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Animal board invited review: The effect of diet on rumen microbial composition in dairy cows



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ABSTRACT

Ruminants play an important part in the food supply chain, and manipulating rumen microbiota is important to maximising ruminants' production. Rumen microbiota through rumen fermentation produces as major end products volatile fatty acids that provide animal's energy requirements, and microbial CP. Diet is a key factor that can manipulate rumen microbiota, and each variation of the physical and chemical composition creates a specific niche that selects specific microbes. Alteration in the chemical composition of forage, the addition of concentrates in the diet, or the inclusion of plant extract and probiotics, can induce a change in rumen microbiota. High-throughput sequencing technologies are the approaches utilised to investigate the microbial system. Also, the application of omics technologies allows us to understand rumen microbiota composition and these approaches are useful to improve selection programmes. The aim of this review was to summarise the knowledge about rumen microbiota, its role in nutrient metabolism, and how diet can influence its composition.

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Implications

This paper reviews the effect of diet on rumen microbiota composition. The rumen microbiota is characterised by Bacteria, Protozoa, Fungi, and Archaea which convert fibrous plant material into high-quality protein (milk and meat). These microbes are related to the host's metabolic function, rumen homeostasis, methanogenesis, and health, which can affect cattle growth and performance. Manipulation of rumen microbiota through the diet can affect feed efficiency, and cattle production and protect the environment by reducing greenhouse gas emissions.

Introduction

The rumen microbiome has a symbiotic relationship with the host and facilitates the utilisation of indigestible plant materials, but its structure changes as a consequence of diet composition. Like other mammals, ruminants do not produce cellulolytic and hemicellulolytic enzymes to digest plant compounds, but rumen microbes perform this function (McCann et al., 2014; Gruninger et al., 2019; Li et al., 2019). Rumen is often described as a "black box", and the rumen microbiota is considered a new organ com-

posed of trillions of microbes, which are divided into two groups of procaryotes (bacteria and archaea) and two groups of eucaryotes (protozoa and fungi) (Liu et al., 2021). During the last years, the rumen microbiome has become an interesting research topic due to its influence on methanogenesis, metabolic function, rumen homeostasis, and health, which can affect cattle growth and performance (Wang and Guan, 2022). Moreover, the microbiota of the digestive tract affects the health, quantity, and quality of animal products (Zhang et al., 2014; Cholewińska et al., 2020). As major end-products of different metabolic pathways, the whole rumen microbiota produces volatile fatty acids (VFAs), microbial crude protein (MCP), carbon dioxide, and methane through fermentation. Rumen microbial community is highly specialised in degrading lignocellulosic biomass and converting complex fibrous substrates into fermentable saccharides (McCann et al., 2014; Gruninger et al., 2019). The microbiota produces up to 70% of the daily energy requirement for the host and its composition changes could influence the feed digestion and methane emissions (Li et al., 2019; Cholewińska et al., 2020). Diet is a key factor in the rumen microbial composition, and changing it has a cascading effect on rumen microbial metabolism, organic acid profiles, and methane production. The effect of diet has been studied with culture-based and standard molecular methods. During the last decades, the next-generation sequencing approach has allowed a greater understanding of the microbial diversity and metabolic pathways of the rumen environment (de Menezes et al., 2011). Dysfunctional

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fermentation may lead to a loss of energy by producing an excess of ammonia and methane (Hendawey et al., 2022). Thus, influencing rumen microbiota composition through the diet can be beneficial for improving feed efficiency, cattle production and protect the environment (Wang and Guan, 2022).

At its very beginning, the characterisation of rumen microbiota composition relied on traditional culture-based techniques, but they only detected 11% of the rumen microbial population (Zeineldin et al., 2018). These approaches are not suitable to understand the complexity of the microbial community in the rumen. High-throughput sequencing technologies represent more useful tools. The 16S rRNA gene is the most frequently targeted to investigate the microbial consortium and could produce thousands of sequences in a few hours (Sanjorjo et al., 2023). Most of these technologies are based on the Illumina platform (San Diego, CA, USA). To further characterise the functional potential of microbial metagenome, metabolomics, metatranscriptomics, and metaproteomics approaches are now being used (Du et al., 2023). The application of the so-called “-omics” techniques is also useful for the improvement of traits in animal genetic selection programmes (Zeineldin et al., 2018).

Rumen microbiota

Bacteria

Bacteria are the most predominant microorganisms (10^{11} cells/ml), and they have numerous enzymatic activities to digest starch, plant cell walls, protein, and lipids. Firmicutes, Bacteroidetes, and Proteobacteria are the most abundant phyla in the rumen (Gruninger et al., 2019). Interestingly, researchers demonstrated that the rumen shows a “bacterial core microbiota”, which is present in ruminants worldwide and comprises members of different taxa, such as *Prevotella* spp., *Butyrivibrio* spp., *Ruminococcus* spp. members of Ruminococcaceae and Lachnospiraceae families and Clostridiales and Bacteroidales orders (Henderson et al., 2015; Weimer, 2015). Clostridia class belongs to the Firmicutes phylum and is mainly involved in protein degradation; *Fibrobacter* genus, *Ruminococcus flavefaciens*, and/or *Butyrivibrio fibrisolvens* break down cellulose, xylans, and pectins, while *Anaerovibrio lyophilica* decomposes fat and *Megasphaera elsdenii* utilises glucose and lactate (Cabral and Weimer, 2024). Early studies were mainly focused on the digestion of cellulose/hemicellulose, leading to a better knowledge of the biology of the principal cellulolytic bacteria, such as *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Butyrivibrio fibrisolvens* (Gruninger et al., 2019). The Bacilli class degrades proteins. Another phylum is Bacteroidetes, gram-negative bacteria that includes the genus *Prevotella*. *Prevotella bryantii* and *Prevotella ruminicola* belong to this genus, whose many catabolic activities include assisting cellulolytic microbes in plant cell wall degradation with the synergic action of cellulolytic bacteria (Cholewińska et al., 2020). Bacteroidetes are also characterised by the ability to synthesise different vitamins, such as B1, B2, B7, B9, and B12. These vitamins are important for different rumen functions, such as the metabolism of amino acids, lipids, and carbohydrates, and they represent co-factors in essential proteins that support cellular function in the microbial community of the rumen and for the host (Hernández et al., 2022).

Archaea

Archaea (10^6 cells/ml) are responsible for methane production by hydrogenotrophic, methylotrophic, or, in small part, by acetoclastic pathways. *Methanobrevibacter gottschalkii* and *Methanobrevibacter ruminantium* are the most abundant archaea in the

rumen (Gruninger et al., 2019). Most of them use CO₂ and H₂, produced by nonmethanogenic microbes, in the methanogenic process, and the electron donors for hydrogen production are primarily carbohydrates, but also alcohols and organic acids. Thus, archaea prevent excessive production of ethanol and lactate during the fermentation process, utilising these compounds as H₂ donors (Cholewińska et al., 2020).

Protozoa

Rumen protozoa (10^5 – 10^6 cells/ml) belong to the genera *Isotricha*, *Dasytricha*, *Entodinium*, *Diplodinium*, *Endiplodinium*, and *Epidinium*. Protozoa's role in the rumen ecosystem is somehow controversial (Weimer, 2015; Cholewińska et al., 2020; Costa-Roura et al., 2022). Defaunation studies demonstrated that protozoa are not essential for rumen function, but their absence could influence feed digestion (Mizrahi and Jami, 2018). Protozoa can engulf starch granules in the rumen and compete with amylolytic bacteria for substrate, reducing starch fermentation rate and the risk of ruminal acidosis. Also, the rumen ciliate protozoa are associated with methane production via their epi- and endo-symbiotic relationship with methanogens (Gruninger et al., 2019; Solomon et al., 2022).

Fungi

Fungi (10^3 – 10^6 zoospores/ml) show important fluctuation depending on the diet. Neocallimastigomycota is the most dominant phylum in ruminants that includes the genera: *Anaeromyces*, *Caecomyces*, *Cyllamyces*, *Neocallimastix*, *Orpinomyces*, and *Piromyces* (Cholewińska et al., 2020; Sanjorjo et al., 2023). Anaerobic fungi participate in fibre degradation and show a set of enzymes (cellulolytic, hemicellulolytic, glycolytic, and proteolytic) that allow the degradation of a plant's structural polymers and increase the surface area available for the colonisation of other microorganisms. This increase is due to the physical penetration and splitting of the plant tissue by the fungal appressoria as they grow (Akin and Borneman, 1990; Hess et al., 2020). Rumen fungi are usually associated with a high-forage diet and their abundance decreases upon the addition of concentrates (Cholewińska et al., 2020; Gruninger et al., 2019).

Redundancy, resiliency, and host individuality

Weimer (2015) describes the rumen microbial community as redundant, resilient, and dependent on the individuality of the host. In the rumen, indeed, different microbial taxa are involved in the degradation of the same substrate, and these microorganisms compete for its utilisation. This redundancy is not well known but it could be involved in carbohydrate digestion (Gruninger et al., 2019). This suggestion is reiterated by other studies (Taxis et al., 2015; Weimer, 2015), in which changes in the community composition often do not result in substantial shift in fermentation parameters, such as pH and volatile fatty acid concentration (Taxis et al., 2015). The redundancy of rumen microbiota also reflects the resilience of the system: studies demonstrate the ability of rumen microorganisms to restore their structure when subjected to different disturbances, such as temperature or ruminal pH changes (Weimer, 2015). Studies have reported a strong correlation between ruminants' production efficiency (feed conversion ratio, average daily gain, average daily feed intake, total weight gain, residual feed intake, milk production yield/quality and rumen microbial profiles in both beef and dairy cattle (Liu et al., 2021; Wang and Guan, 2022).

The rumen microbiota and nutrient metabolism

Rumen microbiota allows ruminants to convert high and low-quality feedstuff into high-quality MCP through fermentation (McCann et al., 2014). The rumen provides heat and nutrients for microbes, and microorganisms can secrete enzymes that facilitate the degradation of feed. If one of these two factors changes, the regularity of the process would be affected. Thus, regulating this balance may bring benefits for the host.

Rumen fermentation produces VFAs and MCP as major end products, providing a large proportion of host energy and amino acid requirements. Volatile fatty acids are the end product of dietary carbohydrate degradation, and they can be absorbed by rumen epithelium, while MCP arrives in the small intestine as amino acids and peptides. The ruminal protein degradation results in ammonia production, which is used by microbes for growth, and even absorbed in the rumen for its detoxification to urea. Bacteria, such as *Clostridium*, *Bacilli*, and *Proteobacteria*, can break down proteins by secreting proteolytic enzymes. However, these microorganisms can also use non-protein nitrogen compounds such as urea and ammonia to synthesise proteins. Urea is decomposed into ammonia and carbon dioxide, while the carbon chain of amino acids is, again, derived from VFAs.

In the rumen, some bacteria have the potential to degrade fat, such as *Anaerovibrio lyophilica*. The glycerol, derived from fat breakdown, is used for sugar transformation to obtain pyruvic acid. Not all fatty acids undergo a degradation process, they could pass through the digestive system, be absorbed in the intestine, and then used by the host (Lu et al., 2019; Cholewińska et al., 2020).

Firmicutes and Bacteroidetes are the main phyla that characterise the rumen bacterial community, and both participate in fibre degradation and VFA production. Each species in these two phyla has special niches and can be influenced by dietary types (Liu et al., 2020). Forage degradation proceeds through alternate rounds of physical breakdown through chewing, the mixing of substrates with saliva, and rumination. During and in between this mechanical process, the rumen microbial consortium degrades cellulose and hemicellulose of the plant cell wall thanks to the secretion of particular enzymes. Compared to bacteria and protozoa, fungi have an essential role in forage degradation, since they can colonise the surface of plant material and penetrate faster than bacteria (Solomon et al., 2016; Elghandour et al., 2020a, b). Also, fungi are better degraders of lignified tissues and can solubilise phenolic compounds, to a certain degree facilitating the potentially degradable fibre (Osorio-Doblado et al., 2023).

Cellulolytic microbes are important in the rumen, which can influence host fibre digestibility by secreting cellulase. The bacteria *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens* can actively produce cellulases (Osorio-Doblado et al., 2023). A specific fibre source can alter rumen microbiota composition. During the early life of cows, the formulation of an appropriate diet can stimulate the development of the rumen and its microbial community (Liu et al., 2020).

Within the complexity of the whole microbial consortium, cross-feeding is crucial for the efficient utilisation of specific end products. It can occur between members of different microbial taxa, not competing for the same substrate. Due to their inability to produce required end products, they rely, in turn, on other microbes' metabolism. An example of such a process was described by Russell (2002), in which the three predominant cellulolytic species (*Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Fibrobacter succinogenes*) cannot produce branched-chain amino acids unless they have carbon skeleton derived from branched-chain VFA, which are supplied by amino acid-fermenting bacteria (Russell, 2002).

Diet

Diet plays a crucial role as a modulator of microbiota, and it can influence its composition and activity in all stages of cows' growth. Each variation of the physical and chemical composition of the diet creates a particular niche that selects specific microbes (Gruninger et al., 2019). Newbold and Morales (2020) summarised the main targets of rumen microbiome manipulation through the diet: improving the nutritional composition of ruminants' products, improving animal health, preventing the accumulation of toxins, reducing pathogens development, decreasing the environmental impact of livestock (reducing greenhouse gas emissions), optimising VFAs and MCP production and decreasing ammonia production in the rumen.

Researchers have identified a complex rumen microbial consortium involved in the catabolism of the main dietary polymers, which would affect the richness of rumen microbiota and reflect health status and animal production (Zeineldin et al., 2018; Cholewińska et al., 2020). Rumen microbiota changes through the 1st year of calves' life, and various microbial groups start to appear in the rumen. The period of "instability" in the gastrointestinal tract of calves during weaning is an opportunity to manipulate microbiota development (Sanjorjo et al., 2023). The intake of solid feed influences the beginning of rumen fermentation in calves, and changes gastrointestinal microbial components (Diao et al., 2019; Xiao et al., 2020). Diet management during weaning could have important and lasting effects on the composition of the gastrointestinal microbiota of young ruminants, as suggested by Du et al. (2023). Moreover, changes in the diet of calves contribute to the development of rumen morphology and function (Gruninger et al., 2019). Kodithuwakku and colleagues (2022) showed how administering fibre (Timothy hay and psyllium) to calves in the pre-weaning period (3 days of age) can alter rumen microbiota composition. Early fibrous diet supplementation facilitates colonisation of the predominant rumen bacteria found in adults (for example *Prevotella* genus). Also, *Shuttleworthia* spp., *Mitsuokella* spp., and *Selenomonas* spp. had a relative higher abundance in treatment calves and a relative increase of butyrate proportion at 7 days of age. Butyrate and propionate enhance the development of rumen epithelium (Mentschel et al., 2001; Zhang et al., 2018; Kodithuwakku et al., 2022).

Adult ruminant diet is usually based on forages, which provide fibre fractions (mainly pectins, cellulose, hemicellulose, and lignin), and concentrates to balance their energy, nitrogen, amino acids, minerals, and vitamins requirements. Alteration in the chemical composition of forage, or the addition of concentrates in the diet could also alter the composition of the rumen microbial community (Gruninger et al., 2019). A high amount of forage in ruminant's diet reduces the risk of acidosis in the rumen and promotes the growth of specific microorganisms, like cellulolytic ones; conversely, a diet with a high amount of concentrates, decreases ruminal pH and affects negatively bacteria richness and diversity (Sanjorjo et al., 2023). The inclusion of plant secondary metabolites in ruminant diets, such as tannins, can act as a source of stress for the ruminal microbes, while the administration of probiotics (yeasts) can induce an increase in abundance of particular strains of microbes (Costa-Roura et al., 2022) (Table 1).

Total mixed ration vs pasture

Using high-throughput amplicon sequencing, De Menezes et al. (2011) compared the rumen microbial composition of cows fed with total mixed ration (TMR) and cows fed with pasture. Total mixed ration diet was composed of maize silage, concentrate blend, grass silage, molasses, and straw. The results give an insight

Table 1

Summary of the main diet's effects on rumen microbial composition.

Factor	Effects on microbiota composition	References
Pasture	↑Prevotellaceae, Erysipelotrichaceae, Veillonellaceae, Lachnospiraceae	De Menezes et al. (2011)
High-hay	↑Fibrobacteres, <i>Butyrivibrio fibrisolvens</i> , <i>Methanobrevibacter</i> spp., anaerobic fungi	Fernando et al. (2010), Indugu et al. (2017), Cholewińska et al. (2020), Kotz et al. (2021)
High concentrates	↑ <i>Megasphaera elsdenii</i> , <i>Streptococcus bovis</i> , <i>Streptococcus ruminantium</i> , <i>Prevotella bryantii</i> ↓ <i>Butyrivibrio fibrisolvens</i> , <i>Fibrobacter succinogenes</i> , archaea richness	Fernando et al. (2010), Zhang et al. (2017), Cholewińska et al. (2020)
High starch	↑ Succinivibrionaceae, <i>Schwartzia</i> spp.	Indugu et al. (2017)
Alfalfa hay	↑ <i>Prevotella</i> spp., <i>Synergistetes</i> , <i>Pyramidobacter</i> spp., <i>Selenomonas</i> spp., <i>Ruminococcus</i> spp., <i>Marvinbryantia</i> spp., <i>Syntrophococcus</i> spp. ↓ <i>Lentisphaerae</i> , <i>Anaeroplasm</i> spp.	Zhang et al. (2014), Wei et al. (2021)
Cornstalk	↑ <i>Lentisphaerae</i> , <i>Rikenellaceae</i> , <i>Ruminococcaceae</i> , <i>Anaerotruncus</i> spp., <i>Papillobacter</i> spp., <i>Actinopolyspora</i> spp., <i>Bacillus</i> spp., <i>Streptomyces</i> spp., <i>Thermoactinomyces</i> spp. ↓ <i>Veillonellaceae</i>	Zhang et al. (2014)
Chinese ryegrass	↑ <i>Lentisphaerae</i> , <i>Proteobacteria</i> , <i>Lachnospira</i> spp.	
Corn stover (high non-fibre carbohydrates)	↑ <i>Prevotella ruminicola</i> , <i>Streptococcus</i> spp., <i>Treponema</i> spp., <i>Ruminobacter</i> spp., <i>Selenomonas ruminantium</i> , <i>Succinomonas</i> spp. ↓ <i>Ruminococcus</i> spp., <i>Marvinbryantia</i> spp., <i>Syntrophococcus</i> spp.	Wei et al. (2021)
Factor	Effects on microbiota composition	References
Protein content		
Low	↑ <i>Prevotellaceae</i> , <i>Prevotella</i> spp., <i>Prevotella_1</i> , <i>Prevotellaceae_UCG-003</i>	Zhang et al. (2021), Parra et al. (2022)
Middle-low	↑ <i>Prevotella_1</i> , <i>Prevotellaceae_UCG-003</i>	
Middle-high	↑ <i>Rikenellaceae_RC9_gut_group</i> , <i>Christensenellaceae_R-7_group</i> ↓ <i>Bacteroidetes</i> , <i>Fibrobacteres</i>	
High	↑ <i>Rikenellaceae</i> , <i>Spirochaetae</i> , <i>Rikenellaceae_RC9_gut_group</i> , <i>Ruminococcaceae_UCG-014</i> , <i>Ruminococcaceae_UCG-010</i> ↓ <i>Cyanobacteria</i>	
Sugars		
Molasses (beet and cane) in vitro	↑ <i>Lachnospiraceae</i> , <i>Bifidobacteriaceae</i> , <i>Erysipelotrichaceae</i> , <i>Streptococcaceae</i> ↓ <i>Prevotellaceae</i>	Palmonari et al. (2023)
Sucrose	↑ <i>Bacteroidaceae</i> , <i>Lactobacillaceae</i> , <i>Peptococcaceae</i> , <i>PeH15</i> , <i>Endomicrobiaceae</i> , <i>Desulfuromonadaceae</i> , and <i>Marinilabiliaceae</i> ↓ <i>Fibrobacteraceae</i> , <i>Prevotellaceae</i> , <i>Spirochaetaceae</i>	Kheirandish et al. (2022)
Fructose	↑ <i>Streptococcaceae</i> , <i>Victivallaceae</i>	Golder et al. (2014)
High starch + sunflower oil	↑ <i>Prevotella</i> spp.	Zened et al. (2013)
Factor	Effects on microbiota composition	References
Fresh diet + mineral salts	↑ <i>Prevotella</i> spp. ↓ <i>Succinoclasticum</i> spp.	Liu et al. (2017)
Palm oil	↓ Protozoa number	Anantasook et al. (2013)
Plant extracts		
<i>Calliandra leaves</i>	↓ <i>Fibrobacter succinogenes</i> , <i>Ruminococcus</i> spp.	Besharati et al. (2022)
<i>Onobrychis viciifolia</i>	↓ <i>Butyrivibrio fibrisolvens</i> , <i>Streptococcus bovis</i>	

into the impact of different feeding strategies. Indeed, different diets yielded different bacterial and archaeal communities, while protozoa were less affected by the diet. At the phylum level, 90% of sequences were characterised by Firmicutes and Bacteroidetes, while the most prevalent families were Prevotellaceae, Lachnospiraceae, Ruminococcaceae, Fibrobacteriaceae, unclassified Bacteroidales, and Clostridiales. The trial showed a relatively higher abundance of Prevotellaceae, Erysipelotrichaceae, and Veillonellaceae in cows fed with pasture. Veillonellaceae is a propionate-producing bacterial family, which is related to a reduction in methane emissions. The abundance of Veillonellaceae was around three times higher in cows fed with pasture. Interestingly, the Erysipelotrichaceae family was around 15% of the sequences in cows fed the pasture diet versus less than 3% in cows fed other treatments. Fibrobacteriaceae was relatively higher in cows fed TMR, and this reflected the presence of straw in the diet.

Forages:concentrates

In a high-grain diet, the ruminal abundance of Bacteroidetes decreases, while Firmicutes increases. This situation would repre-

sent a dysbiotic condition and a potential loss of function. Bacteroidetes have a greater efficiency in degrading fibre than Firmicutes as reported by El Kaoutari et al. (2013), because Bacteroidetes have a larger range of carbohydrate substrates that can be utilised.

Concentrate-based diets also affect the abundance of many members of the microbial community at the lower taxonomic level: increasing the abundance of amylolytic and lactic acid-utilising species and reducing the relative abundance of fibrolytic species (Plaizier et al., 2018). A decrease in gram-negative bacteria abundance has been reported by Zhang et al. (2014) as a consequence of lower pH in cattle. According to Cholewińska et al. (2020), a comparison of the rumen microbiota composition between animals fed with a high-hay or high-grain diet showed an increase in Fibrobacteres concentration in high-hay, while the relative abundance of *Megasphaera elsdenii*, *Streptococcus bovis*, *Streptococcus ruminantium*, and *Prevotella bryantii* increased in the high-grain group, along with a decrease of *Butyrivibrio fibrisolvens* and *Fibrobacter succinogenes* (Cholewińska et al., 2020). These findings were previously displayed by Fernando et al. (2010), who found a high abundance of bacteria belonging to Fibrobacteres

phylum, *Butyrivibrio fibrisolvens*, and *Methanobrevibacter* spp. in a high-hay diet and the relative abundances of *Megasphaera elsdenii*, *Streptococcus bovis*, *Streptococcus ruminantium*, and *Prevotella bryantii* populations in high-grain diet cows increased. Ruminants fed with a high-fibre diet showed an enriched anaerobic fungi community. The positive correlation between fungi and forage was also described in several other papers (Griffith et al., 2010; Kumar et al., 2013; Mao et al., 2016; Kumar et al., 2015). Kumar et al. (2015) reported that an increase in concentrate percentage in the diet, from 25 to 50%, did not alter the β -diversity of the methanogenic community. Zhang et al. (2017) reported that increasing levels of dietary concentrates, decreased ruminal archaea richness but did not affect its diversity. The archaeal community is less diverse, and less variable compared to other microbial domains, and this community might have the ability to be resilient to dietary changes (Kumar et al., 2015; Zhang et al., 2017).

Starch

Increasing starch content in moderate-grain diets can affect microbiota richness and diversity, but there are differences in starch's influence among studies and even among animals within study. When an excessive amount of starch is included in the diet, a decrease in pH can occur, resulting in an altered functionality of the rumen microbiota (Wang et al., 2009; Dijkstra et al., 2012). The decrease in ruminal pH leads to the release of lipopolysaccharide endotoxins from gram-negative bacteria in the rumen (Monteiro and Faciola, 2020). The reduction of the richness and diversity of microbiota can reduce its functionality and resilience and make it more susceptible to invasions of pathogens (Zhang et al., 2014; Zhang et al., 2017). Despite this, more efficient cows displayed a lower richness and diversity of microbiota, according to Shabat et al. (2016) and Lopes et al. (2021). Zhang et al. (2014) studied the inclusion of a 25% grain mixture in the diet compared to 0% of inclusion. The results displayed an increase in rumen bacteria richness and diversity due to the presence, in the grain-mixed diet, of more available substrates for rumen microbiota than the diet without them. However, Weimer (2015) showed that the functionality of rumen bacteria is shared by different taxa, and the prediction of it based on changes in microbial abundance is not very accurate.

Metatranscriptomics and metagenomics could be useful in understanding the metabolic functions of rumen microorganisms (Kotz et al., 2021). As described before, the presence of non-fibre carbohydrates (NFC) in the diet increases the fermentable energy in the rumen and affects the rumen microbiota. Wei et al. (2021) explored the effects of NFC supplementation on rumen microbiota in cows fed with an alfalfa-based diet, corn stover with high NFC, and corn stover with low NFC diets. Relative abundance of lignocellulose-degrading bacteria, such as *Ruminococcus*, *Marvinbryantia*, and *Syntrophococcus*, increase in the alfalfa diet for the high content of pectin, but a lower abundance of them in the high NFC diet may be related to the effect of the excessive starch degradation and the higher abundance of amylolytic bacteria. Also, the rapid fermentation of starch affects the pH value in the rumen which is detrimental to the fibrolytic capacity. The improved nitrogen conversion in the high NFC diet was related to *Treponema*, *Ruminobacter*, *Selenomonas*, and *Succinimonas* genera increasing. They are negatively correlated to ruminal ammonia and urea nitrogen in the blood, and they are highly efficient in ruminal ammonia utilisation (Wei et al., 2021).

Forage sources

Another important aspect of ruminants' diet is forage source. Zhang et al. (2014) reported a difference in bacteria composition

in cows fed with three different forage sources (alfalfa hay, cornstalk, and Chinese ryegrass). At the phylum level, they did not observe differences between Bacteroidetes and Firmicutes relative abundance, since the three diets did not differ in forage/concentrate ratio (45:55). However, at the family level, Lentisphaerae family had a higher concentration in cornstalk and Chinese ryegrass groups compared to alfalfa hay. This family is involved in cellobiose degradation, and cornstalk and Chinese ryegrass diets had a higher concentration of NDF compared to alfalfa. *Prevotella* spp. increased in the alfalfa hay group might be due to its ability to utilise a broad range of substrates that favoured the growth of this genus in the rumen. Unclassified members of the families Bacteroidales, Rikenellaceae, Ruminococcaceae, and Veillonellaceae were significantly affected by the diet. In a previous study, unclassified Ruminococcaceae and unclassified Bacteroidales had a role in ruminal biohydrogenation, so the authors suggested that the source of forage may have an impact on this metabolic process (Zhang et al., 2014).

Indugu et al. (2017) compared a high-producing dairy farm (Farm 12) and a low-producing farm (Farm 9) which differed in the forage proportion in the diet, the forage type, and the types of byproducts utilised. The Farm 9 diet had a higher proportion of NDF (31.3% DM) and less starch (29.2% DM) compared to the Farm 12 diet, which had a higher starch (32.1% DM) and less NDF (28.3% DM) than the Farm 9 diet. In Farm 12, cows had a higher DM intake compared to Farm 9. The results displayed an increase in the Succinivibrionaceae family and reported the presence of the *Schwartzia* genus in Farm 12. The authors related Succinivibrionaceae to feed efficiency, and *Schwartzia* with milk production (Indugu et al., 2017). Also, Succinivibrionaceae compete with methanogens for hydrogen utilisation to make succinate, a precursor of propionate (Wei et al., 2022). The increase in milk yield of Farm 12 could be related to Succinivibrionaceae which convert succinate to propionate, metabolised in the liver to glucose, a precursor of lactose. Another suggestion that may support the increase of Succinivibrionaceae in the rumen is the starch availability, but little information is available about the correlation between Succinivibrionaceae family and starch (Indugu et al., 2017; Xue et al., 2019). In Farm 9, the higher concentration of NDF in the diet may have contributed to the relative increase of *Fibrobacter* genus and Firmicutes phylum (Indugu et al., 2017).

Protein

Dietary proteins are broken down into peptides, amino acids, and ammonia, used by microorganisms in the rumen to synthesise microbial protein. Excess of ammonia production from protein breakdown leads to metabolic stress in cattle, and an excess of N excretion in urine harms the environment and decreases profitability for farmers (Newbold and Morales, 2020; Lima et al., 2023). Ruminants, indeed, can consume a diet with lower protein, but such composition could affect rumen bacterial populations and influence the growth of non-ammonia-dependant microorganisms. Moreover, a low amount of protein in the diet would impact the upregulation of N recycling from the liver into the rumen. Parra et al. (2022) underlined a correlation between N recycling and feed efficiency, and an association between bacterial activity and feed efficiency. To demonstrate these associations, Parra and his colleagues (2022) studied the effect of low-protein (8.8% CP) and high-protein (13.5% CP) diets on rumen bacteria and the feed efficiency of beef steers. They demonstrated a high *Prevotella* spp. abundance in low protein diet, related to the ability of this genera to scavenge N from different substrates, thus being more competitive and abundant in efficient animals. Xue et al. (2019) demonstrated that *Prevotella* has an important role in the synthesis of amino acids and carbohydrate metabolism. In Parra et al. (2022)

study, the low protein diet increased N recycling from 43 to 85% of N intake, accompanied by a shift of ruminal microbial population, which promoted ureolytic bacterial species. According to the authors, low dietary CP levels would drive the bacteria population to obtain N from different sources, resulting also in an increased urea transport from the liver into the rumen.

Zhang et al. (2021) studied four different levels of protein content in the diet of yaks: low (9.64%), middle-low (11.25%), middle-high (12.48%), and high (13.87%). They found that the high diet showed a negative impact on bacterial diversity and a distinct rumen microbiota compared to middle-high diet. Instead, low and middle-low groups had a similar rumen microbiota. At the phylum level, Firmicutes and Proteobacteria did not differ among the groups, while Bacteroidetes decreased in middle-high compared to low and middle-low. At the genus level, *Prevotella_1* was high in low protein group due to the higher fibre content in the diet. *Rikenellaceae_RC9_gut_group* and *Christensenellaceae_R-7_group* relative abundance increased in the middle-high group, while *Ruminococcaceae_UCG-014* and *Ruminococcaceae_UCG-010* were higher in high. Zhang and his colleagues (2021) confirmed that in a low protein content diet, Prevotellaceae family abundance increased. Rikenellaceae, instead, had a high abundance in a high protein content diet, and they speculated that this family degrades protein for their metabolic activity.

Costa-Roura et al. (2020) studied the effect of a low-protein diet (120 g/kg DM basis) in Holstein bulls compared to a diet with 140 g/kg DM basis. They reported that a high CP content led to an increase in proteolytic bacteria abundance but no effect on fungi and protozoa counts. The limitation of CP in the diet displayed a more complex ruminal microbial community and an increase in functional activity among genera, compared to a high-CP diet.

Sugars

Sugars are non-structural carbohydrates which include monosaccharides (glucose, fructose, and galactose), and disaccharides (sucrose, maltose, and lactose). These sugars are quickly and easily fermented in the rumen (Dong et al., 2021). A high abundance of sugars in cows' diets can affect VFA synthesis and microbial community (Wei et al., 2021).

The rapid fermentation of sugars could result in ruminal acidosis, but they can have a different potential to induce acidosis and dysbiosis in the rumen (Palmonari et al., 2023). Kheirandish et al. (2022) demonstrated how different non-structural carbohydrates could impact the rumen microbial community depending on the sugar source. They added sucrose (SU), native cornstarch, and chemical-modified cornstarch into *in vitro* fermentation using Holstein cows as rumen-content donors in a 2-week experimental design. In the SU group, Bacteroidaceae, Lactobacillaceae, Peptococcaceae, PeH15, Endomicrobiaceae, Desulfuromonadaceae, and Marinilabillaceae families abundance increased, while Fibrobacteraceae, Prevotellaceae, and Spirochaetaceae decreased after 8 h of fermentation (Kheirandish et al., 2022).

Palmonari et al. (2023) described the effects of molasses (mainly composed of simple sugars) on rumen microbial composition in dairy cows fed with cane and beet molasses. The presence of raffinose and sucrose affected the level of Lachnospiraceae, Bifidobacteriaceae, and Erysipelotrichaceae which increased in treatment groups (beet and cane). Also, the higher presence of glucose and fructose increased Streptococcaceae relative abundance in beet and cane molasses groups compared to the control (without molasses addition). Streptococcaceae is a starch degrader and rapidly grows with glucose. Authors related the specific composition of molasses, in terms of sulphate and phosphate presence, to the decrease of the Prevotellaceae family in both treatments (Palmonari et al., 2023). Zhao et al. (2020) displayed that dietary

supplementation of Na₂SO₄ decreased the relative abundance of the *Prevotella 1* genus, a ruminal protein and peptide-degrader bacteria. This supplementation also decreased the concentration of NH₃-N and protein degradation in the rumen, which could affect the Prevotellaceae genera (Zhao et al., 2020).

Previously, Golder et al. (2014) demonstrated the effect of fructose supplementation (0.4% of BW DMI) in a grain-based diet in Holstein Friesian heifers. The relative abundance of Firmicutes, Bacteroidetes, and Proteobacteria phyla changed during the challenge in fructose-fed heifers. In this trial, the increase of readily fermentable carbohydrates was related to the increase in the relative abundance of Streptococcaceae (*Streptococcus bovis*). Victivallaceae family had a high relative abundance in the fructose group, *Megasphaera elsdenii*, *Selenomonas ruminantium*, *Veillonella parvula* belong to this family and utilise lactate (Golder et al., 2014).

Fats

Another feed component that impacts rumen microbial composition is fat. The fat content in ruminants' feed is generally low (up to 5%), but still, lipids play a key role in improving the energetic values of ruminants' diets and modulating methane emissions, particularly in intensive farming systems (Enjalbert et al., 2017). Several studies have shown that unsaturated fatty acids negatively affect microbial composition by decreasing ruminal cellulose degradation and VFA concentration (Enjalbert et al., 2017; Zeineldin et al., 2018). Sears et al. (2024) demonstrated that the inclusion of palmitic acid influences positively bacterial growth and supported fibre-degrading bacteria's activity. *Butyrivibrio fibrisolvens* is assumed to be a main biohydrogenating bacterium in the rumen, and a reduction of this species increases the passage of unsaturated fatty acids to tissues and milk. Biohydrogenation is a detoxification process, necessary to prevent the bacteriostatic effects of polyunsaturated fatty acids (Asma et al., 2013). Asma et al. (2013) tested a combination of starch and sunflower oil supplementation on rumen microbial composition in dairy cows. This combination modified the composition of the bacterial community, which was different compared to the only starch addition. *Prevotella spp.* relative abundance was affected by the treatment, switching from a low starch diet with oil addition to a high starch diet with oil addition, resulting in a two-fold increase. This situation is explained by the ability of *Prevotella* to degrade a wide variety of substrates and its resistance to unsaturated fatty acids, but further research is required to deeply understand the metabolic activities of this genus.

Anantasook et al. (2013) tested the effect of palm oil supplementation on rumen microorganism populations. They observed that in the treatment group, protozoa number decreased and suggested that the high content of C18 fatty acids in palm oil could affected protozoa number.

Minerals

Mineral salts are used in ruminants' diets to supply necessary macro- and micro-nutrients and to improve growth, fattening, milk yield, and metabolism. The mineral residual is partially taken up by systemic circulation, while the other remaining part is absorbed by microbes. Despite the extensive use of mineral supplementation, their effects on the rumen microbiota community have not been thoroughly investigated. Liu et al. (2017) show the impact of mineral salt supplements on the rumen microbiota composition in mature cows and heifers fed with the same diet. The treatment consisted of a fresh diet containing mineral salts (Mg, Co, Cu, Fe, Mn, Se, Zn, I, and Na) for 1 month. 16S rRNA gene sequencing revealed that *Methanobrevibacter spp.* was the most abundant archaea in the rumen, but it was not influenced by mineral salt

in both treatment groups (cows and heifers). In treated animals, *Succiniclasticum* spp. abundance was reduced in adult cows and heifers, while *Prevotella* spp. abundance increased. The decrease of *Succiniclasticum* spp. and the increase of *Prevotella* spp. could reflect a compensatory effect, as described in [Weimer \(2015\)](#) in terms of redundancy of rumen microbiota, but this effect was only observed in adult cows. *Barnesiella* spp. abundance was associated with the loss of *Succiniclasticum* spp. abundance and [Liu et al. \(2017\)](#) proposed that these potential propionate-producing bacteria compensate more or less for propionate production. The trial showed a difference in microbial community composition between heifers and adult cows with the same dietary regime in response to mineral salt supplementation, especially for *Succiniclasticum* and *Prevotella* genera. Authors hypothesise that bacteria distribution patterns, and competition between microorganisms, are regulated by age-dependent effects coupled with mineral-salt-induced pathways. The effect of the same diet in adult cows and heifers reveals the differential bacteria activities depending on age. Indeed, the authors ([Liu et al., 2017](#)) suggested that future research is needed to better understand the metabolic activities and the role of specific taxa using metabolomic and metagenomic approaches.

Plant extracts

Several chemical additives are used to improve feed utilisation and decrease methane production, but the appearance of chemical residue in animal products has stimulated research on natural alternatives that would be useful for livestock farming. The phytochemical compounds contained in plants are a part of ruminants' natural diet, and they can modify the rumen ecosystem and functions. Plant extracts could improve rumen metabolism, decrease rumen methanogenesis and protein degradation, and increase MCP production targeting specific rumen microbes.

Tannins are polyphenolic compounds that have an affinity to bind proteins and create complexes that are stable in the rumen but can dissociate in the intestine. This escape from rumen degradation can be beneficial for metabolism efficiency, increasing dietary energy utilisation, if added at proper doses ([Yanza et al., 2021](#)). Tannins were formerly considered anti-nutritional factors, due to their negative effect on feed intake and nutrient utilisation, but in recent years, several studies demonstrated a positive effect on N metabolism and inhibition of methanogenesis ([Goel et al., 2005](#); [Patra and Saxena, 2011](#); [Beauchemin et al., 2020](#)). The antimicrobial activity of tannins is due to their influences on bacteria cell wall morphology. The toxicity of these plant extracts depends on their concentration and chemical structure ([Goel et al., 2005](#); [Patra and Saxena, 2011](#); [Beauchemin et al., 2020](#)).

For example, an inclusion of 30% of *Calliandra* leaves in the diet reduced *Fibrobacter succinogenes* and *Ruminococcus* spp. without affecting proteolytic bacteria and fungi, while the inclusion of sanfoin condensed tannin (*Onobrychis vicifolia*) affected proteolytic bacteria and could reduce *Butyrivibrio fibrisolvens* and *Streptococcus bovis* abundances. Tannins can inhibit methanogen activity limiting the degree of microbial hydrolysis, reducing H₂ availability, and reducing fibre digestibility ([Besharati et al., 2022](#)).

The effects of tannins on the protozoa community are conflicting. Some studies reported a reduction of protozoa number, especially holotrichs, while other studies displayed no effects ([Patra and Saxena, 2009](#)). [Goel et al. \(2005\)](#) reported the existence of tannin-tolerant and tannin-degrading bacteria in the rumen fluid of bovine, such as *Selenomonas ruminantium* and *Streptococcus* species. *Streptococcus ruminantium* produces tannase and converts tannic acid to gallic acid and finally to acetate and butyrate. Tannase was also detected in a strain of fungus (*Aspergillus niger*) isolated in cattle faecal samples ([Goel et al., 2005](#)). Also, *Streptococcus gallolyticus* and *Streptococcus bovis* can tolerate, at different doses, condensed tannins, as demonstrated by [Krause et al. \(2005\)](#). The effect of tannins on the rumen microbial population depends upon the species of microorganism and the type or source of tannin.

Probiotics

The use of probiotics in farm animals has increased in recent years. Probiotics are defined as direct-fed microbials (live beneficial microorganisms), and in feeding ruminants, probiotics are characterised by bacteria *Lactobacillus* spp., *Bifidobacterium* spp., *Bacillus* spp., *Megasphaera elsdenii*, and *Enterococcus* spp., as well the yeast *Saccharomyces cerevisiae* ([Mingmongkolchai and Panbangred, 2018](#); [Susanto et al., 2023](#)). This inclusion has a positive effect on the growth and homeostasis of the digestive system, but the response of the host depends on the probiotic's strain, age of the animal, animal breed, and other dietary traits of ruminants ([Gaggia et al., 2010](#); [Reuben et al., 2022](#)).

The effects of probiotics include a reduction of pH, related to the production of metabolites such as lactic acid, and a synergistic action against pathogens.

According to [Raabis et al. \(2019\)](#) and [Kulkarni et al. \(2022\)](#), probiotics play an important role in feed efficiency, enhancement of feed conversion ratio, increasing weight gain, and milk production. A specific genus of probiotic bacteria, *Lactobacillus* spp., has also the ability to produce bacteriocins, which negatively affect pathogenic microbes' growth (*Escherichia coli*, *Salmonella typhimurium*, *Streptococcus aureus*, *Clostridium perfringens*). *Lactobacillus* spp., indeed, competitively interacts with pathogens for nutrients and adhesion

Table 2
Summary of the most utilised probiotics in ruminants and their effect on cows' health and production.

Probiotics	Effects	References
<i>Lactobacillus</i> spp.	Stimulate growth and homeostasis of the digestive system	Mingmongkolchai and Panbangred (2018) , Susanto et al. (2023)
<i>Bifidobacterium</i> spp.	Enhancement feed conversion ratio	
<i>Bacillus</i> spp.	Increasing weight gain	
<i>Megasphaera elsdenii</i>	Increasing milk production	
<i>Enterococcus</i> spp.	Acting against pathogens	
<i>Saccharomyces cerevisiae</i>		
<i>Lactobacillus</i> spp.	Produce bacteriocins against <i>Escherichia coli</i> , <i>Salmonella typhimurium</i> , <i>Streptococcus aureus</i> , <i>Clostridium perfringens</i>	Reuben et al. (2022)
	In young ruminants: promote health and animal development	
Yeast	Produce vitamin B	Elghandour et al. (2020a, b)
	Affect negatively the growth of pathogens (<i>E.coli</i>)	
<i>Saccharomyces cerevisiae</i>	Improves ruminal morphology	
	Improves fibre digestibility	
	Increases DM intake	

sites on the intestinal mucosa. Also, Reuben et al. (2022) suggested that it is used in young ruminants to promote health and animal development.

Yeasts produce B vitamins and affect the growth of microorganisms, and, on the other hand, could prevent the development of *Escherichia coli* through the production of glucan and mannan, cell wall components (Elghandour et al., 2020a, b). *Saccharomyces cerevisiae* is one of the most utilised probiotics yeasts in livestock systems, and it improves ruminal pH and fibre digestibility by stabilising ruminal pH and increasing DM intake (Elghandour et al., 2020a, b). The effects of probiotics on rumen microbial composition are not conclusively determined and few studies have tracked the abundance or persistence of these direct-fed microbial products in the rumen over time. Other research is necessary to understand the effects of these fed microbial on animal performance and health (Zeineldin et al., 2018; Cholewińska et al., 2020) (Table 2).

Conclusion

Diet represents one of the major factors affecting the activity, richness, and diversity of the rumen microbial community. It can also affect nutrient utilisation and fermentation end products. Differences in diet composition and feed quality influence microbial community composition and activities at all production levels. Also, the inclusion of additives (tannins) and probiotics in dairy cows' diets can be beneficial for animal health and can decrease the environmental impact of livestock. Despite the fact that many studies did not show any difference across treatments at the phylum level, these results can mask differences in abundance at lower taxonomic levels. The abundance of studies on rumen microbiota and its interaction with diet composition should enhance a meta-analysis approach to evaluate possible relations between specific ruminal taxa and nutritional outputs in a statistically meaningful manner.

The review underlines the lack of research and the reduced knowledge about many rumen microbes and their metabolic activities, which will be improved through “-omics” approaches to deeply understand the connections between metabolic pathways and functions of the rumen microbiome, investigating their role, and better defining the ways diet composition could regulate and manipulate it.

Ethics approval

None.

Data and model availability statement

None. Information can be made available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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A. Palmonari: Writing – review & editing, Conceptualization. **A. Federiconi:** Writing – review & editing, Writing – original draft. **A. Formigoni:** Visualization, Supervision.

Declaration of interest

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