JAST (Journal of Animal Science and Technology) TITLE PAGE
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ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	Spatial distribution of bacterial communities across the gastrointestinal tract of Hanwoo steers
Running Title (within 10 words)	Gastrointestinal microbiota in Hanwoo steers
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Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	This study was supported by the Research Program for Agriculture Science & Technology Development (Project No. PJ012031032016) of the Rural Development Administration, Republic of Korea.
Acknowledgements	Not applicable.
Availability of data and material	
Authors' contributions Please specify the authors' role using this form.	Conceptualization: Lee H, Kim M Data curation: Jeong J, Park C, Choi H Formal analysis: Jeong J, Park C, Choi H ethodology: Lee H, Jeong J, Park C, Choi H, Kim M Software: Jeong J, Park C, Park T Validation: Lee H, Yu Z, Kim M

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	Investigation: Lee H, Jeong J, Kim M Writing—original draft: Lee H, Kim M Writing—review & editing: Lee H, Jeong J, Park C, Part T, Yu Z, Kim M
Ethics approval and consent to participate	All experimental procedures were approved and conducted under the guidelines of the National Institute of Animal Science Institutional Animal Use and Care Committee in Korea (approval no.: 2016-205).

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ABSTRACT

This study aimed to characterize bacterial communities across different gastrointestinal (GI) compartments, including the rumen (liquid and solid fractions), cecum, and rectum in Hanwoo steers fed the same late-fattening diet. A total of 48 digesta samples were collected from 12 Hanwoo steers at slaughter. Bacterial communities were analyzed using metataxonomics targeting the V3-V4 regions of the 16S rRNA gene. Sequencing data were generated on the Illumina MiSeq platform and processed using QIIME2. Distinct bacterial communities were observed among GI compartments and between the liquid and solid fractions of the rumen. The rumen liquid fraction was enriched with taxa known to utilize soluble, readily fermentable carbohydrates, including *Selenomonas*, *Ruminococcoides*, *Anseongellahas*, *Luoshenia*, and *Lentimicrobium*. In contrast, the rumen solid fraction was enriched with taxa known to degrade structural carbohydrates, including *Ruminococcus*, *Segatella*, and *Pseudobutyrivibrio*. The cecum was enriched with taxa known to utilize carbohydrates that escape rumen fermentation, including *Parabacteroides*, *Eubacterium*, *Alistipes*, and *Phascolarctobacterium.* The rectum was enriched with taxa, including *Aristaeella*, *Roseburia*, and *Paraprevotella*, which may be involved in degradation of residual and less fermentable carbohydrates remaining after cecal fermentation. Alpha-diversity analyses revealed greater evenness and a higher Shannon diversity index in the hindgut than in the rumen, whereas Faith's phylogenetic diversity was the highest in the rumen liquid fraction. Principal coordinates analysis based on weighted and unweighted UniFrac distances demonstrated clear separation between rumen and hindgut bacterial communities. Significant differences were also observed between rumen liquid and solid fractions, while microbiota structures in the cecum and rectum were similar. Collectively, these findings indicate that GI compartments and the two rumen fractions of Hanwoo steers harbor distinct bacterial communities shaped by local environmental conditions and substrate availability. Differences between rumen liquid and solid fractions reflect distinct ecological niches within the rumen, whereas the microbial similarity between cecum and rectum likely results from their shared hindgut environment.

Keywords: Hanwoo steers, bacterial community, GI compartments, rumen, hindgut

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Introduction

The gastrointestinal (GI) microbiota, especially that in the rumen, plays a crucial role in ruminant digestion and productivity by mediating enteric fermentation and nutrient utilization [1, 2]. Rumen microbes drive the essential fermentation processes that convert dietary nutrients into volatile fatty acids (VFAs), which serve as the host's primary energy source [3]. Additionally, microbial protein synthesized during ruminal fermentation provides most of the metabolizable protein for ruminants [4]. Hindgut microbes ferment residual substrates that escape ruminal digestion, thereby providing additional nutrients to the host and influencing intestinal function [5]. Collectively, GI microbial activity is closely linked to feed efficiency, growth performance, and carcass characteristics [5-7].

Various factors influence GI microbiota composition, including diet, breed, feed additives, season, and geographic environment [1]. Among these factors, diet exerts a particularly strong influence on the structure of the GI microbiota and fermentation characteristics [3, 8, 9]. Because dietary factors strongly affect the GI microbiota, comparisons among GI compartments can be confounded when animals are fed different diets. Therefore, evaluating the microbiota across different GI compartments under the same dietary conditions is necessary to better understand spatial variations in the bovine GI microbiota.

Previous studies in cattle have primarily focused on the rumen microbiota because of its essential roles in feed digestion, fermentation, microbial protein synthesis, and methane production [1-3]. Most of these studies have focused on the rumen microbiota in the liquid fraction [3], but the solid fraction harbors a substantially different one [10]. Despite its important role in fiber degradation, the microbiota associated with the solid fraction is much less characterized than the liquid microbiota. Although microbiota across the gastrointestinal tract, including the hindgut, have been evaluated in dairy, beef, and crossbreed cattle [11-13], most studies in Hanwoo cattle have focused on the rumen or fecal microbiota [5, 14-17].

Hanwoo is an indigenous Korean cattle breed with distinct genetic and physiological characteristics, including a high marbling potential [5, 16, 17]. Because host breed is an important factor influencing GI microbiota composition, characterization of the GI microbiota in Hanwoo cattle may provide breed-specific insights into their microbial ecology and digestive physiology [1, 14]. However, comprehensive comparisons of microbiota among rumen liquid, rumen solid, cecum, and rectum within the same Hanwoo cattle remain limited. Therefore, this study aimed to characterize and compare microbiota among these four GI environments in Hanwoo steers. We hypothesized that microbiota would differ across GI compartments and between the liquid and solid fractions within the rumen under the same dietary conditions because physicochemical environments and substrate availability vary across the GI tract.

Materials and Methods

Animal ethics

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All experimental procedures were approved and conducted in accordance with the guidelines of the National Institute of Animal Science Institutional Animal Use and Care Committee in Korea (approval no.: 2016-205), and all methods followed the relevant guidelines and regulations.

Animals, diet, and sampling

A total of 12 Hanwoo steers at 24 months of age (mean body weight, 657.75 ± 43.38 kg) were enrolled in this study and fed the same late fattening diet (Table 1) until slaughter at 28 months of age (mean body weight, 775.58 ± 49.20 kg). After slaughter at the experimental abattoir of the National Institute of Animal Science in Korea, we aseptically collected rumen content, cecal digesta, and rectal contents from all 12 steers. The rumen digesta samples were further separated into liquid and solid fractions as previously described [10]. Briefly, rumen digesta samples were squeezed through four layers of cheesecloth to obtain the liquid fraction. Microbial cells from the liquid fraction were collected by centrifugation at 10,000 × g for 20 min at room temperature. The particulate matter retained on the cheesecloth was used as the solid fraction. The rumen solid samples were resuspended in an anaerobic diluent containing 0.15% (v/v) Tween-80 and kept on ice for 2.5 h to facilitate the release of particle-associated microbes. Plant particles were removed by centrifugation at 500 × g for 15 min at room temperature, and the microbial cells were subsequently harvested by centrifugation at 10,000 × g for 20 min at 4 ℃. The resulting cell pellets were resuspended in Tris-EDTA buffer (pH 8.0). Cecal digesta were collected from the distal portion of the cecum, whereas rectal digesta were collected from the distal rectum, approximately 10 cm proximal to the anus. The cecal and rectal digesta samples were separately homogenized and transferred into sterile 50-mL tubes. All 48 samples (12 animals × 4 sample types) were immediately stored at −80°C for microbiota analysis.

Rumen fermentation characteristics

Rumen liquid samples were used for volatile fatty acid (VFA) analysis as previously described [18]. Briefly, ruminal fluid samples were centrifuged at 6,000 × g for 15 min at 4 ℃. The resulting supernatant was mixed with 200 μL of 25% metaphosphoric acid and stored at 4 ℃ for 30 min. After incubation, the mixture was centrifuged again at 6,000 × g for 15 min at 4 ℃. The clarified supernatant was passed through a 0.45-μm membrane filter and transferred into 2-mL screw-cap vials for analysis. VFA concentrations were determined using a gas chromatography system (Trace 1310, Thermo Fisher Scientific, USA) equipped with a flame ionization detector and a capillary column (60 m × 0.25 mm × 0.25 μm; Agilent Technologies Inc., CA, USA). The oven temperature program was set to 80 ℃ for 1 min, then increased at 15 ℃/min to 230 ℃ and maintained for 3 min. Nitrogen was used as the carrier gas at a constant flow rate of 2.2 mL/min.

DNA extraction and metataxonomic analysis

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Metagenomic DNA was extracted from the 48 samples using the repeated bead-beating plus column protocol [19]. Amplicon libraries were prepared using PCR with primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) targeting the V3–V4 hypervariable regions of bacteria and archaea [20]. Libraries were pooled at equimolar amounts and sequenced (2×300 bp paired-end) on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) by Macrogen Inc. (Seoul, Korea).

Raw sequence data were processed using the QIIME2 (version 2025.4) pipeline [21]. Adapters and primer sequences were removed using Cutadapt [22]. DADA2 was used for quality filtering (Q-score > 25), paired-end read merging, and chimera removal [23]. Amplicon sequence variants (ASVs) annotated as unassigned, chloroplast, or mitochondria were excluded from downstream analyses. Denoising statistics are provided in Supplementary Table 1. Taxonomic assignment of ASVs was performed using the pre-trained Hanwoo-specific manually weighted taxonomy classifier (available at https://github.com/6seok/rumanclass) with q2 feature-classifier classify-sklearn in QIIME2. This classifier was constructed by Kang et al. [24] following the taxonomic weighting approach of Kaehler et al. [25]. Briefly, shotgun metagenomic and 16S rRNA gene amplicon sequences derived from the rumen of Hanwoo cattle were combined. These sequences were used to generate taxon class weights reflecting the microbial composition of the Hanwoo rumen with q2-clawback, after removal of mitochondrial and chloroplast sequences. The class weights were then incorporated into a Naïve Bayes classifier trained on the NCBI RefSeq database (downloaded on October 14, 2024) using fit-classifier-naive-bayes.

Alpha-diversity metrics, including Chao1, observed ASVs, observed genera, observed species, evenness, Faith's phylogenetic diversity (PD), Shannon index, and Simpson index, were computed from rarefied ASV BIOM tables. Differences in overall microbiota among GI compartments (hereafter, rumen fluid and solids were considered two separate compartments) were evaluated using principal coordinates analysis (PCoA) based on weighted and unweighted UniFrac distances. Shared and group-specific genera were visualized using Venn diagrams based on the ASV count table.

Statistical analysis

Genera representing >0.5% of the total sequences in more than one GI compartment were considered predominant and used to identify taxa enriched in each GI compartment using the linear discriminant analysis (LDA) effect size method [26]. Differentially abundant taxa and predicted functional profiles were identified using the Kruskal-Wallis test with Benjamini-Hochberg correction for multiple testing, with relative abundance as input data. Features were considered significantly differentially abundant if they met the criteria of an adjusted p-value < 0.05 and a logarithmic LDA score threshold of 2.0. For clarity, only the 10 most abundant genera are presented in figures. Statistical differences in alpha-diversity metrics among GI compartments were assessed using the Kruskal–Wallis test, followed by Dunn's post-hoc test for multiple

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comparisons when appropriate. Differences in beta diversity among GI compartments were assessed using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations [27]. Statistical significance was defined as $p < 0.05$. Spearman rank correlation was used to examine associations between prevalent taxa and rumen fermentation parameters in R (version 4.2.1).

Results

Rumen fermentation characteristics

The rumen fermentation characteristics of the 12 Hanwoo steers featured 26.12 mM acetate, 10.44 mM propionate, 4.45 mM butyrate, 1.33 mM iso-butyrate, 0.87 mM valerate, 2.40 mM isovalerate, 45.60 mM total VFA, an acetate-to-propionate ratio of 3.43, and a pH of 6.59 (Table 2).

Rumen microbiota composition

In the liquid fraction, Bacillota was the most abundant and accounted for 47.0% of the total sequences, followed by Bacteroidota (41.8%), Pseudomonadota (3.6%), Spirochaetota (1.9%), Planctomycetota (1.1%), Synergistota (0.7%), and Thermodesulfobacteriota (0.5%) (Fig. 1A). The remaining phyla each accounted for < 0.5% of the total sequences. At the genus level, the 10 most abundant were *Sodaliphilus* (6.3%), *Succiniclasticum* (5.5%), *Segatella* (4.7%), *Xylanibacter* (4.2%), *Lentimicrobium* (3.5%), *Vescimonas* (3.4%), *Anseongella* (2.5%), *Succinivibrio* (2.0%), *Ruminococcus* (1.8%), and *Aristaeella* (1.8%) (Fig. 1B).

In the solid fraction, Bacillota accounted for 51.5% of the total sequences, followed by Bacteroidota (30.9%), Spirochaetota (12.0%), Pseudomonadota (1.4%), and Actinomycetota (1.1%) (Fig. 1A). The remaining phyla were each represented by < 0.5% of the total sequences. The 10 most abundant genera were *Treponema* (11.9%), *Segatella* (10.2%), *Ruminococcus* (7.9%), *Sodaliphilus* (4.2%), *Arstaeella* (3.4%), *Wujia* (2.4%), *Saccharofermentans* (2.1%), *Succiniclasticum* (2.0%), *Vescimonas* (1.5%), and *Pseudobutyrivibrio* (1.5%) (Fig. 1B).

Cecum microbiota composition

Abundant phyla in the cecum included Bacillota (60.4%), Bacteroidota (29.9%), Verrucomicrobiota (3.3%), Pseudomonadota (1.2%), Spirochaetota (1.2%), and Planctomycetota (1.1%) (Fig. 2A). Among the prevalent genera, the top 10 included *Vescimonas* (6.7%), *Alistipes* (4.1%), *Akkermansia* (3.3%), *Aristaeella* (3.2%), *Eubacterium* (2.5%), *Paraprevotella* (1.8%), *Parabacteroides* (1.5%), *Treponema* (1.1%), *Intestinimonas* (1.1%), and *Paraclostridium* (1.0%) (Fig. 2B).

Rectum microbiota composition

Bacillota (60.4%), Bacteroidota (29.1%), Verrucomicrobiota (3.1%), Spirochaetota (2.3%), Pseudomonadota (1.3%), and Planctomycetota (1.0%) were the abundant phyla in the rectum (Fig.

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2A). Among the prevalent genera, *Vescimonas* (7.5%), *Aristaeella* (4.0%), *Alistipes* (3.6%), *Akkermansia* (3.1%), *Eubacterium* (2.5%), *Paraprevotella* (2.3%), *Treponema* (2.3%), *Parabacteroides* (1.3%), *Intestinimonas* (1.0%), and *Phocaeicola* (0.9%) were the most abundant (Fig. 2B).

Differentially abundant taxa among GI compartments

Anseongella, *Butyrivibrio*, *Lentimicrobium*, *Schwartzia*, *Selenomonas*, *Sodaliphilus*, *Succiniclasticum*, *Xylanibacter*, *Luoshenia*, and *Ruminococcoides* were enriched in rumen liquid ($p < 0.05$) compared to rumen solid (Table 3). In contrast, *Chordicoccus*, *Evtepia*, *Pseudobutyrivibrio*, *Ruminococcus*, *Saccharofermentans*, *Segatella*, *Treponema*, and *Wujia* were enriched in the solid fraction ($p < 0.05$) (Table 3). *Akkermansia*, *Alistipes*, *Eubacterium*, *Ihubacter*, *Intestinimonas*, *Monoglobus*, *Parabacteroides*, *Paraclostridium*, *Peptococcus*, *Phascolarctobacterium*, *Phocaeicola*, and *Romboutsia* were enriched in the cecum ($p < 0.05$), while *Paraprevotella*, *Vescimonas*, *Aristaeella*, and *Roseburia* were enriched in the rectum ($p < 0.05$) (Table 3).

Distinct microbiota among GI compartments

All alpha-diversity metrics differed among the four GI compartments ($p < 0.05$; Table 4). However, pairwise comparisons indicated that the hindgut compartments (cecum and rectum) exhibited significantly greater evenness and Shannon index than the rumen liquid and solid fractions ($p < 0.001$). Conversely, Faith's PD was significantly greater in the rumen liquid fraction than in the hindgut compartments ($p = 0.002$; Table 4).

PCoA based on both weighted and unweighted UniFrac distances showed clear separation of microbiota between the foregut (rumen, both solid and liquid fractions) and the hindgut (cecum and rectum) ($p = 0.001$) (Fig. 3). Within the rumen, the solid and liquid fractions also differed significantly in overall microbial composition ($p = 0.001$). In contrast, the cecum and rectum exhibited similar microbiota structures ($p > 0.05$) (Fig. 3).

Shared and unique taxa among GI compartments

A total of 172 genera were shared among all four compartments (Figure 4). The rumen solid fraction exhibited the largest number of unique genera (n = 71), followed by the rumen liquid fraction (n = 39), rectum (n = 22), and cecum (n = 11). The rumen solid and liquid fractions shared 76 genera exclusively, while the cecum and rectum shared 39 genera exclusively. In contrast, relatively few genera were shared between the rumen and the hindgut (Fig. 4).

Discussion

Host and diet can strongly influence the microbiota across the ruminant GI tract. In this study, we eliminated these confounding factors by comparing the microbiota among GI compartments, allowing a better understanding of the factors that shape the microbiota within each compartment.

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Taxa enriched in the rumen liquid fraction

Many genera were enriched in the rumen liquid fraction, including *Selenomonas*, *Ruminococcoides*, *Anseongellahas*, *Luoshenia*, *Lentimicrobium*, and *Xylanibacter*. Most of these genera contain species that can utilize soluble carbohydrates. The enrichment of *Selenomonas* in the liquid fraction can be explained by its preference for soluble carbohydrates as its major substrate and lactate as a fermentation product [28]. Similarly, *Ruminococcoides*, *Anseongellahas*, *Luoshenia*, and *Lentimicrobium* have been reported to utilize soluble carbohydrates [29-32]. *Xylanibacter ruminicola* (formerly *Prevotella ruminicola*) also utilizes soluble carbohydrates [33]. *Muribaculaceae* are known to produce diverse carbohydrate-active enzymes, and *Sodaliphilus*, as a genus of this family, may also prefer soluble carbohydrates [34, 35]. In addition, *Succiniclasticum* and *Schwartzia* were also enriched in the liquid fraction. They can convert succinate to propionate during rumen fermentation, which may explain their enrichment in the rumen fluid environment [36, 37]. This enrichment aligns with their preference for soluble carbohydrates as primary substrates or their ability to use fermentation products, particularly lactate.

Taxa enriched in the rumen solid fraction

Taxa enriched in the rumen solid fraction included bacteria known to degrade fiber, such as *Ruminococcus* species, as well as those that utilize fiber degradation products, such as *Treponema* species. *Ruminococcus* species are well-known cellulolytic bacteria that adhere firmly to plant fiber surfaces in the rumen through specific adhesion mechanisms, including cellulosome-like complexes and fimbrial proteins [38]. Consistent with previous studies reporting greater abundance of *Ruminococcus* in the solid fraction [39-43], the predominance of *Ruminococcus* in the solid fraction observed in this study is not surprising.

Similarly, *Segatella* and *Pseudobutyrivibrio* have been reported to degrade plant-derived polysaccharides, including hemicellulose, pectin, and xylan [44-46], explaining their enrichment in the solid fraction. Previous studies have also reported that *Segatella*, formerly classified within the genus *Prevotella* [33], is involved in fiber degradation in the rumen [47, 48], and it exhibits greater abundance in the rumen solid fraction [39-41, 43]. *Treponema*, although not cellulolytic, may cross-feed with cellulolytic bacteria by utilizing sugars released by fiber-degrading microbes [48-50]. These findings are consistent with previous studies reporting enrichment of *Treponema* in the solid fraction [39, 40, 43].

Saccharofermentans, *Wujia*, and *Chordicoccus* were also enriched in the rumen solid fraction. They may utilize sugars released from fiber digestion. Indeed, *Saccharofermentans* has been reported to metabolize sugars released during cellulose hydrolysis and to be enriched in fiber-rich rumen environments [51-54]. Although the metabolic characteristics of *Wujia* are not yet fully understood, its isolation using carbohydrate-enriched media containing various sugars such as mannose, galactose, rhamnose, and cellobiose [55] suggests a potential utilization of plant-derived carbohydrates. Similarly, *Chordicoccus* can ferment various carbohydrates including cellobiose,

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xylose, arabinose, mannose, and galactose [56], suggesting a potential role in utilizing plant-derived carbohydrates. These characteristics likely explain their enrichment in the rumen solid fraction.

Taxa enriched in the cecum

The cecum is a site of active hindgut fermentation, where undigested substrates escaping the upper GI compartments and host-derived substrates, such as mucin, are fermented into short-chain fatty acids (SCFAs) [5, 17, 57]. The greater number of taxa identified in the cecum than in the rectum may reflect the greater variety of available substrates in cecal environment [58].

Parabacteroides, *Monoglobus*, *Eubacterium*, *Romboutsia*, and *Phocaeicola* are known to utilize dietary carbohydrates and produce SCFAs [9, 59-63]. Previous studies have consistently reported the predominance of these genera in the cecum of cattle [64-68], reflecting their fitness and high fermentative activity in the cecal environment. Among these, *Eubacterium* produces butyrate, a major energy source for the cecal epithelium [63, 69, 70], whereas *Monoglobus* is specialized in pectin degradation [61].

In contrast, *Alistipes*, *Peptococcus*, and *Intestinimonas* are associated with utilizing protein and amino acids as substrates [71-74]. *Alistipes* mainly produces acetate and succinate and has previously been reported to be more abundant in the cecum than in the rectum [72]. *Peptococcus* metabolizes peptides and amino acids [74], whereas *Intestinimonas* utilizes lysine and fructoselysine, producing butyrate and acetate [71]. Although *Intestinimonas* has been reported to be predominant in the rectum of cattle [75, 76], its enrichment in the cecum suggests a broader ecological distribution in the bovine hindgut.

Akkermansia, a mucin-degrading bacterial genus frequently detected in the hindgut of Hanwoo cattle, may reflect active mucin turnover in the cecum [5, 77]. Likewise, *Phascolarctobacterium*, a succinate-utilizing propionate producer that has also been reported as a predominant cecal taxon in cattle [68, 78], may reflect active microbial cross-feeding. Because *Ihubacter*, which is closely related to *Eubacterium*, was only recently isolated from the human gut [79], studies of its ecological role in the GI tract of ruminants remain limited.

Taxa enriched in the rectum

Some poorly degradable fiber and derivatives can escape fermentation in the rumen and cecum and reach the rectum [5]. In addition, water absorption and fecal accumulation in the rectum may further concentrate these residual substrates. Enrichment of some genera in the rectum likely reflects their ecological adaptation to the distal hindgut environment, including nutrient availability and physicochemical conditions.

Aristaeella is known to utilize cellobiose, xylan, and pectin [80], whereas *Roseburia* and *Paraprevotella* can degrade xylan [81, 82]. In addition, *Vescimonas* has recently been reported to degrade plant fibers [83]. Collectively, these taxa may utilize the residual fiber-derived substrates that would otherwise be excreted and wasted. *Roseburia* and *Paraprevotella* have also been

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consistently detected in cattle feces [9, 84], but few studies have reported the detection of *Aristaeella* and *Vescimonas* in the ruminant hindgut.

Relationship between rumen microbiota and fermentation characteristics

No significant correlation was observed between specific taxa and fermentation parameters, partly reflecting the highly complex and functionally redundant nature of the rumen microbial ecosystem [8, 85]. The rumen harbors complex microbiota in which phylogenetically distinct taxa conduct similar metabolic functions in nutrient degradation and SCFA production. Therefore, shifts in individual taxa might not necessarily alter overall fermentation characteristics.

Microbial diversity across GI compartments

Previous studies showed inconsistent patterns of alpha-diversity metrics between the rumen and the hindgut. In Holstein dairy cattle fed a TMR, Shannon diversity index was higher in the rumen than in the hindgut [11], whereas no significant difference between the rumen and hindgut was observed in Simmental × Holstein crossbred heifers fed a corn-soybean-based diet [12]. This discrepancy may be due to breed, diet, or both.

However, the present study showed that Shannon diversity index was significantly higher in the hindgut than in the rumen. The higher Shannon diversity index in both the cecum and rectum aligns with greater richness and higher evenness in the hindgut microbiota. Because readily degradable substrates are utilized by the rumen microbiota, the hindgut microbiota can access only less degradable substrates that escape rumen degradation, as well as host-derived substrates. The greater substrate variety and more difficult degradation likely create more niches for diverse bacteria. The late-fattening Hanwoo steers were fed high-concentrate diets. The lower Shannon diversity index of the rumen microbiota aligns with the lower evenness and taxa richness. Long-term exposure of the rumen microbiota to starch-rich diets may shift the microbiota toward starch-utilizing taxa, leading to fewer dominant taxa, thereby reducing evenness and richness. In contrast, Faith's phylogenetic diversity was higher in the rumen than in the hindgut. The higher Faith's phylogenetic diversity values indicate that the rumen microbiota hosted a wider range of phylogenetically distinct microbes. In addition, differences in substrate types and availability, and GI conditions between the rumen and hindgut may also influence microbial diversity.

Spatial patterns of GI microbiota among cattle breeds

The spatial patterns of GI microbiota observed in the present study were generally consistent with findings in other cattle breeds. Previous studies in Holstein dairy cattle, Angus steers and Simmental × Holstein crossbred cattle revealed distinct microbial compositions among GI compartments, particularly between the rumen and hindgut [11-13]. Consistent with these studies, the cecal and rectal microbiota in Hanwoo steers showed similar overall community structures, whereas both were clearly distinct from the rumen microbiota [11-13]. These findings suggest that differences in microbial composition between the rumen and hindgut in Hanwoo steers represent

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a general feature of the bovine GI tract rather than a Hanwoo-specific pattern. However, differences in the relative abundance of individual taxa among studies may reflect the effects of breed, diet, age, and management conditions. Because direct comparisons with other breeds under the same experimental conditions were not conducted, the enrichment of specific taxa in each compartment should not be considered unique to Hanwoo cattle.

Core and unique genera across GI compartments

The many genera shared among all four GI compartments suggest a core microbiota that persists along the digestive tract. Consistent with the present study, previous studies have also reported substantial overlap in microbial genera between the rumen and the hindgut [86, 87]. Despite substantial overlap in taxa among GI compartments, predominant microbial taxa differed between the rumen and hindgut, aligning with previous studies [86, 87]. These findings highlight differences in substrate availability and GI physiology between the rumen and hindgut environments. The rumen microbiota is likely shaped by abundant feed supply, whereas the hindgut microbiota relies on substrates escaping ruminal digestion and host-derived substrates.

The shared taxa likely include microbes that can adapt to diverse gut environmental conditions, contributing to fermentation processes throughout the GI tract. However, the shared taxa were identified at the genus level rather than the species level. Because individual genera include multiple species with distinct metabolic capacities, the functional similarity of these shared genera cannot be assumed.

In addition, this study relied on metataxonomic analysis of the rumen and hindgut microbiota, which detects microbial DNA but does not distinguish viable and non-viable cells. Consequently, DNA from lysed or dead rumen microbes may be detected in the hindgut, potentially overestimating shared taxa [88]. Therefore, the true functional core microbiota across compartments should be interpreted with caution. Future studies employing higher-resolution approaches, such as genome-resolved metagenomics or viability-based analyses, are required to identify functional core microbiota at lower taxonomic levels.

Conclusion

The present study demonstrates that bacterial communities vary across different regions of the GI tract, influenced by substrate availability and local environmental conditions. Distinct bacterial profiles were found not only between rumen liquid and solid fractions but also between the foregut and hindgut. In contrast, the cecum and rectum exhibited similar bacterial compositions. These findings provide a baseline for understanding the spatial distribution of GI microbiota in Hanwoo steers and lay the framework for future studies aimed at improving rumen efficiency, animal productivity, and environmental sustainability.

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Table 1. Ingredients and nutritional composition of the experimental diets

Item	Ingredients, % DM
Rice straw	9.35
Corn gluten feed	5.77
Molasses	4.48
Lupin	3.48
Wheat bran	9.95
Coconut kernel meal	5.97
Corn gluten meal	4.48
Steam-flaked corn	49.75
Palm kernel meal	4.98
Vitamin-mineral premix	0.10
Limestone	0.81
Salt	0.44
Sodium bicarbonate	0.44
Total	100
Item	**Chemical composition, % DM**
CP	13.76
CA	5.71
NDF	26.72
ADF	13.98
EE	3.51
NFC[1]	50.30
Gross energy (Mcal/kg)	4.38

DM, dry matter; CP, crude protein; CA, crude ash; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extract; NFC, non-fiber carbohydrate.

[1]NFC = 100 – (% of CP + % of EE + % of CA + % of NDF).

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602 **Table 2. Rumen fermentation characteristics of Hanwoo steers**

Parameter	Mean	SEM
pH	6.59	0.06
Acetate, mM	26.12	3.17
Propionate, mM	10.44	3.37
Butyrate, mM	4.45	1.09
Isobutyrate, mM	1.33	0.14
Valerate, mM	0.87	0.23
Isovalerate, mM	2.40	0.32
A:P ratio[5]	3.43	0.33
Total VFAs	45.61	7.05

603 SEM, standard error of the mean;
604 A:P ratio, acetate/propionate ratio; VFA, volatile fatty acid
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609 **Table 3. Bacterial Taxa identified by LEfSe among GI compartments**

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	Relative abundance (%)							
Taxa	**Rumen liquid**	**Rumen solid**	**Cecum**	**Rectum**	**SEM**	**Class**	**LDA score**	**p-value**
Anseongella	2.54	1.34	0.07	0.07	0.21	Rumen liquid	4.13	<0.001
Butyrivibrio	0.59	0.58	0.03	0.01	0.08	Rumen liquid	3.44	<0.001
Lentimicrobium	3.49	0.80	0.12	0.20	0.29	Rumen liquid	4.26	<0.001
Schwartzia	1.01	0.26	0.02	0.02	0.07	Rumen liquid	3.70	<0.001
Selenomonas	1.41	0.98	0.04	0.07	0.14	Rumen liquid	3.82	<0.001
Sodaliphilus	6.35	4.24	0.03	0.03	0.57	Rumen liquid	4.52	<0.001
Succiniclasticum	5.46	1.97	0.22	0.21	0.38	Rumen liquid	4.45	<0.001
Xylanibacter	4.21	1.22	0.09	0.13	0.32	Rumen liquid	4.33	<0.001
Luoshenia	0.89	0.83	0.09	0.10	0.09	Rumen liquid	3.60	0.003
Ruminococcoides	0.76	0.23	0.46	0.44	0.06	Rumen liquid	3.44	0.004
Chordicoccus	0.32	0.73	0.07	0.05	0.05	Rumen solid	3.58	<0.001
Evtepia	0.45	0.62	0.05	0.04	0.05	Rumen solid	3.53	<0.001
Pseudobutyrivibrio	1.19	1.47	0.03	0.03	0.14	Rumen solid	3.85	<0.001
Ruminococcus	1.82	7.90	0.47	0.57	0.49	Rumen solid	4.58	<0.001
Saccharofermentans	0.54	2.12	0.08	0.02	0.15	Rumen solid	4.07	<0.001
Segatella	4.73	10.19	0.12	0.14	0.68	Rumen solid	4.74	<0.001
Treponema	1.68	11.87	1.14	2.29	0.83	Rumen solid	4.70	<0.001
Wujia	0.48	2.36	0.07	0.09	0.17	Rumen solid	4.06	<0.001
Akkermansia	0.00	0.00	3.26	3.13	0.45	Cecum	4.21	<0.001
Alistipes	0.00	0.00	4.10	3.59	0.31	Cecum	4.32	<0.001
Eubacterium	0.78	0.35	2.55	2.52	0.20	Cecum	4.02	<0.001
Ihubacter	0.15	0.16	0.58	0.57	0.04	Cecum	3.37	<0.001
Intestinimonas	0.13	0.01	1.06	1.05	0.09	Cecum	3.75	<0.001
Monoglobus	0.02	0.00	0.57	0.46	0.04	Cecum	3.47	<0.001

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Parabacteroides	0.72	0.03	1.46	1.35	0.14	Cecum	3.89	<0.001
Paraclostridium	0.04	0.01	1.02	0.71	0.10	Cecum	3.74	<0.001
Peptococcus	0.00	0.00	0.66	0.53	0.05	Cecum	3.54	<0.001
Phascolarctobacterium	0.00	0.00	0.60	0.55	0.05	Cecum	3.51	<0.001
Phocaeicola	0.00	0.00	0.95	0.92	0.08	Cecum	3.70	<0.001
Romboutsia	0.05	0.01	1.01	0.51	0.07	Cecum	3.73	<0.001
Paraprevotella	0.36	0.07	1.82	2.29	0.26	Rectum	4.03	<0.001
Vescimonas	3.39	1.54	6.66	7.50	0.41	Rectum	4.47	<0.001
Aristaeella	1.81	3.36	3.16	3.96	0.23	Rectum	4.04	0.004
Roseburia	0.13	0.16	0.38	0.70	0.07	Rectum	3.42	0.005

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Table 4. Alpha-diversity metrics of GI microbiota across GI compartments in Hanwoo steers

Compartment	Observed ASVs	Evenness	Faith's phylogenetic diversity	Shannon's index	Simpson's index
Liquid	1,835	0.777[b]	241.85[a]	8.41[b]	0.9895
Solid	1,807	0.778[b]	229.11[ab]	8.41[b]	0.9898
Cecum	2,092	0.843[a]	203.10[b]	9.29[a]	0.9942
Rectum	2,095	0.843[a]	200.27[b]	9.29[a]	0.9941
SEM	47.7	0.0063	4.734	0.091	0.0007
P-value[1]	0.0355	< 0.0001	0.0016	< 0.0001	0.0257

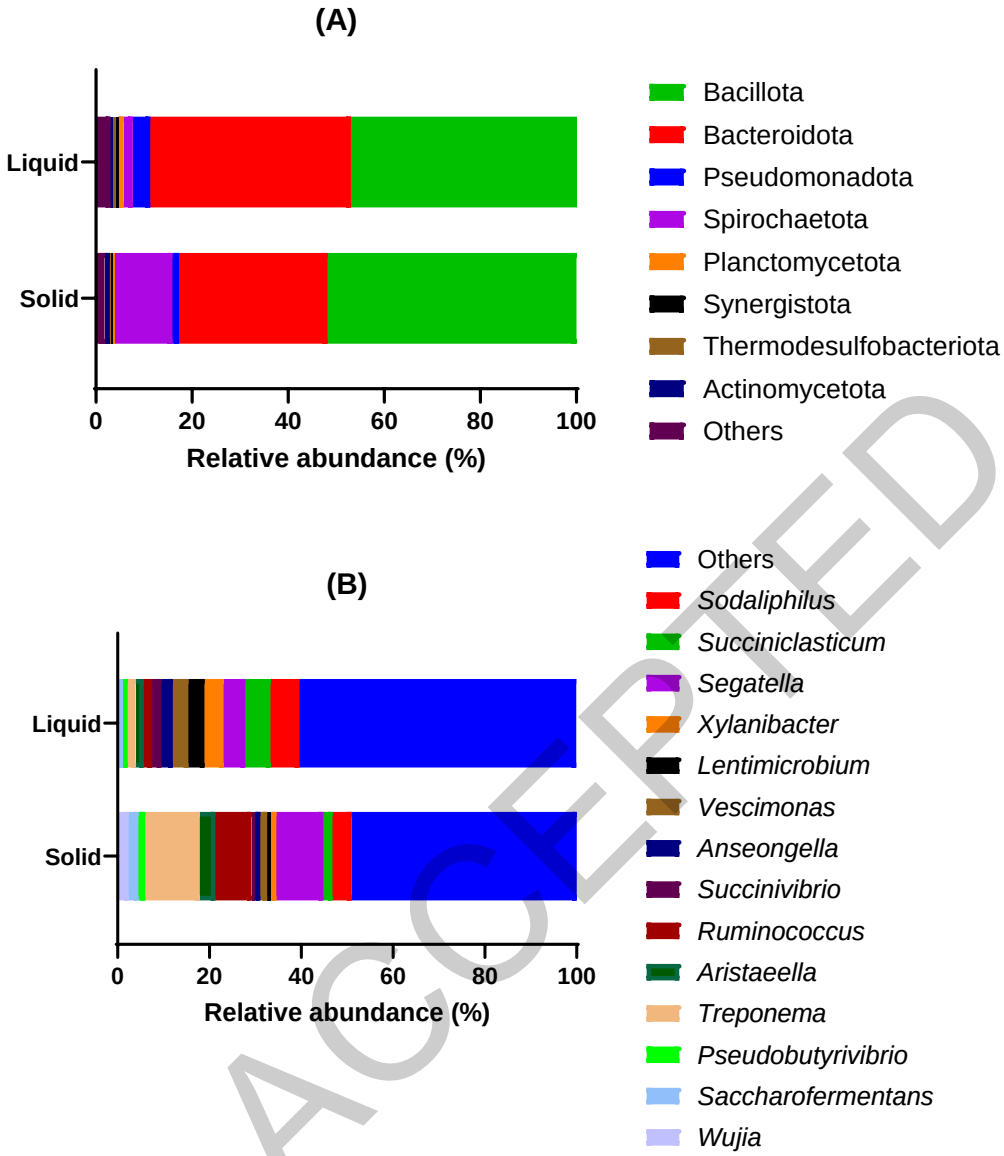
ASV, amplicon sequence variant; SEM, standard error of the mean.

Good's coverage reached 100% for all samples.

[1]*P*-value indicates overall differences among GI compartments.

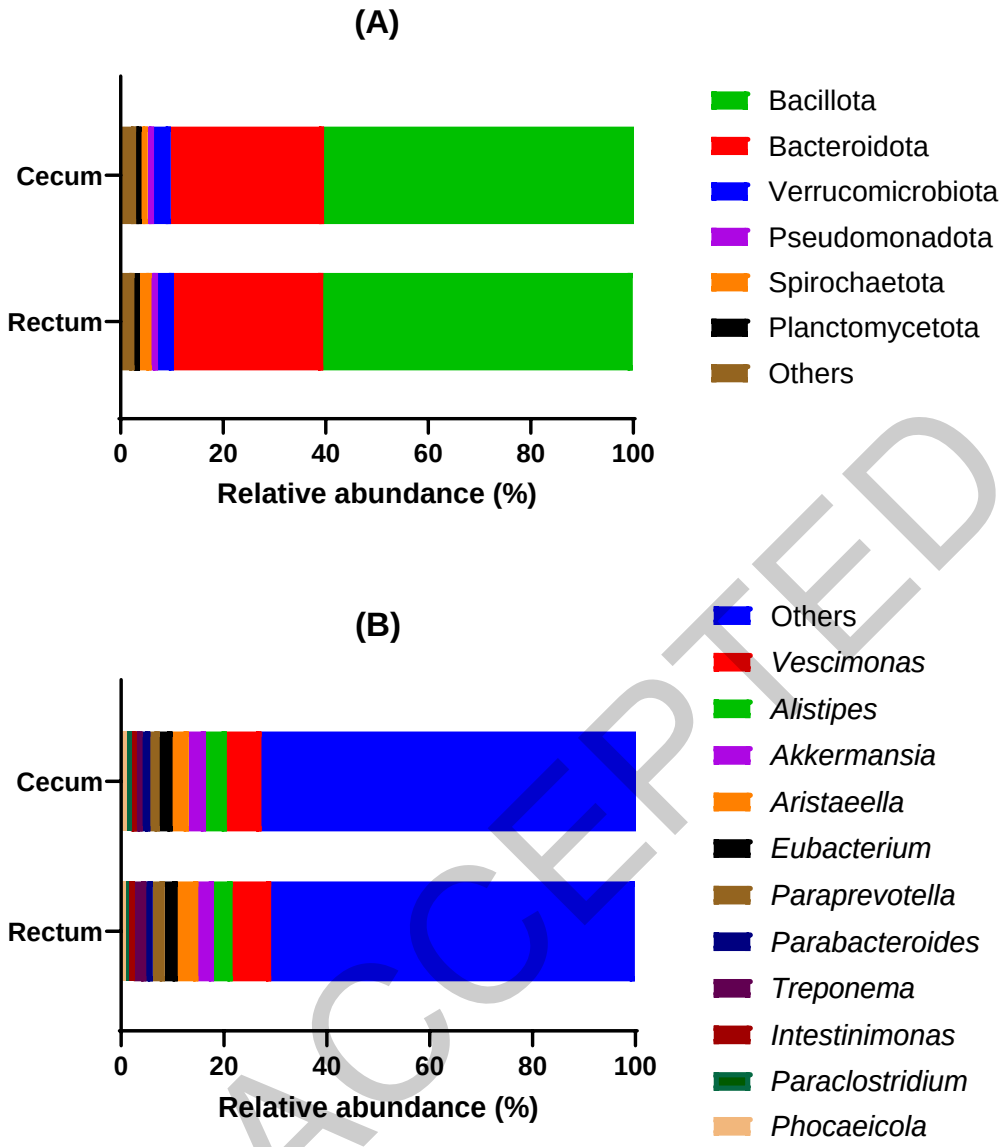
[a,b]Different uppercase letters within a column indicate significant differences based on pairwise comparisons ($p < 0.05$).

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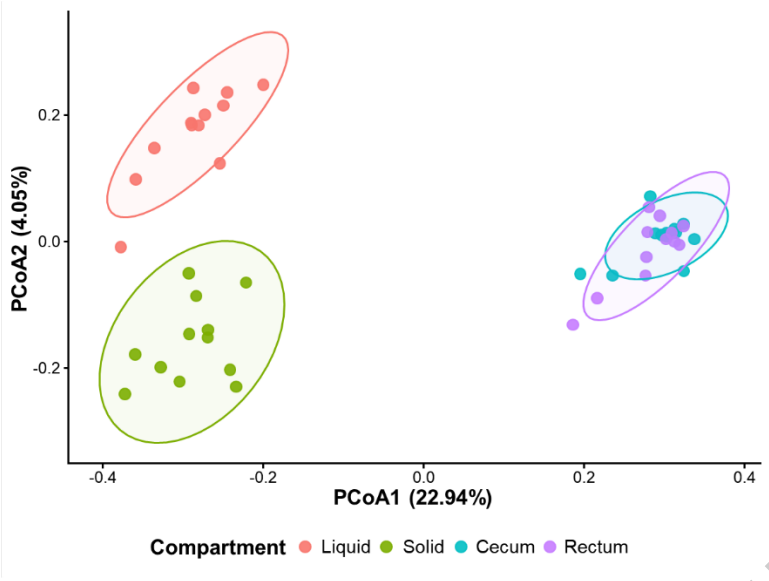
Fig. 1. Relative abundance of predominant bacterial phyla (A) and genera (B) in the rumen liquid and solid fractions of Hanwoo steers. Only phyla with relative abundances ≥ 0.5% are shown. For genera, only the top 10 most abundant taxa were selected independently for each fraction. As some dominant genera were unique to either the liquid or solid fraction, the total number of displayed genera exceeded 10. All remaining taxa, including unclassified taxa, were collectively grouped as “Others”.



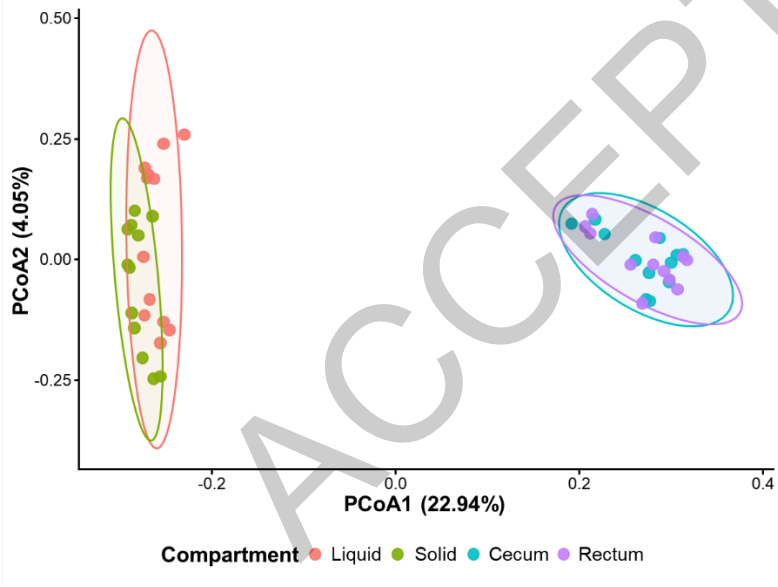
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Fig. 2. Relative abundance of predominant bacterial phyla (A) and genera (B) in the cecum and rectum of Hanwoo steers. Only phyla with relative abundances ≥ 0.5% are shown. For genera, only the top 10 most abundant taxa were selected independently for each compartment. As some dominant genera were unique to either the cecum or rectum, the total number of displayed genera exceeded 10. All remaining taxa, including unclassified taxa, were collectively grouped as "Others".

(A)

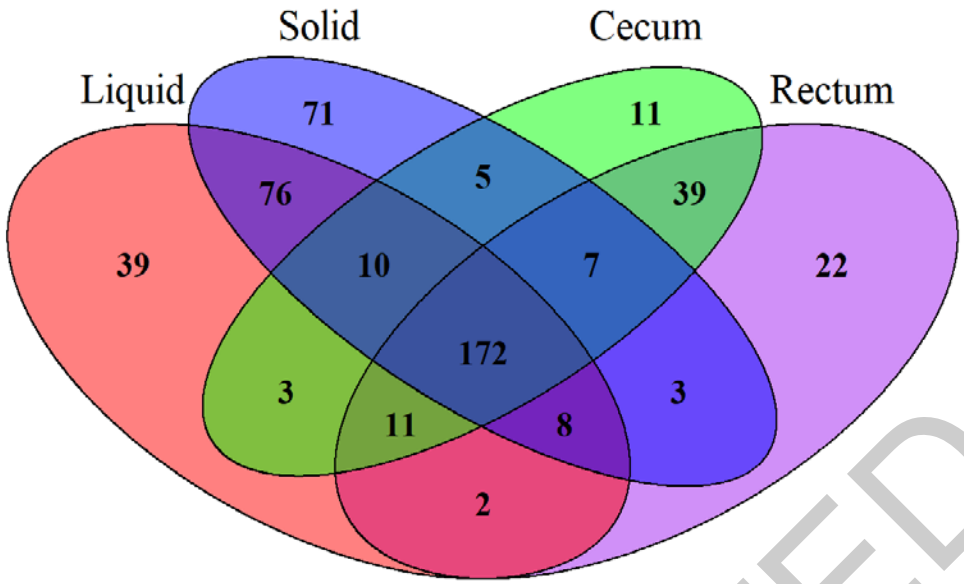


(B)



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Fig. 3. Principal coordinates analysis plots of GI microbiota in Hanwoo steers based on weighted (A) and unweighted (B) UniFrac distances. Both weighted and unweighted UniFrac analyses showed clear separation between the foregut (rumen solid and liquid fractions) and the hindgut (cecum and rectum) microbiota (p = 0.001). Significant differences were observed between rumen liquid and solid (p = 0.001), but not between cecum and rectum (p > 0.05).



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659 **Fig. 4. Venn diagram showing shared and unique genera among the four GI compartments**
660 **(rumen liquid, rumen solid, cecum, and rectum).** The numbers indicate the count of genera
661 detected in each compartment or shared among compartments. Overlapping regions indicate
662 genera shared between two or more compartments, while non-overlapping regions represent
663 compartment-specific genera.

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