



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating (2026): 4.61

VET 2026; 11(6): 53-61

© 2026 VET

www.veterinarypaper.com

Received: 22-03-2026

Accepted: 27-04-2026

Dr. Rajamanickam Kandasamy
Assistant Professor, Department
of Veterinary Physiology and
Biochemistry, Veterinary College
and Research Institute, Salem,
Tamil Nadu, India

Rameshkumar G
Assistant Professor, Department
of Veterinary Physiology and
Biochemistry, Veterinary College
and Research Institute, Theni,
Tamil Nadu, India

Dr. Deepika B
Assistant Professor, Department
of Veterinary Physiology and
Biochemistry, Veterinary College
and Research Institute,
Orathanadu, Tamil Nadu, India

Dr. Siva M
Assistant Professor, Department
of animal Nutrition, Veterinary
College and Research Institute,
Namakkal, Tamil Nadu, India

Dr. Senthilkumar S
Professor, Department of animal
Nutrition, Veterinary College
and Research Institute,
Namakkal, Tamil Nadu, India

Corresponding Author:

Dr. Rajamanickam Kandasamy
Assistant Professor, Department
of Veterinary Physiology and
Biochemistry, Veterinary College
and Research Institute, Salem,
Tamil Nadu, India

Metagenomic insights into rumen microbiome dynamics for enhanced livestock productivity and sustainable rumen manipulation

**Rajamanickam Kandasamy, Rameshkumar G, Deepika B, Siva M and
Senthilkumar S**

DOI: <https://www.doi.org/10.22271/veterinary.2026.v11.i6a.3318>

Abstract

Ruminant animals function as symbiotic superorganisms in which complex microbial communities within the rumen play a pivotal role in digestion, metabolism, and overall host physiology. Traditional culture-dependent approaches have provided limited insights into this ecosystem due to the inability to cultivate the majority of rumen microorganisms. The advent of metagenomics has revolutionized the study of rumen microbiology by enabling culture-independent analysis of microbial diversity, genetic potential, and functional dynamics. This article explores the principles and methodologies of metagenomics, including sequence-based and functional approaches, and highlights their application in understanding rumen microbial ecology. Metagenomic analyses have facilitated the identification of novel carbohydrate-active enzymes (CAZymes), improved knowledge of feed efficiency, and provided insights into methane emissions and microbial detoxification of plant metabolites. Furthermore, the integration of multi-omics approaches offers deeper understanding of microbial interactions and metabolic pathways. Overall, metagenomics serves as a powerful tool for targeted rumen manipulation, aiming to enhance feed utilization, improve animal productivity, and reduce environmental impact, thereby supporting the development of sustainable livestock production systems.

Keywords: Rumen microbiome, metagenomics, feed efficiency, methane mitigation

Introduction

The Ruminant as a Symbiotic Superorganism

Ruminant animals represent one of the most sophisticated examples of symbiotic integration in biological systems, functioning as “superorganisms” in which the host and its resident microbiota operate as a unified metabolic entity. The gastrointestinal tract of herbivorous animals, encompassing both vertebrates and certain invertebrates, harbors highly diverse and dynamic microbial populations that are indispensable for host survival, nutrition, and physiological homeostasis. These microbial consortia perform essential biochemical transformations that the host organism is incapable of executing independently, thereby expanding the metabolic repertoire of the host far beyond its genomic capacity.

A defining feature of herbivorous animals, particularly ruminants, is their inability to produce endogenous cellulolytic enzymes required for the degradation of complex plant structural polysaccharides such as cellulose, hemicellulose, and lignin. Consequently, these animals rely extensively on their gut microbiota to enzymatically hydrolyze lignocellulosic biomass into fermentable substrates. Through microbial fermentation processes occurring primarily in the rumen, complex carbohydrates are converted into volatile fatty acids (VFAs), including acetate, propionate, and butyrate, which serve as the principal energy sources for the host (Flint *et al.*, 2008, Morgavi *et al.*, 2013) ^[2, 11]. In addition to carbohydrate metabolism, rumen microorganisms play a critical role in nitrogen utilization by synthesizing microbial protein from non-protein nitrogen (NPN) compounds such as urea, biuret, and uric acid. This unique capability allows ruminants to thrive on nitrogen-deficient diets and contributes significantly to their nutritional efficiency (Morgavi *et al.*, 2013) ^[11].

Beyond these fundamental roles, the rumen microbiota is involved in a wide spectrum of metabolic and ecological functions that influence host health and productivity. These include lipid metabolism, vitamin synthesis, immune modulation, and the detoxification of plant secondary metabolites. Microbial communities also participate in maintaining rumen homeostasis by regulating pH and redox balance through intricate interspecies interactions. The rumen ecosystem itself is a highly complex, anaerobic fermentation chamber comprising bacteria, archaea, protozoa, anaerobic fungi, and viruses, all of which interact synergistically to sustain efficient digestion and nutrient cycling (Henderson *et al.*, 2016) [4].

In recent decades, there has been increasing scientific and industrial interest in understanding the functional dynamics of rumen microbial communities, particularly in the context of lignocellulose degradation and detoxification of anti-nutritional phytochemicals. These processes are not only fundamental to animal nutrition but also hold significant implications for biotechnology, bioenergy production, and environmental sustainability. With the global demand for livestock-derived products rising steadily due to population growth and changing dietary preferences, improving the efficiency of ruminant production systems has become a critical priority. Enhanced understanding of rumen microbial ecology offers the potential to optimize feed utilization, improve animal performance, and reduce the environmental footprint of livestock systems.

However, traditional approaches to studying rumen microbiology have been constrained by methodological limitations. Historically, knowledge of rumen microbial populations has been derived primarily from culture-dependent techniques. While these methods have provided valuable insights into the physiology and metabolism of certain microbial species, they suffer from a significant limitation known as the “cultivation bias.” It is estimated that only a small fraction—approximately 10-15%—of rumen microorganisms can be cultured under standard laboratory conditions. This leaves the vast majority of microbial diversity unexplored, resulting in an incomplete understanding of the rumen ecosystem and its functional potential (Amann *et al.*, 1995) [1].

The advent of molecular biology techniques has revolutionized the study of microbial ecology by enabling culture-independent analysis of microbial communities. Among these, metagenomics has emerged as a particularly powerful and transformative approach. Metagenomics involves the direct extraction and analysis of total genomic DNA from environmental samples, thereby providing access to the collective genetic material of entire microbial communities. This approach eliminates the need for cultivation and allows for comprehensive characterization of both the taxonomic composition and functional capabilities of the microbiome.

Metagenomic studies typically encompass a series of steps, including DNA extraction, sequencing, assembly, and functional annotation. Early metagenomic investigations relied on clone library construction and Sanger sequencing; however, recent advancements in high-throughput sequencing technologies have dramatically increased the scale and resolution of microbial analysis. Modern platforms, coupled with sophisticated bioinformatics tools, enable the generation of massive datasets that provide detailed insights into microbial diversity, gene abundance, and metabolic pathways (Handelsman *et al.*, 1998) [3].

Although DNA-DNA hybridization was historically regarded as a gold standard for microbial identification, it has largely been supplanted by more efficient and scalable techniques such as polymerase chain reaction (PCR)-based 16S rRNA gene sequencing. This method allows for rapid and accurate identification of microbial taxa based on conserved genetic markers. When combined with next-generation sequencing and advanced computational pipelines, it facilitates high-resolution profiling of complex microbial communities. Improvements in sequencing chemistry, genome assembly algorithms, and annotation frameworks have further enhanced the accuracy and reliability of metagenomic analyses, enabling researchers to reconstruct near-complete microbial genomes and predict their functional roles with minimal bias. One of the most significant advantages of metagenomics is its ability to capture the full spectrum of microbial diversity within the rumen, including both dominant and low-abundance populations. While dominant microbial groups constitute the “core microbiome” responsible for essential metabolic functions, rare taxa often serve as a reservoir of genetic diversity and functional redundancy. These less abundant organisms can play critical roles in adapting to dietary changes, environmental stressors, and the presence of toxic compounds. The identification and characterization of these rare microbial members have been greatly facilitated by deep sequencing approaches, which provide extensive coverage of microbial communities.

Furthermore, metagenomics enables the discovery of novel genes, enzymes, and biosynthetic pathways with significant biotechnological potential. These include enzymes involved in lignocellulose degradation, antibiotic production, stress tolerance, and xenobiotic metabolism. The ability to identify and exploit such genetic resources has far-reaching implications not only for animal nutrition but also for industrial applications such as biofuel production and waste management. Despite its many advantages, metagenomics also presents challenges, particularly in the analysis and interpretation of large-scale sequencing data. The complexity of microbial communities, coupled with the vast volume of genomic information generated, necessitates the use of advanced bioinformatics tools and computational resources. Nevertheless, ongoing developments in data analysis methodologies continue to improve the accuracy and efficiency of metagenomic studies.

In addition to its role in enhancing feed efficiency and animal productivity, the rumen microbiome has important environmental implications. One of the major concerns associated with ruminant livestock is the production of enteric methane, a potent greenhouse gas generated during microbial fermentation. Methanogenic archaea utilize hydrogen and carbon dioxide to produce methane, representing an energy loss for the host and contributing to global climate change. Understanding the microbial pathways involved in methanogenesis is therefore essential for developing strategies to mitigate methane emissions. In this context, the integration of metagenomics with other “omics” approaches, such as metatranscriptomics, metaproteomics, and metabolomics, offers a comprehensive framework for studying the functional dynamics of the rumen ecosystem. These integrative approaches enable researchers to move beyond static descriptions of microbial communities and gain insights into their active metabolic processes and interactions with the host.

Overall, the concept of the ruminant as a symbiotic superorganism underscores the importance of the rumen

microbiome as a central determinant of animal health, productivity, and environmental sustainability. Advances in metagenomics have fundamentally transformed our understanding of this complex ecosystem, providing unprecedented insights into its structure and function. By leveraging these technologies, it is now possible to transition from descriptive studies to targeted manipulation of the rumen microbiome, paving the way for the development of more efficient, resilient, and sustainable livestock production systems.

Metagenomics: Beyond the Limitations of Cultivation

Traditional culture-based methods, while foundational, have reached a point of diminishing returns. The "cultivation barrier" is severe: less than 15% of rumen bacteria are accessible via traditional Hungate techniques. High-resolution data from the Hungate 1000 project confirms that our existing culture collections represent a mere 3.7% of the estimated total microbial numbers in the rumen. This knowledge gap renders traditional methods insufficient for the precision requirements of modern livestock strategy.

The concept of metagenomics was first introduced by Jo Handelsman and colleagues in 1998, marking the emergence of a transformative approach in microbial ecology. Metagenomics has since evolved into a rapidly expanding and multidisciplinary field within environmental biology, aimed at exploring the collective genomes of microbial communities directly from their natural habitats (Handelsman *et al.*, 1998) [3]. This approach overcomes the limitations associated with traditional cultivation techniques and enables comprehensive analysis of microbial diversity, genetic variation, and functional potential. It also provides critical insights into host-microbe interactions, cellular organization, metabolic pathways, and the overall contribution of microbiomes to the physiology of livestock and poultry systems.

Several alternative terminologies are often used interchangeably with metagenomics, including environmental genomics, ecogenomics, community genomics, and megagenomics, all of which emphasize the study of microbial assemblages in their ecological context. Early metagenomic investigations, particularly those conducted in the early 2000s, relied heavily on conventional Sanger sequencing technologies. These studies involved the construction of large-insert genomic libraries such as bacterial artificial chromosomes (BACs), fosmids, and cosmids to capture environmental DNA fragments for subsequent analysis.

In the context of rumen microbiology, initial metagenomic efforts were primarily directed toward the identification of enzymes involved in polysaccharide degradation, reflecting the central role of microbial communities in lignocellulose breakdown. One of the pioneering studies in this area utilized an expression-based metagenomic library derived from dairy cattle rumen samples, leading to the identification of multiple clones exhibiting hydrolytic activity (Qin *et al.*, 2010, Hess *et al.*, 2011) [14, 5]. Since then, extensive research has been conducted, resulting in the discovery of numerous novel fibrolytic enzymes from the rumen metagenome, significantly advancing our understanding of microbial contributions to fiber digestion.

The standard workflow of metagenomic analysis involves several critical steps. These include the collection of representative samples from specific ecological niches, followed by the enrichment of low-abundance microbial populations when necessary. Subsequently, total genomic DNA is extracted from the microbial community, which is

then used to construct metagenomic libraries. These libraries undergo systematic screening and analysis to identify genes of interest and to characterize the functional and taxonomic composition of the microbial ecosystem. Collectively, these methodologies provide a comprehensive framework for investigating complex microbial communities and their roles in diverse biological processes.

Metagenomics data analysis: From Raw Sequences to Functional Insights

The genetic potential of the rumen microbiome is primarily explored using two complementary methodological frameworks: (i) functional metagenomics, which involves high-throughput screening of expression libraries derived from rumen metagenomic DNA, and (ii) sequence-based approaches, which focus on the comprehensive characterization of microbial genomes and gene expression profiles at both DNA (metagenomics) and RNA (metatranscriptomics) levels. These approaches collectively provide insights into the diversity, structure, and functional organization of rumen microbial communities (Figure 1).

Functional metagenomics is centered on the investigation of the collective genetic material of microbial consortia through heterologous expression systems. This involves the extraction of environmental DNA from rumen samples, followed by cloning into suitable vectors such as plasmids, cosmids, fosmids, or bacterial artificial chromosomes (Seshadri *et al.*, 2018, Thomas *et al.*, 2012) [15, 16]. These recombinant constructs are subsequently introduced into surrogate host organisms, most commonly *Escherichia coli*, to enable gene expression. Screening of these expression libraries using activity-based assays facilitates the identification of clones exhibiting desired biochemical functions, such as enzymatic activity or metabolite production. This strategy is particularly valuable for accessing genetic resources from unculturable microorganisms, thereby expanding the scope of functional discovery within the rumen ecosystem.

In contrast, sequence-based metagenomics—often referred to as computational metagenomics—enables in-depth analysis of microbial community composition and functional potential through high-throughput sequencing technologies. Two principal sequencing strategies are employed in this context: small subunit ribosomal RNA (SSU rRNA) gene sequencing (e.g., 16S or 18S rRNA), which provides taxonomic resolution, and whole genome shotgun (WGS) sequencing, which offers a comprehensive view of gene content, metabolic pathways, and functional capabilities. These approaches allow for the identification of microbial taxa, prediction of protein functions, and elucidation of metabolic networks within the rumen microbiome, thereby enhancing our understanding of its structural and functional dynamics.

Sequence-Based Analysis

Sequence-driven metagenomic analysis represents a powerful approach for identifying genes, enzymes, or biomolecules of interest based on sequence similarity and phylogenetic relationships. This methodology involves large-scale sequencing of environmental DNA, often generating extensive datasets comprising millions to billions of sequence reads. Such datasets require robust bioinformatics pipelines and computational tools for effective processing, assembly, annotation, and interpretation.

Metagenomic sequences are typically analyzed through two main strategies. The first involves screening for specific genetic markers or conserved domains associated with known

biochemical functions, enabling targeted identification of genes linked to particular metabolic activities. The second strategy relies on comparative analysis against curated reference databases to assign taxonomic identities and predict gene functions based on sequence homology. This dual approach supports both hypothesis-driven and exploratory analyses, facilitating the discovery of novel genes alongside the characterization of known metabolic pathways.

A range of bioinformatics tools has been developed to support metagenomic data analysis. For instance, MEGAN (MEtaGenome ANalyzer) enables taxonomic and functional interpretation of sequencing datasets by mapping reads to reference databases (Meyer *et al.*, 2008) [9]. Similarly, MG-RAST (Metagenomics Rapid Annotation using Subsystem Technology) provides a web-based platform for automated processing, annotation, and comparative analysis of metagenomic data. The application of such tools has facilitated the identification of numerous novel enzymes, including cellulases and glycoside hydrolases, from rumen

and gastrointestinal microbial communities, highlighting their functional diversity and biotechnological potential.

Function-Driven Analysis

Functional metagenomics provides a complementary, activity-based approach to sequence analysis by enabling the direct identification of genes encoding specific biochemical functions. This strategy involves cloning environmental DNA into suitable expression vectors, followed by transformation into a compatible host organism, typically *E. coli*. Recombinant clones are then screened using functional assays to detect desired enzymatic or metabolic activities. The workflow of functional metagenomic analysis generally includes three major steps: collection of rumen samples, construction of metagenomic DNA libraries, and high-throughput screening of individual clones. Advances in screening technologies, including substrate-specific assays and high-throughput platforms, have significantly enhanced the efficiency and sensitivity of this approach.

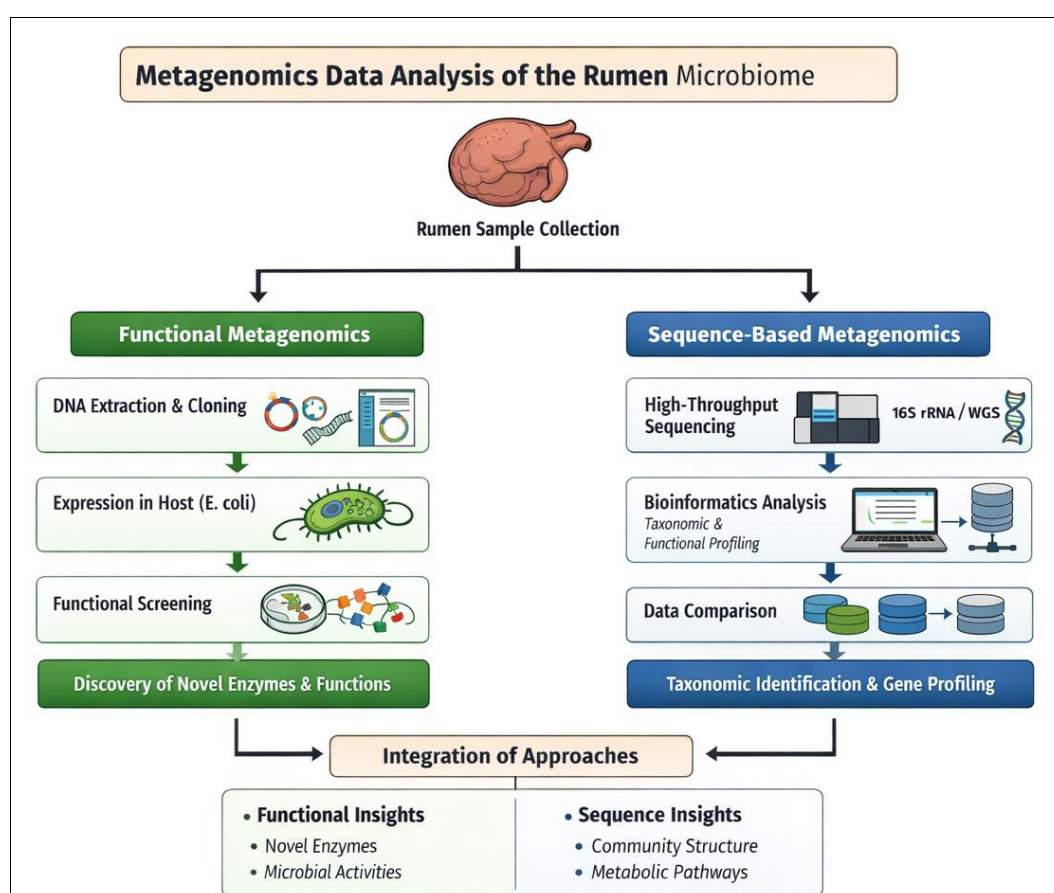


Fig 1: Rumen microbiome metagenomics analysis flowchart

One of the key advantages of functional metagenomics is its ability to identify genes encoding novel proteins that may not exhibit detectable sequence similarity to known genes in existing databases. This enables the discovery of entirely new enzyme classes and metabolic functions that would otherwise remain undetected using sequence-based methods alone. Through such activity-guided screening, a wide range of biologically important molecules has been identified, including novel antibiotics, hydrolytic enzymes, and resistance determinants.

Within the rumen ecosystem, functional metagenomic studies have been particularly successful in uncovering carbohydrate-active enzymes (CAZymes) involved in the degradation of complex plant polysaccharides. These enzymes play a critical

role in lignocellulose breakdown and have significant applications in animal nutrition and industrial biotechnology. For example, novel xylanases belonging to the glycoside hydrolase family 10 (GH10) have been isolated from rumen microbial communities, demonstrating endo- β -1,4-xylanase activity and potential utility in the production of prebiotic xylooligosaccharides.

Overall, the integration of sequence-based and function-driven metagenomic approaches provides a comprehensive framework for exploring the genetic and functional diversity of rumen microbiomes. Together, these methodologies enable a deeper understanding of microbial ecosystem dynamics and support the discovery of novel biomolecules with applications in agriculture, industry, and environmental sustainability.

Application in rumen manipulation

The rumen microbiome is a highly complex and dynamic ecosystem dominated primarily by bacteria, followed by eukaryotes, archaea, and viruses, with Bacteroidetes and Firmicutes being the most abundant phyla and Prevotella the predominant genus (Jami *et al.*, 2012) [7]. A relatively conserved core microbiome exists across ruminants, although diversity varies among species such as cattle, goats, and sheep. Host genetics significantly influences microbial composition, as seen in differences between indigenous and crossbred cattle, including variations in the Firmicutes-to-Bacteroidetes ratio, methanogenic archaea, and protozoal populations. Spatial variation within the rumen (solid vs

liquid fractions) further affects microbial distribution, while diet-particularly high-concentrate versus low-concentrate feeding-plays a crucial role in shaping microbial communities and their metabolic functions. High-concentrate diets favour starch-utilizing microbes and enzymes while reducing fiber-degrading populations and cellulolytic activity. These microbial shifts are accompanied by changes in metabolic pathways, especially enhanced glycolysis and volatile fatty acid production (Palmonari *et al.*, 2024) [12]. Overall, rumen microbial diversity and interactions are governed by a combination of host genotype, diet, and microenvironment, ultimately influencing digestion efficiency, metabolism, and livestock productivity (Table 1).

Table 1: Characterization of Rumen Microbial Diversity and Interactions by metagenomics

Category	Details	Key Findings / Examples	Significance
Microbial Domains in Rumen	Major groups of microorganism's present	Bacteria (dominant) > Eukaryota > Archaea > Viruses & Phages	Indicates bacteria as primary drivers of rumen metabolism
Dominant Bacterial Phyla	Most abundant phyla across ruminants	<i>Bacteroidetes</i> , <i>Firmicutes</i>	Core contributors to digestion and fermentation
Other Important Phyla	Additional microbial diversity	<i>Proteobacteria</i> , <i>Spirochaetes</i> , <i>Fibrobacteres</i> , <i>Verrucomicrobia</i> , <i>Synergistetes</i> , <i>Actinobacteria</i>	Reflects complexity and functional diversity
Dominant Genera	Highly abundant bacterial genera	<i>Prevotella</i> (most dominant), <i>Bacteroides</i> , <i>Clostridium</i>	Key role in carbohydrate and protein metabolism
Core Rumen Microbiome	Conserved microbial structure	~8 phyla, 11 classes, 15 families	Indicates stable microbial ecosystem
Species Comparison (Cattle, Goat, Sheep)	Diversity across species	Total ~22 phyla, 94 families detected	Species-specific microbial adaptation
	Average phyla per animal	Cattle: 16.8, Goat: 11.8, Sheep: 16.9	Reflects variation in microbial richness
Host Genotype Effect	Influence of breed/genetics	Indigenous vs crossbred cattle differences	Host genetics shapes microbiome
	Example (Vechur vs Crossbred)	Higher Firmicutes: Bacteroidetes ratio in Vechur	Linked to higher fat production
	Archaeal composition	Dominated by methanogenic <i>Euryarchaeota</i>	Important for methane production
	Protozoal variation	<i>Entodinium</i> (Vechur), <i>Entamoeba</i> (crossbred)	Indicates host-specific microbial niches
	Diversity comparison	Higher microbial diversity in indigenous cattle	May contribute to resilience and efficiency
Breed-Based Differences (Advanced Study)	Microbial variation by genotype	Japanese Black vs crossbred cattle	Genotype influences microbiome structure
	Cellulolytic bacteria	<i>Fibrobacter succinogenes</i> , <i>Ruminococcus</i> spp. higher in some breeds	Enhances fiber digestion
	Functional genes	Enrichment of cellulose & hemicellulose degradation genes	Improved feed utilization
Rumen Phase Variation	Solid vs liquid fraction differences	Solid: <i>Fibrobacteres</i> , <i>Spirochaetes</i> ; Liquid: <i>Actinobacteria</i>	Spatial heterogeneity in rumen
	Genera distribution	Liquid: <i>Prevotella</i> , <i>Tannerella</i> ; Solid: <i>Butyrivibrio</i> , <i>Blautia</i>	Functional niche specialization
	Variation impact	Solid vs liquid variation (14.9%) > diet (10.5%)	Phase has stronger influence than diet
Dietary Effects (HC vs LC)	High concentrate vs low concentrate diets	HC alters microbial composition significantly	Diet is a major modulator
	Increased in HC diet	<i>Eubacterium</i> , <i>Clostridium</i> , <i>Ruminococcus</i> , <i>Lactobacillus</i> , <i>Bifidobacterium</i>	Promotes starch-utilizing microbes
	Decreased in HC diet	<i>Prevotella</i> , <i>Fibrobacter succinogenes</i> , <i>Bacteroides</i>	Reduces fiber-degrading microbes
Enzymatic Changes (CAZymes)	Carbohydrate-active enzymes variation	↑ GH13, CBM26, CBM34 (starch digestion)	Enhances starch metabolism
	Decreased enzymes	↓ GH5, GH9, GH51 (cellulases)	Reduced fiber degradation
Metabolic Pathways	Changes in fermentation pathways	Increased PK, PFOR, LDH enzymes	Enhanced pyruvate & lactate metabolism
	End products	Increased volatile fatty acids (VFAs)	Improved energy supply to host
Overall Interpretation	Integrated microbiome dynamics	Influenced by host, diet, and rumen environment	Critical for productivity & health

Identification of novel enzymes by metagenomics approach

Rumen microorganisms play a pivotal role in the degradation of plant biomass through the synthesis of a wide spectrum of

carbohydrate-active enzymes (CAZymes). These enzymes are systematically classified into four principal groups—glycoside hydrolases (GHs), glycosyl transferases (GTs), polysaccharide lyases (PLs), and carbohydrate esterases

(CEs)—based on conserved sequence features, structural organization, and catalytic mechanisms (Lombard *et al.*, 2014) [8]. Acting synergistically, these enzyme classes mediate the breakdown of complex plant polysaccharides such as cellulose, hemicellulose, and pectin, thereby enabling ruminants to efficiently utilize fibrous feed resources.

Metagenomic and functional analyses have significantly advanced the identification and characterization of these enzyme systems, revealing their metabolic versatility and highlighting their potential applications in industrial biotechnology, including biofuel production and enzyme engineering (Table-2).

Table 2: CAZyme Classes, Functions, and Metagenomic Insights in Rumen

CAZyme Class	Full Form	Primary Function	Substrate Target	Key Families / Examples	Metagenomic Findings	Significance / Application
GHs	Glycoside Hydrolases	Hydrolysis of glycosidic bonds in carbohydrates	Cellulose, hemicellulose, oligosaccharides	GH2, GH3, GH5, GH9, GH10, GH11, GH43, GH51	Most abundant CAZymes in rumen metagenome; majority of sequences belong to GH families; GH2, GH3, GH43 dominant	Essential for plant biomass degradation; widely used in biofuel and feed industries
GTs	Glycosyl Transferases	Formation of glycosidic bonds (biosynthesis of polysaccharides)	Sugars, glycan chains	Various GT families	Less abundant in rumen metagenome; identified via sequence-based analysis	Important in carbohydrate metabolism and microbial cell wall synthesis
PLs	Polysaccharide Lyases	Cleavage of polysaccharides via non-hydrolytic mechanism	Pectin and uronic acid-containing polysaccharides	Pectate lyases	Identified in smaller numbers (e.g., single genes in studies)	Important for pectin degradation and plant cell wall breakdown
CEs	Carbohydrate Esterases	Removal of ester linkages between lignin and polysaccharides	Lignin-carbohydrate complexes	CE6 and others	Multiple esterases detected via functional metagenomics	Enhance accessibility of cellulose and hemicellulose
CBMs	Carbohydrate Binding Modules	Facilitate binding of enzymes to substrates	Cellulose, hemicellulose	CBM26, CBM34, CBM37	Identified in metagenomic libraries; CBM37 linked to <i>Ruminococcus albus</i>	Improve enzyme efficiency and substrate interaction

Metagenomic investigations have identified several key enzyme functions associated with carbohydrate metabolism. These include carbohydrate-binding modules (CBMs), which facilitate enzyme-substrate interactions; carbohydrate esterases (CEs), which cleave ester linkages between lignin and polysaccharides; glycoside hydrolases (GHs), responsible for hydrolyzing glycosidic bonds in complex carbohydrates; glycosyl transferases (GTs), which catalyze the formation of glycosidic linkages; and polysaccharide lyases (PLs), which are primarily involved in pectin degradation. Collectively, these enzymes contribute to the efficient depolymerization and utilization of plant-derived substrates within the rumen environment.

Functional metagenomic studies have provided valuable insights into the diversity of CAZymes. For instance, screening of rumen metagenomic libraries has led to the identification of multiple enzymatic activities, including endoglucanases, esterases, and cyclodextrinases. Shotgun metagenomic approaches have further revealed a wide array of genes encoding glycoside hydrolases, carbohydrate-binding modules, carbohydrate esterases, and pectate lyases, particularly from fiber-adherent microbial populations. Notably, a relatively small proportion of sequences show high similarity to previously characterized CAZymes, indicating the presence of a substantial reservoir of novel enzymes within the rumen microbiome (Lombard *et al.*, 2014) [8].

Among the identified enzyme families, glycoside hydrolases such as GH2, GH3, GH5, GH9, GH29, and GH39 are major contributors to cellulolytic activity, whereas GH43, GH51, and GH78 are primarily associated with hemicellulose degradation. However, cellulase-associated families such as GH5 and GH9 constitute only a minor fraction of the total glycoside hydrolase pool, while hemicellulase-related families (GH10, GH11, GH26, GH28) collectively represent a small proportion as well. The broad diversity of enzyme sources reflects contributions from multiple microbial taxa, suggesting functional redundancy within the rumen

ecosystem. This redundancy likely serves as an adaptive mechanism, ensuring the essential fermentation processes even when specific microbial populations are affected by environmental changes such as dietary shifts or physiological stress. This functional resilience also explains the relative stability of rumen fermentation patterns despite alterations in diet.

Dietary composition plays a critical role in modulating CAZyme abundance. An increase in dietary roughage has been associated with a higher relative abundance of CAZyme classes, reflecting the enrichment of microbial populations specialized in degrading complex plant polysaccharides. Among CAZymes, oligosaccharide-degrading enzymes are particularly abundant in the rumen metagenome. These include members of GH families such as GH2, GH3, GH5, GH10, GH11, GH36, GH43, GH51, and GH95, with GH2, GH3, and GH43 being especially prevalent (Wang *et al.*, 2013) [18]. This high abundance is consistent with the structural complexity of hemicellulose, which contains diverse side chains requiring a variety of enzymes for complete degradation.

Comparative analyses of glycoside hydrolases and cellulosome-associated genes suggest that initial colonization of plant fiber in the rumen is primarily carried out by microorganisms producing enzymes that target easily accessible side chains of polysaccharides, particularly those belonging to GH2 and GH3 families. In contrast, enzymes responsible for degrading more recalcitrant main chains, such as cellulose (e.g., GH5 and GH9), are less abundant. This indicates a sequential degradation process, where side-chain removal precedes the breakdown of core polysaccharide structures. Carbohydrate-binding modules (CBMs) also play a crucial role by enhancing substrate accessibility and enzyme efficiency through effective binding. For example, CBM37 has been predominantly associated with *Ruminococcus albus*, a key cellulolytic bacterium, and is known for its ability to bind a wide range of polysaccharides in association with

enzymes such as xylanases and esterases.

Functional metagenomic approaches have further enabled the discovery of diverse CAZyme genes across different ruminant species. Examples include β -glucosidases (GH3) and esterases (CE6) from cattle, as well as cellodextrinases from buffalo. Large-scale studies have identified tens of thousands of putative carbohydrate-active genes within the rumen microbiome, with a significant proportion demonstrating enzymatic activity against cellulosic substrates. Additionally, novel enzymes with desirable industrial properties—such as thermostability, pH tolerance, and substrate specificity—have been identified, including esterases, xylanases, lipases, and laccases capable of lignin degradation.

Beyond ruminants, similar metagenomic strategies applied to other livestock systems have revealed additional carbohydrate-active enzymes. For instance, metagenomic analysis of gastrointestinal samples from pigs fed high-fiber diets has led to the identification of multiple genes encoding cellulases, hemicellulases, polygalacturonases, mannanases, and chimeric enzymes, further underscoring the vast diversity and functional potential of microbial enzyme systems in digestive ecosystems (Pope *et al.*, 2010) [13]. In summary, the rumen microbiome represents a rich reservoir of diverse and functionally redundant CAZymes that collectively drive the efficient degradation of plant biomass. These enzymes not only sustain ruminant nutrition but also hold significant promise for industrial and environmental applications.

Gut Microbiota, Feed Efficiency, and Methane Emissions: A Metagenomic Perspective

Investigations into feed efficiency in poultry have demonstrated a strong association between gut microbiota composition and host performance. In a study involving 252 laying hens, comparative analysis using 16S rRNA gene sequencing revealed distinct microbial profiles between birds with divergent feed efficiencies. The caecal microbiota exhibited significantly higher diversity compared to the duodenum and fecal microbiota and showed the most pronounced differences between efficiency groups. Among the differentially abundant taxa, *Lactobacillus* emerged as a key genus, with increased abundance in birds exhibiting superior feed efficiency, along with *Akkermansia*, while *Faecalibacterium* was comparatively reduced. Functional predictions indicated an enrichment of pathways associated with carbohydrate and amino acid metabolism within the caecal microbiome of efficient birds, underscoring the pivotal role of the cecum in nutrient utilization and suggesting the potential application of *Lactobacillus* as a probiotic to enhance performance (Hook *et al.*, 2010) [6].

Complementary insights from shotgun metagenomic analyses of fecal microbiota in birds with contrasting feed conversion ratios (FCR) indicated that bacterial populations dominate the gut ecosystem, accounting for more than 95% of the total microbiota, with minor contributions from eukaryotes, archaea, and viruses. At the phylum level, Proteobacteria, Firmicutes, and Bacteroidetes were consistently predominant. However, variations were observed between efficiency groups, with low-FCR (more efficient) birds exhibiting a higher abundance of Proteobacteria, whereas high-FCR birds showed relatively greater proportions of Firmicutes and Bacteroidetes. Functional annotation revealed broadly similar distributions of genes involved in core metabolic processes such as carbohydrate, amino acid, and protein metabolism across groups. Nonetheless, genes associated with stress response, virulence factors, and cell wall biosynthesis were

generally abundant, while pathways related to sulfur metabolism and flagellar motility were particularly enriched in low-FCR birds, indicating subtle functional differences linked to feed efficiency (Yan *et al.*, 2017) [19].

Methane Emissions and Rumen Microbiome Interactions

Enteric fermentation in ruminants represents a major biological source of methane (CH₄), positioning livestock as one of the leading contributors to anthropogenic greenhouse gas emissions. Methane production, along with nitrogenous waste generated by rumen microorganisms, not only contributes to environmental concerns such as global warming but also represents a loss of feed energy, thereby reducing production efficiency. Consequently, understanding the interplay between rumen microbial composition and host genetics is critical for developing strategies aimed at mitigating methane emissions while maintaining sustainable livestock productivity.

The integration of metagenomics with microbial network analysis has emerged as a powerful approach for characterizing microbial diversity and deciphering complex host-microbe and microbe-microbe interactions. Such approaches provide insights into both the functional potential (DNA level) and active metabolic processes (RNA level) within the rumen ecosystem. Early efforts to establish a link between the rumen metagenome and methane emissions demonstrated that metagenomic profiles could be used to predict methane yield with moderate accuracy. Subsequent studies employing combined metagenomic and metatranscriptomic approaches revealed that variation in methane production is more closely associated with the expression levels of methanogenesis-related genes rather than their mere abundance. Specifically, genes involved in methane metabolic pathways, including those encoding subunits of methyl-coenzyme M reductase and enzymes associated with the CO₂/H₂ reduction pathway, were found to be more actively expressed in high methane-emitting animals (Zhang *et al.*, 2022) [20]. These findings suggest that targeting gene expression may offer a viable strategy for methane mitigation.

In contrast, other studies have reported that the abundance of methanogenesis-related genes at the genomic level is also significantly higher in animals with elevated methane emissions, indicating that both gene abundance and expression contribute to methane output. Integrated multi-omics analyses have further identified specific microbial taxa associated with reduced methane production. For example, *Sharpea* and *Megasphaera* species are more prevalent in low methane-emitting animals and are associated with metabolic pathways involving sugar transport, lactate production and utilization, as well as propionate and butyrate formation. These microbes facilitate a metabolic shift in which carbohydrates are initially fermented to lactate and subsequently converted to butyrate, thereby reducing hydrogen availability for methanogenesis.

Comparative metagenomic studies across different ruminant species and environmental conditions have also provided valuable insights. Animals adapted to high-altitude environments, such as yaks and Tibetan sheep, typically exhibit lower methane emissions and possess microbiomes enriched in genes related to energy and carbohydrate metabolism. In contrast, high methane-emitting animals display a greater abundance of genes associated with methanogenic pathways, including both hydrogenotrophic (CO₂/H₂) and methylotrophic routes (Zhang *et al.*, 2022) [20].

Overall, these findings highlight that metagenomic and metatranscriptomic approaches are essential for understanding both the functional capacity and active metabolic processes of rumen microbial communities (Wallace *et al.*, 2019) [17]. Such knowledge provides a foundation for developing targeted strategies to reduce methane emissions, improve feed efficiency, and enhance the sustainability of ruminant production systems.

Microbial detoxification of anti-nutritional phytometabolites

Herbivorous animals possess a highly specialized gastrointestinal microbiome that has evolved diverse metabolic functions enabling the detoxification of plant secondary metabolites, many of which can exert toxic effects when consumed in large quantities. Numerous bacterial taxa, along with certain fungal species capable of degrading anti-nutritional factors, have been isolated from the digestive tracts of different herbivores. Advances in metagenomic approaches have significantly enhanced the identification of microbial communities and functional genes involved in the biotransformation and detoxification of plant-derived compounds. For example, the detection of phytase-encoding genes within the rumen metagenome of buffalo highlights the microbial contribution to phosphorus mobilization and utilization (Mootapally *et al.*, 2016) [10]. Likewise, metagenomic characterization of the gastrointestinal microbiota of Stephen's woodrat (*Neotoma stephensi*) demonstrated region-specific functional specialization, with the cecum exhibiting an increased abundance of genes associated with the degradation of oxalates and phenolic compounds. Collectively, these observations emphasize the critical role of gut microbiota in facilitating the adaptation of herbivores to diets rich in potentially toxic plant metabolites.

Conclusion: Future Prospects in Rumen Manipulation

The livestock industry must transition toward a "Rumen Microbial Gene Catalogue" to standardize the analysis of high-resolution datasets. The data confirms that our current culture-based knowledge (Hungate 1000) covers less than 4% of the community, necessitating a massive expansion of MAG-based references. Future strategic frontiers include: Organoids: 3D tissue models to study host-microbe signaling in a controlled environment. Ramanome Technology: Single-cell functional analysis to determine metabolic activity without the need for cultivation. The integration of these computational and biological tools into management systems will yield a new generation of ruminants: more robust, highly efficient, and with a significantly reduced environmental footprint.

Conflict of Interest

Not available.

Financial Support

Not available.

References

1. Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological Reviews*. 1995;59(1):143-169. <https://doi.org/10.1128/mr.59.1.143-169.1995>
2. Flint HJ, Bayer EA, Rincon MT, Lamed R, White BA. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nature Reviews. Microbiology*. 2008;6(2):121-131. <https://doi.org/10.1038/nrmicro1817>
3. Handelsman J, Rondon MR, Brady SF, Clardy J, Goodman RM. Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. *Chemistry & Biology*. 1998;5(10):R245-R249. [https://doi.org/10.1016/s1074-5521\(98\)90108-9](https://doi.org/10.1016/s1074-5521(98)90108-9)
4. Henderson G, Cox F, Ganesh S, Jonker A, Young W, Global Rumen Census Collaborators, *et al.* Erratum: Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Scientific Reports*. 2016;6:19175. <https://doi.org/10.1038/srep19175>
5. Hess M, Sczyrba A, Egan RB, Kim TW, Chokhawala H, Schroth G, *et al.* Metagenomic discovery of biomass-degrading genes and genomes from cow rumen. *Science* (New York, N.Y.). 2011;331(6016):463-467. <https://doi.org/10.1126/science.1200387>
6. Hook SE, Wright AD, McBride BW. Methanogens: methane producers of the rumen and mitigation strategies. *Archaea* (Vancouver, B.C.). 2010;2010:945785. <https://doi.org/10.1155/2010/945785>
7. Jami E, Mizrahi I. Composition and similarity of bovine rumen microbiota across individual animals. *PloS One*. 2012;7(3):e33306. <https://doi.org/10.1371/journal.pone.0033306>
8. Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B. The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Research*. 2014;42(Database issue):D490-D495. <https://doi.org/10.1093/nar/gkt1178>
9. Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M, *et al.* The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics*. 2008;9:386. <https://doi.org/10.1186/1471-2105-9-386>
10. Mootapally CS, Nathani NM, Patel AK, Jakhesara SJ, Joshi CG. Mining of Ruminant Microbial Phytase (RPHY1) from Metagenomic Data of Mehsani Buffalo Breed: Identification, Gene Cloning, and Characterization. *Journal of Molecular Microbiology and Biotechnology*. 2016;26(4):252-260. <https://doi.org/10.1159/000445321>
11. Morgavi DP, Kelly WJ, Janssen PH, Attwood GT. Rumen microbial (meta)genomics and its application to ruminant production. *Animal: An International Journal of Animal Bioscience*. 2013;7 Suppl 1:184-201. <https://doi.org/10.1017/S1751731112000419>
12. Palmonari A, Federiconi A, Formigoni A. Animal board invited review: The effect of diet on rumen microbial composition in dairy cows. *Animal: An International Journal of Animal Bioscience*. 2024;18(10):101319. <https://doi.org/10.1016/j.animal.2024.101319>
13. Pope PB, Denman SE, Jones M, Tringe SG, Barry K, Malfatti SA, *et al.* Adaptation to herbivory by the Tammar wallaby includes bacterial and glycoside hydrolase profiles different from other herbivores. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;107(33):14793-14798. <https://doi.org/10.1073/pnas.1005297107>
14. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, *et al.* A human gut microbial gene

- catalogue established by metagenomic sequencing. *Nature*. 2010;464(7285):59-65.
<https://doi.org/10.1038/nature08821>
15. Seshadri R, Leahy SC, Attwood GT, Teh KH, Lambie SC, Cookson AL, *et al.* Cultivation and sequencing of rumen microbiome members from the Hungate1000 Collection. *Nature Biotechnology*. 2018;36(4):359-367.
<https://doi.org/10.1038/nbt.4110>
 16. Thomas T, Gilbert J, Meyer F. Metagenomics - a guide from sampling to data analysis. *Microbial Informatics and Experimentation*. 2012;2(1):3.
<https://doi.org/10.1186/2042-5783-2-3>
 17. Wallace RJ, Sasson G, Garnsworthy PC, Tapio I, Gregson E, Bani P, *et al.* A heritable subset of the core rumen microbiome dictates dairy cow productivity and emissions. *Science Advances*. 2019;5(7):eaav8391.
<https://doi.org/10.1126/sciadv.aav8391>
 18. Wang L, Hatem A, Catalyurek UV, Morrison M, Yu Z. Metagenomic insights into the carbohydrate-active enzymes carried by the microorganisms adhering to solid digesta in the rumen of cows. *PloS One*. 2013;8(11):e78507.
<https://doi.org/10.1371/journal.pone.0078507>
 19. Yan W, Sun C, Yuan J, Yang N. Gut metagenomic analysis reveals prominent roles of *Lactobacillus* and cecal microbiota in chicken feed efficiency. *Scientific Reports*. 2017;7:45308.
<https://doi.org/10.1038/srep45308>
 20. Zhang Q, Guo T, Wang X, Zhang X, Geng Y, Liu H, *et al.* Rumen Microbiome Reveals the Differential Response of and Emissions of Yaks to Feeding Regimes on the Qinghai-Tibet Plateau. *Animals: An Open Access Journal from MDPI*. 2022;12(21):2991.
<https://doi.org/10.3390/ani12212991>

How to Cite This Article

Kandasamy R, Rameshkumar G, Deepika B, Siva M, Senthilkumar S. Metagenomic insights into rumen microbiome dynamics for enhanced livestock productivity and sustainable rumen manipulation. *International Journal of Veterinary Sciences and Animal Husbandry*. 2026;11(6):53-61.

Creative Commons (CC) License

This is an open-access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 International (CC BY-NC-SA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.