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# Rumenology



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*We would like to dedicate this book to everybody who is passionate about the rumen.*

# Foreword

Ruminants thrive from the tropics to the Arctic Circle and serve mankind by making "something from nothing." By readily harvesting and digesting diverse forage resources from inaccessible and nonarable land and forests, and converting otherwise wasted agricultural and industrial by-products and low-cost grain surpluses into milk, meat, and fiber, ruminants make products that are highly prized by humans worldwide. For optimum economic efficiency of production, ruminant producers must assure that both the host ruminant and the microbial population within the rumen receive an adequate but not excessive supply of essential nutrients and energy, appropriate rumen modifiers, and proper animal care, management, and attention to maintain health and productivity. This text includes information and concepts compiled by specialists in microbiology, rumen function, and animal health around the globe. It is intended to supply both students and livestock producers with a framework in rumenology that when applied will help make ruminants more productive and sustainable by enhancing the efficiency of conversion of energy and nutrients from land devoted to either grazing or crop production into useful and valued products while minimizing the adverse effects of ruminant production on the environment.

Fredric N. Owens

# Preface

The motivation for writing and organizing the Rumenology book was based on the lack of literature that reunited all basic and detailed information focusing only on the rumen itself. In order to accomplish this tough task, we invited some of the most renowned "Rumenologists" in the world to write some of the chapters, such as Dr. Fred Owens, Dr. TG Nagaraja, and Dr. Clint Krehbiel. Moreover, this book was organized to support graduate and undergraduate students, as well as scientists, in theirs studies involving the rumen in several disciplines, such as anatomy, biochemistry, physiology, microbiology, digestive metabolism, and animal nutrition. The book starts describing basic features of the rumen like anatomy and physiology and ends showing how rumen models and metabolism studies may play an important role to explore and understand the ruminal dynamics. In addition, chapters from 1 to 11 were organized on purpose in a sequence to make the learning process easier. The Rumenology book will provide to the reader all the basic aspects related to the rumen, and it will help and encourage students and scientists to further understand this fantastic compartment.

Dracena, São Paulo, Brazil — Danilo Domingues Millen

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# Chapter 1
# Anatomy and Physiology of the Rumen

**Claudia Maria Bertan Membrive**

## Introduction

Herbivores can be classified as monogastric or polygastric. Equine, rabbits and elephants represent monogastric herbivores. They have one stomach that does not offer appropriate conditions for fermentative digestion. In these species, the fermentation chambers, which keep a great amount of microorganisms, are represented by the cecum and colon, and both compartments are very developed.

Polygastric herbivores have more than one stomach. In these animals, the true stomach, the abomasum, is preceded by the presence of two to three pre-stomachs. The pre-stomachs consist of an aglandular mucosa and form a compartment where the fermentative digestion occurs exclusively, by the joint action of the microorganisms that live there. The true stomach called abomasum is morphologically and functionally similar to the stomach of monogastric animals, a place of significant enzymatic activity.

Polygastric herbivores can be classified as **Pseudo-ruminants** or **Ruminants**. When they have two pre-stomachs (reticulum and rumen) and a true stomach (abomasum), they are called pseudo ruminants. **Pseudo-ruminants** do not have an omasum and examples are camels, llamas, alpacas and vicunas. **Ruminants** present three pre-stomachs (reticulum, rumen and omasum) and a true stomach (abomasum) and are represented by bovine, sheep, goats, deer, giraffes, reindeer, moose, deer, roe deer and antelopes. After the intake of feed, polygastric herbivores regurgitate it from the ruminoreticular compartment to the oral cavity and chew it again; this mechanism is named rumination. This mechanism, which allows chewing the feed again and reducing it to smaller particles, represents a vital process for the fermentative digestion performed by microorganisms. Figure 1.1 shows the right side view of an adult bovine, illustrating

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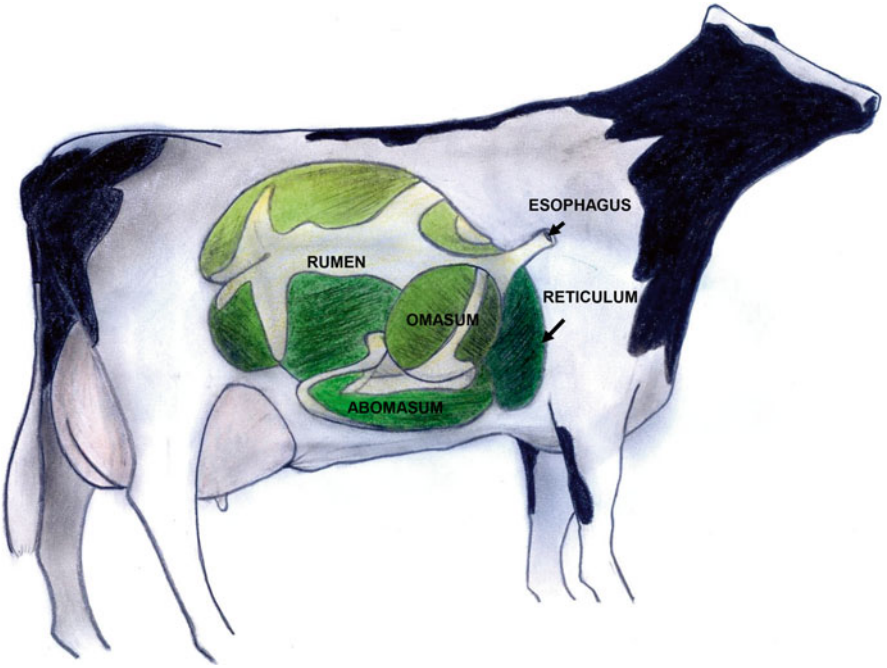


**Fig. 1.1** Right side view of an adult bovine illustrating the different anatomic segments that integrate the digestive tube: ESOPHAGUS, RETICULUM, RUMEN, OMASUM and ABOMASUM

the segments that integrate the digestive tube: esophagus, reticulum, omasum and abomasum. Figure 1.2 illustrates the left side view of an adult bovine, showing the esophagus, reticulum, rumen and abomasum. It is not possible to visualize the omasum from the left side.

Making a functional analogy, the digestive system of equines, monogastric herbivores with well-developed cecum and colon, is not as efficient as ruminants' to convert cellulosic matter into energy. Besides having a broad population of microorganisms in the colon where part of fiber digestion occurs, ruminants expose fibers to ruminal digestion anteriorly, a functional condition that provides a more efficient digestion when compared to equines. The ruminants' extraordinary capacity to take advantage of fibers from feed was summarized by Van Soest: "*grazing ruminants have a well-developed and specialized digestion mechanism that allows the best utilization of fibrous feed when compared to other herbivores*".

Ruminants have a voluminous fermentative chamber represented by the rumen and a wide microorganism population, selected throughout billions of years of evolution according to their biochemical functions. This particularity determines these animals' position as the greatest utilizers of vegetal fibers. The fermentative digestion developed by microorganisms reached its greatest evolution in ruminants.

The general objective of this chapter is to describe the main features of the anatomy and physiology of ruminants' digestive system, especially the rumen. In this

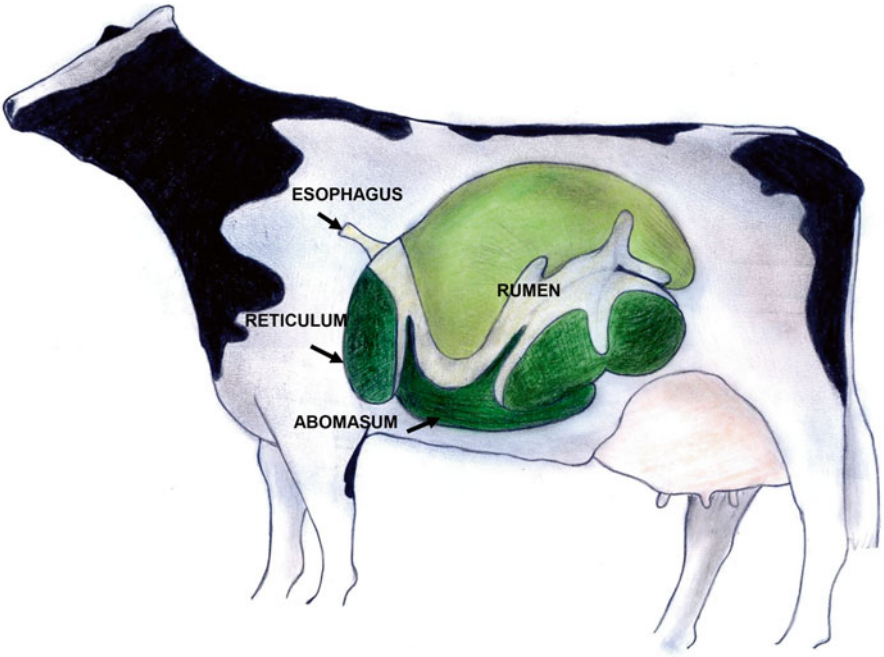


**Fig. 1.2** Left side view of an adult bovine illustrating the different anatomic segments that integrate the digestive tube: ESOPHAGUM, RETICULUM, RUMEN, ABOMASUM

chapter, the anatomical and physiological features of the rumen will be approached integratedly with other compartments that come before and after this extraordinary compartment, which characterizes ruminants as the animals that best utilize fibrous feed when compared to other species. This chapter will provide the understanding of anatomical, mechanical and functional features, and the determination of advantages, limitations and disadvantages of these animals because the rumen is one of the main chambers of the digestive tube.

## Anatomical and Physiological Properties of Ruminants

In ruminants, the extremely low oxygen concentration in the rumen, allowed throughout three billions years, a selection of microorganisms in the digestive system which represented the maximum biochemical yield under anaerobiosis condition. Moreover, there was the selection of a small percentage of facultative aerobic microorganisms whose function is to remove the small amount of oxygen that reaches the rumen with the feed intake, a fundamental mechanism for the preservation of the anaerobic environment of the rumen. It is interesting to point out that if high oxygen concentrations in the rumen had been kept, there

would have been a prioritization of biochemical pathways to form $CO_2$ and water, compounds that would be unable to be utilized as energy substrates by ruminants. The main products formed in the fermentative digestion are short-chain fatty acids (**SCFA**) that are the greatest energy source for herbivores. Ruminants obtain 50–70 % of their energy from SCFA produced in the rumen.

Considering the broad population of microorganisms kept in the digestive system, their short lifecycle and fast proliferation, part of the microorganisms are daily available as protein source in the digestive tube of ruminants. The rumen is anatomically positioned before the abomasum and duodenum. When moving through them, microorganisms are digested as any protein compound of the diet, becoming an extraordinary protein source for the animal.

A lot of microorganisms need ammonium for growth and multiplication. Ammonium can be provided in the animal feeding using sources like urea, ammonium salts, nitrates and other compounds. Microorganisms convert ammonium into amino acids that are utilized to build up microbial protein. Proteins from the diet that were not digested with the microbial protein generated in the rumen when going through the abomasum and the small intestine are digested by a group of proteolytic enzymes, and the available amino acids are readily absorbed. Therefore, a great advantage of ruminants is their capacity to convert ammonium into amino acids that are used to build up microbial protein, utilized as an essential part of the protein that forms the diet. Thus, besides the energetic contribution through SCFA formation, the microorganisms also represent an important protein source.

In the rumen, microorganisms synthetize all vitamins of B and K complexes in sufficient amounts for the animal's maintenance and growth. Under most conditions, ruminants do not require supplementation of these vitamins. The supplementation of vitamins B and K are necessary for calves and lambs, considering that the synthesis of these vitamins is only started when the ruminal microorganism population becomes active.

Moreover, the longest required time for the digestion of structural carbohydrates determined the need to develop fermentative chambers of great volumetric capacity, represented by the reticulum and rumen in ruminants. Although such compartments are differentiated, both together form a single intern chamber. The reticulum has an average volumetric capacity of approximately 9 l and the rumen from 150 to 200 l (Cunningham and Klein 2008).

In the rumen there is a great group of methanogenic archea that produces great amounts of methane ($CH_4$) during the fermentative digestion process. The methane production allows the release of exceeding hydrogen ions inside the rumen to the external environment, an essential condition for the maintenance of ruminal pH. Methane cannot be accumulated in the ruminal cavity; therefore, initially it fills out the dorsal part of the rumen and posteriorly is released from the ruminal chamber to the external environment through a mechanism called "eructation". Approximately 500–1000 l of gases are daily eructed by an adult bovine. In general, rumen gases consist of 0.2 % of hydrogen, 0.5 % of oxygen, 7 % of nitrogen, 26.8 % of methane and 65.5 % of carbon dioxide (Cunningham and Klein 2008). Eructation is a vital and essential physiological mechanism for the survival of ruminants.

## Main Functions of the Digestive System

In monogastric animals, most of the digestion occurs in the duodenum through the action of enzymes produced in the pancreas and duodenal epithelium. Carbohydrates are reduced to monosaccharides (glucose, fructose and galactose) by amylolitic enzymes. Proteins are reduced to amino acids by the action of a group of proteolytic enzymes. Through the action of lipolytic enzymes, lipids are reduced to fatty acids and glycerol. The bloodstream readily absorbs monosaccharides and amino acids. Fatty acids are transported as chylomicrons through the lymphatic system, reaching the bloodstream afterwards. In monogastric animals, glucose represents the main "energetic currency" of the organism.

Ruminants are herbivores characterized by the presence of three aglandular pre-stomachs (reticulum, rumen and omasum) and a glandular stomach (abomasum). Thus, in ruminants, substrates that are part of the feed go first into the ruminoreticular compartment to be available for microorganisms. Before the feed goes on to posterior compartments of the digestive system, the microorganisms digest most of the substrates. Thus, the feed is submitted to fermentative digestion first and then submitted to the action of enzymes produced by the digestive tube and attached glands. It should be noted that the pre-stomachs are totally aglandular, which provides an excellent environment for microorganisms. Thus, the fermentative digestion performed by microorganisms exclusively determines every digestion that occurs in the rumen. The ruminal content presents $10^{10}$–$10^{11}$ bacteria and $10^{5}$–$10^{6}$ protozoas/mL. In the rumen, there is a great number of cellulolytic, amilolytic, proteolytic and lipolytic microorganisms. The fermentative action of microorganisms is not restricted only to structural carbohydrates, but also to non-structural carbohydrates and proteins that are firstly digested in the rumen. The existing microorganisms in the rumen are grouped according to the substrate they predominantly degrade. In general, they are classified as cellulolytic (degrade cellulose), hemicellulolytic (degrade hemicellulose), pectinolytic (degrade pectin), ureolytic (convert urea into $NH_3$), lipolytic (degrade lipids), amilolytic (degrade starch), methane-producing species and ammonia-producing species (Cunningham and Klein 2008).

Structural carbohydrates (cellulose, hemicellulose and pectin) are degraded by a large group of cellulolytic, hemicellulolytic and pectinolytic enzymes. In the rumen, as one of the intermediate phases of the fermentative digestion, there is the production of a great amount of glucose. In ruminants, differently from monogastric animals, glucose produced in the rumen is not readily available as a source of energy to the animal, but it is rapidly utilized by the microorganisms. Thus, glucose produced by bacteria remains in the ruminal environment to be utilized as substrate by them. Microorganisms perform successive degradations that culminate with the production of a group of short-chain fatty acids (SCFA). The main SCFA produced in the rumen are acetic, propionic and butyric acids. They are rapidly transformed in their ionized forms in the rumen and, therefore, commonly mentioned as acetate, propionate and butyrate, respectively. The most produced SCFA is acetate, followed by propionate and butyrate. The proportion of SCFA is altered in function of

the diet composition provided to the animal. The greater the concentrate amount provided to the animal is, the greater the total SCFA production becomes. In addition, the production of propionate is increased when compared to acetate, but it must be pointed out that the acetate production is always the predominant one if rumen pH remains above 5.7 (Cunningham and Klein 2008).

SCFA produced in the rumen are rapidly absorbed by the ruminal wall and get into the bloodstream, where acetate is the main "energy currency" in ruminants. However, some tissues exclusively utilize glucose as energetic substrate, especially the nervous system. This system, which coordinates all the physiological processes of the organism, is not capable of producing or storing glucose. Thus, glucose concentrations in the bloodstream must be constantly kept within a physiological range (35–55 mg/dl in bovines, and 35–60 mg/dl in sheep) to guarantee enough plasmatic glucose concentrations for the nervous system to perform its functions (Cunningham and Klein 2008).

Therefore, considering that glucose produced in the rumen is not available to the animal, and in order to ensure partial maintenance of relatively constant concentrations of glucose in the bloodstream, propionate is converted in glucose and then called glycogenic SCFA. Thus, propionate produced by the rumen is readily absorbed through the ruminal wall, getting into the portal vein, and transformed into glucose when reaching the liver. In ruminants, a second source of glucose is available through carbohydrates that go by the rumen without being digested and reach the duodenum where they are readily digested. The participation of enzymes produced by the pancreas and the duodenal mucosa allows carbohydrate digestion, resulting in a significant amount of glucose. The concentrations of blood glucose in bovines and sheep are naturally lower than those found in monogastric animals, whose glucose is the main "energy currency" of the organism (in humans, the glucose concentrations are kept from 80 to 120 mg/dl).

Butyrate produced in the ruminal environment is mostly utilized as an "energetic currency" inside the rumen itself, where the cells of the ruminal epithelium utilize approximately 95 %. The exceeding butyrate, around 5 %, is absorbed by the ruminal wall, reaches systemic circulation and, in the liver, is converted to acetyl-coA, ketone bodies and long-chain fatty acids that are available in the plasma as lipoproteins. The ketone bodies are also used as "energetic currency" in the organism.

Although ruminants are well equipped to chew fibrous material efficiently, chewing is not efficient in the feed intake phase. Under this circumstance, chewing is enough to mix the feed to saliva, providing a moisture degree that is yet enough to make swallowing possible. Posteriorly, the feed found in the rumen is regurgitated from the ruminoreticular compartment to the mouth through the esophagus, re-chewed, re-salivated and re-swallowed. Together those processes characterize rumination, an essential process for the efficient utilization of fibrous feeds by ruminants. Re-chewing occurs carefully and regularly and is an important stimulus for the production of saliva. Re-chewing during rumination aims to reduce the feed particle size and to form a homogeneous bolus. The reduction of feed into smaller particles is fundamental for bacteria to perform fermentative digestion. According to (Cunningham and Klein 2008), in dairy cows, approximately 20,000–30,000

chewing movements are done daily. It is estimated that ruminants spend 8 h a day ingesting feed and 8 h a day ruminating it. The chemical and physical composition of the feed (fiber, energy and protein content) influences time spent ruminating.

Saliva is the main secretion of the digestive system, and an adult bovine produces 170–180 l of saliva/day. The volume of daily saliva produced depends directly on chewing time. The intake of fibrous feeds provides an abundant production of saliva, which is reduced during the intake of concentrates. The chemical composition of bovine saliva contains 126 mEq/L of sodium, 126 mEq/L of bicarbonate, 26 mEq/L of phosphate, 7 mEq/L of chloride, and 6 mEq/L of potassium. Because it contains a great amount of bicarbonate ions ($HCO_3$), saliva has a fundamental role in the maintenance of ruminal pH. Phosphate becomes important in the process of microorganism multiplication in the rumen (Cunningham and Klein 2008).

In ruminants, the feed intake capacity is influenced by several factors: animal's age (the intake decreases with age), physiological phase (intake reduction in the final third of pregnancy and in the beginning of lactation), sex (females generally ingest less feed than males), production level (the higher the production is, the greater the nutritional demand and intake are), feed availability (for the maximum intake, feed offering is necessary), feed palatability (taste, smell and texture influence the greater or smaller feed intake), feed presentation (natural, ground, granulated, pelletized or bran) and environmental factors (temperature and relative air humidity, stress, population density, trough structure, trough spacing and hygienic-sanitary conditions).

## General Anatomical Aspects of Ruminants' Digestive System

The function of the digestive system is to continuously supply the organism with water, electrolytes, vitamins, proteins, carbohydrates and lipids from feed intake. For the organism to utilize these elements from feed intake, the substrates have to be submitted to a physical (segmentation of feed into smaller particles) and chemical processing (breaking of complex molecules into smaller molecules that can be absorbed). After the chemical processing of the feed, the small molecules generated by the digestion have to be absorbed by the intestinal epithelium to be then available and utilized by the organism.

The ruminants' digestive system consists of a long muscular tube that goes from the mouth to the annus, and of a group of glands attached to this digestive tube. The digestive tube of ruminants comprises the following segments: mouth, pharynx, esophagus, pre-stomachs (reticulum, rumen, omasum), true stomach (abomasum), small intestine (duodenum, jejunum and ileum) and large intestine (cecum, colon and rectum). The rectum is provided with an annal orifice in the caudal portion. The glands attached to the digestive tube are represented by the salivary glands, pancreas, and bile system (which consists of the liver, gallbladder and bile ducts). To understand the ruminal physiology, it is fundamental to understand the general anatomical aspects of ruminants' digestive system. Although this chapter aims to describe

the rumen and the pre-stomachs, the anatomical peculiarities of the mouth and component structures, such as pharynx, esophagus, rumen and reticulum, omasum and abomasum, will be described because they are directly involved in the rumination and eructation processes. The anatomical understanding of these structures is fundamental to understand the functional mechanisms of the rumen.

## *Mouth*

The oral cavity contains different attached elements like the teeth, tongue, and salivary glands. The teeth and tongue are responsible for harvesting and physically reducing the feed. The presence of salivary glands, connected to the oral cavity through ducts, is essential to feed moisture, chewing, and swallowing.

Feed intake consists of prehension, chewing and swallowing. Prehension refers to the introduction of the feed into the oral cavity. Prehension varies according to the different species. In species that utilize teeth to prehend the prey or to fight, like dogs, the opening of the oral cavity is quite broad. In herbivores, in general, the mouth opening is quite small. Considering that bovines ingest small portions of the feed, the relatively small opening of the mouth cavity is not a disadvantage for this species. During feed prehension, the lip muscle movement is important not only for the feed capturing process, but also to promote the emptying of mucosal glands located among the lip muscle fibers. In bovine, there is a ventral buccal gland that ends in the buccal vestibule, which presents a great number of ducts connected to the oral cavity. The bovine oral cavity has a great amount of conical papillae formed by horny and cornified projections pointed cranial-caudally towards the back of the mouth. The function of these structures is to avoid the loss of roughage feed when the animal chews with open lips, which allows a greater displacement of the jaw during chewing.

Another characteristic of the oral cavity of bovine is the hard palate that is connected to the basal lamina due to evolutionary loss of upper incisive teeth. The hard palate is formed by a dozen or more transversal ridges whose protrusions progressively decrease until they finally disappear in the posterior part of the mouth, where ridge borders have numerous papillae. The hard palate is large in bovine and narrower in sheep and goats, species whose tongue is not used for feed prehension.

In bovines, the **TONGUE** is big, large, rough and with great mobility. In sheep and goats, the tongue and the hard palate are less rough when compared to bovine. The ventral side of the tongue is thin and medially attached to the floor of the oral cavity by the tongue frenulum. In the cranial-caudal side, the tongue is divided into three distinct regions: apex, body and root of the tongue, respectively. The dorsal side of the tongue is thick and cornified and presents numerous projections called papillae. Papillae favor the movement and grinding of feed inside the mouth, besides directing the feed towards the esophagus. The tongue is a muscle organ utilized to prehend the feed, intake the water and displaces the feed inside the mouth during chewing. In bovines, the tongue moves the feed on the lower jaw of molar teeth and

also functions as a pump that moves the feed inward the esophagus during the swallowing process. It is important to point out that because bovines have more than one tasting bud per circumvallate papillae, they first select the feed by tasting while other ruminants select the feed by smelling it.

In bovines, the **LIPS** are thick and have strict mobility. In sheep and goats, the lips are thin and flexible and the upper lip has a medial labial division called "filter". This feature allows them to graze close to the ground, characterizing low grazing, which is not possible for bovine. Regarding the animals' capacity to select the feed that they ingest, bovines, bubalines and sheep are classified as non-selectors. In bovines, the relative lip insensitivity favors the non-selectivity and intake of strange bodies that after being ingested can cause lesions in the lower digestive tract. Thus, due to the low selectivity of this species, the utilization of paddocks without strange elements (for example, plastic bags, pieces of barbed wire, nails and others) is recommended. Sheep are also classified as non-selectors. Among domestic ruminants, goats are the most selective ones regarding feed and are considered intermediate selectors. They have greater mobility of the upper lip and a greater percentage of their tongue length is not attached to the floor of the mouth. As a result, a greater proportion of the tongue is loose and can be exposed when compared to non-selective ruminants.

For some time, it was thought that goats' fiber digestion capacity was superior to sheep's and bovines' due to a more efficient fermentative digestion; however, currently it is believed that this is not true because the greater fermentation capacity is due to the intake of better quality feed since this species is very selective when compared to others.

The **TEETH** have the function to mechanically grind and reduce feed to smaller physical particles through chewing. Grinding allows an increase in the feed surface area, which favors a greater area for enzyme action. This preliminary step is fundamental for the chemical and microbiological degradation of the feed. The teeth are also utilized to cut the feed after prehension. Four kinds of teeth are evident according to their location and function. Incisive teeth are the front most ones and are utilized to cut the feed. The canine teeth come after the incisors and are generally used to cut the feed, but they are absent in ruminants. Pre-molar teeth are caudal to canine. After the pre-molars, there are larger teeth called molars. Pre-molar and molar teeth present appropriate size and shape for grinding.

Bovine, sheep and goats present permanent teething consisting of 32 teeth. In the upper jaw, the incisive and canine are absent, and there are 6 pre-molar and 6 molar teeth; therefore, there are a total of 12 teeth. In the place of the upper incisors, bovines present semicircular cuneiform elevations on the surface, which are called dental pads. The dental pads tear the forage when pressed against the lower incisor. The lower jaw has 8 incisors, no canines, 6 pre-molars and 6 molars, totalizing 20 teeth. In bovines, the lower incisors have the shape of a shovel and are located separately from each other, and also have a quite loose implantation, which reduces the lesion risk of the dental pads. During grazing, bovines initially take the grass to the mouth with the help of the tongue and then cut it by pressing the incisors against the dental pad. In ruminants, the upper and

lower dental jaws have uneven width, characterizing unilateral horizontal chewing. Although both sides of the dental arch are utilized, most animals tend to favor one of the sides for chewing.

**SALIVARY GLANDS** release their secretions in the oral cavity through ducts that connect these glands to the oral cavity. Salivary glands are formed by a set of ducts that are internally covered by mucosa and serosa cells. The mucosal cells synthetize a mucous secretion, which is characterized by a group of glycoproteins, called mucin. Salivary mucin consists of albumin, alpha 1-globulin and glycoproteins, and becomes viscous in the presence of water. Mucin gives saliva the viscosity, which is important to reduce the friction between the feed particles and the oral cavity. The serosal cells secrete an aqueous fluid with ions of Na, Cl, and specially $HCO_3$ in great amounts. In the saliva of ruminant animals, the alpha-amylase enzyme is not present; therefore, the saliva is not important for digestion. It should be pointed out that calves and lambs produce lipase in the oral cavity and it reaches the abomasum with the ingested milk. Such enzyme decomposes around 20 % of ester bonds of fats present in the milk, during milking. The amount of secreted saliva by the calf depends on the milk flow that goes by the mouth. When the calf suckles the milk slowly, in milk feeding using bottles, there is a greater saliva production. Milk feeding in buckets makes the milk pass by the mouth faster, reducing saliva production.

Ruminants have a pair of parotid glands, a pair of submandibular glands and a pair of sublingual glands, besides numerous smaller salivary glands in the lips, cheeks, tongue, gums and floor of the oral cavity. The pair of larger salivary glands that produce predominantly serosal secretion does a greater production of saliva. The mandibular gland is located near the jaw angles and produces serosal and mucosal secretion. In ruminants, this gland is larger than the parotid ones and is located deeply. The parotid gland is a pair of serosal gland that is found ventrally to the ear, is particularly developed in herbivores, and secrets a great amount of an alkaline solution. The parotid glands are responsible for over 50 % of the total saliva production. During chewing, due to the pressure of the muscular movement, the salivary glands that are found among the muscular fibers, through the pressure of the muscular movement secret a lot of saliva. The saliva secretion in ruminants is continuous, but the secretion amount increases greatly in the presence of stimuli associated to feeding, rumination and presence of rough feed in the gastric compartments.

During chewing, saliva is mixed to the feed to provide the necessary moisture for the feed to be swallowed. Drier feed requires a greater amount of saliva to be moist, and therefore the saliva amount is changed in function of the feed composition. Saliva consists of a colorless, odorless, and tasteless solution with alkaline pH. According to, bovines produce 110–180 l of saliva daily and it has a pH ranging from 8.2 to 8.2. Sheep produce from 6 to 16 l of saliva a day, and its pH varies from 8.0 to 8.4. Saliva consists of 99–99.5 % of water and 0.5–1 % of dry mass, represented by inorganic and organic compounds, leukocytes, microorganisms and desquamated epithelial cells (Cunningham and Klein 2008).

Ruminants' saliva also presents a great amount of $PO_4$, which is not found in non-ruminant species. Through the swallowing of saliva produced in the oral cavity, $PO_4$ produced in the saliva goes to the rumen where it contributes importantly to the

multiplication of microorganisms that live in the rumen because it is directly involved in the process of ruminal buffering. The high concentration of nitrogen in the saliva of ruminants is particularly important, and it ranges from 9 to 30 mg per each 100 mL. Around 65–70 % of total nitrogen corresponds to urea, which reaches the rumen in significant amounts with saliva. Also, in ruminants, saliva represents a possibility to recycle urea. The exceeding urea in the organism can be directed to the saliva, which is excreted by salivary glands, and be re-directed to the ruminoreticular cavity, increasing nitrogen availability to ruminal microorganisms.

The salivary glands receive parasympathic and sympathic fibers originated in the autonomous peripheral nervous system. The parasympathic stimulation by acetylcholine increases the salivary secretion. The sympathic stimulation through noradrenalin reduces the salivary flow in general.

During feed prehension, ruminants have little elaborated chewing, when the feed is moistened just enough to be swallowed. However, these animals ruminate by regurgitating the feed from the ruminoreticular cavity into the mouth and then through the esophagus. After the feed is regurgitated, the water excess of this material is swallowed and then the animal starts the chewing, which becomes more elaborated. Ruminants spend approximately an average of 8 h ruminating daily. A dairy cow makes around 40,000–50,000 chewing movements/day. Rumination follows the circadian cycle: during the day the animal normally ingests a great amount of feed and ruminates intensively at night, a characteristic that ruminants acquired when they needed to feed themselves during the day to protect themselves from predators during the night, which was a period dedicated to rumination (Cunningham and Klein 2008).

Rumination is an important process to stimulate saliva production. During chewing, the moving muscles compress the salivary glands to help their emptying through a system of ducts that end up in the oral cavity. The abundantly produced saliva is swallowed and sent to the ruminoreticular cavity. The bicarbonate ions have the important function of continuously buffering the ruminal pH. The fermentative digestion in the rumen causes the constant formation of SCFA that reduce ruminal pH. The bovine saliva contributes to the daily infusion of 250 g of $Na_2HPO_4$ and 1–2 kg of $NaHCO_3$. Therefore, the continuous bicarbonate infusion in the rumen through saliva has a buffering function in the ruminal environment so that the pH becomes appropriate for the survival and multiplication of microorganisms, since they in general appreciate ruminal environment with pH ranging from 5.7 to 6.8 (Cunningham and Klein 2008).

## *Pharynx*

The pharynx represents a segment of the passage of feed and air. The pharynx, located between the oral cavity and the esophagus and the choanae and the larynx, is a common region for both the respiratory and digestive organs. During the passage of feed to the pharynx, mechanical factors and reflexes related to the swallowing prevent that

feed gets in the glottis and nasal choanae. The passage of feed into the respiratory tube is avoided by the soft palate that becomes horizontally positioned, and by the larynx elevation, while the epiglottis is positioned against the glottis causing it to close. The muscles of the hyoid bone have a close functional relation with the muscles of the tongue and pharynx, and have an important role in the chewing and swallowing of feed. The pharynx is formed by muscles that cause its narrowing and shortening during swallowing. The pharynx is a segment that has voluntary control in both directions, oral-caudal during swallowing and caudal-oral in regurgitation and eructation, depending on the physiological needs of the ruminants. The pharynx receives and directs the regurgitated bolus to the mouth. It also receives the gas that is expelled in great amounts from the ruminal cavity to the external environment. After the end of swallowing, the passage of air is re-established by the pharynx.

Swallowing is a process that is divided into three phases, the first is a voluntary one and the other two have reflexive nature. In the first phase, called voluntary, the feed, after chewed and transformed into the bolus through the action of tongue muscles, is positioned in the posterior upper part of the tongue. Next, the mouth is shut, chewing is interrupted, breathing is stopped, the tip of the tongue touches the hard palate and the bolus is pressed between the tongue and the pharynx that opens through a contraction of the hyoid bone. In that moment, the feed gets into the pharynx, ending the first phase of swallowing. The second phase of swallowing, called pharyngeal or reflexive, is very short and corresponds to the passage of the bolus through the pharynx. The feed presence in the pharynx stimulates local receptors that send signals through afferent nerve fibers to the swallowing center located in the encephalic trunk. Then, through efferents nerve fibers, the trunk sends stimuli to the muscles that form the pharynx. Under this stimulus, the pharynx muscles contract themselves in the cranial-caudal direction, pushing the passage of the feed from the pharynx to the esophagus, ending the second phase of swallowing. The third phase, called esopharingeal phase, comprises the passage of the feed through the esophagus. This passage occurs through the peristaltic movements that start in the anterior portion of the feed in the esophagus and, when propagating through the esophagus, they push the feed towards the ruminoreticular compartment.

## *Esophagus*

It comprises a muscular tube that extends from the pharynx to the ruminoreticulum. In bovines, the esophagus is 90–105 cm long, from the pharynx to the cardia. The length of the cervical part is 42–49 cm long, and the thoracic part is 48–56 cm long. In sheep, the esophagus is approximately 45 cm long. In this route, the esophagus gets into the thorax, goes through the medianistinical space and finally reaches the abdominal cavity where it connects to the ruminoreticulum. The lumen of the esophagus normally remains closed, making the folds evident on its internal surface. In the passage of the feed, the folds are stretched.

In ruminants' esophagus, there is the formation of functional sphincters such as the cranial esophageal sphincter located in the entrance of the esophagus and

the caudal esophageal sphincter. The cranial and caudal sphincters function alternately, that is, the contraction of the former causes the relaxing of the latter, and the contraction of the latter results in the relaxing of the former. This reciprocal dependence is especially important in eructation. The esophagus is connected to the dorsal part of the common region to both compartments, the rumen and the reticulum.

## *Stomach*

The stomach consists of four chambers through which the feed passes and that are successively called: rumen, reticulum, omasum and abomasum (Figs. 1.1 and 1.2). The first three chambers are known as anterior stomach and were developed to favor the digestion of structural carbohydrates that are part of ruminants' diet. Only the last chamber, the abomasum, is comparable in structure and function to the simple stomach of most animals of other species.

The stomach of an adult bovine is a huge compartment that practically fills up the whole left side of the abdominal cavity, still occupying most of the right abdominal cavity. In an adult bovine, the stomach occupies nearly 75 % of the abdominal cavity, where the rumen corresponds to approximately 6 % of the animal's live weight. The stomach capacity varies greatly with age and animal size. The volumetric capacity of the rumen is 100–150 l in small-sized bovines, 130–160 l in medium-sized bovines, and 120–300 l in large-sized bovines. It is believed that the bovine rumen has an average volumetric capacity ranging from 150 to 200 l (Cunningham and Klein 2008). In sheep, the volumetric capacity of the rumen is approximately 15 l. Considering that the rumen represents the fermentative chamber where most of the digestion happens, it can be assumed that the volumetric capacity of the rumen determines the capacity of feed intake and, consequently, favors a greater productive capacity of the animal. According to DYCE (2004), it is estimated that in bovines the proportion of the different compartments is represented by 80 % of rumen, 5 % of reticulum, 8 % of omasum, and 7 % of abomasum. In small ruminants, represented by sheep and goats, these proportions are different, 75 % of rumen, 8 % of reticulum, 4 % of omasum and 13 % of abomasum.

The celiac artery that branches out irrigating different cavities does the irrigation of the multicavitary stomach of ruminants. The venous vascular system that carries the products of ruminal fermentation absorbed through rumen epithelium, lead to the portal-hepatic vein.

In order to be able to perform their functions, an adequate motor activity of the pre-stomachs becomes fundamental. The movements in the different pre-stomachs aim to fragment particles mechanically, mix existing components inside the compartment, stimulate absorption of short-chain fatty acids, regurgitate feed from the ruminoreticulum to the mouth for rumination takes place, and release gases from the rumen to the external environment through eructation. The ruminants' stomach innervention is autonomous. The sympathic fibers that originate in the celiac plexus form the gastric plexus, right ruminal plexus and left ruminal plexus. The pattern of

the parasympathic innervation is represented by the vagus nerve that is split into dorsal vagus nerve and ventral vagus nerve. The dorsal vagus trunk is especially important for rumen innervation, whereas the ventral vagus trunk is essential for innervation of the reticulum, omasum and abomasum. The sectioning of both trunks eliminates all the motor activity of anterior chambers. The musculature, under parasympathic innervation, assumes a relevant role in the rumen mobility. The development of the muscular layer is associated with the kind of feed ingested by the animal, because the greater the amount of fibrous feed ingested is, the greater the necessity of ruminal motility becomes and, therefore, the greater the development of the muscular layer gets.

For a better anatomical and physiological understanding of the different compartments, they will be described individually.

## Reticulum

As shown in Figs. 1.3 and 1.4, the reticulum comprises a relatively spherical compartment, located cranially to the rumen that presents a volumetric capacity of approximately 9 l in adult bovines. Both compartments are partially separated in the ventral portion through the ruminoreticular fold that forms a big orifice of passage between the rumen and the reticulum when contracted. The rumen and the reticulum

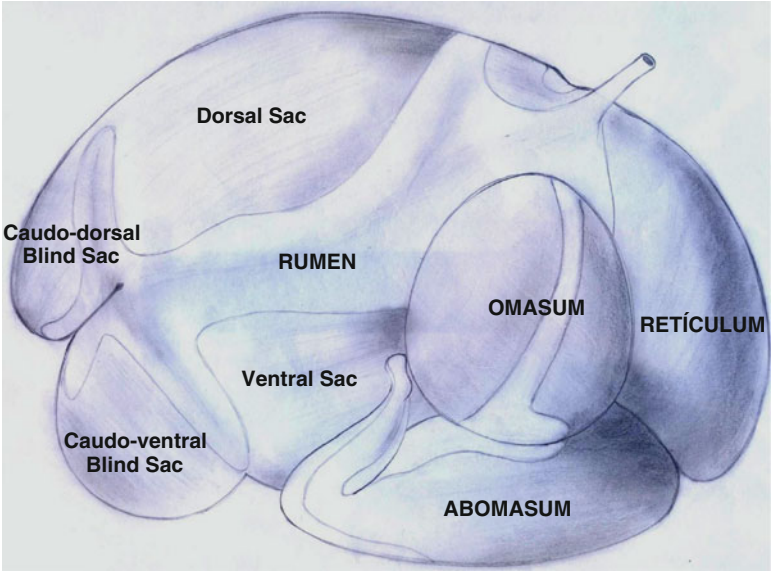


**Fig. 1.3** Right side view illustrating the different anatomic segments that integrate the digestive tube of an adult bovine: the aglandular pre-stomachs (RETICULUM, RUMEN and OMASUM), the glandular stomach (ABOMASUM), as well as Dorsal Sac, Caudo-dorsal Blind Sac, Ventral Sac, Caudo-ventral Blind Sac

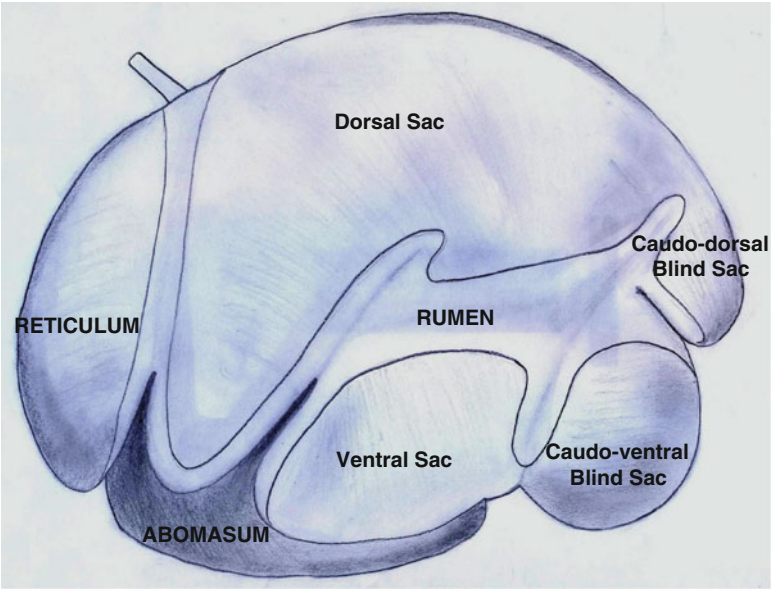


**Fig. 1.4** Left side view illustrating the different anatomic segments that integrate the digestive tube of an adult bovine: the aglandular pre-stomachs (RETICULUM and RUMEN), the glandular stomach (ABOMASUM), as well as Dorsal Sac, Caudo-dorsal Blind Sac, Ventral Sac, Caudo-ventral Blind Sac

freely connect to each other internally. The reticulum is considered a conjugated compartment to the rumen. The reticulum is located extremely close to the diaphragm, distant 2–4 cm from the pericardic bag that constitutes the heart of bovines. The reticulum is located cranially to the rumen, under the sixth and eighth rib and mainly to the left of the median plane.

The esophagus ends in the beginning of the stomach in the limit between the rumen and reticulum, internally presenting a continuation through the esophageal canal, also called esophageal or reticular groove. The cardia is the origin point of the esophageal or reticular groove, which goes ventrally 17–20 cm up to the reticular-omasal orifice. This structure is represented by a groove consisting of spiral fleshy labia where the upper opening is connected to the cardia and the lower opening to the omasum. The cardia is located in the junction of the rumen with the reticulum, and, then, ending in both chambers. In unweaned calves, during the intake of milk, the reticular groove becomes a closed tube that directs the milk from the esophagus to the omasum canal, where the milk goes down to the abomasum. After weaning, the diet changes lead to the decreasing utilization of this via. The mechanisms that act on the closing of the reticular groove will be described later.

The ruminoreticular mucosa is totally deprived of aglandular epithelium and is covered with a rough stratified cutaneous epithelium. The reticulum mucosa has numerous primary folds, approximately 1 cm high, called crests (Fig. 1.5). These

**Fig. 1.5** Reticulum inside view of an adult bovine. The reticulum presents crests with about 1 cm height that design geometric structures of four to six sides and characterize a quite reticulated structure similar to "honeycombs"

structures limit the tetra, penta or hexagonal spaces that are named "reticular cells" and characterize a quite reticulated structure similar to "honeycombs". These structures present short papillae in their interior. This reticulated pattern becomes less regular in the region of the junction with the rumen, gradually mixing itself to the papillated surface of the rumen. The epithelium of the reticular mucosa is stratified and squamous. The keratinized layer becomes important to reduce the abrasion resulted from the rough diet ingested by ruminants.

The reticulum of small ruminants is relatively bigger than the one of bovines. In the covering of the reticulum there are clear differences among the species. In sheep and goats, the crests that limit the four- to six-sided structures are much shorter and present more prominent cut borders. In these species, the papillated ruminal mucosa also invades most part of the reticular wall. In the smaller curvature of the reticulum, there is a reticular-omasal orifice whose function is to promote the passage of particles that are smaller than 1.18 mm to the posterior tract.

## Rumen

According to Figs. 1.3 and 1.4, the rumen consists of a very broad sac-like chamber with an average volumetric capacity of 150–200 l. The microbial digestive capacity of the rumen depends on its volume, among other things. The rumen presents structures represented by thick muscular bands, called pillars, which divide the ruminal

space into dorsal sac, ventral sac, blind dorsal sac and blind ventral sac. The main ruminal pillars surround the organ dividing the main sacs into ventral and dorsal. The coronary pillars, which are smaller, limit the blind caudal sacs. The relative proportions of the sacs that constitute the rumen vary among domestic ruminants. The smaller size of the dorsal sac and the extensive caudal projection of the blind ventral sac give the rumen of sheep and goats an asymmetric aspect when compared to bovine rumen, which has a more symmetric aspect. The interior of the ruminoreticular compartment connects to the esophagus and omasum, through an opening located in the extremities of the reticular groove. The esophagus opens itself dorsally to a region that is common to compartments, rumen and reticulum. Posteriorly, the reticular-omasal orifice links the reticulum to the omasum.

The rumen stretches from the cardia up to the pelvic entrance, from the abdominal roof to the floor. This compartment fills up most of the total left antimerus of the abdominal cavity, and through the caudal-ventral segment it goes through the median plane and reaches the abdominal cavity right half (Figs. 1.6 and 1.7).

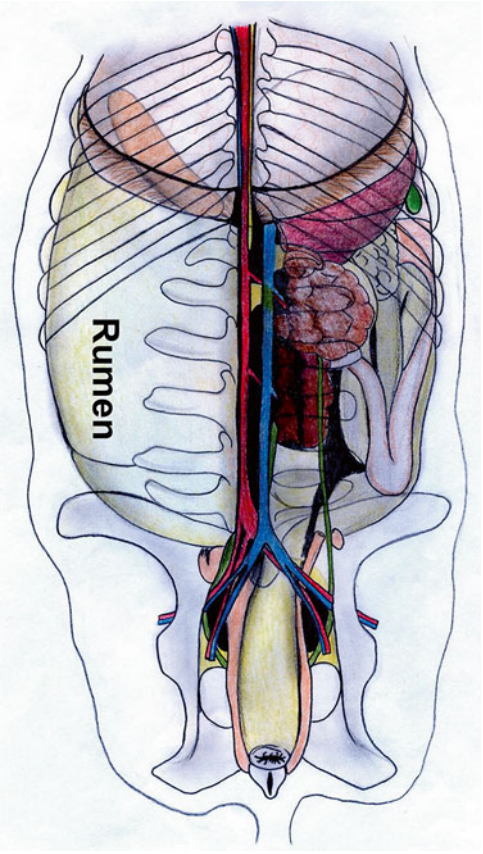


**Fig. 1.6** Dorsal view of the abdominal cavity inside of an adult bovine, illustrating the ruminal compartment that fills up the total left antimerus of the abdominal cavity and reaches the abdominal cavity right half

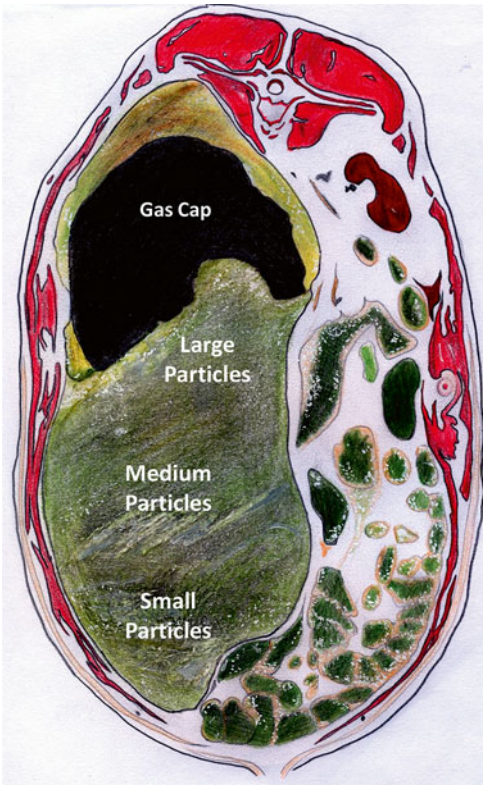


**Fig. 1.7** Caudal view of the cross section of the abdominal cavity of an adult ruminant, illustrating the rumen filling up the left antimerus of abdominal cavity, as well as the stratified organization of the feed particles in the rumen according to the different particle size. Smaller particles are located in the ventral part of the rumen, medium-sized particles stay over the smaller particles, and the larger particles float on the surface of the ruminal content. A gas cap fills up the dorsal part of the rumen

The rumen and the reticulum represent compartments that have lost their gastric glands after undergoing deep phylogenetic changes, in size and shape, caused by the rough and voluminous characteristic of the feed. The relative size of the rumen varies according to the age of the animals, and mainly by the type of diet ingested. The rumen is covered by a stratified keratinized epithelium without glands, and therefore all digestive processes carried out in the rumen are exclusively resulted from fermentative digestion.

The ruminal compartment is covered by papillae (Fig. 1.8), especially developed in the ventral sac. Normally, papillae are bigger and denser inside blind sacs, less numerous and prominent in the ventral sac, and much less developed in the center of the rumen roof and the free borders of the pillars. Individual papillae vary from rounded short elevations, going through conical and linguiform ones, to flattened leaves. These papillae can be up to 1.5 cm long and contain a highly vascularized conjunctive tissue axis consisting of thin collagen fibers and elastic fibers. The ruminants' feeding habits determines the number, distribution and length of papillae. It should be considered that the development of papillae is caused by the trophic action of the feed on the mucosa.

**Fig. 1.8** Rumen inside view of an adult bovine, illustrating the ruminal papillae

Ruminants that intake more concentrates have a more uniform distribution of ruminal papillae in the ruminal mucosa. The adaptive process of the ruminal mucosa (number, size and distribution of the papillae) due to the animal nutrition requires a period of 3–8 weeks. The adaptive mechanism depends on the production of SCFA, butyric and propionic acids, produced during fermentation. The need of a greater amount of blood to absorb these SCFA provides a greater offer of trophic, hormonal and mitogenic agents that reach the papillae for a greater irrigation of the tissue, and determine their greater development. On the other hand, when ruminants' feeding is based on fibers and fermentation induces the production of large amounts of acetate, there is a reduction in the size of the papillae. Thus, in ruminants with great intake great of forage, the ruminal papillae do not present uniform distribution. In the dorsal ruminal wall, the papillae are absent; therefore, in this region absorption of products derived from microbial action does not occur. The SCFA that go through the papillae by simple diffusion reach the vascular system, through the portal-hepatic system reaches the liver, and by the hepatic vein reaches the caudal cava vein.

The ruminal epithelium is deprived of the muscular layer of the mucosa. The characteristics of the papillary covering initially were related to the rough structure of feeds ingested by ruminants. Posteriorly, it was assumed that the presence of ruminal papillae referred to a structure developed to increase the epithelial surface, once the SCFA produced by microbial fermentation are absorbed in the rumen and reticulum. SCFA, water and vitamins of complex B and K are absorbed through the ruminal papillae. Height, thickness and shape of papillae depend on the feed energy composition. Papillae reduce their size when there is an increase in the availability of rough feed or during a drought period. When animals consume high-concentrate diets, the papillae may become longer and larger.

The rumen has the function of providing a compartment with the adequate conditions to allow chemical reduction of feed by microorganisms. In the rumen, the feeds are stratified according to the particle size (Fig. 1.7). The smaller particles, previously submitted to physical reduction of the feed into smaller particles in the mouth, are positioned in the ventral part of the rumen, favoring the passage of these particles to the omasum through the reticular-omasal orifice. The medium-sized particles stay over the smaller particles, and, finally, the larger particles float on the surface of the ruminal content, positioning them on the dorsal part of the rumen. This stratification by particle size allows that larger particles, which are not sufficiently physically degraded and located on the dorsal portion of the ruminal content, to be sent again to the oral cavity. Therefore, the rumen presents ruminal movements that allow regurgitation of bigger particles from the rumen to the mouth, where they can be re-chewed and physically reduced to smaller particles through rumination since only particles smaller than 1.18 mm pass to the posterior digestive tract through the reticular-omasal orifice. After the feed is re-chewed, it returns to the rumen, which has a highly adequate environment for the feed to suffer the bacterial action and be chemically reduced. The ruminal movements also guarantee the eructation process where the gases positioned in the dorsal portion of the rumen are eliminated to the external environment through their passage by the esophagus and oral cavity.

## Omasum

As shown in Fig. 1.9, the omasum has a round shape in bovine and oval shape in sheep similar to the shape of a bean, and is found dorsally right to the reticulum, between the rumen and the liver. The omasum is located to the left between the rumen and the reticulum. The largest part of the omasum is located between the eighth and eleventh ribs. The omasum is relatively smaller in sheep and goats. The volumetric capacity of the bovine omasum is approximately 14–15 l. The omasum interior presents hundreds of semilunar laminas that originate in both sides and from the largest curvature projecting to the smallest one, where there is a more open passage that forms the omasal canal. This characteristic gives the omasum a leafy aspect. In the omasum, there are approximately 12 larger folds and a great number of smaller folds. Besides those larger folds, there are other groups of smaller folds that can be visualized when these laminas are separated or transversely sectioned. The laminas are covered by short keratinized papillae (Cunningham and Klein 2008).

The function of the omasum is not clearly defined. The omasal folds determine a surface area 10 % larger than the rumen, giving the omasal mucosa a great absorptive capacity, especially for water. The absorption capacity of the omasal epithelium is similar to the ruminal papilla capacity. It makes the intake that recently left the ruminoreticular compartment less fluid before reaching the abomasum.

A small orifice, the reticular-omasal sphincter, connects the reticulum to the omasum. A large orifice connects the omasum to the abomasum, called omasal-abomasal sphincter.

**Fig. 1.9** Inside view of the omasum of an adult bovine, illustrating the semilunar laminas that give the omasum a leafy aspect

## Abomasum

As seen in Figs. 1.3 and 1.4, the abomasum is a pear-shaped sac that is bent on the abdominal floor, involving the inferior portion of the omasum behind. In bovines it has an average volumetric capacity of 18 l. In young calves, the abomasum covers a large ventral part of the abdomen, from the coastal arch to just before the pelvis. In adult bovines, the abomasum extends only up to the transversal plane by the first and second lumbar vertebrae. The back part is found in the xiphoidal region, where most part of the organ is located to the left of the median line. The abomasum is a glandular compartment that is similar to the simple stomach of monogastric species. Similarly to the simple stomach, the abomasum is divided into fundus, body and pylorus, even though the border between these parts is not precise. The abomasum of sheep and goats is relatively large when compared to bovine one. Age and pregnancy are factors that influence the size and topographic location of the abomasum.

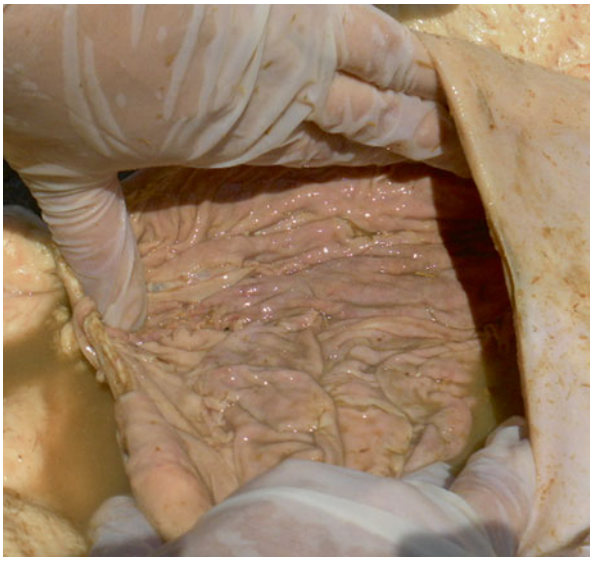

**Fig. 1.10** Inside view of the abomasum of an adult bovine, illustrating the glandular gastric mucosa full of rugae

The abomasum has a mucosa full of rugae like the stomach of other mammals (Fig. 1.10), consisting of glandular gastric mucosa. A very viscous mucous layer coats the pinkish mucosa of the abomasum. The physiological mechanisms that occur in the abomasum are like the mechanisms that happen in the stomach of monogastric animals, a place for intense enzymatic digestion. The presence of rugae increases sixfold the surface area of the abomasum. Ruminants that intake protein-rich feed (concentrate) present a larger glandular portion with a great number of HCl-releasing parietal cells in the abomasum.

The digestive system of ruminant species presents several particularities in the first weeks of life. The understanding of these characteristics, discussed here next, is fundamental to adequate diets in the first weeks of life for these species.

## Characteristics of the Digestive System of a Newborn Ruminant Animal

### *Functioning Mechanism of Esophageal Groove*

In lactating animals it is important that the ingested milk bypasses from the rumen so that it can be properly developed. The presence of milk in the rumen determines inadequate fermentation that can predispose the animal to disorders of the digestive system. Milk bypass from the rumen is possible due to the specifically developed anatomical structure in the digestive system called esophageal groove or reticular groove. This structure consists of muscular pillars that organize themselves on the

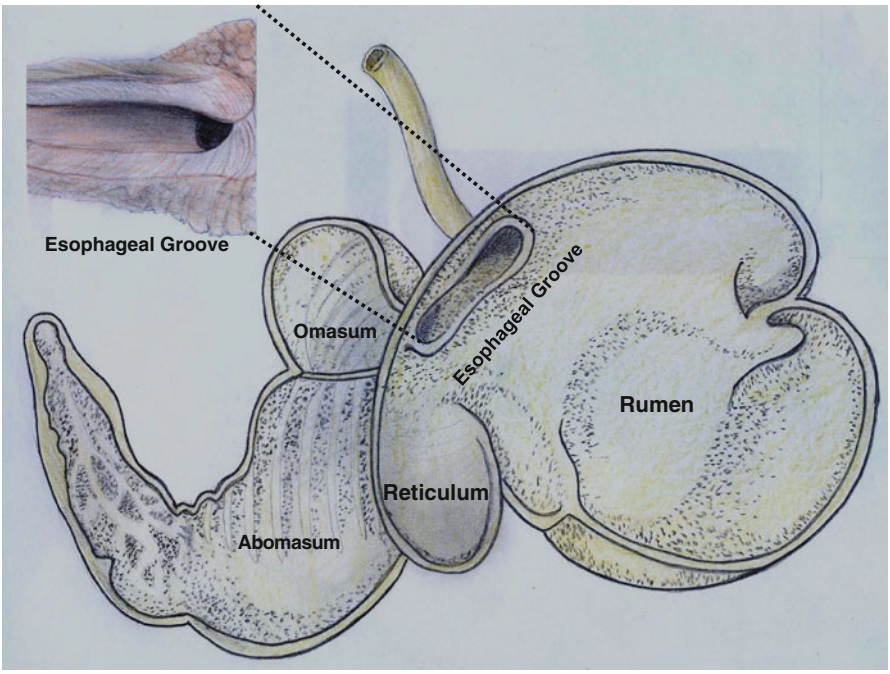


**Fig. 1.11** Inside view of the anatomic segments that integrate the digestive system of a calf in the first weeks of life (reticulum, rumen, omasum and abomasum) illustrating in details the ESOPHAEAL GROOVE, also called RETICULAR GROOVE. This structure consists of muscular pillars that organize themselves on the dorsal wall of the reticulum forming a gutter that runs along this wall from the cardia to the reticular-omasal orifice

dorsal wall of the reticulum forming a gutter that runs along this wall from the cardia to the reticular-omasal orifice. Under specific stimuli, the muscles that form this groove are contracted, so that the muscles arrange themselves in a way that the gutter becomes an almost complete tube. This muscular tube connects the cardia to the omasal canal (Fig. 1.11), making the milk bypass from the rumen and reticulum. Thus, when the groove is contracted, approximately 90 % of the milk that reaches the cardia is directed to the omasum whereas 10 % reaches the rumen (Cunningham and Klein 2008).

The act of milk suckling performed by the calf causes the contraction of the esophageal groove. The closing of this groove is a reflexive action, originated by the calf's "suckling desire" and determined by efferent impulses originated in the brain trunk that reach the esophageal groove through the vagus nerve. When milk goes through the pharynx, it stimulates chemoreceptors that, through afferent fibers represented by the glossopharyngeal nerve, direct this sensorial information to the medulla oblongata. The medulla oblongata sends impulses through efferent fibers, represented by the vagus nerve, causing the closing of the esophageal groove and the relaxation of the reticulo-omasal orifice and omasal canal. The contraction of the groove forms a tempo-

rary tube that connects the cardia orifice to the reticular-omasal orifice, known as esophageal groove. This temporary structure bypasses the milk from the rumen and the reticulum, goes through the omasum to be directly poured into the abomasum, where the milk will be submitted to enzymatic digestion.

The calf or lamb's head position during milk suckling does not seem to affect the efficiency of the esophageal groove closing. However, when compared to bottle, offering milk in a bucket decreases the efficiency of the groove closing, directing a greater amount of milk to the rumen. Therefore, the use of bottles to feed calves is more recommended than the utilization of buckets.

The reflex for the contraction of the esophageal groove is first triggered by the calf's "suckling desire", and after ingestion, milk salts and proteins may increase the stimulus to form the esophageal groove when going through the pharynx. Up to 2 months of age, milk and water go directly from the esophagus to the abomasum; later, when the calf ingests water or milk, this groove starts working less efficiently. This reflex decreases, as the animal gets older.

After weaning, changes in the diet cause a decreasing utilization of this route; however, a part of the soluble nutrients released into saliva during chewing are bypassed by the esophageal groove. In adult animals, when released by the neurohypophysis, the anti-diuretic hormone stimulates the thirst center and affects the esophageal groove bypassing part of the ingested water. It is believed that by action of anti-diuretic hormone most of the water that adult animals intake can be bypassed to the rumen through the esophageal groove. Likewise, the closing of the esophageal groove can be stimulated by copper sulfate, a characteristic that becomes a useful strategy when the introduction of medication in the abomasum is intended without previous dilution in the pre-compartments.

The ingested milk is initially submitted to the action of salivary lipase enzyme, produced by salivary glands, which hydrolyze the butyric and caproic acids. This hydrolysis rapidly occurs before milk reaches the abomasum. The salivary lipase secretion, by salivary glands, is very high in unweaned calves and reduces as forage intake increases. This enzyme progressively decreases the secretion as the animal grows, and practically disappears in the fifth month of the calf's life. When calves suckle the cow's nipples, it stimulates salivary lipase secretion in calves. It was observed that in bucket-fed calves, a smaller amount of salivary lipase is secreted when compared to animals that are fed with nipple bottles.

When the milk reaches the abomasum, renin acts on it causing the coagulation of milk proteins. Milk coagulation in the abomasum by renin is necessary in order to keep milk proteins in the abomasum longer so that they can be initially digested in this compartment. Five minutes after milk intake, milk whey reaches the duodenum.

The only carbohydrates that can be utilized efficiently by a young calf are lactose, galactose and glucose. In the first weeks of life, a calf is not able to utilize sucrose, maltose and starch efficiently. The utilization of starch by a calf results from the fermentation that occurs in the large intestine, where SCFA are produced and utilized as energy source by the calf. However, excessive amounts of starch in calves' diets may cause diarrhea. As these animals grow older, the lactase concentrations gradually decrease due to their smaller dependence on milk, and completely cease when weaning occurs. Nevertheless, if the animals kept on receiving lactose, the lactase activity would not be lost.

**Table 1.1** Approximate percentages of the stomach and pre-stomachs at different ages of a bovine

| | Age in weeks | | | | |
|---|---|---|---|---|---|
| | 0 | 4 | 8 | 20–26 | 34–38 |
| Reticulum—Rumen (%) | 38 | 52 | 60 | 64 | 64 |
| Omasum (%) | 13 | 12 | 13 | 22 | 25 |
| Abomasum (%) | 49 | 36 | 27 | 14 | 11 |

Source: Dárce (1977)

## *Development of Pre-stomachs in the First Weeks of Ruminant's Life*

The development of pre-stomachs in the first weeks of a ruminant's life has three distinct phases: *non-ruminant phase*, which refers to the birth until the first 3 weeks of life; *transition phase*, which comprises 3–8 weeks of life; and finally, *ruminant phase*, established from the 8th week of life. During the transition phase, there are great modifications in the development of pre-stomachs, which are fundamental to make the animal a ruminant.

As seen in Table 1.1, the size of the pre-stomachs in newborn calves is almost the size of the abomasum, with completely different proportions from the ones found in adult ruminants, where the pre-stomachs represent more than 90 % of the total gastric volume. The increase of the pre-stomachs occurs rapidly after the birth.

During the first 3 weeks of life, the calves' diet basically consists of milk. They start the intake of grains and forages in the second week of life, and rumination in the third week. The interruption of feeding solid feedstuffs greatly reduces the rumen development. In calves that are exclusively fed with milk, the pre-stomachs develop later and remain rudimentary for a longer period of time. The composition of the diet provided during this period dictates how fast the pre-stomach development will be.

At birth, the pre-stomachs do not present microorganisms. Immediately after the birth, bacteria existing in the environment quickly colonize them. Some studies have shown that, at 10 days of life, all microorganisms that are necessary to colonize the rumen are already found in the ruminal cavity. As bacterial fermentation starts, bacteria establish a reductive environment in the rumen, creating favorable conditions for the settling of typical microorganisms that developed in the rumen. These organisms have access to the rumen through the intake of forage contaminated with the dam's or other animals' saliva or feces. At the end of the first month of life, calves are already able to digest 75 % of dry matter and 84 % of cellulose when fed with good quality grasses.

After some weeks, with intake of solid feeds like forage and concentrates, the relationship between the different gastric compartments is changed, as shown in Table 1.1. In modern calves' production systems, the early development of the rumen should be stimulated from the first month of life with the utilization of procedures, such as the use of creep feeding, that make calves intake forage and grains,

and become less dependent on milk. However, a single diet containing only grains provided in a large amount or even inadequately can cause rumenitis, that is, lesions or abnormalities in the ruminal epithelium.

These anatomical alterations directly depend on the type of diet fed to the animal, mainly during the transition period, between the first 3 and 8 weeks of life. In calves that receive some kind of coarsely ground concentrate and hay, the volumetric capacity of the ruminoreticulum is four times greater when compared to animals that only consume milk. Moreover, a considerable development of rumen papillae occurs in animals that received solid feedstuffs, whereas the papillae remain rudimentary in animals consuming only milk.

Ruminal papillae reach total development at approximately 2–3 months of age, and their development determines the absorption capacity of the rumen. The development of rumen papillae is stimulated by ammonia, sodium salts and mainly by SCFA, such as propionate and butyrate; and therefore, the earlier the beginning of their production by calves is; the earlier the ruminal epithelium is developed. Also, in order to achieve its maximum functional capacity, the rumen has to develop muscular tissue and this is guaranteed by feeding the calf with good-quality forage.

In calves' intelligent feeding systems, conditions to allow the maximum development of the volumetric capacity of pre-stomachs as well as the development of ruminal papillae should be established. The development of the volumetric capacity depends on feeding of good-quality forages. For the development of ruminal papillae, it is fundamental to provide concentrates, especially those that will be utilized as substrate for propionate production in the rumen. It is important to point out that in first 2 months of a ruminant's life the intake of liquid feed is much easier and preferable when compared to solid feedstuffs. Thus, for calves to become interested in solid feeds in this period, these feeds need to have excellent quality.

## Motor Activity of the Rumen

An adequate motor activity is essential for the ruminoreticular compartment to perform its functions completely. In general, two basic types of movements characterize this activity: propulsive peristaltic movements and mixing movements.

The *propulsive peristaltic movements* aim to push the feed along the different segments that form the digestive tube, passing the feed from one segment to the other consecutively. These movements guarantee the feed transit throughout the digestive tube. The ruminoreticular compartment sends the fragmented feed to the omasum so that the digestive processes continue in the next segments. Therefore, the propulsive peristaltic movements occurring craniocaudally determine this action. However, during the eructation and regurgitation processes, part of the content in the ruminoreticular compartment is sent towards the mouth, and caudo-cranial peristaltic movements become necessary.

The *mixing movements* aim to coordinately rotate the feed in a specific segment of the digestive tube without being pushed to the consecutive compartment. The goal of

these movements in the ruminoreticular compartment is a set of actions: disperse the feed bolus to be digested within the ruminoreticular compartment, mix saliva with the feed ingested causing an efficient balance of ruminal pH, promote the contact of microorganisms with feed fragments to be digested, and help feed fragmentation. The mixing movements are also important to constantly expose new portions of feed ingested to ruminal papillae, guaranteeing an appropriate nutrient absorption. Part of SCFA produced in the rumen is readily absorbed by ruminal papillae.

The ruminal motility follows a coordinate pattern that starts early in a ruminant's life and, except for temporary periods; it persists throughout the animal's life. Under circumstances where this motility is suppressed for a significant period of time, the ruminal functionality gets extremely compromised.

The motor activity of the ruminoreticular compartment is basically controlled by two systems: (a) by the enteric nervous system represented by a large group of neurons distributed along the whole digestive tube, and (b) by the autonomous nervous system where a group of nervous fibers connects the central nervous system (medulla oblongata) to the ruminoreticular compartment.

The enteric nervous system is displayed alongside the whole extension of the digestive tube wall. This system is formed by a more external network of neurons displayed between the muscular layers of the ruminoreticular compartment called *myenteric plexus*, whose main responsibility is to ensure the adequate motor activity of the digestive tube. A second more internal network of neurons is found in the submucosa of the ruminoreticular compartment and forms the *submucosal plexus*, whose principal responsibility is to control the blood flow, which is important in the absorbing activity of the digestive tube. Although the enteric nervous system in general benefits the autonomy of the digestive system of most compartments, the same does not occurs in the ruminoreticular cavity. Therefore, the neurons that integrate the enteric nervous system are connected to sympathetic and parasympathetic nervous fibers of the autonomous nervous system. Generally, by action of noradrenalin, the sympathetic fibers inhibit the motor activity of the digestive system. On the other hand, by action of acetylcholine, the parasympathetic fibers stimulate the motor activity of the digestive system. The vagus nerve has a great control on the motor activity of the rumen because its section causes the interruption of ruminal motility.

Even though the ruminoreticular compartment is provided with an enteric nervous system, the contractions carried out there follow the motility pattern determined by the central nervous system. In the cerebral trunk, specifically in the medulla oblongata, a motility control center determines the frequency and strength of the contractions in the ruminoreticular compartment. This control is established through efferent fibers represented by the vagus nerve. Moreover, there are afferent fibers, also represented by the vagus nerve, that establish a communication connection between the ruminoreticular compartment and the central nervous system. The ruminoreticular compartment presents stretching receptors, found on the ruminal wall and pillars, aiming to capture information on the rumen volume and distension degree. Diets with large amounts of forage cause a higher frequency of contractions when compared to animals fed high-concentrate diets. The rumen and reticulum wall is also provided with chemoreceptors that monitor ruminal pH, SCFA concentration

and ionic force. These receptors capture information from ruminal pH that is sent by afferent via to the motility center in the cerebral trunk, which immediately adjusts the motility in the ruminoreticular compartment. Reductions of ruminal pH cause a decrease in ruminal motility. The normal pH of the rumen ranges from 5.5 to 7.0, depending on the type of diet. When ruminal pH is lower than 5.0, its motility becomes intensely depressed. This response is a protective mechanism considering that fermentation is likely to increase when the feed mixture in the rumen increases. Thus, motility suppression due to pH reduction decreases fermentation allowing SCFA absorption to overcome its production, increasing ruminal pH.

As previously described, the ruminoreticular compartment is divided into compartments or sacs, a division established by the presence of papillary muscles that project themselves from the walls toward the ruminal lumen. These muscles also develop rising and falling movements that occur coordinately with the movements of the ruminoreticular compartment wall.

Two different contraction patterns are evident in the ruminoreticular compartment: the ***primary or mixing contractions*** and the **secondary or eructation contractions**.

The ***primary contractions*** consist of a sequence of very coordinated mechanical events: (a) the reticulum has the first concentric contraction reducing its size approximately 50 % than when relaxed, a determining factor for the liquid reticular content to be pressed toward the center of the rumen during the contraction, mixing itself to the remaining content; (b) the reticulum contracts again (second contraction) reducing almost 100 % of its lumen; this contraction occurs simultaneously to the contraction of the main anterior ruminal fold; (c) after the end of the second reticular contraction, the reticulum opens and the content found in the beginning of the rumen overflows back to the reticular cavity; (d) a peristaltic contraction is started in the cardia region and propagates itself alongside the dorsal ruminal sac craniocaudally, when both longitudinal ruminal folds contract themselves almost simultaneously; these mechanical events push the content of the dorsal sac of the rumen to the ventral sac that is then relaxed; (e) next, there is contraction of the ventral ruminal sac that propagates craniocaudally with consecutive simultaneous contraction of the ventral coronary folds; these mechanical events determine that a great amount of the content in the ventral part of the rumen passes to dorsal part which is relaxed; (f) a contraction starts in the ruminal dorsal sac that propagates caudal-cranially, and (g) finally a contraction begins in the ruminal ventral sac that propagates caudal-cranially. During the contraction of the ruminal ventral sac, the reflux of its content is followed by a long noise within the relaxed dorsal sac; each noise counts for a ruminal contraction.

When they occur, the **secondary contractions** happen immediately after the end of the primary contractions. The secondary contractions usually occur associated with half of the primary contractions, although this relationship may vary in function of the gas formation rate. The triggering stimulus of eructation is the intra-ruminal gas pressure. In the cardia region, there are receptors located in a relatively small area whose stimulation results in deflagration or inhibition of eructation. In secondary contractions, the following mechanical events are evident: (a) the ruminal

content is pushed away from the cardia region in function of both reticular contractions that occurred in primary contractions (items a and b); (b) next, there is a contraction of the ruminoreticular fold and the main cranial fold, a mechanical action that prevents the return of ruminal content to the cardia region; (c) a contraction wave starts in the caudodorsal blind sac and propagates alongside the dorsal sac caudocranially, causing the displacement of the dorsal air bubble in the rumen to the cardia region; (d) the cranial sac is relaxed while the cranial pillar rises and allows liquid ingesta in the rumen to move away from the cardia; (e) then, the caudal sphincter of the esophagus opens while its cranial sphincter remains closed, and gases fill up the esophagus; and (f) finally, the caudal sphincter of the esophagus closes and a peristaltic wave propagates itself caudocranially, displacing the gases through the pharynx.

During and immediately after feed intake, the speed of primary and secondary contractions increase from 50 % to 100 %, and that is more evident in sheep than in bovines. The number of ruminal contractions in five minutes is from 7 to 12 in bovine, 7 to 14 in sheep and 6 to 16 in goats (Cunningham and Klein 2008). These contractions can also be observed by placing the hands on the left paralumbar fossa, which is the commonly utilized method to evaluate ruminal motility. The highest frequency of contractions occurs during the feeding periods and the lowest frequency in resting periods.

## Rumination Mechanisms

Rumination is an innate behavior of ruminants because in newborns, 5- to 8-day-old bovine and 3- to 5- day-old sheep, even only with milk feeding, irregular chewing movements are observed in the absence of feed in the mouth. In general, bovines, sheep and goats start rumination as soon as they start consuming solid feeds since the first week of life.

Rumination is a process in which a small part of the feed found in the ruminoreticular segments goes back to the mouth, passing through the esophagus and pharynx, and then submitted to additional chewing. Rumination process comprises ingesta regurgitation from the rumen and reticulum to the oral cavity, followed by swallowing of the regurgitated liquid, re-chewing of the solid portion, re-salivation and new swallowing. Thus, this process consists of four distinct phases: regurgitation, re-chewing, re-salivation and re-swallowing, consecutively.

When ingesting feed, ruminants chew it very rudimentarily and this little-chewed feed is transported to the ruminoreticular compartment. In this compartment, the feeds absorb water becoming turgid, increase density, are mixed to the pre-existing ruminal content, are fragmented by movements triggered in the ruminoreticular cavity, and are initially digested by the microorganisms existing there. After a determined period, small portions of the ingested feed are redirected to the oral cavity and chewed a second time. In general, rumination starts 30–70 min after the feed intake in bovines and 20–45 min in sheep.

During rumination there is a mechanical fragmentation of the rough regurgitated ruminal content. This process provides conditions for the feed to be sufficiently reduced in size to go from the reticulum to the omasum through the reticulo-omasal orifice. The increase in the feed density also contributes to this process. Therefore, if the diet is composed by feeds containing high-fiber content, the rumination process becomes fundamental for the ruminant because in its absence, the feed would not be fragmented enough to reach the omasum, interrupting its transit throughout the subsequent compartment of the digestive tube. Rumination allows an acceleration of the feed passage through the pre-stomachs; otherwise, the feed would remain there for much longer until becoming reduced to small feed particles. This acceleration of the passage allows the intake of more feed in a determined period of time and, as a result, a greater amount of digestible substrates or nutrients become available to the animal.

The regurgitated feed for re-chewing comes from the dorsal portion of the reticulum and have characteristic size and gravity of the region located between the doughy course mat and the ventrally fluid area. Thus, the ingesta that will be ruminated do not consist of roughage found in the rumen, but of material that previously occupied the mat and underwent some digestive activity. When regurgitation does not occur, the reticular contraction is characterized as biphasic; however, when there is rumination, this contraction becomes triphasic. The extra reticular contraction occurs simultaneously to cardia relaxation and a deep and long inspiration with closed glottis. This generates a negative pressure within the thoracic cavity, contributing to the opening of the esophagus that generates an increase in the negative pressure, making the content next to the esophagus, between the reticulum and rumen, be aspirated from the cardia region and be taken to the esophagus. Then, the esophageal wall contracts aboral-orally by an anti-peristaltic wave with approximate pressure of 80 mmHg in bovines, enabling the feed to transit at a speed of 107 cm/s under this condition, making the esophageal content be transported up to the mouth. A simultaneous expiration with closed glottis helps esophagus emptying. In rumination, there is no participation of the abdominal muscles, differently from vomit regurgitation because in vomiting there is effective participation of abdominal muscles. During regurgitation the animal keeps the mouth and neck stretched. After the regurgitation and swallowing of the aqueous fraction of the regurgitated material, the content in the mouth undergoes intense chewing followed by abundant saliva secretion, especially from the parotid glands. Finally, this content is swallowed by the same physiological mechanisms of swallowing.

In ruminants, rumination periods are alternated with periods of feed intake and rest. The beginning of rumination occurs between 30 to 60 min after feed intake. The number and duration of contraction cycles vary according to feed fiber content, feed particle size, number of meals, and amount of feed intake. The greater the feed fiber content is, the longer the rumination time lasts. The bigger the feed particle size is, the longer the time spent on rumination is. Numerous meals and amounts of ingested feed increase rumination period. In bovines, there are 4–24 rumination periods that last from 10 to 60 min each; therefore, the animals can spend from 7 to 24 h of their day

ruminating. In this species, 360-790 feed boluses, which range from 80 to 120 g/bolus, are generally ruminated. Feed intake and rumination follow a circadian cycle, because feed intake happens mostly during the day whereas rumination predominantly occurs during the night. Goats ruminate from 7 to 8 h daily, concentrating 75 % of this activity during the night.

Rumination is a reflexive mechanism in which some phases like breathing, chewing and swallowing are subordinate to the animals will. The reflexive nature of rumination is proved by the fact that the mechanical stimulation of specific regions of the pre-stomachs trigger rumination partially. The reticulum undergoes an extra contraction to trigger rumination. The mechanical stimulation of the ruminoreticular fold, the reticulum wall, and the reticulo-omasal orifice results in an increase in ruminal motility. These stimuli are captured by sensitive receptors in the pre-stomachs, continue via afferent nervous pathway (vagus nerve) and reach the rumination center found in the medulla oblongata, which coordinates part of the processes that include rumination.

## Eructation Mechanism

Eructation is a physiological process that aims to eliminate gases in the rumen formed by nutrient fermentation. Adult bovines produce 30–50 l of gas/h, whereas sheep and goats produce approximately 5-l/h (Cunningham and Klein 2008). In bovines, $CO_2$ makes up 60–70 % of ruminal gas whereas methane accounts for 30–40 % of it. Furthermore, a bovine produces 0.5–1 l of gas/min.

Eructation is a physiological process in which the gases produced in the rumen and reticulum are eliminated through the mouth, passing through the esophagus and pharynx. Eructation is a mechanism through which gas accumulation and stretching of the dorsal rumen sac trigger a reflexive chain of events that culminate with its expelling. The triggering stimulus for eructation is the intra-ruminal gas pressure. In the cardia region there are receptors concentrated in a relatively small area whose stimulation and stimulus intensity determine eructation.

The occurrence of eructation requires that the cardia region be ingesta-free which does not occur when it gets in contact with the ruminal liquid. Initially, two reticular contractions, occurring in the primary cycle, allow that the cardia region get rid of the ruminal content. Next, there is a contraction of the ruminoreticular pillar and main cranial pillar preventing the ruminal content reflux back to the cardia. There are simultaneous contractions in the dorsal rumen sac and in the cranial and caudal pillars when the reticulum undergoes relaxation. These mechanical events result in the cranial movement. This gas bubble reaches the cardia region and the lower esophageal sphincter opens whereas the upper esophageal sphincter remains closed, favoring the displacement of air from the ruminoreticular cavity to the esophagus. Next, the lower esophageal sphincter is closed and an anti-peristaltic wave starts aboral-orally up to the mouth. The cycle can be rapidly repeated several times as long as gases remain in contact with the cardia.

The strength of gas release is reduced by the contraction of the nasopharyngeal sphincter located in the pharynx, which contracts itself and directs part of the eructated gas towards the trachea and lungs to be absorbed by the blood. This pulmonary mechanism establishes the most common route by aromatic chemical substances to reach the mammary gland; determining undesirable stains in the milk. Therefore, gases can be either released to the atmosphere or transferred to the trachea and directed to the lungs. It is believed that half of the eructated gas is directed to the lungs instead of being expelled by the nostrils. In a 10-min period, bovines eructate 5–8 times, sheep 6 times and goats 4–7 times.

When the eructation process is interrupted or it occurs less often, gas accumulation in the rumen may cause ruminal bloat, which is more common in bovines than in sheep. When the vagus nerve and its ramifications have some lesion, the ruminal motility is directly altered and may cause disorders in eructation, causing ruminal bloat as well.

## Mechanism of Urea Recycling

In ruminants, approximately 60–90 % of nitrogen consumed by the animals is converted into ammonia ($NH_3$) by ruminal bacteria. It is estimated that 50–to 70 % of nitrogen utilized in the synthesis of new bacteria comes from ammonia. Most of the ammonia found in the rumen is ionized ($NH_4$). The ammonia concentration within the bacteria is approximately 15 times greater than the one found in the rumen; therefore, ammonia goes through the bacterial membrane by active transportation.

The balance between ammonia production (availability) and utilization by microorganisms in the rumen during their anabolic and catabolic processes is fundamental for ruminants. In the past two decades, it has been profitable to include inexpensive sources of non-protein nitrogen in ruminants' diets as a substitute of more expensive protein sources, such as soybean meal. The primary non-protein nitrogen source used this purpose is urea. Besides exogenous urea provided in animal diet, some endogenous urea is also produced in the organism of the animal and posteriorly directed to the rumen. As shown in Fig. 1.12, ruminal ammonia is absorbed by the ruminal wall, gets into the bloodstream and is carried to the rumen through the portal-hepatic system. The liver extracts most of the ammonia from the blood, keeping only a small amount of ammonia in the bloodstream. Moderate quantities of ammonia in the organism are considered toxic; therefore, this system allows that only a small amount of potentially toxic ammonia reach the systemic circulation. In the liver, ammonia ($NH_3$) is converted to urea [$(NH_2)_2CO$]. The liver also synthesizes urea from nitrogen originated in the deamination of endogenous amino acids. Most of the urea produced in the liver is excreted in the urine by the kidneys. In monogastric animals, urea is excreted almost exclusively this way. However, in ruminants, urea is also excreted through the rumen. This excretion can occur in two ways: (a) through salivary glands where urea is a compound of saliva and it reaches the rumen when the saliva is swallowed, or (b) by the direct passage of urea from the bloodstream to the ruminal compartment through the ruminal wall.

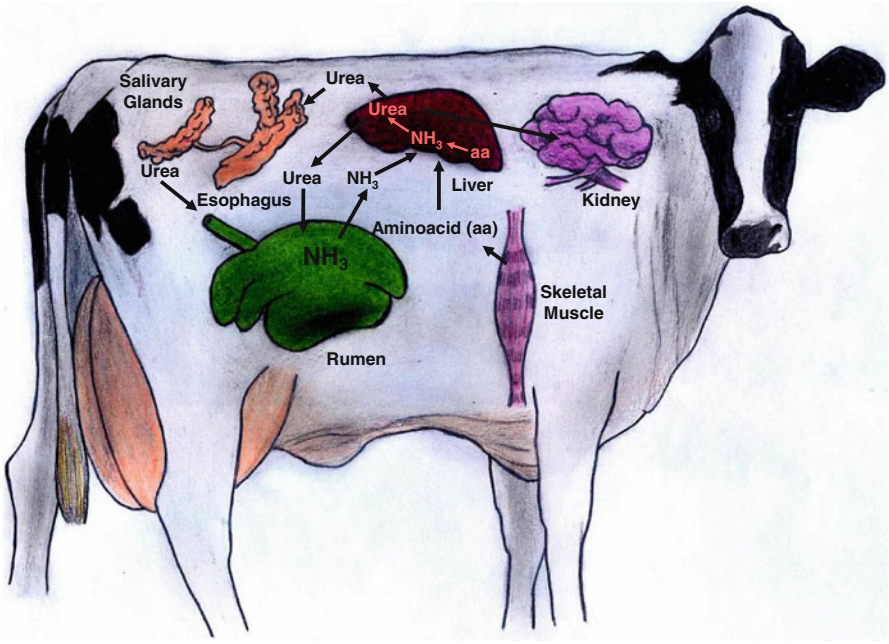


**Fig. 1.12** Circulation of nitrogen through different organs of a ruminant animal. When enough amounts of carbohydrates are available in the rumen, microorganisms are capable of synthetizing protein from a non-protein source, such as urea. This urea is rapidly converted to ammonia ($NH_3$) in the rumen, which is absorbed by the ruminal wall, and carried out by the bloodstream to the liver, where it is converted back into urea. Most of the urea produced in the liver is excreted in the urine by the kidneys. However, in ruminants, urea is also excreted through the rumen. This excretion can occur in two ways: (**a**) through salivary glands where urea is a compound of saliva and it reaches the rumen when the saliva is swallowed, or (**b**) by the direct passage of urea from the bloodstream to the ruminal compartment through the ruminal wall

Thus, significant amounts of urea continuously get into the rumen by three distinct manners: (a) through diet components; (b) through saliva; or (c) by the passage of the molecule present in the bloodstream to the rumen through the ruminal wall. In the rumen, urea is quickly transformed into ammonia and carbon dioxide due to the great amount of urease found in the rumen. The nitrates found in the diet are also rapidly reduced to ammonia. In the rumen, ammonia is immediately available as ruminal nitrogen for the synthesis of microbial proteins. This mechanism is commonly referred to as urea recycling process in ruminants.

Considering that ammonia absorption in the rumen is proportional to its production, and that its production depends on the availability of proteins and carbohydrates in the rumen, the existing relationship between them is essential for the success of diet formulation. The amount of non-protein nitrogen that leaves the rumen and goes into the bloodstream, and the quantity of non-protein nitrogen that reaches the rumen through saliva or by bloodstream depends on the concentrations of ammonia in the rumen. Therefore, when non-protein nitrogen availability in the rumen is relatively high when

compared to carbohydrate availability, a great amount of ammonia is produced inside the rumen, and the main nitrogen flow goes from the rumen to the bloodstream, producing a large quantity of ruminal nitrogen. In this case, there will be great concentrations of blood urea and great losses of nitrogen through the urinary route. This is not economically recommended, because besides the energetic use for urea formation, approximately 12 Kcal/g of nitrogen, there is the loss of nitrogen compounds, making ruminants nutritionally inefficient. However, when carbohydrate availability is high compared to nitrogen availability in the rumen, the main nitrogen flow goes from the bloodstream to the rumen, a circumstance in which the ruminal ammonia concentration is low and most of this urea is excreted to the rumen so that it can be utilized in the synthesis of proteins that will contribute to the host's amino acid needs. Thus, ruminants fed low-protein diets are considered efficient nitrogen keepers. Thus, under ideal conditions, in which the animal is supplied with an appropriate combination of carbohydrate and protein amounts, the main nitrogen flow goes from the bloodstream to the rumen.

After extracellular degradation of proteins that get into the rumen, peptides and amino acids resulting from this digestion are readily captured by ruminal bacteria, showing the low concentration of amino acids in the ruminal flow. Peptides are hydrolyzed when they get into the bacterial cell and most of the amino acids are deaminated. The deamination of valine, leucine and isoleucine results in isobutyrate, isovalerate, and 2-methylbutyrate, respectively. These products, called branched-chain fatty acids, are extremely important for the growth of bacteria that degrade structural carbohydrates. In ruminant feeding, generally there are nitrogen bases that are ingested in small amounts, comprising 5–9 % of nitrogen in forages. When this nitrogen reaches the rumen, bacteria rapidly capture it. A part of it is utilized for the synthesis of the bacterial nucleic acid, but most of it is used in fermentation to produce SCFA, $CO_2$ and ammonia.

Based on its dry weight, the average nitrogen content of ruminal bacteria is 10 %, 75 % of which are amino acids and 25 % nitrogen bases. Ruminal microorganisms require energy to multiply. In general, almost all microorganisms utilize carbohydrates as energetic source, very few species use protein, and no species has the capacity to utilize fat as energetic source. The greater the carbohydrate degradability in the rumen is, the more energy for microbial growth will be available. In the rumen, non-structural carbohydrates determine a greater microbial production when compared to structural carbohydrates. Thus, diets containing highly degradable ruminal starch provide more energy to microorganisms, which will present faster multiplication, increasing its population.

## Energetic Metabolism in Ruminants

SCFA production from substrate fermentation in the rumen is the greatest energetic source for ruminants, providing at least 50 % of the total amount of digestible energy. The relative concentrations of main SCFA are essential for energy utilization by ruminants. Ruminants have to perform gluconeogenesis to obtain the most part of

their glucose, and propionate is the greatest glucose source for ruminants. On the other hand, acetate, as well as butyrate, is also used as energetic source, but for oxidative metabolism and for lipogenesis. Diets that increase propionate production, and as a result decrease acetate concentration, are related to reduction of milk fat.

As shown in Fig. 1.13, when reaching the rumen, forage and grains, make several substrates available, especially cellulose, hemicellulose and starch. These substrates are utilized by a large group of bacteria that, through fermentative digestion, transform substrates initially into glucose and then into SCFA, mainly acetate, butyrate and propionate. Nutrients that are not digested in the rumen are pushed to the small intestine where they undergo enzymatic digestion by pancreatic, hepatic and enteric enzymes, and their final products are absorbed by the portal circulatory system whereas the non-digestible portion of the feed is excreted through feces. SCFA produced in the rumen are readily absorbed by the ruminal epithelium to reach the systemic circulation. Acetate is the main energetic substrate and is available to the animal as energy. Acetate is converted to triglycerides in the adipocytes where it is stored as fat, as well as transformed into fat in the mammary gland. Propionate is converted into glucose in the liver, and is the main source of glucose for ruminants. Glucose is utilized as an energetic source in the muscular tissues and others. In the mammary gland, glucose is converted into lactose and it is fundamental for increasing milk production. Butyrate is mostly utilized by ruminal epithelial cells (95 %) and the rest of it (5 %) goes to the bloodstream where it is converted into ketone bodies (ketones) and long-chain fatty acids in the liver. Ketones are available as energetic source for ruminants and, like acetate, are also converted to triglycerides in adipocytes and in the mammary gland of these animals.

In summary, acetate is utilized by the liver in a very small amount, and most of it is used for the oxidation to generate ATP and for the synthesis of acetyl-CoA, which is utilized in lipid synthesis. Propionate is almost totally sequestered by the liver where it is utilized as an extremely important substrate for gluconeogenesis, transforming it into glucose. Butyrate is oxidized in several tissues for energy production.

## Final Considerations

The digestive system of ruminants is extraordinarily efficient in the utilization of vegetal feed. This characteristic makes ruminants a great promising source of animal protein supply to human population. The worldwide demand for foods has been growing quickly and proportionally to population increase. Under low-efficiency conditions of meat and milk production, the only way to increase productivity is by increasing the number of animals and area destined to livestock farming. However, as this is an undesirable practice due to the obvious negative economic and environmental implications, it is evident how important it is to increase meat or milk production without necessarily increasing the number of animals.

A lot of advances have been achieved aiming the genetic improvement and animal nutrition and many other areas. In order to improve production levels, a greater number

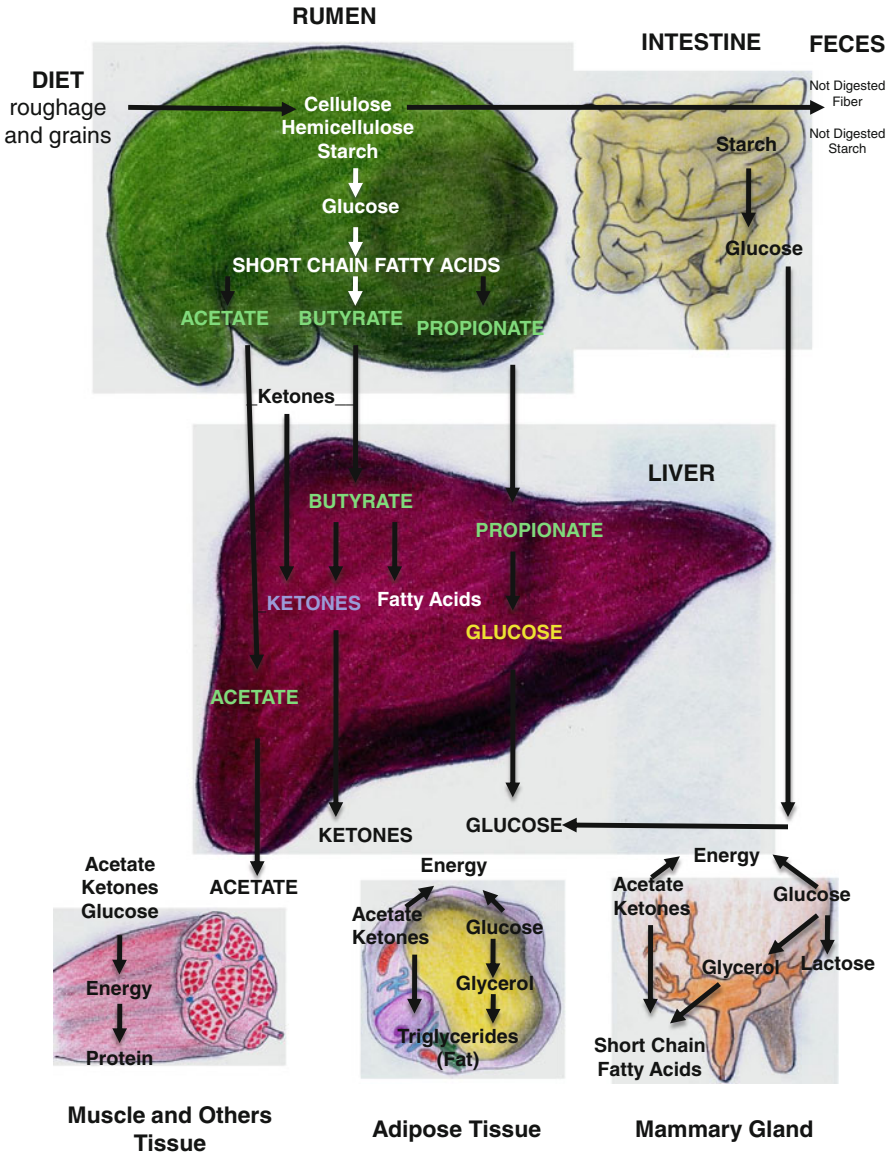


**Fig. 1.13** Schematic representation of the feed energy utilization by the ruminant animal (Adapted from Wattiaux and Armentano in http://babcock.cals.wise.edu/downloads/de_html/ch03.en.html). The roughage and the grains, which compose the diet of a ruminant animal, when reach the rumen provide several substrates; specifically CELULLOSE, HEMICELULLOSE AND STARCH. Such substrates are utilized by a large group of bacteria, which beyond the fermentative digestion, transform the substrates first in glucose and after in short chain fatty acids specially ACETATE, BUTIRATE and PROPIONATE

of animals are being fed significant amounts of concentrate while on pasture. Likewise, it is common nowadays the use of feedlot systems to improve performance and produce more meat and milk in shorter periods of time. However, diet formulation for ruminants has to be based on a great knowledge of the physiology of the digestive system. It should be considered that, even though it represents one of the most extraordinary symbiotic mechanisms between microorganisms and host, the ruminal environment is also represented by a fermentative chamber that requires a set of ideal conditions that should be kept relatively stable.

The management of the ruminal environment is a practice established to increase the utilization of this symbiotic relationship, however, the established modifications have to be always very well dimensioned.

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# Chapter 2
# Microbiology of the Rumen

**T.G. Nagaraja**

## The Ruminal Ecosystem

The rumen, or more appropriately the reticulo-rumen, is a large chamber (50–100 l capacity in adult cattle) in which the ingested feed is first subjected to microbial digestion. The rumen is an ideal microbial habitat because the conditions that exist are conducive for the survival and growth of microorganisms. The temperature remains relatively constant (36–40°). Water the animal drinks and the only exocrine secretion that rumen receives, saliva, provide a moist environment required for microbial growth. Ingested food provides the energy and other nutrients needed for microbial growth and activity. Normal reticulo-ruminal motility (peristalsis and antiperistalsis) helps mix the contents, which brings microbes into contact with fresh substrate. The end products of fermentation are removed by absorption (acids) into the blood or eructation (gases). Absorption coupled with the buffering effect provided by salivary secretions help regulate ruminal pH.

The rumen ecosystem, often referred to as a continuous culture system, operates as a biological fermentation unit, like a 'chemostat', under well-defined conditions that are extremely important regulators of the types, number and biochemical activities of the microorganisms (Fig. 2.1). There is more or less continuous availability of substrate (at least in grazing cattle), removal of end products (by absorption, eructation or passage), and passage of undigested and waste products. The contents in the rumen are heterogeneous, consisting of complex mass of digesta, which may float (forage) or sediment at the bottom (grain) depending on the density of the feed, liquid fraction with microbial cells and fine feed particles, and a free gas cap in the dorsal sac.

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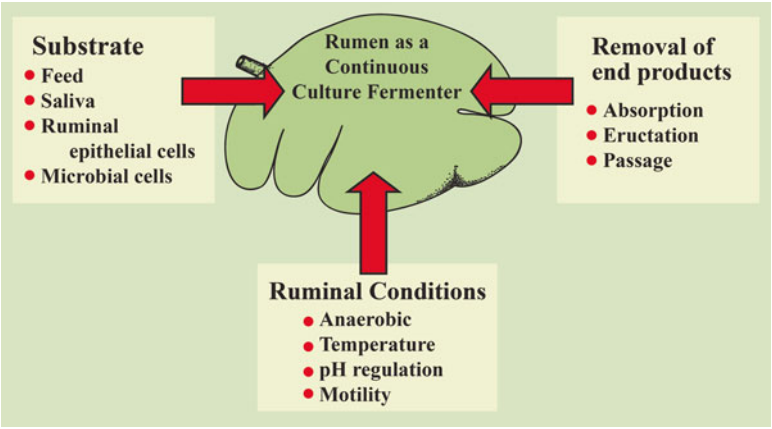


**Fig. 2.1** Rumen as a continuous culture system

## Anaerobiosis and Its Consequences

The ruminal environment is anaerobic with gas phase composed mostly of carbon dioxide (~65 %) and methane (~35 %), although small amounts of other gases ($H_2$, $N_2$, $O_2$, etc) are present. Carbon dioxide is produced by microbial activities and by neutralization of acids by bicarbonate that enters the rumen from saliva and blood. The trace amounts of $O_2$ and $N_2$ are contaminants from air that enters via feed and water. The absence of oxygen makes the rumen highly anaerobic with a redox potential of 150 to −350 mV. Therefore the type of metabolism that takes place in the rumen is fermentation where the final electron acceptor is organic (major) or inorganic (minor) compounds, and not the oxygen. The anaerobic condition imposes two major restrictions. One is the energy yield from substrates (moles of ATP/mole of glucose) is considerably lower (no electron transport phosphorylation) than aerobic metabolism (4 vs. 38). Because of the limited ATP yield there is rapid throughput of substrates to sustain microbial growth. Secondly, the substrates are only partially oxidized, which allows the animal to absorb the products (acetate, propionate and butyrate) as energy source and the reducing equivalents generated during fermentation are disposed by producing reduced products like lactate and propionate, and taken up by methanogens to produce methane.

## Interactions Between the Host and the Microbes

The microorganisms in the rumen and the ruminant animal live in symbiotic relationship. In addition to providing nutrients to the microbes, the host also contributes to the maintenance of physical and chemical conditions for optimal microbial fermentation. In return, microorganisms provide energy, protein, and vitamins to the host (Table 2.1).

**Table 2.1** Symbiotic relationship between the ruminal microorganisms and the host animal

| Host contributions and their outcome to the microbes | Microbial contributions and their outcome to the host |
|---|---|
| Mastication and rumination of the feed breakdown large feed particles into smaller particles (comminution), which increase surface area for microbial attachment and digestion | Host is absolutely dependent on microbes to digest fiber. Only microbes have fibrolytic enzymes to degrade cellulose and hemicelluloses |
| Salivary input to the rumen provides aqueous environment necessary for microbial growth, nutrients (nitrogen), but more importantly supplies the major buffering compounds (bicarbonates and phosphates) essential for regulation of ruminal pH | Microbes can use nonprotein nitrogen (urea, nitrate, nucleic acids) as source of ammonia and synthesize amino acids and protein |
| Ruminal contractions (peristalsis and antiperistalsis) help mix the digesta, which brings microbes into contact with fresh substrate, and facilitates passage of digesta to make room for additional feed | Production of fermentation products, particularly VFA, which serve as the major source of energy to the host |
| Removal of fermentation products by eructation (gases) and absorption (acids) are critical for maintaining optimal conditions (pH) for microbial growth | Production of microbial cells which in the lower tract (abomasums and small intestine) serve as the major source of protein and vitamins |

## The Ruminal Microorganisms

The rumen is inhabited by a multitude of microorganisms. Many of these organisms colonize and grow and are considered indigenous, and hence are termed "normal flora", also called autochthonous microbiota. These microbes, for the most part, live in harmony with the host. Additionally, the ruminal flora includes microbiota, referred to as allochthonous, that do not get established (colonization and growth), are dormant and in transit. These are derived largely from ingested food and water and to a small extent from swallowed air or from another habitat of the host (e.g., skin, respiratory tract, or reproductive tract). The nonindigenous microbes also include a variety of gastrointestinal pathogens that may colonize and grow to establish infections. Also, some members of the normal flora could assume pathogenic roles (opportunistic pathogens) when the ecosystem is perturbed in some way or when a breach occurs in the integrity of the ruminal wall.

The microbial population in the rumen includes members that belong to all three domains, Eubacteria (Bacteria), Archaea (Methanogens), and Eukarya (Protozoa and Fungi). The rumen ecosystem contains the following types of microorganisms: Bacteria, Protozoa, Fungi, and Bacteriophages (Table 2.2).

### *Ruminal Bacteria*

Rumen has a dense population of bacteria with numbers ranging from $10^8$–$10^{11}$ per g of contents. The number is reflective of the digestibility of the feed, which is why bacterial counts in grain-based diets are 10- to 100-fold higher than the forage based

**Table 2.2** Types of ruminal microorganisms

| Types | Domain | Number (per ml or g of ruminal contents) | Percentage of total microbial mass |
|---|---|---|---|
| Bacteria | Eubacteria | $10^9$–$10^{11}$ | 40–90 |
| Methanogens | Archaea | $10^5$–$10^8$ | 2–4 |
| Protozoa | Eukarya | | 0–60 |
| Flagellates | | $10^2$–$10^3$ | |
| Ciliates | | $10^4$–$10^6$ | |
| Fungi | Eukarya | – | 10 |
| Bacteriophages | – | $10^{11}$–$10^{12}$ | – |

diets. Majority of the bacteria is obligately anaerobic although facultative bacteria do exist. Anaerobic bacterial counts are typically about 1000-fold higher than facultative bacterial counts. Most of the facultative bacteria in the rumen are nonindigenous and transient population carried into the rumen through feed and water. Ruminal bacteria are predominantly gram-negative accounting for 80–90 % of the population. Certain species of ruminal bacteria (*Butyrivibrio fibrisolvens*) have typical gram-positive cell wall structure (bilayer with thick peptidoglycan) but stain gram negative. The proportion of gram-positive bacteria increases in grain-fed animals (20–30 % of the total). Morphologically, ruminal bacteria are rod-, spherical- or spiral- shaped organisms, and majority is rod shaped organisms. Spiral shaped organisms, belonging to the genus *Treponema*, constitute only a small fraction (<1 %) of the bacterial population.

Based on the distribution of bacteria in the rumen, they can be categorized into bacteria that float freely in rumen fluid and bacteria that are adherent to feed particles (loosely or tightly), protozoal cells, fungal sporangia, or epithelial cells (Fig. 2.2). The free-floating bacteria constitute a minor component (~30 %) of the total bacterial population. Bacteria associated with feed particles are the major component (70 %) of the total bacterial population. Bacteria attached to ruminal epithelial cells, called epimural bacteria, constitute a small fraction of the total bacterial population in the rumen. Bacteria that float freely or attached to feed particles participate actively in the digestion of feedstuffs. However, epimural bacteria do not make any significant contribution to the ruminal digestion. Many of the epimural bacteria are facultatively anaerobic and produce urease enzyme. Because ruminal wall is highly oxygenated and allow diffusion of urea from the blood into the rumen, it is speculated that the role of epimural bacteria is to maintain anaerobiosis by scavanging any oxygen that otherwise would have diffused into the rumen, and hydrolyze urea. Also, epimural bacteria may digest the cells that get sloughed off from the wall.

Historically, much of the information on numbers and kinds of ruminal bacteria has been obtained by cultivation-based methods (Fig. 2.3). Isolation of bacteria in pure culture has enabled identification of the biochemical activities and fermentation products produced. Recently, applications of cultivation-independent techniques, particularly based on 16S rRNA gene sequence analyses, have indicated that the number of bacterial species in the rumen is vastly underestimated. It is generally believed that culture-based procedures have only identified about 10 % of bacterial species present in the rumen.

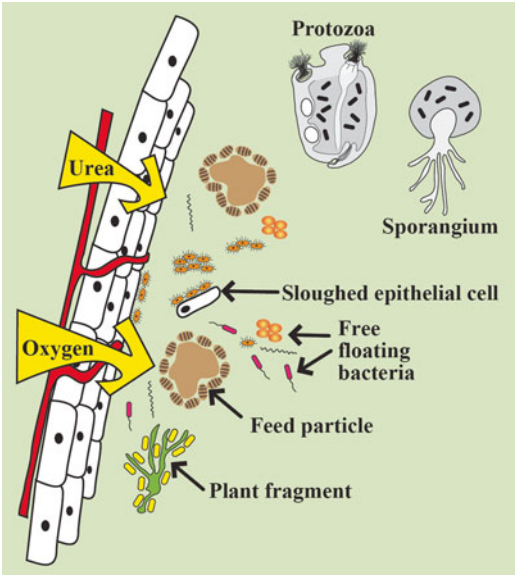


**Fig. 2.2** Diagrammatic representation of the distribution of bacteria in the rumen

## *Methanogens*

Methanogens are members of the Archaea domain and phylogenetically differ from bacteria protozoa and fungi. They lack peptidoglycan and have unusual lipid structures. Methanogens constitute about 2–4 % of the bacterial population in the rumen. Methanogens that have been cultured from ruminal contents belong to only few genera and species, although methanogens outside the rumen habitat have been classified into as many as 23 genera and hundreds of species. Methanogens in the rumen that have been cultivated belong to five genera and seven species: *Methanobacterium formicium*, *Methanobacterium bryantii*, *Methanobrevibacter olleyae*, *Methanobrevibacter millerae*, *Methanobrevibacter ruminantium*, *Methanomicrobium mobile*, *Methanoculleues olentangyi*, and *Methanosarcina barkeri*. Cultivation-independent methods that have assessed archaeal population in ruminal contents have revealed abundance of a variety of methanogenic genera and species. Based on cultivation independent method (16S rRNA gene sequence analyses), majority of ruminal methanogen belongs to three genus-level groups. They are *Methanobrevibacter*, *Methanomicrobium*, and a large group of uncultured methanogens. Ruminal ciliated protozoa harbor methanogens on the outside surface (ectosymbionts) and inside the cell (endosymbionts) based on characteristic fluorescence of cofactors ($F_{350}$ and $F_{420}$). Based on 16S rRNA genes analyses, majority of the methanogens associated with ciliated protozoa belong to the same three groups described in ruminal contents. The most prevalent and best characterized methanogen in the rumen is *Methanobrevibacter ruminantium*.

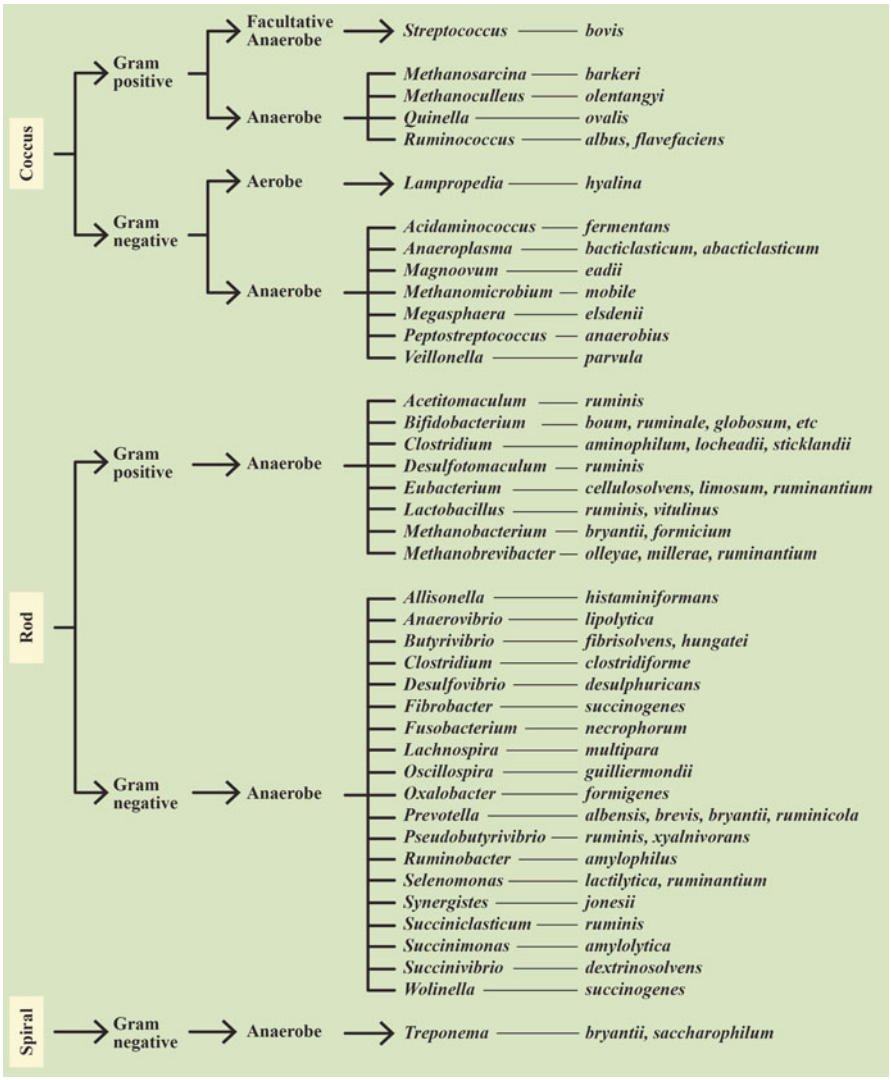


**Fig. 2.3** Classification of ruminal bacteria based on morphology and oxygen relationship

Most species of methanogens can grow using $H_2$ and formate as their energy sources and use the electrons derived from $H_2$ and formate to reduce $CO_2$ to form methane (Table 2.3). Some species can use methyl groups from methanol, methylamines, or acetate (*Methanosarcina barkeri*) to produce methane. However, the amount of acetate used by methanogens is insignificant because the acetate-utilizing methanogens do not survive in the rumen because their growth rates are slower than the rate of passage of contents.

**Table 2.3** Substrates for methanogenesis in the rumen

| Substrates | Reactions | | |
|---|---|---|---|
| **Major** | | | |
| $H_2$ and CO | $4H_2 + CO_2$ | → | $CH_4 + 2H_2O$ |
| Formate | 4HCOOH | → | $CH_4 + 3CO_2 + 2H_2O$ |
| **Minor** | | | |
| Methanol | $4CH_3OH$ | → | $3CH_4 + CO_2 + 2H_2O$ |
| Methylamine | $4CH_3NH_2Cl + 2H_2O$ | → | $3CH_4 + CO_2 + 4NH_4Cl$ |
| Dimethylamine | $2(CH_3)_2NHCl + 2H_2O$ | → | $3CH_4 + CO_2 + 2NH_4Cl$ |
| Trimethylamine | $4(CH_3)_3NHCl + 2H_2O$ | → | $3CH_4 + CO_2 + 2NH_4Cl$ |
| Acetate | $CH_3COOH$ | → | $CH_4 + CO_2$ |

## *Ruminal Protozoa*

Protozoa were the first rumen microorganisms to be discovered (by Gruby and Delafond in 1843), which is not surprising because of their conspicuous cell size (up to 200 μM in length) and active motility. Like bacteria, protozoa in the rumen are anaerobic. Certain species of ciliated protozoa, similar to other anaerobic protozoa like Trichomonads, possess an organelle, called hydrogenosomes, which are implicated in oxygen utilization in the rumen. These structures make protozoa somewhat aerotolerant and are also responsible for the role ciliated protozoa play in scavenging of oxygen to maintain anaerobiosis. Protozoa in the rumen are broadly classified into flagellates and ciliates, depending on whether they have flagella or cilia. Flagella are larger in length and thickness compared to cilia and function in motility. Cilia are small and thin, more numerous and function in motility and also aid in ingestion of food. Flagellates are smaller in size (3–12 μM), fewer in number ($10^2$–$10^3$ per ml), and utilize only soluble nutrients; therefore, their contribution to overall ruminal fermentation is insignificant.

***Ciliated Protozoa***. Ciliated protozoa make up the majority of protozoa in the rumen and range in size from 10 × 20 to 120 × 200 μM. Ciliated protozoa are highly specialized, single cells and have structures and features similar to animals. A single cell is bounded by a pellicle or skin and has internal structures that can be described as digestive tract (mouth, cytopharynx, anus, etc.), urinary tract (contractile vacuoles), skeletal structures (skeletal plates), etc. They are binucleated with one being large (macronucleus) and the other being small (micronucleus).

Based on morphological features (ciliary arrangement, location of the macronucleus, and absence, presence, and size and number of skeletal plates), ciliates are grouped under different genera and species. There are two broad groups of ciliated protozoa that differ in morphological features (Fig. 2.4a and b) and functional roles (Table 2.4). The 'holotrichs', belonging to the order *Trichostomatida*, have cilia covering the entire or almost the entire surface. The group contains two of the moist common genera, *Isotricha* and *Dasytricha*. The entodiniomorphid ciliates, belonging to the order *Entodiniomorphida*, have cilia at the anterior end and may or may

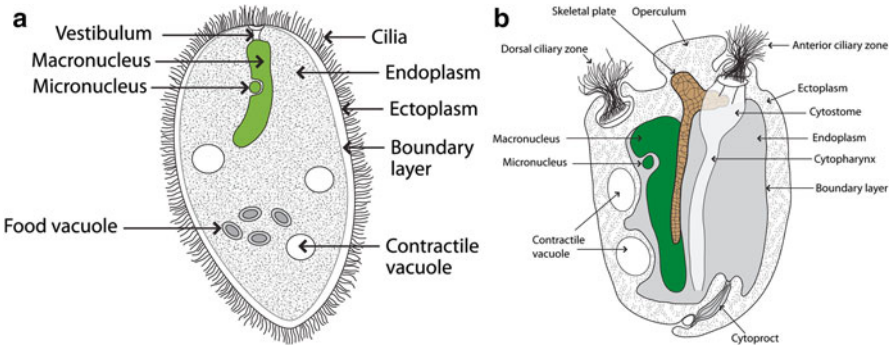


**Fig. 2.4** (**a**) A diagrammatic representation of a holotrichid ciliated protozoon. (**b**) A diagrammatic representation of an entodiniomorphid ciliated protozoon

**Table 2.4** Comparison between holotrichid and entodiniomorphid ciliates

| Characteristics | Holotrichs | Entodiniomorphs |
|---|---|---|
| **Morphology** | | |
| Ciliary arrangement | Distributed on the entire (or almost the entire) surface of the cell | Restricted ciliary zones |
| Ectoplasm | Thin | Thick |
| Nucleus | Located in the endoplasm<br>Spherical or oval shaped macronucleus | Located in the ectoplasm<br>Rod shaped with or without lobes. Useful in species identification |
| Skeletal plates | Absent | Present. Number and location is useful in generic identification |
| Number | $1–10 \times 10^4$ | $1–10 \times 10^5$ |
| Proportion | 10–25 % | 75–90 % |
| Diurnal variation | Two- to fourfold increase in number in 1–2 h after feeding | Numbers generally decrease following feeding |
| Function | Do not hydrolyze structural polysaccharides (Cellulose, hemicellulose) | Do hydrolyze structural polysaccharides |
| Substrates fermented | Starch, pectin, soluble sugars, proteins | Cellulose, hemicellulose, starch, pectin, soluble sugars, proteins |
| Genera (common) | *Isotricha*<br>*Dasytricha*<br>*Charonina* | *Entodinium*<br>*Diplodinium*<br>*Eudiplodinium*<br>*Ostracodinium*<br>*Metadinium*<br>*Polyplastron*<br>*Ophryoscolex*<br>*Epidinium* |

not have a zone or rows of cilia at the dorsal end of the cell. The group contains a several genera, including the most prevalent genus, *Entodinium*. Holotrichid protozoa are much more motile than entodiniomorphid ciliates. The entodiniomorphs are more numerous in the rumen compared to holotrichs. The diurnal variations in population size of the holotrichs and entodiniomorphs in the rumen differ. Generally, holotrichid population increases 1–2 h following feeding and then decline to the prefeeding concentration. The explanation is large numbers of holotrichid ciliates are sequestered on the reticular wall and or settled at the bottom of the rumen and migrate to the rumen in response to some sort of chemical stimulation (nutrient) provided by the feed that enters the rumen. In contrast, entodiniomorphs decrease following feeding, attributed to dilution of ruminal contents, and then increase gradually to reach the prefeeding concentration.

Several genera and hundreds of species of ciliated protozoa have been identified in the rumen. Generally, description of the ciliated protozoal population in the rumen is done at the generic level. Ciliated protozoa generally reproduce asexually by binary fission. In some instances protozoa may exchange genetic material by conjugation and then undergo division. All ciliates actively ingest bacteria and digest the cells. In fact, bacteria are the major source of protein for ciliates.

***Contribution to Ruminal Digestion***. Ciliated protozoa constitute an important fraction of the total microbial mass in the rumen and they actively participate in ruminal digestion. They possess full complement of hydrolytic enzymes to ferment the major components of the feedstuffs. However, our knowledge of the biochemical activities of ruminal protozoa is somewhat limited because of the difficulty in cultivating them *in vitro*. The impact of ciliated protozoa on ruminal digestion and fermentation products depends on their concentration and generic composition of the population. The estimates of generic composition based on numbers may not be meaningful because of large differences in cell size or volume. For example, the genus *Entodinium* based on the number accounts for 60–80 % of the total protozoa. However, based on volume, the *Entodinium* accounts for 10–40 % of the total. It is possible that the voluminous ciliated protozoa (*Isotricha*, *Metadinium*, *Polyplastron*, *Ophryoscolex*, and *Epidinium*) contribute to a greater extent to the metabolic activity of the total protozoa.

Holotrichid ciliates are primary users of soluble sugars, while entodiniomorphs use a large variety of substrates. Most entodiniomorphs, with the exception of small entodinia, are able to ingest small plant particles and use cell wall carbohydrates. All entodiniomorphid ciliates have high amylase activity to digest engulfed starch granules. Some of the engulfed starch may be stored as granules or in the skeletal plate as amylopectin. Digestion of the engulfed protein occurs within the cells. Compared to bacteria, ciliated protozoa are less able to transport amino acids into the cell. One of the commonly used approaches to study the role of ciliated protozoa in ruminal digestion is to eliminate them from the rumen, a process called defaunation. Because ciliated protozoa are preferentially retained in the rumen, their contribution to the postruminal microbial protein supply is not as important as bacteria. In fact, their overall value to the host is a subject of some debate. The effect of the presence or absence of ciliated protozoa on the ruminant host may depend greatly on the diet and

numbers and kinds of protozoa. In animals fed low-protein diet, elimination of protozoa may increase protein supply. An increase in microbial protein synthesis because of increased bacterial numbers (lack of predation) and decrease in protein degradation result in increased flow of protein into the small intestine. Also, in ciliate-free animals, the cell wall and organic matter digestibilities are lowered resulting in lower absorbed energy than faunated animals. In animals fed high-grain diets, presence of ciliated protozoa may be beneficial by controlling the bacterial numbers and slowing down the rate of starch fermentation. The presence of ciliated protozoa results in a more stable ruminal fermentation

## *Ruminal Fungi*

Anaerobic fungi, as a member of the ruminal microbial population, were discovered in 1975 by Colin Orpin. Before the discovery, fungal structures (spores) were mistaken to be flagellated protozoa. Since the discovery in the rumen, anaerobic fungi have been isolated from the gut contents of a number of animal species.

***Types and Morphology***. Based on morphological features, fungi are classified into two broad groups: Yeasts and Molds.

Yeasts are single celled organisms and molds are multicellular forming a network of filaments called hyphae. Hyphae are tubular structures and are collectively called mycelium. Functionally, fungi in the rumen are broadly classified into two groups (Fig. 2.5). The group 1 consists of fungi that are facultatively anaerobic or aerobic, are transient and do not contribute to ruminal digestion. The second group consists of obligately anaerobic fungi that are indigenous to the rumen and contribute to ruminal digestion. Yeasts or yeast-like cells in the rumen range in concentration from $10^2$ to $10^3$ per g of contents and are generally believed to be transient population and make no significant contribution to the ruminal fermentation. However, the number of yeasts increases in the rumen of animal that is acutely acidotic with lactic acid accumulation. The increase is probably because of availability of highly fermentable sugars. The functionally important fungi are referred to as Chytridomycete because they are placed in a phylum, Chytridomycota, which includes fungi that reproduce with motile zoospores. So far five genera and several species have been identified in the rumen and other gut regions of cattle and other animals. The genera are recognized based on the type of sporangium and thallus development, type of rhizoid development, and the number of flagella on the zoospores.

***Life Cycle***. The life cycle of chytridomycete fungi in the rumen consists of two stages (Fig. 2.6); a motile, flagellated zoospore stage in ruminal fluid, and a non-motile, mycelial stage, referred to as thalli, which is associated with solid feed particles. The zoospores that float freely in ruminal fluid reach plant fragments, possibly mediated by chemotactic response to soluble nutrients, attach and encyst (looses flagella). The encysted zoospore germinates by first forming a germ tube, which then grows and branches into a rhizoidal structure or thallus in *Anaeromyces*,

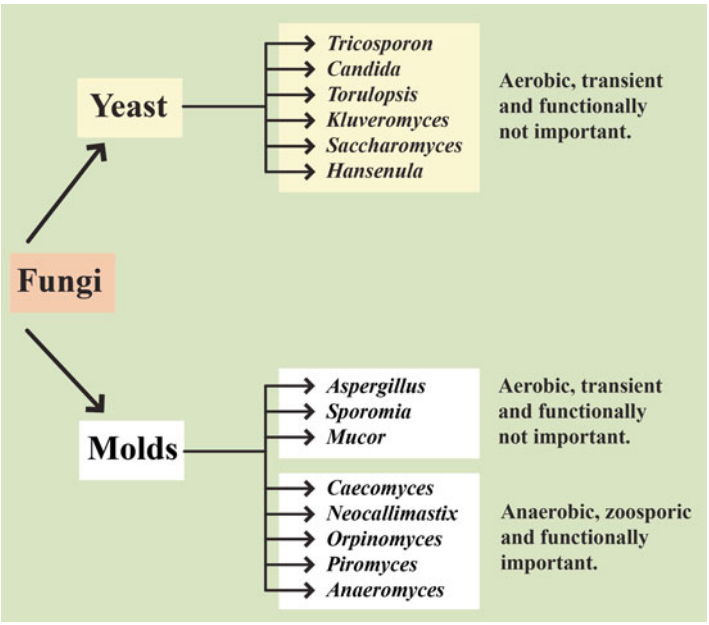


**Fig. 2.5** Morphological and functional classification of ruminal fungi

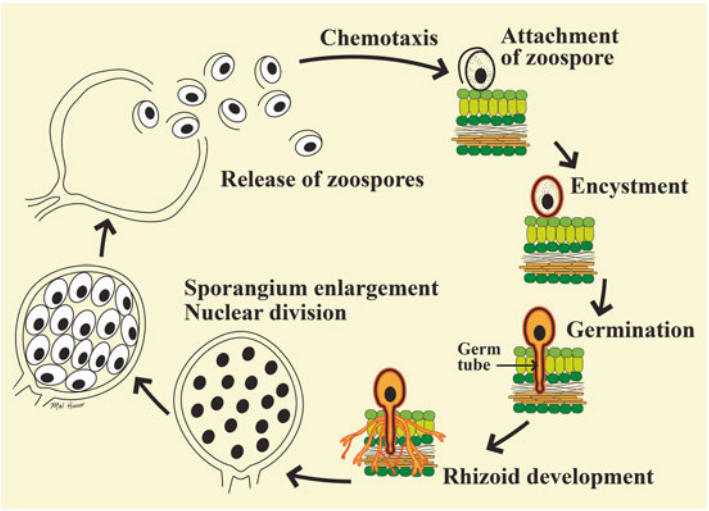


**Fig. 2.6** Life cycle of ruminal fungi

*Orpinomyces*, *Neocallimastix*, and *Piromyces* or into a spherical body called holdfast in *Caecomyces*. At the same time, the encysted spore grows into a sporangium, a sac like structure. The nucleus in the sporangium undergoes mitotic division and each nucleus encloses cytoplasm to develop into a flagellated zoospore. The sporangium then ruptures to release the spores and the cycle is then repeated. Based on the type of sporangium development, ruminal fungi can be monocentric (single sporangium per thallus) or polycentric (multiple sporangia per thallus). In monocentric fungus (*Neocallimastix*, *Piromyces*), the nucleus is retained in the encysted zoospore which enlarges into a sporangium, which is called endogenous sporangium development or exogenous sporangial development, where the nucleus migrates out of the zoospore and the sporangium develops in the germ tube or sporangiophore. In monocentric fungus, the mycelium is anucleated. In polycentric fungus (*Anaeromyces*, *Caecomyces*, *Orpinomyces*), the nucleus from the encysted zoospore migrates into the mycelium where it undergoes mitotic division and as a result the mycelium is polynucleated. The sporangia are formed on sporangiophore singly or in groups of up to six.

***Contribution to Ruminal Digestion***. The contribution of fungi to microbial mass is difficult to assess because of the two-stage life cycle and the extensive growth of the thalli within plant fragments. The counts of zoospores ($10^3$–$10^4$ per ml) and the sporangia on feed particles do not reflect fungal mass. Using chitin as a marker, it has been estimated that fungi account for up to 10 % of the total microbial mass in the rumen. The relative contribution of fungi to ruminal digestion is not known. Experiments in which ruminal fungi were eliminated from the rumen or greatly reduced have provided evidence for the contribution of fungi to the digestion and overall fermentation of carbohydrates in the rumen. Ruminal fungi produce the hydrolytic enzymes required to break down the major components of plant biomass. The enzymes include cellulases, hemicellulases, pectin lyases, amylases, and proteases. Although fungi can break down pectin, they are unable to use the degradation products. Also, ruminal fungi produce phenolic esterases (*p*-coumaroyl and feruloyl) that can break cross linkages between hemicelluloses and lignin, which allow the fungus to have increased access to hemicelluloses. The rhizoidal development of the thalli is able to penetrate plant tissue better than bacteria and protozoa, which may lead to greater degradation of forage. Ruminal fungi are able to use a wide range of di or monosaccharides. All ruminal fungal species can utilize glucose, cellobiose, and lactose but are unable to use arabinose. Very few fungi are able to utilize maltose, galactose, mannose, ribose, rhamnose, trehalose, and melezitose. It is surprising that many fungi are unable to utilize sugars like arabinose, galactose, mannose, and ribose, which are common constituents of plant carbohydrates. Based on pure culture studies, ruminal fungi, like bacteria, have a mixed acid fermentation. They can metabolize hexoses or pentoses to produce acetate, formate, lactate, ethanol, $CO_2$, and $H_2$. Because fungi produce the major precursors of methane (Formate and $H_2$); the fermentation profile is altered (less ethanol, lactate, and $H_2$) in the presence of methanogens. The shift in fermentation products, as a result of interspecies $H_2$ transfer, leads to an increased ATP production, which increases fungal biomass, enzyme production and rate and extent of substrate utilization.

It is generally recognized that with forage-based diets, particularly of low quality plant biomass, ruminal fluid has a lot of zoospores and a substantial portion of plant fragments in the rumen are also colonized with fungi. These observations have led to the suggestion that fungi may contribute to a greater extent to the digestion of fibrous plant material. *In vitro* fermentation studies with pure cultures of ruminal fungal species, depending on the feedstuffs, up to 75–90 % of cell wall degradations have been observed.

### *Bacteriophages*

Bacteriophages are viruses that infect bacteria. They are composed of nucleic acids (DNA or RNA, either single or double stranded) and protein. When a phage attacks a bacterial cell, it first attaches to a receptor on the bacterial surface and then injects the nucleic acid into the cell. Once the DNA enters the cell, one of two processes happens, lysis or lysogeny. In the lytic process, viruses replicate (synthesis and assembly) and result in lysis and release of phages. In the lysogenic process, the phage DNA gets incorporated into the host cell DNA. The phage remains latent and does not cause lysis. Such a state is called lysogeny. The inserted phage DNA is called prophage and is passed on to the daughter cells when the host cell divides. Prophage may confer new properties to the host cell or may get excised from the bacterial chromosome and enter a lytic phase. Based on electron microscopic observation, a large and diverse population of phages has been described in the rumen. The number of phage particles in the rumen could range from $10^7$ to $10^{11}$ per ml or g of ruminal contents. Both lytic and lysogenic phages and phages specific to several bacterial species have been identified. No known function has been identified of any temperate phages and it is possible that lytic phages contribute to nutrient recycling and have some influence on the species or strain composition of the bacterial population in the rumen.

## Microbiology of Ruminal Digestion

The major components of ruminant diets are polymers and include carbohydrates, nitrogenous (protein and nonprotein) substances, lipids, and lignins. The polymers, except for lignins, are hydrolyzed to monomers, which are then metabolized to various fermentation products, chiefly acids and gases, depending on the microbial species. The extent to which the polymers are degraded in the rumen depends on the feedstuff and length of retention in the rumen. Lignins are polymer of phenolic compounds and are virtually indigestible feed component. Because lignins are located in the plant cell wall and are covalently linked to hemicelluloses, there is a negative relationship between lignin content and the extent of fiber degradation in the rumen. In bacteria and fungi, plant polymers (cellulose, hemicelluloses, pectin, starch, protein, and lipids) are hydrolyzed extracellularly to small oligomers (<6-monomer length) and monomers, which are then transported into the cell for

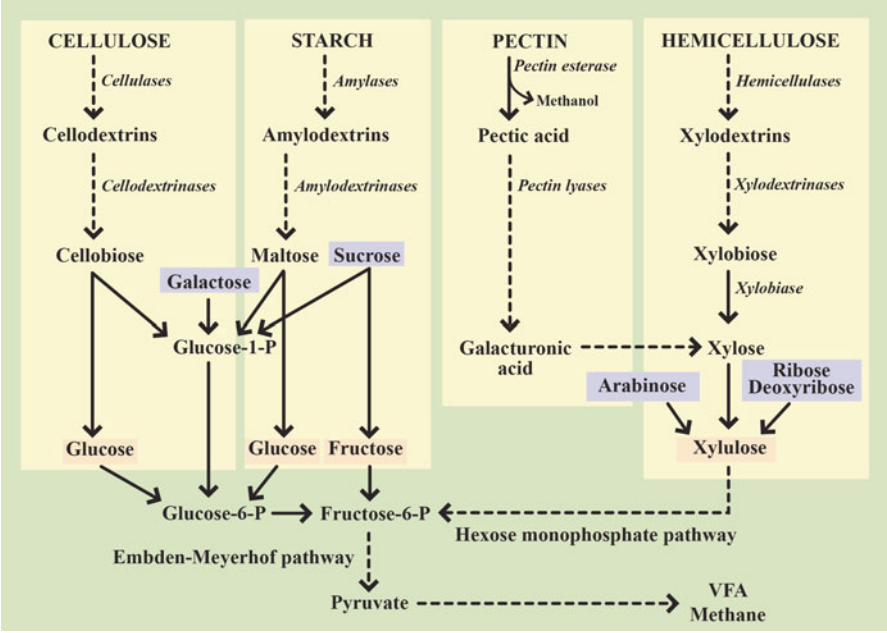


**Fig. 2.7** Carbohydrate fermentation in the rumen

further metabolism into fermentation products. In ciliated protozoa, the degradation of the polymer takes place inside the cell because feed particles are ingested and then digested in a food vacuole within the endoplasm.

***Carbohydrate Fermentation***. Feed carbohydrates include polysaccharides and simple sugars. Polysaccharides may be structural (cellulose, hemicelluloses, and pectin) or non-structural (starch) (Fig. 2.7). Bacteria, ciliated protozoa, and fungi produce a variety of glycosyl hydrolases that breakdown the glycosidic bonds to produce oligosaccharides first and then the di- and monosaccharides. The first step in fiber degradation is the attachment of microbes to the feed particles and the attachment is mediated by capsule, and in some cases specific binding protein may be involved. Several species of ruminal bacteria and ciliated protozoa and all species of ruminal fungi possess cellulolytic activities. The three bacterial species that are considered to be most abundant in the rumen are *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens*. Interestingly, all three are not proteolytic. Some of the noncellulolytic bacteria are capable of utilizing cellodextrins produced by cellulolytic bacteria. The major cellulolytic bacteria can also digest hemicelluloses and pectin. Noncellulolytic bacteria that can digest hemicelluloses include *Prevotella* sp. (*albensis*, *brevis*, *bryanti*, and *rumincola*), *Butyrivibrio fibrisolvens*, *Pseudobutyrivibrio xylanivorans*. The major enzymes involved in hemicelluloses degradations are endoxylanases and several debranching enzymes with arabinofuranosidase being the most important. Although pectin is a structural

polysaccharide it is completely digested in the rumen. The major pectinolytic bacteria include *Prevotella* sp., *Lachnospira multiparus*, *Streptococcus bovis*, and *Trepnema* sp. (*bryantii* and *saccharophilum*). Although *S. bovis* is pectinolytic, it does not utilize the products of pectin degradation (D-galacturonic acid). Similarly ciliated protozoa can breakdown pectin but cannot utilize the products. The predominant pectinolytic enzyme is pectin lyase. Starch is rapidly digested in the rumen and the extent of digestion depends on the grain type and the degree of grain processing. The major amylolytic bacteria in the rumen include *Ruminobacter amylophilus*, *Selenomonas ruminantium*, *Streptococcus bovis* and species of *Lactobacillus* and *Bifidobacterium*. The major enzymes involved are alpha-amylase and debranching enzyme, pullulanase. Entodiniomorphid protozoa engulf starch granules and ferment it slowly, which in a way contributes to slowing down the rate of starch fermentation in the rumen. Fungi have amylase activity but it is believed to contribute minimally because fungal population decreases in grain-fed animals.

Many of the polymer-fermenting bacteria, ciliated protozoa, and fungi can ferment disaccharides and monosaccharides produced from initial hydrolysis. Additionally, rumen has active sugar fermenting bacteria that belong to the genera *Streptococcus*, *Bifidobacterium*, *Lactobacillus*, and *Treponema*. Of these, *S. bovis* and *Lactobacillus* sp. are important because of their propensity for explosive growth and production of lactic acid in situations where rumen is presented with excessive fermentable carbohydrate. Quantitatively, glucose and xylose are the predominant sugars presented to the microbes and Embden-Meyerhof or hexose monophosphate or pentose phosphate pathways metabolize them, with pyruvate as the major intermediate product. The types of fermentation products produced from pyruvate depend on the microorganism and ruminal conditions like pH and dilution rates (Fig. 2.8). The fermentation products produced in the rumen include acetoin, butanediol, acetate, formate, ethanol, lactate, succinate, propionate, butyrate and valerate. Formate is utilized by methanogens to produce methane. Lactate and succinate are intermediate products because lactate-utilizing and succinate-utilizing bacteria further metabolize them, respectively. Succinate is decarboxylated to propionate and the major organism involved is *Selenomonas*, which is why succinate does not accumulate in the rumen. Lactate is metabolized to acetate, propionate, and butyrate by two major lactate-utilizng bacteria, *Megasphaera elsdenii* and *Selenomonas ruminantium*. If rate of lactic acid production exceeds the rate of fermentation, then lactate accumulates in the rumen, a condition called lactic acidosis.

***Protein and Non-Protein Nitrogen Fermentation***. Many of the carbohydrate-fermenting bacteria are also proteolytic. The most active proteolytic bacteria are *Prevotella* sp., *Ruminobacter amylophilus*, *Butyrivibrio fibrisolvens*, *Streptococcus bovis*, *Selenomonas ruminantium*, and *Megasphaera elsdenii*. Proteins are broken down into polypeptides and then to short peptides and amino acids (Fig. 2.9). The rate of peptide breakdown depends on amino acid composition. The peptide fermentation in the rumen is a two-step process. In the first step, dipeptidyl peptidases cleave dipeptides from the n-terminal end of the polypeptides, followed by cleaving of dipeptides into amino acids by dipeptidase. A common bacterial species that has been shown to contain high dipetidyl peptidase activity is *Prevotella ruminicola*. The dipeptidase

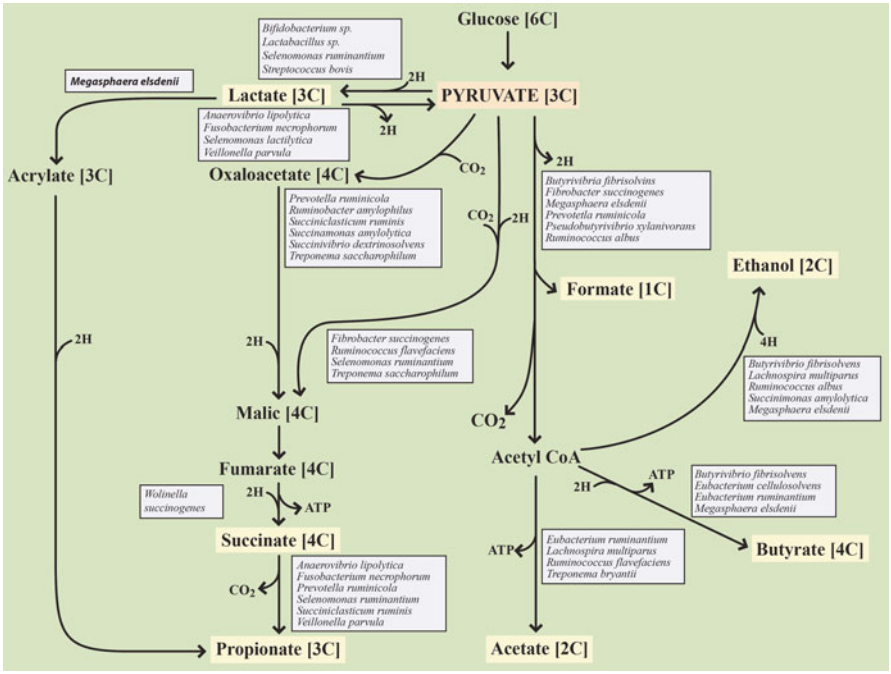


**Fig. 2.8** Pyruvate metabolism in ruminal bacteria

activity has been detected in many bacterial species and ciliated protozoa. Ruminal fungi also possess aminopeptidase activity but the extent of fungal contribution to peptide fermentation is not known. The rumen has very little free amino acids, which suggests that amino acids are rapidly fermented in the rumen. Deamination is probably the most common mode of amino acid fermentation and almost all proteolytic bacteria are involved in deamination. The rumen also has a specialized group of bacteria called 'hyperammonia producers' that do not ferment carbohydrates but can hydrolyze small peptides and deaminate amino acids. So far four species have been identified as hyper ammonia producers: *Peptostreptococcus anaerobius*, *Clostridium sticklandii*, *Clostridium aminophilum*, and *Fusobacterium necrophorum*. Therefore, amino acid deamination in the rumen can be carried out by bacteria that have low activity but are high in numbers or by bacteria that are low in numbers but have high activity. Ciliated protozoa also have a significant role in deamination.

Urea is rapidly fermented in the rumen and the source of urease activity is believed to be epimural bacteria. The microbial species involved in fermentation of nucleic acids or nitrates is poorly understood. Neither ciliated protozoa nor fungi have any ureolytic activity. Most of the rumen bacteria are capable of using ammonia as a nitrogen source. Therefore, hydrolysis of NPN such as urea, nitrates or nucleic acids is beneficial to ruminal fermentation. Glutamic dehydrogenase and glutamine synthetase -glutamine synthase are key enzymes involved in ammonia assimilation by ruminal bacteria.

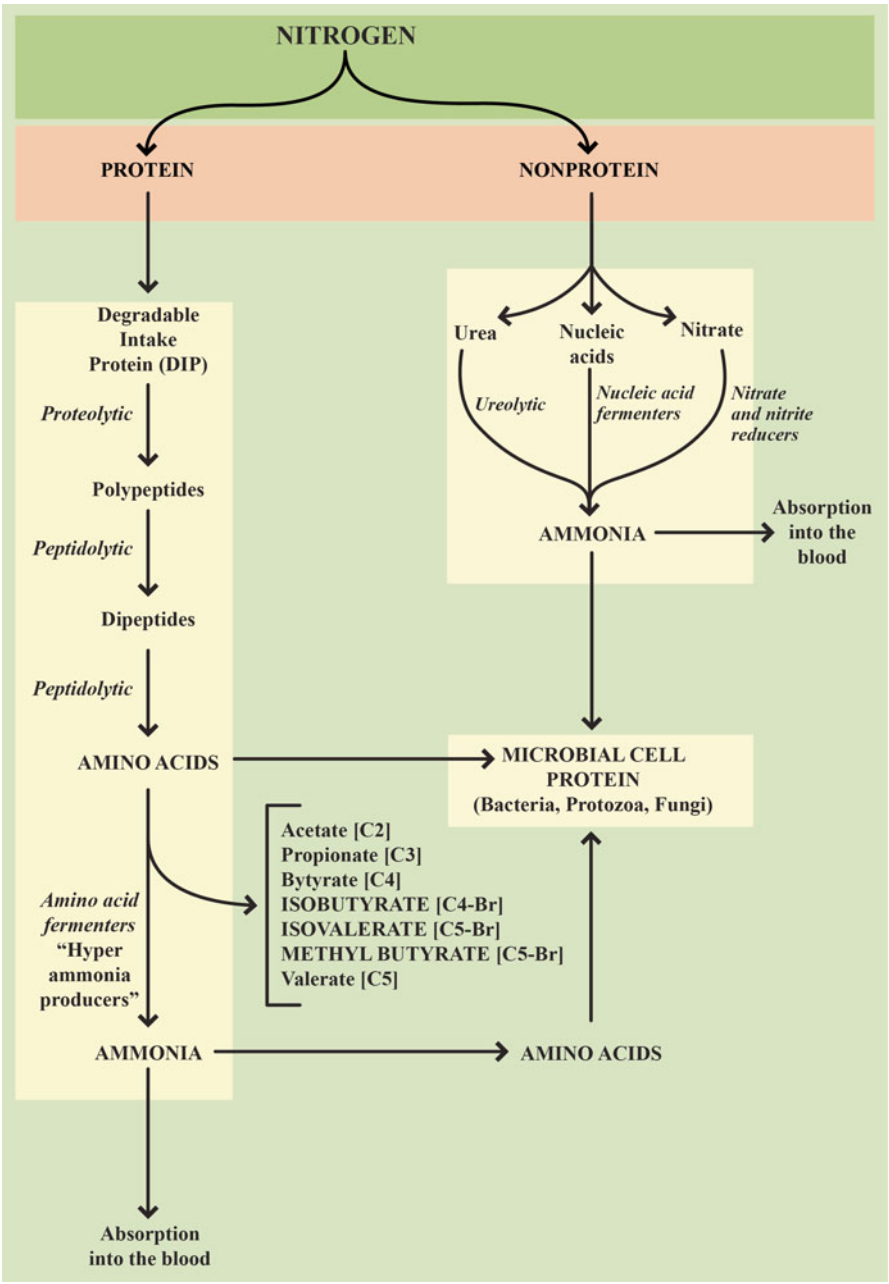


**Fig. 2.9** Overall scheme of nitrogen fermentation in the rumen

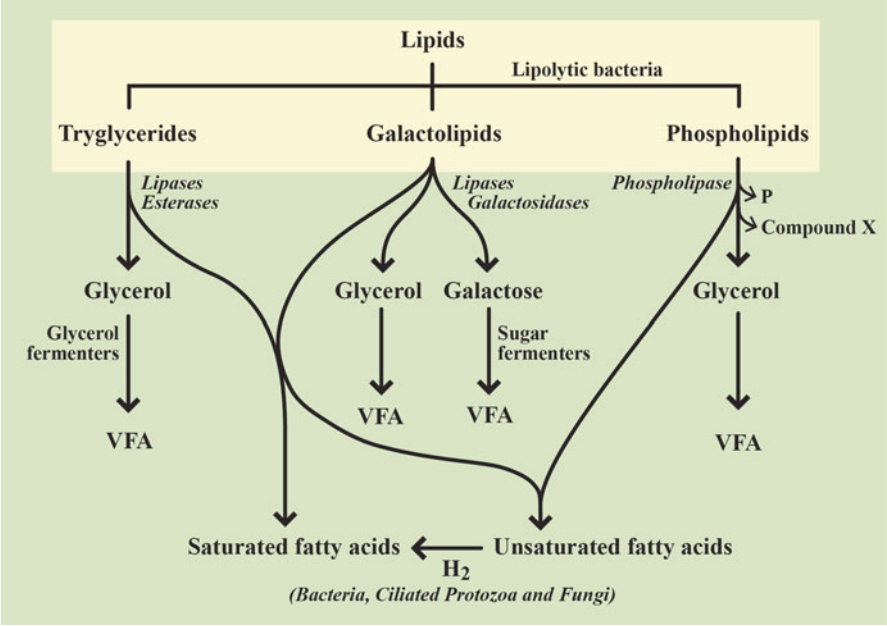


**Fig. 2.10** Lipid fermentation in the rumen

***Lipid Fermentation***. The predominant lipids in ruminant feeds are galactolipids, triglycerides, and phospholipids. The lipids are rapidly hydrolyzed by lipases or esterases produced by bacteria and ciliated protozoa (Fig. 2.10). Fungal contribution to lipid fermentation is not known. The major lipolytic bacteria in the rumen are *Anaerovibrio lipolytica* and *Butyrivibrio fibrisolvens*. Certain strains of *Treponema* also have lipolytic activity. The products of lipid hydrolysis are glycerol and fatty acids and galactose in case of galactolipids. Glycerol is rapidly fermented (*A. lipolytica*, *B. fibrisolvens*, and *Selenomonas ruminantium*) to acetate, propionate and butyrate. The long chain fatty acids are not degraded in the rumen but may get incorporated into cellular lipids by the microbes. In anaerobic ecosystems other than the gut, oxidation of free fatty acids is carried by sulfate-reducing bacteria or by a specialized group of bacteria called obligate $H_2$-producing (proton-reducing) acetogenic bacteria. In the rumen, if the fatty acids are unsaturated a process called biohydrogenation will hydrogenate them. The two major unsaturated fatty acids in ruminant diets are linoleic (*cis*-9, *cis*-12-C18:2) and linolenic acid (*cis*-9, *cis*-12, *cis*-15-C18:3). The prerequisite for biohydrogenation is that the fatty acid has a free carboxyl group. The unsaturated fatty acids are isomerized (*cis* to *trans*) before sequential addition of hydrogen to saturate each double bond to produce stearic acid (C18:0). Biohydrogenation leads to some transient intermediates and the two that have received a great deal of attention are conjugated linoleic acid (CLA) and *trans*10 and 11 fatty acids. A range of positive health benefits have been ascribed to CLA and

both CLA and *trans*-fatty acids, particularly *trans*-10 inhibit lipogenesis in mammary glands and are contributing factors in low milk fat syndrome. Both bacteria and ciliated protozoa are involved in biohydrogenation. However, protozoa may not be a major contributor because defaunation decreases biohydrogenation only slightly. Bacterial species that have been shown to biohydrogenate include *Butyrivibrio hungatei*, *B. proteoclasticus* (formerly *Clostridium proteoclasticum*) and *Propionibacterium acnes*. Biohydrogenation may be a detoxification mechanism because unsaturated fatty acids are more toxic than saturated fatty acids to microbes.

## Microbial Interactions

Fermentation of feedstuffs in the rumen is the result of coordinated activities of multitude of microorganisms. Because microbes compete for nutrients and space in the rumen, it is not surprising that there are different types of interactions. A major interaction that exists is cross feeding among microbes that result in a relatively more complete utilization of feedstuffs into the final fermentation products of VFA and methane. The major components of ruminant feedstuffs are cellulose, hemicelluloses, pectin, starch, proteins and lipids. Lignin is also plant polymer but it is not degraded by the microbes and in fact acts as a physical barrier to restrict digestion of cell wall polysaccharides. Based on the type of substrates metabolized, ruminal microbes can be grouped into three metabolic groups (Fig. 2.11). The group 1 includes fermentative or hydrolytic bacteria, protozoa and fungi that breakdown complex polymers (carbohydrate, proteins, and lipids) initially into oligomers and monomers, and finally to acids (mainly acetate, propionate and butyrate), alcohols (ethanol and methanol), and gases (mainly $H_2$ and $CO_2$). Among fermentative microbes, some are polymer fermenters (Group IA) that can convert polymer to the final fermentation products and monomers (Group IB) that cannot breakdown polymer but can utilize monomers to produce acids, alcohols, and gases. The fermentative bacteria include bacteria that ferment carbohydrates, proteins, and lipids. The second group includes the archaeal domain, methanogens (Group II) that convert $H_2$ and $CO_2$ or acetate to methane. The rumen also contains another metabolic group of bacteria, called homoacetogens (Group III), which can convert $H_2$ and $CO_2$ to acetate. Acetogens are not functional in the rumen because of their inability outcompete methanogens for hydrogen.

The following are some of the well-documented interactions in the rumen:

1. Interactions between fibrolytic and proteolytic microbes. This interaction is the basis of protein or nonprotein nitrogen supplementation of animals consuming low quality forages. The products of protein degradation, branched chain fatty acids and ammonia, are important growth factors for fibrolytic bacteria and the interaction results in higher degradation of fiber.
2. Interactions among succinate producing fibrolytic or amylolytic bacteria and succinate-utilizing bacteria (*Selenomonas ruminantium*). This interaction explains why succinate, a product of many bacterial species, does not accumulate in the rumen and instead becomes propionic acid.

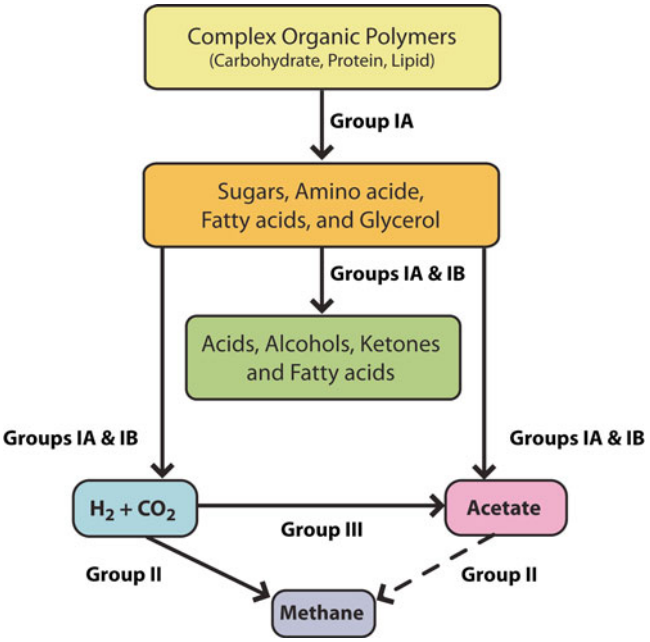


**Fig. 2.11** Microbial groups and stages in ruminal fermentation

3. Interactions among lactic acid producing microbes (bacteria, protozoa, and fungi) and lactate-utilizing microbes (bacteria and protozoa only). This interaction explains why lactic acid does not accumulate in the rumen unless the rate of production exceeds the rate of utilization, as in lactic acid acidosis.
4. Interaction, referred to as interspecies hydrogen transfer, between hydrogen producing microbes (bacteria, protozoa, and fungi) and hydrogen-utilizing bacteria, mainly methanogens. This interaction results in change in fermentation products (more acetate and less reduced fermentation products like lactate, ethanol, succinate, and propionate) and higher ATP yield (because of more acetate) by fermentative bacteria because of the hydrogen utilization by methanogens.

Another major interaction is the predatory role of ciliated protozoa that prey on bacteria and fungal spores. In the absence of bacteria (defaunated rumen) bacterial and fungal spores increase 10- to 100-fold. Ciliated protozoa engulf bacteria, which serves as a major source of nitrogen.

## Ruminal Microbial Development in the Calf

A newborn calf is functionally a monogastric animal, with the abomasum serving as the primary site of digestion. The development of the rumen into a functional organ in the neonate requires a period of growth and development involving transitional changes from the undeveloped organ to the fully functional rumen. The transitional

development is characterized by a number of changes in the anatomy, physiology, and microbiology of the rumen. The stimulation of ruminal anatomical and physiological development by ruminal fermentation product suggests an interrelationship between the ruminal development and the microbial activity. The length and extent of the changes are affected by several conditions, most importantly the consumption of dry feed. Factors that influence establishment include but are not limited to type of diet, water, dirt, bedding material and proximity of other ruminants.

The rumen of a calf at birth is practically a sterile organ. Microbial colonization of the digestive systems of newborn ruminants follows a typical succession in that bacteria proliferate in the fluid phase immediately after birth and colonize the ruminal wall within 36–48 h. These fluid-phase bacteria facilitate the subsequent sequential colonization of the fluid by fungi, followed by protozoa, which result in the establishment of a complex consortium that eventually develops in the fluid, on feed particles and on ruminal epithelial surfaces. Once established, they are generally stable and will change only when the nutrients are changed.

***Establishment of Ruminal Bacteria***. In the first few days of birth, anaerobic bacteria inhabit the rumen predominantly. Facultative bacterial counts are greatest at 1–3 weeks of age and then decrease steadily as the calf ages and the number of anaerobic bacteria increases during the first 3 weeks. Although the neonatal bacterial population is predominantly anaerobic, the predominant genera observed in the neonate are quite different from those in adult ruminants. The sequence of appearance of much of the ruminal bacteria appears to be primarily dependent upon the diet. Even though isolated calves develop ruminal bacterial populations, the source and establishment of many ruminal bacteria are not certain. Early research identified the predominate bacteria isolated from neonates as coliforms, lactobacilli, streptococci, and gram negative, facultative, anaerobes. Cellulolytic bacteria have been detected at relatively high numbers in the rumen of calves at 3 days of age. Ruminal methanogens have also been detected in calves at 3 days of age and in high numbers ($10^8$/g) in calves at 1 week of age. Therefore, the initial establishment of cellulolytic bacteria in the rumen seems to be independent of the amount of cellulose digested or kind of diet fed.

***Establishment of Ruminal Protozoa***. Flagellated and ciliated protozoa establish themselves at different times and establishment may be influenced by several factors. In general, flagellated protozoa become established more easily than ciliates, but their contribution to the ruminal development is not significant. Ruminal establishment of ciliated protozoa in the young calf requires some form of contact with faunated ruminants, and calves that were isolated from mature ruminants usually do remain defaunated. Ciliates may be established as early as 1 week of age; however, complete establishment of a ruminal protozoal population requires several months. Ciliates probably establish themselves according to their sensitivity to low ruminal pH. *Entodinium*, being the least sensitive to low pH, is usually established first, followed by *Diplodinium* and then holotrichs.

***Establishment of Ruminal Fungi***. Ruminal fungi typically appear in the rumen in 7–8 days after birth, which means fungi can survive in the rumen in the absence of plant materials. In the first 2 weeks, rumen will have saliva and sloughed epithelial

cells and some milk that leaked from the reticular groove. Some of the species typically seen in the adult ruminant (*Neocallimastic*, *Piromyces*, etc.) have been isolated from the rumen of the newborn. Subsequent development of the fungal population is to a large extent is dependent on consumption of solid feed. It is not certain whether fungal establishment in a newborn requires contact with or in close proximity of adults. Because fungi have been detected in saliva and feces and it is likely that they can be transmitted from the adults to the new born.

***Ruminal Fermentative Changes***. The milk fed neonate has a ruminal pH of approximately 6.0 with little postfeeding diurnal variation. As the calf consumes dry feed and ruminal function develops, the ruminal pH steadily drops to pH 5–5.5. Age at which this decline is observed is diet and intake dependent, however it is usually observed between 4 and 5 weeks of age in calves offered dry feed from birth. Weaned calves consistently have a lower pH than nonweaned calves of the same age. This drop in ruminal pH is generally linked to increased ruminal microbial activity, and corresponds to the subsequent increase of ruminal VFA concentrations. The pH appears to stay low only a week or two and then gradually increases to pH 6.0 or higher at approximately 10 week of age. This increase may be due to increased absorption of VFA as the rumen matures and possibly to increased salivary secretion. The increased dry feed consumption and change in diet from milk to dry feed also causes a shift in the VFA produced. Acetate decreases and propionate increases decreasing the acetate:propionate ratio. This decreased acetate:propionate ratio is probably a result of increased dry feed entering the rumen stimulating increased amylolytic activity, resulting in more propionate production, combined with a decrease in acetate producing facultative bacteria. Molar proportions of butyrate increase with the age of the calf. This increase may be due to the increased production of butyrate from lactate fermentation due to the low ruminal pH of the young calf. In addition, the rate of neonatal absorption of ruminal butyrate is low. Molar proportions of isobutyrate and isovalerate tend to decrease with the age of the calf. These branch-chained fatty acids are growth factors for cellulolytic bacteria, and the decline of these acids may be due to increased cellulolytic activity in the calf. Ruminal lactate concentrations steadily increase until 3–4 weeks of age, then drop to <0.5 mM by 12 week of age. Dry feed consumption changes the microbial composition away from a facultative, lactate producing population towards a lactate utilizing anaerobic population. This population shift is probably responsible for the steady decline in ruminal lactate concentration that often occurs after 4 weeks of age. Ruminal ammonia-N concentrations generally decrease as calves age. Decreased ruminal ammonia-N is most likely a result of increased ruminal bacterial utilization, and increased ruminal absorption.

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# Chapter 3
# Ruminal Fermentation

**Fredric N. Owens and Mehmet Basalan**

## Classification of Animals by Foods or Feeds Consumed

### *Carnivores, Omnivores, and Herbivores*

Diet selection by animals is dictated largely by capacity of the digestive tract and the ability of enzymes available within their digestive tract to digest or ferment the primary nutrients in a diet (carbohydrates, protein, lipid) to simpler compounds that can be absorbed and metabolized by mammalian tissues. Lacking amylases that attack starch in grains, starch must be gelatinized to be well used by carnivores (cats, dogs). The small capacity of the digestive tract of carnivores, of young animals, and omnivores (poultry, swine, humans) limits their ability to consume bulky, fibrous feeds. In contrast to carnivores and most omnivores, adult herbivores have a digestive tract large enough to maintain fibrous feeds for a time period sufficiently long for anaerobic fiber-digesting microbes resident within their digestive tract to ferment cell walls (hemicellulose and cellulose) to volatile fatty acids (VFA, primarily acetic, propionic, and butyric acids). These products in turn are absorbed and metabolized by the host animal. This capacity allows herbivorous animals to thrive when their diet contains forages and feeds that are bulky and rich in fiber and water.

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## *Non-ruminant Herbivores Versus Ruminants*

Non-ruminant herbivores (horses, rabbits, hares) have an enlarged cecum and colon where anaerobic microbes ferment cell walls to VFA. Because these organs for fermentation are located posterior to the small intestine of non-ruminant herbivores, the microbes involved with post-gastric fermentation excrete most microbial products that could have nutritional value to the host animal (protein, vitamins, phosphorus, sulfur, ammonia) in feces. Nevertheless, post-gastric fermentation allows VFA derived from fermentation to be absorbed, so post gastric fermentation enhances energy recovery from fermentation of nutrients that escape intestinal digestion.

In ruminants, fermentation occurs in the reticulo-rumen, a site anterior to gastric pouch (the abomasum) and the small intestine. Most of VFA are absorbed through the ruminal or omasal wall. In addition, the microbial mass (containing protein, vitamins, phosphorus, sulfur) together with dietary feed components that resist or escape ruminal fermentation become available for digestion and absorption when passed to the abomasum (true stomach) and intestines. Because the site of fermentation is located ahead of the small intestine, ruminants make efficient use of protein and other nutrients synthesized by microbes in the rumen. In addition, ruminants recycle certain essential nutrients (N, S, P) to the rumen that, together with salivary buffers, provide nutrients and maintain growth conditions conducive to growth and activity of anaerobic microbes. In addition to fermenting fibrous components not digested by mammalian enzymes, ruminal microbes will detoxify many substances prevalent in certain plants and herbs that can prove toxic for non-ruminants. As with non-ruminant herbivores, capacity to graze allows all herbivores to both harvest forages from plants found in areas inaccessible for mechanical harvest and to obtain energy and protein from fiber-rich byproducts inedible by omnivores. Through using such products and grazing forage, herbivores increase the supply of calories available for human consumption.

Ruminants are adapted to a wide variety of environmental and dietary conditions. They thrive when fed diets are high or low in moisture content. They thrive when diets are rich in fiber or rich in starch. Thus, surplus grains as well as numerous byproducts of food, feed, and fuel production and products of agricultural and industrial manufacturing that are undesirable or inedible by humans or other animal species can be converted by ruminants into foods valued in the human diet. Milk and meat derived from ruminants provide high quality protein and possess flavors and textures preferred by humans. The capacity of ruminants to convert fiber-rich, undesired, or surplus feeds into desired food products while forming additional products (wool, mohair, leather, manure) prized by humans worldwide depends largely upon the rumen fermentation process. This chapter is an attempt to outline normal rumen function and dysfunction, to discuss potential methods for altering rumen function, and to outline some of the research methods employed to increase our understanding about and control of fermentation within the rumen.

## Fermentation

Fermentation is defined as an anaerobic (without using oxygen) cellular process whereby organic foods are converted to simpler compounds and energy is released. Fermentation is common among many microbial species and even by mammalian muscle tissues when muscles act anaerobically. During fermentation, acid fermentation end-products accumulate over time. Through inhibiting continued microbial metabolism, acid accumulation gradually slows and stops fermentation stabilizing the product (i.e., silage or pickles). But when bases or buffers are present or added to neutralize these acids or when acids are removed by being absorbed as from the rumen, or when basic compounds are released during fermentation (as from proteolysis) to neutralize acid, fermentation can continue and persist. Feeds or foods can be preserved when stored anaerobically in the presence of acids that are weak (as from fermentation) or strong acids or under basic conditions (as with added ammonia or strong bases). Either strong acids or basis will minimize bacterial activity so that fermentation will be minimal or nonexistent.

### *Extent of Fermentation*

When not halted by accumulation of acid or base, microbial fermentation will persist. If allowed to continue for days or weeks, most of the carbon and hydrogen from organic compounds (except for lignin and other polyphenols) will be converted to methane and carbon dioxide, the nitrogen present will be released as ammonia, and sulfur will be released as hydrogen sulfide. Thereby, fermentation allows the organic wastes within biogas (methane) fermenters or at sewage plants to be fully degraded. Released methane can be combusted to release energy. In contrast to such extensive fermentation, fermentation within the rumen differs: time is limited and end-products (VFA) are continuously removed. This allows most of the energy in end-products to be used by the host animal. Fiber, defined as any product that cannot be digested by mammalian enzymes, and other organic compounds are fermented by microbes in the rumen to yield products useful for mammalian tissues with limited loss of energy as methane and heat.

### *Types of Fermentation*

Fermentation processes used industrially are classified into two types—batch systems or continuous flow systems and into two classes—open or closed depending on whether microbes from the environment are allowed to enter the fermentation vessel.

## Batch Fermentation Systems

Typically industrial batch fermentation vessels are closed and substrates often are sterilized to avoid epiphytic microbes. This allows fermentation to proceed with selected microbes to yield enzymes or other products. When desired products have accumulated, the entire mass is harvested and the products of interest are isolated and marketed. To release glucose from starch from grain for generation of ethanol, batches of ground starch first are hydrolyzed and the released sugars are fermented with yeast or enzymes. Numerous organic compounds (nutrients, enzymes, hormones) of industrial and nutritional interest are generated through batch fermentation systems.

Silage formation is a batch fermentation system that often relies on the epiphytic microbial population though specific inoculants often are added to speed or direct fermentation to specific products (primarily lactic and acetic). These acidify moist forages or grains to preserve the mass for later use. Similarly, organic or inorganic acids can be added to preserve feeds or foods (pickling). The accumulation of acid end-products inhibits further fermentation and preserves the ensiled mass so long as oxygen is excluded. Upon re-exposure to oxygen, microbes will catabolize the acids, generate heat, and begin to oxidize the mass.

Often fermentation will be incomplete. If the amount of carbohydrate is insufficient to produce enough acid to inhibit microbes or if the substrate is strongly buffered, microbial degradation will continue yielding various spoilage products (ammonia, butyric acid) that reflect substantial loss of nutrients.

Fermentation of silage is classified as homolactic or heterolactic depending on the products generated. With homolactic fermentation by lactobacilli, D+ and L-lactic acid and its derivatives are the sole organic products. Other microbial species can form a wide variety of other products (ethanol, VFA) from lactate. In the rumen fermentation is largely heterolactic with numerous intermediate products (succinic acid, malic acid, hydrogen, ethanol, as well as lactate) being formed. These compounds typically are reduced further by fermenting microbes or by other microbes so that the primary products of ruminal fermentation are VFA, carbon dioxide, reduced (hydrogenated) fatty acids, and other products that were used as electron or hydrogen (NADH) acceptors (e.g., methane, nitrite or ammonia, and hydrogen sulfide), and the synthesized microbial mass. The mass of microbes generated during fermentation generally is limited by (1) the supply of energy (ATP) derived from fermentation or by (2) availability of other required nutrients (e.g., ammonia).

Many microbial species require only energy plus a source of carbon and a source of nitrogen plus trace amounts of minerals. From these compounds alone, many microbial species will thrive by synthesizing all needed organic substances for growth and reproduction. This process often is called “de novo” or “from nothing.” In contrast, other microbial species require pre-formed organic substances that vary in complexity (specific amino acids or fatty acids, vitamins) for growth. These microbes thrive by assembling compounds they synthesize with other nutrients obtained from their environment or diet into microbial organic matter. For example, when grown in pure culture (absence of other microbial species), most species of ruminal bacteria that digest cellulose require a source of branched-chain fatty acids

or amino acids for growth. However, supplementing the ruminant's diet with these nutrients is not necessary because these same compounds are produced and released by other bacterial species within the rumen so the inherent supply is adequate.

### Continuous Flow Systems

When feed material is provided to a fermentation vessel at frequent intervals and output is continuous or semi-continuous, the system is classified as being continuous. Most waste digestion systems and the rumen are semi-continuous due to frequent addition of substrates and open so that fermentation is performed by both epiphytic (environmental) present within the feeds provided as well as endophytic (digestive tract) microbes inherent within the digestive tract. Products may be continuously removed from fermentation vessels at specific points or removed selectively. Unless readily stirred, continuous fermentation systems develop multiple strata or layers that will differ in chemical composition. Within the rumen, entwined forages form a floating mat or raft that is situated partially submerged atop the liquid mass. Slow but continuous mixing and churning, as occurs within the rumen, inoculates freshly consumed feeds and forages, maintains close contact between microbes and substrates, exposes acids to the rumen wall for removal by absorption, and allows fermentation gases to be cleared. Ethanol generation from sugar cane and biogas (methane) generators typically employ continuous flow fermentation systems.

## *Fermentation Within the Gastro-Intestinal Tract*

Microbes (bacteria, protozoa, yeast, fungi) within the digestive tract are responsible for fermentation. These microbes, whether epiphytic or endophytic relate to other organisms or animals in either a mutualistic (sharing), a synergistic (beneficial to host) or a parasitic (harmful to the host) fashion. All microbes have preferred conditions for maximum growth being mesophylic (body temperature) or thermophylic (high temperature), with preferred or required substrates, and typically yielding specific end-products from a given substrate. Specificity of substrates and products and microscopy were used extensively in the past to identify and classify microbes that often revealed substrates and products as well as metabolic pathways. Today, microbial classification generally is based on genetics instead.

Being largely anaerobic, the complete digestive tract of mammals and other fiber-digesting organisms like termites is an active site of fermentation. Without microbes, fibrous compounds and lignin from plants would accumulate within the environment. (Perhaps coal and petroleum reserves developed at an age before or at a faster rate than microbial degradation could ferment those substances further.) Acidity within the stomach and addition of antibiotics or other modifiers can help inhibit or control the fermentation process, but because the GI tract is an open system, the amount and extent of control imposed by the host is

limited. Fortunately, fierce competition with microbes already present in the rumen and in the digestive tract of non-ruminants typically prevents invasion by epiphytic or pathogenic microbes. Anaerobic bacteria line the digestive tract of all animals (except within gnotobiotic animals delivered by Caesarian section and maintained under sterile conditions). Fermenting microbes are particularly active at locations within the digestive tract where they have an appropriate supply of nutrients, favorable growth conditions, and retention of digesta within an organ (e.g., cecum, large intestine, rumen) is sufficiently long for fermentation to continue so that microbes can multiply faster than they are washed along. With fermentation being a reductive process, organic products within the rumen are reduced to organic acids (VFA), carbon dioxide, and methane. Some products are absorbed (VFA, $CO_2$), others are eructed or belched (methane and $CO_2$), others are passed to the small intestine (microbial mass and remaining carbohydrates, protein, lipids, ash) for digestion, and the remainder is excreted in feces.

Energy (ATP; NADH; NADPH) that is released during fermentation or metabolism is available for use by microbes for their growth and multiplication. Compared with respiration where oxygen or other electron acceptors (sulfur, metals) are freely and readily generate ATP from NADH through the electron transport chain, fermentation is anaerobic and the amount of other electron acceptors limited. Consequently, the quantity of energy released during fermentation is quite limited compared with respiration in the presence of oxygen or other electron acceptors (nitrate, sulfate). With aerobic respiration, carbon dioxide and water are the major products. But during fermentation, carbon dioxide, methane, and a very limited amount of water is produced. Products of fermentation will differ with the substrate being used, the organisms or tissue involved, synergistic metabolism among multiple microbial species, and conditions of fermentation. Indeed, within the rumen, fermentation products can differ drastically with ruminal pH conditions likely due largely to shifts in microbial species (Owens and Goetsch 1988; RAGFAR 2007).

## *Characteristics of Fermentation Within the Rumen*

Within non-ruminant animals, gastric acidity (HCl) hinders the entry of non-spore forming microbes that are sensitive to acid. With ruminants, any microbes in the diet meet an acid barrier only when leaving, not when entering, the rumen. Being an open system, the rumen continuously receives microbes with feed and water being consumed. As a result, the microbial population in the digestive tract might be considered "uncontrolled." Uncontrolled fermentation is evident considering various maladies characteristic of ruminants due to accumulation of specific fermentation products (lactic acid causing acidosis; gases causing bloat; nitrate and sulfide toxicoses).

Compounds enter the rumen largely from two sources—when swallowed (feed plus saliva) and by diffusion through the rumen wall. Feed components carry

not only organic solids and liquids but also water and a limited amount of oxygen. Feed, saliva, and ruminated (re-chewed ruminal contents) products enter the reticulo-rumen through the esophagus. Exchange across the stratified ruminal wall depends on the relative osmolality of the rumen versus blood. Certain blood components (urea, bicarbonate, a limited amount of water and oxygen) may enter the rumen through diffusion from blood. Urea enters the rumen both via saliva and by diffusion through the rumen wall. When hydrolyzed, urea yields ammonia, a source of N for ruminal microbes plus carbon dioxide. The ruminal entry rate of urea by diffusion is enhanced by hydrolysis to ammonia by ureolytic bacteria imbedded within the rumen wall. Diffusion of the released ammonia, being basic, into the rumen mass is accelerated by a low ruminal pH. Likewise, bicarbonate enters the rumen both via saliva and due to exchange with VFA during absorption (VFA ions being exchanged for bicarbonate). Note that ruminal pH conditions can alter extent of nutrient recycling into the rumen through the stratified epithelium of the rumen wall.

Removal of products of fermentation from the rumen follows three routes—eructation, diffusion into the blood or the lymphatic stream, and flushing of liquefied digesta (chyme) containing particulate matter to the omasum. Part of the carbon dioxide produced will diffuse from the rumen into the blood stream. But most carbon dioxide, almost all of the methane (due to its low solubility in blood), and most hydrogen sulfide is removed from the rumen in the form of a gas by eructation (belching). Organic acids (VFA, lactate-that is not considered to be volatile like most other short chain fatty acids) are removed from the rumen largely through the ruminal epithelium by passive or attenuated absorption. Other absorbed compounds (ammonia, ionized minerals) leave the rumen via blood or the lymphatic stream depending on their solubility. Microbial products and undigested feed components that are sufficiently dense and small in size are flushed with liquid digesta from the rumen to the omasum via the reticulo-omasal orifice.

## The Rumen—An Ideal Media for Anaerobic Microbes?

Conditions maintained within the rumen by the animal are similar to those of an ideal commercial fermentation vessel (Table 3.1).

### *Characteristics of Ruminal Microbes*

Facultative bacteria and aerobic yeasts readily consume oxygen that enters the rumen with feed or water or diffuses into the rumen from blood. This helps to maintain strict anaerobic conditions within the reticulo-rumen preferred by ruminal microbes. With this semi-continuous fermentation system, microbial strains are selected or evolve to thrive best given the substrate and environmental conditions provided. Specific factors inherent to an individual strain of ruminal microbes can provide a selective

**Table 3.1** Ruminal characteristics conducive to microbial growth

| | |
|---|---|
| 1. | Fresh substrate and water provided on a regular and frequent basis |
| 2. | Supplemental nutrients (urea, sulfur, phosphorus) recycled via saliva or by diffusion through the rumen wall |
| 3. | Particle size reduction through mastication and rumination |
| 4. | Sufficient water to maintain a fluid or liquid mass |
| 5. | Continuous mixing and churning of ingesta with rumen contents by strong muscular contractions |
| 6. | Stratification of ruminal contents with a floating raft for prolonged retention time of slowly fermented, longer, and buoyant particles |
| 7. | Retention time of particles that is sufficiently long for microbial growth and replication |
| 8. | Temperature of 38–42 °C ideal for growth of mesophylic microbes |
| 9. | pH between 5.5 and 7.0 due to bicarbonate input via saliva and bicarbonate exchange with absorbed ionized acids |
| 10. | Osmolality maintained through exchange with blood fluids |
| 11. | Removal of end products by eructation, attenuated diffusion into blood, and flushing to the omasum |

advantage over other ruminal strains or over epiphytic microbes that enter the rumen with feed and water as outlined by Russell (1984) and shown in Table 3.2.

Those microbial species that multiply faster than being washed from the rumen or dying will increase in number; conversely, microbes that fail to multiply faster than they are lost from the rumen population will decrease in population. As a consequence, competition among ruminal microbes is vigorous, continuous, and cutthroat. Although genetically altered "superbugs" might be developed, it is unlikely that ruminal inoculation would prove successful in altering the ruminal population unless the organism (a) can utilize some unused or underused substrate (e.g., lignin, biuret, oxalate) found in the rumen, (b) can tolerate or degrade a compound that inhibits growth of competing ruminal microbes (e.g., mimosine), (c) produces some compound that inhibits growth of competitors (e.g., bacteriocins, methyl-glyoxal), or (d) operates synergistically with some other ruminal microbes to increase growth efficiency. Despite this vigorous competition among microbial strains, the ruminal population remains sufficiently diverse so that the population can shift readily when the diet is changed. Culture studies indicate that microbial shifts to a new substrate are completed within a few days if energy intake is stable. But when energy intake by an animal fluctuates, as often occurs when diet composition is changed, microbial populations can fluctuate wildly until substrate supply and ruminal conditions become stabilized. Consequently, limiting or restricting the supply of feed for ruminants to avoid overconsumption of a new diet (e.g., one that is rich in concentrate) helps to reduce the wide fluctuations in end-products that can induce acidosis. In order to enhance steady state conditions in the rumen, many feedlots establish and adhere to a very regimented feeding schedule so that each meal is delivered to a given pen of cattle at precisely the same time each day.

Though the ruminal population within any individual animal is quite diverse, each individual animal appears to maintain a very specific mix of microbes according to

**Table 3.2** Factors providing advantages to specific microbial strains found within the rumen

| |
|---|
| 1. Ability to ferment those substrates present in feeds consumed |
| 2. High affinity for and, in some cases, ability to attach to available substrates |
| 3. Capacity to metabolize diverse types of substrates |
| 4. High yield of ATP from fermented feeds |
| 5. A maintenance energy requirement that is low |
| 6. Rapid replication (capacity to multiply faster than being flushed from the rumen) |
| 7. Capacity to survive fluctuations in temperature, pH, and osmolality |
| 8. Ability to store energy to remain viable between meals |
| 9. Low metabolic cost for ammonia and substrate uptake |
| 10. Capacity to attach to the rumen wall to use diffusing substrates (urea, oxygen) |
| 11. Association with floating particles to avoid washout (most evident with protozoa) |

population studies. This mixture of microbes will be characteristically different from that present in the rumen of other cows fed the same diet. Following ruminal evacuation and transfer of ruminal contents, the ruminal population will return to the population characteristic of the individual cow within several days (Weimer et al. 2010). This indicates that in addition to substrate supply, other factors (e.g., water intake, rate of eating, extent of chewing and rumination, saliva flow, ruminal pH, ruminal mixing and stratification, ruminal retention times for liquids and particulates, VFA concentrations and absorption, reticulo-omasal opening times) that are characteristic of an individual animal must play a role in regulating the microfloral population within that individual. Similarly, within a set of cattle, only a few animals will possess certain bacterial strains (e.g., Megaesphaera). And rumen fluid from only certain individual animals and not others within a set of steers similar in genetics and nutritional background will readily and consistently produce lactic acid when provided with glucose or starch. Explanations that can explain these animal-to-animal differences in the presence and activity of ruminal microbes remain unknown.

The population and distribution among various classes of bacteria will shift and change depending on the availability of, not if energy and essential nutrients. Ultimately, the microbial population will increase so that all of the readily available energy will be fermented within the available time. This concept that nutrients are fully fermented is based on the microbiological principle that whenever the supply of energy for microbial growth is increased, the microbial population capable of fermenting that energy automatically will increase to use that energy. Consequently, no available energy should ever exist within the rumen except when microbes encounter (1) deficiencies or excesses of of specific nutrients, (2) antimicrobial compounds or drugs, (3) time is insufficient for fermentation, (4) substrates are inaccessible due to presence of some physical barrier, or (5) following diet engorgement.

Though one might expect the population of cellulose digesting bacteria in the rumen to be lower when forage-poor (concentrate-rich) diets are fed, culture counts indicate that the population of cellulose fermenting bacteria remains relatively stable regardless of diet. This presumably reflects the capacity for cellulolytic microbes to use certain substrates besides cellulose as source of energy. In contrast, the population of microbes capable of fermenting starch increases markedly when diets rich in starch are fed; this in turn decreases the relative prevalence of cellulose-digesting bacteria even though the absolute population of cellulose-digesting bacteria remains stable.

## *Types of Ruminal Microbes*

Specific microbes within the rumen differ in class (bacteria, archaea, protozoa, fungi, yeasts), population, the classes of compounds they ferment, and the products that they produce (Hungate 1966). Some organisms cultured from the rumen are opportunists that are consumed coincidentally with feed or water. However, the majority of rumen organisms multiply within the rumen and, so long as they multiply more rapidly than they are flushed from the rumen, become inherent residents. Billions of bacteria are found within each ml of ruminal contents though the relative population size of various species will vary depending on available substrates and ruminal conditions. Only about half of the bacterial species found in the rumen have been cultured, classified, and identified.

Concentrations of culturable microbes per ml of ruminal fluid can vary daily and with time of the day depending on the supply of available nutrients and dilution by consumed water and saliva. Within the rumen, certain strains of bacteria (facultatives that use diffusing oxygen; ureolytics that cleave diffusing urea) line the ruminal epithelium. But most bacteria are either free floating in the ruminal liquid phase or can be found attached to particles. Attached bacteria can prove difficult to dislodge and count. Though attachment of microbes to fiber is required for fiber digestion, even cellulose digesting bacteria must detach and float freely in order to colonize freshly consumed feeds. This delay for colonization can explain why nutrient fermentation or gas production exhibits a "time lag" before reaching a maximum rate after a new feed is added.

Microbes involved with degradation of feed components and catabolism of monomers (sugars, amino acids, fatty acids) have received the greatest research attention. Compared with aerobic organisms, anaerobic ruminal microbes require critical culture conditions. Although some ruminal microbes are facultative and can survive with oxygen present, most are strict anaerobes and cannot survive in the presence of oxygen. Facultative microbes readily remove oxygen consumed with feed and water and the small amount of oxygen that diffuses into the rumen from the blood stream to maintain anaerobic conditions within the rumen. Presence of oxygen would permit oxidative respiration that would increase ATP yield from substrates and thereby increase the yield of microbial mass, a change that might prove useful under certain conditions. However, oxidative respiration within the rumen would deprive the animal of energy. If substrates were fully oxidized during respiration, not merely partly oxidized to yield the VFA that provide the ruminant with energy, supply of energy for the host animal would be

reduced. To maintain a balance between the supply of energy and the supply of protein for the host, some researchers have theorized that ruminal outflow of liquid and rumen turnover might be regulated based on its protein content. This is supported by the finding that protein content of duodenal chyme across a wide variety of forage and concentrate diets is surprisingly constant. Perhaps this concept could be employed to relieve partially the bulk fill limits to feed intake and increase ruminant productivity under certain conditions.

Traditionally, bacteria were classified based on substrates used and their fermentation products. Today with accessibility of genetic identification, genetics rather than metabolism are used for classification. Genetic typing links bacterial types based on genetic relationships and evolutionary background and can help identify species that possess certain useful genes that may be expressed. However, genetic information alone fails to provide information about degree that specific genes are used and active metabolism of a species. Certain bacterial strains with similar metabolism often are revealed to not be related genetically whereas other strains closely related genetically will differ markedly in their substrate preference and end products. Though a bacterium must possess a specific gene if it is to perform certain reactions or digest a specific compound, gene presence alone fails to reflect activity or metabolism of the microbes being characterized or even viability. Indeed, mRNA prevalence rather than DNA should more closely reflect metabolic activity.

When isolated in pure culture, a given bacterial strain often requires a specific nutrient for growth (e.g., most cellulose digesting bacteria require branched chain fatty acids or amino acids) that yield these acids and many will produce compounds (e.g., succinate, ethanol) not found within the rumen where mixed cultures exist. Cross-feeding among mixed cultures, where one species will produce or metabolize products of other species results in and readily explains differences apparent between results of pure culture studies and ruminal metabolism. Indeed, synergism among bacterial strains and synergism between protozoa and bacteria typically increases extent of ruminal digestion and alters the ratios of end-products (especially methane and VFA) of fermentation. Synergism between the numerous bacterial classes and strains within the rumen often renders data related to specific requirements for and products of a microbial strain when grown in isolation completely invalid and inapplicable under ruminal conditions where substrates and products represent the composite metabolism of a diverse mixture of bacterial strains. Various classes and strains of ruminal microbes have been outlined in Chap. 2 of this book.

## Ruminal Fermentation

Physical aspects and microbial types found within the rumen are described in Chap. 2 of this book. This chapter will cover the degradation of feedstuffs as well as formation of specific products during the ruminal fermentation process. Within the rumen, substrates typically are degraded to monomers that subsequently are fermented or metabolized quite rapidly to carbon dioxide and methane. An overview of this process is presented in Table 3.3 and Fig. 3.1.

**Table 3.3** Substrates and products of ruminal fermentation

| Feed component | Polymer | Monomer(s) | Products of fermentation |
|---|---|---|---|
| Carbohydrates | | | |
| Starch | Amylose | Maltose, Glucose | VFA, esp. propionate |
| | Amylopectin | | |
| Neutral detergent fiber | | | |
| Cellulose | B1,4-glucosan | Cellobiose, Glucose | VFA |
| Hemicellulose | Pentosan | Pentose sugars | VFA, esp. acetate |
| Crude fat | Triglycerides | Fatty acids | Saturated fatty acids |
| | Phospholipids | Glycerol | Propionate, P |
| | Galactosides | Galactose | VFA |
| Sugars | | | VFA |
| Crude protein | | | VFA |
| True protein | Protein | Amino acids | Ammonia & VFA |
| Non-protein N | – | Urea | Ammonia and $CO_2$ |
| DNA, RNA | Nucleic acids | Purines, pyrimidines | Ammonia and $CO_2$ |
| Crude ash | – | Minerals | Reduced minerals |

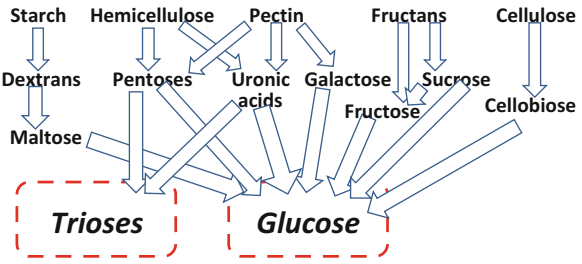



**Fig. 3.1** Ruminal conversion of polymers to simple sugars for fermentation

## *Limits to Ruminal Digestion*

Most feedstuffs are composed of a mixture of chemically diverse components physically encapsulated within plant or animal cell walls. Before internal components can be degraded, physical structures or barriers designed to protect plant or animal tissues from microbial or insect attack must be breached or fractured so that microbes and enzymes have access to components internal to the seed or tissue. External or encasing barriers include the pericarp of grains and oilseeds and the primary and secondary cell walls of plants and animal tissues. Within cereal grains, additional physical barriers (e.g., protein-coated or encapsulated starch granules) and hydrophobic regions (e.g., starch granules imbedded in prolamin) provide additional protection from enzymatic digestion. Likewise, within plant cell walls, the more digestible fiber components (e.g., hemicelluloses) usually are cross-linked with indigestible lignin that in turn serves to guard the fiber complex from attack.

### Feed Processing

Physical barriers are breached to varying degrees by mechanical processing of feedstuffs before a feedstuff is fed to animals. Feed processing methods can vary from simple methods of comminution to reduce the particle size (grinding), crushing (rolling) that fractures particles with or without added moisture or heat (steam rolling, flaking, extrusion), microbial acidification that softens particles and structures (fermentation), or application of chemicals, enzymes, or microbes (base, acid, or enzyme treatment or inoculation). Because ruminants chew food being consumed, no grinding method will precisely match the particle exposure and sizes entering the rumen.

A reduction in particle size increases the rate of fermentation primarily through exposing a greater surface area for bacterial attachment for digestion. Mean particle size and its distribution differ among various processing methods (rolling versus grinding). Slicing or abrasion through a hammer mill results in a much wider diversity in particle size than crushing between rollers of a roller mill. Rollers result in both fewer fine particles and fewer coarse particles than grinding. Thus, rolling is preferred for producing a milled product of consistent size. The shear forces generated by rolls set at differential speeds will shred the edges of particles exposing more surface area for microbial attachment and fermentation. Screening diets or processed feeds through multiple sieves that differ in hole size (e.g., Penn State Separator; Z-box) gives an indication of particle size of an ingredient or a mixed diet (length being estimated by horizontal shaking; diameter by vertical shaking). Wet filtration can differentiate particles by density. Sieving methods usually are employed to appraise "effective fiber," an estimate of the capacity of forages to stimulate rumination and saliva input, and be responsible for bulkiness that can limit feed intake. Through comparing the particle size of screened feeds and orts one can quantify the degree that an individual animal or a group of animals is sorting their feed.

Geometric mean diameter is commonly used as an index of particle size and potential for digestion. Coarser particles of a feed typically are less rapidly fermented and, when not retained for a sufficient time in the rumen, will be less extensively fermented than finer feed particles. However, geometric mean diameter provides only a single index of the total spectrum of particle sizes, not the relative prevalence of the coarse and fine particles. If only the coarser particles resist fermentation and digestion, then some measurement of particles above a specific size or geometric mean diameter would seem preferable for predicting rate of digestion. One method for calculating the amount of surface area exposed from sieved samples was outlined by Baker and Herman (2002).

## *Chewing to Reduce Particle Size of Feeds*

Feeds consumed by ruminants are chewed to reduce particle size and increase the surface area available for microbial or enzymatic attachment or attack. The amount of time that ruminants will chew their feed prior to swallowing appears directly related

to the amount of time an animal needs to produce sufficient saliva so that moistened feed can be swallowed. As a result, adult ruminants with larger more developed and active salivary glands produce copious amounts of saliva so they consume their diet rapidly and swallow their feed with considerably less chewing than young ruminants. Consequently, the need for and benefit from processing grain and forage is greater for adult ruminants than young, growing animals. Similarly, moist feeds generally are consumed more rapidly with less extensive chewing than dry, coarse feedstuffs. In contrast, high moisture feeds and forages can be readily swallowed with little chewing. Because they have smaller mouths and consume smaller bites and they spend more time chewing, the extent and effectiveness of chewing prior to swallowing, is greater for smaller ruminant species (sheep, goats) than larger species. Hence, the digestibility response to feed processing generally is less for the young and for smaller ruminant species than for adults and for larger ruminant species.

## *Rumination and Particle Retention*

During rumination, moistened partially fermented feed particles are re-chewed. Because ruminated particles are moist, particle size reduction is more extensive than with dry particles. The circular jaw motions during rumination allow molars to grind and pulverize particles via both shear and crush forces. Churning of ruminal contents by the extensive ruminal muscles also reduces particle size by abrasion among feed particles and by contact with the rumen wall.

The duration of time animals spend ruminating is proportional to the quantity of fiber floating in the ruminal raft. This is because the rumination reflex is stimulated by sensory (scratch) receptors in the cardia area of the rumen. Dry, fibrous particles that enter the rumen being buoyant will float and become entangled in the floating fibrous mat in the rumen. In contrast, dense particles, intact grains and concentrates can readily sink in rumen fluid and often are flushed from the rumen to the omasum rapidly. Within the rumen, particles near the omasal orifice will be swept from the rumen with fluid whenever the omasal orifice is open. Because most particles leaving the rumen and found in feces are smaller than 1.14 mm, omasal filtration in the past was proposed to be one method by which the rumen selectively retained larger feed components for prolonged fermentation. However, whole grains (>6 mm geometric mean diameter) often are found in feces of cattle fed grain and concentrate particles can be recovered from the abomasum during or immediately after a meal. This presumably reflects the fact that ruminal location, not omasal filtration, dictates the likelihood that a specific feed particle will leave the rumen. Location of particles within the rumen varies with density or buoyancy of the particle, its size, and its entanglement with the ruminal mat. The optimal specific gravity of particles for ruminal exit is between 1.0 and 1.2; particles with greater density (e.g., metal, sand) will settle and be retained longer in the ventral rumen while lighter, more buoyant particles float in the rumen and be retained longer. Continual mixing and churning of ruminal contents pumps liquid digesta containing particles from the reticulum over

the ruminal mat allowing digesta to percolate through the mat. Size and shape of the particle as well as composition and thickness of the ruminal mat size dictate the degree that particles are caught in the mat and selectively retained within the rumen. Though forage concentration in the diet and other forage characteristics associated with particle size and forage type will alter the thickness and porosity of the raft, these factors have received limited research attention. Ruminal stratification along the horizontal plane with the ruminal mat that is present when coarse fibrous materials are fed is widely recognized. However, when ruminants are fed concentrate-rich diets or only finely ground and dense fiber, the rumen may have no ruminal raft.

In addition, to horizontal stratification, vertical stratification within the rumen also seems evident. Undigested particles leave the rumen by passing through the omasal orifice. That orifice is located near the junction of the reticulum and rumen in close proximity to the point that the esophagus enters the rumen. (See discussion of the cardia and the closure of the esophageal groove in Chap. 1 of this book.) Being near the point where feed, water, and remasticated boluses enter the rumen, digesta near the cardia has higher moisture content than other portions of the rumen, likely due to incomplete ruminal mixing of salivary fluids and drinking water from this area with the full contents of the rumen. Rapid omasal exit of fluid from this area shortens the ruminal retention time for such fluids as well as any dense, fine particles found within this region of the rumen. A shortened ruminal retention time for consumed water than for total water within the rumen also may be the result of horizontal and vertical stratification of ruminal contents. How diet and degree of ruminal fill physically alter stratification and retention time need further research attention.

## *Digestion and Fermentation of Various Feed Components*

Most components of feeds and forages exist as polymers (Table 3.3). In contrast, microbial fermentation involves degradation of monomers. The extent to which polymers are degraded to monomers within the rumen differs with substrate type. Before being fully fermented, carbohydrates must be hydrolyzed to glucose or trioses that subsequently are fermented; intact proteins are hydrolyzed to amino acids that are rapidly deaminated forming ammonia and linear or branched chain VFA; nucleic acid polymers are hydrolyzed to purines, pyrimidines and phosphorus; and accessible lipids are partially hydrolyzed to free fatty acids and glycerol within the rumen. Extent of degradation of a polymer also differs with the time for and accessibility of components for microbial or enzymatic attack. Once released, monomers or dimers are rapidly fermented if microbes can generate ATP from their fermentation. (Lack of ATP yield can explain why triglycerides composed of saturated lipids are not readily or rapidly hydrolyzed.) In discussion below various factors that influence the rate and extent of degradation of polymers will be outlined and fermentation of monomers to various products will be discussed. Monomer degradation always occurs internal to microbial cells, but much of the degradation of polymers occurs externally. However, this distinction is not universal. For example,

protozoa act as micro-ruminants continually engulfing and digesting both small feed particles and bacteria. And certain polymers (proteins, soluble starch) may be internalized intact before being degraded by certain species of ruminal bacteria. In contrast, coarser feed components (e.g., fiber), due to their immense size relative to bacteria, are attacked by enzymes external to but yet associated with bacteria. The shortened polymers or dimers when released are immediately internalized by adjacent bacteria, cleaved, and fermented.

## *Degradation of Polymers*

Food components (e.g., sugars, amino acids, some polymers) that are soluble in rumen fluid as well as the soluble cell contents released when cell walls are breached are readily and rapidly attacked. True digestion of the non-starch cell contents in the total digestive tract is very high (98 %). Relative to true digestion, apparent digestion values always are lower because some fecal material originates from the animal's body, not just the diet. So fecal matter includes not only the residue remaining from undigested feed but also metabolic matter from the animal due to incomplete re-digestion and resorption of secreted enzymes and lipids as well as sloughed intestinal cells that are abraded by passing digesta. Extent of degradation of polymers to monomers both in the rumen by microbial digestion and in the intestines by secreted enzymes will vary among polymer types as well as characteristics and accessibility of the polymer. Because microbes and enzymes are transported in an aqueous media, any components that inhibit water access (hydrophobicity) hinder both the rate and extent of digestion.

### Starch Hydrolysis

Of the dietary starch consumed, from 40 % to 90 % typically disappears before leaving the rumen. Rate of degradation of starch will vary with microbial accessibility of the starch granules (faster with finely ground grains), degree to which fed starch is embedded within a hydrophobic protein matrix (slower from more vitreous or flint corn or sorghum grains than from softer grains; slower for corn and sorghum than for other cereal grains), the degree of degradation of the prolamins that encapsulate starch granules (faster with fermented grain or silage), and time for ruminal fermentation (less with shorter ruminal retention time). High feed intake of diets rich in NDF, through reducing ruminal retention time of particles, reduces the extent of ruminal digestion of both concentrate and forage particles. Electron microscope photos from McAllister et al. (1994) clearly illustrate that ruminal bacteria bore holes into starch granules to hydrolyze starch. Rate of degradation also differs with starch structure. Amylopectin, with a branched structure exposes more non-reducing terminal glucose molecules for endoglucanase attack than the more linear amylose. Hence, amylopectin is more

rapidly and extensively fermented than amylose. Extent of ruminal starch digestion is considerably greater for small grains (oats, barley, rye) than for grains that have a larger fraction of starch in the vitreous or flint form (sorghum grain, dent or flint corn). Starch gelatinization (i.e., disruption of starch granules through heat and steam processing of grains) increases the amount of starch exposed for digestion and increases rate and extent of starch digestion, especially for grains with a high proportion of vitreous starch. However, during the flaking process, surface smearing of the starch with melted prolamin may retard microbial access to some of the starch present. With ensiled high moisture grain or corn silage, the fermentation process disrupts surface proteins and decreases hydrophobicity so rate and extent of starch digestion within the rumen are increased. Increased exposure for attack by ruminal bacteria can explain the greater ruminal starch digestion of steam flaked and high moisture maize than dry rolled maize (84 % and 78 % versus 68 % of dietary starch averaged across published trials with feedlot cattle). In contrast, with lactating cows, ruminal starch digestion with these same processed grains has been reported to be 57, 87, and 52, all being lower due to shorter retention time for ruminal digestion combined with the time lag involved with moistening of consumed dry feeds.

Within the intestine, hydrolysis of starch by intestinal beta amylase yields the dimer maltose that subsequently is hydrolyzed to glucose by maltase. In contrast, degradation of starch within the rumen may not involve maltase but instead appears to be driven primarily by endoglucoamylases (that cleave glucose links within the chain) and exoglucoamylases (that cleave glucose from the reducing ends) yielding glucose directly, not with a maltose intermediate.

Both bacteria and protozoa possess amylases. Because protozoa engulf small feed particles, finding amylase internal in protozoa that hydrolyze starch is not surprising, but amylase also has been located within ruminal bacteria. Nevertheless, photomicrographs indicate that digestion of starch granules usually involves external or surface-bound amylases. By increasing the concentration of free amylase in rumen fluid, microbial lysis has been proposed to increase the rate of glucose release and thereby might increase the likelihood of ruminal lactic acidosis. That will be discussed further in Chap. 5 of this book.

### Cell Wall Degradation

Plant cell walls are the least extensively digested organic components found in feeds. Chemical links between hemicellulose and lignin through ferulate ester and ferulate ether bonds both physically and chemically restrict microbial access to cell wall components and retard or inhibit cell wall digestion. Although free hemicellulose is fermented more rapidly than cellulose in vitro, the extent of degradation of these two components of NDF from grasses in the total digestive tract in vivo typically is quite similar in magnitude. Presumably, this reflects steric interference with attack of closely adjacent structures. Treatment with base (sodium hydroxide; calcium oxide) cleaves some of the linkages between hemicelluloses and lignin and deacetylates lignin. Likewise, grasses or legumes

with lower lignin content (e.g., brown midrib mutants) have an increased rate of digestion of NDF and contain less indigestible NDF. Pectin, more prevalent in legume than grass cell walls, is fermented by pectinases within the rumen even though pectin appears to resist degradation during fermentation of ensiled legumes. Lignin, a polyphenol and not truly a carbohydrate, resists anaerobic digestion but, as mentioned earlier, can be degraded aerobically. Lignin can be attacked or solubilized to a limited degree by ruminal fungi presumably through the action of peroxidases, but lignin is not digested by bacterial or protozoal enzymes. Lignin is not uniformly distributed within all plant tissues; more lignified fragments or tissues are more resistant to attack. Because lignin is not extensively fermented or digested, virtually all dietary lignin is excreted in feces. Hence, lignin can be used as an indigestible marker internal to feeds to calculate the digestibility of other feed components by difference. When all feces are collected, lignin also can be used as an indicator of feed intake of grazing ruminants if lignin intake is quantified through analysis of a representative sample of clipped forage samples or of esophageal samples. Differences in its size of lignin polymers and reduction in size can complicate plant analysis and lead to incomplete recovery of lignin from feces.

Degradation of the pentosans in pectin and hemicelluloses, like degradation of the hexosan cellulose, appears to involve complexes of multiple enzymes generally classed as hemicellulases and beta-glucanases. Like cellulases, these enzymes presumably are surface bound or associated with the glycocalyx surface films of specific bacterial strains. The ratios of specific pentose sugars in hemicellulose (arabinose and xylose), whether through steric hindrance or cross linking, can alter the susceptibility of hemicellulose to hydrolysis. Likewise, cellulose exists in both an amorphous and a crystalline type with various cellulolytic bacteria differing in their capacity for fermenting the more resistant crystalline cellulose. Pentosans appear to be degraded directly to component monomer pentose sugars whereas cellobiose, 1,6-beta linked glucose, is an intermediate in cellulose degradation. Furthermore, pentosans appear to be largely converted metabolically to hexose and triose through the pentose cycle before being fermented.

Bacteria must attach to cell walls before digestion can begin. Attachment may be limited because both feed particles and bacteria appear to be negatively charged. Based on in vitro studies, additions of bicarbonate, biotin, and trace minerals appear to accelerate bacterial attachment to feed particles. Methods to accelerate the rate of attachment of ruminal microbes to fiber deserve further research attention because reducing the time lag before fermentation begins allows rate and extent of ruminal fiber digestion to be enhanced. Fractures of the cell wall or pores in cell tissues help to accelerate cell wall hydrolysis. Because the cellulolytic microbes within the rumen associated with plant tissues typically are found internal to plant tissues, they are envisioned to digest fiber from the inside outward. Such an internal location also helps protect bacteria from protozoal predation. Alternatively, their internal location helps to prevent bacteria from being dislodged during sample preparation for microscopic examination.

## Lipid Hydrolysis

Lipids isolated from oilseeds and animals are primarily tri-acylglycerides while lipids of growing plants and forages include substantial amounts of phospholipids and galactolipids. Triglycerides are hydrolyzed to a varying degree within the rumen. Lipids within oilseeds are hydrolyzed only partially to diglycerides and monoglycerides while triglycerides added to the diet as an oil appear to be completely hydrolyzed to glycerol and free fatty acids within the rumen. Heat treatment of oilseeds reduces the extent of lipid degradation within the rumen, likely due to some alteration of proteins associated with the seed, not due to some direct impact on the oil. Ruminal lipolysis appears more extensive when oils are dosed more frequently; this reflects the concept that hydrolysis by ruminal bacteria or protozoa is less aggressive for lipids than of carbohydrates. Released long-chained fatty acids that are saturated are largely inert within the rumen. Compounds (amino acids; vitamins) can be coated or complexed with lipid; being protected from microbial attack, such compounds are marketed widely as "ruminal escape" compounds or complexes. In contrast with the largely insoluble long chain fatty acids, medium chain length fatty acids can prove toxic to some species of ruminal bacteria. The ruminal population of protozoa can be decimated (partially or completely defaunated) through feeding proper dosages of lauric and myristic acids.

Although free fatty acids released during lipolysis that are saturated are not degraded further within the rumen, most unsaturated fatty acids are fully or partially saturated by ruminal bacteria and protozoa. Hydrogenation of unsaturated fatty acids is one process by which ruminal microbes dispose of excess hydrogen. Disposal of reducing equivalents is a persistent bottleneck for anaerobic microbes. Besides biohydrogenation, other methods that ruminal microbes employ for hydrogen disposal include methane formation from carbon dioxide and sulfate or nitrate reduction to hydrogen sulfide and to nitrite or ammonia. These processes are retarded slightly when unsaturated fatty acids or triglycerides are included in a ruminant's diet. The quantitative significance of biohydrogenation of unsaturated fatty acids as a method for hydrogen disposal appears minor compared to these other reactions likely is due to the limited supply of unsaturated lipids in the typical diet and the limited number of sites to hydrogenate.

During microbial biohydrogenation of linoleic and linolenic acids in the rumen, byproducts including conjugated trans-fatty acids (conjugated linoleic acid; CLA) are formed that are absorbed and may be further metabolized and deposited or secreted by ruminants. Being a general class of fatty acids, conjugated fatty acids or their precursors of multiple types exist that differ in the location of their double bond, the structure of that double bond (cis or trans), and the number of double bonds. These factors alter biological activity. Certain CLA possess anti-cancer activity; others depress fat synthesis by the mammary gland and reduce the percentage of fat in secreted milk. Industrially, trans-fatty acids are produced when unsaturated plant oils are hydrogenated. Hydrogenation decreases fluidity and increases shelf life of food products. However, industrially produced trans-fatty acids have adverse effects on cardiovascular health. In contrast, the trans-fatty acid

CLAs found in milk and meat from ruminants, being potent anticancer compounds, are highly desirable and healthful food components.

The fatty acid composition of deposited triglycerides in all species of animals reflects the composition of those fatty acids synthesized by the body when merged with those fatty acids absorbed from the small intestine. As intake of fat increases, biosynthesis by the liver in non-ruminant species or by depot tissues of animals decreases so that synthesized fatty acids comprise a smaller portion of the lipid deposited. Including unsaturated fats in diets for swine and poultry can result in "soft" or "oily" fat. This is not the case for ruminants because of extensive biohydrogenation of dietary fats by microbes in the rumen. This in turn means that the fatty acids present in depot fat or milk fat from ruminants is more saturated than fat from poultry or swine. Because intake of large amounts of fat containing saturated fatty acids has been correlated with certain cardiovascular diseases, intake of fat from ruminant animals as well as certain tropical (e.g., palm and coconut) oils, that are even more saturated than fat from ruminants, should be limited according to certain health professionals.

Nearly half of the fatty acids in milk or meat from ruminants are mono- or poly-unsaturated fatty acids. Nevertheless, increasing the concentration of unsaturated and omega-3 fatty acids in the human diet even further has been suggested to have health benefits for some individuals. To increase the concentration of unsaturated or selected fatty acids in ruminant tissues, the postruminal supply of unsaturated fatty acids can be increased or the prevalence of desaturases could be increased. Supply can be increased by feeding coated lipids or heat-treating oilseeds. However decreasing the saturation of ruminant fats simultaneously increases its potential for oxidative rancidity that in turn shortens the shelf-life of milk, milk products, and meat. Increasing the dietary supply of certain antioxidants (e.g., vitamin E) that are deposited in tissues or excreted with milk helps to retard oxidative rancidity and lengthen the shelf life of ruminant products.

Net energy content per unit of weight is greater for lipids than for protein or carbohydrate. Consequently, increasing the concentration of dietary lipid to as much as 6–8 % of feed dry matter will increase net energy content of the diet. This in turn often decreases feed (but not energy) intake. Concurrently, adding fat to the diet often increases carcass value by increasing the dressing percentage (ratio of carcass to live weight) and may increase deposition of intramuscular fat to enhance meat flavor. Dietary lipid concentrations above 8 % often result in decreased fat digestibility, perhaps due to insufficient saponification or lack of lipase in the small intestine. Supplemental lipids often decrease digestibility of fiber, as well. Whether this decrease is due to lipid physically coating fiber particles or antimicrobial activity of fatty acids is uncertain. High intakes of fat from certain unsaturated oilseeds (sunflower, cottonseed) and from fish oils occasionally have had adverse effects on milk and on the flavor of cooked or re-heated meat, probably associated with oxidative rancidity.

The amount of fat included in diets formulated on a least-cost basis will vary with the relative cost of net energy from fat versus other sources of energy. When cost per unit of net energy is lower for vegetable or animal fat than other sources of energy including fat in a diet will reduce cost of gain. In addition, when dietary

bulkiness and rumen fill limits energy intake, as early in lactation of high producing dairy cows, added fat, through increasing energy intake, often increases the level of milk production. Addition of various liquids (fat, molasses, water, distillers' solubles) or of moist feed (wet byproducts, silages) to a diet often is desired to reduce separation of fine particles from the fibrous components of the diet. By preventing diet sorting by livestock and avoiding segregation of diet components during mixing or in the feed manger, the incidence of metabolic disorders (bloat, acidosis) usually is reduced. Diet sorting, though difficult to detect with group-fed ruminants, can lead to imbalanced diets whereby some animals in a pen will select a high concentrate diet leaving others with the residual fiber or vice versa. Diet sorting also can be reduced by decreasing the supply of excess feed, reducing the diversity in particle size of the various feed ingredients, or by providing adequate bunk space so that all animals within a pen can eat simultaneously. To reduce heat stress of high producing lactating cows, fat often is added to the diet. Because fat is not fermented as extensively is carbohydrates in the rumen, heat from ruminal fermentation is reduced and the heat increment during metabolism also is lower for fat than for carbohydrates because fatty acids can be deposited directly avoiding the need for fatty acid synthesis from carbohydrate that generates heat.

For high producing lactating cows, including oilseeds (soybeans, cottonseed) as a source of dietary fat in the diets helps to increase energy density of the diet and feed intake early in lactation when heat production is high and bulk fill may limit feed intake. Instead of oilseeds, most lipid included in feedlot diets is supplemented as vegetable oil, tallow of various grades, or blends of vegetable and animal fats. Antioxidants (e.g., ethoxyquin) usually are included in such fat sources to avoid oxidative rancidity that can reduce feed intake due to altered odors and flavors of feeds. Byproducts of ethanol production also contain substantial amounts of fat; this can explain partially why such products appear to have more net energy per unit of weight than the grains used to produce ethanol. Further discussion of ruminal digestion and metabolism of fat is available from Palmquist and Jenkins (1980), Bauchart (1993), and Bauman and Lock (2006), and in Chap. 4 of this book.

### Protein Hydrolysis

Crude protein, by definition, is nitrogen content of a sample multiplied by 100/16 = 6.25 (based on the fact that peptide-linked amino acids chains contain about 16 % N). Because N is used as the index of protein content of a food or feed mixture, crude protein includes numerous compounds such as non-protein N (urea, biuret, ammonium salts, amino acids, and purines), as well as the peptide-linked amino acids of true protein. True proteins in turn differ in amino acid content, structure, and composition. Structure and amino acid composition in turn alter solubility of a protein in various solvents, one standard method used to classify proteins. Among various protein types, proteins more soluble in water or salt solutions (albumens and globulins) are more extensively degraded by ruminal bacteria than less soluble protein types (glutamines, glutelins). This supports the

general concept that compounds that are soluble in ruminal fluid are more likely to be attacked and degraded by ruminal bacteria. Because protein solubility also varies with pH of the solvent, differences in ruminal pH, through altering protein solubility, also alters the extent of ruminal degradation of a protein sources. Strangely, soy proteins generally are more soluble at a neutral pH whereas the proteins from corn grain tend to be more soluble at a lower pH. In contrast to bacteria that digest soluble protein and attach to and attack feed particles from the outside, protozoa engulf feed particles (as well as bacteria) and readily digest all protein types. Predation of bacteria by protozoa leads to intra-ruminal degradation of bacterial protein, a very inefficient process energetically. As a result, ruminal defaunation (reduction or obliteration of protozoa through dietary manipulation or additives) often improves energetic efficiency and reduces the amount of dietary true protein that needs to be supplemented. The population of protozoa within the rumen typically is lower for ruminants fed concentrate-rich than forage-rich diets. This difference should reduce the amount of true protein degraded in the rumen, increase the supply of both dietary and microbial protein that reaches the small intestine, and decrease the need for dietary protein. Nevertheless, the crude protein content of diets fed to feedlot cattle has tended to increase in recent years, particularly when the grain being fed is extensively processed (e.g., steam flaked). Much of this added protein is urea that is degraded to ammonia within the rumen. Precisely why the added ammonia might be beneficial is uncertain. Certainly, additional ammonia is needed when grains are processed to meet the increased need of ruminal bacteria for growth with more extensive ruminal fermentation of starch. In addition, ammonia will serve as a base to neutralize ruminal acids and increase urinary output. This in turn will increase water intake and ruminal fluid turnover. Synthesis of urea also may prove beneficial for maintaining a proper blood acid-base balance. Dietary urea also appears to reduce meal size and increase meal frequency, factors that may increase mastication time and production of saliva, and extent of ruminal and total tract digestion.

Cyclic proteins (e.g., ovalbumin) with no exposed terminal amino acids are not degraded by bacteria. Likewise, processing methods that block terminal amino acids, that amalgamate proteins to reduce solubility, or that encapsulate protein or amino acids can retard degradation of dietary proteins. Surprisingly, proteins with a longer chain length often are more rapidly and extensively degraded than shorter peptides, perhaps due to more rapid or efficient uptake of longer amino acid chains by certain species of bacteria. Strangely, many of the actively proteolytic bacterial species in the rumen cannot use amino acids for growth. This indicates that some proteolytic species must be using the liberated amino acids as an energy source for generating ATP for microbial growth and multiplication. However, the quantitative importance of protein as an energy source for growth of ruminal microbes appears limited. Some models developed to predict microbial yield from ruminal fermentation are based only on carbohydrate and ignore any contribution of ATP from degraded protein or fat.

Like proteolytic enzymes of mammals, the proteolytic enzymes of ruminal microbes are of two general types: those that attack amino acids at the end of a protein chain (exopeptidases) and those that cleave peptide linkages between

specific amino acids or amino acid types (endopeptidases). With mammals, the activity of specific endopeptidases can be inhibited by providing peptides or peptide analogs that cannot be hydrolyzed. Such inhibitors have failed to reduce proteolytic activity within the rumen. Some researchers have interpreted this to mean that ruminal microbes possess diverse types of proteases and thereby cannot be inhibited. However, any inhibitor that is not internalized by microbes would not be located at the primary site of proteolysis.

Diets for ruminants often contain non-protein nitrogen (NPN) products as a source of ammonia for bacterial growth. Whether derived from the diet or from recycling to the rumen via saliva or diffusion from blood through the rumen wall, urea is hydrolyzed rapidly to ammonia and carbon dioxide by high populations of ureolytic bacteria in the rumen. Ruminal entry of urea from blood is enhanced by facultative ureolytic bacteria associated with the ruminal wall that hydrolyze urea to ammonia near the rumen wall; released ammonia is rapidly ionized and removed into the rumen because ruminal pH is always lower than the pK (9.3) of ammonia.

Rate of urea hydrolysis within the rumen is reduced by adaptation of ruminants to diets containing urea. Adaptation of animals to urea diets results in a reduced meal size but an increased meal frequency, changes should help reduce the incidence of ammonia intoxication. Urea hydrolysis also is subject to end-product inhibition. Consequently, most cases of urea (ammonia) intoxication involve animals that are not adapted to diets containing urea or animals gaining access to a diet that contains an excessive amount of urea. With abrupt intake of urea and hydrolysis to ammonia, ruminal ammonia concentrations can exceed 100 mg/dl. When combined with a high ruminal pH that speeds ammonia absorption, ammonia toxicity (often erroneously called urea toxicity) can occur. Ruminal fluid has limited base buffering capacity above a pH of 6.9, so ammonia, acting as a weak base (pK = 9.3), readily elevates ruminal pH. This ruminal pH increase is relevant because ruminal absorption always is greater for non-ionized compounds (ammonia, free fatty acids) than for their ionized counterparts (ammonium, ionized fatty acids). Ammonia will be absorbed more rapidly than the ammonium ion. (Conversely, as ruminal pH drops, more fatty acids are protonated and thereby are more rapidly absorbed from the rumen.) Ammonia intoxication can be avoided by dosing animals with an acids (e.g., vinegar) that, by reducing the amount of protonated ammonia, will reduce ruminal absorption. The liver readily and actively detoxifies any ammonia in blood to urea and prevents its accumulation. Presumably, ammonia uptake by the lymphatic system, by circumventing liver detoxification, is responsible for elevated blood ammonia levels that cause recombency and death associated with ammonia (urea) toxicity.

To retard rate of ammonia release within the rumen and avoid ammonia toxicity, modified NPN sources and slow-release encapsulated urea often are substituted for urea in the diet. Like urea, uric acid from poultry waste also is degraded to ammonia that can result in ammonia intoxication, but other NPN sources or complexes (biuret, triuret, cyanuric acid) are slowly degraded by ruminal microbes. Microbes usually adapt to such products after several days or weeks. Coated urea

products, though useful to prevent ammonia toxicity, have proven to be no more useful than urea as a source of ammonia for bacterial growth or for ruminant production. Urea recycling to the rumen is extensive when a diet contains an adequate concentration of either digested protein or urea. Slowing or attenuating the ammonia release rate of NPN compounds in the rumen to match the rate of carbohydrate degradation, though an intriguing theory supported by lab experiments and batch culture fermentation studies, is not supported by trials with animals. This likely is due to the high extent and efficiency of recycling of urea to the rumen. Under field conditions, high meal frequency (often 6–12 meals) and continuous digestion of a large mass of digesta in the rumen accumulated from previous meals helps to avoid asynchrony between carbohydrate and protein availability for ruminal microbes (Fig. 3.2). Through gradually releasing its ammonia, encapsulated urea also may help buffer ruminal pH postprandially. Thereby, attenuated ammonia release may inhibit decreases in ruminal pH that inhibit activity of cellulose digesting ruminal bacteria.

Similar to proteins, peptides are degraded to amino acids when incubated with ruminal fluid. Calculated as crude protein minus ammonia and precipitable protein, peptides differ in amino acid composition. Even though concentrations of free amino acids usually are too low to be detected in rumen contents, peptides often reach detectable concentrations in rumen fluid indicating that peptides are less rapidly degraded than amino acids. Though peptides appear to be required for growth of certain isolated strains of ruminal bacteria, the background concentrations of peptides in rumen fluid when combined with cross feeding among bacterial species appear to supply more peptides than needed to meet nutrient requirements of the mixed microbial cultures found in the rumen. Nucleic acids from RNA and DNA in the diet or synthesized by microbes within the rumen, if free, are degraded to purines and pyrimidines within the rumen. Nucleic acids also can be re-used for nucleic acid synthesis by ruminal microbes. A schematic of ruminal N interchange was developed by Satter (1978) as shown in Fig. 3.3 and a summary of nitrogen metabolism of ruminants was compiled by Owens and Bergen (1983).

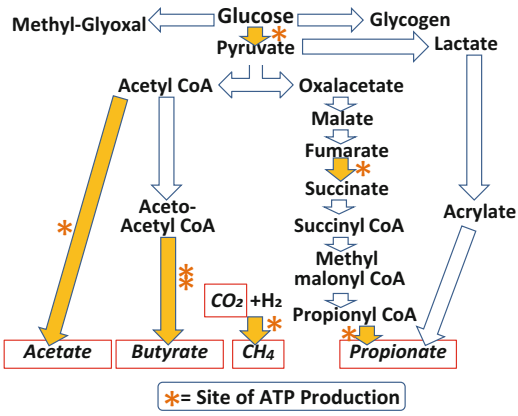



**Fig. 3.2** Ruminal conversion of available sugars to fermentation products. See Baldwin and Allison (1983) and Wolin (1960) for further details

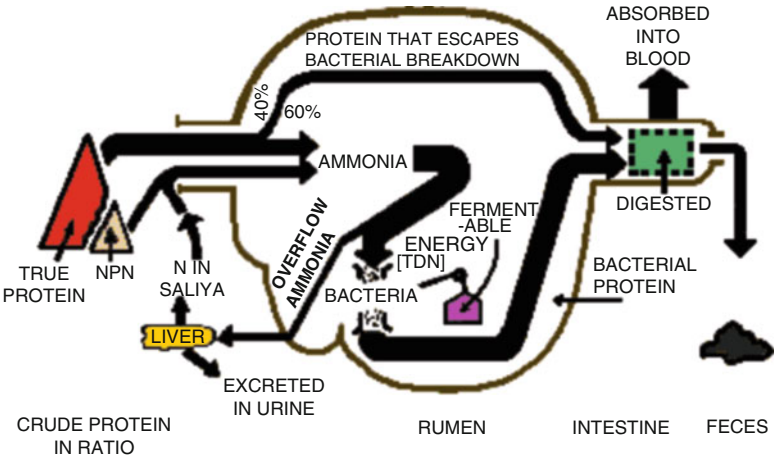



**Fig. 3.3** Schematic summary of nitrogen utilization by the ruminant. Source: Satter 1978. Minnesota nutrition conference proceedings

## Minerals

Availability of monovalent and divalent cations to microbes within the rumen differs with their solubility; soluble cations are readily available while insoluble cations and anions (e.g., defluorinated phosphate; sulfides; some carbonates) are not. However, minerals that can be solubilized by acid in the abomasum and absorbed in the intestines (e.g., defluorinated phosphates) can be used when they are recycled to the rumen via saliva. Similarly, RNAse produced in high concentrations by the ruminant pancreas, degrades microbial nucleic acid polymers. These phosphates may comprise as much as 4 % of bacterial dry matter. Liberated phosphorus is recycled to the rumen. Within the rumen, soluble sulfates from water or consumed feed are partially reduced to sulfide within the rumen for disposal of excess hydrogen. These sulfides also react with various divalent cations (e.g., copper, zinc) forming sulfides that are insoluble and thereby are not available for microbes in the rumen and are not absorbed from the intestine. In a similar fashion, selenate and selenite are partially reduced to selenide within the rumen. These also complex with cations reducing the availability of those cations. When present at high concentrations and when ruminal pH is low, hydrogen sulfide gas is released in the rumen. When inspired into the lungs during eructation of gases, hydrogen sulfide damages lung tissues and together with absorbed sulfate can cause polioencephalomalacia and possibly other persistent lung problems including emphysema.

Mineral supplements provided to ruminants typically are provided as inorganic salts, but minerals chelated with amino acids or protein also are marketed widely. Chelation with amino acids or protein may increase bioavailability of certain minerals through avoiding the formation of insoluble salts; chelation also may help to

avoid antimicrobial effects of certain cations in the rumen. However health, nutritional or performance benefits of chelated or protein bound minerals over inorganic forms of the same mineral have seldom been demonstrated. Acid conditions of the abomasum and upper small intestine appears sufficient to solubilize most mineral salts although certain insoluble salts (sulfides, some carbonates) may not become soluble and thus are not absorbed. For optimal cellulose digestion, supplementation with certain minerals (e.g., cobalt to speed bacterial attachment) may prove beneficial. Because ionophores stress sensitive microbes by increasing their energy requirements by causing influx of sodium (or calcium), higher ruminal concentrations of certain cations may increase their effectiveness.

### Monomer Fermentation

Polymer degradation yields a mixture of monomers (amino acids, glucose, monovalent cations, some divalent cations). Although all of these monomers can be absorbed passively through the ruminal wall, the ruminal concentrations of amino acids and glucose found in the rumen usually are so low that direct absorption is quantitatively insignificant. Such low ruminal concentrations reflect rapid catabolism of these compounds by ruminal microbes. In contrast, ammonia, sodium, and potassium are readily absorbed through the rumen wall. As with certain polymers, degradation of certain monomers can be an adaptive response of the ruminal population. Thus, in vitro findings may not prove applicable in vivo when ruminal microbes are not adapted to the novel substrate being tested (e.g., amino acid derivatives). Ruminal microbes exhibit an immense ability to adapt to and degrade any organic substance that can be reduced. Note that fully saturated compounds (e.g., fatty acids, polyethylene glycol) and insoluble substances (e.g., protein complexes, plastics) generally resist ruminal fermentation. This permits such compounds to be used as coating materials to enhance ruminal escape of dietary substances of interest.

## *Glucose*

Stepwise conversion of glucose to VFA is illustrated in Fig. 3.2. Once liberated within the rumen, glucose normally is catabolized very rapidly. Some bacterial species accumulate glucose, polymerize it to bacterial glycogen, particularly when those microbes are facing a shortage of some nutrient (e.g., ammonia deficiency) that limits microbial growth. When bacteria containing glycogen or when protozoa containing engulfed starch particles are flushed from the rumen, these stored polymers serve to supplement other polysaccharides that escape ruminal digestion. Thereby, microbial polymers of glucose serve as a source of glucose for the host ruminant to metabolize. Glycogen storage by bacteria is considered to be an energetically inefficiency process. By sequestering glucose, glycogen synthesis uses ATP and glycogen storage reduces the supply of energy immediately available for

growth of a bacterial species. As a result, bacterial yield and yield of microbial protein for the animal is reduced. A second more sinister pathway some ruminal bacteria employ when supplied with an excess of glucose is synthesis of methyl glyoxal, a compound that is toxic to most bacteria (Russell 1998). Appearance of methyl glyoxal in the rumen often precedes ruminal stagnation and ruminal acidosis.

Although the concentration of free glucose in the rumen of productive ruminants usually is very low, often too low to be detected, concentrations can increase to more than 100 mg/dl following grain engorgement. When ruminal glucose concentrations become abnormally high, certain bacterial strains (e.g., Streptococcus bovis) that are inefficient at competing with other rumen microbes under normal conditions, will grow rapidly and produce a copious amount of lactic acid. This can result in subclinical or clinical ruminal acidosis. The elevated ruminal osmolality associated with the high concentrations of free glucose, volatile fatty acids and glucose in the rumen, draws liquids into the rumen. Dehydration of the ruminal epithelium and high localized acid concentrations can damage ruminal tissues resulting in erosion of the stratified epithelium of the rumen, damage the rumen wall, and result in sepsis that can lead to liver abscesses.

When glucose is incubated with ruminal bacteria being grown in pure culture, a wide diversity of fermentation products (succinate, malate, hydrogen gas, ethanol, methanol) often are produced by individual strains (Hungate 1966). But when incubated with mixed cultures or with the complete spectrum of ruminal microbes, the diversity of end-products is markedly reduced due to cross-feeding among bacterial species. Normal end-products of glucose fermentation in the rumen are four major volatile fatty acids (VFA), acetate (2 carbons), propionate (3 carbons), butyrate (4 carbons), valerate (5 carbons) in addition to carbon dioxide and methane.

When starch-rich feedstuffs with very rapid fermentation rates are fed or following engorgement of starch-rich feeds, ruminal acidosis can result. Accumulation of acids, often but not always including lactic acid, reduces ruminal pH, hinders activity of acid-sensitive ruminal microbes (this includes all cellulose digesting bacteria), and halts ruminal motility. When ruminal pH falls below about 5.5, the condition is classified as subacute acidosis; a ruminal pH below 5.0 is diagnosed as acute acidosis and often proves fatal. More detailed discussion and summaries relating to the causes, treatment, and prevention of ruminal acidosis can be found in various publications (Owens et al. 1998; Schwartzkopf-Genswein et al. 2003; Oetzel 2007; RAGFAR 2007) and in Chap. 5 of this book.

## *Fermentation Balance Based on VFA Production from Glucose*

Because most of the energy available for ruminal microbes from diets fed to ruminants is derived from carbohydrate, ruminal conversion of glucose, pentoses, and trioses to volatile fatty acids is the primary source of energy (ATP) for ruminal microbes (Fig. 3.2). If one ignores the carbohydrate converted to microbial organic matter, one can calculate a "fermentation balance" that fully describes the conversion

**Table 3.4** Fermentation balance based on VFA produced from glucose

| | Fermentation products | | |
|---|---|---|---|
| | Acetate | Propionate | Butyrate |
| Molar ratio of products | A | P | B |
| Glucose used, moles | 0.5A | 0.5P | B |
| Gas yield, moles | A | 0 | 2B |
| Carbon dioxide, moles | .5A | .25P | 1.5B |
| Methane yield, moles | 0.5A | −.25P | 0.5B |
| NADH produced, moles | 2A | −1P | 2B |
| ATP yield, moles | 2.5A | 2.75P | 3.5B |
| Potential microbial yield at 10 g/mole ATP | 25A | 27.5P | 35B |
| Glucose energy used, mcal | 0.3365A | 0.3365B | .673B |
| Energy in VFA, mcal | .2094A | .3672P | .5243B |
| Methane energy lost, mcal | .1054A | −.0527P | .1054B |
| ATP energy, mcal | .0175A | 0.01925P | .0245B |
| Heat + ATP, energy | .0217A | .022P | .0433B |
| Heat loss, mcal | .0042A | .00275P | .0188B |

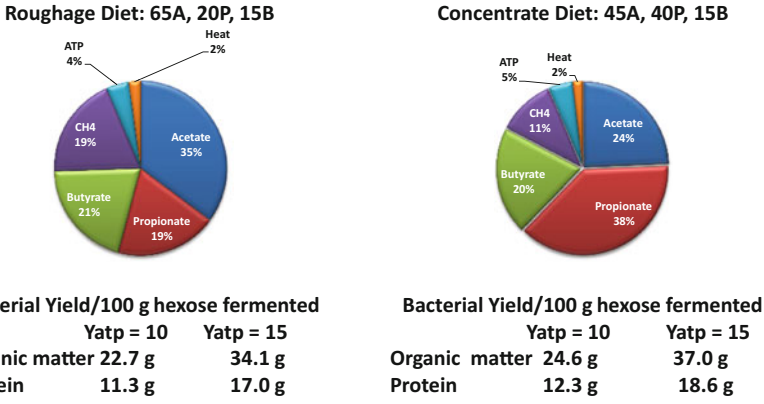
of glucose to VFA (Wolin 1960), the amounts of gases (carbon dioxide plus methane), and the presumed yield of ATP that is available for microbial growth (Table 3.4). Based on these balance equations and the relative oxidation state of the substrates and products, glucose fermentation must yield a specified mixture of VFA and gases. The VFA ratio in turn will vary with substrate type, substrate concentration, and fermentation conditions, particularly pH. The ratios of the VFA produced stoichiometrically dictates the specific amount and the composition of the gases released, energy retention in the fermentation products, and the yield of ATP available for microbial growth. These relationships are illustrated in Table 3.4. By inserting mole ratios of the end products of fermentation that are observed, i.e., acetate, propionate, and butyrate, for A, P and B into each of the formulas of Table 3.4, glucose use and energy yields can be calculated per mole of glucose fermented. Note that these are ratios of VFA produced. The exact ratio of VFA in a sample of ruminal fluid can differ slightly from true ratios produced if and when the rates of ruminal absorption of various VFA differ (Firkins et al. 2006). Consequently, considering VFA concentrations to represent relative VFA production rates can prove erroneous though the magnitude of error generally is reasonably small. To calculate total yield of products daily from a specified diet, one also must determine the total amount of carbohydrate that has been fermented within the rumen or the total amounts of VFAs formed. This can be calculated from disappearance of carbohydrate from the rumen (intake of carbohydrate minus outflow—at the abomasum or duodenum—of carbohydrate).

Though a mixture of VFA is formed during fermentation of glucose, the composition of end-products dictates the amount of hexose that has been fermented, the energetic efficiency of fermentation, and the amount of ATP

available for generating microbial organic matter. The amount of ATP generated, that varies with the VFA ratios formed, must not be confused with the efficiency with which that ATP is used by microbes for growth (Yatp or grams of microbial cells per mole of available ATP).

As shown in Table 3.4, each butyrate (4 carbon atoms) mole formed requires one mole (180 g) of glucose (6 carbon atoms) or 162 g of polymerized glucose as cellulose or starch considering the water involved with hydrolysis. In contrast to only 1 mole of butyrate, 2 moles of acetate (2 carbon atoms) and of propionate (3 carbon atoms) can be formed from a single mole of glucose. All the residual carbon from fermentation of glucose must be lost as gas (carbon dioxide plus methane). Consequently, for each mole of butyrate produced, 2 moles of gas are released. Furthermore, with formation of acetate and butyrate, reducing equivalents (NADH) are formed that must be handled. This excess hydrogen is used primarily to convert carbon dioxide to methane. Four moles of reducing equivalent are used for each mole of methane formed from carbon dioxide. During production of propionate, no gas is generated but unlike acetate and butyrate, some of the excess hydrogen generated during their production is used to form propionate. Consequently, the amount of methane formed from carbon dioxide during fermentation depends on the amount of excess hydrogen (NADH) produced during fermentation of glucose to acetate or butyrate. Other hydrogen acceptors (sulfur, nitrate, unsaturated fatty acids) also can serve as an alternative hydrogen sink and reduce methane production slightly. In essence, the greater the ratio of acetate and butyrate to propionate, the higher the total yield of gas and the higher the proportion of methane within that gas (Fig. 3.4).

Amount of ATP generated differs depending on the ratios among the VFA produced (Table 3.4). Amounts of ATP formed during production of acetate, propionate, and butyrate, after considering the ATP yield from conversion of carbon dioxide to methane using the NADH generated, will be 2.5, 2.75, and 3.5 moles, respectively. On the basis of glucose, for each mole (180 g) of glucose or 162 g of




| Bacterial Yield/100 g hexose fermented | | |
|---|---|---|
| | Yatp = 10 | Yatp = 15 |
| Organic matter | 22.7 g | 34.1 g |
| Protein | 11.3 g | 17.0 g |

| Bacterial Yield/100 g hexose fermented | | |
|---|---|---|
| | Yatp = 10 | Yatp = 15 |
| Organic matter | 24.6 g | 37.0 g |
| Protein | 12.3 g | 18.6 g |

**Fig. 3.4** Fate of fermented energy with roughage and concentrate diets and bacterial yields with Yatp or 10 or 15 g organic matter synthesized per mole of hexose fermented

hexosan fermented, considering the different yields of VFA from glucose, will release 5, 5.5, or 3.5 moles of ATP when the fermentation end product is acetate, propionate, or butyrate, respectively. Alternatively, on the basis of gas production, for each mole of gas released, production of acetate and butyrate yields 2.5 and 1.75 moles of ATP, respectively, but with propionate production, because no gas is released, ATP yield per unit of gas is infinite. Consequently, gas production alone, though suitable as a general index of extent of fermentation and energy value of various feedstuffs, provides an incomplete picture of the amount of ATP generated or the amount of microbial mass formed. Through simultaneously monitoring VFA concentrations and gas yields, potential yield of microbial mass can be estimated thanks to these stoichiometric relationships.

Growth of microbes within the rumen typically is limited by supply of energy (ATP). Carbohydrate conversion to the VFA ratios found during fermentation of fiber and of concentrate feeds should yield 4–4.6 moles of ATP per mole of hexose fermented. With de novo growth (being supplied with only hexose and ammonia), potential maximum cell yield or Yatp calculates to be between 20 to 29 g dry cells per mole of ATP; when supplied with pre-formed organic substances, Yatp should average about 20 % greater or about 29 (Hespell and Bryant 1979). These estimates are based on the calculated requirements for ATP for synthesis or assembly of all polymers found in various strains of bacteria and will differ with chemical composition of the bacteria.

Protein synthesis represents the major expenditure of ATP by bacteria, but lipid biosynthesis also is expensive. The fact that measured Yatp usually is 10–15g or dry cells per mole of ATP, considerably less than the theoretical amount required for assembly of components indicates that a substantial portion of the ATP used by bacteria is expended for functions other than growth (maintenance and replacement of lysed cells). Measured Yatp always is lower than maximum Yatp simply because bacteria, like all organisms, must expend energy for maintenance. Yatp also can differ when bacterial cells differ in chemical composition; strains richer in protein and lipid require more ATP for cell assembly and thereby have a lower Yatp (less cell yield per mole of ATP). In addition, specific nutrient deficiencies that increase energy requirements for bacterial maintenance (e.g., lower pH, slower passage rates, greater protozoal predation, low ammonia concentrations whereby more energy is required for uptake) or conditions that increase lipid, protein, or carbohydrate content of the mixed bacterial population within the rumen all can decrease microbial yield through lowering the Yatp. Bergen (1977) and Dewhurst et al. (2000) have outlined the numerous factors that can alter Yatp as well as laboratory methods for measuring the yield of microbial protein derived from ruminal fermentation.

Generally, growing bacteria synthesize only 10–15 g of bacterial dry mass from each mole of ATP (Yatp = 10–15). This is only 35–50 % of the theoretical maximum Yatp estimates of 20–29 above. This means that between 22 and 42 g of bacteria could be formed from every mole of glucose (180 g) fermented if Yatp is 9–15 and ATP yield is 4.5–5. This equals a bacterial mass yield of equal to 22–42 % of the

organic matter fermented from glucose depending on the specific VFA being produced and the Yatp.

The fraction of glucose energy that is retained within the VFA and becomes available for absorption and metabolism by the animals is equal to 62 % for acetate, 78 % for butyrate, but 109 % for propionate. The value for propionate exceeds 100 % due to transfer of hydrogen to propionate from acetate and butyrate. Methane loss per mole of VFA formed is similar for butyrate and acetate, but per mole of glucose fermented is less per mole of acetate than with acetate than per mole of butyrate produced (16 % versus 31 %). Methane loss would be negative if only propionate were produced! Because high concentrate diets yield a higher proportion of propionate during fermentation, increasing the concentrate level in a ration generally reduces methane production (Fig. 3.4). Other dietary alterations that can decrease methane yield include providing additives that alter the ratio of VFA (e.g., ionophores, malate), management to increase ruminal escape of carbohydrate or decrease the extent of ruminal digestion (e.g., high feed intakes, supplemental tannins), specific compounds that inhibit growth or specific steps in metabolism of methane-producing archea within the rumen, and diet components that serve as hydrogen sinks in the rumen (nitrate, sulfate, unsaturated fatty acids). Note that some of these alterations conserve energy for the animal and thereby should increase efficiency of ruminant production while other alterations do not.

After one subtracts the energy in VFA and methane from the gross energy originally present in glucose, the remainder must be released as either heat or ATP. Some of the energy converted to ATP also is released as heat during microbial metabolism through some energy is retained within polymers synthesized by microbes. Unfortunately, most of the energy used for polymer synthesis by microbes is lost before being of use to the animal due to polymer hydrolysis within the digestive tract prior to absorption by the animal. The minimum heat lost during glucose fermentation as a fraction of gross energy content of glucose based on these relationships will range from 0.8 % for propionate to 2.7 % for butyrate.

The microbial mass and thereby the amount of microbial protein that becomes available for the animal depends on (1) supply of nutrients for microbes (typically limited by energy—ATP—or ammonia), (2) efficacy with which energy from ATP is converted to microbial organic matter and protein in the rumen versus being used for microbial maintenance functions (to maintain cell functions and survive between meals) and (3) degree to which microbial cells lyse or are cannibalized by other microbes within the rumen versus being flushed to the abomasum. The latter two combined represent the overall Yatp within the rumen. Efficiency of microbial protein synthesis, like efficiency of animal growth, is greater when microbes are flushed through the rumen rapidly (harvested younger) so more of the available ATP is used for growth and not for specific maintenance functions (maintaining osmotic pressure, internal turnover of compounds). By increasing the proportion of ATP used for growth rather than maintenance, faster turnover of ruminal microbes causes microbial efficiency (Yatp) to increase and, if extent of fermentation remains constant, the size of the microbial population and microbial yield will increase. Yield of microbial protein for the animal in turn is the multiple of the microbial population and the outflow (or dilution

rate) of microbes from the rumen. By increasing outflow rate, efficiency of production of microbial mass will be increased. But microbial efficiency (Yatp) must not be confused with microbial yield. Whether the total yield of microbial mass is or is not increased as microbial efficiency (Yatp) increases depends on the degree that faster ruminal outflow reduces the quantity of organic matter digested (and yield of ATP) within the rumen. An increase in outflow from the rumen, though it always increases efficiency of microbial growth, may or may not increase ruminal output of microbes. Numerous additional factors that can alter microbial protein yield in the rumen were outlined by Firkins et al. (2006).

Ruminal microbes exist in multiple pools within the rumen. Some strains are attached to or embedded within the ruminal wall, others are attached to either grain or forage particles, while others freely float within the liquids within the rumen. With formation of a rumen mat, forage particles are retained for a longer time (have a lower dilution rate) within the rumen than liquids. In contrast, fine and dense feed particles are readily flushed with fluids from the rumen. Dilution rate of ruminal fluids always is greater than dilution rate of particles from the rumen, particularly when a ruminal raft exists. Liquid dilution rate increases when fluid input from saliva and possibly from drinking water increases. Consequently, an increase in rumination time, through increasing salivary input, generally increases efficiency of microbial growth, particularly of free floating microbes, and particle size reduction will speed passage of particles. As liquid dilution rate increases, particle dilution rate also increases because particles near the reticulo-omasal orifice are swept from the rumen by liquids.

Although bacteria attached to feed particles might be expected to be the primary type found in the rumen, even cellulolytic bacteria must detach and flow with liquids to colonize new feed particles. Most measurements indicate that between 40 % and 60 % of ruminal bacteria are associated with particles in the rumen with the remainder being associated with the liquid fraction of rumen contents. Because ruminal dilution rate always is lower for particles than for rumen liquids, one would expect that greater attachment to particles with higher forage diets should decrease efficiency of converting fermented organic matter to microbial protein. Yet, estimates of microbial organic matter per unit of organic matter truly fermented (organic matter apparently fermented minus microbial organic matter) indicate the opposite. True microbial efficiency always appears greater when diets contain more forage. Though no explanation for this dilemma is obvious, this could reflect an accelerated liquid turnover with forage than with concentrate diets, reduced protozoal predation of attached than of free bacteria, a greater maintenance cost for free floating than attached bacteria due to a lower pH that increases the energy requirement of microbes or the inconsistency in supply of available energy from concentrate during the time interval between meals.

A "time lag" is evident between the time that forage particles are introduced into the rumen or added to an in vitro flask and the time that fermentation of the added forage begins. Factors that could speed attachment of microbes deserve further research attention. Lasting several hours with some forages, this lag markedly shortens the time available for digestion. In turn this increases ruminal fiber fill and, given a limited time for digestion, decreases the extent that organic matter is digested in the rumen.

### Amino Acids

All amino acids that are not linked or guarded from attack are extensively degraded in the rumen to ammonia, carbon dioxide, VFA, and branch chain fatty acids. Degradation of amino acids can proceed either through decarboxylation yielding the amine plus carbon dioxide or by oxidative deamination yielding carbon chains that subsequently will be catabolized to VFA. Decarboxylation appears more prevalent with concentrate diets. Certain decarboxylated amino acids (e.g., histamine, tyramine) when absorbed can have adverse effects on feed intake and on metabolism of the host animal (e.g., laminitis). Oxidative deamination is more prevalent with forage-based diets. This difference presumably is due to higher prevalence of microbes involved with decarboxylation (e.g., Allisonella histaminiformans) with ruminal conditions and a low rumen pH from feeding of concentrate diets.

When grown in pure culture, some ruminal bacteria including most that digest cellulose require a source of branch chain fatty acids (isobutyrate, isovalerate, 2-methylbutyrate) or of their respective amino acids precursors (valine, leucine, and isoleucine) for growth. Theoretically, the supply of these amino acids might be inadequate with diets very low in true protein or in these specific amino acids. Indeed, concentrations of these branch-chained fatty acids within the rumen normally are low with low protein diets. However, supplementation with these compounds typically has not improved productivity or microbial cell yields from the rumen indicating that the ruminal supply must have been adequate, presumably from release of these fatty acids during ruminal degradation of protein of dietary and microbial origin.

As much as 40 % of the ruminal degradation of hydrolyzed protein has been attributed to several species of hyper-ammonia producing bacteria (HAP) that catabolize amino acids or peptides as a source of energy. Through degrading dietary protein, peptides, and amino acids, the HAP reduce the supply of protein for the host animal. Many of these strains are inhibited by ionophores. This may explain why ionophores may reduce a ruminant's need for dietary protein.

Though most ruminal bacteria can use ammonia as their sole source of nitrogen, results of some laboratory studies indicate that supplying amino acids in addition to urea can increase microbial organic matter yield. This may reflect a reduced need of ATP for amino acid biosynthesis (and a higher Yatp) of bacteria that can incorporate amino acids from the media. In vitro laboratory studies have indicated that strains of ruminal bacteria that typically digest structural carbohydrates are unable to use amino acids but instead thrive when will use and thrive when ammonia serves as their sole source of N for growth. In contrast, providing peptides in vitro has increased growth rates of bacteria that digest nonstructural carbohydrate. This resulted in the proposal that two-thirds of the N for bacteria digesting cell contents should be provided by intact protein or peptides in diet formulation programs (Russell et al. 1992). This also has led to the suggestion that source of ruminally degraded N (true protein versus NPN) as well as its level can alter microbial yields. In contrast, subsequent continuous culture and in vivo trials have found little if any improvement in digestibility or in bacterial growth or efficiency from providing additional peptides to rumen fluid when

the ammonia supply was adequate except at very rapid bacterial growth rates. Consequently, the precise conditions remain unclear whether or when peptides might limit digestion or bacterial growth in the rumen if the supply of ammonia is adequate.

The response of cattle to source of supplemental N is opposite from the suggestion that intact protein should prove most beneficial with diets rich in non-structural carbohydrate. Instead, production responses to intact protein sources over NPN are common for cattle fed forage-based diets but rare among cattle fed concentrate diets. Indeed, for cattle fed grain-based diets, urea can readily and fully displace the need for supplemental protein. Reasons for this discrepancy between in vitro and in vivo responses remain unclear though "energy spilling metabolic reactions," increased lysis and protozoal predation leading to greater turnover of bacterial constituents, and differences in glycogen synthesis may be involved.

## *Altering the Products of Ruminal Fermentation*

For growing-finishing cattle, a high ratio of propionate to other VFA, often achieved with feeding of starch-rich diets, is considered desirable energetically probably due to a reduced ruminal loss of energy as methane that often exceeds 6 % of digested energy from a diet. In contrast, a high propionate to acetate ratio for lactating cows is considered undesirable because it is associated with an increased retention of energy by body tissues leaving less energy for milk production. Likewise, milk fat concentration can be depressed by elevated propionate production. Diets that yield higher propionate to acetate ratios also are more likely to lead to metabolic problems (subclinical or clinical acidosis). These factors as well as attempts to decrease ruminal release of methane as a greenhouse gas have led to various approaches to alter the end-products of ruminal fermentation.

Ratios of VFA produced within the rumen can be altered by adjusting the composition of the diet, level of feed intake, and through manipulation of the types of microbes or their activity within the rumen. Increased knowledge of the degree to which specific ruminal microbes possess desired or undesired characteristics can be examined through genetic profiling techniques as described by Firkins (2010). Specific methods that might be used to control or alter ruminal populations include (1) administering specific chemical compounds that inhibit undesired microbial strains (selective antibiotics or ionophores, plant extracts, essential oils, fatty acids, bacteriophages, bacteriocins), (2) increasing the populations of species desired in the rumen through administration of probiotics, antibiotics, oligosaccharides, direct fed bacteria, yeasts, or fungal products, or (3) enhancing the availability of nutrients either within feeds (enzyme or microbial inoculants for feed) or within the rumen (specific fed enzymes, inoculation with fibrolytic or toxin-degrading microbes). These approaches have been described by Nagaraja (2012), and more information can be found in Chap. 6 of this book.

## Lab Procedures to Appraise Ruminal Fermentation of Feeds and Forages

### *Ruminal Digestion Measurements*

In vivo measurements of energetics, digestion, and performance are expensive, time-consuming, logistically complex, require access to multiple animals, and utilize a large amount of test material. As a result, laboratory methods are used extensively by researchers as a proxy for in vitro trials. The source of inocula preferably is ruminal liquor obtained from animals previously adapted to a diet similar to that being tested by in vitro procedures. When cannulated animals are not available as donors, rumen fluid from slaughter plants or fecal matter has been used as a substitute for fresh rumen fluid. Attempts to freeze rumen fluid for later use generally have not proven successful.

Results based on in vitro disappearance may differ from in vivo measurements for multiple reasons.

(a) Particle size distribution. Obtaining a distribution in particle size of a feed to match that of particles entering or retained within the rumen is difficult. The physical characteristics of esophageal samples are almost impossible to simulate through typical mechanical rolling or grinding procedures.
(b) Particle retention. Batch or in situ methods retain all feed particles for the full duration of the fermentation. In contrast, feedstuff components in vivo are segregated by density and particle size into multiple fractions that are selectively retained for various lengths of time. Combining an average rate of digestion with an average rate of passage to calculate the proportion of a feed component should be fermented in the rumen overlooks these physical characteristics that result in selective retention and passage of different fractions and components.
(c) Product accumulation and nutrient additions. With in vitro procedures, pH declines as acids accumulate, but within the rumen the fatty acid produced are absorbed. Buffers, minerals, and urea are continuously added from saliva and exchange through the rumen wall in vivo, activities difficult to simulate in vitro.
(d) Adaptation to substrates. Within a short term batch incubation system, unlike the rumen, the time available for microbes to adjust or adapt to a specific substrate is very limited. Addition of new feeds, chemicals, or compounds may temporarily shock fermentation within the rumen, but within several days the ruminal population will shift to accommodate a substrate change.
(e) Recovery of undigested products. Though called "digestion" in vitro or in situ measurements are estimates of "disappearance" of the test material. Following fermentation, material typically is recovered by filtration. Compounds that disappear are assumed to have been digested. Yet, during fermentation, cell contents and minerals become soluble and size of particles often is reduced to the point that it may not be recovered by filtration or retained within a Dacron bag. In situ bags with a larger pore size will permit more particles to escape through the pores. Because finely ground or pulverized feeds have more small particles, disappear-

ance and presumed digestibility will be greater, so care must be exercised when interpreting in situ disappearance values. In contrast with excessive loss from Dacron bags, microbes that attach tightly to or are located internal to feed components will not be fully dislodged during filtration or pepsin digestion. Consequently, disappearance of materials in vitro may not truly equate with ruminal digestion of that material. Although a "blank" fermentation in which rumen fluid is incubated without addition of substrate often is employed in an attempt to quantify the amount of undigested residue that is present with the inoculated rumen fluid or has entered a Dacron bag, death and lysis of microbes during incubation without a substrate over time may lead to an overestimates of digestibility.

Despite these shortcomings, comparative results still can prove useful to compare feeds or grain processing methods or to screen samples or compounds for more detailed in vivo testing. In vivo results may or may not match with in vitro results. Unfortunately, confirmation of results based on two or more laboratory procedures often is considered to be "verification" of a specific concept or observation. Any in vitro or in situ finding, despite its consistency, must be appraised through in vivo testing to assess its validity, veracity, and applicability in vivo. Usefulness and limitations of various in vitro and in situ procedures were outlined by Owens and Goetsch (1988).

## *Batch Methods*

In vitro procedures. The most common method for estimating ruminal digestion, the Tilley and Terry (1963) procedure, involves incubation of a feedstuff or compound with buffered rumen fluid in a sealed, anaerobic vessel maintained at 39 °C. To remove microbes generated during incubation and attached to the residue, digestion of the residue with a pepsin-HCl solution usually follows ruminal incubation of the test material. The amount of a material that remains in the vessel following an extended incubation time period (12, 24, 48 h) and pepsin incubation is considered to be not digested. Extent of digestion is calculated by difference from that present in the vessel at the start of the fermentation period. Digestion is calculated as input minus residue divided by input. Residues typically are recovered by filtration. As a result, any compounds that become soluble and "disappear" are classified as being digested. Care must be used to maintain viability of rumen samples used for inoculation and to provide adequate conditions for ruminal microbes (sufficient ammonia and minerals, buffering to avoid an acid accumulation that reduces pH). To adjust for differences in rumen fluid and in incubation conditions, disappearance often is adjusted by comparing values within a run against values for "standard" samples known to have a high and a low in vivo digestibility.

In situ procedures. Disappearance of feed components from porous Dacron bags incubated in the rumen of fistulated animals is another common method for estimating ruminal digestion. Pore size of the Dacron bags is critical because if pore size too small (under 20 μm), entry of protozoa is reduced whereas if pore

size is too large (over 50 μm), particles small in size can pass through without being digested (wash-out) or after being partially digested. Again, standards of known in vivo ruminal digestibility should be used to adjust for differences among in situ runs due to variability in ruminal conditions among animals and among days within an animal, both of which alter in situ disappearance. Washout of small particles placed into bags often can be estimated by measuring disappearance of dry matter from bags rinsed with water, but because particle size also decreases during fermentation, initial washout may underestimate subsequent loss of small particles from bags. In comparative studies, some research has indicated that washed particles from forages have an in vitro digestion rate similar to those particles retained within the bags, but this concept needs further study for feeds or diets that contain particles that differ in both particle size and digestibility; compared with dried forages, dried grains typically pulverize more readily when ground. With some feedstuffs (e.g., oats grain) and oil-rich feeds, films may form that will clog pores and inhibit water movement through the bag that is essential both to inoculate the feed and remove products of digestion.

## *Yield of Specific Products or Substrate Disappearance*

End-products of digestion include released gases (methane and carbon dioxide), volatile fatty acids, and microbial components. Through measuring yields of these products, both rate and extent of digestion can be monitored over time.

### In Vitro Gas Production

Carbon dioxide and methane production curves over time can be separated mathematically so that rate and extent of digestion of multiple "pools" can be quantified. Through measuring various nitrogen containing products as well, protein yield (the sum of microbial protein and undegraded feed proteins) and of undigested cell contents also can be calculated. One advantage of gas production measurements is that rates as well as extent of digestion can be evaluated continuously over time. The gas production procedure has proven very useful to evaluate energy availability of novel feedstuffs in developing countries. Although such batch fermentation do not directly match the continuous fermentation conditions within the rumen, the size of various "pools" and their relative rates of gas production when matched with in vivo estimates of production can help guide nutritionists in making appropriate diet modifications to improve productivity. Limitations as noted above include making appropriate correction for products included with the rumen fluid as an inoculum, auto-degradation of microbes with long-term incubations, retention of all particles for the full incubation period, release of carbon dioxide from the buffered incubation media during fermentation, and lack of potential for microbial adaptation to substrates being fermented.

**Rusitec**

Through maintaining rumen fluid over time and repeatedly adding and removing Dacron bags containing various substrates, disappearance of individual feed components can be estimated. Maintaining and operating this semi-continuous culture system can become quite complex and time consuming but in contrast with other incubation systems, this procedure allows time for microbes to adapt to specific feeds when normal microbial activity is retained.

## *Continuous Flow Fermenters*

Fermenters equipped with automated feeding systems, designed to recover both liquid and particulate effluent separately, and equipped to monitor and control pH and other parameters have been engineered in an attempt to simulate ruminal fermentation of specific feeds and to test feed additives. As with the Rusitec system, devices are complex and often fail to maintain typical conditions found with fresh ruminal contents (pH, gas production rates and ratios, in vitro digestion rates, protozoal numbers and activities) for more than 1 week.

## *In Vivo Measurements*

The live ruminant animal is the gold standard for quantifying rate and extent of digestion and absorption, products of metabolism, and energetic efficiency. Often smaller ruminants (sheep, goats) are used as proxies for larger ruminants to reduce cost and the amount of space and feedstuff needed, but species differences must be borne in mind when extrapolating among different ruminant species and even within a species (lactating dairy cows versus non-lactating pregnant heifers versus finishing feedlot steers). Through collecting urine and feces, apparent digestion and retention of nutrients can be calculated. By using indigestible markers inherent in diet components or added to a diet, digestion and retention can be calculated so that total excreta need not be collected. Similarly, metabolism chambers that collect expired gases can quantify whole animal metabolism. Live animals often are surgically equipped with portals to monitor intake, digestion, and metabolism. With esophageal fistulas, composition of forage selected by grazing animals, effects of rumination on regurgitated digesta, and the eructation complex can be studied. Ruminal cannulas provide a window that allows research scientists to monitor the populations and activity of ruminal microbes, characteristics and products of fermentation (pH, temperature, passage), and ruminal motility. Cannulas in the abomasum, the small intestine, and the large intestine permit study of the site and extent of nutrient digestion and passage. Isolated ruminal sacs and tissue culture methods allow study of absorption of specific nutrients, and catheters at specific locations within the digestive tract or isolated tissues (mammary gland; hind limb; fat tail; isolated organs or tissues) yield data regarding net absorption of specific nutrients

and tissue metabolism. Devices to continuously monitor important ruminal factors (e.g., pH, temperature, motility, ammonia) provide a record of ruminal conditions for researchers and can help detect ruminal problems of individual animals within a producing herd that need additional nutritional or medical attention.

## Epilogue

Our current scientific understanding of ruminant metabolism, production, and health and practical production practices is based largely on results of in vitro and in vivo measurements. Future improvements in productivity and health of ruminants are dependent on deepening our understanding of ruminant activity, digestion, and metabolism. Past efforts have been largely based on chemical and microbiological measurements with the more difficult physical measurements often being ignored. Targeted application of science and technology in ruminant production will help to reduce the environmental footprint of ruminants, unique animals that both harvest and efficiently convert unused and underutilized feeds and wastes into healthful and desirable products that enhance human life worldwide.

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# Chapter 4
# Lipid Metabolism in the Rumen

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## Introduction

The increase of energy concentration in rations formulated for ruminants has become an important topic while demands for increasing productivity grows, especially in finishing periods for beef cattle and sheep, and strategically for dairy cows, depending on the stage of lactation. Seeking for alternatives to excessive starch inclusions, several studies have been pointing out as potential solution the utilization of compounds that contain high-energy concentration, since its limitations are understood and its benefic properties maximized.

Therefore, lipids are back to the ruminants' nutrition scenario playing an extremely important role, because they help to reduce heat and acid production in the rumen, as ruminal microorganisms do not use lipids as energy source for growth. There is a great availability of natural lipids from industry coproducts, and recently from bioenergy and biofuel, besides protected lipid sources. On the other hand, the limitation of adding lipids to ruminants' diets, due in part to its negative effect on fiber digestibility, brings up the discussion of positive and negative associative effects between feedstuffs used for ruminants' diets composition.

Consumers' demand for changes in beef and milk fatty acid profile also challenges nutritionists in terms of diet formulation, and its consequence to lipid metabolism. The objective of this chapter is to present the most consistent results, and known metabolic pathways, so that a reader may become updated, and develop new challenges with regional coproducts or new ways to add concentrated lipid sources in the ration of ruminants. Also, advantages and limitations of feeding protected lipids will be considered in this chapter in order to help the reader with nutritional management and decision-making. The idea is to integrate the inclusion of lipids in ruminant's diets in economic evaluations, assessing its real cost-benefit.

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Thus, in this chapter, the approach on lipid metabolism in the rumen will initially consist of general aspects (definition, importance, classification and lipid-rich feed sources); ruminal microbiology emphasizing the main microorganisms that digest lipids (peculiarities and mode of action); digestion, metabolism and incorporation of microbial lipids; biohydrogenation process (concept, particularities, factors that interfere in its dynamics and final fatty acid profile); lipid sources utilized in ruminant nutrition and how they can interfere in the ruminal dynamics regarding digestibility, biohydrogenation, passage rate and utilization of short-chain fatty acids by the animals.

The topic of this chapter also welcomes technological innovations to integrate the set of impacts from lipids addition in ruminants' diets, interacting in ruminal dynamics and, consequently, in beef and milk products.

## Definition, Importance and Classification of Lipids and Lipid-Rich Feed Sources in Ruminant Nutrition

Lipids are little soluble in water and soluble in organic solvents. Complete oxidation of these compounds provides an average of 9.45 Kcal/g, or 2.25 times more energy than the average provided by carbohydrates and proteins. Oils, fats, waxes, steroid hormones, cholesterol, liposoluble vitamins, and phospholipids (cellular membranes) are included in this class. Besides feeding functions and others, lipids mechanically protect against shocks (fat tissue) and are thermal insulators and sealers.

The term fat is utilized to denominate compounds which are rich in long-chain fatty acids (FA) including triglycerides, phospholipids, non-esterified FA (NEFA) and calcium salts (NRC 2001). In chemical analysis, fats are organic compounds extracted by ether. Ether removes liposoluble components such as mono, di and triglycerides, free FA, liposoluble vitamins, steroids, saponins, waxes and some liposoluble pigments from a sample. True fat, called triglyceride, is a chemical compound formed by one glycerol (consisting of three carbons) with a FA linked to each of the carbons. Fatty acids can present variable structure, and that differentiates fats among themselves. They vary in chain length, usually containing 16–22 carbons, and can be saturated or unsaturated. Saturated FA present all hydrogen atoms of their molecules linked to one carbon atom. Unsaturated FA has one or more double bonds because not all their linkages are filled with hydrogen atoms. The main FA found in the ruminants' diets are presented in Table 4.1.

The simplest lipids are called neuter lipids or triacylglycerol. Their structure consists of glycerol and three molecules of long-chain FA. Phospholipids are little more complex and functionally more important because they form structural elements in cell membranes, and one of the most abundant phospholipids in animal tissue is phosphatidylethanolamine.

Forage plants and many seeds generally have a small amount of lipids, from 4 % to 6 %, found mainly as glycolipids and phospholipids. Some authors state that this value may vary from 18 % to 50 % in oleaginous plants, and in this case, the predominant

**Table 4.1** Type, structure, common name and formula of main FA of ruminants' diets

| Type | Structure | Common name | Formula |
|---|---|---|---|
| Saturated | $C_{16}H_{32}O_2$ | Palmitic acid | C16:0 |
| Saturated | $C_{18}H_{36}O_2$ | Stearic acid | C18:0 |
| Unsaturated | $C_{18}H_{35}O_2$ | Oleic acid | C18:1 |
| Polyunsaturated | $C_{18}H_{34}O_2$ | Linoleic acid | C18:2 |
| Polyunsaturated | $C_{18}H_{33}O_2$ | Linolenic acid | C18:3 |

form is triglycerides. Still, within these proportions, vegetal fat content may vary according to plant part, growth stage and material processing. With respect to the predominance of FA, forage plants contain a greater proportion of linolenic acid (C18:3), whereas grains and oleaginous plant seeds predominantly have linoleic and oleic (C18:1 cis 9) acid.

Nutritionally, lipids can be characterized as reserve lipids (mainly triglycerides in seeds), leaf lipids (galactolipids and phospholipids) and a mixture of other water-soluble molecular structures (waxes, carotenoids, chlorophyll). Lipids found in forage plants are mainly represented by galactolipids and phospholipids, whereas fat found in animals and cereal grains of oleaginous plants are basically triglycerides. Most FA from forage plants and vegetables are unsaturated (generally more than 70 %) and mainly represented by linoleic (cis-9, cis-12, 18:2) and linolenic (cis-9, cis-12, cis-15, 18:3) acids.

Besides seeds, triglycerides are abundant in animal fat tissues, but its contents in forage plants are insignificant. Diglycerides found in plant leaves are mainly galactolipids that involve glycerol, galactose and unsaturated FA, which is generally more polarized than triglycerides, but contain lower energy concentration than it would be estimated by factor 2.25 utilized to calculate TDN (total digestible nutrients). When ingested by an animal, galactolipids and other esterified lipids (mainly triglycerides) are extensively hydrolyzed by lipases associated with a bacterial cellular membrane, releasing glycerol, galactose and a mixture of saturated and unsaturated long-chain FA.

Furthermore, according to Grainger et al. (1961), the fat content of coproducts resulted from the oil extraction of whole cottonseed also varies considerably (3 % to 24 %), which can be another benefit for ruminants considering that oil addition to the diet may help mitigate enteric methane by decreasing ruminal methanogenesis.

## Microorganisms that Digest Lipids

### *Ruminal Metabolism of Lipids*

Bacteria are not capable of utilizing FA as energy source and probably not for any structural function. Bacterial lipid content (found mainly in membranes) is around 10 % of its dry weight and represented by phospholipids (30–40 %), NEFA

(approximately 40 %) and other ether-soluble molecules that include neuter lipids (triglycerides) and non-saponin lipids.

Regarding FA profile, more than 90 % are saturated and represented mainly by palmitic and stearic acids. Ruminal bacteria synthesize most of their long-chain FA from sugars but are incapable of synthesizing polyunsaturated FA so that their presence in membranes is insignificant (less than 5 %) and originated from ruminal fluid. Bacteria also synthesize FA with odd number of carbons (15–17) and branched-chain FA. The unsaturated FA has the property of rapidly adhering itself in free surfaces including the surface of bacterial cells and feed particles. Therefore, part of them can penetrate and be incorporated to lipids of bacterial membranes.

## *Lipid Hydrolysis in the Rumen*

As pointed out before, herbivores' diets normally have low lipid content due to the small lipid amount (2–5 %) of the vegetal sources used to formulate these diets. The dietary characteristics of these feedstuffs have demanded metabolic adaptations and methods to preserve essential FA. Vegetal lipids are extensively modified by ruminal fermentation, and, consequently, digested lipids differ from ingested ones.

Rumen is intolerant to high fat levels that can interfere in fermentation. This situation contrasts with a newly-born ruminant that consumes milk containing 30 % fat (dry matter basis) or more, which represents 50 % or more of its caloric intake. In most metabolic systems, FA is derived from glucose. However, glucose from diet is scarce in ruminants' metabolism; and as a result, these animals have developed important mechanisms for its preservation, such as the absence of pathways to convert glucose into FA. Approximately 90 % of fat synthesis in ruminants occurs in the adipose tissue. The liver, which is important for lipogenesis in several non-ruminant species, contributes with only 5 % of lipogenesis in ruminants.

In the rumen, diet lipids are intensely modified by their hydrolysis and biohydrogenation and significantly affect microbiology and physiology of this degradation site (Jenkins 1993). According to Bauman et al. (2000), lipids are subjected to two important transformations in the rumen; the first one is the hydrolysis of ester chains catalyzed by microorganism's lipases. It occurs rapidly after lipids reach the rumen and is carried out by extracellular enzymes of ruminal microorganisms with release of FA, glycerol and other molecules, according to their origin. The released glycerol is readily utilized by ruminal bacteria that produce, in general, propionic acid (Jenkins 1993). The author states that despite the benefit to the host, FA are not utilized as energy source by ruminal bacteria, because they are highly reduced compounds as less than 1 % of FA are degraded to $CO_2$ and short-chain FA in the rumen. An important point is that FA has a "saving" energy effect for ruminal microorganisms through their incorporation to their membranes and cytoplasm, avoiding energy waste for synthesis de novo (Bauchart et al. 1990).

The second transformation is the biohydrogenation of unsaturated FA, an important topic in this chapter; because the understanding of partial or total transformation of

unsaturated FA into saturated FA has been a great challenge for metabolism studies lately. The understanding of mechanisms will only serve for posterior recommendations in feeding management with decision-making about lipid inclusion in the diets as well as the method to provide them to ruminants.

## *Rumen Biohydrogenation of Lipids*

Biohydrogenation of unsaturated FA involves several biochemical steps as different species of bacteria catalyze several chemical and biochemical reactions.

For years, the scientific community only knew *Butyrivibrio fibrisolvens* as the bacterium that was capable of performing biohydrogenation; however, with advances in research, it was observed that a large number of ruminal bacteria present this characteristic. Bauman et al. (1999) and Pariza et al. (2001) also cited *Anaerovibrio lipolytica* and *Propionibacter* among the main bacteria responsible for biohydrogenation. Moreover, rumen bacteria is divided into two groups, according to biohydrogenation reactions and final products: group A is formed by bacteria that hydrogenate linoleic acid (C18:2) into C18:1 trans-11 (elaidic acid, an isomer form of oleic acid), which is the final product; and group B consists of bacteria that utilize C18:1 trans-11 as one of the main substrates, generating stearic acid (C18:0) as the final product. Figure 4.1 show the steps of linoleic acid biohydrogenation, where the Cis-12 double-chain isomerization represents the initial step.

The phenomenon promoted by bacteria described in the literature as a self-defense mechanism against unsaturated FA toxicity receives direct influence of feed passing rate by the reticulum-rumen, because it greatly depends on voluntary intake; type of fiber ingested (from forage or coproducts, such as whole cottonseed) as well as concentrate to forage ratio. Oliveira and Millen (2014) reported that most nutritionists of feedlot cattle in Brazil utilize rations containing, on average, 79 % concentrates, which contain a significant portion of coproducts (whole cottonseed, soybean hulls, as well as residues from regional agroindustry such as citric pulp). Moreover, understanding lipid input in the rumen becomes a little more complex and, therefore, receives influence from several interactions and associative effects.

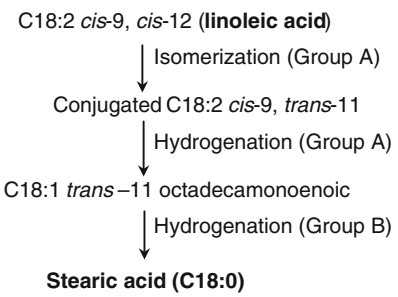


**Fig. 4.1** Scheme of linoleic acid biohydrogenation (adapted from Hobson and Stewart 1997)

Thus, the toxic effect of unsaturated lipids on ruminal microorganism, mainly on GRAM positive bacteria that digest cellulose and on protozoa can be mitigated if several interactions that involve hydrolysis and biohydrogenation processes are understood.

Then, to avoid this toxic effect, microorganisms promote unsaturated FA hydrogenation, rapidly after their release in the rumen (Jenkins 1993). The initial step of ruminal FA biohydrogenation is an isomerization reaction in which a double bond with cis configuration is converted into trans, but the same does not happen if FA is linked to a carboxyl group as it occurs in FA soaps. In the case of linoleic acid, which has double bonds with *cis* configurations (C18:2 *cis*-9, *cis*-12), isomerase action results in the production of C18:2, *cis*-9, *trans*-11 (Fig. 4.1). In the following steps of biohydrogenation sequence, monounsaturated FA C18:1, *trans*-11 and stearic acid (C18:0, saturated; Jenkins 1993; Bauman et al. 1999) are produced through the action of reductases. Thus, depending on the passage rates and biohydrogenation, FA with varied unsaturation levels may leave the rumen. However, in average, 70 % of FA that reach bovines' duodenum is non-esterified saturated FA (Bauchart 1993).

Biohydrogenation rate of FA in the rumen can be reduced by several factors, such as high content of grains with high content of linoleic FA (for example, sunflower seed; Beam et al. 2000), low nitrogen content (Gerson et al. 1983), reduction of feedstuffs particle size (Gerson, et al. 1988), increase of forage maturity (Gerson et al. 1986) and inclusion of ionophores (Fellner et al. 1997) to the diet. High contents of linoleic FA in feeds can cause different levels of intoxication that temporarily paralyze biohydrogenation process and also form a "biofilm" around the fiber particle, which would partially avoid its degradation and growth of fibrolytic bacteria. Likewise, any factor that affects the bacterial group that ferments structural carbohydrates, like the presence of low-quality forages and low-nitrogen content, will negatively affect ruminal biohydrogenation because these microorganisms are responsible for part of this process as well. On the other hand, reduction of particle size of either forage or concentrate ingredients may lead to greater variations in ruminal pH. Based on this fact, if eventually rumen pH decreases below 5.7, fiber degradability is impaired and consequently biohydrogenation rate is reduced, because bacteria that ferment structural carbohydrates are sensitive to low pH and, therefore, their activity is compromised. The feeding of diets containing high content of concentrate ingredients decreases lipolysis and biohydrogenation rates as well as it modifies the profile of intermediates of this process, which increases the proportion of unsaturated FA from trans-10 group reaching the duodenum. These changes are probably due to integrated effects of pH reduction and alteration of ruminal bacterial species composition. Moreover, the carboxyl group of FA has to be free to be biohydrogenated. Finally, the effect of adding ionophores to ruminants' diets is well-known, because the populations of gram-positive bacteria, which include fibrolytic bacteria that digest cellulose, are reduced, decreasing the biohydrogenation rate.

However, saponification has been one of the most utilized alternatives to protect FA from ruminal biohydrogenation. Ruminal dissociation level of FA soaps, which

would allow their biohydrogenation, depends on ruminal pH values and pKa of FA that compose them, which is calculated by Henderson-Hasselbach equation (Sukhija and Palmquist 1990). Thus, the lower the ruminal pH and pKa of FA are, the greater the dissociation becomes in the rumen (by pass fat). Thus, if FA soaps are included in diets that promote ruminal fermentation at healthy appropriate levels (between pH 5.7 and 6.8); it is likely that a greater flow of unsaturated FA reaches the small intestine. Because of that, providing fat as insoluble calcium salts prevent biohydrogenation.

Based on absorptive properties of unsaturated FA and on observations that hydrogenation activity of ruminal fluid is undeniable, it is concluded that enzymes responsible for biohydrogenation are found in membranes of bacteria attached to feed particles. In addition, experimental evidence also indicates that, although *Butyrivibrio fibrisolvens* is one of the bacteria that has the highest biohydrogenation capacity; this activity depends on the conjugated activity with more than one ruminal bacterial species, for example, *Fusocillus* sp. However, it has not been clearly defined up to now the reasons by which some species of ruminal bacteria perform biohydrogenation. As unsaturated FA are toxic to many ruminal bacteria, the most probable function is related to detoxification. However, biohydrogenation can also be a way to drain reduction equivalents ($H_2$ or NADH) from the ruminal environment.

## *Lipids Interference on Ruminal Dynamics*

The inclusion of lipids in ruminants' diets, beyond the fat content that is naturally observed in forage sources, can interfere in fermentation dynamics and also affect the metabolism of other nutrients in the rumen, such as the proteins. As consequence of lipid addition at levels greater than 7 % (dry matter basis) in ruminants' diets, there is reduction of protein digestion and as a result ruminal ammonia concentration is decreased. It is also observed that there is an increase in protein synthesis efficiency, which is attributed to the reduced numbers of ruminal protozoa that are bacterium predators and have greater ruminal retention time. Therefore, the metabolizable protein, which considers the sum of microbial protein and rumen undegradable protein absorbed in the small intestine, may be changed and some adjustments in the diet may be necessary, as there will be a decrease in ammonia supply for bacteria and reduction of protozoa population.

It is important to point out that according to the classification, which was adopted in 1996 by several committees and researchers, bacteria are divided into two big groups: the ones that degrade structural carbohydrates and the ones that degrade non-structural carbohydrates; the former are dependent of ammonia as the sole source of nitrogen, so, if there is a reduction of ammonia availability, there will be a smaller production of microbial protein and smaller outflow of amino acids to be absorbed by the small intestine. On the other hand, the antimicrobial effect and alterations of ruminal fermentation caused by lipids can be reduced by adding hay

or fiber to the diet. It is believed that this reduction occurs due to the adhesion of fat to fiber particles, avoiding their contact and direct effect on bacteria.

Diet FA and lipids synthesized by ruminal bacteria, mainly consisted of stearic (C18:0) and palmitic acids (C16:0) and only 15–20 % of monounsaturated acids (Bauchart 1993), reach the small intestine where long-chain FA absorption occurs. In bovines, the average intestinal absorption of unsaturated FA is greater than saturated FA (92 % vs. 80 %; Bauchart 1993) and true digestibility decreases as lipid intake increases (Palmquist 1991). After absorption, FA are re-esterified to glycerol and transported by lipoproteins through lymph and bloodstream until peripheral tissues to be deposited in the cellular membrane (phospholipids) or adipocyte cytoplasm (triglycerides) or oxidized for energy production (Bauchart 1993).

Unsaturated FA formation in mammals occurs by the action of enzymes called desaturases. Bovines have four desaturases with broad chain-length specificity designated $\Delta^9$, $\Delta^6$, $\Delta^5$ and $\Delta^4$-acyl-CoA-desaturases. In animals, desaturases will occur until C9, and will not continue beyond that due to the absence of $\Delta^{12}$ and $\Delta^{15}$ desaturases, only found in plants. Because of that, $\Delta^{9–12}$ octadecadienoic acid is considered an essential FA, and has to be provided through diet because it is a fundamental precursor of prostaglandins.

The control of $\Delta^9$ desaturase activity is a promising method to manipulate ruminants' fat tissue composition. According to Smith et al. (1998), when high-grain diets are fed, the $\Delta^9$ desaturase activity is inhibited. Yang *et al*. (1999) observed $\Delta^9$ desaturase inhibition through cyclopropenoic acid found in whole cottonseed and other cereal meals. However, Medeiros (2002), estimated greater $\Delta^9$ desaturase activity for feedlot animals when compared to grazing cattle.

## *Conjugated Linoleic Acid—CLA*

Nowadays, there has been continuous interest in increasing concentration of some specific isomers of linoleic acid in ruminants' tissues, generally known as conjugated linoleic acid (**CLA**; Pariza et al. 2001). The denomination CLA corresponds to several geometric and positional isomers of octadecadienoic acid or linoleic acid (Pariza et al. 2001). Linoleic acid is an unsaturated FA of 18 carbons with double bonds in positions 9 and 12, both at same cis spatial orientation (same side). In CLA, the double bonds are conjugated, which means that they are separated only by one simple bond and, according to Chouinard et al. (1999), isomers with double bonds in positions 7–9, 8–10, 9–11, 10–12, 11–13, and 12–14 with different cis and trans orientations have already been identified. However, the predominant isomer is *cis*-9, *trans*-11, which represents 80–90 % of CLA found in feedstuffs (Fig. 4.1).

The CLA was identified more than 40 years ago, but just since the 1980s it has received great interest in research, after Dr. Michael Pariza from the University of Wisconsin in the United States, and his collaborators, identified it as a substance with potent anti-carcinogenic activity in hamburger lipids (Ha et al. 1987). Several studies after that proved this activity (Pariza et al. (2001); Ip and Scimeca 1997; Ip

et al. 1994) and found various other ones related to human health like reduction of atherosclerosis, and immunomodulatory effect (Hayek et al. 1999). The CLA effects on nutrient partition was also observed with reduction of fat content in milk (Baumgard et al. 2000), body fat deposition (Park et al. 1997), and increase of bone mineralization.

In bovines, C18:2 *cis*-9*, trans*-11 is formed as a first intermediate in ruminal biohydrogenation process of linoleic acid by ruminal bacteria (Bauman et al. 1999; Pariza et al. 2001). The next step in linoleic acid biohydrogenation sequence is the production of monounsaturated FA C18:1 *trans*-11 (Fig. 4.1). When biohydrogenation is incomplete due to low pH values, for example, C18:2 *cis*-9, *trans*-11 leaves the rumen and is absorbed and incorporated in animal products and tissues. The C18:2 *cis*-9*, trans*-11 can also be synthesized from FA C18:1 *trans*-11 through endogenous pathway by action of enzyme $\Delta^9$-desaturase. Bauman et al. (1999) state that it seems to be the main formation pathway of C18:2 *cis*-9*, trans*-11 found in ruminants' adipose tissue. The passage of FA C18:1 *trans*-11 from the rumen to the omasum and, consequently, to the small intestine also increases when biohydrogenation is reduced (Fig. 4.1), which helps support the theory that $\Delta^9$-desaturase has a fundamental role in the accumulation of this FA in ruminants' fat.

Unsaturated FA seems to be preferentially metabolized into C18:2 *trans*-10*, cis*-12 in the rumen for some specific types of diets. Diets that have unsaturated fat, high-concentrate content, finely ground forages and ionophore addition result in higher values of C18:2 *cis*-10*, trans*-12 (Bauman et al. 1999). According to this author, fat from American bovine beef contains proportionally more C18:2 *trans*-10*, cis*-12 than milk. Usually high-concentrate diets for finishing cattle can partially explain this fact, because *Butyrivibrio fibrisolvens* bacterium is gram positive and sensitive to low ruminal pH and, thus, the beginning of biohydrogenation process is inhibited as this bacterium is responsible for isomerization and hydrogenation processes, among others (Fig. 4.2).

The mechanisms through which CLA affects carcinogenesis are not totally cleared out and may vary according to site, age, exposure time and carcinogenesis stage (Pariza et al. 2001). Several studies have suggested that CLA act through antioxidant mechanisms (Ip et al. 1991), pro-oxidizing cytotoxicity (Schonberg and Krokan 1995), nucleotide synthesis inhibition (Shultz et al. 1992), proliferative activity reduction (Ip et al. 1994), and inhibition of the segment related to DNA that has the carcinogenic activator (Liew et al. 1995).

Lipid synthesis alterations in milk and adipose tissue seem to be specifically associated with isomer C18:2 *trans*-10*, cis*-12 (Baumgard et al. 2000; Park et al. 1997). Baumgard et al. (2000) verified a reduction of 42 % in fat content of milk from cows in which isomer *trans*-10*, cis*-12 of CLA was infused, whereas the infusion of similar amounts of isomer *cis*-9*, trans*-11 of CLA did not affect milk fat content. According to Doyle (1998), CLA effects on lipid metabolism are attributed to the increased activity of enzymes like carnitine palmitoyltransferase and hormone-sensitive lipase, which participate in beta oxidation and intracellular lipid hydrolysis for posterior release in the bloodstream, respectively. Associated with these effects, there is a decrease of lipoprotein lipase enzyme activity, which is

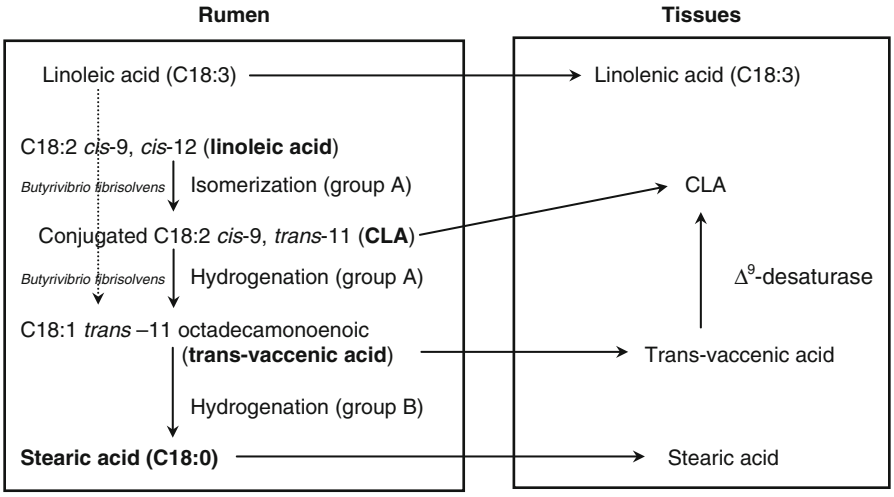


**Fig. 4.2** The CLA formation process in ruminant tissues due to the ruminal influence (Adapted from Robson and Stewart 1997)

involved with the entrance of FA into adipocytes (Doyle 1998). Pariza et al. (2001) state that there is evidence that C18:2 *trans*-10, *cis*-12 induces pre-adipocyte apoptosis.

Average CLA concentration in vegetal feedstuffs is inferior to 1.0 mg/g and in bovine products (beef and milk) is 3–7 mg/g of lipids (Chin et al. 1992). In bovine products, CLA concentration varies in function of several factors, such as source and amount of lipids in the diet (Griinari et al. 2000), concentrate to forage ratio in diet (French et al. 2000) and breed of animals (Lawless et al. 1999).

In studies involving beef cattle, Mir et al. (2000) observed that CLA content increased from 0.21 % to 1.48 % of total FA (635 % over control) in muscular lipids (*biceps femoris*) of bovines consuming diets containing 6 % sunflower oil when compared to those fed diets without lipid supplementation. Enser et al. (1999) added 6 % of linseed oil to bovine's diet and observed that CLA content increased almost three times (35.6 vs 11.3 mg/100 g) in the muscular tissue when compared to animals supplemented with FA soaps.

Similarly to linoleic acid, according to Bauman et al. (2000), linolenic acid biohydrogenation starts with an isomerization followed by a reduction and ends with stearic acid formation. In feedstuffs, the predominant form is alpha C18:3 (cis-9, cis-12, cis-15 octadecatrienoic acid). Alpha-linolenic biohydrogenation in the rumen produces conjugated cis-9, trans-11, cis-15 octadecatrienoic acid as the predominant initial isomerization product, and this is followed by reduction of the cis double bonds. Therefore, trans-11 octadecamonoenoic acid becomes a common intermediate product in the biohydrogenation of both alpha linolenic and linoleic acid (Fig. 4.2).

## Influence of Fat Supplements on Ruminal Metabolism

There are several fat sources that can be utilized in ruminants' diets, such as: soybean oil, rumen-protected fats commercially available, and whole oleaginous seeds. The oil content from forages and several grains, despite usually low, have high proportions of polyunsaturated FA, particularly linolenic acid (C18:3) and linoleic acid (C18:2), which are essential FA in the diet because they cannot be synthesized by animals and human beings.

Fat inclusion in ruminants' diets to increase energy intake is not always an effective method, once high-levels of fat may reduce dry matter digestion in the rumen which, consequently, causes lower energy availability. If ruminal microorganism capacity for hydrogenation is exceeded, unsaturated FA can be accumulated in the rumen and potentially interfere in fermentation (Viñoles et al. 2009).

### *Relationship Between Lipid Properties and Ruminal Metabolism*

When lipid hydrolysis rate and unsaturated FA release in the rumen exceed rumen biohydrogenation capacity, it's expected a fiber digestibility decrease, increase of propionic acid production, and reduction of methane and short-chain FA in the rumen (Chalupa et al. 1986).

According to Van Soest (1994), the reduction of ruminal pH due to changes in forage to concentrate ratios, often results in microbial populations shifts and, as a result, in fermentation end-products. Likewise, Griinari and Bauman (1999) observed that low-fiber diets increased the proportion of trans-10, cis-12 CLA isomer in milk fat.

Factors like diet composition and genetic group will direct influence ruminants fat tissue composition. According to French et al. (2000), grazing animals present a greater amount of polyunsaturated FA, resulting in greater unsaturated to saturated ratios than animals fed high-concentrate diets. Moreover, a greater amount of CLA was observed for grazing animals when compared to feedlot animals fed either high levels of concentrate or corn silage based diet.

Nurnberg et al. (1988) observed that grazing cattle doubled the n-3 C18:3 concentrations in adipose tissue when compared to feedlot animals. Similarly, Enser et al. (1998) reported that grazing bullocks presented a polyunsaturated to saturated ratio almost 3 times greater than those fed concentrate based diets. On the other hand, Medeiros (2002) observed greater unsaturated FA concentrations in adipose tissues of feedlot cattle fed high-concentrate diet when compared to grazing animals. The author explained that due to the high-energy content of the diet used, there was a ruminal pH reduction, and consequently, ruminal biohydrogenation was negatively affected.

The divergent results related to production systems are probably due to the presence of high unsaturated FA concentration in temperate grasses, conditions under which

the experiments were carried out, and the fact that a great amount of concentrate really decreases ruminal biohydrogenation which does not seem to happen when low- and moderate-concentrate diets are fed. Also, it is believed that diet effects are greater in the composition of subcutaneous fat tissue than in the intramuscular portion (De Smet et al. 2000).

According to Huerta-Leidenz et al. (1993), *Bos indicus* cattle present less saturated subcutaneous FA profile than *Bos taurus* cattle, and these values are also observed for 2-way-cross calves from *Bos indicus* dams when compared to 2-way-cross calves from *Bos taurus* dams. Moreover, De Smet et al. (2000) observed quite high content of unsaturated FA in the adipose tissue of Belgian Blue bovines. The values were 46 %, 36 % and 18 % for saturated, monounsaturated and poly-unsaturated FA, respectively, and these values were close to the ones reported for swine.

Bovine carcass contains, in average, 44 % of saturated FA, 45 % of monounsaturated FA and 5 % of polyunsaturated FA (Duckett 2001). Myristic (C14:0) and palmitic (C16:0) saturated FA are considered hypercholesterolemic (Hegsted et al. 1965), whereas monounsaturated and polyunsaturated FA are considered effective in the reduction of cholesterol and low-density lipoprotein (LDL) concentration in human blood (Mattson and Grundy 1985; Grundy 1989), except for *trans* isomers of monounsaturated FA, specially C18:1, which has been associated with high risk of cardiovascular diseases (Williams 2000).

Oleic monounsaturated FA (C18:1) contributed to improve cooked beef flavor, according to Dryden and Marchello (1970). The concentration of linoleic (C18:2) and linolenic (C18:3) polyunsaturated greater in muscles than in subcutaneous fat tissue (Bas and Sauvant 2001) FA is and drastically increases when bovines are fed either cereal-rich and seed-rich diets or diets with high content of these FA (Marmer et al. 1984; Demeyer and Doreau 1999). There is not much interest in the increase of FA concentration in carcass due to their adverse effects on flavor, color and beef stability related to lipid oxidation (Morrissey et al. 1998).

Intensification of bovine production systems has indicated feedlot finishing as a factual and economically viable strategy, mainly for high-quality beef production. Likewise, recommendations to reduce operating costs of feedlots indicate the utilization of rations containing higher amounts of concentrates (grains or coproducts, mainly). This decision-making demands greater accuracy and care with diet formulation. According to Millen et al. (2009), 98 % of feedlot cattle nutritionists in Brazil, and according to Vasconcelos and Galyean (2007), 100 % of feedlot cattle nutritionists in the United States, include ionophores in their formulations. Ionophores inhibit the growth of gram-positive bacteria that are directly involved in ruminal biohydrogenation, including *Butyrivibrio fibrisolvens*. Ionophore addition inhibited linoleic acid biohydrogenation which resulted in reduction of stearic FA and increased C18:1 monounsaturated concentrations in ruminal contents.

The diet of ruminants fed only with forages has low lipid content (from 1 % to 5 % of diet dry matter). Higher lipid levels are obtained by adding either animal or vegetal fat, or seeds or fat-rich vegetal residues. However, ruminal fermentation is negatively affected if lipid content is greater than 7 % of diet dry matter. The explana-

tion for this effect is supported by two theories. One of them is associated with adsorptive property of unsaturated FA, which in excess would form a hydrophobic coating on the bacterial cell, preventing its metabolism or adhesion to feed particles. Another theory proposes the existence of a direct toxic effect as these FA incorporate themselves to the bacterial membrane and change their fluidness and permeability. Higher levels of lipids could be added only as saturated fat or protected from ruminal fermentation as calcium soaps.

## *Strategies for Feeding Lipids and Its Use in Ruminants' Nutrition*

Lipid utilization in ruminants' diets is involved in the hypothesis of several studies, in which the objective is to increase the energy content, mainly for dairy cattle diets. However, recent studies have shown that lipids supply also changes the amount and composition of carcass FA profile in beef cattle (Bas and Sauvant 2001), which, according to Duckett (2001), are the main responsible for beef quality, specially palatability and stability. However, in function of some factors, there are marked differences in the FA profile of lipids that were ingested and the one observed in bovine products (beef or milk), which is resulted from ruminal fermentation and its metabolism (Jenkins 1993; Demeyer and Doreau 1999).

Unsaturated FA seem to be preferentially metabolized into C18:2 trans-10, cis-12 in the rumen for some specific types of diets. According to Bauman et al. (1999), diets that contain unsaturated fat, high-energy content, finely ground forage and ionophores result in greater values of C18:2 cis-10, trans-12, which explains the greater unsaturated FA concentrations in beef fat of U.S.A. cattle.

Lipogenesis alterations of adipose tissue seem to be specifically associated with C18:2 trans-10, cis-12 isomer (Baumgard et al. 2000). Park et al. (1997) provided 0.5 % of CLA in diets for mice and observed 60 % reduction of body fat and 5 % increase in muscular mass compared to the group that received only oil addition to the diet. Moreover, the same authors tested the effects of three isomers on the body composition of mice and observed fat content reduction, and increase in water, protein and body ash when feeding diets supplemented with C18:2 trans-10, cis-12 isomer. Conversely, cis-9, trans-11 isomers did not significantly affect body composition. As previously described, Doyle (1998) verified that CLA effects on lipid metabolism are attributed to the increase in the activity of carnitine palmitoyltransferase enzymes and hormone-sensitive lipase, which participated in beta oxidation and intracellular lipid hydrolysis, respectively.

Economically, an important effect of CLA is the improvement of feed efficiency or feed to gain ratio (Pariza et al. 2001). It would be expected that this was a result of reduced fat synthesis by ruminants. However, when diets for mice were supplemented with C18:2 trans-10, cis-12 isomer, which is supposed to reduce fat synthesis, lower feeding efficiency in mice was observed (Cook et al. 2000; cited by Pariza et al. 2001). The feeding of both isomers (cis-9, trans-11 and trans-10, cis-12)

resulted in feed efficiency that was 55 % higher than in animals that did not consume CLA, indicating that there is a synergic effect between these isomers.

Mcguire et al. (1998) fed diets containing the same protein content for beef cattle with addition of high- and low- oil corn (7.04 % and 4.89 %, respectively), and observed that increases in linoleic acid content resulted in a significant increase of CLA content in *Longissimus dorsi* muscle, especially when diets contained greater forage content (20 %). Mir et al. (2000) observed a CLA increase from 0.21 % to 1.48 % of total FA in muscular lipids (*Bíceps femoris*) of bovines fed dieta containing 6 % of sunflower oil (dry matter basis). Sunflower seeds contain approximately 40 % of lipids and, in average, 65 % of them consists of linoleic acid (Coppock and Wilks 1991).

In addition, improved reproductive performance of bovines is the result of adequate nutrient intake. The nutritional and metabolic state of a reproducing cow affects its endocrine parameters, patters of follicular growth and luteal activity, and uterine secretory activity. These effects influence the onset of puberty, reestablishment of post-partum cyclic activity, establishment and maintenance of pregnancy. Nutrition acts directly as well as indirectly in bovine reproductive performance. In high-producing animals, such as dairy cows of specialized breeds, nutrition during transition period influences the occurrence of metabolic disorders and uterine diseases, which end up altering the probability of cows conceiving in the next lactation. The manipulation of diet composition and feeding management to increase energy supply for cows generally results in improvement of reproductive performance of these animals (Butler and Canfield 1989).

To further improve reproductive performance of cows, the diets of these animals should be supplemented with polyunsaturated FA sources, which increases energy intake, alters prostaglandin F-2-alpha secretion in the uterus, affects follicular growth dynamics, and improves luteal function, which many times results in fertility improvement. Therefore, the high dry matter intake (high net energy intake) may increase hepatic circulation of progesterone and its catabolization by the liver, which affects LH pulsatility, and consequently the follicular growth, among others.

Also, negative energy balance, which is the difference between ingested energy intake and required energy amount for maintenance and production, is mainly present in the last 2 weeks of pregnancy and first 4–8 weeks of lactation. During the beginning of lactation, nutrient distribution mechanisms give priority to milk production in detriment of reproductive functions. According to Beam and Butler (1999), the delay on ovarian cyclical activity resurgence is directly related to the animal's energy balance. The same author also reports that, in dairy cows, the first postpartum ovulation occurs approximately 10–14 days after the lowest negative net energy balance value.

According to Rice (1991), excessive weight loss in the beginning of lactation period may result in anestrus of beef cows, mainly those females presenting low body condition score. Moreover, undernutrition caused whether by lack of feed availability or feed intake inability to meet the animals' requirements inhibits estrus

signs and reduces responses of neural centers to excitatory stimuli due to reduction of the amount of estradiol receptors.

Nutritional management aiming to minimize intensity and duration of negative energy balance can improve reproductive performance, as Grant and Albright (1995) concluded that feed availability is more important and the first factor to affect energy intake. Therefore, ruminants must always have available feed, and diets have to have great acceptability and high quality to ensure maximum intake of dry matter. However, dry matter intake is physically limited at the end of pregnancy and beginning of lactation, which compromises total energy intake and reproductive performance.

Among the numerous strategies to alter energy intake in bovines, the addition of fat supplements to ruminants' diet is often used to increase diet energy concentration and improve animal performance. Beef cattle and dairy cattle's diets without any fat addition contain approximately 2–3 % of vegetal long-chain FA, predominantly mono and polyunsaturated FA. It is important to point out that for these unsaturated FA to have benefic effects on bovine reproduction, they have to resist ruminal biohydrogenation and be absorbed in the small intestine as they were provided in the diet. In general, supplementation with fat sources in dairy and beef cows increases blood cholesterol and progesterone levels, as well as increases growth of ovarian follicles.

Cows supplemented with fat in the post-partum period have dominant follicles larger in diameter and, potentially, with greater ovulation capacity. If FA utilization aims to increase diet energy density, they generally have benefic effect on the reproductive system of dairy cows when provided in pre-partum period as well. Cullens et al. (2005) evaluated the effect of adding source of FA rich in linoleic acid in the form of Ca soap in different stages of lactation on the reproductive parameters of dairy cows. The animals fed with FA source in peripartum period increased 3 % of conception rate. Juchem et al. (2004) evaluated the effect of adding Ca soaps, from palm oil or a mixture of linoleic acid and monoenoic trans acid, on dairy cows' diets during the end of pregnancy until the first 70 days of lactation, and observed similar effects in milk production, but the fat source containing trans FA reduced fat synthesis by the mammary gland and milk production corrected for fat; however, conception rate was increased with FA combination.

Likewise, lactating dairy cows consuming high-energy diets (approximately 44 % of non-fibrous carbohydrates) may present a decrease in milk fat content due to ruminal acidification. Although the feeding of high-energy diets increases propionate and decreases acetate proportion in the rumen, which would already affect fat production by the mammary gland, the high content of non-fibrous carbohydrates can also cause ruminal pH reduction or fluctuation, decreasing activity of fibrolytic ruminal microorganisms, which are sensitive to pH values equal or lower than 5.7 and responsible for acetate production in the rumen, reducing even more the fat production by the mammary gland. Likewise, milk fat reduction may also occur when unsaturated FA, mainly the C18:2 trans-10, cis-12, arrives in the mammary gland, as previously described in this chapter.

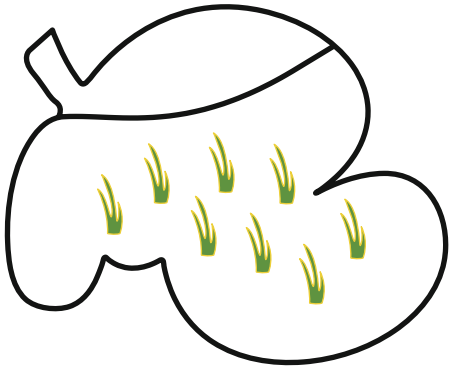

**Fig. 4.3** Biofilm formed by fat excess in diets of ruminants. The yellow lines surrounding the fiber particles represent the fat excess effect

According to Sniffen et al. (1992), some types of fats during supplementation may alter the composition and physico-chemical properties of milk, as in the case of those with high content of unsaturated FA. Thus, for lactating dairy cows, protein content can be reduced in the diets containing high lipid content because of reduced microbial synthesis, once lipids are not utilized as energy source for microbial growth or, according to Wu and Huber (1994), due to the decrease in amino acid availability in the mammary gland.

Unsaturated lipids have a toxic effect on bacteria that degrade cellulose in the rumen and as a result reduce acetate to propionate ratio and acetic acid supply, a direct precursor of 50 % of milk fat (Chalupa et al. 1986). The influence of lipids on ruminal microorganisms also occurs when there are free FA, which can form insoluble salts as well as a physical barrier on the feed, called "biofilm" (Fig. 4.3), making microbial colonization difficult and reducing the daily dry matter intake (Jenkins 1995).

Therefore, it is important to consider the lipid source and its inclusion level in ruminants' diets to minimize the effects on microorganisms and ruminal fermentation, considering a daily intake of approximately 3 %. The addition of whole oleaginous seed in the diets reduces the negative effects of lipids on fermentation due to their smaller contact with ruminal microorganisms (Byers and Schelling 1993). Whole soybean is an excellent energy and protein source for lactating cows; however, if high amounts are fed, milk fat content may be negatively affected. This is explained by the fact that oleaginous seeds, in general, have high content of unsaturated FA, such as oleic, linoleic and linolenic acids (Van Nevel and Demeyer 1988).

Cant et al. (1993) concluded that feeding lactating dairy cows with diets containing some type of fat source reduces milk protein content. According to the authors, lipids inhibit insulin activity besides reducing mammary blood flow of protein. Lipids increase energy efficiency for milk synthesis without increasing amino acid extraction by mammary gland, resulting in depression of milk protein content. This suggests that decreases in milk protein concentrations with fat addition can be the result of insufficient amino acid supply for the mammary gland to perform greater synthesis of milk protein, which is needed to follow the increase of milk production

stimulated by fat supplementation. Anyway, protein reduction can be attributed to reduction of microbial growth, as described previously.

## Intestinal Availability of Lipids

Forage-based diets for ruminants usually have low-lipid content (1–4 % of diet dry matter), represented mainly by galactolipids and triglycerides. Higher levels can be obtained by adding some fat sources or oleaginous seeds to the diet, since fat content is not over 7 % of diet dry matter, which would inhibit ruminal fermentation through a mechanic inhibition of cellulolytic microflora action (biofilm, Fig. 4.3), besides the toxic effect of unsaturated FA on bacterial cellular membranes (Kozloski 2002; NRC 2001).

More than 70 % of FA found in galactolipids and triglycerides of seeds are unsaturated (mainly oleic, linoleic and linolenic). A fraction of them is incorporated to bacterial lipids whereas a high FA proportion is biohydrogenated and flows from the rumen to the abomasum as free saturated FA without being utilized by ruminal microflora (Kozloski 2002; Hess et al. 2008). An alternative to increase the flow of unsaturated FA to the small intestine is the use of rumen-protected fat sources, which correspond to long-chain FA that become free in a process of triglyceride scission in vegetable oils. The FA react with calcium salts, united as a salt (R-COO-Ca), popularly known as calcium soaps (NRC 2001).

The utilization of essential FA as rumen-protected supplement (calcium salts) can also serve as a tool to increase ruminants' reproductive efficiency. In some cases, a commercial long-chain rumen-protected FA supplement is capable to supply all energy requirements not fulfilled by the rest of the diet and, therefore, have positive influence on animal body condition score, fertility rate and milk production (Ghoreishi et al. 2007).

Most FA that reach the small intestine are attached to feed particles. Bile provides biliary salts and lecithin, and pancreatic juice provides enzymes to convert lecithin into lysolecithin and bicarbonate in order to increase pH (Davis 1990). Lysolecithin and biliary salts release FA from feed particles and bacteria, and this allows the formation of micelles. Once micelles are formed, they are taken by epithelial cells to the jejunum where FA are re-esterified into triglycerides and then stored in chylomicrons (Demeyer and Doreau 1999).

Ferlay et al. (1993) extensively reviewed the literature on lipid digestion using data obtained on FA disappearance between the duodenum and ileum or between the duodenum and feces. Studies based on lipid disappearance along the tract were not used because typically overestimate FA digestibility in small intestine and provide questionable results (Doreau and Ferlay 1994). This is usually related to wrong estimates of FA intake, and because their flow into the duodenum is generally greater than the ingested amount. Thus, Doreau and Ferlay (1994) reported a great variation for FA digestibility, from 55 % up to 92 %, but this variation was not reported for FA intake. In reality, dairy cows' capacity to absorb long-chain FA is

very high and may exceed 1 kg/day (Doreau and Chilliard 1997). In monogastric animals, FA individual digestibility is reduced when the size of FA chains increases; however, digestibility increases when the numbers of double bonds increase (Lessire et al. 1992). Although similar patterns are observed in ruminants, the differences are small (Ferlay et al. 1993). Digestibility does not differ significantly between saturated FA with 16 and 18 carbons, and is smaller for those saturated FA with longer chain when compared to polyunsaturated FA. Doreau and Ferlay (1994) reported that average digestibility were 0.77, 0.85, 0.83 and 0.76 for FA of 18 carbons with zero, one, two and three double bonds, respectively

Another approach to investigate FA digestibility is abomasal infusion of FA. These studies generally have obtained similar digestibility results. The exception is the linoleic acid digestibility in which the abomasal infusion reveals slightly higher values. This is probably related to inappropriate measurements of C18:3 in diets without fat supplementation because the flow of this FA from the rumen to the small intestine is minimum. Avila et al. (2000) evaluated the effect of fat supplementation with different sources varying saturated and unsaturated FA proportions. The increase in unsaturation level of fat sources infused into abomasum did not affect digestibility of FA 18:1 cis-9, 18:2 or 18:3 that varied from 0.67, to 0.64, and to 0.76, respectively.

The utilization of rumen-protected fats in dairy cows' diets have raised interest in the effect of FA calcium salts in digestion and fat absorption. In an attempt to model metabolism and intestinal digestion of FA in ruminants, Chalupa et al.(2003) proposed that digestion coefficient of FA derived from calcium salts are substantially greater than free FA that reach the small intestine. However, there is little support for this in the literature published up to now. In general, it's considered that the use of Ca salts has little or no effect on apparent digestibility of individual FA in the small intestine (Møller 1988; Wu et al. 1991; Ferlay et al. 1993). When smaller differences come up, they are related to differences in FA profiles of compared supplements, and the existence of small variations of digestibility among individual FA as discussed above.

The FA digestibility is reduced for all active fats in the rumen with hydrogenation. If it were possible to produce a rumen-protected fat source composed mostly by unsaturated FA, the problem involving fat sources digestibility would be reduced. Calcium salts of unsaturated FA are relatively inert in the rumen and have greater intestinal digestibility than saturated FA. Most FA found in feedstuffs are from the 18-carbons family like linoleic acid (C18:2) and linolenic acid (C18:3), which are active in the rumen. The inert stearic acid portion (C18:0) in the rumen found in feedstuffs is very little, unless hydrogenated FA or tallow are provided.

## Dynamic of Lipid Absorption

Although it is not the objective of this chapter, the description of lipid absorption after abomasum could not be left out because some aspects can help in the recommendations whether or not to utilize supplementary lipids, natural or protected one.

Then, analyses of FA from lipids that reach the small intestine have shown that they are very similar to the ones that leave the rumen. Therefore, there is no significant absorption or modification of medium-chain and long-chain FA in the omasum or abomasum (Noble 1981). As a consequence of ruminal metabolism, lipids that get into the small intestine consist of saturated FA, mainly stearic and palmitic acids.

The total lipid amount that reaches the duodenum is generally greater than the ingested amount. In high-forage diets, this difference is more significant. Lipid supplements utilized in ruminants' diets can result in greater, equal or smaller post-ruminal flow in relation to the ingested amount of FA due to the diversity of effects that they can have on lipid microbial synthesis and ruminal metabolism (Demeyer and Doreau 1999). Approximately 80–90 % of lipids that reach the small intestine are free FA attached to feed particles (Davis 1990; Doreau and Chilliard 1997). The rest of lipids are microbial phospholipids and small amounts of feed triglycerides and glycolipids (esterified FA), which are hydrolyzed by intestinal and pancreatic lipases (Doreau and Ferlay 1994).

When supplementary fats are provided to dairy cows, increases in triglyceride flow from rumen to omasum will be observed only when encapsulated lipids are provided. Calcium salts of FA are the predominant source of protected lipids provided to ruminants and they dissociate at some level in the rumen, but dissociation is much greater in the abomasum where pH is lower. Therefore, protected lipid supplements with calcium salts compose the free FA pool that reaches the small intestine.

Fat absorption in the small intestine occurs in the jejunum and micelle formation is the key for efficient absorption of FA in all species (Davis 1990). In non-ruminants, monoacylglycerols are necessary to form micelles (Doreau and Chilliard 1997).

## Influence of Lipid Supplementation on Dry Matter Intake

Optimal inclusion level of supplementary fat in ruminants' diets is smaller than 3 % of diet dry matter. Then, if the goal is to maximize the use of forage-based diets, limited to 2 % of dry matter intake, or prevent substitution of forage intake by supplementary fat intake, it is not recommended to exceed 4 % of total availability of diet energy with fat supply (Hess et al. 2008). On the other hand, if the idea is to supplement ruminants that consume total mixed rations (TMR) with some fat source, the maximum limit of addition becomes 7 % of diet dry matter.

In dairy cows' feeding, lipids are included in the diet to increase energy density, improve energy balance and promote increments in animal reproduction and immunity. The effects of fat supplementation in the diets offered to dairy cows depend on some factors, such as: amount of carbons, presence and number of unsaturation, formation of complexes with other substances and fat physical form. Fat excess in diet, at levels higher than 7 % of diet dry matter, can cause the formation of biofilm mainly around forage particles (Fig. 4.3), which would negatively impact ruminal

fermentation, especially the production of acetate by fibrolytic microorganisms, and would reduce dry matter intake.

There are several fat physical forms that can be provided to dairy cows, such as oleaginous seeds, animal fat, mixture of animal and vegetal fats, dry fat in granules and rumen-protected fat (NRC 2001). Vegetal fats mostly have great amounts of unsaturated FA. Soybean, cottonseed and sunflower have high linoleic acid content. Forage and linseed have high linolenic acid contents. Saturated FA are more common in animal fats like tallow. It is important to understand these variations in lipid composition because their reactivity in the rumen will be different, and so their digestibility. Saturated FA are less available in the intestine than unsaturated FA like stearic acid (C18:0). Therefore, lipid supplements that increase the flow of stearic acid into the small intestine will provide less energy to the cow. Palmitic acid (C16:0) is also a saturated FA, but it seems to be relatively better absorbed in the small intestine than stearic acid, probably due to the size of its carbon chain and greater solubility. Saturated triglycerides that go through the rumen have low digestibility in the small intestine. As cited before, triglycerides are not fermented in the rumen and, therefore, are not energy source for ruminal microorganisms.

Calcium salts seem to reduce feed intake more than saturated fat sources, especially when provided over acceptable levels. This confirms the biological and nutritional sense that a low-producing cow that receives a diet containing more than of 3 % of lipids (dry matter basis) will reduce dry matter intake, as energy excess will not be used for milk production, and eventually there will be a nutrient unbalance (Palmquist 1994).

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# Chapter 5
# Ruminal Acidosis

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## Introdution

### *Dynamics of Stable Fermentation vs. Unstable Fermentation*

Ruminants are herbivores that have developed themselves in biosphere in order to consume forages (grasses and legumes), which are characterized by the high content of cell wall; although there are variations related to ruminants' species regarding their capacity to select plants with smaller contents of these compounds (Van Soest et al. 1991). The intake of this type of diet keeps the reticulum-rumen (main digestive compartment of these animals) with a set of physical and chemical characteristics that are considered appropriate to maintain the animal's ruminal microbiota and health. However, due to the need to increase (beef and milk) production, ruminants have been subjected to different diet conditions, from those that they evolved consuming, which may contain large amounts of carbohydrates that quickly ferment in the rumen and cause a series of digestive and metabolic disorders like ruminal and metabolic acidosis, rumenitis, bloat, liver abscesses and laminitis (González et al. 2012).

Rumen pH is influenced by mechanisms that determine the production of short-chain fatty acids (**SCFA**) and those that promote their buffering. Dietary

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carbohydrate fermentation by ruminal microbiota represents the main form of SCFA (mainly acetic, propionic and butyric acids) and lactic acid production, which due to its pKa (in average 4.8 for SCFA and 3.8 for lactic acid) tend to donate protons to the ruminal environment (when rumen pH is above 6.2). Thus, SCFA production is the main mechanism of ruminal pH reduction.

Under normal dietary conditions, which means with appropriate proportion of physically effective fiber, ruminal pH is kept relatively close to neutrality (6.5–6.7) because buffering mechanisms are fully active, especially salivary secretion, and also because non-fibrous carbohydrate concentration in the diet is low.

The final products of ruminal fermentation vary according to the type of diet offered to the animals, basically due to specificity and metabolic pathways used by different populations of microorganisms that digest each dietary carbohydrate (fibrous or non-fibrous). Forage-based diets are rich in cellulose, intermediary in soluble sugars and poor in starch (Kaufmann et al. 1980). Therefore, cellulolytic and saccharolytic bacteria will be the most active and consequently cellulose digestion and soluble sugar fermentation by these microbial communities will result in great production of acetate (Owens and Goetsch 1988). On the other hand, in starch-rich diets, amylolytic bacteria represent the most of the microbial population and compete for substrates (soluble carbohydrates, products of starch fermentation and hemicellulose) under ruminal pH lower than normally observed when high-forage based diets are fed, resulting in an increase in propionate production (Kaufmann et al. 1980; Fig. 5.1).

As more starch and soluble sugars are added to the diet of ruminants, whose microbiota is not adapted to metabolize such substrates, ruminal pH reduction is frequently observed, which is also due to the lack of ruminal epithelial adaptation. Likewise, in ruminal pH lower than 5.6, strong organic acids, like lactate (pKa = 3.86); that were not detected before in the rumen because they are interme-

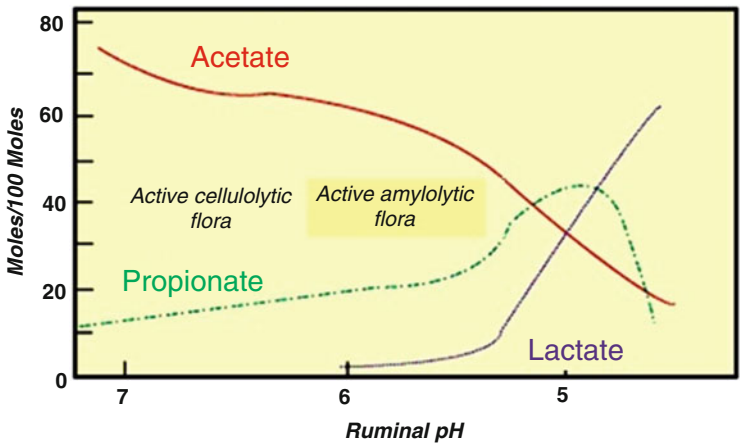


**Fig. 5.1** Relationship of ruminal pH with proportions of acetate, propionate and lactate. Adapted from Kaufmann et al. (1980)

diary metabolites of non-fibrous carbohydrate fermentation, which results in propionate production (pKa = 4.8); accumulate in the rumen, especially when the rumen epithelium is not fully developed to absorb fermentation products of the new diet. The consequences of ruminal acidification process can range from decrease of dry matter intake up to the animal's death.

Therefore, microbiota and ruminal epithelium have to be properly adapted to the respective dietary substrate so that stable fermentation occurs. Then, abrupt changes in the diet, particularly from high-fiber to high-concentrate or high-starch diets, causes an imbalance of the symbiotic relationship between the ruminal microbial population and the host. This occurs because the microbial population that lives in the rumen adapt itself more quickly than the ruminal epithelium to new nutritional situations; therefore, there is an accumulation of acids in the rumen, once the production rate of these acids by bacteria will be greater than the absorptive capacity of the ruminal epithelium. That is the beginning of the most studied nutritional disorder in ruminants: ruminal acidosis.

## *Absorption of Short-Chain Fatty Acids by the Rumen*

The inclusion of high amounts of starch in ruminants' diets increases SCFA production, as well as the production of lactic acid by ruminal microorganisms, which can drastically reduce ruminal pH, resulting in digestive problems like acidosis (Preston 1998).

Great ruminal pH fluctuations are always associated with clinical and subclinical acidosis, and the latter has been the subject of several studies in the past years because it is one of the metabolic diseases that have affected beef and dairy cattle operations mostly when there high-concentrate diets are fed. The great challenge is to produce the largest amount of SCFA in the rumen up to the point that the ruminal epithelium will be able to absorb them in order to prevent acid accumulation, mainly lactic acid, to avoid pH reduction to the point of compromising fermentative processes, which negatively impacts animal performance. Therefore, it is important to deeply understand the mechanisms by which SCFA are absorbed by the ruminal epithelium, and how acids accumulation occurs in the rumen, in order to establish the most appropriate nutritional strategy to avoid acidification.

Moreover, animals consuming high-energy diets inevitably have more intense ruminal fermentation and greater SCFA production, which lowers rumen pH. Therefore, some additional buffer has to participate in the ruminal content metabolism to increase pH within the range considered normal for the microbiota and the host. One of the greatest sources of buffering agents is saliva, because it contains a large amount of phosphate and bicarbonate ($\mathbf{HCO_3^-}$) that are fundamental to buffer ruminal pH, maintaining its equilibrium. Besides that, the mechanism of $HCO_3^-$ input in the rumen is directly related to SCFA absorption and ruminal acidification.

In general, there are two input mechanisms of $HCO_3^-$ in the rumen: through salivation, which is already well known, and through the ruminal epithelium, which has been very studied in the last years. Under normal conditions, approximately 50 % of $HCO_3^-$ reaches the rumen through saliva and the other 50 % gets to the rumen through the ruminal epithelium when there is SCFA absorption. Likewise, when the ruminal wall absorbs SCFA, ruminal epithelium releases a molecule of $HCO_3^-$ into the rumen, and it contributes to the buffering of the ruminal contents, keeping it at more comfortable levels for the animals.

In the rumen, SCFA can be protonated, which means non-dissociated (**HSCFA**), and non-protonated or ionized, which means dissociated (**$SCFA^-$**). In other words, the amount of HSCFA and $SCFA^-$ will determine the amount of free $H^+$ in the rumen, which is responsible for its acidification. The equilibrium between the amount of HSCFA and $SCFA^-$ is given by the value of pKa for each acid, which is the pH value of a determined solution where the molecules (in this case SCFA or lactic acid) are found 50 % in its protonated form (HSCFA) and 50 % in its non-protonated form ($SCFA^-$).

Considering that the average pKa of SCFA is 4.8, it is possible to calculate through Henderson-Hasselbalch equation that for pH 2.8, 3.8, 4.8, 5.8 and 6.8, SCFA release their protons at rates of 1 %, 10 %, 50 %, 90 % and 99 %, respectively. Therefore, the lower the ruminal pH gets, the greater SCFA percentage in its protonated form (HSCFA) becomes, whereas the higher the ruminal pH is, the higher SCFA percentage in its non-protonated form ($SCFA^-$) gets. Thus, SCFA act like buffering components within the rumen because they sequester protons ($H^+$) from the ruminal environment when pH decreases and provide protons as pH increases (Aschenbach et al. 2010). However, if we consider that average pKa of SCFA is 4.8, in a very intense ruminal acidification (like in the case of clinical acidosis in which the ruminal pH is very close to 4.8), the contribution of SCFA protonation mechanism for rumen buffering is relatively low because in this pH range only 50 % of SCFA are HSCFA and the other 50 % will be in its dissociated or ionized form ($SCFA^-$).

Besides SCFA, lactic acid is also found in the rumen in its protonated (**LACH**) and non-protonated or dissociated form (**$LAC^-$**). The main difference from SFCA is that pKa of lactic acid is 3.86, which means that only when ruminal pH reaches the value of 3.86, 50 % of lactic acid will be protonated (LACH) and 50 % will be dissociated ($LAC^-$). Therefore, lactic acid is a strong acid, differently from the main SCFA found in the rumen, which are considered weak acids. Lactic acid is 10 times stronger than SCFA commonly found in the rumen, and thus, when accumulated in the rumen, it can compromise rumen health and fermentative processes. Due to the dissociation constant of lactic acid under normal conditions, as well as in clinical and subclinical acidosis conditions, the predominant form of lactic acid in the rumen will always be $LAC^-$, which means that every lactic acid produced in the rumen releases a $H^+$ in the ruminal environment. In addition, when lactic acid is absorbed, it does not contribute with $H^+$ withdrawal from the ruminal environment. Moreover, when compared to SCFA, lactic acid is much less absorbable and many times does not need an increase in its production rate to accumulate in the rumen (Williams and Mackenzie 1965). Thus, the easiest form to prevent lactic acid accumulation is to avoid great

ruminal pH fluctuations (<5.6), reducing the proliferation of lactate-producing microorganisms and increasing the population of lactate-consuming bacteria.

It is important to remember that depending on the ruminal pH, SCFA may be linked, or not, to $H^+$; then, it can be concluded that HSCFA, when absorbed by the rumen wall, take $H^+$ proton with them, and then $H^+$ concentration in the ruminal environment decreases, and consequently, acidification reduces. However, it's noteworthy to point out that, in general, whenever ruminal pH is over 4.8, there will be a greater amount of $SCFA^-$ in the rumen that do not carry $H^+$ to bloodstream during its absorption, which do not reduce acidification.

As discussed before, one way for $HCO_3^-$ to get into animals' rumen is through SCFA exchange during absorption through the ruminal epithelium, but not every SCFA is exchanged by $HCO_3^-$ when absorbed because there are differences in absorption mechanisms between HSCFA and $SCFA^-$. When protonated in the ruminal fluid, HSCFA are absorbed by lipophilic diffusion, which means that they are absorbed by the rumen wall (papilla and epithelium) through membranes without exchange with $HCO_3^-$ during absorption. Moreover, SCFA that are absorbed by lipophilic diffusion are high-lipophilicity molecules because they are found in their protonated form (HSCFA).

In the dissociated form, $SCFA^-$ are electrically charged molecules and are more unstable in the rumen. Besides, permeability of ruminal epithelium lipid layer for electrically charged molecules ($SCFA^-$) is extremely low, and because of that passive diffusion through membranes has been attributed only to molecules in their protonated forms (HSCFA; Walter and Gutknecht 1986; Gabel et al. 2002). Therefore, there is an alternate responsible mechanism for $SCFA^-$ absorption through membrane proteins. $SCFA^-$ that are mostly molecules with less lipophilicity seems to be bicarbonate dependent for absorption, which means that when a $SCFA^-$ is absorbed, a molecule of $HCO_3^-$ is released into the ruminal fluid (Aschenbach et al. 2009).

Regarding absorption mechanisms of lactic acid, it is not very clear why the absorption of this molecule is so low when compared to SCFA. Probably, the fact that lactic acid has lower pKa, when compared to SCFA, makes it release more $H^+$ ions for the ruminal environment. Likewise, absorption rate by the ruminal wall either from a SCFA or lactate is smaller when they are dissociated. There is evidence that the lactic acid in dextrorotatory configuration (D-Lactate) has a little higher pKa (3.96) than in levorotatory configuration (3.76; L-Lactate), indicating that L-Lactate is still less absorbed by the rumen wall, influencing reduction of ruminal pH more drastically.

Thus, it is possible to observe that there are different absorption mechanisms of SCFA by the ruminal epithelium, whose main cause is SCFA protonation ($SCFA^-$ or HSCFA). It is impressive how the physiology and biochemistry of ruminants work perfectly under very intense ruminal acidification conditions, because as ruminal pH decreases, SCFA themselves act as buffer sequestering $H^+$ ions from the ruminal environment through its protonation, and when absorbed they carry these $H^+$ ions from the ruminal environment, reducing acidification. It is important to point out that when protonated, SCFA as well as lactate are more absorbed than their dissociated forms. Besides, the SCFA that are in a dissociated form ($SCFA^-$), when absorbed by the ruminal epithelium, do not carry $H^+$ with them during

absorption, but in this case the ruminal epithelium releases a molecule of $HCO_3^-$ into the rumen, which will link itself to free $H^+$ ions in the ruminal fluid, forming water and $CO_2$.

## Etiology

### *Definition*

Acidosis can be defined as a decrease of alkaline bases content found in body fluids in relation to acid contents (hydrogen ions; Stedman 1982, cited by Owens et al. 1998). This process starts when a non-adapted animal (normally fed with forage-based diets) consumes great amounts of readily fermentable carbohydrates, which most of the times is starch.

### *Causes*

There are several causes of ruminal acidosis, but they always converge to one single point: excessive intake of readily fermentable carbohydrates. Among the most common causes, it is possible to mention: (1) SCFA accumulation in the rumen due to the excessive dry matter intake of (normally observed in dairy herds); (2) quick ruminal fermentation (diets containing high starch contents or extensively processed corn); (3) abrupt diet change or even feeding of low-fiber diets that can lead to disequilibrium between the increase of microbial population, its utilization of substrates and absorption of organic acids by the ruminal epithelium; and finally, (4) diets containing high-fat content (which make fiber degradation difficult). Regarding feeding management, there are two preponderant points for the occurrence of acidosis: (1) erratic distribution during daily and/or weekly feeding, associated with the high-density stocking rate of pens or paddocks, where bunk space availability for animals is reduced, which causes high competition to obtain feed and then dry matter intake may fluctuate on a daily basis; and (2) when the mixed ration is not uniformly blended, which may not mask less palatable feeds and allow sorting of feed by the animals. Also, in this case, less palatable feeds or fine particles may go to the bottom of the bunk.

### *Ruminal Acidosis: Microbiological and Biochemical Focus*

According to Chaps. 2 and 3 of this book, the ruminal environment of a healthy animal has low concentration of free glucose and lactate, and ruminal pH between 5.7 and 6.8. However, from the moment ruminal acidification starts, an inverse

situation is found. In general, acidosis development first occurs abruptly with an increase in intake of rapidly fermentable carbohydrates followed by quick ruminal fermentation that changes the profile of ruminal microbial population. After feed intake, starch utilized by specific bacteria in the digestion of rapidly fermentable carbohydrates is converted into glucose. Normally, glucose is found at very low ruminal concentrations (160 mg/dl, Slyter 1976; Horn et al. 1979) and, therefore, is not considered an important intermediary metabolite in the rumen. When glucose is free in the rumen due to the quick hydrolysis of readily fermentable carbohydrates, some non-competitive bacteria may grow very fast. This increase generates an appropriate condition for the fast growth of lactate-producing bacteria, mainly *Streptococcus bovis* and *Lactobacillus* ssp. species. Consequently, more lactate is produced (acid that is ten times stronger than SCFA) and pH of ruminal content can vary from 5.5 to 4.0.

*Streptococcus bovis* is a facultative anaerobic microorganism normally found in the rumen, cecum and colon of ruminants. In animals fed forage, the population of this bacterium is $10^4$–$10^7$/g of ruminal content; however, in animals fed excessive amount of rapidly fermentable carbohydrates, its population can reach $10^{11}$/g of ruminal content (Nagaraja and Titgemeyer 2007). Even if other species of ruminal bacteria can utilize starch, the relative success of *S. bovis* is due to its fast multiplication in the rumen, as it duplicates every 24 min in cases of abrupt diet change with fast degradation of starch from cereals (McAllister et al. 1990, cited by Nagaraja and Titgemeyer 2007). When rumen pH is close to neutrality, this bacterium produces acetate, formate and ethanol from glucose. However, when rumen pH is lower than 5.6 and a high amount of potentially fermentable substrates are available; *S. bovis* starts homolactic fermentation, mainly L-Lactate (Russell and Hino 1985; Finlayson 1986, cited by Nagaraja and Titgemeyer 2007).

This change occurs because of the type of enzymes utilized by *S. bovis* to convert pyruvate in other metabolic compounds. In this bacterium, pyruvate is transformed into lactate by lactate dehydrogenase and pyruvate hydrogenase; or converted by pyruvate formate lyase into acetyl CoA that is then converted into acetate or ethanol. The increase in lactate production with consequent reduction of acetate and ethanol production occurs because the microorganism allows that intracellular pH reach 5.5, which is optimal for maximum activation of lactate dehydrogenase (Russell and Hino 1985) and pyruvate hydrogenase, increasing competition for pyruvate and its conversion into lactate.

Buffering mechanisms of SCFA produced in the rumen include: the action of saliva through bicarbonate and phosphate ions that buffer approximately 30 % of total produced SCFA; the action of bacteria that utilize lactate (mainly *Megasphaera elsdenii* and *Selenomonas ruminantium*); the absorptive action of the ruminal epithelium that buffers approximately 50 % of total SCFA produced in the rumen by SCFA absorption, which contributes to reduce their concentration; and the passing of SCFA to the omasum/abomasum and lower digestive tract which receives approximately 15 % of produced SCFA (González et al. 2012). Thus, ruminal pH at a certain time after feeding will be determined by the equilibrium of these mechanisms just described and the acid production (SCFA and lactate).

Normally, in diets that have high-fiber content (NDF>40 %) and moderate amount of starch (<20 %), ruminal pH is maintained over 6.2; because ruminal fermentation of fiber, and acid production in this case, is performed slowly by the ruminal microbiota, and at the same time, because of the effect that fiber has stimulating ruminal motility, rumination and saliva production (Van Soest et al. 1991).

However, when high-concentrate diets (over 65 % concentrate and/or more than 45 % of non-fibrous carbohydrates) are fed ad libitum once daily, or without appropriate adaptation, there will be quick starch digestion in the rumen which allows high SCFA production associated to the limited amount of fiber, producing less saliva. As a consequence, ruminal pH can be reduced to values lower than 6.0, causing ruminal acidosis (clinical or subclinical; Van Soest et al. 1991).

When ruminal pH is higher than 6.0, in general it means that there is low availability of starch and/or sugars in the ruminal fluid, and therefore, there is low lactate production and, consequently, the amount of lactate-utilizing bacteria is also reduced. Besides that, produced SCFA and lactate are constantly absorbed by the ruminal epithelium and then the ruminal pH remains stable. However, when diet is suddenly altered, the high intake of non-fibrous carbohydrates allows quick growth of amylolytic bacteria (due to greater glucose availability), which, under this condition, promote quicker SCFA production in the rumen, and these SCFA reduce ruminal pH to values lower than 6.0. When ruminal pH is lower than 6.0, gram-positive bacterium *Streptococcus bovis* starts glucose fermentation by utilizing a lactate dehydrogenase enzyme, which is activated by the reduction of intracellular pH. Due to this change, *S. bovis* has greater energy use efficiency per time unit despite being less efficient per mole of fermented glucose (2 x 4 mole of ATP/glucose). Thus, *S. bovis* starts growing quickly in the rumen (it duplicates every 24 min) under conditions of abrupt diet change (McAllister et al. 1990) and, therefore, ferments more glucose and produces more lactate, reducing pH more drastically (Fig. 5.2).

Considering that lactate is a strong acid (pKa = 3.8), which means that it donates protons (H+ ions) to the rumen environment more intensely than SCFA, the increase of its production and, consequently, its concentration in the ruminal fluid reduces more drastically the ruminal pH. While ruminal pH is maintained higher than 5.2, the permanence of lactate-utilizing bacteria in the rumen, such as *Megasphaera elsdenii* and *Selenomonas ruminantium*, collaborate to buffering ruminal pH; however, with pH reduction under 5.2, there is a decrease of lactate-utilizing bacteria growth, which leads to lactate accumulation in the rumen, and consequently to ruminal acidosis.

The ruminal pH reduction to values under 5.2, besides decreasing the growth of lactate-utilizing bacteria, it also negatively affects growth of *S. bovis* bacteria; however, as a consequence, there is the growth of *Lactobacillus* bacteria that are more homolactic and tolerant to low pH, and become the main bacterial group for this pH range. This condition increases lactate production and its accumulation in the rumen even more, aggravating ruminal acidification (Nagaraja and Titgemeyer 2007). Thus, when pH is below 5.2 and when the growth of lactate-utilizing bacteria ceases, the rumen gets into stasis and consequently metabolic acidosis can occur and even cause the animal's death (Fig. 5.2).

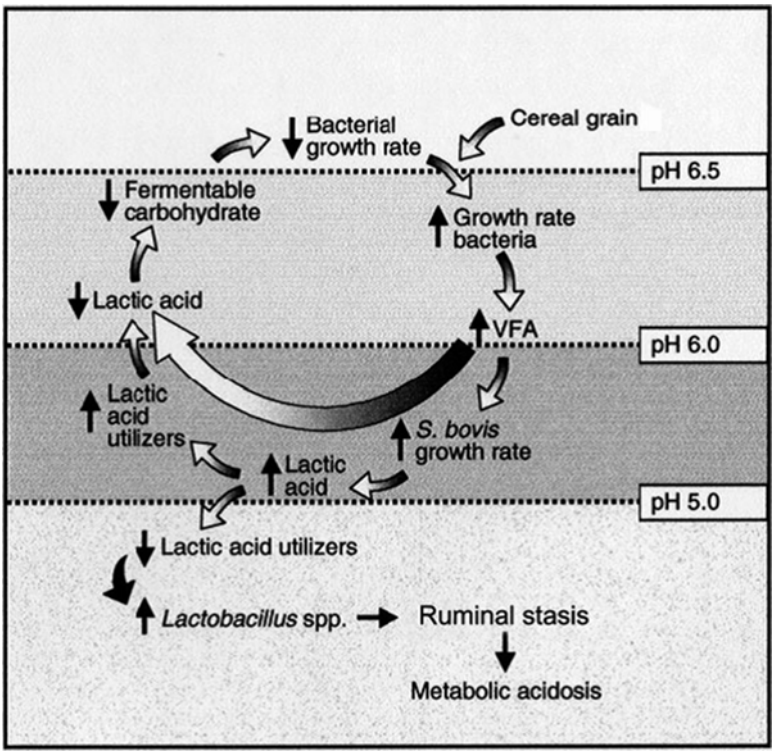


**Fig. 5.2** Changes on fermentation patterns and microbial population at different ruminal pH levels due to the feeding of high-energy diets. Adapted from Schwartzkopf-Genswein et al. (2003)

There is a series of bacteria that ferment lactic acid in the rumen, including *Megasphaera elsdenii*, *Selenomonas ruminantium* ssp. *lactilytica*, *Anaerovibrio lipolytica*, *Fusobacterium necrophorum*, *Peptostreptococcus asaccharolyticus*, *Propionibacterium acnes* and *Veillonella parvula*. The most active of these species in the rumen of animals fed concentrate-rich diets are *Megasphaera elsdenii* and *Selenomonas ruminantium* (Nagaraja and Titgemeyer 2007). Considering that *Megasphaera elsdenii* is able to ferment 60–80 % of lactate in the rumen, this bacterium is probably the most important microorganism that ferments lactic acid and therefore, has a fundamental role in preventing lactate accumulation in the rumen of animals adapted to high-grain diets (Nagaraja and Titgemeyer 2007).

Besides the action of lactate-utilizing bacteria in the rumen, the presence of ciliated protozoa has been considered favorable to control rumen acidification, once this kind of microorganisms engulfs starch granules and slowly ferments them, or converts them into reserve carbohydrates. These actions decrease starch availability for amylolytic bacteria, mainly for lactate-producing ones. Likewise, protozoa feed themselves with bacteria which also contribute to reduce ruminal acidification. However, there is also a lot of controversy on the real effect of ruminal protozoa on

the control of starch metabolism in the rumen, due to the high sensitivity of this kind of microorganism to ruminal pH lower than 6.0 (Nagaraja and Titgemeyer 2007).

Studies conducted some decades ago allowed concluding that ciliated protozoa population in the ruminal fluid is eliminated or drastically reduced in animals fed starch-rich diets. However, more recently, it has been noticed that although a diversity of protozoa is reduced when high-grain diets are fed, the number of some genera (*Entoninium* spp.) can remain extremely high. *Entodinium* spp. can reduce the risk of subclinical acidosis because they utilize lactic acid and moderate the starch digestion rate in the rumen (Newbold et al. 1987; Williams and Coleman 1992).

Moreover, ruminal acidosis can predispose rumen to other nutritional and metabolic disorders like rumenitis, parakeratosis, liver abscesses and laminitis. Changes in the ruminal microbiota profile associated with pH reduction decrease the growth and lead some bacterial species to death, mainly gram-negative ones. As a result, lipopolysaccharides (LPS) from the outer membrane of these bacteria, as well as other toxins, are released and reach the bloodstream, where they can translocate and cause liver abscesses and laminitis.

## *Acute Acidosis* Versus *Subacute Acidosis*

There are two kinds of acidosis, clinic (acute) and subclinic (subacute). In clinic acidosis, pH is drastically reduced and lactic acid concentration is high. It is considered that ruminal pH 5.2 is the threshold between clinic and subclinic acidosis (Owens et al. 1998). Moreover, it is suggested that acute acidosis starts when ruminal concentration of lactic acid reaches 40 mM (both as isomer D and L; Owens et al. 1998; Galyean and Rivera 2003).

In animals that present this nutritional disorder, the difference between blood and ruminal fluid osmolality causes acid migration from the rumen to bloodstream, exhausting blood buffering capacity, formed by bases, represented mainly by bicarbonate (Galyean and Rivera 2003). Blood osmolality causes swelling in the animal's legs and laminitis (Nocek 1997). In more severe cases of clinic acidosis, there may be an influx of water from the blood to the rumen and the animal can die if blood homeostasis is not re-established.

Animals suffering from subclinic acidosis have high osmolality (>350 mOsm) in ruminal fluid and that impair digestion of fiber (240–265 mOsm) and starch (280–300 mOsm; Garza et al. 1989). Because of osmolality increases, abomasum motility is compromised, impairing the removal of produced acids and exacerbating ruminal acidification even more, which may cause rumenitis and liver abscesses. Therefore, the altered motility or tonicity can cause dry matter intake fluctuations (Owens et al. 1998). A lot of studies have concluded that great variations or fluctuations of dry matter intake by cattle fed with high-concentrate diets can cause digestive disorders like acidosis (Fulton et al. 1979; Britton et al. 1989) and reduce feedlot performance (Stock et al. 1995); and this effect is greater in the beginning of the feeding period (Krehbiel et al. 1995; Soto-Navarro et al. 2000). Schwartzkopf-Genswein et al. (2004) observed that cattle with fluctuating intakes spent more time at ruminal pH below 5.5,

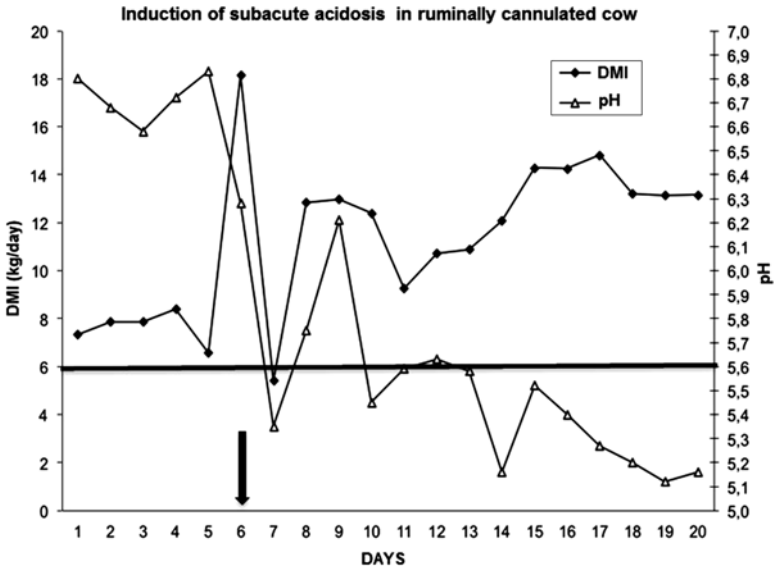


**Fig. 5.3** Voluntary daily dry matter intake (DMI) and ruminal pH of ruminally cannulated cow induced to subacute acidosis. Initially, a sugarcane-and-urea- based diet (14 % of CP) was provided until day 5. From day 6, an isoprotein diet containing 73 % concentrate (high-moisture rolled corn grain silage, soybean meal, supplement and fresh-chopped sugarcane) was fed, which is indicated by the *bold arrow*. The *horizontal line* indicates the limit of acidosis initial pH (5.6), measured 3 h after feeding (Pacheco, unpublished data)

which can already be considered subclinic acidosis (Owens et al. 1998; Galyean and Rivera 2003) and still severely inhibit fiber digestion (Yang 2002). Similarly, Cooper et al. (1999) reported that animals with fluctuating feed intake spent less time eating.

Regarding subclinic or subacute acidosis, the external signs are rarely found and indicatives are related to reduction of dry matter intake and performance (DiLorenzo 2004). The pH of 5.6 is considered the threshold between subclinical acidosis and a healthy rumen (Owens et al. 1998; Galyean and Rivera 2003). Reduction of dry matter intake occurs concomitantly with low ruminal pH as observed in Fig. 5.3 (Pacheco, unpublished data). Based on this fact, considering that the animals do not consume much feed for one or two days, maybe as a way to decrease ruminal acidity, in the beginning of the third day they will be starving. Therefore, the animal will be avid for feed and will consume it in excessive amounts in a short period of time, contributing for a new cycle of pH reduction. Thus, the animal suffering from subacute acidosis presents fluctuating dry matter intake for days. Because cattle in commercial feedyards are fed in group pens, and also due to the different individual susceptibility levels to this digestive disorder, the occurrence of subclinic acidosis can be masked.

To diagnose ruminal acidosis, the concentration of lipopolysaccharide (LPS) endotoxin released from the outer membrane of gram-negative bacteria can be measured in the blood of animals suffering either from subclinic or clinic acidosis. The increase in lipopolysaccharide (LPS) endotoxin concentration is associated with

immunological disorders, inflammatory responses and alteration of blood vessel permeability, which allows translocation of potentially harmful bacteria to the animal's bloodstream. As a consequence, there is an increase in laminitis, rumenitis and other inflammations and infections (Gozho et al. 2006).

Another more precise method to detect and compare the intensity of subclinical acidosis in research studies is by finding out the minimum ruminal pH reached when a given diet is fed and verifying how long it remains low. This approach is based on the observation that ruminal pH varies throughout the day, and it is lower (Nadir) 2–6 h after morning feeding. Therefore, the simple average daily pH can mask the occurrence of subacute acidosis. However, it is necessary to utilize ruminal probes (data loggers) to continuously monitor pH to elaborate a consistent pH curve and not to interfere in the feeding behavior of the evaluated animals.

The comparison between the two types of acidosis can be better understood by gathering the observations on changes that occurred in ruminal fermentation and the respective consequences to the animal, based on the literature review done by Nagaraja and Titgemeyer (2007) summarized in Table 5.1.

**Table 5.1** Comparison of acute and subacute acidosis in cattle

| Item | Acute | Subacute |
|---|---|---|
| Clinic signals | Present | Absent |
| Mortality | Yes | No |
| Ruminal changes | | |
| Ruminal pH | <5.0 | 5.0–5.5 |
| Total organic acids | Increased | Increased |
| Lactic acid | Increased (50–120 mM) | Normal (0–5 mM) |
| SCFA | Lower than normal (100 mM) | High (150–225 mM) |
| Microbes | | |
| Gram-negative bacteria | Decrease | Unaltered |
| Gram-positive bacteria | Increase | Unaltered |
| *Streptococcus bovis* | Initial Increase | Unaltered |
| *Lactobacillus* spp. | Increase | Increase |
| Lactate producers | Increase | Increase |
| Lactate utilizers | Decrease | Increase |
| Blood changes | | |
| pH | Decrease (<7,35) | Unaltered, low decrease |
| Lactate | Increase, isomer D | Normal |
| Bicarbonate | Great reduction (<20 mEq/L) | Normal, transient reduction |
| Sequels | | |
| Rumenitis | Yes | Yes |
| Laminitis | Yes | Yes |
| Polioencephalomalacia | Yes | Yes |
| Liver abscesses | Yes | Yes |

Adapted from Nagaraja and Titgemeyer (2007)

Subacute acidosis mainly affects high-production dairy cattle. Despite consuming lower concentrate diet when compared to feedlot beef cattle, high-production dairy cows normally have greater dry matter intake, which results many times in greater concentrate intake than beef cattle, besides the fact that they are fed for longer periods. Thus, the development of subacute acidosis in dairy cows can cause economic losses because these animals have successive productions throughout their lives, and then significant losses during the lactations, as this kind of disorder does not present specific signals or symptoms.

According to Stock and Britton (1996), almost all beef cattle suffer from subacute acidosis sometime during finishing period in commercial feedlots in the Unites States. Researchers from the University of Nebraska calculate that the losses are approximately US$13.00 per head a year due to lower dry matter intake and consequently poorer animal performance. This data becomes relevant to the Brazilian conditions as the number of feedlots and concentrate proportions in the diet increase annually. Millen et al. (2009) carried out a survey with feedlot beef cattle nutritionists in Brazil and 52 % of the interviewees answered that acidosis or problems related to acidosis (laminitis and bloat) are the second biggest issue related to feedlot beef cattle's health after respiratory problems (56 %).

## Predisposition Factors

### *Types of Carbohydrates in Diet*

Diets commonly fed to beef and dairy cattle in highly intensive commercial operations are characterized by high inclusions of quickly fermentable carbohydrates from grains and coproducts from food and biofuel industries. Among the types of carbohydrates with quick ruminal fermentation are pectin (found in citrus pulp, soybean hulls and beet pulp) and starch (present in several types of cereal grains).

Marino et al. (2011) compared the acidification potential among citrus pulp, finely-ground corn and high-moisture corn silage of cows cannulated in the rumen and fed diets containing approximately 70 % concentrate. The authors observed that animals fed citrus pulp presented pH values above 6.0 throughout a 12-h period after feeding, whereas animals fed finely-ground corn and high-moisture corn silage had pH value around 5.6–5.7 in a 2–6-h after feeding period. The animals fed high-moisture corn silage had lower daytime pH. Despite being degraded in the rumen, pectin is a form of carbohydrate with lower potential for acidification when compared to starch due to microorganisms that degrade it produce acetate which does not have lactate as intermediary metabolite in its formation.

On the other hand, it is expected that animals fed diets containing cereal grain and, consequently starch, present greater predisposition to acidosis. Thus, the potential for acidification of starch depends on the concentration of this carbohydrate in the diet and its ruminal degradation rate. Starch reduction rate into glucose is related to degradation rate and varies according to grain type, processing and starch form (Owens

et al. 1997; Huntington et al. 2006; cited by González et al. 2012). Grains that are more extensively processed or with starch that is less bound to the protein matrix tend to ferment more quickly and, therefore, are more likely to develop acidosis. According to Owens et al. (1997) it is desirable to increase starch digestion rate without increasing the risk of acidosis and without causing dry matter intake reduction.

Based on starch ruminal degradation rate, grains can be ranked from fastest to slowest degradations and, thus, it is possible to infer the respective potential for acidification in the following order: oats, wheat, barley, high-moisture corn silage, steam-flaked corn, dry-rolled corn, whole corn grains and whole sorghum grains (Herrera-Saldana et al. 1990; Huntington 1997, 2006; Offner et al. 2003; González et al. 2012). Chapter 8 of this book brings more details on the different grain processing effects on ruminal metabolism.

Likewise, fiber level, especially physically effective NDF, which is responsible for rumination and, consequently, for buffering the ruminal fluid, should be carefully taken into consideration at the time of diet formulation for ruminants fed high content of quickly fermentable carbohydrates. Moreover, inclusion of coproducts in the diets (fibrous and starch-free coproducts) can decrease the minimum necessary amount of NDF from forage; because fermentation rate and ruminal lactate production will be reduced, decreasing the chances to develop ruminal acidosis.

## *Behavioral and Social Aspects*

According to Dado and Allen (1995), an animal's intake behavior encompasses time spent eating, ruminating, resting, as well as feeding and rumination efficiencies. This behavior varies according to the physical consistency of the feed because during grazing, pasture is apprehended by the tongue and cut by inferior incisive teeth. On the other hand, concentrates are caught by the tongue and sucked by the mouth due to their small particle size so that cutting is not needed even though there are chewing movements (Albright 1993). Therefore, animals fed high-concentrate diets tend to intake greater amounts of feed in a shorter period of time, to chew ingested feed less and to ruminate it less. Together, these factors contribute to reduce ruminal pH.

Thus, bunk management directly influences animals' intake behavior. The most adopted ones are: traditional management (*ad libitum*), restrict management or restrict feeding and slick bunk management. In *ad libitum* management, a small daily leftover is expected, allowing animals to feed themselves without any restriction. However, Pritchard and Bruns (2003) proposed restrict feeding (based on proportions of metabolizable energy) in which animals intake approximately 5–10 % less feed than in *ad libitum* feeding. These researchers observed smaller daily variations of feed delivery and, therefore, smaller fluctuations of dry matter intake, one of the causes of acidosis. Slick bunk management programs leave the bunk empty at specific times of the day, mainly in the beginning of the morning. This is intended to provide the exact amount that the animal is supposedly able to consume so that

there are no excessive intakes and it is possible to reduce feeding costs without negatively affecting performance.

These same authors also reported animal training and feeding monitoring as critical points of these systems. Restriction can have an unexpected contrary effect, stimulating the occurrence of subacute acidosis because the animal has a smaller number of daily meals and ingests a greater feed amount each time it feeds itself (Zinn 1995), which might result in greater daily variations of ruminal pH (Schwartzkopf-Genswein et al. 2003).

Physical limitations like bunk space can influence the way animals behave. It has long been known that cattle have gregarious behavior, which is structured on hierarchy levels. The social hierarchical order determines priority to access sources like feed and receptive females for which individuals of the same group compete (González et al. 2008). Therefore, the development of acidosis can also be involved in a hierarchy in which dominant animals that get to the bunk first generally eat more than what was calculate for each animal in a pen. Thus, those that wait to feed themselves sometimes eat a great amount of feed and sometimes eat less, as well as they may consume greater amounts of feed in a shorter period of time. Based on this fact, subordinate animals are more likely to present dry matter intake fluctuations and, therefore, are more susceptible to develop acidosis, besides compromising their own development regarding variations of daily weight gain. In commercial feedlots operations, feed (or bunk space) is the most common source of dispute among animals. Competition increases as feed availability decreases and aggression is observed many times right after ration delivery (González et al. 2012).

Similarly to the previously discussed restriction, competition for feed may result in less time spent eating and increase the amount of feed consume in a short period of time (Olofsson 1999). González and Schwartzkopf-Geinswein (unpublished data, cited by González et al. 2012) observed that calves compensated the shorter time spent on daily intake by increasing in 75 % the intake rate of a mixture consisting of 43 % of barley grains and 57 % of barley silage in *ad libitum* feeding. The same authors calculated salivation reduction from 3 to 1 mL/g of ingested dry matter when intake rate increased, resulting in a saliva production reduction of 22 L daily.

Hence, competition for feed is a key factor for acidosis occurrence in the herd, and the negative impacts on ruminal pH are greater in the beginning of the feeding period (González et al. 2008).

## *Repeated Exposures to Acidosis Conditions*

Dohme et al. (2008) induced subacute acidosis in two groups of cows (high risk: animals in the beginning of lactation fed a diet containing 45 % of forage; and low risk: last third of lactation and 60 % forage diet) three times with a 10-day interval between the challenge periods. Induction consisted of a 50 % restriction of *ad libitum* feeding on the previous day of the challenge and supply of 4 kg of a mixture

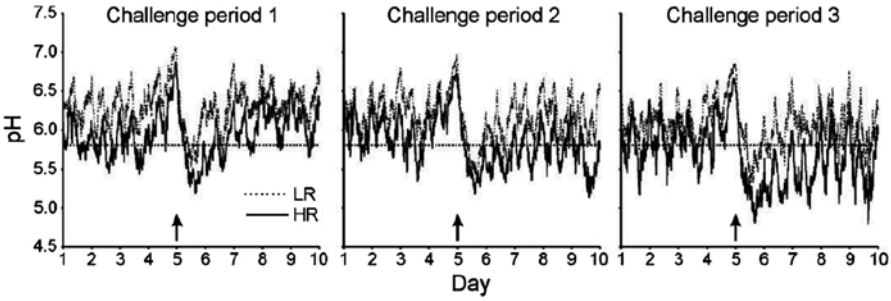


**Fig. 5.4** Ruminal pH of high-risk cows (HR) and low-risk cows (LR, see the text for the definition of risks) submitted to three acidosis induction periods. Periods consisted of 3 days of normal feed delivery (day 1–3), on the fourth day there was a 50 % dry matter intake restriction of *ad libitum* feeding, whereas induction consisted of a supply of 4 kg of a mixture (50 % barley, 50 % wheat) an hour before the first daily feeding on the fifth day, as shown by the arrows (Adapted from Dohme et al. 2008)

(50 % barley, 50 % wheat) an hour before the first daily feed delivery. The authors observed an increment in the severity of subacute acidosis with challenge repetitions. Between the first and second challenge, pH was back to normal level, although 10 days were not enough to normalize ruminal pH between the second and third challenge periods (Fig. 5.4).

Ruminal microbiota disequilibrium caused by nutrient restriction (supply of only 50 % of dry matter consumed *ad libitum* before the challenge) as well as the elimination of cellulolytic bacteria explains most of the occurrence of this disorder with animals' exposure to the challenges. Moreover, researchers questioned the possibility of possible damages caused to the ruminal epithelium that consequently would decrease the absorptive capacity of this organ and would increase the chances of SCFA accumulation.

The information on the previous paragraphs of this section is a reflection that repeated management errors, based on amount of feed delivered and mixtures that were not uniformly blended (simulated by adding grains previously to feed delivery), cause an increase in acidosis severity. In addition, in research, recovery periods between challenges have to be long enough to allow complete recovery of ruminal fermentation patterns as well as to decrease residual effects of previous periods in the case of crossing-over or Latin square designs.

Repeated exposures to rations or feeds that potentially cause digestive discomfort can cause aversion and activate a mechanism named "metabolic memory". Phy and Provenza (1998) observed that lambs reduced their preference for barley when frequently or excessively fed with this grain. Likewise, Phy and Provenza (1998b) observed that lambs fed with diets that potentially caused acidosis preferred the ones that had sodium bicarbonate addition. Dohme et al. (2008) observed a decrease in the voluntary intake of a barley and wheat mixture after the first acidosis induction. Furthermore, Pacheco (2010) decided not to carry out a third induced acidosis challenge in rumen-cannulated cows by drastically changing diets from 100 % forage to 73 % concentrate fed *ad libitum* because, after

observing the reduction of voluntary intake of the high-concentrate diet between the first and second challenge periods, a smaller acidosis severity could occur if a third period was included in the study, and, therefore, it would compromise the evaluation of preventive feed additives to acidosis.

For that reason, it is expected that an animal learns from negative discomfort experiences to avoid excessive intake of a ration or feed that potentially causes acidosis. However, depending on the diet composition, quality of diet mixing (uniformly blended or not), and feeding management, maybe the acquired experience is not enough to avoid this nutritional disorder as the animal is not able to avoid the disorganized growth of ruminal microorganisms when they are still destabilized by previous acidotic stresses.

## *Adaptation of Ruminal Epithelium*

Formulated diets with high-energy content are destined to feedlot programs that aim to maximize animal performance through a constant increase of daily weight gain, hot carcass weight, and dressing percentage.

The absence of gradual adaptation to high-concentrate diets, amount of feed offered, and the number of meals distributed throughout the day, can determine acidosis onset. Moreover, acidification can be potentialized according to the daily variations of animal intake. When it has intake fluctuations, small areas of the rumen are damaged and as these variations persist, papillae compromising degree increases and proportionally their absorption capacity of SCFA decreases. Sodium monensin can be added to feedlot diets because it selects and inhibits certain lactate-producing bacteria and, therefore, reduces daily variations of feed intake (Cooper and Klopfenstein 1996) and consequently decreases incidence of deaths caused by digestive problems (Parrott 1993; Vogel 1996).

On the other hand, when cattle are gradually adapted to high-concentrate diets, lactic acid accumulation is prevented; however, ruminal pH will still remain low due to a greater production of SCFA. Some authors consider pH 5.5 the threshold (Slyter 1976; Nagaraja and Titgemeyer 2007) for subclinical acidosis, when lactate-producing bacteria, such as *Streptococcus bovis* and *Lactobacillus* spp., proliferate. Simultaneously, the growth of lactate-utilizing bacteria (*Megasphera elsdenii* and *Selenomonas ruminantium*) is inhibited when pH is below 5.5 (Russell and Hino 1985). Thus, regardless of diet type, when ruminal pH drops under 5.5, lactic acid accumulation is expected. This accumulation is frequently observed in abrupt changes from high-forage diets to high-energy diets, which means that without the appropriate adaptation, a greater incidence of lesions in the ruminal epithelium can occur.

For a greater utilization of ingested feed by ruminal microorganisms, besides the maximum production and absorption of SCFA from these products, development and integrity of papilla are necessary. If the absorptive capacity of the ruminal wall

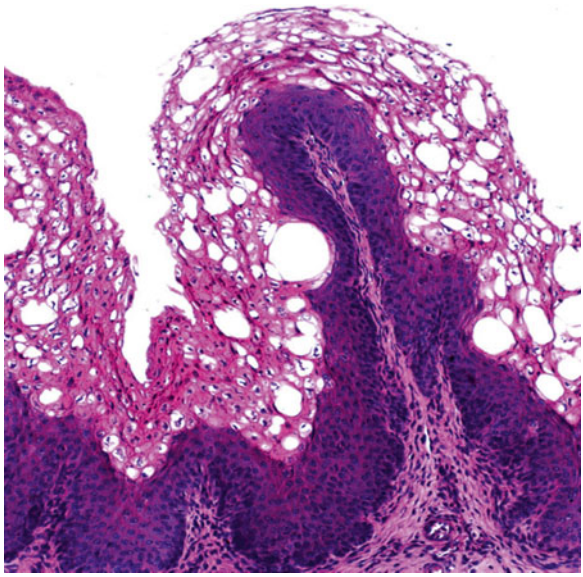

**Fig. 5.5** Ruminal papillae with vacuolated cells due to lactate accumulation in the ruminal fluid. Adapted from Costa et al. (2008)

is compromised by some disorder started by acidosis, as rumenitis, the animal's ability to try to keep its rumen under stable conditions will be affected (Fig. 5.5).

During acidosis, the biggest changes occurring in the rumen are due to osmolality when compared to variations regarding hydrogen concentrations (Owens et al. 1998). In function of acidosis process, high-osmotic pressure in the rumen reduces SCFA absorption rate by ruminal epithelium and papilla, which would be more quickly absorbed under lower osmotic pressure conditions (Tabaru et al. 1990).

When the rumen has greater osmotic pressure than blood, water may flow quickly from the blood through the ruminal wall as a way to keep the homeostasis. This fast influx of water causes papillae swelling and stains in the ruminal epithelium as described by Eadie and Mann (1970). Damages caused to ruminal epithelium under high-osmotic pressure can be the first step to the development of future abscesses, as some ruminal microorganisms like *Fusobacterium necrophorum* can migrate through blood portal system and cause abscesses in the liver. Moreover, attempts to recover the damaged tissues may originate metabolic disorders secondary to acidosis, like hyperkeratosis, which is caused by the thickening of ruminal wall epithelium through the process of papillae keratinization. As a consequence, SCFA absorption may be inhibited while lesions in the epithelium are still present (Krehbiel et al. 1995). Depending on the lesion severity, it can take ruminal epithelium six months to one year to recover.

According to Scott (1975), high osmolality condition (>300 mOsm) combined to abomasum distensions makes the removal of fluid and SCFA produced in the rumen difficult. Besides that, in situations of osmolality over 350 mOsm there is inhibition of the action of fiber- and starch-digesting bacteria. This hypertonicity generally reduces contraction frequencies in the rumen (Carter and Grovum 1990) and consequently decreases input and output flows of ruminal fluids, and then ruminal acidification is potentialized.

## *Organism Reactions Against Acidosis Development*

In recent surveys carried out by Millen et al. (2009) and Oliveira and Millen (2014), it was reported that more than 50 % of Brazilian feedlots assisted by surveyed nutritionists did not control feed amount provided to each pen, adopting the continuous delivery system (kg offered per pen is not controlled), which can collaborate to the increase of dry matter intake variation and contribute to the development of acidosis.

Animals fed diets delivered by this system have to receive feed more times daily (and less feed in each feeding), and need fresh clean water. Thus, abrupt pH variation or reduction is avoided; otherwise there is maximization of activities of lactate dehydrogenase and pyruvate hydrogenase enzymes, which favor the conversion of pyruvate into lactate. Moreover, feedlot animals fed high-concentrate diets have limitations in saliva production due to the low intake of roughage sources.

Saliva produced by rumination is a source of approximately half of available bicarbonate in the rumen whereas the other half comes from $SCFA^-$ exchange via absorption through ruminal epithelium. Therefore, the smallest bicarbonate input via saliva due to the lower content of forage ingredients in a diet stimulates the increase in bicarbonate derived from blood as a way to avoid pH reduction in the rumen due to excessive fermentation (Table 5.2). Thus, base excess in the blood is reduced and if respiratory and renal systems are inefficient, the animal can develop metabolic acidosis.

Under acidosis, an animal's physiological system tries to alleviate the processes that are causing, or will be able to cause, losses to the host organism. As a way to avoid damages that can alter the normal functions of the animal, chemoreceptors of the ruminal wall capture information of abnormal conditions of the rumen and through the vague nerve it reaches the hypothalamus. When the information that the homeostasis condition is compromised arrives, reactions like decrease in ruminal wall motility and a smaller number of biochemical processes occur in response to this undesirable condition in the ruminal environment. As a result, dry matter intake is reduced and less substrate will reach the rumen of these animals daily, while the conditions are not again favorable for the development of healthy fermentation (pH between 5.7 and 6.8). Because of these actions, the intermediary production of lactate is controlled and production and absorption rates of SCFA tend to equilibrate themselves.

**Table 5.2** Effects of different forage contents in the diet on physiological responses of cattle

| | % of hay in the diet | | | |
|---|---|---|---|---|
| Physiological Parameters | 80 | 60 | 40 | 20 |
| Time spent chewing, min/day. | 1040 | 970 | 820 | 520 |
| Saliva, L/day | 196 | 189 | 174 | 143 |
| Bicarbonate, kg/day | 2.4 | 2.3 | 2.2 | 1.8 |

Adapted from Mertens (2001)

Moreover, when ruminal pH is under 5.7, there is a decrease of ruminal epithelium permeability to input of ammonia from bloodstream. This occurs because the growth rate of microorganisms is negatively affected due to the lower ammonia input to the ruminal environment, resulting in slower production of SCFA.

Another form to control problems related to greater intakes of high-energy diets, due to low saliva secretion and lactic acid accumulation, is the partial starch replacement for pectin. The substitution of cereal grains rich in quickly fermentable carbohydrates, such as corn, for feeds rich in highly digestible structural carbohydrates, such as soybean hulls and citrus pulp, has been utilized to prevent disorders in ruminal functioning (Ipharraguerre et al. 2003), because ruminal fermentation of structural carbohydrates does not produce acids that are capable of reducing ruminal pH drastically, like lactate (Hall 2001). Pectin, classified as a quickly fermentable non-fibrous carbohydrate, can be utilized as an energy source, and in smaller proportions keeps diet effective NDF content without decreasing concentration of ruminal acetate and milk fat content. Specific microbial enzymatic systems are required to degrade pectin in the rumen because ruminants do not produce pectinase. Therefore, the end product of pectin fermentation in the rumen is acetate that is a weak acid and does not have lactate as intermediary in its biochemical pathway, which can contribute eventually to reduce variations of ruminal pH.

Pedroso et al. (2007) evaluated the substitution of coarsely-ground corn grain for soybean hulls in rations that contained corn silage as the main forage source, and citrus pulp as part of the energy source. Thirty-six Holstein cows in mid-lactation were utilized to evaluate three levels of substitution of coarsely-ground corn grain for soybean hulls in the ration: 0 %, 10 % and 20 %. The inclusion of soybean hulls to the diets did not affect dry matter intake, milk production or milk production corrected to 3.5 % fat; however, it linearly increased total milk fat production (kg/day) and linearly decreased milk-urea-N concentration. Thus, the use of this coproduct as corn substitute in the diet of dairy cows that produce approximately 28 kg of milk a day may be interesting if its price is as competitive as corn.

Another strategy that ruminants utilize to prevent acidosis conditions involves protozoa. Protozoa are good indicators of ruminal acidosis because they control excessive fermentation of the rumen by predating bacteria, sequestering starch granules and releasing them slowly. However, marked reduction of their populations is observed under both forms of acidosis because the pH is under its survival level (ideal pH = 6.5). Because of this change in the profile of ruminal microorganisms related to acidosis, which shifts end-products resulted from fermentation, like the presence of D-lactate and SCFA produced in excess, other alterations can be found such as the presence of endotoxins, tiramins, histamines, ethanol and methanol (Koers et al. 1976; Slyter 1976).

## Metabolic Disorders Related to Acidosis

### *Rumenitis/Hyperkeratosis*

Rumenitis is characterized by the development of inflammatory alterations in the ruminal epithelium and in subjacent tissues of ruminants fed with cereal-rich diets and inappropriate forage levels. In most cases, ruminal epithelium infection occurs after mechanic or chemical damage. The most common mechanic damage is reticulum and rumen perforation by metallic pointy objects that are trapped in the reticulum. The most frequent chemical cause is the high concentration of acids produced in the rumen. Rumenitis induced by acids can be acute, verified after an episode of lactic acidosis; or chronic or subacute, as a result of long feeding with cereal-rich diet with inappropriate amount of forage. This last condition is also called ruminal hyperkeratosis, in which ruminal papilla become dark, big, thick, irregular and compressed against each other due to the formation of abnormal keratin layer deposited on papillae surfaces (Fig. 5.6). In cases of acute rumenitis, papilla, responsible for approximately 95 % of organic acid absorption in the rumen, cannot resist to low pH and stop proliferate, resulting in cellular death. Therefore, the areas of the epithelium affected by excessive acidification may lose absorptive function partially or totally (Figs. 5.7 and 5.8), contributing even more to acid accumulation in the rumen.

In chronic rumenitis as well as in acute one, inflamed epithelium becomes susceptible to invasions of opportunistic ruminal microbes such as *Fusobacterium necrophorum, Arcanobacterium pyogenes* and some fungus species. Rumenitis

**Fig. 5.6** Ruminal hyperkeratosis: agglutinated papilla due to excessive keratinization. Source: personal archive

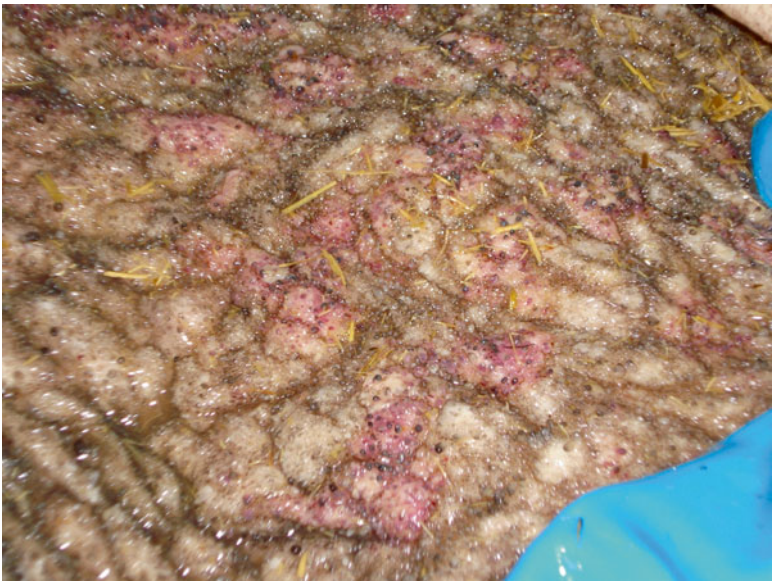

**Fig. 5.7** Absence of papilla in ruminal epithelial wall due to intense acidification and presence of rumenitis. Source: personal archive

**Fig. 5.8** Ruminal papilla with compromised function due to lesions. Source: personal archive

caused by opportunistic fungi, particularly from *Aspergillus* species, is almost always secondary to ruminal acidosis. The mycelial structure of the fungi allows fungal invasion and dispersal in ruminal wall, resulting in hemorrhagic lesions in the rumen (Nagaraja et al. 1996). Mycotic rumenitis can also occur as a consequence of intense utilization of oral antibiotics or intake of feed contaminated with fungi, and its damage degree depends on its extension and lesion location in the rumen (Nagaraja and Chengappa 1998).

## *Liver Abscesses*

Liver abscesses, in general, are consequence of infections in ruminal wall. The relationship between ruminal acidosis and liver abscesses in feedlot beef cattle has already been well documented (Jensen et al. 1954). Lesions caused in the ruminal epithelium by altered conditions of pH, osmolality and temperature, besides ruminal microflora disruption caused by the development of acidosis, allow the emergence of certain microorganisms like *Aspergillus* spp. and *Fusobacterium necrophorum.*

The term "rumenitis-liver abscess complex" has been utilized due to the high correlation between liver abscesses and ruminal lesions. Induced rumenitis by acid accumulation and damages to the surface of the ruminal epithelium are generally associated with high intake of concentrate ingredients (Jensen et al. 1954; Fell et al. 1972). Therefore, ruminal wall damaged by acidity or penetration of foreign bodies becomes susceptible to invasion and colonization by *F. necrophorum*. After colonization occurs, *F. necrophorum* invades and goes through the ruminal epithelium until reaching portal circulation, and then, when reaching and settling in the liver, it causes abscesses (Fig. 5.9). Among ruminal bacteria, *Fusobacterium necrophorum* is the most common one found in rumenitis and liver abscesses (Nagaraja and Chengappa 1998). Liver abscesses sporadically occur in the most part of animals, but it is more common in cattle fed high-concentrate diets. It can occur at all ages and in all types of cattle, including dairy cows, but its greater economic impact is on feedlot beef cattle whose incidence ranges from 2 % up to 90 % or 95 %, presenting averages of 20–30 % in most feedlot animals in the USA (Nagaraja et al. 1996). In addition, this is the greatest cause of liver condemnation at slaughter.

The main economic effect of liver abscesses is related to poorer animal performance and reduction of hot carcass weight, and consequently dressing percentage. In general, liver abscesses are direct consequences of erroneous feeding practices. Therefore, diets provided to animals play a fundamental role in its incidence. Practices like drastic diet energy increase without proper adaptation and wrong nutritional management, characterized by irregular feedings (regarding volume or frequency) may cause ruminal acidosis and rumenitis as well as result in a greater incidence of liver abscesses (Nagaraja et al. 1996). Almost all bacte-

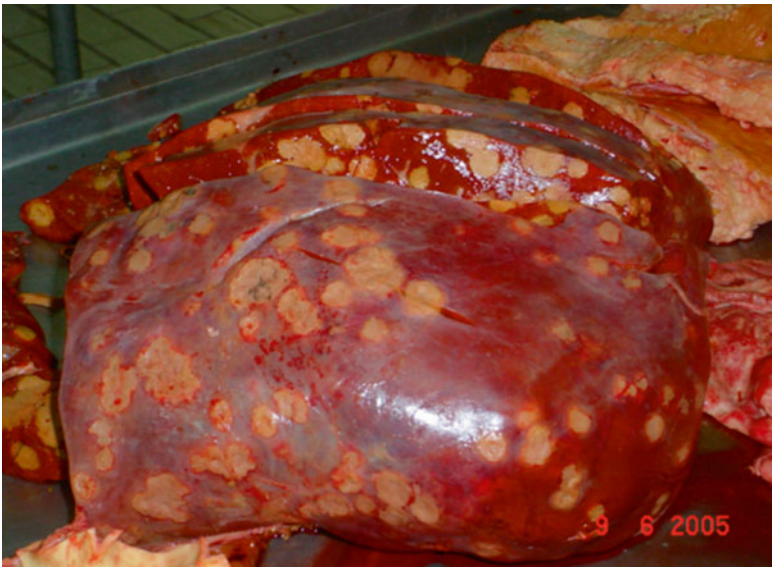


**Fig. 5.9** Liver affected by a great amount of abscesses. Source: Dr. T.G. Nagaraja

riological studies on liver abscesses concluded that *Fusobacterium necrophorum* regularly found in the rumen is the primary causing agent; and *Arcanobacterium (Actinomyces) pyogenes* is the second most frequent isolated pathogen (Nagaraja and Chengappa 1998).

## *Bloat*

Considered a non-infectious disease, bloat develops through the accumulation of gases produced in the ruminoreticulum in response to fermentation process. According to Cole et al. (1945), bloat should be classified according to its cause. In general, there are two causes for the development of bloat: acute bloat normally caused by the nature of feed, and chronic one due to abnormality of the animal's physiological state.

The development of bloat, characterized by pressure formed in the ruminoreticulum compartment, can be caused by the lack of normal mechanisms of the animal to remove gas excess through eructation due to the presence of pathogens or obstruction conditions. However, the main cause of bloat is the excessive intake of high-energy feeds like grains, which produce a great amount of gases due to fermentation and the animal is not capable of removing them from the rumen by eructation.

Symptoms like left flank abnormally swollen, back arched, labored breathing, vomiting, tongue hanging out of the mouth, frequent urination and defecation followed by death are common in animals affected by bloat.

Frothy bloat occurs when the eructation mechanisms of the animals are harmed and there is gas excess produced by feed fermentation that cannot be released by the animal (Majak et al. 2003). The fast release of soluble proteins in the rumen causes the formation of a biofilm (foam) which trapes gases and causes frothy bloat (Fig. 5.10; Clarke and Reid 1974; Pinchak et al. 2005).

In cases in which animals are not cannulated, the increase of ruminal volume caused by gas pressure can make animals' breathing difficult and result in cardiorespiratory failure and consequently death.

## *Laminitis*

As already described in the chapter, one of the consequences of clinic or subclinic acidosis is laminitis. As cited by Norlund (1995) and Gentile et al. (1986), in dairy operations where cows are in free-stalls, the main challenge is subclinic acidosis. Therefore, in the rumen of these animals there is little lactate accumulation, but even so this environment is more acid and makes the animal present symptoms of subclinic acidosis like variation of dry matter intake, weight loss, diarrhea and laminitis.

**Fig. 5.10** Release of gases and ruminal content through cannula by an animal with frothy bloat. Source: personal archive

Diffuse aseptic pododermatitis or laminitis is, then, strongly related to acidosis, causing alterations in the hemodynamics of peripheral microvasculature (Boosman et al. 1989). Therefore, for years, several theories have been developed to explain the events that cause laminitis. Substances that are vaso-active (like histamine and endotoxins) are released during ruminal pH decrease as the result of bacterial death and tissue degradation. These substances cause vasoconstriction and dilatation, which destroys the peripheral microvasculature of corium laminar (Brent 1976; Mgassa et al. 1984).

The evolution of acidosis to laminitis is associated with several systemic phenomena; however, the cascade of events starts from the reduction of ruminal pH. The effects of low pH pathogenically impair the rumen, liver and gastrointestinal system, affecting animals' hemodynamics and predisposition to laminitis. Episodes of clinic acidosis drastically change animal's hemodynamics and predispose it to short and severe episodes of acute laminitis. When these episodes are constantly repeated, they may cause a predisposition to ruminal hyperkeratosis, infiltration of pathogens and liver abscesses at several degrees of severity. In other words, change in osmolality of gastrointestinal tract and vascular destruction may result in irreversible laminitis (Nocek 1997).

According to the review carried out by Nocek (1997), laminitis is a process that happens in four phases. The first one is associated with ruminal pH reduction and posterior systemic pH decrease. This systemic pH reduction results in a greater irrigation of the digital region of the animal and increases whole body blood flow. However, when the disorder happens, the release of endotoxins and histamine may also occur, resulting in continuous processes of vascular constriction and dilatation that can cause non-physiological arteriovenous derivations obstructing blood passage through vases and contributing to blood pressure increase. As a consequence of the first phase, the second one is characterized by ischemia, which is defined as localized anemia due to occurrence of arteriovenous derivations causing hypoxemia in the interdigital tissue and reducing the oxygen flow to it. In phase three, due to vasoconstriction and reduced amount of nutrients reaching the tissue, it starts dying causing degeneration of the corium and death of the laminar region associated with basal lamina. In the fourth and last phase there is physical damage of basal lamina resulting in separation of germinating stratus and corium. These separations cause rupture between the dorsal and lateral lamina and the hoof tissue support. In the end, the process causes separation of the laminar tissue and the pedal bone changing its natural positioning, which results in compression of softer tissues and makes them susceptible to any damage. This compression causes bleeding, thrombosis and at last tissue necrosis, characterizing a case of irreversible laminitis.

Although Donovan et al. (2004) did not find a direct relationship between acidosis and subclinic laminitis, several authors like Nocek (1997), Stone (2004) and Cook et al. (2004) reported that acidosis is the trigger that causes laminitis and this can be controlled by increasing or changing the type of fiber source used in the diet. Nocek (1997) also reports that an appropriate proportion of structural and non-structural

carbohydrates as well as a sufficient amount of effective fiber in the diet should be provided to promote adequate ruminal function and to maximize energy intake, preventing laminitis.

## Implications

Ruminal acidosis can affect ruminants in general due to several factors. However, the understanding of these factors is essential so it is possible to adopt the best nutritional strategies for each case in order to prevent clinic or subclinic acidosis more efficiently.

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# Chapter 6
# Control and Manipulation of Ruminal Fermentation

**Paulo Henrique Mazza Rodrigues**

## Introduction

### *Importance of Fermentation for Animals*

Species evolution has shaped an infinite variability of different life forms. In higher animals, especially in mammals, the variability of forms and functions that the digestive system has been taking over is intriguing. It has allowed animals to effectively take advantage of a broad variety of ingested feed. Particularly in herbivores and omnivores, the development of a fermentative chamber found in the digestive system (some anatomists prefer to call it digestive tract) has permitted the symbiotic association with other life forms such as microorganisms. These unicellular organisms like bacteria, protozoa and fungi, at a point in the evolutionary scale, occupied the niche provided by the digestive system, underwent a continuous and intense process of selective pressure and ended up adapting to the specific conditions of this fermentation chamber. On the other hand, through symbiosis, they were able to offer an important enzymatic and metabolic apparatus that is not found in any other higher organism.

Early in the evolutionary scale, nature placed this fermentative chamber in a more distal position in the digestive system, in the organs known as cecum and colon. This happened to rabbits that may have come up 55–35 million years ago, and to equines that appeared 35–25 million years ago. Similarly to swine and even humans, those herbivores present a post-gastric fermentative chamber and, therefore, are called cecum-colon fermenters. This chamber allows the breaking of complex carbohydrates into volatile fatty acids (nowadays they are called short-chain fatty acids—SCFA), which are used for energy generation, or even for the growth of body tissues after its absorption by the intestinal epithelium. However, this chamber located there

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does not allow the utilization of other fermentation products such as the protein structure of microbes that develop themselves there. This high quality microbial protein is then lost in feces because the organs that could have digested and absorbed it, like the stomach and the small intestine, were left behind.

In early evolutionary scale, probably between 25 and 5 million years ago, nature shaped this fermentative chamber in a more anterior position of the digestive system, allowing the emergence of ruminants. These animals can, through fermentation, utilize not only the energy of intermediate metabolites (mainly acetic, propionic and butyric acids) originated in the degradation of complex polymers (cellulose, hemicellulose, pectin and others), but also from the cellular structure produced by microorganisms. This intriguing fermentation apparatus, called ruminoreticulum, has started providing such important advantages for animals having it that it has kept on differentiating itself and improving itself. This apparatus has received a great evolutionary importance given its size and complexity, which are not seen in the digestive system of any other animal group (Table 6.1).

Certainly ruminants were not the only animals that developed pre-gastric fermentation. Colobus monkeys, found in the equatorial jungles of Africa, and the Tree Kangaroo, found in the Papua New Guinea jungles, in the north of the Australian continent, also have a fermentation chamber located before the stomach. Even a bird called Hoatzin found in the Amazon jungle possesses a fermentation chamber in the digestive system, which has its own capacities. Although other animals have developed pre- or post-gastric fermentation chambers, undoubtedly ruminants were the ones that most benefitted from this organ, allowing them to spread throughout the most varied ecosystems of the planet, from the freezing tundra in the Artic Polar Circle to the hottest and driest deserts in Africa.

## *Importance of Fermentation for Humans*

Before keeping on studying ruminal fermentation, it is important to learn that other fermentative processes are part of our lives. Human beings, somehow, take direct advantage of the post-gastric fermentation that occurs in their own digestive

**Table 6.1** Contribution of short-chain fatty acids from pre-gastric fermentation (ruminoreticulum) or post-gastric fermentation (cecum and colon) to provide the daily energy needs (in percentage) of different animal species

| | Energy origin (%) | |
|---|---|---|
| Species | Pre-gastric | Post-gastric |
| Rat | – | 5 |
| Human | – | 8 |
| Rabbit | – | 30 |
| Equine | – | 30 |
| Swine | – | 25 |
| Sheep | 70 | 8 |
| Bovine | 63 | 9 |

Bergman (1990)

system. Out of all energy utilized by a person, approximately 5–10 % comes from fermentation that occurs in the cecum and colon (Table 6.1). Human beings also take advantage of fermentation done by ruminants. The history of our own species' emergence is entangled with the exploration of these animals through hunting which has been done for at least hundreds of thousands of years. Moreover, we have interacted with ruminants more closely. Around 20 thousand years BCE, a lot of communities adopted transhumance as a lifestyle. They would follow migrating herds, slaughtering animals according to their needs. Approximately 10 thousand years BCE, humans developed herding and effectively domesticated these animals.

Indirectly, we, human beings, have also benefitted from fermentative processes to enhance the nutritional and organoleptic characteristics of our food. Bread is the oldest known product from a fermentation process. Developed in Egypt around 3 thousand years BCE, the ancient Egyptians already knew how to use yeast to improve the characteristics of wheat, enhancing its flavor and consistency. Although wine, beer, pickles and fermented cabbage are presently considered fine expensive foods, they were, in reality, practical and important solutions to preserve and improve the characteristics of a lot of foods, some of which available only in specific seasons of the year. Therefore, our ancestors already knew how to utilize fermentative processes even without knowing the microorganisms that performed them.

## *Symbiosis Between the Ruminant Host and Its Microbiota*

A ruminant is a product of the symbiosis between the host organism, for example a cow, and its ruminal microbiota. In this process, both parts are benefited. However, for this interaction to be possible, both have to fulfil specific functions. The microbiota contributes with an enzymatic package that is responsible for the digestion and ruminal synthesis reactions. The presence of digestive bacterial enzymes, which are not produced by any higher animal species, allows utilizing sugars contained in complex polysaccharides like cellulose and hemicellulose. Moreover, the presence of other bacterial enzymes allows the synthesis of essential amino acids and hydro soluble vitamins. Once in the small intestine, these nutrients may be digested, if necessary, and absorbed by the host. Such characteristic guarantees the importance of ruminants to food production for humans.

However, a vertebrate host does not have a passive position in the symbiotic relation process. On the contrary, a cow provides basic conditions for the adequate microbial growth. Moist is guaranteed through salivation and frequent ingestion of water. The maintenance of water in the rumen is also important. The omasum has a fundamental role in this process, working as a valve that controls the flow of liquids and solids through the rumen. The temperature, produced by microorganisms, as well as produced and kept close to 39 °C by the host, allows optimal conditions for the occurrence of enzymatic reactions. Anaerobiosis is an essential part of the process since the presence of oxygen is toxic to many of these microorganisms. The presence of oxygen could allow the growth of aerobic bacteria that are capable of

unfolding sugars into $CO_2$ and $H_2O$, without resulting in the production of intermediate metabolites (short-chain fatty acids). If that occurred, only bacteria (aerobic bacteria) would be benefited, but not the host organism.

The host also provides constant supply of substrate for microorganisms to unfold. A grazing ruminant can spend up to 8 h a day doing it. This amount of time spent on a meal aims to make the ingested feed have the greatest possible amount of nutrients available to both parts. Other 8 h can be spent on rumination of the ingested feed. This process decreases the size of feed particles, allowing a greater adhesion surface as well as a larger attack surface for digestive microbial enzymes. The ruminal movement through peristaltic movements also gives to the microorganisms a greater opportunity of being in contact with the feed. Likewise, this ruminal movement allows feed to be displayed in the rumen in a form of strata. Bigger and less dense particles are retained in the top of the rumen digesta (raft or mat), which gives to the microorganisms more time for digestion. On the other hand, smaller and more dense particles (part of that already digested), are placed in the bottom of the rumen and are more susceptible to flow out of this organ through the reticulo-omasal orifice.

The removal of products and metabolites resulting from fermentative processes is as important as the removal of non-digested feed particles. Short-chain fatty acids, hydrogen ions and gases have to be respectively absorbed, neutralized and eructed. Accumulation of these compounds in the rumen would prevent the continuity of chemical reactions in this organ. Finally, the acid-base balance of this entire complex system has to be buffered to avoid brusque pH variations that cause inactivation and even death of microorganisms as well as damage to the host's health.

## *Ruminal Fermentation from Management Perspective*

Modern systems of milk and meat production demand more and more individual production. Properties with more productive animals have lower housing cost per unity of produced milk or meat. Moreover, feeding cost to maintain each animal is diluted since more milk or meat are produced with more productive animals at the same maintenance cost when compared to less productive animals. Maximum milk or meat productions require high-energy diets.

Necessarily, a conventional diet for lactating cows consists of approximately 18 % crude protein, 4 % ether extract and 6 % ash on dry matter basis. Although these values may vary a little, maybe a little more for beef cattle, they still mean that approximately 28 % (=18 % + 4 % + 6 %) of the diet will be imperatively committed to these three components. Therefore, there is a 72 % space that should contain non-structural carbohydrates (sugars and starch), which are the main energy source of the diet, and the fibrous fraction (cellulose, hemicellulose and lignin). Thus, it is easy to conclude that fiber and energy are excluding factors of the diet, that is, when one increases, the other decreases. System that aim to reach maximum milk or meat production demand great amounts of energy and, therefore, the diets are poor in fiber.

Moreover, the ruminal fermentative process involves countless complex chemical degradation and synthesis reactions. For millions of years, under the evolutionary process, nature has developed and has selected more energetically favorable chemical reactions (or at least the microorganisms that performed them), favoring the main partners involved in the symbiotic process. Although ruminal fermentation is a very efficient process, it still results in losses. These losses can be energy released as heat as well as metabolites (methane, for example), or important nutrients like nitrogen.

In order to avoid deviations of the activity that occurs in the rumen with consequent damages to the animal health as well as to avoid losses in the fermentative process, it is important that a nutritionist be able to control and even manipulate the process.

Even though there is not a consensus among specialists, we understand that there is a differentiation between the terms "control" and "manipulation". While "control" is about the capacity to stop or accelerate a process, "manipulation" means the capacity of giving new directions to this process, that is, finding new ways for it to occur. Control generally reminds us of the use of more readily available methods for nutritionists whereas manipulation reminds us of methods that are more elaborated and originated from scientific innovation. Regardless of the interpretation, nowadays nutritionists have tools that help the control as well as the manipulation of fermentation that happens in the rumen.

## Objectives of Ruminal Fermentation Control and Manipulation

Several benefits can be obtained when the ability to control and manipulate ruminal fermentation is dominated. A better fermentative efficiency with consequent improvement of productive efficiency may be reached by decreasing losses and avoiding damages to animal health; the latter are caused by digestive disorders or by toxins found in feed. It is also possible to aim the improvement of nutritional and organoleptic characteristics of animal products. It is even possible to increase the concentration of nutritional principles that are beneficial to the health of consumers of animal products. For example, "nutraceutical" is a term that has been recently coined to describe foods that have natural principals and are beneficial to the health of their consumers. Moreover, the control and manipulation of fermentation has gotten attention from the communication media recently, once these techniques have potential to decrease eventual losses to the environment caused by these productive systems, minimizing residue accumulation in the soil, water reservoirs and atmospheres.

Although a lot is known and many benefits have been achieved up to the present moment with this knowledge, the control and manipulation of ruminal fermentation are open to new discoveries. Seen from the perspective of human knowledge evolution, we are still beginning the development of this promising area of knowledge.

## *Acidosis Control*

All animals have an aqueous corporal system that needs to be regulated. Blood pH is accurately regulated within variations of 0.01 pH unity. Small pH alterations of this fluid, which is normally around 7.4, may cause death. This demands an intricate system of acid-base equilibrium of blood and interstitial liquids. Ruminants possess a second aqueous "pool" in the rumen which has a complex system of acid-base regulation that, although it allows greater pH variations (from 5.5 to 7.0), also needs to be regulated. Ruminal pH content is the result of a series of factors that promote input or production of acids, and of factors that cause its neutralization or clearance from the rumen. Acid supply is affected by the amount, pH and composition of ingested feed. Its control is done by neutralization through saliva or feed, and by removal of these acids from the rumen by the ruminal wall or through the reticulo-omasal orifice. Failure to control ruminal acid-base equilibrium can result in smaller feed utilization (smaller digestion of fiber and smaller microbial protein production), damages to the animal health (ruminal hyperkeratosis, rumenitis, liver abscesses, abomasal dislocation, laminitis, diarrhea, etc.) and, therefore, impairment of its productive efficiency (decreased weight gain, reduced milk production, and decreased milk fat percentage). Although acute acidosis may have serious consequences to the animal health, subacute acidosis, generally invisible to the producer, can have a greater economic impact on the efficiency of productive systems.

## *Alteration of Short Chain Fatty Acids Proportion*

Since the 1940s, it has been known that feed ingested by ruminants is fermented in the rumen, and those metabolites, which are produced there, partly depend on the kind of ingested feed. There are, at least, ten short-chain fatty acids produced in the rumen and quantitatively the three most important ones are acetic, propionic and butyric acids. When the characteristics of the diet or its ingestion form are altered, the total amount of these organic acids produced in the rumen is changed and also the relative proportion among them. While propionic acid production occurs through two specific metabolic pathways, production of acetic and butyric acids happens through a different one. Thus, the acetic acid production pathway is related to the butyric acid one, which is different from the two production processes of propionic acid. Besides the different products (short-chain fatty acids) and the metabolic pathways, the energy efficiencies of each are different as well. Table 6.2 shows the recovery efficiency of energy contained in the substrate (glucose) when it is fermented into acetic, propionic or butyric acid.

Not only do ruminal metabolic pathways and resulting energy efficiencies of the fermentative process differ among the resulting short chain fatty acids, but also the way those short chain fatty acids are utilized, after absorbed by the host organism,

**Table 6.2** Efficiency of substrate conversion (glucose) in ATPs/glucose molecule, expressed as kcal/mol of glucose or in relative energy efficiency, when glucose is metabolized into acetic, propionic or butyric acids

| | Energetic efficiency | | |
|---|---|---|---|
| Metabolite | ATPs/glucose molecule | Kcal/mol | Relative efficiency (%) |
| Acetic acid | 20 (=2×10 ATPs) | 418 (=2×209 kcal) | 62 % |
| Propionic acid | 36 (=2×18 ATPs) | 772 (=2×386 kcal) | 109 % |
| Butyric acid | 27 (=1×27 ATPs) | 510 (=1×510 kcal) | 78 % |

Orskov and Ryle (1990)

differ. Certainly these three fatty acids can be utilized in animal tissues to generate the necessary energy for several metabolic processes required for maintenance and other productive objectives (growth, lipid deposition or milk production), despite the different energetic results. However, it is also important to know that these metabolites will be part of the animal tissue components, differing from the host's tissues of which they will be part. The non-oxidized acetic acid for energy generation will be utilized for lipogenesis (fat synthesis), which may be performed in the fat tissue (body fat production) as well as in the mammary gland of ruminants (milk fat production). The non-oxidized propionic acid in tissues is important because it is the only short-chain fatty acid that can be converted into glucose, which occurs in the liver. Although glucose is not the main energy source for ruminants, it is still important in the energetic metabolism of specific tissues like erythrocytes and the nervous tissue that use only glucose as energy source under normal conditions. It is also essential to know that the greater or smaller availability of these metabolites in the circulatory system may alter the metabolism of animal tissues, favoring or not the accumulation of reserve tissues (fat tissue).

Therefore, a nutritionist must have in mind that ruminal fermentation management may alter not only the productive efficiency but also the chemical and nutritional composition of foods originated from it (meat, milk and others).

## *Methanogenesis Reduction*

Ruminants have a great advantage over monogastric animals since their digestive process is able to release energy contained in the cellulosic material from roughage through carbohydrate fermentation by enzymes of the microbiota that is found in the ruminoreticular system. However, carbohydrate fermentation results not only in short-chain fatty acids (acetic, propionic and butyric acids), but also in less desirable products like heat and methane gas that represent energy loss to the animal.

The quantification of diet energy loss through eructation in ruminants has long been reported in the literature. The first studies are from the 1950s and aimed to investigate the inefficiency of specific diets and to find solutions to reduce it. The natural concern of a ruminants' nutritionist is to obtain the best possible feeding

efficiency, that is, the best relationship between weight gain and dry matter intake, resulting in high productivity. Under this perspective, the reduction of energy loss through gas eructation is important because an animal can lose 2–12 % of gross energy from the diet through methane eructation.

However, with the recent discovery that methane is a potent greenhouse effect gas, new areas of research have been explored. It is known that methane has the capacity to absorb infrared radiation 23 times greater than carbon dioxide and, considering 10 years of life in the atmosphere, contributes with approximately 18 % of all warming potential that presently occurs in the world. Thus, inventories have been published by competent organs in several countries which have committed themselves to reduce and control pollutant emissions.

It is known that there are three main sources of methane emitted in the world: natural ones (swamps, oceans and termite populations), the ones linked to energy generation and deject (gas burnings, coal, waste ponds and landfills) and the ones related to agricultural activities (rice plantations and herds). Approximately 16 % of all methane emission in the world per year comes from livestock activity (73 % comes from ruminant herds).

Therefore, all methane generated by herds of cattle, buffalos, sheep and goats in the world cannot be unconsidered and, regarding the concern of global warming, strategies that minimize eructation and consequent methane emission to the environment are more and more relevant.

## *Control of Protein Degradation*

Ruminal action on ingested feed has been puzzling ruminants' nutritionists for a long time. In the 1960s, experiments were carried out with animals receiving their ration through fistulas placed in the duodenum, deviating one or more sources of this feed from the pathway which was until then mandatory through the rumen. In one of these pioneer experiments, it was demonstrated that animals that received good quality protein source directly in the duodenum, without going through the rumen, had better productive performance when compared to animals that received the same amount of the same protein source through feeding, which went through the rumen. Since then, a sequence of digestive and ruminal metabolic phenomena has become known. For example, partial protein degradation occurs in the rumen producing ammonia and carbon chain. This nitrogen source, ammonia, is reutilized for the synthesis of the new protein, now with microbial origin, having a new amino acid profile. The quality of this amino acid profile is more stable with better or worse quality than the original source, depending on the initial quality of this original source. Although this transformation has an advantage (change of amino acid profile), in fact it implies in losses, because not all nitrogen degraded into ammonia is reincorporated to the true microbial protein. Thus, not all ingested nitrogen reaches the small intestine as true protein, because part of it is lost in feces as ammonia, or in the urine after the conversion of ammonia into urea by the liver.

Every time a dietetic protein is extensively degraded in the rumen, part of it is invariably lost; therefore, this fraction is not useful for productive purposes. The logical reached conclusion is that because not every degraded dietetic protein can be reconverted into microbial protein, it would then be valuable if it were possible to control ruminal protein degradation, just allowing degradation of the protein amount that the rumen could synthesize again. Many techniques have been tried and others have been developed to attempt to limit and control the extension of protein degradation in the rumen. Only a few of them will be shown in this chapter.

### *Toxin Inactivation*

Among the theories that explain the emergence of ruminoreticulum in the evolution scale of ruminants is its capacity to detoxify undesirable components found in plants. Technicians know well the problems caused by anti-nutritional factors, found in raw soybean ingestion, or the effects of gossypol, found in cottonseeds, when these kinds of feed are ingested by monogastric animals. For ruminants, these toxic compounds have less harmful consequences due to the detoxifying effects of the ruminal microbiota.

Yet, several other toxic compounds found in plants can be harmful to ruminants, especially beef cattle grazing in arid regions. Ruminal manipulation can be done to inactivate these toxins. A well-succeeded example of this purpose is the discovery of *Synergistes jonesii* in Hawaiian goats. Even ingesting considerable amounts of leucaena (*Leucaena leucocephala*), these goats did not show intoxication signs by mimosine, a toxic amino acid found in great amounts in this plant. The toxicity caused by mimosine causes goiter with thyroid gland enlargement, hair loss, reduction in appetite and in reproductive performance. It has been demonstrated that the bacterium had the ability to inactivate the active toxic principle of mimosine. Therefore, ruminal content transfer of animals resistant to this toxin was able to provide resistance to animals that were sensitive to it before, found in other continents.

## Ruminal Fermentation Control and Manipulation Methods

Control or management of ruminal fermentation can be done in different ways, from substrate variation, i.e., through the diet formulated and intrinsic properties of the feed that consist it; through the methods used to deliver feed to the animals (feeding management); by the chemical or physical processing or treatment of nutrients found in the feed as well as the action of additives that select ruminal microbiota and/or modulate metabolic pathways responsible for its activities. Fermentation can also be controlled and manipulated by chemical principles naturally found in feed and other products. More recently, there has been a search to develop ways to control or

manipulate ruminal fermentation through immunological methods, active (vaccines) or passive (antibodies) ones. Finally, it is still possible to manipulate ruminal fermentation through specific microorganisms. Because it is a very broad and complex subject, only the most common and promising methods of fermentation control and manipulation have been approached in this chapter.

## *Carbohydrate Composition and Proportion*

Nutritionally, fiber can be defined as a fraction of polysaccharides and lignin of feeds that cannot be digested by digestive enzymes of mammals, and that occupies some space in the gastrointestinal tract of animals. However, this definition is only valid for monogastric animals. For ruminants, which symbiotically associate with unicellular microorganisms, fiber is better defined as the slowest digested portion of carbohydrates found in plants. Therefore, latter is a more vague definition than the former, making fiber a chemical, physical and nutritionally non-uniform entity, and a very complex subject.

Fiber mainly consists of cellulose, hemicellulose and lignin. It is important to remember the difference between structural carbohydrates and fibrous carbohydrates. The former literally constitute the structure of the vegetal wall and are represented by cellulose, hemicellulose, lignin and pectin. However, fibrous carbohydrates are essentially non-soluble and represented by cellulose, hemicellulose and lignin, but not by pectin, which is a soluble structural carbohydrate.

Frequently, total carbohydrates represent more than 65 % of diet dry matter of beef cattle or lactating dairy cows. Total carbohydrates are represented by fiber (NDF) and non-structural carbohydrates. When degraded by microbial enzymes, carbohydrates produce acids that are responsible for the decrease of ruminal pH. However, they can also stimulate rumination, salivation and, consequently, ruminal buffering. Therefore, the carbohydrate effect of increasing or decreasing ruminal pH will depend essentially on the type of carbohydrate.

Non-structural carbohydrate fraction, represented by sugars and starch, is mainly found in concentrate feedstuffs. Due to the reduced particle size, these carbohydrates do not have the capacity to stimulate rumination, contributing little to ruminal buffering through saliva. On the other hand, they have a faster or normally more extensive degradation rate than fibrous fraction, even though it varies from one source to another. Thus, this class of carbohydrates contributes more to ruminal pH decrease than its maintenance.

Fibrous fraction, mainly found in forage, has a digestible portion and a non-digestible one. Similarly to non-structural carbohydrates, digestible fraction is also converted into organic acids that reduce ruminal pH. The non-digestible fraction is capable of stimulating rumination and, therefore, favors ruminal buffering. Besides promoting dilution of more fermentable components of the feed, it increases ruminal motility, resulting in a greater mixture of the contents in this organ. This greater mixing causes a greater contact of the content with the epithelium, making absorption of SCFA easier.

**Table 6.3** Ruminal degradation rate (%/h), degradation extension (%) and effective degradability (%) of two energy sources submitted to different treatments[a]

| | Characteristic | | |
|---|---|---|---|
| Feed | Rate | Extension | Degradability |
| Corn grain | | | |
| Coarsely ground | 4.0 | 100.0 | 47.3 |
| Finely ground | 7.8 | 96.3 | 66.3 |
| High moisture silage | 13.3 | 93.6 | 81.7 |
| Sorghum grain | | | |
| Coarsely ground | 3.2 | 98.1 | 47.5 |
| Finely ground | 3.7 | 99.8 | 51.0 |
| High moisture silage | 2.8 | 96.7 | 44.4 |

[a]Source: Passini et al. (2002)

Although the total amount of fiber and non-structural carbohydrates in the diet influences ruminal content pH, the degradation rate and extension of these fractions vary a lot among feed types and, in a smaller proportion, within the same feed. A variation between 30 % and 90 % is observed in the dry matter ruminal degradation of different feeds. The dry matter ruminal degradation of total mixed rations has presented variations between 30 % and 67 %. In Table 6.3, some characteristics of ruminal degradation of concentrates commonly utilized in cattle production feeding are observed.

Degradation extension is the total proportion of a nutrient that has the potential to degrade itself when retention time in the rumen is not a limiting factor. The greater carbohydrate degradation rate and extension in the rumen are, the greater conversion of these carbohydrates into SCFA is; when they dissociate themselves, they release hydrogen ion and reduce ruminal pH. Not only is the amount of these acids altered, but the proportion between the several produced acids is also changed. When forage based diets are fed, acetic acid is generally produced in greater amount, followed by propionic and then by butyric acid. On the other hand, when availability of highly fermentable carbohydrates in the diet increases, the production of all acids increases as well, even though the production of propionic acid increases in a greater proportion, making its concentration in the rumen close or even the same as acetic acid.

Nutritionists try to understand why the proportion and composition of carbohydrates of the diet, after affecting ruminal pH, result in alteration of the proportion of SCFA produced. This understanding is essentially important since changes in this proportion result in alterations of fermentative efficiency, as shown before. It is true that the proportion of these acids depend on the type of predominant bacteria in the ruminal environment. Moreover, it is also true that the type of predominant bacteria depends on the ruminal pH. Therefore, it can be concluded that high availability of substrate would stimulate fermentation with pH reduction and consequent selection of specific groups of bacteria (mostly Gram positive) that would have propionic acid as preferred end product. It has been observed that changes in fermentation

conditions may alter the proportion of SCFA even if time was not enough for changes in the microbial population. Thus, ruminal pH would affect microbial metabolic pathways before the microbial population itself. In other words, strong fermentation from fermentable carbohydrates would result in a great production of hydrogen ions, which need to be removed from the rumen to avoid the inhibition of fermentation itself. Propionic acid works as a drain of hydrogen ions, because when it is produced, more hydrogen ions leave the rumen incorporated to this acid. Thus, the more carbohydrates are available, the more hydrogen ions (that reduce pH) are produced, and the more hydrogen ions there are, the greater the favoring of propionic acid production pathway is. It is also important to remember that the greater propionic acid production is, the greater ruminal fermentative efficiency is. There is no doubt that this generates other important consequences like ruminal pH disorders. However, this is a topic that will be addressed in Chap. 5 of this book.

It is also possible to manipulate ruminal fermentation utilizing other types of carbohydrates. Pectin is a soluble structural carbohydrate, therefore, non-fibrous. Because it consists of galacturonic acid monomers, pectin is preferably degraded in acetic acid, but not in lactic acid, regardless of rumen pH. By-products of fruit processing industry like citrus pulp, apple residues and even beet pulp are very rich in pectin. When added to diets of lactating dairy cows, citrus pulp increases milk fat, probably due to the increase in acetate:propionate ratio. However, because it also has high contents of soluble carbohydrates, citrus pulp can keep or even alter ruminal pH. Therefore, in citrus pulp-rich diets, the percentage of milk fat does not represent a good indicator of ruminal fermentation conditions.

In the figure shown below (Fig. 6.1), addition of citrus pulp to the diets of cannulated Girolando cows increased the acetate:propionate ratio. Regarding ruminal pH, the diet with pulp addition sometimes increases or decreases pH, but always keeps it more stable.

## *Feed pH and Neutralization Capacity*

Feed has intrinsic characteristics that can promote greater input of pre-formed acids, causing decreases in ruminal pH or even promoting uptake of hydrogen ions from the rumen, buffering it. Feed capacity to provide resistance to pH changes is called buffer capacity. This resistance occurs in both ways, avoiding pH increase when it is relatively low, and preventing pH reduction when it is relatively high. Feed buffer capacity does not depend on its pH. Feed with low pH can provide high or low resistance to pH variations and vice-versa. Feed buffer capacity is calculated by reducing feed sample pH in a solution to 4.0 by adding acids to it. Posteriorly, the amount of base milliequivalent that has to be added to this solution to increase pH to 6.0 is evaluated. The greater the amount of weak acids and bases as well as its salts present in the feed is, the greater the amount of bases that must be added to the solution to increase pH from 4.0 to 6.0 is; therefore, the greater buffer capacity of that feed is. Feed buffer capacity is given by the sum of buffer capacity of the

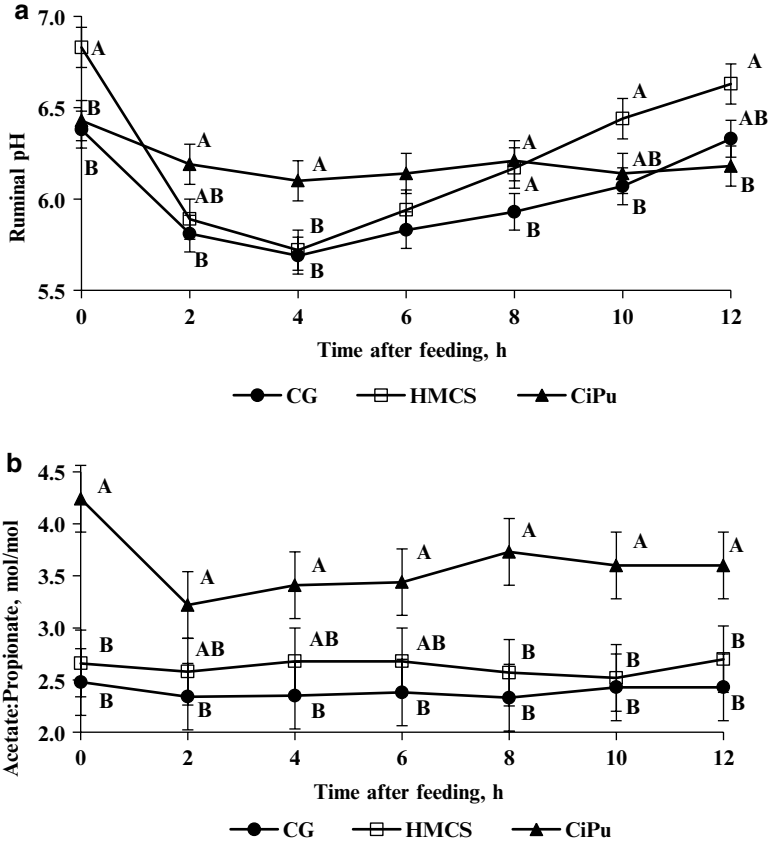


**Fig. 6.1** Values of pH and acetate:propionate ratio in ruminal fluid of animals submitted to different feed sources and processing (*GC* coarsely ground corn grain, *HMCS* high moisture corn silage, *CiPu* citrus pulp). The comparison between the treatments and the letters should be done within each time after feeding

soluble compounds added by cation exchange capacity of the fibrous fraction. Cation exchange capacity is the fiber ability to link metallic ions ($K^+$, $Ca^{++}$, $Na^+$ and $Mg^{++}$) to its surface. When pH lowers, fiber exchanges cations by hydrogen ions, keeping pH more stable. When pH increases and more cations are available, fiber sites are loaded with new cations. Cation exchange capacity varies a lot depending on the type of feed, and it is generally greater in leguminous forages than in grasses.

As it can be observed in Table 6.4, the fermentation process that occurred in corn silage lowers this feed pH. Thus, produced acids increase resistance to variations of this pH, and, consequently, buffer capacity also increases. Alfalfa fresh plants have a relatively high pH, and the amount of weak acids and bases as well as its salts that are present there (malic, fumaric and other acids) cause high resistance to these pH variations. Therefore, it is concluded that feed preserved by fermentation (silage) are likely to reduce and keep ruminal pH low whereas fresh feed or kept by dehydration (hay) tend to increase and keep ruminal pH high.

**Table 6.4** pH and buffer capacity of some feedstuffs[a]

| Feed | pH | Buffer capacity[b] |
|---|---|---|
| Corn | | |
| – Fresh | 5.20 | 19.1 |
| – Silage | 3.90 | 39.7 |
| Alfalfa | | |
| – Fresh | 6.10 | 48.7 |
| – Pre-dry silage | 4.74 | 70.5 |

[a]Source: Partially adapted from Erdman (1988)
[b]Calculated in milliequivalents needed to increase pH of 100 g of feed from 4.0 to 6.0

In fermented feed, like corn silage, low pH and high buffer capacity may hinder adequate ruminal pH maintenance. Corn silage ingestion with pH 3.0 by an animal that has ruminal pH of 6.0 would need 33 g of equivalent sodium bicarbonate per kilo of ingested silage dry matter for the animal to keep its ruminal pH at 6.0. On the other hand, a cow would not need the additional amount of bicarbonate if it were ingesting fresh feed or hay that had pH close to 6.0.

Feed buffer capacity varies a lot according to the feed type, plant physiological stage at the time of harvest and others. There is a controversy among researchers if this characteristic affects acid-base balance in the rumen under normal conditions. Some believe that its effect on rumen final pH is quite reduced. Most of buffering by feed occurs at pH lower than 5.0. This pH is below the functional amplitude of healthy animals' rumen (from 5.5 to 6.8). It is estimated that the contribution of feed buffer capacity represents a fifth of saliva buffer capacity.

## *Lipids*

Ruminal metabolism of lipids has already been described in Chap. 4 of this book, so this chapter will focus only on the specific effects of these compounds on ruminal fermentation. The effects and interactions of these compounds in the rumen as well as the transformations that they undergo in this organ are extensive and complex, especially regarding lipids formed by polyunsaturated fatty acids.

Ruminants' feeding naturally contains lipids rich in unsaturated fatty acids, which are, after extensively hydrolyzed, biohydrogenated by ruminal microbiota. Due to its high energetic concentration, there is a lot of interest in the use of lipids in animal nutrition, especially for dairy cows. However, as a result of high ingestion of these fatty acids, and their consequent effects on ruminal metabolism, it is common to observe undesirable effects, especially by decrease in fiber degradation. Thus, lately, emphasis has been given on how to minimize the action of lipids on the rumen, such as utilizing processes of protection through the formation of calcium soaps, artificial hydrogenation, lipid encapsulation or ingestion in the form of whole grains. With it, it has been tried to minimize or even to null the effects, undesirable or not, of lipids in the rumen.

Therefore, with immediate utilization or not, it is important to know the effects that lipids have on ruminal fermentation. These nutrients, especially polyunsaturated fatty acids after lipolysis process, can work as alternate electron acceptors. Hydrogen produced in fermentation process would be taken from the rumen through another way that was not through methane gas production. Thus, unsaturated fatty acids would compete for hydrogen destination like $CO_2$, resulting in reduction of methane production. This phenomenon is especially interesting nowadays due to global warming concern. There is still the possibility that this group of compounds (polyunsaturated fatty acids) has toxic and specific effects against methanogenic arqueas, which are the ruminal microorganisms responsible by methane production. The result would be the inactivation of these ruminal microorganisms, what would decrease methanogenesis (chemical reduction of $CO_2$ to form methane), redirecting hydrogen to propionic acid, therefore, improving efficiency of ruminal fermentation.

Although it has been shown that manipulation of polyunsaturated fatty acids really increases ruminal concentration of propionic acid, other researchers believe that this happens due to inhibition of cellulose degradation, which would have acetic acid as the main end product, and not because of the specific effect against methanogenic arqueas.

Hence, considering that lipid action on microbiota is not specific, the beneficial effect caused by controlling methanogenic arqueas would be counterbalanced through cellulolytic activity inhibition, by inactivating cellulolytic bacteria. Thus, improvement of ruminal fermentation efficiency would cause lower production of fermentation products without resulting in liquid gains. This way, the beneficial effect of these acids to act as alternative hydrogen acceptors would be all that would be left. However, their real capacity to receive hydrogen is questionable due to its high molecular weight.

In a simple stoichiometric calculation, the amount of unsaturated fatty acids that would be necessary to serve as alternative drain to all ruminal hydrogen of an animal, which would be producing 150 g of methane/day, was calculated. The total lipid amount that would have to be ingested reaches 5.295, 2.625 or 1.744 g/animal/day, if the lipid source has oleic ($C_{18:1}$), linoleic ($C_{18:2}$) or linolenic ($C_{18:3}$) acid as an exclusive source of fatty acid. Any person that works with nutrition knows that this amount is too high to be ingested by a beef or dairy cattle and, if ingested, it will certainly cause problems. Therefore, use of lipids as a way to reduce methane production and, thus, decrease energy losses, would be like the use of a huge truck to transport a small garbage bag from our neighborhood to a landfill.

To heat up the discussion, studies showed that unsaturated fatty acids have beneficial activity, similar to monensin, when reducing ruminal deamination activity without inhibiting proteolytic activity.

There is no doubt that the use of lipids may be an important tool for nutritionists by increasing energetic density of ruminants' diet. Moreover, if lipids have other advantages on ruminal fermentation efficiency, it is still open for discussion.

## *Feeding Frequency and Total Mixed Rations*

Nutritionists who follow a specific philosophy believe that length of time that rumen pH remains low is more important to trigger acidosis than minimum value of ruminal pH after digestion. Coherently, the increase in number of times that an animal is fed daily, i.e., increase in feeding frequency, would decrease the length of time in which ruminal pH remains low, and therefore, it would reduce the consequences of acidosis. However, it is more prudent to admit that damages caused by acidosis are proportional to at least two different characteristics of ruminal pH: (1) length of time of low pH, and (2) intensity of pH reduction. This is the reason why research studies that use continuous data collection tools for ruminal pH content have great perspectives to generate new information on acidosis. For example, pH area below a determined value is easily obtained with the use of these tools and associates length of time of low pH to intensity of pH reduction at the same time.

The use of complete diets (also known as TMR—"Total Mixed Ration") has increased the frequency in which animals ingest feed. When mixing forage and concentrate, the ingestion rate of concentrate is lower than if it were ingested separately. This helps avoid great ruminal pH variations throughout the day. If correctly prepared, total mixed rations also prevent cattle from sorting feed particles, forcing forage intake. Among other advantages, it also allows the inclusion of fiber-rich by-products as whole cottonseed and soybean hulls that would not be ingested if offered separately.

Moreover, feed moisture seems to affect saliva production but not very consistently. It is believed that the low moisture of hay is partially responsible for its higher capacity of stimulating rumination and, therefore, providing greater buffering of ruminal environment when compared to silages.

Saliva removes hydrogen ions in solution by combining buffering and alkalization processes through bicarbonate and phosphate ions. In phosphate system, hydrogen ions incorporated to phosphate ions ($H_2PO_4^-$) are removed through the flow by the reticulo-omasal orifice. In bicarbonate system, hydrogen ions react with bicarbonate producing carbonic acid. Then, this acid is degraded to carbon dioxide and water. $CO_2$ leaves the rumen through eructation, whereas water to which hydrogen ions got incorporated can be utilized.

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$$

Buffering capacity of saliva results most exclusively from the sum of buffering capacities of bicarbonate and phosphate ions in which the former is the main buffer. As bicarbonate $pK_a$ is 6.1, and phosphate ion is 7.2, under pH conditions close to neutrality, phosphate ions gain more importance. Under physiological conditions, bicarbonate is responsible for approximately 82 % of saliva buffering capacity, although this proportion varies with the pH of the ruminal content.

The use of total mixed rations is extensively spread among feeders of both beef and dairy cattle. The utilization of dry forages (hay and straw), even in small amounts, are recommended by many nutritionists almost as "mandatory feed" for feedlot animals' diets. Such tools help the control of ruminal fermentation, avoiding acidosis and associated processes.

## *Adaptation to Diet*

Short-chain fatty acids produced by ruminal fermentation leave the rumen through absorption through the ruminal wall or liquid flow through the reticulo-omasal orifice. Acids that leave the rumen through wall absorption do it mainly in a non-ionized form, under physiological conditions. Therefore, low ruminal pH favors absorption of acids that carry hydrogen ions once they are absorbed. Absorption rates of acetic, propionic and butyric acids are similar at neuter pH, close to 7.2. However, at low pH, close to 4.5, there is an increase in the absorption rate of butyric and propionic acids, but not of acetic acid. The increase in absorption rate with pH reduction is greater for butyric acid, intermediate for propionic acid and null for acetic acid (Table 6.5). This is due to the fact that butyric acid is almost completely metabolized by the ruminal wall and, therefore, there is no need for accumulation of this acid. Propionic acid is partially metabolized by this tissue whereas the acetic acid is almost not. This makes acetic acid, at high acid concentration in the rumen, the principal acid responsible for low pH condition.

The surface area of ruminal epithelium affects absorption rate of organic acids produced by this organ. Absorption rate is smaller when ruminal liquid volume is greater since its area/volume relationship is smaller. Moreover, size of ruminal papillae affects this absorption surface. Papillae length can be manipulated by diet composition. The greater the amount of concentrate offered to the animal is, the greater the stimulus to increase the length of these papillae is. Butyric and propionic acids are the main responsible acids for this stimulus. Therefore, the gradual increase in diet energetic density decreases the incidence of subsequent acidosis. A more detailed discussion of SCFA absorption and rumen papillae development is found in Chap. 5 of this book.

For beef cattle, several researchers have been evaluating different adaptation protocols to high-energy diets, including different recommendations for animals of European or Indian origin. In general, these protocols are implemented in the first three weeks in feedlot, with addition of approximately 10 % units of TDN (total

**Table 6.5** Effect of ruminal pH on absorption rate of SCFA (%/h) through the ruminal epithelium[a]

| | Ruminal pH | |
|---|---|---|
| Fatty acid | 7.2 | 4.5 |
| Acetic | 31.0 | 31.0 |
| Propionic | 35.0 | 68.0 |
| Butyric | 28.0 | 85.0 |

[a]Source: Adapted from Allen (1997)

digestible nutrients) to the diet each week, for example. In this case, adaptation periods longer than 21 days are usually not recommended once this practice could impair animal performance from compensatory gain, and reduce the time to which animals will be fed the finishing diet, considering the short period of time that animals normally spend in feedlot (no longer than 90 days in Brazil or 150 days in the United States of America). On the other hand, adaptation periods shorter than 14 days may not prepare beef cattle appropriately to receive finishing diets rich in energy, which can impact the animals' performance and ruminal health negatively as well.

For dairy cows, this adaptation practice is known as "warming" and is carried out in the last weeks before calving. Committees of nutritionists specialized in dairy cows recommend that diet for dry cows contain around 1.25 Mcal of net energy for lactation (dry matter basis). However, in the last days of pregnancy, intake is quite suppressed whereas the energy demands for fetal maintenance increase. Therefore, it is recommended that the energy density be increased, which may reach 1.4 Mcal of net energy for lactation (dry matter basis) during the last weeks of pregnancy. This warming practice avoids accelerated movement of body reserves that could result in ketosis as well as provides greater development of ruminal papillae, helping decrease acidosis incidence in cows in the beginning of lactation.

Therefore, the dry period can be divided into two different phases: the first one called maintenance (1.25 Mcal of net energy for lactation [dry matter basis]), and the second one called warming (1.40 Mcal of net energy for lactation [dry matter basis]). In cows that dried under ideal body conditions, substitution of maintenance diet by warming diet can be done at 21 days before the expected delivery date. In thin cows presenting low body condition scores, diet substitution can be anticipated so that these animals have the chance to replace reserves during this phase, even though this is not very easy.

## *Buffers and Neutralizers*

The term buffer has been frequently utilized indiscriminately since it is confused with substances that have neutralizing or alkalinizing capacity. Buffer is the substance in aqueous solution that causes effective resistance to pH variations when an acid or base is added to the medium. In order to be considered a buffer, a substance has to be a weak acid or base as well as its salts, be hydro soluble and have equivalence point ($pK_a$) close to the system to be buffered. For example, sodium bicarbonate is a hydro soluble salt that has $pK_a$ equal to 6.2. This $pK_a$ is quite close to the rumen physiological pH; therefore, it is considered a buffer. On the other hand, magnesium oxide (MgO) does not have defined $pK_a$ and has varying solubility. Due to that, it is considered neutralizing.

Some physical-chemical characteristics of buffers and neutralizers utilized to control pH of ruminants' gastrointestinal tract are shown in Table 6.6. Besides the cited characteristics, the buffer must also be palatable, easy to mix to the diet and not hygroscopic, i.e., must not have high water retention capacity. Sodium bicarbonate is

**Table 6.6** Chemical properties of some buffers and neutralizers utilized for dairy cows[a]

| | Characteristics | | |
|---|---|---|---|
| Source | $pK_a$ | Neutralizing capacity (meq/g) | Water solubility (g/100 mL) |
| Buffers | | | |
| Sodium bicarbonate | 6.25 | 12.2 | 6.9 |
| Potassium bicarbonate | 6.25 | 10.1 | 22.4 |
| Potassium carbonate | 6.25 and 10.25 | 20.2 | 112.0 |
| Sodium sesquicarbonate | 6.25 and 10.25 | 13.3 | 13.0 |
| Trona mineral | 6.25 and 10.25 | 11.1 | 13.0 |
| Neutralizer | | | |
| Magnesium oxide | – | 49.6 | Varying |

[a]Source: Adapted from Erdman (1988)

the most utilized buffer with magnesium oxide as neutralizer. Magnesium oxide, when associated with bicarbonate, would have the role of leading to a longer neutralizing action than buffers, especially in the posterior digestive system (intestines). The action of potassium bicarbonate is similar to the one of sodium bicarbonate. Potassium carbonate is less palatable, and sodium carbonate is too hygroscopic to be mixed to dairy cows' feed; calcium carbonate, also known as limestone, is little soluble and then has little buffering action, despite its alkalinizing effect in small and large intestines. Sesquicarbonate is a mixture of bicarbonate and sodium carbonate, besides being alkalinizing agents. Trona is a raw mineral that originates sodium bicarbonate, especially in the United States.

Normally, fixed doses of buffer/neutralizer agents in the diet are recommended (Table 6.7). For example, the sodium bicarbonate dose is around 110–225 g per cow a day. The dosage can also be calculated in percentage of the diet. Mixtures with 25–33 % of magnesium oxide and 75–67 % of sodium bicarbonate are recommended for the dosage of 0.75–1.0 % of diet dry matter. The amount can be based on diet ADF content so that the lower the diet ADF concentration is, the greater the amount of added buffer should be. It is recommended 44 g of sodium bicarbonate or 20 g of magnesium oxide for each ADF percentage unit lower than the minimum fiber requirement. For example, if a diet contains 14 % of ADF and the minimum recommended is 19 %, then it is necessary to offer 220 g of sodium bicarbonate $[220 = 44 \times (19 - 14)]$ or 100 g of magnesium oxide $[100 = 20 \times (19 - 14)]$ to avoid reduction of milk fat contents.

Another practical criterion that may be utilized to determine the buffer amount to be incorporated to a diet is its final pH. Studies have shown that the optimal intake of diets based on silage would be reached with pH 5.6, amplitudes between 5.0 and 6.0. Increasing amounts of buffers could be added until the diet reached pH 5.6.

Evidently, better responses related to dry matter intake, productive performance (weight gain or milk production) and fat content in milk with inclusion of

**Table 6.7** Recommendation of commonly utilized buffers/neutralizers for dairy cows[a]

| Product | Dose (g/day) |
|---|---|
| Sodium bicarbonate | 110–225 |
| Potassium carbonate | 270–410 |
| Calcium carbonate | 115–180 |
| Sodium sesquicarbonate | 160–340 |
| Sodium bentonite | 450–900 |
| Magnesium oxide | 50–90 |

[a]Source: Adapted from Erdman (1988)

buffers/neutralizers in ruminants' diets are expected when the diet has low fiber content and reduced particle size, and high content and/or high degradation rate of non-structural carbohydrates; is rich in moist feed (silages), and poor in hay; and also when the animals are susceptible to heat stress.

## *Ionophore and Non-Ionophore Antibiotics*

Ionophores are a class of chemical compounds that obtained considerable success as feed additives. The use of ionophores, like monensin, in animals is not recent. Its beginning dates back to 1970s aiming to increase efficiency of feed utilization. The advantages of using it for dairy cattle are related to increase in milk production, greater feed efficiency and reduction of metabolic disorders like clinical and subclinical ketosis, bloat and acidosis. For beef cattle, ionophore benefits are related to the reduction of dry matter intake, greater weight gain, better feeding efficiency and reduction of morbidity and mortality. For both categories, they also help to reduce the amount of nutrients excreted in feces, as well as reduce the emission of gases by the ruminants.

Despite the great success of ionophore antibiotics as feed additive, the potential of non-ionophore antibiotics like avoparcin, flavomycin, tylosin, bacitracin and virginiamycin that can improve animal performance by altering fermentative characteristics of the rumen cannot be ruled out.

Basically, ionophores act on the cellular membrane of ruminal microorganisms, interfering in normal ionic flow through the membrane and dissipating proton and cation gradients, which are systems responsible for the input of important nutrients (amino acids, sugars and other ions) against a concentration gradient. Thus, due to its reduced size, a bacterium has little energy storage capacity in the form of organic compounds (starch, lipids and others). Therefore, they store energy for their vital processes through a charge difference between the interior and exterior of the cell. This is done by transporting and storing hydrogen ions with positive charge outward the cell. When they come back into the cell, these ions allow ATP re-synthesis or nutrient absorption. If this difference of charge concentration between the interior and exterior is inadvertently undone, the stored energy is then dissipated and the bacterium is harmed. In order to perform their function efficiently, ionophores

form stable complexes with cations, including hydrogen ions, and are capable of dividing themselves between the external surface and membrane interior, i.e., they must have lipophilic or hydrophilic properties, depending on the presence or absence of cation complexation. When found in non-complexed form, this molecule places itself on the internal or external membrane surface. While complexed with cation, it becomes lipophilic, and as a result it goes through the membrane, allowing that cation transfer occur at a sufficiently high rate. This property makes these molecules harm some bacterial groups, but not others. Bacteria, which have an electron transport system coupled to proton extrusion and/or ATP synthesis, will have better survival conditions, despite greater energy demands. Bacteria that depend on ATP phosphorylation at substrate level via ATPase will be harmed.

Therefore, ionophores cause changes in rumen microbiota, favoring Gram-negative bacteria and negatively affecting Gram-positive ones. When they have selective action on ruminal bacteria, they also change the fermentation profile of the rumen. Another possibility is that these effects originate especially from direct alterations in preferential metabolic pathways of some bacteria. In other words, when allowing proton input inwards the bacterium, ionophores would force the selection of preferential metabolic pathways that favor the production of propionic acid, which are, as seen before, more energetically efficient.

As a result of this basic effect, several other secondary effects would be found, such as: modifications of SCFA production, feed intake, gas production, nutrient digestibility, protein utilization, ruminal filling and passing rate. The abundant literature on ionophore effects on these parameters show that the obtained results have been quite varied, a phenomenon that can be explained, partly, by different experimental conditions.

It is common to observe, as a result of monensin utilization in beef cattle, an average decrease of 6.4 % in feed intake without great responses to daily weight gain, which may result in an improvement of feed to gain ratio of 7.5 %. In dairy cattle, the effects of monensin on milk production are varied and inconsistent.

Although ionophores may increase ruminal pH when this parameter is relatively low, these ruminal fermentation modifiers tend to reduce even more the percentage of milk fat when added to cows' diets. The cause of milk fat percentage reduction due to the lack of fiber is still not fully understood; likewise, the effect of ionophores on this factor is also not completely known. Anyway, ionophores decrease acetate:propionate ratio in the rumen and can affect ruminal lipid biohydrogenation as well. Regardless of the theory accepted to explain the milk fat percentage reduction, ionophores affect the conditions of ruminal fermentation and, therefore, fat content in milk.

Cows that are more susceptible to reduction of milk fat percentage with addition of ionophores are those in the end of lactation period and under marginal conditions regarding ruminal fermentation profile and milk fat percentage. The first factor is easily explained by the main source of milk fat origin of cows that are in the beginning and in the end of lactation. Cows in the beginning of lactation are more dependent of fat found in tissues, whereas cows in the end of lactation depend on the *De Novo* synthesis in the mammary gland. Considering the second factor, the reduction

of acetate:propionate ratio caused by ionophores would have a greater impact in cows with marginal ratios, close to 2.2, than with higher ones.

The utilization of ionophores as feed additives for cattle has become fundamental in ruminal fermentation manipulation, increasing efficiency of utilized diets. Especially in beef cattle, the tendency to increase utilization of diets containing high content of readily fermentable carbohydrates is obvious in order to obtain greater daily weight gain. Thus, there is the decrease of days needed for animal finishing. In this case, ionophore addition guarantees animal health because it controls microorganisms related to acidosis development. However, there are sanitary and food safety issues related to their use as it has been discussed worldwide for some years.

The European Community has been restricting the utilization of antibiotics and coccidiostats as feed additives for cattle. Some pharmaceutical principles are not found for commercialization anymore, and others are gradually being withdrawn from the market. Despite the lack of scientific proof, this caution was taken to prevent the possible relation between the increase of resistant microorganism incidence to antibiotics, observed in human medicine, and the use of these substances in animal feeds. Moreover, there is the increasing demand from the consuming market for natural and healthy vegetal and animal foods that come from productions that utilize less synthetic substances as possible. For this reason, important producers and exporters of animal feed have to pay attention to the new reality of the world market.

## *Active and Passive Immunization*

Some researchers have defended the utilization of immunity concept as a potential tool for ruminal fermentation manipulation. Calves and sheep have already been immunized against lactic-producing bacteria like *Streptococcus bovis* and *Lactobacillus* spp. In general, these vaccines were efficient to keep feed intake, maintain ruminal pH, decrease ruminal concentration of lactate, and reduce populations of *Streptococcus bovis* and *Lactobacillus* spp. after challenge with high-grain diet. Sheep also present lower incidence of severe diarrhea when compared to control animals.

However, other researchers have shown that passive immunization principle has potential to originate new feed additives. Within this principle, polyclonal antibody preparations (**PAP**) have been developed specifically for some bacteria found in the ruminal environment, as well as to be added to animal diet. To produce polyclonal antibodies, hens are immunized intramuscularly against inactivated antigens. Hens' immune system reacts producing specific antibodies (IgY) for each antigen, which are transferred to egg yolk when still in the bird's oviduct. Thus, immunoglobulins can be extracted from egg yolk through several techniques and added to ruminants' diet.

Immunoglobulins Y, produced by chicken eggs, have fundamental characteristics to act in the ruminal environment, such as: resistance to pH up to 4.5, temperature up to 120 °C, and proteolysis. Even after its breaking, they seem not to lose the linking

capacity to the microbial agent. It is believed that this resistance to proteolysis may be related to the presence of disulfide bonds in the composition of immunoglobulins, which are more difficult to be broken by proteolytic enzymes.

Studies with these antibodies have also shown that they are efficient to reduce ruminal concentrations of target bacteria for which they had been developed. However, data from ruminal metabolism concentrations are not so encouraging. In only one experiment, out of six reported studies, ruminal pH improved. In this study, PAP produced against *S. bovis*, *F. necrophorum* and three strains of proteolytic bacteria was as efficient as monensin to keep ruminal pH after 4 h of feeding. Both products presented ruminal pH higher than the control group. Another experiment carried out by the same group of researchers showed that beef cattle that received PAP had the same productive performance than those that received monensin. They also observed that feeding PAP significantly reduced the incidence of rumenitis when compared to feeding of monensin.

An important characteristic of this product is that, differently from monensin, PAP does not seem to change ruminal fermentation profile (total concentration and molar proportion of SCFA) and, therefore, does not change the energy efficiency of fermentation. Studies in our laboratories have shown that product efficiency depends on its presentation form (solid or liquid), kind of reached acidosis (lactic or by SCFA surcharge) as well as on other experimental characteristics.

## *Antimethanogenic Drugs*

Several drugs have been tested as potential inhibitors of ruminal methanogenesis. However, limitations to the use of these compounds have still superseded by their effective beneficial effects. For example, chloroform and chloral hydrate, when used for that purpose, may cause hepatic and nervous lesions. Moreover, these compounds are not available for utilization because they can be used as illegal drugs. Amichloral, trichloroacetamide, bromochloromethane and 2-bromoethanesulfonic acid (BES) effectively reduce methanogenesis, but their effects are transitory and do not last long, probably due to the adaptation of methanogenic population to these compounds. Halogenic methane analog and 9.10 antrachinone reduce methanogenesis by uncoupling electron transfer in methanogenic arqueas. This effect seems to be long-lasting but the technique is still in the initial development stage. In addition, it is not uncommon to observe undesirable effects on feed digestibility using these different drugs.

Although methanogenesis results in emission of an undesirable pollutant for the Earth environment (methane), it is necessary to acknowledge that this activity removes an important metabolite from the ruminal environment (hydrogen) by eructation. If this metabolite accumulated in the rumen, fermentation and, consequently, ruminal digestion would be prevented. Thus, every time that methanogenesis blocking or reduction is considered, other hydrogen elimination pathways from the rumen have to be available. Therefore, the literature has been pointing out lipids, organic

acids like malate and fumarate and even nitrate and sulfate as potential electron acceptors (hydrogen removers). The limitations found until now for the effective use of these alternative pathways of hydrogen disposal are that lipids and organic acids should be ingested in great amounts in the diet, making their use economically unfeasible. For nitrate and sulfate ions, the limitation is that reduction of these compounds in the rumen, a process that causes hydrogen removal, would result in conversion of toxic products to ruminants. Recent studies carried out with these products have shown solutions for some of these limitations. For example, the utilization of adaptation protocols of ruminal microbiota as well as industrial techniques of particle capsuling, which would provide slow release of the active principle, present themselves as possible methods to be used in the near future.

There is no doubt that the development of new drugs with effective capacity to manage ruminal fermentation is still one of the most promising study fields of ruminants.

## *Yeast and Other Probiotics*

The endorsed definition of probiotics is live microbial feed supplements that beneficially affect the host, improving intestinal microbial balance. They are microbial cultures, microbial extract cultures, enzymatic preparations or a combination of them. The American FDA (Food and Drug Administration) named these kinds of probiotics "direct-fed microbial" (DFM). The most known of these additives for ruminants' nutrition are from fungal and bacterial cultures. Microbial cultures or probiotics can be presented as live yeasts such as *Saccharomyces cerevisiae*. It is believed that it can result in development of cellulolytic bacteria like *F. succinogenes* and *R. albus*, besides stimulating lactate utilization by *M. elsdenii*, which could result in increase of ruminal acetate production.

Whether or not yeasts and other probiotics have beneficial effects on adult cattle is one of the most polemic topics in ruminants' nutrition, mainly because of the opinion of experts in the area who state that these additives have inconsistent effects on animal performance, especially since there is no evidence that these agents may establish themselves as significant and viable members in the ruminal environment.

## *Tannins, Saponins and Other Natural Principles*

Some secondary compounds found in plants, like saponins and tannins, can modify ruminal fermentation. Tannins are polymers of phenolic compounds with different molecular weighs and capable of complexing macromolecules, especially proteins. Tannins can reduce nutrient degradation due to the complexation with this protein, enzymes and other macromolecules. This complexation has been considered beneficial sometimes as it reduces protein degradation in the rumen (bypass effect) and

decreases damages caused by bloat. Its effects on the rumen show great variability of observed responses, probably because of the great diversity of chemical forms of this compound (physical structure and molecular weight), its concentration in plants, value of ruminal pH and others. For example, it is known that the molecular structure of condensed tannin provides stronger and more irreversible bonds with protein than hydrolysable tannins, which can be degraded by microorganisms.

Recent studies have pointed out that tannins can also reduce ruminal methanogenesis, possibly by inhibiting the development of methanogenic arqueas. In addition, some studies have shown that tannins increase the propionate molar proportion, decrease acetate proportion, but also result in reduction of total SCFA production. This indicates that such effect on ruminal fermentation may be not so specific against undesirable microorganisms (methanogenic arqueas), resulting in general and unspecified impairment of fermentation. Although the scientific community has positively seen that tannins have potential to be used for methane gas mitigation, this technological alternative still needs more accurate experimental confirmation.

Saponins are glycosides that have the property to change plasmatic cellular membrane permeability. When ingested by ruminants, these compounds can have inhibitory effect on bacterial and protozoa populations, resulting in decrease of acetate:propionate ratio, which is desirable, but also reducing total production of SCFA.

Anyway, even if it is proved that these compounds have beneficial effects, this alternate technology still needs to be improved with information such as which forage containing tannin or saponins could be utilized, how much of it could be ingested, and how it could be incorporated to animal feeding systems, among others.

## Principles for Monitoring the Results of Rumen Manipulation

Due to the great number of factors that influence ruminal fermentation, it is difficult to predict the characteristics of fermentation conditions only through diet composition. When preparing the diet, there are oscillations in chemical composition and variations in ingredient weighing as well as lack of mixture homogeneity. Moreover, even if the diet is well controlled, cattle still can reject big particles of feed, as they are very selective animals. Considering this sum of errors, the ingested diet is very likely to be different from the offered diet, which is different from the formulated one. A nutritionist has to consider the possibility of continuous monitoring of animal response due to the offered diet to sharply adjust the adequate feeding. For this purpose, indicators are proposed to help this evaluation. Some are so complex that demand collaboration from universities or research institutes whereas others are so simple that can be performed by a technician or even employees, presenting immediate results. Overall, indicators should not be utilized isolated. The association of two or more indicators will provide more reliable results.

**Evaluation of particle siz'e**: Combined to diet NDF values, and obtained by laboratorial analyses, the size of feed particles can be used to assess diet appropriateness for fiber. A particle separator has been developed by Penn State University to

**Table 6.8** Distribution of particle size (%) recommended for the Penn State particle separator[a]

| | Feed | | |
|---|---|---|---|
| Sieve | Corn silage | Haylage | Total mixed rations |
| Upper sieve (>20 mm) | 3–8 | 10–20 | 2–8 |
| Middle sieve (8–20 mm) | 45–65 | 45–75 | 30–50 |
| Lower sieve (4–8 mm) | 20–30 | 30–40 | 10–20 |
| Bottom pan (<4 mm) | <10 | <10 | 30–40 |

[a]Source: Adapted from Heinrichs (1996)

quantify the distribution of particle size of forage feed and total mixed ration. The particle separator consists of an upper sieve with 20 mm holes (0.75 in.), a middle sieve with 8 mm holes (0.31 in.), a lower sieve with 4 mm holes (0.16 in.), and a bottom pan. The recommendation of particle proportion that is retained in each sieve is presented in Table 6.8

**Ruminal pH**: Ruminal pH can be monitored by a simple potentiometer or tapes of indicator dyes. Sample collection should be done preferably by rumenocentesis because collection using esophageal or nasal-esophageal probe is frequently contaminated with saliva, overestimating ruminal pH value. Rumenocentesis is done by inserting a needle in the left side of the animal, half height of the torso, behind the last rib, and by draining ruminal liquid with a syringe. Users of this technique state that the liquid obtained from a single area of the rumen is very representative. Sample collection time depends on the offered diet. In diets with concentrates and forage offered separately, samples should be collected two hours after concentrate feeding. Animals fed with total mixed rations (TMR) should be sampled 4–7 h after the first bunk access. At least 6–10 animals per group should be sampled to obtain a representative data. Values of pH can vary from 5.5 to 7.0, and 6.0–6.5 are considered optimal values. Animals with rumen pH over 5.9 are considered normal. Groups whose 30 % of animals' pH ranges between 5.6–5.8 are considered marginal and those whose 30 % of animals' pH is lower than 5.5 are considered abnormal.

**Fecal pH**: This technique has the advantage of making fecal sampling easier when compared to ruminal liquid collection. With this technique, it is believed that fecal pH is representative of ruminal pH. However, diets with high amounts of starch or low ruminal starch digestibility can cause atypical fermentation of this starch in the large intestine, which would generate wrong results. Fecal pH should be measured seconds after defecation, or even when sampling is done in the rectum; otherwise, there is alkalization of feces when exposed to the environment.

**Ruminal acetate:propionate ratio**: Evaluation of acetate:propionate ratio in the ruminal liquid is quite expensive and demands sample fixing in acid, freezing and analysis by a specialized laboratory. The kind of acid used for sample fixing depends on the methodology utilized by the laboratory. Time between feeding and sample collection as well as the number of sampled animals must be similar to the utilized methodology in order to evaluate ruminal pH. When compared to pH, the acetate:propionate ratio has the advantage of being less influenced by

saliva contamination since the proportion of these acids is not affected by their own buffering capacity. Even dilution of the ruminal liquid does not influence the result because the interpretation is given by the acid relation with another acid and not by the total concentration of each acid. The normal values of acetate:propionate ratio range from 3.0:1 to 2.5:1. Values lower than 2.2:1 are considered abnormal.

**Milk composition**: the percentage of milk fat or fat:protein ratio in milk has the advantage of utilizing a non-invasive method. Moreover, fat:protein ratio is not influenced by the time samples were collected. The sample has to consist of milk from all milking times, mainly if milking intervals are not equally spaced. Milk from longer milking interval tends to have less fat content. The ideal sample should have proportional amounts regarding the production of each milking. As this would demand a lot of work, equal volumes of milk from each milking are accepted. Special care should be taken to obtain a quite homogenous sample that does not form milk froth. Modern techniques that utilize NIRS methodology are sensitive to the breaking of fat globules caused by excessive homogenization. Samples from expansion tanks demand even greater care since homogenization cannot be complete. The samples should be fixed in special preservatives and kept refrigerated for future analysis. The minimum acceptable value of milk fat from Holstein cows is approximately 3.3 % and fat:protein ratio should not be lesser than 1.1:1. Milk fat content or fat:protein ratio as an indicator of diet appropriateness is more important for cows in the middle and in the end of lactation than in the beginning, since the main source of milk fat from cows in the beginning of lactation is that one mobilized from reserve tissues.

**Percentage of ruminating animals**: Diet NDF, chewing activity and ruminal pH are directly related factors. The percentage of ruminating animals in the herd at a given time can be utilized as another indicator of fiber appropriateness in the diet if used carefully. At least 50 % of the animals are expected to be ruminating between 2 to 4 h after feeding. Normal herds may present up to 70–80 % of animals ruminating at a determined time.

**Fecal shape**: Fecal characteristics carry very valuable information for the attentive observer. Through them, it is possible to evaluate feed utilization and speculate about the balance of fermentative and digestive processes in the gastrointestinal tract. Ideal feces of a lactating cow have the shape of a small volcano. When falling to the ground, they keep themselves together, forming a small depression on its surface. Animals receiving low-fiber diets containing great amounts of concentrates, present excessively moist and soft feces due to water sequestration caused by high osmolality of gastrointestinal lumen. On the other hand, animals that have lower dry matter intake, but with non-excessive ruminal fermentation, may present harder feces or annealed ones due to greater absorption of water in the large intestine and to peristaltic movements. In general, fecal piles should have a minimum of 5.0 cm.

**Sodium bicarbonate intake**: Although bicarbonate is naturally little palatable to ruminants, it's possible that under acidosis conditions, they might try to consume this additive aiming greater ruminal comfort. A test providing sodium bicarbonate ad libitum to the animals separated from their diet can be carried out

anytime. An average sodium bicarbonate intake higher than 100 g/animal/day indicates the occurrence of acidosis and some measures have to be implemented to control it. Special care should be taken for avoiding losses of sodium bicarbonate in the bunks due to wind action during intake measurement, since it is a very fine powder.

**Others**: Animals fed low-fiber diets frequently present cyclic intake. Normal intake days can be followed by days of low intake or even lack of it. These animals may become excessively thin despite the high-energy diet offered. In addition, some considerable increase of the incidence of some diseases in the herd can be observed.

## Conclusions

Fermentation is a process present in our lives, in which there's an association with desirable microorganisms in a way to obtain a lot of benefits. Under animal nutrition perspective, fermentation implies in the transformation of less valuable compounds into more valuable ones, and because of that, it is considered an efficient process. Although it is efficient, this process implies in losses that can be avoided or at least minimized through its control and manipulation. Control of fermentative deviation as well as reduction of losses is possible if there is a full understanding of the complex mechanisms involved in this process.

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# Chapter 7
# Use of Virginiamycin in Cattle Feeding

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## Introduction

Virginiamycin is a growth promoter, antibiotic, non-ionophore additive produced by a specific strain of *Streptomyces virginiae*, isolated in Belgium in 1954 (De Somer and Van Dijck 1955). The antimicrobial action of virginiamycin is due to a natural combination of two components, factors M and S that together are primarily active against Gram-positive bacteria.

Since its finding, virginiamycin has been utilized successfully in production systems of bovines, swine and poultry in most varied environments, management and feeding conditions. Virginiamycin can be used routinely within each production system without withdrawal periods.

In the past decades, several researchers have shown the effective action of virginiamycin to inhibit the growth of certain types of bacteria in the digestive system, including the rumen. When provided appropriately and ingested according to recommended levels, virginiamycin becomes an important tool to increase average daily gain and improve feeding efficiency of growing and finishing cattle; regardless of production system, pasture or feedlot; to reduce prevalence and incidence of subclinical acidosis and hepatic abscesses in cattle experiencing feeding challenge; besides increasing the production of milk and its components, especially fat and protein, in lactating cows.

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## Origin and Mode of Action

The bacterial genus *Streptomyces* is predominantly found in soil and has the ability to produce different antibiotics and other natural products with broad applications in the pharmaceutical and agrochemical industry. More than 500 *Streptomyces* species have been described (Kämpfer 2006). Nowadays, *Streptomyces* represents the most important genus of antimicrobial-producing bacteria, including antibacterial, antifungal, anti-parasite products and a broad group of bioactive compounds, including immune suppressors (Watve et al. 2001). In 1954, De Somer and Van Dijck isolated virginiamycin from a culture of a specific strain of *Streptomyces virginiae* (De Somer and Van Dijck 1955; Fig. 7.1). Virginiamycin was initially called "Antibiotic 899" or "Staphilomycin".

Like every streptogramin, virginiamycin is a natural mixture of two distinct chemical compounds, a peptolid, with molecular weight 525 (factor $M_1$; $C_{28}H_{35}N_3O_7$) and a macrocyclic lactone, with molecular weight 823 (factor $S_1$; $C_{43}H_{49}N_7O_{10}$; Cocito 1979; Fig. 7.2).

Virginiamycin antibacterial activity depends on the synergistic interaction of the two compounds, factors $M_1$ and $S_1$. Each factor individually is active against a series of Gram-positive microorganisms, but with bacteriostatic action. Once the virginiamycin factors are combined, in the correct proportion, the antibacterial activity is potentialized, and it becomes bactericidal (Van Dijck et al. 1957; Vanderhaeghe and Parmentier 1960).

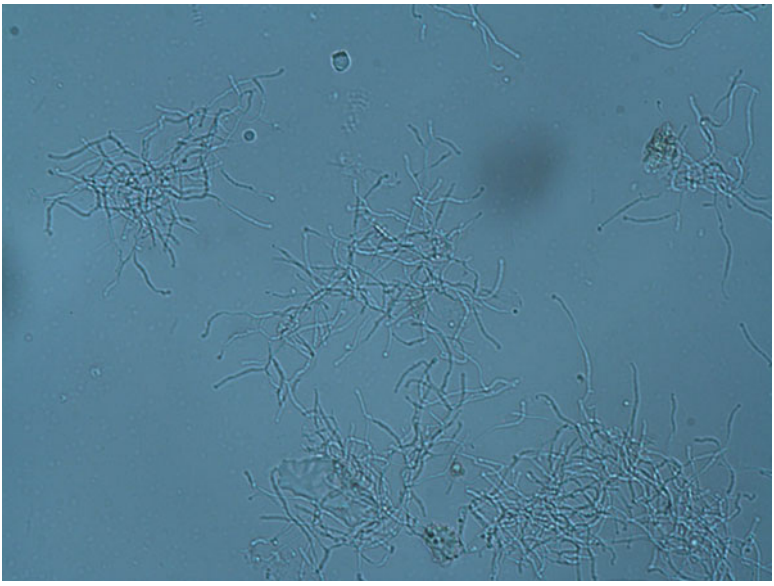

**Fig. 7.1** *Streptomyces* spp culture by Phibro Animal Health Corporation

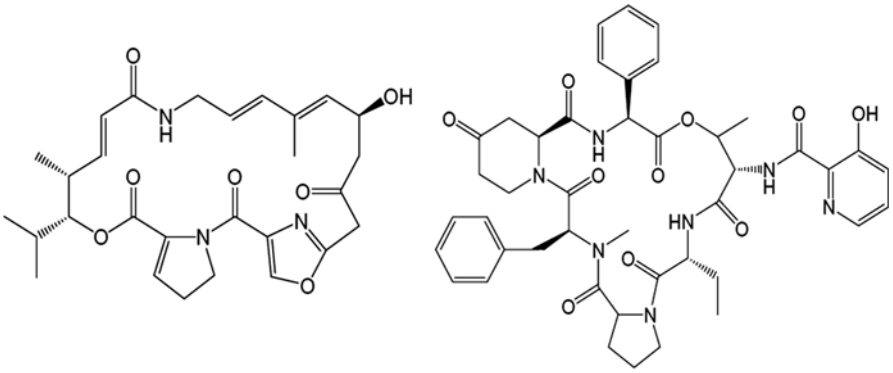


**Fig. 7.2** Chemical structure of virginiamycin factors $M_1$ and $S_1$. Adapted from Gottschall et al. (1987)

**Table 7.1** Antimicrobial activity of virginiamycin and its components against *Bacillus subtilis*

| Factors | Minimum inhibitory concentration[a] (μg/mL) |
|---|---|
| M | 0.5 |
| S | 0.4 |
| M + S | 0.04 |

[a]Minimum inhibitory concentration for virginiamycin against *Bacillus subtilis*

Adapted from Van Dijck (1969)

Van Dijck (1969) demonstrated the synergistic action of both virginiamycin compounds. Minimum inhibitory concentrations for *Bacillus subtilis* are 0.5 and 0.4 μg/ml for factors M and S, respectively (Table 7.1). When both factors are combined, the minimum inhibitory concentration for *B. subtilis* becomes 0.04 μg/ml, which demonstrates that the synergistic activity of both compounds together is ten times higher than for each of the compounds separately.

Virginiamycin penetrates through the cell wall of Gram-positive bacteria linking itself to ribosomal subunits in the cytoplasm and, consequently, inhibiting the formation of peptide bonds during protein synthesis (Cocito 1979; Cocito and Chinali 1985; Di Giambattista et al. 1989). Metabolic processes are interrupted in the microorganism, resulting in the inhibition of multiplication and eventually cellular death.

During the translation phase, the message contained in messenger RNA is decoded and a protein is formed. For the protein synthesis to complete, besides the correct and efficient reading of messenger RNA, it is necessary that portion 50S as well as 30S of ribosomes connect to each other, forming ribosome 70S. In cytoplasm, virginiamycin factor M binds itself to the portion 50S of the ribosome, causing alterations in its conformation, which prevents transporter RNA from binding to

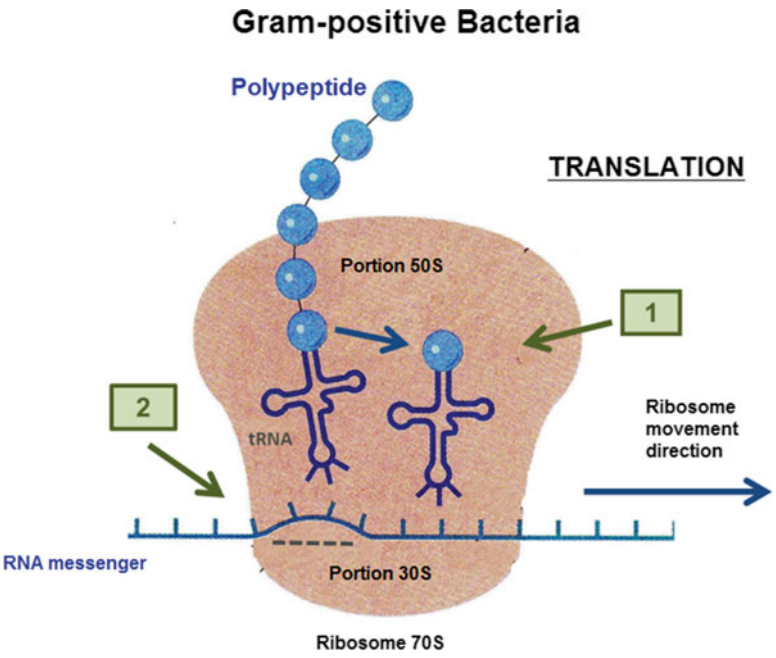


**Fig. 7.3** Translation phase of protein synthesis in Gram-positive bacteria. Adapted from Cocito and Chinali (1985)

messenger RNA/ribosome complex, ending the reading of the genetic code (mechanism 1; Fig. 7.3). Once linked to portion 50S, factor M potentiates the action of factor S which prevents elongation of the peptide chains when binding to the ribosome, causing the release of incomplete peptides (mechanism 2; Fig. 7.3). In the presence of both factors, virginiamycin effects on bacterial cell are irreversible.

Another very unique characteristic of streptogramins, very well described for virginiamycin, is the phenomenon called bacteriopause (Parfait et al. 1981; Chinali et al. 1981), which refers to the irreversible action of virginiamycin in the presence of both factors M and S. Cocito et al. (1979) demonstrated the action of virginiamycin on in vitro bacterial growth across time in the presence and absence of the molecule. Cocito et al. (1979) demonstrated in his experiment (Fig. 7.4.) that null bacterial growth or protein synthesis across time was observed on treatment D (blank; no bacteria, no inhibitors), treatment C (the bacterial culture was in contact with virginiamycin throughout the study) and treatment B (virginiamycin withdrawal at time 0). In the same Fig. 7.4., intense protein synthesis is detected in treatment A (control; with bacteria and without antibiotics). Moreover, treatment B maintained the same response and bacterial growth intensity as treatment C. In this case, demonstrating that the inhibitory effect on protein synthesis remains the same after withdrawal of the virginiamycin. Once the protein synthesis is interrupted irreversibly, the virginiamycin effect remains the same after withdrawal from the culture media.

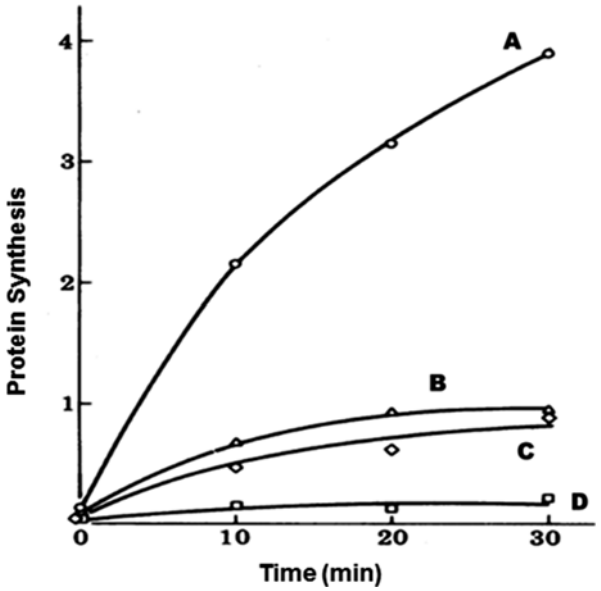


**Fig. 7.4** Effect of virginiamycin on the growth of bacteria. Adapted from Cocito et al. (1979). *A* = control treatment; with bacteria, without virginiamycin *B* = treatment with virginiamycin withdrawal at time 0 *C* = continuous treatment with virginiamycin *D* = blank, without bacterial culture and without virginiamycin

## Antimicrobial Activity

In general, virginiamycin is active against Gram-positive bacteria (Table 7.2) with some cocci as exception; virginiamycin is not active against Gram-negative bacteria (Table 7.3) because virginiamycin cannot penetrate the bacterial external membrane, which makes molecule passage to the cytoplasm impossible. Eukaryotes, e.g., fungi, yeasts, algae, plants are not sensitive to virginiamycin.

In monogastric animals, several studies evaluated the potential of virginiamycin on in vitro and in vivo bacterial populations. The results of these studies suggested that one of primary effects of VM is balancing the gastro-intestinal flora in such a way that less nutrients are degraded/used by flora itself and there is an increased availability for the animal. In in vitro studies, carried out with ileum content of growing piglets, Dierick et al. (1981) indicated that virginiamycin inhibits urea and acid decarboxylation, sparing the amount of available essential amino acids and reducing the formation of ammonia and other amines.

Lindsey (1985) reported that incubation of chicken intestinal content with virginmiamycin reduced bacterial metabolism of glucose, transformed into lactate, and thus increasing the amount of available metabolizable energy for animals. Also, in another study, Henderickx et al. (1981) demonstrated that virginiamycin increases the growth rate in monogastric animals through changes in metabolic activities of

**Table 7.2** Minimum inhibitory concentration (MIC) of virginiamycin against Gram-positive bacteria. Adapted from Van Dijck (1969); Hedde et al. (1982); Lechtenberg (1988)

| Microorganism | MIC (μg/mL) |
|---|---|
| *Actinomyces* spp. | 0.10 |
| *Bacillus subtilis* NCTC 8226 | 0.04 |
| *Brucella abortus* | 75.00 |
| *Clostridium bifermentans* NCTC 2914 | 0.25 |
| *Clostridium fallax* NCTC 8380 | 5.00 |
| *Clostridium hystolyticum* NCTC 503 | 0.12 |
| *Clostridium oedematiens* NCTC 538 | 1.00 |
| *Clostridium septicum* NCTC 547 | 0.12 |
| *Clostridium sporogenes* | 0.25 |
| *Clostridium tertium* NCTC 541 | 0.20 |
| *Clostridium tetani* NCTC 279 | 0.05 |
| *Clostridium tetani* NCTC 5404 | 0.50 |
| *Clostridium welchii* NCTC 8081 | 0.50 |
| *Clostridium welchii* NCTC 8359 | 0.50 |
| *Corynebacterium pseudotuberculosis* | 0.04 |
| *Corynebacterium xerosis* | 0.03 |
| *Lactobacillus acidophilus* ATCC 4962 | 0.50 |
| *Lactobacillus casei* ATCC 7469 | 0.50 |
| *Lactobacillus fermenti* ATCC 9338 | 0.12 |
| *Lactobacillus leichmannii* ATCC 7830 | 0.30 |
| *Lactobacillus plantarum* ATCC 8014 | 0.70 |
| *Listeria monocytogenes* ATCC 984 | 1.50 |
| *Mycobacterium phlei* | 2.00 |
| *Mycobacterium smegmatis* | 7.00 |
| *Mycobacterium tuberculosis* $H_{37}Rv$ | 1.00 |
| *Mycoplasma arthritidis* PG6 | 3.00 |
| *Mycoplasma fermentans* V58N | 2.00 |
| *Mycoplasma gallisepticum* S6 | 0.05 |
| *Mycoplasma hominis* IV37NN | 3.50 |
| *Nocardia asterioides* NCTC 6761 | >100.00 |
| *Sarcina lutea* (*Kocuria rhizophilia*) | 0.03 |
| *Staphylococcus aureus* ATCC 6538P | 0.20 |
| *Staphylococcus* spp. | 0.80 |
| *Streptococcus pneumoniae* | 0.07 |
| *Streptococcus pyogenes* ATCC 8668 | 0.07 |
| *Streptococcus pyogenes* | 0.06 |
| *Streptococcus viridans* NCTC 779 | 15.00 |

the intestinal microflora, resulting in increase of protein and metabolizable energy levels, and altering intestinal mucosa permeability in order to stimulate local absorption of nutrients.

In ruminants, any effect that virginiamycin may have on ruminal fermentation is related to changes in populations of bacterial and protozoan species that inhabit the rumen. Nagaraja and Taylor (1987) demonstrated the susceptibility and resistance

**Table 7.3** Minimum inhibitory concentration (MIC) of virginiamycin against Gram-Negative bacteria. Adapted from Van Dijck (1969); Hedde et al. (1982); Lechtenberg (1988)

| Microorganism | MIC (μg/mL) |
|---|---|
| *Escherichia coli* ATCC 9661 | >100.00 |
| *Escherichia coli* W | 75.00 |
| *Escherichia coli* 110-37 | 50.00 |
| *Escherichia coli* 637 | >100.00 |
| *Fusobacterium necrophorum* | 1.00 |
| *Haemophilus aegyptius* NCTC 8502 | 0.05 |
| *Haemophilus influenzae* NCTC 4560 | 0.25 |
| *Haemophilus influenzae* (27 clinical isolates) | 0.85 |
| *Haemophilus parapertussis* NCTC 5952 | 0.25 |
| *Haemophilus pertussis* NCTC 8189 | 0.40 |
| *Haemophylus pertussis* clinical isolates | 0.25 |
| *Klebsiella edwardii* NCTC 7242 | 50.00 |
| *Moraxella lacunota* NCTC 7784 | 0.05 |
| *Neisseria catarrhalis* NCTC 3622 | 0.30 |
| *Neisseria gonorrhoeae* | 0.12 |
| *Neisseria meningitides* NCTC 8339 | 0.10 |
| *Neisseria meningitides* (4) | 0.40 |
| *Pasteurella pestis* | 3.00 |
| *Pasteurella pseudotuberculosis* | 15.00 |
| *Pasteurella* spp. (4) | 1.30 |
| *Proteus mirabilis* | >100.00 |
| *Pseudomonas fluorescens* | 15.00 |
| *Shigella flexneri* | >100.00 |

of ruminal bacteria cultivated in vitro in the presence of several additives, including virginiamycin (Table 7.4). In general, microorganisms that produce lactic acid, butyric acid, formic acid and hydrogen are susceptible to virginiamycin, and bacteria that produce succinic acid or ferment lactic acid are resistant. Virginiamycin has shown to be very efficient in the inhibition of lactic-acid producing microorganisms (e.g., *Lactobacillus* and *Streptococcus*) without interfering in the growth of lactic-acid consuming micro-organisms like *Megasphaera elsdenii*.

*Fusobacterium necrophorum* is an important etiological agent of hepatic abscesses in cattle (Kanoe et al. 1976; Berg and Scanlan 1982; Scanlan and Hathcock 1983; Lechtenberg et al. 1988). The damage caused to the liver results in losses due to the reduction of daily weight gain and condemnation of beef cuts and offal (Foster et al. 1970). Virginiamycin is efficient in the control of this pathogen (Lechtenberg et al. 1988).

## *Action on Organic Acid Production*

The action of virginiamycin on the production of short-chain fatty acids is well described in several in vitro (Nagaraja et al. 1987; Clayton et al. 1999) and in vivo (Coe et al. 1999; Hill et al. 2002) studies in beef as well as in dairy cattle.

**Table 7.4** Susceptibility of ruminal bacteria to virginiamycin (MIC). Adapted from Nagaraja and Taylor (1987)

| Microorganism | MIC (μg/mL) |
|---|---|
| Main lactic acid-producing bacteria | |
| *Bifidobacterium boum* | 0.38 |
| *Bifidobacterium globosum* | 0.75 |
| *Eubacterium cellulosolvens* | 1.50 |
| *Eubacterium ruminantium* | 1.50 |
| *Lachnospira multiparis* | 0.75 |
| *Lactobacillus ruminis* | 1.50 |
| *Lactobacillus vitulinus* | 1.50 |
| *Selenomonas ruminantium* D | –[a] |
| *Selenomonas ruminantium* HD1 | - |
| *Streptococcus bovis* 7H4 | 3.00 |
| *Streptococcus bovis* JB1 | 0.75 |
| *Main butyric acid-producing bacteria* | |
| *Butyrivibrio fibrosolvens* | 6.00 |
| *Eubacterium cellulosolvens* | 1.50 |
| *Eubacterium ruminantium* | 1.50 |
| *Megasphaera elsdenii* | – |
| *Selenomonas ruminantium* B385 | 1.50 |
| Main formic acid-producing bacteria | |
| *Bacteroides ruminicola* | – |
| *Bacteroides succinogenes* | – |
| *Eubacterium ruminantium* | 1.50 |
| *Lachnospira multiparis* | 0.75 |
| *Ruminococcus albus* | 0.38 |
| *Ruminococcus flavefaciens* | 0.75 |
| *Treponema bryantii* | 12.00 |
| Main hydrogen-producing bacteria | |
| *Lachnospira multiparis* | 0.75 |
| *Megasphaera elsdenii* | – |
| *Ruminococcus albus* | 0.38 |
| *Ruminococcus flavefaciens* | 0.75 |
| *Selenomonas ruminantium* D | – |

[a]indifferent, the highest concentration of antibiotic tested was 48 μg/mL

When ruminants are fed with high-energy diets, the primary effect of virginiamycin is on the concentration of lactic acid in the rumen. Sugar-rich and starch-rich diets as well as those grain-based or vegetal-based ones contain a great amount of highly fermentable carbohydrates in the rumen. Under these conditions, bacteria like *Streptococcus bovis* grow rapidly, causing a great production of organic acids, mainly d-lactate, and consequently reducing ruminal pH. With pH reduction, proliferation of several bacterial Lactobacillus species is triggered. *Lactobacillus* is highly efficient for lactic acid production, which reduces ruminal pH even more,

making the rumen more acid. Therefore, a quick introduction of highly fermentable feeds can result in acidosis caused by the excess of organic acid production in the rumen and subsequent reduction of pH (Britton et al. 1986). Likewise, sudden alteration of concentrate levels in the diet can cause rumenitis predisposing animals to other problems like laminitis and hepatic abscesses throughout the feeding period (Brent 1976).

At normal pH levels, ruminal bacteria are capable of converting lactic acid into utilizable energy as short-chain fatty acids. When pH goes lower than 5.8, this activity is inhibited and the lactic acid accumulates. Lactic acid accumulation in the rumen and consequent pH reduction decreases feed intake by the animal, which results in productive performance losses of milk or beef and poorer feeding efficiency (Slyter 1976).

Virginiamycin is active against *Streptococcus bovis* and *Lactobacillus ruminis*, preventing the development of harmful levels of lactic acid in the rumen (Muir and Barreto 1979; Dutta and Devriese 1981; Nagaraja and Taylor 1987). Boselli et al. (1993) evaluated the effect of virginiamycin in vivo on pH and concentration of ruminal lactic acid on 500 kg LW heifers. These animals were supplemented with 0, 75, 150, or 250 mg of virginiamycin daily and were equipped with fistula for ruminal fluid collection at 0, 2, and 4 h after feeding. Animals were fed with high starch diet (barley basis) to create an acidosis challenge. In this study, virginiamycin supplementation increased ruminal pH and intake significantly in response to the reduction of lactic acid concentration (Tables 7.5 and 7.6, respectively).

**Table 7.5** Effect of different doses of virginiamycin on ruminal pH. Adapted from Boselli et al. (Boselli et al. 1993)

| Doses | Hours after feeding | | | |
|---|---|---|---|---|
| | | 2 | 4 | Average |
| 0 | 6.97 $^{a, x}$ | 5.83 $^{b, x}$ | 5.59 $^{b, x}$ | 6.13 $^{x}$ |
| 75 | 6.96 $^{a, x}$ | 5.90 $^{b, x}$ | 5.63 $^{b, xy}$ | 6.17 $^{xy}$ |
| 150 | 6.86 $^{a, x}$ | 6.22 $^{b, y}$ | 5.88 $^{b, yz}$ | 6.32 $^{yz}$ |
| 250 | 7.03 $^{a, x}$ | 6.37 $^{b, y}$ | 6.01 $^{b, z}$ | 6.47 $^{z}$ |

$^{a,b}$ Means with different superscripts on the same rows, differ ($P<0.05$); $^{x,y,z}$ Means with different superscripts on the column, differ ($P<0.01$)

**Table 7.6** Effect of different doses of virginiamycin doses on lactic acid concentration (mg/100 ml). Adapted from Boselli et al. (Boselli et al. 1993)

| Doses | Hours after feeding | | | |
|---|---|---|---|---|
| | | 2 | 4 | Average |
| 0 | 124.40 $^{b, z}$ | 260.73 $^{a, z}$ | 147.34 $^{b, y}$ | 177.49 $^{c, z}$ |
| 75 | 55.22 $^{b, y}$ | 107.15 $^{a, y}$ | 45.10 $^{b, x}$ | 69.16 $^{b, y}$ |
| 150 | 10.99 $^{b, x}$ | 63.92 $^{a, x}$ | 21.21 $^{b, x}$ | 32.04 $^{a, x}$ |
| 250 | 22.37 $^{b, xy}$ | 69.67 $^{a, x}$ | 31.73 $^{b, x}$ | 41.22 $^{a, x}$ |

$^{a,b,c}$ Means with distinct superscripts on the same row, differ ($P<0.05$); $^{x,y,z}$ Means with distinct superscripts on the same column, differ ($P<0.01$)

**Table 7.7** Effect of virginiamycin on ruminal parameters of in vivo and in vitro experiments

| Reference | Bovine/Method | Ruminal Parameters[1] | | | | |
|---|---|---|---|---|---|---|
| | | Acetate | Propionate | Butyrate | Lactate | pH |
| Hedde et al. (1980) | Beef; in vitro | … | (+) | (−) | (−) | (+) |
| Hedde et al. (1980) | Beef; in vivo | … | (+) | … | (−) | (+) |
| Van Nevel et al. (1984) | Beef; in vitro | (−) | (+) | (=) | (−) | … |
| Nagaraja et al. (1987) | Beef; in vitro | (−) | (+) | (−) | (−) | (+) |
| Clayton et al. (1999) | Dairy; in vitro | (=) | (=) | … | (−) | (+) |
| Hill et al. (2002) | Dairy; in vivo | (+) | (=) | … | (−) | (+) |

[1] (+) Increase; (−) Reduction; (=) No alteration; … Non-characterized results

**Table 7.8** Effect of virginiamycin on ruminal populations of *Streptococcus bovis*, *Lactobacillus* spp. and *Fusobacterium necrophorum* during the adaptation period to high-concentrate diet. Adapted from Coe et al. (1999)

| Forage: concentrate ratio | 100/00 | 30/70 | 15/85 | 0/100 |
|---|---|---|---|---|
| | (d −2 to 1) | (d 2 to 4) | (d 5 to 7) | (d 8 to 10) |
| Control | | | | |
| *S. bovis* ($10^6$ cfu [a]/g DM[a]) | 19.5 | 136.6 | 45.6 | 29.9 |
| *Lactobacillus* ($10^7$ cfu /g MS) | 4 | 100.30[b] | 491.80[b] | 542.40[b] |
| *F. necrophorum* ($10^6$ MPN[a]/g DM) | 0.9 | 6.60[b] | 17.80[b] | 12.70[b] |
| Virginiamycin (175 mg/day) | | | | |
| *S. bovis* ($10^6$ cfu /g DM) | 8.1 | 9.2 | 5.7 | 2.4 |
| *Lactobacillus* ($10^7$ cfu /g DM) | 8.3 | 7.30[c] | 75.30[c] | 93.40[c] |
| *F. Necrophorum* ($10^6$ MPN/g DM) | 0.8 | 0.80[c] | 1.00[c] | 0.30[c] |

[a] *cfu*: colony forming units, *DM*: dry matter, *MPN*: most probable number. [b, c] Means on the same column for each variable followed by distinct superscripts differ among themselves ($P<0.05$)

Besides reducing lactic acid concentration in the rumen, virginiamycin supplementation positively changes the production of other organic acids, especially propionic acid in beef cattle (Hedde et al. 1980; Van Nevel et al. 1984). Hill et al. (2002) observed an increase in acetic acid production when virginiamycin was added to the feeding of lactating dairy cows fed with corn silage-based diet (Table 7.7).

Coe et al. (1999) evaluated the effect of virginiamycin supplementation on fermentation patterns and ruminal microbiota of beef cattle submitted to fast adaptation to high-grain diet (Table 7.8). Animals that were supplemented with virginiamycin during the transition period, which changed from 100 % forage to 100 % concentrate in 8 days, were able to keep populations of *Lactobacillus* spp., *Streptococcus bovis* and *Fusobacterium necrophorum* 5 and 40 times smaller, respectively.

Reduction of population of these microorganisms decreased the acidosis risk in animals during the experimental period as the lactate concentration in the rumen also decreased, and also reduced the prevalence and incidence of liver abscesses due

**Table 7.9** Effect of virginiamycin supplementation on average ruminal pH and percentage of herds with subacute acidosis. Adapted from Salgado and Gomez (2006)

| Treatment | Average Ruminal pH | % Herds With acidosis |
|---|---|---|
| Virginiamycin | 6.18 ± 0.010[a] | 0.00%[a] |
| Control | 5.77 ± 0.012[b] | 67.00%[b] |

[a, b] Means with different superscripts in the same column, differ ($P < 0.05$)

to the control of the main etiologic agent of this disorder, *F. necrophorum*. Virginiamycin is also registered as an additive to prevent liver abscesses in feedlot cattle in the USA and Australia.

In dairy cattle's diets, virginiamycin has also shown to be an excellent inhibitor of lactic acid production in the rumen, resulting in the increase of ruminal pH without compromising the production of other short-chain fatty acids (Clayton et al. 1999; Hill et al. 2002). Salgado and Gomez (2006) evaluated the virginiamycin effect on ruminal pH and subacute acidosis incidence in dairy cows. That experiment utilized 1861 animals from several distinct herds, and collected ruminal liquid through ruminocentesis after four hours of feeding for immediate analysis of pH. Moreover, the authors considered herds whose more than 20 % of animals presented pH lower than 5.6 as herd with acidosis (Table 7.9). The herds that were supplemented with virginiamycin presented higher ruminal pH, over 6.0, four hours after feeding, reducing the percentage of herd with acidosis to zero.

## Performance Results

### *Beef Cattle*

In general, the inclusion of virginiamycin in finishing diets of feedlot cattle modulates rumen pH (Godfrey et al. 1992) reducing the risk of lactic acidosis (Rogers et al. 1995). Moreover, virginiamycin addition has been shown to increase ruminal propionate concentration (Coe et al. 1999) improving dietary energy utilization (Salinas Chavira et al. 2009). When virginiamycin is included in feedlot cattle rations, average daily gain (ADG) is improved on average by 4–8 %, feed conversion (feed to gain, F:G) is reduced by 5–10 % and liver abscess (LA) incidence and severity is significantly reduced when compared to negative control diets.

Rogers et al. (1995) evaluated different virginiamycin doses in feedlot cattle trials. Pooled analyses of four studies providing virginiamycin at 11.0, 19.3, and 27.6 mg/kg of dry matter (DM) in the complete diet indicated that growth and feed conversion were linearly improved ($P < 0.05$; Figs. 7.5 and 7.6; respectively). Overall the incidence and severity of LA were reduced ($P < 0.01$) by feeding virginiamycin at either 19.3 or 27.6 mg/kg. The estimated effective dose for reducing LA prevalence was established in the 16.5–19.3 mg/kg range.

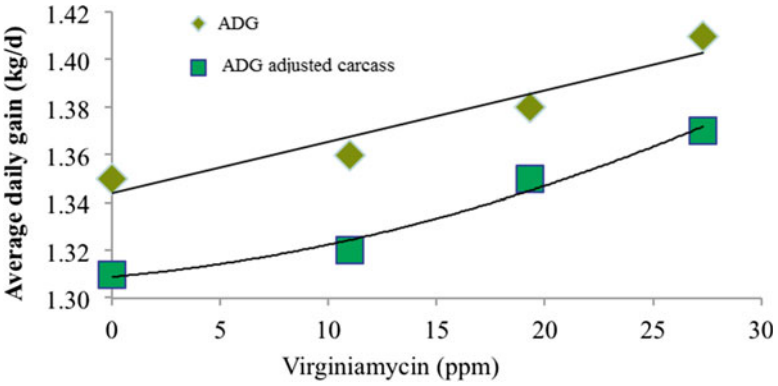


**Fig. 7.5** Average daily gain (ADG) and average daily gain adjusted for carcass (ADG adjusted carcass) of cattle fed finishing diets containing different doses of virginiamycin (0, 11.0, 19.3 and 27.6 ppm). Adapted from Rogers et al. (1995)

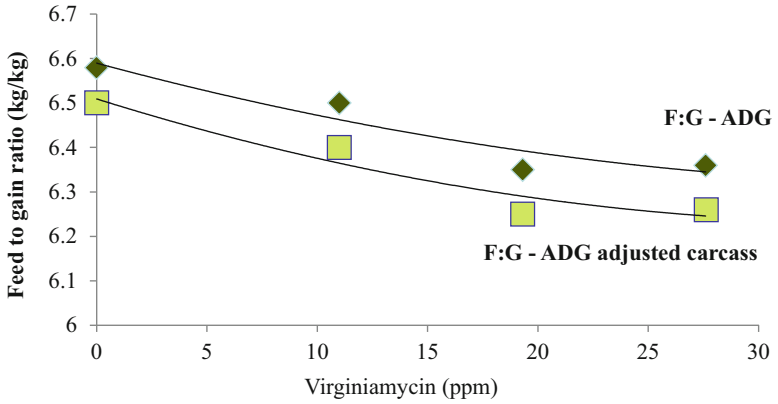


**Fig. 7.6** Feed to gain ratio based on average daily gain (F:G–ADG) and feed to gain ratio calculated according to average daily gain adjusted for carcass (F:G–ADG adjusted carcass) of cattle fed finishing diets containing different doses of virginiamycin (0, 11.0, 19.3 and 27.6 ppm). Adapted from Rogers et al. (1995)

## *Use in Combination with Ionophores for Beef Cattle*

Virginiamycin is considered to improve feed efficiency and the rate of growth in cattle through modulation of the rumen environment, potentially improving nutrient digestion and absorption. Ionophores in general are considered to improve feed efficiency in cattle through modulation of the rumen environment and through modulation of feed intake. Thus, feeding virginiamycin in combination with ionophores could lead to additive improvements in feed efficiency due to their complimentary modes of action.

Sodium monensin is commonly utilized in beef cattle diets for growth promotion or for coccidiosis control. In some countries, including Brazil, the combined use of virginiamycin and an ionophore is allowed if the latter is included for coccidiosis control. Recent trials conducted in several countries and under different management conditions have reported performance improvements (weight gain rate, feed efficiency) when virginiamycin and monensin are used in combination compared to when monensin is used alone (Sitta et al. 2011; Gorocica et al. 2014; Kawas et al. 2015). Similarly, Nunez et al. (2008) observed that the combination of virginiamycin and salinomycin, another ionophore utilized in feeding of beef cattle in Brazil, resulted in better animal performance when compared to animals fed only salinomycin. Table 7.10 summarizes field studies on the effect of feeding virginiamycin in combination with monensin on feedlot performance.

Ives et al (2002) evaluated diets with or without wet corn gluten feed containing no antibiotics, 17.5 ppm of virginiamycin or 25 ppm of monensin + 10 ppm of tylosin.

**Table 7.10** Effects of virginiamycin supplementation when fed in combination with monensin or alone on performance variables of finishing cattle

| Trial | Breed | Initial weight, kg | Days on feed | Treatment[1] | DMI[2] | ADG[2] | HCW[4] | F:G[5] |
|---|---|---|---|---|---|---|---|---|
| Phibro Tech. Sv. (Canada), (2013) | Continental x Brittish crossbreds | 442.8 | 134 | MN (33 ppm MN + 11 ppm TY) | 9.4 | 1.38 | 372 | 6.76 |
| | | | | VMMN (33 ppm MN + 25 ppm VM) | 9.7 | 1.53 | 384 | 6.34 |
| Sitta et al. (2011) | Nellore | 330.0 | 102 | MN, 30 ppm | 9.2 | 1.33 | 255 | 6.9 |
| | | | | VMMN, 15 ppm VM + 30 ppm MN | 8.98 | 1.44 | 258 | 6.21 |
| Benatti et al. (2013) | Nellore | 348.3 | 110 | VM, 25 ppm | 10.11 | 1.36 | 286 | 7.45 |
| | | | | VMMN, 25 ppm VM + 30 ppm MN | 9.42 | 1.37 | 286 | 6.88 |
| Gorocica et al. (2014) | Zebu x continental crossbreds | 267.7 | 130 | MN (400 mg/hd/day) | 9.44 | 1.67 | 301 | 5.65 |
| | | | | VMMN (200 mg VM + 400 mg MN/hd/day) | 9.38 | 1.74 | 306 | 5.38 |
| Kawas et al. (2015) | Zebu x continental crossbreds | 276 | 100 | MN (400 mg/hd/day MN) | 7.94 | 1.32 | 247 | 6.03 |
| | | | | VMMN (250 mg VM + 300 mg MN/hd/day) | 7.86 | 1.4 | 252 | 5.63 |
| Lizarraga et al. (2015) | Continental x Brittish crossbreds | 197 | 80 | MN (180 mg/hd/day MN) | 9.32 | 1.08 | 173 | 8.63 |
| | | | | VMMN (200 mg VM + 180 mg MN/hd/day) | 8.59 | 1.18 | 178 | 7.28 |

[1]Treatment; *MN*: monensin; *VM*: virginiamycin; *TY*: tylosin. [2]*DMI*: dry matter intake; [3]*ADG*: average daily gain; [4] *HCW*: Hot carcass weight; [5]*F:G*: feed to gain ratio

In diets with or without wet corn gluten feed, animals receiving virginiamycin increased propionate concentration in the rumen when compared to animals that did not receive additives or were supplemented with monensin + tylosin.

## *Grazing Cattle*

Virginiamycin modulates the rumen environment altering the fermentation pattern so that more nutrients become available for digestion in the small intestine (Kook et al. 1999; Van Nevel et al. 1984; Van Nevel and Demeyer 1992; Ives et al. 2002; Salinas Chavira et al. 2009). The virginiamycin-modulated rumen environment is conductive to improvements in cattle productivity under diverse feeding and management conditions, including pasture-based systems.

When fed to grazing cattle, virginiamycin has been shown to increase weight gain rate in periods with abundant forage offering (rainfall season) as well as in drought periods, when forage availability is compromised quantitatively and qualitatively (Valle et al. 2013; Goulart 2010; Justiniano et al. 2014).

Ionophores are commonly used in grazing animals to control coccidiosis and improve weight gain rate (Hersom and Thrift 2015). However, virginiamycin appears to be more effective improving growth rate in pasture based cattle than ionophores. Ferreira et al. (2012) compared virginiamycin and salinomycin vs. only-mineral supplemented Nellore bulls during the rainy season. The virginiamycin supplemented group had 25 % greater ADG ($P < 0.05$) than the control group and 9.3 % numerically greater ADG ($P > 0.05$) than the salinomycin group. Similar results were reported by Goulart (2010). This author conducted two experiments comparing virginiamycin vs. salinomycin and a negative control in different locations and time periods. In study 1 (conducted at Sao Paolo State, from January to May), the virginiamycin group had greater ($P < 0.05$) ADG than the control, while for the salinomycin group ADG was intermediate and not statistically different from the other treatments. In study 2 (conducted at Mato Grosso do Sul Federal University, from May to August) virginiamycin led to greater ($P < 0.05$) ADG than salinomycin, and tended ($P = 0.09$) to be greater than the Control group. These trials are summarized on Table 7.11.

**Table 7.11** Average daily gain of Nellore bulls rotationally grazed and supplemented with minerals with our without antimicrobials in Brazil

| Trial | Control | SM[1] | VM[2] | VM vs. control | VM vs. SM |
|---|---|---|---|---|---|
| Ferreira et al. (2011) | 0.513[a] | 0.589[ab] | 0.644[b] | 26 % | 9 % |
| Goulart, 2010 (study 1) | 0.580[a] | 0.620[ab] | 0.675[b] | 16 % | 9 % |
| Goulart, 2010 (study 2) | 0.487[ab] | 0.478[a] | 0.518[a] | 6 % | 11 % |

[1]Salinomycin. Dosage used: In study 1, 0.23 mg/kg BW; In study 2 and 3, 0.30 mg/kg BW
[2]Virginiamycin. Dosage used: In study 1, 0.24 mg/kg BW; In study 2 and 3, 0.30 mg/kg BW
[a, b] Means with a common superscript within the same row are not statistically different ($P > 0.05$)

On average, virginiamycin increased ADG by ≈ 100 g/day when compared to a negative control and by ≈ 50 g/day when compared to ionophore-supplemented minerals.

The inclusion of virginiamycin in supplements for grazing cattle has been studied under different seasons (dry and rainy), supplementation strategies (mineral only; mineral and protein; energy and protein), duration of supplementation (2–5 months), etc. A summary of 12 trials is presented in Table 7.12. Basic information is included to provide appreciation on the variety of conditions under which virginiamycin response has been evaluated. On average, cattle supplemented with virginiamycin gained ≈ 103 g/day more than control animals.

**Table 7.12** Summary of trials conducted in Brazil to evaluate the effect of virginiamycin supplementation of grazing cattle on average daily gain (ADG)

| Trial[1] | ADG control, kg/d | ADG VM, kg/d | Dose, mg/kg BW | Dif. VM vs. Control, % | Season[2] | N | *P* value | Initial BW | D[3] |
|---|---|---|---|---|---|---|---|---|---|
| Valle et al. (2013) | 1.000 | 1.200 | 0.53 | 20 % | R | 50 | <0.05 | 330 | 70 |
| Ferreira et al. (2012) (Exp.1) | 0.398 | 0.391 | 0.31 | −2 % | D | 30 | >0.05 | *350* | *NA* |
| Ferreira et al. (2012) (Exp. 2) | 0.398 | 0.431 | 0.62 | 8 % | D | 30 | >0.05 | *350* | *NA* |
| Florez et al. (2014) | 0.280 | 0.390 | 0.56 | 39 % | D | 24 | >0.05 | 233 | 70 |
| Goulart (2010) | 0.643 | 0.709 | 0.30 | 10 % | R | 309 | 0.02 | 236 | 133 |
| Bruning (2013) (Exp. 1) | 0.302 | 0.377 | 0.45 | 25 % | T | 32 | >0.05 | 223 | 99 |
| Siqueira et al. (2014) (Exp. 1) | 0.711 | 0.768 | 0.40 | 8 % | R | 40 | 0.16 | 205 | 75 |
| Costa et al. (2013) | 0.859 | 0.978 | 0.40 | 14 % | R | 80 | <0.01 | 253 | 127 |
| Bruning (2013) (Exp. 2) | 0.336 | 0.427 | 0.44 | 27 % | T | 32 | <0.05 | 225 | 99 |
| Justiniano et al. (2014) | 0.103 | 0.377 | 0.45 | 266 % | D | 24 | <0.05 | 250 | 101 |
| (Arrigoni et al. (2010)) | 0.407 | 0.571 | 0.38 | 40 % | T | 75 | <0.05 | 160 | 91 |
| Siqueira et al. (2014) (Exp. 2) | 0.807 | 0.866 | 0.40 | 7 % | R | 40 | 0.16 | 205 | 75 |

[1]List of References provided in the "References"

[2]Season = *D*, Dry; *T*, Transition dry–rainy; *R*, rainy

[3]*D* = Days of the trial; *NA* = Not available

## *Dairy Cattle*

When virginiamycin is included in diets of lactating dairy cows, fat-corrected milk increases on average 0.6 kg/day, and milk fat content increases by 0.15 % (Clayton et al. 1999; Chavarria-Solis 2008; Erasmus et al. 2008; Duran-Leyva 2009; Silva et al. 2013; Desantadina et al. 2015).

A research study was conducted to evaluate the effect of virginiamycin on acidosis and milk production under commercial conditions in Australia (Clayton et al. 1999). Since sodium bicarbonate ($NaHCO_3$) has been used to buffer rumen pH aiming to ameliorate rumen acidosis (Solorzano et al. 1989) it was hypothesized that combining $NaHCO_3$ and virginiamycin would result in greater rumen acidosis control due to their different modes of action. There was no evidence of any significant interaction between the $NaHCO_3$ and virginiamycin. Cows fed virginiamycin tended ($P=0.09$) to have a higher ruminal pH than did cows not fed virginiamycin. Virginiamycin treated cows had greater rumen and fecal pH even considering that ruminal pH was relatively high throughout the trial. Virginiamycin-treated cows tended ($P=0.09$) to produce more milk per day (0.62 L) and the effect was consistently observed throughout the trial. It was concluded that improved rumen health was associated with higher and more stable rumen pH leading to greater milk production.

In Brazil, Silva et al. (2013) conducted a study to determine the effect of virginiamycin and its interaction with rumen-protected fat (PF) on milk production and composition in cows supplemented and maintained on pasture. No interactions between virginiamycin and PF were detected ($P>0.40$) for milk production or composition. Four-percent corrected milk production was 0.7 L/day greater ($P<0.05$) in virginiamycin supplemented cows (Table 7.13). Results have also been reported in

**Table 7.13** Effects of adding Virginiamycin to lactating dairy cows' diets on protein and milk fat contents

| Reference | Without virginiamycin | | | | With virginiamycin | | | | Increment | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Protein | | Fat | | Protein | | Fat | | Protein | | Fat | |
| | % | kg | % | kg | % | kg | % | kg | kg/d | % | kg/d | % |
| Clayton et al. (1999) | 3.44 | 0.80 | 4.26 | 0.99 | 3.40 | 0.81 | 4.26 | 1.02 | 0.013 | 1.61 | 0.026 | 2.66 |
| Lean et al. (2000) | 3.00 | 0.64 | 4.68 | 1.00 | 3.24 | 0.67 | 4.93 | 1.03 | 0.035 | 5.46 | 0.029 | 2.87 |
| Valentine et al. (2000) | 3.06 | 0.84 | 3.45 | 0.95 | 3.04 | 0.85 | 3.41 | 0.95 | 0.007 | 0.81 | 0.003 | 0.32 |
| Erasmus et al. (2008) | 3.08 | 1.17 | 3.52 | 1.34 | 3.14 | 1.22 | 3.81 | 1.48 | 0.048 | 4.12 | 0.137 | 10.22 |
| Solis et al. (2013) | 3.19 | 0.83 | 3.28 | 0.85 | 3.30 | 0.86 | 3.63 | 0.94 | 0.028 | 3.35 | 0.090 | 10.58 |
| Silva et al. (2013) | 3.25 | 0.55 | 3.77 | 0.64 | 3.21 | 0.56 | 3.84 | 0.67 | 0.009 | 1.69 | 0.031 | 4.87 |
| Desantadina et al. (2015) | 3.21 | 0.89 | 3.34 | 0.92 | 3.24 | 0.92 | 3.46 | 0.98 | 0.031 | 3.49 | 0.057 | 6.22 |

In general, the addition of Virginiamycin to lactating dairy cows' diets increased protein and milk fat contents in 2.93 % and 5.39 %, respectively

totally mixed ration (TMR) fed cows (Duran-Leyva 2009). Multiparous Holstein cows were fed a TMR diet balanced to meet or exceed their requirements with either no antibiotic (CT group) or 300 mg/cow/day of virginiamycin (VM group). The inclusion of virginiamycin increased milk production ($P<0.01$).

Monensin is an ionophore widely used to increase milk yield in many countries including the USA, Argentina, Mexico, and Brazil. Feeding virginiamycin in combination with monensin may lead to additional benefits in the rumen and in the animal health and productivity due to their different and possibly complementary modes of action.

To investigate the potential interaction between monensin and virginiamycin, Erasmus et al. (2008) conducted a trial in the Republic of South Africa. Milk production from cows supplemented with either only monensin or only virginiamycin did not differ from control cows ($P>0.10$). However, cows in the virginiamycin and monensin treatment had the greatest milk production among treatments and it was different ($P<0.10$) from the virginiamycin or monensin alone treatments. Though not statistically different ($P>0.10$) compared to the control, cows in the virginiamycin and monensin treatment produced 2.3 kg/day of additional milk. The authors hypothesized that the combined positive effects of the two additives on stabilizing feed intake and rumen fermentation, together with a potential post-ruminal effect of virginiamycin, could have contributed to the observed responses in production.

In a study conducted in Costa Rica (Chavarria-Solis 2008), grazing Holstein cows were used to evaluate the effects of virginiamycin and monensin supplementation. Additives were fed alone or in combination at a 300 mg/hd/day each. Overall results are in agreement with the Erasmus et al. (2008) trial in which the greatest fat-corrected milk and milk fat content were observed when virginiamycin and monensin were fed in combination.

Desantadina et al. (2015) conducted a trial to determine the effect of virginiamycin use on milk production and milk components on commercial dairy farms in Argentina. Virginiamycin supplemented cows had numerically greater 4 % fat corrected milk production during the first 130 days of lactation (1.1 L/day, $P=.14$). Specifically, virginiamycin-supplemented cows had greater fat-corrected milk on d 52, 78 ($P<0.10$) and 101 ($P<0.05$) than monensin-supplemented cows. The authors concluded that the addition of virginiamycin in diets containing monensin increases fat-corrected milk during peak- to mid-lactation.

Summarizing, results from Australia (Clayton et al. 1999), the Republic of South Africa (Erasmus et al. 2008), Costa Rica (Chavarria-Solis 2008), Mexico (Duran-Leyva 2009) and Argentina (Desantadina et al. 2015) indicate that when included in diet of lactating cows, in both grazing- or totally enclosed- production systems, virginiamycin increases energy corrected milk production (on average 0.6 kg/day across all trials) by increasing milk yield, milk fat content, or a combination of both. These results have been observed in trials where virginiamycin is fed alone or in combination with monensin.

The positive effects of virginiamycin supplementation to lactating calves on growth rate and feed efficiency has been documented (Parigi-Bini 1980; Skrivanova et al. 1994; Skrivanova et al. 1996).

**Table 7.14** Performance of dairy calves receiving or not receiving virginiamycin in the first months of life

| | Age | | Control | | | Virginiamycin | | | Increment | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | Final | Initial weight | Final weight | ADG | Initial weight | Final weight | ADG | Final weight | ADG |
| Reference | (days) | | (kg) | (kg) | (kg/d) | (kg) | (kg) | (kg/d) | (kg) | (%) |
| Skrivanova et al. (1994) | 28 | 126 | 48,00 | 162.00 | 1.16 | 50.00 | 172.00 | 1.24 | 8.00 | 6.89 |
| Skrivanova et al. (1996) | 21 | 91 | 51,60 | 88.00 | 0.52 | 52.60 | 92.40 | 0.57 | 3.40 | 9.61 |

Parigi-Bini (1980) reported that when virginiamycin was included in the diet of veal calves (initial BW, 53.6 kg) for 118 days, growth rate and feed efficiency were increased by 5 %. The author reported that in an accompanying metabolism trial, diet protein and fat digestibility were numerically improved, although differences did not reach statistical significance likely due to the limited number of replicates used in the trial.

Skrivanova et al. (1994) evaluated the effects of virginiamycin supplementation to veal male calves (BW = 48 kg; 8 weeks of age) for 14 weeks (Table 7.14). The virginiamycin dose evaluated was 80 mg/hd/day. Calves supplemented with virginiamycin gained 8 extra kg of BW compared to non-supplemented calves in the experimental period (≈80 g /day). Although not statistically different, virginiamycin supplemented calves had greater feed efficiency (≈3 %) in part due to their increased digestibility of dietary crude protein ($P < 0.05$).

In a follow up study with female young calves (Table 7.12), Skrivanova et al. (1996) evaluated the effects of virginiamycin and bovine somatotropin (bST) on growth rate. Female calves ($n = 5$ per treatment; BW = 50 kg) 2 weeks old were individually penned and fed skimmed twice per day until they were weaned at 7 weeks of age. The trial lasted 70 days. The virginiamycin treated calves received 80 mg/hd/day whereas the control (CT) calved did not receive virginiamycin. Daily gain rate and feed efficiency were improved by 9.6 % and 2.3 % respectively in virginiamycin compared to CT. However, differences were not statistically different, likely due to the limited number of replicates used in the trial.

Taken together these results suggest that including virginiamycin in lactating calves at a dose of 80 mg/hd/day increases growth rate and feed efficiency.

## Safety and Toxicity

### *Animal Safety*

In a study carried out in the USA by FDA (Food and Drug Administration), Hedde et al. (1982) evaluated the safety of Virginiamycin administration to feedlot cattle. In that study, 18 male and female cattle were supplemented with a dose 4 and 20 times

**Table 7.15** Performance of crossbred cattle (*Bos indicus* x *Bos taurus*) receiving 0, 200, 700 and 1,100 mg/head of Virginiamycin daily during 112-day toxicity evaluation. Adapted from Davidson et al. (1987)

| | Virginiamycin (mg/head/day) | | | |
|---|---|---|---|---|
| Parameters | Zero | 200 | 700 | 1.100 |
| Average daily gain, kg | 1.208 | 1.321 | 1.354 | 1.101 |
| Daily dry matter intake, kg | 8.872 | 9.015 | 8.829 | 8.030 |
| Feed to gain ratio, kg/kg | 7.674 | 7.049 | 6.654 | 7.685 |

greater than the recommended one, which means that animals whose recommended dose was 250 mg/head, received 1000 and 5000 mg/head of Virginiamycin daily for 23 weeks. The animals receiving 4 or 20 times the recommended daily dose of Virginiamycin over a 23 week period did not experience adverse an effect on average daily gain, dry matter intake health and behavior. There was no adverse treatment related effect with respect to mortality, gross pathology, or histopathology reported. This study provides important supporting data for safe virginiamycin supplementation in cattle feeding.

In another study utilizing 36 crossbred animals (*Bos indicus* x *Bos taurus*), Davidson et al. (1987) administered 200, 700, and 1100 mg/head/day of Virginiamycin daily for 112 consecutive days. Tolerance to high virginiamycin dosing was evaluated for 28 days, utilizing 6000 mg/head/day. In that study, in addition to the laboratory analyses of urine, blood and feces during the adaptation period and every 4 weeks throughout the experiment, all animals were thoroughly inspected through physical examination twice a day. At the end of the experiment, all animals were slaughtered and necropsy was carried out in order to identify possible treatment related pathology (Table 7.15).

No adverse effects were observed with respect to clinical signs, clinical pathology, or gross pathology in the tested groups up to, and including, the 6000 mg virginiamycin/animal/day dosing. The results of this study demonstrate administration rates substantially higher than normally recommended (up to 30 times the recommended dosage) are not expected to result in adverse clinical or pathological effects in cattle.

## *Environment*

Due to the potential excretion of virginiamycin in the feces of treated animals, it is important there are no significant adverse environmental effects resulting from the use of this molecule.

In cattle, poultry and swine, there is very limited systemic absorption of virginiamycin from the gastrointestinal tract. Metabolism is rapid and the majority of the ingested dose is excreted (>94 %) mainly in feces. The excreted virginiamycin is rapidly degraded in the environment after elimination by the animal.

In one specific study reviewed by FDA USA and summarized in the publicly available "Environmental Assessment For NADA 140-998", researchers added virginiamycin to cattle feces, free from pharmaceuticals, in a concentration of 34.9 mg/kg of feces, then evaluated the biological potency after 0, 1, 3, 5, 7, 10 and 14 days. Within 7 days, only 13.2 % (4.6 ppm) of the initial microbiological activity level remained, and on the tenth day, the activity was lower than the standard curve. In that study, it was concluded that the half-life of virginiamycin in bovine feces is 2.5 days. According to Gottschall et al. (1988) virginiamycin half-life in the rumen is approximately 8 h.

Molecular biodegradation was evaluated in different soil types for 64 days using 14C-virginiamycin. After samples were extracted from day 1–64, they were analyzed by high performance liquid chromatography (HPLC), and the result indicated that Virginiamycin is extensively degraded in the soil, transforming into a series of smaller organic components. No single degradation product was detected at a concentration higher than 10 % of the initial one.

## Claims and Posology

Virginiamycin can be administered safely within recommended levels for feedlot, grazing cattle and dairy cattle. According to Phibro Animal Health, the dose of virginiamycin ranges from 100 mg up to 340 mg/head/dayay, beef and dairy cattle, in dependent upon country registration. In some countries such Australia, virginiamycin has been authorized and used in therapeutic form for acidosis control

Current claims DEPENDING OF COUNTRY APPROVAL are:

- Improve feed efficiency and increase weight gain rate.
- Accelerated growth rate and feed efficiency.
- Increase of milk production and quality.
- Acidosis control.
- Acidosis prevention and control.
- Acidosis reduction.
- Reduction of liver abscess incidence.

## Final Considerations

The effects of virginiamycin are very clear and known in the rumen, where specific bacteria are balanced allowing a favorable rumen flora, improving feed efficiency and better production (LW gain and milk). New research studies showing the effect of virginiamycin not only in the rumen but also in the intestine are very promising. A good part of the observed results in dairy and milk cattle can be explained by intact, healthy villi with high absorption capacity of nutrients.

In the past 60 years, since its discovering in Belgium, Virginiamycin still offers a lot of opportunities for its application not only in animal production but also in other types of industries involving from the most complex fermentation processes up to the simplest ones like the production of ethanol and fuels.

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# Chapter 8
# Grain Processing for Beef Cattle

**Flávio Augusto Portela Santos, Rodrigo da Silva Marques, and João Ricardo Rebouças Dórea**

## Introduction

Rations with higher concentrate content lead to greater average daily, better feed to gain ratio, greater fat deposition on carcasses, increased dressing percentage and lower operating costs in feedlot, making the activity more profitable (Preston 1998; Nunez et al. 2008; Carareto et al. 2010). Because of these reasons and the high cost of energy of preserved forage, worldwide concentrate-rich diets have been studied in the countries that have significant production of beef and milk in feedlots (Santos et al. 2011). In general, cereal grains represent the main energy source in rations for cattle finished in feedlot (Huntington 1997; Owens et al. 1997; Santos et al. 2011).

Starch makes up 60–70 % of most cereal grains (Rooney and Pflugfelder 1986), and, therefore, it is fundamental to optimize the use of this nutrient to obtain high feed efficiency from feedlot animals fed high-grain diets (Theurer 1986; Huntington 1997; Owens et al. 2005). Starch digestibility is affected by several factors, especially:

(a) Type of cereal grain: among cereal grains, starch digestibility may vary drastically due to factors such as the presence and association level of protein matrices with starch granules, compaction level of starch granules in endosperm, contents of amylopectin and starch amylose and grain outer layer (Rooney and Pflugfelder 1986; Philippeau and Michalet-Doureau 1998; McAllister et al. 2006).
(b) Processing methods: Different processing methods may, in smaller or greater scale, break or solubilize protein matrices associated with starch granules, cause starch gelatinization and also increase grain surface area and, thus, facilitate access of amylolytic enzymes to starch granules (McAllister et al. 2006).

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Cereal grain processing particularly aims to increase starch digestibility in the digestive system and, therefore, raise the energy content of cereal grains (Zinn et al. 2002), which, in general, results in improved feed efficiency in dairy and beef cattle (Owens et al. 1997). Taking corn grain as an example, a difference in starch digestibility in the whole digestive system is considerable when different processing methods of this cereal grain are compared. According to Owens and Soderlund (2007), starch digestibility of dent corn in the whole digestive system of growing and/or finishing beef cattle is lower for whole grains (87.08 %), intermediate for dry-rolled grains (91.03 %) and greater for high-moisture grains (99.25 %) or steam-flaked grains (99.09 %).

In certain regions of the world, like in South America, the production and utilization of hard corn or *flint* with high proportion of vitreous endosperm is predominant. The greater the proportion of vitreous endosperm in the grain is, the lower the starch digestibility becomes (Philippeau and Michalet-Doureau 1998; Correa et al. 2002, 2006). In recent studies carried out in Brazil with flint corn and Zebu cattle finished in feedlot, the reported values of total starch digestibility were from 72.7 % to 81.8 % for whole corn, 81.2 % to 85.7 % for dry-rolled corn and 93.3 % to 98.7 % for steam-flaked corn (Carareto et al. 2010; Marques et al. 2011; Peres 2011; Gouvea et al. 2012). These values of starch digestibility obtained with whole flint corn or dry-rolled corn are much lower than the ones reported for dent corn by Owens and Soderlund (2006). Moreover, the increases in starch digestibility with steam-flaking corn were greater with flint corn than dent corn.

## Properties of Cereal Grain Starch

Cereal grains consist of an external protective layer called pericarp, rich in fiber and with low digestibility. Internally, there is a starch-rich endosperm and an oil-rich germen (Rooney and Pflugfelder 1986; McAllister et al. 2006).

Starch is a polysaccharide whose function is to reserve energy for plants, found mainly in the endosperm of cereal grains like corn, wheat, rice, sorghum, barley and oats, among others; in tubers like potatoes and sweet potatoes; and in roots like cassava. Two main types of glucose polymers form starch molecule: amylose and amylopectin. Amylose is a linear polymer with 1-4 alpha bonds among its glucose units. Amylopectin is a longer and more branched polymer with linear D-glucose chains (1,4 alpha) and branching points (1,6 alpha) every 20–25 glucose molecules. Amylose and amylopectin molecules are kept together by hydrogen bonds, resulting in starch granules with highly organized structure. The granules are formed by the depositing of growth rings that consist of alternate layers of amorphous and crystalline regions. Crystalline regions are mainly composed of amylopectin whereas amorphous regions consist of amylose mainly (Rooney and Pflugfelder 1986; Nocek and Tamimga 1991; McAllister et al. 2006).

Cereal grains are classified as waxy when amylose:amylopectin ratio is lower than 15 %, normal when amylose represents 16–35 % of starch granule, and high-amylose

when amylose represents more than 36 % of the granule. Amylose-rich starch is less digestible than amylopectin-rich starch. The exact function of amylose in corn granule is unknown. When heated in water, waxy materials swell more than non-waxy ones, indicating that amylose has the role to restrict granule swelling. It is possible that amylose molecules cause an increase in numbers of intermolecular hydrogen bonds, which could be a limiting factor to granule swelling and enzymatic hydrolysis. Although several studies on non-ruminants showed that amylose:amylopectin ratio is negatively correlated to starch digestion, the impact of this variable in ruminal digestion has not been well determined (Rooney and Pflugfelder 1986; McAllister et al. 2006).

When exposed to polarized light, starch granules present a characteristic shadow known as "Malta Cross". This phenomenon is known as birefringence. Starch granules may undergo a process called gelatinization. In that case, there is birefringence loss, i.e., there is irreversible loss of its native structure in function of some applied energy that will be responsible for breaking intermolecular hydrogen bonds. Gelatinization can be caused by several factors, such as: thermal, mechanical, chemical agents or a combination of them. During gelatinization, granules absorb water, swell, expose the amylose part and become more susceptible to enzymatic degradation (Rooney and Pflugfelder 1986).

Retrogradation can be considered the opposite of gelatinization, which means the re-association of starch molecules that were separated during gelatinization. Hydrogen bonds are formed between amylose and the amylopectin part, however starch that undergoes this process does not have a pseudo-crystalline characteristic like *in natura* starch. The degree of retrogradation depends on several factors like the structure of amylopectin and amylose, grain moisture, temperature, acting agents in linkages like lipids, and starch concentration. Retrogradation phenomenon is more associated with amylose, and grains may have reduced digestibility after undergoing this process. For the occurrence of retrogradation, moisture and high temperature during grain storage are necessary (Rooney and Pflugfelder 1986; McAllister et al. 2006).

Digestibility of cereal-grain starch is affected by two main factors: cereal grain type and processing method. Intrinsic factors to cereal grain type are: amylose:amylopectin ratio (Rooney and Pflugfelder 1986), grain vitreousness (Correa et al. 2002) and mainly the presence of protein matrix embedding starch granules (McAllister et al. 2006).

The ranking of grains regarding starch digestibility is oats, wheat, barley, corn and sorghum. Oats, wheat and barley grain proteins do not form dense protein matrices strongly associated with starch granules, which characterizes their high starch digestibility. In the case of corn and especially sorghum, there is the presence of dense and low digestibility protein matrix strongly associated with starch granules, which limits the action of amylolytic enzymes in the rumen as well as in the intestines (Rooney and Pflugfelder 1986; Herrera-Saldana et al. 1990).

Less intense processing methods such as cracking, coarse grinding or dry-rolling are sufficient to optimize starch digestibility of grains like oats, wheat and barley. However, in the case of corn and sorghum, more intense processing methods like

steam-flaking and high- moisture grain silage are necessary to break and solubilize protein matrices, and optimize starch digestion (Theurer 1986; Huntington 1997; Owens et al. 1997; Owens and Soderlund 2006).

In North America, almost all cultivated corn is dent (*Dent—Zea mays ssp. Indentata*) whereas in South America, flint corn cultivation is predominant (*flint—Zea mays ssp. Indentura*; Correa et al. 2002). Dent corn grains have soft porous starch and low density. During the plant physiological maturity process, there is grain moisture loss and the soft floury endosperm reduces its volume more than the endosperm vitreous layers, originating denting by wrinkling the endosperm on the top of the kernel (Figs. 8.1 and 8.2). Flint corn grains have hard endosperm that occupies almost its whole volume and low proportion of floury endosperm.

**Fig. 8.1** Dent corn with typical denting on top of the kernel and floury endosperm

**Fig. 8.2** Flint corn with rounded kernel top and high proportion of vitreous endosperm

Vitreousness is defined as the proportion of vitreous endosperm in relation to total endosperm.

Endosperm hardness is determined by grain protein composition and compact arrangement among starch molecules. Starch granules within cells are embedded in a protein matrix whose density varies with cell location in the grain. The matrix is sparse and fragmented in the floury endosperm, and dense and well developed in the vitreous region. In the floury portion, starch granules are more susceptible to enzymatic attack. The interaction with protein may reduce starch susceptibility to enzymatic hydrolysis, decreasing the digestibility of this carbohydrate (Harmon and Taylor 2005).

Ruminal bacteria preferably colonize starch granules that are more exposed within the protein matrix. As fermentation occurs, starch is totally digested and the protein matrix remains intact. The protein type that forms the protein matrix also influences starch susceptibility to the action of digestive enzymes. Zein proteins are less digestible than gluteins (McAllister et al. 2006).

The higher the vitreousness of corn grain is, the lower the ruminal starch degradability gets. In a study carried out by Correa et al. (2002), Brazilian hybrids, representing extreme grain hardness, were compared to corn hybrids cultivated in the United States (Fig. 8.3). Vitreousness of Brazilian hybrids in mature stage varied from 64.2 % to 80 % of endosperm, an average of 73.1 %. In North-American hybrids, vitreousness ranged from 34.9 % to 62.3 % of endosperm, with an average of 48.2 %. The less vitreous Brazilian hybrid presented greater vitreousness than the most vitreous American hybrid. In that study, the correlation between vitreousness and ruminal degradability was negative and high, and it was similar to the one observed by French researchers working with another population of plants (Philippeau and Michalet-Doureau 1998).

Therefore, the energy values of whole, ground or cracked flint corn cultivars, utilized in South America, must be lower than the tabulated values of NRC (1996)

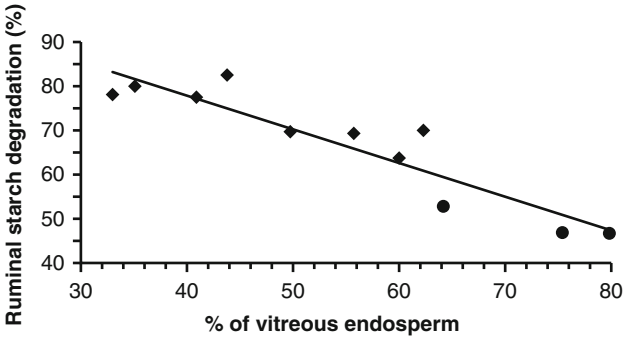


**Fig. 8.3** Grain vitreousness and ruminal availability of starch evaluated in situ in American (*filled diamond*) and three Brazilian (*filled circle*) hybrids. Availability = 108.2 − 0.7605 * Vitreousness. $R^2 = 0.87$. Availability (% of starch) = A + B [Kd/(Kd + Kp)]. Kp = 0.08/h. Adapted from Correa et al. (2002)

obtained from studies on dent corn in North America. Due to the greater vitreousness, hard corn cultivars are expected to present greater positive responses to more intense grain processing than dent corn cultivars.

## Ruminal Digestion of Starch

Regardless of the adopted processing method and type of cereal grain (corn, sorghum, barley, wheat or oats), the rumen is the main digestion site of starch in cattle with production of short-chain fatty acids (**SCFA**) and microbial protein besides $CO_2$ and methane. In growing and finishing cattle, when corn was dry-rolled, provided whole, flaked or ensiled containing high-moisture, respectively, 63.80 %, 68.34 %, 84.05 % and 86.55 % of ingested starch was digested in the rumen and, respectively, 70.15 %, 79.20 %, 84.74 % and 87.24 % of total digested starch was digested in the rumen (Owens and Soderlund 2006).

The first step in starch ruminal fermentation process is its hydrolysis through the action of enzymes secreted by ruminal microorganisms, mainly amylolytic bacteria. They tend to be predominant in the rumen of animals fed high-starch diets (Yokoyama and Johnson 1988). The main identified amylolytic bacteria in the rumen are *Selenomonas ruminantium, Streptococcus bovis, Ruminobacter amylophilus, Prevotella ruminicola, Succinomonas amylolytica* and *Butyrivibrio fibrisolvens* (Cotta 1988). Degradation by these bacteria involves the action of several enzymes (Alpha-amylase; Beta-amylase; Amyloglucosidase; Isoamylase; Phosphorylase; Pullulanase; McAllister et al. 2006) that can break alpha 1-4 and alpha 1-6 linkages when acting together. After starch molecule degradation, resulting mainly in maltose and glucose, saccharolytic bacteria will ferment these substrates quickly through glycolytic pathway to produce pyruvic acid. This is the intermediate step where all carbohydrates have to go through before being converted into SCFA, $CO_2$, and $CH_4$ (Yokoyama and Johnson 1988).

Protozoa also have an important role in ruminal digestion of starch, not only because it digests starch, but also because it performs this process more slowly than bacteria. Protozoa encapsulate starch particles, being able to store them for long periods of time. This can help to avoid sudden decreases of ruminal pH (Yokoyama and Johnson 1988).

## Intestinal Digestion of Starch

It's noteworthy to point out that excellent reviews on digestion site, emphasizing starch intestinal digestion in ruminants, have been published in the last 3 decades (Owens et al. 1986; Harmon 1992, 2009; Huntington 1997; Owens and Soderlund 2006). In this section, the digestion of starch granules in the small and large intestines will be described.

### *Small Intestine*

Although the rumen is the main site of starch digestion, considerable amounts of this nutrient may reach the small intestine of cattle fed with grain-rich diets. A steer that daily consumes 10 kg of dry matter of diet containing 80 % corn will be ingesting approximately 5.8 kg of starch a day. Based on data by Owens and Soderlund (2006), in the case of ensiled high-moisture corn with 86.55 % of ruminal digestibility, 0.78 kg of starch will reach the small intestine whereas in the case of dry-rolled corn with 63.80 % of ruminal digestibility, 2.1 kg of starch will bypass to the small intestine of this animal to be digested. These amounts of starch are much inferior to the ones that reach the rumen and, regardless of the corn processing method, starch digestion in the small intestine is not complete, ranging from only 58.83 % with dry-rolled corn and 64.64 % with whole shelled corn to 92.48 % with steam-flaked corn and 94.86 % with ensiled high-moisture corn for growing or finishing steers (Owens and Soderlund 2006). There is still not a consensus on the subject, but the main suggested causes for this limitation of starch digestion in the small intestine are: (a) enzymatic limitation, (b) limitation of glucose absorption capacity, (c) insufficient time for digestion in small intestine, (d) enzyme difficulty to access starch granules (Russell et al. 1981; Owens et al. 1986; Harmon 1992; Huntington et al. 1997; Huntington et al. 2006; Harmon 2009).

Starch digestion in the small intestine occurs in two distinct phases: (a) digestion in the intestinal lumen through the action of pancreatic alpha-amylase enzyme, and (b) digestion in villi or brush borders by intestinal carbohydrases (Huntington et al. 2006; Harmon 2009).

Optimal pH for the action of pancreatic alpha-amylase enzyme in the intestinal lumen is approximately 6.9 (Russell et al. 1981). This enzyme acts on alpha 1-4 glycosidic bonds producing maltose, maltotriose and several limit dextrins that occur due to the alpha 1-6 glycosidic linkage of amylopectin (Harmon 2009). The process of starch digestion until glucose is completed in intestinal villi by intestinal carbohydrases with maltase and isomaltase activities (Huntington et al. 2006; Harmon 2009).

Larsen et al. (1956) were probably the first to mention the possible limitation of pancreatic alpha-amylase enzyme to total digestion of starch in the small intestine. In the following decade, Karr et al. (1966) and Little et al. (1968) corroborated the hypothesis of enzymatic limitation to explain the reduction of starch digestibility when the amount of this nutrient was increased in the small intestine through feeding starch-richer diets or through abomasal infusion, respectively.

Concentration and secretion of pancreatic alpha-amylase enzyme can be nutritionally manipulated; however, the exact regulatory mechanisms in ruminants are still not known (Harmon 2009). Initially it was logically believed that the presence of starch in the small intestine would be the main factor to stimulate alpha-amylase secretion by the pancreas because starch-rich diets had stimulated greater enzymatic

secretion than forage-rich diets. However, with the study by Russell et al. (1981) it became clear that in previous studies there was a misunderstanding between starch intake and energy intake and that the latter is a determining factor for the secretion of pancreatic alpha-amylase.

Huntington et al. (2006) and Harmon (2009) discussed the possible effect of the presence of protein in the small intestine to stimulate pancreatic alpha-amylase secretion. According to Harmon (2009), the main control system seems to be the same one for energy intake. However, energy intake increases the production and flow of microbial protein, which stimulates alpha-amylase secretion.

The lack of specific response from the pancreas to the presence of starch in the small intestine made Huntington (1997) study on the limitation of alpha-amylase as a possible cause for the absence of total starch digestion in the small intestine, as initially proposed by Larsen et al. (1956).

On the other hand, Owens et al. (1986) rejected this hypothesis based on the lack of plateau occurrence when the relation between the starch supply (g/kg of body weight) and starch digestion (g/kg of body weight) in the small intestine was studied. The authors suggested that with typical feedlot diets, the size of particles containing starch might be a more limiting factor to the digestion of this nutrient in the small intestine. This hypothesis has been confirmed by studies on dairy cows carried out by Oba and Allen (2003a, b) and Taylor and Allen (2005).

Glucose absorption does not seem to be a limiting factor to starch digestion in the small intestine (Huntington 1997; Huntington et al. 2006). Transporters mediate the absorption process. Until recently, it was believed that sodium-dependent glucose transporter SGLT1 mediated intestinal glucose absorption with energy utilization (ATP) during the process. More recently, there has been evidence that transporter GLUT2 may be the main glucose absorption pathway. Through this pathway there is not energy use to absorb glucose. This pathway can be what once was believed to be a process of diffusion or paracellular absorption (Harmon 2009).

## *Large Intestine*

Starch that is not digested in the small intestine is again submitted to fermentation and action of microbial enzymes in the large intestine with production of SCFA, microbial protein, methane and heat.

Surely all ingested starch that reaches the large intestine has lower digestibility. In the case of dry-rolled corn, starch digestibility in the large intestine is 56.32 % versus 24.8 % for ensiled high-moisture corn and 20.47 % for steam-flaked corn. Due to the high ruminal digestibility, and consequently to high small intestinal digestibility of ensiled high-moisture corn and steam-flaked corn, starch that reaches the large intestine with these materials is composed almost only by starch indigestible fraction.

## Cereal Grain Processing Methods

Man has practiced grain processing for animal feeding for several years and its goal is to improve the utilization of some feedstuffs by ruminants, mainly by cattle (Hale 1973; Orskov 1986). There are several manners to process grains, and according to Hale (1973), the methods are classified into dry and wet processing. Therefore, cracking, grinding, roasting, and pelleting are examples of dry processing; and ensiling high-moisture grain, steam-flaking, exploding and pressure cooking are the most typical wet processing methods.

In a survey conducted with 29 consultants responsible for nutritional management of 18 millions of feedlot cattle in the USA, Vasconcelos and Galyean (2007) reported that typical rations contained approximately 91 % of concentrate and that all corn utilized in the ration went through some kind of processing, and the most common ones were steam-flaking, high-moisture grain ensiling, and dry-rolling. Millen et al. (2009) utilized a similar survey protocol with Brazilian nutritionists, and according to this study, typical rations utilized in Brazilian feedlots contained an average of 71.2 % of concentrate. Around 59 % of the nutritionists stated that they included 51–80 % of cereal grain in the diets. For 79.3 % of the interviewed nutritionists, corn was the first option of cereal grain whereas sorghum was the first option for 20.7 % of the nutritionists. The most utilized grain processing method was fine grinding, adopted as the first choice by 54 % of the nutritionists, whereas 38.7 % of them utilized grain cracking and 6.5 % used coarse grinding as their first processing options. In a recent survey, Oliveira and Millen (2014) reported that: 81.8 % of Brazilian nutritionists formulate diets ranging from 71 % to 90 % concentrate; corn is still the most utilized grain; 75.5 % of nutritionists include this grain in amounts between 51 % and 80 % of diet dry matter; and the main grain processing method is coarse grinding followed by fine grinding.

### *Grinding*

Grinding aims to reduce grain particle size to allow greater utilization of its ingredients and improve logistics of ration preparation. According to McKinney (2006), the physical principles involved in grinding or particle size reduction are: (1) compression, (2) impact, (3) friction and (4) shearing; and most utilized equipment uses a combination of these principles.

Hammer and roller mills are the most common types. Hammer mills use impact and friction whereas roller mills utilize shearing and compression to reduce particle size (McKinney 2006). Hammer mills are more utilized in Brazilian feedlots (Millen et al. 2009).

In a hammer mill, the particle size is determined by the opening size of the utilized screen. In a roller mill, which can have 1, 2 or even 3 pairs of corrugated rollers, particle size of the processed material is determined by the differential speed between

the rollers. The greater this differential is, the greater the applied shearing force gets (McKinney 2006).

Comparatively, both types of mill have advantages and disadvantages (McKinney 2006):

Hammer mills produce particles with rounder shape whereas roller mills produce more cubic shapes. Rounder particles require less time spent mixing to produce a homogeneous mixture with vitamins, drugs or mineral mixtures. Hammer mills are more efficient than roller mills to finely grind more fibrous grains like oats, barley and others. They are also more appropriate to finely grind corn and sorghum grains than rollers.

Roller mills better process wetter materials than hammer mills, produce less dust and less fine particles due to the cubic shape of particles, produce more homogenous material regarding particle size and have greater yield with lower power consumption when the objective is to obtain a large particle size, greater than 1.8 mm.

According to Mendes and Mendo (2009), with the use of rollers instead of hammers, there is a 90 % reduction of power consumption per ton of processed grain. The whole system has low rotation and the rollers touch the grains only once, resulting in more homogenous particles than in hammer mills. However, power consumption should be verified more specifically because, according to McKinney (2006), the advantage for energy consumption of roller mills when compared to hammer mills only occurs when the material is coarsely ground, larger than 1.8 mm. When the goal is to process cereal grains with small particle size, equal to or smaller than 0.6 mm, the power consumption and yield of both mills are equivalent.

Both mill types are efficient to increase grains contact surface area, but they only partially break the protein matrix that embeds starch granules and are not effective for starch gelatinization. The increase in starch digestibility is determined by the reduction of particle size (Corona et al. 2005; Pedroso et al. 2010; Carareto et al. 2010; Marques et al. 2011). There is no official rule to classify grinding level of cereal grains in function of particle size. Litherland (2006) proposed the following ruling utilizing a system with four screens (Table 8.1).

In studies on processing methods of flint or hard corn grains carried out in Brazil (Peres 2011; Carareto et al. 2010; Gouvea et al. 2012), grinding that results in medium particle size equal to or smaller than 1.3 mm is considered fine grinding, whereas coarse grinding presents particle size over 2 mm. These patterns are close to the ones considered in the Brazilian national market.

**Table 8.1** Classification of grinding level in function of particle sizes according to Litherland (2006)

| Screen | Size | Corn |
|---|---|---|
| Screen 1 | >4.5 mm | Whole or coarse |
| Screen 2 | >2.2 mm | Cracked |
| Screen 3 | >1.1 mm | Ground |
| Screen 4 | >0.5 mm | Finely ground |
| Pan | <0.5 mm | Degree of starch |

## *High-Moisture Grain Silage*

According to surveys conducted by the *Journal of Animal Science* from 1910 to 2010, the first study comparing high-moisture corn to low-moisture corn for beef cattle was published in 1958 (Beeson and Perry 1958). In 1976, the University of Oklahoma, in the United States, organized a symposium on the utilization of high-moisture grain silage for cattle (High-Moisture Grain Symposium 1976), which reunited several researchers in the field and it is until nowadays considered a classic literature on the topic.

In Brazil, this technique was introduced in the early 1980s in the state of Paraná, initially for swine feeding and later for dairy and beef cattle (Kramer and Voorsluys 1991); however, the first scientific studies were published in the 1990s (Jobim 1996; Jobim et al. 1997, 1999).

High-moisture grain silage consists of grain harvesting right after physiological maturation, material grinding (optional), silage storing, compacting and sealing to keep anaerobiosis (Fox 1976; Jobim and Reis 2001; Costa et al. 2002; Mader and Rust 2006).

Grains are considered physiologically mature when dry matter production reaches its maximum point. Dry matter accumulation in corn grains occurs until moisture content is reduced to approximately 35 %, and it may remain around 40 % in some hybrids (Mader and Rust 2006). The presence of a black layer on the grain base is a determining characteristic of corn physiological maturity (Costa et al. 2002). In practice, depending on the utilized cultivar, weather conditions and silage process, the variation range of moisture content of ensiled material considered acceptable is from 25 % to 35 % (Jobim and Reis 2001; Costa et al. 2002; Owens and Zinn 2005; Mader and Rust 2006).

Once physiological maturity is reached, dent corn grains lose approximately 0.5–1 % of moisture a day, depending on the environmental conditions. This results in a short-harvesting window, a factor that becomes a great challenge for large-scale processing. Moisture loss seems to be faster in flint or hard corn when compared to dent corn cultivars (Da Silveira 2009) resulting in an even shorter harvesting window for these materials.

In sorghum grains, the moisture loss is faster than in corn because the grains in the panicle are exposed to sunlight (Mader and Rust 2006). According to Defoor et al. (2006), the harvesting window for dent corn is from 7 to 14 days against 2 to 5 days for sorghum.

In the case of sorghum, due to the short harvesting window and the little consistent results on animal performance with high-moisture grain harvesting and silage, the process of dry grain reconstitution with water has been preferred, followed by aerobic germination from 1 to 5 days, material grinding and silage (Defoor et al. 2006).

In general, some procedures and principles similar to the ones utilized for any forage conservation must be adopted for grain silage. Care should be taken regarding harvest, loading, silage storage, sealing and, after some weeks, high-moisture

grain removing from silo (Jobim and Reis 2001; Costa et al. 2002). In special silos with oxygen exhaustion, corn can be ensiled whole, whereas in trench silos or silage buns, the material should be ground in hammer or roller (dry-rolled) mills before ensiling (Mader and Rust 2006).

There are several advantages of high-moisture grain silage when compared to harvesting, storing and feeding dry corn to cattle (Self 1976; Jobim and Reis 2001; Costa et al. 2002; Owens and Soderlund 2006), such as: (a) reduction of losses until harvesting by plagues and rodents, (b) reduction of losses during harvesting, and decrease in losses by attacks of rats and insects during storing, (c) lower storing cost and anticipation of crop harvesting from planting area allowing anticipated planting of new crop at the end of the summer, and finally, (d) the best animal performance when compared to dry-processed corn.

The nutritional value of high-moisture corn silage is greater than the one of dry-ground grains in hammer or roller mills (Corah 1976; Owens et al. 1997; Defoor et al. 2006; Owens and Soderlund 2006). The main determining factor of this nutritional value is the greater starch digestibility of ensiled material in the rumen as well as in the intestines and total digestive system than the same materials when dry-ground (Owens and Zinn 2005; Owens and Soderlund 2007). The increase in starch digestibility occurs mainly due to the solubilization of grain protein matrix, especially prolamines, through the action of proteolytic microbial enzymes as well as by the action of organic acids during the fermentation process in the silo (Prigge 1976; Rooney and Pflugfelder 1986; Owens and Zinn 2005; Defoor et al. 2006).

In the case of sorghum reconstitution, aerobic germination process before grinding and ensiling is fundamental for the protein matrix solubilization process. During the 1 to 5-day process of grain aerobic germination, gibberellin-like hormones migrate to the aleuronic zone and stimulate the release of proteases and amylases that start the solubilization process of protein matrix and starch endogenous hydrolysis. This process ceases when the material is ensiled and then microbial enzymes and organic acids produced during the fermentative process act and keep acting on the dense protein matrix of sorghum.

Owens and Zinn (2005) emphasized that the ideal moisture content between 26 % to 31 % and time spent fermenting in the silo are both critical factors for the maximum starch ruminal digestion and feed efficiency of animals fed ensiled high-moisture corn. Jobim and Reis (2001) worked in Brazil with flint or hard corn cultivars and suggested that the ideal harvesting point is when grains present 32–35 % moisture.

Sprague (1976) evaluated the correlation ($r = 0.73$) between moisture content and soluble N content (soluble N in ethanol) on silage samples of high-moisture corn from several feedlots in the United States (Table 8.2).

Prigge 1976 studied the effect of storing time on soluble N content on ensiled ground high-moisture corn samples (Table 8.3).

Based on the data from Tables 8.2 and 8.3, it is clear that protein matrix solubilization is greater in more moist materials and the solubilized amount increases with storing time.

**Table 8.2** Effect of silage moisture content of high-moisture grain on soluble N content (% of total N)

| Silage moisture (%) | Soluble N (% of total N) |
|---|---|
| 19.9–24.5 | 30.65 |
| 25.4–29.2 | 33.53 |
| 29.9–32.9 | 59.70 |

Sprague (2006)

**Table 8.3** Effect of storing time on soluble N content (% of total N) of high-moisture corn grain silage

| Storing (days) | Soluble N (% of total N) |
|---|---|
| 0 | 15.8a |
| 6 | 21.9b |
| 12 | 24.3b |
| 28 | 33.1c |
| 56 | 38.2d |

Prigge (1976)

According to Hicks and Lake (2006), the soluble protein content of ensiled material is a good indicator of starch digestibility. Benton et al. (2005) carried out an *"in situ"* study to evaluate the effect of storing time on dry matter and protein degradability of ensiled high-moisture corn grain. Dry matter degradability increased rapidly in the first 28 days of silage storing, but it continued increasing after 327 days of ensiling. Ruminal degradability of protein behaved similarly to dry matter.

Hicks and Lake (2006) reported that in a commercial American feedlot that has produced a great amount of ensiled high-moisture corn grains since 1973 with optimal control on quality, the content of soluble protein in silage increased as described by Benton et al. (2005). The average observed values for soluble protein (% of crude protein) were 10–15 % for dry-rolled corn, 50–60 % for high-moisture rolled corn silage (processed in roller mills), and 60–80 % for high-moisture finely ground corn silage (processed in hammer mills).

Owens and Zinn 2005 also reported that causes that lead to worse feed efficiency of cattle fed ensiled corn grains with moisture content between 20 % to 24 % when compared to those fed dry ground corn (by hammer or roller mills) are unclear.

Another important factor in the quality of high-moisture corn grain silage is its particle size. The ideal particle size represents the balance between starch digestion optimization and acidosis occurrence. Small particles allow better material compacting in the silo and greater starch digestibility; however, they may increase acidosis incidence (Hicks and Lake 2006). According to the authors, in the commercial feedlot Hitch Enterprise in the USA, the goals for high-moisture corn grain silage are: (a) compacting with 1 tractor for every 5000 bushels per hour (127 tons per hour); (b) corn must be ground in the maximum 18 h after reaching feedlot; (c) grain moisture between 28 % to 30 %, (d) utilization of 3 screen-system (4.75, 2.0 and 1.0 mm) and collecting pan in order to obtain ground material containing less than 2.5 % of whole grains and less than 20 % of fine particles in the collecting pan; and (e) sampling of material being ground at least 4 times a day to determine particle size.

### *Steam-Flaking*

Corn steam-flaking started in the USA in 1962 with a field experiment conducted in a commercial feedlot by John Matsushima, a former University of Colorado professor (Matsushima 2006). The first article on steam-flaking sorghum and barley was published in the Journal of Animal Science in 1966 (Hale et al. 1966).

Steam flaking is an intense process that demands greater quality control compared to other processing methods like grinding and dry rolling. In this process, the grain is exposed to steam for 30–60 min in a vertical stainless steel chamber. In this phase, the grain absorbs water until reaching 18–20 % of moisture. Next, it is flaked between pre-heated rollers adjusted to obtain the desired density. The rollers are heated in consequence of the passage of grains that have been exposed to steam (Theurer et al. 1999; Zinn et al. 2002).

During corn and sorghum steam flaking, through the action of high temperature and moisture, there is starch gelatinization, which consists of a rupture of intermolecular hydrogen bonds and consequent change of the native structure of starch molecule. There is also an increase of the surface area of the grains now subject to microbial attack and mainly, there is the breaking of the dense protein matrix that involves grain starch granules, resulting in greater starch digestion in ruminants' digestive system (Theurer et al. 1999; Zinn et al. 2002; Owens and Soderlund 2006).

According to reviewed studies, there is an ideal intensity range of steam-flaking process for corn and sorghum grains for beef cattle. The recommendations for finishing feedlot cattle fed grain-rich diets are steam-flaking corn or sorghum to obtain density between 310 to 360 g/l (Zinn 1990; Reinhardt et al. 1998; Swingle et al. 1999; Theurer et al. 1999; Brown et al. 2000a; Zinn et al. 2002; Sindt et al. 2006; Hales et al. 2010). Less processed materials (greater densities) do not present satisfactory results because they do not increase starch digestibility sufficiently. Excessively processed materials (lower densities) also harm animal performance, probably because they increase the risks of ruminal acidosis besides resulting in higher processing costs. Zinn et al. (2002) reviewed 64 studies and determined the relationship between flake density and percentage of ruminal starch digestion and total tract starch digestion (Figs. 8.4 and 8.5).

According to Zinn et al. (2002) corn grains have to be exposed to steam for 30 min and then flaked to obtain density of 310 g/L.

## Effect of Grain Processing on Starch Digestion Site and Energy Efficiency

Huntington (1997) reviewed articles published from 1986 to 1995 that presented digestibility coefficient of corn and sorghum grains submitted to several processing methods to feed dairy cows and beef heifers and steers. According to his

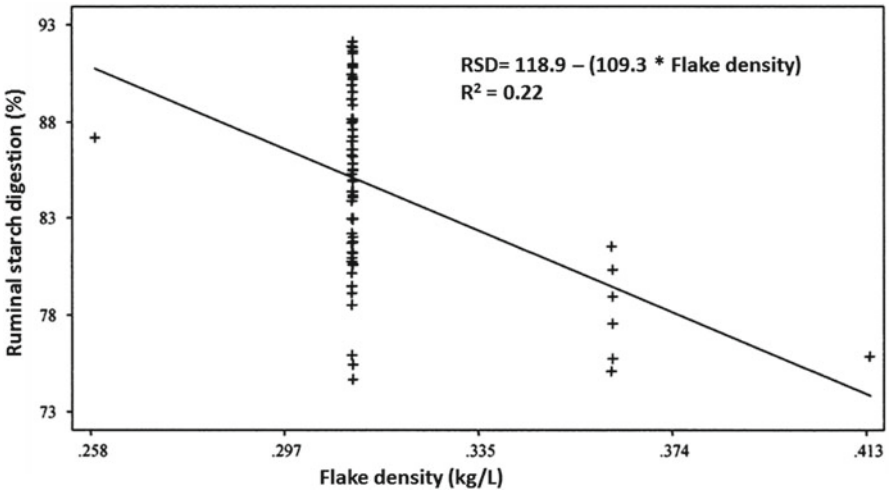


**Fig. 8.4** Relationship between final flake density and ruminal starch digestion (RSD; Adapted from Zinn et al. 2002)

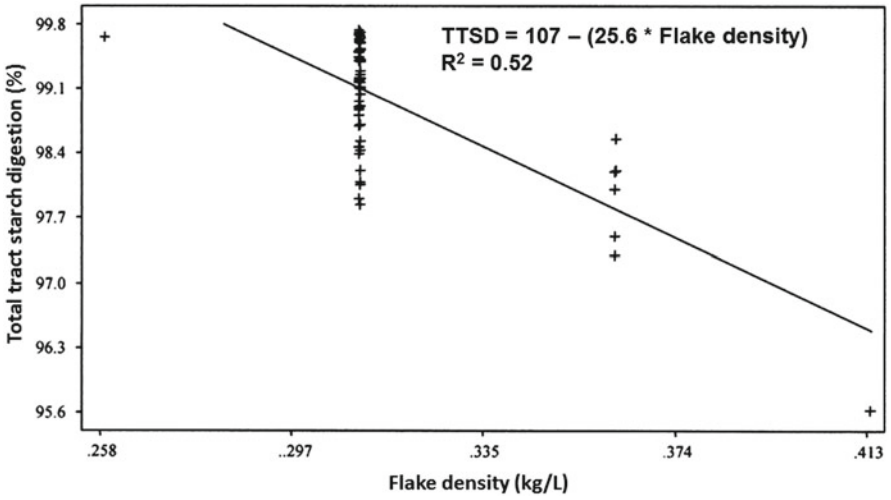


**Fig. 8.5** Relationship between the final flake density and total tract starch digestion (TTSD; Adapted from Zinn et al. 2002)

review, dry-rolled corn as well as sorghum presented lower values of starch digestibility in the rumen and in the total digestive system when compared to steam-flaked grains or ensiled high-moisture grains. When the processing method was dry rolling, starch digestibility in the rumen and in the total digestive tract was greater for corn than for sorghum. Steam flaking process practically eliminated the differences between sorghum and corn regarding starch digestibility in total digestive tract system.

**Table 8.4** Influence of corn grain processing on starch and fiber digestion in different compartments of the digestive system of beef steers in feedlot (% of nutrient that goes into the compartment)

| | Whole corn | Dry-rolled corn | High- moisture corn | Steam-flaked corn |
|---|---|---|---|---|
| Starch digestion in rumen, % | 68.34[b] | 63.80[b] | 86.55[a] | 84.05[a] |
| Starch digestion in small intestine, % | 64.64[b] | 58.83 [b] | 94.86[a] | 92.48[a] |
| Starch digestion in large intestine, % | 32.09[ab] | 56.32[a] | 24.80[b] | 20.47 [b] |
| Starch digestion in rumen + small intestine, % | 86.60[ab] | 83.67 [b] | 99.07[a] | 98.48[a] |
| Starch digestion in small intestine + large intestine, % | 52.99[c] | 72.16 [b] | 93.10[a] | 94.33[a] |
| Starch digestion in total digestive tract system, % | 87.08[c] | 91.03 [b] | 99.25[a] | 99.09[a] |
| NDF digestion in rumen, % | 33.43[bc] | 48.07[a] | 18.48[d] | 27.71[c] |
| NDF digestion in small intestine + large intestine, % | 2.43 | 9.95 | 15.50 | 19.89 |
| NDF digestion in total digestive tract system, % | 38.10[cd] | 50.83[a] | 34.27[d] | 44.39[bc] |

[a,b,c,d]Means without common superscript on the same row, differ ($P<0.05$). Adapted from Owens and Soderlund (2006)

Owens and Soderlund (2006) compiled data from experiments published between 1990 and 2006 on grain processing, in which corn starch digestibility in different compartments of the digestive system was measured in beef steers (Table 8.4).

When corn is provided whole without processing or dry-rolled, starch digestibility decreases as it passes by the compartments of the digestive system, which means that digestibility is higher in the rumen, intermediate in the small intestine and low in the large intestine. However, more intense processing methods like high-moisture grain ensiling and steam-flaking increase ruminal starch digestion compared to dry processing methods, but increase even more the digestion of starch that reaches the small intestine. Starch digestibility of high-moisture corn grain silage and steam-flaked corn in the large intestine is very low due to the fact that almost all-digestible starch of these materials has been digested in the rumen and small intestine.

As shown in Table 8.4, the most intense corn processing methods change starch digestion site, increasing total starch portion that is digested in the rumen in detriment of small intestine. In beef cattle fed high-moisture corn or steam-flaked corn, approximately 85 % of total starch digestion occurs in the rumen. The explanation for improved animal performance when more extensively processed corn and sorghum grains are fed is that total tract starch digestibility is higher, generating more energy for the animal. Another factor that may contribute to a better performance, especially in the case of young animals, is the increase of microbial protein synthesis in the rumen, which results in greater flow of metabolizable protein for the intestine.

Energy efficiency is greater when starch is digested in the small intestine compared to starch that is fermented in the rumen, due to reduction of energy losses by heat generation and lack of methane production (Owens et al. 1986; McLeod et al. 2001). However, scientific data that support or quantify these differences are still scarce in the literature (McLeod et al. 2006).

Fermentation heat is calculated as the difference between substrate fermentation heat and fermentation heat of end products. According to the data reviewed by McLeod et al. (2006), losses by fermentation heat in the rumen vary from 3 % to 12 % of digestible energy against a 0.6 % loss of digestible energy through heat generated by breaking glycosidic linkages in the small intestine.

Ninety to 95 % of total methane produced by ruminants comes from ruminal fermentation and this loss can represent 3–18 % of ingested digestible energy (McLeod et al. 2006). According to these authors, a calf that consumes 6 kg of starch with 80 % of ruminal digestibility would lose from 1.7 to 2.4 Mcal of starch digestible energy or 6.8–9.6 % of ingested energy of starch as methane.

McLeod et al. (2001) infused a solution of corn starch hydrolysate in the rumen or in the abomasum of beef steers fed forage-based diets at 1.5 times the maintenance energy demand. Partial efficiency (Kr) of starch metabolizable energy conversion in tissue energy was calculated as the increase in energy retained over the basal diet, divided by metabolizable energy supplied by infused starch. This efficiency value reflects direct and indirect heat losses associated with digestion, absorption and assimilation of substrate in tissue. Partial efficiency value for infused starch in the rumen was 0.48, against 0.60 of infused starch in the abomasum. Partial efficiency value of 0.60 when adjusted for the disappearance of starch hydrolysate in the small intestine of 88 % (Branco et al. 1999), results in a maximum theoretical partial efficiency value of 0.68. Adopting partial efficiency range from 0.60 to 0.68 for starch supplied in the small intestine, and average loss of 10 % of digestible energy related to ruminal production of methane, the total energy efficiency of fermented starch in the rumen ranges only from 65 % to 72 % of digested starch in the small intestine, according to McLeod et al. (2006).

In the large intestine, besides losses through methane and fermentation heat, every protein found in microbial cells is lost in feces (Owens and Zinn 2005). This increases the importance of optimization of starch digestion in the small intestine, where methane production is negligible.

Non-processed (whole) or little processed (cracked, rolled or coarsely ground) corn grains present smaller ruminal starch digestion than more processed materials, resulting in significant starch amounts passing to the small intestine. This could be considered an energetic advantage for these processing methods, but it does not occur due to low starch digestibility in the small intestine: only 64.64 % for whole shelled corn and 58.83 % for dry-rolled corn (Table 8.4). As a result, significant amounts of starch go to the large intestine, where digestibility is even lower and more energy is lost since microbial protein formed in the large intestine is completely excreted in feces. Thus, total gastrointestinal tract starch digestibility is low when corn grains fed non-processed or processed as dry-rolled.

According to the simulation done by Huntington et al. (2006), increasing starch passing from the rumen to be digested in the small intestine of cattle will be signifi-

cantly more efficient only if starch digestibility that reaches the small intestine is at least 75 %. According to these authors, starch digestibility values of corn or sorghum grains in the small intestine of this magnitude are only observed when starch intake is low or when the animal is fed either high-moisture ensiled grains or steam-flaked grains.

In feedlot beef cattle, digestibility of ensiled high-moisture or steam-flaked corn in the small intestine ranges from 92 % to 95 % (Table 8.4). Based on the simulation carried out by Huntington et al. (2006), animals fed ensiled high-moisture corn or steam-flaked corn could benefit from a greater passing of this highly digestible starch to the small intestine. Owens and Zinn 2005 simulated the effects when there was an increase of 20 % in the passing of dry-rolled corn or steam-flaked corn starch into the small intestine. Theoretically, this increase with dry-rolled corn would result in reduction of energy efficiency. On the other hand, the same increase percentage with steam-flaked corn or ensiled high-moisture corn would increase the supply of absorbable energy for the animal in 2.4 % and 4.5 %, respectively.

However, all this theoretical advantage when changing the starch digestion site to the small intestine can be lost if this extra absorbed glucose is not incorporated into animal carcass (Owens and Soderlund 2006). Until now, little is known about how and where glucose that disappears in the animal's small intestine is utilized. The fact that part of the glucose that disappears in the small intestine cannot be recovered in the portal vein has been intriguing nutritionists (Huntington et al. 2006). This can occur due to microbial fermentation of glucose in the small intestine, utilization of glucose by visceral tissues (Huntington et al. 2006) or direct or indirect energy conversion of glucose into omental and visceral fat. According to the studies reviewed by McLeod et al. (2006), the increase of glucose supply in the small intestine has stimulated the growth of animal's fat tissue, mainly visceral and omental fat. If animal performance is improved, based on increasing weight gain due to greater starch intestinal flow, as consequence of visceral and omental fat increase, there will not be carcass gain, and then there is no advantage for the production system (Owens and Soderlund 2006).

Until now there is no technique that allows processing corn and sorghum grains intensively and control the flow of highly digestible starch to the small intestine. Every attempt to feed less-processed grains in order to increase starch escape to the small intestine has resulted in smaller starch digestion in the total digestive system and poorer animal performance (Huntington 1997).

## Grain Processing Methods and Animal Performance

### *Whole Corn Grain*

Whole corn grains can be provided to feedlot cattle. This practice allows either the inclusion of lower levels or forage to the diet or simply no addition of a roughage source (Loerch and Gorocica-Buenfil 2006).

Owens et al. (1997) carried out an extensive review of the literature on cereal grain processing for feedlot cattle. In compiled studies, experimental diets containing whole corn grain for feedlot cattle, in average, consisted of 6.0 % forage (dry matter basis), but diets containing cracked, dry-rolled or coarsely ground corn had 7.9 % forage, in average. Feed to gain ratio was better for animals fed whole corn grains (5.95 vs. 6.57) than those fed cracked, dry-rolled or coarsely ground corn. The energy value of whole dent corn was higher (3.56 vs. 3.26 Mcal of metabolizable energy per kg of diet dry matter) than coarsely ground corn.

Gorocica-Buenfil and Loerch (2005) compared the performance of feedlot cattle fed diets containing whole corn grain or cracked corn grain with 5 % or 18 % of corn silage in the diet (dry matter basis). It was not observed any effect of grain processing or roughage inclusion on average daily gain and feed efficiency. On the other hand, in diets containing 12 % forage, Corona et al. (2005) reported that fecal starch content was higher (25.91 % vs. 19.60 %) and average daily gain was lower (1.24 kg vs. 1.36 kg) for animals fed whole corn grain when compared to those fed dry-rolled corn.

The feeding of diets without any forage source generally results in lower dry matter intake, which may lead to lower average daily gain in feedlot animals. Utley and McCormick 1975 compared diets containing cracked corn and 20 % of peanut hulls as forage source (dry matter basis) to diets containing whole corn grain without any forage source. Daily dry matter intake (11.95 kg vs. 8.49 kg) and average daily gain (1.42 kg vs. 1.16 kg) were lower in animals fed whole corn based diets without forage. However, the feeding of whole corn grain without forage improved feed efficiency (0.119 vs. 0.136).

Traxler et al. (1995) compared diets containing whole corn grains without any forage source to cracked corn based diets containing 40 % forage in the growing period and 15 % forage during finishing period. The animals fed whole corn grain without forage had lower dry matter intake, similar average daily gain and better feed efficiency than animals fed cracked corn based diets containing a forage source.

In a study conducted by Marques et al. (2011) with flint corn in finishing diets for Nellore bulls, fecal starch content was 32.28 % and starch digestibility in the total gastrointestinal tract was only 72.74 % when a whole corn grain based diet was fed without any forage source. The addition of only 3 % of sugarcane bagasse (dry matter basis) as forage source increased daily dry matter intake (8.42 kg vs. 10.16 kg) and average daily gain (1.197 kg vs. 1.587 kg).

## *Dry Processing—Grinding*

Regarding dent corn utilized in North America, there is no advantage of fine grinding when compared to coarse grinding for feedlot cattle fed high-grain diets. Corona et al. (2005) did not observe any advantage of fine grinding when compared to rolling (coarse grinding in roller mills) for feedlot cattle fed dent corn-rich diets. Besides not improving performance, dent corn fine grinding results in high proportion of very

small particles, which have high rates of ruminal degradation, which increase the risk of metabolic diseases (Owens and Soderlund 2006).

Considering flint corn utilized in Brazil and in a great part of South America, fine grinding results in lower fecal starch content and better performance of Nellore bulls than rolling. In studies by Peres (2011), the animals consuming finely ground corn based diets (1.1–1.3 mm average particle size) presented lower fecal starch content (18.75 % vs. 24.78 %), greater average daily gain (1.36 kg vs.1.16 kg) and better feed efficiency (0.160 vs. 0.141), and in the study carried out by Carareto et al. (2010), cattle fed the same finely ground corn based diets presented lower fecal starch content (9.68 % vs. 20.03 %), lower daily dry matter intake (9.37 kg vs. 10.18 kg) and better feed efficiency (0.121 vs. 0.108) when compared to those animals fed dry-rolled corn based diets (3.0–3.2 mm average particle size). In both studies, clinical signs of metabolic diseases were not observed. In the study by Peres (2011) no animal presented liver abscess, regardless of corn processing method. In the research carried out by Carareto et al. (2010) no animal fed finely ground corn presented liver abscess, however, 2.1 % of animals fed dry-rolled corn presented liver abscesses.

In the case of sorghum grains as well as flint corn, due to the dense protein matrix found in the grain endosperm, fine grinding has been preferred in Brazil than coarse grinding in hammer or roller mills.

When corn or sorghum grains were ground (by hammer or roller mills), sorghum presented 90 % of energy value of dent corn (2.94 vs. 3.26 Mcal of metabolizable energy per kg of diet dry matter; respectively), according to data compiled by Owens et al. (1997). In Table 8.5, five compiled studies compared sorghum and corn ground in roller mills fed to finishing beef cattle.

In general, cattle receiving dry-rolled corn based diets presented lower dry matter intake (−3.9 %), greater average daily gain (+5.8 %) and higher feed efficiency (+10.3 %) than animals fed with dry-rolled sorghum.

**Table 8.5** Effects of replacing dry-rolled sorghum for dry-rolled corn in the diets of finishing cattle on feedlot performance

| Reference | Concentrate content % of DM[a] | DM intake variation, % | ADG[b] variation, % | Gain:Feed ratio variation, % |
|---|---|---|---|---|
| Stock et al. (1987) | 90 | −1.9 | +5.7 | +7.6 |
| Stock et al. (1987) | 90 | −4.4 | +5.1 | +9.0 |
| Stock et al. (1990) | 100 | −4.0 | 0 | +4.8[c] |
| Stock et al. (1990) | 90 | 0 | +8.5 | +9.5[c] |
| Stock et al. (1991) | 88 | −5.5 | +14.4 | +21.1[c] |
| Sindt et al. (1993) | 85 | −7.7 | +1.3 | +9.7[c] |
| *Average* | *90.5* | *−3.9* | *+5.8* | *+10.3* |

[a]DM: dry matter
[b]ADG: average daily gain
[c]Significant at ($P<0.05$)

## *High-Moisture Grain Silage*

In the past years, the utilization of high-moisture corn grain silage for ruminant feeding has been increasing in Brazil. The benefic effect of this processing method on the ruminal, intestinal and total tract starch digestibility when compared to grinding or dry rolling is consistent with the reviewed literature (Huntington 1997; Owens and Soderlund 2006). However, there is great inconsistency with respect to animal performance results with this grain processing method. According to data reviewed by Owens et al. (1997), the feeding of high-moisture corn silage or high-moisture sorghum silage did not improve feedlot cattle performance consistently when compared to dry rolling.

Partially, the data inconsistency is due to the variation of moisture content in high-moisture grain silage, because once ensiled materials with moisture content that are lower than 24 % may result in poorer animal performance than the one obtained with dry-processed grains (Owens and Zinn 2005).

American studies comparing high-moisture grain silage to dry-rolling for finishing cattle published since 1995 (not included in the review by Owens et al. (1997)) were compiled in Table 8.6. In all these studies, the moisture content of ensiled grain varied from 28 % to 35 %. In studies by Ladely et al. (1995) high-moisture corn grains were ground, whereas in other experiments the grains were rolled, and then ensiled.

In experiments carried out in Brazil utilizing flint corn, the feeding of high-moisture grain silage based diets to feedlot cattle decreased dry matter intake and increased average daily gain when compared to finely ground or rolled corn (Table 8.7). The increment in feed efficiency reported in national studies when high-moisture flint corn was ensiled was greater than the one reported with dent corn (Table 8.6).

**Table 8.6** Effects of replacing dry-rolled corn for high-moisture corn grain silage in the diets of finishing cattle on feedlot performance

| Reference | Concentrate content % of DM[a] | DM intake variation, % | ADG[b] variation, % | Gain to feed ratio variation, % |
|---|---|---|---|---|
| Ladely et al. (1995) | 90.0 | −15.8[c] | 0 | +17.7[c] |
| Ladely et al. (1995) | 90.0 | −6.2[c] | +2.6 | +11.3[c] |
| Huck et al. (1998) | 90 | −3.8 | −1.1 | +3.4 |
| Scott et al. (2003) | 92.5 | −6.6[c] | −2.0 | +5.0[c] |
| Scott et al. (2003) | 92.5 | −0.9 | +0.5 | +1.8 |
| Corrigan et al. (2009) | 92.5 | −9.9[c] | +1.2 | +12.3[c] |
| *Average* | *91.2* | *−7.2* | *+1.2* | *+8.6* |

[a]DM: dry matter
[b]ADG: average daily gain
[c]Significant at ($P<0.05$)

**Table 8.7** Effects of replacing finely grinding or dry-rolled flint corn for high-moisture flint corn silage in the diets of finishing cattle on feedlot performance (national data)

| Reference | Concentrate content % of DM[a] | DM intake variation, % | ADG[b] variation, % | Gain to feed ratio variation, % |
|---|---|---|---|---|
| Silva et al. (2007) | 60 | −18.10[c] | −1.00 | +22.0[c] |
| Henrique et al. (2007) | 80–88 | −2.71 | +6.25 | +5.9[c] |
| Carareto et al. (2010) | 80–88 | −7.56[c] | +11.00[c] | +19.4[c] |
| *Average* | | *−9.5* | *+5.4* | *+ 15.8* |

[a]DM: dry matter
[b]ADG: average daily gain
[c]Significant at ($P<0.05$)

## *Steam-Flaking*

According to the review by Owens et al. (1997), in finishing diets for feedlot cattle, steam-flaked corn reduced daily dry matter intake in 11.6 % (9.45 kg vs. 8.35 kg), did not affect average daily gain (1.45 kg vs. 1.43 kg) and improved feed efficiency in 12 % when compared to dry-rolled corn. In the case of sorghum, steam flaking reduced dry matter intake in 17 % (10.47 kg vs. 8.68 kg), did not affect average daily gain (1.43 kg vs. 1.40 kg) and increased feed efficiency in 17.4 % when compared to dry rolling. Sorghum grains have a more intense protein matrix involving starch granules than corn grains and that results in lower digestibility of sorghum starch. Therefore, it is expected that steam flaking causes greater increase of energy value in sorghum than in corn, which was confirmed by Owens et al. (1997) who reported that steam flaking increased metabolizable energy of sorghum in 21 % (3.56 vs. 2.94 Mcal per kg of diet dry matter) and of corn in 14.4 % (3.73 vs. 3.26 Mcal per kg of diet dry matter). According to Zinn et al. (2002), NRC (1996) underestimates the energetic value of steam-flaked corn and overestimates the value of dry-rolled corn. According to the authors, appropriate steam flaking process involving dent corn grains results in an increase of 15 % of net energy for maintenance and 18 % of net energy for gain when compared to dry-rolling process.

More recent studies have also confirmed the advantages of corn steam-flaking over dry-rolling consistently (Table 8.8). In general, in these studies, steam-flaked corn density was between 310 and 387 g/L (Zinn 1990; Brown et al. 2000b; Zinn et al. 2002; Sindt et al. 2006; Hales et al. 2010).

The increment of 12.3 % in feed efficiency with steam flaking is in accordance to the value of 12.0 % reported by Owens et al. (1997) who reviewed data published from 1974 to 1995. However, in studies reviewed by Owens et al. (1997), the determining factor of greater feed efficiency of animals fed steam-flaked corn based diets was the reduction of 11.6 % in dry matter intake without negatively impacting average daily gain, whereas in most recent studies, compiled in Table 8.8, the determining factor of greater feed efficiency was the increase of 8.3 % of average daily gain with average dry matter intake reduction of only 3.4 %.

**Table 8.8** Effects of replacing dry-rolled corn for steam-flaked corn in the diets of finishing cattle on feedlot performance

| Reference | Concentrate content % of DM[a] | DM intake variation, % | ADG[b] variation, % | Gain to feed ratio variation, % |
|---|---|---|---|---|
| Barajas and Zinn (1998) | 88.0 | −10.1 | +8.2 | +19.8[c] |
| Huck et al. (1998) | 85.0 | 0 | +7.7 | +8.6[c] |
| Brown et al. (2000a) | 90.0 | −1.2 | +17.7 | +19.8[c] |
| Brown et al. (2000b) | 90.0 | 0 | +8.2 | +7.8[c] |
| Scott et al. (2003) | 92.5 | 0 | +3.4 | +4.3[c] |
| Scott et al. (2003) | 92.5 | 0 | +10.2 | +8.4[c] |
| Macken et al. (2004) | 93.0 | −1.5 | +15.4 | +16.6[c] |
| Corona et al. (2005) | 88.0 | −8.1 | +4.4 | +17.6 % |
| La Brune et al. (2008) | 92.0 | −0.9 | +14.0 | +12.1[c] |
| Leibovich et al. (2009) | 90.0 | −6.8 | +1.3 | +9.0[c] |
| Corrigan et al. (2009) | 92.5 | −8.9 | +0.6 | +11.7[c] |
| *Average* | *90.3* | *−3.4* | *+8.3* | *+12.3* |

[a]DM: dry matter
[b]ADG: average daily gain
[c]Significant at ($P<0.05$)

The studies reviewed by Owens et al. (1997) and the ones compiled in Table 8.8 were carried out in North America, predominantly with *Bos taurus* cattle and dent corn that presents smaller vitreousness than flint corn utilized in South America. Due to the lower starch digestibility of flint corn cultivars (Correa et al. 2002), greater benefits are expected from steam flaking of these materials when compared to cultivars of dent corn (Santos et al. 2011). Four experiments were carried out at University of São Paulo (Peres 2011; Carareto et al. 2010; Marques et al. 2011; Gouvea et al. 2012) to study the effects steam-flaked flint corn on the performance of Zebu cattle finished in feedlot with diets that contained from 68.8 % to 85 % (dry matter basis) of corn (Table 8.9).

As reported for sorghum (Owens et al. 1997), flint corn steam-flaking caused an increase in feed efficiency of cattle finished in feedlot greater than the reported for dent corn. The positive effect of flint corn steam flaking in feed efficiency was two-fold greater when compared to the effect reported for dent corn by Owens et al. (1997; 24.8% vs. 12.0%) and with data compiled in Table 8.8 (24.8 % vs. 12.3 %).

Melo (2015) conducted a study to determine the effect of corn grain processing methods (steam-flaking or grinding) in finishing diets containing 4 %, 7 %, 10 % and 13 % of roughage NDF on performance, and incidence of rumen lesions of feedlot cattle. It was used 240 22-months-old Nellore bulls in this study, and cattle fed steam-flaked corn performed better than those fed ground corn regardless of level of roughage NDF. Also, cattle receiving steam-flaked corn consumed less feed and had heavier carcasses than animals fed ground corn independent of NDF roughage level. On the other hand, rumenitis incidence was not affected by either processing methods or level of roughage NDF in the finishing diets. In a companion study

**Table 8.9** Effects of replacing finely ground or dry-rolled or whole flint corn grain for steam-flaked flint corn in the diets of finishing Nellore cattle on feedlot performance

| Reference | Concentrate content % of DM[a] | Processing method | DM intake variation, % | ADG[b] variation, % | Gain to feed ratio variation, % |
|---|---|---|---|---|---|
| Peres (2011) | 88.0 | Dry-rolling | −2.3 | +22.4[c] | +25.5[c] |
| Carareto et al. (2010) | 80.0–88.0 | Dry-rolling | −9.0[c] | +14.7[c] | +25.9[c] |
| Marques et al. (2011)[d] | 94.0–97.0 | Whole grain | −18.3[c] | −1.0 | +20.7[c] |
| Marques et al. (2011)[e] | 94.0–100.0 | Whole grain | 0.0 | +30.0[c] | +28.7[c] |
| Gouvea et al. (2012) | 88.0 | Fine grinding | −7.90 | +12.5[c] | +23.3[c] |
| *Average* | *80.0–100.0* | | *−7.5* | *+15.7* | *+24.8* |

[a]DM: dry matter
[b]ADG: average daily gain
[c]Significant at ($P<0.05$)
[d]Steam-flaked corn with 6 % forage vs. average value of whole corn grain with 3 % or 6 % of forage in the diet
[e]Steam-flaked corn with 6 % forage vs. whole corn grain with 0 % of forage in the diet

involving 16 cannulated Nellore bulls, Melo (2015) observed greater concentration of propionate in cattle fed steam-flaked corn when compared to those fed ground corn regardless of level of roughage NDF. Likewise, the feeding of steam-flaked corn reduced NDF digestibility, and consequently the acetate production in the rumen. However, no main effect of either processing method or forage NDF level was observed for mean pH and total short-chain fatty acids concentration in the rumen. When fed high-energy diets, it seems that cattle regulate intake according to the fiber content in the diet, because animals fed 4 % of forage NDF consumed less feed (on dry matter basis) than those that were fed diets containing higher levels of roughage NDF regardless of grain processing method, which helps to control rumen fermentation, as well as incidence of rumen lesions, as a smaller amount of substrate will be available to fermentation in the rumen. More studies and different research centers are necessary to determine more safely the real benefit of steam flaking of flint corn in Brazil.

In all studies compiled in Table 8.8, corn was the main source of energy, included from 64.0 % to 82.5 % of diet dry matter. The addition of coproducts to the diets like citrus pulp, soybean hull, corn gluten feed, among others, reduces its starch content. The question is: does the addition of coproducts to the diets reduce the positive effect of steam flaking on animal performance?

Vander Pol et al. 2008 reported that in diets containing 30 % (dry matter basis) of wet distillers grains with solubles, steam flaking did not increase the efficiency of feedlot cattle when compared to dry rolling. Corrigan et al. (2009) reported that while growing doses (0 %, 15 %, 27.5 %, and 40 % of diet dry matter) of wet distillers grains with solubles were added to the diets of finishing cattle, the increment

in feed efficiency caused by steam-flaking when compared to dry-rolling was drastically reduced (+11.6 %, +9.4 %, 0 %, and −1.1 %; respectively).

On the other hand, in two studies conducted by Scott et al. (2003) with feedlot cattle receiving diets containing 32 % of wet corn gluten feed (dry matter basis), steam-flaked corn increased feed efficiency (+6.7 % and +10.4 %) when compared to rolled corn in diets without corn coproduct addition (+4.3 and +8.4 %). Leibovich et al. (2009) did not observe a difference in response to steam flaking compared to dry rolling when the diets contained either 0 % or 15 % (dry matter basis) of wet distillers sorghum grains.

In the study by Macken et al. (2004), the increment in feed efficiency promoted by steam-flaked corn when compared to dry-rolled corn in finishing diets of feedlot cattle was greater (+16.6 % vs. +8.1 %) when diets did not contain 30 % of corn bran (dry matter basis). Likewise, in the study by Gouvea et al. (2012) with flint corn, the substitution of 50 % of cereal grain for pellets of citrus pulp in the diet reduced the positive effect of steam flaking in feed efficiency of finishing beef cattle from +23.3 % to +11.1 % when compared to fine grinding.

Based on the discussion above, the response to steam flaking may vary according to the source and inclusion level of coproducts to the diet of finishing beef cattle.

Regarding sorghum, its nutritional value for feedlot cattle is lower than corn, when both grains are dry-processed. However, when both grains are steam-flaked, the difference of energy value between the grains is smaller or inexistent. According to Owens et al. (1997), the values of metabolizable energy for dry-rolled corn and dry-rolled sorghum were 3.26 vs. 2.94 Mcal per kg diet dry matter, respectively; and for steam-flaked corn and steam-flaked sorghum the values were 3.73 vs. 3.56 Mcal per kg diet dry matter, respectively.

Schake et al. (1976), Brandt et al. (1992) and Huck et al. (1998) reported that animals fed steam-flaked sorghum presented similar average daily gain and feed efficiency when compared to animals fed steam-flaked corn. In a second experiment carried out by Huck et al. (1998) and in a study by Zinn (1991), steam-flaked corn was superior when compared to steam-flaked sorghum.

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# Chapter 9
# Net Nutrient Flux Across the Portal-Drained Viscera and Liver of Ruminants

**Clinton R. Krehbiel, Rufino Lopez, and Matt J. Hersom**

## Introduction

Digestion of feedstuffs and absorption of nutrients are the steps through which animals obtain nutrients from the environment needed to maintain life. Following digestion, absorbed nutrients which are water soluble enter the bloodstream and are transported to the liver by the portal venous system. In addition to exogenous nutrients absorbed from the portal venous system, endogenous circulating metabolites derived from peripheral tissues reach the liver via the hepatic artery. As part of this system, the liver is the central regulator of metabolism that controls the levels of nutrients that will be distributed to the body and ensure that needs are met for maintenance, growth, lactation, reproduction, and physical activities of animals (Seal and Reynolds 1993).

The functional role played by tissues of the portal-drained viscera (**PDV**; reticulo-rumen, omasum, abomasum, small and large intestines, cecum, pancreas, spleen, and mesenteric and omental fat) has a considerable energetic cost and a variable quantity of substrates are metabolized by these tissues (Huntington and Reynolds 1987). Therefore, changes in metabolism of nutrients by the PDV can change the proportions and the absolute quantities of metabolites that become available to the peripheral tissues and consequently can influence the metabolism of the whole animal. Surgical methods have been established and employed to determine

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the net exchange of nutrients across the PDV and liver, and to determine their contribution to whole-body metabolism and energy expenditure. This chapter will summarize the literature regarding net nutrient flux across the PDV and liver relative to some specific production scenarios of ruminant animals.

## Net Flux Measurements

### *Fick Principle*

Net nutrient flux measurement across the PDV and hepatic tissues uses multicatheterized animals and employs the Fick Principle. The Fick Principle assumes that the removal or release of any substance by an organ is equal to the blood flow through that organ multiplied by the venous-arterial concentration difference of the substance as it passes through (Katz and Bergman 1969a; Webster 1974). Blood flow through the PDV can be measured by a primed, continuous infusion of para-aminohippurate (**PAH**; Symonds and Baird 1973; Bergman 1975; Huntington 1982) or other markers (Fries and Connor 1961; Bensadoun and Reid 1962). Venous-arterial concentration differences are used to calculate net flux; therefore, positive rates denote net release of a metabolite and negative rates denote net removal (or uptake) of a metabolite (Huntington and Eisemann 1988). In order for net flux calculations to be accurate, near steady state conditions and accurate blood flow measurements should be obtained (Chase et al. 1977), or adequate samples should be collected across the day to account for diurnal variation (Whitt et al. 1996).

Katz and Bergman (1969a) developed a single indicator-dilution technique to simultaneously measure both portal and hepatic venous blood flow. This method involves the placement of chronic indwelling catheters into a mesenteric vein, hepatic portal vein, hepatic vein and an artery (Fig. 9.1), and infusion of PAH (or other marker) into the mesenteric vein to measure downstream dilution. Simultaneous blood samples are then slowly withdrawn from the hepatic portal, hepatic venous and arterial catheters (Zierler 1961). Plasma and whole blood flow rates through the PDV and liver can be calculated as $BF = IR^{PAH}/Cv^{PAH} - Ca^{PAH}$, where BF represents the plasma or whole blood flow rate through the tissue (mL/min), $IR^{PAH}$ is the infusion rate of PAH (mg/min), and $Cv^{PAH}$ and $Ca^{PAH}$ are the PAH concentrations (mg/mL) in venous and arterial plasma or whole blood, respectively. Portal and hepatic vein flow rates are measured directly and hepatic artery flow can be calculated as the difference between hepatic and portal blood flow. Net flux of metabolites and hormones across the PDV, hepatic, and total splanchnic (PDV + liver) vascular beds can be calculated as PDV flux = PBF × (Cp − Ca), Hepatic flux = PBF × (Ch − Cp) + ABF × (Ch − Ca), and Total splanchnic flux = PDV flux + hepatic flux, where ABF and PBF are the blood flow rates (l/h) in the artery and portal vein, and Ca, Cp, and Ch are the metabolite or hormone concentrations in the arterial, portal, and hepatic blood samples, respectively. Several researchers have employed this

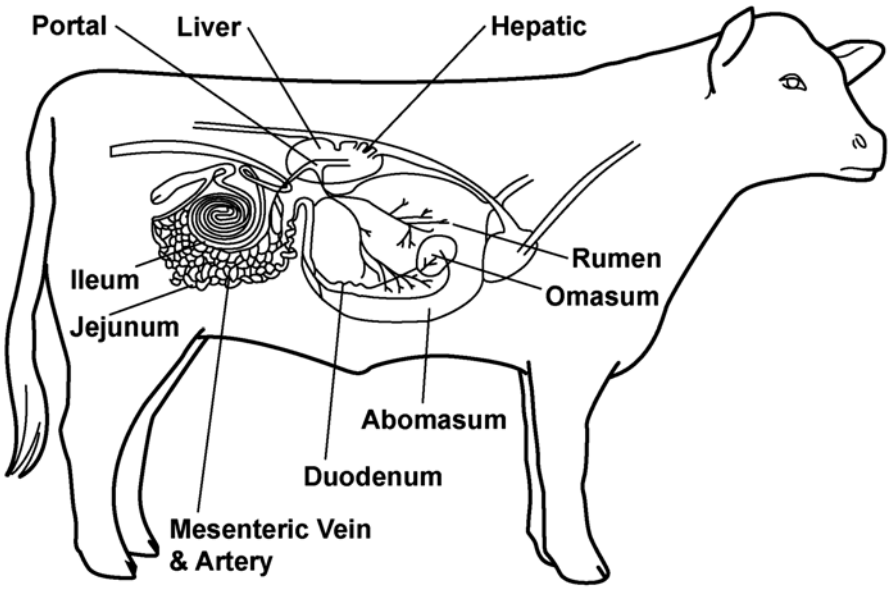


**Fig. 9.1** Anatomical location of catheters places in a hepatic vein, hepatic portal vein, and mesenteric vein and artery to determine net flux of nutrients and hormones across the portal-drained viscera and liver

technique (Katz and Bergman 1969b; Baird et al. 1975; Huntington 1989). Metabolite extraction by the liver can be calculated for each metabolite and hormone as Hepatic flux/(PBF × Cp) + (ABF × Ca), where ABF, PBF, Ca, and Cp are the same as described above (Brockman et al. 1975).

Bergman (1975) noted that experimental errors using the Fick Principle could be large when venous-arterial concentration differences are small and blood flow is high. The proper placement and maintenance of chronic indwelling catheters is critical for interpretation of hepatic and portal flux measurements (Gross et al. 1988). Measurements of portal absorption can underestimate actual absorption from the gut lumen because a proportion of the arterial supply of these nutrients is metabolized during a pass through PDV tissues (Bergman 1975). Bergman (1975) concluded that a combination of venous-arterial concentration differences and blood turnover measurements using infusions of radiolabelled substrates to trace metabolism would provide a more detailed description of absorption than either method used alone. Nevertheless, the use of net nutrient flux across the PDV and liver is a good approach to studying metabolism as it allows measurements to be taken with minimal imposition to the normal physiological state of the animal.

## *Surgical Procedures*

Surgical procedures for placing catheters for net flux measurements have been described by Huntington et al. (1989) and Ferrell et al. (1991) and detailed by D.L. Harmon (University of Kentucky; personal communication). Animals should be worked gently and calmly, which minimizes stress of handling prior to, during, and after surgery. For opening, a paracostal incision of approximately 35–40 cm (cattle) or 25 cm (sheep) is made 3–5 cm caudal to the last rib and approximately 10 cm below the lumbar transverse process. Muscle layers and peritoneum are cut. Bleeding from transected vessels is stopped by clamping, suturing, or cauterizing. The intestines are covered with sterile towels soaked in warm sterile saline. Hand retractors are used to move the intestines to visualize the base of the liver. A rib retractor elevates the rib cage for exposure of the area. Starting at the gall bladder, the insertion of the lesser omentum is separated by blunt dissection from the liver dorsally. In cattle, a lymph node 1–3 cm wide is attached to the lateral surface of the portal vein at the liver. The lymph node is removed by digital blunt dissection and the portal vein just caudal to the liver is freed of surrounding pancreas and connective tissue. When complete, 2–4 cm of the lateral surface of the portal vein is visible. A finger can be inserted completely around the medial surface. The liver is palpated cranial to the gall bladder to feel indentations corresponding to hepatic vessels for hepatic catheterization. Real-time ultrasound may be used to scan the liver and identify the vena cava and hepatic vasculature.

*Portal Vein Catheterization*. Insertion and an anchor suture site are selected on the wall of the portal vein. Number 2 nonresorbable suture swaged to a large atraumatic half circle needle is passed through a 7-mm bite of the lateral wall of the portal vein and one arm of the suture is firmly tied around the silicone sheath of the portal catheter. The tip of a 0.96-mm wire guide is placed in a 15-gauge, 5-cm needle, and the lateral surface of the portal vein is punctured cranial to the anchor suture. The guide is then threaded into the vein. The guide is retained by squeezing the vein, the needle is removed completely from the guide, and the catheter is inserted into the vein by threading it over the guide. A polished teflon catheter is inserted into the vein along the visceral surface of the left lobe of the liver. Location of the tip is checked by palpation and determining free patency with a syringe. The catheter is further secured by tying it to the surface of the pancreas with three interrupted sutures. An alternative approach is to simply puncture the portal vessel with a 14-guage needle and insert a tygon catheter (Ferrell et al. 1991). This catheter is secured as described above.

*Hepatic Vein Catheterization*. The diaphragmatic surface of the liver is punctured with a 14-gauge, 5-cm long hypodermic needle over a hepatic vein, usually 2–6 cm from the lateral edge of the liver. Ultrasound may be used to help see the needle and watch it puncture the hepatic vein 2–6 cm from the lateral surface. A wire guide or (alternatively) small teflon tubing is inserted through the needle and into the hepatic vein. A larger catheter is then threaded over the wire guide and the wire guide

removed. Cuffs, visible outside the liver, are used to suture the catheter to the liver with 0 nonresorbable suture swaged to an atraumatic half circle needle. A second approach is to pass the tygon catheter with no cuffs through the 14-gauge needle into a hepatic vein. The catheter can then be secured to the liver. The tip of the catheter can be marked to ensure the appropriate placement in the vessel.

*Mesenteric Vein and Arterial Catheterization*. Tygon catheters are placed in one or two mesenteric veins and a mesenteric artery located either at the ileo-cecal junction or mid jejunum. Location of catheters at these sections of the small intestine are to assure maximum distance from the portal vein as a marker will be infused into the mesenteric vein catheters and a greater distance from the portal vein means greater mixing of the marker in the blood stream. The omentum is gently pulled dorsally and cranially by grasping the ventral edge within the visceral cavity. The small intestine is then exteriorized and displayed on a surgical drape. Towels soaked with saline are used for protecting the intestines and keeping them moist. Catheters are inserted into smaller branches between the major venous arcade and the small intestine. The vein and artery are cleared of surrounding tissue by blunt dissection. Several individual sutures 5- to 7-cm apart are used to lead the catheters toward the portal vein and away from the small intestine.

To allow for measurement of blood flow and net flux across the mesenteric-drained viscera (**MDV**), or post-stomach tissues of the PDV, a double-catheter apparatus can be inserted through a branch of the anterior mesenteric vein as described by Huntington (1989). The catheter for sampling of mesenteric blood must be introduced at a point where the capillary drainage from the small intestine first enters the main mesenteric arcade and the tip inserted to a point prior to the junction with the ileocecal vein. A second catheter, for infusion of the downstream dilution marker (PAH), must be inserted approximately 20–25 cm upstream of the sampling catheter.

*Closing*. Intestines are rinsed with sterile saline, blood clots and other detached tissue are removed, and a final rinse with sterile saline is administered before returning intestines to the visceral cavity and repositioning the omentum. All catheters are routed through the abdominal muscles and under the skin to the midpoint of the back. Catheters exit at that point and are stored in a cloth pouch secured on the back of the animal with tag cement. Before exteriorization, catheters are swabbed with an antiseptic to minimize the possibility of infection in the exit wound. The peritoneum, muscle layers, and skin are sutured separately with a continuous interlocking stitch. The operative site is cleaned, sprayed with germicide, and the incision is coated with an antiseptic gel. The animal is placed in a recovery stall.

The animal should be allowed to fully regain reflexes before moving to permanent housing. The animal is treated with an antibiotic and analgesics immediately post surgery. Further antibiotic treatment is dependent on rectal temperature of the animal. The animal should be observed for signs of infection and any inflammation or abnormality recorded and treated. Daily feed intake should be monitored to assure that adequate nutrients are consumed. Sutures can be removed 7–10 days following surgery.

### *Intragastric Infusion*

The alteration of nutrients in feedstuffs by microbial fermentation in the rumen makes the nutrition of ruminants difficult to study. Both volatile fatty acids (**VFA**) and microbial protein can vary in amounts produced depending on the composition and degradability of the diet (Orskov et al. 1979b). Orskov et al. (1979b) developed the technique of intragastric infusion so that ruminants can be maintained solely on infusions of purified nutrients into the digestive tract. The technique of intragastric infusion offers distinct advantages over conventional feeding by removing the confounding effect of microbial fermentation of feedstuffs (Gross et al. 1990).

Several researchers have infused VFA and protein sources into the digestive tract of ruminants (Annison et al. 1957; Leng and Leonard 1965; Judson and Leng 1973; Weekes and Webster 1975; De Jong 1982), but maintaining sheep (Gross et al. 1990; Orskov et al. 1979a) and cattle (MacLeod et al. 1982) on total intragastric infusion of nutrients has been less extensively studied. Normally, VFA and a buffer solution are infused into the rumen and a protein source is infused into the abomasum. Other nutrients such as glucose, lipids, ketone bodies, and peptides can be infused in addition to VFA and protein (Asplund et al. 1985; Istasse et al. 1987).

The mechanics of the intragastric infusion technique are described in detail by Orskov et al. (1979b) for use in lambs. MacLeod et al. (1982) reported few problems in adapting the same technique to mature cows and steers. One problem was that an increase in osmotic pressure to 400 mosmol/L was associated with a fall in pH. They corrected the problem by diluting the ruminal contents with an additional infusion of water.

Extrapolation of data obtained with intragastrically infused ruminants must be carefully evaluated since the amount of indigestible material passing through the intestines is small and thus tissue nutrient metabolism may be altered (Owens 1987). Time, cost, and difficulty in maintaining animals by infusion are other disadvantages. Nevertheless, the intragastric infusion model in ruminants has potential for evaluating interactions between different nutrients and determining nutrient absorption and utilization by the PDV and liver tissues.

## Net Nutrient Flux

The gastrointestinal tract and liver account for substantial proportions of whole-body heat production (40–65 %; Burrin et al. 1989; Reynolds and Tyrrell 1989); thus, factors that affect their metabolism are of paramount importance to heat loss and energy balance. Because of their high rate of metabolism and heat energy combined with their central role in the assimilation and processing of dietary nutrients, diet-induced differences in whole-body tissue energy gain might be attributed to differences in mass and metabolism by visceral organs (Reynolds et al. 1991a). Reynolds et al. (1991a) studied the effects of forage-to-concentrate ratio on energy

metabolism in growing heifers. Blood flow through the PDV and liver was greater when heifers were fed 75 % alfalfa versus 75 % concentrate. Increased PDV and liver $O_2$ uptake accounted for 44 % and 72 % of heat increment for the 75 % concentrate and 75 % alfalfa diets, respectively. Greater PDV uptake of $O_2$ accounted for 72 % of the decrease in whole-body tissue energy gain of heifers fed 75 % alfalfa versus 75 % concentrate when metabolizable energy (**ME**) intake was equal, resulting in the conclusion that greater utilization of ME for whole-body tissue energy in beef heifers fed diets lower in forage content and having greater ME density was predominantly caused by changes in PDV $O_2$ consumption. These differences in metabolic activity in visceral tissues might be attributed to differences in the work of digestion and absorption as well as the components of absorbed ME. This concept of metabolic "toll-keeping" as described by Reynolds (2001) is supported by comparisons of net nutrient release by the PDV with estimates or measurements of rate of absorption of those nutrients from the lumen of the digestive tract.

As defined, the PDV represents an anatomical and vascular aggregation of heterogeneous tissue types. In the case of glucose and amino acids, whereas some absorptive metabolism may occur in the small intestinal enterocytes, those cells represent a small proportion of the total PDV. Many other PDV tissues such as the rumen and hindgut epithelium, gut muscle, pancreas, and adipose have substantial glucose and amino acid requirements. As previously suggested, any use of these nutrients from arterial blood will mask an equivalent release into venous blood. To obtain measurements of gross or "unidirectional" rates of nutrient removal or release by splanchnic tissues, measurements of net flux must be combined with isotopic labeling to trace metabolism of a specific nutrient (Bergman 1975).

The effect of diet on blood flow through the organs of the gastrointestinal tract and liver is of great importance. Blood flow from the gastrointestinal tract assimilates absorbed nutrients for delivery to the liver. Metabolism of nutrients by the liver and flux out of the liver dictates the nutrients and their concentrations available for use by peripheral tissues. Several studies have been conducted to determine the effects of diet on blood flow and net flux of nutrients and oxygen consumption across the PDV and liver. Several of these experiments are summarized below.

## *Roughage Diets*

*Blood Flow*. Blood flow across the PDV, liver, and arterial blood flow was not affected by forage type (Patil et al. 1996; Park et al. 1997). Similarly, Goetsch et al. (1997a) reported that arterial, portal, or hepatic blood flow was not affected by forage type or particle lengths even though dry matter intake (**DMI**) was affected. In addition, inclusion of corn or alfalfa hay in hay diets increased total DMI but did not affect blood flow (Goetsch et al. 1997b). In contrast, significant increases in DMI (400 g/day) of alfalfa pellets increased PDV blood flow by 31 % in sheep (MacRae et al. 1997) and an increase of 250 g/day of hay increased PDV blood flow by 14 % in ewes (Han et al. 2002). Similarly, Reynolds et al. (1991b) reported a near

equal increase in portal and hepatic blood flow with increased DMI. A 39 % increase in DMI resulted in a 46 % and 44 % increase in portal and hepatic blood flow, respectively (Reynolds et al. 1991b). The response in blood flow to DMI seems to vary between forage versus concentrate diets. In contrast to the aforementioned experiments, Huntington et al. (1996) reported decreased portal and hepatic blood flow in steers when dietary concentrate was increased from 27 % to 63 % with a 1 kg difference in DMI between the two diets. Seal et al. (1992) also reported decreased mesenteric and portal blood flow between all forage and 50:50 forage:concentrate diets. Therefore, it is apparent that blood flow across the PDV and liver responds to dietary type in addition to DMI.

Blood flow responds to changes in DMI and energy content of the diet. As indicated, differences in forage type, processing, or supplementation do not appear to have much effect on blood flow. However, when the roughage content of the diet is replaced by concentrates blood flow decreases. Huntington et al. (1996) noted that as diets increased from 0 % to 90 % concentrate, hepatic blood flow decreased from 850 to 795 L/h, PDV blood flow from 750 to 620 L/h, and MDV blood flow from 270 to 250 L/h. Apparently differences in fermentation in the rumen and potentially post-ruminal digestion can have significant effects on blood flow. Reynolds et al. (1991a) suggested that changes in blood flow in response to forage:concentrate ratio occur too rapidly to be attributed solely to changes in tissue mass. In addition to mass, intake changes result in differences in metabolic activity of visceral tissues. For example, it is well established that varying dietary fiber content alters the pattern of VFA absorbed and associated hypertrophy of the ruminal epithelium. Webster (1980) suggested that acetate and butyrate stimulate hypertrophy of ruminal epithelium, thereby increasing energy costs when higher roughage diets are fed. Therefore, differences in metabolic activity of visceral tissues related to work of digestion and patterns of nutrient absorption may alter blood flow across portal and hepatic tissues (Reynolds et al. 1991a).

*Oxygen Consumption*. Oxygen consumption or the energy expenditure by splanchnic tissues constitutes a major portion of the maintenance energy requirements of animals (Crooker et al. 1991). Oxygen consumption by the PDV was similar for lambs consuming alfalfa, ryegrass-wheat, or bermudagrass hay (Park et al. 1997), lambs consuming warm- or cool-season grass hay with or without alfalfa hay (Patil et al. 1996), and lambs consuming ryegrass-wheat hay with supplemental corn or alfalfa (Goetsch et al. 1997b). An increase in PDV oxygen consumption was observed in lambs consuming ground and pelleted bermudagrass or ryegrass-wheat hay (Goetsch et al. 1997a). Han et al. (2002) reported increased oxygen consumption by the PDV of ewes consuming hay with additional infused urea or casein, and a linear increase in PDV oxygen consumption with increasing dietary bulk. Ruminal oxygen consumption was not affected by ruminal nutrient infusion or dietary bulk, suggesting increased oxygen consumption by post-ruminal tissues. Because metabolism of the splanchnic tissues (PDV and liver) is driven by DMI, they make up a substantial proportion of whole body oxygen consumption (Reynolds 2001). Increasing DMI of a 75 % alfalfa diet increased total splanchnic tissue oxygen

consumption, and accounted for 72 % of the increase in whole body oxygen consumption (Reynolds et al. 1991a). Splanchnic tissue oxygen consumption was similar for lambs consuming ryegrass-wheat hay alone or supplemented either with corn and/or alfalfa hay (Goetsch et al. 1997b). The inclusion of 20 % alfalfa hay in the diet of lambs consuming either warm- or cool-season hay increased splanchnic tissue oxygen consumption by 16 %. In addition, grinding and pelleting alfalfa hay increased splanchnic tissue oxygen consumption by 17 % in lambs consuming either bermudagrass or ryegrass-wheat hay (Goetsch et al. 1997a).

Oxygen consumption by the PDV and total splanchnic tissues generally increases with increasing DMI. Similar to blood flow, increased DMI does not appear to be solely responsible for increased oxygen consumption by the splanchnic tissues; increased dietary bulk also increased oxygen consumption. Increasing the amount of nutrients available for digestion, absorption, and metabolism increases the workload of the splanchnic tissues, and thereby increases energy consumption by those tissues. Greater dietary bulk also increases mechanical workload.

*Flux of Nitrogenous Nutrients*. Release of ammonia N by the PDV was increased by the inclusion of alfalfa in ryegrass-wheat hay diets and warm-season grass hay diets (Goetsch et al. 1997b; Patil et al. 1996, respectively). The inclusion of alfalfa in the diet provided additional ruminally fermentable N that was absorbed across the rumen. Increasing DMI of a 75 % alfalfa diet by 39 % increased ammonia N release by 45 % from the PDV of heifers (Reynolds et al. 1991b). In contrast, grinding and pelleting grass hays resulted in a decreased release of ammonia N from the PDV (Goetsch et al. 1997a), attributed to a decrease in ruminal protein degradation due to an increased rate of passage when the hay was processed. Infusion of 8.5 g/day of urea and 33 g/day of casein into the rumen increased PDV ammonia N flux in ewes by 10.4 mmol/h compared with ewes only infused with urea (Han et al. 2002). Huntington et al. (1996) reported no difference in PDV release of ammonia N in steers consuming 73 % or 47 % roughage diets. The PDV release of ammonia N was offset by a net removal of ammonia N by the liver that resulted in a net utilization of ammonia N by the splanchnic tissues (Patil et al. 1996; Goetsch et al. 1997a, b). Removal of ammonia N by the splanchnic tissues increased with increasing DMI (Reynolds et al. 1991b). In general, increasing ruminally degradable N via increased DMI or supplementation increases the release of ammonia across the PDV and subsequent removal of ammonia N by the liver.

Unlike ammonia N PDV flux, urea-N PDV flux is generally negative (net removal) and not affected by forage type (Goetsch et al. 1997a; Park et al. 1997), form of the diet (Goetsch et al. 1997a), or addition of corn or alfalfa to ryegrass-wheat hay diets (Goetsch et al. 1997b). In contrast, Huntington et al. (1996) reported decreased urea-N removal by the PDV when concentrate level was increased. This may have resulted from greater ruminal fermentation with increasing level of concentrate and more efficient use of ruminal ammonia N by the ruminal microorganisms. The liver releases urea N that results in a positive flux of hepatic urea N (Patil et al. 1996; Goetsch et al. 1997a, b). Alfalfa hay or the inclusion of alfalfa in ryegrass-wheat hay increased hepatic urea-N flux (Park et al. 1997; Goetsch et al.

1997b, respectively). Increased liver urea-N release was reported to account for 16 % of the increase in oxygen consumption by the liver with increased intake of 75 % alfalfa diets (Reynolds et al. 1991a). Release of urea N by the liver, which is greater than the removal by the PDV, resulted in a net release of urea N across splanchnic tissues (Patil et al. 1996; Goetsch et al. 1997a, b). Release of urea N from splanchnic tissues would supply N for recycling (Huntington et al. 1996).

Release of alpha-amino N (**AAN**) from PDV was 56 % greater in alfalfa hay diets compared with ryegrass-wheat or bermudagrass hay diets (Park et al. 1997). Ryegrass-wheat hay had 11 % greater AAN PDV flux than bermudagrass hay diets (Goetsch et al. 1997a). Inclusion of alfalfa into warm- or cool-season grass hay diets increased AAN PDV flux compared to grass hay diets only (Patil et al. 1996). However, the inclusion of corn or alfalfa into ryegrass-wheat hay diets resulted in similar AAN PDV flux in wethers. Similarly, Reynolds et al. (1991b) and Huntington et al. (1996) reported no differences in PDV AAN flux in cattle consuming diets that differed in forage:concentrate ratio. Interestingly, increasing dietary bulk by feeding increasing amounts of hay coupled with infusion of urea and casein resulted in a linear increase of amino acid MDV flux in ewes (−3.03, 6.45, 12.21 mmol/h; low, medium, high bulk, respectively; Han et al. 2002). Due to a low availability of energy for microbes, casein added to the rumen may have been rapidly degraded and absorbed as ammonia so that the net release of amino acids increased linearly as the amount of hay in the diet increased. Increased MDV flux of amino acids likely resulted from increased synthesis of microbial cell protein (Han et al. 2002). Hepatic removal of amino acids can be used as possible gluconeogenic precursors or urea cycle intermediates (Reynolds et al. 1991b).

Flux of nitrogenous nutrients across the PDV appears to be primarily driven by crude protein content of the diet. Increasing crude protein in the diet by increasing intake or supplementation of high protein sources increased PDV release of ammonia N and AAN. The source of the increase in ammonia N is likely the rumen when ruminal digestible protein is supplied. Likewise, the increase in AAN might be from increased microbial flow to the small intestine or increased flow of ruminal undegradable protein. Removal of amino acids by the liver is related to need for synthesis of glucose, urea, and protein. Elevated ammonia absorption or decreased glucogenic precursors absorption can increase hepatic uptake of amino acids. High PDV release and hepatic removal of ammonia increases ureagenesis as well as N required from blood amino acids. The carbon skeletons provided by the sources of AAN can be utilized as gluconeogenic precursors. Urea N removal by the PDV is primarily a result of recycling urea N to the rumen. However, the increased release of urea N from the liver for recycling is a source of energy expenditure incurred by the splanchnic tissues. Adequate ruminally degradable protein in the diet may have the potential to decrease energy expenditure by the liver.

*Flux of Energetic Nutrients*. Because of ruminal fermentation little starch reaches the lower gastrointestinal tract for digestion and absorption when high-roughage diets are fed. When starch is digested in the lower gastrointestinal tract is used by the tissues of the gastrointestinal tract. Therefore, the PDV is generally a net utilizer

of glucose, and removal of glucose across the PDV occurs in roughage-based diets (Patil et al. 1996; Goetsch et al. 1997a; Han et al. 2002). In the preceding reports, PDV glucose flux was not affected by grass hay type or the inclusion of alfalfa hay, level of bulk in the diet, or ruminal infusion of urea and casein. In addition, Reynolds et al. (1991b) reported that increasing DMI of an alfalfa diet increased PDV removal of glucose. In contrast, Huntington et al. (1996) reported decreased glucose removal by the MDV when concentrate level was increased from 27 % to 63 %. Net release of glucose by the MDV indicates increased starch flow to the small intestine and increased post-ruminal digestion of starch when concentrate levels were increased in the diet. Because PDV glucose flux is negative when roughage diets are grazed or fed, hepatic glucose flux and the subsequent splanchnic tissue flux must be positive to maintain adequate glucose concentrations for metabolic needs of peripheral tissue. In the studies of Patil et al. (1996) and Goetsch et al. (1997a), which utilized animals that had similar nutrient requirements, hepatic and total splanchnic glucose flux were similar regardless of diet type, supplementation, or diet form. However, Reynolds et al. (1991b) reported a 55 % increase in hepatic and a 32 % increase in total splanchnic release of glucose when alfalfa intake increased by 39 %. Release of glucose from the liver and total splanchnic tissues in light of extraction of glucose by the PDV indicates substantial gluconeogenesis.

The primary gluconeogenic precursor that arises from ruminal fermentation is the VFA propionate. Because VFA are a product of ruminal fermentation, the PDV releases VFA (Huntington et al. 1996; Patil et al. 1996; Goetsch et al. 1997a; Han et al. 2002). Level of nutrition does affect acetate and propionate PDV flux. Inclusion of alfalfa hay in warm- or cool-season grass hay diets increased propionate PDV flux (Goetsch et al. 1997a). Likewise the addition of urea or casein to grass hay diets increased propionate PDV flux (Han et al. 2002). Acetate release by the PDV was decreased when roughage level was decreased from 73 % to 37 %, but propionate was not affected (Huntington et al. 1996). Because propionate is a gluconeogenic precursor, the liver removes it, whereas acetate is used for lipid synthesis and is released from the liver on a net basis. Extraction of propionate was not affected by hay type, supplementation (Patil et al. 1996), or form of the diet (Goetsch et al. 1997a), and low total splanchnic release of propionate resulted from roughage diets (Huntington et al. 1996; Patil et al. 1996; Goetsch et al. 1997a).

Lactate in portal blood can come from two sources, ruminal absorption and glycolysis in the post-ruminal digestive tract (Reynolds and Huntington 1988a; Eisemann et al. 1997). Contribution of lactate from either ruminal or adipose sources is not well defined. In general, lactate from adipose tissue is modulated by the nutritional status of the animal (fasting or fed) and also by the degree of obesity. During prolonged fasting conditions or low levels of circulating glucose, adipose tissue increases lactate production, particularly in the mesenteric tissue, and contributes to hepatic gluconeogenesis. In contrast, under conditions of high insulin, adipose tissue increases circulating levels of lactate and facilitates glycogen synthesis in the liver. The ruminal contribution of lactate from ruminants consuming roughage-based diets may be minimal. However, Reynolds et al. (1991b) reported increased PDV lactate release in heifers with increased intake of 75 % alfalfa diets.

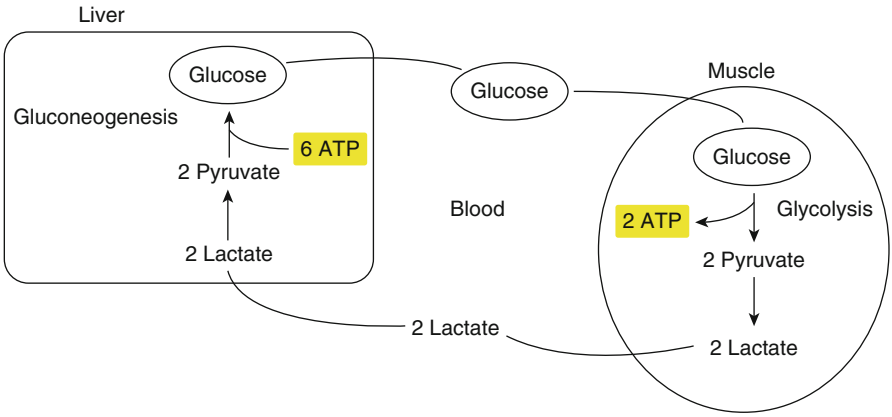


**Fig. 9.2** The Cori cycle

Interestingly, PDV lactate release was similar between 75 % alfalfa and 75 % concentrate diets. Han et al. (2002) reported similar PDV lactate flux between diets differing in bulk from hay intake and nutrients supplied by infusion. Ruminal contribution to net PDV lactate flux varied from 21 % in low bulk, low nutrient supplementation to 40 % in medium bulk, high nutrient supplementation. Goetsch et al. (1997a) reported a 50 % increase in PDV flux of lactate when bermudagrass and ryegrass-wheat hay was pelleted as opposed to coarsely chopped. The liver extracts nearly all lactate resulting in removal by the liver equal to PDV flux and therefore splanchnic flux is near zero (Goetsch et al. 1997a). Removal of lactate by the liver is an important factor in gluconeogenesis and transamination in the Cori cycle (Fig. 9.2).

Flux of energetic nutrients across the PDV appears to be entirely diet dependent. Roughage diets increase the uptake of glucose by the PDV with some exceptions. Addition of concentrate sources into the diet decreases the removal of glucose by the PDV. Because of the large uptake of glucose by the PDV, the liver synthesizes most (90 %) of the glucose for peripheral use, although the kidney makes some (10 %). Net flux of VFA and lactate are diet dependent. Release of VFA and lactate by the PDV is counterbalanced by uptake by the liver for metabolism into substrates that are used by peripheral tissues. Acetate is an exception because it can be utilized directly for fatty acid synthesis in peripheral tissues.

## *Concentrate Diets*

*Blood Flow*. Eisemann et al. (1996) examined the pattern of blood flow change in growing steers as they aged and gained BW. Portal vein and liver blood flow increased concomitant with DMI, age, and BW. Blood flow reached a plateau at 400 kg of BW and 400 days of age. Increased DMI of a 75 % concentrate diet

increased portal and hepatic blood flow by 41 % and 39 % in heifers and 33 % and 29 % in steers (Reynolds et al. 1991b, 1992). Burrin et al. (1989) fed pelleted diets that were 67 % corn, 20 % alfalfa at either ad libitum or at maintenance levels to growing lambs and measured blood flow. Arterial, portal, and hepatic blood flows increased 44 %, 5 %, and 21 %, respectively, in ad libitum fed lambs during the 21 days feeding period; whereas, blood flows in maintenance-fed lambs decreased 32 %, 7.5 %, and 16 % from days 0 to 21. Increases from low to medium (39 %) and medium to high (33 %) DMI resulted in 40 % and 47 % increase in PDV blood flow and 79 % and 30 % increase in total splanchnic blood flow in steers, respectively (Lapierre et al. 2000).

Differences in corn and sorghum processing (dry-rolled vs. steam-flaked) resulted in similar mesenteric, ruminal, portal, and hepatic blood flows (Alio et al. 2000; Theurer et al. 2002). Different undegradable intake protein (**UIP**) supplements to corn, pelleted corncob diets had no effect on arterial blood flow (Bonhert et al. 1999). Supplementation with UIP supplements decreased portal blood flow by 14 % and hepatic blood flow by 17 % compared with soybean meal (Bonhert et al. 1999). Intraruminal infusion of VFA regardless of concentration level increased portal blood flow in sheep by 23 % compared with non-infused sheep (Kristensen et al. 2000). However, Krehbiel et al. (1992) observed no increase in portal or hepatic blood flow with increasing intraruminal infusion of butyrate. Lobley et al. (1998) reported that infusion of amino acids into the mesenteric vein increased arterial blood flow by 44 % and decreased portal and hepatic blood flow by 11 % and 7 %, respectively, compared with pre-infusion blood flow. Reasons for variation in blood flow with various nutrients are not clear. In general it appears that increasing energy substrates increases portal and hepatic blood flow, whereas increasing postruminal amino acids decreases portal and hepatic blood flow.

*Oxygen Consumption*. Oxygen consumption by the PDV and liver in growing steers increased as steers aged, DMI increased, and steers gained BW (Eisemann et al. 1996). Liver oxygen consumption was greater than PDV oxygen consumption and total splanchnic tissue oxygen consumption was 58 % of whole body oxygen consumption at 236 kg of BW and 66 % of whole body oxygen consumption at 522 kg of BW (Eisemann et al. 1996). Differences in DMI of concentrate have been reported to affect oxygen consumption by the PDV, liver, and total splanchnic tissues. Maintenance level of intake resulted in a 30 % reduction in PDV oxygen consumption in lambs after 21 days and no change in PDV oxygen consumption in ad libitum-fed lambs during a 21-day feeding period (Burrin et al. 1989). Liver oxygen consumption increased by 29 % in ad libitum fed lambs and decreased by 29 % in maintenance-fed lambs after 21 days; total splanchnic tissue oxygen consumption was unchanged in ad libitum fed lambs, but was decreased by 30 % in maintenance-fed lambs after 21 days compared to day 0 values (Burrin et al. 1989). Reynolds et al. (1992) reported a 44 % increase in DMI (g/kg of $BW^{0.75}$) by growing steers resulted in 30 %, 39 %, and 34 % increase in PDV, hepatic, and total splanchnic tissue oxygen consumption, respectively. Lapierre et al. (1999) reported a linear increase in $CO_2$ production with increasing intake of a 64 % corn diet. Infusion of amino acid solution into the mesenteric vein resulted in a 35 % increase in oxygen

consumption by the PDV and a 29 % increase in liver oxygen consumption (Lobley et al. 1998). In each of the preceding studies, increasing DMI increased oxygen consumption by substantial amounts. In addition, supplying additional nutrients by supplementation or infusion that increased the digestive and absorptive load for the PDV increased oxygen consumption. In these experiments similar trends were also observed for blood flow through the PDV, liver, and total splanchnic tissues. Therefore, increases in blood flow are often associated with increases in oxygen consumption by splanchnic tissues. One exception might be increasing amino acid concentrations in the mesentery.

*Flux of Nitrogenous Nutrients*. Ammonia N flux across PDV in growing steers varied curvilinearly as steers aged, increased DMI, and gained BW (Eisemann et al. 1996). Peak ammonia N release from the PDV appeared to be at 87 g/kg of $BW^{0.75}$ (Eisemann et al. 1996). Removal of ammonia N by the liver exhibited a nearly inverse response to PDV ammonia N flux. The inverse relationship of PDV and hepatic flux resulted in a low release of ammonia N by total splanchnic tissues (Eisemann et al. 1996). Whitt et al. (1996) examined PDV and hepatic ammonia N flux during a 24-h period in steers fed twice daily. Ammonia N PDV flux peaked 1.5 h after feeding and decreased to daily average (131 mmol/h) by 5 h after feeding. Hepatic ammonia N flux was a mirror image of PDV flux in steers. The removal of ammonia N by the liver is essential because of ammonia's potential toxicity. Reynolds et al. (1991b, 1992) reported that a 45 % increase in DMI of high-concentrate diets resulted in 31 % and 43 % increased PDV release and hepatic uptake of ammonia N, respectively. Similarly, differences in low and high DMI resulted in a 33 % increase in ammonia N PDV release and a 35 % increase in hepatic removal, whereas increasing DMI from medium to high increased ammonia N PDV release by 19 % and increased hepatic removal by 16 % (Lapierre et al. 2000). Splanchnic tissue flux of ammonia N was unaffected by DMI (Reynolds et al. 1992; Lapierre et al. 2000).

Differences in grain processing did not affect PDV, hepatic, or splanchnic ammonia N flux (Alio et al. 2000; Theurer et al. 2002). Furthermore, addition of energy in the form of glucose or starch through abomasal infusion to a high-concentrate diet did not alter PDV release of ammonia N in beef heifers (Huntington and Reynolds 1986). Bonhert et al. (1999) reported a decrease in ammonia N PDV flux with increasing UIP supplementation in lambs. The decrease in PDV ammonia N release was coupled with a decreased hepatic removal of ammonia N, but similar total splanchnic flux.

Urea N removal by PDV increased in growing steers as they increased DMI, age, and $BW^{0.75}$ (Eisemann et al. 1996). Hepatic flux exhibited a more dramatic increase in the rate of release, and splanchnic flux increased in the overall rate of release as steers aged, increased DMI, and gained BW (Eisemann et al. 1996). In steers fed twice daily, PDV urea-N flux resulted in a net uptake by the PDV after the initial feeding of the day and release of urea N after the second feeding, resulting in a mean daily uptake of urea N by the PDV (Whitt et al. 1996). Hepatic urea-N flux changed from release to uptake 7 h after the initial morning feeding and uptake continued until approximately 4 h before initial morning feeding the next day (Whitt et al. 1996).

Differences in DMI did not affect PDV uptake of urea N; however, hepatic release of urea N increased by 31 % between low and medium DMI and 15 % between steers on medium and high DMI (Lapierre et al. 2000). Because of the similarity in PDV flux, splanchnic urea N flux was not affected by level of DMI. In contrast, in two studies by Reynolds et al. (1991b, 1992) PDV uptake of urea N increased by 53 % and 70 % with high intake of concentrate diets compared with low intakes. Release of urea N from the liver was also significantly increased in cattle consuming greater amounts of high-concentrate diets (Reynolds et al. 1991b, 1992); and subsequent release of urea N was also increased with increased DMI.

Increasing steam-flaked corn bulk density increased PDV urea N uptake (Alio et al. 2000), but grain processing (dry-rolled vs. steam-flaked) did not affect hepatic urea-N flux, and subsequent total splanchnic urea-N flux did not differ between grain processing methods in growing steers (Alio et al. 2000; Theurer et al. 2002). Intraruminal infusion of butyrate did not affect urea N PDV, hepatic, or total splanchnic flux (Krehbiel et al. 1992). Likewise, abomasal infusion of glucose or starch did not affect urea N PDV uptake (Huntington and Reynolds 1986). Increases in amino acid infusion or UIP feeding increased urea N PDV uptake in lambs (Lobley et al. 1998; Bonhert et al. 1999, respectively). Hepatic urea-N flux increased with infusion of amino acids by increasing the release rate of urea N (Lobley et al. 1998). Feeding UIP vs. ruminally degradable protein decreased urea-N release in lambs (Bonhert et al. 1999).

As steers aged, increased DMI, and BW, PDV flux of AAN increased and greater amounts of AAN were released to the liver (Eisemann et al. 1996). Hepatic flux of AAN resulted in uptake of AAN at a lower rate than PDV release but hepatic uptake increased as steers aged and increased DMI (Eisemann et al. 1996). The resulting total splanchnic flux indicated a release of AAN in steers at 60 g/kg $BW^{0.75}$, but splanchnic flux was near zero at 114 g/kg $BW^{0.75}$ (Eisemann et al. 1996). Whitt et al. (1996) reported that AAN PDV, hepatic, and total splanchnic flux was not sensitive to time of feed intake or time of day. Lapierre et al. (2000) reported a linear increase in AAN PDV flux between low, medium, and high levels of DMI in steers. Similarly, Reynolds et al. (1991b, 1992) reported 56 % and 65 % increased PDV release of AAN in cattle consuming 45 % more of concentrate diets on a DM basis. Hepatic flux was not different between low, medium, and high levels of intake despite differences in PDV flux (Lapierre et al. 2000). However, the two studies of Reynolds et al. (1991b, 1992) reported increased hepatic uptake of AAN when cattle consumed greater amounts of concentrate diets. Differences in PDV flux between DMI levels resulted in an increased total splanchnic tissue release of AAN with high levels of intake (Reynolds et al. 1991b, 1992; Lapierre et al. 2000). Amino acids released from PDV and removed by the liver could be deaminated and carbon skeletons used for gluconeogenesis. Increased release of amino acids from total splanchnic tissues could be used for protein synthesis in peripheral tissues.

Dry-rolled and steam-flaked corn diets resulted in similar PDV, hepatic, and total splanchnic flux of AAN (Alio et al. 2000). However, decreasing flake density increased PDV release of AAN (Alio et al. 2000), which may have resulted from the increased ruminal fermentation of starch and increased microbial cell protein

synthesis. Microbial protein synthesis in the rumen and flow to the small intestine may have increased with decreasing flake density. Infusion of additional amino acids into the mesenteric vein increased release of AAN and increased removal of AAN by the liver above basal levels (Lobley et al. 1998). A second infusion of amino acids following the first infusion increased AAN PDV and hepatic flux above basal levels, but not to the extent that the initial amino acid infusion produced (Lobley et al. 1998).

The high rate of metabolism of the liver can be attributed to its' high rate of protein turnover (Reynolds 2001). The high rate of protein turnover in the liver is a result of the synthesis of constitutive and exports proteins (Connell et al. 1997; Reynolds 2001). Export proteins synthesized by the liver such as albumin can be used as anabolic sources for peripheral tissues. In addition, export and other constitutive proteins synthesized by the liver can act as temporary repositories of amino acids to mediate variation in amino acid supply (Lobely et al. 1998).

In cattle consuming concentrate-based diets the flux of nitrogenous nutrients appears to be influenced by DMI. Increasing DMI increases the PDV release of ammonia N and AAN. Increased PDV release of ammonia N and AAN are probably a result of increased availability of nutrients for absorption. Ammonia N uptake by the liver counterbalances PDV release; however, AAN uptake by the liver is not always as great as PDV release and release of AAN from total splanchnic tissues can be observed. Uptake of urea N by the PDV, most likely by the rumen, increases with increased DMI. Increased fermentable carbohydrates of high-concentrate diets increases the need of N by the ruminal microbes for microbial protein synthesis. A good example of this is the increased urea N uptake by the PDV of steers consuming diets of lower flake density on steam-flaked sorghum diets.

*Flux of Energy Yielding Nutrients*. Portal-drained visceral, hepatic, and total splanchnic flux of VFA were all affected by increases in DMI in growing steers (Eisemann et al. 1996). Increasing acetate release by the PDV and liver resulted in increasing release of acetate by total splanchnic tissues for peripheral use. Because cattle deposit more fat as they age when consuming high-energy diets, the supply of acetate required by peripheral tissues increases. Likewise, PDV release of propionate increased as steers aged and increased DMI (Eisemann et al. 1996). Hepatic flux increased in a similar fashion, but the liver removed propionate resulting in a near zero splanchnic flux of propionate (Eisemann et al. 1996). Butyrate PDV, hepatic and total splanchnic flux varied by less than 50 mmol/h as steers increased age and $BW^{0.75}$ (Eisemann et al. 1996).

Glucose flux across the PDV in growing steers remained relatively stable as steers gained BW, increased DMI, and aged (Eisemann et al. 1996), but did increase slightly at approximately 92 kg $BW^{0.75}$. However, because of the increasing DMI and flux of gluconeogenic precursors, hepatic release of glucose increased as steers gained $BW^{0.75}$ (Eisemann et al. 1996). Subsequent splanchnic release of glucose also increased with BW but leveled off at 92 kg $BW^{0.75}$. Whitt et al. (1996) reported a removal of glucose by PDV in steers consuming a 64 % concentrate diet. Increased PDV removal of glucose implies utilization of glucose exceeded absorption of glucose by intestinal tissues. In contrast, increasing level of DMI linearly increased

PDV glucose flux in growing steers (Lapierre et al. 2000). Examination of within day variation showed that PDV glucose flux was released for only 3 h after the second feeding of the day (Whitt et al. 1996). Mean hepatic glucose flux indicated a release of glucose by the liver (Whitt et al. 1996), but removal occurred for 10 h during the day after the first of two feedings of steers. Therefore, a positive daily total splanchnic flux was observed in steers on high-energy diets (Whitt et al. 1996). Similarly, the increase in DMI and gluconeogenic precursors resulted in an increase in hepatic glucose flux and total splanchnic glucose flux (Reynolds et al. 1991b, 1992; Lapierre et al. 2000). Addition of intraruminal butyrate or increases in UIP did not affect PDV, hepatic, or splanchnic flux of glucose in steers or sheep, respectively (Krehbiel et al. 1992; Bonhert et al. 1999). However, Huntington and Reynolds (1986) reported increased PDV glucose release with abomasal glucose infusion compared with infusion of starch. Lower PDV glucose release associated with starch infusion implies digestion of starch to glucose may be the rate-limiting step in glucose absorption, potentially due to lack of pancreatic amylase or limit dextrins.

Release of lactate by the PDV was similar in growing steers as they increased DMI, BW, and age (Eisemann et al. 1996). Net lactate flux indicated removal of lactate by the liver in growing steers, but remained stable with increases in DMI and BW (Eisemann et al. 1996). The subsequent total splanchnic flux indicated removal of lactate, and was constant across the growth period. In contrast, Reynolds et al. (1991b, 1992) reported increased release of lactate by the PDV with increasing DMI of concentrate diets. However, cattle on a lower DMI level of concentrate diets had hepatic lactate removal greater than cattle on high DMI (Reynolds et al. 1991b, 1992). Because of greater PDV release, total splanchnic release of lactate was greater for cattle consuming greater amounts of concentrate diets (Reynolds et al. 1991b, 1992).

Flux of NEFA across the PDV, liver, and splanchnic tissues have been reported to be DMI dependent (Reynolds et al. 1992; Lapierre et al. 2000). Steers consuming a medium level of DMI had greater release of NEFA by the PDV compared with low or high DMI steers. However, hepatic removal of NEFA was greatest in low DMI steers followed by medium and then high DMI steers (Lapierre et al. 2000). The increased liver removal of NEFA increased with decreasing intake, in relation with increased arterial concentrations. However, the amount of NEFA extracted by the liver was insufficient to affect elevated circulating concentrations resulting from increased mobilization of lipid reserves during low fed intake. Total splanchnic flux was indicative of the extent of uptake of NEFA by the liver. In contrast, Reynolds et al. (1992) reported greater release of NEFA by the PDV and increased uptake by the liver for steers on a high level of intake, but splanchnic uptake was not different between steers on low and high levels of intake. Release of NEFA by PDV could result from mobilization of visceral lipid deposits or potentially from PDV absorption of medium-length NEFA.

Flux of energy yielding nutrients is significantly affected by differences in intake in ruminants fed concentrate-based diets. Volatile fatty acids released by the PDV increases with increasing DMI. Hepatic and total splanchnic flux is mostly dependent

on individual VFA. The liver removes butyrate and propionate whereas acetate is released. The PDV, even on high-concentrate diets, generally removes glucose. However, on high-concentrate diets greater release of glucose from the liver is possible because of greater concentrations of gluconeogenic precursors (propionate, amino acids, lactate). The effect of diet on lactate and NEFA metabolism is more variable than glucose. Release of lactate by the PDV is variable, but liver utilization remains nearly complete.

## Conclusions

Tissues of the portal-drained viscera and the liver are key to the digestion, absorption, transport, metabolism, and recycling of nutrients required for the maintenance, growth and lactation of ruminant animals. Changes in blood flow, oxygen consumption and net nutrient flux across the portal-drained viscera and liver ultimately drives animal production. The contribution of these tissues to whole-body nutrient use and energy expenditure can be measured employing the Fick Principle in animals prepared with chronic indwelling catheters. Level of intake and diet type affects the nutrient profile and concentration that is ultimately absorbed by the gastrointestinal tract. The portal-drained viscera requires nutrients and release of nutrients from the portal-drained viscera varies with feed intake and diet type. The liver is an important thoroughfare for nutrients between the portal-drained viscera and peripheral tissues. Depending on portal-drained viscera release, peripheral needs, and nature of the nutrient, the liver can alter the concentration of nutrients that exit relative to the concentration that enters the liver. Ultimately the concentrations of nutrients that leave the liver dictate animal maintenance and productive functions.

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# Chapter 10
# Rumen Models

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## Introduction to Ruminal Kinetics

When ruminants consume some sort of feed, especially forage, a part of the organic matter ingested returns to the mouth to be chewed, and then swallowed again (Russell 2002). Consequently, part of this feed is degraded by ruminal microorganisms, which produce short-chain fatty acids (**SFCA**) that will supply the energy requirements of the host; whereas the particles that were not degraded, especially those smaller than 1.18 mm, by pass to the omasum via reticular-omasal orifice. In general, the facts just described is what happens during the day as a result of feeding of ruminants; however, it's needed to understand the fermentation dynamic of various feedstuffs, as well as their rate of passage through the rumen and nutritional implications related to animal performance.

Nowadays, nutritionists worldwide have been using several types of feedstuffs in the diets of ruminants' animals, and also different types of processing to increase feed nutrients availability to the animal. However, the potential digestion of feedstuffs utilized to feed ruminants, regardless of type (forages or concentrates) or processing method, is controlled by two factors: rate of passage ($k_p$) and rate of degradation ($k_d$), both expressed as $\%.h^{-1}$.

Moreover, it's know that feed consumed can leave the rumen either by degradation ($k_d$), when it's transformed in SFCA and then absorbed by the rumen epithelium, or by passage to the omasum via reticular-omasal orifice ($k_p$), when it's not utilized as

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substrate by microorganisms. The sum of these two factors is called rate of disappearance ($_{kt}$), and it is expressed by the following equation:

$$Kt = Kp + Kd$$

The $k_d$ of each ingredient that is part of a diet formulated to ruminants may be altered either by some type of feed processing (e.g. steam-flaking) or by the $k_p$. Likewise, the $k_p$ may be affected by diet type or feeding habit (feeding frequency, meal size, time spent ruminating); forage source; particle size of diet ingredients, mainly forages; and diet content of neutral detergent fiber (**NDF**) and acid detergent fiber (**ADF**).

Diets containing higher contents of roughage (e.g., 50 % concentrate/50 % roughage) promote greater rumen volume when compared to animals consuming diets containing higher energy contents (e.g.: 86 % concentrate/14 % roughage). Moreover, ruminal volume does not mean capacity, but the space a diet will take in the rumen, regardless of its dry matter content. Ruminants regulate their dry matter intake either by physical limitation or rumen fill, typically when high-forage diets are provided, or by chemical stimuli, when rumen wall's chemoreceptors are activated due to the accumulation of SCFA and consequently low pH, which takes place when high-concentrate diets are offered.

Based on the facts just described, diets formulated for ruminants containing different ingredients, as well as different chemical composition, will lead to different ruminal volume, and as a result to different $k_p$ *and* $k_d$. Typically, high-forage diets contain less energy (55–60 % total digestible nutrients [**TDN**] or 0.64–0.70 Mcal of net energy for gain per kg of diet dry matter) than high-concentrate diets (75–80 % TDN or 1.22–1.30 Mcal of net energy for gain per kg of diet dry matter), because it provides less energy per kg of dry matter ingested. Therefore, when ruminants consume diets containing less energy (higher forage content) the ruminal volume will increase. On the other hand, when high-concentrate diets are fed (lower forage content), the ruminal volume will decrease, despite increasing the amount of substrate (dry matter basis) available for fermentation.

The greater the diet NDF content is, the greater the ruminal volume becomes (Fig. 10.1b), indicating greater rumen fill. In this case, the frequency and strength of ruminal contractions (internal ruminal pressure) will depend on NDF content of the diet provided. Therefore, when diet NDF content is greater, the strength of ruminal contractions will be increased. As a result, rumen motility will increase, as well as become more often, leading to greater mixture of the digesta in the rumen, which increases $k_p$ of liquids and undegraded feed smaller than 1.18 mm to the omasum via reticular-omasal orifice. On the other hand, high-energy diets containing lower NDF content will decrease ruminal volume (Fig. 10.1a). As consequence, strength of ruminal contractions will be decreased, and contractions will occur less often, decreasing motility and $k_p$. Thus, while high-forage diets promotes greater ruminal volume and accelerate the passage of nutrients through the rumen, high-concentrate diets makes $k_p$ slower, giving more opportunities for the microorganism to attach and degrade the substrate.

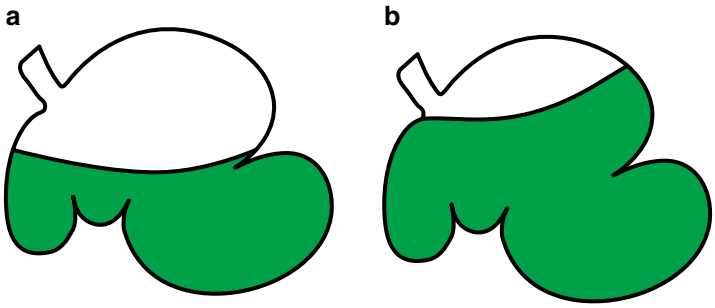



**Fig. 10.1** Illustrations of the rumen from an animal consuming either high-concentrate (**a**) or high-forage diet (**b**), which presents greater ruminal volume

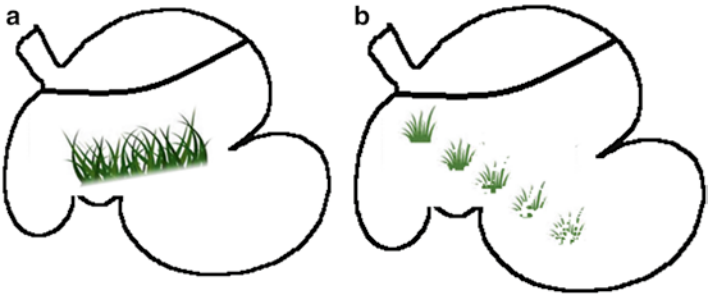



**Fig. 10.2** Illustration of how different diet particle sizes affect rumen motility. The greater the diet particle size is, the greater the strength of ruminal contractions becomes (**a**), increasing $k_p$; and vice-versa (**b**)

Besides the amount of roughage in the diet, another factor that determines the $k_p$ of the digesta through the rumen and intestines is the roughage particle size (Fig. 10.2). Roughage sources with small particles presents greater surface area to rumen microbes attachment, which reduces the lag time of microorganisms that degrade fiber, and in this case, leads to greater $k_d$ than roughage sources with large particles; as small particles have shorter retention time in the rumen, which results in decreased stimuli of rumen motility and $k_p$. Moreover, small particles have the capacity to absorb water more quickly (once rumen is a liquid environment). As a result, these particles become heavier and go to the bottom of the rumen, leading to decreased stimuli of rumen motility as well (Fig. 10.2b).

The particle size of a diet and its capacity to stimulate rumen motility can be controlled by monitoring the amount of physical effective NDF (**peNDF**) expressed as percentage of diet dry matter. Therefore, it's possible to evaluate the particle size of a given diet and manipulate both $k_p$ and $k_d$. High-forage diets with large particles have greater peNDF and promote greater rumen volume that leads to greater stimulation of rumen motility and increased $k_p$.

Another important fact that must be considered is the $k_p$ of liquids and small particles when diets contain high peNDF content. Diets containing higher contents

of roughage have greater peNDF concentration, increasing $k_p$ of rumen contents, especially liquids and those ingredients with particles smaller than 1.18 mm, which are mostly concentrate particles that when fermented in the rumen produces propionate, an important glucose precursor for the energetic metabolism of animals.

Regardless of feed processing that are adopted in ruminant diets, the greater the $k_p$ is, the lower the $k_d$ of small particles becomes; because those particles flow at faster rates to the omasum via reticular-omasal orifice, which reduces their chances of ruminal degradation by microorganisms and decreases SCFA production, as part of them will be digested and absorbed by the intestines.

If $k_t = k_d + k_p$, it's possible to observe that $k_d$ and $k_p$ are negatively correlated; moreover, when $k_p$ increases, the $k_d$ decreases and vice-versa. Thereby, it's possible to manipulate the $k_d$ of a given diet to improve nutrients utilization in the rumen and intestines.

Grain type or processing method also will modify the $k_d$ of rumen contents. Less processed feedstuff has lower $k_d$ in the rumen, especially when high-forage diets are fed, when compared to more processed feed ingredients. Therefore, besides the low $k_d$ presented by the less processed feed ingredient itself, the feeding of high-forage diets will decrease $k_d$ even more. Cole et al. (1976a) evaluated the influence of roughage content on the site and extent of digestion of whole shelled corn by beef steers, and observed that animals receiving diets containing 14 % forage had decreased ruminal starch digestibility when compared to those animals that did not receive any source of forage in the diet (67.9 % vs. 89.0 %, respectively). However, regarding starch disappearance in the intestines, cattle fed diets containing 14 % of roughage decreased starch digestibility in the rumen and in the total gastrointestinal tract when compared to those receiving 0 % of roughage in the diet. Moreover, animals fed diets containing 14 % of roughage showed reduction of dry matter and cellulose digestibility when compared to those receiving diets with 0 % of roughage. These authors suggested that the decreased nutrients digestibility when 14 % roughage diet was fed might be due to an increased $k_p$ of rumen contents. In other words, increasing fiber inclusion level in diets of beef steers promoted greater rumen volume indicating greater rumen fill, which in turn increased $k_p$ of nutrients and decreased $k_d$ both in the rumen and in total gastrointestinal tract. This shows that when whole shelled corn is included in diets containing higher fiber content, changes on the site and extent of starch, cellulose and dry matter digestion will be expected.

On the other hand, the more intense the processing method is (e.g. dry rolled *vs*. steam-flaking), the greater the $k_d$ of ingredients that compose the diet becomes, both in the rumen or in the total gastrointestinal tract. Furthermore, if high-forage diets are fed, there will be significant changes on rumen $k_d$ and dry matter content. Therefore, Cole et al. (1976b) evaluated the influence of roughage content and corn processing method on site and extent of digestion by beef steers. The authors evaluated the inclusion of 0 % and 21 % of roughage in diets containing either dry-rolled or steam-flaked corn, and observed that feeding of steam-flaked corn improved both dry matter and organic matter digestibility in the rumen and total gastrointestinal tract. Also, it was reported increases in starch digestibility in the rumen, intestines and total gastrointestinal tract (91.6 % vs. 71.7 %; 88.4 % vs. 76.2 %; 99 % vs.

93.6 %; respectively) when steam-flaked corn was fed, showing that grain processing may alter site and extent of nutrients digestion.

Regardless of processing method, these authors related that steers receiving the 21 % roughage diet had greater dry and organic matter intakes, expressed in kilos, than those steers fed diets without roughage; however, despite greater intakes, steers fed 21 % roughage presented lower digestibility of dry and organic matter. In fact, it's suggested that increasing of intake may be related to the increase of rumen $k_p$, which in turn may have decreased the $k_d$ of diet organic matter and dry matter. Likewise, roughage inclusion at level of 21 % also decreased starch and cellulose digestibility in the intestines and total gastrointestinal tract, suggesting that increasing roughage levels may lead to faster rumen $k_p$, which may alter the site and extent of nutrients digestion.

Therefore, based on the facts just described, it's possible to suggest that either roughage source or its inclusion levels may impact ruminal kinetics, altering the digesta $k_p$ through the rumen and intestines, which changes $k_d$ of feed ingredients that compose the diets and finally affects animal performance.

## Introduction to Mathematical Models

Mathematical modeling is the science that portrays biological phenomena in mathematical term. In the last 40 years, the acknowledgement of modeling has increased drastically inside and outside research institutions. Its application ranges from predictions of earthquake to optimization of fuel efficiency in war tanks.

The most common models in animal agriculture is "ration formulation and evaluation", where one can elaborate and analyze the impact of diet manipulation on performance outputs, such as milk production and gain per day. With the advance of biological understanding and computer technology, these "simple" models became more complex by integrating multiple variables, such as microbial protein production, nitrogen excretion and many others.

Currently one can optimize a diet for a specific herd at specific physiological state to maximize profitability and minimize environmental impacts. Today's models embrace what is known as the whole system approach.

## Model Types, Description and Organizational Hierarchy

France and Thornley (1984) proposed a system to categorize mathematical models:

*Static* or *Dynamic*: the main difference is the time factor, a dynamic model will give results over a period of time, such as the radiation of the sun throughout the day and it requires differential equations. A static model gives onetime result, such as body mass index (BMI) of a person at a given height and weight.

*Deterministic or Stochastic*: the difference refers to the type of results given. A deterministic model gives exact results, such as the BMI of a person; on the other hand, stochastic model takes into account probability and variance, such as the estimated BMI (with standard deviation) of a person given the averages for that gender, age group and any other variable.

*Mechanistic or Empirical*: the difference refers to the relationship of state variables within the model. In a mechanistic model relationship among variables are understood, such as knowing the passage rate of starch, which can then be used to estimate the amount of starch in the rumen. Empirical model would claim knowledge of the amounts of starch in the rumen and in the duodenum and through this knowledge builds a relationship such as starch passage rate.

Scientists have developed models for various reasons: (1) to understand how a system works, (2) to simulate an experiment that would be difficult to conduct due to time or money constraints, (3) to formulate a hypothesis and experimental scheme before beginning an experiment and (4) to use as a decision tool for a business. Despite the reason, the approach to modeling requires combination of many factors which all contribute to the overall system; this arrangement involves two processes: reduction and integration (Table 10.1). Reduction could be used when one wish to know what physiological process determine the growth rate of an animal at different feed intake, and integration could be applied to understand how these difference in growth rates can influence total greenhouse gases production.

Hierarchy systems have three important properties:

1. Each level has its own language or principles. For example, the terms used at the cell level have little meaning at the production level.
2. The relationship between levels is not symmetrical. For example a car (higher level) to run well has to have its parts (lower level) working properly, but not vice versa. If a car is taken apart it will not run, but the parts will work fine.
3. The integration of items from lower level will compose the higher level. Understanding and discoveries made at a given level can be connected to the next higher level. Description on organ $(i-1)$ metabolism can provide explanation on an observed fact at animal level $(i)$.

**Table 10.1** Organizational hierarchy of biological systems

| Level | | Description of level | |
|---|---|---|---|
| ← Reduction | $i+1$ | Production | Integration → |
| | I | Animal | |
| | $i-1$ | Organs | |
| | $i-2$ | Tissues | |
| | $i-3$ | Cells | |
| | $i-4$ | Macromolecules | |

## Concepts and Dynamic Representation

All rumen models presented in this chapter simulate digestion, absorption and nutrient outflow of the rumen. Metabolism and production are predicted based upon knowledge of physiological process and nutrients fed to the animal. In addition of helping research, these models can lead animal production towards a more sustainable system, by improving animal performance and product quality, as well as reducing nutrient excretion to the environment.

For this chapter I have chosen three models (MOLLY, CNCPS and COWPOLL) to illustrate their differences in structure and application. Please for greater detail on the development of these models read the original manuscripts.

Baldwin and colleagues (1970) were the first to develop a complete mechanistic and dynamic rumen model in sheep. Later on, Baldwin et al. (1987a, b, c) created a model for lactating dairy cows called MOLLY.

Russell and colleagues (1992) developed the Cornell Net Carbohydrate and Protein System (CNCPS), a static model for beef and dairy cattle.

Dijkstra and colleagues (1992) developed a rumen model based on a series of dynamic, deterministic and nonlinear equations; later in 2001, Mills and colleagues incorporated ruminal and hindgut methane production; in 2004, Kebreab and colleagues integrated this rumen model to a whole-animal model and added nitrogen and phosphorus excretion; and in 2006, Bannink and colleagues developed a new stoichiometry for ruminal fermentation; this whole-animal model is known as COWPOLL.

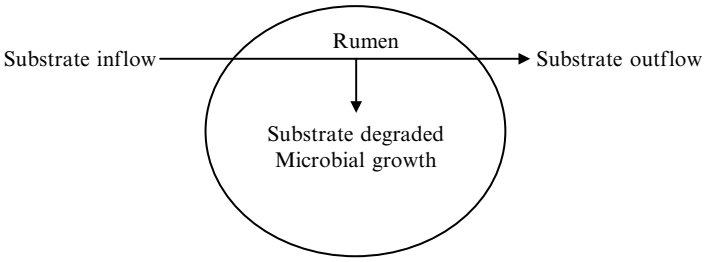
Diet (structure and type of feed offered), animal (feed intake, genetic potential for performance) and microorganism activities are the main factors that influence rumen function, as well as their interactions, for example rumen pH, volume of rumen content and absorptive rumen wall capacity.

Despite of different mathematical approach, available rumen models share common principles on rumen activity, and they are:

- Feed particle degradation
- Substrate utilization by microorganism
- Microbial mass production
- Formation of VFA and ammonia
- $CO_2$ and $CH_4$ synthesis
- Intra-ruminal microbial recycling
- Outflow rate of rumen content

As a result of complex interactions among all factors cited above; in the last 50 years scientist have attempted to develop mechanistic and dynamic models. Empirical and static models lack the ability to integrate these factors when representing rumen function.

Substrate degradation depends on the interaction between microorganisms and substrate; this process is driven by enzymes; and this is the fundamental element in dynamic modeling. In addition, dynamic models represent the effects of all factors




Substrate outflow = substrate inflow * $k_p$ / ($k_p$+ $k_d$)

Substrate degraded = substrate inflow * $k_d$ / ($k_p$ + $k_d$)

Microbial growth = Substrate degraded * $Y_m$ (g microbial matter/ kg degraded substrate)

where $k_d$ = rate of digestion and $k_p$ is rate of passage

**Fig. 10.3** General representation of empirical and static rumen model (adapted from Bannink et al. 2006a, b)

mentioned above on microbial population size and substrate quantity in the rumen, thus allowing for estimations on microbial growth, VFA synthesis and absorption, and substrate degradation.

Empirical and static models utilize substrate rate of degradation ($k_d$) and rate of passage ($k_p$) as input to predict substrate outflow, substrate degradation and microbial growth (Orskov and McDonald 1979). These models do not represent any interaction between substrate and microorganism (Fig. 10.3).

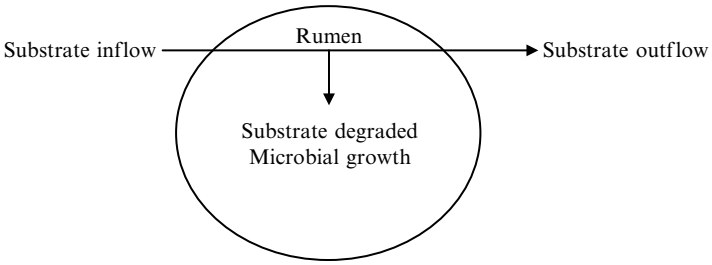
Models such as, CNCPS and similar approaches (Lescoat and Sauvant 1995; Danfær et al. 2006) are described as (semi-) mechanistic and static. These models allow for predictions of passage rate and degradation, and microbial growth in relation to various rumen fermentation conditions, even with a more mechanistic representation than in empirical approach, these models still do not account for the relationship between microbial activity and substrate availability (Fig. 10.4).

Mechanistic and dynamic models (France et al. 1982; Baldwin et al. 1987a, b, c; Dijkstra et al. 1992) calculate microbial population size and substrate availability which are included in the calculations of rate of passage and degradation, microbial growth and rumen outflow. Substrate degradation and microbial activity dependency are described by enzyme kinetics (Fig. 10.5).

## Examples of Rumen Models

### *"MOLLY"—More than a Dairy Cow Model*

MOLLY (Fig. 10.6) is a dynamic and mechanistic model for dairy cow developed by Baldwin (1987a, b, c) and last updated in 2007. Molly has been used by researches worldwide to identify areas of knowledge deficiency (Freetly et al.

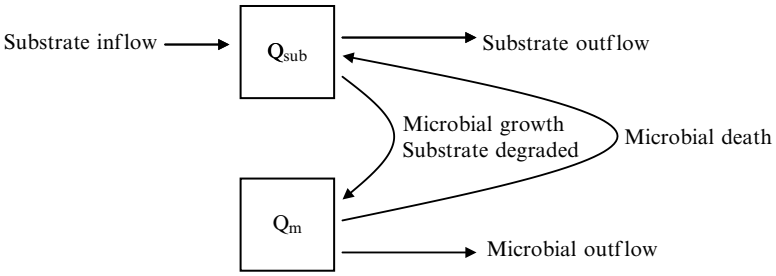



Substrate outflow = substrate inflow * $f(k_d, k_p)$

Substrate degraded = substrate inflow * $f(k_d, k_p)$

Microbial growth = Substrate degraded * $f(k_d, k_p, Y_m)$

**Fig. 10.4** General representation of (semi-) mechanistic and static rumen model (adapted from Bannink et al. 2006a, b)




$Q_{sub}$ = kg or mole of degradable substrate

$Q_m$ = g of microbial matter

Substrate outflow = $f(Q_{sub})$

Substrate degraded = $f(Q_{sub}, Q_m)$

Microbial growth = $f(Q_{sub}, Q_m)$

Microbial death = $f(Q_{sub}, Q_m)$

Microbial outflow = $f(Q_{sub}, Q_m)$

**Fig. 10.5** General representation of mechanistic and dynamic rumen model (adapted from Bannink et al. 2006a, b)

1993a), as a teaching tool for students (Johnson et al. 2008), to estimate methane ($CH_4$) emissions (Kebreab et al. 2008) and in many other scenarios.

As an example; ruminal $CH_4$ production in MOLLY follows the assumption that remaining hydrogen from microbial growth, biohydrogenation of unsaturated fatty acids and production of glucogenic VFA (propionate and valerate) produced initially during fermentation of carbohydrate and protein to lipogenic VFA (acetate

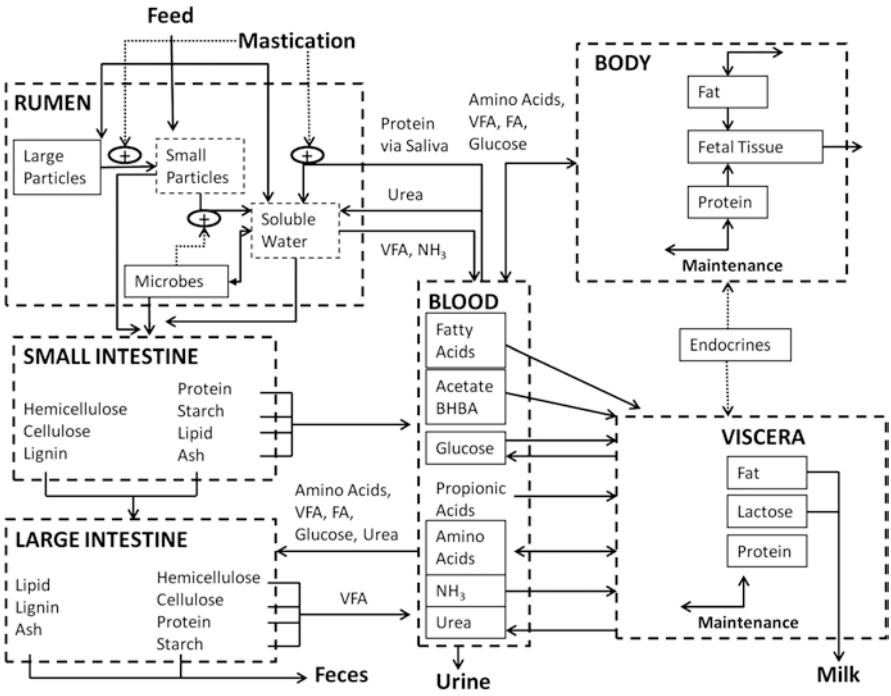



**Fig. 10.6** Schematic of MOLLY. Figure adapted from Hanigan et al. (2006). *Boxes* with *dashed lines* represent compartments; *boxes* with *solid lines* represent pools; *arrows* represent fluxes; and Ы⊕ represent activators

and lactate) will be totally used by methanogenic bacteria. This assumption is described mathematically by the equation bellow:

$$CH_4E = 0.211 * \left(DCsHy + DRAaHy - DHyMi - DHyFlF + 2\,RLaAc - RLaPr\right) / 4,$$

where,

$CH_4E$: total methane production, Mcal,
DCsHy: hydrogen formation from soluble carbohydrate fermentation,
DRAaHy: hydrogen released due to amino acid fermentation in the rumen,
DHyMi: hydrogen use to support rumen microbial growth,
DHyFlF: hydrogen use to saturate unsaturated fatty acids,
RLaAc: rate of acetate formation from lactate,
RLaPr: the rate of propionate formation from lactate.

Even though MOLLY was developed with lactating dairy cow dataset, MOLLY's ability to predict $CH_4$ in feedlot steers has been recently challenged with current U.S. feedlot data (Kebreab et al. 2008) and the conclusions were that MOLLY was

more precise and accurate than the other models tested; approximately 99.6 % of the predictions errors were random and there was an absence of mean bias.

## *"CNCPS"—Cornell Net Carbohydrate and Protein System*

The Cornell Net Carbohydrate and Protein System (CNCPS) was developed by Russell et al. (1992), Sniffen et al. (1992) and Fox et al. (1992). The CNCPS differently than MOLLY is not based on dynamic differential equations, but has the advantage of including various environmental and management condition, as well as feeds (Fox et al. 2000).

The latest version CNCPSv6 (Fig. 10.7) was re-designed from the previous version v5, and currently considers physiological functions, such as growth and lactation; and anatomical compartments, such as rumen and mammary gland as objects. In addition carbohydrate pools were expanded and now include sugars, soluble fibers and organic and VFA. Ruminal lypolization and biohydrogenation, and absorption of fatty acids in the small intestine were integrated into the fat model.

As mentioned earlier one of the advantages of this model is to predict output excretion, here are some of the equations used for lactating cows:

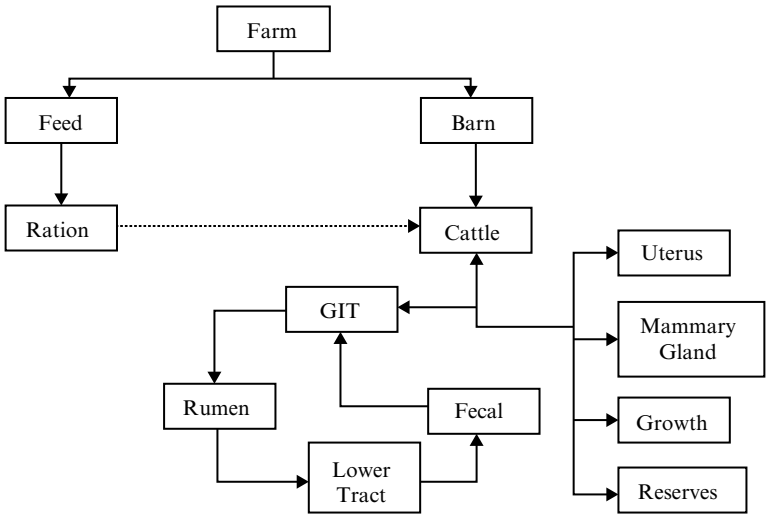



**Fig. 10.7** Re-designed object-oriented CNCPSv6 structure. Figure obtained from Tylutki et al. (2007). *GIT* gastrointestinal tract

**Manure Production**

*Total Feces Production*

$$\text{TFDM} = \text{IDM}/\ \text{FDM}, \text{when milk production is higher than } 45.4 kg\ /\ d$$

where,
TFDM: total fecal dry matter from indigestible feed, g/day
IDM: indigestible dry matter, g/day
FDM: fecal dry matter, kg/day
*Total Urine Production*

$$\text{TU} = \left(3.55 + 0.16 \times \text{DMIA} + 6.73 \times \text{CPIA} - 0.35 \times \text{MILKA}\right) \times \text{AU}$$

where,
TU: total urine, kg/day
DMIA: dry matter intake per AU, kg/day
CPIA: crude protein intake per AU, kg/day
MILKA: daily milk production per AU, kg/day
AU: animal unit, body weight, kg / 454

**Partitioning of Fecal and Urinary Nitrogen Excretion**

*Fecal Nitrogen*

$$\text{FN} = \left(\text{FFN} + \text{BFN} + \text{MFN}\right)$$

where,
FN: total fecal nitrogen, g/day
FFN: fecal nitrogen from indigestible feed, g/day
BFN: bacterial fecal nitrogen (bacterial cell wall), g/day
MFN: metabolic fecal nitrogen, g/day
*Urinary Nitrogen*

$$\text{UN} = \left(\text{BEN} + \text{BNA} + \text{NEU} + \text{TN}\right)$$

where,
UN: total urinary nitrogen, g/day
BEN: excess bacterial nitrogen, g/day
BNA: bacterial nucleic acids, g/day
NEU: inefficiency of nitrogen use, g/day
TN: degraded tissue nitrogen, g/day

The static equations of the rumen sub model of CNCPS are well described in Russell et al. (1992) and Sniffen et al. (1992). Basically the rumen sub model

consists of equations to estimate degradation and passage of several feed fractions, and equations to represent growth and outflow of microorganism. In 1996, Pitt and colleagues updated the sub model, yet predictions of pH, VFA molar proportions and starch degradation still not accurate.

## *"COWPOLL"*

When the rumen compartment was first developed by Dijkstra et al. (1992) they included some aspects that were not addressed in previous models, such as microbial substrate preference, effect of pH on microbial activity, VFA and ammonia absorption; and inclusion of amylolytic and fibrolytic microbes. A detailed scheme of microbial mechanism is shown on Fig. 10.8.

COWPOLL is a mechanistic and dynamic model such as MOLLY. One of the main differences between the rumen compartments of these models is the number of groups of microbes, MOLLY uses 1 group and COWPOLL separates the microbial community into 3 groups: amylolytic, cellulolytic and protozoa. Using dairy cattle data COWPOLL's rumen showed to be more precise and accurate than the other models tested (Kebreab et al. 2008); random sources of prediction contributed with 95 % of the total error.

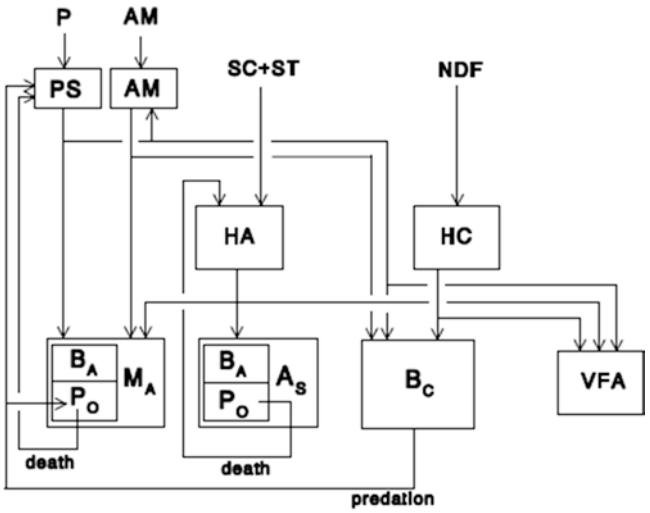



**Fig. 10.8** Detailed schematic of COWPOLL's microbial mechanism. Figure obtained from Bannink et al. (1997). *AM* ammonia, *P* protein, *PS* soluble protein, *SC* soluble carbohydrate, *ST* starch, *NDF* neutral detergent fiber, *M* microbial mass, *VFA* volatile fatty acids, *AS* storage polysaccharides of amylolytic microbes, *BA* amylolytic bacteria, *BC* cellulolytic bacteria, *HA* soluble rumen hexose originating from SC and ST, *HC* soluble rumen hexose originating from NDF, *MA* amylolytic microbes, *PO* Protozoa

## Conclusion

Particle size dynamics, rate of passage and degradation, and feeding dynamics are areas that still need further refinement. Also animal and microorganism genomics data will soon be incorporated into rumen models for a more realistic rumen simulation.

Innumerous rumen model comparisons using MOLLY (Kohn et al. 1995; Bannink et al. 1997; Offner and Sauvant 2004; Kebreab et al. 2008), CNCPS (Fox et al. 2000; Offner and Sauvant 2004) and COWPOLL (Kebreab et al. 2008) are available in the literature. These manuscripts tested these models with different breed, physiological stages and inputs.

A common conclusion noted among modeling manuscripts is that dynamic and mechanistic models are capable of more detailed and more realistic analysis of the effects of nutrition on rumen function than empirical and static models. In this new era where producers have to minimize environmental impacts and at the same time maintain profitability; mechanistic models not only allow producers to inspect results associated to diet or management manipulation, as well as the limits associated with these changes.

Another benefit of mechanistic and dynamic models is that producers are able to simulate different options to mitigate environmental impact, and therefore choose which one or which combination fits better to its own production system.

I would like to conclude this chapter with one of my favorite modeling quote by George Box (1979) "All models are wrong, but some are useful".

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# Chapter 11
# Planning and Analyzing Digestibility Experiments

**Nicolas DiLorenzo**

## Introduction

Researchers in the animal sciences rely heavily in the use of analytical techniques that optimize the use of the experimental resources. Specifically in the field of ruminant nutrition, the most expensive resource is often the experimental unit itself. Availability of animals (and in particular ruminally or duodenally cannulated animals) can be the limiting factor in many research institutions. Thus, designing the correct experiment to answer the key research questions becomes imperative in order to make an efficient use of the animal resource. The development of assays based on principles of chemistry and physics combined with the application of mathematical and statistical tools provided the basis for most of the techniques used nowadays in ruminant nutrition laboratories. Several of these techniques were developed in the 70s and 80s and the solid science principles behind their development make them valid even 30 or 40 years from their first description. However, several scientific and technological advances have been made in the last decades, which can be adapted to the existing protocols to improve the accuracy or reduce the associated cost of ruminal nutrition research, whether that cost is in the form of supplies or labor.

The objective of this chapter is to review some key aspects of the planning and execution of studies designed to answer questions related to ruminal metabolism and digestive function. The chapter is divided into four sections based on the specific objectives of the experiment to be designed: (1) In vivo digestibility studies, (2) In situ digestibility studies, (3) In situ/in vitro studies: the three-step procedure, (4) In vitro studies. Several protocols are provided with practical tips to improve the accuracy and repeatability of each technique as well as a brief discussion on statistical considerations regarding experimental designs and number of replicates needed.

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The discussion of each technique focuses on the basic aspects of ruminal metabolism without a clear distinction between beef or dairy cattle nutrition. Although the principles are similar in both types of production systems, some unique aspects relevant to each may limit the adoption of certain techniques.

## Studies Designed to Determine Nutrient Digestibility In Vivo

One of the greatest limitations of studies aimed at measuring the digestibility in vivo is the recovery of the indigestible marker. That issue is discussed in detail in this section along with some alternative markers that have been recently adopted in ruminal metabolism studies. Another aspect that becomes apparent after reviewing the currently available techniques is the lack of assays that accurately quantify the contributions of postruminal digestion processes to total tract digestibility. This is particularly important in animals fed high-forage diets, where a significant proportion of it can undergo postruminal fermentation.

In most livestock systems, feed costs represent the largest proportion of the total costs of production. Thus, as nutritionists we place special interest in the efficient utilization of feed resources in an attempt to improve the efficiency of utilization of such expensive inputs. In order to improve the productivity of a livestock system, two main approaches could be followed: (1) to improve the efficiency of utilization of the nutrients digested and absorbed towards productive processes (milk production, weight gain, etc.), or (2) to increase the digestibility of nutrients. Often, combinations of both mechanisms are responsible for the increases in performance observed, especially when considering dietary interventions or growth promoting technologies. Implicitly under the second approach (improved digestibility) we could find the effects of minimizing wasteful processes such as methane production, or wasteful deamination of dietary proteins, which in turn can lead to improvements in total tract digestibility of dietary energy and protein, respectively.

If we consider the possible fates of nutrients entering the gastrointestinal tract, our goal as nutritionists is to maximize nutrient utilization while minimizing the amount of nutrients excreted or diverted towards non-productive processes. Although not always obvious, the minimization of the diversion of nutrients towards non-productive processes can be extremely important under specific circumstances. Imagine for example the hypothetical case of a byproduct of an industrial process (ethanol production, high-fructose corn syrup, etc.) that can be used as feed for ruminants. Now let us pretend that we are able to modify the production process in such way that we can increase the digestibility of the fibrous fraction of it. If we design a study to measure total tract digestibility of nutrients in this byproduct when obtained from the original or the modified production process, we will likely find that the digestibility of the fiber fraction, whether neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**) or both in the byproduct obtained from the modified

process is greater than that observed with the 'original' byproduct. Unless we obtain some other measurements, such as growth performance, methane production, volatile fatty acids (**VFA**) concentrations, ruminal $NH_3$-N, etc., concurrent with total tract digestibility of fiber, we could easily arrive to the erroneous conclusion that the process modification led to a byproduct with higher nutritive value. In the particular example that we are using to illustrate the point, an increase in fiber digestibility could lead to a greater proportion of methane production resulting in the diversion of energy towards a non-productive, and in this case, environmentally harmful process. Concurrent measurements of methane production and growth performance could, in the particular example used above, help elucidate whether an increase in nutrient digestion was accompanied by improved nutrient utilization. The point with this example is that nutrient digestibility of feedstuffs, although highly correlated with animal performance, may not always provide a complete picture regarding nutrient utilization.

The relationship between feed intake, total fecal output and digestibility of the whole diet is given by the following equation (Dove 2010):

$$\mathrm{I} = \mathrm{F} / \left(1 - \mathrm{D}\right)$$

where I = total feed intake, F = total fecal output, and D = coefficient of digestibility for the diet. From this equation is easy to determine that we need to know at least two of the three components to be able to calculate the third. Often in confinement situations feed intake can be measured by weighing the amount of feed offered and weighing the orts, thus in these situations fecal output is either measured by total feed collection or estimated by the use of an internal or external marker. When fecal output is estimated by the use of markers, nutrient digestibility can be calculated by the following formula:

$$\begin{aligned}&\text{Apparent nutrient digestibility}, \% = 100 - 100 \\ &\times \left[ \frac{\text{concentration of marker in feed}}{\text{concentration of marker in feces}} \times \frac{\text{concentration of nutrient in feces}}{\text{concentration of nutrient in feed}} \right]\end{aligned} \tag{11.1}$$

***Total Fecal Collection***. The use of total fecal collection to measure total tract apparent digestibility relies on the ability to precisely quantify daily fecal output, daily feed intake, and to obtain a representative sample of both feed and feces. Digestibility is simply calculated by doing a ratio of nutrients excreted to nutrients consumed, which represents the percentage of undigested material, as indicated in the following equation:

$$\begin{aligned}&\text{Apparent nutrient digestibility}, \% \\ &= \left[ \left( \text{nutrient intake} - \text{nutrient in feces} \right) / \text{nutrient intake} \right] \times 100\end{aligned} \tag{11.2}$$

Note that in both equations described we refer to "apparent" nutrient digestibility because, unless we perform specific analyses to measure the endogenous contribution of nutrients (mainly nitrogen) in the feces, we cannot separate these from the actual undigested dietary nutrients.

Undoubtedly, this method is considered the golden standard in terms of total tract digestibility measurement and as a result it is frequently used by ruminant and non-ruminant nutritionists. Its improved accuracy over other techniques stems from the elimination of errors associated with the incomplete recovery of the marker when digestibility markers are used, as well as errors associated with fecal and feed sampling, and posterior analysis to determine marker concentration. Some of the constrains associated with the use of total collection techniques are: (1) the need for facilities that allow the collection of manure separately from urine (typically done in metabolism crates); or (2) the use of fecal collection bags, which requires daily or twice daily weighing of total fecal output and subsampling for DM content and nutrient analysis. The collection of fecal output not contaminated with urine, whether by using metabolism crates or by using males fitted with a fecal collection bag, is critical for the determination of apparent nitrogen digestibility.

The main source of error in the determination of apparent total tract digestibility using total fecal collection is the failure to collect a representative sample to be analyzed for nutrient composition after the entire fecal output has been weighed. Table 11.1 summarizes the results of several studies in which total fecal collection was used to determine apparent total tract digestibility, showing the standard error of the mean (**SEM**) for some measurements and number of observation per treatment used. From this table we can observe that typical studies conducted using total fecal collection use between four and eight animals per treatment or main effect, yet achieving a relatively low SEM. Although total fecal collection represents a more accurate determination of total tract digestibility when compared with techniques that rely on indigestible markers, its use is not frequently reported in the literature, especially in studies involving bovine species. Probably this is because of the large volume of fecal output produced daily in bovine species, which difficult the collection. The use of total fecal collection in small ruminant studies is much more frequently reported, probably because of the opposite reason: a smaller need for space to house animals in metabolism crates, and a smaller volume of daily fecal output when compared with bovine species.

***Use of Indigestible Markers***. The success in measuring apparent total tract digestibility using digestibility markers is greatly dependent on the choice of marker to use, dosing method (if an external marker is used) and the protocol for collection of feed and fecal samples. Reviewing the literature it appears that the most widely used external marker is chromic oxide ($Cr_2O_3$), however some concerns exist regarding its ability to mix well with ruminal contents and to flow through the gastrointestinal tract at a similar rate to that of digesta (Titgemeyer 1997). In addition, in the United States $Cr_2O_3$ is not approved as a dietary additive by the Food and Drug Administration (Titgemeyer et al. 2001). Titanium dioxide ($TiO_2$) has been proposed as a potential digestibility marker and satisfactory results have been

**Table 11.1** Summary of studies conducted to determine apparent total tract digestibility (aTTD) of nutrients using total fecal collection

| | | Standard error of the mean for aTTD[a] (as % intake) of: | | | | |
|---|---|---|---|---|---|---|
| Source | n[c] | OM | CP | NDF | Exp. design[b] | Fecal collection method |
| Bossuyt et al. (1996) | 8 | – | 0.08 | 1.23 | LS | Metabolism crates |
| Corrigan et al. (2009) | 6 | 2.0 | – | 1.6 | SB | Fecal collection bags |
| Doreau et al. (2011) | 6 | 1.14 | – | 2.62 | LS | Metabolism crates[d] |
| Farmer et al. (2004) | 4 | 1.7 | – | 2.4 | RCBD | Fecal collection bags |
| Gilbery et al. (2007) | 4 | 1.6 | 2.5 | 1.3 | RCBD | Metabolism crates |
| Lardy et al. (2004) | 4 | 0.70 | 0.52 | 0.83 | LS | Metabolism crates |
| Li et al. (2011) | 8 | 0.89 | 1.63 | 2.41 | LS | Individual tie-stalls[e] |
| Reed et al. (2007) | 4 | 1.1 | 2.0 | 0.9 | LS | Fecal collection bags |

In all the studies the experimental unit was the animal (only *Bos taurus* or *Bos indicus* species included)

[a]Apparent total tract digestibility

[b]Experimental design: *LS* Latin square, *SB* switchback, *RCBD* randomized complete block design

[c]Number of experimental units per treatment or main effect mean

[d]Feces and urine could not be separated in the crates

[e]Because heifers were used; total urine collection was done from the bladder using indwelling balloon catheters directed through tubing into a bucket to avoid contamination of feces

obtained in terms of recovery and in comparison with digestibility estimates obtained with other markers (Titgemeyer et al. 2001; Myers et al. 2004; Pina et al. 2009). In addition, the use of ytterbium chloride has been successfully used as a digestibility and digesta flow marker (Prigge et al. 1981; Musimba et al. 1987). The main disadvantages of using ytterbium chloride as a marker are the cost and the more elaborate analytical procedure required for dosing of marked feed.

In the late 1980s and early 1990s, the n-alkanes method was developed (Dove and Mayes 1991; Dove 2010) to address one of the biggest problems in grazing cattle: how to accurately measure herbage intake. The n-alkanes are long-chain (C25 to C35) saturated hydrocarbons present in the cuticular wax of plants, and are for the most part indigestible. The odd-numbered chain length alkanes (especially C29, C31, and C33) are present in much greater amounts than the even-numbered chain length in grassland species, which led to the suggestion that could be used to calculate digestibility. However fecal recoveries of n-alkanes are less than 100 %, which makes them not ideal for use as digestibility markers (Dove 2010). To overcome this, Dove and Mayes (1991) suggested the use of oral doses of a synthetic even-chain alkane of similar recovery of a plant alkane of adjacent carbon-chain length. In essence, the plant alkane functions as an internal marker to provide an estimate of digestibility, while the dosed alkane functions as the external, fecal output marker (Dove 2010).

**Table 11.2** Summary of studies conducted to determine apparent total tract digestibility (aTTD) of nutrients using digestibility markers

| | | Standard error of the mean for aTTD[a] (as % intake) of: | | | | |
|---|---|---|---|---|---|---|
| Source | n[c] | OM | CP | NDF | Exp. design[b] | Digestibility marker used |
| Corona et al. (2006) | 8 | 0.93 | 1.62 | – | LS | $Cr_2O_3$ |
| DiLorenzo et al. (2011) | 8 | 1.35 | 3.36 | 1.27 | RBD | $Cr_2O_3$ |
| Elizalde et al. (1998) | 4 | 2.3 | 3.2 | 1.95 | LS | $Cr_2O_3$ |
| Elizalde et al. (1999) | 4 | 1.41 | – | 3.22 | LS | $Cr_2O_3$ |
| May et al. (2009) | 8 | 1.44 | – | 6.28 | RBD | $Cr_2O_3$ |
| May et al. (2010) | 12 | 2.10 | 2.76 | 5.56 | RBD | $Cr_2O_3$ |
| Mazzenga et al. (2009) | 4 | 2.7 | 2.7 | 3.5 | LS | AIA[d] |
| Montgomery et al. (2004) | 6 | 1.7 | – | 4.5 | LS | $Cr_2O_3$ |
| Pina et al. (2009)[e] | 8 | 1.13 | 1.29 | 1.83 | LS | $Cr_2O_3$ |
| Pina et al. (2009)[e] | 8 | 1.13 | 1.29 | 1.83 | LS | $TiO_2$ |
| Rotger et al. (2006) | 4 | 3.05 | 3.38 | – | LS | $Cr_2O_3$ |
| Scholljegerdes and Kronberg (2010) | 6 | 0.11 | 0.43 | 0.21 | CRD | $TiO_2$ |
| Uwituze et al. (2011) | 6 | 2.1 | 2.0 | 6.1 | RBD | $Cr_2O_3$ |
| Vander Pol et al. (2009) | 5 | 1.8 | – | 3.2 | LS | $Cr_2O_3$ |
| Wang et al. (2009) | 8 | 0.4 | 0.5 | 0.4 | LS | $Cr_2O_3$ |
| Winterholler et al. (2009) | 6 | 3.5 | 2.29 | 3.55 | RCBD | AIA[d] |
| Zinn et al. (2003) | 4 | 0.4 | 0.6 | – | LS | $Cr_2O_3$ |

In all the studies the experimental unit was the animal (only *Bos taurus* or *Bos indicus* species included)

[a]Apparent total tract digestibility

[b]Experimental design: *LS* Latin square, *CRD* completely randomized, *RCBD* randomized complete block design, *RBD* randomized block design

[c]Number of experimental units per treatment or main effect mean

[d]Acid insoluble ash

[e]Evaluation of $Cr_2O_3$ and $TiO_2$ as digestibility markers was done simultaneously. No significant difference ($P>0.34$) found between markers

The potential of a novel digestibility marker based on indigestible fiber (Rodriguez et al. 2006; Ferreira et al. 2009) has been investigated and it will be briefly discussed later in this chapter.

Table 11.2 shows a summary of research studies using external markers to assess apparent total tract digestibility. From Tables 11.1 and 11.2 we can calculate the standard deviations ($\mathbf{SD} = \sqrt{n} * \text{SEM}$) of each of the variables measured in order to provide a rough estimate of the precision of the average value reported. When doing so, the first conclusion that we arrive at is that the precision in studies using total fecal collection to determine digestibility, seem to be more accurate than those using markers. This is not surprising given that the use of markers will add a few

**Table 11.3** Differences in digestibility values between two treatments means (*d*) to be detected using Eq. (11.3) with average standard deviations (SD) calculated from Tables 11.1 and 11.2 for each variable and equation parameters fixed at: significance (α) = 0.05, power (1 − β) = 0.8

| | | Difference in treatment means (*d*) to be detected[a] | | |
|---|---|---|---|---|
| Method used to calculate digestibility | n[b] | OM | CP | NDF |
| Total fecal collection[c] | | | | |
| | 4 | 6.7 | 6.8 | 9.0 |
| | 6 | 5.2 | 5.3 | 7.0 |
| | 8 | 4.4 | 4.5 | 5.9 |
| Use of indigestible marker[d] | | | | |
| | 4 | 9.8 | 11.2 | 18.2 |
| | 6 | 7.6 | 8.7 | 14.1 |
| | 8 | 6.4 | 7.3 | 12.0 |

[a]Solving for *d* in the following formula (Snedecor and Cochran 1989): $n = 1 + 2C \times (S/d)^2$, where *n* = number of experimental units per treatment, *S* = standard deviation, *d* = difference in magnitude between treatment means to be detected, and *C* = constant determined based on the level of significance (α) and the power (1 − β) of the test. In this example C = 7.85 (α = 0.05, β = 0.2)
[b]Number of experimental units per treatment mean
[c]Average standard deviations obtained from all studies reported in Table 11.1 were used for the calculations. Values were 2.91, 2.98, and 3.94 for OM, CP, and NDF digestibility, respectively
[d]Average standard deviations obtained from all studies reported in Table 11.2 were used for the calculations. Values were 4.30, 4.90, and 7.98 for OM, CP, and NDF digestibility, respectively

extra error sources to the determination when compared to total fecal collection, such as fecal sampling error and analytical error associated with marker concentration measurements. Consideration of typical SEM reported in digestibility studies is very important to determine the optimal number of replicates per treatment in the experimental design, based on the expected difference between treatments (Table 11.3). This will be discussed in detail in a subsequent section.

Internal markers have the advantage of being intrinsically mixed with the diet and thus they can offer an advantage. However most internal markers are based on the use of an indigestible component usually abundant in the fiber fraction (Van Keulin and Young 1977; Thonney et al. 1979), and thus, its use in high-grain diet is often limited because of the low amount recovered in feces (Titgemeyer 1997). However, quantification of internal marker concentrations in feed and feces are often less expensive laboratory procedures and can be successfully used to estimate total tract digestibility (Mazzenga et al. 2009; Winterholler et al. 2009; Mc Geough et al. 2010).

When using external markers, one of the most frequent questions that the investigator faces is whether to dose once or twice daily (if the marker is not mixed with the feed). Early research conducted using $Cr_2O_3$ show inconsistent results regarding whether significant diurnal variation exists when feeding the marker once vs. multiple times daily. McGuiee et al. (1966) concluded that no significant variation in excretion patterns was found when feeding $Cr_2O_3$ once daily (at 0800 h) vs. feeding

the same daily amount divided in six meals, while Prigge et al. (1981) and Brisson et al. (1957) found a significant diurnal variation in marker excretion pattern when dosing $Cr_2O_3$ once vs. twice or six times daily. All three publications concluded that the variation in daily excretion of the marker can be overcome by an appropriate fecal sampling schedule and analysis of a composite sample. To demonstrate the importance of a proper sampling schedule to account for the variation in marker excretion, Theurer et al. (1981) conducted a study to test the effects of collecting fecal samples twice vs. six times daily when dysprosium was mixed with the diet to use as a digestibility marker. Theurer et al. (1981) concluded that collecting fecal samples for 2 days, six times per day yielded similar digestibility coefficients than those obtained from sampling during 6 days, twice daily, with both sampling methods having a total of 12 fecal samples per animal.

Considering that the marker needs to be fed for at least 7 days before the first fecal collection takes place (Table 11.4), dosing the marker twice daily can be time consuming if animals are to be processed for dosing every day. When ruminally cannulated animals are in a tie-stall or stanchion, the marker can be easily dosed twice daily without major efforts. However, if animals need to be removed from their pen to be dosed via a balling-gun, depending on the number of animals to be dosed, the disruption in intake patterns can offset any benefits of dosing the markers twice daily. Studies have yielded satisfactory results when dosing the marker twice daily or once daily as long as a proper fecal sample protocol is conducted to account for the variation in marker excretion (Table 11.4). A recommended fecal sample collection protocol is included in Table 11.5.

***Newer digestibility markers***. Researchers are in continuous search for digestibility markers that provide reliable results at a reasonable cost of analysis. Lignin is not a suitable marker for digestibility studies because of its low recovery in feces as a result of degradation within the gastrointestinal tract (Titgemeyer 1997). However when lignin was enriched with phenolic groups not commonly found in cattle diets, a modified and enriched hydroxyphenyl propane was created by a group of Brazilian researchers naming this compound LIPE® (Rodriguez et al. 2006; Ferreira et al. 2009). Subsequent evaluations of this marker, comparing it with digestibility values obtained from total fecal collection or using a series of internal and external markers, yielded satisfactory results in studies conducted with sheep and cattle (Rodriguez et al. 2006; Ferreira et al. 2009). While the initial studies using this new marker appear very promising, more research is needed to validate the marker and determine its recovery across a wide range of diets and feed intake levels.

***Statistical considerations***. Choosing the correct number of replications per treatment is one of the most important aspects of the experimental design. Because of the amount of work involved in studies aimed at measuring nutrient digestibility in which usually the experimental unit is the animal, a careful calculation of the minimum number of animals needed per treatment is essential to optimize research resources. For example, if we are assessing the effects of two treatments on nutrient digestibility, we could use the following formula (Snedecor and Cochran 1989) to

**Table 11.4** Common parameters used in sample collection protocols of studies conducted to determine apparent total tract digestibility of nutrients using external digestibility markers

| Source | Adaptation period, d[a] | Marker feeding, d[b] | Marker dosing, times/d | Fecal collection, d[c] | Fecal samples/d[d] |
|---|---|---|---|---|---|
| Corona et al. (2006) | 10 | 10 | In feed[e] | 4 | 2 |
| DiLorenzo et al. (2011) | 39 | 7 | In feed[f] | 4 | 2 |
| Elizalde et al. (1998) | 8 | 6 | 2 | 6 | 4 |
| Elizalde et al. (1999) | 16 | NR[g] | 2 | 5 | 3 |
| May et al. (2009) | 12 | 7 | 1 | 3 | 4 |
| Montgomery et al. (2004) | 18 | 7 | 1 | 4 | 3 |
| Pina et al. (2009) | 7 | 6 | 1 | 5 | 1 |
| Rotger et al. (2006) | 14 | 6 | In feed[h] | 3 | 2 |
| Scholljegerdes and Kronberg (2010) | 22 | 4 | 2 | 2 | 4 |
| Uwituze et al. (2011) | 13 | 7 | 1 | 3 | 4 |
| Vander Pol et al. (2009) | 16 | 3 | 2 | 4 | 3 |
| Wang et al. (2009) | 11 | 7 | 2 | 5 | 3 |
| Zinn et al. (2003) | 10 | 10 | In feed[i] | 4 | 2 |

[a]Number of days of adaptation period to diets or facilities before first fecal sample was collected
[b]Number of days or external digestibility marker feeding before first fecal sample was collected
[c]Duration of the fecal collection period in days
[d]Number of fecal samples collected per day during the fecal collection period
[e]Marker was continuously dosed by mixing with feed at a concentration of 0.3 %, DM basis
[f]Marker was continuously dosed by mixing with feed at a concentration of 0.25 %, DM basis
[g]Not reported
[h]Marker was continuously dosed by mixing with feed at a concentration of 0.1 %, DM basis
[i]Marker was continuously dosed by mixing with feed at a concentration of 0.4 %, DM basis

estimate the number or replicates needed when error variance and expected differences between treatments are known:

$$n = 1 + 2C \times \left(S / d\right)^2 \tag{11.3}$$

where $n$ is the number of experimental units per treatment, $S$ is the standard deviation, $d$ is the difference in magnitude (same units as $S$) between treatment means to be detected and $C$ is a constant determined based on the level of significance ($\alpha$) and the power ($1-\beta$) of the test. Values for this constant can be obtained in statistics books, but as a reference to use in our example below, the $C$ value for $\alpha = 0.05$ and $\beta = 0.2$ is 7.85. Note that the previous considerations are for the comparison of two treatments means. If more than two treatments are used, an adjustment for multiple comparisons should be made to the above equation which will lead to a greater $n$ needed maintaining all parameters constant.

**Table 11.5** Protocol for the collection of samples to determine apparent total tract digestibility in cattle in confinement using an indigestible external marker

| | |
|---|---|
| 1. | Day 0: Bring cattle into the research facilities to be used for digestibility measurements to adapt for at least a week before the beginning of marker dosing |
| 2. | Day 7: Begin dosing the indigestible marker ($Cr_2O_3$ or $TiO_2$) and continue until the last day of fecal collection. Marker should be dosed at a rate of 0.25 % of the diet DM if mixed with the feed or 10 g/day twice daily, 5 g in the morning and 5 g in the afternoon, if dosed using gelatin capsules (via cannula or balling gun). Marker dosing can be lowered to 7.5 g/day or 0.15 % of the diet DM in high-concentrate diets because the higher digestibility in the total tract may concentrate the marker in the feces. When dosing via balling gun, make sure that the capsule is swallowed before the animal is released |
| 3. | Day 13: Begin collecting feed samples for a total of 4 continuous days. Record daily feed delivered and orts for each experimental unit. If the marker is fed mixed with the diet, take a sample of orts daily for marker concentration analysis. One way to avoid the collection of orts is by limit-feeding during the 4-day collection period, if the objective of the study allows it. Store samples at −20 °C |
| 4. | Day 14: Begin collecting fecal samples from individual animals for 4 continuous days. Samples can be collected by rectal grab or from the floor, carefully avoiding contamination with soil material. Collect samples twice daily[a] (in the morning and afternoon) and freeze at −20 °C |
| 5. | Dry feed and fecal samples at 55 °C for 72 h and grind through a 2-mm screen |
| 6. | Composite feed samples delivered to each experimental unit on an equal-amount basis to obtain at least 200 g of dry sample. Thus, all four samples collected during the week will combine into one feed composite sample |
| 7. | Composite fecal samples within experimental unit on an equal-amount basis to obtain at least 200 g of dry sample. Thus, all eight samples collected during the week will combine into one fecal composite sample. |

This protocol was developed based on the discussions from this chapter regarding marker dosing, adaptation period and sample collection times

[a]If for logistic reasons marker cannot be dosed twice daily and instead is fed once daily, then fecal collection should be conducted every 6 h for 4 continuous days to account for the variation in daily marker excretion

Using Tables 11.1 and 11.2 as a guide we can calculate the SD for the different variables measured ($SD = \sqrt{n} * SEM$). For example for OM digestibility, a range of SD from 1.4 to 4.90 can be calculated in the studies conducting total fecal collection reported here (Table 11.1). Using Eq. (11.3) to calculate the number of replicates needed to detect a treatment difference of 4 percentage points in OM digestibility, we conclude that we need three replicates/treatment when SD = 1.4 and 24 replicates/treatment when SD = 4.9. This simple exercise highlights the importance of proper error control in the experimental design and analytical method, as well as the need to calculate the number of replicates needed for a given difference in treatment means to be detected.

If we average all the calculated SD values for each of the digestibility variables in Tables 11.1 and 11.2, **assuming all these studies represent samples from the same population**, we can solve Eq. (11.3) to determine the value of *d* that we can detect when using different replicates/treatment and with $\alpha = 0.05$ and $\beta = 0.2$.

Table 11.3 shows the results of conducting such mathematical exercise and can serve as a guide for the animal science researcher designing experiments to measure apparent total tract digestibility.

We can draw several conclusions from the data in Table 11.3:

1. The difference in treatment means that we can significantly detect for OM and CP digestibility are increased by approximately 50 % when using digestibility markers vs. doing total fecal collection. This same difference is increased by 100 % for NDF digestibility. This may be related to the external marker's inherent inability to mimic the dynamics of fibrous material in the gastrointestinal tract. A proof of this may be the fact that in the two studies cited in Table 11.2 that used acid insoluble ash as an internal marker (Mazzenga et al. 2009; Winterholler et al. 2009), the SEM for NDF digestibility does not seem to increase much from that of OM and CP digestibility. For the most part, the opposite is found in the studies that use $Cr_2O_3$ or $TiO_2$ as a marker.
2. Most digestibility studies conducted using markers use six replicates per treatment or more, which would allow them to detect a difference (with $P<0.05$) between treatment means of 7.6, 8.7 and 14.1 percentage points in total tract digestibility of OM, CP and NDF, respectively.
3. For both total fecal collection and marker-assisted digestibility measurements, the gain in detection capability increases to a greater extent when moving from 4 to 6 replicates per treatment than when moving from 6 to 8 replicates per treatment. This is not a surprise since the number of replicates is affected by the square root when solving for $d$ in Eq. (11.3). However this reminds us of the greater effect that a more precise estimate of SD can have on decreasing the SEM (by proper error control methods), over that achieved by increasing the number of replicates.

The use of proper laboratory techniques is critical, especially when dealing with markers, since the additive nature of experimental errors at the different steps (marker dosing, fecal and feed sampling, sample compositing, and analytical procedures) will contribute to a more "imprecise" estimation of the true standard deviation of the populations. The last paragraph of this section deals with practical considerations that lead to a more precise measurement of total tract digestibility. Finally, when choosing the experimental design in digestibility studies, a Latin square design can provide advantages in terms of control of inherent animal-to-animal variability in total tract digestibility. This is evidenced by the larger number of studies designed as a Latin square vs. any other design. However, an obvious disadvantage of such design is that the overall duration of the experiment usually increases when conducting a Latin square.

***Practical considerations when conducting in vivo digestibility studies***. Below are a few practical considerations that have been gathered from experience and from the analysis of the literature in the subject. These, along with the protocol in Table 11.5, are intended to aid the researcher in the planning and execution of digestibility studies.

1. If marker is to be delivered in a gelatin capsule via a balling gun, make sure the animal has swallowed the capsule before releasing from the chute. Any amount of marker that is not consumed by the animal needs to be accounted for in the digestibility equation.
2. If feeding the marker mixed with the feed, total daily consumption of marker needs to be accurately determined. Restricting feed intake during marker delivery has been used successfully to achieve this (Zinn et al. 2003; Corona et al. 2006). However, if ad libitum intake is required for the experiment, the amount of feed refused daily needs to be recorded. In addition if there is evidence of feed sorting by the animal, it may be necessary to analyze feed refusals for marker concentration and subtract total amount left in the bunk from offered amount of marker.
3. Typically with high-concentrate diets, the beginning of feed and fecal collections is offset by 1 day to account for average total tract retention times with such diets. However when conducting digestibility studies using low-quality hay, an offset of 2 days can be considered (Winterholler et al. 2009) to account for the longer dietary retention time in the total tract.
4. Because of the diurnal variation in marker and nutrient excretion, caution needs to be exercised during sample compositing. It is recommended to grind all samples collected and then create a composite on an equal dry weight basis.

## Studies Designed to Determine Nutrient Digestibility In Situ

The use of techniques to measure nutrient digestibility in situ has been essential in the development of nutritional models for ruminants. One of the greatest contributions of these techniques was in the area of protein requirements, in which measurements of protein degradation allowed the researcher to differentiate between meeting the nitrogen needs of the rumen microorganisms and meeting the protein needs of the ruminant host (Vanzant et al. 1996; Stern et al. 1997). In situ techniques require the use of animals cannulated in rumen, duodenum or both, depending on the objective. One common feature of in situ techniques is that they rely on the use of nylon or Dacron polyester bags with a pore size such that does not allow the feed sample to leave the bag, but that allows microorganisms and enzymes to enter the bag and digest the feed. The in situ methods described in this section are: (1) the mobile bag technique, (2) the in situ ruminal digestibility technique, (3) the three-step in situ/in vitro technique.

***Mobile bag technique***. This technique was originally developed to measure protein digestibility in pigs but has been modified to be used to determine postruminal digestion in ruminants (Stern et al. 1997). The most common use of the mobile bag technique is to determine apparent intestinal (small and large) digestibility of nitrogen when digestibility markers are not used. Thus one of the advantages is the elimination of complicated fecal collection schedules and any errors associated with

**Table 11.6** Protocol to determine apparent intestinal digestibility by the mobile bag technique

| | |
|---|---|
| 1. | Feed a minimum of two ruminally and duodenally cannulated ruminants a constant diet for at least 14 days before the beginning of the study |
| 2. | Grind samples through a 2 mm screen, place in Dacron polyester bags (5 × 10 cm, 40–60 μm of pore size) providing a maximum ratio of sample size:surface area of 10 mg of DM/cm$^2$, and heat-seal bags |
| 3. | Preincubate bags in the rumen for a time that resembles the mean ruminal retention time (MRT) of the feed sample. Mean retention time (in h) can be derived from the ruminal rate of passage of the feed ($K_p$, in $h^{-1}$) as follows: MRT = 1/$K_p$. When using forages, consider adding a 10-h lag to the MRT and incubate samples for a total of 75 % of the lag-added MRT. Concentrate feeds can be preincubated for the MRT |
| 4. | After ruminal incubation wash bags with cold tap water doing six cycles of rinsing of 2 min each. After removing excess water, dry bags at 55 °C for 48 h. Bags can be frozen for subsequent washing and drying if they are not going to be processed immediately for duodenal insertion |
| 5. | At this point, bags can be opened and pooled (within feed sample and ruminal incubation time) for subsequent postruminal incubation. If the same bag ruminally preincubated is to be used for duodenum insertion, make sure enough material is in the bag to be able to have remnant sample after intestinal digestion |
| 6. | Weight the amount of ruminally preincubated sample in the mobile bag and perform a sample dry matter analysis (100 °C for 24 h) on a separate sample to account for moisture present after drying at 55 °C. Never insert mobile bags with sample dried at 100 °C as heat damage will alter results |
| 7. | Insert bags through the duodenal cannula at a rate of one bag every 6 min. Include a blank bag to account for microbial attachment during transit through the lower gastrointestinal tract |
| 8. | Beginning 8 h after the insertion of the first bag through the duodenal cannula, start monitoring fecal excretions for the presence of bags. Collect bags immediately after excretion and freeze (−20 °C) until all bags from one animal have been excreted |
| 9. | Thaw bags at room temperature and rinse gently with cold tap water for a total of 10 s and squeeze to remove excess water. Repeat this process for a total of five rinsing cycles of 10 s each |
| 10. | Dry samples at 55 °C for 48 h and analyze for nutrient content. Correct for moisture content in the samples by drying at 100 °C |
| 11. | Apparent intestinal digestibility of nutrients can be calculated subtracting the amount of each nutrient found in feces from the amount entering the small intestine |

marker recovery and laboratory analysis to calculate marker concentrations. Logically to determine site of digestion it is imperative to have access to cattle fitted with both ruminal and duodenal cannulas, which can be the first limitation for the use of this technique. Procedures for the mobile bag technique have been extensively described in the literature (Stern et al. 1997; Vanzant et al. 1996, 1998) and are summarized in Table 11.6; however a few modifications have been introduced as a result of research conducted to optimize the method (Loveday et al. 2006). The basis of this method is the use of small nylon or Dacron polyester bags loaded with the feed sample to be tested. The bags are preincubated in the rumen prior to introducing them in the duodenum. Ruminal preincubation time has varied in several studies according to the substrate incubated but this time should simulate the mean

ruminal retention time of the substrate in the bag (Stern et al. 1997; Haugen et al. 2006b; Loveday et al. 2006). The effect of ruminal preincubation time on postruminal digestibility of nitrogen has been discussed by Haugen et al. (2006a, b) and it is concluded that each feedstuff should have a distinct ruminal preincubation time, which should be similar to the mean retention time (**MRT**) estimated from the following equation (Klopfenstein et al. 2001):

$$\mathrm{MRT} = 1 / \mathrm{K_p} \tag{11.4}$$

where MRT is the mean retention time (in h) and $K_p$ is the rate of passage (in $h^{-1}$) of the feedstuff to evaluate. In the case of forages, Klopfenstein et al. (2001) proposed the following equation to calculate $K_p$:

$$\mathrm{k_p}\ \left(\% / \mathrm{h}\right) = 0.07 \times \mathrm{IVDMD} - 0.20 \tag{11.5}$$

where **IVDMD** is the in vitro dry matter (**DM**) digestibility expressed in percentage and measured by following the protocol first developed by Tilley and Terry (1963) and subsequently modified to include urea in the McDougall's buffer (Klopfenstein et al. 2001). In vitro measurements of digestibility will be discussed in the next section of this chapter.

In order to obtain the total mean retention time to use in ruminal preincubations, Klopfenstein et al. (2001) proposed a modification based on the fact that forages (unlike concentrate feeds) may not be readily available for passage out of the rumen. Thus, a lag value of 10 h was proposed by Klopfenstein et al. (2001) to determine total mean retention time (**TMRT**) of forages. This value should be added to the MRT calculated by Eq. (11.4), to obtain the TMRT, which represent the time (in hours) at which bags used in the mobile bag technique should be incubated. This lag time should not be added to the MRT when concentrate feeds are being preincubated in the rumen. In fact some studies have shown that in concentrate feeds the contribution of ruminal preincubation to subsequent intestinal digestibility is negligible because of the extensive proteolytic activity of the small intestine (Stern et al. 1997). Studies conducted to optimize the mobile bag technique procedures in forage-based diets proposed that 75 % of the TMRT should be used instead of TMRT as the ruminal preincubation time to more closely mimic the kinetics of ruminal digestion of nitrogen (Haugen et al. 2006a, b).

Following the ruminal preincubation, the bags are removed and washed with cold tap water to remove the ruminal content attached to the exterior of the bag and to minimize microbial attachment to the bags and samples.

The original protocols for the mobile bag technique include a pepsin-HCl digestion incubation step to simulate abomasal digestion. However, in a review of studies using the mobile bag technique, Stern et al. (1997) concluded that pepsin-HCl incubation has no effect on intestinal digestion of nitrogen if a ruminal preincubation takes place. Similarly Loveday et al. (2006) found no effect of pepsin-HCl incubation on intestinal

disappearance of DM and CP in samples that had been preincubated in the rumen, and thus concluded that this step should be removed from the protocol. After ruminal incubation, bags should be washed by five cycles of 1 min each to remove most of the bacterial N attached to the bag and then dried at 55 °C for 48 h (Vanzant et al. 1998). Bags can be frozen after removal from the rumen if they are not going to be washed and dried immediately (Haugen et al. 2006b).

Bags preincubated in the rumen can be pooled on an animal and feed sample basis to ensure that sufficient material is present in the mobile bags prior to intestinal digestion (Loveday et al. 2006). Take into account that after post-abomasal digestion, sufficient sample should be recovered in the feces to able to do all the analyses for nutrient content. Also keep in mind that weighing of samples added to each bag to be inserted through the duodenal cannula is imperative to be able to calculate intestinal digestibility. A subsample of each ruminally preincubated feed should be dried at 100 °C to determine sample dry matter, but this sample should be discarded. Never use samples dried at 100 °C for ruminal or intestinal incubation because heat damage is likely to affect the results. Bags preincubated in the rumen can be inserted in the duodenal cannula at a rate of 5–6 min per sample (Haugen et al. 2006b) starting immediately after the morning feeding (Loveday et al. 2006) or 2 h postfeeding (Haugen et al. 2006b). A minimum of 5 min between the insertion of each bag is needed to allow for the movement of the previous bag an avoid compaction. Because of the continuous influx of digesta into the small intestine, the timing of insertion of bags in the duodenum relative to feed delivery is not likely to impact the results significantly.

The collection of bags incubated in the small intestine can be done in the ileum (if a ruminant with an ileal cannula is used) or in the feces. For practical purposes, most studies have used fecal recovery of mobile bags yielding acceptable results, however especial measures have to be taken to account for microbial contamination of bags. For intestinal CP degradation, because of the small amount of nitrogen that may remain in the sample after passage through the small intestine, the contribution of microbial contamination in the hindgut can be significant affecting the results if bags are not properly washed, leading to underestimation of intestinal CP degradation (Stern et al. 1997). Depending on the size of the bags used, the first bag should appear in the feces approximately 8 h after insertion through the duodenal cannula, with larger bags having a shorter MRT in the post-abomasal digestive tract (Loveday et al. 2006). Average transit times of approximately 13 h have been reported for bags of 5 × 10 cm containing 1.25 g of air-dry forage ground through a 2-mm screen, inserted in the duodenum and collected in the feces (Haugen et al. 2006b). Once recovered in the feces, bags should be frozen immediately (−20 °C) as they are excreted until all bags from the one animal are collected (Haugen et al. 2006b; Loveday et al. 2006). This is to prevent further degradation of nutrients in the bag after excretion. Once all bags are collected and frozen, they should be thawed at room temperature and washed with cold tap water to remove fecal materials and bacterial contents. Loveday et al. (2006) analyzed the effects of several washing

protocols and concluded that washing with cold tap water for a total of 10 s repeating the process for a total of five rinsing cycles of 10 s each was not significantly different from the results with longer washing cycles. After washing, samples can be dried at 55 °C for subsequent nutrient content analyses.

The mobile bag technique provides a relatively easy and fast way to measure small intestine (if ileal cannula is present) or post-abomasal (if collecting bags in the feces) digestibility of feedstuffs. The need to standardize the procedures used in this technique in order to create a reliable database was recognized by Stern et al. (1997). Much progress has been made since then in identifying the factors that affect the variability of the technique (Klopfenstein et al. 2001; Loveday et al. 2006; Haugen et al. 2006a). Based on the previous discussion of factors affecting the use of the mobile bag technique, a detailed protocol is provided in Table 11.6 to determine intestinal digestibility of nutrients.

**In situ ruminal digestibility**. Ruminal incubations of polyester bags have been used to measure rate and extent of nutrient digestion. The contribution of this technique to modern ruminant nutrition has been considerable, and data generated by this technique has been essential in digestion models based on ruminal degradation kinetics such as the Cornell Net Carbohydrate and Protein System, or the 2001 edition of "Nutrient Requirements of Dairy Cattle" by the National Research Council.

The most common use of the in situ digestibility technique is to determine the rate and extent of DM and CP digestibility (Stern et al. 1997; Bach et al. 1998); however, models have also been developed to determine in situ forage digestibility (Mertens and Loften 1980). To determine rate and extent of CP and DM digestibility it is imperative to divide the incubated feed into three fractions: (1) the immediately soluble and thus assumed to be readily digestible fraction (fraction A); (2) the potentially digestible fraction (fraction B); and (3) the undegradable fraction (fraction C). In order to determine these fractions, Dacron polyester bags (40–60 μm of pore size) containing the feedstuff to test (air-dried, or dried at 55 °C) are incubated in the rumen for different periods. Once removed from the rumen the bags are rinsed and the material that "disappears" from the bag is considered digested.

Fraction A is determined by soaking the bags with feed for 15 min in warm (39 °C) water. Any material leaving the bag is considered soluble and thus assumed digested. While digestibility and solubility are often considered synonymous, this may not always be true for all feedstuffs. For more information on this specific issue of solubility and digestibility the reader is encouraged to consult the study by Bach et al. (2008). Fraction C is determined as the remainder after an incubation time well beyond that required to reach the full extent of potential digestibility. This is typically achieved with ruminal incubations of at least 48 h for concentrate feeds and at least 72 h for forages. Fraction B (potentially digestible) is then calculated by subtracting the A and C fractions to the total material incubated. To facilitate calculations, these fractions are usually measured as percentages of the total amount of material incubated.

There are two key components in the calculation of the extent of ruminally degradable protein (**RDP**): the rate of degradation ($\mathbf{K_d}$) and the rate of passage ($\mathbf{K_p}$). Those parameters are "competing" in the rumen to either digest or remove the feed

material ingested. The relationship between those parameters and the extent of ruminal CP degradation (RDP) is given by the following equation (Bach et al. 1998):

$$\begin{array}{l}\text{Extent of ruminal CP}\\ \text{degradation (or RDP)} = \end{array} \underset{\uparrow}{\text{Soluble CP}} + \underset{\uparrow}{\text{degradable CP}} \times \left[K_d / (K_d + K_p)\right] \quad (11.6)$$

(Fraction A) (Fraction B)

The rate of passage $k_p$ is usually measured or estimated based on the MRT reported in previous studies with similar diets and intakes. However, the degradation rate ($K_d$) is measured based on the analysis of residual N in ruminally incubated Dacron polyester bags. Two main approaches have been used to determine $K_d$: linear and nonlinear models. The most commonly used nonlinear method is that described by Ørskov and McDonald (1979) in which the disappearance of CP is a function of incubation time and rate of degradation as described in Eq. (11.7) below:

$$\text{CP disappearance} = A + B \times \left(1 - e^{-Kd \times t}\right) \quad (11.7)$$

where A is the soluble CP fraction (% of CP), B is the potentially degradable CP fraction (% of CP), $K_d$ is the degradation rate constant ($h^{-1}$), and t is the ruminal incubation time (h).

The most commonly linear approach to determine the rate of ruminal CP or DM disappearance is that described by Mathers and Miller (1981), in which $K_d$ of the potentially degradable CP or DM is calculated as the slope of the regression line of the natural logarithm of residual N (or DM) vs. incubation time. This method involves the incubation of bags for several times and the individual analysis of remaining CP or DM. Same as what was discussed for the mobile bag technique, the incubation of material in the rumen should be conducted considering a maximum sample size:bag surface area of 10 mg of DM/$cm^2$ to ensure proper contact of incubated feed with ruminal fluid (Vanzant et al. 1998; Loveday et al. 2006; Galyean 2010). To calculate $K_d$, the first (0 h) and last incubation time (48, 72 or 96 h, depending on feedstuff) is considered fractions A (soluble) and C (undegradable), respectively. Thus, to determine the slope of the regression line of the natural logarithm, only the material remaining after all other incubation times is used, which represents the fraction B, or potentially degradable. An example calculation of $K_d$ using the slope of the regression line of the natural logarithm is presented below:

Figure 11.1 shows the results of samples that have been suspended in the rumen using Dacron polyester bags at several incubation times (see Table 11.7 for details). After analyzing the bags for remaining N content, data points corresponding to 0 and 72 h are removed from the $K_d$ analysis as they represent fractions A and C, respectively. The remaining data points represent fraction B (potentially degradable

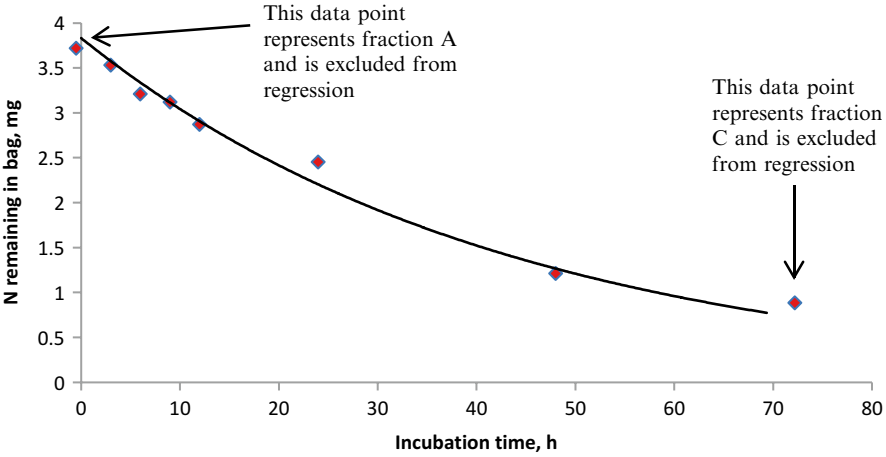


**Fig. 11.1** Example of the determination of $K_d$ using the slope of the regression line of the natural logarithm

CP) and are transformed using the natural logarithm and a regression line is fitted. The absolute value of the slope of the regression line represents the $K_d$. The absolute value of the slope is used because when fitting remaining N values, the direction of the slope in the regression will yield a negative value.

While both linear and nonlinear methods are commonly found in the literature when determining $K_d$, the choice of the method may depend on the type of feedstuff incubated. Bach et al. (1998) analyzed several mathematical models used to calculate kinetics of protein degradation and concluded that the choice of model should be based on the analysis of residuals vs. fitted and lack-of-fit tests to assess the validity of the model to describe the degradation patterns. It is noteworthy that the study by Bach et al. (1998) included only meat and bone meal and soybean meal as protein sources, thus, testing of mathematical models across a wider range of feeds may be needed.

Note that the point made in Step 4 of Table 11.7 about placing bags in reverse order of incubation applies only to steady-state conditions this is, when cattle are consuming feed continuously (e.g., a dairy cow fed ad-libitum). When it is of research interest to determine the digestibility of a feedstuff relative to feeding times (e.g., a feedlot steer fed once or twice daily), then the reverse order incubation may not be suitable. The advantage of placing bags in reverse order is that it creates minimal disturbance to the rumen contents every time a new bag is added and it allows for the washing of all bags at the same time when they are removed from the rumen.

**Table 11.7** Protocol to determine in situ ruminal digestibility using Dacron polyester bags

| | |
|---|---|
| 1. | Feed a minimum of two ruminally and duodenally cannulated ruminants a constant diet for at least 14 days before the beginning of the study |
| 2. | Grind samples through a 2 mm screen, place in Dacron polyester bags (40–60 μm of pore size) providing a maximum ratio of sample size:surface area of 10 mg of DM/$cm^2$, and heat-seal bags |
| 3. | Soak all bags in warm (~39 °C) water for 15 min immediately before ruminal incubation |
| 4. | Incubate bags in the rumen for 0, 3, 6, 9, 12, 24, 48, and 72 h. The 0 h bags should be soaked for 15 min in warm (~39 °C) water only. Different incubation times can be used depending on feedstuff incubated. To facilitate the procedure, bags should be placed in the rumen in reverse order of incubation time so that all bags are removed at once at the end of the longest incubation time. Place 1 empty and sealed bag for each incubation time to account for bacterial and feed attachment to the bag |
| 4. | After ruminal incubation wash bags with cold tap water doing six cycles of rinsing of 2 min each. After removing excess water, dry bags at 55 °C for 48 h |
| 5. | Weigh bags to determine DM remaining. To determine remaining CP at each time point bags can be opened to measure total N by combustion or Kjeldahl method. Alternatively the entire bag (and blanks) can be digested by adding 15 mL of concentrated sulfuric acid, 3.5 g of $K_2SO_4$ + 0.4 g $CuSO_4$ (catalyzer) and heating to 400 °C for 2 h. After digestion, total N can be determined by Kjeldahl. Content of N in blank bags is subtracted from the N in bags of the respective incubation time |
| 6. | Calculate the rate of degradation ($K_d$) by using one of the methods described previously: linear or nonlinear approach. Material disappearing from 0-h bags represents the soluble fraction A and material remaining after the last incubation time represents the undegradable fraction C |
| 7. | Once $K_d$ is determined, ruminal in situ extent of CP or DM degradation can be calculated using Eq. (11.6) |

## Three-Step In Situ/In Vitro Digestibility

Ruminal degradation of protein often provides an incomplete picture for the researcher and nutritionists. Protein sources (microbial or dietary) entering the small intestine can have different digestibility coefficients based on factors such as heat or chemical pre-treatment. The development of protein sources, mainly soybean meal, with increased degree of ruminal protection or bypass potential has been an active area of research and revenue for feed companies. However, a protein that is so protected from ruminal degradation that ends up bypassing the entire GI tract is also useless for the ruminant. As a result, more emphasis is placed nowadays in intestinally absorbable dietary protein (**IADP**; Stern et al. 1997) which results from a combination of the ruminal undegradability and intestinal digestibility coefficients. The three-step procedure (**TSP**) has been developed to provide a more complete picture of protein degradation in ruminants.

The TSP was developed by Calsamiglia and Stern (1995) to provide an alternative to the more labor-intensive methods of estimating intestinal digestion of proteins, which require the use of surgically prepared animals. The TSP is a relatively rapid and less expensive method and has been adopted by the NRC (2001) as the reference method for intestinal digestibility of proteins (Gargallo et al. 2006). The original protocol of the TSP (Calsamiglia and Stern, 1995) involves a ruminal incubation of the protein source for 16 h in a Dacron polyester bag. The bags are then washed with cold tap water and dried at 55 °C for 48 h. The remaining material, which is essentially rumen undegradable protein (**RUP**), is then incubated for 1 h in a pepsin/HCl solution at 38 °C in a shaker incubator. After the pepsin/HCl incubation, a pancreatin/$KH_2PO_4$ solution is added to the tubes and incubated for an additional 24 h at 38 °C in a shaker incubator. After the pancreatin incubation, the fermentation is stopped and undigested proteins are precipitated with trichloroacetic acid. Samples are then centrifuged and the supernatant is analyzed for N content. The digestibility of RUP is then calculated as trichloroacetic acid-soluble N divided by the amount of N in the sample.

While the original TSP was widely used after its development in 1995, it presented two major issues: (1) trichloroacetic acid is highly corrosive and toxic acid for humans and the environment (Gargallo et al. 2006), and (2) the use of trichloroacetic acid prevented the method from further analyses to determine individual amino acid digestibility. As a result, a modification was proposed by Gargallo et al. (2006) to reduce the cost and labor associated with the original method to eliminate the use of trichloroacetic acid. Briefly, the modification of the method involved the use of Dacron polyester bags (Ankom R510, Ankom Technology, Macedon, NY) and a Daisy$^{II}$ incubator (Ankom Technology). This allowed the elimination of the trichloroacetic acid from the method because the undigested protein remained in the bag instead of being precipitated and centrifuged. As a result, in the modified method the digestibility of RUP is calculated by subtracting the N remaining in the bag from that found after the 16-h ruminal incubation.

The modification of the method by Gargallo et al. (2006) was later coupled with the use of an immobilized digestive enzyme assay to be able to measure the individual amino acid digestibilities of RUP by analyzing the amino acid concentrations in the feed (Boucher et al. 2009). In addition, Boucher et al. (2009) tested the possibility of using Ankom F57 (Ankom Technology) bags in an attempt to reduce the pore size from 50 μm (R510 bags) to 25 μm (F57 bags). Boucher et al. (2009) concluded that Ankom R510 bags should be used instead of Ankom F57 during the enzymatic incubations because the reduced pore size may lead to clogging of pores and inconsistent results.

A protocol to determine IADP based on the original TSP procedure by Calsamiglia and Stern (1995) with the modifications by Gargallo et al. (2006) is provided in Table 11.8. Several practical comments are included based on personal communications from Dr. Martin Ruiz Moreno.

Note that the procedure described in Table 11.8 can be used to calculate IADP under the assumption that the material remaining in the bag after 16 h of incubation represents the RDP in the feed sample. For an increased precision, the researcher should calculate RDP using the techniques described previously and detailed in

**Table 11.8** Protocol to determine intestinally absorbable dietary protein using the three-step procedure

| | |
|---|---|
| 1. | Grind samples through a 2 mm screen, place 0.5 g in Dacron polyester bags (40–60 μm of pore size and 5 × 10 cm) and heat-seal bags |
| 2. | Soak all bags in warm (~39 °C) water for 15 min immediately before ruminal incubation |
| 3. | Incubate bags in the rumen for 16 h |
| 4. | After ruminal incubation wash bags with cold tap water until runoff is clear. After removing excess water, dry bags at 55 °C for 48 h and weigh to determine DM remaining |
| 5. | Place a maximum of 24 bags in a Daisy$^{II}$ incubator jar (Ankom Technology), fill with 2 L of a pepsin/HCl (pH = 1.9) solution and incubate for 1 h at 39 °C under constant rotation. Include a blank bag to account for enzyme residue in the bag after final wash |
| 6. | Empty the jar and add 2 L of a pancreatin/$KH_2PO_4$ buffer (pH = 7.75). Add 50 ppm of thymol to prevent microbial growth. Incubate bags for 24 h at 39 °C under constant rotation |
| 7. | Rinse the bags with tap cold water until the runoff is clear |
| 8. | Digest the entire bag (and blanks) to determine total N by the Kjeldahl method |

Refer to the publications by Calsamiglia and Stern (1995) and Gargallo et al. (2006) for further details on reagents and methodology

Table 11.7, since the use of multiple incubation times will provide a better estimate of $K_d$ than the residue of a single incubation point.

Finally, the development of an in situ method to determine forage RUP by Mass et al. (1999) deserves some attention. The need for a rapid and labor-efficient method to determine the undegradability (or bypass) faction of the CP forages motivated the researchers at the University of Nebraska to develop the modified in situ neutral detergent insoluble nitrogen method (Mass et al. 1999). Briefly, the method involves the in situ incubation of samples at three different times, and the subsequent neutral detergent extraction of the residue to remove microbial N. This method has been proved accurate for estimating forage RUP yielding results similar to those using accepted microbial correction methods such as purines (Mass et al. 1999; Klopfenstein et al. 2001).

## In Vitro Studies to Determine Nutrient Digestibility and Fermentation Profile

Measuring digestibility in vitro has been always a very attractive option to the more expensive and labor-intensive methods. Since the development of the early in vitro procedures by Tilley and Terry (1963), hundreds of studies have been conducted using this technique and several modifications to the procedure have been reported. While in vitro digestibility conducted in batch cultures can be an excellent tool to screen potential additives or to obtain a quick estimate of energetic content of a forage or byproduct, the procedure has several limitations that need to be recognized:

1. It is a static system and does not account for the effect of rate of passage.
2. The incubation fluid can acidify rapidly as there is no absorption of VFA produced.

3. There is a great effect of particle size on digestibility estimates, thus all ingredients tested need to be ground to the same particle size.
4. If gas produced is not properly vented, pressure build up can affect microbial fermentation during long incubation times.
5. Only liquid-associated bacteria are included in the inoculum when using strained ruminal fluid in traditional in vitro systems. Considering that 70 % of the ruminal bacteria are found in biofilms on the surface of feed particles (Russell 2002), the contribution of this group in the overall digestion process can be underestimated.

In particular, the use of batch culture incubations can present a few advantages, such as to provide the opportunity to measure total gas production and total VFA produced. Because VFA accumulate in vitro, measuring VFA concentration at the end of batch culture incubations is a true reflection of production, which is not always the case in vivo because absorption across the rumen wall can be significant. It is also for this reason that is necessary to include a buffer in the incubation fluid to prevent a drop in pH that can affect the fermentation, yielding a final incubation pH similar to that expected in vivo with a similar diet to that used in vitro. One of the greatest challenges of the design of batch culture incubations for in vitro studies is how to determine the optimal proportion of buffer to ruminal fluid, and how to determine the correct amount of feed and additive to add. To address the first problem, it is common to conduct pilot studies to determine the final pH after the desired incubation time, which should match the ruminal pH typical of an animal consuming the same diet. Buffer:ruminal fluid proportions of 3:1 and 4:1 for substrates comprised of concentrate and forage, respectively, are commonly found in the literature. The issue of how much feed to incubate has been extensively studied and agreement exists, based on some of the early work by Tilley and Terry (1963), that the relationship should be between 0.5 and 1 g of dried substrate per 50 mL of incubation fluid. Considering that the ruminal fluid is diluted four or five times by the buffer, an incubation of 0.5 g of substrate per 50 mL of volume can be comparable to an animal consuming 5 kg of DM per day and with a ruminal volume of 100 L. Finally, on the subject of calculating how much of an additive to add, two considerations can be made to simulate in vivo conditions: to include it as a proportion of the substrate incubated, or as a proportion of the incubation volume. Both approaches have been found in the literature. However, for the testing of additives with low inclusion rates, it is preferable to add them to the batch culture dissolved in polar or nonpolar solvents, or in suspensions, to improve the precision. A typical example of this is the use of the ionophore monensin in batch culture incubations. It is common to include monensin as a liquid dissolved in ethanol (Kung et al. 2000; Quinn et al. 2009; Smith et al. 2010) because the low inclusion rate in diets of ruminants would make it impossible to weigh the small amount required per bottle or flask. To illustrate with an example: if a 100 mL bottle containing 1 g of substrate is to be incubated, in order to test a monensin dose of 400 mg/animal/day, one could make the assumption that once ingested with the feed this monensin amount will be diluted in a ruminal volume of 100 L. Thus, the final concentration in the batch culture incubation fluid should be 400 mg/100 L = 4 mg of monensin/L.

**Table 11.9** Protocol to determine in vitro digestibility using 50-mL polycarbonate tubes

| | |
|---|---|
| 1. | Grind samples through a 2 mm screen, place 0.5 g in a 50-mL polycarbonate tube. Place a stopper with a one-way release valve (rubber policeman). Alternatively the stopper can be fitted with a 16-gauge needle for continuous gas release |
| 2. | Prepare a McDougall buffer (McDougall 1948) making sure that the $CaCl_2$ is added prior to use and that the pH is reduced to 6.8–7.0 by bubbling with $CO_2$ |
| 3. | Collect ruminal fluid staining through four layers of cheesecloth and transport to the laboratory maintaining a warm temperature and anaerobic conditions |
| 4. | Inoculate tubes with 36 mL of a 3:1 or 4:1 mixture of buffer:ruminal fluid. Flush tubes with $CO_2$, place stopper and incubate for 24 h at 39 °C under constant agitation. Note: make sure to incubate one or two blank tubes without any substrate |
| 5. | After incubation, place tubes on an ice-water bath for 15 min to stop the fermentation. Centrifuge for 15 min at 2000 × *g* and discard the supernatant. At this point tubes can be frozen to continue with the next steps later |
| 6. | Prepare a pepsin/HCL solution as indicated by Galyean (2010), add 36 mL to the tubes and incubate for 48 h at 39 °C under constant agitation |
| 7. | Label filter papers, place in a 100 °C oven for a minimum of 12 h and record weight. Note: Make sure to use ashless paper if OM digestibility is to be determined |
| 8. | Filter the contents of the tube through a filter paper rinsing with deionized water to ensure that all the material is collected in the filter. Dry filter papers containing undigested material for 24 h at 100 °C and weigh |
| 9. | Calculate in vitro dry matter digestibility as follows:<br>IVDMD (%) = 100 × [initial dry sample weight × (residue × blank)/initial dry sample weight)] |

Modified from Tilley and Terry (1963), and Galyean (2010)

One of the key concepts to consider when designing experiments involving batch cultures is the need for proper replication. Because of the variation that exists from day to day in the inoculum (ruminal fluid), it is recommended that for a proper replication, batch culture incubations should be conducted in at least 2 (preferably 3) separate days. While each treatment can be replicated in several bottles within each day, the true replicate should be considered to be the incubation day, and bottles within day can be considered subsamples of an experimental unit. Values from each of the subsamples can be averaged within incubation day for the final statistical analysis.

The technique modified from Tilley and Terry (1963) involves an incubation with ruminal fluid:buffer mix, followed by a centrifugation step and removal of supernatant. The tube with undigested material is then filled with pepsin/HCl solution and incubated for 24 h at 39 °C. The residue is then filtered on a filter paper and weighed after drying. As with many other techniques, the inclusion of blank tubes without any substrate added is essential to account for inoculum and buffer contributions to the final DM.

The procedure in Table 11.9 can be modified by scaling everything up (substrate and incubation fluid) to allow the subsampling of incubation fluid for VFA or $NH_3$-N analyses. In addition, if more material is incubated, the remains can be analyzed for fiber content to determine NDF or ADF digestibility. A useful modification of the procedure in Table 11.9 is to conduct the incubations in 125-mL serum bottles

crimp-sealed with butyl rubber stoppers to allow the collection of total gas produced and further analysis of gas composition (Quinn et al. 2009; May et al. 2010; Smith et al. 2010).

Finally, an in vitro method that has been adopted as the standard to determine metabolizable protein in European nutrition models is the *Streptomyces griseus* procedure (Aufrére et al. 1991). This method was later modified by Abdelgadir et al. (1997) by removing carbohydrases and successfully validated using the in situ procedure to adapt its use to measure forage RDP (Klopfenstein et al. 2001).

## Conclusions

Measuring digestibility of feeds is essential for the development of nutritional strategies in ruminant nutrition. As a result, a great number of publications over the years have been devoted to the development or modification of techniques aimed at accurately, rapidly and inexpensively measuring digestibility. The most commonly used techniques were reviewed and practical recommendations based on experience are provided in the discussion of each method. The golden standard to measure apparent digestibility in the total tract continues to be the total collection of feces. However, several methods have been developed to provide insights not only on digestibility of nutrients in the total tract, but also on kinetics of feed degradation, ruminal fermentation profiles, and postruminal digestibility. While the in vitro digestibility provides a rapid and relatively inexpensive alternative, future research efforts should concentrate on the improvements of this technique to account for the contribution of feed particle-associated bacteria to the in vitro digestion process.

Ruminant nutrition research is a challenging and dynamic field where new feedstuffs constantly become available as a result of the evolving agricultural industries, yielding byproducts suitable for animal feeding. The combinations of in situ/in vitro assays to develop rapid and cost-effective assays that accurately model all the digestive processes should be a research priority in order to allow the nutritionists to cope with the changes in the cattle feeding industry.

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