American Journal of Animal and Veterinary Sciences

Original Research Paper

Improving Rumen Fermentation by Inoculation of Cellulolytic Bacteria Isolated from Anoa, Bison, Muntjak and Timor Deer Faecal

[1]Sri Suharti, [1]Erika Julian Cahyani, [1]Iksan Qodri Pramarta, [2]Agus Budiansyah, [2]Syafwan and [1]Komang Gede Wiryawan

[1]Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Jl. Agatis Kampus Fapet IPB Dramaga, Bogor, West Java, Indonesia
[2]Faculty of Animal Science, Jambi University, Jl. Jambi-Muara Bulian KM 15 Mendalo Indah, Muaro, Jambi 36361, Indonesia

Article history
Received: 03-07-2023
Revised: 04-09-2023
Accepted: 12-09-2023

Corresponding Author:
Sri Suharti
Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Jl. Agatis Kampus Fapet IPB Dramaga, Bogor, West Java, Indonesia
Email: sri_suharti@apps.ipb.ac.id

Abstract: Tropical endemic herbivores such as anoa (*Bubalus depressicornis*), bison (*Bos javanicus*), muntjak (*Muntiacus muntjak*), and Timor deer (*Rusa timorensis*), can digest various types of fibrous feed because their digestive tract has high cellulolytic bacteria and those can be found in their feces. Previously, we isolated and identified cellulolytic bacteria from the feces of anoa, bison, muntjak, and Timor deer. This study aimed to examine the use of a consortium of cellulolytic bacteria originating from feces of anoa, bison, muntjak, and Timor deer in the *in vitro* rumen fermentation and their effect on digestibility and fermentation properties. The *in vitro* study was conducted using beef cattle rumen fluid with 4 treatments and 5 replications in a Completely Randomized Design (CRD). The treatment including Control (T0) = Forage: Concentrate 60: 40, T1 = T0+10^5 CFU/mL cellulolytic bacteria, T2 = T0 + 10^6 CFU/mL cellulolytic bacteria, T1 = T0+10^7 CFU/mL cellulolytic bacteria. Variables measured were dry and organic matter digestibility, crude fiber digestibility, rumen pH, formation of Ammonia (NH_3), Volatile Fatty Acids (VFA), microbial population, and methane estimation. The results showed that the addition of cellulolytic bacterial consortium isolated from anoa, bison, muntjak, and Timor deer feces increased dry matter digestibility, crude fiber digestibility, total VFA production, and total bacterial population. Rumen pH, ammonia production, proportional VFA, and methane were similar among treatments. Inoculation of the cellulolytic bacteria consortium at level 10^6 CFU/mL was the optimum level to improve fiber digestibility and had a beneficial effect for ruminants that fed high-fiber feed.

Keywords: Cellulolytic Bacteria, Rumen Fermentation, Microbe Population, Tropical Herbivore

Introduction

Forages are the main feedstuff for ruminants as ruminants' main source of energy is plant carbohydrates. Particularly for ruminants fed a high-forage diet, rumen microorganisms play a significant role in feed decomposition and fermentation. Nutritionists are concerned with altering rumen microbial composition to optimize feed utilization and ruminant. Forages as a ruminant diet consist of grass, legume, and agricultural by-product that contains cellulose, hemicellulose, lignin, and pectin (Ribeiro *et al*., 2016).

Ruminants can consume feed fiber that has been enzymatically degraded in the rumen by rumen microorganisms. Cellulolytic enzymes such as endo-1,4-glucanase, cellobiohydrolase, and -glucosidase combine to degrade cellulose. Endoxylanase, β-xylosidase, and glucuronoxylan hydrolases are required for the breakdown of hemicellulose. Another essential stage in the breakdown of plant cell walls is the removal of the side chains from the xylan backbone by the enzymes α-l-arabinofuranosidases, feruloyl esterases, and -glucuronidases (Terry *et al*., 2019). The rumen of mature

ruminants naturally contains a lot of microbes such as bacteria, protozoa, and fungi that can digest feed fiber including, cellulose, hemicellulose, or xylan. Endoglucanases, exoglucanases, and glucosidases are produced by the primary cellulolytic bacteria Ruminococcus flavefaciens, Ruminococcus albus, and Fibrobacter succinogenes, which combine to break down cellulose in the rumen (Hua *et al*., 2022).

In the rumen, the anaerobic ruminal environment and the associated bacteria are necessary for the breakdown and digestion of fiber (Torres-Salado *et al*., 2023). Fiber digestibility is significantly influenced by the variety and density of rumen microbes. Furthermore, the ability of the rumen to digest fiber is influenced by the interactions between several microbes (Terry *et al*., 2019). Additionally, the administration of fibrolytic bacteria with cellulolytic activities that collaborate with rumen microbial communities' hydrolases can enhance the rumen's ability to use fiber, particularly in ruminants with high forage intake.

As a tropical nation, Indonesia is home to numerous native herbivores that are fed lignocellulose feeds such as grass, tree leaves, rice straw, and legumes. These herbivores may adapt to the environment with greater nutrient and shelter quality (Naqibzadeh *et al*., 2021). The capacity of those herbivores to consume forage-based feed suggests that they have cellulolytic bacteria in their gastrointestinal tract. Our previous study isolated cellulolytic bacteria from the feces of tropical endemic herbivores including anoa (*Bubalus depressicornis*), bison (*Bos javanicus*), muntjak (*Muntiacus muntjak*), and Timor deer (*Rusa timorensis*). The isolates were gram-positive coccus facultative anaerobes that could ferment starches like cellulose , glucose and sucrose. The phylogeny tree and cellulolytic bacteria isolated from anoa and bison feces were molecularly identified and showed relationships to *Enterococcus faecium* (Suharti *et al*., 2023). *Enterococcus faecium* includes lactic acid bacteria which also have cellulolytic activity. Moreover, *Enterococcus* species are suitable for usage in food, feed, and probiotics due to their antimicrobial capabilities (Hanchi *et al*., 2018). Azzaz *et al*. (2022) reported when administered to lactating Holstein cows, the probiotic strain of *E. faecium* isolated from fresh dairy products enhanced *in vitro* nutritional breakdown as well as cows' feed digestion, milk output, and feed efficiency at a level of 2 g/kg DM feed).

The capability of cellulolytic bacteria (*E. faecium*) isolated from those herbivores in the cellulose degradation in the rumen environment has to be studied in more depth. The goal of this study was to examine how adding a cellulolytic bacteria consortium from anoa, bison, muntjak, and Timor deer feces affected *in vitro* rumen profiles like pH, Volatile Fatty Acids (VFA), ammonia (NH_3) production, digestibility, microbe population, and methane estimation.

Materials and Methods

Cellulolytic Bacteria Isolates Preparation

Anoa, bison, muntjak, and Timor deer feces were the sources of the cellulolytic bacteria used in this investigation. Brain Heart Infusion (BHI) medium was used to culture all isolates. The media's ingredients include 3.7 g of BHI powder, 0.1 g each of glucose and starch, 0.5 mL of haemin, 0.05 mL each of resazurin and resazurin, and 1 mL each of Carboxy Methyl Cellulose (CMC) dissolved in 100 mL distilled water. After the mixture was heated, CO_2 was flushed through it to keep the anaerobic condition, and 0.1 g of HCl-cysteine was added. Then, 9 mL of BHI broth was placed in a test tube and sterilized in an autoclave for 15 min at 121°C (Suharti *et al*., 2021). One mL of the culture of all isolates grew in media and incubated at 39°C, then the bacteria population was counted by roller tube method.

In vitro Incubation

Two local cattle rumens were sampled for rumen fluid from the slaughterhouse and kept in anaerobic condition, blended in equal amounts. Double thicknesses of cheesecloth were used to filter the combined rumen fluid and maintained in an anaerobic condition by CO_2 flushing until inoculation.

The procedure of *in vitro* incubation according to Suharti *et al*. (2019). About 500 mg of feed as the substrate, 39.5 mL of McDougall buffer, and 10 mL of rumen fluid were added to each 100 mL fermentation tube before being incubated at 39°C in a shaker water bath. The feed as substrate consists of Napier grass 30%, palm leaf meal 30%, and concentrate mix 40%. Using a 4×5 completely randomized design, the experiment included 4 treatments, comprising T0 = Napier grass: Palm leaf meal: Concentrate = 30: 30: 40 (Control), T1 = control + 10^5 CFU/mL cellulolytic bacteria isolate, T2 = control + 10^6 CFU/mL cellulolytic bacteria isolate and T3 = control + 10^7 CFU/mL cellulolytic bacteria isolate.

The nutrient composition of Napier grass, Palm leaf meal, concentrate mix, and control ratio was shown in (Table 1) The variables measured were population of rumen bacteria, total protozoa, dry matter digestibility, organic matter digestibility, crude fiber digestibility, ammonia (NH_3) concentration, VFA total and its proportion (acetate, propionate, butyrate, valerate), as well as methane estimation.

Table 1: Nutrient composition of Napier grass, palm leaf meal, and concentrate mix and control ration

Nutrients (%)	Napier Grass (NG)	Palm Leaf meal (PL)	Concentrate Mix (CM)	Total ration (Control)
Dry matter	94.14	93.89	87.30	91.33
Ash	8.52	19.34	14.55	14.18
Ether Extract (EE)	1.37	3.18	2.78	2.48
Crude Protein (CP)	14.49	13.59	11.59	13.17
Crude Fiber (CF)	30.63	18.06	24.82	24.54
Nitrogen-Free Extract (NFE)	44.99	45.47	46.25	45.64
Total Digestible Nutrient (TDN)	49.54	56.14	51.33	52.24

Note : NG: PL: CM = 30: 30: 40

Sample Selection and Measurement

After 4 h, samples from an aliquot were taken for the analyses of pH, VFA, NH_3, protozoa, and total bacteria. After 48 h, samples from an aliquot were taken for the analyses of dry matter, organic matter, and crude fiber digestibility.

According to Ogimoto and Imai (1981), the quantity of protozoa was counted under a microscope. Trypan Blue Formalin Saline (TBFS) and rumen fluid totaled 0.5 mL each and this mixture was further diluted five times. TBFS was made up of 900 mL of distilled water, 2 g of trypan blue, 100 mL of formaldehyde 35%, and 8 g of NaCl. Protozoa were counted directly on 16 divisions using a measurement chamber (Brand, Germany; 0.0625 mm^2, 0.02 mm^2 deep) (40×) under a microscope and calculated using the following formula: $P = ((1/(0.1\times0.0625\times16\times5))\times1000\times n \times d$, where P is the number of ciliates per 1 mL rumen contents, n is protozoa number and d is sample dilution.

The total number of bacteria was calculated in accordance with Ogimoto and Imai (1981) using the roller tube method and modified Rumen-Fluid-Glucose Cellobiose Agar (RGCA). The following components were present in the RGCA media: 30 mL of rumen fluid, 0.2 g of glucose, 0.2 g of cellobiose, 0.1 g of cysteine, 0.1 mL of resazurin 0.1% solution, 15 mL of solution I of the mineral mix, 15 mL of solution II of the mineral mix, 2 g of bacto agar and 40 mL of distilled water. 1 mL of an 8% Na_2CO_3 solution, 1 g of bacteria, 0.3 g of yeast extract, 0.2 g of starch-soluble yeast extract, 0.4 g of $NaHCO_3$, and 1 mL of sodium lactate make up the composition of the solution. 0.5 mL of the rumen sample was combined with 45 mL of the anaerobic dilution solution, which was then diluted 10 times in the Hungate tube. The 0.5 mL sample from dilutions 6-10 was added and then the petri plates holding the RGCA media were rotated. At a temperature of 37-40°C, for 48 h, samples were incubated. The total number of bacteria in the sample was calculated using the equation: $C \times10n /0.05\times0.1$, where C is the colony-forming unit count and n is the number of dilutions.

The microdiffusion method was used to measure the amount of NH_3 in the sample (Masterson, 2014). 1 mL of 2% boric acid with mixed indication was in the inner chamber. 1 mL of filtered rumen fluids and one mL of saturated sodium carbonate solution were each added to the outer chamber's opposing sides. The Conway micro-diffusion cell's lid was fixed and the outer chamber's contents were mixed by gradually rotating the assembly. 1 h was spent incubating the Conway dish at 38°C plus or minus 1°C. Ammonia from the rumen fluids was released when it was combined with sodium carbonate and during incubation, this ammonia was captured by the boric acid in the center chamber. Following an hour, the inner chamber's contents were measured.

Gas chromatography (Chrompack CP9002, Netherlands, flame ionized detector, Capillary column type WCOT Fused Silica 25 m 0.32 mm, oven temperature: Conditioning at 60°C and running at 115°C and nitrogen as a gas carrier) was used to analyze the total VFA concentration and proportion of VFA in accordance with Suharti *et al*. (2021) by using gas chromatography (Chrompack CP9002, Netherlands, flame ionized detector, Capillary column type WCOT Fused Silica 25 m ×0.32 mm, oven temperature: Conditioning at 60°C and running at 115°C and nitrogen as a gas carrier). Using the formula 0.45 (C2) - 0.275 (C3) + 0.4 (C4), where C2 = acetate, C3 = propionate, and C4 = butyrate, methane production was determined from the molar proportion of VFA (Moss *et al*., 2000).

In Vitro Dry Matter Digestibility (IVDMD), *In Vitro* Organic Matter Digestibility (IVOMD), and crude fiber digestibility were measured after 48 h incubation. The IVDMD and IVOMD were determined by using the Tilley and Terry (1963) method.

Data Analysis

Analysis of variance (ANOVA) was used to evaluate the data from the *in vitro* measurements and Duncan's post hoc analysis was used to compare the treatment means. It was decided that a probability level of 5% qualified as statistically significant. SPSS version 16 was used to conduct the statistical analysis (IBM).

Results

Rumen Total Bacteria, Cellulolytic Bacteria, and Protozoa Population

The addition of cellulolytic bacteria isolates at the level 10^5 CFU up to 10^7 CFU/mL increased ($p\leq0.05$)

the rumen total bacteria population, but did not affect the population of cellulolytic bacteria and total protozoa (Table 2).

Rumen Dry Matter, Organic Matter and Crude Fiber Digestibility

The addition of cellulolytic bacteria isolates at levels 10^6 and 10^7 CFU/mL increased ($p \leq 0.05$) the digestibility of dry matter and crude fiber. The organic matter digestibility was similar among treatments (Table 3).

Rumen Fermentation Characteristics, Proportion of VFA and Methane Estimation

The addition of cellulolytic bacteria isolates at level 10^5 up to 10^7 CFU/mL increased ($p \leq 0.05$) total VFA production.

The pH value, NH_3 concentration, proportional VFA (acetate, propionate, butyrate, valerate), and estimation of methane were similar among treatments (Table 4).

Table 2: Effect of cellulolytic bacteria inoculation on rumen total bacteria, cellulolytic bacteria, and total protozoa population

	Treatment			
Variables	Control (C)	C +10^5 CFU/mL cellulolytic bacteria	C +10^6 CFU/mL cellulolytic bacteria	C +10^7 CFU/mL cellulolytic bacteria
Total bacteria (log CFU/mL)	7.57±0.12^b	7.85±0.15^a	7.81±0.20^a	7.87±0,13^a
Cellulolytic bacteria (log CFU/mL)	5.63±0.11	5.67±0.16	5.73±0.09	5.75±0.09
Protozoa (log cell/mL)	5.52±0.11	5.50±0.00	5.52±0.08	5.46±0.09

Note: Means in the same row with different superscripts differ significantly ($p \leq 0.05$). Control ration = Napier grass: Palm leaf meal: Concentrate mix = 30: 30: 40

Table 3: Effect of cellulolytic bacteria inoculation on rumen dry matter, organic matter, and crude fiber digestibility

	Treatment			
Variables	Control (C)	C +10^5 CFU/mL Cellulolytic bacteria	C +10^6 CFU/mL cellulolytic bacteria	C +10^7 CFU/mL cellulolytic bacteria
Dry matter digestibility/DMD (%)	44.45±0.85^c	44.73±0.59^{bc}	45.81±1.26^{ab}	46.39±0.57^a
Organic matter digestibility/OMD (%)	42.79±1.13	42.59±1.08	43.89±1.54	44.21±0.82
Crude fiber digestibility (%)	24.30±1.40^b	25.01±2.85^b	28.98±1.16^a	28.45±2.90^a

Means in the same row with different superscripts differ significantly ($p \leq 0.05$). Control ration = Napier grass: Palm leaf meal: Concentrate mix = 30: 30: 40

Table 4: Effect of cellulolytic bacteria inoculation on rumen fermentation characteristics, the proportion of VFA and methane estimation

	Treatment			
Variables	Control (C)	C +10^5 CFU/mL cellulolytic bacteria	C +10^6 CFU/mL cellulolytic bacteria	C +10^7 CFU/mL cellulolytic bacteria
pH	6.79±0.09	6.70±0.06	6.76±0.19	6.76±0.17
NH_3 (mM)	11.81±1.86	13.13±1.61	11.79±1.36	12.73±1.08
VFA total (mM)	119.60±7.06^c	132.17±5.05^b	135.12±2.96^{bc}	139.80±3.47^a
Proporsional VFA (%mM)				
Acetate/C2 (%)	47.44±8.61	51.75±8.11	52.56±5.90	55.78±11.34
Propionate/C3 (%)	2.33±1.97	11.90±1.59	11.40±1.48	13.04±2.23
Butirate/C4 (%)	8.82±1.39	8.19±1.12	7.55±1.23	8.52±1.27
Valerate C5 (%)	1.84±0.14	2.05±0.43	1.65±0.36	1.80±0.29
Estimation of Methane/CH_4 (mM)	21.49±3.86	23.29±3.58	23.54±2.69	24.92±4.96

Note: Means in the same row with different superscripts differ significantly ($p \leq 0.05$). Control ration = Napier grass: Palm leaf meal: Concentrate mix = 30: 30: 40

Discussion

Rumen Total Bacteria, Cellulolytic Bacteria and Total Protozoa Population

The increasing rumen total bacteria population suggests that the addition of cellulolytic bacteria consortia isolated from anoa, bison, muntjak, and Timor deer feces did not have an impact on rumen bacterial growth, but could increase rumen bacterial accretion. Anoa, bison, muntjak, and Timor deer feces contain cellulolytic bacteria isolates that may persist in the rumen ecosystem and may not compete with protozoa or other rumen microbes.

Our cellulolytic isolates' bacteria naturally came from the gastrointestinal tracts of the herbivores from which they were isolated. The isolates may be able to adapt and survive in the rumen system. This finding, which was consistent with protozoa data that were comparable across treatments, indicates that there was no competition between the cellulolytic bacteria isolates with the rumen protozoa. Our isolates from anoa and bison feces were identified as *Enterococcus faecium* which includes Lactic Acid Bacteria (LAB). Innoculation of LAB to the rumen will affect ruminal fermentation activity by rumen microbe. A previous study suggested that the inoculation of LAB as a feed addition can have an impact on the ruminant's microbial environment and help the animal's natural rumen microbial community to emerge (Ramaswami *et al*., 2005). *Enterococcus faecium* addition levels of at least 2.67×10^6 CFU/mL demonstrated excellent potential for promoting the growth of rumen bacteria to boost the glucogenic propionate energy source for host ruminants (Pang *et al*., 2014). Mamuad *et al*. (2019) suggested that the highest levels of total bacteria and *Ruminococcus flavefaciens* were produced by supplementation with 0.1% *E. faecium* SROD, while the highest levels of total fungi and *Fibrobacter succinogenes* were produced by supplementation with 1.0% *E. faecium* SROD. Another function of LAB, including Pseudomonas spp., was the production of Lactobionic Acid (LBA), which was utilized in the pharmaceutical, food, medical, cosmetic, and chemical sectors, for example, in the development of skincare and anti-aging products (Narala *et al*., 2022).

Rumen Dry Matter, Organic Matter and Crude Fiber Digestibility

The inclusion of isolates from the cellulolytic consortium bacteria may have enhanced the digestibility of dry matter and crude fiber due to the overall increase in bacteria. The rumen bacteria are crucial for the digestion of feed. A large variety of enzymes from the ruminal microbiome are available to the host to break down the intricate plant cell walls and potentially harmful chemicals. Wang and McAllister (2002) suggested that the rumen was known to be the site of several different enzyme activities, including those that degrade plant cell wall polymers (such as cellulases, xylanases, β-glucanases and pectinases), amylases, proteases, phytases and those that degrade specific plant toxins (such as tannases).

Moreover, the increase in fiber digestibility following the addition of cellulolytic bacteria isolates suggests that the isolate developed in the rumen system and broke down the feed fiber. Kazemi-Bonchenari *et al*. (2013) reported the improvement of Neutral Detergent Fiber (NDF) digestibility of the sheep which was fed symbiotic *E. faecium* and inulin. The addition of *E. faecium* and endo-1,4-β-xylanase also improved the nutrient digestibility of the pig fed with diets based on the corn-soybean meal (Nguyen *et al*., 2017). Azzaz *et al*. (2022) stated that milk production, feed efficiency, and *in vitro* nutrient degradation were all increased when cows were given daily supplements of *E. faecium* (both isolated and commercial) at a rate of 2 g/kg DM feed. The different result reported by Saeidi *et al*. (2021) that feeding adult horses fructooligosaccharides and *E. faecium* reduced triglyceride and cholesterol contents and decreased fecal pH, but had no effect on nutrient digestibilities.

Typically, the rumen cellulolytic bacteria break down cellulose by adhering to a cellulosome, then extracellular structure. Four steps could be used to define the adherence: Non-motile bacteria first colonize the substrate before randomly adhering to areas of the plant cell wall, then they bind selectively to receptors on the substrate with ligands or adhesins and finally, they multiply to form colonies on potentially digestible substrate sites (Miron *et al*., 2001). The majority of cellulases were Glycosyl Hydrolases (GH), which could hydrolyze the glycosidic linkages found within carbohydrates (Krause *et al*., 2003). The glucosides' C-O, C-N, or C-C bonds were broken by the hydrolases to create sugar and other substances, whereas in order to create cellulose's monomers, the cellulases primarily dissociated the 1,4-glycosidic linkages between the glucosyl moieties (Hua *et al*., 2022). Krause *et al*. (2003) stated that three basic cellulase types-endoglucanases (endo-1,4-D-glucan hydrolases), exoglucanases (exo-1,4-D-glucan cellobiohydrolases) and-glucosidases (-D-glucosidases) coordinately hydrolyze cellulose into monomeric glucose units.

Rumen Fermentation Characteristics, Proportion of VFA and Methane Estimation

Volatile Fatty Acid (VFA) production increased with the presence of cellulolytic bacteria consortium isolates due to the improvement of dry matter and crude fiber digestibility. The fermentation in the rumen is accelerated by enhancing rumen bacteria with cellulolytic isolates addition. Mamuad *et al*. (2019) showed the same result

that the addition of 0.1% *E. faecium* SROD improved total VFA production. Jiao *et al*. (2017) also reported that supplementation of *E. faecium* combined with *Saccharomyces cerevisiae* stimulated rumen fermentation and increased total VFA production, modifying the proportional VFA with a greater proportion of acetate and a smaller proportion of propionate.

The rumen pH value of all treatments was monitored at the normal range of about 6.70-6.79. No variations in rumen pH may indicate that the rumen environment has not been altered by the presence of cellulolytic bacteria isolates. In order to maximize the microbial process in the rumen's ability to degrade and ferment feed, pH stability is crucial. When the rumen's pH is between 6.0 and 6.7, normal fiber digestion takes place. According to Saeed *et al.* (2023), the ideal range for the pH of the rumen for the microbial digestion of protein and fiber was 6.0-7.0. Fiber digestibility was significantly impacted as rumen pH drops below 6.0. Acidosis, also known as Sub-Acute Acidosis (SARA), can cause animals to become "off-feed" and pose serious issues as the rumen pH falls below 5.5 (Fu *et al*., 2022). Previous research by According to Goto *et al*. (2016), rumen fluid in SARA cattle with low pH and high levels of lactic acid may be improved by administering 20-50 g of a multi-strain Bacteria Probiotic (BP) for seven days. According to Basso *et al*. (2014), lambs fed a meal containing Lactic Acid Bacteria (LAB) including either Lactobacillus Buchneri (LB) alone or a combination of LB and Lactobacillus plantarum had no effects on ruminal pH.

The similar NH_3 concentration among treatments indicated that the cellulolytic bacteria did not influence the feed protein degradation and ruminal N-metabolism level. Ammonia was produced in the rumen as a result of feed protein and non-protein nitrogen being broken down by ruminal bacteria.

Conclusion

It can be concluded that the inclusion of a cellulolytic bacterial consortia derived from feces of anoa, bison, muntjak and Timor deer increased dry matter digestibility, crude fiber digestibility, total VFA production, and total bacterial population. Methane, individual VFAs, rumen pH, and ammonia production were comparable among treatments. Based on fiber digestibility and VFA production, inoculation of the cellulolytic bacteria consortium at level 10^6 CFU/mL was the optimum level to improve fiber digestibility, and VFA production and had a beneficial effect for ruminants fed high-fiber feed.

Acknowledgment

The Biochemistry and Microbiology Laboratory, Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University (Bogor Agricultural University) is where the authors' work can be found. The authors thank all of the lab staff there.

Funding Information

With contract number 4011/IT3.L1/PT.01.03/M/T/2022, bogor agricultural university-Indonesia's "hibah riset kolaborasi universitas Jambi-IPB university" provided funding for this study.

Author's Contributions

Sri Suharti, Agus Budiansyah, Syafwan, and Komang Wiryawan: Study conception and design, data collection, analysis, and interpretation of results, drafted manuscript preparation and reviewed the final of the manuscript.

Erika Julian Cahyani and Iksan Qodri Pramarta: Data collection, analysis and interpretation of results.

Ethics

This article was original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues were involved.

References

Azzaz, H. H., Kholif, A. E., Murad, H. A., & Vargas-Bello-Pérez, E. (2022). A newly developed strain of *Enterococcus faecium* isolated from fresh dairy products to be used as a probiotic in lactating Holstein cows. *Frontiers in Veterinary Science*, (*9*), 989606. https://doi.org/10.3389/fvets.2022.989606

Basso, F. C., Adesogan, A. T., Lara, E. C., Rabelo, C. H. S., Berchielli, T. T., Teixeira, I. A. M. A., ... & Reis, R. A. (2014). Effects of feeding corn silage inoculated with microbial additives on the ruminal fermentation, microbial protein yield and growth performance of lambs. *Journal of Animal Science*, *92*(12), 5640-5650. https://doi.org/10.2527/jas.2014-8258

Fu, Y., He, Y., Xiang, K., Zhao, C., He, Z., Qiu, M., ... & Zhang, N. (2022). The Role of Rumen Microbiota and Its Metabolites in Subacute Ruminal Acidosis (SARA)-Induced Inflammatory Diseases of Ruminants. *Microorganisms*, *10*(8), 1495. https://doi.org/10.3390/microorganisms10081495

Goto, H., Qadis, A. Q., Kim, Y. H., Ikuta, K., Ichijo, T., & Sato, S. (2016). Effects of a bacterial probiotic on ruminal pH and volatile fatty acids during subacute ruminal acidosis (SARA) in cattle. *Journal of Veterinary Medical Science*, *78*(10), 1595-1600. https://doi.org/10.1292/jvms.16-0211

Kazemi-Bonchenari, M., Ghasemi, H. A., Khodaei-Motlagh, M., Khaltabadi-Farahani, A. H., & Ilani, M. (2013). Influence of feeding synbiotic containing Enterococcus faecium and inulin on blood metabolites, nutrient digestibility and growth performance in sheep fed alfalfa-based diet. *Scientific Research and Essays*, *8*(21), 853-857.

Hanchi, H., Mottawea, W., Sebei, K., & Hammami, R. (2018). The genus *Enterococcus*: Between probiotic potential and safety concerns-an update. *Frontiers in Microbiology*, *9*, 1791. https://doi.org/10.3389/fmicb.2018.01791

Hua, D., Hendriks, W. H., Xiong, B., & Pellikaan, W. F. (2022). Starch and cellulose degradation in the rumen and applications of metagenomics on ruminal microorganisms. *Animals*, *12*(21), 3020. https://doi.org/10.3390/ani12213020

Jiao, P. X., Liu, F. Z., Beauchemin, K. A., & Yang, W. Z. (2017). Impact of strain and dose of lactic acid bacteria on *in vitro* ruminal fermentation with varying media pH levels and feed substrates. *Animal Feed Science and Technology*, *224*, 1-13. https://doi.org/10.1016/j.anifeedsci.2016.11.005

Krause, D. O., Denman, S. E., Mackie, R. I., Morrison, M., Rae, A. L., Attwood, G. T., & McSweeney, C. S. (2003). Opportunities to improve fiber degradation in the rumen: Microbiology, ecology and genomics. *FEMS Microbiology Reviews*, *27*(5), 663-693. https://doi.org/10.1016/S0168-6445(03)00072-X

Mamuad, L. L., Kim, S. H., Biswas, A. A., Yu, Z., Cho, K. K., Kim, S. B., ... & Lee, S. S. (2019). Rumen fermentation and microbial community composition influenced by live *Enterococcus faecium* supplementation. *Amb Express*, *9*(1), 1-12. https://doi.org/10.1186/s13568-019-0848-8

Masterson, B. (2014). Conway's Microdiffusion Analysis: Eighty years on and still counting!. *The Biochemist*, *36*(1), 34-39. https://doi.org/10.1042/BIO03601034

Miron, J., Ben-Ghedalia, D., & Morrison, M. (2001). Invited review: Adhesion mechanisms of rumen cellulolytic bacteria. *Journal of Dairy Science*, *84*(6), 1294-1309. https://doi.org/10.3168/jds.S0022-0302(01)70159-2

Moss, A. R., Jouany, J. P., & Newbold, J. (2000). Methane production by ruminants: Its contribution to global warming. In *Annales de Zootechnie* (Vol. *49*, No. 3, pp. 231-253). EDP Sciences. https://doi.org/10.1051/animres: 2000119

Naqibzadeh, A., Sarhangzadeh, J., Sotoudeh, A., Mashkour, M., & Thomalsky, J. (2021). Habitat Suitability Modeling for Wildlife Management Objectives by Using Maximum Entropy Method. *J Human, Earth and Futur*, *2*(4), 371-381. https://doi.org/10.1139/CJAS-2016-0197

Narala, V. R., Zagorska, J., Sarenkova, I., Ciprovica, I., & Majore, K. (2022). Acid whey valorization for biotechnological lactobionic acid bio-production. *Journal of Human, Earth and Future*, *3*, 46-55. https://doi.org/10.28991/HEF-SP2022-01-04

Nguyen, D. H., Lee, K. Y., Tran, H. N., Upadhaya, S. D., Jeong, Y. J., & Kim, I. H. (2017). Influence of *Enterococcus faecium* and endo-1, 4-β-xylanase supplementation on growth performance, nutrient digestibility, fecal microflora, fecal gas emission and meat quality in finishing pigs fed with diets based on corn-soybean meal. *Canadian Journal of Animal Science*, *98*(1), 126-134. https://doi.org/10.1139/CJAS-2016-0197

Ogimoto, K., & Imai, S. (1981). *Atlas of Rumen Microbiology*. Japan Scientific Societies Press. https://www.cabdirect.org/cabdirect/abstract/19820894286

Pang, D. G., Yang, H. J., Cao, B. B., Wu, T. T., & Wang, J. Q. (2014). The beneficial effect of *Enterococcus faecium* on the *in vitro* ruminal fermentation rate and extent of three typical total mixed rations in northern China. *Livestock Science*, *167*, 154-160. https://doi.org/10.1016/j.livsci.2014.06.008

Ramaswami, N., Chaudhary, L. C., Agarwal, N., & Kamra, D. N. (2005). Effect of lactic acid producing bacteria on the performance of male crossbred calves fed roughage based diet. *Asian-Australasian Journal of Animal Sciences*, *18*(8), 1110-1115. https://doi.org/10.5713/ajas.2005.1110

Ribeiro, G. O., Gruninger, R. J., Badhan, A., & McAllister, T. A. (2016). Mining the rumen for fibrolytic feed enzymes. *Animal Frontiers*, *6*(2), 20-26. https://doi.org/10.2527/af.2016-0019

Saeed, O. A., Sani, U. M., Sazili, A. Q., Akit, H., Alimon, A. R., & Samsudin, A. A. (2023). Profiling of Fatty Acids and Rumen Ecosystem of Sheep Fed on a Palm Kernel Cake-Based Diet Substituted with Corn. *Agriculture*, *13*(3), 643. https://doi.org/10.3390/agriculture13030643

Saeidi, E., Yarahmadi, H. M., Fakhraei, J., & Mojahedi, S. (2021). Effect of feeding fructooligosaccharides and *Enterococcus faecium* and their interaction on digestibility, blood and immune parameters of adult horses. *Journal of Equine Veterinary Science*, *99*, 103410. https://doi.org/10.1016/j.jevs.2021.103410

Suharti, S., Firliani, I., Mawar, M., Sekar, V., & Wiryawan, K. G. (2019). *In vitro* ruminal fermentation, microbial population, fatty acid profile in cattle in the presence of sapindus rarak extract combined with oils microencapsulation. *Adv. Anim. Vet. Sci*, *7*(9), 798-805. https://doi.org/10.17582/journal.aavs/2019/7.9.798.805

Suharti, S., Novrariani, N., & Wiryawan, K. G., (2023). Morphological, Biochemical and Molecular Identification of Cellulolytic Bacteria from the Feces of Tropical Endemic Herbivores. *Submitted*. https://doi.org/10.13057/biodiv/d240742

Suharti, S., Oktafiani, H., Sudarman, A., Baik, M., & Wiryawan, K. G. (2021). Effect of cyanide-degrading bacteria inoculation on performance, rumen fermentation characteristics of sheep fed bitter cassava (Manihot esculenta Crantz) leaf meal. *Annals of Agricultural Sciences*, *66*(2), 131-136. https://doi.org/10.1016/j.aoas.2021.09.001

Terry, S. A., Badhan, A., Wang, Y., Chaves, A. V., & McAllister, T. A. (2019). Fibre digestion by rumen microbiota-a review of recent metagenomic and metatranscriptomic studies. *Canadian Journal of Animal Science*, *99*(4), 678-692. https://doi.org/10.1139/cjas-2019-0024

Tilley, J. M. A., & Terry, D. R. (1963). A two-stage technique for the *in vitro* digestion of forage crops. *Grass and Forage Science*, *18*(2), 104-111. https://doi.org/10.1111/j.1365-2494.1963.tb00335.x

Torres-Salado, N., Ayala-Monter, M. A., Sánchez-Santillán, P., & Almaraz-Buendía, I. (2023). Effect of the addition of cellulolytic bacteria to ruminal bacteria on *in vitro* fermentation characteristics. *Revista Colombiana de Ciencias Pecuarias*, *36*(1), 22-32. https://doi.org/10.17533/udea.rccp.v35n4a5

Wang, Y., & McAllister, T. A. (2002). Rumen microbes, enzymes and feed digestion-a review. *Asian-Australasian Journal of Animal Sciences*, *15*(11), 1659-1676. https://doi.org/10.5713/ajas.2002.1659