### academicJournals

Vol. 10(14), pp. 456-464, 14 April, 2016 DOI: 10.5897/AJMR2016.7627 Article Number: 0ACAF2358006 ISSN 1996-0808 Copyright © 2016 Author(s) retain the copyright of this article http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Review

### Ruminal microorganism consideration and protein used in the metabolism of the ruminants: A review

Tiago Neves Pereira Valente<sup>1</sup>\*, Erico da Silva Lima<sup>2</sup>, Wallacy Barbacena Rosa dos Santos<sup>3</sup>, Andréia Santos Cesário<sup>3</sup>, Cássio Jardim Tavares<sup>1</sup>, Ítalo Lacerda Fernandes<sup>1</sup> and Marco Antônio Moreira de Freitas<sup>1</sup>

> <sup>1</sup>Instituto Federal Goiano, Posse Campus /GO, Brazil. <sup>2</sup>Master's Degree Studies Program in Environmental Health at FMU, São Paulo/SP, Brazil. <sup>3</sup>Instituto Federal Goiano, Morrinhos Campus /GO, Brazil.

> > Received 16 June, 2015; Accepted 7 March, 2016

A great diversity of species of microorganisms are present in the rumen environmental with specific functions in the degradation of carbohydrates, protein and lipids. However, the knowledge of the interactions between the different species of microorganisms in the rumen ecosystem and their specific substrates were used to improve nutritional management and can increase production of meat or milk. A balanced nutritional management is very important. When inappropriate feedstuffs are used on diet formulation for cattle, there is a decrease in the growth of microorganisms in the rumen. And the availability of the use of protein synthesized in rumen for all metabolisms of the animal.

Key words: Microorganism growth, microbial protein, proteolysis.

### INTRODUCTION

The rumen is a suitable environment for the development of a large number of anaerobic microorganisms, having unique characteristics such as temperature around 38 to 42°C (Pourazad et al., 2015). But normally, the temperature was more commonly found to be 39°C (Hoover and Miller, 1991; Kim et al., 2014; Yazdi et al., 2015). In the rumen, a redox potential was found (-350 mV, with fluctuations between -250 and -450 mV), with result of a strong ambient environment due to the lack of oxygen. The rumen is usually well buffered, due to the presence of bicarbonates and phosphates founded in continuous flow of saliva (Puggaard et al., 2011; Røjen and Kristensen, 2012; Storm et al., 2014). Saliva production can be high in a cow, reaching over 180 L/day. However, the pH can vary due the nature of the diet, but typically found between 5.5 and 7.0 when ruminants are fed with predominantly on forage diet (Aschenbach et al., 2014). According to Pourazad et al. (2015), highly fermentable diets are rapidly converted to volatile fatty acid (VFA) in the rumen. The resulting release of protons can constitute a challenge to the ruminal ecosystem and animal health. Although increased acid production is a nutritionally desired effect of increased concentrate feeding, the accumulation of

\*Corresponding author. E-mail: tiago.valente@ifgoiano.edu.br

Author(s) agree that this article remains permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> protons in the rumen is not. Because pH values below 6.0 can reduce the survival of bacteria degrading cellulose and synthesis protein. Similar results were observed by Russell and Strobel (1987). Rumen fermentation appeared to continue unimpeded, but when the mean rumen pH was 5.4 it resulted in reduced fiber digestion and less microbial protein flowing to the small intestine (Hills et al., 2015). In the gas phase, carbon dioxide and methane were gases that are more present. Oxygen varies from less than 0.1% depending on the amount of water and feed ingested. The osmotic pressure of rumen contents ranges between 260 and 340 moles with an average value of about 280 moles. Values greater than 350 can cause stop rumination process and a decrease in the absorption of VFA (Russell, 2002).

### RUMEN MICROBIAL DIVERSITY

The ruminal microorganisms are composed mainly by three groups of anaerobes; by bacteria, protozoa and fungi. The populations of each group and their species are directly influenced by the type of feedstuff provided to the ruminant host. The rumen-reticulum represents more than 50% of the digestive capacity, consisting 10<sup>10</sup> bacteria, 10<sup>6</sup> protozoa and 10<sup>4</sup> fungi per milliliter of rumen content (Theodorou and France, 2005; Krause et al., 2014). The microbial population has three important nutritional functions for animals: (1) Digestion and fermentation of carbohydrates, such as cellulose and starch, with consequent production of VFA. (2) Amino acid (AA) synthesis from non-protein nitrogen (NPN) from the diet or from recycling via saliva or ruminal and diffusion from dietary rumen degradable protein. (3) The synthesis of B-complex vitamins and vitamin K. Microbial populations can be subdivided into those free in liquid phase, those linked to food particles, and those attached to the ruminal epithelium (Cheng and McAllister, 1997).

### Bacteria

The classification of ruminal bacteria may vary depending on the substrate offered the animal, according to Koslozki (2002), as follows:

(1) Fermenters of structural carbohydrates: Degrade cell wall components of plants, especially cellulose and hemicelluloses, have a relatively slow growth rate and depend on ammonia and branched-chain fatty acids for the synthesis of proteins (isovalerate, isobutyrate and 2-metilbutyrate), for example, *Ruminococcus albus* (Suen et al., 2011), *Fibrobacter succinogenes, Ruminococcus flavefaciens*.

(2) Fermenters of non-structural carbohydrates (CNE): Associate the particles of cereal grains or granules of starch and can use ammonia, AA or peptides (PEP) for

synthesis of proteins. As we have increased our knowledge to modify the microbial population to enhance production efficiency and animal health. Because lactic acidosis is an animal health issue related to rumen function, it became an early target for fermentation modifications (including the use of ionophores) (Krause et al., 2014). Much of the development of lactic acidosis (acute, subacute, and chronic) has been linked to increasing populations of Lactobacillus species. Ruminobacter amylophilus and Streptococcus bovis, after increased availability of fermentable soluble starch in the rumen (Plaizier et al., 2012).

(3) Proteolytic: Most of the ruminal bacterial species degrade proteins. However, there are a few species that have a proteolytic activity far more intense than the others, for example, *Peptostreptococus* species; higher *Prevotella ruminicola* and *Streptococcus bovis*.

(4) Methanogenics: Are the most strictly anaerobic rumen. Produce methane from CO<sub>2</sub> and H<sub>2</sub> fermentative activity derived from other species. Methane  $(CH_4)$ emissions from ruminant livestock are 90% made from enteric fermentation. Reduction of carbon dioxide to CH₄ is critical for efficient ruminal fermentation, because it prevents the accumulation of reducing equivalents in the rumen (McAllister et al., 2015). The methanogenics are members of the Kingdom Archae (Russell, 2002), for examples: Methanobacterium species and Methanobrevibacter species. The relationship between methanogens and protozoa is expected. Studies have shown that reducing protozoa populations would reduce CH<sub>4</sub> production indirectly (Leahy et al., 2010; Hook et al., 2010; Wright and Klieve, 2011; Attwood et al., 2011). Second McAllister et al. (2015) methanogens exist in a symbiotic relationship with rumen protozoa and fungi and within biofilms associated with feed and the rumen wall. Studies of the ecology of ruminal methanogenesis are important in ruminant nutrition and identifying ways for its mitigation. According to Hünerberg et al. (2015), several factors such as fast passage rate or cattle fed high-grain diets can decrease CH<sub>4</sub> emissions. However, lowering ruminal pH alone is, therefore, not an effective CH<sub>4</sub> mitigation strategy. Other examples of CH<sub>4</sub> mitigation occur when add lipid in the ration of ruminants, methane emission would reduce by 2.2 to 5.6% (Beauchemin et al., 2008; Martin et al., 2010).

(5) Lactic: Growing under conditions of low rumen pH and using, among others, lactic acid as an energy substrate. Marx et al. (2011), for example: *Megasphaera elsdenii* is a lactate-utilizing bacterium whose ruminal abundance has been shown to be greatly elevated during milk fat depression. Second Weimer et al. (2015) studied the effects of the addition of *M. elsdenii* and concluded milk yield and composition were not affected by dosing. Ruminal pH, VFA and lactate did not differ between dosed and control cows, although acetate-to-propionate ratio declined in both groups and butyrate increased after dosing with *M. elsdenii*. The results confirm that

establishing exogenously added bacterial strains in the rumen is difficult, even for strains previously isolated.

(6) Pectinolitics: Ferment pectin. Although pectin is a polymer of a structural nature, its fermentation, as well as the characteristics of bacteria that use it, are similar to those that ferment the non-structural carbohydrates, for example, *Succinivibrio dextrinosolvens* and *Lachnospira multiparus*.

(7) Lipolytic: Hydrolyze triglycerides into glycerol and fatty acids such as *Anaerovibrio lipolytica*; despite the importance of understanding lipolysis and its impact on subsequent biohydrogenation of polyunsaturated fatty acids by rumen microbes. According to Prive et al. (2013), the enzymes had higher activities at neutral to alkaline pH and had higher hydrolytic activity against caprylate (C8:0), laurate (C12:0), and myristate (C14:0).

(8) Ureolitics: Adhered to the ruminal epithelium, hydrolyze urea and release ammonia in the rumen, as *Enterococcus faecium* (Khattab and Ebeid, 2014).

### Protozoa

Protozoa can contribute with more than half of the rumen microbial mass (Van Soest, 1994). However, the flow out of the rumen protozoa is not proportional to its number in the rumen. Protozoa are retained because of biomass selectively in the rumen (Williams et al., 2008). The removed of rumen protozoa increases the flow of microbial and dietary protein to duodenum. In animals feed diets of low nutritional value and high in fiber, evidence the increase in the number of protozoa. Some protozoa are cellulolytics. The main sources of energy supply to the protozoa are carbohydrates, with preference for the use of starch and sugars, compared to diet high level of fiber. The main source of nitrogen (N) is the protein feedstuff, or microbial available through engulfment of bacteria in rumen environment. Protozoa use insoluble proteins rather than soluble proteins, while bacteria did not use ammonia for AA synthesis (Dijkstra et al., 1998).

Many types of protozoa in rumen have different participations on ruminal fermentation, some being beneficial to ruminants and not others (Williams et al., 1991; Williams and Coleman, 1997). In vivo experiments have established that mixtures of protozoa ciliates decrease the flow of ammonia N of the stomach into the intestine (Ivan et al., 2000). Despite the greater flow of N ammoniac in animals without protozoa (named defaunated), compared with the non-removed rumen protozoa, second Veira (1986), most of the experiments showed a lower growth rate due to the elimination of rumen ciliates protozoa. Defaunation decreased NH<sub>3</sub>-N concentration and increased the conversion efficiency of blood urea N and protein-derived NH<sub>3</sub>-N conversion into microbial protein in the rumen. According to Firkins et al. (2007) more studies should be done for characterization

of protozoa interactions with proteolytic and deaminating bacterial populations. For example, dairy cattle have greater intakes of readily available carbohydrate combined with increased ruminal passage rates, decreased protozoa biomass relative to bacterial biomass and increase the efficiency of protozoa growth. Thus, reducing the negative effects of bacterial predation, compared with the beneficial effects that protozoa have on stabilizing the entire microbial ecosystem. The addition of lipid in the ration of ruminants negatively affects protozoa population (Szumacher-Strabel et al., 2004; Varadyova et al., 2007; Szumacher-Strabel and Cieslak, 2012).

Belanche et al. (2015) study the effect of diet and absence or presence of rumen protozoa on the rumen microbial community in lambs. In studying the presence of protozoa buffered the effect of diet on the rumen bacterial population. Faunated animals fed alfalfa hay had a greater abundance of *F. succinogenes*, anaerobic fungi and methanogens, as well as an enhanced rumen bacterial diversity. Cellulolytic bacteria were more abundant in solid-associated bacterial fractions.

### Fungi

The anaerobic fungi are part of the natural microorganisms of the rumen and its occurrence is known since the 1970s (Orpin and Joblin, 1997). The 3 species of anaerobic fungi, second Krause et al. (2014), Neocallimastix frontalis, Sphaeromonas (now named Caecomyces) communis, and Piromonas (now named Piromyces) communis. Fungi degrade cellulose more efficiently than the main species of ruminal cellulolytics bacteria. There is evidence that the ruminal inoculation with fungi may improve digestion and fiber ingestion (Grenet and Barry, 1988), because fungi are capable to digest cellulose and hemicelluloses, even when these carbohydrates are present in lignified cell walls. The greater ability of fungi in relation to bacteria is in breaking the cell wall of plants. Therefore, it is comprehensible that the greater participation of the roughage in the diet of ruminants, the greater the contribution of the fungus in the digestive process. Second Faichney et al. (1997) in relation with N available made anaerobic fungi contributed only 11 to 35 g N/kg of the microbial-N in the rumen and 7 to 27 g N/kg of the microbial-N flowing to the duodenum.

# TECHNIQUES FOR STUDIES OF FEEDSTUFF DEGRADATION BY RUMINAL MICROORGANISMS

The evaluation of digestion parameters in ruminant animals is assumed for techniques *in situ* or *in vitro* (Broderick and Cochran, 2000; Krizsan et al., 2013; Ramin et al., 2013). Among these techniques, the *in situ* 



Figure 1. Photomicrographs (X 100) of F57 textile (a), of nylon textile (b) and NWT (c) (Valente et al., 2011b).

evaluations have been more recommended, because the measurements are performed in the rumen, where the degradation process is supposed to be more reliable than in vitro (Zhou et al., 2012). In addition, the in situ evaluation avoids the accumulation of final products of fermentation, which can affect degradation as sometimes observed under in vitro environments. On the other hand, the mathematical modeling of the in situ degradation profiles allows the estimation of different parameters of the rumen dynamics, including rate and extension of degradation, the effectively degraded fraction by ruminal microorganisms. Different textiles have been suggested to make the bags used in the in situ evaluations, such as nylon (50 µm), F57 (Ankom®) and non-woven textile (NWT, 100 g/m<sup>2</sup>) (Casali et al., 2009; Valente et al., 2011a, b). In order to consider a textile useful for ruminal incubation, bags must present porosity thin enough to avoid loss of intact or non-degraded particles and be wide enough to allow the inflow of rumen fluid and microorganisms and the outflow of degradation products and to assure that microbial activity inside bags is similar to that observed in the ruminal environment (Valente et al., 2015). Analyze photomicrographs zoom (X 100) can be observed in Figure 1.

The geometrical structures are similar for F57 and NWT and differ of nylon bag arrangement. However, the objective of both bags is not to avoid the inflow of microorganisms and the outflow of final products of fermentation, important for knowledge, and the ruminal degradation profile by microorganisms in rumen studies (Valente et al., 2015).

### NITROGENOUS COMPOUNDS METABOLISM IN THE RUMEN

The quality of ingested protein by ruminants and their AA profile may be advantageous or not for the ruminal microorganism. Ruminal bacteria can use and transform low quality proteins into high quality proteins for the cattle

(microbial proteins). However, proteolysis activities were beneficial to the ruminal bacteria during the fermentation process for producing proteins of high biological value (Mackie and White, 1990; Rodríguez et al., 2007).

Ruminal bacteria are responsible for converting about 60 to 90% of N consumed by cattle in ammonia source. Studies indicated that about 50 to 70% of N from bacterial origin can be derived from ammonia (Kosloski, 2002). Using ammonia marked <sup>15</sup>N, reveal the relative importance of the N sources of microbial origin and depending on the animal diet (Nolan and Dobos, 2005) found results in 40, the 95% of N of microbial origin from the ammonia dietetics, after turnover rate recycle in rumen environmental. Many bacteria can use ammonia as a source of N for their development, but the concentrations of ammonia in the rumen present considerable fluctuations (Figueiras et al., 2015). Recent research showed that high concentrations of ammonia in the rumen liquid can result from a decrease in transport of urea from the blood to the rumen (Bannink and Tamminga, 2005). Second Abdoun et al. (2007) independent spend the energy urea throughout epithelium recycling because of movement of blood urea.

The concentration of urea in the visceral port system is always less than the arterial. This N was used in the synthesis of microbial protein and urea circulated through the gastrointestinal tract. But Lindsay and Reynolds (2005) note that the microbial protein formed in the large intestine possibly be lost in the feces, although urea reaching the rumen and may be used for protein formation or simply pass and be absorbed in the small intestine. Bacteria require at least 1% of N for its maintenance, and the urea recycling can occur either via saliva or by diffusion through the rumen wall (Van Soest, 1994).

According to Russell et al. (1992), ammonia in excess may be lost as urea by animal urine. However, diets containing low degraded protein in the rumen (RDP), ammonia concentration can be much smaller. The ruminant retains urea in body, avoid urea loss, mainly



**Figure 2.** Lactic acid bacteria growth on cellulose (1) on starch (2) with different nitrogen sources after 24 hours (A - control; B - casein; C - soy peptone; and D - urea. The fluid was X 100 diluted). (photo: Carvalho et al., 2011).

through the kidneys. With low protein urea diets, various mechanisms seem to reduce renal excretion of urea, being redirected to the ruminal environment, when the availability of the RDP is low, this can limit microbial growth (Obara et al., 1991; Marini and Van Amburgh, 2003).

Increase in development of microorganisms in the rumen occurs when supplementation with N are realized (Figueiras et al., 2010; Costa et al., 2011). Satter and Slyter (1974) initially studied levels of microbial protein production and concluded that the levels of production were constant with levels of 13 to 14% of CP in the diet. However, the variations in turnover and in rates of fermentation can cause variation in microbial production. And after study, inside the rumen N can be absorbed through the epithelium and the assimilation of ammonia is typically mediated by glutamate dehydrogenase (GDH) or glutamine synthetase (GS). The assimilation of ammonia by GDH is advantageous when the concentration of ammonia is high and carbohydrates (ATP) are limiting. While the GS way is only advantageous when the concentration of ammonia is low and ATP of carbohydrates is not the limiting factor (Russell, 2002).

# INFLUENCE OF TYPE OF SUPPLEMENTATION ON RUMEN DEGRADATION

In experiment with cattle, Bailey et al. (2012) observed the effects of supplemental energy sources on nutrient digestion. Supplementation with glucose tended to decrease ruminal neutral detergent fiber (NDF) digestibility. Otherwise, more precision of the rate of increasing casein supplementation increased ruminal concentrations of NH<sub>3</sub>, acetate, and propionate. Supplemental energy decreased plasma urea-N concentration, but casein level did not affect it. In a study with cattle to evaluate the effects of supplementation with different sources of energy and nitrogenous compounds on the interaction of the *in vitro* growth and production of bacteriocin of lactic acid bacteria Carvalho et al. (2011), selected microorganisms according to the energy sources and nitrogenous compounds and concluded that starch promote growth of lactic acid bacteria when compared to cellulose. Supplementation with true protein (soy peptone and casein) stimulated the growth of these bacteria when compared to without supplementation with nitrogenous compounds. Moreover, urea addition does not stimulate the growth of lactic acid bacteria. Sources of true protein increase the competition between non-structural and structural carbohydrates fermenting bacteria (Figure 2).

### MICROBIAL PROTEIN CONSIDERATIONS

The average composition of ruminal bacteria is approximately 62.5% crude protein (CP), 21.1% carbohydrates, 12% fat and 4.4% ash at the base of the dry matter (DM) (Russell et al., 1992). According to Russell (2002), increasing the production and flow of rumen microbial protein to the gastrointestinal tract are necessary to maximize livestock production and to reduce their dietary protein requirements. Most of the AA absorbed by ruminants are derived from microbial protein synthesized in the rumen. Dietary metabolizable protein requirements are met by absorption in the small intestine thought microbial protein or dietary protein is not degraded in the rumen (RUP). In ruminant nutrition, maximizing the microbial protein flow to the small intestine, allows in most cases to increase production efficiency. Indeed, utilization of formulated diets, rich in RUP, do not allow optimal growth for animals, because of their deficiency in lysine and methionine (Ali et al., 2009).

Concentration of fermentation end-products	Isolate Gram +	Total VFA (mmol I <sup>-1</sup> **)	А	Р	В	lb (%)	F	S	lv
High	C48	79.9 <sup>a</sup>	47.8	8.1	7.8	5.3	4.37	4.8	21.5
	C51	76.6 <sup>a</sup>	54.2	7.1	7.3	5.1	1.57	1.5	23.0
	C33	73.3 <sup>a</sup>	55.4	7.9	5.8	5.9	2.37	nd	22.3
	R34	71.0 <sup>a</sup>	37.4	3.7	5.9	5.3	1.78	1.7	44.0
	C11	68.4 <sup>a</sup>	69.1	11.8	6.2	7.2	1.32	nd	4.2
	R36	66.7 <sup>a</sup>	50.3	11.3	5.1	5.1	1.48	nd	26.5
	C47	64.4 <sup>a</sup>	49.2	8.4	7.7	5.8	1.95	nd	26.7
	C37	62.5 <sup>a</sup>	65.9	13.9	6.8	6.5	1.69	nd	5.0
	R40	60.6 <sup>a</sup>	37.8	10.2	5.5	4.0	9.48	nd	32.9
	R90	60.2 <sup>a</sup>	64.9	9.7	5.4	9.7	3.63	1.5	4.9
	R15	59.6 <sup>a</sup>	67.4	10.1	6.9	8.2	1.94	0.5	4.7
	R23	53.7 <sup>a</sup>	61.8	12.0	8.3	9.5	5.14	nd	3.0
Medium	C89	49.2 <sup>b</sup>	57.1	9.5	7.3	6.0	14.9	2.5	2.4
	C34	45.5 <sup>b</sup>	63.3	14.6	7.2	7.2	3.00	nd	4.5
	C122	45.0 <sup>b</sup>	57.9	10.4	7.3	5.6	13.44	2.9	2.2
	R50	44.2 <sup>b</sup>	66.8	10.1	7.3	7.8	2.89	nd	4.9
	R61	44.0 <sup>b</sup>	39.0	7.3	6.6	4.4	Nd	nd	42.5
	C114	43.6 <sup>b</sup>	51.4	10.4	6.5	7.8	21.72	nd	2.0
	C118	41.6 <sup>b</sup>	55.7	10.1	7.4	7.9	12.63	3.2	2.6
	R51	39.0 <sup>b</sup>	50.1	16.9	9.0	6.8	13.43	1.6	1.9
	C54	38.3 <sup>b</sup>	51.5	16.5	8.8	8.3	12.86	nd	1.8
	C117	34.5 <sup>b</sup>	49.4	15.0	2.6	13.6	8.69	6.3	4.1
Low	R21	30.6 <sup>c</sup>	71.5	9.0	8.7	5.9	Nd	nd	4.7
	R60	30.3 <sup>c</sup>	66.0	8.4	13.5	6.2	Nd	nd	5.8
	R91	25.0 <sup>c</sup>	20.2	10.2	3.5	56.2	8.02	nd	1.6
	R63	22.9 <sup>c</sup>	64.7	11.6	10.3	7.3	Nd	nd	5.9
	R107	21.5 <sup>°</sup>	58.8	15.4	3.6	13.5	6.59	nd	1.9
	C116	19.3 <sup>°</sup>	50.0	17.2	nd	15.4	10.61	6.6	nd
	R96	16.9 <sup>c</sup>	52.2	17.3	4.5	11.6	11.13	nd	3.0
	R97	12.2 <sup>c</sup>	47.8	25.7	nd	7.3	19.05	nd	nd

**Table 1.** Fermentation products (mmol l<sup>-1</sup>) of hyper-ammonia-producing bacterial isolates\* obtained from Nellore steers.

VFA: Volatile fatty acid; A: acetate; P: propionate; B: butyrate; Ib: isobutyrate; F: formate; S: succinate; Iv: isovalerate; nd : not detected. Values are provided as the mean  $\pm$  standard deviation of the mean. \*The isolates were grown in anaerobic mineral medium supplemented with 15 g  $\Gamma^1$  trypticase for 24 h at 39°C. \*\*Averages of total volatile fatty acids followed by different letters in the same column differ at 5% probability by the Scott-Knott test (Bento et al., 2015).

#### PROTEOLYSIS IN THE RUMINAL ENVIRONMENT

Understand of characteristics of the ruminal microbial population has opened new avenues of microbial ecology, such as the existence of hyperammoniaproducing bacteria (HAB) and how they can be used to improve N efficiency in ruminants (Krause et al., 2014). The excess of proteolysis by bacteria in the rumen can be detrimental (Bento et al., 2015), isolation and characterization of HAB in animals fed tropical diets or supplemented with rumen-degradable proteins. This work investigated the bacterial community diversity of the rumen of Nellore steers fed tropical forages, with or without casein supplementation (Table 1). Most bacteria produced a variety of VFA from trypticase fermentation, with a predominance of acetic acid, propionic acid, butyric acid, isobutyric acid, isovaleric acid and formic acid. All the hyper-ammonia-producing bacteria were Gram-positive, by ranking VFA as high, medium and low production for concentration of fermentation endproducts.

The proteolysis in the rumen can be beneficial to the animal host if the products are transformed into digestible microbial protein. The PEP are intermediates in the ruminal bacteria conversion of protein ingested ammonia and are a point at which the rate of breakage can be controlled. The rate of protein digestion in the rumen often exceeds the capacity of microorganisms to incorporate the AA released. This imbalance results in deamination and loss of ammonia through the rumen wall, one of the main causes of inefficient N retention by ruminants (Falconer and Wallace, 1998; Krause et al., 2014).

The predominant proteases in ruminal contents are modified by diet and also vary greatly between animals, these variations are due mainly to the amount of soluble protein in diet. The proteolysis is an important function of ruminal microorganisms because it provides PEP and subsequently, AA for growth and energy production. Mixed cultures of bacteria use PEP faster than AA and ammonia is captured by passive diffusion (Russell and Strobel, 2005).

Microbial protein synthesized in the rumen represents 40 to 90% of the protein that reaches the small intestine, although more than 50% of this microbial protein synthesis can be degraded to ammonia in the rumen. Microbial N recycling in the rumen occurs as a result of both break and degradation of protozoa and bacteria. According to Lapierre and Lobley (2001), around 45 to 60% of microbial N can be derived from ruminal ammonia. But, they believe that this N is recycled several times, increasing the chance of converting 20 to 50%. This explains how the production of N from the hepatic urea in some circumstances may be more important than dietary N.

The pool of PEP, AA and ammonia in the rumen tends to increase after 2 to 4 h pots ingestion of the feedstuff. Certainly, the concentration of PEP can range from 10 to 150 mg N/l, and that of AA from 0.1 to 16 mg N/l. However and according to Nolan and Dobos (2005), this variation depends on the protein degradability and conditions for microbial growth (Krause et al., 2014).

Recycling within the rumen microbial matter is extensive and affects the feedstuff conversion efficiency, normally because energy is required for re-synthesis of microbial protein and microbial protein is degraded submitted to deamination. Maximizing the microbial protein synthesis and pass their flow for duodenum (Argyle and Baldwin, 1989; Fatehi et al., 2015).

# EFFECT OF PROTEIN SOURCES ON MILK PRODUCTION AND COMPOSITION

The use of supplemental with AA protected rumen degradation has been useful in lactation cows feeding high production to meet the needs of protein and providing satisfactory results in the production of milk, especially during the first weeks of lactation. Infusions of casein in the abomasum or intestine, increases the milk production (Chalupa and Sniffen, 1991; Larsen et al., 2015; Hills et al., 2015). However, in Brazil according to Santos and Greco (2007) from 127 compared 88 experiments with cows in milk production, where the soybean meal was replaced partly or wholly by RUP rich sources, have concluded that only 17% of cows showed an increase in milk production due to the processing of soybean meal. The possible explanation of the absence of positive effect can be related with the reduction of rumen microbial protein synthesis by lack of RDP; or low quality of the RUP source in terms of essential AA balance or still low digestibility of RUP sources in the small intestine.

### CONCLUSIONS

The efficient nitrogen metabolism in ruminants depends on complex interaction energy and various nutrients in the gastrointestinal tract and tissues. Nitrogen is not used efficiently by ruminal microorganism, unless concurrency in the diet with supplements rich in energy. Bacteria, fungi and protozoa, are important in the digestion of fiber in feedstuff. However, stimulating the growth of microorganisms in the rumen increased and high quality protein can be absorbed in the small intestine of the animal, resulting in better production. However, it is not necessary to provide excessive protein in the diet because it is lost through urine, which economically is not desirable, for efficient production systems.

### Conflict of interests

The authors have not declared any conflict of interests.

### ACKNOWLEDGEMENT

The authors thank IFGoiano, Goiás (Brazil) for the financial support

### Abbreviations

AA, Amino acids; ATP, adenosine triphosphate; CNE, non-structural carbohydrates; CP, crude protein; CH<sub>4</sub>, methane; DM, dry matter; GDH, glutamate dehydrogenase; GS, glutamine synthetase; HAB, hyperammonia-producing bacteria; N, nitrogen; NPN, nonprotein nitrogen; PEP, peptides; RDP, protein degraded in the rumen; RUP, protein is not degraded in the rumen; VFA, volatile fatty acid.

#### REFERENCES

Abdoun K, Stumpff F, Martens H (2007). Ammonia and urea transport across the rumen epithelium: A review. Anim. Health Res. Rev. 7:43-59.

Ali CS, Sharif M, Nisa M, Javaid A, Hashmi N, Sarwar M (2009).

Supplementation of ruminally protected proteins and amino acids: feed consumption, digestion and performance of cattle and sheep. Int. J. Agric. Biol. 11:477-482.

- Argyle JL, Baldwin RL (1989). Effects of amino acids and peptides on rumen microbial growth yields. J. Dairy Sci. 72:2017-2027.
- Aschenbach JR, Penner GB, Stumpff F, Gabel G (2014). Ruminant nutrition symposium: Role of fermentation acid absorption in the regulation of ruminal pH. J. Anim. Sci. 89:1092-1107
- Attwood GT, Altermann E, Kelly WJ, Leahy SC, Zhang L, Morrison M (2011). Exploring rumen methanogen genomes to identify targets for methane mitigation strategies. Anim. Feed Sci. Technol. 166:65-75.
- Bailey EA, Titgemeyer EC, Olson KC, Brake DC, Jones ML, Anderson DE (2012). Effects of supplemental energy and protein on forage digestion and urea kinetics in growing beef cattle. J. Anim. Sci. 90:3492-3504.
- Bannink A, Tamminga S (2005). Rumen function. In: Quantitative aspects of ruminant digestion and metabolism. Dijkstra J, Forbes JM, France J.(eds). 2 ed. pp. 263-288.
- Beauchemin KA, Kreuzer M, O'Mara F, McAllister TA (2008). Nutritional management for enteric methane abatement: a review. Aust. J. Exp. Agric. 48:21-27.
- Belanche A, De la Fuente G, Pinloche E, Newbold CJ, Balcells J (2015). Effect of diet and absence of protozoa on the rumen microbial community and on the representativeness of bacterial fractions used in the determination of microbial protein synthesis. J. Anim. Sci. 90:3924-3936.
- Bento CBP, Azevedo AC, Detmann E, Mantovani HC (2015). Biochemical and genetic diversity of carbohydrate-fermenting and obligate amino acid-fermenting hyper-ammonia-producing bacteria from Nellore steers fed tropical forages and supplemented with casein. BMC Microbiol. 15:28.
- Broderick GA, Cochran RC (2000). *In vitro* and *in situ* methods for estimating digestibility with reference to protein degradability. In: Theodorou MK, France J (Eds.). Feeding systems and feed evaluation models. Wallingford: CAB Int. pp. 53-85.
- Carvalho IPC, Detmann E, Mantovani HC, Paulino MF, Valadares Filho SC, Costa VAC, Gomes DI (2011). Growth and antimicrobial activity of lactic acid bacteria from rumen fluid according to energy or nitrogen source1. Braz. J. Anim. Sci. 40(6):1260-1265.
- Casali AO, Detmann E, Valadares Filho SD, Pereira JC, Cunha MD, Detmann KD, Paulino MF (2009). Estimation of fibrous compounds contents in ruminant feeds with bags made from different textiles. Braz. J. Anim. Sci. 38:130-138.
- Chalupa W, Sniffen CJ (1991). Protein and amino acid nutrition of lactating dairy cattle. The Veterinary Clinics of North America. In: Sniffen CJ & Herdt TH. (Eds). pp. 353-372.
- Cheng KJ, McAllister TA (1997). Compartmentation in the rumen. In: Hobson PN, Stewart CS (Ed.). The rumen microbial ecosystem. London: Chapman & Hall. Pp. 492-522.
- Costa VA, Detmann E, Paulino MF, Valadares Filho SD, Henriques LT, Carvalho IP, Valente TN (2011). Consumo e dinâmica ruminal da fibra em detergente neutro em bovinos em pastejo no período das águas recebendo suplementação com nitrogênio não-proteico e/ou proteína verdadeira. Braz. J. Anim. Sci. 40(12):2805-2814.
- Dijkstra J, France J, Tamminga S (1998). Quantification of the recycling of microbial nitrogen in the rumen using a mechanistic model of rumen fermentation processes. J. Agric. Sci. 130(1):81-94.
- Faichney GJ, Poncet C, Lassalas B, Jouany JP, Millet L, Doré J, Brownlee AG (1997). Effect of concentrates in a hay diet on the contribution of anaerobic fungi, protozoa and bacteria to nitrogen in rumen and duodenal digesta in sheep. Anim. Feed Sci. Technol. 64:193-213.
- Falconer ML, Wallace RJ (1998). Variation in proteinase activities in the rumen. J Appl. Microb. 84:377-382.
- Fatehi F, Krizsan SJ, Gidlund H, Huhtanen P (2015). A comparison of ruminal or reticular digesta sampling as an alternative to sampling from the omasum of lactating dairy cows. J. Dairy Sci. 98:3274-3283.
- Figueiras JF, Detmann E, Paulino MF, Valente, TNP, Valadares Filho SC, Lazzarini I (2010). Intake and digestibility in cattle under grazing supplemented with nitrogenous compounds during dry season. Braz. J. Anim. Sci. 39(6):1303-1312.

Figueiras JF, Detmann E, Valadares Filho SC, Paulino MF, Batista ED,

Rufino LM, Valente TN, Reis WL, Franco MO (2015). Desempenho nutricional de bovinos em pastejo durante o período de transição seca-águas recebendo suplementação proteica. Arch. Zootec. 64(247):269-276.

- Firkins JL, Yu Z, Morrisson M (2007). Ruminal Nitrogen Metabolism: Perspectives for Integration of Microbiology and Nutrition for Dairy. J. Dairy Sci. 90(1):E-16.
- Grenet E, Barry P (1998). Colonization of thick walled plant tissues by anaerobic fungi. Anim. Feed Sci. Technol. 19(1):25-31.
- Hills JL, Wales WJ, Dunshea FR (2015). Invited review: An evaluation of the likely effects of individualized feeding of concentrate supplements to pasture-based dairy cows. J. Dairy Sci. 98:1363-1401.
- Hook SE, Wright ADG, McBride BW (2010). Methanogens: Methane producers of the rumen and mitigation strategies. Archaea Volume 2010, Article ID 945785. 11 p.
- Hoover WH, Miller TK (1991). Rumen digestive physiology and microbial ecology. The Veterinary Clinics of North America. In: Sniffen CJ & Herdt TH (Eds). Pp. 311-325.
- Hünerberg M, McGinn SM, Beauchemin KA (2015). Impact of ruminal pH on enteric methane emissions. J. Anim. Sci. 93(4):1760-1766.
- Ivan M, Neill L, Forster R, Alimon R, Rode LM, Entz T (2000). Effects of Isotricha, Dasytricha, Entodinium, and total fauna on ruminal fermentation and duodenal flow in wethers fed different diets. J. Dairy Sci. 83(4):776-787.
- Khattab MSA, Ebeid HM (2014). Isolation of *Enterococcus Faecium* and *Enterococcus Cecorum* from Bovine Rumen Using Modern Techniques. J. Anim. Prod. Adv. 4(10):514-519
- Kim DH, McLeod KR, Klotz JL, Koontz AF, Foote AP, Harmon DL (2014). Evaluation of a rapid determination of fasting heat production and respiratory quotient in Holstein steers using the washed rumen technique. J. Anim. Sci. 91:4267-4276
- Kosloski GV (2002). Bioquímica de ruminantes. 140p.
- Krause DO, Nagaraja TG, Wright AD, Callaway TR (2014). Boardinvited review: Rumen microbiology: Leading the way in microbial ecology. J. Anim. Sci. 91:331-34.
- Krizsan SJ, Jančík F, Ramin M, Huhtanen P (2013). Comparison of some aspects of the *in situ* and *in vitro* methods in evaluation of neutral detergent fiber digestion. J. Anim. Sci. 91:838-847.
- Lapierre H, Lobley GE (2001). Nitrogen recycling in the ruminant: a review. J. Dairy Sci. 84:223-236.
- Larsen M, Galindo C, Ouellet DR, Maxin G, Kristensen NB, Lapierre H (2015). Abomasal amino acid infusion in postpartum dairy cows: Effect on whole-body, splanchnic, and mammary amino acid metabolism. J. Dairy Sci. 98:7944-7961.
- Leahy SC, Kelly WJ, Altermann E, Ronimus RS, Yeoman CJ, Pacheco DM, Li D, Kong Z, McTavish S, Sang C, Lambie SC (2010). The genome sequence of the rumen methanogen *Methanobrevibacter ruminantium* reveals new possibilities for controlling ruminant methane emissions. PLoS ONE 5:8926-8943.
- Lindsay DB, Reynolds CK (2005). Metabolism of the portal-drained viscera and liver. In: Quantitative aspects of ruminant digestion and metabolism. Dijkstra J, Forbes JM, France J. (eds). 2 ed. pp. 311-343.
- Mackie RI, White BA (1990). Symposium: Rumen microbial ecology and nutrition: Potencial impact on nutrient output. J. Dairy Sci. 73:2971-2995.
- Marini JC, Van Amburgh ME (2003). Nitrogen metabolism and recycling in Holstein heifers. J. Anim. Sci. 81:545-552.
- Martin C, Morgavi DP, Doreau M (2010). Methane mitigation in ruminants: from microbe to the farm scale. Animal 4:351-365.
- Marx H, Graf AB, Tatto NE, Thallinger GG, Mattanovich D, Sauer M (2011) Genome sequence of the ruminal bacterium *Megasphaera elsdenii*. J. Bacteriol. 193(19):5578-5579.
- McAllister TA, Meale SJ, Valle E, Guan LL, Zhou M, Kelly WJ, Henderson G, Attwood GT, Janssen PH (2015). Ruminant nutrition symposium: Use of genomics and transcriptomics to identify strategies to lower ruminal methanogenesis. J. Anim. Sci. 93(4):1431-1449.
- Nolan JV, Dobos RC (2005). Nitrogen transactions in ruminants. In: Quantitative aspects of ruminant digestion and metabolism. Dijkstra J, Forbes JM, France J. (eds). 2 ed. pp. 177-206.

- Obara Y, Dellow DW, Nolan JV (1991).The influence of energy-rich supplements on nitrogen kinetics in ruminants. In: Tsuda T, Sasaki T, Kawasashima R. (eds) Physiology Aspects of digestion and metabolism in ruminants. New York, pp. 515-539.
- Orpin CG, Joblin KN (1997). The rumen anaerobic fungi In: Hobson PN. and Stewart CS (Ed.). The rumen microbial ecosystem. 2 ed. London: Chapman and Hall. pp. 140-195.
- Plaizier JC, Khafipour E, Li S, Gozho GN, Krause DO (2012). Subacute ruminal acidosis (SARA), endotoxins and health consequences. Anim. Feed Sci. Technol. 172:9-21.
- Pourazad P, Khiaosa-ard R, Qumar M (2015). Transient feeding of a concentrate-rich diet increases the severity of subacute ruminal acidosis in dairy cattle. J. Anim. Sci. (in Press).
- Prive F, Kaderbhai NN, Girdwood S, Worgan HJ, Pinloche E, Scollan ND, Huws SA, Newbold CJ (2013). Identification and Characterization of Three Novel Lipases Belonging to Families II and V from *Anaerovibrio lipolyticus* 5ST. PLoS ONE 8(8): e69076.
- Puggaard L, Kristensen NB, Sehested J (2011). Effect of decreasing dietary phosphorus supply on net recycling of inorganic phosphate in lactating dairy cows. J. Dairy Sci. 94:1420-1429.
- Ramin M, Krizsan SJ, Jančík F, Huhtanen P (2013). Short communication: measurements of methane emissions from feed samples in filter bags or dispersed in the medium in an in vitro gas production system. J. Dairy Sci. 96:4643-4646.
- Rodríguez R, Sosa A, Rodríguez Y (2007). Microbial protein synthesis in rumen and its importance to ruminants. Cuban J. Agric. Sci. 41(4):287-294.
- Røjen BA, Kristensen NB (2012). Effect of time duration of ruminal urea infusions on ruminal ammonia concentrations and portal-drained visceral extraction of arterial urea-N in lactating Holstein cows. J. Dairy Sci. 95:1395-1409.
- Russell JB (2002). Rumen Microbiology and its role in ruminant nutrition, Ithaca, NY, 119 p.
- Russell JB, O'connor JD, Fox DG, Van Soest PJ, Sniffen CJ (1992). A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. J. Anim. Sci. 70:3562-3578.
- Russell JB, Strobel HJ (1987). Concentration of ammonia across cell membranes of mixed rumen bacteria. J. Dairy Sci. 1987:7097-7112.
- Russell JB, Strobel HJ (2005). Microbial energetics. In: Quantitative aspects of ruminant digestion and metabolism. Dijkstra J, Forbes JM, France J. (Eds). 2 ed. pp. 229-261.
- Santos FAP, Greco LF (2007). Digestão pós ruminal de proteínas e exigências de aminoácidos para ruminantes. In: Rennó FP.(Ed.) Simpósio Internacional Avanços em técnicas de pesquisas em nutrição de ruminantes. pp.122-159.
- Satter LD, Slyter LL (1974). Effect of ammonia concentration on rumen microbial protein production *in vitro*. Br. J. Nutr. 32:199-208.
- Storm AC, Kristensen NB, Røjen BA, Larsen M (2014). Technical note: A method for quantification of saliva secretion and salivary flux of metabolites in dairy cows. J. Anim. Sci. 91:5769-5774.
- Suen G, Stevenson DM, Bruce DC, Chertkov O, Copeland A, Cheng JF, Detter C, Detter JC, Goodwin LA, Han CS, Hauser LJ (2011). Complete genome of the cellulolytic ruminal bacterium *Ruminococcus albus*. J. Bacteriol. 193(19):5574-5575.
- Szumacher-Stabel M, Cieslak A (2012). Dietary possibilities to mitigate rumen methane and ammonia production. In: Guoxiang Liu (Ed.), Greenhouse Gases, Capturing, Utilization and Reduction. In: Tech, pp. 199-239.

- Szumacher-Strabel M, Martin SA, Potkanski A, Cieslak A, Kowalczyk J. (2004). Changes in fermentation process as the effect of vegetable oil supplementation in vitro studies. J. Anim. Feed Sci. 13:215-218.
- Theodorou MK, France J (2005). Rumen microorganisms and their interactions. In: Quantitative aspects of ruminant digestion and metabolism. Dijkstra J, Forbes JM, France J. (eds). 2 ed. pp. 207-228.
- Valente TN, Detmann E, Queiroz AC, Valadares Filho SD, Gomes DI, Figueiras JF (2011a). Evaluation of ruminal degradation profiles of forages using bags made from different textiles. Braz. J. Anim. Sci. 40(11):2565-2573.
- Valente TN, Detmann E, Valadares Filho SD, Cunha MD, Queiroz AC, Sampaio CB (2011b). *In situ* estimation of indigestible compounds contents in cattle feed and feces using bags made from different textiles. Braz. J. Anim. Sci. 40:666-675.
- Valente TNP, Detmann E, Sampaio CB (2015). Review: Recent advances in evaluation of bags made from different textiles used in situ ruminal degradation. Can. J. Anim. Sci. 95:493-498
- Van Soest PJ (1994). Nutritional Ecology of the Ruminant. Ithaca: Cornell University Press, 476 p.
- Varadyova Z, Kisidayova S, Siroka P, Jalc D (2007). Fatty acid profiles of rumen fluid from sheep fed diets supplemented with various oils and effect on the rumen ciliate population. Czech J. Anim. Sci. 52:399-406.
- Veira DM, (1986). The role of ciliate protozoa in nutrition of the ruminant. J. Anim. Sci. 63:1547-1560.
- Weimer PJ, Cabral LS, Cacite F (2015). Effects of ruminal dosing of Holstein cows with *Megasphaera elsdenii* on milk fat production, ruminal chemistry, and bacterial strain persistence. J. Dairy Sci. 98:8078-8092.
- Williams YJ, Rea SM, Popovski S, Pimm CL, Williams AJ, Toovey AF, Skillman LC, Wright AD (2008). Reponses of sheep to a vaccination of entodinial or mixed rumen protozoal antigens to reduce rumen protozoal numbers. Br. J. Nutr. 99:100-109.
- Williams AG, Coleman GS (1997). The rumen protozoa. In: Hobson PN, Stewart CS. (Eds.). The rumen microbial ecosystem. London: Blackie Academic. 2:73-139.
- Williams PE, Tait CA, Innes GM, Newbold CJ (1991). Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. J. Anim. Sci. 69:3016-3026.
- Wright ADG, Klieve AV (2011). Does the complexity of the rumen microbial ecology preclude methane mitigation? Anim. Feed Sci. Technol. 166:248-253.
- Yazdi MH, Mirzaei-Alamouti HR, Amanlou H (2015). Effects of heat stress on metabolism, digestibility, and rumen epithelial characteristics in growing Holstein calves. J. Anim. Sci. (in Press).
- Zhou B, Meng Q-X, Ren L-P, Shi F-H, Wei Z, Zhou Z-M (2012). Evaluation of chemical composition, *in situ* degradability and *in vitro* gas production of ensiled and sun-dried mulberry pomace. J. Anim. Feed Sci. 21:188-197.