REVIEW ON ANIMAL MICROBIOME: IT'S IMPORTANCE

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Abstract: The microbiome of hosts, also known as microflora or microbiota, is routinely defined as all the microorganisms inhabiting a specific environment, and these terms are often used interchangeably. Gut microbial communities, composed of bacteria, ciliate and flagellate protozoa, anaerobic fungi, and viruses, play a vital role in nutritional, physiological, immunological, and protective functions of the host. The rumen is one of the most extensively studied and well-documented gut ecosystem because of the importance of ruminants to human nutrition and the major role played by rumen microbes in nutrition of the ruminant animal. Volatile fatty acids, principally acetate, propionate, and butyrate, are the major products of rumen microbial fermentation and are absorbed and used as energy sources by the host. The interaction between the host and microbes in the rumen is synergistic, in that the host provides heat, moisture and food, while the microorganisms produce protein and by-products of digestion, to use by the host. Bacteria are the most abundant microbes in the foregut of ruminant animals, with approximately 10^{10} – 10^{11} cells/ml and over 200 species. The lower-gut microbiota diverge in composition according to intestinal segment, likely reflecting differences in physical, chemical, and biological conditions in each compartment. The association between the host and the microbiome is affected by a large number of abiotic and biotic factors. Culture-dependant identification techniques rely on various selective and enrichment culture conditions in order to replicate the microbes' natural environment. Culture-independent methods, or, more specifically DNA-based methods of identification and detection of microorganisms, allow the examination of microbial communities at a molecular level.
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1. INTRODUCTION

The microbiome of hosts, also known as microflora or microbiota, is routinely defined as all the microorganisms inhabiting a specific environment, and these terms are often used interchangeably. The term microbiota has been used historically, and, most likely, the suffix "-biota" was used to define "living organisms in an ecosystem." The term microbiome was coined in the "-ome" and "-omics" era "to signify the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease". Although "-om" and "-ome" surely have meanings in linguistics and biology, the authors proposed that "the -ome idea is borrowed from the multitude of terms already ensconced into English or the scientific lingua franca," rather than being derivations from Greek or Sanskrit. In contrast, others proposed to define microbiota as the microbial taxa and the term microbiome as the catalog of these microbes and their genes. Either term can be used to describe microbial communities, and the holistic "-ome" approach also includes their genetic information (Bleich and Fox, 2015).

Gut microbial communities, composed of bacteria, ciliate and flagellate protozoa, anaerobic fungi, and viruses, play a vital role in nutritional, physiological, immunological, and protective functions of the host (Chaucheyras-Durand and Ossa, 2014). The microbiomes, including bacteria, fungi, and viruses, live within and upon all organisms and have become a growing area of research. With the advances of new technologies it is now possible to entangle complex microbial communities found across animal kingdoms (Bahrndorff *et al*., 2016).

One of the main beliefs about the symbiosis of microbes and animals is that they choose their host and it is not related to probabilities. It is notable that mostly microbes weight in some species is heavier than their brains and absolutely, they have DNA and it is observed in the different situations that it can affect the physiology. Furthermore, predigestion is one of gut microbiome duties which scientists have found could lead to dysphoria on the dietary decision for their hosts. We live in a microbial world. The germs colonize the intestine and the outer surface of the animal as well as some reproductive organs. Some animals even have specialized organs that have selected groups of microbes. In general, despite the dispersal of microbes,

the relationship between animals and microbes is not random (Bolooki and Jafarzadeh Shirazi, 2019). The microorganisms in the digestive tracts of ruminant livestock have a profound influence on the conversion of feed into end-products which can impact on the animal and the environment. As the livestock sector grows in numbers and productivity particularly in developing countries, there will be an increasing need to understand these processes for better management and use of both feed and other natural resources that underpin the development of sustainable feeding systems (McSweeney and Mackie, 2012).

Recent advances in molecular biology have provided new possibilities to investigate complex microbial communities and it has become clear that the vast majority of bacteria living in/on other animals cannot be cultured. It is now commonly accepted that at least 80% of the total bacterial species in the human gut cannot yet be cultured (Bahrndorff *et al*., 2016).Conventional culture-based techniques such as isolation, enumeration, and nutritional characterization have provided significant information on the diversity of the rumen microbiota. In fact, more than 200 species of bacteria and at least 100 species of protozoa and fungi inhabiting the rumen have been identified by culture-based techniques. Nevertheless, over the last 10 years the development of highthroughput sequencing techniques has allowed for a considerable increase in knowledge of the microbial diversity of the rumen ecosystem. Indeed, even if culture-based techniques are successful in isolating key representatives of rumen bacteria, archaea, and fungi, they are not well suited for characterizing the overall microbial diversity, because a vast majority of rumen species are not yet culturable (Chaucheyras-Durand and Ossa, 2014).

The culture-independent analysis of the host microbiome can be obtained by either metagenomic approaches or amplicon sequencing using specific marker genes (Caporaso *et al*., 2012). High-throughput DNA sequencing approaches provide an attractive and cost-effective approach to investigate the composition and functions of the host microbiome (Bahrndorff *et al*., 2016). Amplicon sequencing provides a targeted version of metagenomics with a specific genetic region shared by the community members of interest. The amplified fragments derive from universal primers and are usually assumed to produce sequence read abundance that reflects the genetic diversity in the studied sample and hence sequence read abundance should reflect the genetic diversity in the studied sample. The amplified fragment typically contains phylogenetic or functional information, such as the 16S ribosomal RNA gene. 16S rRNA gene sequences are well studied and provide excellent tools for microbial community analysis, but other functional marker genes can also be used (Caporaso *et al*., 2012). During the last 15 years, the advent of routine gene sequencing technologies and the availability of large public databases for comparative analysis have allowed for rapid identification of new bacterial isolates on the basis of their 16S/18S rRNA (*rrs*) gene sequences (Chaucheyras-Durand and Ossa, 2014).

Subsequent taxonomy profiling of the entiremicrobial communities is conducted by comparisons to reference sequences or by *de novo* clustering of specific regions of sequences. Functional profiling of metagenomics is more challenging since major parts of the metagenomic data remain insufficiently characterized and frequently samples are contaminated by host DNA or traces from the diet. Compared to both culture-dependent and more traditional molecular approaches such as sequencing of clone libraries and DGGE, amplicon sequencing approaches allow a more in depth analysis of the complete microbiome and are less restricted to the number of samples to be investigated (Bahrndorff *et al*., 2016).

The ability to study the entire microbiome from complex communities such as the rumen has been impaired by classical methods. Culture-based techniques accounted for only 10 to 20% of the bacterial species present in the rumen. However, the recent advances in nucleic-acid-based techniques, namely high-throughput DNA sequencing, provided the means to study rumen and gut ecology. Recent efforts to study the rumen microbiome have focused on analyzing the ruminal microbial communities (i.e., identification and quantification), whereas microbial activities are extrapolated based upon measuring changes in microbial communities (AlZahal *et al*., 2017). Therefore, the objectives of this paper are to review composition of the animal microbiome and their importance, and to highlight the identification techniques of the animal microbiome.

2. THE MICROBIOME OF ANIMALS

The number of microorganisms sharing the human body is thought to outnumber human cell numbers by a factor of ten and the combined microbiome usually contains 100x more genes than its host. The microbiome also plays a major role in human health (Cho and Blaser, 2012). In recent years the microbiome of a number of vertebrate nonhuman species has been sequenced including livestock (Isaacson and Kim, 2012). Insects are the most diverse and abundant groups of animals on earth and have colonized many different habitats. It is therefore not surprising that insect species are also inhabited by large and diverse microbial communities playing a pivotal role for insect biology. Many insect species are

inhabited by a large and diverse assembly of microorganisms, where especially the microbial communities in the intestinal tract have received much attention (Wong *et al*., 2013).

The microbiome of other groups of invertebrates has also been established although for a limited number of species. Studies have compared the microbiome of different species of marine invertebrates with or without photosynthetic symbionts (Bourne *et al*., 2013). Given the relative proportion of microbes and microbial genes in the animal body as well as the fidelity of these relationships in the animal generation, researchers have referred to these groups as "holobiont" and suggest that holobionts are valid units of choice in animal evolution (Bolooki and Jafarzadeh Shirazi, 2019).

2.1. Composition of the Microbiota

Many animals across a wide range of orders have a portion of their digestive tract adapted to accommodate a microbial population which aids in digestion and provides a variety of nutritional and health benefits. Microbial populations have been described in the gut of herbivores, omnivores and carnivores and in all zoological classes. This complex, mixed, microbial culture (comprising bacteria, ciliate and flagellate protozoa, anaerobic phycomycete fungi and bacteriophage) forms a closely integrated ecological unit with each other and the host animal, as well as playing a vital role in the nutritional, physiological, immunological and protective functions of the host. Development of microbial populations in the digestive tract of higher animals commences soon after birth and involves a complex process of microbial succession and many microbial – host interactions which, eventually resulting in dense, stable microbial populations inhabiting characteristic regions of the gut (McSweeney and Mackie, 2012).

On the phylum level, the gut bacteria are similar in mammals, for example, in humans and mice. However, this does not apply to the species level. Presence and importance of species specific microbiota, even within rodent species, was demonstrated three decades ago and was also shown more recently. Of the more than 50 bacterial phyla (29 of which have cultured representatives), humans and mice are colonized mainly by Firmicutes (a phylum containing bacteria like clostridia, lactobacilli, streptococci and staphylococci), Bacteriodetes (like Bacteroides, Porphyromonas), Actinobacteria (like Actinomyces, Streptomyces), and Proteobacteria (which contain Enterobacteriaceae like E. coli or Helicobacter spp.). Composition and complexity vary at different regions of the body, with the highest number of species being found in the colon (mainly Firmicutes and Bacteroidetes in mice and humans) and only a few species in the acid-secreting stomach or the genital tract (Bleich and Fox, 2015). The microbiomes, including bacteria, fungi, and viruses, live within and upon all organisms and have become a growing area of research. With the advances of new technologies it is now possible to entangle complex microbial communities found across animal kingdoms. Recent advances in molecular biology have provided new possibilities to investigate complex microbial communities (Bahrndorff *et al*., 2016).

2.2. The rumen microbiome and their importance

The rumen is one of the most extensively studied and well-documented gut ecosystem because of the importance of ruminants to human nutrition and the major role played by rumen microbes in nutrition of the ruminant animal. The ruminant foregut or stomach has evolved into three pregastric fermentation chambers (rumen, reticulum and omasum) of which the rumen is by far the largest. Ingested plant material is hydrolysed and fermented in the rumen, and microbial cells and undigested plant particles pass into the abomasum where gastric digestion begins. The most distinctive feature of ruminants, rumination, where foregut digesta is regurgitated, rechewed and reswallowed in a frequent regular pattern repeated up to 500 times per day and enables reduction in particle size (comminution) and exposure of maximal surface area to microbial attack. The mutualistic microbial fermentation is based on digestion of the plant cell wall by cellulases and hemicellulases, synthesis of microbial proteins from poor quality dietary (forage) protein and nonprotein nitrogen mainly via ammonia as precursor, synthesis of vitamins B and K, as well as detoxification of phytotoxins and mycotoxins. In turn, the host animal provides a mechanism for the selection and harvesting of feed, maintaining a high level of nutrient supply (10–18 percent dry matter), temperature regulation (38–41 °C), pH control (6–7) by buffer in saliva, osmotic control (250–350 milliosmole) and removal of soluble inhibitory end-products of digestion as well as undigested particulate matter (residence time 1–2 days) and microbial cells, and provision of some nutrients (urea, phosphate and bicarbonate through saliva and the rumen wall) (McSweeney and Mackie, 2012).

The forestomachs of ruminant animals contain a great diversity of prokaryotic (bacteria), archaea, virus and eukaryotic (protozoa and fungi) micro-organisms that together breakdown and ferment the feed ingested by the host animal (Yáñez-Ruiz *et al*., 2015). The rumen can be viewed as an anaerobic and methanogenic fermentation chamber that contains microorganisms that have the ability to utilise, and increase the productivity of, cellulolytic feeds (i.e. straw, hay, silage and grass) (Matthews *et al*., 2019). The rumen

microbiome is a phylogenetically diverse consortium of anaerobic bacteria, fungi, methanogenic archaea, ciliate protozoa, and viruses. This microbial cohort contains cellulolytic, hemicellulolytic, amylolytic, proteolytic, and biohydrogenating (lipolytic) species, exhibiting a high level of functional redundancy, and is capable of effectively degrading host-indigestible plant fiber. Volatile fatty acids (VFAs), principally acetate, propionate, and butyrate, are the major products of rumen microbial fermentation and are absorbed and used as energy sources by the host. Ruminally derived VFAs can meet up to 70% of the host's energy needs, and thus their production is essential to animal performance (O'Hara *et al*., 2020). There are considerable benefits associated with understanding rumen function, as rumen dynamics are almost solely responsible for providing nutrients to the hostanimal. Figure 1shows the gastrointestinal tractof the bovine animal, where the rumen and itsmicrobiota play a particularly important role inthe degradation of feedstuffs. As a result of fermentingfeedstuffs, carbon dioxide (CO2) andhydrogen (H2), which are the main electron acceptorsand donors of the ecosystem, are produced inthe rumen (Matthews *et al*., 2019).

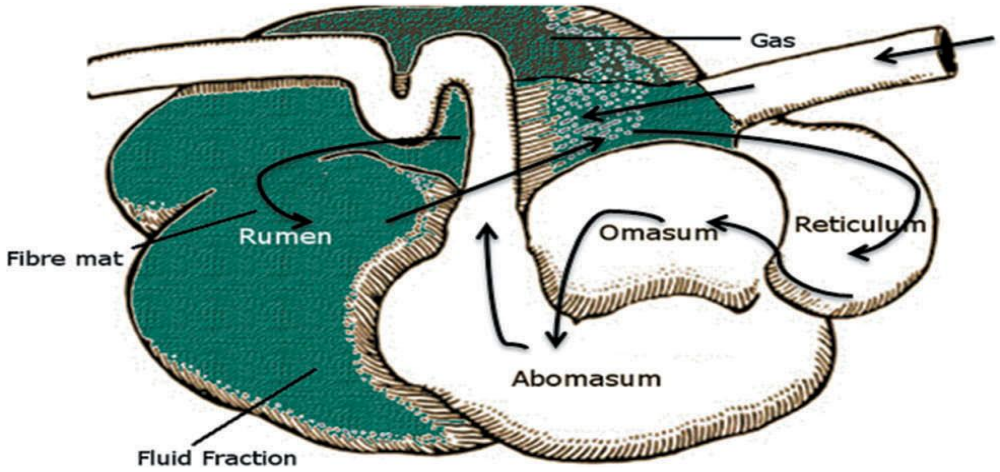

Figure 1: The ruminant gastrointestinal tract (Matthews *et al*., 2019).

Many animals across a wide range of orders have a portion of their digestive tract adapted to accommodate a microbial population which aids in digestion and provides a variety of nutritional and health benefits. Microbial populations have been described in the gut of herbivores, omnivores and carnivores and in all zoological classes. This complex, mixed, microbial culture (comprising bacteria, ciliate and flagellate protozoa, anaerobic phycomycete fungi and bacteriophage) forms a closely integrated ecological unit with each other and the host animal, as well as playing a vital role in the nutritional, physiological, immunological and protective functions of the host. Development of microbial populations in the digestive tract of higher animals commences soon after birth and involves a complex process of microbial succession and many microbial – host interactions which, eventually resulting in dense, stable microbial populations inhabiting characteristic regions of the gut. The rumen is one of the most extensively studied and well-documented gut ecosystem because of the importance of ruminants (cattle, sheep, goats, camels and yak) to human nutrition and the major role played by rumen microbes in nutrition of the ruminant animal (McSweeney and Mackie, 2012).

Ruminants, through the action of their microbiota, can utilize components that the human body cannot break down, namely lignocellulose. Lignocellulose is the most abundant carbon polymer on the planet, with the rumen having a central role in releasing this vast energy store. The rumen ultimately uses lignocellulose to make products (i.e. milk and meat) that are then available to humans to consume as a nutrient dense food source. The interaction between the host and microbes in the rumen is synergistic, in that the host provides heat, moisture and food, while the microorganisms produce protein and by-products of digestion, such as the aforementioned VFAs, for use by the host.The degradation of microbes by the host has also been described in literature, with microbes utilised for their protein, lipid and starch content.The complex rumen ecosystem consists of bacteria, archaea, ciliate protozoa, fungi, bacteriophage and viruses (Matthews *et al*., 2019).

Bacteria are the most abundant microbes in the foregut of ruminant animals, with approximately 10^{10} – 10^{11} cells/ml and over 200 species.The composition of the bacteria found in the rumen is dictated by a number of factors including preference for certain substrates (i.e. diet), energy requirements, and resistance to certain metabolic end-products that may be toxic to some species (Matthews *et al*., 2019). The rumen bacterial composition is mainly Gram negative when animals are being fed high forage diets, with more Gram positive bacteria, such as Lactobacillus, present in animals fed high grain diets, with ruminal pH levels dropping after the consumption of easily fermented carbohydrates (Oetzel, 2003).

Due to the high forage diets of ruminants, particularly those in receipt of grass-based diets, cellulose digesters are an important part of supplying the animal with vital nutrients. These bacteria degrade cellulose and hemicellulose, the main components of plant fibre.The ability to degrade cellulose is strongly dependant on the type of forage, crop maturity and the accessibility of the cellulolytic bacterial communities.The matrix of plant fibre is complex, composed of β-1, 4 linked glucose residues for cellulose and β-1, 4 linked xylose for hemicellulose, requiring the coordination of a number of hydrolytic enzymes in order to break it down.Although there are many cellulose degrading bacteria, Fibrobacter succinogenes and Ruminococcus albus are the most desirable cellulose degraders. Their ability to digest cellulose is much higher than that of other cellulolytic bacteria (Koike and Kobayashi, 2009).

Fermentation end products of cellulolytic bacteria include acetate, butyrate, propionate and CO2. Hydrogen, ethanol, succinic acid, formic acid and lactic acid are also formed but are quickly used by other bacteria.Starch is also an important constituent of the ruminant diet, in particular for highly productive dairy cows. High grain diets result in an increase in the amount of starch in the rumen. Streptococcus bovis, an amylolytic bacterium, is normally present in low numbers in cows fed high forage diets or cows adapted to grain diets over a course of time and in high abundance in un-adapted cows that consume high grain diets. S. bovis has a lower pH optimum for growth than many other bacteria, and its high abundance following consumption of high grain diets is attributed to a sudden increase in glucose levels in the rumen and the loss of protozoa due to the more acidic environment created by high grain diets (Santos, *et al*., 2014).

More specifically, lactic acid is produced from starch and, as lactic acid is not metabolized by the animal, it is instead absorbed through the rumen wall causing an increase in lactic acid in the blood and reduced blood P^H. If the diet of the animal is changed too quickly, there is also an accumulation of VFAs found in the rumen, having a detrimental effect on the microbiota and the host animal. These severe and sudden changes lead to a decrease in rumen pH and an increase in S. bovis and Lactobacillus species.Some anaerobic bacteria acquire energy from the degradation of pectin, with the most important pectinolytic species, Lachnospira multiparus, Prevotella ruminicola and Butyrivibrio fibrisolvens, being capable of reducing pectin to oligogalacturonides, yielding large quantities of acetate, a VFA important in bovine metabolism.Citrus by-products such as citrus pulp are used widely in ruminant feeding systems and contain a high percentage of pectin substances (Santos, *et al*., 2014).

Archaea, in general, have a broad spectrum of unusual and distinctive metabolisms, enabling them to survive in a variety of different environments. Rumen archaea are strictly anaerobic and are the only known microorganisms present in the rumen capable of producing methane.Such archaea are referred to as methanogens. Archaea are found in the rumen in the range of 10^6 to 10^8 cells per ml, accounting for less than 4% of the microbial community.Archaea are found at the bottom of the trophic chain due to their need to use the end products of fermentation as substrates.The domain Archaea is broken into two different kingdoms; Euryarchaeota, consisting of methanogens and extreme halophiles, and Crenarchaeota, consisting of hyperthermophiles and nonthermophiles.Methanogens found in the kingdom Euryarchaeota require a very low redox potential and are among the strictest anaerobes known (Matthews *et al*., 2019).

According to meta-analysis of global data, 90% of rumen methanogens belong to the following genera(Patra *et al*., 2017); Methanobrevibacter (63.2% of methanogen population), Methanomicrobium (7.7% of methanogen population) Methanosphaera (9.8%) "Rumen Cluster C", now referred to as Thermoplasma (7.4%) and Methanobacterium (1.2%). Most methanogens remove hydrogen gas by reducingCO2 with hydrogen gas to form methane. In contrast, Methanosphaera stadtmanae only produces methane through the reduction of methanol with H2, having one of the strictest energy metabolisms of all methanogenic archaea. Producing methane keeps hydrogen concentrations in the rumen low, allowing methanogens to promote the growth of other species, and enabling a more efficient fermentation.However, methane produced in the rumen is eructated, leading to atmospheric pollution. Efforts to mitigate rumen methane emissions include vaccines (targeting rumen methanogens through the generation of antibodies to selected methanogen antigens that enter via saliva, binding to targets on the methanogens),small-

molecule inhibitors (targets enzymes essential for the growth of methanogens), additives and breeding approaches (Indikova *et al*., 2015).

Ciliate protozoa are found in the range of 10^4 – 10^6cells/ml in rumen fluid and are responsible for 30to 40% of overall fibre digestion.They are alsorelatively active in lipid hydrolysis and can producehydrogen via their hydrosomes.TheEntodinium genus is the most dominant protozoanin high grain diets. This genus rapidly degradesstarch, engulfing it and converting it to an iodophilicstorage polymer.Degradation occursthrough a combination of debranching, amylaseand glucosidase enzymes. More research may beneeded in order to determine their immediate rolein methanogenesis and therefore create a betterunderstanding of the value of manipulating thispopulation as a means of reducing methane emissionsin ruminants (Matthews *et al*., 2019).

Amoebae can represent an important reservoir for bacteria in the environment, but their role in the rumen is unclear. In the vegetative cycle (multiplication by binary fission), amoeba, similar to ciliate protozoa, survive by ingesting bacteria through phagocytosis.While further research is necessary in order to ascertain the role of amoeba in the rumen, it is known that some bacteria can survive phagocytosis by protozoa and live as endosymbionts (Indikova *et al*., 2015).

Rumen fungi (10^3 – 10^6 zoospores/ml) are anaerobic, falling into the class Neocallimastigomycetes, consisting of 6 previously recognised genera (Anaeromyces, Caecomyces, Cyllamyces, Neocallimastix, Orpinomyces and Piromyces) with 21 known species and, using molecular techniques, 2 recently discovered genera Oontomycesand Buwchfawromyces (Dagar *et al*., 2015).

Bacteriophages are obligate pathogens of bacteria and occur in dense populations of approximately 10^7 – 10^9 particles per gram of digesta in the rumen.As is the case for other populations, bacteriophage abundances are also influenced by external sources, meaning they may also be controlled through different strategies. The bacteriophage and virus population found in a sample is referred to as the virome. The high number of rumen bacteriophage suggests that they may have an important function in the balance of the rumen system, but there is little known about the effect of the rumen virome on the system it inhabits. Viruses, however, have been shown to be a driving factor for evolution of many microbial systems in different environments, often facilitating horizontal gene transfer (Matthews *et al*., 2019).

Symbiotic microbiomes can be beneficial to the hosts in many ways, including dietary supplementation, host immune system, and social interactions (Ridley *et al*., 2012). The symbionts need not to be completely dependent on the host and animal-microbial interactions can be flexible and facultative and the host can carry different symbionts at different times (Weiss and Aksoy, 2011). Fermentative microbes, mainly bacteria, hydrolyse plant polymers (starch, cellulose, hemicellulose, pectins and protein) to short oligomers and monomers. These soluble substrates are transported into the microorganism by specific transport mechanisms and fermented, resulting in synthesis of microbial cells and production of fermentation end-products (acetate, propionate, butyrate, carbon dioxide and hydrogen). Hydrogen and formate are produced by many microbes in the rumen where the hydrogen is quantitatively converted to methane by methanogenic archaea, resulting in undetectable or low levels of free hydrogen in the gas phase. Although acetogenic and syntrophic bacteria that also consume hydrogen have been isolated, they are of minor quantitative importance in the rumen. The predominant microorganisms in the rumen are obligate anaerobes. Fermentation of feedstuffs in the rumen yields short-chain volatile fatty acids, primarily acetic, propionic and butyric acids, carbon dioxide, methane, ammonia and occasionally lactic acid. Some of the change in free energy is used to drive microbial growth, but heat is also evolved (McSweeney and Mackie, 2012).

Table 1: Fermentative properties of ruminal bacteria**(**Chiba**,** 2009):

Species	Function*	Products
Fibrobacter (Bacteroides) succinogenes	C, A	F, A, S
Ruminococcus albus	C, X	F, A, E, H, C
Ruminococcus flavefaciens	C, X	F, A, S, H
Butyrivibrio fibrisolvens	C, X , PR	F, A, L, B, E, H, C
Clostridium lochheadii	C, PR	F, A, B, E, H, C
Streptococcus bovis	A, S, SS, PR	L, A, F
Ruminobacter (Bacteroides) amylophilus	A, P, PR	F, A,S
Prevotella (Bacteroides) ruminocola	A, X, P, PR	F, A, P, S

Succinimonas amylolytica	A, D	A, S
Selenomonas ruminantium	A, SS, GU, LU, PR	A, L, P, H, C
Lachnospira multiparus	P, PR, A	F, A, E, L, H, C
Succinivibrio dextrinosolvens	P, D	F, A, L, S
Methanobrevibacter ruminantium	M, HU	M
Methanosarcina barkeri	M, HU	M, C
Treponema bryantii	P, SS	F, A, L, S, E
Megasphaera elsdenii	SS, LU	A, P, B, U, CP, H, C
Lactobacillus sp.	SS	L
Anaerovibrio lipolytica	L, GU	A, P, S
Eubacterium ruminantium	SS	F, A, B, C
Oxalobacter formigenes	O	F, C
Wolinella succinogenes	HU	S, C

* C = cellulolytic; X = xylanolytic; A = amylolytic; D = dextrinolytic; P = pectinoiytic; PR = proteolytic; L = lipolytic; M = methanogenic; GU = glycerol-utilizing; LU = lactate-utilizing; SS = major soluble sugar fermenter, HU = hydrogen utilizer; O = oxalate-degrading.¶ F = formate; A = acetate; E = ethanol; P = propionate; L = lactate; B = butyrate; S = succinate; V = valerate; CP = caproate; H = hydrogen; C = carbon dioxide; M = methane.

Table 2: Summary of physical, chemical, and microbiological characteristics of the rumen ecosystem**(**McSweeney and Ma*ckie*, 2012).

Physical criteria	**Range characteristics**
pH	5.5–6.9 (mean 6.4)
Redox potential	-350 to – 400 mV
Temperature	38-41 °C
Osmolality	250-350 milliosmole/kg -1
Dry matter	10-18%
Chemical criteria	**Range characteristics**
Gas phase (%)	CO2, 65; CH4 27; N2 7; O2 0.6; H2 0.2
Volatile fatty acids (mmol L-1)	Acetate 60-90
Nonvolatile acids (mmol L-1)	Propionate 15-30
Amino acids and oligopeptides	Butyrate 10-25
Ammonia	Branched-chain and higher 2-5
Soluble carbohydrates	Lactate < 10
Insoluble polysaccharides	< 1 mmol L-1 present 2-3 h post feeding
Dietary (cellulose, hemicelluloses, pectin)	2-12 mmol L-1
Endogenous (mucopolysaccharides)	< 1 mmol L-1 present 2-3 h post feeding
Lignin	Always present
Minerals	Always present
Trace elements/vitamins	Always present
Growth factors	High Na; generally good supply
	Always present; good supply of B vitamins
	Good supply; branched-chain fatty acids, long-chain fatty acids, purines, pyrimidines, other unknown
Microbiological* criteria	**Range characteristics**
Bacteria	10^{10}-10^{11} g-1 (> 200 species)
Ciliate protozoa	10^{4}-10^{6} g-1 (25 general)
Anaerobic fungi	10^{3}-10^{5} g-1 (5 general)
Bacteriophage	10^{7}-10^{9} g particles ml-1

*Diversity is based on culturable microbes. *Note:* mmol L = millimole per liter; mV = millivolts.

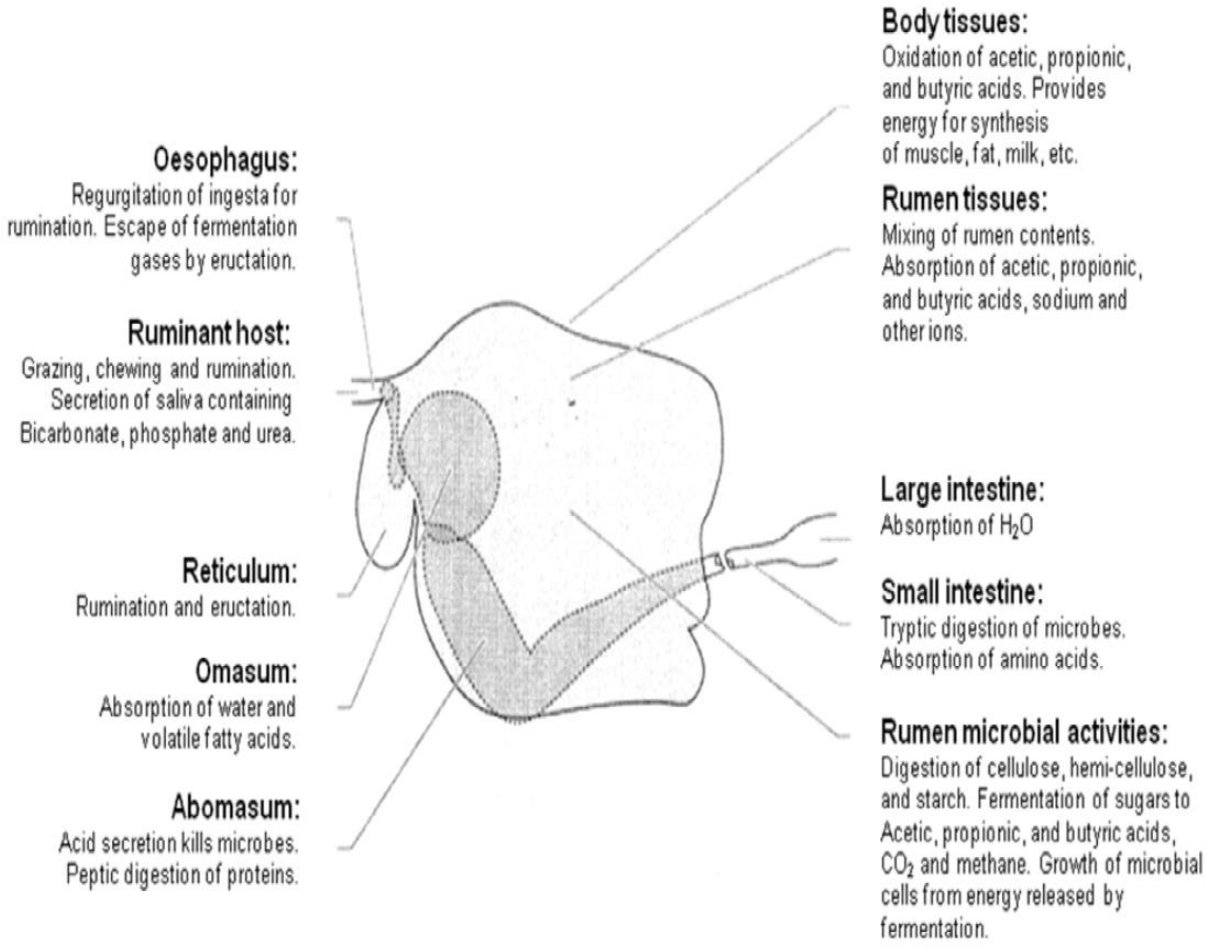

Figure 2: Summary diagram describing interrelationships between the ruminant forestomach, its resident microbial population and the host animal **(**McSweeney and M*ackie*, 2012).

2.3. The Lower-Gut Microbiome

The lower gut is defined as the post-gastric intestinal tract and thus consists of both the small intestine and the hindgut regions. Bacteria are present at levels of 10^{12}–10^{14} cells/ml in the hindgut digesta (cecum, colon, rectum) of cattle. Microbial fermentation in the hindgut may be responsible for up to 30% of cellulose and hemicellulose degradation in ruminants, though smaller figures have also been proposed. Lower dietary energy production in the hindgut compartments is likely due to a combination of factors, including reduced retention time of digesta in the hindgut compartments versus in the rumen, as well as the fact that substrates entering the cecum and colon already have been partially digested by enzymes in the rumen (microbial) and small intestine (host and microbial). The lower-gut microbiota diverge in composition according to intestinal segment, likely reflecting differences in physical, chemical, and biological conditions in each compartment. The jejunum is a major site of post-ruminal protein and carbohydrate digestion and absorption, with *Firmicutes* (up to 90%) being the predominant phyla detected here. The hindgut regions, the cecum and colon, have similar functions, with *Firmicutes* and *Bacteroidetes* dominating their microbial communities. Augmenting the hypothesized importance of the lower-gut microbes to animal performance, several taxa in both the small and large intestine have been related to feed efficiency status,

with abundances of *Butyrivibrio, Pseudobutyrivibrio*, *Prevotella*, *Anaeroplasma*, *Paludibacter*, *Faecalibacterium*, and *Succinivibrio* in the hindgut, and that of *Butyrivibrio* in the jejunum (O'Hara *et al*., 2020).

Fermentation of carbohydrates leads to the production of principally short chain fatty acids (SCFA) resulting in uptake of ammonia as a source of nitrogen for microbial growth. The production of SCFA (mainly acetate, propionate and butyrate) from fermentation of non hydrolysable oligo- and polysaccharides improves gut epithelial cell proliferation, thereby increasing intestinal tissue weight, with changes in mucosal morphology. Several mechanisms are involved in the growth-stimulating role of SCFA on animal intestines. For example, collagenous and non-collagenous protein syntheses in mucosa are both stimulated by butyrate. According to them, butyrate may affect intestinal muscles by directly acting at the molecular level on myocytes. Growth of the intestinal epithelium, which is the largest mucosal surface of the human body and in chicken, is very important since it is heavily implicated in intestinal permeability through regulation by the intestinal epithelial barrier and its intercellular tight junctions (Niba *et al*., 2009).

Interest in the role of commensal gut microflora within the GI tract is presently growing, particularly due the advantage which the fermentative properties of beneficial gut microflora could confer on gut health. Gut microflora could also benefit the host by the fermentation of poorly digestible feed ingredients and the production of short chain fatty acids (SCFA) (Niba *et al*., 2009).Unlike in the rumen, where there remains incongruence over the presence of any robust host immune mechanisms that propagate gut health, the lower-gut regions are highly active in terms of immune function, with the mucosal immune system comprising physical (mucosal/epithelial layers) and chemical (antimicrobial peptides, secretory IgA) barriers, as well as pattern-recognition receptors (for example toll-like receptors, TLRs) and containing a wide array of immune cells that contribute to host defense (Hooper, 2012). As such, with the lower gut regions known to be vital to immune system development in monogastric animals (O'Hara *et al*., 2020).

The primary functions of the GI tract were believed to be limited to the digestion and absorption of nutrients and electrolytes, and to water homeostasis. Excretion of waste products of metabolism and toxic substances and safe containment of micro-organisms present are also functions of the GI tract. However, a better understanding of microbial activity within the GI tract and the introduction of molecular techniques in studies of gut microbial ecology has brought about renewed interest in gut function in relation to microbial activities. GI microflora compete with the host for other nutrients, stimulate rapid turnover of absorptive epithelial cells, require an increased rate of mucus secretion by intestinal goblet cells, and stimulate immune system development and inflammatory responses (Niba *et al*., 2009).

A recent study of functional metagenomic profiles derived from the ileal tissue of *Lactobacillus*-dominant calves showed elevated expression of genes involved in "leukocyte and lymphocyte chemotaxis" and the "cytokine/chemokine-mediated signaling pathway" (Malmuthuge *et al*., 2019). Gut microflora could also benefit the host by the fermentation of poorly digestible feed ingredients and the production of short chain fatty acids (SCFA). For the most part, dietary breakdown takes place through physical and enzymatic digestion by the host animal. The most important part of hydrolysis by enzymes takes place in the small intestines. However, a larger proportion of digestion, which takes place by microbial fermentation in non-ruminants, occurs in the large intestine. Furthermore, fermentation in non-ruminant animals occurs to the largest extent in the large intestines (caecum and colon), mainly due to the longer transit time of the diet in this part of the GI tract. In chicken particularly, a major portion of microbial fermentation is concentrated in the caeca. Therefore, improvements in fermentative activities within the gut will depend on the inclusion of ingredients that can escape the host's digestive enzymes in the small intestines and be available for fermentation by microflora in the large intestines (Niba *et al*., 2009).

3. FACTORS AFFECTING THE ANIMAL MICROBIOME

To begin with all microorganisms were seen as pathogens causing infectious diseases to the host. The host immune system of eukaryotes was built to eliminate these intruders, but at the same time tolerating its own molecules. However, we now know that the association between eukaryotic hosts and the microorganisms is far more complex. With the advances in molecular biology, such as next generation sequencing, it is now possible more specifically to address the association between a host and its microbiome. In animals the association between the host and its microbiome can take many forms and includes symbiotic and pathogenic associations (Weiss and Aksoy, 2011).

The association between the host and the microbiome is affected by a large number of abiotic and biotic factors and can involve the immune system, nutrition, reproduction, communication, and many other systems of the host (Cerf-Bensussan and Gaboriau-Routhiau, 2010; Staubach *et al*., 2013). The number of studies addressing the role of the microbiome on

animal health is limited and almost entirely restricted to human studies. However, a large number of studies have addressed the role of single bacterial symbionts on animal fitness. There is now a growing interest in understanding what factors can affect the microbiome of animals in order to understand how fitness is affected and to explain differences between ecosystems, species, and/or populations. The composition of the bacterial communities of animals including invertebrates and vertebrates seems to be shaped by multiple factors, such as the host genotype, life stage, laboratory rearing, and the ecological and physiological conditions. Further, recent studies have proposed that the microbiome impacts the nutritional supplementation, tolerance to environmental perturbations, and maintenance and/or development of the immune system (Bahrndorff *et al*., 2016).

Diet, genetics, age, gender, and geography are among the determinants of rumen microbial composition and function; however, influence of diet is the best studied to date (O'Hara *et al*., 2020). Studies have also suggested that abiotic factors can affect the microbiome of disease vectors and thus vector competence of the host (Wei *et al*., 2014). The recent interest in the importance of the microbiome on tolerance to environmental perturbations has revealed the presence of single bacterial species and mainly endosymbionts with large impact on, for example, temperature tolerance (Feldhaar, 2011). Temperature can affect the host directly or indirectly through either abundance of the symbiont or efficiency of transmission to the offspring (Prado *et al*., 2009).

The accumulation of acidic fermentation products, such as short-chain fatty acids, is suspected to decrease the luminal pH, leading to changes in microbial composition and damage to the gut epithelium, with detrimental effects on animal productivity and health. While clear relationships between the ruminal microorganisms and acidosis have been demonstrated relationships between hindgut acidosis and the changes of lower-gut microbiota in the ruminant remain poorly understood (O'Hara *et al*., 2020). The composition of the bacteria found in the rumen is dictated by a number of factors including preference for certain substrates (i.e. diet), energy requirements, and resistance to certain metabolic end-products that may be toxic to some species (Matthews *et al*., 2019). Microbial populations can vary with diet! Perhaps, reflecting substrate availability; e.g., Populations of cellulolytic bugs are depressed in animals fed diets rich in grain. Environmental conditions in the "fermentation vat" can have profound effects: Rumen fluid normally has pH between 6 and 7. But, may fall if large amounts of soluble carbohydrate are consumed. If pH drops to about 5.5, protozoal populations become markedly depressed because of acid intolerance. More drastic lowering of rumen pH, as can occur with grain overload, can destroy many species (Chiba, 2009).

4. TECHNIQUES FOR THE IDENTIFICATION OF THE ANIMAL MICROBIOME

4.1. Culture-dependant approaches

Conventional culture-based techniques such as isolation, enumeration, and nutritional characterization have provided significant information on the diversity of the rumen microbiota (Chaucheyras-Durand and Ossa, 2014). The gastrointestinal tract of ruminant animals has a wide range of extremities, making it difficult to replicate conditions for optimal growth. While specific microbes can grow and can be characterised, a large percentage are unculturable in vitro and cannot be grown on laboratory media. Culture-dependant techniques rely on various selective and enrichment culture conditions in order to replicate the microbes' natural environment. Culturing anaerobes is quite difficult due to the need to exclude oxygen, the slow growth of the microbes and the complexity of other growth requirements (Matthews *et al*., 2019).Using the solid phase of rumen contents can pose many problems when attempting to culture microbes. Many microbes adhere to particulate matter and are thus difficult to separate. Methylcellulose solution can be used to encourage detachment of bacteria from feed particles by providing a readily available feed source (Fessenden, 2016).

The overwhelmingly anaerobic microbiota of gut ecosystems could be accessed and studied only with the use of sophisticated anaerobic techniques allowing to create oxygen-free conditions in order for the anaerobes to grow (Tajima and Aminov, 2015). Traditional methods of classifying rumen bacteria were based on the standard bacterial identification methods; morphology, shape and Gram stain. Nutritional requirements and fermentation end products were also used as a means of classification. Roll tubes came to be employed to grow and isolate anaerobic species, and were used instead of conventional agar plates. The phylum Firmicutes and family Lachnospiraceae were the most commonly cultured bacteria from the rumen (Matthews *et al*., 2019).

Rumen fistulation

Stomach tubing

Slaughter

Figure 3: Rumen sample collection (Sinead, 2017).

4.2. Culture-independent approaches

Culture-independent methods, or, more specifically DNA-based methods of identification and detection of microorganisms, allow the examination of microbial communities at a molecular level. Metagenomic analysis allows the description of a microbial community by high throughput sequencing technology. Methods associated with this type of analysis include 16S rRNA and Internal Transcribed Spacer (ITS) amplicon sequencing, used for bacterial and fungal communities, respectively, or, shotgun sequencing, where DNA fragments are sequenced randomly, regardless of the microbe from which they came. Targeting of the mcrA gene has also been suggested in recent studies as a means of identifying methanogens (Luton *et al*., 2016). The molecular microbial ecology methods used were 16S rRNA hybridization, competitive PCR, denaturing gradient gel electrophoresis (DGGE), 16S rDNA clone libraries, real-time PCR (also called quantitative PCR, qPCR) and metagenomic Analysis (Tajima and Aminov, 2015).

Although these methods identify unculturable microbes, they do not provide a strain for further study. They may, however, provide insights that would allow the culturing of species that, thus far, have not been cultured. Understanding community structure is an important part in the recognition of how microorganisms are affected by the environment which they live in and how they in turn affect the host organism. DNA sequencing technologies have transformed research of microbial and animal ecosystems. They have completely changed the approach involved in identifying microorganisms and the limitations, outlined above, associated with culture-dependant studies. Several platforms are available to achieve results; these differ only in small details, and all follow the same basic protocol of template preparation and clonal amplification, followed by rounds of parallel sequencing (Reuter *et al*., 2015).

16S rRNA amplicon sequencing requires the use of primers in order to identify the presence of specific bacteria and archaea. 18S target regions can also be used in identification of protozoa but may run the risk of amplifying bovine DNA, thereby affecting results. The ITS region found in fungi is the most widely used region to study the ecology of fungi. Various primers have been designed in order to yield the highest diversity. 16S sequencing is useful as it provides a good phylogenic marker and there is a large database of sequences to assist in data analysis. It can identify highly conserved regions that are identical in all bacteria, with the use of a single primer pair. However, primer specificity is key and lack thereof will contribute to bias in detection of a target organism.

Often, it is difficult to choose or design a primer that is specific to diverse groups, such as Firmicutes (Chaucheyras-Durand and Ossa, 2014).Incorrect PCR conditions, such as improper annealing temperatures during the amplification step in 16S, may result in false amplification. Additionally, 16S rRNA sequencing only gives information on bacterial populations, not fungal, viral, protozoa or amoeba (Matthews *et al*., 2019).

5. CONCLUSIONS

The microbiome of hosts, also known as microflora or microbiota, are all the microorganisms inhabiting a specific environment. Gut microbial communities, composed of bacteria, ciliate and flagellate protozoa, anaerobic fungi, and bacteriophages, play a vital role in nutritional, physiological, immunological, and protective functions of the host. Volatile fatty acids, principally acetate, propionate, and butyrate, are the major products of rumen microbial fermentation and are absorbed and used as energy sources by the host. The interaction between the host and microbes in the rumen is synergistic, in that the host provides heat, moisture and food, while the microorganisms produce protein and by-products of digestion, such as the aforementioned Volatile fatty acids, for use by the host. Bacteria are the most abundant microbes in the foregut of ruminant animals followed by ciliate protozoa. Lower dietary energy production in the hindgut compartments is likely due to a combination of factors, including reduced retention time of digesta in the hindgut compartments as well as the fact that substrates entering the cecum and colon already have been partially digested by enzymes. The association between the host and the microbiome is affected by a large number of abiotic and biotic factors and the consequence of the factors should be studied in order to minimize negative consequences on the host. Cultural identification of the anaerobic microbiota of gut ecosystems could be accessed and studied only with the use of sophisticated anaerobic techniques allowing to create oxygen-free conditions in order for the anaerobes to grow. Culture-independent methods, or, more specifically DNA-based methods of identification and detection of microorganisms, should be carried out at a molecular level as most of the organisms are difficult to culture.

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