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*Corresponding author

Silvano Focardi, Department of Environmental Sciences, Università di Siena, Via Mattioli, 4, 53100 Siena, Italy

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Review Article

Development and Maturation of Microbiota in Cow Rumen, Plant-Fibers Degradation and Influences on the Immune System and Cow Health

Silvano Focardi[1*] and Milva Pepi[2]

[1]*Department of Environmental Sciences, Università di Siena, Via Mattioli, 4, 53100 Siena, Italy*

[2]*Stazione Zoologica Anton Dohrn, Fano Marine Centre, Viale Adriatico, 1-N, 61032 Fano, Italy*

Abstract

Rumen is part of the forestomach of ruminants and plays a key role in the conversion of feed into metabolites that are absorbed and used by the host. The rumen is also the place of formation of proteins of microbial origin, which represent a source of energy for the host animal. From a functional point of view, ruminants are monogastric at birth as they have an undeveloped forestomach system. Microbial communities in the rumen first show colonization by bacteria, followed by that of methanogenic Archaea and then anaerobic fungi and protozoa. In newborn calves, molecular-based techniques evidenced initial rumen colonisation by facultative anaerobic bacteria, as the phyla Proteobacteria and Firmicutes, with genera *Enterococcus* and *Streptococcus* and the species *Escherichia coli*, followed by Archaea within a few hours after birth. These early colonizers utilize the oxygen available in the rumen, thus creating an anaerobic environment conducive to the growth of rigorous anaerobic communities, including *Bifidobacterium* and *Bacteroides.* The strict anaerobic bacterial community, including cellulolytic and proteolytic bacteria, establishes and dominates the rumen microbiome within the first two weeks of life. The entire microbial community allows ruminants to use ligno-cellulosic materials and non-protein nitrogen to produce high-quality food. Importantly, these close anaerobic bacterial communities in the rumen of newborns play an essential role in the development of the mucosal immune system. A healthy rumen leads to healthy ruminants with optimal performance. It is worth highlighting the importance of the microbiome in maintaining the health of cattle and its potential in alleviating disease. This mini-review described the development of the cow microbiome in the rumen, the degradation abilities and influence of the feed on the rumen microbiota, and the microbiota effects on the cow's immune system and health.

Introduction

Ruminants define mammals in the Order Artiodactyla, also termed mammals with toes and hooves, of the Suborder Ruminantia. The word ruminant comes from the Latin *ruminare*, which means chewing over again, or chewing the cud [1]. The stomach of ruminants includes four compartments or chambers called reticulum, rumen, omasum and abomasum (Figure 1). The three compartments reticulum, rumen and omasum are lined with non-glandular mucous membranes while the abomasum, represents the gastric compartment and is lined with glandular mucosa. The abomasum resembles the human stomach in terms of function. The rumen forms the largest compartment and together with the reticulum give rise to the sites of anaerobic fermentation. In the rumen there are coronary grooves that give rise to sacs, with a cranial groove that separates the reticulum and rumen. In some cases, including cattle, the two compartments are easily distinguished with the reticulum having a honeycomb appearance. These compartments are lined with finger-like protrusions called papillae, which absorb nutrients such as volatile fatty acids produced by the rumen microbiota. The shape of the finger-like protuberances represents a natural increase in the absorbent surface of the reticulum and rumen. These compartments are often referred to as reticulo-rumen because together they function in the ruminal cycle coordinating contractions to support the functions of belching and rumination. The action of contractions during the ruminal cycle allows various processes to occur, including the inoculation of new foods with microorganisms, the distribution of the final products of digestion for absorption by the mucosal papillae and the passage of digesta to the omasum. By the process of eructation, ruminants release gases from the reticulo-rumen that are produced during anaerobic fermentation. During eructation, gas passing up the esophagus and into the trachea and the lungs to be respired [1]. Rumination involves carrying a digestive bolus along the esophagus, the regurgitate, into the mouth where the digestive bolus, the cud, is chewed. The cud is finally swallowed again and the process continues with another regurgitated bolus for the chewing of the cud by rushing and grinding of the particles by the molars. Chewing the cud increases the surface area of the feed particles, especially the fibrous material, to improve microbial digestion. The act of chewing also stimulates the production of saliva and the buffers present in the saliva help maintain the pH of the rumen when the bolus is re-ingested. Digesta leaves the reticulum through the reticulo-omasal orifice. The omasum, with its numerous leaves or laminae, controls the flow of the digesta to the abomasum. The abomasum is the gastric, glandular, stomach-like compartment of non-ruminants, with secretion of chloridric acid and pepsinogen and a pyloric sphincter that regulates the flow of digesta from the abomasum to the duodenum [1].

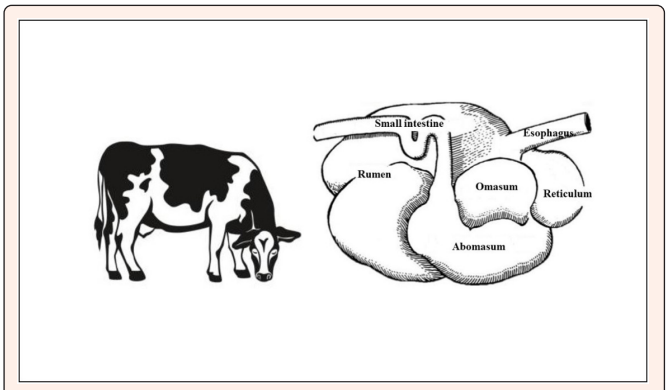

Figure 1: Ruminant and stomach of the ruminant with four compartments or chambers: reticulum, rumen, omasum, and abomasum.

Feeding Regimen and Microbial Communities in the Gastrointestinal Tract from Neonatal to Adult Calves

At birth, ruminants are functionally monogastric with the abomasum and intestines that serve as their major digestion sites, whereas the other components of the gastrointestinal tract constitute a primitive forestomach system, including the rumen, reticulum and omasum [2]. The rumen is the least developed portion during the first month of life [3]. The formation of a fully mature gastrointestinal tract needs the development of the whole sytem of the reticulo-rumen and the associated microbiota [4]. In the rumen, microbial communities show a sequential pathway of colonization where bacteria start first, followed by methanogenic archaea, anaerobic fungi and protozoa as the last group of colonizers [5,6]. Detection of the microbiota composition of rumen in new-born calves, molecular-based techniques investigations showed initial colonization by facultative anaerobic bacteria, with detection of *Enterococcus* spp., *Streptococcus* spp. and *E. coli*, as well as Archaea, within few hours after birth [7,8]. Nevertheless, rumen colonizers of neonatal calves include active bacterial communities at birth [9]. In one-week-old calves, active complex-carbohydrate-fermenting bacterial species were also identified, albeit in the absence of solid substrates in the diet [9]. As a common tract, these initial gut microorganisms utilise the available oxygen, thus originating an anerobic environment favouring growth of strict anaerobic gut bacterial genera, as genera *Bifidobacterium* and *Bacteroides* [10,11]. From the metabolic point of view, the strict anaerobic bacterial communities comprehend cellulolytic and proteolytic bacteria, that will dominate the gut microbial communities within the first two weeks of life (Table 1) [7,12-14]. Investigations based on metagenomic sequencing have shown that at birth the rumen of the newborn calf is colonized by bacteria for the 99.9 ± 0.5%. No Archaea and protozoa are detected in the calf rumen at birth, while fungi and viruses together represent about 0.1% of total identified rumen microbiota [9]. During the first weeks after birth, calves are still suckilg milk and the rumen is not functional and the suckled milk by-pass it, due to closure of the esophageal groove by reflex action. In this period the proportions of the rumen are considerably lower, moreover the wall villi responsible for the absorption of nutrients are not yet developed. Some rumen bacterial strains essential for mature rumen function have been observed to be detected even one day after birth, before ingestion of plant material and long before the rumen is active. With age, it is possible to observe a more diverse but homogeneous and specific mature community, compared to the more heterogeneous and less diverse first communities (Table 1) [7,15].

All the changes that occur with age in the structural and physiological characteristics of the rumen are related to the development of rumen microorganisms, as microbial metabolites are important for the development of the rumen wall villi [16,17]. A rapid colonization of the rumen of newborn animals appeared close to birth by aerobic and facultative anaerobic microorganisms originating from mother and the surrounding environment, followed by a gradual decrease of these microbial communities, until a constant level at between six and eight weeks of age, with a successive gradual and complete subtitution by anaerobic microbial taxa [6]. Experiments conducted with a classic cultural approach, evidencing only a small portion of the total microorganisms, highlighted that cellulolytic bacteria emerged in ruminants three to five days after birth and became abundant in two to three weeks [6]. Further investigations on ruminal microbial communities using molecular methods have highlighted the presence of bacteria typical of mature animals in calves of fourteen and forty-two days, therefore still at the stage of pre-ruminants (Table 1) [7,12]. The rumen microbiome at the early feeding is a critical point and has a similar importance to the weaning period and may enhance the rumen development and facilitate weaning transition. Host microbial interactions during early rumen development in neonatal calves may be coordinated by microRNAs (miRNAs), and this phenomenon may be applicable to early gut development of all mammalian species. Rumen colonization began during the birthing process and the pre-ruminant rumen microbiota was highly active and ready to ferment a solid diet even from the first week of life. The volatile fatty acids produced by the early microorganisms were associated with the rumen tissue metabolism and the development of the epithelium, according to interactions with the host transcriptome and microRNAome [9]. In Table 1, the progressive colonization by microorganisms of the rumen gastrointestinal tract and related feeding regime were detailed from birth to the adult stadium. Related changes in the microbial communities in the rumen, based on age and different feeding regimes were moreover described.

Table 1: Effect of early feeding regimen and age on the initial establishment and development of microbial communities in the gastrointestinal tract of neonatal to mature calves.

Feeding Regime	Age	Effect on Microbial Community	References
heat-treated colostrum	within the first 12 hours of life	pathogens inhibition, including *Escherichia coli* and *Shigella* sp. and increase of growth of *Bifidobacterium* sp.	[18,19]
heat-treated colostrum	in 51 hour-old dairy calves	Firmicutes and Proteobacteria were the predominant phyla, with genera *Escherichia*, *Enterococcus*, *Streptococcus*, *Lactobacillus* as the most representatives; an increase in *Bifidobacterium* sp. was observed	[19-21]
colostrum feeding, milk replacer and starter concentrated-based diet	1 to 3 day-old calves	rumen bacterial community dominated by Proteobacteria and *Streptococcus*-related that are rapidly replaced by strictly anaerobic bacterial taxa	[7,22]
whole milk	1 week-old calves	*Ruminococcus flavefaciens*, using milk as a substrate	[9]
whole milk	1-4 weeks-old pre-weaned calves	increase of the abundance of typically milk-utilizing bacteria as those of the genera *Lactobacillus*, *Parabacteroides*, and *Bacteroides*	[23]
feeding milk	2 weeks-old calves	increased the abundance of *Ruminococcus flavefaciens*, a fibrolytic bacterium in the rumen	[24]
silage suplementation to milk	2 weeks-old dairy calves	increase in archaeal diversity as well as fungal richness	[25]
solid feed intake, a constant or gradual supply of concentrate and ad-libitum hay in addition to milk feeding	2 to 3 weeks of life of calves	increased abundance of amylolytic and fibrolytic bacteria, such as *Succinovibrionaceae*, *Fibrobacteraceae*, and *Prevotellaceae*	[7,22,24,26-28]

milk plus starter concentrate based diet	pre-weaned calves	higher abundance of the genus *Methanosphaera* and lower abundance of the genus *Methanobrevibacter*	[23]
restricted liquid diet	6 weeks-old calves	Bacteroidetes	[12]
	2 month-old, pre-weaned calves	Bacteroidetes	[7]
	in the rumen of pre-weaned calves	the same dominant phyla as found in the rumen of mature post-weaned calves, including Bacteroidetes, Firmicutes, and Proteobacteria	[29]
	in the rumen microbial community from pre- to post-weaned state	increase in the abundance of Firmicutes and Proteobacteria and a decrease in the abundance of Bacteroidetes	[30]
	pre- and post-weaned calves	genus *Prevotella* dominates the rumen microbial community	[30,31]
reduction of milk and an increasing supply of solid feed to young calves	at weaning, from 6 to10 weeks	decrease in the abundance of Bacteroidetes and a subsequent increase in Firmicutes	[31]
	early weaned calves	higher number of species *Fibrobacter succinogenes* and *Ruminococcus albus*	[32]
solid feed intake	in mature rumen, 2 month to 2 years old cattle	high dominance of strains of the genus *Prevotella*	[7,12]
solid feed intake	mature rumen and body weight gain in calves	*Ruminococcus* spp. abundance was positively correlated with provided feed	[30]

Microorganisms Present in the Rumen and their Enzymatic Activites

Molecular investigations allow the description of rumen microbiome that resulted composed, in order of abundance, by bacteria, protozoa, Archaea and fungi, with bacteria and Archaea that are essential to the viability of the ruminant host [33]. The bacterial phyla Firmicutes and Bacteroides are the most abundant and include the important fibre degraders as the cellulose-degrading species *Fibrobacter succinogenes*, *Ruminococcus flavefaciens* and *Ruminococcus albus* [34], and the hemicellulose-degrading genera *Prevotella*, *Butyrivibrio* and *Pseudobutyrivibrio* [35]. These bacteria represent the core genera that were found in almost all ruminants and may have a fundamental role in the metabolism and function of the rumen [29,35]. Bacterial strains of the genera *Prevotella*, *Butyrivibrio* and *Ruminococcus* represent dominant species in the colonization and degradation of the hemicellulose portion of the fibre, as they comprise a broader fibre-related enzymatic repertoire that could enable them to digest the available fibre [34]. Due to the enzymes of cellulolytic and hemicellulosolytic bacteria, such complex substrata are degraded and several rumen bacteria thrive on the resulting degradation products, as in the case of cellulolytic bacteria such as *Treponema bryantii* [36]. Other rumen microorganisms that utilize secondary fermentation products of other microorganisms, are represented by bacteria of the species *Selenomonas ruminantium* and members of the *Succinovibrionaceae* family (Table 2) [37,38]. Rumen is the first compartment of the gastrointestinal tract, where plant digestion allows conversion of plant fibers into chemical compounds that are absorbed and digested by hosts [3]. In this compartment of the gastrointestinal tract, plant biomass is exposed to specialized microorganisms that degrade the plant fibers and, in the meantime, stable and favorable condition for microbial growth are provided [39].

The capability of gut microorganisms to digest their plant feed enables ruminants to convert cellulose and hemicellulose, complex polysaccharides constituting major part of the plant biomass, into nutritive substrates [33,40]. Cellulolytic bacteria, sulfur-reducing bacteria, and methanogenic Archaea constitute major functional groups of rumen microbiota that become established during the first few days of life [41]. The feature to digest their plant feed depend by metabolism of microbial communities residing in the rumen compartment localized in the upper part of the gastrointestinal tract. The function of the rumen microbiome is tightly linked to host physiology, along with the phases of rumen epitelium development [42,43]. This phenomenon involves the modulation of host gene regulation by short chain fatty acids produced by bacteria [9,15]. In microbial communities in general, it should be noted that microorganisms present in low quantities does not mean that they are less important within the microbial community. In the case of the rumen, although very few cellulolytic species have been isolated from the rumen microbiome, they prevail over other species that use the products of the digestion of primary fibers carried out by the same cellulolytic species. As an example, the key cellulolytic bacterium *F. succinogenes*, although it has been detected in all ruminants, represents 0.5% of the entire bacterial community. The fundamental aspect is that *F. succinogenes* feeds the metabolism of other microorganisms composing microbiota by producing soluble sugars and succinate from cellulose [44,45]. The importance of the low abundant species has also emerged in the case of the Archaea. Indeed, although this domain is usually present in the rumen as small proportions of the total rumen microbiota, it includes methanogenic Archaea which are the only methane producers in the rumen ecosystem and are part of the ruminant microbiome core [33,35]. The genera *Methanobrevibacter* and *Methanosphaera* were detected in 100% and 60% of ruminants, respectively, although they occupied only a small part of the entire microbiota population [15]. In ruminants, the digestion process of fibers is the result of an important symbiosis between the host animal and the microbial communities living in the rumen. The activity of the host animal lies in furnishing the plant substrates and in promoting degradation by microorganisms through repeated grinding of plant substrates through rumination with the aim of increasing the surface area of access to better allow the metabolic activity of the microorganisms. Host animals also provide a supportive environment that promotes microbial activity during hydrolysis of plant fibers, such as stable pH, constant temperature and continuous mixing [34].

The successive deconstruction of fibers and their fermentation are carried out by microorganisms present in the rumen, allowing the release of the energy stored complex substrata represented by plant carbohydrates, otherwise inaccessible to the animal, converting them to shorth-chain fatty acids that are absorbed by the animal through the rumen wall. Moreover, these same microorganisms serve as an important protein source for the animal upon later digestion in the gastrointestinal tract [33]. The fiber degradation process is the basis of subsequent metabolic processes in the rumen ecosystem. Cellulose degradation is the most critical step as cellulose is the most resistant polysaccharide to degradation present in vegetable fiber. The degradation of hemicellulose and other polysaccharides and the microorganisms involved are also of fundamental importance [34]. The microbial enzymes involved in the degradation of fibers are represented by multiple glycosidic hydrolases adapted to deconstruct the complex structure of plant biomass, and these enzymes were encoded by the microbial genomes [34]. The bovine rumen microbiota is composed for the 95% of the microorganisms by bacteria and a minority, the 2-5%, is composed by Archaea, and eukaryotic protozoa and fungi were detected at low values of 0.1-1% of the total microbioma [33]. Key fiber-degrading bacteria were detected in the rumen just few days after birth, with the rumen ecosystem functioning with respect to the degradation of fibers already in this period [7]. Further investigation evidenced that fiber degrading bacteria were present just after birth [7,8]. These observations confirm the importance of the fiber-degrading microorganisms with the role of founders of the rumen microbial community. Studies conducted in different animals and countries have shown a preserved ruminant nucleus microbiome composed of microorganisms that degrade cellulose and hemicellulose in all ruminants [35]. A selection toward a general and specialized for gastrointestinal tract core microbiome in ruminants was moreover evidenced [46]. Cellulose is characterized by an insoluble and crystalline chemical structure where cellulose chains tightly packed together with extensive internal and interchain hydrogen bonding, originating an insoluble, crystalline chemical structure. Inside the plant cell walls, cellulose fibrils are strictly embedded in a matrix of hemicellulose, mostly composed by xylan, mannan, xyloglucan and β-glucan, along with the other plant components lignin and pectin. In its natural state,

embedded into a matrix, cellulose is even more recalcitrant to degradation [47]. An efficient plant fiber degradation can be obtained by the activity of a multiplicity of enzymes, both cellulases and hemicellulases, which, depending on sequence, functional and structural properties, are grouped in different families [48]. The degradation of cellulose is a slow process, with an incomplete degree of degradation. During the process of cellulose degradation, the cellulose fibrils are attacked at the end of the chain by exoglucanases that originate from different families of glycoside hydrolases and are higly important enzymes for cellulose breakdown. A second group of cellulases that act in concert with the exoglucanases are the endoglucanases, which cleave the cellulose chain internally [34].

Hemicellulose is the second major component of the plant cell wall and it is composed by various polysaccharides including xylan as the most representative. The latter is composed of xylose, containing xylobiose units, branched with different side-chain sugars. Xylanases are the xylane-degrading enzymes including endoxylanases, that cleave the main chain internally, and exoxylanases, that cleave at the chain ends, and side-chain cleavage enzymes, with kinetics of xylan degradation that are relatively rapid [34]. Starch is a biopolymer of glucose representing a storage polysaccharide and a source of carbon in plant biomass. Amylases can hydrolyse starch and are divided in α-amylases that act randomly on starch substrates and in β-amylases that act only from the non-reducing end of the chain. Debranching enzymes allow to achieve the complete degradation of starch, with maltose as the main product of starch degradation. Maltose is a disaccharide composed of two α-linked molecules of glucose, as opposed to the cellobiose unit of cellulose, which is a β-linked glucose. Additional glycoside hydrolases families can hydrolyze pectin and other polysaccharides present in lower amounts in the plant biomass, along with the activity of accessory enzymes such as carbohydrate esterases, polysaccharide lyases and lytic polysaccharide monooxygenases [48]. The aromatic lignin polymer is degraded by different classes of oxidative enzymes, such as peroxidases and laccases, mainly produced by fungi [34,49]. Interactions were found between bacteria, Archaea and protozoa in the rumen. Methanogens are known to colonize protozoa and this mutualistic relationship is believed to increase methane formation in the rumen. These associations can be non-specific or occuring at strain level. Some positive associations were instead detected between bacterial and protozoal groups, as in the case of the associations of protozoa *Isotricha* and *Dasytricha* with bacteria of the genus *Fibrobacter*. *Fibrobacter* has been reported to decrease in abundance in animals in which protozoa have been eliminated, indicating that there may be a mutually beneficial relationship between these protozoa and bacterial strains of the genus *Fibrobacter* [35]. In Table 2, the different microorganisms involved in fibers degradation, belonging to the different superkingdoms, were described according to their enzymatic properties.

Table 2: Microorganisms present in the rumen belonging to the superkingdoms Bacteria, Archaea, and Eukaryota, and their enzymatic activites.

Role in Rumen	Phylum	Family	Genus	Species of Isolated Strains	Enzymes	References
Bacteria						
cellulose degraders (crystalline cellulose degradation)	Firmicutes	*Oscillospiraceae*	*Ruminococcus*	*R. flavefaciens*	cellulosomal system complex, multiplicity of protein assembly	[46,50]
	Firmicutes	*Oscillospiraceae*	*Ruminococcus*	*R. bromii; R. albus*	carbohydrate-binding molecules, cellulosome	[33,51,52]
	Firmicutes	*Lachnospiraceae*	*Butyrivibrio*	*B. fibrisolvens; B. hungatei; B. proteoclasticus*	polysaccharide-degrading enzymes which initiate the breakdown of pectin, starch and xylan	[35,53]
	Fibrobacteres	*Fibrobacteraceae*	*Fibrobacter*	*F. succinogenes*	outer-membrane vescicles degrading cellulose and other polysaccharides	[54-56]
	Bacteroidota	*Prevotellaceae*	*Prevotella*	*P. bryant; P. ruminicola; P. brevis*	polysaccharide-utilization, locus mechanism for cellulose-degradation	[53,57]
	Proteobacteria	*Succinivibrionaceae*	*Succinivibrio*	*S. dextrinosolvens*	glucose fermentation produces acetic and succinic acids and many strains posses the enzymes for nitrogen utilization	[35,58]
			Ruminobacter	*R. amylophilus*		
hemicellulose degradation	Bacteroidota	*Prevotellaceae*	*Prevotella*	*Prevotella* spp.	enzymes for degradation of complex hemicellulose types in the cell wall, without cellulosome, and the produced sugars will be used by all microorganisms	[29,59,60]
	Firmicutes	*Lachnospiraceae*	*Butyrivibrio*	*Butyrivibrio* spp.		
	Firmicutes	*Lachnospiraceae*	*Pseudobutyrivibrio*	*Pseudobutyrivibrio* spp.		
	Firmicutes	*Oscillospiraceae*	*Ruminococcus*	*Ruminococcus* spp.		
	Fibrobacteres	*Fibrobacteraceae*	*Fibrobacter*	*Fibrobacter* spp.		
starch degradation	Proteobacteria	*Succinivibrionaceae*	*Succinimonas*	*S. amylolytica*	produce free enzymatic subunits, degrades resistant starch by producing amylosome complex analogous to cellulosomes	[60-62]
	Proteobacteria	*Succinivibrionaceae*	*Ruminobacter*	*R. amylophilus*		
	Bacteroidota	*Prevotellaceae*	*Prevotella*	*P. ruminicola*		
	Firmicutes	*Streptococcaceae*	*Streptococcus*	*S. bovis*		
	Firmicutes	*Selenomonadaceae*	*Selenomonas*	*S. ruminantium*		
	Firmicutes	*Lachnospiraceae*	*Butyrivibrio*	*B. fibrisolvens*		
	Firmicutes	*Oscillospiraceae*	*Ruminococcus*	*R. bromii*		

Archaea						
methanogens	Euryarchaeota	*Methanobacteriaceae*	*Methanobrevibacter*	*M. smithii*	uses CO_2, formate and H_2 as substrates for methane production	[63]
				(Methanobacterium) Methanobrevibacter ruminantium	contains a formate dehydrogenase that uses F_{420} as the electron acceptor with formate as substrate	[64]
			Methanosphaera	*M. stadtmanae*	uses hydrogen to reduce methanol to methane	[65]
Fungi (Eukaryota)						
cellulose and hemicellulose degradation	Chytridiomycota	*Neocallimastigaceae*	*Neocallimastix*	*Neocallimastix* spp.	fungi active on both cellulose and hemicellulose, rich in cellulases and hemicellulases; in A. robustus and N. californiae, fungal cellulosomes with an independent origin for dockerin modules	[66-69]
			Caecomyces	*Caecomyces* spp.		
			Piromyces	*Piromyces* spp.		
			Anaeromyces	*Anaeromyces* spp.		
			Orpinomyces	*Orpinomyces* spp.		
			Cyllamyces	*Cyllamyces* spp.		
			Anaeromyces	*A. robustus*		
			Neocallimastix	*N. californiae*		
Protozoa (Eukaryota)						
cellulose degradation	Ciliophora	*Ophryoscolecidae*	*Entodinium*	*Entodinium* spp.	cellulolytic activity, cellulose degraders	[70,71]
			Epidinium	*Epidinium* spp.		
			Epidinium	*E. ecaudatum*		
			Eudiploidinium	*E. magii*		
			Ostracodinium	*O. dilobum*		
			Epidinium	*E. ecaudatum*	enzymes cellulanases produced by the cellulolytic protozoa	[72,73]
			Polyplastron	*P. multivesiculatum*		
			Diploplastron	*D. affine*		
			Metadinium	*M. medium*		

Gut Microbiota and Development of the Ruminant Animal Immune System

The process establishing a strict anaerobic bacterial community in the gastrointestinal tract of new born calves plays an essential role in mucosal immune system development, and is therefore, a critical phase for the host. After the initial gastrointestinal tract colonisation, the continuous exposure to specific microorganisms is necessary to ensure to the ruminal host the energy for its own metabolism, health, and the mucosal immune system maturation [74]. The whole microbial community of the gut microbiota allow development of the mucosal epitelium and immune system of the ruminant animal. The mucosal epithelial cells delineate the upper part of gastrointestinal tract and represent the cells responding first to the resident microorganisms [75]. Various physical and chemical barriers and receptors of pattern recognition are present in the mucosal immune system, enhabling mucosal epithelium to coexist with its resident symbiotic microorganisms and providing protection against potential invading pathogens [76]. It is noteworty to point out that signalling cascades are essential for maintaining the intestinal omeostasis, its integrity, the capability to express antimicrobial peptides, and modulation of the mucosal barrier functions and immune responses. The Mucosa-Associated Lymphoid Tissues (MALTs) generally initiate the immune response at the mucosal surface level [75]. In ruminants, the initiation of mucosa-associated lymphoid tissues in utero occurs before the development of the microbial communities [77]. In utero, the lymphoid tissue associated with the mucosa is able to originate specific immune responses through secretory IgA production, althought IgA^+ and IgG^+ cells develop in Peyer's patches only after birth, as in utero infections are absents [77]. The germinal centres of Peyer's patches require exposure to the microbiome of the gastrointestinal tract for their complete development [78]. The gut microbiome provides cues for the production of a wide variety of preimmune B cells (Figure 2). In addition to the role played by the gut microbiome, the signals may come from colostrum, intensive milk feeding or milk replacer based-diet or from environmental toxins. These compounds exert a strong influence on the development of the mucosal immune system in neonatal calves [79]. As an example, during the first life phases, an extended colostrum feeding resulted in an increase of active mucosa-associated bacterial strains *Lactobacillus* spp. and *Escherichia coli*, with a consequent upregulation of the expressions of serotonin and adrenergic receptors genes in the intestines of calf (Figure 2) [80]. Serotonin and adrenergic receptors are involved in the regulation of glucagon-like peptide-2 secretion by enteroendocrine L cells, causing a decrease of the apoptosis of epithelial cells, a reduction of the motility and permeability of the gut, and an increase in mesenteric blood flow, in intestinal growth, and nutrient in absorption [81]. In the colon, a positive correlation was observed between increases in concentrations of *Lactobacillus* spp. and *E. coli* and serotonin receptor gene expression, suggesting that just the early feeding modalities may affect the host-microbiota interactions and may play a critical role in host immune system in new-born calves [80].

Maturation of the intestinal immune system needs of an important provision of nutrients [82]. The pre-weaning period is a very delicate step for maturation of the intestinal mucos immune system in calves [83]. The host is capable to identify commensal microorganisms by Toll-Like Receptors (TLRs) and bacteria attached to mucosa can alter the expression of toll-like receptors, causing activation of Pattern Recognition Receptors (PRRs). The latter play a crucial role in the proper function of the innate immune system, mediating the initiation of antigen-specific adaptive immune response of the host-immunity and release of inflammatory cytokines [84,85]. Pathogen-dependent activation of toll-like receptors signalling activates inflammatory responses [84]. As an example, it was observed an age-dependent decrease in mucosal toll-like receptors gene expression and an increase in T lymphocytes $CD3^+$, $CD4^+$, and $CD8^+$ cells in the mucosa of the jejunum and ileum of calves [85]. The consequence of these changes evidenced a decrease in the innate immunity and an increase in the adaptive immunity. It is thus possible that the age-dependent downregulation of the innate immunity protect the hosts from harmful inflammatory responses [86]. Toll-like receptors can act as the primary mechanism of innate immunity in neonatal calves and are replaced over time by innate immune mechanisms dependent on antimicrobial peptides and protect the animal from unnecessary inflammatory responses [85].

Bovine trancriptome investigations evidenced that the host-microorganisms interactions play a crucial role in regulating the gastrointestinal tract development. In fact, a positive correlation between the numbers of gene copy of *Lactobacillus* spp. or *Bifidobacterium* spp. and expression levels of microRNAs (miRNA) was highlighted. MicroRNAs act as promoters of gastrointestinal tract development, including miR-15/16 for the immune cells development, miR-29 for maturation of dendritic cells, and miR-196 for lymphoid tissue development. In the meantime, the microbial-driven transcriptional regulation of developing rumen in calves via miRNAs, with three miRNA-mRNA pairs involved in the development of rumen, was also suggested [9,74]. Figure 2 describes the activation of immune responses in mucosal surface of calves, dependent by the Mucosa-Associated Lymphoid Tissues (MALTs).

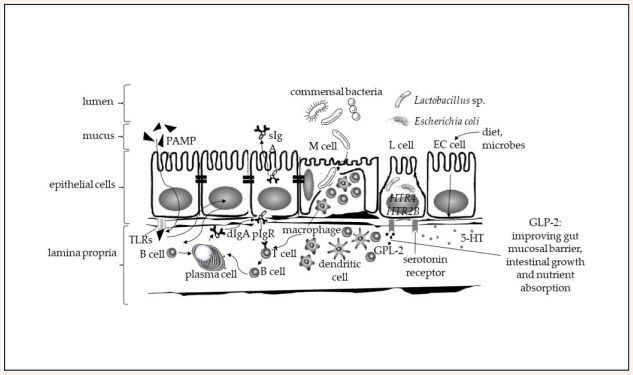

Figure 2.

Figure 2: Activation of immune responses in mucosal surface of calves, dependent by the Mucosa-Associated Lymphoid Tissues (MALTs). Microfold (M) cells transport microbial antigens from the luminal surface to the underlying MALT cells, where they stimulate specific T- and B-lymphocytes, resulting in the production of dimercic immunoglobulin A (dIgA) by B cells, which form a complex with the polymeric Ig receptor (pIgR) and enter the cell of mucosa and are then translocated as secretory immunoglobulin A (sIgA) to the apical epithelial surface. Pathogen-Associated Molecular Patterns (PAMPs) can alter the expression of tool-like receptors and activate host immunity. Upregulation of 5-hydroxytryptamine receptor 4 (HTR4) and 5-Hydroxytriptamine Receptor 2B (HTR2B) genes expression by mucosa-associated bacteria. These genes code for the serotonin receptors that regulate glucagon-like peptide-2 (GLP-2) secretion by enteroendocrine L cells via interaction of 5-hydroxytryptamine/serotonin (5-HT) with serotonin receptors. Abbreviations: PAMPs, pathogen-associated molecular patterns; dIgA, dimeric immunoglobulin A; sIgA, secretory immunoglobulin A; pIgR, polymeric Ig receptor; TLRs, toll-like receptors; EC cell, enterochromaffin cell; 5-HT, 5-hydroxytryptamine/serotonin; HTR4, 5-hydroxytryptamine receptor 4; HTR2B, 5-hydroxytryptamine receptor 2B; GLP-2, glucagon-like peptide-2.

An intensive milk or milk replacer feeding during the pre-weaning period favoured the expression of long noncoding RNAs influencing the synthesis of tight junction proteins in the jejunal mucosa of calves [87]. The cells of the mucosal barrier are strictly maintained and protected by these tight junctions, whose breakage can give rise to the so-called leaky gut syndrome (Figure 3). The mucous barrier normally protects the ruminal host's passage of luminal contents, including microorganisms and their products in case of leaky gut syndrome, pathogenic bacteria can cross the mucosal barrier, triggering inflammation. Dendritic cells and macrophages react to these bacteria. The innate immune cells release cytokines that exert pro-inflammatory (tumor necrosis factor and interferon-γ) and anti-inflammatory (interleukin-13) effects. If pro-inflammatory signals dominate and signal to the mucosal epithelium, myosin light chain kinase can be activated to cause barrier dysfunction through the leak pathway, allowing an increase in the amount of luminal material presented to immune cells. In the absence of appropriate immune regulation, immune activation may cause further pro-inflammatory immune activation, cytokine release, and barrier loss, resulting in a self-amplifying cycle that can result in disease [75,88]. Figure 3 describes how the breaking of the tight junctions allows the transport of pathogens and the activation of inflammatory responses by the host ruminant.

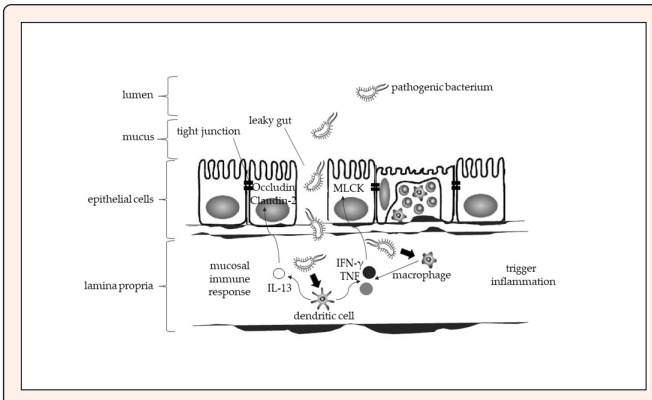

Figure 3.

Figure 3: The breaking of the tight junctions allows the transport of pathogens and the activation of inflammatory responses by the host ruminant. The figure shows how dendritic cells and macrophages react to these materials. These innate immune cells release cytokines that exert pro-inflammatory (TNF and interferon-γ) and anti-inflammatory (IL-13) effects. If pro-inflammatory signals dominate, MLCK can be activated to cause barrier dysfunction through the leak pathway, resulting in a self-amplifying cycle that can result in disease. Abbreviations: IFN, interferon; M, macrophage; MLCK, myosin light chain kinase; IL, interleukin; TNF, tumor necrosis factor.

Gut Microbiota and Ruminant Health

The rumen is perhaps the most diverse and complex microbial ecosystem of the gastrointestinal tract of animals. In ruminants, interactions between the gut microbiome, diet, host genetics and health are critical to the development of better animal health and animal production along with better food quality, that is more respectful, environmentally friendly and efficient. A healthy gut leads to healthy ruminants with optimal performance. Ruminants can digest a wide range of forages, resulting in no competition for edible food for humans. However, the rumen microbiota can give rise to concerns, as in the case of protozoa carrying out proteolysis that can lead to low nitrogen efficiency, or regarding excess rumen ammonia which, if not recovered by the rumen microbiota for its own protein synthesis, it can be absorbed and expelled by the animal into the environment. The formation of methane, a greenhouse gas, in the rumen by methanogenic Archaea may contribute to climate change. Furthermore, the biohydrogenation of rumen fatty acids by rumen microorganisms leads to a higher quantity of saturated fat in the milk and meat of ruminants than in monogastric animals [89]. The microbiome naturally associated with a healthy cattle has been characterized and therapies such as probiotics and prebiotics have shown efficacy against dysbiosis, characterized by a disruption of the microbiome resulting in an imbalance in the microbiota, changes in their functional composition and metabolic activities, or a shift in their local distribution. Dysbiosis is most commonly reported as a condition in the gastrointestinal tract, particularly during Small Intestinal Bacterial Overgrowth (SIBO) or Small Intestinal Fungal Overgrowth (SIFO) [90]. The co-evolution of gastrointestinal tract in ruminants and the colonising microbiome is essential for the cow health. Disturbances result in an imbalanced symbiosis, leading to gut microbial dysbiosis which can induce several enteric disorders [90].

Birth and the pre-weaning period are critical periods due to the high susceptibility of neonatal calves to a vast variety of bacterial and viral infections, which cause diarrhoea that represents the major cause of death in neonatal calves. A decreased incidence of diarrhoea was correlated with a higher abundance of *Faecalibacterium* in faecal samples of one-week-old calves and in the large intestine of three-week-old calves. *F. prausnitzii* promotes antiinflammatory responses, maintains intestinal homoeostasis and produces butyrate in the large intestine. A high abundance of this species during the pre-weaning period may provide health benefits to the neonates by decreasing their susceptibility to enteric infections. The practice of a microbiota transplantation to stabilise the gut microbiome was applied

in ruminants by transferring the rumen microbiome of adult animals orally to young calves. Although the overall microbiome structure was not affected, the incidence of calf diarrhoea decreased [74]. Feeding milk containing drug residues to the preweaned calves resulted in lower abundance of genes involved in regulation and cell signalling, stress response and nitrogen metabolism [91]. Moreover, the direct treatment of calves with antibiotics may also result in the emergence of antibiotic-resistant bacterial strains [91]. A decrease in the prevalence of multidrug-resistant fecal *E. coli* has been observed with increasing age of calves, indicating that the underdeveloped digestive system of neonatal calves favors the growth of resistant bacteria due to limited competition for resources [74]. Supplementation of calf diet with zinc oxide reduced the incidence of diarrhoea from days 1-3, increased the abundance of the beneficial genera *Faecalibacterium* and *Lactobacillus* within the first seven days of life and improved the immunity by increasing the concentrations of serum immunoglobulins (IgM and IgG). Moreover, when zinc methionine was supplemented, a prolonged reduction in diarrhoea was observed from days 1-14, and increased abundances of *Faecalibacterium* after 7 days and *Ruminococcus* in two weeks were detected [92]. Thus, the essential role of zinc in the treatment of neonatal calf diarrhoea was evidenced. Calcium propionate supplementation increased the body weight and decreased the relative abundance of Bacteroidetes in both pre- and post-weaning calves, increasing the phylum Proteobacteria, with the family *Succinivibrionaceae*, and the genus *Methanobrevibacter* in the post-weaning group [93]. These studies suggest that microbial manipulations are easier to perform during early life, and these effects may persist longer when manipulations are performed in early life of ruminants [74].

Conclusion

The rumen microbiota plays a key role in the metabolism, the immunological system and health of rumen hosts. Aspects related to the rumen microbiota can offer important insights to improve the relationships between animals and the microbiota and possibly to achieve animal welfare and thus obtain safe and good quality products to be consumed by humans. Therefore, future research should focus on ruminant health, in particular regarding immune responses, and on aspects such as the ecological and metabolic activity of the microbiota based on advanced technologies and predictive modeling approaches.

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