Current Research in Food Science 9 (2024) 100845

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Effects of yeast cultures on meat quality, flavor composition and rumen microbiota in lambs

Xiongxiong Li [a], Yanchi Wang [a], Jinlong Xu [b], Qitian Yang [a], Yuzhu Sha [a], Ting Jiao [b,**], Shengguo Zhao [a,*]

[a] College of Animal Science and Technology, Gansu Agricultural University, Lanzhou, 730070, China
[b] College of Grassland Science, Key Laboratory of Grassland Ecosystem, Gansu Agricultural University, Lanzhou, 730070, China

ARTICLE INFO

Handling Editor: Siyun Wang

Keywords:
Muscle amino acids
Fatty acids
Flavor substances
Rumen microbiota
Yeast culture

ABSTRACT

Since the banning of antibiotics, the use of feed additives to improve meat quality to satisfy people's pursuit of high quality has become a research hotspot. Yeast culture (YC) is rich in proteins, mannan oligosaccharides, peptides, and yeast cell metabolites, etc., and its use as a feed additive has a positive impact on improving meat quality. So the study aimed to provide a theoretical basis for YC improving mutton flavor and quality by detecting and analyzing the effects of YC on muscle physicochemical properties, amino acids, fatty acids, flavor composition, expression of related genes, and rumen microbiota of lambs. A total of 20 crossbred F1 weaned lambs (Australian white sheep♂ × Hu sheep♀; average 23.38 ± 1.17 kg) were randomly assigned to 2 groups, the control group (CON) and the 1.0% YC supplemented group (YC) (n = 10), and were reared in separate pens. The experiment had a pre-feeding period of 10 d and a treatment period of 60 d. After the experiment, 6 lambs in each group were randomly selected for slaughtering. The results showed that dietary YC supplementation increased rumen total VFA and acetate concentrations ($p < 0.05$), and muscle carcass fat (GR), a* value, intramuscular fat (IMF), lysine (Lys), arginine (Arg), nonessential amino acid (NEAA), oleic acid (C18:1n9c), and eicosanoic acid (C20:1) contents were significantly increased ($p < 0.05$), while cooking loss and γ-linolenic acid (C18: 3n6) were decreased ($p < 0.05$). Furthermore, we found that dietary YC improved the types of flavor compounds, and the key flavor substances such as hexanal, nonanal, styrene, benzaldehyde, p-xylene, and 1-octen-3-ol contents were changed ($p < 0.05$). Additionally, the expression of fat metabolism related genes *PPARγ*, *FASN*, and *FABP4* were increased. Adding 1% YC to lamb diets increased profits by 47.70 CNY per sheep after 60 d of fattening. All of which indicated that YC could improve meat quality, especially flavor, which may be related to the regulation of the relative abundance of rumen microorganisms *Bacteroidota*, *Prevotella_7*, *Succiniclasticum* and *Lachnospiraceae_NK3A20_group*.

1. Introduction

In recent years, with the rapid development of animal husbandry, intensive farming has become the main mode to improve economic benefits. However, intensive feeding practices and excessive concentrate inputs can lead to excessive fat deposition, which seriously affects feed utilization and meat quality, thereby influencing the tenderness, color, and flavor of the meat (Dong et al., 2007; Yang et al., 2022). Therefore, to ensure healthy growth of sheep and improve product quality and safety, researchers have selected bioactive substances such as plant extracts, herbs and probiotics as additives to improve the mutton quality, such as amino acids composition, flavor substances and functional components (Low et al., 2021). Yeast culture (YC), as a safe and environmentally friendly functional microbial preparation, has attracted considerable attention.

YC is a by-product formed by drying under conditions that maintain the activity of fermentation products, following anaerobic fermentation in a specific culture medium. It is primarily rich in proteins, β-glucans, manno-oligosaccharides, peptides, yeast cell metabolites, and digestive enzymes (H. Wang et al., 2023). These components can have beneficial nutritional and health effects on animals (Z. Ma et al., 2023; Newbold and Rode, 2006). Therefore, as a safe and efficient green feed additive,

* Corresponding author.
** Corresponding author.
E-mail addresses: tanxianyi81@163.com (Q. Yang), jiaot@gsau.edu.cn (T. Jiao), zhaosg@gsau.edu.cn (S. Zhao).

https://doi.org/10.1016/j.crfs.2024.100845
Received 20 June 2024; Received in revised form 6 September 2024; Accepted 9 September 2024
Available online 11 September 2024

YC has been widely used in livestock industry (Poppy et al., 2012; Wagner et al., 2016). Recent research results have shown that YC can improve rumen fermentation characteristics (Dias et al., 2018; S. Li et al., 2016), animal immunity function (Broadway et al., 2015; Mahmoud et al., 2020), the degradation rate of nutrients (Titi et al., 2008), and ultimately enhance production performance (Z. Ma et al., 2023; Ogbuewu and Mbajiorgu, 2023). Additionally, YC can positively improves meat quality (Y. Z. Liu et al., 2019; Swyers et al., 2014), which is an important economic property.

Fatty acid and amino acid content and composition are key nutritional factors affecting meat quality. Amino acids serve as flavor enhancers and flavour precursor substances (X. Zhang et al., 2023), and fatty acids significantly influence meat flavor, tenderness, and juiciness (Guo et al., 2020). Flavor is an important indicator for evaluating the quality of mutton, which includes both aroma and taste. Aroma is produced through chemical reactions of flavor precursors, with major aromatic compounds being aldehydes, alcohols, ketones, acids, and esters (Utama et al., 2018). Unsaturated fatty acids (UFAs) have a significant impact on the formation of flavor compounds and meat quality (F. Wang et al., 2024). In addition, host genes are also involved in the regulation of meat and fatty acid metabolism. For example, de novo synthesis (*ACACA*) is a key regulator of fatty acid synthesis and oxidation. Peroxisome proliferator-activated receptors (*PPAR*) is a key regulatory factors that mainly regulated lipids transport and metabolism via PPAR signaling pathway (Guo et al., 2020). Fatty acid-binding proteins(*FABP)* has an affinity for long-chain fatty acids, allowing it to preferentially bind and transport long-chain fatty acids (LCFAs) (McIntosh et al., 2019). Fatty acid synthase (*FASN)* is the gene encoding a fatty acid synthase that controls the ab initio biosynthesis of LCFAs (Buchanan et al., 2016). Studies have found that the interaction between the host and microbiota affects fat deposition in muscle (B. Wang et al., 2021). The content and composition of meat flavour are related to changes in microbiota, especially in ruminants, where the microbiota directly or indirectly influences host metabolism. However, studies on YC regulation of rumen microbes, which further affects muscle quality and flavour in lambs, are relatively rare. Therefore, this study investigated the effects of YC on muscle carcass traits, amino acid and fatty acid profiles, flavor characteristics, and expression of muscle fat metabolism-related genes of lambs, as well as their interaction with the microbiota. The aim was to determine YC impact on rumen microbiota, host fat metabolism genes, fatty acid composition and content, and volatile flavor compounds in lambs. This research provides a basis for evaluating nutritional value, industrial development, and utilization of YC for mutton.

2. Materials and methods

2.1. Yeast culture

YC was provided by Sichuan Runge Biotechnology Co., LTD., and the nutritional active ingredients: nucleotide≥ 1.5%, mannooligosaccharides ≥1.5%, water ≤10.0%, yeast cell count ≥4×10^9 CFU/g.

2.2. Animals, diets, and experimental design

Weaning lambs, from a well-managed and large-scaled farm in Linxia, Gansu Province, China, were approved by the Animal Committee of Gansu Agricultural University (Approval No. GSAU-2nd-AST-2023-053). A total of 20 healthy Australian white sheep♂ × Hu sheep♀-crossbred F1 weaned lambs (average 23.38 ± 1.17 kg) were randomly divided into 2 groups, the control group (CON) and the 1.0% YC supplemented group (YC) (n = 10), and were reared in separate pens, with one replicate per sheep. The YC was added according to the recommended amount of Sichuan Runge Biotechnology Co. and the study of Wang et al. (L. W. Wang, Liu, Y. B., Zhao, J., Wang, B., Te, R., … He, J. F., 2023). The experimental diets were a total mixed pellet diet formulated by the Agricultural Industry Standard Mutton Sheep Feeding Standard of the People's Republic of China (Table 1), and lambs were fed full mixed pellet feed twice daily (08:00 and 18:00), with ad libitum intake and free access to water through fresh tap water. For feed formulation, YC was uniformly mixed into the basal diet at a ratio of 1.0% to prepare a total mixed pellet diet. The experiment had a pre-feeding period of 10 d and a treatment period of 60 d, and after the experiment, 6 lambs were randomly selected for slaughtering in each group. The slaughter procedures were performed according to operating procedures of livestock and poultry slaughtering for sheep and goats (NY/T 3469-2019, Ministry of Agriculture, China).

2.3. Sample collection and processing

After slaughtering, the longissimus dorsi (LD) samples were taken from between the 12th and 13th ribs on the left half carcass. Part of the sample was used to determine muscle pH, cooking loss, and drip loss, etc., while the remaining portion was stored at −20 °C for the determination of muscle amino acids, fatty acids, and volatile flavor compounds. Another portion of the sample was taken in a 2 mL freezing tube and stored at −80 °C for the determination of the expression of the relevant genes. In addition, the rumen fluid was immediately filtered through four layers of sterile gauze into sterile freezing tubes and then placed in a liquid nitrogen tank and brought back to the laboratory for storage at −80 °C for rumen fermentation and 16S rRNA sequencing analysis.

2.4. Meat quality analysis

2.4.1. Analysis of muscle carcass traits and physicochemical indexes

Live weight was measured and recorded 12 h before slaughter. Carcass weight was the weight of the skin, head, hooves, tail and internal organs removed after slaughter. The carcass fat content (GR) by the vernier caliper and the tissue thickness at 11 cm from the midline of the dorsal ridge were determined. Backfat thickness was measured by vernier caliper between the 3rd and 4th ribs at 6–8 cm from the midline (Y. Ma et al., 2023). Measurement of a* (redness), b* (yellowness) and L* (brightness) values of mutton using a colorimeter (CR-10 plus, Konica Minolta, Japan) and the pH using a pH meter (Testo 205, Testo, Germany). Association of Official Analytical Chemists methods (AOAC, 2005) for determination of intramuscular fat content. A thermometer was inserted into the lamb wrapped in a polyethene bag, and the water bath temperature was reached to 70 °C, take out the room temperature and cool to 35 °C, and mutton tenderness was determined by a shear force. Additionally, the lamb samples were steamed in a water bath at 85 °C for 30 min to calculate the cooking loss (Ren et al., 2023). Sampled along the muscle fiber direction, weighed (W1), then suspended in

Table 1
Basic diet formulation.

Items	Diets (%)	Nutrient levels	
Corn	32.0	digestive energy DE/ (MJ/kg)	11.02
Wheat bran	6.0	H_2O/%	11.76
Grass meal	10.0	CP/%	16.43
Wheatgrass	10.0	Ash/%	8.16
Soybean meal (43%)	5.0	salinity	0.96
Cottonseed meal (46%)	5.0	Ca/%	1.23
Bran	8.0		
Corn germ meal	15.0		
Alcohol grains	5.0		
Premix[a]	4.0		

[a] Composition (per kg of dry matter): 100,000–500 000 IU of vitamin A, 50,000–200 000 IU of vitamin D_3, ≥500 IU of vitamin E, Fe 1500–7000 mg, Cu 300–750 mg, Mn 1000–5000 mg, Zn 1500–4000 mg, I 20–30 mg, Se 5–20 mg, Co 8–35 mg.

plastic bottles and placed at 4 °C for 24 h, weighed (W2), and calculated according to the following formula. Drip loss (%) = [(W1−W2)/W1] × 100.

2.4.2. Analysis of meat amino acid composition

An appropriate amount of sample was weighed and placed into a 50 mL hydrolysis tube, add 20 mL of 1 + 1 HCL, and then hydrolyse at 110 °C for 22 h in an electric blast drying oven. After removing and cooling, transfer to a 25 mL colorimetric tube with constant volume. Subsequently, 50 μL of the sample was accurately taken into a 5 mL centrifuge tube, and put into a vacuum drying oven for 2 h at 60 °C, After drying, the sample was filled with water to 1.0 mL and mixed well. The solution was filtered using a 0.45 μm membrane filter into an auto-sampler vial and analyzed by liquid chromatography (Agilent-1260). The column was 4.6 mm*100 mm*2.7 μm, the column temperature: 40 °C.

2.4.3. Analysis of fatty acid composition

According to the method previously reported by our team (Wu et al., 2022), the LD samples were thawed and ground, 1.0 g was weighed into a 10 mL stopper tube, to which 0.7 mL of 10 mol L^{-1} KOH solution and 5.3 mL of anhydrous methanol were added. The test tube was shaken for 5 s every 20 min in a 55 °C water bath for 1.5 h, then cooled to below room temperature with tap water. Following this, 0.58 mL of 12 mol L^{-1} H_2SO_4 solution was added, and the above steps were repeated. After completion, 3 mL of n-hexane was added, mixed well, and transferred to a centrifuge tube, which was then centrifuged at 3000 r•min^{-1} for 5 min. The supernatant was collected and filtered through an organic phase filter membrane into a sample vial, and heat and concentrate 2 mL of the sample at 45 °C to about 1.5 mL. The relative percentage of each fatty acid was determined by gas chromatograph (GC-2010 plus; Shimadzu Company, Japan). The determination was performed on an SPTM-2560 capillary column (100 m × 0.25 mm × 0.2 m). The chromatographic conditions were as follows: detector inlet temperature was 250 °C. Nitrogen was used as the carrier gas with a flow rate of 1.0 mL/min, and the injection volume was 1.0 μL. The relative percentage of each fatty acid was determined by peak area normalization, and the average percentage of each fatty acid was calculated.

2.4.4. Analysis of meat volatile flavor compounds

For the determination of flavor compound content using Gas Chromatography-Mass Spectrometry (GC-MS), 10 g of meat emulsion was weighed and placed into a 10 mL headspace vial. 20% sodium chloride was added, stirred with a glass rod, the bottle mouth was sealed, and the thermostat was placed at a constant temperature of 80 °C for 1 h. The aging solid-phase microextraction (SPME) head was inserted into the headspace vial through the rubber septum of the cap, and the fiber head was pushed to adsorb for 40 min, and the SPME head was adsorbed at 250 °C for desorption, resolved for 5 min, followed by manual spectral analysis (R. Liu, Wang, K , Xu, J, Shi, X, Zhang, H, & Xue, C. , 2018). Chromatographic conditions: the injection port temperature was 250 °C, the carrier gas was He gas, and the initial temperature was 50 °C, keep for 1 min, and raise the temperature to 220 °C at 3.5 °C/min for 20 min. Qualitative analysis: compounds were matched to both the NIST Library (107k compounds) and the Wiley Library (320k compounds, version 6.0) by computer search. Only identification results with a match and purity greater than 800 (maximum 1000) were reported; Quantitative: relative percentages are calculated using the peak area normalization method.

Relative Odor activity values (ROAVs) were obtained according to the method of Wang et al. (Y. Wang et al., 2022) following the formula below:

$$ROAV = 100 \times (Ci/Cmax) \times (Tmax/Ti)$$

where Ci and Ti represent the relative content and odor threshold of target compounds, respectively; Cmax and Tmax denote the relative content and odor threshold of the compounds that contribute most to the flavor, respectively, μg/kg^{-1}. The ROAV of the compound with the greatest flavor contribution is defined as 100. Compounds with ROAV≥1 are considered key volatile aroma compounds of the sample, and compounds with 0.1 < ROAV <1 are considered to have a modifying effect on the flavor of the sample (Bi et al., 2022).

2.4.5. Expression of genes related to fat metabolism

The method of total RNA extraction was referred to Wang et al. (F. Wang et al., 2024). Approximately 100 mg of frozen tissue sample was placed in a mortar (with continuous addition of liquid nitrogen), ground into a fine powder (without visible particles), and then transferred to a centrifuge tube. Then, 1 mL AG RNAex Pro Reagent was added and let stand for 5 min (blow until the lysate is transparent); centrifuged at 12, 000 g for 10 min. After that, the follow-up test operation was carried out according to the kit guide. After the extraction, the concentration and purity of 2 μL RNA samples were detected by ultramicro spectrophotometer (Thermo Nano drop-2000). RNA concentration (ng/μL) = OD260 × dilution factor × 40. Reverse transcription kits (HiScript II Q RT SuperMix for qPCR; The cDNA was synthesized in Nanjing, China. After cDNA synthesis, Primer5.0 software was used to design gene primers (β-actin as internal reference gene), and the primer information is shown in Table 2. The relative quantification of the relevant genes was performed using a real-time fluorescence quantitative PCR instrument. Reaction conditions: pre-denaturation at 95 °C for 30 s; cycling reaction at 95 °C for 10 s and 60 °C for 30 s for 40 cycles; solubilization curves (95 °C for 15 s, 60 °C for 60 s and 95 °C for 15 s). Reaction system: 20 μL system containing 2 × Cham Q Universal SYBR qPCR Master Mix, cDNA template and upstream and downstream primers. β-actin was used as the internal reference gene for correction, and the data were analyzed by the method of $2^{-\Delta\Delta CT}$.

2.5. Rumen fermentation characteristics

The pH value was recorded with a pH meter (P611, Shanghai, China) and a glass electrode immediately after the rumen fluid extraction. The ruminal NH_3-N concentrations were measured following colorimetric methods as described using a 721-type spectrophotometer (TU-1901); Volatile fatty acids (VFAs) were determined by gas chromatography (GC-2010 Plus; Shimadzu, Kyoto, Japan)(X. Li et al., 2022).

2.6. DNA extraction and 16S rRNA sequencing

After the rumen fluid DNA was extracted by bacterial DNA extraction kit (Omega, Shanghai, China), DNA concentration and purity were determined using a NanoDrop 2000 UV–VIS spectrophotometer (Thermo Scientific, Wilmington, USA). Then, the V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified with primer pairs 338F: 5′- ACTCCTACGGGAGGCAGCA-3′ and 806R: 5′- GGACTACHVGGGTWTCTAAT-3′. The PCR amplification products were purified with an Omega DNA purification kit (Omega Inc., Norcross, GA, USA) and paired ends (2 × 250 bp) sequencing was performed at Illumina novaseq600, and raw sequences underwent filtration using an in-house program.

Clean reads were subjected to feature classification to identify amplicon sequence variants (ASVs), and the ASVs conuts less than 2 in all samples were filtered, Then, species annotation was performed by comparing the ASVs to the sequences in the SILVA (Release 138.1) database at a confidence threshold of 70% (Quast et al., 2012) to analyze microbial community structure and species clustering. The alpha analysis of each sample was examined using QIIME2 software, and beta diversity calculations were performed using principal coordinate analysis (PCoA). Bacteria with LDA scores (>2.5) were selected to exhibit the differential abundances between the CON and YC groups, and t-tests in Metastats software were used to compare the species abundance data

Table 2
Primer information.

Items	(5′-3′)	Length/bp	Tm/°C	Gene Sequence No.
β-actin	F:AGCCTTCCTTCCTGGGCATGGA R:GGACAGCACCGTGTTGGCGTAGA	113	60 °C	NM_001009784.3
PPARγ	F: CTTGCTGTGGGGATGTCTCA R: TTCAGTTGGTCGATGTCGCT	104	60 °C	NM_001100921.1
FASN	F: AAGGCAGTCTGATCGTGAGC R: GCTCCTTGTACACGTCACCA	147	60 °C	XM_027974304.2
FABP4	F:TCCTTCAAATTGGGCCAGGAAT R: GGTAGCAGTGACACCGTTCAT	189	60 °C	NM_001114667.1
LPL	F: CCTGGAGTGACGGAATCTGTG R: CCACGATGACGTTGGAGTCT	160	60 °C	NM_001009394.1
ACACA	F: TATCTTATGACTAGAGCCTCCGT R: GTCTGAGCAGATATCCACTTCCA	177	60 °C	NM_001009256.1

between groups, which screened the different bacterial genera between the two groups.

2.7. Statistical analyses

The independent sample T-test in IBM SPSS Statistics 22 software was used to statistically analyze. Spearman correlation test was used to calculate the correlation between muscle amino acids, fatty acids, genes related to fat metabolism, and key flavor substances, the same method was used for the correlation between key flavor substances and rumen microbes (Top10). The range of correlation coefficient (r) was from −1 to 1. r > 0 and <0 represented positive correlation and negative correlation, respectively. The |r| value denoted the correlation degree among variables. In particular, r = −1, 0 and 1 meant a completely negative correlation, uncorrelated and a completely positive correlation, respectively.

3. Results

3.1. Carcass and muscle physical traits

As shown in Table 3, the body weight before slaughter, carcass weight, carcass fat content (GR), a* values and intramuscular fat (IMF) contents were significantly higher ($p < 0.05$) and cooking loss was significantly lower ($p < 0.05$) in the YC group than those in the CON group.

3.2. Volatile flavor precursors of muscle

3.2.1. Amino acid composition

Compared to the CON, dietary YC supplementation significantly increased NEAA content in LD muscle ($p = 0.017$), and the TAA and EAA contents tended to increase ($0.05 < p < 0.10$), the contents of lysine (Lys) and arginine (Arg) were increased by 7.98% and 13.61%, respectively ($p < 0.05$) (Table 4).

Table 3
Analysis of carcass and meat physical traits.

Items		CON	YC	*P* value
Body weight before slaughter, kg		36.68 ± 0.78^{b}	41.35 ± 0.49^{a}	0.002
Carcass weight, kg		18.96 ± 0.69^{b}	21.32 ± 0.12^{a}	0.015
Dressing percentage, %		51.66 ± 0.82	51.58 ± 0.47	0.451
carcass fat content (GR), mm		10.49 ± 0.29^{b}	11.58 ± 0.03^{a}	0.031
Back fat thickness, mm		4.39 ± 0.57	4.74 ± 0.28	0.616
pH value	45 min	6.49 ± 0.04	6.54 ± 0.09	0.581
	24 h	6.52 ± 0.06	6.61 ± 0.08	0.409
Meat color $_{45\ min}$	L*	29.04 ± 0.55	27.74 ± 0.29	0.103
	a*	17.08 ± 0.21^{b}	18.70 ± 0.47^{a}	0.035
	b*	6.85 ± 0.34	7.33 ± 0.34	0.354
Intramuscular fat (IMF), %		2.66 ± 0.09^{b}	4.64 ± 0.48^{a}	0.015
Shear force, N		57.65 ± 2.07	54.29 ± 3.13	0.173
Drip loss, %		4.94 ± 0.27	4.83 ± 0.27	0.509
Cooking loss, %		22.92 ± 0.90^{a}	19.05 ± 1.06^{b}	0.049

Values are expressed as means ± SEM (n = 6); CON = control group; YC = yeast culture group. ^{a,b}The values within a row with different superscripts are significantly different ($p < 0.05$).

3.2.2. Fatty acids composition

A total of 36 kinds of fatty acids were detected, including 16 kinds of saturated fatty acids (SFAs), 9 kinds of monounsaturated fatty acids (MUFAs), and 11 polyunsaturated fatty acids (PUFAs) (Table 5). Dietary YC supplementation decreased the contents of octanoic acid (C8:0), heptadecanoic acid (C17:0), arachidic acid (C20:0), henicosanoic acid (C21:0), tricosanoic acid (C23:0) and lignoceric acid (C24:0) in SFAs ($p < 0.05$), and increased the contents of oleic acid (C18:1n9c) and eicosenic acid (C20:1) in MUFAs ($p < 0.05$), but decreased the content of gamma linolenic acid (C18:3n6) in PUFAs ($p < 0.05$). Compared to the CON, the MUFAs content in the YC group was increased by 18.75% ($p = 0.006$).

3.3. Volatile compound composition

The CON and YC groups were detected 35 and 41 volatile compounds, respectively, including aldehydes, alcohols, ketones,

Table 4
Effects of dietary YC supplementation on muscle amino acids (mg/kg).

Items	CON	YC	*P* value
Essential amino acids (EAAs)			
Isoleucine (Ile)	0.95 ± 0.02	0.94 ± 0.03	0.716
Valine (Val)	1.02 ± 0.01	1.02 ± 0.01	0.907
Methionine (Met)	0.32 ± 0.01	0.34 ± 0.00	0.196
Leucine (Leu)	1.55 ± 0.04	1.55 ± 0.03	0.963
Phenylalanine (Phe)	0.79 ± 0.02	0.77 ± 0.02	0.565
Lysine (Lys)	1.63 ± 0.01^{b}	1.76 ± 0.02^{a}	0.014
Threonine (Thr)	0.82 ± 0.02	0.86 ± 0.02	0.464
EAAs	6.97 ± 0.05	7.31 ± 0.12	0.055
Nonessential amino acids (NEAAs)			
Cysteine (Cys)	0.01 ± 0.00	0.02 ± 0.00	0.158
Histidine (His)	0.56 ± 0.03	0.59 ± 0.01	0.426
Arginine (Arg)	1.47 ± 0.00^{b}	1.67 ± 0.02^{a}	0.001
Tyrosine (Tyr)	0.55 ± 0.00	0.59 ± 0.02	0.153
Serine (Ser)	0.71 ± 0.00	0.73 ± 0.02	0.387
Proline (Pro)	0.39 ± 0.03	0.49 ± 0.05	0.161
Glycine (Gly)	1.10 ± 0.06	1.06 ± 0.02	0.477
Alanine (Ala)	1.10 ± 0.01	1.11 ± 0.03	0.752
Aspartate (Asp)	1.68 ± 0.00	1.76 ± 0.02	0.079
Glutamic acid (Glu)	2.64 ± 0.06	2.75 ± 0.07	0.305
NEAAs	10.21 ± 0.08^{b}	10.91 ± 0.16^{a}	0.017
Total amino acid (TAA)	17.18 ± 0.06	18.22 ± 0.28	0.059
Flavor amino acid (DAA)	7.47 ± 0.07	7.71 ± 0.12	0.153
Sweet amino acid (SAA)	5.72 ± 0.06	6.04 ± 0.15	0.111

Values are expressed as means ± SEM (n = 6); CON = control group; YC = yeast culture group; DAA = Asp + Glu + Gly + Ala + Ile; SAA = Gly + Ala + Ser + Pro + Lys + Thr. ^{a,b}The values within a row with different superscripts are significantly different ($p < 0.05$).

Table 5
Effects of dietary YC supplementation on muscle fatty acids (%).

Items	CON	YC	*P* value
Saturated fatty acid (SFA)			
Butyric acid, C4:0	0.70 ± 0.06	0.51 ± 0.08	0.140
Caproic acid, C6:0	0.12 ± 0.01	0.08 ± 0.01	0.056
Caprylic acid, C8:0	0.13 ± 0.01^{a}	0.07 ± 0.01^{b}	0.017
Capric acid, C10:0	0.11 ± 0.01^{b}	0.16 ± 0.00^{a}	0.032
Undecanoic acid, C11:0	0.26 ± 0.03	0.20 ± 0.03	0.282
Lauric acid, C12:0	0.13 ± 0.05	0.16 ± 0.02	0.548
Myristic acid, C14:0	1.89 ± 0.53	2.39 ± 0.17	0.415
Pentadecanoic acid, C15:0	0.22 ± 0.02	0.34 ± 0.03	0.202
Palmitic acid, C16:0	20.33 ± 1.50	23.79 ± 0.33	0.141
Margaric acid, C17:0	1.64 ± 0.14^{a}	1.09 ± 0.11^{b}	0.035
Stearic acid, C18:0	13.39 ± 1.29	12.13 ± 0.40	0.403
Arachidic acid, C20:0	0.09 ± 0.01^{a}	0.06 ± 0.00^{b}	0.014
Heneicosanoic acid, C21:0	0.17 ± 0.05^{a}	0.08 ± 0.01^{b}	0.144
Behenic acid, C22:0	0.10 ± 0.00	0.09 ± 0.01	0.333
Tricosanoic acid, C23:0	5.17 ± 0.60^{a}	2.91 ± 0.41^{b}	0.036
Lignoceric acid, C24:0	0.06 ± 0.01^{a}	0.04 ± 0.00^{b}	0.016
Monounsaturated fatty acid (MUFA)			
Myristoleic acid, C14:1	0.09 ± 0.01	0.13 ± 0.03	0.210
Palmitic acid, C15:1	0.19 ± 0.07	0.12 ± 0.02	0.375
Palmitoleic acid, C16:1	1.29 ± 0.20	1.52 ± 0.04	0.360
Margaroleic acid, C17:1	0.50 ± 0.04	0.49 ± 0.01	0.858
Trans-9-Elaidic acid, C18:1n9t	2.88 ± 0.42	4.16 ± 0.51	0.125
Cis-9-Elaidic acid, C18:1n9c	32.25 ± 0.69^{b}	38.33 ± 1.22^{a}	0.012
Cis-11-Eicosenoate acid, C20:1	0.10 ± 0.00^{b}	0.11 ± 0.00^{a}	0.049
Erucic acid, C22:1n9	0.37 ± 0.05	0.32 ± 0.05	0.531
Nervonic acid, C24:1	0.79 ± 0.07^{a}	0.42 ± 0.03^{b}	0.012
Polyunsaturated fatty acid (PUFA)			
Trans-Linolelaidic acid, C18:2n6t	0.33 ± 0.06	0.19 ± 0.02	0.065
Cis-Linoleate acid, C18:2n6c	12.95 ± 1.43	9.90 ± 1.22	0.181
γ-linolenic acid, C18:3n6	0.12 ± 0.01^{a}	0.08 ± 0.00^{b}	0.027
Arachidonic acid, C20:4n6	0.08 ± 0.00	0.07 ± 0.01	0.229
Docosahexaenoic acid, C22:6n3	0.33 ± 0.04	0.25 ± 0.04	0.204
α-Linolenic acid, C18:3n3	0.32 ± 0.06	0.23 ± 0.01	0.246
Eicosadienoate acid, C20:2	0.23 ± 0.02	0.22 ± 0.01	0.869
Eicosatrienoate acid, C20:3n6	0.43 ± 0.00	0.33 ± 0.04	0.107
Eicosatrienoic acid, C20:3n3	0.05 ± 0.00	0.05 ± 0.01	0.670
Docosadienoic acid, C22:2	0.29 ± 0.03	0.21 ± 0.03	0.198
Eicosapentaenoic acid, C20:5n3	0.08 ± 0.01	0.06 ± 0.01	0.276
Saturated fatty acid (SFA)	44.49 ± 1.15	44.08 ± 0.92	0.794
Unsaturated fatty acid (UFA)	53.60 ± 1.19	57.26 ± 2.33	0.234
Monounsaturated fatty acid (MUFA)	38.45 ± 0.36^{b}	45.66 ± 1.28^{a}	0.006
Polyunsaturated fatty acid (PUFA)	15.15 ± 1.55	11.60 ± 1.38	0.163

Values are expressed as means ± SEM (n = 6); CON = control group; YC = yeast culture group. ^{a,b}The values within a row with different superscripts are significantly different ($p < 0.05$).

hydrocarbons, esters, and heterocycles. The YC group detected 6 more volatile compounds than the CON group (Fig. 1.). Compared to the CON, dietary YC significantly increased the contents of 2-octenal, (E), decane, 2-methyl-, nonane, 4,5-dimethyl- ($p < 0.05$) and significantly decreased the contents of benzaldehyde, benzaldehyde, 3-ethyl-, 1-dodecen-3-ol, 1-octen-3-ol, cyclooctyl alcohol, styrene, cyclodecane and 5-hepten-2-one, 6-methyl- ($p < 0.05$) (Table S1).

To further evaluate the aroma contribution of volatile compounds, ROAV was determined (Table 6). In both the CON and YC groups, nonanal had the highest odor contribution, with a ROAV value of 100. ROAV analysis was performed on other compounds, and compounds with ROAV>0.1 were retained. A total of 22 and 24 key flavor substances were identified in the CON and YC groups, respectively. Among them, nonanal had the highest ROAV in the YC group, followed by hexanal, heptanal, 2,4-decadienal, (E, E)-, octanal, 2-decenal, (E)-, 2-undecenal, benzene, 1,3-dimethyl- and furan, 2-pentyl-2-, all with ROAV>1. The ROAV values of 2-octenal, (E)-, octanal, and benzene, and 1,3-dimethyl- were higher in the YC group than those in the CON group.

3.4. Expression characteristics of genes related to fat metabolism

As shown in Fig. 2., dietary YC supplementation significantly increased the expression of *PPARγ*, *FASN*, *FABP4*, and *ACACA* genes in LD muscle ($p < 0.01$), but had no significant effect on *LPL* gene expression ($p > 0.05$).

3.5. Rumen fermentation characteristics

As shown in Table 7, the total VFA and acetate concentrations in the YC group were significantly higher than those in the CON group ($p < 0.05$), but there was no significant effect on pH and NH_3-N ($p > 0.05$).

3.6. Rumen bacterial microbiota

A total of 956,099 reads were obtained and 817,890 clean reads were obtained after quality control and splicing, yielding an average of 68,158 clean reads per sample. Based on the weighted_unifrac distance algorithm, Principal coordinates analysis (PCoA) was used to show the separation of bacterial communities in the two groups. We can observe that the two eigenvalues PC1 and PC2, which lead to the largest differences between samples, which are 13.00% and 11.62%, respectively, with R2 = 0.104, $p = 0.057$, which indicates that the two communities was basically separated (Fig. 3A), but YC supplementation had no significant effects on rumen bacterial diversity (Shannon index and Simpson index) and abundance (ACE index and Chao index) ($p > 0.05$) (Table S2). The ASV dilution curves demonstrated that the sequencing depth obtained in this study was sufficient to characterize the bacterial microbiota of the samples (Fig. 3B). In addition, the Venn diagram showed that the two groups shared 695 OTUs, while the YC and CON groups had 3127 and 2502 exclusive OTUs, respectively (Fig. 3C). At the taxonomic level, a total of 29 phyla and 471 genera were detected in the rumen microbiota. At the phylum level, *Bacteroidetes, Firmicutes* and *Actinobacteria* were the dominant bacteria (Fig. 3D), we carried out metastats analysis on bacteria with relative abundance >0.1% found that the relative abundance of *Bacteroidota* ($p = 0.04$) was significantly

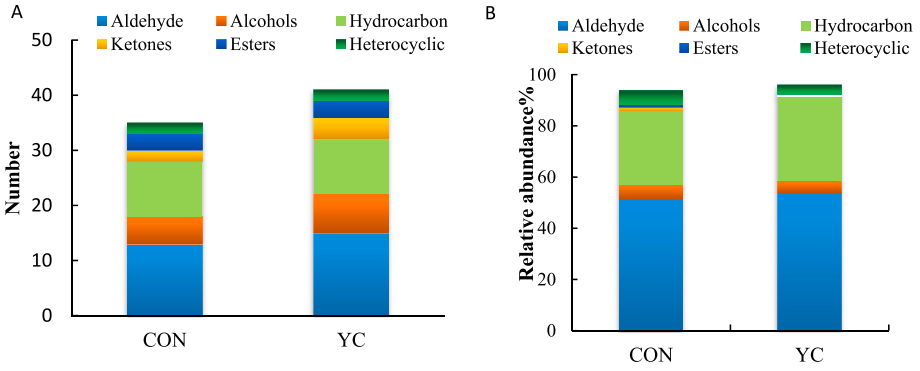

Fig. 1. Numbers (A) and relative contents (B) of volatile flavor compounds in two groups.

Table 6
ROAV of volatile flavor compounds in two groups.

Compound	CAS	Odor Threshold (ug/kg) [a]	ROAV		Odor Description (Y. Wang et al., 2023)
			CON	YC	
Aldehyde					
Hexanal	66-25-1	5	29.31	28.71	Grassy, green, fresh, tallow, fat
Heptanal	111-71-7	2.8	11.60	22.15	Grassy, green, fat,
Nonanal	124-19-6	1.1	100.00	100.00	Floral, aldehyde-like, citrus, soapy, fried fragrant, roasted fragrant
Benzaldehyde	100-52-7	350	0.16	0.10	Almond, burnt sugar, sweet
Decanal	112-31-2	3	0.88	0.66	Fresh grease, fruity, soapy
2,4-Decadienal, (E, E)-	25152-84-5	0.027	51.34	49.09	
2-Octenal, (E)-	2548-87-0	4	0.36	2.34	Meaty, nutty, grease, cucumber flavor, umami
Octanal	124-13-0	3.4	9.52	22.47	Grease, citrus, soapy
Dodecanal	112-54-9	10	0.06	0.05	
Benzeneacetaldehyde	122-78-1	2	0.24	0.28	Sweet, intense floral
2-Decenal, (E)-	3913-81-3	0.3		43.23	
2-Undecenal 2-	2463-77-6	0.78		9.47	
Alcohols					
1-Octen-3-ol	3391-86-4	45	0.02	0.01	earth, fat, floral, green, herb
2-Octen-1-ol, (E)-	18409-17-1	20	0.00	0.09	
Hydrocarbon					
Toluene	108-88-3	527	0.20	0.18	Bitter almond, glue, paint, solvent
Ethylbenzene	100-41-4	22000	0.00	0.00	
o-Xylene	95-47-6	450	0.11	0.23	
p-Xylene	106-42-3	1000	0.06	0.05	
Benzene, 1,3-dimethyl-	108-38-3	5.5	2.51	4.80	
Styrene	100-42-5	65	0.12	0.07	plastic, rubber, solvent
Ketones					
5-Hepten-2-one, 6-methyl-	110-93-0	50	0.04	0.04	
Cyclooctanone	502-49-8	280	0.03		
Esters					
Hexanoic acid, ethyl ester	123-66-0	55.33	0.07	0.06	Apple peel, banana, brandy, cheese, overripe fruit
Octanoic acid, ethyl ester	106-32-1	19	0.13	0.10	
Heterocyclic					
Furan, 2-pentyl-2-	3777-69-3	5.8	7.27	3.64	Floral, fruit, green, green bean

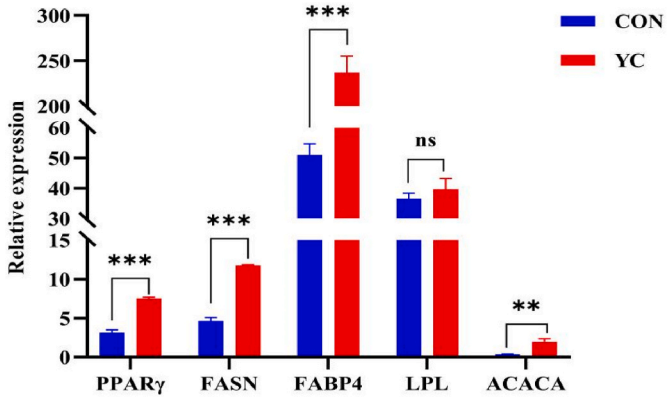

Fig. 2. Effects of dietary YC supplementation on the expression of genes related to muscle fat metabolism. All values were expressed as means ± SEM, n = 6. CON = Control group; YC= Yeast culture group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

higher and *Proteobacteria* ($p = 0.02$) was significantly lower in the YC group than that of the CON group, and *Firmicutes* was decreased by 7.65% in the YC group ($p = 0.65$). At the genus level, *Prevotella_7*, *Prevotella* and *Lachnospiraceae_NK3A20* were the dominant genera (Fig. 3E). Metastats analysis with relative abundance >0.1% found that the relative abundance of *Prevotella_7, Succiniclasticum, unclassified_Selenomonadaceae* and *Olsenella* were significantly increased in the YC group ($p < 0.05$), while the relative abundances of *Lachnospiraceae_NK3A20_group*, *unclassified_Prevotellaceae* and *unclassified_Lachnospiraceae* were significantly decreased in the YC group ($p < 0.05$). LEfSe difference analysis was performed to examine the rumen microorganisms identified in the two groups, the main microbial in the YC group were *f_F082*, *s_uncultured rumen bacterium*, and *g_uncultured* (Fig. 3F).

Table 7
Effects of dietary YC supplementation on rumen fermentation.

Items	Treatments		SEM	*P*-value
	CON	YC		
pH	6.19	6.26	0.21	0.884
NH_3-N, mg/100 mL	5.35	5.55	0.16	0.601
Total VFA, mmol/L	77.13[b]	97.90[a]	5.23	0.030
Acetate, mmol/L	44.47[b]	57.29[a]	5.23	0.044
Propionate, mmol/L	19.36	24.28	3.34	0.312
Butyrate, mmol/L	9.43	11.42	2.21	0320
Valeric acid	1.55	2.31	0.29	0.223
Acetate/propionate ratio	2.68	2.37	0.10	0.640

Values are expressed as means ± SEM (n = 6); CON = control group; YC = yeast culture group. [a,b]The values within a row with different superscripts are significantly different ($p < 0.05$).

3.7. Analysis of economic benefits

According to the market price of mutton in China, and considering only the cost of feed ingredients for each group, excluding management and disease prevention costs. We found that adding 1% YC to lambs diet increased profits by 47.70 CNY per sheep after 60 d of fattening (Table 8).

3.8. Correlation analysis

The correlation analysis between the key volatile flavour components and the amino acids and unsaturated fatty acids were analyzed by Spearman's coefficient model, which showed highly significant correlations between the key volatile flavour components and the amino acids and fatty acids (Fig. 4.). Specifically, heptanal, 2-octenal, (E)-, octanal, o-xylene, benzaldehyde, and benzene 1,3-dimethyl- showed significant positively correlated with Cys, Arg, Tyr, Pro, Ala, Asp, Glu, Val, Met,

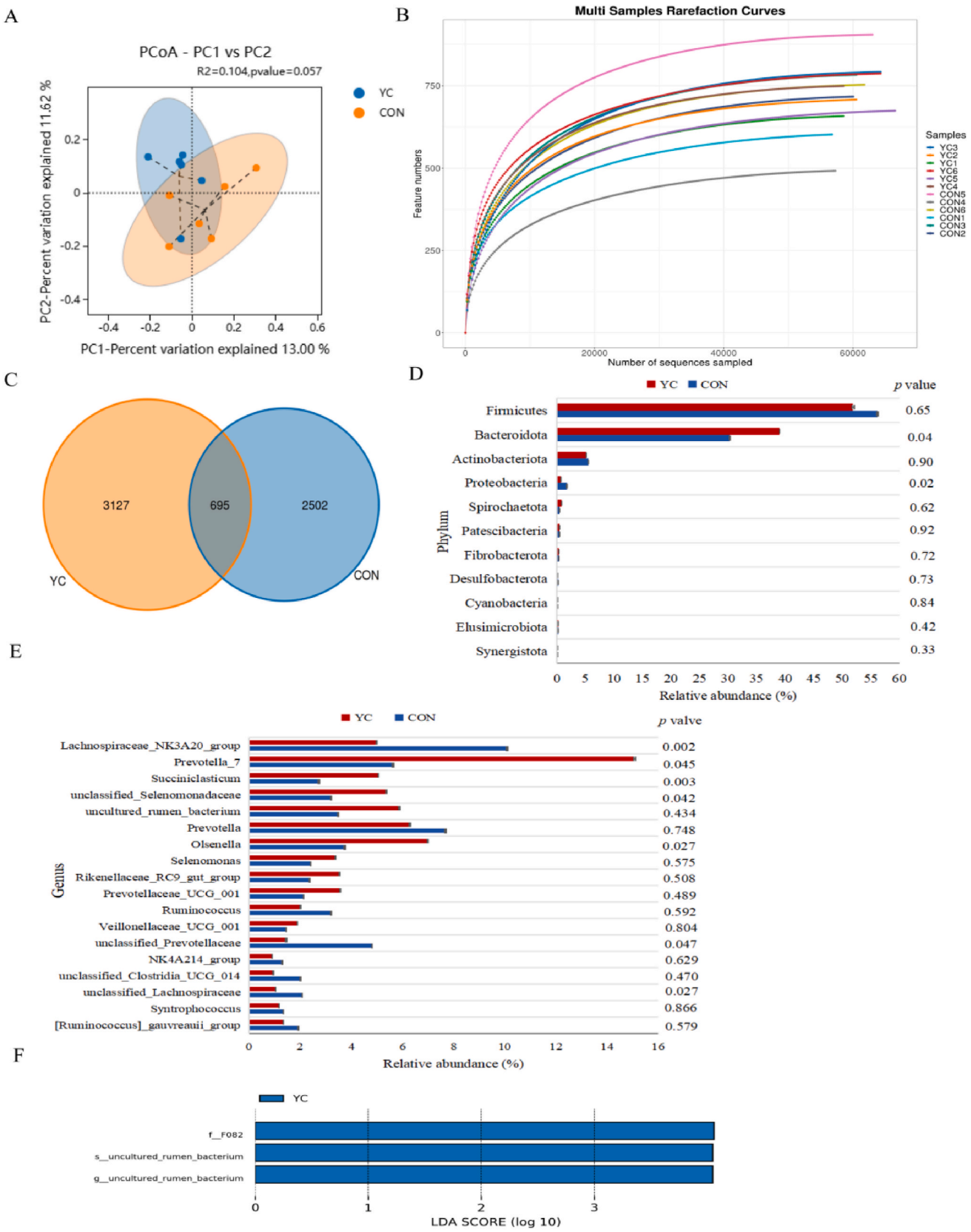

Fig. 3. Effects of dietary YC supplementation on rumen microbial composition. (A) PCoA analysis; **(B)** dilution curve analysis; **(C)** OTU-Venn analysis; **(D)** Relative abundance at the phylum levels of ruminal microbiota; **(E)** The relative abundances at the genus levels of ruminal microbiota; **(F)** Significantly different bacterial taxa identified by the linear discriminant analysis effect size (LEfSe). All values were expressed as means ± SEM, n = 6. CON = Control group; YC= Yeast culture group.

Table 8
Analysis of economic benefits.

Items	Treatments		*P*-value
	CON	YC	
Daily weight gain, kg	0.290 ± 0.012	0.332 ± 0.020	0.038
Unit price of feed, CNY/kg	3.20	3.30	–
Purchase price, CNY/kg	30.00	30.00	–
Total weight gain, kg	17.43 ± 0.66	19.90 ± 1.07	0.044
Weight gain amount, CNY	522.9 ± 29.74	597.0 ± 19.86	0.010
Feed cost, CNY	270.6 ± 15.74	297.0 ± 11.50	0.320
Gross profit, CNY	252.3 ± 29.74	300.0 ± 19.86	0.029

Cost of input = feed unit price × feeding days; Cost of output = daily weight gain × purchase price of live sheep; Profit = cost of output - cost of input.

Leu, Lys, and Thr, and significant negatively correlated with C24:1, C18:2n6t, C18:2n6c, C18:3n6, C22:6n3, C20:3n6, C22:2 ($r > 0.7$, $p < 0.05$). In contrast, 1-octen-3-ol, toluene, styrene, 5-hepten-2-one, 6-methyl- were significantly negatively correlated with Cys, Arg, Tyr, Pro, Ala, Asp, Glu, Val, Met, Leu, Lys, and Thr, and significantly positively correlated with C24:1, C18:2n6t, C18:2n6c, C18:3n6, C22:6n3, C20:3n6, C22:2 ($r > 0.7$, $p < 0.05$).

Correlation of fat metabolism related genes with key volatile flavour substances showed that *PPARγ*, *FASN*, and *FABP4* were significantly positively correlated with heptanal, 2-octenal, (E)-, octanal, and benzene 1,3-dimethyl-, and significantly negatively correlated with 1-octen-3-ol, toluene, styrene, 5-hepten-2-one, 6-methyl- ($r > 0.7$, $p < 0.05$) (Fig. 5A.). Further analysis of key volatile flavor substances and top 10 ruminal bacterial genera revealed that *Prevotella* was significantly negatively correlated with styrene, p-xylene and dodecanal ($r > 0.6$, $p < 0.05$), hexanal and benzaldehyde were significantly positively correlated with *uncultured_rumen_bacterium*, but significantly negatively correlated with *Lachnospiraceae_NK3A20_group* ($r > 0.6$, $p < 0.05$) (Fig. 5B.).

4. Discussion

The YC used in this study had a yeast cell count of $\geq 4 \times 10^9$ Cfu/g, which produced abundant cell metabolites, including organic acids, oligosaccharides and vitamins. YC products were added to the total mixed pellet diet, the live yeast was inactivated during the pelleting process. Because under high temperature conditions, yeast cannot survive the pelleting process without special protection techniques (Aguirre-Guzmán et al., 2002; Shurson, 2018). In this study, the results obtained from supplementing with YC were attributed to the yeast fermentation metabolites and culture medium action, rather than live yeast.

Previous studies found that adding YC to broiler diets had no significant effect on meat quality (Hoque et al., 2021; Sun and Kim, 2019). Our study reached different conclusions, revealing that adding YC to sheep improved carcass characteristics (body weight before slaughter, carcass weight and GR) and meat quality (a*, IMF and cooking loss). IMF is closely related to meat quality and is also an important economic indicator of muscle quality (Joo et al., 2013). In this study, dietary YC supplementation significantly increased the IMF content, and reduced cooking loss, thereby improving water-holding capacity and juiciness. IMF content is positively correlated with muscle juiciness, moisture, and tenderness, reasonable IMF content helps improve meat quality and flavor (Schumacher, DelCurto-Wyffels, Thomson and Boles, 2022). Meat color is an important appearance indicator of meat quality, higher redness values (a*) indicate a more vibrant color and fresher meat. We found that YC supplementation significantly increased muscle a* and improved meat color, which was consistent with previous research (Lin et al., 2022; Milewski et al., 2011; J. Zhang et al., 2021).

Peptides and amino acids produced from protein hydrolysis can enhance meat flavor through processes such as Maillard reaction and Strecker degradation (Gan et al., 2019). Amino acids crucial for lamb flavor include tryptophan, threonine, arginine, lysine and leucine (M. Li et al., 2019). Previous studies have found that lysine can enhance appetite resistance and participate in fat metabolism, and arginine may promote IMF synthesis by up-regulating the expression of key fat generating genes in muscle (Tan et al., 2011). In this study, dietary YC supplementation the contents of lysine (Lys) and arginine (Arg) were increased by 7.98% and 13.61% compared to the CON, respectively, and the expressions of *PPARγ*, *FASN*, *FABP4*, and *ACACA* genes related to fat metabolism were significantly increased. These results indicate that YC regulates the expression of genes related to lipid metabolism, altering the contents of amino acids and IMF, and ultimately achieve the regulation of the body lipid metabolism. The higher the amino acid score, the better the nutritional value of the food (Berrazaga, Micard, Gueugneau and Walrand, 2019). In this study, YC supplementation of the TAA and EAA contents showed an increasing trend. In summary, adding YC to the diet increases muscle nutritional value and has the potential to improve muscle flavor. The imbalance in fatty acid ratios can easily lead to heart and other diseases, and fatty acids in muscles are also precursors for flavor formation (Arshad et al., 2018). It is well known that high content of SFAs in meat products can affect cholesterol metabolism (Madruga et al., 2013). For fatty acids, adding YC significantly reduced the contents of octanoic acid (C8:0), heptadecanoic acid (C17:0), arachidic acid (C20:0), henicosanoic acid (C21:0), tricosanoic acid (C23:0) and lignoceric acid (C24:0) in SFAs. This indicates that YC supplementation can lower the SFAs content in muscle, resulting in lamb meat that is more beneficial to human health. At the same time, the contents of oleic acid (C18:1n9c) and eicosenic acid (C20:1) in MUFAs were significantly increased in the YC group, which was beneficial to improve the muscle nutritional quality. In addition, Oleic acid (C18:1n9c) is the most important MUFA in sheep and plays a positive role in the absorption of other fatty acids (F. Wang et al., 2024). It can prevent cardiovascular diseases by maintaining low levels of high density lipoprotein (HDL) and low density lipoprotein (LDL), which is beneficial to human health (Nkukwana et al., 2014). MUFA have functions such as protecting the heart, lowering blood sugar, regulating blood lipids and preventing memory decline (de Souza Vilela et al., 2021). The MUFA content increased by 2.5% in the YC group in this study, indicating that YC could enhance the healthcare function of mutton. Studies have reported that the expression of the *FABP4* genes is related to fat content and meat quality to some extent (Y. Wang et al., 2016), the *FASN* gene has an important role in fat deposition, whereas the *ACACA* and *PPAR* genes play a role in lipid metabolism (Pecka-Kiełb et al., 2021). In this study, the expression levels of *PPARγ*, *FASN*, *FABP4*, and *ACACA* were significantly increased in the YC group, which may be one of the reasons for the increased of muscle IMF and MUFA contents. However, the specific mechanism of the increase needs to be further investigated from a molecular perspective.

Meat flavor is considered the most important factor affecting palatability. Flavor compounds in mutton include aldehydes, alcohols, ketones, and esters (J. Liu et al., 2022). Aldehydes are primarily produced by the oxidation of fats, have low threshold values, strong fruit and flower flavor, and contribute greatly to meat flavor (Rasinska et al., 2019). In this study, the highest content of aldehydes was detected, key aldehydes with ROAV>1 include nonanal, hexanal, heptanal, 2,4-decadienal, (E, E), octanal, 2-decenal, (E)-, 2-undecenal. Hexanal has a grassy flavor, but in high levels it has a rancid, gamy taste, and 2-decenal, (E)- with orange blossom, rose, and citrus aromas, as well as a fatty aroma. The addition of YC had no significant effect the levels of nonanal and heptanal, but it significantly reduced the concentration of hexanal and increased the level of 2-decenal, (E)-. Benzaldehyde alters the overall flavor of mutton (ROAV >0.1), with an almondy, caramel and sweet flavor. It is produced from the degradation of phenylalanine or linolenic acid oxidation, The addition of YC the benzaldehyde content was significantly reduced. Alcohols are generally produced by fatty acid derivatives, lipid oxidation, or the reduction of carbonyl compounds, and can impart distinct flavors to meat (D. Wang et al., 2022). We found

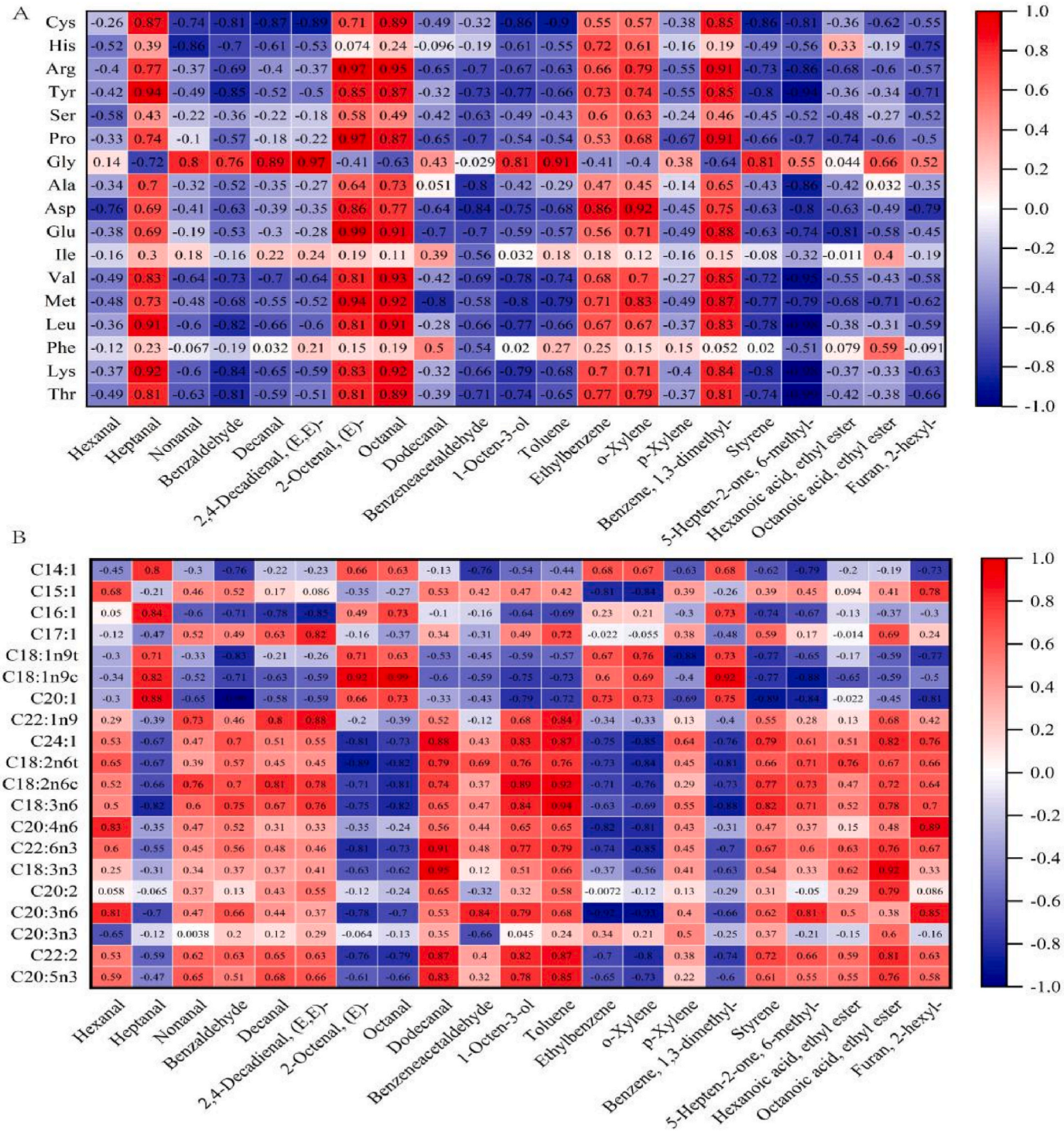

Fig. 4. Correlation analysis. (A) Correlation analysis between key volatile flavor compounds and amino acids. (B) Correlation analysis between key volatile flavor compounds and unsaturated acids. The values represent correlation coefficients. Red represents positive correlation and blue represents negative correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

that the addition of YC decreased the contents of 1-octen-3-ol and cyclooctyl alcohol, while increased the contents of cyclohexanol, 1-ethyl - and 2-octen-1-ol, (E)-, indicating that YC altered the content of volatile flavouring substances. Given the relatively low threshold of 1-octen-3-ol and 2-octen-1-ol (E), they significantly impact meat flavor, the decrease of 1-octene-3-ol content may be related to the reduction in γ-linolenic acid levels, and it has been shown that alcohols were mainly formed by the oxidation of linoleic acid degradation products (Bai et al., 2022). Hydrocarbons have relatively high thresholds and contribute less to flavor. The key hydrocarbons (ROAV>0.1) detected in both groups of this study included toluene, ethylbenzene, o-xylene, p-xylene1, and benzene, 1,3-dimethyl-, and dietary YC supplementation significantly reduced the content of styrene, which has plastic, rubber and solvent, and can improve the mutton flavor (Luo et al., 2019). Lys, Arg, 18:1n9c, C20:1, *PPARγ*, *FASN*, and *FABP4* were significantly positively correlated with heptanal, 2-octenal, (E)-, octanal, ethylbenzene, o-xylene and

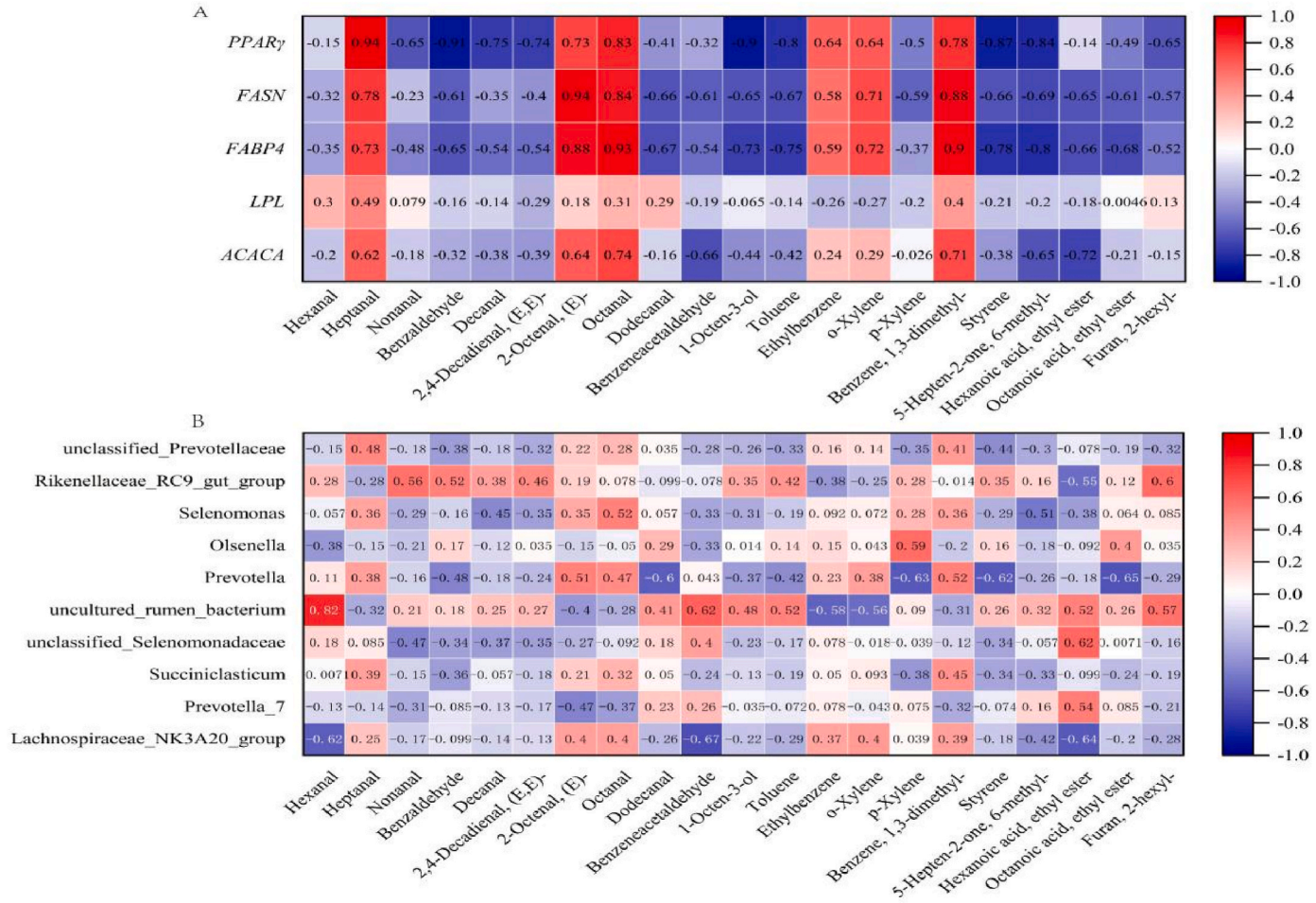

Fig. 5. Correlation analysis. (A) Correlation analysis between key volatile flavor compounds and fat metabolism related genes. (B) Correlation analysis between key volatile flavor substances and rumen bacteria. The values represent correlation coefficients. Red represents positive correlation and blue represents negative correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

benzene 1,3-dimethyl-. These results indicated that dietary YC supplementing could improve the mutton flavor by regulating some amino acids and fatty acids contents of flavor precursors, and the expression of genes related to lipid metabolism.

Research has found that changes in the rumen microbiota have a significant impact on the quality traits of ruminant meat (X. Zhang et al., 2022). Therefore, we performed 16S rRNA sequencing and found that YC had no significant effect on rumen microbial richness and diversity, but changed the relative abundance of bacteria genera. Compared to the CON, the relative abundance of *Bacteroidota* in the YC group was significantly higher, while the relative abundance of *Firmicutes* was significantly lower. *Bacteroidetes* was related to body fat metabolism (Kallus and Brandt, 2012), and we have previously found that YC promotes intramuscular fat deposition, which may be related to the increased relative abundance of *Bacteroidetes* , but the specific mechanisms still require further study. At the same time, it has been reported that the ratio of *Firmicutes* to *Bacteroidetes* was related to fat deposition in tissues (Walters et al., 2014). Therefore, we hypothesized that YC affects fatty acid metabolism in muscle and promotes intramuscular fat deposition by altering rumen microbiota, which would affect the fatty acid and amino acid contents of the flavor precursors, and consequently muscle quality and flavor (Fig. 6.).

At the genus level, dietary YC mainly increased the relative abundance of *Prevotella_7* and *Succiniclasticum*, and decreased the relative abundance of *Lachnospiraceae_NK3A20_group* and *unclassified_Prevotellaceae*. *Prevotella* effectively degraded hemicellulose and starch (Lv et al., 2019), and the ability of yeast to stimulate the growth of fibre digesting bacteria. These results indicated that dietary YC supplementation stimulated *Prevotella* to degrade fiber effectively, which provides energy for fat metabolism, protein metabolism and carbohydrate metabolism (Newbold and Rode, 2006). In this study, the rumen fluid Total VFA and acetate concentrations were increased under the YC treatment, indicated that YC promoted microbial fermentation to produce more VFA by increasing the number and metabolic capacity of VFA

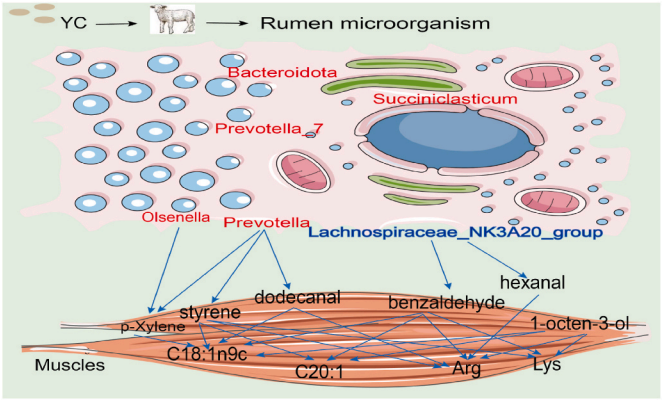

Fig. 6. Effects of YC on rumen microbiota and muscle fatty acids, amino acids and flavor substances. Arrows indicate the correlation.

producing microorganisms (Doležal et al., 2012). Ruminal *Succiniclasticum* plays a crucial role in energy harvest by converting succinate into propionate (van Gylswyk, 1995). A study reported that *Lachnospiraceae_NK3A20_group* was involved in the biohydrogenation of C18:2c9c and a-linolenic acid in two beef cattle breeds (Conte et al., 2022). We found a significant negative correlation *Lachnospiraceae_NK3A20_group* with hexanal and benzaldehyde, indicating that *Lachnospiraceae_NK3A20_group* may affect muscle flavor. In conclusion, YC can change rumen microbiota structure in lambs, and affect muscle quality and flavor by affecting fatty acid metabolism.

5. Conclusions

Addition of 1% YC to the diet increased muscle GR, IMF and the beneficial fatty acids such as oleic acid (C18:1n9c) and eicosanoic acid (C20:1) contents, while decreased the content of some SFAs. At the same time, the levels of lysine (Lys) and arginine (Arg) that affect the muscle flavour were increased, and the levels of the key flavor substances such as hexanal, nonanal, styrene, benzaldehyde, paraxylene, and 1-octen-3-ol were changed. Additionally, the expression of fat metabolism related genes *PPARγ*, *FASN*, and *FABP4* were increased. For economic benefits, adding 1% YC increased profits by 47.70 CNY per sheep after 60 d of fattening. In summary, adding of YC to lamb diets can increase farming profits, improve meat quality, and particularly mutton flavor, which may be related to the regulation of the relative abundance of *Bacteroidota*, *Prevotella_7*, *Succiniclasticum*, and *Lachnospiraceae_NK3A20_group*.

Ethics statement

This experiment was carried out in strict accordance with the Guidelines for the Care and Use of Laboratory Animals of the Gansu Agricultural University and was approved by the Laboratory Animal Ethics Committee of the Gansu Agricultural University (Approval No. GSAU-Eth-AST-2023-035).

Funding

This research was funded by the Technology Innovation Guidance Plan of Gansu Province Science and Technology Plan project - LuGan Science and Technology cooperation topic, grant number 22CX2NA005; Guanghe County cattle and sheep industry quality and efficiency technology demonstration project, grant number LXZC21520230202 and Gansu educational technology innovation project, grant number 2021A441.

CRediT authorship contribution statement

Xiongxiong Li: Writing – original draft, Writing – review & editing, Methodology. **Yanchi Wang:** Formal analysis, Data curation. **Jinlong Xu:** Investigation. **Qitian Yang:** Data curation. **Yuzhu Sha:** Writing – review & editing, Methodology, Conceptualization. **Ting Jiao:** Supervision, Methodology, Conceptualization. **Shengguo Zhao:** Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The authors would like to thank Pro. Shengguo Zhao, Pro. Ting Jiao, Dr. Yuzhu Sha, and Dr. Yanchi Wang for their help in the successful completion of this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2024.100845.

References

Aguirre-Guzmán, G., Ricque-Marie, D., Cruz-Suárez, L.E., 2002. Survival of agglomerated Saccharomyces cerevisiae in pelleted shrimp feeds. Aquaculture 208 (1), 125–135. https://doi.org/10.1016/S0044-8486(01)00711-6.

Arshad, M.S., Sohaib, M., Ahmad, R.S., Nadeem, M.T., Imran, A., Arshad, M.U., Amjad, Z., 2018. Ruminant meat flavor influenced by different factors with special reference to fatty acids. Lipids Health Dis. 17 (1), 223. https://doi.org/10.1186/s12944-018-0860-z.

Bai, S., You, L., Ji, C., Zhang, T., Wang, Y., Geng, D., Luo, R., 2022. Formation of volatile flavor compounds, maillard reaction products and potentially hazard substance in China stir-frying beef sao zi. Food Res. Int. 159, 111545. https://doi.org/10.1016/j.foodres.2022.111545.

Bi, J., Li, Y., Yang, Z., Lin, Z., Chen, F., Liu, S., Li, C., 2022. Effect of different cooking times on the fat flavor compounds of pork belly. J. Food Biochem. 46 (8), e14184. https://doi.org/10.1111/jfbc.14184.

Broadway, P.R., Carroll, J.A., Sanchez, N.C., 2015. Live yeast and yeast cell wall supplements enhance immune function and performance in food-producing livestock: a review (†,)(‡). Microorganisms 3 (3), 417–427. https://doi.org/10.3390/microorganisms3030417.

Buchanan, J.W., Reecy, J.M., Garrick, D.J., Duan, Q., Beitz, D.C., Koltes, J.E., Mateescu, R.G., 2016. Deriving gene networks from SNP associated with triacylglycerol and phospholipid fatty acid fractions from ribeyes of angus cattle. Front. Genet. 7, 116. https://doi.org/10.3389/fgene.2016.00116.

Conte, G., Dimauro, C., Daghio, M., Serra, A., Mannelli, F., McAmmond, B.M., Mele, M., 2022. Exploring the relationship between bacterial genera and lipid metabolism in bovine rumen. Animal 16 (5), 100520. https://doi.org/10.1016/j.animal.2022.100520.

de Souza Vilela, J., Alvarenga, T., Andrew, N.R., McPhee, M., Kolakshyapati, M., Hopkins, D.L., Ruhnke, I., 2021. Technological quality, amino acid and fatty acid profile of broiler meat enhanced by dietary inclusion of black soldier fly larvae. Foods 10 (2). https://doi.org/10.3390/foods10020297.

Dias, A.L.G., Freitas, J.A., Micai, B., Azevedo, R.A., Greco, L.F., Santos, J.E.P., 2018. Effects of supplementing yeast culture to diets differing in starch content on performance and feeding behavior of dairy cows. J. Dairy Sci. 101 (1), 186–200. https://doi.org/10.3168/jds.2017-13240.

Doležal, P., Dolezal, J.E., Szwedziak, K., Dvořáček, J., Zeman, L., Tukiendorf, M., Havlicek, Z., 2012. Use of Yeast Culture in the TMR of Dairy Holstein Cows, 2, pp. 51–56.

Dong, S.K., Gao, H.W., Hou, X.Y., Kang, M.Y., Lassoie, J.P., Long, R.J., Xu, G.C., 2007. Farmer and professional attitudes to the large-scale ban on livestock grazing of grasslands in China. Environ. Conserv. 34 (3), 246–254. https://doi.org/10.1017/S0376892907004213.

Gan, M., Shen, L., Fan, Y., Guo, Z., Liu, B., Chen, L., Zhu, L., 2019. High altitude adaptability and meat quality in Tibetan pigs: a reference for local pork processing and genetic improvement. Animals 9 (12). https://doi.org/10.3390/ani9121080.

Guo, Y., Guo, X., Deng, Y., Cheng, L., Hu, S., Liu, H., Wang, J., 2020. Effects of different rearing systems on intramuscular fat content, fatty acid composition, and lipid metabolism-related genes expression in breast and thigh muscles of Nonghua ducks. Poultry Sci. 99 (10), 4832–4844. https://doi.org/10.1016/j.psj.2020.06.073.

Hoque, M.R., Jung, H.I., Kim, I.H., 2021. Effect of yeast culture (Saccharomyces cerevisiae) supplementation on growth performance, excreta microbes, noxious gas, nutrient utilization, and meat quality of broiler chicken. J. Poultry Sci. 58 (4), 216–221. https://doi.org/10.2141/jpsa.0190144.

Joo, S.T., Kim, G.D., Hwang, Y.H., Ryu, Y.C., 2013. Control of fresh meat quality through manipulation of muscle fiber characteristics. Meat Sci. 95 (4), 828–836. https://doi.org/10.1016/j.meatsci.2013.04.044.

Kallus, S.J., Brandt, L.J., 2012. The intestinal microbiota and obesity. J. Clin. Gastroenterol. 46 (1), 16–24. https://doi.org/10.1097/MCG.0b013e31823711fd.

Li, M., Yang, R., Zhang, H., Wang, S., Chen, D., Lin, S., 2019. Development of a flavor fingerprint by HS-GC-IMS with PCA for volatile compounds of Tricholoma matsutake Singer. Food Chem. 290, 32–39. https://doi.org/10.1016/j.foodchem.2019.03.124.

Li, S., Yoon, I., Scott, M., Khafipour, E., Plaizier, J.C., 2016. Impact of Saccharomyces cerevisiae fermentation product and subacute ruminal acidosis on production, inflammation, and fermentation in the rumen and hindgut of dairy cows. Anim. Feed Sci. Technol. 211, 50–60. https://doi.org/10.1016/j.anifeedsci.2015.10.010.

Li, X., Wang, Z., Qin, W., Gao, X., Wu, J., Zhao, S., Jiao, T., 2022. Effects of oregano essential oil, cobalt and synergistic of both of them on rumen degradation rate and fermentation characteristics for corn silage. Ital. J. Anim. Sci. 21 (1), 1476–1488. https://doi.org/10.1080/1828051X.2022.2121666.

Lin, Y., Yu, C., Ma, Z., Che, L., Feng, B., Fang, Z., Wu, D., 2022. Effects of yeast culture supplementation in wheat-rice-based diet on growth performance, meat quality, and gut microbiota of growing-finishing pigs. Animals 12 (17). https://doi.org/10.3390/ani12172177.

Liu, J., Han, L., Han, W., Gui, L., Yuan, Z., Hou, S., Alanazi, A.M., 2022. Effect of different heat treatments on the quality and flavor compounds of black Tibetan sheep meat by HS-GC-IMS coupled with multivariate analysis. Molecules 28 (1). https://doi.org/10.3390/molecules28010165.

Liu, R., Wang, K., Xu, J., Shi, X., Zhang, H., Xue, C., 2018. Effects of compound Chinese herbal additives on the types and contents of volatile flavor compounds in mutton. Anim. Husb. Vet. Med. 50 (10), 38–42.

Liu, Y.Z., Lang, M., Zhen, Y.G., Chen, X., Sun, Z., Zhao, W., Qin, G.X., 2019. Effects of yeast culture supplementation and the ratio of non-structural carbohydrate to fat on growth performance, carcass traits and the fatty acid profile of the longissimus dorsi muscle in lambs. J. Anim. Physiol. Anim. Nutr. 103 (5), 1274–1282. https://doi.org/10.1111/jpn.13128.

Low, C.X., Tan, L.T., Ab Mutalib, N.S., Pusparajah, P., Goh, B.H., Chan, K.G., Lee, L.H., 2021. Unveiling the impact of antibiotics and alternative methods for animal husbandry: a review. Antibiotics 10 (5). https://doi.org/10.3390/antibiotics10050578.

Luo, Y., Wang, B., Liu, C., Su, R., Hou, Y., Yao, D., Jin, Y., 2019. Meat quality, fatty acids, volatile compounds, and antioxidant properties of lambs fed pasture versus mixed diet. Food Sci. Nutr. 7 (9), 2796–2805. https://doi.org/10.1002/fsn3.1039.

Lv, X., Chai, J., Diao, Q., Huang, W., Zhuang, Y., Zhang, N., 2019. The signature microbiota drive rumen function shifts in goat kids introduced to solid diet regimes. Microorganisms 7 (11). https://doi.org/10.3390/microorganisms7110516.

Ma, Y., Han, L., Raza, S.H.A., Gui, L., Zhang, X., Hou, S., Alkhalil, S.S., 2023. Exploring the effects of palm kernel meal feeding on the meat quality and rumen microorganisms of Qinghai Tibetan sheep. Food Sci. Nutr. 11 (6), 3516–3534. https://doi.org/10.1002/fsn3.3340.

Ma, Z., Wu, Z., Wang, Y., Meng, Q., Chen, P., Li, J., Shan, A., 2023. Effect of yeast culture on reproductive performance, gut microbiota, and milk composition in primiparous sows. Animals 13 (18). https://doi.org/10.3390/ani13182954.

Madruga, M., Dantas, I., Queiroz, A., Brasil, L., Ishihara, Y., 2013. Volatiles and water- and fat-soluble precursors of Saanen goat and cross Suffolk lamb flavour. Molecules 18 (2), 2150–2165. https://doi.org/10.3390/molecules18022150.

Mahmoud, M.M., Youssef, I.M.I., Abd El-Tawab, M.M., Bakr, H.A., Eissa, N.A., Hassan, M.S., Sobiech, P., 2020. Influence of probiotic and yeast culture supplementation on selected biochemical and immunological parameters of growing lambs. Pol. J. Vet. Sci. 23 (1), 5–12. https://doi.org/10.24425/pjvs.2019.131413.

McIntosh, A.L., Atshaves, B.P., Martin, G.G., Landrock, D., Milligan, S., Landrock, K.K., Kier, A.B., 2019. Effect of liver fatty acid binding protein (L-FABP) gene ablation on lipid metabolism in high glucose diet (HGD) pair-fed mice. Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1864 (7), 985–1004. https://doi.org/10.1016/j.bbalip.2019.03.009.

Milewski, S., Zaleska, B.J. J.o.A., Sciences, F., 2011. The effect of dietary supplementation with Saccharomyces cerevisiae dried yeast on lambs meat. Quality 20, 537–545.

Newbold, C.J., Rode, L.M., 2006. Dietary additives to control methanogenesis in the rumen. Int. Congr. 1293, 138–147. https://doi.org/10.1016/j.ics.2006.03.047.

Nkukwana, T.T., Muchenje, V., Masika, P.J., Hoffman, L.C., Dzama, K., Descalzo, A.M., 2014. Fatty acid composition and oxidative stability of breast meat from broiler chickens supplemented with Moringa oleifera leaf meal over a period of refrigeration. Food Chem. 142, 255–261. https://doi.org/10.1016/j.foodchem.2013.07.059.

Ogbuewu, I.P., Mbajiorgu, C.A., 2023. Meta-analysis of Saccharomyces cerevisiae on enhancement of growth performance, rumen fermentation and haemato-biochemical characteristics of growing goats. Heliyon 9 (3), e14178. https://doi.org/10.1016/j.heliyon.2023.e14178.

Pecka-Kiełb, E., Kowalewska-Łuczak, I., Czerniawska-Piątkowska, E., Króliczewska, B., 2021. FASN, SCD1 and ANXA9 gene polymorphism as genetic predictors of the fatty acid profile of sheep milk. Sci. Rep. 11 (1), 23761. https://doi.org/10.1038/s41598-021-03186-y.

Poppy, G.D., Rabiee, A.R., Lean, I.J., Sanchez, W.K., Dorton, K.L., Morley, P.S., 2012. A meta-analysis of the effects of feeding yeast culture produced by anaerobic fermentation of Saccharomyces cerevisiae on milk production of lactating dairy cows. J. Dairy Sci. 95 (10), 6027–6041. https://doi.org/10.3168/jds.2012-5577.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41 (D1), D590–D596. https://doi.org/10.1093/nar/gks1219.

Rasinska, E., Rutkowska, J., Czarniecka-Skubina, E., Tambor, K., 2019. Effects of cooking methods on changes in fatty acids contents, lipid oxidation and volatile compounds of rabbit meat. LWT 110, 64–70. https://doi.org/10.1016/j.lwt.2019.04.067.

Ren, Y., Zhaxi, Y., Ciwang, R., Wang, Z., Liu, M., 2023. Responses of rumen microorganisms and metabolites to different roughage of domesticated Tibetan sheep. Front. Microbiol. 14, 1247609. https://doi.org/10.3389/fmicb.2023.1247609.

Schumacher, M., DelCurto-Wyffels, H., Thomson, J., Boles, J., 2022. Fat deposition and fat effects on meat quality-A review. Animals 12 (12). https://doi.org/10.3390/ani12121550.

Shurson, G.C., 2018. Yeast and yeast derivatives in feed additives and ingredients: sources, characteristics, animal responses, and quantification methods. Anim. Feed Sci. Technol. 235, 60–76. https://doi.org/10.1016/j.anifeedsci.2017.11.010.

Sun, H.Y., Kim, I.H., 2019. Dietary supplementation of mixed yeast culture derived from Saccharomyces cerevisiae and kluyveromyces maxianus: effects on growth performance, nutrient digestibility, meat quality, blood parameters, and gut health in broilers. J. Poultry Sci. 56 (2), 140–147. https://doi.org/10.2141/jpsa.0180052.

Swyers, K.L., Wagner, J.J., Dorton, K.L., Archibeque, S.L., 2014. Evaluation of Saccharomyces cerevisiae fermentation product as an alternative to monensin on growth performance, cost of gain, and carcass characteristics of heavy-weight yearling beef steers. J. Anim. Sci. 92 (6), 2538–2545. https://doi.org/10.2527/jas.2013-7559.

Tan, B., Yin, Y., Liu, Z., Tang, W., Xu, H., Kong, X., Wu, G., 2011. Dietary L-arginine supplementation differentially regulates expression of lipid-metabolic genes in porcine adipose tissue and skeletal muscle. J. Nutr. Biochem. 22 (5), 441–445. https://doi.org/10.1016/j.jnutbio.2010.03.012.

Titi, H.H., Dmour, R.O., Abdullah, A.Y., 2008. Growth performance and carcass characteristics of Awassi lambs and Shami goat kids fed yeast culture in their finishing diet. Anim. Feed Sci. Technol. 142 (1), 33–43. https://doi.org/10.1016/j.anifeedsci.2007.06.034.

Utama, D.T., Lee, C.W., Park, Y.S., Jang, A., Lee, S.K., 2018. Comparison of meat quality, fatty acid composition and aroma volatiles of Chikso and Hanwoo beef. Asian-Australas. J. Anim. Sci. 31 (9), 1500–1506. https://doi.org/10.5713/ajas.17.0902.

van Gylswyk, N.O., 1995. Succiniclasticum ruminis gen. nov., sp. nov., a ruminal bacterium converting succinate to propionate as the sole energy-yielding mechanism. Int. J. Syst. Bacteriol. 45 (2), 297–300. https://doi.org/10.1099/00207713-45-2-297.

Wagner, J.J., Engle, T.E., Belknap, C.R., Dorton, K.L., 2016. Meta-analysis examining the effects of Saccharomyces cerevisiae fermentation products on feedlot performance and carcass traits1,2,31Use of trade names in this publication does not imply endorsement by Colorado State University or the authors or criticisms of similar products not mentioned.2Mention of a proprietary product does not constitute a guarantee or warranty of the products by Colorado State University or the authors and does not imply its approval to the exclusion of other products that may also be suitable.3Partial sponsorship of this study was provided by Diamond V, Cedar Rapids, IA 52404. Prof. Anim. Sci. 32 (2), 172–182. https://doi.org/10.15232/pas.2015-01438.

Walters, W.A., Xu, Z., Knight, R., 2014. Meta-analyses of human gut microbes associated with obesity and IBD. FEBS (Fed. Eur. Biochem. Soc.) Lett. 588 (22), 4223–4233. https://doi.org/10.1016/j.febslet.2014.09.039.

Wang, B., Wang, Y., Zuo, S., Peng, S., Wang, Z., Zhang, Y., Luo, H., 2021. Untargeted and targeted metabolomics profiling of muscle reveals enhanced meat quality in artificial pasture grazing tan lambs via rescheduling the rumen bacterial community. J. Agric. Food Chem. 69 (2), 846–858. https://doi.org/10.1021/acs.jafc.0c06427.

Wang, D., Zhang, J., Zhu, Z., Lei, Y., Huang, S., Huang, M., 2022. Effect of ageing time on the flavour compounds in Nanjing water-boiled salted duck detected by HS-GC-IMS. LWT 155, 112870. https://doi.org/10.1016/j.lwt.2021.112870.

Wang, F., Sha, Y., Liu, X., He, Y., Hu, J., Wang, J., Huang, W., 2024. Study of the interactions between muscle fatty acid composition, meat quality-related genes and the ileum microbiota in Tibetan sheep at different ages. Foods 13 (5). https://doi.org/10.3390/foods13050679.

Wang, H., Su, M., Wang, C., Li, D., Li, Q., Liu, Z., Ma, Y., 2023. Yeast culture repairs rumen epithelial injury by regulating microbial communities and metabolites in sheep. Front. Microbiol. 14, 1305772. https://doi.org/10.3389/fmicb.2023.1305772.

Wang, L.W., Liu, Y.B., Zhao, J., Wang, B., Te, R., et al., 2023. Effects of dietary yeast cultures supplementation on slaughter performance and MuscularContents of amino acid and functional component in small-tail han sheep. Animal husbandry and feed science 44 (3), 18–26.

Wang, Y., Bu, X., Yang, D., Deng, D., Lei, Z., Guo, Z., Yu, Q., 2023. Effect of cooking method and doneness degree on volatile compounds and taste substance of pingliang red beef. Foods 12 (3). https://doi.org/10.3390/foods12030446.

Wang, Y., He, Y., Liu, Y., Wang, D., 2022. Analyzing volatile compounds of young and mature docynia delavayi fruit by HS-SPME-GC-MS and rOAV. Foods 12 (1). https://doi.org/10.3390/foods12010059.

Wang, Y., Hui, X., Wang, H., Kurban, T., Hang, C., Chen, Y., Wang, J., 2016. Association of H-FABP gene polymorphisms with intramuscular fat content in Three-yellow chickens and Hetian-black chickens. J. Anim. Sci. Biotechnol. 7, 9. https://doi.org/10.1186/s40104-016-0067-y.

Wu, T., Yang, F., Jiao, T., Zhao, S., 2022. Effects of dietary oregano essential oil on cecal microorganisms and muscle fatty acids of luhua chickens. Animals 12 (22). https://doi.org/10.3390/ani12223215.

Yang, Z., Liu, C., Dou, L., Chen, X., Zhao, L., Su, L., Jin, Y., 2022. Effects of feeding regimes and postmortem aging on meat quality, fatty acid composition, and volatile flavor of longissimus thoracis muscle in sunit sheep. Animals 12 (22). https://doi.org/10.3390/ani12223081.

Zhang, J., Wan, K., Xiong, Z.B., Luo, H., Zhou, Q.F., Liu, A.F., He, H., 2021. Effects of dietary yeast culture supplementation on the meat quality and antioxidant capacity of geese1. J. Appl. Poultry Res. 30 (1), 100116. https://doi.org/10.1016/j.japr.2020.100116.

Zhang, X., Deng, Y., Hu, S., Hu, X., Ma, J., Hu, J., Wang, J., 2023. Comparative analysis of amino acid content and protein synthesis-related genes expression levels in breast muscle among different duck breeds/strains. Poultry Sci. 102 (1), 102277. https://doi.org/10.1016/j.psj.2022.102277.

Zhang, X., Han, L., Hou, S., Raza, S.H.A., Gui, L., Sun, S., Aloufi, B.H., 2022. Metabolomics approach reveals high energy diet improves the quality and enhances the flavor of black Tibetan sheep meat by altering the composition of rumen microbiota. Front. Nutr. 9, 915558. https://doi.org/10.3389/fnut.2022.915558.