Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

REVIEW ARTICLE

Role of diverse fermentative factors towards microbial community shift in ruminants

T.O. Faniyi[1], M.J. Adegbeye[2], M.M.M.Y. Elghandour[3], A.B. Pilego[3], A.Z.M. Salem[3], T.A. Olaniyi[4], O. Adediran[1] and M.K. Adewumi[1]

1 Department of Animal Science, Faculty of Agriculture and Forestry, University of Ibadan, Ibadan, Nigeria
2 Department of Animal Science, College of Agriculture, Joseph Ayo Babalola University, Ikeji-Arakeji, Nigeria
3 Faculty of Veterinary Medicine and Animal Sciences, Autonomous University of the State of Mexico, Toluca, México
4 Federal College of Animal Health and Production Technology, Moor Plantation Ibadan, Ibadan, Nigeria

Keywords
diet, fermentation, microbial shift, pH, rumen.

Correspondence
Abdelfattah Z.M. Salem, Faculty of Veterinary Medicine and Animal Sciences, Autonomous University of the State of Mexico, Instituto Literario 100 Ote, PO 50000, Toluca, México.
E-mails: salem@uaemex.mx; asalem70@yahoo.com

2018/2362: received 17 December 2018, revised 12 January 2019 and accepted 21 January 2019

doi:10.1111/jam.14212

Summary

Besides the liver, rumen is one of the most important components of metabolism in ruminants. However, the microbes residing in the rumen are influenced by several complex factors such as diet, which result in fluctuations in the rumen pH. Rumen pH affects feed intake and feed digestibility, subsequently causing microbial shift in the individual members of microbial community residing in the foregut and hindgut. This in turn causes an increase in lipopolysaccharide concentration, among other factors, in the gut fluid and animal blood. Irrespective of diet fed to animals, Firmicutes would probably be the most dominant in high grain diet while Bacteroidetes are dominant in hay diet, and both have a relative abundance of about 80% or more at times. The shift in microbial population is not limited to adult ruminants alone but also occur in calves. Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria were the most abundant in both hay and concentrate diet of newly weaned calves. Prolonged, depressed pH, causes subacute ruminal acidosis. This leads to compromise in the integrity of both foregut and hindgut of ruminants, eventually causing structural changes in the gut physiology. Furthermore, diet containing C-12:0 and C-14:0, which are medium-chain fatty acids, were toxic to rumen protozoa. Phytochemical content in some plant residues when fed to animals also causes shift in microbial population. Therefore, foregut and hindgut pH stability is important for ruminant health and for optimal productivity.

Introduction

The bionetworks of microbes consisting of bacteria, ciliate protozoa, anaerobic fungi, bacteriophages, viruses, and methanogens, found in the foregut of ruminants, make it possible for them to digest a wide range of ingredients of plant, animal and chemical origin. Examples are grasses, legumes, grains, oil and oil seeds, crop by-product, urea and ammonia sulphate which varies in biochemical composition. In order to meet the demand for animal protein all over the world, and to improve the quality of animal products, ruminant nutritionists supplement diet by offering grains and concentrates to livestock. In intensive systems of ruminant production, large quantities of easily fermentable carbohydrates are incorporated into the diet to support high milk yields or fast weight gain (Liu *et al.* 2014) in developed countries. A similar method has been adopted in developing countries to an extent. Indeed, rapidly fermentable starch improved productivity. However, this improvement resulted in alteration of rumen parameters such as rumen pH, ammonia nitrogen, affected rumen microbial population and caused unintended health challenges. Hence, there is an urgent need for us to better understand how rumen pH affects microbial population and ruminant productivity. A state of the rumen where there are favourable

microbes, large rumen papillae, efficient nutrient absorption, good barrier function and good fibre digestibility could be termed as rumen health. This highlights the importance of adequate feeding, microbial balance and gut structural integrity of foregut and hindgut.

Optimal rumen pH is vital for persistence and stability of rumen microbes (Penner 2016), because rumen microorganisms are very sensitive to little changes in pH level, which occurs due to type of feed consumed. Diet composition is fundamental to the hierarchical structural changes in bacterial populations (Welkie *et al.* 2010). The relationship between diet, rumen pH and rumen microbes is triangular, and each influences the another. The state and pattern of this relationship in the rumen exerts great influence on animal productivity, feed digestibility, animal health and ruminant environmental footprint. Most profound changes in the community structure occur during the weaning period, and, in adult animals, during dietary changes such as the switch from roughage to high grain diet or transfer from hay/concentrate to pasture feeding (Tajima *et al.* 2000).

Evaluations of the rumen during feeding trial often result in alteration in rumen fermentation characteristics and microbial community. Furthermore, when rumen microbes are assessed by culture-based techniques, it is often assumed that all the important microbes are discovered, and this frequent in some developing countries due to the lack of appropriate molecular equipment. However, molecular-based evaluation gives a more holistic approach in describing various functional groups of microbes and differences in the microbial diversity of complex ecosystems (Roesch *et al.* 2007; Franzolin and Dehority 2010) and this gives us the opportunity to assess how microbes are largely influenced under different feeding and environmental conditions. Several reports used in this review have demonstrated that microbes in the gut change based on the diet they consume, but to the best of our knowledge, none has been able to summarize in addition to diet, the influence of pH on shift in microbial diversity and animal health. Hence, this review is meant to evaluate the effect of rumen pH, diet and fatty acid on rumen microbial community, animal health, digestibility and productivity in ruminants fed different diets. References would be made to changes at phylum, genus and individual microbe level, with molecular-based comparison and few culture-based references.

Microbial population in ruminants

The rumen ecosystem is predominated by bacteria, protozoa, fungi, archaea and bacteriophages and is diverse when grouped into phylum and genus. Each of these microbes produces and utilizes different substrates during fermentation, which are beneficial or detrimental to animal health, productivity and environment (methane production). Degradation of diets by rumen microbes can be said to be regimented due to their preference for different feed structures and substrates. Similarly, individual variation of animals plays an important role in deciding the rumen microbiome of ruminants even when fed similar diets (Franzolin and Dehority 2010; Kala *et al.* 2017), a condition that could probably be attributed to genetic difference.

In ruminants, Firmicutes and Bacteroidetes are frequently the predominant bacteria irrespective of the diet, while the proportion of Proteobacteria and Fibrobacteres depends on the diet consumed (Firkins 2010). Similarly, in pre-ruminant animals, Verrucomicrobia, Synergistetes, Firmicutes, Bacteroidetes, Proteobacteria and Fusobacteria are present and Bacteroidetes, Firmicutes and Proteobacteria are the dominant microbes (Kala *et al.* 2017). The population of Fibrobacteres is at a very low level (0·01%) in pre-ruminants (Kala *et al.* 2017), which could be because they have not started taking solids and they do not have a functional rumen yet. However, rumen microbial communities of pre-ruminant calves are capable of maintaining a stable community function and metabolic potentials and should therefore not be considered rudimentary (Kala *et al.* 2017).

Rumen fungi play an important role in dietary fibre digestibility; this is possible through the penetration of fungal mycelia into plant particles, thereby weakening them and promoting rapid reduction in particle size and providing greater access for other micro-organisms (Li *et al.* 2012); this is more efficient at higher pH. An increase in the percentage of rapidly degradable starch in the diet generally favours the development of protozoa as long as the rumen pH is not below 5·5 (Leng 2014). However, ciliate protozoa could be completely eliminated if the pH falls below 5·0 (Goad *et al.* 1998; Martin *et al.* 2002), a process known as defaunation. Protozoa in the rumen are more susceptible to decline under low pH. Nevertheless, *Entodinium* sp. is more tolerant to low pH than other genera of rumen protozoa (Mackie *et al.* 1978; Gebeyehu and Mekasha 2013).

Rumen pH

Ruminal pH is a fermentation parameter that quantifies the state of acidity and alkalinity of the rumen. This is a predisposing factor for rumen health, microbial stability and shift, and a prerequisite for optimal microbial proliferation. Its state could be used to predict the type of diet fed to animals, and its degree of increase or decrease could be used to predict the rate of fermentation. The rumen pH influences all aspect of rumen function in

Artiodactyla. It is important in rumen development and calf health (Kala *et al.* 2017) because it influences microbial growth, microbial shift, microbial stability, cellulose digestion, biohydrogenation, methanogenesis, defaunation and volatile fatty acid (VFA) absorption rate and animal health. The impact of rumen pH makes it a 'valiant or villain' in any ruminant production system. Rumen pH constantly changes whenever ruminants are fed because it is greatly influenced by the composition of the diet. For instance, in cattle fed once daily, ruminal pH decreases after feeding for a period of a few hours, and then increases again because of VFA removal, rumination and salivation (Lyle *et al.* 1981). Generally, pH ranges between 5·5 and 7·0 (Lyle *et al.* 1981) and occasionally up to 7·5 (Krause and Oetzel 2006), perhaps when high forage is consumed. Notwithstanding, for optimal rumen function, Kamra (2005) and Ososanya *et al.* (2013) stated that rumen pH should range between 6·00 and 6·80. This is needed for rumen ecological stability to aid 'peaceful' coexistence in the rumen microbial community, and for the continual symbiotic relationship between microbes and animals.

A major consequence of pH below 6·0 is the decrease in fibrolytic activity (Chiba 2014). This is caused by the microbe's inability to maintain the pH inside their cell when ruminal pH is low (Chiba 2014). Furthermore, if the pH is below 5·0 or above 7·8, rumen ciliated protozoa cannot survive (Rode 2000). In addition, rumen pH lower than 5·5 leads to digestive disturbance known as ruminal acidosis. This affects dry matter intake, and subsequently microbial growth and animal production and causes several secondary complications. An increase in VFA production greater than absorption will result in a drop in rumen pH, which will cause disruption of rumen microbiota (Clarke 1977). However, the highest absorption of VFAs occurs under low pH, i.e. from 5·6 to 5·8 (Plaizier *et al.* 2008).

Diet and rumen microbial shift

Grains (highly fermentable starch) and their by-products have been used in feeding trials and in farms (feedlot and dairy) to improve animal productivity and maximize the genetic potential of animals. This feeding management may be helpful in enhancing cost-efficiency in the short term, but they have considerable consequences for animal gastrointestinal health (Liu *et al.* 2014). Acidosis, diarrhoea, villi wear out and production of endotoxins in aggregate decrease animal performance and all these anomalies have implications on animal welfare and profitability. The change from high forage to high concentrate diet is accompanied by a significant alteration in ruminal microbial populations (Silva and Leão 1979). This is due to changes in the biochemical structure of feed ingredients, preference of microbes for a particular substrate and overall changes in the rumen pH; especially in a diet containing more than 60% concentrate (Gaafar *et al.* 2009; Grilli *et al.* 2016).

High grain diet consumed by ruminants could cause rumen pH to drop below 5·8 and 5·6 for dairy and beef, respectively, for a period of at least 3 h due to imbalance between production of VFA and their absorption by the rumen walls (Cherdthong *et al.* 2010). During subacute rumen acidosis induced by high grain diet or sudden change from high forages to a grain-based diet, rumen bacteria shift caused a decline in the population of Bacteroidetes which are Gram negative (Cardo 2015), and correlate with an increase in ruminal lipopolysaccharide (LPS) (Khafipour *et al.* 2009), which leads to a drastic shift of microbes to Gram-positive lactic acid producers such as *Streptococcus* sp. and *Lactobacillus* sp. in the rumen, caecum and colon of overfed animals (Mao *et al.* 2013; Ososanya *et al.* 2013; Petri *et al.* 2013) (Table 1).

Rumen pH and foregut microbial shift

The dietary characteristics of feed consumed by animals affect ruminal pH and rumen microbial community members, which subsequently influence the concentration of ammonia nitrogen and the proportion of individual VFA. In other words, there exists an interaction between diet, rumen pH, microbial population, microbial shift and animal productivity. Therefore, it is inaccurate to discuss changes in rumen pH and microbial shift without including diet, because they are conjoined players in ruminants.

In Metzler-Zebeli *et al.* (2013), 0, 30 and 60% grains were included in the diet of goats, which led to the variation in the rumen pH at 6·4, 6·0 and 5·5 respectively. There were reductions in *Prevotella, F. succinogenes,* Enterobacteriaceae and *Clostridium* cluster XIV at pH 5·5. However, *Lactobacillus* sp. growth increased as the pH was decreasing. This could be attributed to their preference for starch and ability of lactic acid utilizers and producers to cope with lower pH. Furthermore, there was prevalence of *Prevotella* at pH 6·0 than at pH 6·4 and 5·5. This is in contrast to a microbial report of Kim *et al.* (2016) that observed a decrease in the growth of *Prevotella* with increasing grain in the diet. A possible reason might be because *Prevotella* is an extremely versatile genus that has a range of substrate that could be utilized as carbon and energy sources in degrading starch, cellulose, hemicelluloses and pectin (Kim *et al.* 2016). There was an increase in *F. succinogenes* at pH 6·0 (30% grain) than at pH 6·4 (0% grain) and 5·5 (60% grain), which contradicts results from other studies (Russell *et al.* 2009;

Table 1 Summary of the influence of diet on ruminant animals

Type of diet	Effect on animal	Animal species	Method of evaluation	References
High forages to high grain diet	Decrease in Bacteroidetes and increased rumen lipopolysaccharide concentration	Cattle	Molecular method	Khafipour *et al.* (2009)
Grain-based diet at 0, 30 and 60%	Reduction in fibrolytic bacteria, Holotrich protozoa, and increased lactobacilli, entodiniomorphid protozoa and decreased pH in the rumen	Goat	Molecular for bacteria and microscopic method for protozoa	Metzler-Zebeli *et al.* (2013)
Grain-based diet at 0, 30 and 60%	12- to 33-fold increase in the colon of Enterobacteriaceae 1·9-fold increase in *Fibrobacteres succinogenes* Increased colonic pH	Goat	Molecular for bacteria and microscopic method for protozoa	Metzler-Zebeli *et al.* (2013)
Hay diet and calf starter concentrate	Increase in Firmicutes and decrease in Bacteroidetes in calf starter-fed calves	Calves	Molecular method	Kim *et al.* (2016)
Grain and hay diet	Increased epithelial *Firmicutes*, fourfold increase in *Butyrivibrio* in high grain-fed animal and predominance of *Prevotella* in hay-fed goats	Goat	Molecular method	Liu *et al.* (2015)
Grain-based diet	Microbial disruption Increased blood, rumen, caecum and faecal lipopolysaccharide	Cattle	Molecular method	Plaizier *et al.* (2012)
Dietary oil palm by-product containing medium-chain fatty acid C12:0 and C14:0	Microbial shift Reduced protozoa population	Goat	Microscopic method	Abubakr *et al.* (2012)

Carberry *et al.* 2012). It could possibly be due to higher ruminal glucose resulting in energy availability due to amylolytic activity of other ruminal bacteria on the grain, which might have supported the proliferation of *F. succinogenes* (Metzler-Zebeli *et al.* 2013). A shift towards the growth of entodiniomorphid protozoa was observed as the pH was reducing with increasing grain. This could be attributed to chemotaxis of entodiniomorphid protozoa towards consumption of starch granules, the availability of which increases with increasing proportion of starch source. Moreover, there was a concomitant decrease in holotrich protozoa as entodiniomorphid protozoa was increasing. This trade-off in the protozoa growth may be attributed to the reduced availability of soluble carbohydrate due to the consumption of starch granules by entodiniomorphid protozoa, which would have increased holotrich, had it been available. This is in agreement with Rode (2000).

The shift in microbial population is not limited to adult ruminant alone; it also occurs in calves. When feeding dietary forage and calf starter to calves that were just weaned, the relative abundance of Firmicutes was about 60% in concentrate-fed calves whereas it was about 52% in hay-fed calves (Kim *et al.* 2016). On the contrary, Bacteroidetes accounted for just 20% of the population in concentrate-fed calves while it was about 35% in hay-fed calves.

Using a molecular-based technique, Liu *et al.* (2015) evaluated changes in the epithelial microbial population of goats using high grain diet and hay. Firmicutes, *Butyrivibrio* and *Ruminococcus* were higher in high grain diet while *Prevotella* was predominant in hay-fed animals. *Butyrivibrio* were about fourfold higher in concentrate-fed animals than in hay-fed animals. This is because many *Butyrivibrio* species are amylolytic (Shriver *et al.* 1986), thus the increase in concentrate-fed animals. Other benefits of increased epithelial *Butyrivibrio* would be the release of butyrate close to the epithelium, which might enhance butyrate bioavailability for the host, which is useful for rumen epithelial proliferation (Sakata and Tamate 1978; Liu *et al.* 2015). Microbial shifts are not limited to goat, cattle, and pre-ruminants, but also occur in buffaloes. The pH was 6·88 and 7·03 when buffaloes were fed high concentrate and low concentrate diet respectively (Shen *et al.* 2004) (Table 1).

Microbial shift in the hindgut

Changes in the diet and the corresponding effect on rumen pH and microbial community do not affect the foregut only. Alteration in diet composition results in both numerical and qualitative variations in the supply of substrates to the microbes in the large intestine (Lin *et al.* 2015). Studies of Liu *et al.* (2014) and Kim *et al.* (2016) on goats showed that changes occur in the hindgut too. Ensuring hindgut health is of utmost importance to ruminants. For instance, caeca fermentation accounts for 12% of total VFA production in sheep (Louis *et al.*

2007). Therefore, hindgut fermentation can provide VFA for cattle, goat, buffalo, and contribute to overall quality and quantity of energy used by animals, thus improving feed efficiency. Changes in foregut microbial balance could also affect the hindgut microbial population. The study of Metzler-Zebeli *et al.* (2013) shows that there was variation of the colon microbes when the colon pH was at 8·5, 8·1 and 7·0 in goats fed diets containing 0, 30 and 60% starch respectively. It was observed that *Clostridium* cluster XIV, *Prevotella*, Enterobacteriaceae, and *F. succinogenes* were present in the colon except for *Lactobacillus* sp. The absence of *Lactobacillus* sp. in the colon could mean that the rumen is the only niche in the gastrointestinal tract of ruminants that lactic acid microbes can grow or be found. Secondly, it could be that *Lactobacillus* sp. cannot thrive under the colonic pH, which is high. The growth of *Clostridium* cluster XIV, *Prevotella*, Enterobacteriaceae and *F. succinogenes* growth increased when pH was 7·0 than at pH 8·1 and 8·5. Furthermore, there were 33- and 12-fold increases in the colon growth of *F. succinogenes* in goats fed 60% starch compared with goats fed 0 and 30% respectively. Furthermore, there was a 1·88-fold increase in the growth of Enterobacteriaceae in 60% starch-fed animals compared with others. The increased colon in *F. succinogenes* could be attributed to the poor digestibility of fibre in the rumen due to low rumen pH (5·5). Therefore, when there is an increase in the individual microbes in the rumen, there would be a corresponding decrease in the colon for such microbes. This is because of the low substrate that would be available for such microbes in the colon as a result of efficient extraction of nutrients from the diet.

Rumen pH, lipopolysaccharide and ruminant health

Rumen bacteria are broadly divided into Gram positive and Gram negative due to their cell wall composition. The latter make up to 51% of the total viable bacterial counts within the rumen (Faichney 1968) and they are susceptible to lysing (Caldwell and Bryant 1966), during prolonged depression of pH. During acidosis, the shift in microbial population leads to the death of many microbes, including Gram-negative microbes, such as *F. succinogenes*. This scenario is caused by the accumulation of VFA which has lower pH. Besides, *F. succinogenes* is a fibre-degrading microbe which does not thrive efficiently under low pH. Hence, upon death, it releases embedded LPS into the rumen (Biomin 2018). It is important to know that LPS toxicity varies among bacterial species (Wells and Russell 1996). Microbes such as *Megasphaera elsdenii, F. succinogenes, Prevotella* sp. and *Bacteroides* sp. are less toxic than LPS of *Escherichia coli*, and *Salmonella* sp. (Plaizier *et al.* 2012). Interestingly, during fast growth of Gram-negative bacteria, there could be large 'shedding' of endotoxins.

Damages caused by endotoxins are facts and no fictions. The Plaizier *et al.* (2012) study shows that in subacute rumen acidosis-induced animals, the concentration of endotoxins in blood, rumen, ileum, caecum and faeces. Could increase up to 20, 35, 27.5, 7 and 7 fold respectively, compared to healthy animals (Plaizier *et al.* 2012). High LPS during acidosis may be attributed to the death of *F. succinogenes* (which is Gram negative) and important fibrolytic microbes, which could not tolerate the low level of rumen pH. Thus, abundance of LPS is a litmus test for the death of Gram-negative microbes. Absorption at this high concentration into the peripheral blood would put the animal's health in a precarious state and cause economic loss in the long run for farmers, especially those in developed countries.

Prolonged depression of rumen pH has varying consequences on ruminant health. Low rumen pH for prolonged periods can negatively affect feed intake, microbial metabolism and nutrient degradation. This results in acidosis, inflammation, laminitis, diarrhoea, bloat, milk fat depression, abomasal displacement, fatty liver, abscessed liver, increased mycotoxin absorption and sudden death syndrome (Enemark 2008; Qi *et al.* 2010; Chaucheyras-Durand *et al.* 2012). The increase in mycotoxin is due to the reduction in the number of protozoa, which is one of the most important mycotoxin-degrading agents (Zebeli and Metzler-Zebeli 2012). It could also cause a shift in the colonic and caeca microbial population, and anomalies such as increased vacuoles in cell layers, mitochondrial swelling, apparent intercellular tight junction erosion and sparse and messy villi in the anatomy of the hindgut (Liu *et al.* 2014). The prolonged depression of pH caused by the accumulation of VFA in grain-fed ruminants is partly due to the poor absorption of organic acids from the rumen. The poor absorption is a result of morphological alteration, and numerical reduction of gut villi-a channel which nutrient is absorbed into the blood.

Lameness is a major concern in modern dairy and beef production, and its occurrence is clearly linked between acidosis and the inflammation of the lamellar tissue of the hoof—a condition known as laminitis. This not only causes problems by itself, but is also the first step for other conditions such as sole ulcers and white line haemorrhages (Cherdthong *et al.* 2010). It is thought that laminitis is due to lower systemic pH during acidosis and substances such as histamine (involved in immune response) and endotoxins entering the bloodstream (Cherdthong *et al.* 2010) through lowered rumen wall integrity (Schaumberger 2015). The aggregation of this may result in increased and unregulated absorption of harmful and beneficial fluids into the body, eventually

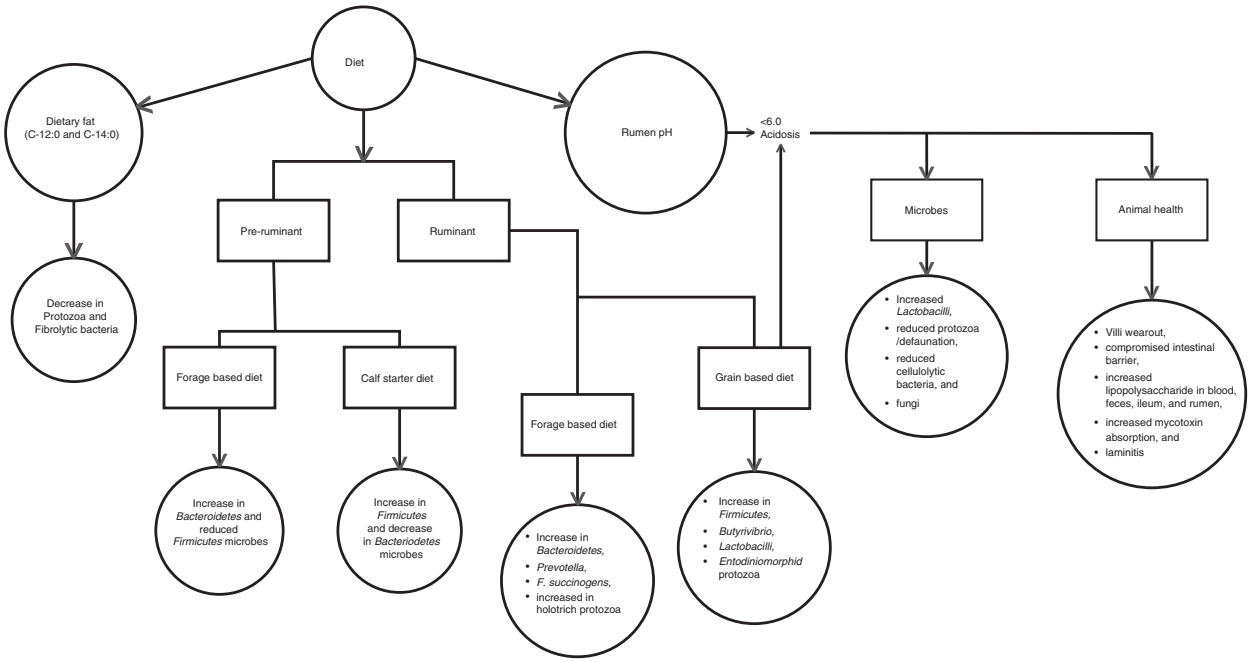

Figure 1 Factors affecting Microbial Shift and their effect on Ruminant.

putting pressure on highly active organs such as the liver that metabolizes them. Eventually, if there is liver failure/malfunction, it would give room for endotoxins to overwhelm the cow's biological function (Biomin 2016), which is practically involved in all metabolic and some hormonal regulatory activity. Therefore, control of LPS (endotoxins) and its production may be vital to the eradication of laminitis (Cherdthong *et al.* 2010). In other words, controlling the incidence of acidosis and excessive lysing of Gram-negative microbes will help to reduce the challenge caused by endotoxins (Fig. 1).

Rumen pH and feed intake and digestibility

Diet fed to animals is altered from time to time depending on the season, physiological state, purpose of production and sometimes to reduce cost of feeding. This modification affects the proportion of metabolite produced during fermentation and the quality and quantity of animal product and animal health. The gastrointestinal microbial community is not only influenced by available substrate but also by the gut environment, with pH playing a major role (Schaumberger and Reisinger 2015).

Ruminal pH has implications for the microbial ecosystem, frequently leading to an undesired population shift, which results in inefficient digestion of feedstuffs (Carberry *et al.* 2012). Low pH may lead to reduced feed efficiency. Fibrolytic microbes are more susceptible to low pH, compared with other microbes, because of their inability to maintain the pH inside their cell (Chiba 2014). Fibre digestibility and feed intake were reduced in steer fed pelleted barley when ruminal pH declined to about 5·5 (Sahoo and Soren 2012). Thus, a pH near neutrality between 6 (Sarwar *et al.* 1999) and 7·5 is optimal for fibre digestibility. Fibre digestion was reduced when pH was 5·8 compared with 6·2 (Russell *et al.* 2009), an indication that rumen microbes responsible for the breakdown of cellulose in the rumen are very sensitive to slight changes in rumen pH. Furthermore, ruminal acid detergent fibre (ADF) digestion decreased by 3·6% for every 0·1 decrease in pH below 6·3 (Weimer 1996). ADF digestibility is a measure of fibre digestibility and feed quality. Hiltner and Dehority (1983) and Erdman (1988) resolved that prolonged exposure of cellulolytic bacteria to low pH has little effect on the subsequent ability to digest cellulose. However, ruminal pH needs to remain higher than 6·0 long enough to permit growth rates that exceed the passage rate (Nagaraja 2012). The increase in rumen pH is not only a function of dietary fibre intake but could also be an implication of inadequate feed intake (Chiba 2014).

Although there is limitation to fibre digestibility when ruminal pH is <6·0 (Erdman 1988; Mouri˜no *et al.* 2001; Palmonari *et al.* 2010), using molecular techniques has shown that even cows with very low pH can maintain normal populations of cellulolytic bacteria. Still, if the pH drops below 6·0, the population and growth of cellulolytic bacteria and the ruminal fungi decline (Palmonari *et al.* 2010). This impairs fibre digestibility and results in

changes in the bacterial population and eventually leads to the overproduction of lactic acid—a much stronger acid involved in acute acidosis (Palmonari *et al.* 2010) due to the growth and dominance of acidophilic bacteria such as lactic acid bacteria, is implicated.

Prolonged depression of pH leads to metabolic disorders such as acidosis, which could be either acute rumen acidosis or subacute rumen acidosis and could also alter the feeding pattern and reduce feed intake (Palmonari *et al.* 2010). A short stint of subacute rumen acidosis (<30 min) did not reduce neutral detergent fibre digestibility; conversely, repeated bouts of 4 h did affect neutral detergent fibre digestibility (Palmonari *et al.* 2010). At those thresholds, fibre digestion is reduced and noticeably affects fat concentration in milk, invariably reducing the milk quality and quantity of other milk products such as butter. Furthermore, poor fibre digestibility and lower feed efficiency resulting from depressed pH translate into increased cost of production and high cost per unit of animal product. Thus, producers and nutritionists should pay particular attention to rumen pH in order to optimize neutral detergent fibre digestibility of forages (Cardo 2015).

Dietary fat on microbial shift

Supplementation of diets with oils has a significant effect in shaping the microbial community in the rumen (Lillis *et al.* 2011). Fat supplements, which contain unsaturated fatty acid, given to ruminants to modulate rumen activity could have negative effects on the growth of microbes such as protozoa and fibrolytic bacteria (Enjalbert *et al.* 2017). In the study of Abubakr *et al.* (2012), which involved the use of dietary fat of oil palm origin, there were reductions in the microbial population of protozoa. The diets, which reduced the protozoa population, contained lauric acid (C-12:0) and myristic acid (C-14:0) which are medium-chain fatty acids while the control had no C-12:0 and C-14:0. In addition, C12:0 has a strong influence in decreasing protozoa counts compared with C14:0 (Hristov *et al.* 2012). It could be deduced that medium-chain fatty acids were toxic to rumen protozoa. This is due to the ability of C-12:0 and C-14:0 to reduce pH, and their ability to dissociate (Adegbeye *et al.* 2018). Furthermore, this is because in their dissociated form, they can infiltrate into the lipid membrane of cells and dissociate in the intracellular environment, leading to microbes not maintaining a neutral pH, depletion of cellular ATP and cell death (Ricke 2003). The infiltration of the cell membrane is due to the ability of dietary fat to alter cell or plasma membrane composition (De Pablo and De Cienfuegos 2000), which alters the fluidity of the cell membrane and the membrane protein which act as a receptor, thereby permitting the medium-chain fatty acid to breach the selective medium integrity of the membrane (Adegbeye *et al.* 2018). Although the palm kernel cake diet contained higher C-12:0 and C-14:0 (53·41 and 16·21 g per 100 g) of fatty acid than other palm products used which had 0·02 and 0·05 g per 100 g of C-12:0 and C-14:0 for decanter cake, and 0·01 and 0·81 g per 100 g of C-12:0 and C-14:0 for control diet +5% palm oil, they still elicited the same effect on the protozoa (2·1 × 105) compared with the control (6·2 × 105). This reduction may not be attributed to pH because all the animals fed the same dietary source had the same number of protozoa. It can be deduced that a little trace of C-12:0 and C-14:0 can cause an antiprotozoal effect. The decline in the population of protozoa would promote an increase in fibre digestion due to increased bacteria, especially cellulolytic, biomass and due to decreased phagocytosis (Abubakr *et al.* 2012).

Conclusion

Understanding the dynamism of rumen microbes and how they are affected by rumen pH will go a long way in influencing the way in which diet is formulated, type of ingredients to use, while being wary of the implications of these diets on all animal welfare. Irrespective of the type of diet offered to animals, a core group of microbes exists in the rumen at the phylum level; notwithstanding, diet and rumen pH cause a shift in microbial population at the individual level. Firmicutes might probably be the most dominant in high grain diet while Bacteroidetes are dominant in hay diet, with a relative abundance of about 80% or more at times. Others that vary with diet are Fibrobacteres, Actinobacteria, and Proteobacteria, which increase with higher fibre in the diet.

Anatomically, low pH compromises both the foregut and hindgut of ruminants, by eroding the villi, and they cause structural changes in the gut physiology, which may be responsible for the accumulation of VFA in the rumen when fed grain- or concentrate-based diets due to slow absorption of VFA caused by short and few gut villi. Microbiologically, diet and pH cause shift in the rumen, colon, caeca, and influence the concentration of endotoxins in the gut. Thus, ensuring hindgut health should be a priority to ruminant ecologists and nutritionists, especially for the enormous amount of VFA that hindgut fermentation provides for ruminants. The experimental animal also determines the type and population of microbes in the gut even when fed the same quality and quantity of diet, which may be due to the difference in the genetic potential.

The addition of grain or starch to diet might enhance the growth of cellulolytic microbes better than when very high fibre or grain is used alone. Fibrobacteres can thrive well if the fermentable starch ingredient in the diet is not

more than 35%. Furthermore, starch will provide the energy needed for the growth of fibrolytic microbes. Thus, diets containing fermentable starch below 35% are not toxic to the growth of Fibrobacteres. In addition, reports on the effect of diet on gut anatomy are limited, especially in ruminants. Hence, further research should include histology and gut morphology of the foregut and hindgut of ruminants.

Conflict of Interest

The authors declare no conflict of interest.

References

Abubakr, A.R., Alimon, A.R., Yaakub, H., Abdullah, N. and Ivan, M. (2012) Digestibility, rumen protozoa, and ruminal fermentation in goats receiving dietary palm oil by-products. *J Saudi Soc Agric Sci* **12**, 147.

Adegbeye, M.J., Elghandour, M.M.Y., Faniyi, T.O., Perez, N.R., Barbabosa-Pilego, A., Zaragoza-Bastida, A. and Salem, A.Z.M. (2018) Antimicrobial and antihelminthic impacts of black cumin, pawpaw and mustard seeds in livestock production and health. *Agroforestry System*. https://doi.org/10.1007/s10457-018-0337-0.

Biomin (2016). What's wrong with my herd? Part 2: Endotoxins science and solutions: a magazine of biomin®. *Rumin Issue* **37**, 1–15.

Biomin (2018). Star performance from your forage: the link between endotoxins and mycotoxins. science and solutions: a magazine of biomin®. *Rumin Issue* **53**, 1–12.

Caldwell, D.R. and Bryant, M.P. (1966) Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. *Appl Environ Microbiol* **14**, 794–801.

Carberry, C.A., Kenny, D.A., Han, S., McCabe, M.S. and Water, S.M. (2012) Effect of phenotypic residual feed intake and dietary forage content on the rumen microbial community of beef cattle. *Appl Environ Microbiol* **78**, 4949–4958.

Cardo, L. (2015). Tackling acidosis the dangers of SARA, science and solutions: a magazine of biomin®. *Rumin Issue* **25**, 1–15.

Chaucheyras-Durand, F., Chevaux, E., Martin, C. and Forano, E. (2012) Use of yeast probiotics in ruminants: effects and mechanisms of action on rumen pH, fibre degradation, and microbiota according to the diet. *Intechopen Sci* **7**, 119–152.

Cherdthong, A., Wanapat, M., Kongmun, P., Pilajan, R. and Khejornsart, P. (2010) Rumen fermentation, microbial protein synthesis and cellulolytic bacterial population of swamp buffaloes as affected by roughage to concentrate ratio. *J Anim Vet Adv* **9**, 1667–1675.

Chiba, L. (2014) Rumen microbiology and fermentation. *Animal Nutrition Handbook*, pp 57–79. Auburn, AL: Self-published.

Clarke, R.T.J. (1977) Protozoa in the rumen ecosystem. In *Microbial Ecology of the Gut* ed. Clarke, T.T.J. and Bauchop, T. pp. 251–275. New York: Academic Press.

De Pablo, M.A. and De Cienfuegos, G.A. (2000) Modulatory effects of dietary lipids on immune system functions. *Immunol Cell Biol* **78**, 31–39.

Enemark, J.M.D. (2008) The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): a review. *Vet J* **176**, 32–43.

Enjalbert, F., Combes, S., Zened, A. and Meynadier, A. (2017) Rumen microbiota and dietary fat: a mutual shaping. *J Appl Microbiol* **123**, 782–797.

Erdman, R.A. (1988) Dietary buffering requirement of the lactating dairy cow: a review. *J Dairy Sci* **71**, 3246–3251.

Faichney, G.J. (1968) Volatile fatty acids in the caecum of the sheep. *Aust J Biol Sci* **21**, 177–180.

Firkins, J.L. (2010) Reconsidering rumen microbial consortia to enhance feed efficiency and reduce environmental impact of ruminant livestock production systems. *R Bras Zootec* **39**, 445–457.

Franzolin, R. and Dehority, B.A. (2010) The role of pH on the survival of rumen protozoa in steers. *R Bras Zootec* **39**, 2262–2267.

Gaafar, H.M.A., Mohi El-Din, A.M.A., Basiuoni, M.I. and El-Riedy, K.F.A. (2009) Effect of concentrate to roughage ratio and baker's yeast supplementation during hot season on performance of lactating buffaloes. *Slovak J Anim Sci* **42**, 188–195.

Gebeyehu, A. and Mekasha, Y. (2013) Defaunation: effects on feed intake, digestion, rumen metabolism and weight gain. *J Agric Res* **2**, 134–141.

Goad, D.W., Goad, C.L. and Nagaraja, T.G. (1998) Ruminal microbial and fermentative changes associated with experimentally induced sub-acute acidosis in steers. *J Anim Sci* **76**, 234–241.

Grilli, D.J., Fliegerov, K., Kopecný, J., Lama, S.P., Egea, V., Sohaefer, N., Pereyra, C., Ruiz, M.S. *et al.* (2016) Analysis of the rumen bacterial diversity of goats during shift from forage to concentrate diet. *Anaerobe* **42**, 17–26.

Hiltner, P. and Dehority, B.A. (1983) Effect of soluble carbohydrates on digestion of cellulose by pure cultures of rumen bacteria. *Appl Environ Microb* **46**, 642–648.

Hristov, A.N., Callaway, T.R., Lee, C. and Dowd, S.E. (2012) Rumen bacterial, archaeal, and fungal diversity of dairy cows in response to ingestion of lauric or myristic acid. *J Anim Sci* **90**, 4449–4457.

Kala, A., Kamra, D.N., Kumar, A., Agarwal, N., Chaudhary, L.C. and Joshi, C.G. (2017) Impact of levels of total digestible nutrients on microbiome, enzyme profile and degradation of feeds in buffalo rumen. *J Pone* **12**, e0172051.

Kamra, D.N. (2005) Rumen microbial. Special Section: Microbial diversity. *Current Sci* **89**, 124–135.

Khafipour, E., Krause, D.O. and Plaizier, J.C. (2009) A grain-based sub-acute ruminal acidosis challenge causes

translocation of lipopolysaccharide and triggers inflammation. *J Dairy Sci* **92**, 1060–1070.

Kim, Y.-H., Nagata, R., Ohtani, N., Ichijo, T., Ikuta, K. and Sato, S. (2016) Effects of dietary forage and calf starter diet on ruminal pH and bacteria in Holstein calves during weaning transition. *Front Microbiol* **7**, 1–12.

Krause, K.M. and Oetzel, G.R. (2006) Understanding and preventing sub-acute ruminal acidosis in dairy herds: a review. *Anim Feed Sci Tech* **126**, 215–236.

Leng, R.A. (2014) Interactions between microbial consortia in biofilms: a paradigm shift in rumen microbial ecology and enteric methane mitigation. *Anim Prod Sci* **54**, 519–543.

Li, R.W., Connor, E.E., Li, C., Baldwin, R.L. and Sparks, M.E. (2012) Characterization of the rumen microbiota of pre-ruminant calves using metagenomic tools. *Environ Microbiol* **1**, 129–139.

Lillis, L., Boots, B., Kenny, D.A., Petrie, K., Boland, T.M., Clipson, N. and Doyle, E.M. (2011) The effect of dietary concentrate and soya oil inclusion on microbial diversity in the rumen of cattle. *J Appl Microbiol* **111**, 1426–1435.

Lin, B., Henderson, G., Zou, C., Cox, F., Liang, X., Janssen, P.H. and Attwood, T. (2015) Characterization of the rumen microbial community composition of buffalo breeds consuming diets typical of dairy production systems in Southern China. *Anim Feed Sci Tech* **207**, 75–84.

Liu, J., Xu, T., Zhu, W. and Mao, S. (2014) High-grain feeding alters caecal bacterial microbiota composition and fermentation and results in caecal mucosal injury in goats. *Br J Nutr* **112**, 416–427.

Liu, J., Bian, G., Zhu, W. and Mao, S. (2015) High grain feeding causes strong shifts in ruminal epithelial bacterial community and expression of Toll-like receptor genes in goats. *Front Microbiol* **6**, 1–10.

Louis, P., Scott, K.P. and Duncan, S.H. (2007) Understanding the effects of diet on bacterial metabolism in the large intestine. *J Appl Microbiol* **102**, 1197–1208.

Lyle, R.R., Johnson, R.R. and Wilhite, J.V. (1981) Rumen characteristics in steers as affected by adaptation from forage to all concentrate diets. *J Anim Sci* **53**, 1383–1390.

Mackie, R.J., Gilchrist, A.M. and Roberts, A.M. (1978) Microbiological and chemical changes in the rumen during the stepwise adaptation of sheep to high concentrate diets. *J Agric Sci* **90**, 241–254.

Mao, S.Y., Zhang, R.Y., Wang, D.S. and Zhu, W.Y. (2013) Impact of sub-acute ruminal acidosis (SARA) adaptation on rumen microbiota in dairy cattle using pyrosequencing. *Anaerobe* **24**, 12–19.

Martin, C., Fonty, G. and Michalet-Doreau, B. (2002) Factors affecting the fibrolytic activity of the digestive microbial ecosystems in ruminants. In *Gastrointestinal Microbiology in Animals* ed. Martin, S.A. pp. 1–17. Trivandrum: Research Signpost.

Metzler-Zebeli, B.U., Schmitz-Esser, S., Klevenhusen, F., Podstatzky-Lichtenstein, L., Wagner, M. and Zebeli, Q. (2013) Grain-rich diets differently alter ruminal and colonic abundance of microbial populations and lipopolysaccharide in goats. *Anaerobe* **20**, 65–73.

Mouriño, F., Akkarawongsa, R. and Weimer, P.J. (2001). pH at the initiation of cellulose digestion determines cellulose digestion rate *in vitro*. *J Dairy Sci* **48**, 848–859.

Nagaraja, T.G. (2012). *A microbiologist's view on improving nutrient utilization in ruminants*. pp. 135–160: Gainesville, FL: Florida ruminant nutrition symposium. University of Florida.

Ososanya, T.O., Odubola, O.T. and Shuaib-Rahim, A. (2013) Intake, nutrient digestibility and rumen ecology of West African Dwarf sheep fed palm kernel oil and wheat offal supplemented diets. *Int J Agric Sci* **3**, 380–386.

Palmonari, A., Stevenson, D.M., Mertens, D.R., Cruywagen, C.W. and Weimer, P.J. (2010) pH dynamics and bacterial community composition in the rumen of lactating dairy cows. *J Dairy Sci* **93**, 279–287.

Penner, G.B. (2016). Influence of microbial ecology in the rumen and lower gut on production efficiency of dairy cows. *Tri-State Dairy Nutrition Conference*, 18–20 April 2016, Fort Wayne, IN. pp. 75–81.

Petri, R.M., Schwaiger, T., Penner, G.B., Beauchemin, K.A., Forster, R.J., McKinnon, J.J. and McAllister, T.A. (2013) Changes in the rumen epimural bacterial diversity of beef cattle as affected by diet and induced ruminal acidosis. *Appl Environ Microbiol* **79**, 3744–3755.

Plaizier, J.C., Krause, D.O., Gozho, G.N. and McBride, B.W. (2008) Subacute ruminal acidosis in dairy cows: the physiological causes, incidence and consequences. *Vet J* **176**, 21–31.

Plaizier, J.C., Khafipour, E., Li, S., Gozho, G.N. and Krause, D.O. (2012) Subacute ruminal acidosis (SARA), endotoxins and health consequences. *Anim Feed Sci Tech* **172**, 9–21.

Qi, M., Jakobar, D. and McAllister, T.A. (2010) Rumen microbiology. In: *Animal and plant productivity Encyclopaedia of life support systems-(EOLSS)* ed. Hudson, R.J. pp. 161–176. Oxford: EOLSS.

Ricke, S.C. (2003) Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poult Sci* **82**, 632–639.

Rode, L.M. (2000) Maintaining a healthy rumen – an overview. *Adv Dairy Tech* **12**, 101–108.

Roesch, L.F., Fulthorpe, R.R., Riva, A., Casella, G., Hadwin, A.K.M., Kent, A.D., Daroub, S.H., Camargo, F.A.O. *et al.* (2007) Pyrosequencing enumerates and contrasts soil microbial diversity. *ISME J* **1**, 283–290.

Russell, J.B., Muck, R.E. and Weimer, P.J. (2009) Quantitative analysis of cellulose degradation and growth of cellulolytic bacteria in the rumen. *FEMS Microbiol Ecol* **67**, 183–197.

Sahoo, A. and Soren, N.M. (2012) Phytochemicals and gut microbial populations in nonruminants. In *Dietary Phytochemicals and Microbes* ed. Patra, A.K. pp. 379–389. Dordrecht, Heidelberg, New York, London: Springer.

Sakata, T. and Tamate, H. (1978) Rumen epithelial cell proliferation accelerated by rapid increase in intraruminal butyrate. *J Dairy Sci* **61**, 1109–1113.

Sarwar, M., Mahmood, S., Nisa, M.U. and Bilal, Q. (1999) Factors affecting digestibility of feeds in ruminant. *Int J Agric and Biol* **1**, 1560–8530.

Schaumberger, S. (2015) The peril of on/off mycotoxin risk management. Science and Solutions: a magazine of Biomin®. *Rumin Issue* **25**, 1–12.

Schaumberger, S. and Reisinger, N. (2015). Endotoxins in cows: an underestimated risk? Biomin® *Rumin Issue* **09**, 1–8.

Shen, Z., Seyfert, H.M., Lohrke, B., Schneider, F., Zitnan, R. and Chudy, A. (2004) An energy rich diet causes rumen papillae proliferation associated with more IGF type1 receptors and increased plasma IGF-1 concentrations in young goats. *J Nutr* **134**, 11–17.

Shriver, B.J., Hoover, W.H., Sargent, J.P., Crawford, J.R. and Thayne, W.V. (1986) Fermentation of a high concentrate diet as affected by ruminal pH and digesta flow. *J Dairy Sci* **69**, 413–418.

Silva, J.F.C. and Leão, M.I. (1979) *Fundamentos de nutrição de ruminantes*, p 384. Piracicaba: Livroceres.

Tajima, K., Shozo, A., Ogata, K., Nagamine, T., Matsui, H., Nakamura, M., Aminov, R.I. and Benno, Y. (2000) Rumen bacterial community transition during adaptation to high-grain diet. *Anaerobe* **6**, 273–284.

Weimer, P.J. (1996) Why don't ruminal bacteria digest cellulose faster? *J Dairy Sci* **79**, 1496–1502.

Welkie, D.G., Stevenson, D.M. and Weimer, P.J. (2010) ARISA analysis of ruminal bacterial community dynamics in lactating dairy cows during the feeding cycle. *Anaerobe* **16**, 94–100.

Wells, J.E. and Russell, J.B. (1996) Why do so many ruminal bacteria die and lyse so quickly? *J Dairy Sci* **79**, 1487–1495.

Zebeli, Q. and Metzler-Zebeli, B.U. (2012) Interplay between rumen digestive disorders and diet induced inflammation in dairy cattle. *Res Vet Sci* **93**, 1099–1208.