

Metagenomic investigation of the fecal microbiota in Lakor goats of different sexes with a basic diet of field grass

INSUN SANGADJI¹*, RONY MARSYAL KUNDA², PRASETYARTI UTAMI³

¹Department of Animal Husbandry, Faculty of Agriculture, Universitas Pattimura. Jl. Ir. M. Putuhena, Ambon 97233, Maluku, Indonesia.
Tel./fax.: +62-911-322653, *email: insunsangadji@gmail.com

²Department of Biotechnology, Faculty of Sciences and Technology, Universitas Pattimura. Jl. Ir. M. Putuhena, Ambon 97233, Maluku, Indonesia

³Department of Biology, Faculty of Sciences and Technology, Universitas Terbuka. Jl. Cabe Raya, South Tangerang 15437, Banten, Indonesia

Manuscript received: 28 January 2025. Revision accepted: 15 April 2025.

Abstract. Sangadji I, Kunda RM, Utami P. 2025. *Metagenomic investigation of the fecal microbiota in Lakor goats of different sexes with a basic diet of field grass.* Biodiversitas 26: 1861-1869. Gut microbiota play a crucial role in the health and productivity of ruminants, including goats. This study investigated the composition and diversity of fecal microbiota in Lakor goats of different sexes fed a basic diet of field grass using a metagenomic approach. A total of 10 samples were collected from male and female goats of 5 samples each, and high-throughput sequencing of the 16S rRNA gene was performed to analyze the microbial community structure. Results revealed significant differences in microbiota composition between sexes, which may be influenced by hormonal differences, feeding behavior, and metabolic needs. This study suggests that sex influences the composition of fecal microbiota in Lakor goats. Moreover, it revealed that sex affects the diversity and composition of the microbiota, with significant differences at the family and species levels. Alpha diversity analysis showed that female goats had higher microbial diversity than male goats, with a Shannon index of 2.03 in females and 1.33 in males, indicating a more complex microbial community in females. The microbial community composition in male goats was dominated by species such as *Romboutsia timonensis* and *Clostridium* sp., whereas female goats showed a more balanced microbial distribution, with the dominance of genera such as *Pradoshia*, *Eubacterium*, and *Lysinibacillus*. Phylogenetic analysis showed that female goats have a more stable microbial community, supporting more efficient fiber fermentation and Short-Chain Fatty Acid (SCFA) metabolism. Proximate test results showed differences in feed nutrient content between the wet and dry seasons, with higher crude protein content in the wet season, potentially affecting feed quality and microbiota. Overall, this study highlights the importance of considering sex in microbiota studies to optimize feeding strategies and health management for Lakor goats. These findings suggest that sex-specific dietary interventions and probiotic strategies could improve feed utilization, immune function, and productivity in Lakor goats.

Keywords: Fecal microbiota, field grass diet, Lakor goats, metagenomics, sex differences

INTRODUCTION

The gut is often referred to as a "black box" because of its diverse and complex microbial communities. The gut microbiota itself is considered a distinct organ, comprising trillions of microorganisms whose genetic material surpasses that of the host's cells by several hundredfold (Mao et al. 2015; Elolimy et al. 2018). These microbial genes play crucial roles in influencing host nutrient absorption and overall health through specialized metabolic processes. As a result, the ruminal microbiota has a significant impact on the host's digestion and metabolic functions. The gut microbiota of ruminants, particularly goats, plays a critical role in various physiological functions, including nutrient digestion, metabolism, and immune response (Liu et al. 2021). Goats, as part of the Caprinae subfamily, are known for their ability to efficiently utilize fibrous plant material, largely due to the presence of symbiotic microorganisms in their gastrointestinal tract (Wang et al. 2017). These microbes help break down complex carbohydrates into short-chain fatty acids, which serve as a primary energy source for the host (Jami and Mizrahi 2012; He et al. 2019). Consequently, a better understanding of the composition and diversity of the gut microbiota is essential for improving the health,

productivity, and nutritional efficiency of goats (Shabana et al. 2020).

The microbiota composition can be influenced by a range of factors, including diet, age, genetics, and environmental conditions (Yuan et al. 2020; Elbir and Alhumam 2022). Among these, sex has recently emerged as an important factor affecting gut microbial diversity and composition (Zhi et al. 2022; Cao et al. 2023). The sex of an animal may influence the gut microbiota through hormonal differences, which affect immune function, metabolism, and feeding behavior (Haro et al. 2016). However, while there is growing interest in sex-specific differences in microbiota across various animals, limited research has focused on these differences in ruminants, such as Lakor goats, particularly those consuming a natural diet of field grass. Understanding the microbial complex and its interactions is a crucial aspect of ruminant nutrition research. This knowledge serves as a foundation for exploring the relationship between microbes and the digestive efficiency, metabolism, and overall health of ruminant animals.

The Lakor goat is a protected breed under the Ministry of Agriculture Decree No. 2912/Kpts/OT.140/6/2011, and its distribution is highly restricted to Lakor Island. According

to Rumanta et al. (2020), this breed is believed to have originated from a cross between the Etawah goat lineage and the Kacang goat, with genetic dominance of the Etawah. Kunda et al. (2020) noted that their body coloration typically consists of solid hues mixed with black and white, gray, brown, or black-and-white spots, while the head is predominantly black. Lakor goats primarily feed on field grass, making them a suitable model for studying the gut microbiota under natural dietary conditions. Unlike commercial goat breeds that often receive formulated feed, Lakor goats provide a unique opportunity to investigate how a natural, fibrous diet interacts with sex to shape the gut microbiota, a subject that has not been studied before in this breed. Given that diet is a primary driver of microbial diversity in ruminants, this study also seeks to explore how sex-specific factors interact with dietary habits to influence gut health and function in Lakor goats.

Research on fecal microbiota in goats has largely focused on more common breeds such as Boer, Peanut, or Saanen, while no studies have been conducted on Lakor goats. Studies exploring differences in microbiota composition by sex are also limited, especially in the context of natural grass-based diets. Using a metagenomic approach, this study investigated the fecal microbiota of male and female Lakor goats, focusing on how sex impacts microbial composition and diversity. Specifically, it aimed to explore the fecal microbiota diversity and composition of male and female Lakor goats using a metagenomic approach. Metagenomics allows for a comprehensive analysis of the microbial community by sequencing the 16S rRNA gene (Do et al. 2018; Dominguez et al. 2022). These findings have potential implications for understanding the role of the microbiota in Lakor goat health and could inform strategies for optimizing nutrition and management based

on sex. Future research should explore the functional consequences of these microbial differences and their impacts on Lakor goat performance and welfare.

MATERIALS AND METHODS

Study area, animal selection, and sample collection

This study did not require animal ethics commission approval because it used noninvasive samples. The goat samples used in this study were of the same age (± 12 months). They kept together on the same farm, with access to a diet of field grass in the pasture (Figure 1). This stage maintains normal microbiota behaviors and matches environmental factors that affect the microbiota. During the sampling process, it was ensured that the goats were healthy and did not receive any medication. Fecal samples from 10 individual goats were collected during the rainy season (July to August) from five farmers (two goats per farm, one male and one female) and were collected aseptically and immediately stored at -80°C until further processing and analysis.

Procedures

DNA extraction and sequencing

The ZYMO RESEARCH Quick-DNA Microbe Miniprep Commercial Kit (D6010; Zymo Research Corp., Irvine, CA, USA) was used for genomic DNA extraction and purification. The DNA concentration was measured using a NanoDrop spectrophotometer (Thermo Scientific), with approximately $1.8 \mu\text{g}$ of extracted DNA needed for 16S rRNA gene amplification.

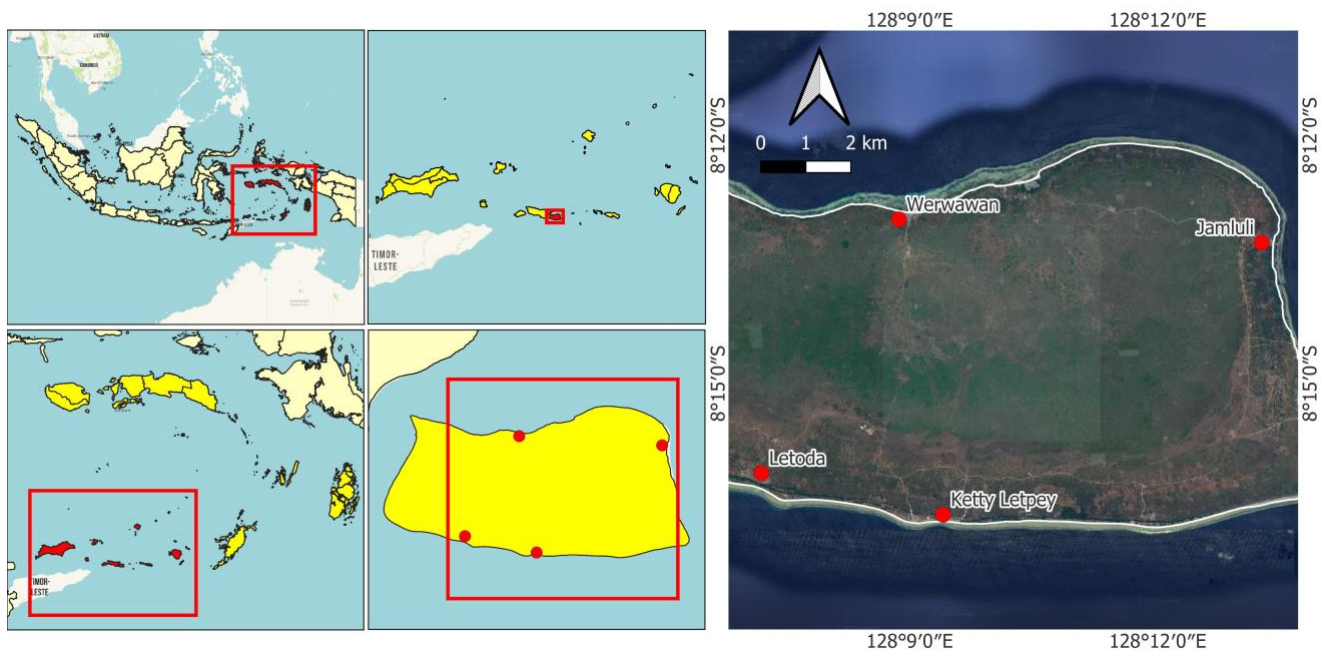


Figure 1. Map of the sampling locations. Fecal samples were collected from Lakor goats at four locations in Lakor Island, Southwest Maluku District, Maluku, Indonesia

The 16S rRNA gene library (V3-V4 regions) was created using universal Forward (5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3') and Reverse (5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATCC-3') primers (Kameoka et al. 2021). The PCR protocol for NGS analysis involved pre-denaturation at 95°C for 3 min, followed by 25 cycles of denaturation (95°C, 30 s), annealing (55°C, 30 s), and elongation (72°C, 30 s), and a final elongation step at 72°C for 5 min. A 410-bp DNA fragment was observed using 1.5% (w/v) agarose gel electrophoresis, after which the PCR product was purified with magnetic beads and sequenced on an Illumina MiSeq platform 2 × 250 bp (Genoscreen, France).

NGS-metagenomic library preparation and sequencing

The NGS procedure was performed using the Illumina preparation for fecal and swab samples, metagenomic protocol. A paired-end library with an approximate insert size of 410 bp was constructed using primers targeting the V3-V4 regions of 16S rRNA. The quality and quantity of the metagenomic library were assessed using an Agilent 2200 TapeStation with the Genomic DNA ScreenTape Assay (Agilent Technologies Inc., Santa Clara, CA, USA). Libraries were pooled in equal ratios and sequenced over 600 cycles on the MiSeq platform (Macrogen, Seoul, Korea) using v3 reagents (2 × 300 bp paired-end reads). PhiX bacterial metabiome DNA (10%) was added as an internal control. Paired-end reads were stored in FASTQ format, which was automatically demultiplexed, with Macrogen handling Nanopore adapter operations.

Proximate tests

Select field grasses from four grazing areas on Lakor Island. Obtain several random sections of grass to obtain a representative sample. Clean the samples of dirt or soil, cut them into small pieces, and homogenize them. The grass samples were dried at low temperatures (approximately 60°C-70°C) to avoid the loss of volatile nutrients. Fresh grass samples, after being in the oven and made into flour, will then be tested for proximate content. The proximate test consisted of testing the water, ash, fat, protein, carbohydrate, and crude fiber contents, with each repetition performed twice (duplo) (Yellavila et al. 2015).

Data analysis

Raw sequence data were processed using QIIME2 (Bolyen et al. 2019) for quality filtering, taxonomic classification, and diversity analysis. Alpha and beta diversity metrics were calculated using the Shannon index and Wilcoxon Tests to compare between two sex groups in the study of fecal microbiota. Principal Coordinate Analysis and Permutational Multivariate Analysis of Variance were used to assess differences in the microbiota composition according to sex.

RESULTS AND DISCUSSION

Microbial diversity and composition

The results show that male and female goats have diverse gut microbial communities. Male goats were dominated by three consecutive families from high to low, i.e., Peptostreptococcaceae, Aerococcaceae, and Staphylococcaceae, while three microbial families dominated female goats consecutively, i.e., Peptostreptococcaceae, Carnobacteriaceae, and Bacillaceae (Figure 2.A). However, significant differences were observed in the relative abundance of certain bacterial taxa according to sex. The results of this study showed that the abundance of bacterial species in male goats successively from high to low abundance was dominated by species i.e., *Romboutsia timonensis*, *Facklamia lactis*, *Paraclostridium tenue*, and *Jeotgalicoccus psychrophilus*. The abundance of bacterial species in female goats from high to low abundance was dominated by species i.e., *Pradoshia eiseniae*, *Desemzia incerta*, *Paraclostridium tenue*, *Lysinibacillus odyseeyi*, *Jeotgalicoccus psychrophilus*, *Lysinibacillus odyseeyi*, and *Romboutsia timonensis* (Figure 2.B). Barcode 13 i.e., male Lakor goats; barcode 14 i.e., female Lakor goats.

Alpha diversity analysis

The alpha diversity index was analyzed using the Shannon Index approach, which combines two approaches, richness (number of species) and evenness (evenness of species distribution in the community), with the interpretation of high values indicating an even number of species. The results of the alpha diversity analysis showed that female goats (barcode 14) had more complex and stable microbial community diversity than male goats (barcode 13). The alpha diversity value of barcode 13 was 1.33, while that of barcode 14 had an alpha diversity index of 2.03 (Figure 3). Statistical analysis shows that the Shannon index of fecal microbiota diversity of Lakor goats was significantly different between the sexes ($W = 85$, $p = 0.021$). The median Shannon index for male goats was 3.8 (IQR: 3.2-4.1), while for females it was 4.5 (IQR: 4.0-5.0), indicating that female goats have higher microbiota diversity than male goats.

The microbial community composition of Lakor goats

The proportion of microbial community in the metagenomic analysis is the relative quantitative ratio of different microbial groups (taxa) present in a sample. The proportion was calculated based on the number of reads or the frequency of genetic sequences identified at a particular taxonomic level. Proportion visualization is performed using a Krona chart, which displays proportions in the form of hierarchical circles. The interpretation of the proportion of microbial communities showed that in barcode 13 (Figure 4.A), the microbial community is dominated highest by class Clostridia and lowest by class Bacilli. The remaining 18% were other Bacillia, while in barcode 14 (Figure 4.B), the proportion of microbial communities is dominated by class Bacilli and lowest by class Clostridia.

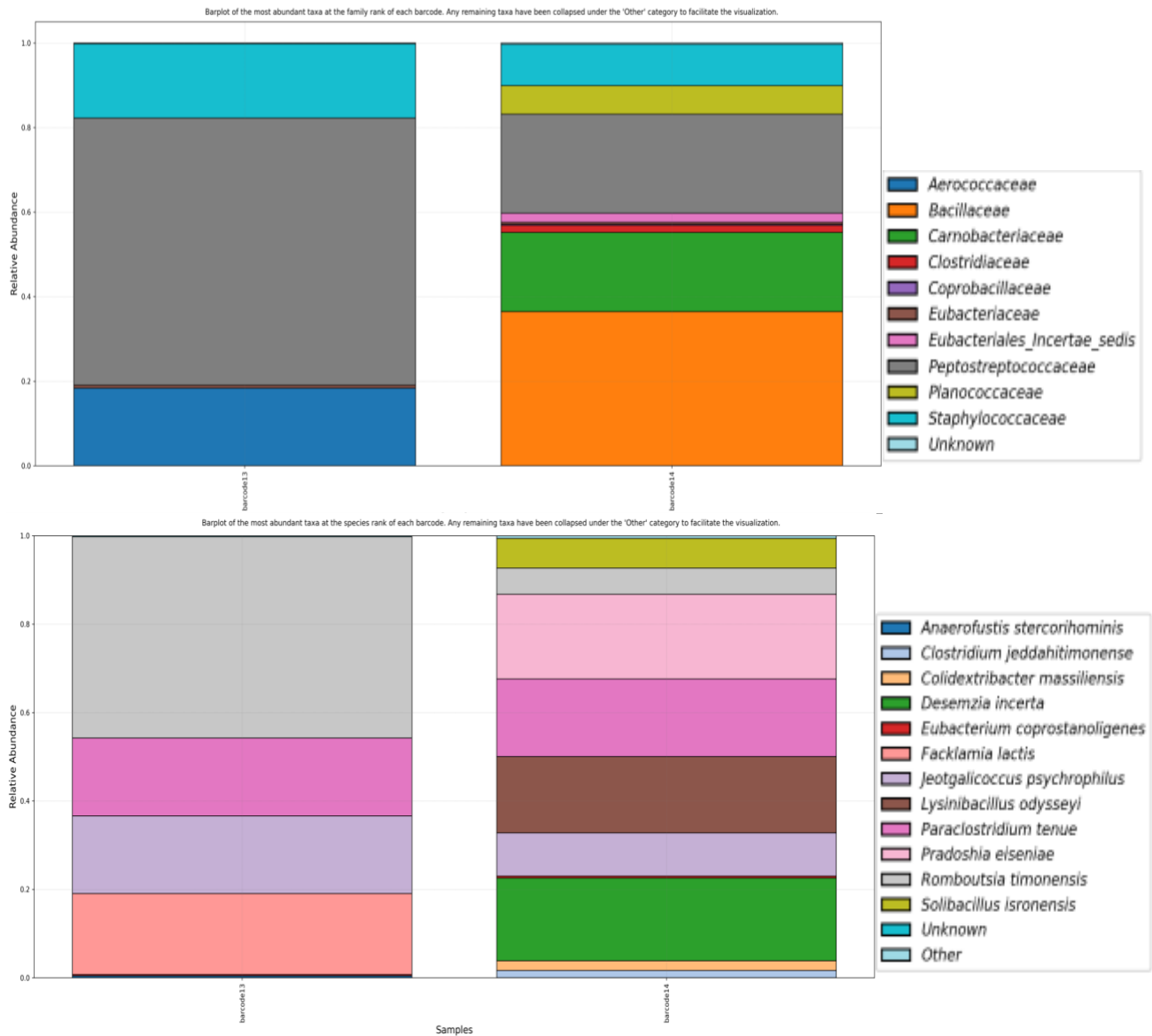


Figure 2. A. The abundance of dominant families in the fecal microbiota of the Lakor goat breed; B. The abundance of dominant species in the fecal microbiota of the Lakor goat breed

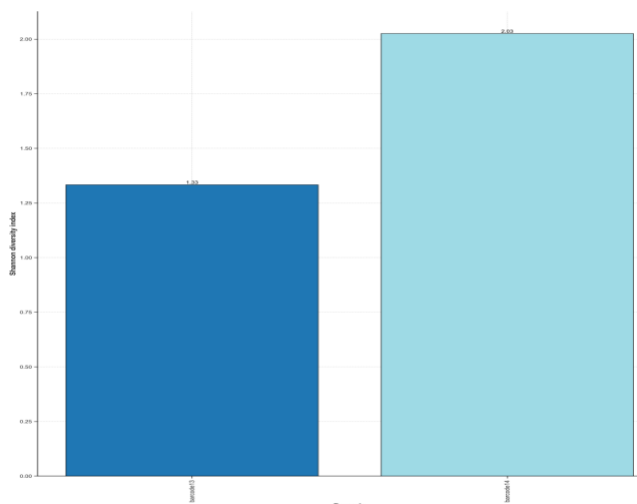


Figure 3. The alpha diversity index of Lakor goats

Phylogenetic species-level composition

Phylogenetic analysis at the species level in the metagenomic study of Lakor goats was conducted by comparing specific 16S rRNA gene sequences to identify microorganisms at the species level. The results were organized in the form of a phylogenetic tree that showed that, based on taxonomic richness (species richness), the microbial species in male goats (barcode 13) were more diverse than those in female goats (barcode 14). However, genetic diversity results show that female goats (barcode 14) are more dominant, compared to male goats (barcode 13) (Figure 5). Phylogenetic analysis also allows comparative studies between individuals and between sexes in goat populations or under environmental conditions to understand microbial adaptation to specific habitats better. Based on phylogenetic analysis, barcode 13 has a species cluster with longer branches, compared to barcode 14, which shows a higher level of genetic diversity. Genetic homogeneity data

showed that barcode 14 was categorized as a species cluster with shorter branches, indicating higher genetic homogeneity than barcode 13.

Forage nutrient concentrations (proximate test)

The results of the proximate analysis of the nutrient content of forage on pastures on Lakor Island indicate that forage production and the capacity of natural pastures fluctuate according to the season. Based on statistical analysis shows that the highest production and highest forage nutrient quality occurred during the rainy season and were lowest at the end of the dry season (Table 1).

Discussion

This study confirmed that sex influences the composition of the gut microbiota in mammals, including goats. The results showed that the gut microbial community in male and female Lakor goat breeds was diverse at the family and species levels. Male Lakor goats had distinct gut microbial communities dominated by Peptostreptococcaceae, Aerococcaceae, and Staphylococcaceae. Peptostreptococcaceae plays a key role in protein fermentation and short-chain fatty acid production, supporting gut function and metabolism, especially in the rumen and jejunum of goats (Guerra et al. 2022; Zeng et al. 2024). Aerococcaceae aids in fermenting complex carbohydrates, enhancing nutrient absorption in ruminants, whereas Staphylococcaceae, although known for pathogens, includes nonpathogenic strains that promote gut health, immune function, and nutrient breakdown (Mao et al. 2015; Xu et al. 2021). These results are consistent with previous studies, which have indicated that hormonal differences between males and females can influence microbial communities (Org et al. 2016). Testosterone, for instance, has been associated with the promotion of the growth of certain bacterial groups that enhance fiber digestion, which may explain the higher abundance of these bacteria in males.

The observed differences in microbial composition between male and female goats could also be attributed to variations in metabolic requirements and feeding behavior. Male goats generally have higher energy demands owing to greater body mass and muscle development, which may result in differences in dietary intake and digestion processes. These factors may contribute to the selection of specific microbial communities that efficiently extract energy from fibrous plant material. In contrast, female goats, especially those in the reproductive phase, may have distinct metabolic priorities that favor the growth of different bacterial taxa, such as Bacteroides, which are involved in breaking down complex carbohydrates into easily absorbable nutrients (Cao et al. 2023).

In this study, the results of the alpha diversity analysis revealed significant differences between the microbial communities of female (barcode 14) and male (barcode 13) goats. The diversity index in female goats was 2.03, whereas that in male goats was only 1.33. The higher Shannon index value in female goats indicates a more complex and balanced microbial community with a more even distribution of species. In contrast, lower values in male goats indicate simpler microbial communities with possible dominance by a few specific species.

Table 1. Nutrient compositions of natural pasture forage during the rainy and dry seasons in Lakor Island

Feed ingredients	Seasons	DI (%)	Nutrient composition (100% DI)				
			AC	CP	CF	DF	EWN
Field grass	Rainy	46.46	11.71	8.94	4.80	25.43	49.12
	Dry	35.57	11.27	6.36	4.20	30.50	47.67

Note: DI: Dry Ingredients; CP: Crude Protein; CF: Crude Fat; AC: Ash Content; DF: Dietary Fiber; EWN: Extracts Without Nitrogen

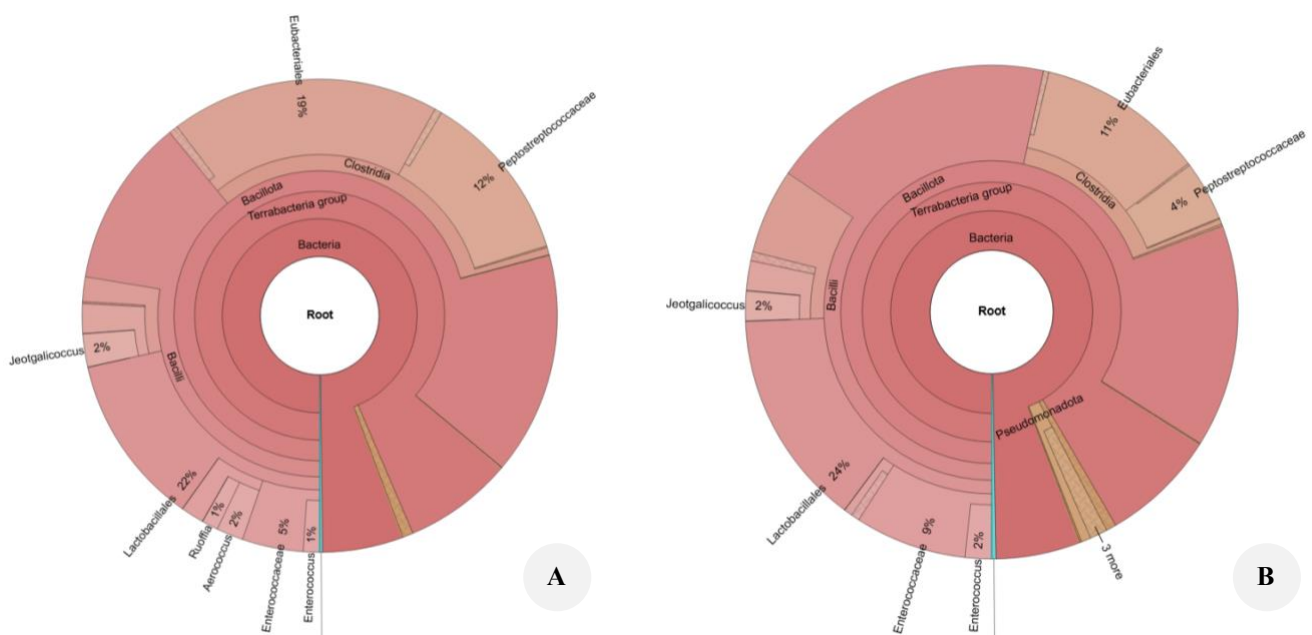


Figure 4. Microbial community proportions of Lakor goats: A. Barcode 13; B. Barcode 14

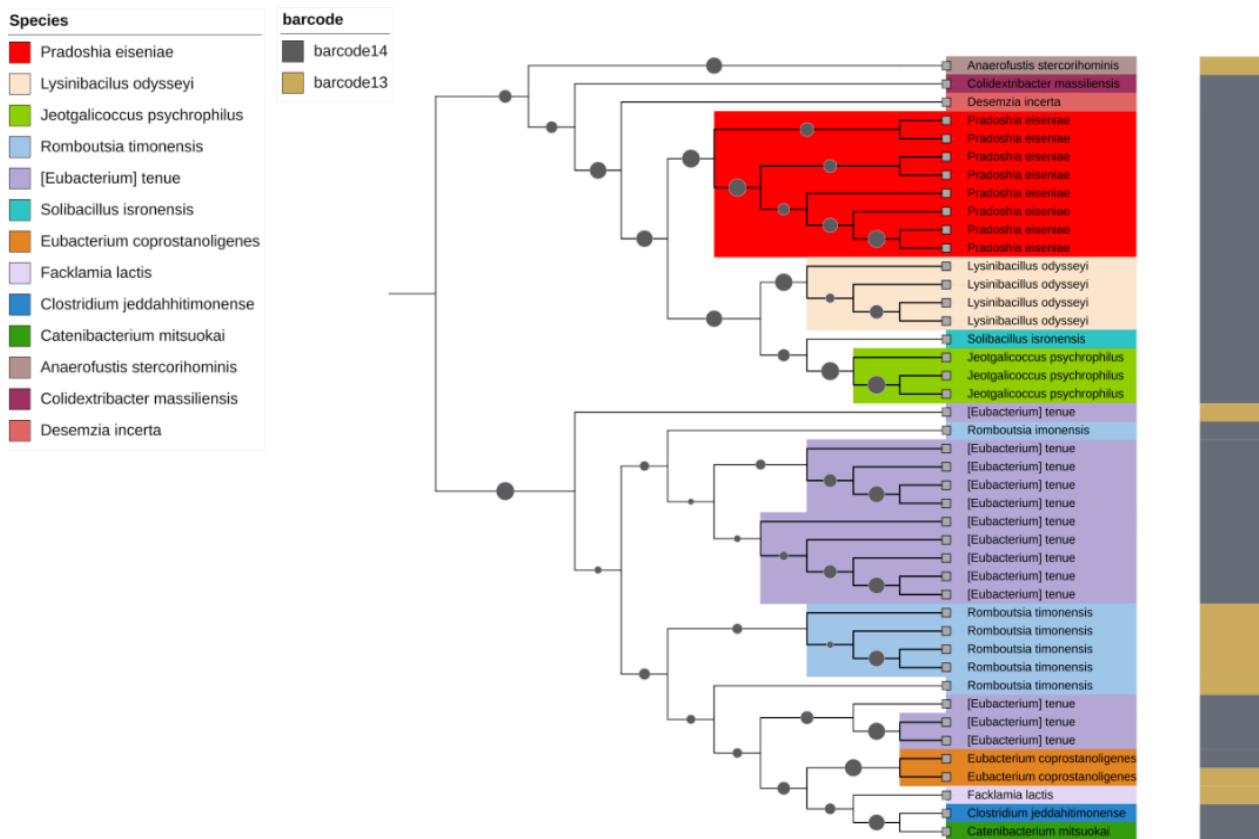


Figure 5. Phylogenetic species-level composition of Lakor goats

The results of this study indicate that the sample size used still considers statistical power to ensure that the differences observed are not just the result of chance. These results are in line with the research findings of Lozupone et al. (2012), who recommended that ≥ 10 samples per group are often sufficient for microbiota exploratory studies with alpha and beta diversity analysis. Various biological factors, such as differences in body physiology between male and female goats, sex hormones, and microbial environmental conditions in the digestive tract, may cause these differences in diversity indices. Previous studies have shown that sex differences can affect microbiota profiles, mainly due to variations in diet, metabolism, and hormones (Cao et al. 2023; Khairunisa et al. 2023). The hormone estrogen in female animals favors higher microbial diversity, whereas in males, androgen levels may direct the dominance of certain species (Santos-Marcos et al. 2023). These findings suggest that even minor differences in microbial populations could have significant functional implications for nutrient utilization and overall health.

Phylogenetic analysis revealed significant differences in microbial diversity between barcodes 13 and 14. Barcode 13 exhibited a dominance of certain species, such as *Romboutsia timonensis* and *Clostridium* sp., indicating a microbial community with low diversity and an overrepresentation of dominant species within the ecosystem. In contrast, barcode 14 displayed a more even and complex microbial distribution, with the involvement of genera such as *Pradoshia*, *Eubacterium*, and *Lysinibacillus*, which are

recognized for their roles in fiber fermentation and Short-Chain Fatty Acid (SCFA) metabolism. The *Pradoshia* genus is closely associated with various metabolic functions in the rumen microbiome (Anderson and Fernando 2021); *Eubacterium* plays an important role in rumen fermentation, breaking down complex carbohydrates into SCFAs that support ruminant energy metabolism (Gharechahi et al. 2021); and the genus *Lysinibacillus* plays an important role in digesting fiber, improving ruminant nutritional efficiency, and supporting feed efficiency and animal health (Do et al. 2018). Higher microbial diversity at barcode 14 reflects greater microbial community stability, allowing the microbial ecosystem to support a wider range of metabolic functions, such as fiber digestion and protection against pathogens (Huang et al. 2024). The low microbial diversity at barcode 13 may be attributed to physiological and hormonal influences in male goats, which often limit the colonization of certain microbes (Org et al. 2016). The dominant presence of species such as *Romboutsia timonensis* at barcode 13 suggests a specific role for these microbes in protein metabolism, which may be related to the metabolic requirements of males. This analysis confirms the importance of diverse microbial communities in promoting farm animal health and productivity.

One possible explanation for the differences in microbiota composition between the sexes is hormonal regulation. Hormones such as estrogen and testosterone have been shown to directly and indirectly influence the gut microbiota by modulating immune responses, altering

gut permeability, and affecting microbial metabolism (Haro et al. 2016). For example, estrogen has been associated with a more diverse and balanced microbial community. In comparison, testosterone can promote the growth of specific bacterial groups that enhance energy extraction from the diet (Org et al. 2016). These hormonal effects may explain why the male and female goats in this study exhibited distinct microbial profiles, despite being fed the same basic diet. Several studies detail that estrogen is known to increase microbiota diversity by stimulating the growth of beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium*, which have roles in gut health and energy metabolism (Qi et al. 2021). Conversely, testosterone tends to reduce microbial diversity by increasing the prevalence of potential pathogenic bacteria, such as *Bacteroides* and *Clostridium*, which may contribute to gut inflammation (Ma et al. 2024). The effects of these hormones are mediated through androgen and estrogen receptors expressed in gut tissues, which influence mucus production and the regulation of mucosal immune responses (Gomez et al. 2015). In addition, these hormones can also modify Short-Chain Fatty Acid (SCFA) production, which plays a role in energy balance and gastrointestinal health in ruminants (Shin et al. 2015). Thus, sex hormone balance may be a key factor in regulating gut microbiota homeostasis and metabolic health in ruminants.

Moreover, the dominance of Bacillota (synonym Firmicutes) in male and female goats aligns with previous studies on ruminants, where these phyla play crucial roles in fiber digestion and SCFA production (Wassan et al. 2023). SCFAs, such as acetate, propionate, and butyrate, are the primary energy sources for ruminants and contribute significantly to their overall energy balance. The observed differences in microbial taxa between sexes could influence SCFA production, potentially leading to differences in energy metabolism and fat deposition. Future research should include SCFA quantification to understand better the functional impact of the microbial differences observed in this study.

Based on the results of the proximate analysis (Table 1), the composition of forage in the Lakor Island pasture exhibited significant variation between the rainy and dry seasons. During the rainy season, the Dry Ingredient (DI) content was 46.46%, compared with 35.57% in the dry season, indicating lower forage moisture due to abundant water availability (Pereira et al. 2021; Núñez et al. 2022). The Crude Protein (CP) content was also higher in the rainy season (8.94%) than in the dry season (6.36%), reflecting better forage quality during the rainy season due to optimal forage growth (Widodo et al. 2023; Cooke et al. 2024). In contrast, Extracts Without Nitrogen (EWN) and Dietary Fiber (DF) contents were higher in the dry season, indicating that structural carbohydrates accumulate as an adaptation to environmental stress (Dias e Silva and Filho 2021). Small fluctuations were observed in Ash Content (AC) (11.71% rainy season, 11.27% dry season) and Crude Fat (CF) content (4.80% rainy season, 4.20% dry season), reflecting the stability of the forage mineral and lipid content (Capstaff and Miller et al. 2018). Overall, forage in the wet season had better nutrient quality, supporting the

nutritional needs of livestock more optimally. In contrast, declining forage quality in the dry season highlights the importance of supplementary feeding strategies to maintain livestock productivity on Lakor Island (Pereira et al. 2021; Cooke et al. 2024). The vegetation structure that dominates natural pastures on Lakor Island is similar to Moa Island and is almost the same in every village. The dominant forage species are *Ischaemum indicum*, *Themeda arguens*, and *Hyparrhenia rufa* (jaragua). Other grass species found were *Paspalum conjugatum*, *Imperata* spp., *Eragrostis amabilis*, *Panicum repens*, and *Setaria anceps*. Tatipikalawan (2022) found that the comparison of the botanical composition of natural pastures in 3 villages on Moa Island, Southwest Maluku Regency, was dominated by grasses 93.16%, legumes 0.87%, while the remaining 5.07% were weeds.

The potential effects of sex-specific behavioral patterns on microbiota composition are also worth considering. Previous research has suggested that males and females may differ in their grazing patterns, food selection, and rumination behavior (Dominguez-Pino et al. 2024). These behavioral differences could alter the exposure of the gut microbiota to different types of plant matter, thereby influencing microbial selection and community composition. Several studies on ruminants have demonstrated sex-based differences in microbiota composition. For instance, Tardiolo et al. (2025) found that male and female sheep harbored distinct microbial communities, with males exhibiting a higher abundance of fiber-digesting bacteria such as *Ruminococcus* and *Fibrobacter*, likely due to differences in feeding strategies. Similarly, Guo et al. (2022) explore the sex differences in the structure and function of rumen microbiota in Tibetan goats. Analysis of rumen microbiota structure showed significant differences based on sex, with females having higher microbiota diversity than males ($P > 0.05$). At the phylum level, Firmicutes and Bacteroidetes were the dominant phyla in the rumen of Tibetan goats, but the proportion of Firmicutes was significantly higher in females than in males ($P < 0.05$). These findings confirm that sex factors can affect rumen microbiota composition, VFAs metabolism, and nutrient transport gene expression, which have implications for sex-based nutritional strategies in ruminant management.

Although it provides valuable insights into the composition of the fecal microbiota of Lakor goats, this study has some limitations, i.e., the relatively small sample size, which may limit the generalizability of the findings to a wider population of Lakor goats. This study only focused on fecal microbiota, which may not fully represent the entire gut microbial community, especially in the rumen, where fermentation mainly occurs, so that the functional profile of the microbial community has not been comprehensively studied. In addition, environmental factors such as seasonal variations in temperature, humidity, and forage availability may have influenced the microbiota composition but were not extensively controlled. Lastly, although there were sex-related differences, this study did not assess the potential impact of other physiological factors such as age, reproductive status, or stress levels on the gut microbiota. Future research with larger sample sizes,

rumen microbiota analysis, and functional metagenomic approaches is needed to provide a more comprehensive understanding of the microbiota dynamics in Lakor goats.

Overall, the findings of this study highlight the complex interplay between sex, diet, and gut microbiota composition in Lakor goats. Although the goats were fed the same basic diet of field grass, their microbiota profiles diverged based on sex, underscoring the importance of considering sex in microbiome studies. Understanding how sex influences microbial communities in livestock is critical for developing targeted nutritional and management strategies that optimize health and productivity (Kaur et al. 2023). For instance, tailoring feeding regimens or probiotic supplementation based on sex-specific microbiota profiles could enhance nutrient absorption and improve the overall goat well-being. From a practical perspective, these insights could guide precision livestock farming approaches, such as adjusting dietary formulations to support optimal rumen fermentation efficiency in males and females separately. Additionally, selective use of feed additives or prebiotics to modulate microbial composition may help improve feed conversion ratios, reduce methane emissions, and enhance disease resistance, ultimately leading to more sustainable and efficient goat production systems.

ACKNOWLEDGEMENTS

The authors would like to thank the Biotechnology Laboratory, Faculty of Science and Technology, Universitas Pattimura, Ambon, Indonesia, for the stool sample preparation process, the Staff and Head of the Genomics Laboratory, Veterinary Research Center, National Research and Innovation Agency (BRIN) for the genomic analysis process, and Barnabas Gairtua and Alberthus Sairudy for the Lakor goat feces collection process. The authors declare that this research did not receive financial assistance from any institution.

REFERENCES

- Anderson CL, Fernando SC. 2021. Insights into biosynthetic gene cluster diversity in rumen via genome-resolved metagenomics. *Commun Biol* 4 (1): 818. DOI: 10.1038/s42003-021-02331-7.
- Bolyen E, Rideout JR, Dillon MR et al. 2019. Reproducible, interactive, scalable, and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 37 (8): 852-857. DOI: 10.1038/s41587-019-0209-9.
- Cao Y, Feng T, Wu Y et al. 2023. The multi-kingdom microbiome of the goat gastrointestinal tract. *Microbiome* 11 (1): 219. DOI: 10.1186/s40168-023-01651-6.
- Capstaff NM, Miller AJ. 2018. Improving the yields and nutritional qualities of forage crops. *Front Plant Sci* 9: 535. DOI: 10.3389/fpls.2018.00535.
- Cooke AS, Machekano H, Gwiriri LC, Tinsley JHI, Silva GM, Nyamukondiwa C, Safalaoh A, Morgan ER, Lee MRF. 2024. Nutritional feed gap: Seasonal variations in ruminant nutrition and knowledge gaps in relation to food security in Southern Africa. *Food Secur* 17 (1): 73-100. DOI: 10.1007/s12571-024-01509-1.
- Dias e Silva TP, Filho ALA. 2021. Sheep and goat feeding behavior profile in grazing systems. *Acta Sci Anim Sci* 43 (1): e51265. DOI: 10.4025/actascianimsci.v43i1.51265.
- Do TH, Dao TK, Nguyen KHV, Le NG, Nguyen TMP, Le TL, Phung TN, van Straalen NM, Roelofs D, Truong NH. 2018. Metagenomic analysis of bacterial community structure and diversity of lignocellulolytic bacteria in Vietnamese native goat rumen. *Asian-Australas J Anim Sci* 31 (5): 738-747. DOI: 10.5713/ajas.17.0174.
- Domínguez FF, Crisanto MEV, Castro RLS, Rojas LV, Cuba VMB, Santos GRS, Ramos CAL, Mialhe E. 2022. Metagenomic analysis of the intestinal microbiome in goats fed cactus- and *Salicornia*-based diets. *Open Vet J* 12 (1): 61-68. DOI: 10.5455/OVJ.2022.v12.i1.7.
- Domínguez-Pino M, Mellado S, Cuesta CM, Grillo-Risco R, García-García F, Pascual M. 2024. Metagenomics reveals sex-based differences in murine fecal microbiota profiles following chronic alcohol consumption. *Intl J Mol Sci* 25 (23): 12534. DOI: 10.3390/ijms252312534.
- Elbir H, Alhumam NA. 2022. Sex differences in the fecal microbiome composition and function of dromedary camels in Saudi Arabia. *Animals* 12 (23): 3430. DOI: 10.3390/ani12233430.
- Elolimy AA, Arroyo JM, Batistel F, Iakiviak MA, Loor JJ. 2018. Association between residual feed intake and the abundance of ruminal bacteria and biopolymer hydrolyzing enzyme activities during the periparturient period and early lactation in Holstein dairy cows. *J Anim Sci Biotechnol* 9: 43. DOI: 10.1186/s40104-018-0258-9.
- Gharechahi J, Vahidi MF, Bahram M, Han J-L, Ding X-Z, Salekdeh GH. 2021. Metagenomic analysis reveals a dynamic microbiome with diversified adaptive functions to utilize high lignocellulosic forages in the cattle rumen. *ISME J* 15 (4): 1108-1120. DOI: 10.1038/s41396-020-00837-2.
- Gomez A, Luckey D, Taneja V. 2015. The gut microbiome in autoimmunity: Sex matters. *Clin Immunol* 159 (2): 154-162. DOI: 10.1016/j.clim.2015.04.016.
- Guerra V, Tiago I, Aires A, Coelho C, Nunes J, Martins LO, Veríssimo A. 2022. The gastrointestinal microbiome of browsing goats (*Capra hircus*). *PLoS One* 17 (10): e0276262. DOI: 10.1371/journal.pone.0276262.
- Guo X, Sha Y, Lv W, Pu X, Liu X, Luo Y, Hu J, Wang J, Li S, Zhao Z. 2022. Sex differences in rumen fermentation and microbiota of Tibetan goat. *Microb Cell Fact* 21 (1): 55. DOI: 10.1186/s12934-022-01783-8.
- Haro C, Rangel-Zúñiga OA, Alcalá-Díaz JF, Gómez-Delgado F, Pérez-Martínez P, Delgado-Lista J, Quintana-Navarro GM, Landa BB, Navas-Cortés JA, Tena-Sempere M, Clemente JC, López-Miranda J, Pérez-Jiménez F, Camargo A. 2016. Intestinal microbiota is influenced by gender and body mass index. *PLoS One* 11 (5): e0154090. DOI: 10.1371/journal.pone.0154090.
- He B, Jin S, Cao J, Mi L, Wang J. 2019. Metatranscriptomics analysis of Hu sheep rumen microbiome reveals novel cellulases. *Biotechnol Biofuels* 12: 153. DOI: 10.1186/s13068-019-1498-4.
- Huang Q, Xing J, Tang F, Ren J, Wang C, Xue F. 2024. Recombinant *Lactiplantibacillus plantarum* modulates gut microbial diversity and function. *BMC Microbiol* 24: 423. DOI: 10.1186/s12866-024-03570-4.
- Jami E, Mizrahi I. 2012. Composition and similarity of the bovine rumen microbiota across individual animals. *PLoS One* 7 (3): e33306. DOI: 10.1371/journal.pone.0033306.
- Kameoka S, Motooka D, Watanabe S, Kubo R, Jung N, Midorikawa Y, Shinozaki NO, Sawai Y, Takeda AK, Nakamura S. 2021. Benchmark of 16S rRNA gene amplicon sequencing using Japanese gut microbiome data from the V1-V2 and V3-V4 primer sets. *BMC Genomics* 22 (1): 527. DOI: 10.1186/s12864-021-07746-4.
- Kaur H, Kaur G, Gupta T, Mittal D, Ali SA. 2023. Integrating omics technologies for a comprehensive understanding of the microbiome and its impact on cattle production. *Biology* 12 (9): 1200. DOI: 10.3390/biology12091200.
- Khairunisa BH, Heryakusuma C, Ike K, Mukhopadhyay B, Susanti D. 2023. Evolution of the understanding of rumen methanogen ecophysiology. *Front Microbiol* 14: 1296008. DOI: 10.3389/fmicb.2023.1296008.
- Kunda RM, Volkandari SD, Rumanta M, Kakisina P. 2020. Polymorphism of the Growth Hormone (GH) gene in lakor goats from lakor island, southwest Maluku Regency. *Buletin Peternakan* 44 (4): 194-199. DOI: 10.21059/buletinpeternakan.v44i4.58934.
- Liu K, Zhang Y, Yu Z, Xu Q, Zheng N, Zhao S, Huang G, Wang J. 2021. Ruminal microbiota-host interaction and its effect on nutrient metabolism. *Anim Nutr* 7: 49-55. DOI: 10.1016/j.aninu.2020.12.001.
- Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. 2012. Diversity, stability and resilience of the human gut microbiota. *Nature* 489 (7415): 220-230. DOI: 10.1038/nature11550.
- Ma Z, Zuo T, Frey N, Rangrez AY. 2024. A systematic framework for understanding the microbiome in human health and disease: From basic principles to clinical translation. *Signal Transduct Target Ther* 9 (1): 237. DOI: 10.1038/s41392-024-01946-6.

- Mao S, Zhang M, Liu J, Zhu W. 2015. Characterizing the bacterial microbiota across the gastrointestinal tract of dairy cattle: Membership and potential function. *Sci Rep* 5: 16116. DOI: 10.1038/srep16116.
- Núñez L, Hirigoyen A, Durante M, Arroyo JM, Cazzuli F, Bremm C, Jaurena M. 2022. What factors control the crude protein content variation of a basaltic “campos” native grassland of South America. *Agronomy* 12 (8): 1756. DOI: 10.3390/agronomy12081756.
- Org E, Mehrabian M, Parks BW, Shipkova P, Liu X, Drake TA, Lulis AJ. 2016. Sex differences and hormonal effects on the gastrointestinal microbiota composition in mice. *Gut Microbes* 7 (4): 313-322. DOI: 10.1080/19490976.2016.1203502.
- Pereira GF, Neto JVE, dos Santos Difante G, da Silva Lagos Cortes Assis LC, de Oliveira Lima P, da Silva Santos R. 2021. Production and quality of tropical grasses at different regrowth intervals in the Brazilian semi-arid. *Acta Sci Anim Sci* 43 (1): e52842. DOI: 10.4025/actascianimsci.v43i1.52842.
- Qi X, Yun C, Pang Y, Qiao J. 2021. The impact of the gut microbiota on the reproductive and metabolic endocrine system. *Gut Microbes* 13 (1): 1-21. DOI: 10.1080/19490976.2021.1894070.
- Rumanta M, Kunda RM, Volkandari SD, Indriawati I, Kakisina P. 2020. Genetic characterization and phylogenetic study of Lakor goat from Southwest Maluku Regency based on mitochondrial COI gene. *Vet World* 13 (6): 1209-1220. DOI: 10.14202/vetworld.2020.1209-1220.
- Santos-Marcos JA, Mora-Ortiz M, Tena-Sempere M, López-Miranda J, Camargo A. 2023. Interactions between the gut microbiota and sex hormones and their relationship with sexual dimorphism in metabolic diseases. *Biol Sex Differ* 14 (1): 4. DOI: 10.1186/s13293-023-00490-2.
- Shabana II, Albakri NN, Bouqellah NA. 2020. Metagenomic analysis of the fecal microbiota of sheep and goats of the same ages. *J Taibah Univ Sci* 15 (1): 1-9. DOI: 10.1080/16583655.2020.1864930.
- Shin N-R, Whon TW, Bae J-W. 2015. Proteobacteria: Microbial signature of dysbiosis in gut microbiota. *Trends Biotechnol* 33 (9): 496-503. DOI: 10.1016/j.tibtech.2015.06.011.
- Tardiolo G, La Fauci D, Riggio V, Daghighi M, Di Salvo E, Zumbo A, Sutura AM. 2025. Gut microbiota of ruminants and monogastric livestock: An overview. *Animals* 15: 758. DOI: 10.3390/ani15050758.
- Tatipikalawan JM. 2022. Keberlanjutan pengembangan peternakan kerbau Moa di Pulau Moa Provinsi Maluku. [Dissertation]. Universitas Gadjah Mada, Yogyakarta. [Indonesian]
- Wang H, Zheng H, Browne F, Roehe R, Dewhurst RJ, Engel F, Hemmje M, Lu X, Walsh P. 2017. Integrated metagenomic analysis of the rumen microbiome of cattle revealed the key biological mechanisms associated with methane traits. *Methods* 124: 108-119. DOI: 10.1016/j.jymeth.2017.05.029.
- Wassan JT, Wang H, Zheng HJ. 2023. Recent advances in phylogenetic analysis for studying the rumen microbiome. *Curr Bioinform* 19 (3): 250-263. DOI: 10.2174/1574893618666230605120615.
- Widodo S, Shiddieqy MI, Wahyono T, Widiawati Y, Muttaqin Z. 2023. Analysis of the correlation between the nutrient content, digestibility, and gas production of forages in Indonesia. *Adv Anim Vet Sci* 11 (11): 1770-1778. DOI: 10.17582/journal.aavs/2023/11.11.1770.1778.
- Xu Q, Qiao Q, Gao Y, Hou J, Hu M, Du Y, Zhao K, Li X. 2021. Gut microbiota and their role in the health and metabolic diseases of dairy cow. *Front Nutr* 8: 701511. DOI: 10.3389/fnut.2021.701511.
- Yellavala SB, Agbenorhevi JK, Asibuo JY, Sampson GO. 2015. Proximate composition, mineral content, and functional properties of five lima bean accessions. *J Food Secur* 3 (3): 69-74. DOI: 10.12691/jfs-3-3-1.
- Yuan X, Chen R, Zhang Y, Lin X, Yang X. 2020. Sexual dimorphism of gut microbiota at different pubertal status. *Microb Cell Fact* 19 (1): 152. DOI: 10.1186/s12934-020-01412-2.
- Zeng Y, Mou H, He Y, Zhang D, Pan X, Zhou L, Shen Y, Guangxin E. 2024. Effects of key rumen bacteria and microbial metabolites on fatty acid deposition in goat muscle. *Animals* 14 (22): 3225. DOI: 10.3390/ani14223225.
- Zhi W, Tang K, Yang J, Yang T, Chen R, Huang J, Tan HS, Zhao J, Sheng Z. 2022. Gut microbiota of Hainan black goat. *Animals* 12 (22): 3129. DOI: 10.3390/ani12223129.