Archivos de Medicina Veterinaria
ISSN: 0301-732X
archmv@uach.cl
Universidad Austral de Chile
Chile

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Archivos de Medicina Veterinaria, vol. 46, núm. 3, 2014, pp. 349-361
Universidad Austral de Chile
Valdivia, Chile

Arch Med Vet **46, 349-361 (2014)**

REVIEW ARTICLE

Rumen microorganisms and fermentation

Microorganismos y fermentación ruminal

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RESUMEN

El rumen es un ecosistema complejo donde los nutrientes consumidos por los rumiantes son digeridos mediante un proceso de fermentación realizado por los microorganismos ruminales (bacterias, protozoos y hongos). Dichos microorganismos están en simbiosis, debido a su capacidad de adaptación e interacción, y mientras el rumiante proporciona el ambiente necesario para su establecimiento estos proporcionan energía al animal, la que proviene de los productos finales de la fermentación. Dentro del rumen, los microorganismos coexisten en un entorno reducido y a un pH cercano a la neutralidad. Estos microorganismos fermentan los sustratos presentes en la dieta del rumiante (azúcares, proteínas y lípidos). Sin embargo, el proceso de fermentación no es 100% eficaz, ya que durante la fermentación existen pérdidas de energía, principalmente en forma de gas metano (CH_4), el que representa un problema medioambiental, ya que es un gas de efecto invernadero. Por consiguiente, para mejorar la eficiencia de los sistemas de producción de rumiantes se han establecido estrategias nutricionales que tienen como objetivo manipular la fermentación ruminal mediante el uso de aditivos en la dieta, como monensina, sebo, tampones, compuestos de nitrógeno, probióticos, etc. Estos aditivos permiten cambiar el proceso de fermentación y mejorar la eficiencia animal, además disminuyen la pérdida de energía. El objetivo de este trabajo es revisar los procesos fermentativos que tienen lugar en el rumen y aplicar los fundamentos de estos en el desarrollo de nuevas estrategias nutricionales que pudieran ayudar a mejorar los procesos de digestión, de manera que se alcance una máxima producción.

Palabras clave: aditivos, microorganismos ruminales, simbiosis.

SUMMARY

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, and fungi. A symbiotic relationship is found among different groups of microorganisms due to the diverse nature of these microbial species and their adaptability and interactions also coexist. The ruminant provides the necessary environment for the establishment of such microorganisms, while the microorganisms obtain energy from the host animal from microbial fermentation end products. Within the ruminal ecosystem, the microorganisms coexist in a reduced environment and pH remains close to neutral. Rumen microorganisms are involved in the fermentation of substrates contained in thedietof the animals (carbohydrates, proteins and lipids). However, the fermentation process is not 100% effective because there are energy losses mainly in the form of methane gas (CH_4), which is a problem for the environment since it is a greenhouse gas. In order to improve the efficiency of ruminant production systems, nutritional strategies that aim to manipulate ruminal fermentation using additives in the diet such as monensin, tallow, buffers, nitrogen compounds, probiotics, and others have been used. These additives allow changing the ruminal fermentation process in ways that produce better growth efficiency while decreasing energy loss. The purpose of this review is to contribute to a better understanding of the fermentation processes taking place in the rumen, providing information that can be applied in the development of new nutritional strategies for the improvement of the digestion process to achieve maximum production.

Key words: additives, ruminal microorganisms, symbiosis.

INTRODUCTION

The rumen is a complex ecosystem where nutrients consumed by the microorganisms such as bacteria, protozoa, and fungi are digested anaerobically. The main end products of fermentation are volatile fatty acids (VFAs) and microbial biomass, which are used by the host ruminant. The interaction between microorganisms and the host animal results in a symbiotic relationship that allows ruminants to digest diets rich in fiber and low in protein. In the rumen the environment favors the microorganisms to provide the enzymes necessary to digest the nutrients. Ruminants have the ability to convert the low quality fibrous materials into products such as meat, milk and fibers, which are useful to humans. The ability of ruminal microorganisms to produce the enzymes necessary for fermentation processes allows ruminants to efficiently obtain the energy contained in forages (Burns 2008). However, the ruminal fermentation process is not completely efficient because it produces some final products such as methane gas (Kingston-Smith *et al* 2012) and excess ammonia (Russell and Mantovani 2002). Ruminants such as cattle, sheep, and goats have

Accepted: 20.03.2014.

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evolved to use fibrous food efficiently (Oltjen and Beckett 1996). The anatomical adaptation of their digestive system allows them to use cellulose as an energy source without requiring external sources of vitamin B complex (Russell and Mantovani 2002) or essential amino acids because ruminal microorganisms are able to produce such products (Cole *et al* 1982). Thus, a symbiotic relationship exists within the rumen providing the necessary environment for the establishment of microorganisms and substrates required for their maintenance. In turn, the microorganisms provide nutrients to the host ruminant to generate energy (Russell and Rychlik 2001). The continued increase of the human population has increased the need for more and better animal products. For this reason, in the field of animal production, most efforts have been directed to increase ruminant production using biotechnological tools to manipulate the ruminal ecosystem. For example, the use of additives in the diet such as monensin, tallow, buffers, nitrogen compounds, probiotics, etc., allow manipulation of the ruminal fermentation process to maximize the production efficiency while decreasing energy loss, for example, methane which pollutes the environment. The objective of this review is to describe the fermentation processes in the rumen so that current knowledge can be applied in the development of nutritional strategies for improving animal production.

PHYSICOCHEMICAL PROPERTIES OF THE RUMEN

The ruminant digestive system is composed of reticulum, rumen, omasum, and abomasum. The rumen is mainly where the major fermentation processes are held (Tharwat *et al* 2012). Enzymes present in the rumen are produced by microorganisms. These enzymes are used to digest and ferment food eaten by ruminants, thus, the rumen is considered as a fermentation vat (Aschenbach *et al* 2011). The main factors influencing the growth and activity of ruminal microbial populations are temperature, pH, buffering capacity, osmotic pressure and redox potential. These factors are determined by environmental conditions. The rumen temperature is maintained in the range of 39 to 39.5 °C (Wahrmund *et al* 2012) and may increase up to 41 °C immediately after the animal eats because the fermentation process generates heat (Brod *et al* 1982). The pH depends on the production of saliva, the generation and absorption of short-chain fatty acids (SCFA), the type and level of feed intake, and the exchange of bicarbonates and phosphates through the ruminal epithelium (Aschenbach *et al* 2011). Thus, these factors determine both pH and buffering capacity in the reticule ruminal environment. The pH constantly changes (Russell and Strobel 1989), but it usually remains in the range of 5.5 to 7.0 (Krause and Oetzel 2006), depending on the diet and buffering capacity of saliva, because saliva production is a constant process that provides bicarbonates and phosphates into the rumen. Furthermore, reticuloruminal secretions also possess buffering capabilities, so this environment does not only depend of the buffering capacity of saliva, which has a pH of 8.2 (Krause and Oetzel 2006). Generally, the bacterial intracellular pH remains near 7.0, which decreases considerably when the cell is under acidic environment. Likewise, microbial enzymes are sensitive to changes in pH, for example, inhibition of bacterial growth under acidic pH. This may be due to the imbalance of intracellular hydrogen ions (Russell and Wilson 1996). The osmotic pressure in the rumen depends on the presence of ions and molecules, which generate a gas tension (Lodemann and Martens 2006). The ruminal fluid osmolality is approximately 250 mOsm/kg. Ruminal fermentation processes may depend on the environmental conditions and the type of diet, so these factors may influence the osmotic pressure of the rumen. Immediately after feed intake, the osmotic pressure increases from 350 to 400 mOsm and then decreases gradually over a period of 8 to 10 hours. The osmotic pressure increases with the presence of VFAs produced by fermentation processes and has a direct relationship with the pH in diets rich in carbohydrates (Lodemann and Martens 2006).

RUMINAL MICROORGANISMS

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). The microbiota is formed by ruminal bacteria, protozoa, and fungi, at concentrations of 10^{10}, 10^{6}, and 10^{4} cells/ml, respectively. Bacterial populations are most vulnerable to the physicochemical properties of the rumen (McAllister *et al* 1990).

RUMINAL BACTERIA

The rumen contains a variety of bacterial genera (table 1), which constitute the majority of the microorganisms that live in anaerobic environment (Pitta *et al* 2010). The competition between bacteria in the rumen is determined by several factors, among which are the preference for certain substrates, energy requirements for maintenance, and resistance to certain metabolism products that can be toxic (Russell *et al* 1979).

CELLULOSE-DEGRADING BACTERIA

The ruminant diet is based on plant-based feed consumption. Because cellulose is the main component of the cell wall of these plants, cellulolytic ruminal microorganisms play an important role in animal nourishment (Russell *et al* 2009). Cellulose is digested in the rumen (Michalet-Doreau *et al* 2002). The ability to degrade cellulose depends mainly on the type of forage, crop maturity, and the members of the cellulolytic bacterial

Table 1. Characteristics of principal ruminal bacteria.
Características de las principales bacterias ruminales.

Microorganisms	Gram stain	Morphology	Fermetation products	Reference
Cellulose-degrading bacteria				
Fibrobacter succinogenes	Negative	Bacillus	Succinate, Acetate, Formate	(Ivan *et al* 2012)
Butyrivibrio fibrisolvens	Negative	Bacillus curve	Acetate, Formate, Lactate, Butyrate, H_2, CO_2	(Weimer 1996)
Ruminococci albus	Positive	Cocci	Acetate, Formate, H_2, CO_2	(Michalet-Doreau *et al* 2002)
Clostridium lochheadii	Positive	Bacillus (espores)	Acetate, Formate, Butyrate, H_2, CO_2	(Weimer 1996)
Amylolytic bacteria				
Bacteriodes ruminicola	Negative	Bacillus	Formate, Acetate, Succinate	(Cotta 1988)
Ruminobacter amylophilus	Negative	Bacillus	Formate, Acetate, Succinate	
Selenomonas ruminantium	Negative	Bacillus curve	Acetate, Propionate, Lactate	(Cotta 1992)
Succinomonas amylolítica	Negative	Oval	Acetate, Propionate, Succinate	
Streptococci bovis	Positive	Cocci	Lactate	(Cotta 1988, McAllister *et al* 1990)
Lipolytic bacteria				
Anaerovibrio lipolytica	Negative	Bacillus	Acetate, Propionate, Acetate	(Fuentes *et al* 2009)
Lactate-degrading bacteria				
Selenomonas lactilytica	Negative	Bacillus curvado	Acetate, Succinate	(Brown *et al* 2006)
Megasphaera elsdenii	Positive	Cocci	Acetate, Propionate, Butyrate, Valerate, H_2, CO_2	
Pectin-degrading bacteria				
Lachnospira multiparus	Positive	Bacillus curve	Acetate, Formate, Lactate, H_2, CO_2	(Duskova and Marounek 2001)
Ruminal archaea (methanogens)				
Methanobrevibacter ruminantium	Positive	Bacillus	CH4 (of H_2+CO_2 or Formate)	(Yanagita *et al* 2000, Hook *et al* 2010)
Methanomicrobium mobile	Negative	Bacillus	CH4 (of H_2+CO_2 or Formate)	
Lactic acid-utilizing bacteria				
Megasphaera elsdenii	Negative	Cocci	Lactate	(Counotte and Prins 1981)

communities (Fondevila and Dehority 1996). To ensure maintenance and growth of cellulolytic bacteria, optimal ruminal conditions are required. A neutral pH near neutrality between 6 and 9 is best, while a pH less than 5.5 affects fiber digestibility (Weimer 1996). A temperature of 39 °C affects the adhesion ability of bacteria to feed particles (Michalet-Doreau *et al* 2001), while the presence of extracellular cellulase enzymes (Weimer 1996) to break β-glycosidic bonds (1-4) of the biopolymer provides sugars for use by microorganisms (Wedekind *et al* 1988). In addition, the presence of ionized calcium (Ca^{+2}) favors the establishment of such bacteria, except for *F. succinogenes* (Morales and Dehority 2009). 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. For a*mylolytic bacteria* (table 1), 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. Although these diets for ruminants have been effective as a fermentable energy source, they are also associated with metabolic disorders such as acidosis (Gressley *et al* 2011), low-fat milk syndrome and liver abscesses (Owens *et al* 1998).

In ruminants fed mainly forage, this bacterial species is found at a concentration of 10^4-10^7cells/grams. In addition, when the fermentable sugar concentration increases, its concentration may be greater than 10^{11}/grams in ruminal contents (Nagaraja and Titgemeyer 2007). Furthermore, *S. bovis* ferments glucose to provide acetate, formate, and ethanol as a final product. 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). To avoid this situation, it is necessary to gradually introduce fermentable carbohydrates in the ruminant diet. This dietary management allows *S. bovis* not to produce lactic acid rapidly. Thus, the growth of other starch-degrading bacteria, such as *Bacteroides ruminicola*, *Ruminobacter amylophilus*, *Selenomonas ruminantium* and *Succinomonas amylolytica* which produce other VFAs such as formate, acetate, propionate, and succinate, is also promoted so that an imbalance of homeostasis in the biochemical pathways in the ruminal environment is avoided (Cotta 1992).

LACTATE-DEGRADING BACTERIA AND LACTATE- BACTERIA

They have a very important role in the rumen (table 1) mainly in those ruminants that are fed with high grains in the diet. These bacteria metabolize lactic acid and control its accumulation, which helps to keep the pH in the proper range (Mackie and Heath 1979). This type of bacteria increases when the diet consists of approximately 70% concentrate (Brown *et al* 2006).

PECTIN-DEGRADING BACTERIA

They are important because the pectin represents 10-20% of total carbohydrates in forages used in ruminant nutrition (table 1). Pectin is fermented by both bacteria and protozoa (Dehority 1969) and the main bacteria that perform this function are *Butyrivibrio fibrisolvens*, *Prevotella ruminicola*, *Bacteroides ruminicola* and *Lachnospira multiparus*. These ruminal bacteria produce and release pectinolytic enzymes into the ruminal environment; pectin lyases are the primary enzymes that hydrolyze the pectin in oligogalacturonoides (Duskova and Marounek 2001).

RUMINAL ARCHAEA OR METHANOGENS

They depend on the growth, maintenance, and activity of a diverse population of microorganisms (table 1). However, microbial activity is the main source of greenhouse gases in agriculture (Mosoni *et al* 2011), such as methane gas. Methane is an 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 fiber fermentation, mainly cellulose, and 20% of methane is generated by the decomposition of manure (Vergé *et al* 2007). These percentages can vary depending upon the composition of ruminant diet (Rotz *et al* 2010). Methanogenesis is a necessary process because it is a way to maintain low H^+ concentrations in the ruminal environment by reducing CO_2 (Bodas *et al* 2012). Oxidative processes, for example, glycolysis occur in the rumen, reduced substrates are generated this process. For example, cofactors such as NADH, which is re-oxidized to NAD^+ to complete sugar fermentation, are produced. NAD^+ is regenerated by electron transfer through electron acceptors other than O_2, such as CO_2, sulfate, nitrate, or fumarate. Electron transport is linked to ATP generation as a result of an electron gradient generated across the membrane where these cofactors are present (Moss *et al* 2000). The production of H^+ is a thermodynamically unfavorable process controlled by the potential of the electron carriers (Moss *et al* 2000). Although H^+ is one of the main end products of the fermentation of bacteria, protozoa, and fungi, it does not accumulate in the rumen because it is rapidly used by some microorganisms that are a part of the ecosystem. In the rumen, there exists an interrelationship 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, where there is an association between species that produce and utilize H^+ (Walker *et al* 2012). Methanogenesis is the main sink of H^+ removal (Moss *et al* 2000). Methane is generated by methanogenic bacteria utilizing the carbon dioxide and hydrogen (van Zijderveld *et al* 2011). When H^+ is not used by the methanogens, NADH can be reoxidized by a dehydrogenase to produce ethanol or lactate. This process occurs rapidly in animals fed with high amounts of fermentable sugars (Moss *et al* 2000).The methanogens belong to the domain *Archaea* (Morgavi *et al* 2010) and the phylum *Euryarchaeota*. 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_3OO^-) groups, such as methanol and acetate that act as electron acceptors (Liu and Whitman 2008).

PROTEOLYTIC BACTERIA

In the rumen, the forage proteins and structural polysaccharides are degraded by 50-70% by the action of microorganisms. Ruminal proteolysis is carried out by enzymatic production of ruminal microorganisms by protein hydrolysis processes, degradation of peptides, and amino acid deamination (Cotta and Hespell 1986). The main bacterial species with proteolytic activity are *Bacteroides amylophilus*, *Bacteroides rutminicola*, and *Butyrivibrio fibrisolvens* (Cotta and Hespell 1986). This activity has also been reported in *Streptococcus bovis* and *Prevotella albensis* (Sales-Duval *et al* 2002).

LIPOLYTIC BACTERIA

Lipids are modified by microbial fermentation, and the unsaturated fatty acids present are transformed into saturated fatty acids in the rumen. Ruminal microorganisms transform lipids by two major pathways, lipolysis and biohydrogenation. The major types of lipids in the diet are triglycerides, phospholipids, and galactolipids (Jenkins *et al* 2008). The lipids in the rumen are first hydrolyzed by microbial lipases. These lipases break the ester bonds, releasing fatty acids (Liu *et al* 2009). After lipolysis, unsaturated fatty acids are biohydrogenated due to the presence of H^+ produced by ruminal microorganisms (Jenkins *et al* 2008). The rate of lipolysis depends mainly on the type of lipids present in the diet (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 (van Nevel and Demeyer 1996). *A. lipolytica* produces two hydrolytic enzymes, one of which is bound to the cell membrane and the other is extracellular (Henderson and Hodgkiss 1973), the activity of these bacteria decreases in high concentrate diets, due to the drop in pH (Loor *et al* 2004), the main function of *B. fibrisolvens* is to biohydrogenate polyunsaturated fatty acids (Maia *et al* 2010).

LACTATE-DEGRADING BACTERIA

Lactate is an intermediary product of ruminal fermentation, which is metabolized to VFAs. In diets rich in starch, the population of this type of bacteria capable of using lactic acid is increased (Counotte and Prins 1981). *Megasphaera elsdenii* is the main species responsible for lactic acid metabolization; thus, it has an important role in the prevention of acidosis during the adaptation period when ruminants are fed diets high in concentrate (Counotte *et al* 1981).

PROTOZOA IN THE RUMEN

Protozoa constitute 40-80% of the biomass, most abundant (table 2) of which are the orders *Entodiniomorphida* and *Holotricha* (Firkins *et al* 2007, Yáñez-Ruiz *et al* 2004). The flow of ruminal protozoa to the ruminant abomasum is less than that of bacteria, since they are retained in the feed particles (Hook *et al* 2012). Holotrichs can assimilate soluble sugars and keep some of them in reserve polysaccharides; thus, this protozoa can decrease the risk of acidosis after consuming foods with high concentrations of easily digestible sugars (van Zwieten *et al* 2008).

CELLULOLYTIC PROTOZOA

Approximately 90% of total protozoa belong to the genus *Entodiniomorphida*, many of which are involved in the hydrolysis and fermentation of cellulose (Yáñez-Ruiz *et al* 2004). In *in vitro* studies with cultured protozoa, it was observed that crystalline cellulose is degraded mainly by protozoa of the genera *Polyplastron* and *Eudiplodinium* and to a lesser degree by *Epidinium* (Fondevila and Dehority 2001). Besides having the ability to digest cellulose,

Table 2. Main ruminal protoza and fungi.
Principales protozoarios y hongos ruminales.

Protozoa	Fermentation products	Reference
Cellullolytic protozoa		
Enoploplastron triloricatum	Reducing sugars	(Coleman *et al* 1976)
Eudiplodinium maggii		
Diploplastron affine		
Epidinium ecaudatum		
Diplodinium monacanthum		
Diplodinium pentacanthum		
Proteolytic protozoa		
Entodinium caudatum	Amonium, VFA	(Ivan *et al* 2000)
Eudiplodinium medium	Amonium, VFA	(Forsberg *et al* 1984)
Fungi		
Cullulolytic fungi	Fermentation products	Reference
Neocallimastix frontalis	Lactate, Formate, Acetate, Succinate, Ethanol	(Moniello *et al* 1996)
Piromyces communis	Celobiose, celooligosacarides,	(Dashtban *et al* 2009)
Orpinomyces joyonii	Glucose	(Hodrova *et al* 1995)

Diploplastron affine has amylolytic activity; due to its ability to produce amylolytic enzymes, including two isoforms of α-amylase and maltase, it produces maltose, maltotriose, and glucose (Wereszka and Michalowski 2012). *Proteolytic protozoa:* In the ruminal environment, soluble proteins are mostly degraded by bacteria and protozoa (Hino and Russell 1987). The proteolytic activity of ruminal bacteria is 6 to 10 times greater than that of protozoa (Brock *et al* 1982).

RUMINAL FUNGI

Fungi (table 2) 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 microaerotolerants and are attached to feed particles through a system of rhizoids (Denman *et al* 2008). Ruminal fungal populations are favored by the consumption of fibrous forage that is mainly highly lignified. Ruminal fungi are present in the duodenum, cecum, and feces and are removed when ruminants are fed high concentrations of rapidly fermentable sugars; however, the fungi quickly proliferate once the feed concentration is increased (Grenet *et al* 1989).

CELLULOLYTIC FUNGI

Ruminal fungi (table 2) are able to produce enzymes that hydrolyze cellulose and xylans. Their enzymatic activity is variable, depending on their phylogenetic origin and especially their rhizoidal structure, but it has been postulated that some species, such as *Neocallimastix frontalis*, *Piromyces joyonii*, and *Orpinomyces communis*, can more efficiently digest structural polysaccharides than cellulolytic bacterial species in monoculture (Bernalier *et al* 1992). 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. For example, *N. frontalis* has the ability to solubilize small lignin fractions of the plant cell wall, allowing the bacteria access to the cellulose (Borneman *et al* 1991).

MANIPULATION OF RUMINAL FERMENTATION

Biotechnology has been used to modify ruminal fermentation by promoting or diminishing certain fermentation processes, leading to a higher efficiency in animal productivity. This biotechnological management can be categorized into the following five groups: 1) modification of diet and fermentation profile, 2) transformation of food before consumption, 3) manipulation of ruminal microorganisms, 4) use of microorganism fermentation activators, and 5) use of substances with ruminal activity.

MANAGEMENT OF DIET AND MODIFICATION OF FERMENTATION PROFILE

Some diet changes may improve the microbial fermentation profile. For example, the inclusion of legumes to the ruminant diet can have positive results in terms of microbial activity and fermentation end products. Legumes are a good source of protein; rich in amino acids, vitamins, and minerals; and good substrates of cellulolytic microorganisms for growth and enzyme function (Galindo and Marrero 2005). On the other hand, forages of low nutritional value exist which contain high amounts of lignincellulose, low concentrations of fermentable sugars, and proteins of low quality; these characteristics affect the microbial activity and thus the profile of the fermentation products. For example, the use of rice straw decreases cellulolytic, proteolytic, and amylolytic bacterial populations and the total protozoa population; if these agricultural byproducts are treated with urea, the microorganism can use it as a substrate, leading to an increase of these populations and improvement of the fermentation profile (Wanapat 2000).

FOOD TRANSFORMATION BEFORE CONSUMPTION

Food transformation before consumption involves all of the physical and chemical treatments applied to food before being consumed by the ruminant. Physical treatments include heating (Brandt *and* Klopfenstein 1986) and varying the particle sizes (Schadt *et al* 2012). On the other hand, chemical treatments are the best used methods, including the use of enzymes such as cellulases and xylanases (Giraldo *et al* 2007). The ruminant diet is based on forage consumption, in ruminant production systems, anything that improves the nutritional value of forage with a high fiber concentration and low digestibility can increase the productivity of ruminants (Giraldo *et al* 2007). Researchers have shown that supplementing the cattle diet with fibrolytic enzymes significantly improves the use of nutrients and increases ruminant efficiency. The primary enzymes used for this objective are xylanases (Goncalves *et al* 2012) and cellulase (Morgavi *et al* 2001), which are purified from fermentation cultures of both bacteria and fungi (Beauchemin *et al* 2003). Several of these fibrolytic enzymes have been evaluated as additives in ruminant diets and were originally developed as additives in silage, hay, and agricultural byproducts (Tang *et al* 2008). For example, Tang *et al* (2008), proved that fibrolytic enzymes supplementation increased *in vitro* digestibility of dry matter and *in vitro* organic matter digestibility of maize stover, maize stover silage, rice straw and wheat straw. The exogenous enzymes that improve fibrolytic activity favor the access of ruminal microbes to the cell wall

matrix, thus favoring fiber digestion (Nsereko *et al* 2000). Cellulose and hemicellulose are the main polysaccharides that form part of the cell wall (Hindrichsen *et al* 2006). These polysaccharides are insoluble; but in the presence of enzymes, such as amylases, proteases, and pectinases, they are converted to soluble sugars so that they can be used by ruminal microorganisms. Furthermore, these enzymes may have secondary activities (Beauchemin *et al* 2003). The improvement in ruminant efficiency due to the use of fibrolytic enzymes is attributed to improved ruminal forage digestion, which results in an increase in dry matter digestibility and voluntary intake, increasing the digestible energy that is used by the ruminant (Beauchemin *et al* 2003, Krueger *et al* 2008). Also cellulase has been shown to increase protein degradation of forages *in vitro*, because proteins are more available to proteolytic enzymes (Kohn and Allen 1992). Since intake of fibrolytic enzymes improve the forage digestibility, it increases the total digestible nutrients and consequently changes concentration and proportion of VFA, therefore, ruminant productivity improves (Pinos-Rodríguez *et al* 2002, Pinos-Rodríguez *et al* 2008). The enzyme products with this activity used in ruminant nutrition are usually produced by fungi such as *Trichoderma longibrachiantum*, *Aspergillus niger*, *Penicillium funiculosum* (Wallace *et al* 2001) and *A. oryzae,* as well as *Bacillus* spp. bacteria (Beauchemin *et al* 2003).

MANIPULATION OF RUMINAL MICROORGANISMS

Despite the importance of ruminal microorganisms, protozoa are not vital but are important for these animals. It has been shown that protozoa can be eliminated from the ruminal environment, defaunation causes changes in the characteristics of ruminal digestion, such as an increase in the bacterial density, degradation of starch, and decreasing propionate and methane concentrations; in addition, fiber digestion is affected (Morgavi *et al* 2010). Because protozoa are H^+ producing microorganisms, this ion is used by methanogens to reduce CO_2 to CH_4, it is considered that the removal of protozoa decreases methanogenesis due to the reduction of available H^+ for methanogens (Mosoni *et al* 2011). Moreover, defaunation causes changes in the production of ammonia and the VFA profile (Ozutsumi *et al* 2005). The amount of ammonia is less in defaunated animals due to decreased proteolysis (Firkins *et al* 2007). Therefore, although protozoa are not essential for ruminant growth, its presence is important because of its ability to degrade the main components, which is an important role in the fermentation process (Coleman 1985). In addition, the decrease in postprandial pH is regulated by protozoa (Belanche *et al* 2011) because they modulate amylolytic bacterial populations, which use glucose from starch as a substrate for its fermentation processes to give lactate as one of its main products (Mendoza *et al* 1993). Belanche *et al* (2011), showed that presence of rumen protozoa increased rumen total VFA and ammonia-N significantly, when the ruminal protozoa are present. Hence there exists a positive response on fiber digestibility and VFA concentration to fautation, especially when structural carbohydrates represent the principal dietary component, this confirms the actions of protozoa to fribrolytic activity (Belanche *et al* 2011). Nevertheless, it remains unclear whether this increment in fiber digestibility is attributed specifically to the protozoal activity, or to synergy between all ruminal microorganisms (Chaudhary *et al* 1995).

USE OF MICROORGANISM FERMENTATION ACTIVATORS

Probiotics are microorganisms that are not of ruminal origin but can be adapted to ruminal conditions and improve the ruminal fermentation process. Probiotics are defined as live microorganisms or components of microbial cells that have beneficial effects on the host, since they regulate the intestinal flora to improve animal health, these products are named "direct-fed microbial" instead of probiotic (Krehbiel *et al* 2003). The probiotics used in ruminant feed mainly include fungi and bacteria, which have replaced conventional antibiotics as growth promoters (Lila *et al* 2004). Specific strains of bacteria are used for this purpose in animal feed (Donohue 2006). In beef cattle production systems, antibiotics are used to decrease the frequency of metabolic disorders, improve efficiency, and reduce ruminal acidosis, however, the use of some unnatural antibiotics is restricted (Zerby *et al* 2011). Some bacterial species of the genera *Lactobacillus* and *Bifidobacterium* have been shown to have good results as probiotics in ruminants, increasing levels of weight gain and efficiency (Whitley *et al* 2009). Furthermore, it has been seen that these lactic acid bacteria are able to reduce the risk of enteric disease caused by *E. coli* and *Salmonella* spp. (Stephens *et al* 2007). The fungi *Aspergillus oryzae*, *Saccharomyces cerevisiae* (Beharka *et al* 1991), and *Candida levica* 25 (Marrero *et al* 2011) have been used as probiotics as well as food additives to improve the ruminal fermentation process. *A. oryzae* improves the use of lactate by several bacteria such as *Megasphaera elsdenii* and *Selomonas ruminantium* (Beharka *et al* 1991), thus avoiding the drop in pH immediately after eating (Moya *et al* 2009). *S. cerevisiae* favors the metabolism of ruminal microorganisms (Oeztuerk *et al* 2005) because it increases the ruminal pH or decreases the time in which the pH is less than 5.6 (Thrune *et al* 2009), increases the ratio of butyrate and propionate (Pinos-Rodríguez *et al* 2008), and increases the digestibility of dry matter and neutral detergent fiber (Lila *et al* 2004). Also, yeast cultures stimulate the use of H^+ by acetogenic bacteria, thereby decreasing methane production (Chaucheyras *et al* 1995). The presence of *Saccharomyces cerevisiae* favors the establishment of fibrolytic bacteria as *F. succinogenes* and *R. albus* (Callaway and Martin 1997), and decreases

the population of lactate-producing microorganisms and those using H^+ to produce CH_4 (Lila *et al* 2004). In addition, *S. cerevisiae* stimulates propagation of the fungus *N. frontalis* and lactate production using *M. elsdenii* and *S. ruminantium* (Chaucheyras *et al* 1995), preventing ruminal pH decreases (Lynch and Martin 2002), improving fiber fermentation, and increasing propionate production (Lila *et al* 2004). All of these results suggest that these additives are beneficial in terms of weight gain (Tricarico *et al* 2007) and milk production (Dann *et al* 2005). Furthermore, DFM decreased fecal excretion of *E.coli* O157:H7 from infected calves, therefore a possible application for DFM might be to reduce shedding of this pathogen from cattle (Krehbiel *et al* 2003). In ruminants, probiotics have shown positive effects in cattle at weaning and increased production of dairy cows, nutrient absorption, efficiency, weight gain in beef cattle as well as greater resistance to gastrointestinal diseases (Krehbiel *et al* 2003).

USE OF SUBSTANCES WITH RUMINAL ACTIVITY

The use of substances with ruminal activity refers to the use of additives or supplements added to animals with the objective to potentiate the fermentation processes taking place in the rumen, thus, greater efficiency in the utilization of food is achieved.

NITROGENOUS SUPPLEMENTS

Feeding ruminants with agricultural byproducts is an economical option to cover the energy requirements from fiber and maintenance of the animal; however, the crude protein content is not adequate, which requires supplementation with nitrogenous compounds (Farmer *et al* 2004). The use of non-protein nitrogen such as urea is a good strategy to cover this need (Currier *et al* 2004), but its benefit in efficiency is lower when the animal is supplemented with low quality forage with natural sources of protein. Furthermore, supplementation with crude protein (CP) to ruminants improves fiber digestibility because the population of cellulolytic bacteria is increased (Currier *et al* 2004). Schauver *et al* (2005) suggest that providing a protein supplement daily to cows grazing low quality forage increases body weight (BW) and decreases the grazing time. Also, there exists a synergy between protein and sugar supplementation, because the microbial CP synthesis is improved to a greater extent when protein is supplied in addition to sugar rather that when they are provided separately.

USE OF PH BUFFERING SUBSTANCES

Increased propionate levels at the expense of acetate are generally associated with high concentrate fattening rations. Excessive lactic acid accumulations can occur on high concentrate diets and produce a pH drop. The use of pH buffering compounds has the ability to maintain the ruminal pH necessary to provide good microbial activity. For this purpose, mineral salts such as carbonates, bicarbonates, and phosphates of sodium, potassium, calcium, and magnesium are used. These compounds maintain a constant pH and promote the population of cellulolytic bacteria, thereby increasing the digestibility of dry matter and protein synthesis (Galindo and Marrero 2005) and preventing acidosis.

IONOPHORES

Chemically, ionophores are polyether carboxylic antibiotics, which initially were used as anticoccidial agents in poultry. These compounds are produced by various strains of *Streptomyces* and include moensin, lasalocid, salinomycin, and narasin (Bergen and Bates 1984). These compounds are used as growth promoters (Bretschneider *et al* 2008). In the 1970s, the US Food and Drug Administration approved the use of the ionophore monensin as an additive in ruminant diets. However, as the use of antibiotics in animal feed may leave residues of these compounds in animal products allowing the development of pathogens resistant to antibiotics, Europe has prohibited the use of sodium monensin since January of 2006 (Zawadzki *et al* 2011). Monensin is an antibiotic produced by the fungus *Streptomyces cinnamonensis* (Li *et al* 2009), which acts mainly by affecting Gram-positive bacteria. Monensin binds to the cell membrane of these bacteria, causing monovalent proton ion exchange, a decrease in intracellular K^+ and Na^+ accumulation, and a loss of cellular energy (Ellis *et al* 2012). Ruminal acidosis is one of the main and most common metabolic disorders in cattle. This disorder is caused by a diet with a high proportion of concentrate, which is currently used in animal production and is related to lactic acid production, but excessive production of VFA may be a more important contributor to chronic acidosis problems in dairy cows, because it causes a pH below 5.5 (McGuffey *et al* 2001). Ionophores also have been used in ruminants due to their ability to reduce the risk of bloating, increase the efficiency in the animal (Bergen and Bates 1984, Bretschneider 2010), reduce the production of methane (gas trapped in bubbles which produce the bloat) by controlling the bacteria that produce H^+ used in dioxide reduction and acetate (Bretschneider 2010), increase the production of propionate, and improve the acetate:propionate ratio (Russell and Mantovani 2002), thereby increasing the holding energy (Marrero *et al* 2011). In ruminants that are fed high concentrate diets, ionophores usually reduce feed intake and improve animal weight gain. On the other hand, when the ruminant diet contains significant concentrations of cellulose, as in the case of forage, ionophores do not reduce consumption and improve weight gain, improving feed conversion in both cases (Bergen and Bates 1984). Monensin is one of the most used ionophores in ruminant nutrition. The main benefit observed with the use of this

ionophore is the increase in the molar proportion of propionic acid at the expense of a decrease in the acetic acid and butyric acid concentrations produced in the rumen. These changes in the profile of VFAs are related to a decrease in methane concentration (Bergen and Bates 1984).

TALLOW

It is an abundant and low cost source of supplemental fat used to increased dietary energy density for dairy cows (Ruppert *et al* 2003).The objective of tallow supplementation in the diets of dairy and beef cattle is to increase the energy density to improve milk production and metabolic efficiency of cattle (Grummer and Carroll 1991). It has been demonstrated that native tallow did not negatively affect ruminal fermentation, nutrient digestibility, dry matter intake, or milk production when supplemented in typically recommended amounts (e.g., 2 to 3% of dry matter) to high producing dairy cows when the basal diet consisting of alfalfa hay or silage as the principal forage (Ruppert *et al* 2003), so the dairy cattle industry routinely adds fat to promote milk production (Appeddu *et al* 2004). On the other hand, one experiment showed that supplementation of either tallow or choice white grease to a diet in which corn silage was the only forage decreased dry matter intake, milk yield, milk fat percentage, and the ruminal acetate to propionate ratio, but neither fat source affected in situ dry matter intake or neutral detergent fiber disappearance (Onetti *et al* 2001). Another important effect of fat supplementation is an improved reproductive development and reduced risk of metabolic disorders, due to the removal of fat from body stores (Dann *et al* 2005). In addition, inadequate energy intake during the prepartum and early lactation periods is associated with metabolic disorders such as ketosis (Dann *et al* 2005), fatty liver, and low reproductive response (Douglas *et al* 2007). If high producer cows are supplemented with fat, energy consumption is increased and the effect of a negative energy balance is reduced, thereby improving the health of the dairy cows (Grummer and Carroll 1991). During lactation, feed high in available protein, starch, and /or sugar are commonly supplemented to promote pre weaning gains in offspring and to prevent excessive BW losses of grazing cows and ewes. Casals *et al* (1999) reported that 4 wk old lambs increased their weights when supplying fat to ewes consuming a high forage diet. However, an excess (> 3-5%) of tallow in the diet has been associated with a decrease in the palatability and digestibility of fiber. These negative effects occur due to a toxic effect of the long chain fatty acids on ruminal microorganisms, mainly bacteria (Henderson 1973). On the other hand, when the amount is not exceeded, a small increase of fat may improve bacterial growth by incorporating the dietary fatty acids and reducing the need for synthesizing them (Amorocho *et al* 2009). There also have been reports that fattening steers with tallow supplementation causes a decrease of ciliated protozoa populations, however, yellow grease supplementation did not affect numbers of protozoa in steers fed either sorghum or corn diets (Towne *et al* 1990).

CONCLUSION

Ruminal fermentation is the result of metabolism of bacteria, fungi, and protozoa that are present in this environment. Their metabolic pathways are interwoven so that the end product or intermediate metabolites of any type of microorganism is the substrate of another, thus achieving the end products of ruminal fermentation necessary for ruminant nutrition. This situation creates interdependence between different microorganisms, and these emerging consortia facilitate their establishment. Modern meat and milk productions tend to use diets that can be challenging for ruminants, or rather for ruminal microorganisms. The microorganisms' responses to these challenges are not always beneficial to the animal. So, experts have speculated on ways to improve ruminal fermentation to increase animal production. The use of additives can manipulate the ruminal ecosystem and ruminal microflora in order to improve production. Because ruminal microorganisms are crucial for proper animal nutrition, it is important to generate innovative knowledge in the study of ruminal fermentation and microbial ecosystems to improve the ruminant feeding process. Today, molecular tools are being developed to better understand the symbiosis between microorganisms and ruminants. In addition, the environmental impact due to cattle production is a current concern. Therefore, the use of additives in the diet to improve the efficient use of nutrients and thus reduce the final products that affect the atmosphere are being developed. Prospects indicate that the use of combinations of additives can be added to ruminant rations. These additives will improve animal production with minimal possible damage to the environment. Thus, molecular techniques will identify microbial population Holotrichos changes during fermentation so that it may be possible to manipulate and stimulate the presence of microorganism populations capable of providing a better nutritional benefit to the animal.

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