

## **Nutrient Requirements and Metabolism of Rumen Microorganisms**

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### ***Introduction***

Many mammals are herbivorous and consume plant material high in cellulose. Consequently, these animals have evolved a close symbiotic relationship with the microorganisms which reside in their gut which aid the digestion of highly fibrous plant material for the host. The ruminant animal has evolved a specially adapted digestive system to enable, for the best part, relatively efficient breakdown of feedstuffs and is split into four different compartments, the reticulum, rumen, omasum and abomasum. The rumen is the main site of microbial digestion and is perhaps best described as a large fermentation vat which contains a complex array of different microorganisms which act synergistically to break down feed for the host animal. After extensive fermentation by the resident microbes, the products of fermentation, mainly organic volatile fatty acids (VFAs) and microbial protein then become available to the host. Up to 80% of an animal's energy requirements may be met by the production of VFAs and, depending on the diet, microbial protein leaving the rumen may account from between 50 and 90% of the protein that enters the small intestine which is available to the host. Conditions in the rumen are strictly anaerobic, although small trace amounts of oxygen may be found, particularly in close proximity to the rumen wall and in ruminal gas. Temperature is maintained between 38 to 42°C which enables optimum growth of the microbes present and if animals are fed a balanced ration of forage and grain the pH lies between 5.8 and 6.4 which is a favourable environment for the growth of a wide variety of different microorganisms. Problems may occur however, if there is a sharp decrease in pH which may cause a marked change in the composition of the microflora. This may have significant consequences for the productivity and health of the host animal and in some instances these effects may not be realized until several weeks after the initial change. The aim of this paper is to describe the different microorganisms present in the rumen, their nutrient requirements and metabolism, what roles they play and how a perturbation or an imbalance in the microbial population may lead to several metabolic disorders which can have a direct impact on productivity and health.

### ***The rumen microbial ecosystem***

The rumen is perhaps the best characterized gut microbial ecosystem of all, and for many years nutritionists, microbiologists and physiologists have been studying the rumen with the aim of maximizing productivity and improving overall host health through manipulating the rumen and its microbial ecosystem. The rumen contains a complex and diverse array of anaerobic bacteria, archaea, ciliate protozoa, anaerobic fungi, bacteriophage (viruses) and mycoplasmas. The role of the bacteria, protozoa and fungi in the breakdown of feedstuffs have been well documented, particularly for the bacteria, and although the bacteriophage and mycoplasmas have no known role in feed breakdown, due to their parasitic nature they can have a direct effect on the composition, dynamics and activity of the bacterial community.

The protozoa are found at concentrations of  $10^4 - 10^6$  cells/ ml rumen contents and due to their relatively large size can account for up to 50% of the biomass. They primarily play a role in predation of other microorganisms, resulting in a contribution to nitrogen recycling, although some are able to digest starch and plant particles. Twenty-five different genera have been identified to date and divided into two different groups on the basis of their morphological traits, the holotrichs (*Isotrichidae*) and the entodiniomorphs (*Orphyroscolecidae*). Protozoa are of particular interest because they live in a symbiotic relationship with the methanogenic archaea, the methanogens profiting from hydrogen produced by the protozoa and the protozoa profiting by hydrogen removal. Ciliate protozoa, unlike the bacteria, are not essential for ruminal fermentation, as defaunation (removal of the protozoal population) has no drastic effect on overall fermentation. Consequently, defaunation has been examined as a potential method for either reducing methane production or of improving the flow of nitrogen from the rumen to the small intestine due to a decrease in nitrogen recycling. Protozoa are generally more sensitive to dietary changes than the bacterial population and there appears to be greater host animal to animal variation in the protozoal population than with bacterial populations. Protozoal diversity also tends to be reduced in browsing ruminants. This is thought to be due to these animals feeding on more fibrous foods. It is perhaps also interesting to note that the protozoa are the last microorganisms to colonise the rumen of young animals and as a consequence their presence is taken as a sign of rumen maturity.

Although the anaerobic fungi only make up a very small percentage of the total microbial population they are thought to act as the initial colonizers of plant material and play an important role in plant cell wall weakening due to their high hemicellulase and cellulose activity and the use of their rhizoids to pull apart the plant fibre. In so doing, they increase the rate of cellulose digestion by the bacteria as the bacteria are able to gain access more easily. Numbers generally are in the region of  $10^3-10^5$  zoospores/ml and they are particularly numerous on fibrous diets although they tend to be sensitive to sharp decreases in pH and therefore tend to be reduced on high concentrate diets.

The majority of bacteria found in the rumen are strictly anaerobic and the total number of bacteria present in the rumen is in excess of  $10^{10}$  cells/ g of rumen contents. Only a very small percentage of bacteria from the rumen (1 to 10% depending on diet) may be cultivated under present laboratory conditions which may lead to a misrepresentation of the true ruminal bacterial community using culture based techniques which tend to select for Gram negative organisms from the *Cytophaga-Flexibacter- Bacteroides* phylum. However, due to the development of molecular techniques and the use of clone libraries this may now be corrected. To date more than 200 different species have been identified and this list is growing as more information is derived from non-culture based microbial community analysis and these organisms are added to the list. Most bacteria are classified on the basis of their fermentative capability - even those which cannot be cultured may be assigned putative roles on the basis of their relatedness to known microorganisms. The main substrates of digestion in the rumen are non-structural carbohydrates (starch, sugar, pectin), structural carbohydrates (hemicellulose, cellulose) and nitrogen containing compounds (protein, peptides, amino acids, ammonia). Thus the main

classifications on the basis of fermentative capacity are amylolytic (starch-degrading), cellulolytic (fibre degrading) and proteolytic (protein degrading) organisms. Other hydrolytic, fermentative and hydrogenotrophic bacteria are also present. It should be noted that no single species of bacterium has the ability to display all of the enzymatic properties required to degrade all dietary components, although many have evolved so that they can utilize more than one substrate. During the fermentation process one organism may produce several different fermentation products which may in turn be utilized as a growth substrate by another organism, resulting in a cascade event, where everything is interlinked. This has led to interspecies dependence and interaction in the rumen with each microorganism carrying out a specific role and filling a particular ecological niche. Interactions between dietary particles, other microorganisms and even the host animal are therefore important factors which may affect the composition of the microbial population. Any perturbation or sudden alteration of the composition of the microbial community can have very far-reaching consequences in terms of overall host health and productivity. Diet in particular can have a significant effect upon the diversity and population sizes of different groups of bacteria and can affect the relative proportions of Gram negative to Gram positive organisms. Bacterial numbers tend to be higher on high grain diets than on high forage diets, although this may be a simple reflection on ease of enumeration as bacteria attached to feed particles can be more difficult to count than bacteria associated with the liquid phase. When a high amount of concentrate is included in the diet, the Gram positive amylolytic bacteria can also tend to proliferate.

### ***Amylolytic bacteria***

A large number of ruminal bacteria, protozoa and fungi are able to use starch or the intermediate products of starch degradation. Many species of ruminal bacteria actively degrade starch and/ or utilize the intermediate products of starch degradation (amyloextrins, maltose, and glucose; Figure 1, Nagaraja & Titgemeyer, 2007), forming lactate as an end product of fermentation. In some instances, particularly in animals fed a high concentrate diet, the proportion of amylolytic bacteria can account for as much as 90% of the total culturable bacterial population, although it is hard to say at this moment the exact contribution that each genus or species makes to overall amylolytic activity and the formation of lactic acid. The numerically predominant starch degrading organisms with the highest amylolytic activity and the fastest growth rates are *Ruminobacter amylophilus*, *Streptococcus bovis* and *Selenomonas ruminantium*. In addition, isolates of *Bifidobacterium*, *Butyrivibrio fibrisolvens*, *Clostridium*, *Eubacterium ruminantium*, *Lactobacillus*, *Mituoskella*, *Prevotella*, *Succinimonas*, and *Succinivibrio* may all exhibit amylolytic, amyloextrinase and/or maltose utilizing activity. However, not a single one of these bacteria is equipped with the complete array of digestive enzymes for complete starch breakdown and thus the maximal digestion of starch to monosaccharides requires synergistic interaction among several bacterial species. Cotta (1992) demonstrated that co-culture of *S. bovis*, *B. fibrisolvens* or *Prevotella ruminicola*, with *S. ruminantium* led to high growth rates and complete digestion of starch, again demonstrating the degree of interspecies interaction and dependence which may be found in the rumen. Most of these organisms involved in starch breakdown are relatively easy to grow under laboratory

conditions and experiments with gnotobiotic lambs fed starchy diets in which a defined bacterial flora was established and normal growth and ruminal function was achieved, led to the suggestion that it is probable that the major bacteria involved in starch digestion have been identified and isolated (Hobson et al, 1981). These experiments also demonstrated that amylolytic protozoa and fungi are not essential elements for ruminal starch utilization to occur as they were not included in the inoculum.

*S. bovis* has been implicated as the "bad guy" in terms of excessive lactic acid production in the rumen. A sudden increase in rapidly fermentable carbohydrate in the diet may in some instances cause the proliferation of this Gram positive organism which when energy is readily available will switch to the production of lactate. Even though this population can increase, it does not maintain high levels for a long period of time, so is probably best regarded as an initiator organism in that it can produce the conditions whereby an increase in the lactic acid bacteria can occur, and an increase in its numbers is generally regarded as the first step in the chain of events which can lead to the downward spiral into acute lactic acidosis. It should be noted however, that if the animal is allowed to adapt to the inclusion of concentrate in the diet, or active dry yeast is included in the diet, this organism will not get the chance to proliferate to the same extent.

Even though not essential for starch breakdown to occur, ruminal protozoa do play a role in engulfing and ingesting starch particles which may also have bacteria attached to their surface. This engulfment process is believed to limit access to the starch by the rapidly fermenting amylolytic bacteria and slows its degradation and the consequent lowering of ruminal pH. The rate of starch uptake varies greatly with species, with *Entodinium* spp. engulfing starch grains very rapidly. Nearly all of the larger entodiniomorph protozoa are amylolytic. Starch is broken down to maltose and then glucose and either used as an energy source or stored in the ectoplasm. The rate of starch uptake and breakdown is governed by the concentration of starch or amylopectin inside the protozoa.

### ***Fibrolitic bacteria, fungi and protozoa***

Fibre breakdown in the rumen is catalysed by a complex community of fibrolitic microorganisms. Fibrolitic bacteria tend to degrade the more readily digestible fibrous structures and rely on help from the ruminal fungi to weaken the plant cell wall. Thus optimal fibre degradation and ruminal fermentation will occur when ruminal conditions produce an environment conducive to the growth of these organisms.

The major fibrolitic bacteria include the Gram negative organism *Fibrobacter succinogenes* and two species of Gram positive bacteria, *Ruminococcus albus* and *Ruminococcus flavefaciens*. Fibrolitic activity and growth of these organisms are severely affected by low pH. *In vitro* incubations have shown that below pH 6 the growth and enzymatic activity of these major fibrolitic bacteria is inhibited. This is of particular note when animals are fed high concentrate diets where ruminal pH is regularly below pH 6. Unlike most ruminal bacteria which can ferment carbohydrates and are capable of using numerous

monosaccharides and disaccharides as growth substrates, *F. succinogenes* and the ruminococci are nearly solely restricted to cellulose and its hydrolytic products as growth substrates.

*F. succinogenes* possesses a complex battery of fibrolytic enzymes and is one of the few microorganisms isolated from the rumen which is capable of digesting crystalline cellulose. Endocellulase, endoxylanase and licheninase activity from several glycosyl hydrolase families have all been identified in this organism. This organism can interact synergistically with non-cellulolytic bacteria during forage digestion. Co-culture of *F. succinogenes* with the hemicellulolytic bacterium *Prevotella ruminicola* resulted in a 2-fold increase in the breakdown of orchard grass (Osborne and Dehority, 1989). *Ruminococcus albus* and *Ruminococcus flavefaciens* also possess a large number of glycosyl hydrolases involved in the breakdown of cellulose and hemicellulose, several which have been isolated, purified or cloned. Both *R. albus* and *R. flavefaciens* have high xylan degrading activity. At least seven different endoglucanases have been identified in *R. albus* as well as a  $\beta$ -glucosidase. The cellulose system of *R. flavefaciens* is composed of several endoglucanases, an exoglucanase and a cellodextrinase.

Other important bacteria involved in fibre breakdown include the *Butyrivibrio* and *Prevotella* spp. Although cellulolytic strains of *Butyrivibrio* have been isolated from the rumen, this trait is generally lost upon cultivation under laboratory conditions. It does however retain its ability to rapidly utilize xylans and an abundance of xylanase genes have been identified. This group of organisms is thought to be one of the most metabolically versatile ruminal bacteria and can use simple sugars, starches, pectic polysaccharides and other non-cellulolytic polymers for growth. It is also one of the main organisms involved in ruminal biohydrogenation and the metabolism of linoleic acid (LA) but again is an organism sensitive to decreases in ruminal pH. *Prevotella* spp. are numerically predominant under several different dietary regimes and in some instances can comprise 60% of the total bacterial population. They can exclusively degrade the non-cellulose components of plant cell walls and possesses several xylanases and are an important contributor to xylan degradation in the rumen. These organisms are also of particular interest in the breakdown of dietary protein and peptides by virtue of their high proteolytic and peptidolytic activity. *Lacnospira multiparus* is the most major pectinolytic bacterium and possesses both an endo-acting pectate lyase and an exo-acting polygalacturanase digalacturonohydrolase which cleaves polygalacturonate to galacturonate residues.

The fungi have an important role in fibre digestion because they are able to penetrate both the cuticle and cell wall of lignified tissue, suggesting the presence of cutinase activity (Akin, 1986). When incubated with barley straw, increased degradation was observed by the fungi when compared with fibrolytic bacteria (Joblin et al, 1989). Filamentous growth by the fungi aids its ability to penetrate plant tissue. These organisms have a broad range of highly active fibrolytic enzymes and are the only rumen microorganisms with exocellulase activity. They have the capacity to attack all carbohydrate components of the cell wall and can slowly solubilize lignin. However, they are relatively slow growing and their ability to persist in the rumen is limited by their growth rates which are much lower than the rumen

dilution rate. The best characterized ruminal fungi are the *Neocallimastix* spp. which are highly efficient at degrading crystalline cellulose. Fibrolytic activity and growth of this organism may be enhanced by co-culture with hydrogen-utilizing methanogens, but may be repressed by *Ruminococcus* spp. This antagonistic effect only affects the cellulases of the fungi but not its growth, therefore only cellulolytic activity is affected and appears to be due to the production of extracellular proteins which bind either to the cellulose substrate or the fungal cellulase. Numbers of anaerobic fungi tend to be highest on highly fibrous diets and diminish with increasing concentrate (Gordon, 1984).

Approximately 25 - 33% of fibre breakdown in the rumen is protozoal. Defaunation, or removal of the protozoa, results in a decrease in fibre breakdown (Bonhomme, 1990; Ushida et al, 1990). All of the rumen entodiniomorphid protozoa, except for *Entodinium* spp. possess cellulase activity, with highest activity in *Eudoplodinium maggi*. Xylanases are also present and a broad range of glycosidase activities are observed.

Thus fibre breakdown is carried out by a complex consortium of different microorganisms, key members of which can be significantly influenced by changes in diet or by interactions with other microorganisms.

### ***Proteolytic Bacteria***

The breakdown of dietary protein in the rumen is a complex process that involves many different microbes that provide the necessary enzymes to hydrolyse peptide bonds (figure 2, Reproduced from Walker et al, 2005). The bacteria are the main contributors to protein breakdown, but protozoa and fungi may also play a role. Protein is first broken down to oligopeptides, then small di- and tripeptides, amino acids and finally ammonia and these substrates are then used as a source of nitrogen for microbial growth and protein synthesis. The ruminant is relatively unique in that the majority of protein which is actually available to the host is in fact derived from the microbial population. Unfortunately problems can occur when proteolysis occurs in excess of microbial requirements. Ammonia is formed which, again if in excess of microbial requirements, is absorbed across the rumen wall, metabolized to urea in the liver and excreted, resulting in a loss of nitrogen from the system and a problem in terms of environmental nitrogen pollution. Several factors may affect the rate and extent of protein degradation. These include the type of protein, in terms of its structure and solubility; interactions with other nutrients, particularly carbohydrate availability; the composition of the microbial population which may in turn be affected by diet, ruminal dilution rate and ruminal pH; and plant proteinase activity, which may also contribute to the breakdown of its own cell protein.

One of the key organisms involved in the breakdown of protein in the rumen are the *Prevotella* sp. Not only is this group of organisms numerically predominant but they are involved in every step of the proteolytic cascade and they are the only ruminal organisms which have been identified to date that possess dipeptidyl peptidase (DPP) activity. This is of particular importance as this is the main mechanism by which oligopeptides are broken down in incubations with

whole rumen fluid. Not many bacteria possess this type of activity - even in other microbial ecosystems the common bacterial oligopeptidase activity is aminopeptidase activity whereby the oligopeptide is broken down by sequential removal of a single amino acid from the N-terminus. DPP activity is seen more in mammalian systems and a dipeptide is removed in a sequential manner from the N-terminus of the oligopeptide. This results in the formation of smaller di- and tripeptides which are further degraded by separate di- and tripeptidases to free amino acids. Inhibitors which targeted the DPP activity of the *Prevotella* were effective at reducing peptide breakdown and subsequently ammonia production during *in vitro* incubations.

The HAP (Hyper Ammonia Producing) bacteria are also of particular note due to their high deaminase activity. Although not numerically predominant, they have a real impact on deamination due to their high rates of ammonia formation. The HAP group of organisms is very diverse but one defining characteristic is that they are all sensitive to monensin and the majority are assacharolytic and rely on the breakdown of amino acids as a source of carbon and nitrogen for growth. A decrease in their populations leads to a decrease in ammonia production and improved nitrogen retention.

As we enter a more environmentally aware era, strategies to reduce excessive proteolysis, peptidolysis and deamination and improve nitrogen retention and the flow of nitrogen from the rumen to the small intestine are being studied.

#### ***Effect of diet on certain microbial populations***

The ruminant evolved to be able to utilize fresh forage diets but because of a need to increase productivity in intensive farming systems ruminants are now fed diets which contain high quality forages and concentrate in order to meet their high demands for nutrients. Diets of this type can cause a significant perturbation of the microbial ecosystem, particularly if any change in the ration is done suddenly. In many cases, high concentrations of dietary starch and low concentrations of effective fibre are factors which may lead to a reduction in pH and a greater predisposition to digestive disorders like acidosis which can affect the health and productivity of the host.

#### ***Acute Lactic Acidosis***

Normal concentrations of lactate in the rumen are less than 5 mM, however under conditions of acute acidosis they may exceed 100 mM, and the relative proportions of D(-) and L(+)lactate changes as the pH decreases. Below pH 5, the D(-) isomer may make up 50% of the lactate present and as this isomer is less readily metabolized than the L-isomer its accumulation increases and the host has difficulty in clearing it from it's system, this may result in acute lactic acidosis. The sequence of events and the role the ruminal microbial population plays in the onset of acute acidosis have been well described (Nocek, 1997, Figure 3). Carbohydrate overload resulting from a sudden increase in the quantity of grain in the ration can lead to the accumulation of large amounts of lactic acid in the rumen and a very sharp decline in ruminal pH. To start with, the microbial ecosystem responds well to the increased supply of readily available carbohydrate and there is an

increase in the total bacterial population, fermentation and VFA production - unfortunately this also results in a drop in pH and rumen motility. One consequence of a decrease in motility is a decrease in rumination and less production of saliva leading to a reduction in the buffering capacity of the rumen (Crichlow and Chaplin, 1985). If the *S. bovis* population manages to take off due to its ability to out-compete other members of the bacterial populations due to its fast doubling time and high amylolytic activity this can cause a relatively rapid change in the balance and composition of the normal microflora. A shift can occur from one where Gram negative and Gram positive organisms are in balance to one where Gram positive lactate producers (*S. bovis*, and *Lactobacillus* sp.) predominates. If this occurs, ruminal pH can decrease further such that the fibrolytic bacteria are detrimentally affected and even the lactate utilizing organisms (*M. elsdenii*, Selenomonads), which up until now had been coping at keeping the levels of lactate under control, are unable to keep abreast of the situation and lactate begins to accumulate. This drops the pH even further and when the pH drops below pH 5.5, no fibrolytic and relatively few saccharolytic bacteria survive, the lactate utilisers begin to decline leading to even greater accumulation of lactate until conditions become too extreme even for *S. bovis* (pH<5) and they die off. Once this point is reached, the Lactobacilli can really take over and continue to produce lactic acid as the pH drops. *Mitsuokella* sp. may also proliferate, producing excess D-lactate at high rates. This downward acidotic spiral continues until a state of acute acidosis is reached, the consequences of which may even result in death of the animal. Also associated with very low pH is the death and lysis of the normal microflora which lead to the release of lipopolysaccharides from the cell wall and endotoxins from the interior of the bacterial cells which may trigger inflammatory responses and also lead to the activation of metalloproteinases (MMPs) which are normally involved in the normal growth and repair of the hoof, but which when overexpressed may actually lead to the destruction of the normal hoof structure leading to a laminitic event. However, secondary events like laminitis may not be seen until several weeks after the initial grain insult. The potential for experiencing the onset of laminitis is also elevated due to the production of vasoactive amines which can cause ischaemia of the lamellae which make up part of the hoof structure. Destruction of the lamellae can result in pedal bone rotation and lameness. One of the main vasoactive amines associated with the onset of laminitis is histamine which is produced by *Allisonella histaminiformans*. Other vasoactive amines are produced by the streptococci and lactobacilli, which can make up a predominant percentage of the population under these conditions.

Generally cases of acute acidosis occur very infrequently and can be regarded as the extreme end of the spectrum. More frequently, cases of subacute ruminal acidosis will be observed in dairy herds.

#### ***Subacute Ruminal Acidosis (SARA)***

Subacute Ruminal Acidosis (SARA) would be considered the more common of the two states of acidosis and can be characterized by a rumen pH between 5 and 5.5, where the total VFA concentration has been increased due to increased available carbohydrate and the VFA profile has been shifted towards the production of propionic acid and butyric acid at

the expense of acetic acid and accumulated levels of lactic acid in the rumen fluid do not exceed 5 to 10 mM. Whereas the acute acidotic state is defined as occurring when ruminal pH is below 5 and where lactate accumulation can occur to levels far higher than 40 mM (Owens et al, 1998). If a ruminal sample was obtained and compared under the microscope between an animal experiencing SARA and one experiencing acute acidosis a marked difference in the composition of the microflora would be observed. Generally in the acute acidotic state, no protozoa are present due to the low pH (Goad et al, 1998) and the flora is mostly comprised of Gram positive bacteria - the majority of which are lactic acid bacteria. A Gram negative organism, *Mitsuokella* sp. which can also produce high levels of D-lactic acid, can also proliferate under these conditions. In the SARA animal, generally the total number of bacteria has increased but the Gram positive and Gram negative organisms are still relatively in balance, although the Gram positive organisms are beginning to proliferate. The lactate utilizing population is able to keep tee with the production of lactate and consequently it does not accumulate to the levels observed in the acutely acidotic animal. Protozoal numbers may be decreased and a shift in the composition may be observed towards more acid resistant protozoa like the *Isotricha* and *Entodinium* sp from the more general diverse mixture (Nagaraja & Towne, 1990). DNA fingerprint analysis of the microbial banding pattern profile can pick up differences in the banding patterns as we move through a transition period from a high fibre diet to a normal dairy ration containing concentrate. Whether this technique would be sensitive enough to pick up differences between an acutely acidotic animal and an animal experiencing SARA is highly likely, but remains to be tested.

If the ruminal pH of a normal dairy cow is measured continuously throughout the day using in-dwelling pH probes, there are points in time when that animal could be described as experiencing sub-acute ruminal acidosis and this disorder is perhaps more common than some people are aware, as there are no outward visible signs that that particular animal is experiencing any problems. Although the results are not as severe in SARA animals as those observed in animals suffering acute acidosis, they do take their toll on animal health and productivity, affecting intake patterns, leading to weight loss, diarrhea and lameness. There may even be effects on the milk with a depression in milk fat percentage being observed. Increased incidences of liver abscesses may also be observed due to the presence of *Fusobacterium necrophorum* which uses D-lactate as a carbohydrate source, and in grain fed animals numbers of this organism can increase dramatically.

### ***Stabilising ruminal pH and improving fibrolytic activity***

Sudden and abrupt changes in the diet can cause an imbalance in the microbial population which in turn can have an effect upon host health and productivity. Sound management practices such as gradual changes in the ration and maintaining a balance between the amount of effective fibre and grain in the diet is a key factor in obtaining a stable ruminal microflora. Several different chemicals and feed additives may be used as a means of helping to stabilise ruminal pH. Chemical buffers such as bicarbonate or bentonite may be added to the feed, antimicrobials eg ionophores, growth promoting antibiotics, which may

affect the proliferation of Gram positive organisms, can be used as a means of reducing any potential explosion of Gram positive organisms responsible for the onset of acidosis. Natural alternative additives such as DFMs may also be used and live yeast has been shown to be particularly effective at stabilizing ruminal pH; elevating the average mean pH throughout the day and reducing the time spent in the low pH range (<6.0) which is particularly detrimental to the growth and metabolic activity of the fibrolytic bacteria.

Yeast has a particularly positive effect on ruminal pH through a variety of different modes of action (Chaucheyras-Durand et al, 2007). If included in the feed as active dry yeast (ADY), where the yeast is dried in such a manner so that they are still metabolically active, they can compete against the amylolytic bacteria for sugars, thus reducing their growth and proliferation and as a consequence the production of lactate (Walker, personal observation). In addition this would appear to slow the release of sugars (similar to what is observed when protozoa engulf starch particles), thus the availability of the carbohydrate is slowed resulting in no large bursts or spikes in the production of VFAs, thereby helping to stabilize ruminal pH. The growth and metabolic activity of lactate utilizing bacteria is also increased due to the production of metabolites from the yeast which appear to stimulate these organisms. Thus lactate production and accumulation is reduced, also impacting on ruminal pH.

Fibrolytic bacteria are also stimulated through the addition of ADYs again through a variety of different modes of action. These organisms are sensitive not only to drastic decreases in ruminal pH but small amounts of oxygen can also affect their growth. Although the rumen is regarded as an anaerobic system, small pockets of oxygen can be found. Live yeasts are effective at scavenging any available oxygen and as a consequence improve anaerobiosis in the rumen thereby creating an optimum environment for the growth of the fibrolytic community. Certain metabolites produced by the yeast may also stimulate the growth of fibre degrading organisms. By slowing the availability of carbohydrates and sugars, not only is pH elevated and stabilized, but these fibrolytic bacteria may also be subjected to catabolite repression and by reducing carbohydrate overload, enzymatic activity of these organisms is also increased.

Overall, all these effects on the fibrolytic population in terms of growth and metabolic activity can result in increased dry matter intake and increased meal frequency leading to improved productivity. Even on high fibre containing diets a positive effect on the fibrolytic community is observed upon the inclusion of yeast due to a stabilization of ruminal pH and a reduction in lactate production. Generally pH is not regarded as an issue on high fibre diets, but a recent study by Guedes et al, (2007) demonstrated that ruminal pH can decline after feeding maize silage and that live yeast may help to prevent a sharp decline in pH. Less lactate production was also observed in the presence of yeast. The type and nature of the forage also had an effect upon its degradability, and yeast was particularly effective at improving the digestibility of low quality maize silages as well as having a less pronounced stimulatory effect on the digestibility of silages which were regarded as highly degradable. This effect may not only be due to increased growth of the fibrolytic

bacteria but also due to improved growth of the anaerobic fungi which are also known to be positively enhanced by the addition of yeast. Unfortunately changes in the microbial community were not evaluated in this study.

To conclude, diet can have a significant effect upon the composition and stability of the ruminal microbial population and subsequently on ruminal fermentation. As ruminal fermentation is an essential component of nutrient input for the animal, any disturbance or disruption of the microflora may have an effect on the health and productivity of the host animal. Therefore it is important to achieve a balanced and stable microbial population. By including active dry yeast in the diet we can stabilize ruminal pH and we can have a positive effect upon the fibrolytic population and an increase in DMI and digestability may be observed.

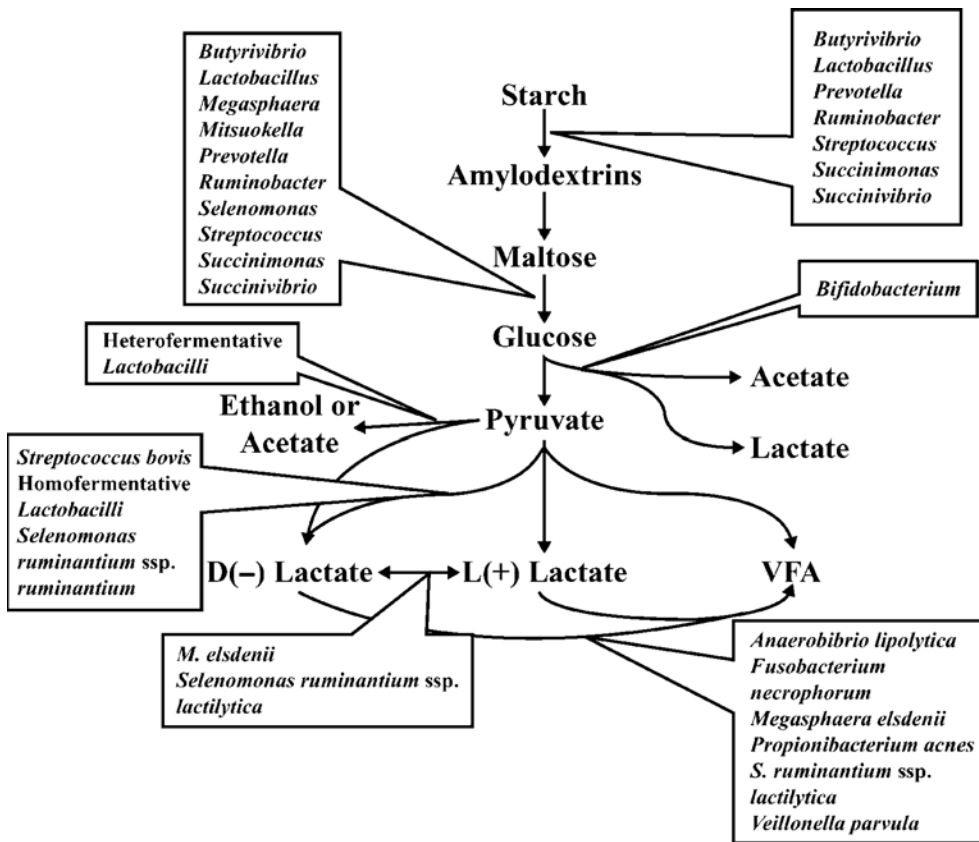


Figure 1. Schematic depicting the organisms involved in the breakdown of starch and the production of lactate (Reproduced from Nagaraja & Titgemeyer, 2007)

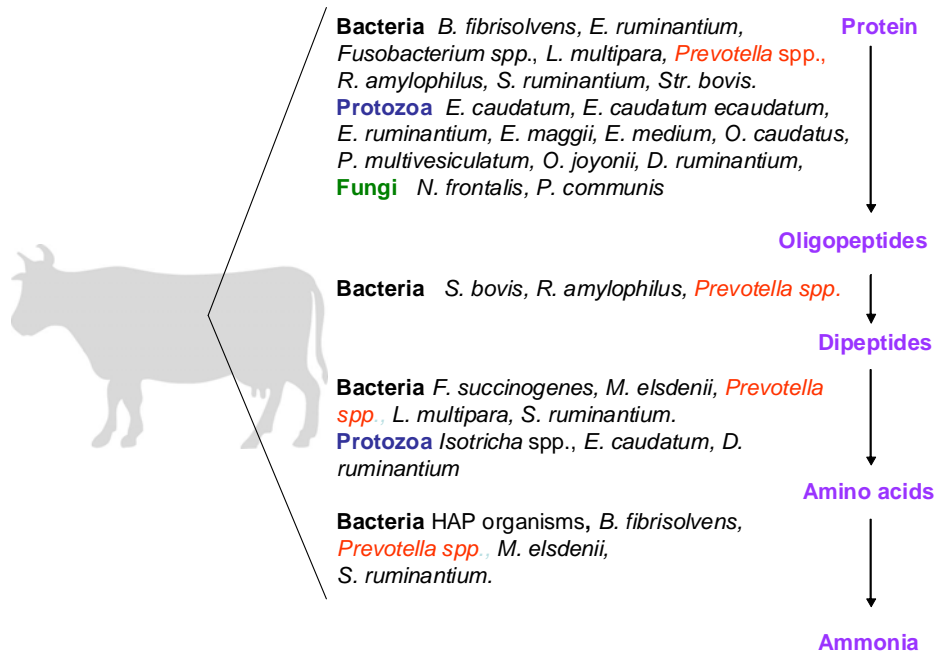


Figure 2. Schematic depicting the breakdown of protein and the ruminal microorganisms involved (Reproduced from Walker et al, 2005).

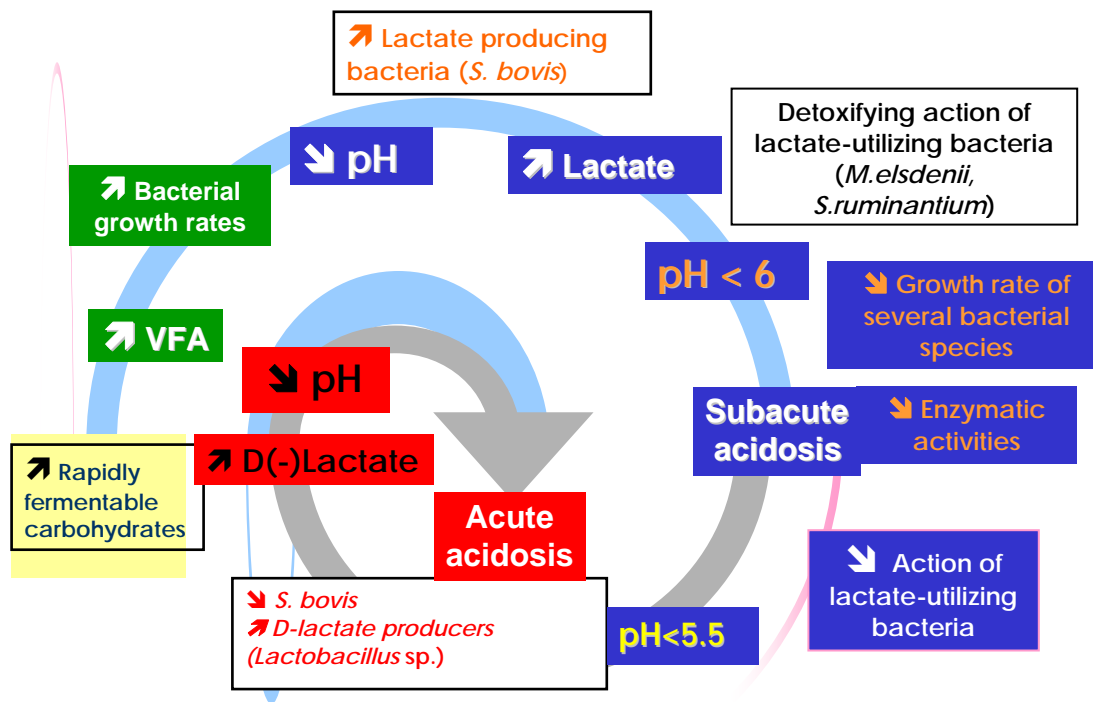


Figure 3. Schematic depicting the microbial events associated with the onset of acidosis.

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