



Investigation of rumen microbiota using 16S rRNA-based metagenomic analysis in Hanwoo cattle

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Abstract

Rumen microbes ferment various feeds consumed by ruminants, producing volatile fatty acids that can be utilized by the host ruminants. Since 16S rRNA gene amplicon sequencing was developed, previously unculturable rumen microbes have been identified. Using this method, many studies on rumen microbiota in the field of ruminant nutrition have been conducted to expand our understanding of various factors that influence rumen microbiota such as diet, breeds, and regions. Because these factors impact the composition of rumen microbiota, understanding rumen microbiota in Korean native Hanwoo cattle is crucial for optimizing rumen fermentation and improving animal productivity. This review discusses recent 16S rRNA-based metagenomic studies on rumen microbiota, focusing on various factors including different rumen sampling methods, cannulation surgery, feeding systems, breed, and marbling score in Hanwoo cattle.

Keywords: 16S rRNA gene, Amplicon sequencing, Rumen microbiota, Hanwoo cattle

INTRODUCTION

The rumen microbiota in ruminants play an important role in breaking down various nutrients through a complex fermentation process [1]. Among the rumen microbes, bacteria are the most dominant and produce volatile fatty acid as fermentation products, which can be utilized by the host animals [1]. On the other hand, rumen archaea produce methane gases, which represent a loss of feed gross energy [2]. After culture-independent 16S rRNA gene-based sequencing was developed, nearly all rumen microbes can now be detected, contributing to understanding the roles of rumen microbiota in ruminants [3,4].

Hanwoo, a native Korean beef cattle breed, comprises four distinct breeds: brown Hanwoo, black Hanwoo, jeju black Hanwoo, and brindle Hanwoo [5]. Among these four breeds, brown Hanwoo is the most prevalent breed in Korea. While many studies have investigated the rumen microbiota of different ruminant breeds across various regions [6], there are relatively few studies on the rumen microbiota of Hanwoo cattle. Because rumen microbiota composition can be affected by factors such as diet, breeds, and regions [1,6], studies on rumen microbiota of Korean native Hanwoo cattle would be crucial to optimize rumen fermentation and improve animal productivity in this breed.

Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions

The article is prepared by a single author.

Ethics approval and consent to participate

This article does not require IRB/IACUC approval because there are no human and animal participants.

This review aimed to examine the composition of rumen microbiota through the 16S rRNA gene amplicon sequencing and discuss the factors affecting the rumen microbiota in Hanwoo cattle.

RUMEN MICROBIOTA COMPOSITION IN HANWOO CATTLE

Rumen cannulation is a surgical technique commonly used to collect rumen contents for nutritional or rumen microbial studies in ruminants [7]. Alternatively, a non-invasive stomach tubing technique can be employed to collect rumen contents [8]. Previous studies have shown that the composition of rumen microbiota analyzed through stomach tubing is similar to that of rumen microbiota analyzed via rumen cannulation [9–12]. Nonetheless, the stomach tubes used in these studies are too thick and not suitable for Hanwoo cattle, which have a smaller body size compared to other cattle breeds. Hence, Song et al. [8] developed a new stomach tube specifically for collecting rumen samples from Hanwoo cattle. This study comparatively assessed the composition of rumen microbiota in Hanwoo steers fed a diet of 80% concentrate and 20% mixed hay using two different rumen sampling methods and identified similar microbial composition [8]. In this study, Bacteroidetes and Firmicutes were predominant phyla, while *Succiniclacticum*, *Butyrivibrio*, *Ruminococcus*, and *Selenomonas* were predominant genera (Table 1).

A recent study examined the impact of cannulation surgery on the composition of rumen microbiota in Hanwoo steers fed a total mixed ration (TMR) [7]. This study analyzed the longitudinal changes in the rumen microbiota of Hanwoo steers and identified that some major taxa and microbial diversity were affected by rumen cannulation surgery [7]. Bacteroidetes and Firmicutes were predominant phyla, while *Prevotella*, *Succiniclacticum*, *Treponema*, *Butyrivibrio*, *Saccharofermentans*, and *Ruminococcus* are predominant genera (Table 1).

Heat stress adversely influences Hanwoo productivity by reducing feed intake. Baek et al. [13] evaluated the rumen microbiota composition of Hanwoo steers exposed to heat stress. For the experiment, Hanwoo steers fed a diet of 60% concentrate and 40% rice straw were individually housed in respiration chambers equipped with temperature and humidity control systems [13]. After 6 days of exposure to heat stress, there was a decrease in Ruminococcaceae, which includes fibrolytic bacteria, while Lactobacillaceae, which includes lactate-producing bacteria, increased [13]. Bacteroidetes and Firmicutes were predominant phyla, while Prevotellaceae, Paraprevotellaceae, Porphyromonadaceae, Ruminococcaceae, Veillonellaceae, Lachnospiraceae, Erysipelotrichaceae, Clostridiaceae, Christensenellaceae, Mogibacteriaceae, and Succinivibrionaceae were predominant families (Table 1). Within the archaea domain, *Methanobrevibacter* was the most dominant genus, accounting for 89.4% to 99.3% of the total sequences. *Methanosphaera* (0.2%–2.2%), and Methanomassiliococcaceae (0.5%–9.4%) were also identified (Table 1).

Lee et al. [14] evaluated the impact of different feeding systems (separate feeding vs. TMR) on the rumen microbiota of Hanwoo steers. These Hanwoo steers were fed a diet of 85% concentrate and 15% forage in the separate feeding system, or TMR. Prevotellaceae was more abundant in the separate feeding group, while Rikenellaceae was more abundant in the TMR group [14]. Bacteroidetes and Firmicutes were predominant phyla in both feeding systems. Within

Table 1. Factors affecting rumen microbiota in Hanwoo cattle

Factor	Diet	Animal	Rumen microbiota composition	References
Rumen sampling method	80% concentrate, and 20% mixed forage (45% tall fescue, 45% orchardgrass, and 10% Kentucky bluegrass)	Brown Hanwoo steers (605 ± 18 kg)	1) No difference between cannulation and stomach tube methods 2) Dominant phyla: Bacteroidetes, Firmicutes 3) Dominant genera: <i>Succiniclasticum</i> , <i>Butyrivibrio</i> , <i>Ruminococcus</i> , <i>Selenomonas</i>	[8]
Cannulation surgery	Total mixed ration (TMR)	Brown Hanwoo steers (341 ± 20 kg)	1) Some major taxa and microbial diversity were affected by rumen cannulation surgery. 2) Dominant phyla: Bacteroidetes, Firmicutes 3) Dominant genera: <i>Prevotella</i> , <i>Succiniclasticum</i> , <i>Treponema</i> , <i>Butyrivibrio</i> , <i>Saccharofermentans</i> , and <i>Ruminococcus</i>	[7]
Heat stress	60% concentrate, and 40% rice straw	Brown Hanwoo steers (235 ± 24 kg)	1) Ruminococcaceae decreased by heat stress 2) Lactobacillaceae increased by heat stress 3) Dominant phyla: Bacteroidetes, Firmicutes 4) Dominant families: Prevotellaceae, Paraprevotellaceae, Porphyromonadaceae, Ruminococcaceae, Veillonellaceae, Lachnospiraceae, Erysipelotrichaceae, Clostridiaceae, Christensenellaceae, Mogibacteriaceae, Succinivibrionaceae 5) Dominant methanogen: <i>Methanobrevibacter</i>	[13]
Feeding system	1) 85% concentrate and 15% forage (separate feeding) 2) Total mixed ration	Brown Hanwoo steers (507 ± 67 kg)	1) Prevotellaceae was abundant in the separate feeding group 2) Rikenellaceae was abundant in the TMR group 3) Dominant phyla: Bacteroidetes, Firmicutes 4) Dominant methanogen: <i>Methanobrevibacter</i>	[14]
Breed	Total mixed ration	Brown and jeju black Hanwoo steers (400 ± 24 kg)	1) <i>Ruminococcus</i> was greater in brown Hanwoo steers than in jeju black Hanwoo steers 2) Dominant phyla: Bacteroidetes, Firmicutes	[15,16]
Marbling content	89% concentrate and 11% forage	Brown Hanwoo steers (an average weight of 626 kg)	1) Dominant taxa in high-marbling score group: RFP12, Verrucomicrobia, <i>Oscillospira</i> , Porphyromonadaceae, <i>Paludibacter</i> 2) Dominant taxa in low-marbling score group: <i>Olsenella</i> 3) Dominant phyla: Bacteroidetes, Firmicutes 4) Dominant genus: <i>Prevotella</i>	[17]

the archaea domain, *Methanobrevibacter* was the most dominant genus, accounting for more than 90% of the total sequences, while *Methanosphaera*, *Methanomicrobium*, and *Methanimicrococcus* each represented less than 1% of the total sequences (Table 1).

A recent study assessed the rumen microbiota composition of brown Hanwoo steers and Jeju black Hanwoo steers, both fed the same TMR diet and housed in the same barn [15]. Firmicutes and Bacteroidetes were the dominant phyla, each accounting for more than 40% of the total sequences in both breeds, while fibrolytic *Ruminococcus* were significantly greater in brown Hanwoo steers compared to jeju black Hanwoo steers (Table 1) [16]. These findings demonstrated that fiber digestibility might be lower in jeju black Hanwoo steers.

Hanwoo cattle have been selectively bred to exhibit a high marbling content [5]. Kim et al. [17] assessed the association between rumen microbiota and marbling score in Hanwoo steers that were fed a late fattening diet consisting of 89% concentrate and 11% forage before slaughter. This study revealed distinct differences in the overall rumen microbiota between the high-marbling score group and the low-marbling score group [17]. RFP12, Verrucomicrobia,

Oscillospira, Porphyromonadaceae, and *Paludibacter* were greater in high-marbling score group than in the low-marbling score group, whereas the reverse held true for *Olsenella* (Table 1). Bacteroidetes and Firmicutes were dominant phyla across all Hanwoo steers, while *Prevotella* was the most dominant genus across all Hanwoo steers (Table 1).

CONCLUSION

Recently, numerous studies have employed the 16S rRNA gene amplicon sequencing to examine the rumen microbiota in ruminants. Nonetheless, there are few studies focusing on the rumen microbiota of Hanwoo cattle. The present study reviewed the rumen microbiota associated with various factors, including different rumen sampling methods, cannulation surgery, feeding systems, breed, and marbling score in Hanwoo cattle. The findings from these studies could potentially contribute to optimizing rumen fermentation based on the feeding system, improving fiber digestibility in jeju black Hanwoo, and enhancing marbling score of Hanwoo cattle.

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01206

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