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Running Title (within 10 words)	ADY on rumen fatty acids and rumen bacterial community diversity
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Abstract

Active dry yeast (ADY) is frequently utilized as a probiotic to promote the growth and health of ruminants. However, it is not clearly established whether ADY influences and engages in the metabolism of fatty acids (FA) through interactions with rumen microbial communities. This study was to evaluate the effects of ADY on rumen FA and rumen bacterial community diversity in finishing bulls. Twenty Yanbian cattle were randomly divided into two groups (10 bulls in each). The control group (CON) received a basal diet, while the treatment group (ADY) received a basal diet supplemented with ADY (Levucell SC, *Saccharomyces cerevisiae* CNCM I-1077, 1.0 g/bull/day, viable count $\geq 8.0 \times 10^9$ CFU/g). After the 100-day finishing trial, rumen fluid samples were collected to analyze rumen fermentation parameters, medium- and long-chain FA composition, and bacterial DNA sequencing. The results demonstrated that ADY noticeably increased the proportions of propionate, C18:1n9c, C18:2n6c, C20:1, and total monounsaturated fatty acids (MUFA) in rumen fluid ($p < 0.05$). ADY supplementation tended to decrease the Simpson ($p = 0.087$) and Shannon ($p = 0.052$) indices. NMDS analysis revealed significant differences in beta diversity between the CON and ADY groups (PERMANOVA: $R^2 = 0.104$, $p = 0.041$). Furthermore, ADY supplementation effectively regulated lactate-utilizing and volatile fatty acid (VFA)-producing bacteria ($p < 0.05$). Correlation analysis demonstrated that VFA-producing bacteria (*Christensenellaceae R-7 group* and *Schwartzia*) were correlated with the proportion of propionate ($p < 0.05$), and the members of the Lachnospiraceae and Ruminococcaceae (*Lachnobacterium*, *Lachnospiraceae AC2044 group*, *Lachnospiraceae UCG-006*, *Ruminococcaceae UCG-002*, *Ruminococcaceae UCG-010*, and *uncultured bacterium Ruminococcaceae*) were noticeably correlated with C18:1n9c, C18:2n6c, C20:0, C20:1, and total MUFA ($p < 0.05$). In conclusion, these findings suggest that ADY supplementation modulates the composition of rumen bacterial communities in finishing bulls, potentially contributing to a more favorable rumen FA profile characterized by increased propionate and MUFA.

Keywords (3 to 6): active dry yeast, finishing bulls, fatty acids, rumen, bacteria

Introduction

Studies indicate that active dry yeast (ADY) enhances growth and production performance in ruminants by increasing feed intake, improving feed digestibility, and bolstering animal immunity [1,2]. As a prevalent probiotic in ruminant feed additives, the functional mechanism of ADY is mainly reflected in maintaining or restoring rumen microbial stability, particularly when high-concentration feeds might induce microecological disorders [1,2].

Currently, the action mechanism of ADY enhances animal growth and production performance including improved dry matter intake, milk quality, and meat quality has been widely discussed, and the changes in fatty acids (FA) and microorganisms in the rumen are at least involved in [2-4]. The rumen is the distinctive digestive organ of ruminants. It hosts a large, complex, and diverse array of microorganisms, among which rumen bacteria are particularly active in lipid metabolism, including fat decomposition, biohydrogenation, and de novo synthesis of FA [5]. The rumen FA mainly includes short-chain (also known as volatile fatty acids, with less than 6 carbon atoms), medium-chain (with 6-12 carbon atoms), and long-chain (with more than 12 carbon atoms) fatty acids, which are the crucial energy sources for ruminants. Volatile fatty acids (VFA) mainly come from the fermentation of carbohydrates by rumen microorganisms. Medium-chain and long-chain fatty acids mainly come from the decomposition of dietary lipids by microbial lipase and microbial synthesis in rumen [6]. It was reported that ADY modifies the type and proportion of VFA by altering cellulolytic and lactate-utilizing bacteria in rumen [7-9]. This is mainly because ADY provides a more favorable environment for cellulolytic bacteria, and the metabolites produced by ADY support the growth and function of lactate-utilizing bacteria [2]. Our prior studies suggest that gastrointestinal medium-chain FA may participate in the regulation of appetite-related hormones (such as Ghrelin), thus increasing dry matter intake (DMI) in finishing bulls fed with ADY [3,4]. Additionally, ADY influences the rumen biohydrogenation processes of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), potentially crucial for altering FA composition in meat and milk [10-12]. However, the extent to which ADY affects and participates in FA metabolism through its interaction with rumen bacterial communities remains to be conclusively established.

To date, studies on the impact of ADY on rumen medium- and long-chain FA are scarce. Therefore, this study aims to establish a basis for investigating the mechanisms by which ADY supplementation enhances rumen FA metabolism. It evaluates the effects of ADY on rumen fermentation parameters, medium- and long-chain FA, and rumen bacterial community diversity in finishing bulls. Additionally, it explores the correlations between changes in rumen bacteria and FA.

Materials and Methods

The trial was conducted from November 2020 to February 2021 at Longjing Mule Animal Husbandry Co., Ltd., Jilin Province, China. ADY (Levucell SC, *Saccharomyces cerevisiae* CNCM I-1077, viable count ≥ 8.0×10^9 CFU/g) was purchased from Lallemand Animal Nutrition Company, France.

Animals, diets, and management

Twenty Yanbian cattle (bulls) weighing 485 ± 38 kg were divided into two groups (control and treatment groups, each consisting of 10 bulls) according to the method of completely randomized design. The control group (CON) was fed a basal diet. The treatment group (ADY) received a basal diet supplemented with ADY. ADY was top-dressed for the treatment diet at the manufacturer's recommended dosage of 1.0 g/bull/day throughout the trial. The trial lasted over 100 days, including 10 days of pre-feeding and 90 days of formal trial. Prior to the trial commencing, all bulls were dewormed and tethered in tie stalls using neck straps. The bulls were provided with total mixed ration (TMR) at 05:00 and 17:00 every day, and fresh water was continuously obtained throughout the trial. The ingredient and nutritional composition of the basal diets are detailed in Table 1.

Sample collection

During the trial period, feed samples were collected regularly by the quartering method, and all samples were evenly mixed. Meanwhile, fecal samples (approximately 200 g) were collected from the rectum after the morning feeding for seven days before the end of the experiment. The feces from each cattle were mixed. The collected feed and fecal samples were dried at 65°C for 72 hours, and then ground to pass through a 1-mm screen for analysis of apparent digestibility. At the end of the trial, following a 12-hour fast, all bulls were transported by truck to a commercial slaughterhouse (Yanji, Jilin Province, China) for slaughter. Rumen fluid sample was collected after slaughter and filtered through four layers of gauze for the analysis of fermentation parameters, medium- and long-chain FA composition, and bacterial DNA sequencing.

Apparent digestibility

The feed and feces samples were analyzed for dry matter (DM), organic matter (OM), ether extract (EE), neutral detergent fiber (NDF), and acid detergent fiber (ADF) according to AOAC methods [13]. The apparent digestibility was calculated by the endogenous indicator method [acid insoluble ash (AIA)] as described by Diao et al. [14].

Rumen fermentation parameters and rumen fatty acids

The pH value of rumen fluid was instantly determined after collection using a rapid pH analyzer (ST3100, Ohaus, NJ, USA). Lactic acid content was measured by a UV-visible spectrophotometer (UV759CRT, Yoke Instrument, Shanghai, China) according to Luan et al. [15]. Additionally, a volume of 1 mL of rumen fluid was mixed with 0.2 mL of 25% (w/v) metaphosphoric acid solution containing 2-ethylbutyrate and centrifuged at 10,000 rpm for 15 minutes for VFA analysis by a gas chromatograph (GC-1120; Sunny Hengping Instrument, Shanghai, China) [15]. The contents of medium- and long-chain FA in the basal diet and rumen fluid were measured by capillary gas chromatography, as described by O'Fallon et al. [16].

Ruminal bacteria DNA extraction and sequencing

The TGuide S96 Magnetic Soil/Stool DNA Kit (Tiangen Biochemical Technology Co., Ltd., Beijing, China) was used to extract total microbial DNA from rumen fluid samples. DNA sequencing was conducted as previously described [17]. DNA purity and concentration were measured using the multi-mode reader. After the quantitative measurement of DNA samples, a total of 16 samples were qualified (eight samples in each group). Full-length 16S rDNA sequencing was amplified using the universal primers: 27F (5'-AGRGTTTGATYNTGGCTCAG-3') and 1492R (5'-TASGGHTACCTTGTTASGACTT-3'). The polymerase chain reaction (PCR) system and cycling parameters refer to the previous methods [17]. PCR amplification products were measured by Qubit4 (Thermo Fisher Scientific, Waltham, United States), then purified, quantified, and homogenized to construct an amplicon sequencing library [18]. The marker genes were sequenced on a PacBio Sequel II platform (Pacific Biosciences, Menlo Park, United States).

Sequence data processing and analysis

The Sequence data processing and analysis of this study was carried out with the support of the BMK Cloud (Biomarker Technologies Co., Ltd., Beijing, China). Briefly, the raw reads generated from sequencing were filtered and demultiplexed to generate circular consensus sequencing (CCS) reads by SMRT LINK (version8.0) (minPasses ≥ 5; minPredictedAccuracy ≥ 0.9). Then, the CCS sequences were assigned to the corresponding samples according to barcodes by LIMA (version 1.7.0). The CUTADAPT v2.7 (error rate 20%) was used to filter and remove CCS readings without primers and beyond the length range (1200bp-1650bp) by identifying forward primers and reverse primers. For obtaining clean reads, the UCHIME algorithm (version 8.1.3) was used to detect and remove chimera sequences [19]. Subsequently, the sequence with similarity ≥ 97.0% was clustered by using USEARCH (v.10.0) to obtain the operational taxonomic units (OTUs), and filtering out OTUs with reabundace < 0.005% [20]. Taxonomy annotation of the OTUs was based on the RDP classifier (version 2.2.4) and using the SILVA database (Release 132) with a confidence threshold of 80% [21]. The abundance information of OTU was normalized by using the sequence number standard corresponding to the sample with the fewest sequences, and the alpha diversity and beta diversity were analyzed according to the normalized output data [18]. The raw data of 16S rDNA sequencing in this manuscript are deposited in the NCBI database (accession number PRJNA949540)

The Venn diagram was displayed with R software v3.1.1 (VennDiagram-v1.6.9) [22]. Species abundance was generated by QIIME2 (v.2020.6) and mapped by PYTHON2 (matplotlib-v1.5.1) [16]. The standard diversity indexes obtained by QIIME2 (v.2020.6) include Chao1, ACE, Shannon and Simpson indexes for alpha diversity analysis [23]. The difference of alpha diversity between the two groups was tested by one-way analysis of variance, and the box plots of alpha diversity index was obtained by using R software v3.1.1 (picante, v1.8.2). Non-Metric Multidimensional Scaling (NMDS) and the Bray-Curtis distance algorithm were employed for the dimension reduction ranking analysis of beta diversity by QIIME v1.8.0 (principal_coordinates.py), and permutational multivariate analysis of variance

(PERMANOVA) was used to test the significant differences in beta diversity between treatment groups [24].

Statistical analysis

For the data of apparent digestibility, rumen fermentation parameters, and rumen medium-chain and long-chain fatty acids, the Shapiro-Wilk test was used to determine whether the data followed normal distribution. For the data with a normal distribution, one-way ANOVA was performed, while for the data with non-normal distribution, the Mann-Whitney test was used to determine the significance of the difference. All the above data was statistically analyzed on SPSS 21.0 (SPSS Inc., Chicago, IL, USA). $p \leq 0.05$ indicates a significant difference, while differences with $0.05 < p \leq 0.1$ are considered trends.

Differential abundance of genus and species were analyzed by Wilcoxon rank sum test using PYTHON2 (scipy v 0.14.1) [25]. Pearson's correlation matrix was calculated for the significantly different rumen bacteria (genus level) and FA in rumen fluid. Correlation heat maC ps were visualized using ORIGIN v9.8.0 (CorrelationPlot) [26]. The correlation coefficient ranges from -1 to +1, representing a spectrum from strong negative to strong positive correlation. Correlations with $p < 0.05$ and $p < 0.01$ are considered significant and extremely significant, respectively.

Results

Apparent digestibility

As shown in Table S1, ADY tended to increase the apparent digestibility of NDF ($p = 0.063$), whereas it did not influence the apparent digestibility of DM, OA, ADF, and EE ($p > 0.05$).

Rumen fermentation parameters

As shown in Table 2, compared with the CON group, the proportion of propionate in the ADY group increased significantly, while the ratio of acetate to propionate decreased significantly (all $p < 0.05$), and the proportion of isobutyrate showed a downward trend ($p = 0.083$). However, ADY did not significantly impact the pH, concentrations of lactic acid, total VFA, or the proportions of other VFA ($p > 0.05$).

Rumen medium- and long-chain fatty acids

As shown in Table 3, twenty-one types of FA were identified in this study. Additionally, ADY significantly increased the proportions of oleic acid (C18:1n9c), linoleic acid (C18:2n6c), eicosenoic acid (C20:1), and total MUFA ($p < 0.05$), tended to increase the proportions of pentadecenoic acid (C15:1) and total FA concentration (both $p = 0.95$), tended to decrease the proportions of SFA (p=0.065), and decreased the proportion of arachidic acid (C20:0) in the rumen fluid ($p < 0.05$). No significant effects were observed on the other FA ($p > 0.05$).

Rumen bacterial diversity

A total of 1,097 OTUs from 16 samples were obtained by performing OTU clustering on nonrepetitive sequences based on 97% similarity. The results from the OTU analysis were used to generate Venn diagrams (Figure 1), which illustrate the quantities of OTUs shared or unique between different groups.

The total numbers of OTUs in the CON and ADY groups was 1,040 and 1,038, respectively, with 981 mutual OTUs between the two groups, representing 89.43% of all OTUs.

The alpha diversity index of the rumen bacterial community is depicted in Figure 2. Although the ACE and Chao1 indices demonstrated no significant differences between the two groups ($p > 0.05$) (Figure 2A, 2B), ADY supplementation tended to lower the Simpson ($p = 0.087$) and Shannon ($p = 0.052$) indices (Figure 2C, 2D). Beta diversity was employed to compare the bacterial communities across the groups using NMDS analysis based on Bray-Curtis distance (Figure 3). NMDS plots indicated that the points representing rumen fluid microbiota in the two treatments were distinctly separated (PERMANOVA: $R^2 = 0.104$, $p = 0.041$).

Rumen bacterial composition

At the phylum level, the relative abundances of Firmicutes (CON vs ADY = 73.89% vs 71.39%) and Bacteroidetes (CON vs ADY = 16.53% vs 19.73%) were the dominant bacteria in the CON and ADY groups (Figure 4A). In addition, the relative abundances of Ruminococcaceae (CON vs ADY = 21.42% vs 21.34%), Lachnospiraceae (CON vs ADY = 19.84% vs 17.69%), Acidaminococcaceae (CON vs ADY = 10.35% vs 15.11%), Christensenellaceae (CON vs ADY = 14.02% vs 6.89%), and Rikenellaceae (CON vs ADY = 7.17% vs 7.29%) were also dominant in the CON and ADY groups at the family level (Figure 4B). The dominant bacterial genera mainly included *Succiniclasticum* (CON vs ADY = 10.34% vs 15.05%), *Christensenellaceae R-7 group* (CON vs ADY = 13.95% vs 6.87%), *Rikenellaceae RC9 gut group* (CON vs ADY = 6.77% vs 7.05%) (Figure 4C). The dominant bacterial species mainly included *uncultured bacterium Christensenellaceae R-7 group* (CON vs ADY = 13.81% vs 6.74%), *uncultured bacterium RikenellaceaeRC9 gut group* (CON vs ADY = 7.85% vs 11.46%), and *uncultured bacterium Succiniclasticum* (CON vs ADY = 6.78% vs 7.03%) (Figure 4D).

Furthermore, rank sum test was used to identify bacteria with significantly different abundance from genus and species level between the two treatments (Figure 5). The relative abundances of *Ruminococcaceae UCG-002*, *FD2005*, *Lachnobacterium*, *Schwartzia*, *Schwartzia succinivorans*, *uncultured_bacterium Ruminococcaceae UCG-002*, *Lachnobacterium bovis*, *uncultured bacterium FD2005*, *Solobacterium sp.*, and *Desulfovibrio sp*. were increased in the ADY group ($p < 0.05$). In contrast, the relative abundances of *Christensenellaceae R-7 group*, *Ruminococcaceae UCG-010*, *Lachnospiraceae AC2044 group*, *Coprococcus 1*, *uncultured bacterium Ruminococcaceae* (genus), *Lachnospiraceae UCG-006*, *uncultured bacterium Christensenellaceae R-7 group*, *uncultured bacterium Ruminococcaceae UCG-010*, *uncultured bacterium Lachnospiraceae AC2044 group*, *uncultured bacterium Coprococcus 1*, *uncultured bacterium Ruminococcaceae* (species), and *uncultured bacterium Lachnospiraceae UCG-006* were decreased in the ADY group (all $p < 0.05$).

Correlation analysis of rumen bacteria

Pearson correlation analysis of significantly different bacterial relative abundance (genus level) and FA proportion in rumen fluid was shown in Figure 6. *Christensenellaceae R-7 group* ($r = -0.57$; $p < 0.05$) was

significantly correlated with C3:0. *Schwartzia* was positively correlated with C3:0 (r = 0.67; $p < 0.01$), C18:2n6c (r = 0.50; $p < 0.05$), and C20:1 (r = 0.46; $p < 0.05$). *Lachnobacterium* was positively correlated with C18:1n9c (r = 0.33; $p < 0.05$) and total MUFA (r = 0.29; $p < 0.05$), whereas *Lachnospiraceae AC2044 group* was negatively correlated with C18:1n9c (r = -0.57; $p < 0.01$), C20:1 (r = -0.69; $p < 0.01$), total MUFA (r = -0.56; $p < 0.01$). Furthermore, *Lachnospiraceae UCG-006* (r = 0.59; $p < 0.05$), *Ruminococcaceae UCG-010* (r = 0.56; $p < 0.05$), and *uncultured bacterium Ruminococcaceae* (r = 0.61; $p < 0.05$) were noticeably correlated with rumen C20:0. *Ruminococcaceae UCG-002* was notably correlated with C18:2n6c (r = 0.64; $p < 0.01$), C20:0 (r = -0.68; $p < 0.05$), and C20:1 (r = 0.64; $p < 0.01$).

Discussion

ADY (*Saccharomyces cerevisiae* CNCM I-1077), a commercially available yeast product, is widely used in ruminant farming. Our companion studies have demonstrated the impact of ADY on growth performance, meat quality, and serum indices of finishing bulls [4,27].

The current research revealed that ADY notably increased the proportion of propionate and decreased the ratio of acetate to propionate, consistent with previous findings [8,9,28]. ADY can increase propionate production in the rumen by stimulating lactate-utilizing and VFA-producing bacteria through its metabolites so that bulls could use energy more effectively [2]. This is because propionate is the main source of glucose supply for ruminants and the key precursor of gluconeogenesis. It will be quickly absorbed by rumen papilla and used as an energy source [29]. Furthermore, ADY supplementation significantly enhanced the proportions of C18:1n9c, C18:2n6c, C20:1, and total MUFA in rumen fluid. ADY can influence biohydrogenation through the modification of rumen microorganisms, thereby increasing the levels of unsaturated fatty acids (UFA) [30-32]. Troegeler et al. [30] found that supplementing 0.5 or 5.0 g/d of live yeasts in dairy cow diets elevated the ratio of UFA in the rumen, including C18:1n9c and C18:2. Similarly, the study by Julien et al. [31] indicated that 5.0 g/d of live yeast supplements enhanced the accumulation of *trans*-11 C18:1 and inhibited the formation of C18:0 in the rumen. The results of these studies are in alignment with our findings. Due to the increased proportion of C18:1n9c, C18:2n6c, C20:1, and total MUFA in the rumen, it means that these beneficial FA for humans may pass through the rumen and deposit more in beef. Additionally, UFA such as C18:1n9c, C18:2n6c, and C20:1 is closely related to the formation of intramuscular fat [33]. In our companion paper, ADY improved the fat deposition and meat quality in Yanbian cattle [27], which may be closely related to ADY's regulation of rumen FA composition.

To further explore the relationship between rumen FA and bacteria with ADY supplementation, we examined the effect of ADY on the bacterial community in rumen samples of finishing bulls using full-length amplicon sequencing of 16S rRNA gene. Analysis of microbial diversity indices from rumen fluid samples in the two groups indicated that ADY tended to decrease the Shannon and Simpson indices. The potential mechanism by which ADY influences microbial diversity may involve the consumption of trace

oxygen attached to ruminal food particles, thereby developing an anaerobic condition and swiftly fermenting substrates, which could cause competitive inhibition with other microbes [34]. Liu et al. [28] observed that adding 4 g/head/day of ADY to a low-concentrate diet resulted in decreased Shannon and Simpson indices, corroborating our findings. Furthermore, Beta diversity is an indicator used to compare species diversity between different communities or ecosystems. It describes the difference in species composition between two communities or ecosystems, mainly considering the quantity and abundance of species. NMDS analysis results demonstrated a notable disparity in colony structure between the CON and ADY groups, and the samples in each group were close, indicating substantial alterations in microbial community structure following the addition of ADY. A previous study of beta diversity of cattle supplemented with 0.8g/ head/day ADY also showed that the points of rumen bacteria in the ADY and blank group were clustered in separate quadrants in the PCA and PCoA plots [35]. The reason for the difference in bacterial flora structure caused by ADY may be that it can eliminate oxygen and provide nutrients, thus forming a rumen environment conducive to cellulolytic and lactate-utilizing bacteria [1,2].

The Wilcoxon rank sum test analysis demonstrated that ADY supplementation effectively modulates the composition of rumen bacteria. For example, ADY was observed to increase the relative abundance of *Ruminococcaceae UCG-002*, while decreasing that of *Ruminococcaceae UCG-010* and *uncultured bacterium Ruminococcaceae* in this study. All these genera are members of the Ruminococcaceae family. Consistent with our results, supplementing the diet with 4 g/head/day of ADY led to an increase in the abundance of *Ruminococcaceae UCG-002* in the rumen of beef cattle [28]. Conversely, a study involving rumen-cannulated cattle demonstrated that 15 g/day of live yeast resulted in an increased abundance of *Ruminococcaceae UCG-010* [36]. These variable results could be attributed to differences in the status of the animals or the dosage of ADY used. Similarly, the ADY group showed an increase in the abundance of *FD2005*, *Lachnobacterium*, while the abundance of *Coprococcus 1*, *Lachnospiraceae UCG-006*, and *Lachnospiraceae AC2044 group* decreased. The genera mentioned are all members of the Lachnospiraceae family, with past studies also demonstrating the great potential of yeast products to modulate members of this family [36-38]. Ruminococcaceae and Lachnospiraceae are core anaerobic bacteria in the gastrointestinal tract, playing crucial roles in the degradation of cellulose and hemicellulose, and converting them into VFA [39-41]. ADY may influence these bacteria by enhancing the binding affinity between anaerobic microorganisms (i.e., cellulolytic bacteria) and feed particles, as well as creating a more conducive environment for these bacteria through deoxygenation [42-43]. Moreover, we found that ADY supplementation tended to enhance the apparent digestibility of NDF (Table S1). In summary, the results of this study illustrate that ADY can regulate the members of the Ruminococcaceae and Lachnospiraceae families in the rumen, thus effectively enhancing fiber degradation in finishing bulls.

Additionally, the relative abundances of *Schwartzia*, *Schwartzia succinivorans*, *Solobacterium sp*., and *Desulfovibrio sp*. were significantly increased with ADY supplementation. *Solobacterium sp*. (e.g.,

Selenomonas ruminantium) and *Desulfovibrio sp*. (e.g., *Desulfovibrio desulfuricans*) are recognized as lactate-utilizing bacteria [2]. ADY is acknowledged for its crucial role in maintaining a healthy rumen environment by stimulating these lactate-utilizing bacteria [34]. *Schwartzia* (*Schwartzia succinivorans*) exists in rumen fluid and specifically ferments succinate quantitatively to propionate [44]. Conversely, the reduced relative abundance of *Christensenellacee R-7 group* was observed with ADY supplementation. *Christensenellacee R-7 group* mainly participates in the metabolism of amino acids, peptides and lipids of the host [45]. This bacterium was relatively abundant in the rumen of growth-retarded yak, mainly producing acetate and butyrate as fermentation end products in the rumen, which would have a negative impact on feed efficiency [45,46]. A previous study indicated that the abundance of *Christensenellaceae R-7 group* was negatively correlated with propionate concentration in the gut of broilers [47]. We also observed that the relative abundances of *Schwartzia*, and *Christensenellacee R-7 group* can be considerably correlated with the proportion of propionate. These findings indicate that ADY could influence the relative abundances of lactate-utilizing and VFA-producing bacteria, thereby potentially improving rumen fermentation in finishing bulls.

ADY has the capability to modify the microbial ecology of the gastrointestinal tract and may impact lipid metabolism [2]. In ruminants, dietary lipids release free FA, glycerol, and small amounts of mono- and diglycerides through the action of microbial lipases upon entry into the rumen [48]. These FA are then bio-hydrogenated by rumen microorganisms. Bacteria in the *Butyrivibrio* group have been identified as the primary agents responsible for biohydrogenation in the rumen over recent decades [49,50]. Additionally, recent studies have indicated that other bacteria, such as certain species from the Lachnospiraceae and Ruminococcaceae families, may also participate in ruminal biohydrogenation [51-53]. Interestingly, our findings suggest that changes in genera from the Lachnospiraceae and Ruminococcaceae families with ADY supplementation are strongly correlated with C18:1n9c, C20:0, C20:1, and total MUFA. Notably, *Lachnobacterium* and *Lachnospiraceae AC2044 group* were noticeably correlated with C18:1n9c and total MUFA. *Lachnobacterium* has been shown to be highly correlated with *trans*-11 C18:1, total biohydrogenation intermediates, and total octadeca-carbon FA in the rumen of lambs [54]. An *in vitro* study revealed that *Lachnospiraceae AC2044 group* might be involved in the rumen biohydrogenation of octadeca-carbon FA [55]. Moreover, *Lachnospiraceae UCG-006*, *Ruminococcaceae UCG-002*, *Ruminococcaceae UCG-010*, and *uncultured bacterium Ruminococcaceae* were found to have significant correlations with C18:2n6c, C20:0 or C20:1 in this study. These results confirm that alterations in genera from the Lachnospiraceae and Ruminococcaceae families are significant in the metabolism of ruminal FA with ADY supplementation, although the exact roles in ruminal biohydrogenation remain to be fully elucidated.

Conclusion

In conclusion, supplementation with ADY at 1.0 g/bull/day can enhance the proportion of propionate, C18:1n9c, C18:2n6c, C20:1, and total MUFA in the rumen fluid of bulls. Additionally, ADY effectively improves rumen fermentation by regulating VFA-producing bacteria (*Schwartzia*, and *Christensenellaceae R-7 group*). Furthermore, members of the Lachnospiraceae and Ruminococcaceae families (*Lachnobacterium*, *Lachnospiraceae AC2044 group*, *Lachnospiraceae UCG-006*, *Ruminococcaceae UCG-002*, *Ruminococcaceae UCG-010*, and *uncultured bacterium Ruminococcaceae*) may play a significant part in the ADY-regulated rumen FA composition. These findings suggest that ADY supplementation modulates the composition of rumen bacterial communities in finishing bulls, potentially contributing to a more favorable rumen FA profile characterized by increased propionate and MUFA.

Acknowledgments

Not applicable.

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Tables and Figures

Table 1. Ingredient and nutritional composition of basal diets (% of DM)

Ingredient composition	Content (% of DM)	Nutritional composition[1]	Content (% of DM)
Corn silage	42.00	Dry matter	86.45
Corn meal	40.00	Crude protein	11.95
Soybean meal	8.00	Ether extract	3.60
DDGS	3.10	Neutral detergent fibers	26.60
Corn germ meal	3.00	Acid detergent fibers	14.61
Bacterial protein feeds	1.00	Calcium	0.39
Sodium bicarbonate	1.00	Phosphorus	0.32
Soybean oil	0.50	Sodium chloride	0.40
Salt	0.40	Fatty acid composition [3]	
Compound premix[2]	1.00	C8:0-C12:0	0.11
Total (%)	100.00	C13:0-C15:1	0.43
		C16:0-C17:1	21.05
		C18:0	3.60
		C18:1n9c	26.52
		C18:2n6c	36.66
		C18:3n3	2.39
		C18:3n6	1.86
		C20:0-C21:0	2.88
		C22:0-C24:0	4.50
		NEg[4] (Mcal/kg DM)	1.24

[1] The value reported for nutritional composition of diets was calculated based on the nutrient analysis from ingredient samples.

[2] Supplied per kilogram of product. Fe: 500 mg; Cu: 1 000 mg; Zn: 2 400 mg; Mn: 1500 mg; I: 10mg; Co: 7 mg; Se: 45 mg; vitamin A: 500 000 IU; vitamin D: 150 000 IU; vitamin E: 400 mg.

[3] Proportion of total fatty acids

[4] NEg (net energy for growth) was estimated from the analyzed value of the dietary ingredients [based on Ministry of Agriculture of P.R. China (2018)]

Table 2. Effects of ADY supplementation on rumen fermentation parameters of finishing bulls

Items	CON[1]	ADY[2]	SEM[3]	p-value
pH	6.17	6.24	0.034	0.195
Lactic acid (mg/100mL)	1.69	1.56	0.071	0.355
Acetate (%)	64.73	62.41	0.823	0.166
Propionate (%)	18.56	22.51	0.878	0.019
Isobutyrate (%)	2.48	1.96	0.249	0.308
Butyrate (%)	9.44	9.00	0.364	0.564
Isovalerate (%)	2.98	2.61	0.154	0.083
Valerate (%)	1.81	1.52	0.099	0.150
Acetate / Propionate	3.53	2.86	0.152	0.020
Total VFA (mmol/L)	47.97	49.35	2.405	0.786

[1] CON, the control group cattle feed control diets. [2] ADY, the treatment group cattle feed control diets containing active dry yeast. [3] SEM, standard error of the means.

516 **Table 3.** Effects of ADY supplementation on the proportion of medium- and long-chain fatty acids in
517 rumen fluid of finishing bulls (%)

Items	CON[1]	ADY[2]	SEM[3]	p-value
Lauric acid, C12:0	0.30	0.31	0.020	0.718
Tridecanoic acid, C13:0	0.09	0.11	0.008	0.150
Myristic acid, C14:0	2.21	2.36	0.149	0.626
Myristoleic acid, C14:1	0.28	0.26	0.015	0.557
Pentadecanoic acid, C15:0	1.94	1.90	0.119	0.878
Pentadecenoic acid, C15:1	1.10	0.88	0.067	0.095
Palmitic acid, C16:0	30.56	31.23	1.020	0.757
Palmitoleic acid, C16:1	0.01	0.02	0.002	0.140
Heptadecanoic acid, C17:0	1.09	0.99	0.058	0.415
Octadecanoic acid, C18:0	17.36	14.74	0.803	0.105
Octadecenoic acid, C18:1n9c	9.86	14.35	1.011	0.020
Linoleic acid, C18:2n6c	5.17	8.46	1.063	0.010
γ-Linolenic acid, C18:3n6	8.41	6.60	0.609	0.161
α-Linolenic acid, C18:3n3	3.89	3.32	0.222	0.279
Arachidic acid, C20:0	0.93	0.75	0.032	0.002
Eicosenoic acid, C20:1	0.28	0.44	0.034	0.009
Henicosanoic acid, C21:0	1.04	0.86	0.062	0.141
Behenic acid, C22:0	0.64	0.54	0.037	0.152
Erucic acid, C22:1n9	0.22	0.30	0.034	0.280
Tricosanoic acid, C23:0	4.38	3.47	0.286	0.114
Lignoceric acid, C24:0	10.24	8.11	0.676	0.161
SFA[4]	70.78	65.38	1.636	0.065
MUFA[5]	11.75	16.24	0.992	0.017
PUFA[6]	17.47	18.38	1.039	1.000
Total (mg/mL)	0.17	0.22	0.014	0.095

518 [1] CON, the control group cattle feed control diets. [2] ADY, the treatment group cattle feed control diets
519 containing active dry yeast. [3] SEM, standard error of the means.[4] SFA, saturated fatty acids = C12:0 +
520 C13:0 +C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0; [5] MUFA,
521 monounsaturated fatty acids = C14:1 + C15:1 + C16:1 +C18:1n9c + C20:1+ C22:1n9; [6] PUFA,
522 polyunsaturated fatty acids = C18:2n6c + C18:3n6 + C18:3n3.

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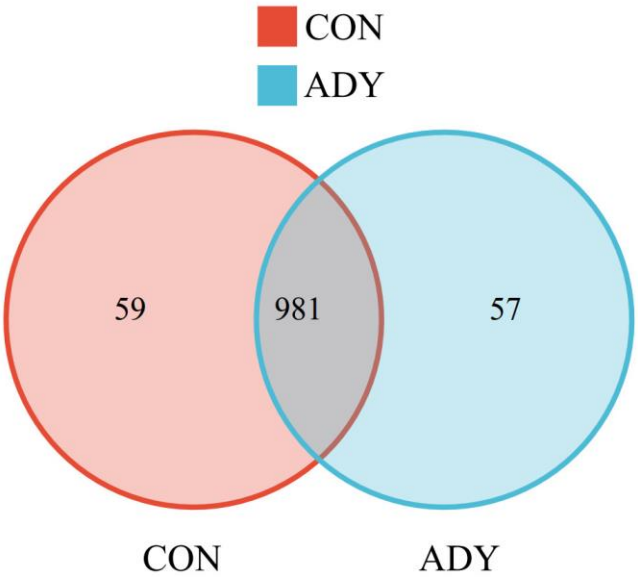

Figure 1. Venn diagram of the number of operational taxonomic units of rumen fluid bacteria in finishing bulls. CON, control group (n = 8); ADY, active dry yeast group (n = 8)

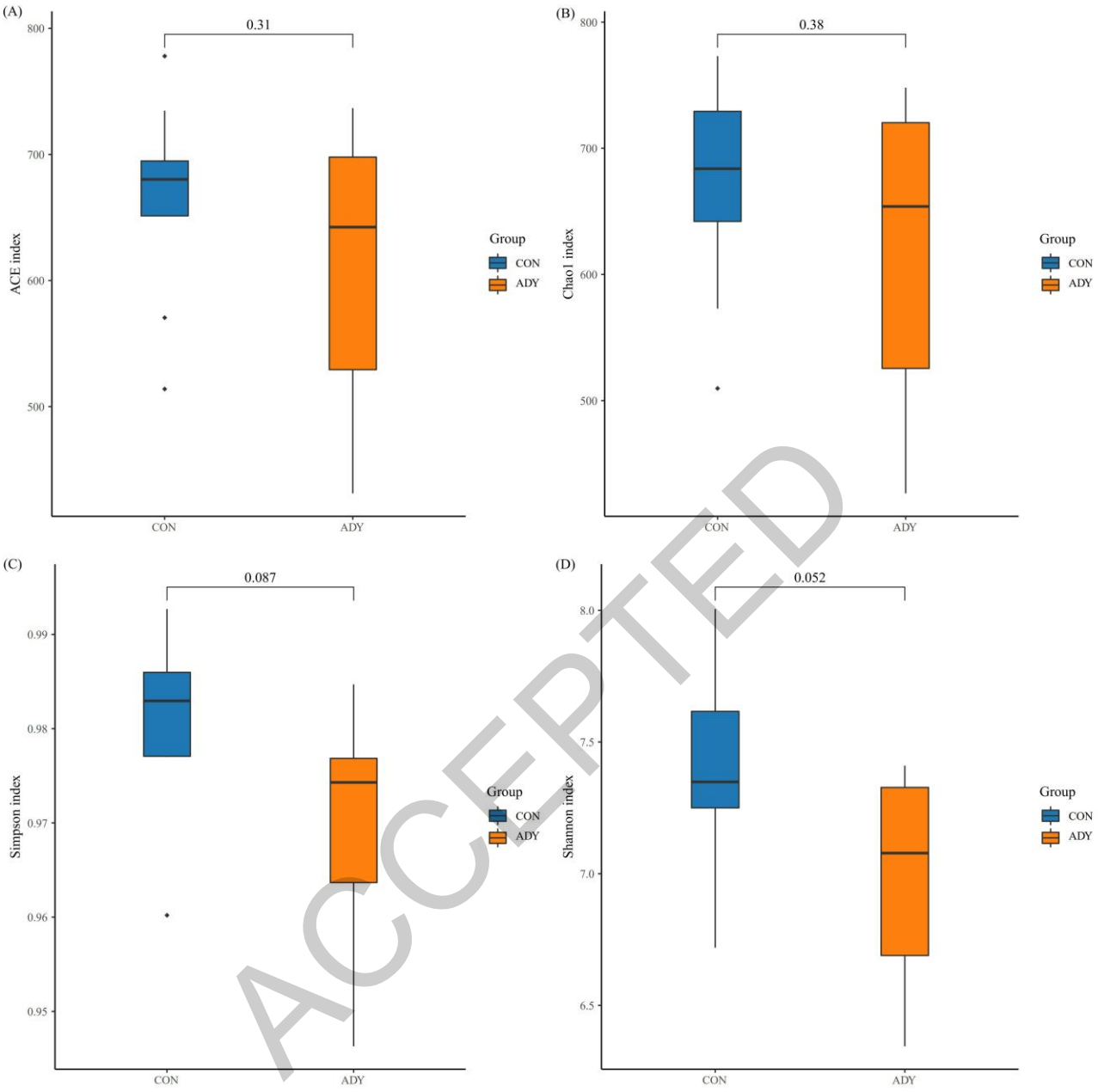

Figure 2. Box plots of alpha diversity indices. (A) ACE, (B) Chao 1, (C) Simpson, and (D) Shannon index values of rumen microbiota of finishing bulls. CON, control group (n=8); ADY, active dry yeast group (n=8).

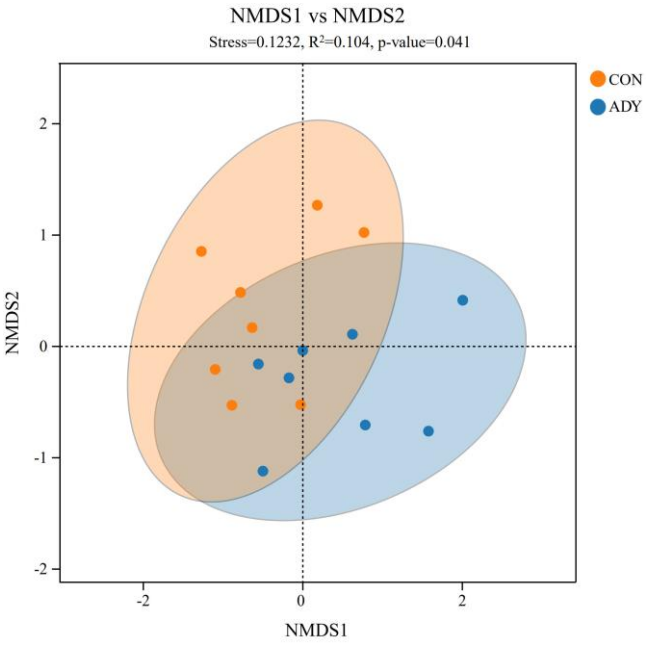

Figure 3. Beta diversity analysis of rumen fluid bacteria through non-metric multidimensional scaling analysis (NMDS). CON, control group (n=8); ADY, active dry yeast group (n=8)

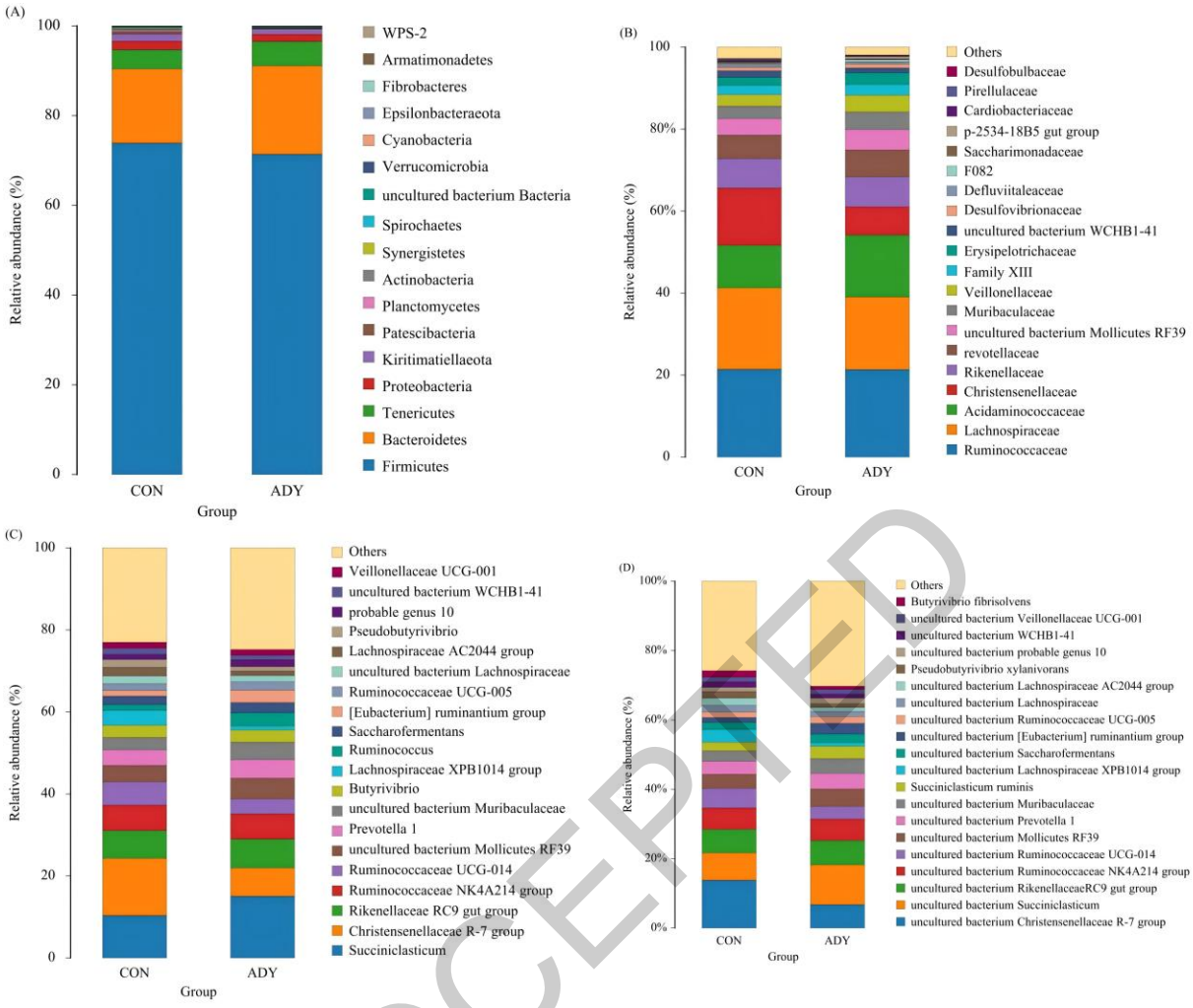

Figure 4. The relative abundance of rumen bacterial community compositions at (A) phylum (B) family, (C) genus, and (D) species levels (top 20). Taxonomy was assigned using the SILVA database version 132. The different colors of the bars represent different species, and the length of the bars represents the proportion of the species. CON, control group (n=8); ADY, active dry yeast group (n=8).

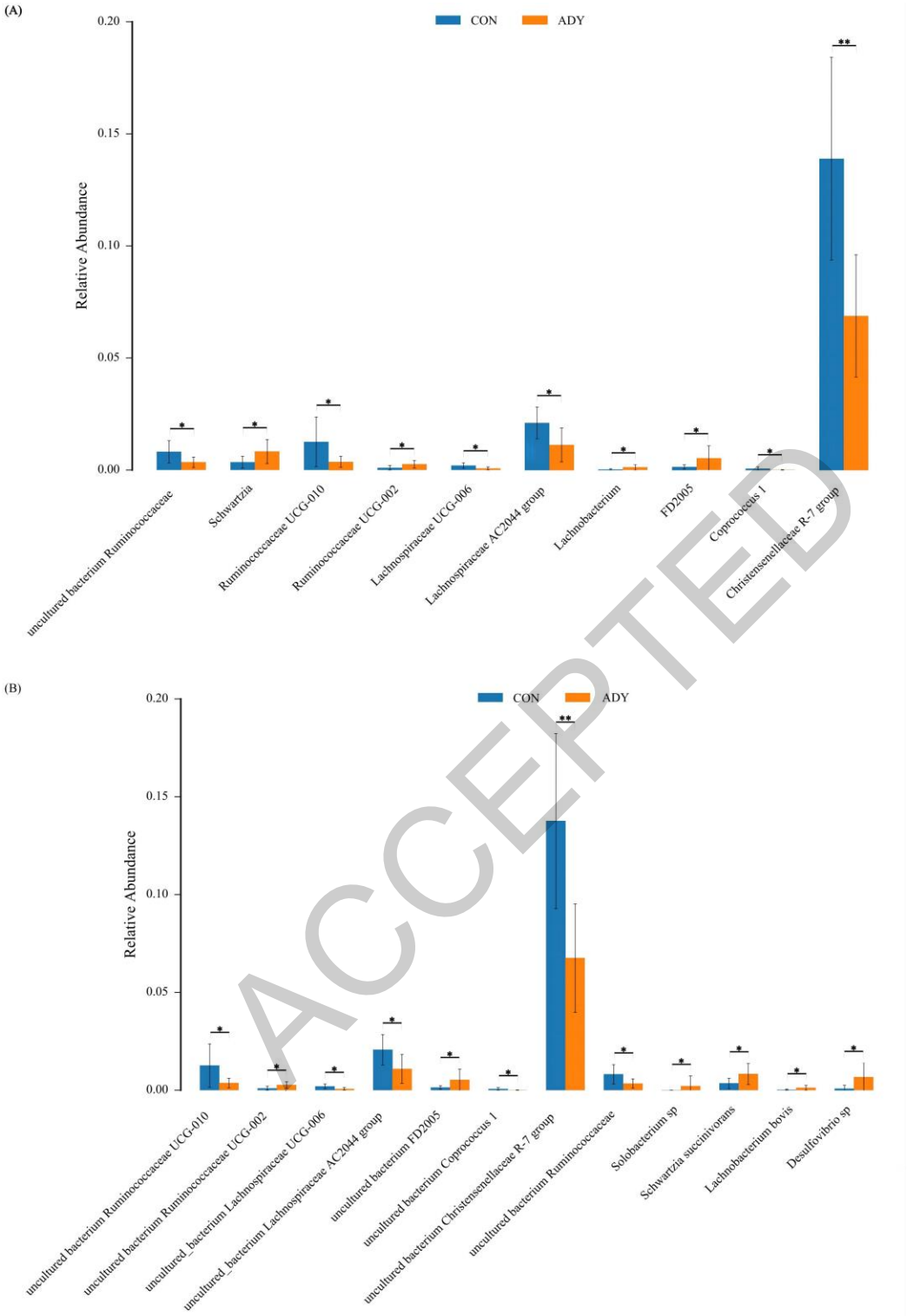

Figure 5. Wilcoxon rank sum test analysis of significantly different rumen bacteria at (A) genus level and (B) species level. CON, control group (n=8); ADY, active dry yeast group (n=8). * $p < 0.05$, ** $p < 0.01$.

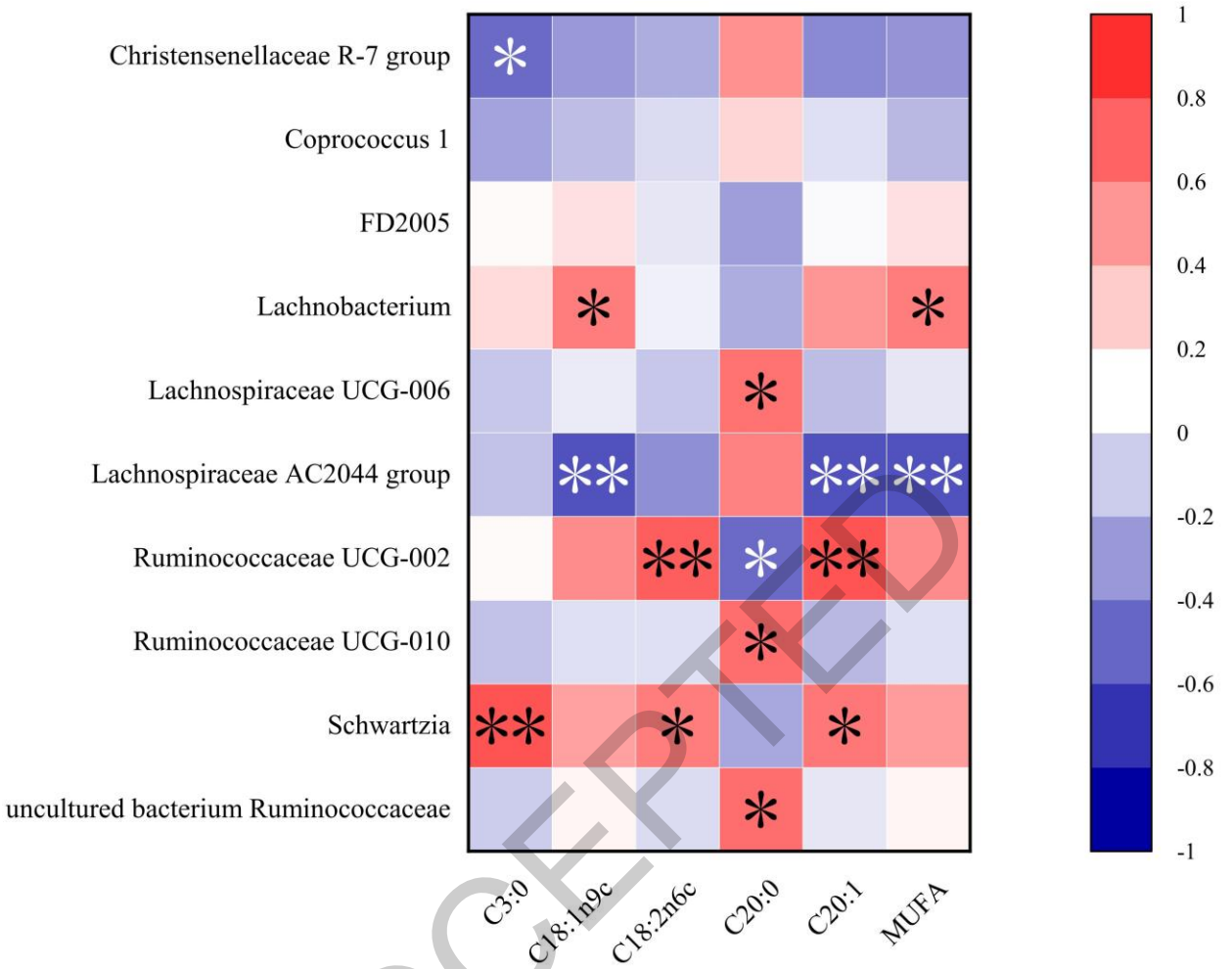

Figure 6. Pearson's rank correlations between significantly differential rumen bacteria (genus level) and fatty acids in rumen fluid of finishing bulls. Pearson's rank correlation coefficient was from –1 to 1. Coefficient (r) > 0 and < 0 represented a positive and negative correlation, respectively. The (r) value denoted the degree of correlation between variables. Only the bacteria with a relative abundance of 0.01%, or higher, in at least one sample were considered. * $p < 0.05$, ** $p < 0.01$.