

# CCP Feeding: Extracting MORE from Feed and Forage

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The main goal for any dairy producer is to be able to extract more value from their herd by improving nutrient utilisation and therefore feed efficiency—or in simple words, “get more from what they feed.” In order to be able to do this, it is necessary to optimise rumen function, gut health, and productivity. One way this can be done is by understanding how the ration or different feed additives can affect the rumen microflora (microbiome) and in turn influence rumen fermentation, fibre digestion, and nutrient utilisation. In addition, the effect the lower gut and gut microbiota may have on feed efficiency and utilisation must not be overlooked, as improvements to total tract digestibility, especially of starch, and improvements to overall gut health can also influence the performance of the host animal. As moves are made to obtain better feed efficiency and utilisation, not only can productivity be improved, but nutrient and energy losses such as nitrogen or methane to the environment can also be reduced as a consequence. With the impact of intensive farming practices on the environment coming under greater scrutiny from government agencies, there is a need to demonstrate that each dairy unit is running as efficiently as possible and in a sustainable manner. By taking steps to maximise nutrient utilisation and feed efficiency and minimise nutrient excretion and losses, profitability can be improved leading to a win-win situation for both the producer and the environment and ensuring that the producer will be able to continue for future generations.

## THE RUMEN MICROFLORA

Compared with other herbivores, the ruminant animal is the most efficient at breaking down plant material by virtue of the rumen microbial ecosystem, which has evolved to work in a synergistic manner to help break down feed and forage for the host animal. Microbial fermentation leads to the formation of volatile fatty acids (VFA) and microbial cell protein which are sufficient to satisfy the energy and protein requirements of the ruminant host to make meat or milk. The VFA are readily adsorbed through the rumen epithelia, whereas microbial protein passes from the rumen into the small intestine as small peptides

or amino acids, where it is then absorbed. Ammonia formed in the rumen from proteolysis is either utilised by the rumen microbes themselves, or passes across the rumen wall and is excreted as urea. Problems can occur however if fermentation is disrupted and the normal equilibrium is upset. Thus a key target is to optimise rumen fermentation, leading to a balanced production of VFA and microbial growth.

The rumen microbial population consists of a complex and diverse array of anaerobic bacteria, archaea (methanogens), ciliate protozoa, anaerobic fungi, bacteriophage (viruses), and mycoplasmas. The role of the bacteria, protozoa, and fungi in the breakdown of feed and forage have been well documented, 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. Therefore they can affect nutrient utilization and rumen fermentation. Indeed, some research groups have isolated and cultivated bacteriophage to target and kill certain bacteria and archaea with the aim of reducing their negative effects on rumen fermentation, feed efficiency, host health, or the environment (Callaway et al., 2008; Buddle et al., 2011).

Under certain dietary conditions, the rumen protozoa can account for up to 50% of the microbial biomass due to their relatively large size. Compared to the bacteria their population numbers are much smaller at only  $1 \times 10^4$  to  $1 \times 10^6$  protozoa/mL. Classified on the basis of their morphology, 25 different genera have been identified to date. The protozoa play a key role in nitrogen recycling through predation of the rumen bacteria, although they can also engulf starch and plant particles and as a result are involved in rumen pH control and fibre breakdown. Although not critical for rumen fermentation to occur, protozoal removal by chemical defaunation can lead to a reduction in fibre digestion by between 25 and 33% due to reduced ruminal hemicellulase and cellulase activity (Bonhomme, 1990; Ushida et al., 1990). The protozoa



also live in a symbiotic relationship with methanogenic archaea, and as a result defaunation has been considered as a way to reduce methane emissions and reduce nitrogen recycling leading to a reduction in the excretion of nitrogen to the environment (Buddle et al., 2011).

Relatively little is known about the anaerobic fungi except that they are one of the first colonisers of plant material when it enters the rumen. They play a key role in plant cell wall weakening and fibre digestion due to their high hemicellulase and cellulase activity and their ability to use their rhizoids to pull apart the plant structure, allowing the fibre digesting bacteria to gain access more easily (Orpin and Joblin, 1997). Numbers generally are in the region of  $10^3$  to  $10^5$  zoospores/mL, and they are particularly numerous on fibrous diets but are sensitive to decreases in rumen pH and are less abundant on concentrate diets (Gordon, 1984).

The rumen bacteria are the best characterized and most numerically abundant type of microorganism, with population sizes measured in excess of  $1 \times 10^{11}$  bacteria/mL of rumen liquor. Within the rumen many different species of bacteria exist and exhibit a wide range of fermentative activities. The main substrates for digestion are non-structural carbohydrates (starch, sugar, pectin), structural carbohydrates (hemicellulose, cellulose), and nitrogen-containing compounds (protein, peptides, amino acids, ammonia). The majority of bacteria are classified on the basis of their key fermentative activity, that is whether they are amylolytic (starch-degrading), cellulolytic (fibre degrading), proteolytic (protein degrading), or lipolytic (fat utilizing) organisms. Other hydrolytic, fermentative, and hydrogenotrophic bacteria are also present. Although many bacteria can utilize more than one substrate, no single species of bacterium has the ability to degrade all available dietary components. This has led to a high degree of interspecies dependence and synergistic action between the different species of rumen microbes. Interactions between dietary particles, other microorganisms, and even the host animal are therefore important factors which may affect the composition of the microbiome and its ability to effectively break down feed and forage.

## STUDYING THE COMPOSITION OF THE MICROBIOME

Fifty years ago the majority of studies on the rumen microbiome were limited to the use of cultivation-based techniques. Invaluable information on the key roles of different bacteria, the protozoa, and the fungi were obtained. Although these cultivable organisms represented only a

very small proportion of the organisms that were present within the microbiome, their fermentative activities can be used as markers for other non-culturable organisms which are closely related and exhibit similar functions and activities. For example, the ruminal *Prevotellae* were originally classified into four different groups on the basis of their phenotypic and genotypic properties (Avgustin et al., 1997). However, through the use of high throughput sequencing analysis, the ruminal *Prevotellae* have been shown to be far more diverse and abundant than previously thought, and genome sequencing has revealed that they have a larger repertoire of enzymes and as a result are able to carry out functions other than simply hemicellulose digestion and peptidolysis. With the rapid advancement in non-culture-based molecular methods for the identification and quantification of previously unknown organisms, and the ability to sequence cheaply and quickly, new research has focused on studying the effects of diet on the microbiome, rumen fermentation, feed efficiency, and animal performance with the ultimate aim of maximising feed utilisation.

## EFFECT OF DIET ON THE RUMEN MICROBIOME

Diet can have a significant effect on the composition of the rumen microbiome as well as nutritional performance and digestion. Perhaps the best characterised effect of diet on the microbiome is the effect of moving from a high forage diet to a high concentrate diet leading to the onset of sub-acute ruminal acidosis (SARA). The effects of SARA on animal performance have been well documented (Plaizier et al., 2008). Productivity can be decreased and butterfat can drop due to decreased dry matter intake and reduced fibre digestion. Manure quality and consistency can be affected, indicating problems with digestion and lameness issues, and elevated inflammatory responses may be observed. These effects are driven mainly by changes in the rumen microbiome and fermentation. An increase in rapidly fermentable carbohydrates in the diet causes a drop in rumen pH due to the increase in fermentation and microbial growth leading to the accumulation of VFA. The time spent under a pH of 5.8, the time taken for recovery, and the number of bouts suffered in a day determine the severity of the SARA incident and negative effects on productivity. A pH of 5.6 is taken as a key indicator of SARA, as below this pH fibre digestion is impaired. Lysis of the normal Gram-negative bacterial population can also occur, and certain microbes have been identified which release particularly potent endotoxins in the form of lipopolysaccharide (LPS) contained in their cell wall, which can stimulate an inflammatory response (Gozho et al., 2007). Changes in the microbiome following a grain challenge measured using molecular techniques gener-



ally support and follow the pattern identified by original cultivation-based techniques. Increases in amyolytic bacteria which form lactic acid, such as *S. bovis*, and lactic acid bacteria, and increases in lactic acid utilising bacteria, such as *M. elsdenii* and *S. ruminantium*, and decreases in fibre digesting bacteria were observed after a grain challenge. Several SARA challenge models have been developed and the effect on the rumen microbiome characterised using a range of different molecular techniques to quantify and identify changes in the diversity, composition, and population sizes of different organisms. One thing that has been demonstrated when using these models is that the method employed to induce acidosis and the substrate used (corn, barley, wheat, alfalfa) can have different effects on the microbiome and the degree of severity of the challenge in terms of pH decrease, time spent under a pH of 5.6, type and site of LPS release, and the fermentation profiles observed (Khafipour et al., 2009; Lettat et al., 2010; Li et al., 2010). How the model is implemented can therefore have an effect on the outcome. By reducing the incidence of SARA and stabilising the rumen microbiome, thus keeping a balanced rumen fermentation, fibre digestion can be improved which can have positive impacts on animal performance in terms of milk yield and components, feed utilisation, and herd health.

### **OTHER FACTORS AFFECTING THE MICROBIOME AND FEED EFFICIENCY**

It has been shown that there is a large degree of animal to animal variation in the composition of the rumen microbiome and that each animal has a core microbiome that it will try to revert back to, demonstrating that the host may also have a role to play in dictating the composition of their microflora. This was nicely demonstrated by Weimer et al. (2010), who performed near-total rumen content swaps between two different dairy cows fed the same diet with the only difference being some significant differences in their pH and fermentation profiles. Within 1 day of the contents being exchanged, VFA patterns and pH had returned to their original values, and within 14 days for one cow and 61 days for the other, the DNA fingerprint of the microbial community had reverted back to its original composition. This degree of “individuality” and “pull” by the core microbiome may explain some of the differences seen in animal to animal performance.

Other factors which can influence feed efficiency and digestibility include the quality of the forage; plant genetics; growing, harvesting, and storage conditions; degree of kernel processing of corn silage; and chop length. Grain digestibility may also be altered by grinding, rolling, and steam flaking. Treatment of the forage or TMR by enzymes

may also significantly improve digestibility and feed efficiency (Holthausen et al., 2011) and cause changes in the rumen microbiome, further increasing fibre digestion (Chung et al., 2012).

### **MANIPULATION OF THE RUMEN MICROBIOME AND RUMEN FERMENTATION WITH FEED ADDITIVES**

A large number of different feed additives, supplements, and growth promoting ionophores have been shown to have positive effects on rumen fermentation in vitro. For some of these products, further in vivo trials have also proven their efficacy, causing positive effects on host performance and feed efficiency. Of these, there are some which alter rumen fermentation by interacting positively with the host microbiome. Perhaps the best characterised are direct-fed microbials (DFM). Originally called “probiotics” because of their positive action on the gut flora, the FDA adopted the term DFM in 1989 to refer to “a source of live (viable), naturally occurring microorganisms,” sources of which include yeast, bacteria, and fungal extracts.

Supplementation with live yeast has been shown to increase fibre digestion and stabilise rumen pH by interacting positively with the rumen flora, leading to increased rumen fermentation, increased microbial growth, and better animal performance (Chaucheyras Durand et al., 2007). Recent studies have used molecular techniques to measure the effect of live yeast on the rumen microbiome and the resulting effects on host performance. AlZahal et al. (in press) investigated the effects of supplementing dairy cows with live yeast during a SARA challenge on performance, rumen pH and fermentation, the microbiome, and the host animal itself. Results showed yeast supplementation led to significant increases in dry matter intake, milk yield, and feed efficiency (+1.7 kg/d, +3.1 kg/d, +4% respectively) when animals were faced with a SARA challenge. Rumen pH was significantly increased and time spent below pH 5.6 was halved. Microorganisms involved in fibre digestion were also significantly increased due to the higher rumen pH. The rumen *Prevotella* spp. were significantly decreased. *Prevotella* spp. have been shown to be associated with SARA under some challenge models (Khafipour et al., 2009) and have been suggested to be an important source of LPS within the rumen responsible for rumen inflammation and the incidence of lameness (Plaizier et al., 2008). Therefore, a reduction in the numbers of *Prevotella* would be expected to have a positive effect on rumen health. The *Prevotella* are also responsible for excessive peptidolysis leading to nitrogen excretion (Avgustin et al., 1997). By decreasing their numbers, nitrogen retention could also potentially be



improved. Another study demonstrated that when the same yeast was included in a combination mixture with some bacterial DFM, similar effects on rumen pH were observed, and rumen and circulating LPS were significantly reduced, probably due to higher rumen pH reducing the lysis of organisms responsible for the formation of LPS (Chiquette et al., 2012). Unfortunately, the effects on the microbiome were not investigated in this study.

Using a different live yeast strain, Pinloche et al. (2013) used high throughput sequencing analysis to demonstrate the effects of live yeast on the microbiome in cows fed a SARA-type diet. Yeast supplemented animals had greater VFA concentration, higher pH, and lower lactate compared with the control. These changes were associated with increased abundance of the main fibre digesting bacteria (*Fibrobacter* and *Ruminococcus*) and increased lactate utilising bacteria (*Megasphaera* and *Selenomonas*), thus confirming earlier cultivation-based analyses and the original proposed mode of action of live yeast.

Bacterial DFM act both in the rumen and in the lower gut, helping to promote gut health and productivity of dairy cows. Bacterial DFM are thought to have a “tonic” effect upon the lactate utilising bacterial population, working by producing low level doses of lactic acid, such that when the animal is faced with a SARA challenge there is a resident population of lactic acid utilising bacteria which can deal with any increases in lactic acid. Studies have demonstrated that the addition of these organisms, either alone or in conjunction with a live yeast may have a positive effect on dry matter intake and milk production, as well as stabilising rumen pH (Nocek et al., 2003; Nocek and Kautz, 2006).

### MOVING FURTHER DOWN THE DIGESTIVE TRACT

In the past researchers have concentrated on the effects of diet on rumen health and efficiency with little attention being given to the lower gut. However it has now been recognised that if lower gut health can be improved and total tract digestibility of nutrients, especially starch, is maximised, the overall performance of the animal can be increased. A recent study by Braman et al. (2014) investigated the effects of combining several strains of bacterial DFM with a live yeast on total tract starch digestibility in commercial herds. Fecal starch content was significantly decreased the longer the herds were on the combined DFM product. Better feed utilisation and digestibility were obtained, leading to greater performance and feed efficiency. SARA has been shown to have an effect not just on rumen health, but also on lower gut health, especially

in regard to levels of LPS which can cause inflammation of the gut lining, stimulating an inflammatory response (Plaizier et al., 2008). Li et al. (2012) studied the effects of a grain-based SARA and an alfalfa pellet SARA challenge on rumen fermentation and endotoxin release in the rumen and caecum. Both models caused a drop in rumen and caecal pH, but only the grain overload caused an increase in starch content in the caecum. Both models also caused an increase in ruminal LPS, but only the grain model increased LPS in the caecum and the faeces. Higher levels of starch in the caeca have the potential to cause a problem with overgrowth of virulent strains of *E. coli* in the lower gut and elevated production of LPS and stimulation of the immune response (Khafipour et al., 2011). The use of bacterial DFM, which can reduce the growth of these type of *E. coli* in the lower gut through competitive exclusion, has the potential to not only increase starch digestion, but also improve lower gut health. Yeast cell walls have also been shown to have positive effects on reducing *E. coli* populations by having a greater affinity for their fimbriae and attaching to the yeast wall and reducing pathogen attachment to the gut mucosa. A relatively new synbiotic product containing bacteria, live yeast, and yeast cell wall has been shown to have positive effects on animal performance through better gut and rumen health. Further research is needed to investigate its effects on both the rumen microbiome and the gut microbiome.

### IMPACT OF IMPROVING FEED EFFICIENCY ON THE ENVIRONMENT

Improving feed efficiency (yield of milk components/unit of feed intake) has been identified as a method of reducing methane produced/unit of milk produced. The average cow in the USA has a feed efficiency of 1.44, producing 72 pounds of energy corrected milk/day with a dry matter intake of 50.1 pounds of feed. A simple and relatively achievable increase in feed efficiency of 7% would lead to a similar reduction in methane produced/unit of milk. Improving feed digestibility and utilization means that there is more energy produced from rumen fermentation and greater microbial growth leading to greater milk yield, increased milk components, and better feed efficiency. Forage quality can have a significant effect on digestibility and passage rate, as can grain processing. The more digestible a ration can be made, the faster the passage rate, and methane output is reduced as a result. Feeding diets high in carbohydrates can also lead to shifts within the fermentation profile and higher propionate production, which acts as an alternative hydrogen sink to methane formation, thus methane emissions can be reduced whilst still maintaining good productivity. However, care should be taken that animals do not enter SARA, and a delicate



balance must be maintained between optimizing rumen health and productivity whilst still reducing methane emissions. Improving feed efficiency and extracting more value from feed and forage is a win-win situation for both the producer and the environmentalist.

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