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Research article

Unveiling the bacterial microbiota profiles across the gastrointestinal tract regions in dairy buffaloes (*Bubalus bubalis*)

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Abstract

Understanding the pivotal role of bacterial communities in the gastrointestinal tract (GIT) of agriculturally significant animals, such as buffalo, on host productivity and health is crucial. However, our knowledge of buffalo GIT bacterial communities remains limited. This study aimed to profile and compare bacterial communities across three distinct GIT regions—forestomach (rumen, reticulum, omasum, abomasum), small intestine (duodenum, jejunum, ileum), and large intestine (cecum, colon, rectum)—in two riverine- type buffaloes using the Illumina MiSeq platform. Fresh samples were collected in triplicate from various GIT sites within two dairy buffaloes reared under identical conditions. Genomic DNA was extracted, and bacterial profiles were analyzed, with sequences annotated using the Green Gene database. The results revealed substantial intra-buffalo variation at lower taxonomic levels, with Bacteroidetes dominating the forestomach and duodenum, while Firmicutes prevailed in the hindgut from the jejunum to the rectum. Comparisons of GIT sites across different buffaloes indicated variations in primary bacterial phyla, with significant taxonomic differences among gut sections in distinct regions but similarities within the same region. This research provides insights into complex microbial communities within the buffalo GIT, contributing to our understanding of buffalo health and productivity.

Keywords: Dairy buffalo; Gastrointestinal tract; Bacteria

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INTRODUCTION

The gastrointestinal tract (GIT) of economically important animals such as ruminants harbor extremely dense and diverse microbial communities that contribute to the overall health (modulation of the immune system) and productivity of the animals. The complex GIT microbiome is comprised primarily of bacteria, particularly in the rumen and large intestine (1,011 cells per gram), where microorganisms actively degrade dietary plant polysaccharides (Flint *et al.,* 2008).

The bacterial microbiota plays an important role in the breakdown of plant fibers (Nyonyo *et al*., 2014) providing microbial crude proteins and volatile fatty acids (VFAs) such as acetate, butyrate, and propionate to the host. The conversion of agricultural by-products and renewable fibrous materials into high-quality foods such as meat and milk is essential in human societies (Mao *et al*., 2015). Thus, in recent years, ruminant microbial community populations and active metabolic pathways have been extensively studied in the fields of animal nutrition, biotechnology, and climatology. However, most of the studies conducted to date have used culture-dependent methods and genetic fingerprinting, which have several drawbacks in terms of evaluating the gut microbiome. Culture-dependent methods or classical microbiology methods allow detection of only approximately 10-11% (Cammack *et al*., 2014) of bacterial phyla as revealed by nucleic acid-based techniques, resulting in inaccurate and incomplete datasets (Fernando *et al*., 2010). Furthermore, fingerprinting methods cannot provide accurate results because of the high richness and diversity of uncultured microorganisms (Costa *et al*., 2015). Aside from the techniques used, previous studies primarily utilized fecal and ruminal samples to assess the GIT microbiome due to the easy and non-invasive sampling procedures (Lee *et al*., 2011; Lettat *et al*., 2012; Kittelmann *et al*., 2013), but the use of these samples does not guarantee the representation of the entire microbial community within the GIT of the animal. Hence, the role of microorganisms in the other segments of the GIT, such as the small and large intestines, remains poorly explored.

Recent studies have investigated the microbiota in the GIT of Brazilian Nelore steers (De Oliveira *et al*., 2013), Chinese Mongolian sheep (Zeng *et al*., 2017), and Holstein dairy cows (Mao *et al*., 2015) using high-throughput next-generation sequence analysis. This approach allowed for a more comprehensive characterization of complex microbial communities, even in the GIT sites in which extreme environmental conditions such as low pH and high enzymatic activity function as filters that select only certain bacteria.

In this study, high-throughput next-generation sequence analysis was employed to explore the composition and phylogenetic distribution of bacterial communities across various gastrointestinal compartments in dairy buffalo. Considering the agricultural and economic significance of buffalo in tropical regions for global milk and meat production, as well as its role in draft power and hide material, this research aims to enhance understanding of GIT-associated bacteria localization. The findings of the study will contribute valuable insights for developing improved livestock management practices and sustainable feeding systems, ultimately enhancing ruminant production efficiency.

MATERIALS AND METHODS

All the experimental procedures, including experimental animal maintenance and sample collection, were conducted following the guidelines of the ethical committee at the Philippine Carabao Center National Headquarters and Gene Pool with the research code AN19004-RC.

Study location

Sample collection and DNA extraction were conducted in the Philippines, while sample analysis took place in Japan.

Animals and sample collection

Samples were collected from two healthy, male, island-born riverine-type buffalo aged 35 months, weighing 464 ± 32 kg. Following standard dairy buffalo production management practices, the animals were reared and maintained at the Gene Pool Farm, Philippine Carabao Center National Headquarters, Science City of Munoz, Nueva Ecija, Philippines. The animals were kept in complete confinement and offered the same diet, composed of rice straw, grower concentrate, and freshly chopped grasses. The ration offered was estimated to provide the amount of protein and energy needed for the growth and maintenance of the animals. Ad libitum clean water was also provided. Feeding was continued until the animals reached the target slaughter weight.

The animals were butchered using a standard procedure in accordance with the Humane Slaughter Guidelines of the National Meat Inspection Services at Animal Products Development Center of the Bureau of Animal Industry, Marulas, Valenzuela City Philippines. Fresh luminal samples (20 g) were collected from different sites of the three GIT regions of each buffalo (forestomach: rumen, reticulum, omasum, and abomasum; small intestine: duodenum, jejunum, and ileum; large intestine: cecum, colon, and rectum; Figure 1). In the rumen, three types of samples were collected: rumen fluid, rumen digesta, and rumen tissue. Rumen samples were filtered using sterilized gauze to obtain both liquid and solid samples (fiber-adherent). Reticulum mucosa was also collected in addition to reticulum digesta. Sampling of the intestine was carefully conducted from the beginning of the small intestine (duodenum) through the end of the large intestine (rectum). Each sample from the different GIT sites was thoroughly mixed before further processing. Three replicates from each site and sample type were placed in sterile centrifuge tubes, a total of 78 samples were immediately frozen in liquid nitrogen, and transported to the laboratory for genomic DNA extraction.

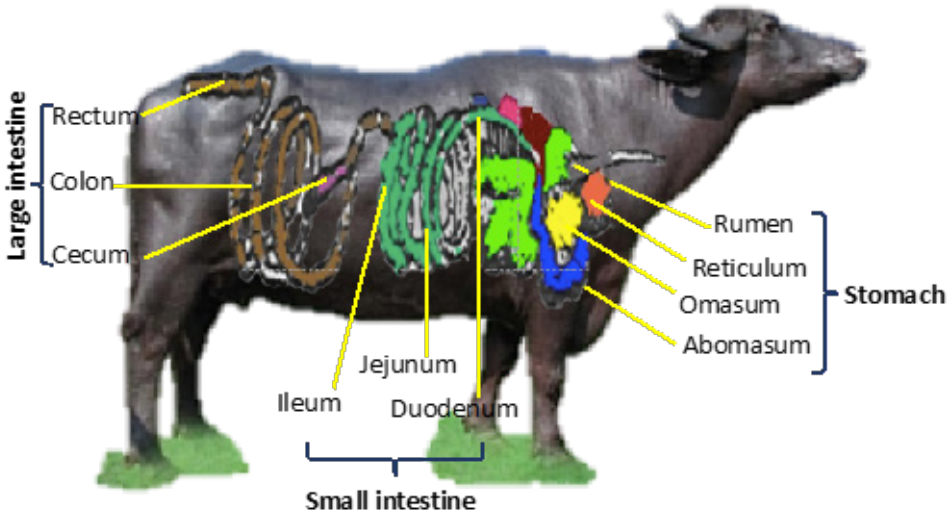


Figure 1 Sampling locations along the buffalo gastrointestinal tract (GIT)

DNA extraction

Rumen and reticulum mucosa samples were scraped to remove attached food particles and then rinsed three times with sterilized phosphate buffered saline (pH 7.0) prior to extraction. Genomic DNA was extracted from the samples using a QIAamp™ Fast DNA Stool Mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's manual for bacterial DNA isolation. A portion of the DNA extracted from each collection site and sample type was pooled. The samples were stored at -20ºC until further analysis. Sixteen DNA samples were subjected to next-generation sequence analysis: 13 samples (rumen fluid, rumen digesta, rumen mucosa, reticulum, reticulum mucosa, omasum, abomasum, duodenum, jejunum, ileum, cecum, colon, and rectum) from buffalo 1 (B1) for intra-buffalo bacterial composition evaluation, and three samples (rumen digesta, ileum and rectum) from buffalo 2 (B2) for inter-buffalo comparison.

Library preparation and sequencing method

Thirteen DNA samples from the GIT of B1 and three from the samples collected from the GIT of B2 were used for the analysis. Prior to sequence analysis, the DNA concentrations were determined using Synergy H1 (Bio Tek) and Quanti Fluor dsDNA Systems (Promega) (Table 1). This was followed by amplification of the V3-V4 hypervariable regions of the bacterial 16S rRNA gene using an *ExTaq* kit (Takara, Otsu, Japan). Two-step tailed PCR was conducted for library preparation. A total of 10 µl of PCR reaction mixture (Table 2) for each sample was amplified using the following PCR conditions: initial denaturation at 94ºC for 2 min, followed by thermal cycles consisting of denaturation at 94ºC for 30 s, 55ºC annealing for 30 s, extension at 72ºC for 10 min (30 cycles), and final extension at 72ºC for 5 min (Table 3). A second PCR was conducted to attach Illumina sequencing adapters and unique dual indices (Table 4). The second PCR reaction mixture (Table 5) was similar to the first except for the concentration of PCR product used (maximum of 5 ng/µl) as DNA

template (Table 6). The annealing temperature was also increased to 60ºC, with 10 amplification cycles performed (Table 7). The concentration of the prepared library was measured using the Synergy H1 and Quanti Fluor dsDNA Systems (Table 8), and quality (Table 9, 10) was confirmed using a Fragment Analyzer and dsDNA 915 Reagent kit (Advanced Analytical Technologies). Sequencing was performed on an Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA) using the 2×300 bp paired-end method according to standard protocols at Bioengineering Laboratory Co. Ltd. in Kanagawa, Japan.

Table 1 Initial concentration of DNA samples from the different site in buffaloes' GIT

Sample Name	Concentration(ng/ul)	Volume(ul)
Rumen-Fluid	1.35	60
Rumen-Digesta	0.377	60
Rumen-Mucosa	0.291	60
Reticulum	0.346	60
Reticulum-Mucosa	0.262	60
Omasum	0.346	60
Abomasum	0.655	60
Duodenum	0.259	60
Jejunum	0.209	60
Ileum	0.985	60
Cecum	1.18	60
Colon	0.736	60
Rectum	0.727	60
Rumen-Digesta-a*	0.487	60
Ileum-a*	2.54	60
Rectum-a*	0.845	60

* Samples collected in the GIT of buffalo 1

Table 2 First PCR reaction mixture for library preparation

Reagent	Concentration	Volume(ul)
Buffer	10X	1.0
dNTP Mixture	2.5mM each	0.8
Forward primer	10uM	0.5
Reverse primer	10uM	0.5
Template	Max 0.5ng/ul*	2.0
ExTaq (TaKaRa)	5U/ul	0.1
DDW		5.1

Table 3 First PCR reaction conditions for library preparation

Temperature	Time	Number of cycles
94°C	2min	
94°C	30sec	
55°C	30sec	30cycles
72°C	30sec	
72°C	5min	

Table 4 Illumina sequencing adapters used in library preparation prior to sequencing

Sample Name	Index1	Index2
Rumen-Fluid	AGCTTCAG	TCGACTAG
Rumen-Digesta	AGCTTCAG	TTCTAGCT
Rumen-Mucosa	AGCTTCAG	CCTAGAGT
Reticulum	AGCTTCAG	GCGTAAGA
Reticulum-Mucosa	AGCTTCAG	CTATTAAG
Omasum	AGCTTCAG	AAGGCTAT
Abomasum	AGCTTCAG	GAGCCTTA
Duodenum	AGCTTCAG	TTATGCGA
Jejunum	GCGCATTA	TCGACTAG
Ileum	GCGCATTA	TTCTAGCT
Cecum	GCGCATTA	CCTAGAGT
Colon	GCGCATTA	GCGTAAGA
Rectum	GCGCATTA	CTATTAAG
Rumen-Digesta-a	GCGCATTA	AAGGCTAT
Ileum-a	GCGCATTA	GAGCCTTA
Rectum-a	GCGCATTA	TTATGCGA

Table 5 Second PCR reaction mixture for library preparation

Reagent	Concentration	Volume (ul)
Buffer	10X	1.0
dNTP Mixture	2.5mM each	0.8
Forward primer	10uM	0.5
Reverse primer	10uM	0.5
PCR product	Max 5ng/ul	2.0
ExTaq (TaKaRa)	5U/ul	0.1
DDW		5.1

Table 6 Second PCR reaction conditions for library preparation

Temperature	Time	Number of cycles
94°C	2min	
94°C	30sec	
60°C	30sec	10cycles
72°C	30sec	
72°C	5min	

Table 7 Primers used in library preparation prior to sequencing

Primer Name	Sequence (5' → 3')
1st-341f_MIX*	ACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNNNN-CCTACGGGNGGCWGCAG
1st-805r_MIX*	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-NNNNN-GACTACHVGGGTATCTAATCC
2ndF	AATGATACGGCGACCACCGAGATCTACAC-Index2-ACACTCTTTCCCTACACGACGC
2ndR	CAAGCAGAAGACGGCATACGAGAT-Index1-GTGACTGGAGTTCAGACGTGTG

Table 8 Sample DNA concentration from library preparation

Sample Name	Concentration(ng/ul)	Liquid Volume(ul)
Rumen-Fluid	24.9	20
Rumen-Digesta	24.6	20
Rumen-Mucosa	19.9	20
Reticulum	25.2	20
Reticulum-Mucosa	22.9	20
Omasum	24.9	20
Abomasum	25.2	20
Duodenum	23.7	20
Jejunum	27.4	20
Ileum	26.7	20
Cecum	23.6	20
Colon	23.3	20
Rectum	22.3	20
Rumen-Digesta-a	22.5	20
Ileum-a	27.6	20
Rectum-a	22.7	20

Table 9 Sequence result of samples from the different GIT sites

Sample Name	Raw Read Number	Q20*(%)	Q30*(%)
Rumen-Fluid	53,935	93.8	86.2
Rumen-Digesta	54,668	94.2	86.9
Rumen-Mucosa	42,503	94.0	86.6
Reticulum	51,459	94.0	86.4
Reticulum-Mucosa	63,030	94.1	86.7
Omasum	61,347	94.0	86.6
Abomasum	45,489	94.3	87.0
Duodenum	64,189	94.0	86.6
Jejunum	46,678	94.3	87.2
Ileum	45,434	94.6	87.8
Cecum	56,419	94.3	87.0
Colon	46,636	94.3	87.0
Rectum	59,136	94.5	87.3
Rumen-Digesta-a	52,539	93.9	86.3
Ileum-a	45,892	94.7	87.8
Rectum-a	59,394	94.4	87.2

Table 10 Number of reads used for QIIME analysis

Sample Name	Raw	Nonchimera
Rumen-Fluid	53,935	28,908
Rumen-Digesta	54,668	30,799
Rumen-Mucosa	42,503	26,358
Reticulum	51,459	28,055
Reticulum-Mucosa	63,030	38,358
Omasum	61,347	35,517
Abomasum	45,489	26,562
Duodenum	64,189	44,835
Jejunum	46,678	33,645
Ileum	45,434	27,940
Cecum	56,419	30,283
Colon	46,636	24,889
Rectum	59,136	30,936
Rumen-Digesta-a	52,539	27,791
Ileum-a	45,892	34,975
Rectum-a	59,394	32,113

Data analysis

Raw fastq files were processed using the fastq_barcode_spliltter of FASTX- Toolkit (ver. 0.0.14). Sequences with an average quality value of <20 were removed using sickle tools (ver. 1.33) and sequences with a length of ≤150 bp and their paired sequences were disabled. The array that passed the quality filtering was merged using the paired-end merge script FLASH (ver.1.2.11). The merging conditions were set to a fragment length of 420 bases, 280 bases length of lead, and a minimum overlap length of 10 bases. To increase the analysis quality, Usearch's Uchime algorithm (ver.8.1.1861) was used to remove chimeric sequences. Operational taxonomic units (OTUs) were selected using the Green-gene database (ver. 13_8) which is included in the Qiime (ver. 1.19.1), a pipeline for bacterial flora analysis with 97% similarity cut-off. All sequences that were not identified as chimeras were extracted and used in subsequent analyses. OTU creation and phylogeny estimation were performed using Qiime's workflow script with no references and default parameters. A heatmap with dendogram was constructed using R software to show relative abundances and the relationships between bacterial communities of the different phyla within the different sites of buffalo GIT. Venn diagrams were created to determine the shared and unique bacterial genera between the three regions of the GIT (forestomach, small intestine, and large intestine).

RESULTS

This study was carried out to obtain a deeper understanding of the composition of bacterial communities within the buffalo GIT. One buffalo (B1) was used for the intra-individual analysis, wherein samples across the whole GIT were subjected to next- generation sequence analysis, and the other buffalo (B2) was used for the inter-individual analysis. For the inter-individual analysis, DNA from luminal samples collected in the rumen, ileum, and rectum of both animals were utilized and compared.

A total of 501,964 high-quality sequences of 16S rRNA gene amplicons were obtained after sequence trimming, quality filtering, and chimera removal using the Illumina MiSeq platform. Sample sequences were classified from phylum to genera using the QIIME (ver. 1.19.1) program, and after annotation, 25 phyla, 50 classes, 87 orders, 147 families, and 176 (53.98 %) genera and unclassified bacteria were detected.

Bacterial composition across the GIT of buffalo

In our study, phylum *Bacteroidetes* dominated the forestomach (rumen fluid, rumen mucosa, reticulum, reticulum mucosa, omasum, and abomasum), including the duodenum, whereas from the jejunum to the rectum, phylum *Firmicutes* was the most abundant (Figure 2a and Figure 3). Phylum *Proteobacteria* was also found to have the highest percentage in the rumen digesta and rumen mucosa among the GIT sites (Figure 2a and Figure 3). In terms of diversity, the duodenum (17 phyla), omasum (15 phyla), and abomasum (14 phyla) were the most diverse sites in the GIT. Moreover, among the phyla with ≥ 0.1% relative abundance in samples from at least one site of the GIT, six (*Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *TM-7*, *Tenericutes,* and *Verrucomicrobia*) were shared across the buffalo GIT; two (*Elusimicrobia* and *Fibrobacteres*) were found in the rumen fluid, omasum, abomasum and duodenum; *LD1* and *SR1* were found in the rumen fluid, omasum and duodenum; and *WPS-2* was only detected in the duodenum and *Chloroflexi* in the jejunum (Table 11). A heatmap of bacterial phyla within the GIT of B1 revealed the most abundant OTUs and their respective abundances at the different sites within the GIT, as well as the relationship between the OTUs (Figure 3). Figure 3 also shows that the bacterial composition within the jejunum and ileum differed from the composition at other sites.

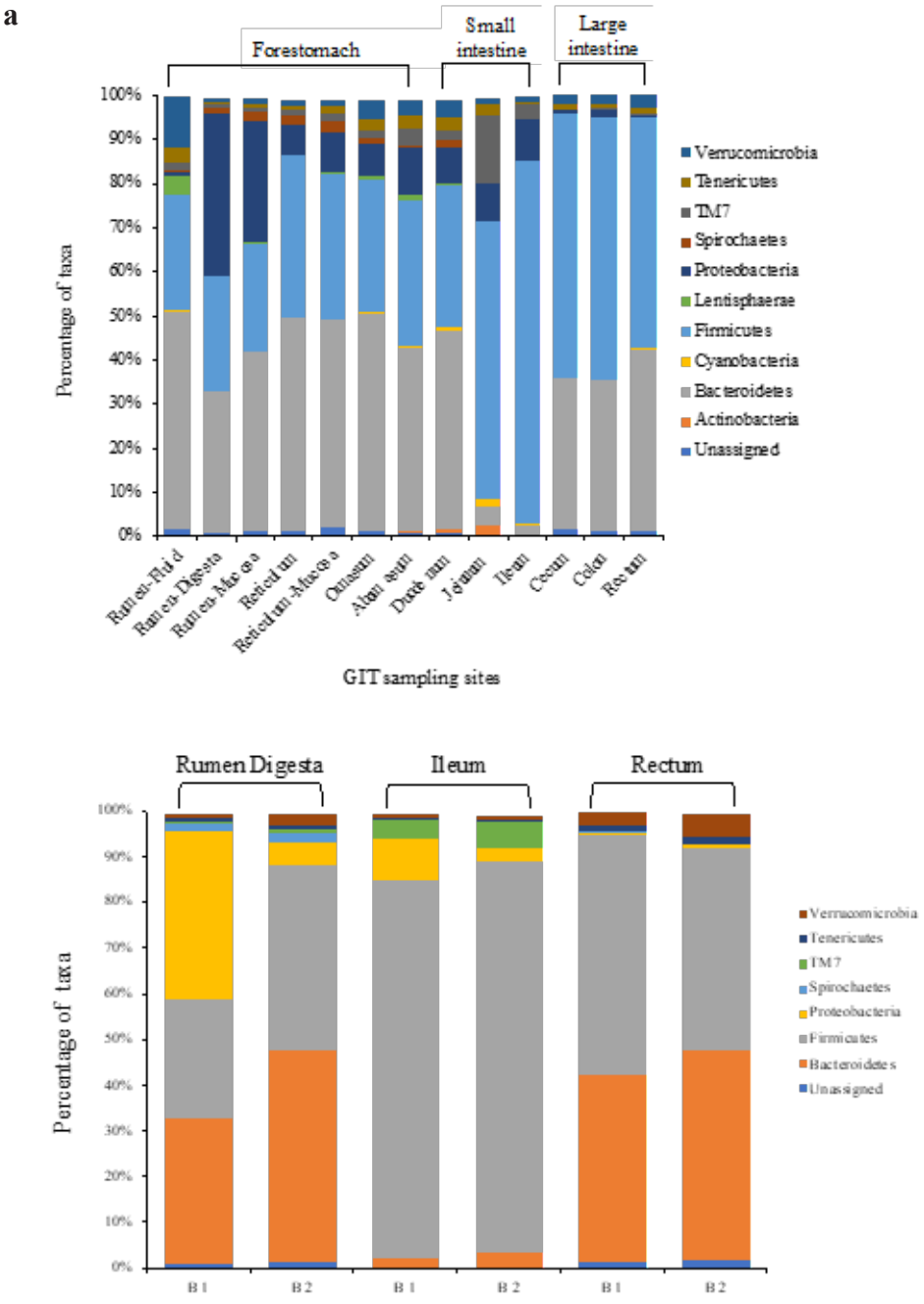


Figure 2 Bacterial taxa composition in Phylum -level. Relative abundances of the most abundant bacterial phyla (a) across the GIT of buffalo 1 (intra- individual evaluation) and (b) specific site in the GIT of buffalo 1 (B1) and buffalo 2 (B2) for the inter-individual comparison. Only taxa with >1% relative abundance in at least one site of the GIT were represented.

Table 11 Summary of the bacterial Phyla localization across the GIT of dairy buffalo 1.

Phylum	GIT sites												
	Rumen Fluid	Rumen Digesta	Rumen Mucosa	Reticulum	Reticulum Mucosa	Omasum	Abomasum	Duodenum	Jejunum	Ileum	Cecum	Colon	Rectum
Actinobacteria	X	X	O	X	X	X	O	O	O	O	X	X	X
Bacteroidetes	O	O	O	O	O	O	O	O	O	O	O	O	O
Chloroflexi	X	X	X	X	X	X	X	X	O	X	X	X	X
Cyanobacteria	O	O	O	X	O	O	O	O	O	O	X	O	O
Elusimicrobia	O	X	X	X	X	O	O	O	X	X	X	X	X
Fibrobacteres	X	X	X	X	X	O	O	O	X	X	X	X	X
Firmicutes	O	O	O	O	O	O	O	O	O	O	O	O	O
LD1	O	X	X	X	X	O	X	O	X	X	X	X	X
Lentisphaerae	O	O	O	O	O	O	O	O	X	X	O	O	O
Proteobacteria	O	O	O	O	O	O	O	O	O	O	O	O	O
SR1	X	X	X	X	X	O	X	O	X	X	X	X	X
Spirochaetes	O	O	O	O	O	O	O	O	X	X	O	O	O
Synergistetes	X	O	O	O	O	O	O	O	O	X	X	X	X
TM7	O	O	O	O	O	O	O	O	O	O	O	O	O
Tenericutes	O	O	O	O	O	O	O	O	O	O	O	O	O
Verrucomicrobia	O	O	O	O	O	O	O	O	O	O	O	O	O
WPS-2	X	X	X	X	X	X	X	O	X	X	X	X	X

o, detected; x, not detected

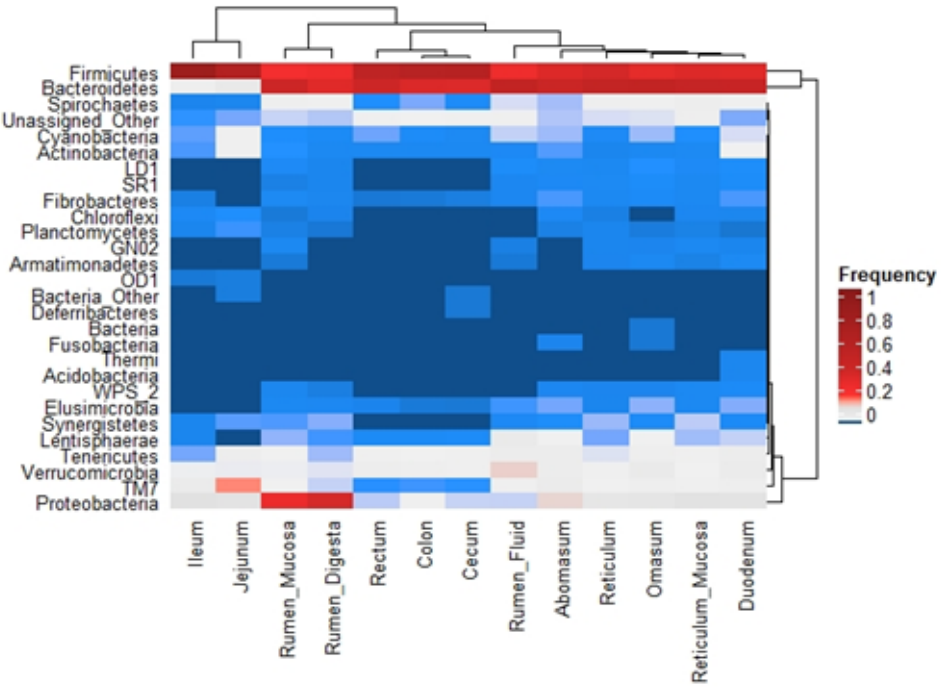


Figure 3 Beta diversity of samples shown in heatmap. The color intensity indicates the abundance of bacteria (Phylum level) in each sampling site of buffalo 1 (B1).

Bacterial composition of the three major phyla at the family level

Firmicutes (43.0%), *Bacteroidetes* (36.2%), and *Proteobacteria* (9.72%) were the most dominant phyla across the GIT of B1; therefore, we further analyzed these phyla at the family level. In phylum *Firmicutes,* the families *Ruminococcaceae* (17.8%), unidentified under order *Clostridiales* (7.38%) and *Lachnospiraceae* were most abundant across the GIT (Figure 4b). *Ruminococcaceae* was highest in the cecum, whereas unidentified (order *Clostridiales*) was most dominant in the jejunum and *Lachnospiraceae* in the reticulum. Families *Peptococcaceae* and *Eubacteriaceae* were only found in the colon and jejunum, respectively (Table 12).

After phylum *Bacteroidetes,* the next most abundant phyla across the GIT consisted primarily of unidentified families under the order *Bacteriodales* (16.4%), *Prevotellaceae* (6.99%), and *BS 11* (5.34%) (Figure 5a). Unidentified family (order *Bacteriodales*) was dominant in the reticulum mucosa, whereas *Prevotellaceae* was most abundant in the reticulum and *BS 11* in the rumen fluid (Figure 5a). Some families belonging to this phylum were found only in the large intestine (*Rikenellaceae*, [*Burnesiellaceae*], [*Odoribacteraceae*], and *p-2534-18B5*) and jejunum (*Flavobacteriaceae*) (Table 13).

The third most abundant phylum was *Proteobacteria,* which was comprised primarily of the families *Enterobacteriaceae* (5.08%), *Succinivibrionaceae* (1.88%), and *Moraxellaceae* (1.42%) (Figure 6a). Family *Succinivibrionaceae* was most abundant in the abomasum, *Enterobacteriaceae* in the rumen digesta, and *Moraxellaceae* in the rumen mucosa. Although phylum *Proteobacteria* was the least abundant among the three dominant phyla, many rare families were only found in the reticulum mucosa, small intestine, and large intestine (Table 14). Families *Rhodocyclaceae* and *Desulfobulbaceae* were found only in the reticulum mucosa. In contrast, in the small intestine, families *Bradyrhizobiaceae*, *Rhizobiaceae*, *Rhodobacteraceae,* and *Sphingomonadaceae* were found only in the duodenum. Families *Aurantimonadaceae*, *Aeromonadaceae*, *Idiomarinaceae*, [*Chromatiaceae*], *Halomonadaceae,* and *Xanthomonadaceae* were found only in the jejunum. Families *Acetobacteraceae*, *Caulobacteraceae,* and *Methylobacteriaceae* were found both in the duodenum and jejunum. In addition, family *Alcaligenaceae* was detected only in the large intestine (cecum, colon, and rectum).

Table 12 Overview of the different family localization under the phylum *Firmicutes* across the dairy buffalo's (B1) GIT.

Family	GIT Sites												
	Rumen Fluid	Rumen Digesta	Rumen Mucosa	Reticulum	Reticulum Mucosa	Omasum	Abomasum	Duodenum	Jejunum	Ileum	Cecum	Colon	Rectum
Bacillaceae	X	O	O	O	O	O	X	X	O	O	X	O	O
Paenibacillaceae	X	X	X	X	X	X	X	X	X	O	X	O	X
Planococcaceae	X	O	O	O	O	X	X	O	O	O	O	O	O
Staphylococcaceae	X	X	X	X	X	X	X	O	O	O	X	X	X
[Exiguobacteraceae]	X	X	X	X	X	X	X	X	O	X	X	X	X
Aerococcaceae	X	X	X	X	X	X	X	X	O	X	X	X	X
Leuconostocaceae	X	O	X	O	X	O	X	O	O	X	X	X	X
Streptococcaceae	X	O	X	O	O	X	X	O	O	X	X	X	X
Turicibacteraceae	X	X	X	X	X	X	X	X	O	O	X	O	X
Order Clostridiales	X	X	X	X	X	X	X	X	X	X	O	O	O
Order Clostridiales	O	O	O	O	O	O	O	O	O	O	O	O	O
Christensenellaceae	O	O	O	O	O	O	O	O	O	O	O	O	O
Clostridiaceae	O	O	O	O	O	O	O	O	O	O	O	O	O
Eubacteriaceae	X	X	X	X	X	X	X	X	O	X	X	X	X
Lachnospiraceae	O	O	O	O	O	O	O	O	O	O	O	O	O
Peptococcaceae	X	X	X	X	X	X	X	X	X	X	X	O	X
Peptostreptococcaceae	X	X	X	X	X	X	X	X	O	O	O	O	O
Ruminococcaceae	O	O	O	O	O	O	O	O	O	O	O	O	O
Veillonellaceae	O	O	O	O	O	O	O	O	O	O	O	O	O
[Mogibacteriaceae]	O	O	O	O	O	O	O	O	O	O	O	O	O
Erysipelotrichaceae	O	O	O	O	O	O	O	O	O	O	O	O	O

O- detected; X- not detected

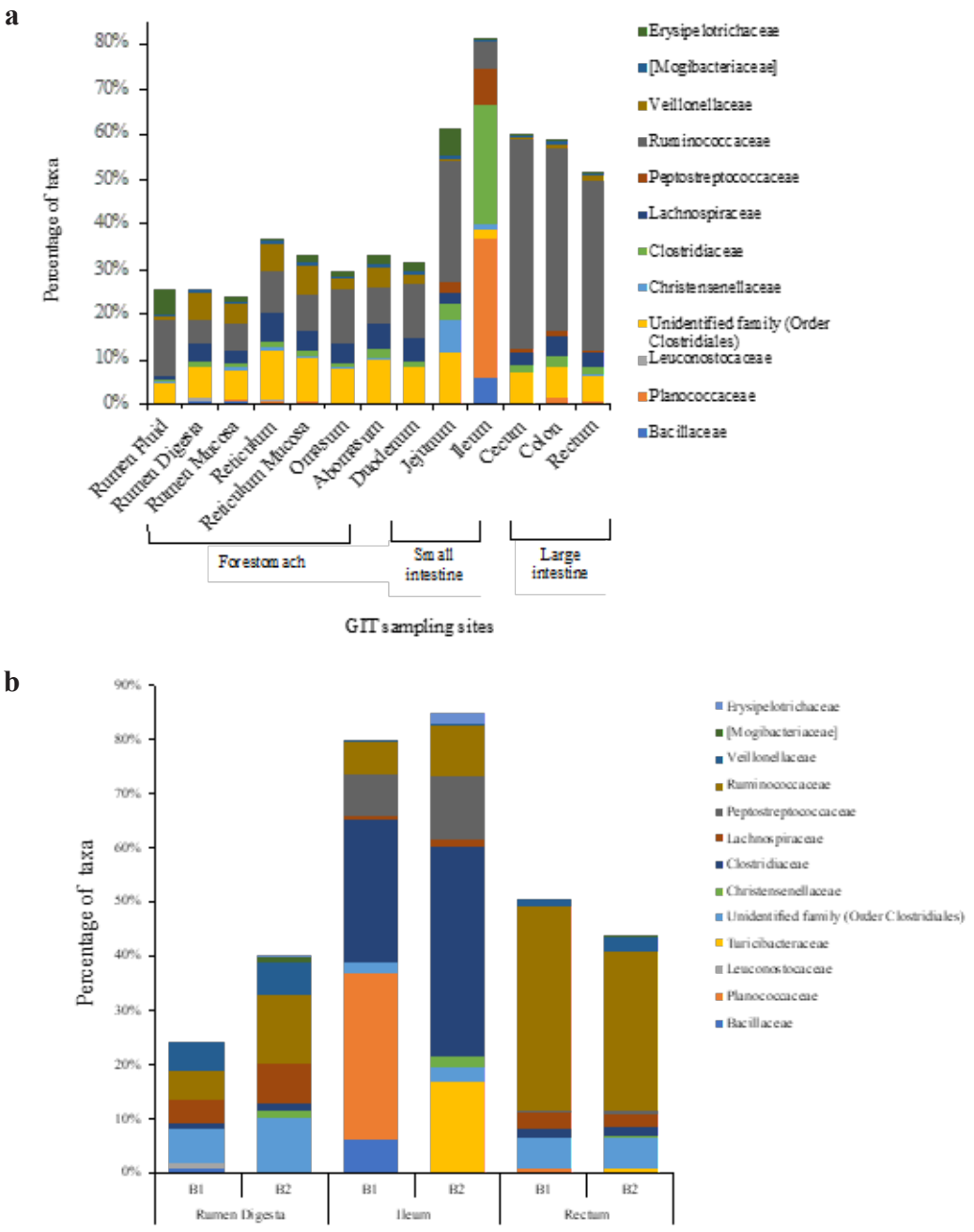


Figure 4 Relative abundance of OTUs within Phylum Firmicutes (a) across the GIT of buffalo 1; (b) specific sites within the GIT of buffalo 1 (B1) and buffalo 2 (B2) for inter-individual comparison Only taxa with >1% relative abundance in at least one site of the GIT are shown.

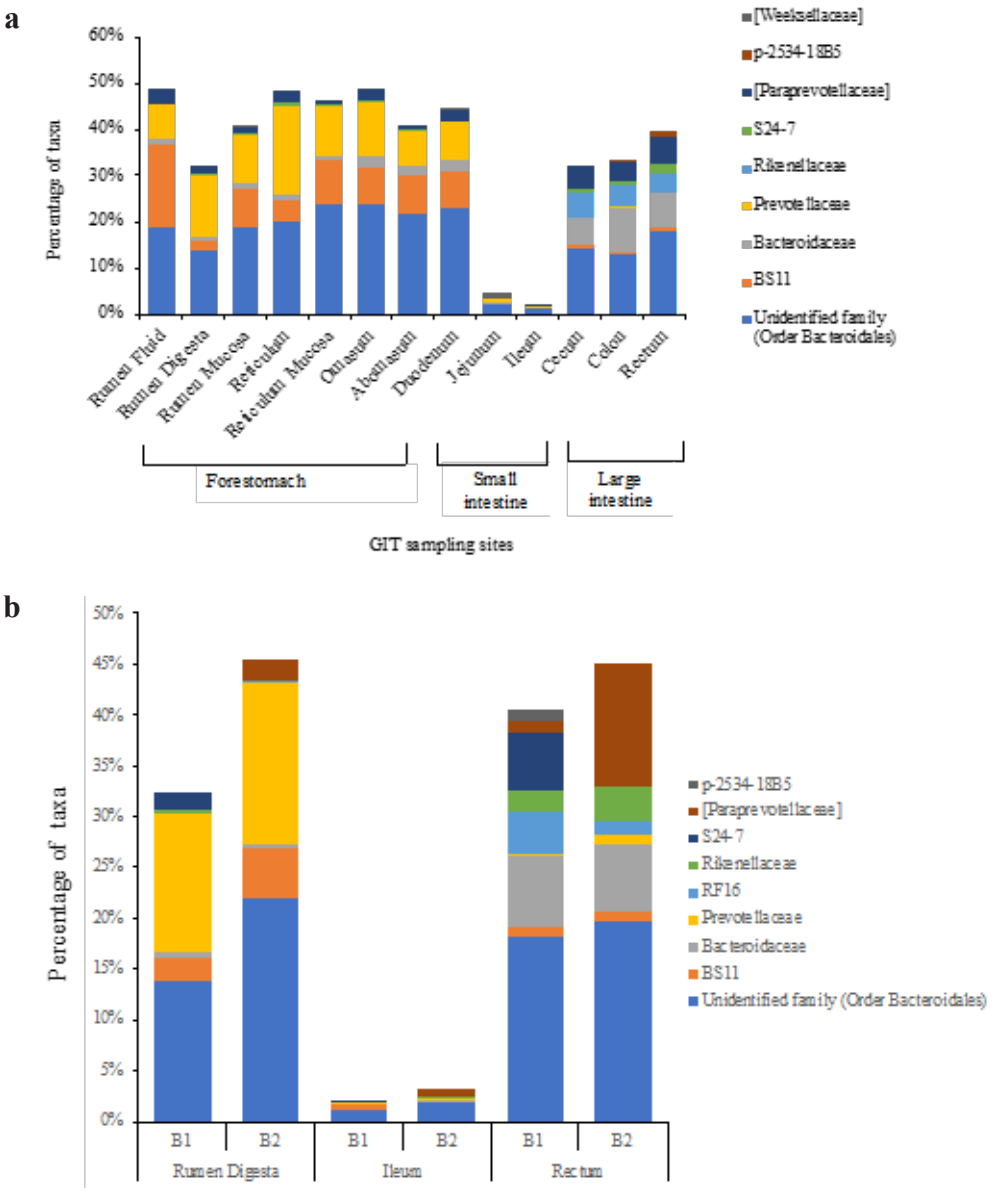


Figure 5 OTUs abundances within the Phylum Bacteroidetes (a) across the GIT of buffalo 1 (B1); (b) specific sites within the GIT of buffalo 1 (B1) and buffalo 2 (B2) for inter-individual comparison Only taxa with >1% relative abundance in at least one site of the GIT are shown.

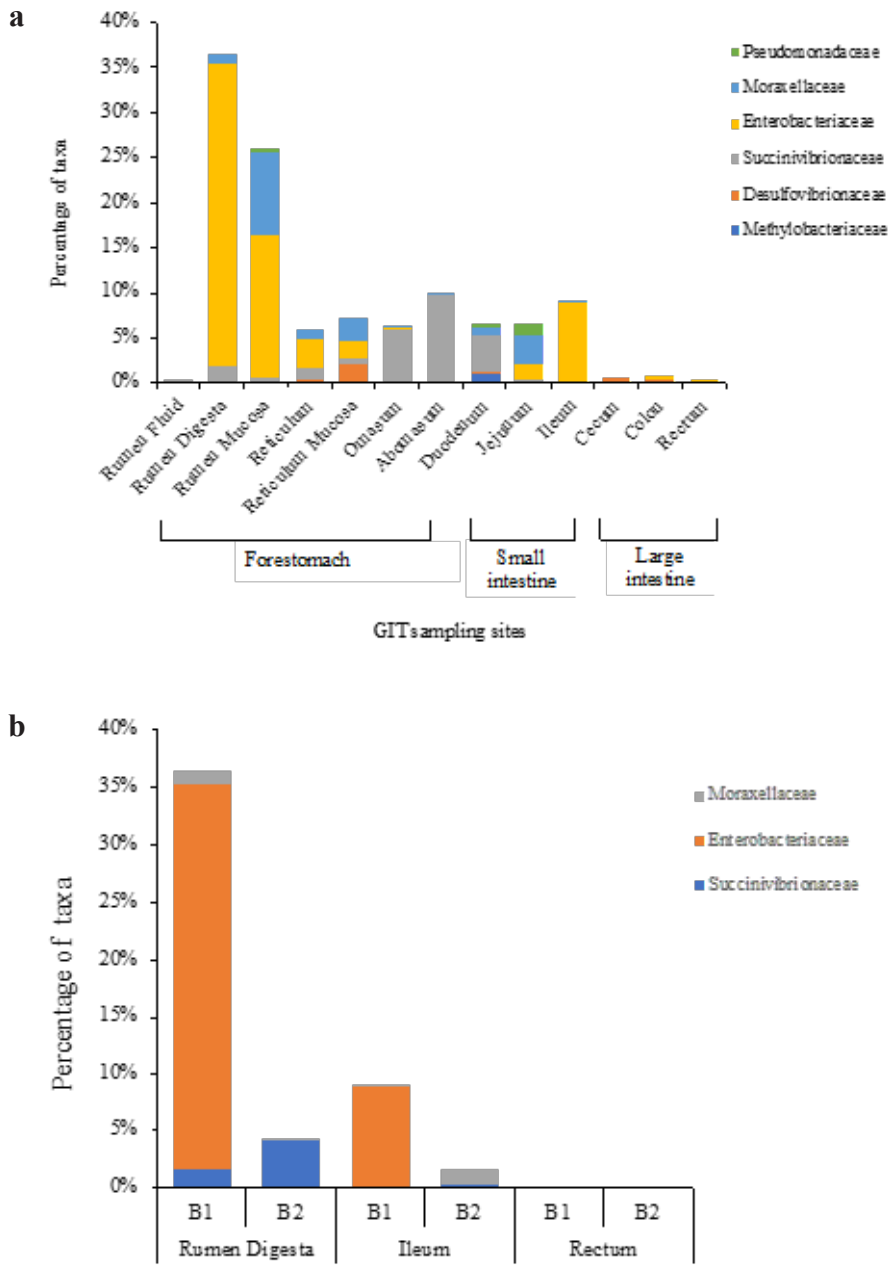


Figure 6 Relative abundance of OTUs within Phylum Proteobacteria (a) across the GIT of buffalo 1 (B1); (b) specific sites within the GIT of buffalo 1 (B1) and buffalo 2 (B2) for inter-individual comparison Only taxa with >1% relative abundance in at least one site of the GIT are shown.

Table 13 Localization of the different families under phylum *Bacteroidetes* across the GIT of dairy buffalo (B1).

Family	GIT Sites												
	Rumen Fluid	Rumen Digesta	Rumen Mucosa	Reticulum	Reticulum Mucosa	Omasum	Abomasum	Duodenum	Jejunum	Ileum	Cecum	Colon	Rectum
Order Bacteroidales;Other	X	X	X	X	X	O	X	O	X	X	X	X	X
Order Bacteroidales	O	O	O	O	O	O	O	O	O	O	O	O	O
BS11	O	O	O	O	O	O	O	O	O	O	O	O	O
Bacteroidaceae	O	O	O	O	O	O	O	O	O	X	O	O	O
Porphyromonadaceae	O	X	X	X	X	X	X	X	X	X	O	O	O
Prevotellaceae	O	O	O	O	O	O	O	O	O	O	O	O	O
RF16	O	O	O	O	O	O	O	O	O	O	O	O	O
Rikenellaceae	X	X	X	X	X	X	X	X	X	X	O	O	O
S24-7	O	O	O	O	O	O	O	O	X	X	O	O	O
[Barnesiellaceae]	X	X	X	X	X	X	X	X	X	X	O	O	O
[Odoribacteraceae]	X	X	X	X	X	X	X	X	X	X	O	O	O
[Paraprevotellaceae]	O	O	O	O	O	O	O	O	O	O	O	O	O
p-2534-18B5	X	X	X	X	X	X	X	X	X	X	X	O	O
Flavobacteriaceae	X	X	X	X	X	X	X	X	O	X	X	X	X
[Weeksellaceae]	X	X	O	X	X	X	X	O	O	X	X	X	X

O- detected; X- not detected

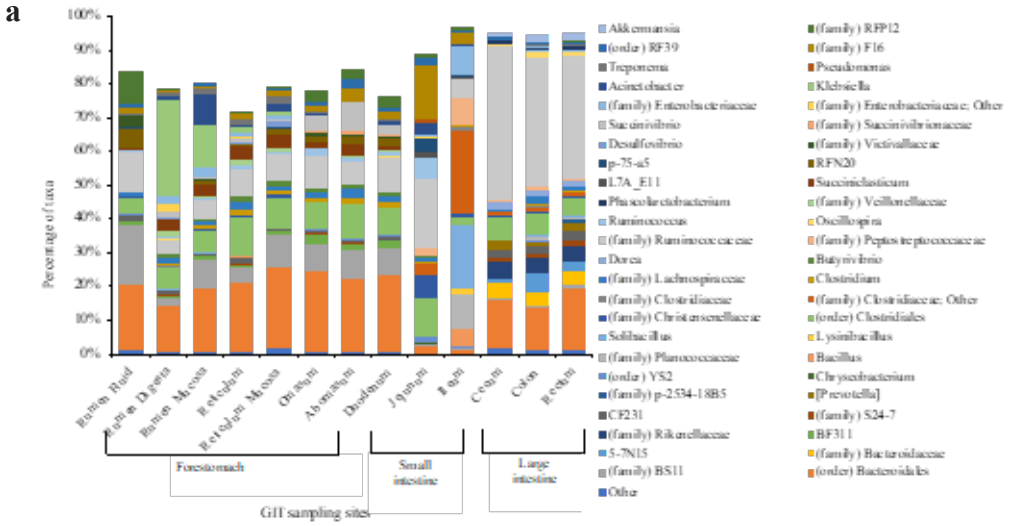
Table 14 Dairy buffalo (B1) GIT localization of the families under phylum *Proteobacteria.*

Family	GIT Sites												
	Rumen Fluid	Rumen Digesta	Rumen Mucosa	Reticulum	Reticulum Mucosa	Omasum	Abomasum	Duodenum	Jejunum	Ileum	Cecum	Colon	Rectum
Class Alphaproteobacteria	O	X	X	X	X	X	O	X	X	X	X	X	X
Caulobacteraceae	X	X	X	X	X	X	X	O	O	X	X	X	X
Order RF32	O	O	O	O	O	O	O	O	O	X	X	O	O
Aurantimonadaceae	X	X	X	X	X	X	X	X	O	X	X	X	X
Bradyrhizobiaceae	X	X	X	X	X	X	X	O	X	X	X	X	X
Methylobacteriaceae	X	X	X	X	X	X	X	O	O	X	X	X	X
Rhizobiaceae	X	X	X	X	X	X	X	O	X	X	X	X	X
Rhodobacteraceae	X	X	X	X	X	X	X	O	X	X	X	X	X
Acetobacteraceae	X	X	X	X	X	X	X	O	O	X	X	X	X
Order Rickettsiales	X	O	O	O	O	X	X	X	X	X	X	X	X
Sphingomonadaceae	X	X	X	X	X	X	X	O	X	X	X	X	X
Alcaligenaceae	X	X	X	X	X	X	X	X	X	X	O	O	O
Comamonadaceae	X	X	O	X	O	X	X	O	O	X	X	X	X
Oxalobacteraceae	X	O	X	O	X	O	X	X	X	X	X	X	X
Rhodocyclaceae	X	X	X	X	O	X	X	X	X	X	X	X	X
Desulfobulbaceae	X	X	X	X	O	X	X	X	X	X	X	X	X
Desulfovibrionaceae	X	O	O	O	O	O	O	O	O	X	O	O	O
Pelobacteraceae	X	O	X	O	X	O	O	X	X	X	X	X	X
Order GMD14H09	X	X	O	O	O	O	X	X	X	X	X	X	X
Campylobacteraceae	X	X	O	X	O	X	X	X	O	X	X	X	X
Aeromonadaceae	X	X	X	X	X	X	X	X	O	X	X	X	X
Succinivibrionaceae	O	O	O	O	O	O	O	O	O	O	X	X	X
Idiomarinaceae	X	X	X	X	X	X	X	X	O	X	X	X	X
[Chromatiaceae]	X	X	X	X	X	X	X	X	O	X	X	X	X
Enterobacteriaceae	X	O	O	O	O	O	O	O	O	O	X	O	O
Halomonadaceae	X	X	X	X	X	X	X	X	O	X	X	X	X
Moraxellaceae	X	O	O	O	O	O	O	O	O	O	X	X	X
Pseudomonadaceae	X	X	O	X	X	X	X	O	O	O	X	X	X
Xanthomonadaceae	X	X	X	X	X	X	X	X	O	X	X	X	X

O- detected; X- not detected

Shared and unique bacterial genera across the GIT of B1

To obtain a more in-depth understanding of the bacterial community composition of the buffalo GIT, we also examined the genus level. Among the identified genera, the most abundant were *Klebsiella*, *Succiniclasticum*, *Solibacillus*, *Succinivibrio*, *Acinetobacter*, *Ruminococcus* and *CF231* (Figure 7a). Furthermore, to identify unique bacterial genera shared between the forestomach, small intestine, and large intestine, we created a Venn diagram (Figure 7e). The large intestine had the highest number of unique genera, followed by the small intestine. There were no unique genera found in the forestomach; however, it shared genus *Akkermansia* with the large intestine. Furthermore, most of the genera shared between the GIT regions (forestomach, small intestine, large intestine) belonged to the phylum Firmicutes (e.g., *Ruminococcus*, *Oscillospira*). Genera shared between the forestomach and small intestine belonged to the phyla *Proteobacteria* and *Firmicutes*, whereas those shared between the small and large intestines all belonged to phylum *Firmicutes*.



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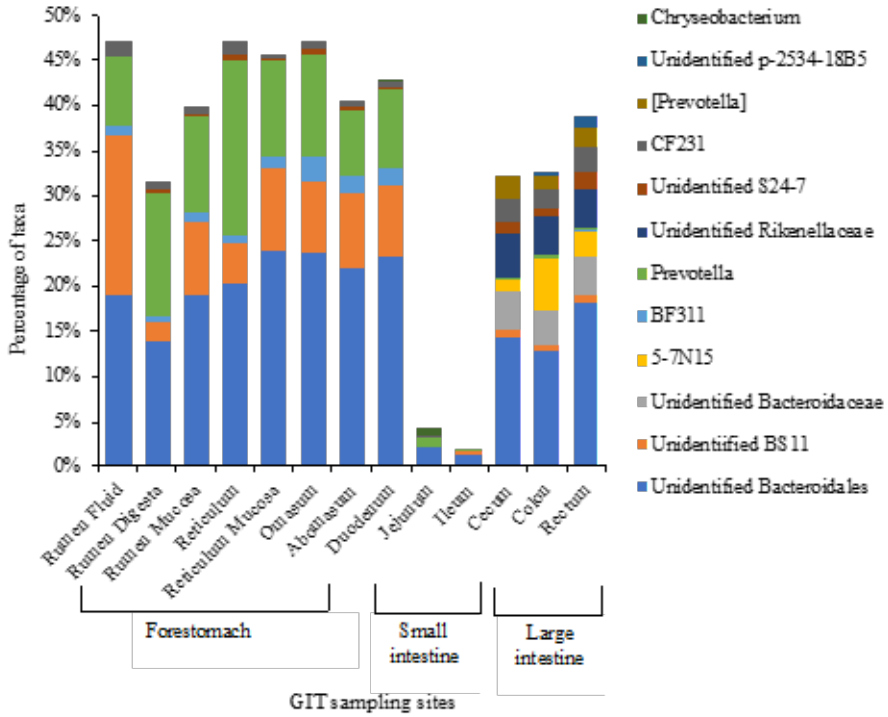
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BF311
5-7N15
Unidentified Bacteroidaceae
Unidentified BS11
Unidentified Bacteroidales
Rumen Fluid
Rumen Digesta
Rumen Mucosa
Reticulum
Reticulum Mucosa
Omasum
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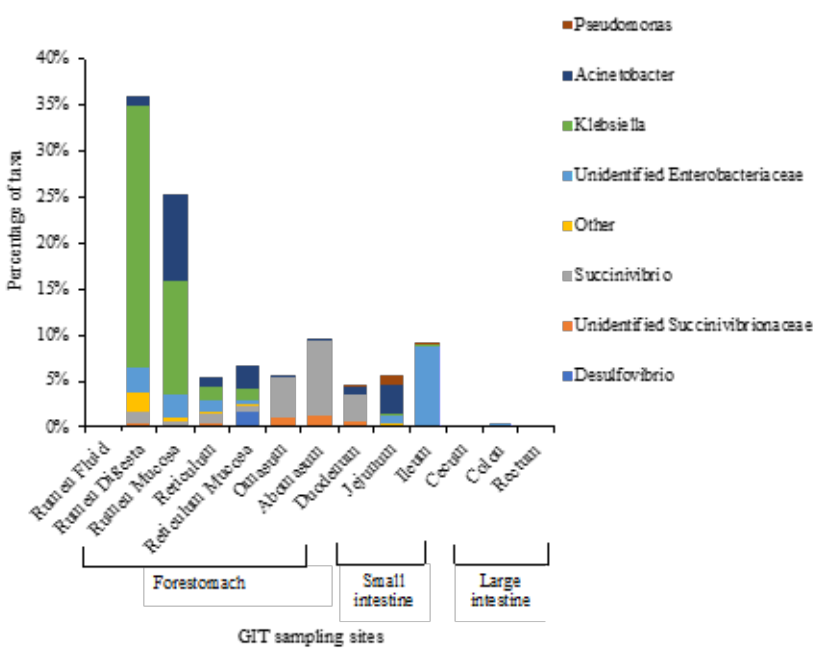
Duodenum
Jejunum
Ileum
Cecum
Colon
Rectum
Forestomach
Small intestine
Large intestine
GIT sampling sites

d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Other
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Rumen Fluid
Rumen Digesta
Rumen Mucosa
Reticulum
Reticulum Mucosa
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Rectum
Forestomach
Small intestine
Large intestine
GIT sampling sites

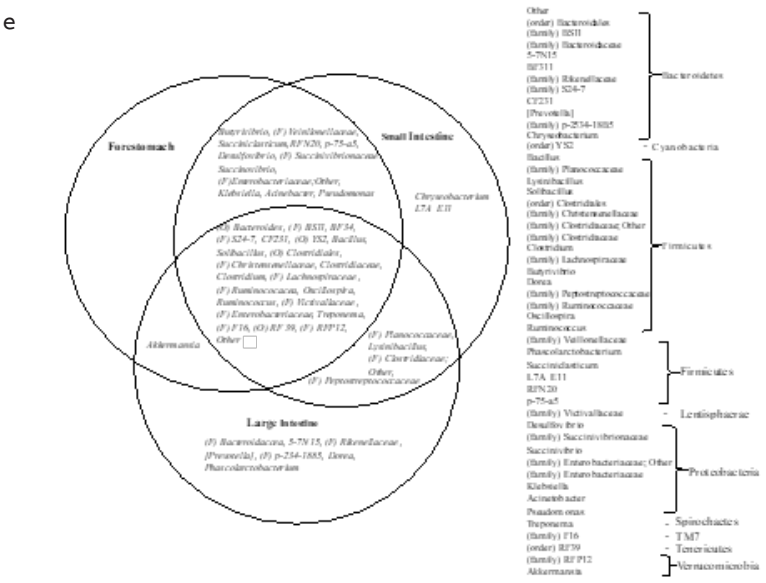

Figure 7 GIT bacterial profile across the GIT of buffalo 1 (B1) at the genus level (a). Relative abundance of OTUs at the genus level of the three major phyla Firmicutes (b) Bacteroidetes (c) Proteobacteria (d) and Venn diagrams of unique and shared bacterial genera between the forestomach, small intestine, and large intestine (e).

Inter-buffalo bacterial community analysis (rumen digesta, ileum, rectum)

Phylum level

For the inter-buffalo analysis, we only used the OTU exhibiting ≥ 0.1% composition in at least one site of the GIT. We found that the bacterial composition of the rumen digesta from the two animals was the same, except that the phyla that dominated in each animal differed. In B1, *Proteobacteria* (36.9%) and *Bacteroidetes* (32.4%) exhibited the highest percent composition, whereas in B2, *Bacteroidetes* (46.2%) and *Firmicutes* (40.5%) dominated. In the ileum, by contrast, *Firmicutes* (B1: 82.6%, B2: 85.7%) was the most abundant phylum, followed by *Proteobacteria* (9.3%) in B1 and *TM7* (5.6%) in B2. In the rectum, the phyla *Firmicutes* (52.2%) and *Bacteroidetes* (41.10%) exhibited the highest percent composition in B1, whereas in B2, *Bacteroidetes* (46.4%) and *Firmicutes* (44.10%). Interestingly, phylum *Spirochaetes* was not in the ileum of B1 or rectum of B2 (Figure 2b).

Genus level

There were differences between the two animals in terms of genera detected. For genera belonging to the phylum *Firmicutes* (Figure 4b), *Bacillaceae*, *Planococcaceae,* and *Leuconostocacceae* were only detected in B1. *Bacillaceae* and *Planococcaceae* were found in three sites (the rumen digesta, ileum, and rectum), whereas *Leuconostocacceae* was only found in the rumen digesta. The genera *Turicibacteraceae* (ileum and rectum), *Christensenellaceae*, *Erysipelotrichaceae* (rumen digesta, ileum, rectum), and [*Mogibacteriaceae*] (rumen digesta) were only present in B2. Regarding genera belonging to phylum *Bacteroidetes* (Figure 5b), *S24-7* was detected only in B1, whereas [*Paraprevotellaceae*] was found only in B2. For phylum *Proteobacteria* (Figure 6B), genus *Enterobacteriaceae* was detected only in B1 (rumen digesta, ileum, rectum).

DISCUSSION

The primary objective of our study was to profile and compare the bacterial community composition across the GIT of dairy buffalo using a culture-independent method, specifically, next-generation sequence analysis using an Illumina MiSeq platform. The bacterial communities within the 13 GIT sites of B1 and three GIT sites of B2 were characterized to determine similarities and differences in the ruminal and fecal bacterial microbiota relative to other GIT sections as well as to characterize the variations in bacterial communities within the selected GIT sites of two animals of the same breed, fed with the same diet, and raised under the same conditions.

Phylum level

The results of the present study showed that the phyla *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* were predominant in all of the GIT samples (Figure 2a), comparable to the results of De Oliveira et al., 2013, (Zeng et al., 2017), and De Menezes et al., 2011. *Firmicutes,* the most abundant phylum across the GIT (Figure 2a and Figure 3) and composed primarily of various genera of fibrolytic and cellulolytic bacteria (Evans et al., 2011), was found at highest abundance from the small intestine down to the large intestine (Figure 3), particularly in the jejunum and rectum. Phylum *Bacteroidetes,* the second most abundant phylum (Figure 2a), is composed of bacteria responsible for complex carbohydrate digestion (Spence et al., 2006; Zeng et al., 2017) and predominated in the forestomach, including the first segment of the small intestine, the duodenum (Figure 3), similar to the results of Zeng et al., 2017 regarding the bacterial microbiome of Mongolian sheep. The third most abundant phylum was *Proteobacteria* (Figure 2a), which is composed of bacteria that catabolize feedstuff components such as grass and corn (Evans et al., 2011). This phylum was found to be in highest abundance within the rumen digesta and rumen mucosa (Figure 3), in contrast to Mongolian sheep (Zeng et al., 2017) and South American folivorous hoatzin (Godoy-Vitorino et al., 2012). Previous results showed that the number of *Proteobacteria* was higher in the hindgut than the foregut, whereas in our study of dairy buffalo, phylum *Proteobacteria* was most abundant in the forestomach (Figure 2a and Figure 3a). The differences in these results are likely due to several factors, including host characteristics such as diet, which is a major determinant of digestive communities (Tajima et al., 2001; Godoy-Vitorino et al., 2012; Ishaq et al., 2012; Perea et al., 2017). Another factor that can lead to variation in the host GIT microbiota is physio-chemical differences in different regions, such as the decline in water content, pH (Mackie et al., 1988), particle size, and VFA concentrations and fluctuations (Sato, 2010). The foregut, for example, is dominated by *Bacteroidetes*, which is related to high cellulolytic activity (Godoy-Vitorino et al., 2012). The midgut or the small intestine, a low-pH environment, is dominated by *Firmicutes*, which have thick peptidoglycan gram-positive cell wall that enable these bacteria to survive under harsh conditions (Bergmann, 2017). In addition, the function of each organ could be a determinant of differential bacterial community structure. A good example of this was provided by a study comparing the foregut and hindgut of hoatzins and cows (Godoy-Vitorino et al., 2012), which revealed strong similarities between the foregut and hindgut of these animals, despite their phylogenetic differences.

Genus level under the major Phylum

To obtain an in-depth understanding of GIT bacterial variations in dairy buffalo, we further analyzed the major phyla at lower taxonomic levels. At the genus level, phylum *Firmicutes* was comprised primarily of the families *Ruminococcaceae*, unidentified bacteria under order *Clostridiales*, and *Lachnospiraceae*, *Dorea, Ruminococcus, Succinuclasticum, Butyrivibrio,* unidentified *Lachnospiraceae,* unidentified *Peptostreptococcaceae*, and *Bacillus* (Figure 7b). Among the predominant taxa, *Ruminococcus* are considered one of the most important cellulose-degrading bacteria in the intestine of herbivores, as they produce large amounts of cellulolytic enzymes such as endoglucanases, exoglucanases, glucosidases, and hemicellulases. Detection of these bacteria in high numbers in the intestinal microbiota of buffalo and cattle is associated with the degradation of fiber in the feed, that is, the degradation of xylan and pectin and utilization of degraded soluble sugars as substrates (Zhang et al., 2016). In addition to the findings of (Zhang et al., 2016), the presence of *Dorea* was found to be positively correlated with the amount of milk protein and the pathways of amino acid biosynthesis.

Phylum *Bacteroidetes*, on the other hand, was predominated by unidentified *Bacteroidales*, *BS11*, *Prevotella*, *5-7NI15*, unidentified *Rikenellaceae*, and unidentified *Bacteroidaceae* (Figure 7c). *Prevotella* bacteria utilize starch and proteins to produce succinate and acetate, and as reported by Xue et al., 2018, this genus is most abundant in the rumen of lactating cows and thus part of the core microbiome.

Regarding phylum *Proteobacteria* family *Klebsiella*, *Acinetobacter*, unidentified *Enterobacteriaceae*, *Succinivibrio*, and unidentified *Succinivibrionaceae* were the most abundant (Figure 7d). Taxa belonging to *Succinivibrionaceae* produce succinate, a precursor of propionate that affects propionate production and thus regulates lactation performance (Xue et al., 2018). The shared genera or the core and pan bacteriomes in our study may interact to affect host performance.

Inter-individual comparison

Inter-animal differences were observed between the two dairy buffaloes. This only implies that even if the animals have the same breed, diet, and environment there were still differences within their GIT bacterial composition. Different individuals have different feed intake and diet preferences which can affect their gut microbiota (Ishaq et al., 2014). Moreover, external factors such as stress might also affect the bacteriome in the animals (Cholewińska et al., 2021).

CONCLUSIONS

In this study, we found out that the composition of the bacterial population comprising the dairy buffalo gastrointestinal tract varies greatly among compartments (intra-individual) at lower taxonomic levels and that there is inter-individual variation within the selected sites. Adjacent compartments were more similar to each other and can be useful as sample representative of changes occurring in the distal compartments such as the rectum which can be use as proxy to assess the GIT bacterial composition of the colon.

However, it is also important to consider that there are differences between these compartments, particularly at lower taxonomic levels. Physio-chemical characteristics of each GIT sites such as intestinal flow rate, redox potential, oxygen concentration, pH level, and availability of nutrients must also be addressed as a factor affecting the host microbiota aside from the physical properties.

Furthermore, we believe that the results of our study provided the first glimpse of the complex GIT bacterial communities within the GIT of dairy buffaloes. The study also revealed the inter-individual bacterial composition variation on GIT sites between two animals of the same breed and raised in the same conditions.

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AUTHOR CONTRIBUTIONS

The corresponding author participated in the collection, analysis, and interpretation of data, as well as in the writing of the manuscript. All authors have participated in the conception and design of this work, editing and reviewing the research report, and making he decision to submit the article for publication.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest that could inappropriately influence or bias the content of the paper.

REFERENCES

Bergmann, G.T., 2017. Microbial community composition along the digestive tract in forage- and grain-fed bison. BMC Vet. Res. 13(1), 253.

Cammack, K.M., Austin, K.J., Lamberson, W.R., Conant, G.C., Cunningham, H.C., 2018. Ruminant nutrition symposium: tiny but mighty: the role of the rumen microbes in livestock production. J. Anim. Sci. 96(10), 4481.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. Qiime allows analysis of high-throughput community sequencing data. Nat. Methods. 7(5), 335-336.

Cholewińska, P., Górniak, W., Wojnarowski, K., 2021. Impact of selected environmental factors on microbiome of the digestive tract of ruminants. BMC. Vet. Res. 17(1), 1-10.
Costa, M.C., Silva, G., Ramos, R.V., Staempfli, H.R., Arroyo, L.G., Kim, P., Weese, J.S., 2015. Characterization and comparison of the bacterial microbiota in different gastrointestinal tract compartments in horses. Vet J. 205(1), 74-80.
de Menezes, A.B., Lewis, E., O'Donovan, M., O'Neill, B.F., Clipson, N., Doyle, E.M., 2011. Microbiome analysis of dairy cows fed pasture or total mixed ration diets. FEMS Microbiol. Ecol. 78(2), 256-265.
de Oliveira, M.N., Jewell, K.A., Freitas, F.S., Benjamin, L.A., Tótola, M.R., Borges, A.C., Moraes, C.A., Suen, G., 2013. Characterizing the microbiota across the gastrointestinal tract of a brazilian nelore steer. Vet. Microbiol. 164(3-4), 307-314.
Evans, N.J., Brown, J.M., Murray, R.D., Getty, B., Birtles, R.J., Hart, C.A., Carter, S.D., 2011. Characterization of novel bovine gastrointestinal tract treponema isolates and comparison with bovine digital dermatitis treponemes. Appl. Environ. Microbiol. 77(1), 138-147.
Fernando, S.C., Purvis, H.T., 2nd, Najar, F.Z., Sukharnikov, L.O., Krehbiel, C.R., Nagaraja, T.G., Roe, B.A., Desilva, U., 2010. Rumen microbial population dynamics during adaptation to a high-grain diet. Appl. Environ. Microbiol. 76(22), 7482-7490.
Flint, H.J., Bayer, E.A., Rincon, M.T., Lamed, R., White, B.A., 2008. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. Nat. Rev. Microbiol. 6(2), 121-131.
Godoy-Vitorino, F., Goldfarb, K.C., Karaoz, U., Leal, S., Garcia-Amado, M.A., Hugenholtz, P., Tringe, S.G., Brodie, E.L., Dominguez-Bello, M.G., 2012. Comparative analyses of foregut and hindgut bacterial communities in hoatzins and cows. ISME J. 6(3), 531-541.
Ishaq, S.L., Wright, A.D., 2014. High-throughput DNA sequencing of the ruminal bacteria from moose (Alces alces) in Vermont, Alaska, and Norway. Microb. Ecol. 68(2), 185-195.
Ishaq, S.L., Wright, A.D., 2012. Insight into the bacterial gut microbiome of the North American moose (Alces alces). BMC. Microbiol. 12, 212.
Kittelmann, S., Seedorf, H., Walters, W.A., Clemente, J.C., Knight, R., Gordon, J.I., Janssen, P.H., 2013. Simultaneous amplicon sequencing to explore co-occurrence patterns of bacterial, archaeal and eukaryotic microorganisms in rumen microbial communities. PLoS ONE. 8(2), e47879.
Lee, J.E., Lee, S., Sung, J., Ko, G., 2011. Analysis of human and animal fecal microbiota for microbial source tracking. ISME J. 5(2), 362-365.
Lettat, A., Nozière, P., Silberberg, M., Morgavi, D.P., Berger, C., Martin, C., 2012. Rumen microbial and fermentation characteristics are affected differently by bacterial probiotic supplementation during induced lactic and subacute acidosis in sheep. BMC Microbiol. 12, 142.
Mackie, R.I., Wilkins, C.A., 1988. Enumeration of anaerobic bacterial microflora of the equine gastrointestinal tract. Appl. Environ. Microbiol. 54(9), 2155-2160.
Mao, S., Zhang, M., Liu, J., Zhu, W., 2015. Characterising the bacterial microbiota across the gastrointestinal tracts of dairy cattle: membership and potential function. Sci. Rep. 5, 16116.
Nyonyo, T., Shinkai, T., Mitsumori, M., 2014. Improved culturability of cellulolytic rumen bacteria and phylogenetic diversity of culturable cellulolytic and xylanolytic bacteria newly isolated from the bovine rumen. FEMS. Microbiol. Ecol. 88(3), 528-537.
Perea, K., Perz, K., Olivo, S.K., Williams, A., Lachman, M., Ishaq, S.L., Thomson, J., Yeoman, C.J., 2017. Feed efficiency phenotypes in lambs involve changes in ruminal, colonic, and small-intestine-located microbiota. J. Anim. Sci. 95(6), 2585-2592.
Sato, H., Shiogama, Y., 2010. Acetone and isopropanol in ruminal fluid and feces of lactating dairy cows. J. Vet. Med. Sci. 72(3), 297-300.
Spence, C., Wells, W.G., Smith, C.J., 2006. Characterization of the primary starch utilization operon in the obligate anaerobe bacteroides fragilis: regulation by carbon source and oxygen. J. Bacteriol. 188(13), 4663-4672.
Tajima, K., Aminov, R.I., Nagamine, T., Matsui, H., Nakamura, M., Benno, Y., 2001. Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. Appl. Environ. Microbiol. 67(6), 2766-2774.

Xue, M., Sun, H., Wu, X., Guan, L. L., Liu, J., 2018. Assessment of rumen microbiota from a large dairy cattle cohort reveals the pan and core bacteriomes contributing to varied phenotypes. Appl. Environ. Microbiol. 84(19), e00970-18.

Zeng, Y., Zeng, D., Ni, X., Zhu, H., Jian, P., Zhou, Y., Xu, S., Lin, Y., Li, Y., Yin, Z., Pan, K., Jing, B., 2017. Microbial community compositions in the gastrointestinal tract of Chinese Mongolian sheep using Illumina MiSeq sequencing revealed high microbial diversity. AMB Express. 7(1), 75.

Zhang, J., Xu, C., Huo, D., Hu, Q., Peng, Q., 2017. Comparative study of the gut microbiome potentially related to milk protein in Murrah buffaloes (Bubalus bubalis) and Chinese Holstein cattle. Sci. Rep. 7, 42189.

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