Streptococcus bovis* ferments glucose to provide acetate, formate and ethanol as final products. However, in high concentrate diets, this species changes its metabolism to provide lactic acid as the final product, which causes a drop of pH to 5.5 that is detrimental to the ruminant (Russell and Hino, 1985). It is necessary to introduce fermentable carbohydrates gradually in the ruminant diet to avoid this situation.

Lactate degrading bacteria

When diet containing high amount of concentrate is being fed, lactate is produced as an intermediary product of ruminal fermentation, which is then metabolized to VFAs. Lactate degrading bacteria (Table 3) have an important role in the rumen fermentation, mainly in those ruminants that are being fed with high grains in the diet. These bacteria metabolize lactic acid and prevent its accumulation, which helps to keep

Table 1: Cellulose degrading bacteria, morphology and its fermentation characteristics.

Microorganisms	Gram stain	Morphology	Fermentation products
<i>Fibrobacter succinogenes</i>	Negative	Bacillus	Succinate, Acetate, Formate
<i>Butyrivibrio fibrisolvens</i>	Negative	Bacillus curve	Acetate, Formate, Lactate, Butyrate, H ₂ , CO ₂
<i>Ruminococci albus</i>	Positive	Cocci	Acetate, Formate, H ₂ , CO ₂
<i>Clostridium lochheadii</i>	Positive	Bacillus	Acetate, Formate, Butyrate, H ₂ , CO ₂

the pH in the proper range. Thus, they have an important role in the prevention of acidosis during the adaptation period when ruminants are fed with high concentrate diet (Counotte *et al.*, 1981). This type of bacteria increase when the diet consists of approximately 70% concentrates (Brown *et al.*, 2006).

Pectin degrading bacteria

Pectin degrading bacteria are important as the pectin represents 10-20% of total carbohydrates in forages used in ruminant nutrition. Pectin is fermented by both bacteria and protozoa. The major bacteria that perform this function are *Butyrivibrio fibrisolvens*, *Prevotella ruminicola*, *Bacteroides ruminicola* and *Lachnospira multiparus*. These ruminal bacteria produce and release pectinolytic enzymes primarily pectin lyases into the ruminal environment that hydrolyze the pectin in to oligogalacturonoides (Duskova and Marounek, 2001).

Proteolytic bacteria

Protein digestion in the ruminant animals can be divided into two phases: (1) digestion in the reticulorumen and (2) digestion in the abomasum and small intestine. Therefore, in ruminant animals, dietary proteins are classified as rumen degradable protein and rumen undegradable protein.

In ruminants, proteins serve as a source of nitrogen for rumen microbes to make their own microbial protein. Microbes can use non protein nitrogenous substances also such as urea for microbial protein synthesis. Urea is 100% degradable in the rumen by microbial urease and can be toxic at higher levels.

Protein in the rumen may be degraded by both proteolytic bacteria (Table 4) and protozoa which produce proteolytic enzymes like proteases and peptidases to cleave peptide bonds in polypeptides and release the free amino acids from proteins. These rumen degraded amino acids release NH_3 and carbon skeleton by deamination process.

Along with volatile fatty acids (from carbohydrates fermentation), rumen microbes synthesize their own microbial protein, which serves as a primary source of protein to ruminant animals. Some amount of ammonia get absorbed directly from rumen and is converted to urea and secreted into the blood as blood urea nitrogen (BUN). Urea can be filtered and recycled to the rumen via saliva or through the rumen wall. The concentration of BUN in ruminants reflects the efficiency of protein utilization.

Proteins that are not degraded by rumen microbes are called “escaped protein”, “bypassed protein” or “rumen undegradable protein” (RUP) and have a low rumen degradation rates. RUP enters the abomasum and small intestine of the ruminant animals for subsequent digestion and absorption. Proteins reaching to the small intestine could be RUP or those from microbial sources. The amino acid needs of the host animal are met by RUP and microbial proteins.

Lipolytic bacteria

In ruminant animals, the lipid content of the diet is low and comes from different sources such as grass, leaves, oil seeds, or cereal grains. The major types of lipids in the diet are triglycerides, phospholipids and galactolipids (Jenkins *et al.*, 2008). Leaf or grass lipids are mainly galactolipids, phospholipids, waxes, pigments and essential oils while oil seed or grain lipids are mainly triglycerides.

Lipids are modified by microbial fermentation in the rumen. Ruminal microorganisms transform lipids by two major pathways *i.e.* lipolysis and biohydrogenation (Table 5). When dietary lipids enter the rumen, hydrolysis of dietary lipids is brought by microbial lipases, which releases glycerol and free fatty acids from the lipid backbone. Glycerol is readily metabolized by the rumen bacteria to form propionic acid. Hydrolysis is a prerequisite for the next step. The rate of lipolysis depends on the type of lipids present in the diet

Table 2: Amylolytic degrading bacteria, morphology and its fermentation characteristics.

Microorganisms	Gram stain	Morphology	Fermentation products
<i>Bacteroides ruminicola</i>	Negative	Bacillus	Formate, Acetate, Succinate
<i>Ruminobacter amylophilus</i>	Negative	Bacillus	Formate, Acetate, Succinate
<i>Selenomonas ruminantium</i>	Negative	Bacillus curve	Acetate, Propionate, Lactate
<i>Succinomonas amylolítica</i>	Negative	Oval	Acetate, Propionate, Succinate

Table 3: Lactate degrading bacteria, morphology and its fermentation characteristics.

Microorganisms	Gram stain	Morphology	Fermentation products
<i>Selenomonas lactilytica</i>	Negative	Bacillus curve	Acetate, Succinate
<i>Megasphaera elsdenii</i>	Positive	Cocci	Acetate, Propionate, Butyrate, Valerate, H_2 , CO_2

Table 4: Proteolytic degrading bacteria, morphology and its fermentation characteristics.

Microorganisms	Gram stain	Morphology	Fermentation products
<i>Bacteroides amylophilus</i>	Negative	Bacillus	NH_3 , Amino acid, VFAs
<i>Bacteroides ruminicola</i>	Negative	Bacillus	NH_3 , Amino acid, VFAs
<i>Butyrivibrio fibrisolvens</i>	Negative	Bacillus curve	NH_3 , Amino acid, VFAs

Table 5: Lipolytic degrading bacteria, morphology and its fermentation characteristics.

Microorganisms	Gram stain	Morphology	Fermentation products
<i>Anaerovibrio lipolytica</i>	Negative	Bacillus	Acetate, Propionate

Table 6: Ruminal archaea (methanogens), morphology and its fermentation characteristics.

Microorganisms	Gram stain	Morphology	Fermentation products
<i>Methanobrevibacter ruminantium</i>	Positive	Bacillus	Methane
<i>Methanomicrobium mobile</i>	Negative	Bacillus	Methane

Table 7: Ruminal protozoa, morphology and its fermentation characteristics.

Protozoa	Fermentation products
Cellulolytic protozoa	
<i>Enoploplastron triloricatum</i>	Reducing sugars
<i>Eudiplodinium maggii</i>	Reducing sugars
<i>Diploplastron affine</i>	Reducing sugars
<i>Epidinium ecaudatum</i>	Reducing sugars
<i>Diplodinium monacanthum</i>	Reducing sugars
<i>Diplodinium pentacanthum</i>	Reducing sugars
Proteolytic protozoa	
<i>Entodinium caudatum</i>	Amonium, VFA
<i>Eudiplodinium medium</i>	Amonium, VFA

(Beam *et al.*, 2000) and the ruminal pH. A pH value less than 6.0 causes slow lipolysis, which decreases as the pH drops (Fuentes *et al.*, 2009). Therefore, lipolysis depends on the type of fermentable substrates in the diet (Nevel and Demeyer, 1996).

Biohydrogenation of unsaturated fatty acids is the second major transformation that dietary lipids can undergo in the rumen. Fatty acids with double bonds are altered due to the presence of H^+ produced by ruminal microorganisms to form more stable fatty acids (Jenkins *et al.*, 2008). Fatty acids such as linoleic acid are converted “conjugated” fatty acids (e.g. conjugated linoleic acid (CLA)) in which the double bonds are not separated by methylene (CH_2) groups, but the position of double bonds is altered and the fatty acids are converted to more stable “trans” fatty acid. Some odd-numbered (e.g., C19:0) and branched-chain fatty acids are also created during this process. For example, linoleic acid (C18:2 n-6), where the double bonds are in the cis position (cis9-cis12), is converted to several isomers of CLAs during this conversion step.

Ruminal archaea or methanogens

Methane (CH_4) is one of the end product of ruminal fermentation and is considered as a loss of total energy consumed by ruminants, representing 6-10% of total energy (Mohammed *et al.*, 2004), which contributes to the greenhouse effect (Garnsworthy *et al.*, 2012). In ruminants 80% of methane is generated during fermentation of fiber, mainly cellulose and 20% of methane is generated by the decomposition of manure (Verge *et al.*, 2007). These percentages can vary depending upon the composition of ruminant diet (Rotz *et al.*,

2010). Production of methane is more when the diet contains more roughage as compared to concentrate diet.

The methanogens (Table 6) belong to the domain Archaea (Morgavi *et al.*, 2010) and the phylum Euryarchaeota. Methane is generated by methanogenic bacteria utilizing the carbon dioxide and hydrogen (Zijderveld *et al.*, 2011). H^+ is one of the main end products of the fermentation process in rumen by bacteria, protozoa and fungi. In the rumen, an interrelationship exists among species producing and utilizing H^+ that is called “interspecies H^+ transfer”. The production of methane in the ruminal environment is a clear example of this process (Walker *et al.*, 2012). Methanogenesis is the main sink of H^+ removal (Moss *et al.*, 2000). Not only CO_2 is used by the methanogens to produce CH_4 , but these microorganisms can also degrade substrates containing methyl (CH_3) or acetyl (CH_3COO) groups, such as methanol and acetate that act as electron acceptors (Liu and Whitman, 2008).

Ruminal protozoa

Rumen protozoa (Table 7) constitute about 50% of the viable biomass in the rumen (Newbold *et al.*, 2015). Majority are ciliates and few are flagellates and are very motile. Among the ciliated protozoa holotrich and entodiniomorphid protozoa, have been studied inside the rumen. Morphological studies have identified more than 250 species of ciliates living in the various ruminants (Williams and Coleman, 1997).

- They engulf bacteria and feed particles and digest carbohydrates, proteins and fats.
- These ciliates play an important role in fiber digestion and the modulation of the fermentation profiles. The rumen protozoa produce fermentation end products similar to those made by the bacteria, particularly acetate, butyrate and H_2 .
- Rumen methanogenic bacteria actually attach and live on the surface of rumen protozoa for immediate access to H_2 .
- They utilize large amounts of starch at one time and can store it in their bodies. This may help to slow down the production of acids that lower rumen pH, benefiting the rumen.
- The enzymes responsible for cellulose and hemicellulose degradation have also been reported in the holotrich protozoa, but the levels are very low compared to those present in the entodiniomorphid protozoa (Williams and Coleman, 1992).
- The enzymatic profile of holotrich protozoa indicates that these have amylase, invertase, pectin esterase and polygalacturonase in sufficiently large quantities for using starch, pectin and soluble sugars as energy source.

Table 8: Ruminal fungi, its morphology and its fermentation characteristics.

Cellulolytic fungi	Fermentation products
<i>Neocallimastix frontalis</i>	Lactate, Formate, Acetate, Succinate, Ethanol
<i>Piromyces communis</i>	Cellobiose, Glucose
<i>Orpinomyces joyonii</i>	Glucose

- The rumen ciliates also have proteolytic activity and produce ammonia and amino acids as end products. Their nitrogen metabolism is based largely on the digestion of engulfed bacteria, although all rumen ciliates contain enzymes capable of digesting plant proteins (Coleman, 1983).

Ruminal fungi

Fungi (Table 8) represent a small proportion (approximately 8%), of the biomass in the ruminal ecosystem (Jenkins *et al.*, 2008), but they do have a role in the digestion of food consumed by the ruminant (Nam and Garnsworthy 2007). Some fungi are microaerotolerant and are attached to feed particles through a system of rhizoids (Denman *et al.*, 2008). Ruminal fungal populations are favoured by the consumption of fibrous forage that are mainly highly lignified. Ruminal fungi are present in the duodenum, cecum and faeces and are removed when ruminants are fed with high concentrations of rapidly fermentable sugars.

The fungal activity helps the ruminal digestion of the plant cell wall. The production of zoospores by chemotaxis allows rapid adhesion to the particles, then the fungi fracture zones of lignified tissues by mechanical action and the nonlignified plant tissues are rapidly degraded (Grenet *et al.*, 1989). Thus, ruminal fungi are particularly important when the ruminant consumes many lignified substrates.

Bacteriophage

Bacteriophages are one of the most important component of the rumen microbial community and are present typically at 10^8 - 10^9 organisms/ml of rumen fluid. These are specific for different bacteria present in the rumen. These are also considered to be obligate pathogens for the bacteria, as bacteriophages are capable of lysing the bacteria.

These phages help in bacterial mass turnover in the rumen, which may be considered not so useful for the animals on different feeding schedules (Klieve and Swain, 1993), but by lysing the bacterial cells, bacterial protein is easily made available to the animals as a source of amino acids.

Oscillospira

Oscillospira is motile and iodophilic in nature. In comparison with bacteria it is larger in size. The cell structure shows filaments filled transversely making partition in the cell. It could not be cultured outside the rumen. Till recently, it was considered to be a yeast. But it possesses similar cell wall structure and composition as in gram negative bacteria. This depicts that this organism is a higher form of bacteria. *Oscillospira guilleromondii* has been found only in the rumen.

Case studies

Cherdthong *et al.* (2010) used four rumen-fistulated male swamp buffalo as 4×4 latin square design to evaluate the effect of feeding urea-treated rice straw(R) with concentrate(C) in different ratio on rumen fermentation, nutrient digestibilities, microbial protein synthesis and cellulolytic bacterial population. Animals were fed urea treated rice straw with concentrate in the ratio of 100:0, 75:25, 50:50 and 25:75, respectively. At the end of experiment they were observed that ruminal microbial counts and variable bacteria were significantly different ($p<0.01$) among treatments. Protozoa and amylolytic bacteria were greatest when urea treated rice straw with concentrate in the ratio of 25:75 (5.8×10^6 and 5.6×10^8 cell/g, respectively). Moreover, cellulolytic bacteria were increased when urea treated rice straw with concentrate in the ratio of 100:0 and 75:25 (10.9×10^9 cell/g).

Wanapat *et al.* (2014) conducted an experiment to determine the effect of roughage to concentrate ratio (R:C) on rumen pH, fermentation and bacterial population in dairy steers. Four rumen fistulated dairy steers (170±20 kg) were randomly assigned according to a 4×4 Latin square design, in which the steers were fed with four dietary treatments with different R:C ratios of 80:20, 60:40, 40:60 and 20:80 respectively. All animals were kept in individual pens and received feed according to the respective R:C ratios at 0.025 body weight/d; urea-treated rice straw (prepared using 3.5 kg urea + 100 kg water sprayed onto 100 kg of rice straw) was used as a roughage source. The experiment was conducted for four periods of 21 days each. At the end of experiment they observed that numbers of protozoa, fungi and proteolytic bacteria were not affected by R:C ratio. Cellulolytic bacteria decreased linearly while amylolytic bacteria increased linearly with 60 and 80 concentrates.

Sinha *et al.* (2017) conducted an experiment to investigate the effect of feeding high and low roughage total mixed ration (TMR) diets on rumen metabolites and enzymatic profiles. Three rumen-fistulated crossbred cattle and buffalo were randomly assigned as per 3×3 switch over design for 21-days. Three TMR diets consisting of concentrate mixture, wheat straw and green maize fodder in the ratios of 60:20:20 (T1), 40:30:30 (T2) and 20:40:40 (T3), respectively were fed to the animals ad libitum. At the end of experiment they observed that the Total number of rumen protozoa were significantly ($p<0.05$) higher in crossbred cattle than buffaloes along with significantly ($p<0.05$) higher population in animal fed with high concentrate diet (T1).

Na *et al.* (2017) conducted a feeding trial to determine the effects of forage-to-concentrate (F:C) ratio on the nutrient digestibility and enteric methane (CH₄) emission in growing goats and Sika deer. Three male growing goats and three male growing deer were respectively allotted to a 3×3 Latin square design with an adaptation period of 7 d and a data collection period of 3 d. Respiration-metabolism chambers were used for measuring the enteric CH₄ emission. Treatments of low (25:75), moderate (50:50) and high (73:27) F:C ratios were given to both goats and Sika deer. During experiment they observed that in both goats and Sika deer, the enteric emission of CH₄ expressed as g/d, g/kg BW^{0.75}, % of gross energy intake (GEI), g/kg DMI and g/kg OMI decreased linearly ($p < 0.05$) with increasing F:C ratios in both goats and Sika deer. However, no difference was observed in enteric CH₄ production expressed as g/kg digested dry matter intake and g/kg digested organic matter intake in both goats and Sika deer.

Zhang *et al.* (2021) conducted an experiment to evaluate the effect of dietary supplementation of 2-nitroethanol (NEOH) in comparison with monensin on methane (CH₄) emission, growth performance and carcass characteristics in female lambs. Sixty female, small-tailed Chinese Han lambs (3.5±0.3 month) were randomly allotted into three dietary treatment groups: (1) Control group fed with basal control diet, (2) monensin group fed with the basal diet supplemented with 40 mg/kg monensin, (3) NEOH group fed with the basal diet supplemented with 277 mg/kg nitroethanol and the feed lotting trial lasted for 70 days. They observed that dietary supplementation of monensin and NEOH did not affect nutrient digestibility in lambs, both monensin and NEOH decreased the calculated CH₄ production (12.7% vs. 17.4% decrease; $p < 0.01$).

CONCLUSION

The rumen microbiota plays an essential role in nutrients acquisition and utilization. The rumen microbial fermentation provides VFAs, microbial protein and vitamins by degrading plant fibers, NPN and other organic matter in the diet. The symbiotic relationship between ruminants and rumen microorganisms is paramount for the conversion of low-quality feed into high-quality end products. The microbiota controls the production efficiency of the animal, with certain pathways (such as those associated with methane production) resulting in energy loss in the animal. Improvement in ruminal fermentation by using additives that can manipulate the ruminal ecosystem and ruminal microflora to increase animal production. High concentrate feeding/diets increase population of proteolytic bacteria and protozoa while high roughage feeding/diets increase population of bacteria and fungus.

FUTURE PROSPECTS

It will be becoming increasingly possible to understand the complexities of the rumen microbiota and subsequent effects on the host animal. Further studies are needed to focus on

specific microbes or metabolites that are linked to particular pathophysiological processes. A better understanding of rumen microbes-host interaction can provide novel insight that inform the development of applicable approaches to improve animal production and health. More feed additives should be evaluated for better ruminal fermentation to improve the performance.

REFERENCES

- Aschenbach, J.R., Penner, G.B., Stumpff, F. and Gabel, G. (2011). Ruminant nutrition symposium: Role of fermentation acid absorption in the regulation of ruminal pH. *J. Ani. Sci.* 89(4): 1092-1107. <https://doi.org/10.2527/jas.2010-3301>.
- Beam, T.M., Jenkins, T.C., Moate, P.J., Kohn, R.A. and Palmquist, D.L. (2000). Effects of amount and source of fat on the rates of lipolysis and biohydrogenation of fatty acids in ruminal contents. *J. Dairy Sci.* 83(11): 2564-2573. [https://doi.org/10.3168/jds.S0022-0302\(00\)75149-6](https://doi.org/10.3168/jds.S0022-0302(00)75149-6).
- Brod, D.L., Bolsen, K.K. and Brent, B.E. (1982). Effect of water temperature in rumen temperature, digestion and rumen fermentation in sheep. *J. Ani. Sci.* 54(1): 179-182. <https://doi.org/10.2527/jas1982.541179x>.
- Brown, M.S., Ponce, C.H. and Pulikanti, R. (2006). Adaptation of beef cattle to high-concentrate diets: Performance and ruminal metabolism. *J. Ani. Sci.* 84(13): 25-33. https://doi.org/10.2527/2006.8413_supplE25x.
- Cherdthong, A., Wanapat, M., Kongmun, P., Pilajun, R. and Khejornsart, P. (2010). Rumen fermentation, microbial protein synthesis and cellulolytic bacterial population of swamp buffalo as affected by roughage to concentrate ration. *J. Ani. and Vet. Advances.* 9(11): 1667-1675.
- Coleman, G.S. (1983). The cellulolytic activity of 13 species of rumen entodiniomorphid protozoa. *J. Protozoology.* 30(3): 36-36.
- Counotte, G.H.M., Prins, R., Janssen, R.H.A.M. and DeBie, M.J.A. (1981). Role of *Megasphaera elsdenii* in the fermentation of DL-[2-13C] lactate in the rumen of dairy cattle. *Applied and Environmental Microbiology.* 42(4): 649-655. <https://doi.org/10.1128/aem.42.4.649-655.1981>.
- Denman, S.E., Nicholson, M.J., Brookman, J.L., Theodorou, M.K. and McSweeney, C.S. (2008). Detection and monitoring of anaerobic rumen fungi using an ARISA method. *Letters in Applied Microbiology.* 47(6): 492-499. <https://doi.org/10.1111/j.1472-765X.2008.02449.x>.
- Duskova, D. and Marounek, M. (2001). Fermentation of pectin and glucose and activity of pectin degrading enzymes in the rumen bacterium *Lachnospira multiparus*. *Letters in Applied Microbiology.* 33(2): 159-163. <https://doi.org/10.1046/j.1472-765x.2001.00970.x>.
- Eisler, M.C., Lee, M.R., Tarlton, J.F., Martin, G.B., Beddington, J., Dungait, J.A. and Winter, M. (2014). Agriculture: Steps to sustainable livestock. *Nature News.* 507(7490): 32-34.
- Fondevila, M. and Dehority, B.A. (1996). Interactions between *Fibrobacter succinogenes*, *Prevotella ruminicola* and *Ruminococcus flavefaciens* in the digestion of cellulose from forages. *J. Ani. Sci.* 74(3): 678-684. <https://doi.org/10.2527/1996.743678x>.

- Fuentes, M.C., Calsamiglia, S., Cardozo, P.W. and Vlaeminck, B. (2009). Effect of pH and level of concentrate in the diet on the production of biohydrogenation intermediates in a dual-flow continuous culture. *J. Dairy Sci.* 92(9): 4456-4466. <https://doi.org/10.3168/jds.2008-1722>.
- Garnsworthy, P.C., Craigon, J., Hernandez-Medrano, J.H. and Saunders, N. (2012). On-farm methane measurements during milking correlate with total methane production by individual dairy cows. *J. Dairy Sci.* 95(6): 3166-3180. <https://doi.org/10.3168/jds.2011-4605>.
- Grenet, E., Breton, A., Barry, P. and Fonty, G. (1989). Rumen anaerobic fungi and plant substrate colonization as affected by diet composition. *Ani. Feed Sci. and Tech.* 26(1-2): 55-70. [https://doi.org/10.1016/0377-8401\(89\)90006-0](https://doi.org/10.1016/0377-8401(89)90006-0).
- Jami, E., Israel, A., Kotser, A. and Mizrahi, I. (2013). Exploring the bovine rumen bacterial community from birth to adulthood. *The International Society for Microbial Ecology Journal.* 7(6): 1069-1079.
- Jenkins, T.C., Wallace, R.J., Moate, P.J. and Mosley, E.E. (2008). Board-invited review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. *J. Ani. Sci.* 86(2): 397-412. <https://doi.org/10.2527/jas.2007-0588>.
- Klieve, A.V. and Swain, R.A. (1993). Estimation of ruminal bacteriophage numbers by pulsed-field gel electrophoresis and laser densitometry. *Applied and Environmental Microbiology.* 59(7): 2299-2303. <https://doi.org/10.1128/aem.59.7.2299-2303.1993>.
- Krause, K.M. and Oetzel, G.R. (2006). Understanding and preventing subacute ruminal acidosis in dairy herds: A review. *Ani. Feed Sci. and Tech.* 126(3-4): 215-236. <https://doi.org/10.1016/j.anifeedsci.2005.08.004>.
- Liu, Y. and Whitman, W.B. (2008). Metabolic, phylogenetic and ecological diversity of the methanogenic archaea. *Annals of the New York Academy of Sciences.* 1125(1): 171-189. <https://doi.org/10.1196/annals.1419.019>.
- Lodemann, U. and Martens, H. (2006). Effects of diet and osmotic pressure on Na⁺ transport and tissue conductance of sheep isolated rumen epithelium. *Experimental Physiology.* 91(3): 539-550. <https://doi.org/10.1113/expphysiol.2005.032078>.
- Mohammed, N., Ajisaka, N., Lila, Z.A., Hara, K., Mikuni, K., Hara, K. and Itabashi, H. (2004). Effect of Japanese horseradish oil on methane production and ruminal fermentation *in vitro* and in steers. *J. Ani. Sci.* 82(6): 1839-1846. <https://doi.org/10.2527/2004.8261839x>.
- Morgavi, D.P., Forano, E., Martin, C. and Newbold, C.J. (2010). Microbial ecosystem and methanogenesis in ruminants. *Animal.* 4(7): 1024-1036. <https://doi.org/10.1017/S1751731110000546>.
- Moss, A.R., Jouany, J.P. and Newbold, J. (2000). Methane production by ruminants: Its contribution to global warming. In *Annales de Zootechnie.* 49(3): 231-253. <https://doi.org/10.1051/animres:2000119>.
- Na, Y., Li, D.H. and Lee, S.R. (2017). Effects of dietary forage-to-concentrate ratio on nutrient digestibility and enteric methane production in growing goats (*Capra hircus hircus*) and Sika deer (*Cervus nippon hortulorum*). *Asian-Australasian Journal of Animal Sciences.* 30(7): 967-971. <https://dx.doi.org/10.5713%2Fajas.16.0954>.
- Nagaraja, T.G. and Titgemeyer, E.C. (2007). Ruminal acidosis in beef cattle: The current microbiological and nutritional outlook. *J. Dairy Sci.* 90: 17-18. <https://doi.org/10.3168/jds.2006-478>.
- Nam, I.S. and Garnsworthy, P.C. (2007). Biohydrogenation of linoleic acid by rumen fungi compared with rumen bacteria. *J. Applied Micro.* 103(3): 551-556. <https://doi.org/10.1111/j.1365-2672.2007.03317.x>.
- Nevel, C.J. and Demeyer, D.I. (1996). Influence of pH on lipolysis and biohydrogenation of soybean oil by rumen contents *in vitro*. *Reproduction Nutrition Development.* 36(1): 53-63.
- Newbold, T., Hudson, L.N., Hill, S.L., Contu, S., Lysenko, I., Senior, R.A. and Purvis, A. (2015). Global effects of land use on local terrestrial biodiversity. *Nature.* 520(7545): 45-50.
- Ozutsumi, Y., Tajima, K., Takenaka, A. and Itabashi, H. (2005). The effect of protozoa on the composition of rumen bacteria in cattle using 16S rRNA gene clone libraries. *Bioscience, Biotechnology and Biochemistry.* 69(3): 499-506. <https://doi.org/10.1271/bbb.69.499>.
- Pitta, D.W., Pinchak, W.E., Dowd, S.E., Osterstock, J., Gontcharova, V., Youn, E. and Malinowski, D.P. (2010). Rumen bacterial diversity dynamics associated with changing from bermudagrass hay to grazed winter wheat diets. *Microbial Ecology.* 59(3): 511-522. link.springer.com/article/10.1007/s00248-009-9609-6.
- Rotz, C.A., Montes, F. and Chianese, D.S. (2010). The carbon footprint of dairy production systems through partial life cycle assessment. *J. Dairy Sci.* 93(3): 1266-1282. <https://doi.org/10.3168/jds.2009-2162>.
- Russell, J.B., Muck, R.E. and Weimer, P.J. (2009). Quantitative analysis of cellulose degradation and growth of cellulolytic bacteria in the rumen. *FEMS Microbiology Ecology.* 67(2): 183-197. <https://doi.org/10.1111/j.1574-6941.2008.00633.x>.
- Russell, J.B. and Hino, T. (1985). Regulation of lactate production in *Streptococcus bovis*: A spiraling effect that contributes to rumen acidosis. *J. Dairy Sci.* 68(7): 1712-1721. [https://doi.org/10.3168/jds.S0022-0302\(85\)81017-1](https://doi.org/10.3168/jds.S0022-0302(85)81017-1).
- Scharen, M., Frahm, J., Kersten, S., Meyer, U., Hummel, J., Breves, G. and Danicke, S. (2018). Interrelations between the rumen microbiota and production, behavioral, rumen fermentation, metabolic and immunological attributes of dairy cows. *J. of Dairy Sci.* 101(5): 4615-4637. <https://doi.org/10.3168/jds.2017-13736>.
- Seymour, W.M., Campbell, D.R. and Johnson, Z.B. (2005). Relationships between rumen volatile fatty acid concentrations and milk production in dairy cows: A literature study. *Animal Feed Science and Technology.* 119(1-2): 155-169. <https://doi.org/10.1016/j.anifeedsci.2004.10.001>.
- Sinha, S.K., Chaturvedi, V.B., Singh, P., Chaudhary, L.C., Ghosh, M. and Shivani, S. (2017). Effect of high and low roughage total mixed ration diets on rumen metabolites and enzymatic profiles in crossbred cattle and buffaloes. *Vet. World.* 10(6): 616-622. <https://dx.doi.org/10.14202%2Fvetworld.2017.616-622>.
- Verge, X.P.C., Dyer, J.A., Desjardins, R.L., Worth, D. (2007). Greenhouse gas emissions from the Canadian dairy industry in 2001. *Agricultural Systems.* 94: 683-693. <https://doi.org/10.1016/j.agsy.2007.02.008>.

- Walker, C.B., Redding-Johanson, A.M., Baidoo, E.E., Rajeev, L., He, Z., Hendrickson, E.L. and Stahl, D.A. (2012). Functional responses of methanogenic archaea to syntrophic growth. *J. The International Society for Microbial Ecology*. 6(11): 2045-2055. <https://www.nature.com/articles/ismej201260>.
- Wanapat, M., Gunun, P., Anantasook, N. and Kang, S. (2014). Changes of rumen pH, fermentation and microbial population as influenced by different ratios of roughage (rice straw) to concentrate in dairy steers. *The Journal of Agricultural Science*. 152(4): 675-685. <https://doi.org/10.1017/S0021859613000658>.
- Wahrmund, J.L., Ronchesel, J.R., Krehbiel, C.R., Goad, C.L., Trost, S.M. and Richards, C.J. (2012). Ruminant acidosis challenge impact on ruminal temperature in feedlot cattle. *J. Ani. Sci*. 90(8): 2794-2801. <https://doi.org/10.2527/jas.2011-4407>.
- Williams, A.G. and Coleman, G.S. (1992). Role of Protozoa in the Rumen. In *The Rumen Protozoa*. Springer, New York, NY. pp. 317-347.
- William, A.G. and Coleman, G.S. (1997). *The Rumen Protozoa*. Springer, The Rumen Microbial Ecosystem. Pp - 73-138. https://doi.org/10.1007/978-94-009-1453-7_3.
- Weimer, P.J. (2015). Redundancy, resilience and host specificity of the ruminal microbiota: implications for engineering improved ruminal fermentations. *Frontiers in Microbiology*. 6: 296. <https://doi.org/10.3389/fmicb.2015.00296>.
- Zijderveld, S.M., Fonken, B., Dijkstra, J., Gerrits, W.J.J., Perdok, H.B., Fokink, W. and Newbold, J.R. (2011). Effects of a combination of feed additives on methane production, diet digestibility and animal performance in lactating dairy cows. *J. Dairy Sci*. 94(3): 1445-1454. <https://doi.org/10.3168/jds.2010-3635>.
- Zhang, Z.W., Wang, Y.L., Chen, Y.Y., Zhang, L.T., Zhang, Y.J., Liu, Y.Q. and Yang, H.J. (2021). The dietary supplemental effect of nitroethanol in comparison with monensin on methane emission, growth performance and carcass characteristics in female lambs. *Animals*. 11(2): 327. <https://doi.org/10.3390/ani11020327>.