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- Review -

Recent Application Technologies of Rumen Microbiome Is the Key to Enhance Feed Fermentation

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Rumen microbiome consists of a wide variety of microorganisms, such as bacteria, archaea, protozoa, fungi, and viruses, that are in a symbiotic relationship in a strict anaerobic environment in the rumen. These rumen microbiome, a vital maker, play a significant role in feed fermentation within the rumen and produce different volatile fatty acids (VFAs). VFAs are essential for energy metabolism and protein synthesis of the host animal, even though emission of methane gas after feed fermentation is considered a negative indicator of loss of dietary energy of the host animal. To improve rumen microbial efficiency, a variety of approaches, such as feed formulation, the addition of natural feed additives, dietary feed-microbes, etc., have taken to increase ruminant performance. Recently with the application of high-throughput sequencing or next-generation sequencing technologies, especially for metagenomics and metatranscriptomics of rumen microbiomes, our understanding of rumen microbial diversity and function has significantly increased. The metaproteome and metabolome provide deeper insights into the complicated microbial network of the rumen ecosystem and its response to different ruminant diets to improve efficiency in animal production. This review summarized some recent advances of rumen microbiome techniques, especially "meta-omics," viz. metagenomic, metatranscriptomic, metaproteomic, and metabolomic techniques to increase feed fermentation and utilization in ruminants.

Key words : Feed fermentation, metaomics, next-generation sequencing, rumen microbiome

Introduction

Ruminants play important role in the production of meat, milk, wool, and leather. So, ruminants are reared under a diverse range of farming systems and environments and are fed a wide variety of diets to improve their production. Ruminants have a complex digestive system and digestion of feed takes place initially in the rumen [52]. Rumen microbiome consists of a wide variety of microorganisms such as bacteria, archaea, protozoa, fungi and virus that are in a symbiotic relationship in a strict anaerobic environment in the rumen [32, 81]. There are more than 200 species of rumen bacteria and their population range is 10^{10} to 10^{11} per g. Anaerobic fungi in the rumen are classified into 6 genera with the range population of 10^3 to10^6 per g, rumen methanogen population is up to 10^9 per g, whole bacteriophage and ciliate protozoa having population ranges of 10^7 to10^9 per g and 10^4 to 10^6 per g, respectively [101]. Bacteria population are most actively involved in the plant fiber degradation, as revealed by the fact that bacteria associated with feed particles account for nearly 50% to75% of the total microbial population [74]. Anaerobic fungi degrade lignocellulosic components of the feed particles. They constitute the smallest proportion (only about 20%) of the rumen microbial biomass [87]. Rumen protozoa play an important role in fiber digestion and modulation of the fermentation profiles by slowing down the production of acids that lower rumen pH [98], benefiting the rumen.

The main end products of fermentation are volatile fatty acids (VFAs) and microbial biomass, which are absorbed by host ruminant and used in energy metabolism and protein synthesis [40]. A brief overview of rumen microbial feed fermentation and their fermentation products are presented in Fig. 1 and Table 1. The other advantages of rumen fermentation are a microbial synthesis of important vitamins and amino acids. Some of the microbes in the rumen utilize the by-products produced during fermentation to produce

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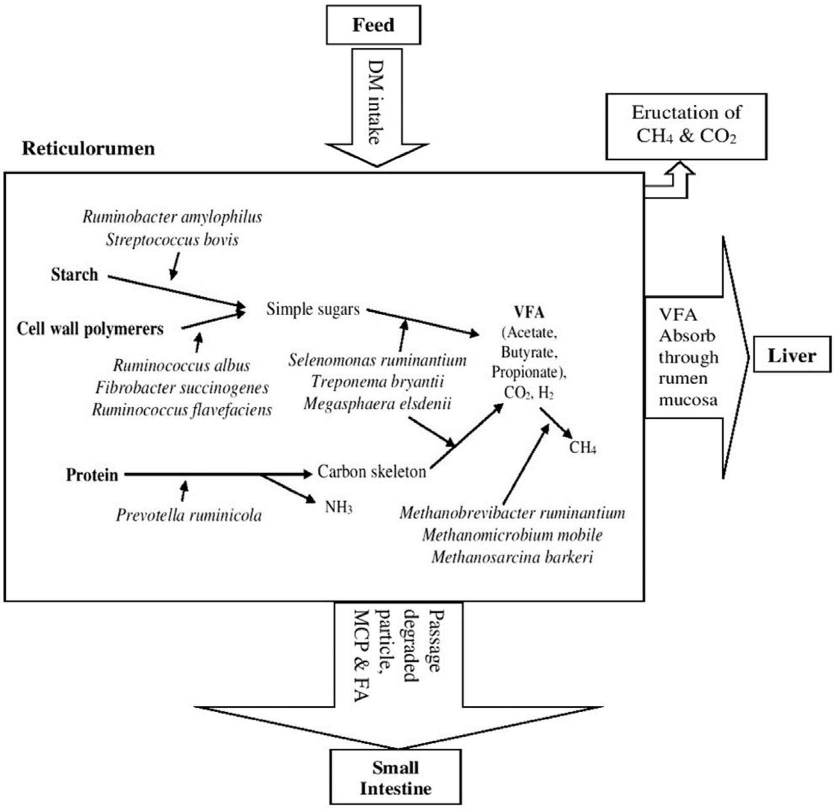

Fig. 1. Overview of rumen microbial feed fermentation and fermentation products. DM: dry matter; VFA: volatile fatty acid; CH4: methane; CO2: carbon dioxide; NH3: ammonia; MCP: microbial crude protein; FA: fatty acids.

methane. Methane production is performed by a group of bacteria known as methanogens. As methane involves in the removal of carbon from the rumen, increased methane production indicates poor animal's performance. This methane eructated by ruminants and represents 2 to 12% dietary gross energy lost to the animal [52] and contributes as a greenhouse gas (GHG) with a global warming potential 28-fold that of carbon dioxide [41] which is responsible for anthropogenic GHG emissions up to 16%[42].

Several studies have been conducted to find out the host-microbial interactions in the rumen reporting associations between the rumen microbiota and feed efficiency in beef [1] and dairy [62, 84] cattle, methane emission in cattle and sheep [2, 3, 94], milk production in dairy cows [2, 3]. Changes in diet influenced the rumen microbiome, methane concentration, and methanogen diversity and abundance in cattle [56]. Dietary microbes, probiotics, also have the potential effect on rumen fermentation and animal performance [22]. In past, our knowledge of rumen biodiversity was limited and entirely dependent on the anaerobic culturing approach. However, recent advancements in molecular techniques especially "meta-omics" created a great scope for the rumen microbiome study.

Though rumen microbiome has played a vital role in ruminant, the knowledge is still limited how rumen microbiome improves feed fermentation or digestibility as well as to reduce methane production. The present review summarizes the knowledge regarding recent advance of rumen microbiome to improve the feed fermentation which enhances our understanding of rumen ecosystems and help to find out possible new approaches that improve microbial fermentation and feed efficiency.

Rumen microbial diversity and feed efficiency

Diet notably affects the microbiome by increasing or decreasing the microbial population into the rumen ecosystem [61, 76, 89]. The rumen microbiome plays a significant role in the metabolism of ruminants via producing 70% of daily energy requirement of animal [4]. So, several managements have been taken to maximize the microbial fermentation in ruminants in order to increase the available VFAs for the host and/or to decrease methane production [78]. Studies have revealed that the rumen microbiome influence feed efficiency in cattle through VFAs production [35]. A very recent study has also reported that variation in volatile fatty acids production, controlled by the rumen microbiota, proportionally related to feed efficiency in cows [93]. Also, differences in the rumen microbial groups that involve in vari-

Table 1. Some important rumen microbes and their fermentation products

Types of microbes	Name of microorganisms	Fermentation products	References
Cellulose-degrading bacteria	*Fibrobacter succinogenes*	Succinate, Acetate, Formate	[46]
	Ruminococcus albus	Acetate, Formate, H_2, CO_2	[72]
	Butyrivibrio fibrisolvens	Acetate, Formate, Lactate, Butyrate, H_2, CO_2	[102]
	Clostridium lochheadii	Acetate, Formate, Butyrate, H_2,CO_2	[102]
Amylolytic bacteria	*Selenomonas ruminantium*	Acetate, Propionate, Lactate	[15]
	Succinomonas amylolitica	Acetate, Propionate, Succinate	[15]
	Streptococcus bovis	Lactate	[14]
	Bacteriodes ruminicola	Formate, Acetate, Succinate	[69]
	Ruminobacter amylophilus	Formate, Acetate, Succinate	[69]
Proteolytic bacteria	*Prevotella ruminicola*	NH_3, VFA	[99]
Lipolytic bacteria	*Anaerovibrio lipolytica*	Acetate, Propionate	[27]
Lactate-degrading bacteria	*Selenomonas lactilytica*	Acetate, Succinate	[7]
	Megasphaera elsdenii	Acetate, Propionate, Butyrate, Valerate, H_2, CO_2	[7]
Lactic acid-utilizing bacteria	*Megasphaera elsdenii*	Lactate	[16]
Pectin-degrading bacteria	*Lachnospira multiparus*	Acetate, Formate, Lactate, H_2, CO_2	[21]
Ruminal archaea (methanogens)	*Methanobrevibacter ruminantium* *Methanomicrobium mobile*	CH_4 (of H_2+CO_2 or Formate)	[37, 105]
Cellullolytic protozoa	*Enoploplastron triloricatum* *Eudiplodinium maggii* *Diploplastron affine* *Epidiniume caudatum* *Diplodinium monacanthum* *Diplodinium pentacanthum*	Reducing sugars	[12]
Proteolytic protozoa	*Entodinium caudatum*	NH_3, VFA	[45]
	Eudiplodinium medium	NH_3, VFA	[25]
Cullulolytic fungi	*Neocallimastix frontalis*	Lactate, Formate, Acetate, Succinate, Ethanol	[75]
	Piromyces communis	Celobiose, celooligosacarides	[17]
	Orpinomyces joyonii	Glucose	[36]

H_2: hydrogen, CO_2: carbondioxide, CH_4: methane, VFA: volatile fatty acid, NH_3: ammonia

ous fermentation pathways may have contributed to the differences in the production of VFAs, which eventually impact on feed efficiency of ruminants. Several others studies have been conducted to assess rumen fermentation as well as CH_4 emission with feeding management. Dietary supplementation of illite at 1% in a dietary dry matter (DM) as a feed additive has a potential effect on increasing VFA production as well as reducing CH_4 emission from Hanwoo steers [5]. The most effective way to reduce CH_4 is to optimize the dietary formulation. Methane emissions from ruminants can be mitigated through proper selection of feed ingredients to be used in the formulation of diets [54]. Different feed ratios considerably affect rumen fermentation especially on pH, ammonia-nitrogen, CH_4, butyric acid, VFA and other metabolite concentrations and microbiome. So, balanced protein and carbohydrate ratios are essential for rumen fermentation [49].

Natural feed additives, rumen microbiome and feed fermentation

As a management strategy to improve animal health and performance, feed additives are commonly used with ruminant feeds [79]. After the banding of antibiotics as growth promoter, several natural feed additives such as plant secondary metabolites, probiotics, and enzymes are using now. Earlier study showed that natural feed additives have significant effect of feed fermentation (Table 2). The alternative supplemental products especially plant extract viz. tannin, saponin, and essential oils significantly increased total microbial population, total volatile fatty acid (VFAs) pro-

Table 2. List of some important natural feed additives and their impact on rumen microbiome and feed fermentation

Natural feed additives	Impacts on rumen microbiome and feed fermentation	References
Dicarboxylic Acids: Aspartate, fumarate and malate	- Reduce rumen CH4 production - Improve the animal energy balance. - Preventing the drop in ruminal pH usually 1 to 2 hr after feeding high-concentrate diets.	[8, 63, 67]
Tanin: Condensed and hydrolysable tannin	- Improved Dry matter intake. - Increased average daily weight gain. - A huge reduction in severity of bloat by reducing microbial activities, biofilm production and ruminal gas production. - Reduced rate of in vitro gas production was also reported. - The decrease in ruminal methane production. - Significant reduction in total number of protozoa at increasing level of tannin inclusion.	[73, 85, 88]
Saponin: Triterpenoid saponin, tea saponin, methanol extract saponin	- A significant reduction of protozoa population in the rumen. - Increase bacterial population. - Increase in microbial biomass with increasing inclusion level. - Increased short-chain fatty acids production at 48 h with increasing inclusion level.	[38, 39, 104]
Probiotics: - *Bacteria (Bacillus, Bifidobacterium, Enterococcus, Lactobacillus, Ruminococcus, Propionibacterium, Megasphaera elsdenii, Prevotella bryantii, etc.)* - Yeast (*Saccharomyces cerevisiae*)	- Increase the total microbial population. - Increases total VFAs production. - Reduces CH_4 production.	[11, 48, 53, 55, 64, 105]
Enzymes: Lysozyme	- Improve *in vitro* rumen fermentation and reduce CH_4 emission.	[6]

VFAs: volatile fatty acids, CH_4: methane

duction, average daily weight gain, milk production and decreased protozoal population and methane production [38, 39, 73, 85, 88, 104]. The highly promising essential oil was *Allium arenarium* oil (garlic oil) which significantly reduced methane production both *in vivo* and *in vitro* by 12% and 36%, respectively [58]. Also, a very recent study strongly supported the earlier report that plant secondary metabolites (PSMs) has significant role on rumen fermentation, CH4 production and rumen bacterial community composition [51]. Probiotics, dietary feed-microbes, are the single or mixed cultures of live microorganisms, which when administered in adequate amounts, confer a health benefit on the host [24]. It was also defined as non-pathogenic and nontoxic live microorganisms that are capable of exerting a beneficial effect on the host animals at the appropriate dosage [23]. Probiotics in particular for ruminants include direct-fed microbes such as bacterial species including *Bacillus, Bifidobacterium, Enterococcus, Lactobacillus, Propionibacterium, Megasphaera elsdeniiand Prevotella bryantii* and yeast (*Saccharomyces cerevisiae*) [90]. Probiotics strengthen the existing rumen microbiome and contribute to improve rumen fermentation and feed efficiency [11, 48, 105]. It may also block the growth of pathogenic organisms, stimulate the immune system through secretion of bacteriocin and modulate microbial balance in the gastrointestinal tract [53]. Some researchers also provided strong support in favor of dietary probiotics. Fumarate reducing bacteria changes the rumen microbial diversity by helping ruminal fermentation and reducing CH_4 emission [65]. Addition of *Lactobacillus mucosae* and cell-free supernatant during the in vitro fermentation of dried brewers grain increases the VFA production and increase the total bacterial population [95]. Likewise, *Enterococcus faecium* SROD increases total VFAs as well as reduces CH_4 production in in-vitro rumen fermentation [53]. In addition to this, lysozyme supplementation may improve in-vitro rumen fermentation and reduce CH_4 emission [6].

The recent advance of rumen microbiome techniques

Among the diverse rumen microbiomes, relatively few of these have been successfully characterized so far based on conventional culture-based methods. Recently with the application of next-generation sequencing (NGS) technologies for studying rumen microbiomes, our understanding of rumen microbial diversity and function has been significantly increased. Recent microbial molecular techniques, especially, quantitative real-time PCR (qRT-PCR) [13, 34, 43, 44, 100, 107] and next-generation sequencing (also called high-throughput sequencing) techniques viz. 454 pyrosequencing [3, 26, 57, 64, 86, 106, 107], Illumina [3, 10, 13, 29-31, 43, 47, 59, 66, 77, 96], Pacific Biosciences (PacBio) [20, 97] and Ion Torrent platform [34, 50, 83, 102], are being successfully used to monitor the population and community composition of ruminal microbes. Recent advances on rumen microbiome and feed fermentation studies focused on "metaomics" technologies viz. metagenomic, metatranscriptomic, metaproteomic and metabolomic studies based on the genome, transcriptome, proteome, and metabolome respectively [9, 60, 70, 71, 82]. The meta-omics and some of their impacts on rumen microbiome and feed fermentation are presented in Table 3.

Through metagenomics, we knew that rumen microbiome acts as a significant component which influences weight gain and to enhances better understanding of microbial ecology as well as host factors that will improve feed efficiency [59]. Metagenomics revealed that the rumen microbial population, as well as microbial community composition, was different between host species [43]. A metagenomics of the camel rumen's microbiome identifies the major microbes responsible for lignocelluloses degradation and fermentation [59]. Metatranscriptome sequencing reveals insights into the gene expression and functional potential of rumen wall bacteria [10] and made a linkage between the active rumen microbiome and feed efficiency in beef cattle [47]. Also, the active bacterial and eukaryotic fibrolytic microbes of rumen of dairy cattle having mixed diet were revealed by metatranscriptomics [44]. The metaproteome and metabolome provide deeper insights into the complicated microbial network of the rumen ecosystem and its response to different animal diets to improve efficiency in animal production. The metaproteomic techniques including 2D SDS-PAGE [80] and mass spectrometric analysis (shotgun peptide sequencing) [18] has potentials for a more complete understanding of the rumen ecosystem which provides complementary information to the other omics technologies. Metaproteomic profiles of rumen samples revealed that Bacteriodetes, Firmicutes and Proteobacteria were the most highly abundant taxonomic phyla in the rumen, which resembled with the most abundant taxonomic phyla determined by16S rRNA studies [33].

Metabolomics study also developed rapidly with the ad-

Table 3. Meta-omics and their impact on rumen microbiome and feed fermentation

Type of omics	Target	Advanced detection techniques	Impact on rumen microbiome and feed fermentation	References
Metagenomics	Genome	DNA sequencing by NGS platform	Revealed the rumen microbial population, as well as microbial community composition and their linkage to improve feed efficiency.	[43, 59]
Metatranscriptomes	Transcriptome	RNA sequencing by NGS platform	Made a linkage between the active rumen microbiome and feed efficiency in ruminant. Also reveals insights into the gene expression and functional potential of rumen wall bacteria.	[10, 44, 47]
Metaproteomics	Proteome	Mass spectrometric analysis	Proteome profiles revealed taxonomic phyla in the rumen which were resembled with taxonomic phyla determined by16S rRNA studies. Also revealed metabolic pathways of some microbes.	[19, 33]
Metabolomics	Metabolome	GC-MS, LC-MS, CE-MS, DFI-MS/MS, ICP-MS, ^{1}H NMR spectroscopy	Identified and quantified different metabolites in rumen samples and discovered some strong relationships between metabolites and certain microbes in the rumen.	[28, 90, 91]

NGS: next-generation sequencing, GC-MS: gas chromatography-mass spectrometry, LC-MS: liquid chromatography-mass spectrometry, CE-MS: capillary electrophoresis-mass spectrometry, DFI-MS/MS: direct flow injection tandem mass spectroscopy, ICP-MS: inductively coupled plasma mass spectroscopy, 1H NMR: proton nuclear magnetic resonance vances of analytical methods of mass spectrometry (MS) and high-resolution nuclear magnetic resonance (NMR) spectroscopy. Quantitative analysis of metabolites has possible by using Gas Chromatography-Mass spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS) or Capillary Electrophoresis-Mass spectrometry (CE-MS) [28]. However, a combination of proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, GC-MS, and direct flow injection tandem mass spectroscopy (DFI-MS/MS) techniques identified and quantified 93 metabolites in rumen samples [90]. Likewise, a combined use of NMR spectroscopy, inductively coupled plasma mass spectroscopy (ICP-MS), GC-MS, DFI-MS/MS and lipidomics with computer-aided literature mining identified 246 ruminal fluid metabolites or metabolite species [91]. So, through the multiple metabolomics platforms and technologies, it should be possible to identify and quantify more and more metabolite species present in rumen samples.

In addition, performing two or more omics technologies together provided strong and concrete data regarding rumen microbes and feed fermentation. An earlier study with the combination of 454 pyrosequencing strategy and MS-based metabolomics technique revealed a significant influence of high grain diet shaping the community structure, diversity, and composition of ruminal bacteria as well as discovered some strong relationships between metabolites and certain microbes in the rumen [67]. Another study based on protein and DNA datasets revealed significant differences between sample fractions and diets and stated similar pattern concerning shifts in phylogenetic composition. The study revealed the presence of 166 carbohydrate active enzymes in varying abundance with analyzing 8163 quantified bacterial proteins [19]. In fine, through the recent advance of rumen microbiome techniques or a combination of two or more meta-omic techniques will be used as potential tools to made a strong linkage among feed, rumen microbiome and animal performance.

Future prospects

Ruminants are the important provider for human's nutrition. So, it is highly needed to increase the production of safe meat and milk. In previous, several steps have been taken to improve it through feed management and the dietary introduction of probiotics but still the result in not reach up to the mark. Moreover, methane emission during feed fermentation indicates the loss of dietary energy. This methane also acts as a greenhouse gas which is an important contributor to global warming. Rumen microbiome plays a significant role in feed fermentation and maintaining the rumen ecosystem. Recent technological advancement especially metagenomic, metatranscriptomic, metaproteomic and metabolomic techniques creates a diverse field for rumen microbiome study. Furthermore, a combination of two or more recent rumen microbiome techniques will find out a new linkage between rumen microbiome and feed fermentation which will improve animal performance.

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초록 : 최근 반추위 미생물 군집의 응용기술을 이용한 사료효율 개선연구

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반추위 속에는 박테리아, 고세균, 프로토조아, 곰팡이 및 바이러스와 같은 다양한 미생물들이 편성의 혐기조건에서 공생하고 있다. 사료의 발효에 중요한 역할을 하고 있는 반추위 미생물은 위내 발효과정에서 에너지 손실에 영향을 주는 메탄의 발생을 제외하면 에너지와 단백질 대사에 필수적인 다양한 휘발성 지방산을 생산한다. 반추위내 미생물의 이용효율을 개선시키기 위해 사료배합비조절, 천연사료첨가제, 생균제첨가 등의 다양한 접근방법들이 사용되고 있다. 최근에 반추위 군집에 대한 메타유전체 또는 메타전사체와 같은 차세대 유전체 해독기술 또는 차세대 시퀀싱 기술의 적용으로 반추위 미생물의 다양성 및 기능에 대한 이해가 크게 증가하였다. 특히 메타단백질체와 메타대사체는 반추위 생태계의 복잡한 미생물네트워크에 대한 더 깊은 통찰력을 제공할 뿐만 아니라, 다양한 반추가축용 사료에 대한 반응을 제공함으로서 생산효율을 개선시키는데 기여하였다. 본 논문에서는 반추위내 사료의 발효와 이용을 향상시키기 위한 메타오믹스 기술, 즉, 메타유전체, 메타전사체, 메타단백질체 및 메타대사체의 최신 응용기술을 요약하고자 한다.