Diet dominates age in shaping the rumen bacteria community and function in dairy cattle

Yangyi Hao
China Agricultural University
Yue Gong
China Agricultural University
Shuai Huang
China Agricultural University
Shoukun Ji
Hebei Agricultural University
Wei Wang
China Agricultural University
Yajing Wang
China Agricultural University
Hongjian Yang
China Agricultural University
Zhijun Cao
China Agricultural University
Shengli Li (haoyangyi0928@163.com)
China Agricultural University

Research

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Abstract

Background

To understand the effects of diet and age on the rumen bacterial community and function, forty-eight dairy cattle at 1.5 (M1.5), 6 (M6), 9 (M9), 18 (M18), 23 (M23), and 27 (M27) months old were selected. The M1.5, M6, and M27 had the high protein and starch dietary, while the M9, M18, and M23 had the high fiber dietary. Fermentation profile, enzyme activity, and bacteria community in rumen fluid were measured.

Results

The acetate to propionate ratio (A/P) at M9, M18, and M23 (high fiber diet) was higher than other ages, and M6 was the lowest ($P < 0.05$). The total volatile fatty acid (TVFA) at M23 and M27 was higher than other ages ($P < 0.05$). The urease at M18 was lower than M1.5, M6, and M9, and the xylanase at M18 was higher than M1.5, M23, and M27 ($P < 0.05$). The α-diversity indexes (Ace and Chao1) of ruminal bacteria increased from M1.5 to M23, while they decreased from M23 to M27 ($P < 0.05$). Thirty-three bacteria were identified as biomarkers of the different groups based on the linear discriminant analysis (LDA) when the LDA score > 4. The variation partitioning approach analysis showed that the age and diet had a 7.98% and 32.49% contribution to the rumen bacteria community variation, respectively. The richness of *Succinivibrionaceae_UCG-002* and *Fibrobacter* were positive correlated with age ($r > 0.60$, $P < 0.01$) and also positively correlated with TVFA and acetate ($r > 0.50$, $P < 0.01$). The *Lachnospiraceae_AC2044_group*, *Pseudobutyrivibrio*, and *Saccharofermentans* has a positive correlation ($r > 0.80$, $P < 0.05$) with diet NDF and negative correlation ($r < -0.80$, $P < 0.05$) with diet CP and starch, which were also positively correlated with the acetate and A/P ($r > 0.50$, $P < 0.01$).

Conclusion

These findings indicated that the quantitative effect of diet and age on the rumen bacteria were 7.98% and 32.49%, respectively. The genera of *Lachnospiraceae_AC2044_group*, *Pseudobutyrivibrio*, and *Saccharofermentans* could be worked as the target bacteria to modulate the rumen fermentation by diet; meanwhile, the high age-correlated bacteria such as *Succinivibrionaceae_UCG-002* and *Fibrobacter* also should be considered when shaping the rumen function.

1. Introduction

Ruminant animals can capture nutrients from roughage by the digestion process of ruminal microorganisms to the cell wall components. The rumen is a complex microbial ecosystem containing a great diversity of bacteria, archaea, viruses, protozoa, and fungi [1, 2]. Within the rumen microorganisms, bacteria are the most abundant species and are the major contributor to digest plants [2]. The bacteria

could convert the feed into volatile fatty acids (VFA), ammonia, and microbial crude protein (MCP), which could further supply nutrients for ruminants [3, 4]. The key role during the degradation of plant is the enzymes, which were encoded and secreted by the microorganisms [5, 6]. The digestive enzyme could catalyze and decompose feedstuff into molecular for animals to use, like amylase could decompose the starch into glucose and futher enhance the ruminants starch digestibility [7]. The exogenous protease could alert the amino acid composition and improve the starch digestibility of corn silage [8]. Bacteria, enzymes, and the VFA, MCP, etc., are closely related and jointly assist in completing the rumen digestive function.

The rumen function and ecosystem stability largely depend on the diversity and complexity of microorganisms [9]. The study about the rumen bacteria has been explored further from the application of high-throughput sequencing technology. Fonty et al. had found that the rumen cellulolytic bacteria of lambs reach a comparable level of the mature rumen at the end of a week after birth [10]. Jami et al. found that the calf was born with some rumen bacteria essential for mature rumen function [11]. The ruminal bacterial community is established before the intake of solid feed, and the increased intake of starter could, in turn, shape this community [12]. Anderson et al. also indicated with the solid feed intaking, and the proteolytic bacteria increased from 1–2% (at the delivery) to 10% (12weeks); meanwhile, the amylolytic bacteria also increased with age [13]. From 6-month to 2-year old, the rumen bacteria community was significantly different with the same diet [11]. However, Bohra et al. showed the rumen bacteria composition varied with the dietary nutritional level [14]. The different roughage sources also altered the rumen microbiome and carbohydrate-active enzyme profile [14], and the change is associated with feedstuffs' nutrients [15]. A meta-analysis showed the bacteria might exert independent effects on various aspects of ruminant performance [16]. Ming-yuan et al. indicated the richness of *Prevotella* in rumen, contributing to improved functions related to branched-chain amino acid biosynthesis and then enhanced the dairy cows' milk protein yield [17]. The bacteria composition, metabolic pathway, and metabolite also different between high and low-yield dairy cows [18]. Diet and age, who is the main driving force to change the rumen bacteria and affect animal performance, has not yet been revealed.

The bacteria composition, enzyme, and the endproduct of VFA, MCP, etc., are the crucially factors shaping the rumen functions and characteristics. Although it has been demonstrated that age and diet influence the ruminal bacterial community. There is no information about the correlation of these elements in different dairy cattle stages and which one is the main pusher to alter the rumen bacteria community and function. Therefore, this study investigates the ruminal bacteria profile and its products in six production stages under different ages and diets. We hope to illustrate the specific diet or age-related bacteria and its' production. Ultimately, to provide the theory basis for dairy cattle precisely feeding and management.

2. Materials And Methods

2.1 Ethics statements

The experimental procedures used in the present study were approved by the Ethical Committee of the College of Animal Science and Technology, China Agriculture University (Protocol number: 2013-5-LZ).

2.2 Animals and sample collection

Animals were selected from a farm in Beijing, China, with the same management system. At the 1.5 (M1.5), 6 (M6), 9 (M9), 18 (M18), 23 (M23), and 27 (M27) months, eight cattle were selected at each period and collected the rumen fluid samples. The animal feed formula and chemical composition of these diets are shown in Table S1. In brief, the M1.5, M6, and M27 had a relatively high diet starch and protein content, while the M9, M18, and M23 had a relatively high fiber diet.

Rumen fluid sample was collected by oral intubation before morning feeding. About 50 mL of rumen liquid from each animal was obtained, with the initial 50 mL (approximately) discarded to avoid saliva contamination. Each sample was separated into two sterile tubes. One was immediately placed in liquid nitrogen and stored at -80℃ for 16S rRNA sequencing and enzyme activity analysis. Another was filtered by four cheesecloth and then stored at -20℃ for fermentation profile analysis.

2.3 Sample analysis

2.3.1 Fermentation profile and enzyme activity

The rumen pH was immediately determined after sample collection using a pH electrode (Model pH B-4; Shanghai Chemical, Shanghai, China). Ammonia-nitrate (NH_3-N) concentration of rumen fluid was measured using the phenol-sodium hypochlorite colorimetry method described by Broderick and Kang [19]. The MCP concentration was detected referred to Negi et al. [20]. The VFA content was measured by gas chromatography (6890 N; Agilent technologies, Avondale, PA, USA) referred to Cao et al. [21]. The urease [22], protease [23], amylase [24], lipase [25], xylanase [25], and dehydrogenase [26] were measured using the SpectraMax 190 Microplate Reader (MD., Newyork, USA) with the commercial kits (Suzhou Grace Biotechnology Co., Ltd, Jiangsu, China).

2.3.2 16S rRNA sequencing

The DNA of rumen fluid samples was extracted using HiPure Stool DNA Kits (Magen company, Guang zhou, China). DNA concentration and purity were monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1ng/μL using sterile water. The V3-V4 region of the 16s rRNA gene was amplified by PCR (denaturation: 94°C for 2 min, followed by 30 cycles at 98°C for 10 s, annealing reaction: 62°C for 30 s and 68°C for 30 s and a final extension at 68°C for 5 min) using specific primer: former primer 341F (CCTACGGGNGGCWGCAG), reverse primer 806R (GGACTACHVGGGTATCTAAT) [27]. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions. The amplicons were quantified using ABI StepOnePlus Real-Time PCR System (Life Technologies, Foster City, USA). The purified amplicons were pooled in equimolar and paired-end sequenced on a PE250 Illumina platform. Paired-end reads were merged using FLASH (V1.2.7,http://ccb.jhu.edu/software/FLASH/) [28]. Low

quality (score ≤ 20) short reads (< 200 bp) and reads containing ambiguous bases or unmatched to primer sequences and barcode tags were filtered to obtain the high-quality clean tags [29] according to the QIIME (V1.9.1, http://qiime.org/scripts/split_libraries_fastq.html) [30] quality-controlled process. The tags were compared with the reference database (Silva database, https://www.arb-silva.de/) using the UCHIME algorithm (UCHIME Algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html) [31] to detect chimera sequences. Then the chimera sequences were removed [32], and the Effective Tags were finally obtained. Sequences analysis was performed by Uparse software (Uparse v7.0.1001, http://drive5.com/uparse/) [33]. Sequences with ≥ 97% similarity were assigned to the same OTUs. The representative sequence for each OTU was screened for further annotation. OTUs abundance information was normalized using a standard of sequence number corresponding to the sample with the least sequences. Subsequent analysis of alpha diversity and beta diversity was performed basing on this output normalized data. For each representative sequence, the Silva Database 132 (http://www.arb-silva.de/) [34] was used based on Mothur algorithm to annotate taxonomic information.

2.4 Statistics

The rumen fermentation profile and enzyme activities were subjected to One-Way ANOVA by SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). Alpha-diversity indices were calculated with QIIME (Version 1.7.0) and analyzed using the Kruskal-Wallis test and Wilcoxon rank test using the "dplyr" package in R. Principal Co-ordinates Analysis (PCoA) and analysis of similarities (ANOSIM) (999 permutations) was performed and visualized using the "ggplot2" package in R (Version 3.6.1). Spearman's rank correlation was used to identify the relationship between the enzyme activity and rumen fermentation profile (VFA, NH_3-N, and MCP); the top 50 abundance bacteria at genus level and its' byproducts (enzyme, VFA, NH_3-N, and MCP) using the "corrplot" package in R. The result was visualized as a heatmap using the R package "pheatmap." All *P*-value was corrected using a false discovery rate of 0.05, as described by Benjamini and Hochberg [35]. The false discovery rate corrected $P < 0.05$ was considered significant. The linear discriminant analysis effect size (LEfSe) [36] was used to determine the difference of rumen bacteria among ages and diets by coupling Kruskal-Wallis Test for statistical significance with additional tests assessing biological consistency and effect relevance. Variation partitioning approach (VPA) was used to evaluate the relative importance of age and dietary nutrients on rumen bacteria community using "vegan" package in R [37]. Spearman's rank correlation and liner regression also used to analysed the relationship between the PC1 (Principal component 1 of the principal coordinate analysis' axis of rumen bacteria) and age or diet nutrients [38].

3 Results

3.1 Rumen fermentation profile and enzyme activity

The rumen pH among the 1.5 M, 6 M, 9 M, and 18 M dairy cattle had no difference, while the ruminal pH value in these groups was higher than the M27 ($P < 0.05$). The NH_3-N content in the M1.5 was the highest ($P < 0.05$). The MCP content at the M6, M23, and M27 was higher than M1.5 and M9, and the M18 was

the lowest ($P < 0.05$). The acetate concentration at M6, M9, and M18 was no different from others, while that at M6, M9, and M18 was significantly higher than M1.5 ($P < 0.05$). Propionate content at M6 and M27 was higher than that at M1.5 and M18 ($P < 0.05$). The rumen butyrate concentration at M1.5 was lower than others ($P < 0.05$). The rumen butyrate at M27 and M6 was significantly higher than M18 ($P < 0.05$). The total VFA concentration at M27 and M23 were higher than others ($P < 0.05$). The acetate to propionate ratio (A/P) at M23, M18, and M9 (high fiber diet) was higher than other ages, and M6 was the lowest ($P < 0.05$).

The dehydrogenase at the M6, M9, and M27 was higher than the M1.5 ($P < 0.05$), while M9, M18, M23, and M27 had no difference. The urease at the M18 was lower than M1.5, M6, and M9 ($P < 0.05$). The protease at the M1.5 was higher than the M9 ($P < 0.05$). The xylanase at the M18 was higher than M1.5, M23, and M27 ($P < 0.05$). The amylase at the M6 and M27 was higher than M1.5 and M9 ($P < 0.05$). The lipase at the M6 was higher than M1.5, M9, M18, and M23 ($P < 0.05$), while that within the M9, M18, M23, and M27 had no difference.

Spearman's rank correlation was performed to study the correlation between enzyme activity and rumen fermentation profile (VFA, NH_3-N, and MCP) (Fig.S1). As a result, we observed that dehydrogenase and amylase were positively correlated with propionate and valerate ($r > 0.50$, $P < 0.01$). Xylanase was negatively correlated with NH_3-N ($r = -0.56$, $P < 0.01$). Rumen amylase was negatively correlated with A/P ($r = -0.57$, $P < 0.01$).

3.2 Rumen bacteria analysis

3.2.1 Rumen bacteria diversity analysis

After sequence trimming, quality filtering, and chimeras removing, a total of 2,575,670 high-quality sequence tags was obtained from all samples. The M1.5, M6, M9, M18, M23, and M27 groups had 453,635 (56,704 ± 1418, mean ± standard deviation), 448,576 (56,704 ± 1803), 408,982 (51,122 ± 1468), 418,263 (52,282 ± 1800), 411,811 (51,485 ± 1583), 434,270 (53,658 ± 1986) tags, respectively (Table S2). The Good's coverages for all samples were more than 99.70%. The alpha-diversity indices, including Chao1, ACE, observed OTUs, Shannon, and Simpson index, were compared among six groups (Fig.S2). Interestingly, the observed OTUs, ACE, and Chao1 values at M18 and M23 were significantly higher than M6 and M9; the M1.5 and M6 were lower than others ($P < 0.05$). Shannon index was increased from M1.5 to M18 but showed no difference between the M18 and M23. Shannon index at the M27 was lower than M18 and M23 ($P < 0.05$). The Simpson index at the M1.5 was higher than other groups ($P < 0.05$). These indexes showed the M18 and M23 had the highest bacteria diversity.

The Venn diagram analysis revealed that 127 amplicon sequence variants (ASV) were shared across the six groups (Fig. 3A). There are 41, 10, 10, 21, 12, and 16 unique ASVs at the M1.5, M6, M9, M18, M23, and M27. The PCoA analysis showed that the M1.5 and M6 separated with others (Fig. 3B), and ANOSIM showed these groups were statistically different ($R^2 = 0.62$, $P = 0.001$).

3.2.2 Rumen bacteria composition analysis

The top ten phyla account for more than 99.9% of bacteria (Fig. S3B). Twenty-two genera were identified as core bacteria, which were identified with a relative abundance > 1% and present in at least 80% of all samples (File. S2). Bacteria with LDA scores higher than four were speculated to have a different abundance across the different groups (Fig. 4A). Finally, 33 bacteria were identified as biomarkers of the various groups, respectively. The unique bacteria at M1.5 were *Proteobacteria*, *Gammaproteobacteria*, *Succinivibrio*, *Lachnospiraceae*, and *Bacteroidaceae* (genus level). *Prevotellaceae*, *Veillonellaceae*, *Selenomonadales*, *Negativicutes*, and *Muribaculaceae* were higher at M6. *Prevotella_ruminicola* and *Lachnospiraceae* (family level) were higher at M9. Some *Firmicutes* phylum bacteria could be the biomarker at M18 (Fig. 4B), like *Clostridia*, *Firmicutes*, *Ruminococcaceae*, and *Christensenellaceae*. *Rikenellaceae* and *Bacteroidales*. The unique bacteria at M23 was *Fibrobacter*. *Succinivibrionaceae* and *Aeromonadales* were higher at M27 ($P < 0.05$).

3.3 Driving factors and the correlations between rumen bacteria and its byproducts

3.3.1 Driving factors of rumen bacteria variation

Variation partitioning approach (VPA) revealed diet and age factors explained 32.49 and 7.98% of rumen bacteria communities' variations (Fig. 5A). The CP, NDF, starch, and EE had a 4.50%, 4.31%, 4.64%, and 5.44% effects on the rumen bacteria community (Fig. 5B). The Spearman rank correlation analysis showed that age and NDF negatively correlated with PC1 (r = -0.66 and − 0.83, $P < 0.01$, respectively). CP, starch, and EE positively correlated with PC1 (r = 0.83, 0.67, and 0.65, $P < 0.01$, respectively).

3.3.2 The correlation between bacteria and its main byproducts

To explore the potential roles of ruminal bacteria on enzyme activity and fermentation profile, we analyzed the relationship between the top 50 abundant genera and their main byproducts (enzyme, VFA, NH_3-N, and MCP) using Spearman correlation analysis (Fig. 6). We found that 23 bacteria were significantly correlated with A/P, acetate, and TVFA ($P < 0.05$). Five genera belong to phyla *Firmicutes*, and eight genera belong to phyla *Bacteroidota*. 13 bacteria genera were significantly correlated with NH_3-N, valerate, and urease ($P < 0.05$). Four genera belong to the *Firmicutes* phyla; five genera belong to the *Bacteroidota* phyla; two genera belong to the *Proteobacteria* phyla. The genera of *Shuttleworthia*, *Oribacterium*, *Prevotellacear_YAB2003_group*, *Succinivibrionaceae_UCG-001* were positively correlated (r > 0.5, $P < 0.05$) with the dehydrogenase, isovalerate, MCP, and propionate, while genera of *Prevotellaceae_NK3B31_group*, *UCG-005*, *Butyrivibrio*, and *Rikenellaceae_RC9_gut-group* were negatively correlated (r < -0.5, $P < 0.05$) with them. Specifically, the genus of *Succinivibrionaceae_UCG-002*, *Treponema*, and *Eubacterium_ruminantium_group* were strongly positive-correlated with acetate (r > 0.73, $P < 0.01$).

The Spearman's correlation coefficient of the top 50 genera with age or diet was in Table S3. We selected five bacteria genera highly correlated with age and diet and also correlated with the VFA (Fig. 7). The *Succinivibrionaceae_UCG-002* and *Fibrobacter* were positively correlated with age ($r > 0.60$, $P < 0.01$) and positively correlated with TVFA and acetate ($r > 0.50$, $P < 0.01$). The *Lachnospiraceae_AC2044_group*, *Pseudobutyrivibrio*, and *Saccharofermentans* has a Spearman's correlation coefficient value > 0.80 with diet NDF and < -0.80 with diet CP and starch ($P < 0.01$), which also positively correlated with the acetate and A/P ($r > 0.50$, $P < 0.01$). These bacteria should be targeted goal when regulated the rumen function based on different ages and diet backgrounds.

4 Discussion

4.1 Rumen fermentation profile and enzyme activity

Rumen pH was affected by the diet chemical composition, and high dietary NDF content could increase the rumen pH (Jiang et al., 2017), while the high grain diet could produce more fatty acids and further reduce the rumen pH [39]. M1.5 group received the lowest NDF and highest grain content diet (Table S1). Still, the incomplete rumen function couldn't produce enough fatty acids making the rumen pH decreased. VFAs are the endproducts of diets' fermentation, and they are also essential for rumen development, production performance, and body metabolism [40–42]. Previous studies indicated diet chemical composition could alter the rumen VFA production [21, 41, 42]. High diet starch content could enhance the rumen propionate concentration [41]. The calf at the age of 1.5M had a lower propionate concentration was due to the immature rumen function, which couldn't produce enough enzyme to degrade the starch into propionate. At 18M, heifer with the lowest diet starch content also had less propionate, caused by the lack of substance, such as starch. A high fiber content diet could enhance the rumen acetate concentration and the A/P value [43]. Also, the A/P is age-related [44]. The M27 group had a lower A/P value than M9 and M18, and the discrepancy indicated the diet takes on a more important role in shape the rumen fermentation.

Non-protein nitrogen could be hydrolyzed into ammonia by urease produced by microbes [45]. The protein is hydrolyzed into amino acids and peptides by protease, and then parts of amino acids also became ammonia by microbial deaminating [45]. A portion of ammonia synthesis MCP via microorganism, the other parts be absorbed into the blood, participating in the rumen nitrogen cycle [46]. Our results indicated with the high protein diet, unmatured rumen absorption function [47] at M1.5 lead to the high NH_3-N content in rumen. MCP acted as a significantly important role in the ruminants production performance and diet CP utilization efficiency. In our study, low CP and energy levels in diet inhibited rumen synthesis MCP (M9 and M18) [48]. Our results indicated dietary protein level, enzyme activity, and matured rumen function were three critical factors for rumen utilization of protein.

4.2 Rumen bacteria composition

Although the rumen bacteria community has been established in the calf period, the change of rumen bacteria still age-related in 6 to 120 months [44]. The observed OTUs and diversity index were increased

with age; however, the decrease from 23M to 27M indicated the dietary had a more decisive influence on rumen bacteria diversity. The transition of rumen bacteria from 23M to 27M was consistent with the Zhigang et al. [49], which indicated the change from high fiber to low fiber diet decreased the rumen bacteria diversity. Jami et al. indicated rumen bacteria community was affected by age and diet [11]. The genus with a relative abundance > 1% and present in at least 80% of all samples was defined as core bacteria (Slifierz et al., 2015). The core bacteria were established during the calf stage, testified by Figure S3A, S3B, and File. S2. However, under the specific age and diet condition, rumen cultured unique genera to finish the particular rumen function in this stage.

Our result indicated the *Gammaproteobacteria* was rich in 1.5M. It was consistent with Rey et al., which stated the *Gammaproteobacteria* was the dominant bacteria (24% relative abundance) in calf at the age of 15–83 d [50]. *Firmicutes* strongly correlated with fiber digestion and could degrade the complex carbohydrates, like cell surface [51, 52]. The *Firmicutes*, *Clostridia*, *Ruminococcaceae* were rich in M18, digesting the high fiber diet [52, 53]. Huws et al. indicated the *Fibrobacteria* was abundant in the rumen bacteria community under the ryegrass diet, which also plays a vital role in forage degradation [54]. M23 had different fiber correlated bacteria, like *Fibrobacteria*, from M18; it's because the roughage type affected these bacteria [55]. Our results also indicated the rumen bacteria composition was concerned with the nutrient level and the feedstuff species [55, 56]. Diet supplement with nitrate could increase the *Succinivibrio*, which worked efficiently in the nitrogen utilization [57, 58]. Under the high CP diet condition, M27 was rich in *Succinivibrionaceae*, while M1.5 and M6 were not. It was due to the *Succinivibrionaceae* was also age-related [44], which may reach a certain abundance under the specific age and dietary conditions to come into play.

4.3 The relationship within the rumen bacteria, enzyme, and VFA

Rumen was the most important workshop for the digestion of the nutritional substance of ruminants. Bacteria played a crucial role in digest and convert plant materials to VFA and MCP [59]. The enzyme, which was secreted by bacteria, could catalyze feedstuff decomposition and nutrients turnover [5]. The acetate, TVFA, A/P, NH3-N, urease, valerate, and xylanase strongly correlated with rumen bacteria in our study. Bacteria act as a processor to connect the diets and these end products. The genus *Fibrobacter* plays a vital role in cellulolytic and converted the feeds into VFA [54, 60]. *Pseudobutyrivibrio* could degrade the complex plant polysaccharides and produce VFA for ruminants to utilize [61]. *Saccharofermentans* belongs to the *Bacteroidetes* phylum, including 116 genes encoding glycosyl hydrolases involved in hemicellulose, pectin, arabinogalactan, starch, fructan, and chitin degradation [62]. These age or diet-related genera could work as the target bacteria to regulated the rumen function under different feeding backgrounds. The age-related bacteria affected the TVFA and acetate, while the diet-related bacteria affected the A/P and acetate. From the age and diet-related bacteria and their relationship with TVFA and A/P, it can be concluded that the diet could change the rumen fermentation type. In contrast, age influences the rumen fermentation ability.

5. Conclusion

Although the rumen bacteria community has already been established at the calf stage, the rumen bacteria composition still changes along with age and diet variation. Our study gave the quantitative effect of diet and age on the rumen bacteria (explained 32.49% vs. 7.98% bacterial community variation, respectively). Comprehensive correlations were observed between rumen bacterial community, microbiota functions, and rumen fermentation capacities. Our results reveal targeting the bacterial community by diet to regulate rumen fermentation is an efficient way, but dairy cattle's age should also be considered. Besides the diet and age, there are more unknown factors affecting the rumen bacteria community of dairy cows, which need to be further explored.

Abbreviations

NH3-N: ammonium nitrogen; MCP: microbial crude protein; TVFA: total volatile fatty acid; A/P: the ratio of acetate to propionate; ASVs: amplicon sequence variants; PCoA: Principal coordinate analysis; VPA: Variation partitioning approach; CP: crude protein; NDF: neutral detergent fiber; EE: ether extract; FDR: false discovery rate.

Declarations

Conflict of interest

The authors declare no conflict of interest.

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Availability of data and materials

The datasets analyzed are not publically available due to ownership by the funding partners, but are available from the corresponding author on reasonable request.

Author contributions

Yangyi Hao: conceptualization, methodology, investigation, writing - original draft. Yue Gong: writing - review & editing, data curation, methodology. Shoukun Ji: writing - review & editing, data curation. Shuai

Huang: review & editing, resources, methodology, investigation. Wei Wang: review & editing, resources, formal analysis, supervision. Yajing Wang: review & editing, data curation. Hongjian Yang: review & editing, data curation, supervision. Zhijun Cao: conceptualization, review & editing. Shengli Li: project administration, supervision.

Ethics approval

The experimental procedures used in the present study were approved by the Ethical Committee of the College of Animal Science and Technology, China Agriculture University (Protocol number: 2013-5-LZ).

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Figures

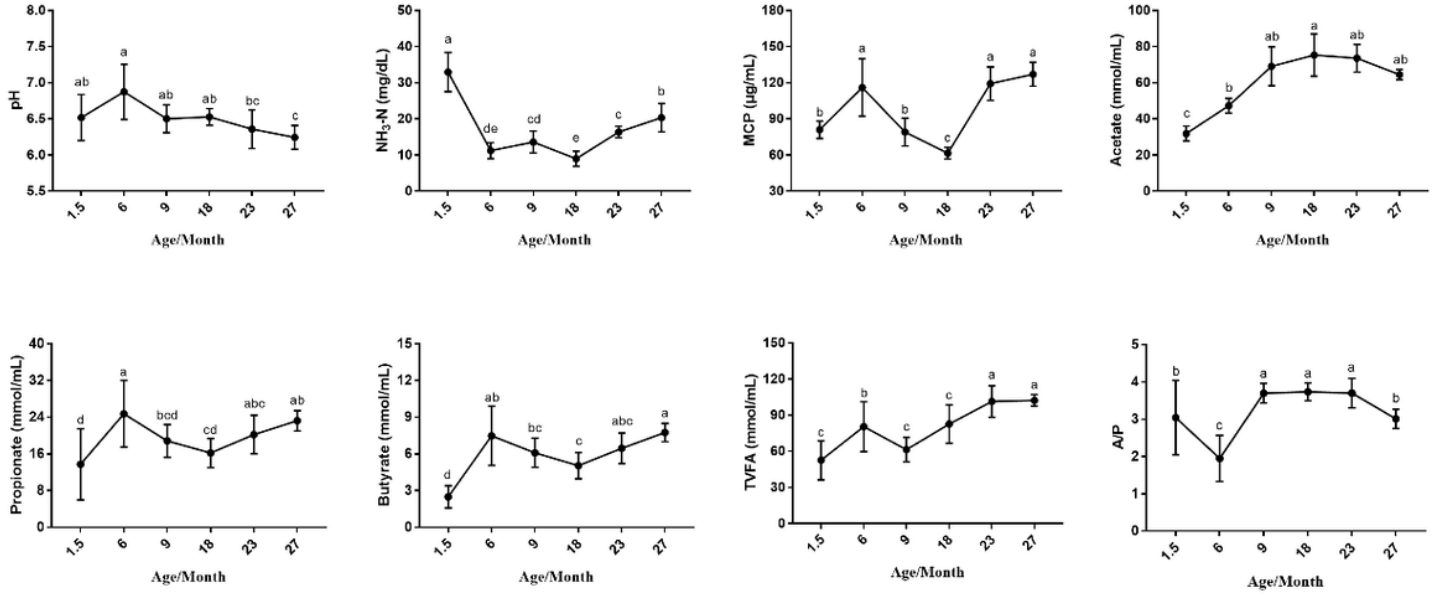

Figure 1

The rumen fluid fermentation profile of dairy cattle NH3-N: ammonium nitrogen; MCP: microbial crude protein; TVFA: total volatile fatty acid; A/P: the ratio of acetate to propionate. The different letters mean that the difference is significant ($P < 0.05$).

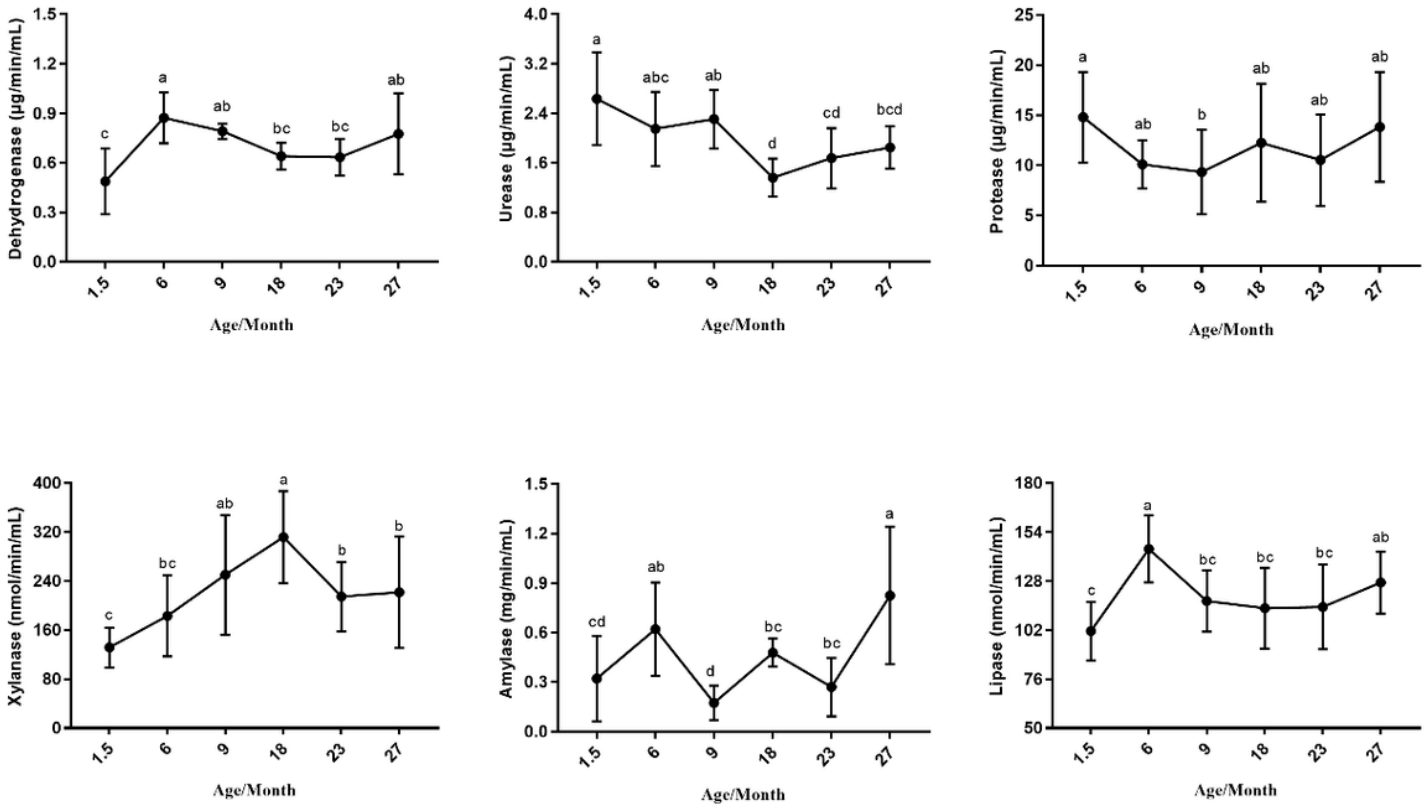

Figure 2

The enzyme activity in rumen fluid of calves and heifers The different letters mean that the difference is significant ($P < 0.05$).

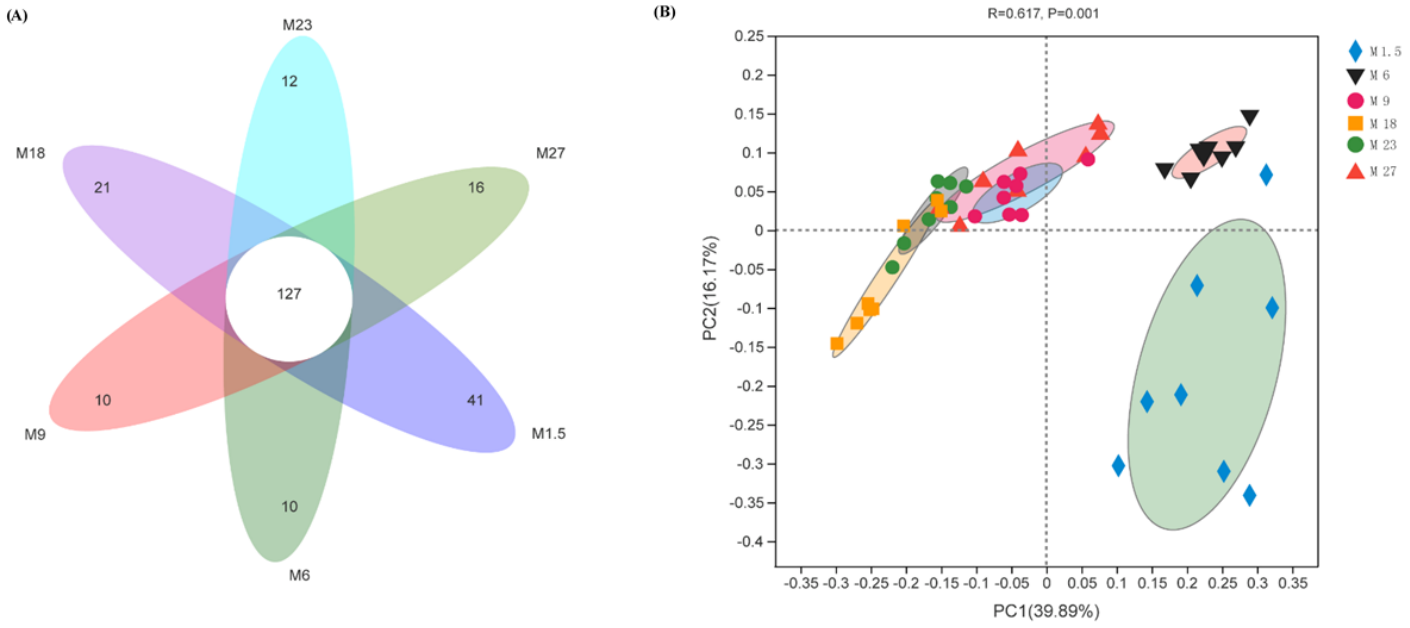

Figure 3

(A) Number of shared and unique bacterial ASVs (amplicon sequence variants) within each age across all stages of calves and heifers. (B) Principal coordinate analysis (PCoA) with Bray-Curtis dissimilarity of the rumen microbial community in different calves and heifers ages at the genus level. 1.5M: 1.5-month-old dairy cattle; 6M: 6-month-old dairy cattle; 9M: 9-month-old dairy cattle; 18M: 18-month-old dairy cattle; 18M: 18-month-old dairy cattle; 23M: 23-month-old dairy cattle; 27M: 27-month-old dairy cattle.

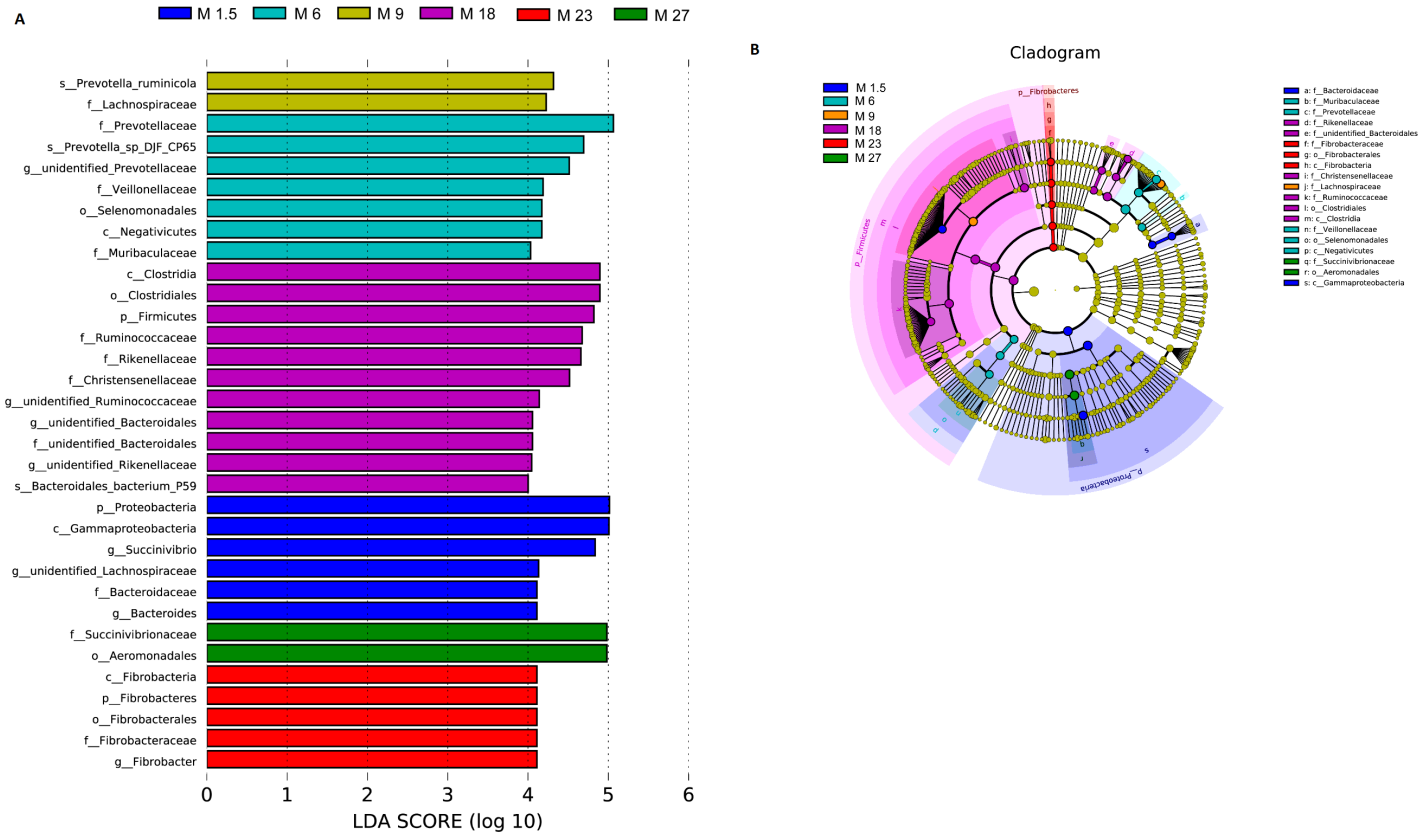

Figure 4

A Histogram of the LDA scores computed for differentially abundant rumen bacteria across the different ages of calves and heifers. Significant differences are defined as $P < 0.05$ and LDA score > 4.0. 1.5M: 1.5-month-old dairy cattle; 6M: 6-month-old dairy cattle; 9M: 9-month-old dairy cattle; 18M: 18-month-old dairy cattle; 18M: 18-month-old dairy cattle; 23M: 23-month-old dairy cattle; 27M: 27-month-old dairy cattle. Figure 4 B The LDA effect size (LEfSe) analysis of bacterial taxa within the different ages of calves and heifers. Cladogram shows significantly enriched bacterial taxa (from phylum to genus level). 1.5M: 1.5-month-old dairy cattle; 6M: 6-month-old dairy cattle; 9M: 9-month-old dairy cattle; 18M: 18-month-old dairy cattle; 18M: 18-month-old dairy cattle; 23M: 23-month-old dairy cattle; 27M: 27-month-old dairy cattle.

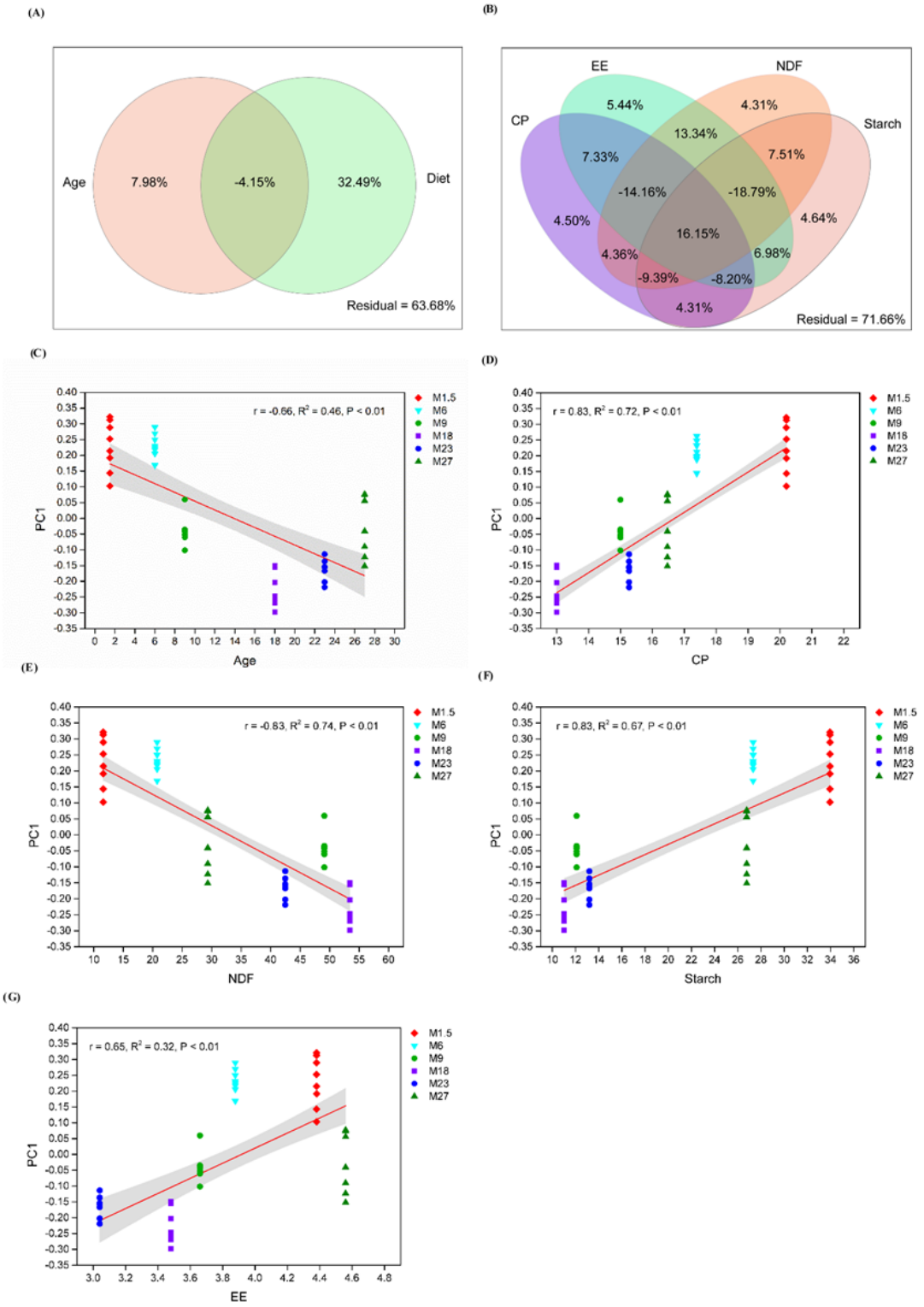

Figure 5

Variation partitioning approach (VPA) analysis the relative importance of (A) diet and age, (B) CP, NDF, starch, and EE on rumen bacteria composition. Scatterplot depicting the correlation of (C) age, (D) CP, (E) NDF, (F) Starch, and (G) EE with the number of PC1. For each sample source, the PC1 values are shown on the y-axis, and the age, CP, NDF, ADF, starch, and EE are on the x-axis. The red line denotes the linear regression line with the gray shading indicating 95% confidence intervals. The Spearman rank correlation

(r), Coefficient of determination (R2), and P values are shown at the top of each panel. PC1: Principal component 1 of the principal coordinate analysis' axis of rumen bacteria. CP: crude protein; NDF: neutral detergent fiber; EE: ether extract.

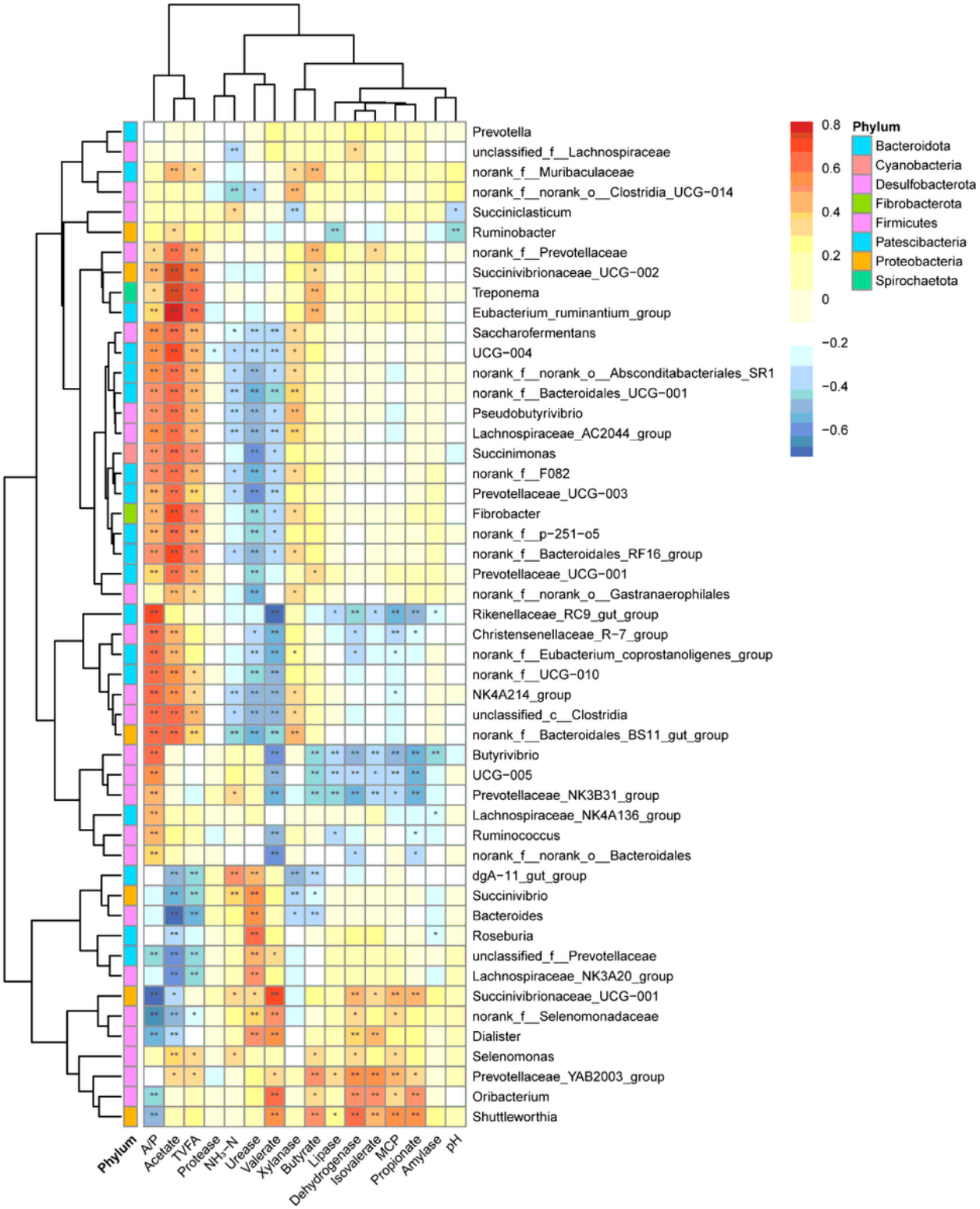

Figure 6

The correlation between bacteria (genus level) and its byproduct. Cells are colored based on Spearman's correlation coefficient: red represents a positive correlation, and blue represents a negative correlation. “*,”

and “**,” indicate FDR (false discovery rate) adjusted P-values <0.05, and <0.01, respectively. NH3-N: ammonium nitrogen; MCP: microbial crude protein; VFA: volatile fatty acid; TVFA: total volatile fatty acid; A/P: the ratio of acetate to propionate.

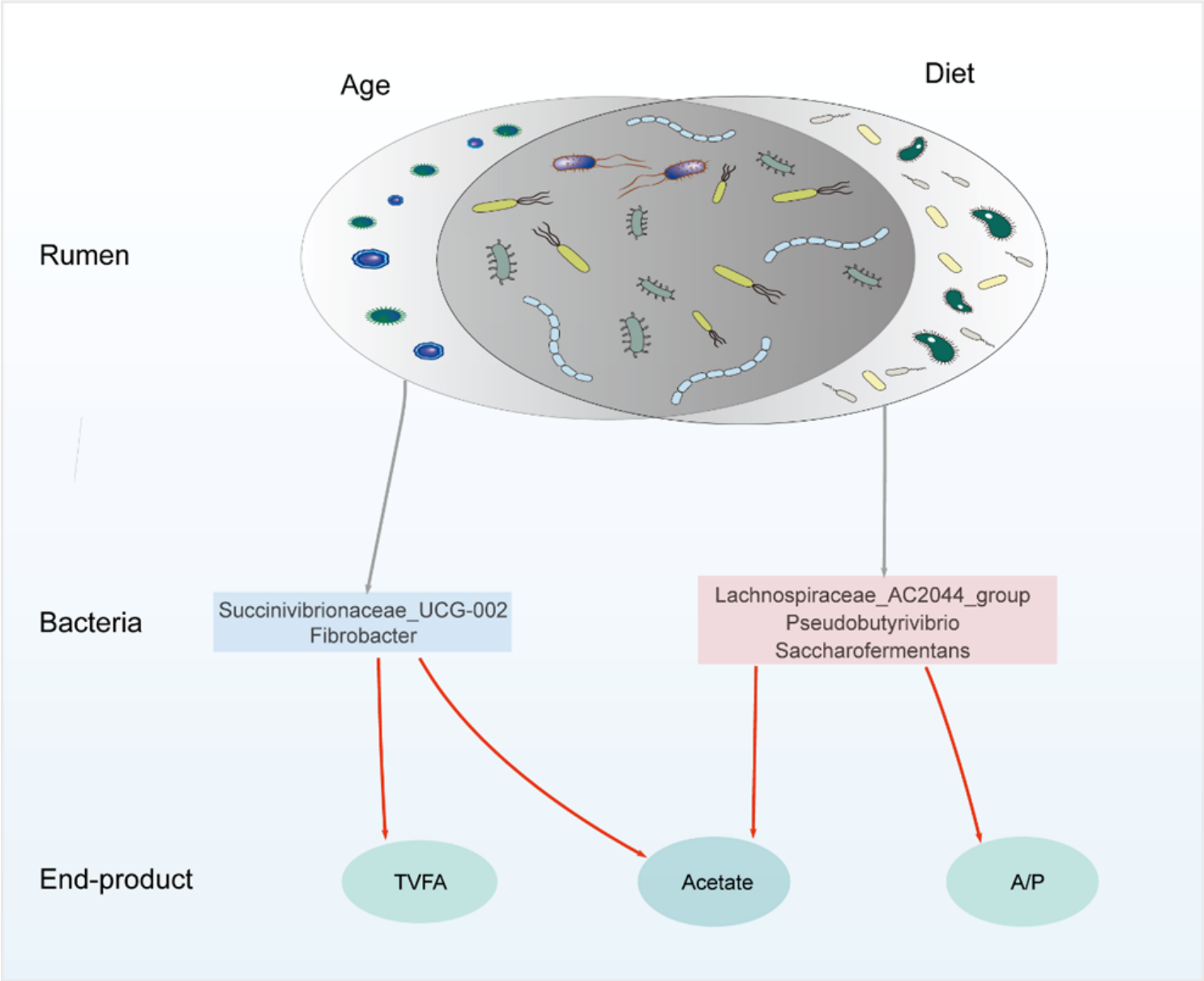

Figure 7

Diagram of the proposed mechanism for age and diet influence on the rumen function. All these bacteria were at the genus level and significantly correlated with TVFA, acetate, and A/P ($r > 0.50$, $P < 0.01$). The Succinivibrionaceae_UCG-002 and Fibrobacter were correlated with age ($r > 0.60$, $P < 0.01$). The Lachnospiraceae_AC2044_group, Pseudobutyrivibrio, and Saccharofermentans has a Spearman's correlation coefficient value > 0.80 with diet NDF and < -0.80 with diet CP and starch ($P < 0.01$). The red line indicated that the bacteria positively correlates with TVFA, acetate, and A/P. Our results give the target bacteria to regulate the rumen function based on different age or diet conditions, promising to provide a theoretical basis for precision feeding of dairy cattle. TVFA: total volatile fatty acid, CP: crude protein, NDF: neutral detergent fiber, A/P: acetate to propionate ratio.

Supplementary Files

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