




# The improved growth performance of *Cyprinus carpio* by dietary yeast culture depends on improvement of intestinal structure and digestive enzyme activities rather than changes of intestinal microbiota composition

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## ABSTRACT

The ban of antibiotic growth promoters can influence the total production of aquaculture. Yeast culture (YC) possesses the ability to enhance immunity and has been supplemented into diet to try to increase production. To investigate the effects of YC on the growth and its mechanisms, four diets supplemented with 0 g/kg (Y0), 10 g/kg (Y1), 20 g/kg (Y2), 30 g/kg (Y3) YC were assigned to *Cyprinus carpio* for 8 weeks (three replicates per diet, 30 fish in each replicate). The results showed that dietary 10 g/kg YC significantly increased weight gain rate (WGR), protein efficiency ratio (PER), specific growth rate (SGR) and reduced feed conversion ratio (FCR) ( $P < 0.05$ ). Also, the apparent digestibility of lipids and proteins was significantly increased by dietary 10 g/kg YC compared with the Y0 group ( $P < 0.05$ ). Meanwhile, fish fed 10 g/kg YC showed significantly higher intestinal villus height and width as well as the digestive enzyme activities of trypsin and lipase than Y0 group ( $P < 0.05$ ). However, no significant differences were found in the composition of intestinal flora between the Y1 and Y0 groups ( $P > 0.05$ ). Whereas, excessive levels of YC significantly reduced abundances of Fusobacteriota and increased that of Proteobacteria ( $P < 0.05$ ). Simultaneously, the growth performance, apparent digestibility of dry matter and protein and intestinal trypsin activity in the Y3 group were also significantly lower than that of Y1 group ( $P < 0.05$ ). In summary, our results supported the applicability of dietary 10 g/kg YC as a growth promoter for common carp through the improvement of intestinal digestive enzymes and intestinal structure but not gut microbial composition, and suggested that excessive levels of YC may cause imbalances in the gut flora, resulting in growth inhibition.

## 1. Introduction

Since the European Union banned antibiotic growth promoters in 2006, many countries have banned the use of them as feed additives (Castanon, 2007). In China, the use of antibiotics in feed was also completely banned in July 2020. Traditional method of adding sub-therapeutic concentrations antibiotics into aqua-feeds to improve feed utilization and weight gain has been limited (Momeni-Moghaddam et al., 2015). So the search for alternative growth and health promoters is necessary. Recently, it is an attractive strategy for feeding various probiotics and prebiotics to enhance the growth performance of aquatic animals (Momeni-Moghaddam et al., 2015). Yeast culture (YC), as a healthy and green biological product, has received heightened attention

(Bu et al., 2019; Ayiku et al., 2020; Burgents et al., 2004; Bai et al., 2022). It contains not only peptide, amino acid, vitamin, but also unknown growth factor and a variety of functional factors such as mannan oligosaccharide (MOS), nucleotide and  $\beta$ -glucan (Zhang et al., 2018). These components ( $\beta$ -glucan, nucleotide, and mannose oligosaccharide) as immunomodulatory constituents could not only enhance the antioxidant capacity of fish, but also act as signaling molecules to regulate the related signaling pathways to adjust immune responses. Some studies have found that YC can improve the antioxidant capacity and immunity of aquatic animals such as gibel carp (*Carassius auratus gibelio*) and grass carp (*Cenopharyngodon idellus*) (Liu et al., 2018; Zhang et al., 2020a). Furthermore, the positive effects of YC on growth performance have also been reported in *Pseudobagrus ussuriensis* and *Pelteobagrus fulvidraco*

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(Hou et al., 2022; Cheng et al., 2019). Previous studies have found that yeast products may promote the release of digestive enzymes, enhance the digestion of nutrients and ultimately improve the growth (Hou et al., 2022; Wang et al., 2022a). It is well known that the gut microbiota has an important effect on animal growth and its health, but there are no enough studies on the effect of YC on the gut microbiota of aquatic animals.

With technological advances in culture-independent techniques, the association between the gut microbiota and host's growth performance has received increasing attention. Studies have shown that some intestinal microbiota are extensively involved in the digestion of food and provide nutrients for the host (Zhang et al., 2022). Carbohydrates and peptides could be fermented by gut microbes into nutrients available to the host (Wang et al., 2021; Karine et al., 2018). Moreover, some functional substances such as vitamin, short-chain fatty acids, and polyamines produced by intestinal bacteria can improve intestinal structure and inhibit the expression of pro-inflammation factors (Timmons et al., 2012; Chen et al., 2015; Itziar et al., 2016). However, some genera of Proteobacteria such as *Aeromonas* and *Burkholderia* may be pathogenic that can increase the production of inflammatory cytokines in human (Emily and Tracy, 2012) and decrease the growth performance of animals (Zhang et al., 2022). Feeding probiotics and prebiotics is an important method for regulating the composition of the intestinal flora to improve intestinal function and growth performance in aquatic animals (Meng et al., 2021; Liu et al., 2018). Numerous studies have reported that yeast products can increase the richness and diversity of gut microbiota, promote the growth of beneficial bacteria, which have a positive effect on animal growth and immunity (Wang et al., 2022a; Zhang et al., 2020a).

Common carp (*Cyprinus carpio*) is the major economic fish in the world, and the production was more than 4 million tons, contributing about 4.2 % to the global aquaculture production (FAO, 2024). The ban of antibiotic growth promoters in aquaculture may influence the total production of common carp. Therefore, it is necessary to find some effective substitutes to improve the health of fish and maintain the efficiency of aquatic production. Recently, some yeast productions as novel dietary supplements have attracted more and more attention in improving fish health and growth (Liu et al., 2018; Zhang et al., 2020a; Yuan et al., 2017; Bu et al., 2019). However, information on the effect of YC on growth performance of *Cyprinus carpio* has not been available until now. Hence, this experiment was designed to investigate the effect of YC on growth performance, intestinal microbiota and digestive ability of common carp, and as well as their interrelationships. Findings from the study provide a scientific basis for the selection of healthy and green growth promoting additives and lay a foundation for the improvement of health diets.

## 2. Materials and methods

### 2.1. Diets and feeding trial

Four isonitrogenous and isolipid (340 g/kg crude protein and 57 g/kg crude fat) experimental diets, containing 0, 10, 20,30 g/kg YC (named as Y0, Y1, Y2 and Y3), were designed to investigate the effect of YC on growth performance, intestine microbiota and digestion of common carp (Table 1) according to previous studies (Bu et al., 2019; Hou et al., 2022). The dry ingredients were ground, sifted through 40 mesh and were mixed with vitamins and minerals by the progressive enlargement method. Water and lipid were added and thoroughly mixed using a blending machine, and then was pressed into 1.5 mm by a laboratory extruder (QL, Henan, China) at room temperature. Finally, the diets were dried in 45 °C oven for 6 h and stored at -20 °C until used.

Common carp from Fisheries Research Institute of Harbin Academy of Agricultural Sciences (Harbin, China) were fed Y0 diet to acclimate the experimental condition for 2 weeks. And then 360 healthy fish were randomly divided into four groups, with each group having three

**Table 1**

Formulation and proximate composition of the test diet (g/kg of dry matter).

Ingredients	Experimental diet (g/kg)			
	Y0	Y1	Y2	Y3
Fishmeal	80	70	60	50
Soybean meal	300	300	300	300
Rapeseed meal	100	100	100	100
Cottonseed meal	50	50	50	50
Corn gluten meal	80	80	80	80
Wheat bran	77	77	77	77
Soybean oil	55	55	55	55
Wheat flour	220	220	220	220
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	20	20	20	20
Choline	3	3	3	3
Vitamin and mineral premix <sup>a</sup>	10	10	10	10
Yeast culture	0	10	20	30
Cr <sub>2</sub> O <sub>3</sub>	5	5	5	5
Proximate composition (% dry matter)				
Crude protein	34.78	34.53	34.36	34.03
Crude lipid	5.77	5.71	5.79	5.74
Moisture	7.23	7.12	7.24	7.12
Crude ash	7.61	7.57	7.46	7.45

<sup>a</sup> Vitamin and mineral mixture were prepared according to Luo et al. (2019).

replicates, and each replicate containing 30 fish. The fish were hand-fed diets to apparent satiation three times a day (08:00, 12:00 and 17:30) for 8 weeks. The water in the tanks (1.0 × 0.5 × 0.6 m, water depth about 0.4 m) was changed once every two days to maintain ammonia-N < 0.1 mg L<sup>-1</sup>. And the temperature and pH were controlled at 24 ± 1°C and 7.0 ± 0.5 respectively. The dissolved oxygen level was maintained above 7 mg L<sup>-1</sup> by continuous aeration in each tank. The photoperiod was set at 12-hours light and 12-hours dark.

### 2.2. Sample collection

All fish in each tank were weighted at the beginning of the experiment. After an 8-week feeding period, the fish were fasted for 24 h. Subsequently, all fish in each aquarium were weighted and counted for calculating the weight gain rate (WGR), specific growth rate (SGR), conversion ratio (FCR) and protein efficiency ratio (PER). Fish from each tank were anesthetized with eugenol (diluted at 1:12000, purchased from Shanghai Reagent Corporation, Shanghai, China) and the weight of viscera and liver tissue were determined. The intestinal samples of three fish per groups were immediately frozen in liquid nitrogen and stored at -80 °C until used. Another three intestinal samples of fish from each group were fixed at 10 % neutral formalin solution for histological analysis.

### 2.3. Analysis and measurement

#### 2.3.1. Proximate composition analysis

The proximate analyses of both feces and diets were carried out in line with the published standards (AOAC, 2000). To measure the dry - matter content, the samples were dried at 105°C until a constant weight was achieved. The crude protein content was determined via the Kjeldahl method. An Auto Kjeldahl System (2300 - Auto - analyzer; FOSS, USA) was employed for this measurement. For the measurement of crude lipid, the Soxhlet extraction method was utilized. As for the ash content, the samples were put into a muffle furnace at 550°C for 24 h for the measurement.

#### 2.3.2. Apparent digestibility analysis

Chromium oxide (Cr<sub>2</sub>O<sub>3</sub>) as an inert indicator was added into diets to estimate the apparent digestibility of dry according to the method of Wang et al. (2009). Feces were collected from the 7th day after the experiment. Feces were collected at 2–4 h after feeding from the bottom of the tank using a siphon for determining apparent digestibility. The

collected feces were stored at  $-20^{\circ}\text{C}$  until used. The  $\text{Cr}_2\text{O}_3$  content of samples was determined by using the method described by Che et al. (2017).

### 2.3.3. Assay for activity of intestinal digestive enzymes

Intestinal digestive enzymes containing trypsin (A080–2–2), lipase (A054–2–1) and alpha-amylase (C016–1–1) were determined by commercial reagent kits (Nanjing Jiancheng Bioengineering Institute, China).

### 2.3.4. Histological analysis

Intestinal samples were preserved in 10 % neutral formalin solution for 48 h, dehydrated in a graded ethanol series, embedded in paraffin. The  $5\ \mu\text{m}$  were stained with hematoxylin-eosin (HE) and observed with an Echo Revolve FL microscope. In our study, for each intestinal region of every fish under each treatment, we observed 3 sections and obtained the data on intestinal villus height (VH), villus width (VW), and muscle thickness (MT) using Image - Pro Plus 6.3 software.

### 2.3.5. Microbiome analysis

After 8-week feeding trial, three hindgut feces from each group were carefully isolated and frozen until the samples were sent to Shanghai Personal Biotechnology Co., Ltd for analysis of the intestinal microbiota. The V3 – V4 region of 16S rRNA was amplified by PCR using forward primer 338 F (5'-ACTCCTACGGGAGGAGCA-3') and reverse primer 806 R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR amplification was carried out on the Illumina MiSeq sequencing platform (Illumina, San Diego, CA, USA) using the NovaSeq-6000 sequencing strategy. The sequencing data were denoised using QIIME2 DADA2 analysis, and then clustered into amplicon sequence variant (ASV) or operational taxonomic unit (OTU) by using Vsearch software analysis. The ASV and OTU were used to analyze the composition of phylum and genus, alpha diversity and beta diversity. And Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) is based on the genetic information on the OTU to predict the full spectrum of the gene function. PICRUSt2 was used to predict the metabolic function of rumen microorganism base on Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs (KO) in this study.

## 2.4. Statistical analysis

All data were analyzed using SPSS software (version 25). The variance homogeneity of the data was assessed using the Levene's equal variance test. One-way ANOVA and Duncan's multiple comparison test were used to determine significant differences between means for data satisfying a normal distribution, and otherwise the Kruskal-Wallis test was used. The results are expressed as mean  $\pm$  standard deviation (SD) and the bar with superscript letters or asterisks indicating differences between groups. The value of statistical significance was set at  $P < 0.05$ .

## 2.5. Calculation and statistical analysis

The following variables were calculated:

$$\text{Weight gain rate (WGR, \%)} = 100 \times (W_t - W_0) / W_0$$

$$\text{Specific growth rate (SGR, \% day}^{-1}\text{)} = (\ln W_t - \ln W_0) \times 100 / t$$

$$\text{Feed conversion ratio (FCR)} = \text{feed consumed} / \text{weight gain}$$

$$\text{Condition factor (CF, g/cm}^3\text{)} = (\text{body weight}) / (\text{body length})^3 \times 100.$$

$$\text{Protein efficiency ratio (PER)} = \text{wet weight gain} / \text{crude protein intake}$$

$$\text{Apparent digestibility coefficient (ADC) of dry matter (\%)} = 100 \times (1 - D_{\text{Cr}} / F_{\text{Cr}})$$

$$\text{Apparent digestibility coefficient (ADC) of nutrition (\%)} = 100 \times [1 - (F/D \times D_{\text{Cr}} / F_{\text{Cr}})]$$

$$\text{Hepatosomatic index (HSI, \%)} = (\text{hepatopancreas weight} / \text{whole body weight}) \times 100.$$

$$\text{Viscera somatic index (VSI, \%)} = (\text{viscera weight} / \text{whole body weight}) \times 100.$$

where  $W_t$  and  $W_0$  are final and initial body weight, respectively,  $D_{\text{Cr}}$  and  $F_{\text{Cr}}$  represent  $\text{Cr}_2\text{O}_3$  contents in diet and feces, respectively.  $D$  and  $F$  represent nutrient contents in diet and feces, respectively.

## 3. Results

### 3.1. Growth performance

The effects of dietary YC on growth performance of common carp were shown in Table 2. The initial body weights of common carp in each group were not significantly different, and after 8 weeks of feeding the experimental diets, the final body weight, weight gain, protein efficiency ratio and specific growth rate in Y1 group was significantly higher than Y0, Y2 and Y3 groups ( $P < 0.05$ ), but no significant difference was found among Y0, Y2 and Y3 groups ( $P > 0.05$ ), noticeably, fish in Y3 group had the lowest final body weight, weight gain, protein efficiency ratio and specific growth rate. For the feed conversion ratio, the significantly lower value was observed in Y1 group than that in Y0, Y2 or Y3 group ( $P < 0.05$ ), and no significant differences was observed among Y0, Y2 and Y3 groups ( $P > 0.05$ ). In addition, there were no significant differences in condition factor, hepatosomatic indices and viscerosomatic index among all the groups ( $P > 0.05$ ). These results revealed that dietary 10 g/kg YC could improve the growth performance of common carp whereas dietary excessive level of YC might weaken the positive effect on growth.

### 3.2. Apparent digestibility

The apparent digestibility of dry matter, crude protein and crude lipid are shown in Table 3. Fish fed diets with YC had higher apparent digestibility of dry matter and crude lipid than fish fed diets without YC ( $P < 0.05$ ), and the highest value was observed in Y1 group ( $P < 0.05$ ).

**Table 2**  
Growth performance of common carp fed different levels of YC.

Index	Diet			
	Y0	Y1	Y2	Y3
IBW (g)	12.5 $\pm$ 0.15	12.1 $\pm$ 0.28	12.80 $\pm$ 0.09	12.61 $\pm$ 0.12
FBW (g)	32.28 $\pm$ 0.62 <sup>a</sup>	38.75 $\pm$ 2.0 <sup>b</sup>	32.33 $\pm$ 1.08 <sup>a</sup>	30.69 $\pm$ 0.80 <sup>a</sup>
WG (%)	158.28 $\pm$ 4.94 <sup>a</sup>	180.93 $\pm$ 6.12 <sup>b</sup>	158.64 $\pm$ 8.67 <sup>a</sup>	145.53 $\pm$ 6.44 <sup>a</sup>
FCR	1.80 $\pm$ 0.07 <sup>b</sup>	1.33 $\pm$ 0.07 <sup>a</sup>	1.77 $\pm$ 0.04 <sup>b</sup>	1.73 $\pm$ 0.07 <sup>b</sup>
SGR (% per day)	1.69 $\pm$ 0.03 <sup>a</sup>	1.82 $\pm$ 0.09 <sup>b</sup>	1.70 $\pm$ 0.06 <sup>a</sup>	1.60 $\pm$ 0.05 <sup>a</sup>
PER	1.64 $\pm$ 0.10 <sup>a</sup>	1.90 $\pm$ 0.15 <sup>b</sup>	1.63 $\pm$ 0.05 <sup>a</sup>	1.60 $\pm$ 0.19 <sup>a</sup>
CF (g/cm <sup>3</sup> )	2.70 $\pm$ 0.10	2.57 $\pm$ 0.89	2.60 $\pm$ 0.07	2.65 $\pm$ 0.06
VSI (%)	9.54 $\pm$ 0.54	9.68 $\pm$ 0.28	9.72 $\pm$ 0.27	9.98 $\pm$ 0.29
HIS (%)	2.44 $\pm$ 0.25	1.93 $\pm$ 0.17	2.14 $\pm$ 0.17	1.97 $\pm$ 0.17

Note. Data represent as mean  $\pm$  SD. Values in the same row with different superscripts are significantly different ( $P < 0.05$ ). PER: Protein efficiency ratio; CF: condition factor; FBW: final body weight; FCR: feed conversion ratio; HSI: hepatosomatic index; IBW: initial body weight; SGR: specific growth rate; VSI: viscerosomatic index; WG: weight gain.

**Table 3**  
Apparent digestibility coefficients (ADC) of nutrient of common carp fed diets with different levels of YC.

Index	Diet			
	Y0	Y1	Y2	Y3
ADC <sub>d</sub> (%)	61.68 ± 2.65 <sup>a</sup>	70.16 ± 0.21 <sup>b</sup>	69.74 ± 2.89 <sup>b</sup>	67.51 ± 1.19 <sup>b</sup>
ADC <sub>l</sub> (%)	56.30 ± 6.09 <sup>a</sup>	72.02 ± 6.21 <sup>b</sup>	65.97 ± 4.89 <sup>b</sup>	68.09 ± 4.47 <sup>b</sup>
ADC <sub>p</sub> (%)	73.59 ± 2.14 <sup>a</sup>	82.46 ± 3.33 <sup>b</sup>	75.19 ± 0.24 <sup>a</sup>	76.68 ± 1.16 <sup>a</sup>

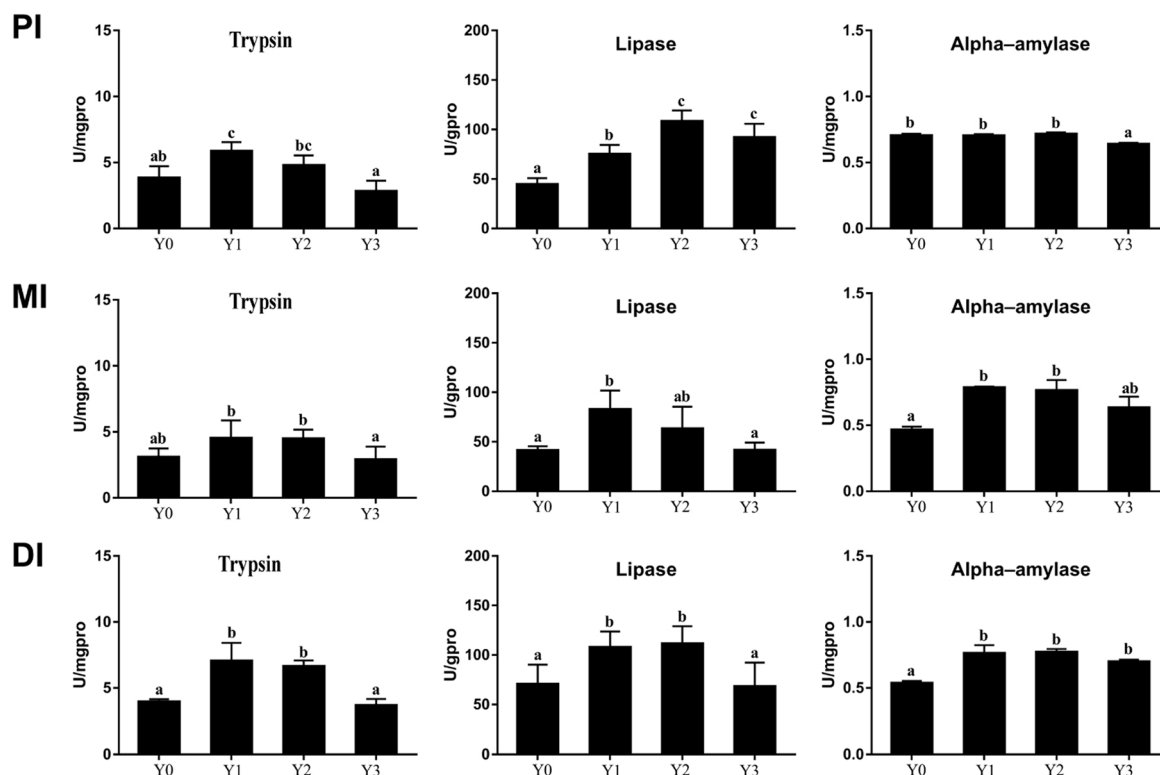
Note. Values in the same row with different superscripts are significantly different ( $P < 0.05$ ). ADC<sub>d</sub>: Apparent digestibility coefficient of dry matter; ADC<sub>l</sub>: Apparent digestibility coefficient of lipid; ADC<sub>p</sub>: Apparent digestibility coefficient of protein.

However, for the crude protein digestibility, the fish in Y1 group had significantly higher value than fish in Y0, Y2 and Y3 groups, but there were not significantly different among Y0, Y2 and Y3 groups. These

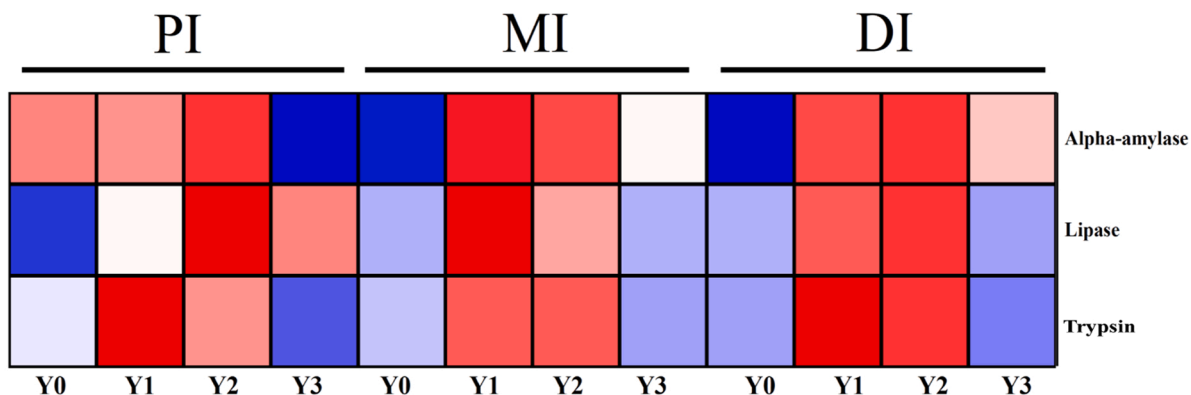
results showed that dietary 10 g/kg YC can significantly improve nutrients availability.

### 3.3. Activity of digestive enzymes

As shown in Fig. 1, dietary YC supplementation for common carp had significant effects on intestinal digestive enzymes activities. The activities of trypsin in proximal intestine (PI), mid intestine (MI) and distal intestine (DI) showed a trend of “low-high- low” pattern with the increase of dietary YC supplementation level, and dietary 10 g/kg YC supplementation had the highest activity of trypsin ( $P < 0.05$ ). The similar trend was also showed in the lipase activities in PI, MI and DI, and fish in Y1 and Y2 had higher activity of lipase than fish in Y0 and Y3 except for PI in Y3. The alpha - amylase activities of MI and DI in dietary YC supplementation groups were significantly higher than that in Y0 group except for MI in Y3 ( $P < 0.05$ ). However, the alpha - amylase



### Intestinal digestive enzymes



**Fig. 1.** The activity of intestinal digestive enzymes in proximal intestine (PI), mid intestine (MI) and distal intestine (DI), respectively, of common carp fed diets with different levels of YC. Values are presented means ± SD. Values with different superscript letters represent significantly different ( $P < 0.05$ ).

activity in PI in Y3 group was significantly lower than that in any other group ( $P < 0.05$ ). These results indicated that dietary appropriate YC supplementation has a positive effect on the activities of intestinal digestive enzymes whereas dietary excessive level of YC might weaken the positive effect on intestinal digestive enzymes activities.

### 3.4. Intestinal morphometric measurement

The histological morphology of three intestinal segments of common carp were shown in Fig. 2. Fish in Y1 and Y2 group increased the villus length of MI and DI than fish in Y0 group, but fish in Y3 group significantly decreased the villus length of DI than fish in Y0 group ( $P < 0.05$ ). Meanwhile, dietary 10 g/kg YC supplementation also significantly increased the villus width in PI and DI, as well as muscle thickness in MI and DI than dietary no YC supplementation ( $P < 0.05$ ). The results showed that dietary appropriate YC supplementation may promote intestinal development and increase intestinal nutrient absorption area.

### 3.5. Intestinal microbiota analyses

To investigate the effect of YC on intestinal flora of common carp, we collected the feces and analyzed their intestinal microbiota changes by high-throughput sequencing. A total of 1370541 valid sequences were obtained for 12 samples in the present study. And, there were 4962 OTUs identified at the 97 % homology cutoff. The rarefaction curves (Fig. 3C) tended to approach the saturation plateau, illustrating that the microbial data in this study was reliable. The data also showed that the number of OTU was increased with the increase of YC addition amounts. The Venn diagram exhibited that there were 115 OTUs shared by four treatment groups, and 194, 362, 1142 and 1312 unique OTUs for the Y0, Y1, Y2 and Y3 groups respectively (Fig. 3B). Alpha diversity indices are presented in Fig. 3A. We observed that the richness index (Chao1 and observed species) and community diversity index (Shannon and Simpson) were increased with increasing dietary YC supplement level from 0 g/kg to 30 g/kg, and especially the change of Shannon index is significantly different ( $P < 0.05$ ). The good coverage showed no

discrimination among four groups ( $P > 0.05$ ). Next, the principal coordinate analysis (PCoA) based on unweighted UniFrac distances was used to study the similarity of microbial communities of fish fed diet with different YC level. As shown in Fig. 4, the points of the Y0 groups are clustered together, while the points of the Y1, Y2 and Y3 groups are relatively scattered. Notably, the most points of Y2 and Y3 groups are relatively far from Y0 group. These findings indicated that the microbiota profiles of common carp might be changed by YC.

We analyzed the relative abundances of the bacteria community composition at the main taxonomic levels among four groups. At the phylum levels (Fig. 5A), we found that the relative abundance of dominant bacteria in four groups were Fusobacteriota, Proteobacteria, Tenericutes, Bacteroidetes, Cyanobacteria and Firmicutes. Compared with the Y0 group, fish in Y1, Y2 and Y3 groups showed decreased abundances of Fusobacteriota and increased abundances of Proteobacteria, Bacteroidetes, Cyanobacteria and Firmicutes, especially in Y3 groups, the abundances of Fusobacteriota and Proteobacteria were significantly changed ( $P < 0.05$ ). At the genus levels (Fig. 6B), the abundant bacterial species in common carp were *Cetobacterium*, *Rhodobacter*, *Aeromonas*, *Burkholderia* and *Polynucleobacter*. With increasing dietary YC supplement level from 10 g/kg to 30 g/kg, the bacterial relative abundance of *Cetobacterium* was decreased, and the bacterial relative abundance of *Rhodobacter*, *Aeromonas* and *Burkholderia* were increased ( $P > 0.05$ ). The results showed that the increase of dietary YC supplementation levels may be more suitable for the growth of intestinal Proteobacteria.

We also used the linear discriminative analysis (LDA) effect size (LEfSe) biomarker discovery tool to identify taxonomic differences among four groups (Fig. 6). The biomarkers of bacterial taxa for the Y0 group were *Cetobacterium* (from genus to phylum levels). No biomarker of bacterial taxa was found in Y1 group. In Y2 group, the biomarkers were *Kerstersia* and *Akkermansia*. And one phylum, one class, five orders, three families and three genera were enriched in the Y3 group, including Proteobacteria (from genus to phylum levels), *Bdellovibrio* (from genus to class levels) and *Aggregatibacter* (from genus to class levels), Enterobacteriales (from family to class levels), Rhodoferrax and

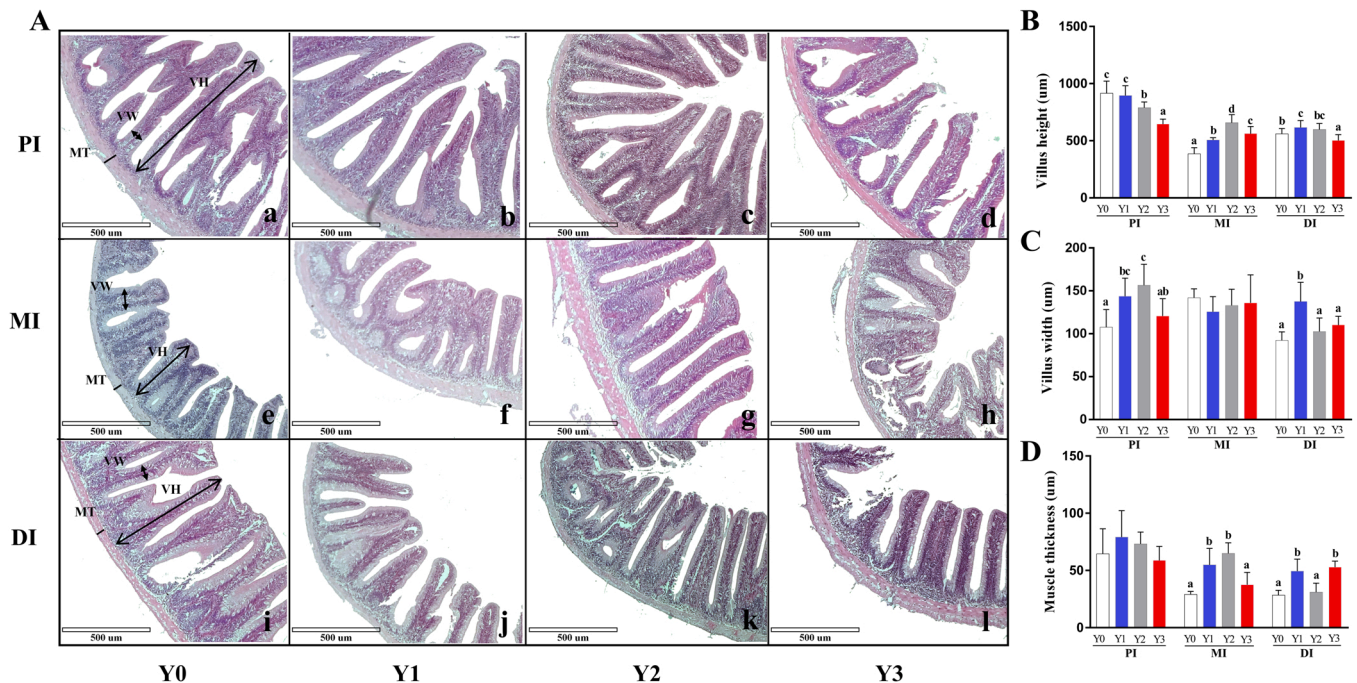
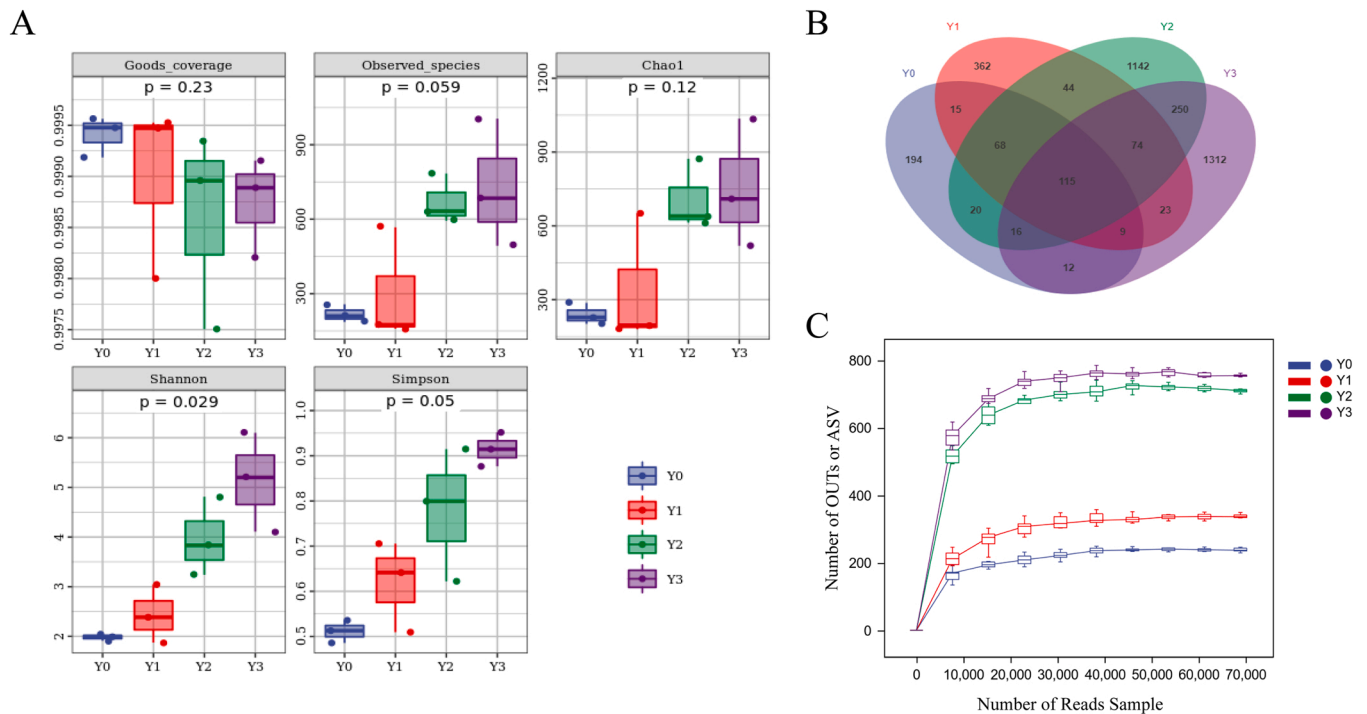
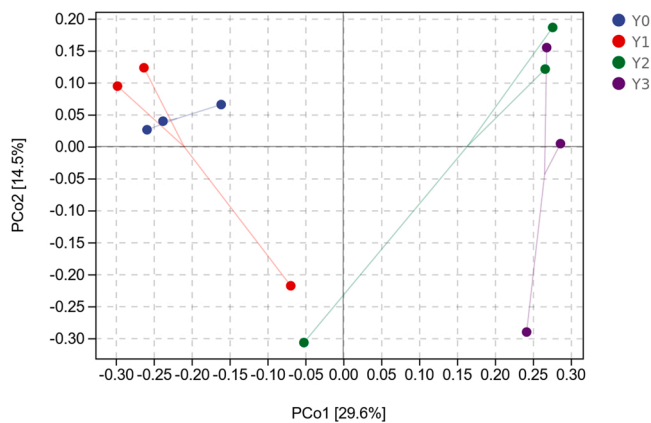


Fig. 2. (A) The intestinal histology of PI, MI and DI of common carp fed diets with different levels of YC. (a - d) PI of common carp in Y0, Y1, Y2 and Y3 groups respectively. (e - h) MI of common carp in Y0, Y1, Y2 and Y3 groups respectively. (i - l) DI of common carp in Y0, Y1, Y2 and Y3 groups respectively. Intestinal morphology analysis of VH (B), VW (C) and MT (D) in fish fed diets with different levels of YC for 8 weeks. Notes: PI, proximal intestine; MI, mid intestine; DI, distal intestine; VH, villus height; VW, villus width; MT, muscle thickness. Values with different superscript letters significantly different ( $P < 0.05$ ).



**Fig. 3.** The diversity and richness indices of bacteria communities (A), Venn diagram (B) and Rarefaction curves of samples (C) of common carp fed diets with different levels of YC.



**Fig. 4.** Principal coordinates (PCoA) analysis of the intestine microbial communities in fish fed diets with different levels of YC (based on unweighted UniFrac distances).

MIZ46 (class).

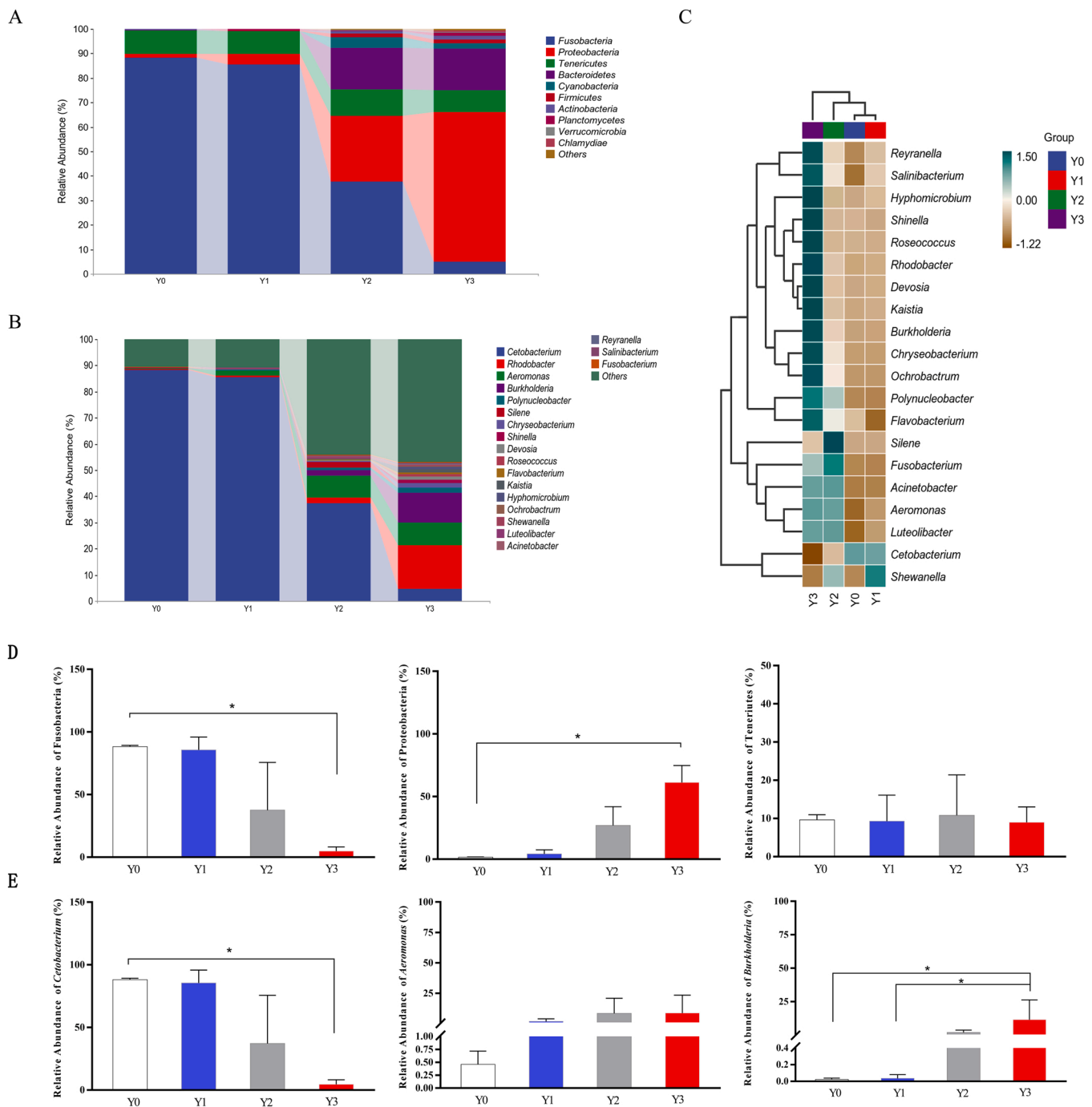
Finally, we used PICRUST2 (Fig. 7) to predict the gut microbial metabolic function. The relative abundance of lipid metabolism and amino acid metabolism (annotated to KEGG level 2) consistently increased with YC supplementation from 10 g/kg to 30 g/kg, while nucleotide metabolism decreased ( $P < 0.05$ ). The pathways of lysine biosynthesis, cysteine and methionine metabolism were significantly downregulated with the increase of YC supplemental level at KEGG level 3.

#### 4. Discussion

Probiotics and prebiotics have been demonstrated to be an effective way to improve the growth performance and health status of aquatic animals (Gibson and Roberfroid, 1995). YC, as a healthy and green biological product, has been proved to improve the growth and gut

health in grass carp and Pacific white shrimp (Liu et al., 2018; Ayiku et al., 2020). In the present study, an evaluation of YC supplementation in common carp was conducted based on growth, gut microbiota and digestive ability. The results showed that dietary 10 g/kg YC significantly increased the apparent digestibility of lipid and protein as well as the growth performance. Besides, dietary 10 g/kg YC promotes the intestinal digestive enzyme activities of trypsin and lipase and increases intestinal villus height and width.

The intestinal microbial components play an important role in the gut immune system, digestion and absorption of auxiliary food and the promotion of nutrient metabolism (Maarten et al., 2018). Several reports have confirmed that probiotics and prebiotics could regulate the diversity and composition of the intestinal microbiota (Ringø et al., 2016). YC, as a mixture formed by yeast fermentation, contains a variety of functional substances such as peptides, amino acids, vitamin A, vitamin B, MOS, nucleotides and  $\beta$ -glucan, which can effectively increase the diversity and richness of the intestinal flora (Wang et al., 2022b; Vera et al., 2022). Present study found that dietary 10, 20 and 30 g/kg YC supplementation also could increase the diversity and richness of intestinal microbiota. Similar study was also reported by Zheng et al. (2020), who found that dietary 20 g/kg yeast extract could significantly enhance intestinal microbial diversity of *Litopenaeus vannamei* and also significantly increase the relative abundance of Proteobacteria. The present study showed that the relative abundance of Proteobacteria also increased significantly in the Y3 group compared to the control group, while the relative abundance of Fusobacteria decreased significantly. Zhang et al. (2020b) suggested that yeast is not the dominant intestinal group in the gut of freshwater fish and its metabolites may inhibit the growth and reproduction of *Cetobacterium*. The reduction of the relative abundance of the *Cetobacterium* may promote the growth and reproduction of facultative anaerobe such as Proteobacteria through altering the anaerobic environment of the gut (Rivera-Chávez et al., 2016) that may lead to the risk of diseases and reduce growth performance (Li et al., 2019; Zhang et al., 2022). Yousefi et al. (2020) believe that higher levels of dietary yeast cell wall (the main components are MOS and beta-glucan) can damage the liver and



**Fig. 5.** The composition of the gut microbial community in fish fed diets with different levels of YC at phylum levels (A) and genus levels (B). (C) Heatmap analysis of intestine microbial on the top 20 genera. The significant differences of main phylum and genus in gut microbial are showed in D and F respectively and asterisks indicate significant differences among different groups (\* $P < 0.05$ , \*\*\* $P < 0.001$ ).

have toxic effects on the organism, possibly resulting in growth inhibition. Indeed, our results complement and extend these previous findings and also provide information on the inhibition of growth performance by the excessive addition of YC, and the adverse impact may be associated with the imbalance of intestinal flora and intestinal dysfunction. Functional prediction of intestinal bacteria by PICRUSt at KEGG level 3 showed that the genes related to lysine biosynthesis, cysteine and methionine metabolism were significantly lower in the Y3 group compared to the Y1 and Y0 groups, which may also lead to the reduced growth performance of the Y3 group. The structure of the intestinal flora between the Y0 and Y1 groups was not significantly different, but the growth performance of Y1 group was significantly higher than that of Y0

group. These means that the improvement of YC on growth performance in common carp was related to the improvement of intestinal digestive enzymes and intestinal structure but not gut microbial composition. However, Ayiku et al. (2020) conducted a similar study in *Litopenaeus vannamei* and received the opposite results. The difference may be due to different fish species, diet formulation and feeding strategy. Common carp is a fish without stomach, and digestive capacity is partly determined by the activity of digestive enzymes. The activity of fish digestive enzymes plays an important role in the digestion and absorption of nutrients. Interestingly, there were significant improvements in protease, lipase and amylase activities in three segments of intestine in fish fed diet with 10 and 20 g/kg YC. Zhang et al. (2020c) also found that

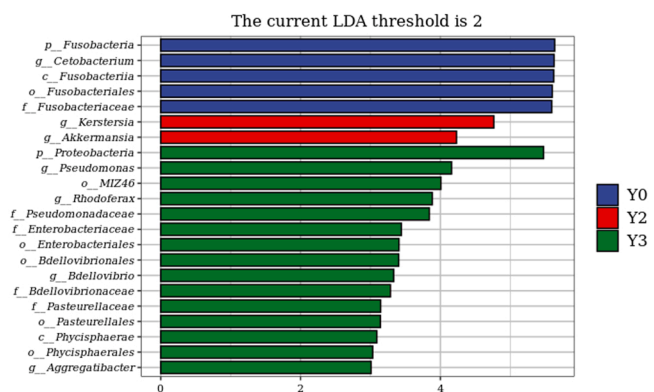


Fig. 6. Linear discriminant analysis (LDA) score were computed for the differential richness of biomarker taxa using LEfSe in the common carp fed diets with different levels of YC.

dietary YC supplementation significantly increased the activities of chymotrypsin and  $\alpha$ -amylase in duodenal chyme of aged laying hens. Yann et al. (2006) indicated that whole yeast and yeast component may improve pancreatic function and promote digestive synthesis. In addition, the composition of glutamine in hydrolyzed yeast also has been shown to improve the activity of mucosal digestive enzymes and absorption function in animals (Boontiam et al., 2022). Otherwise, early study reported that intestinal microbial composition could also affect intestinal digestive functions (Kim et al., 2022). It was reported that microorganisms synthesizing peptidases hydrolyze the same substrates as the enzymes synthesized by the fish digestive tract and the relative activity of peptidases of intestinal microbiota constitutes 20–40 % of the maximum activity of fish own enzymes (Kuz'mina et al., 2015). For instance, *Cetobacterium* is widely and common present in guts of many freshwater fishes, and involved in fermentation of amino acids and carbohydrates and proteolytic as well as synthesis of vitamin B12 that improve host protein digestion efficiency. (Tsuchiya et al., 2008). In the present study, dietary 30 g/kg YC group decreased the relative abundance of *Cetobacterium* compared with the Y0 and Y1 groups, which may

prevent the utilization of protein by fish in Y3 group. In addition, lipase activity in Y3 group was also significantly lower than that in Y1 group, and the reason may be that the higher relative abundance of *Aeromonas* and *Burkholderia* of Proteobacteria in Y3 group. Some studies have suggested that *Aeromonas* and *Burkholderia* as pathogenic bacteria can trigger an immediate and robust inflammatory response (Emily and Tracy, 2012; Zhou et al., 2021), causing intestinal ecological imbalances and metabolic disturbances, ultimately affecting nutrient uptake and utilization by fish (Wang et al., 2022c).

Additionally, intestinal structure was also strongly related to the digestion and absorption of nutrients (Zhang et al., 2022). Ayiku et al. (2020) indicated that the intestinal structural integrity was the basic reflection of nutrition absorption functions. It is known that villi height and width were associated with absorptive area and nutrient uptake efficiency. In the study, the morphological parameters (villi height and width) in three intestinal segments were also improved by dietary 10 g/kg YC supplement compared with Y0 and Y3 groups, illustrating the enhancement of digestive and absorptive area of the brush border membrane. Similar results were also found in a previous study that dietary YC supplementation could significantly improve intestinal villus height and width of hybrid grouper (*♀Epinephelus fuscoguttatus* × *♂E. lanceolatus*) (Wang et al., 2022a). Meanwhile, we also found that dietary 10 g/kg and 20 g/kg YC still increased the MT in MI, which was in consistent with manifestation investigated by Bian et al. (2021). The elevated level of VH, VM, and MT suggests that dietary YC supplementation may have positive effects on intestinal health in common carp. In addition, the upregulation of the relative abundance of Firmicutes, and Bacteroidetes in the intestine indicated a positive effect on the synthesis of endogenous polyamine after YC supplementation (Pegg and Michael, 2010). Previous study demonstrated that the release of polyamines in intestinal lumen of piglets could be improved by yeast, which may promote intestinal epithelial cell growth and differentiation (Zhao et al., 2021).

The digestibility of dry matter, crude protein and crude lipid could reflect the utilization of fish to feedstuff. In the present study, the results showed that fish in Y1 group had significantly higher apparent digestibility coefficient (ADC) of dry matter, crude protein and crude lipid than fish in Y0 group. However, with the further increase of YC additive

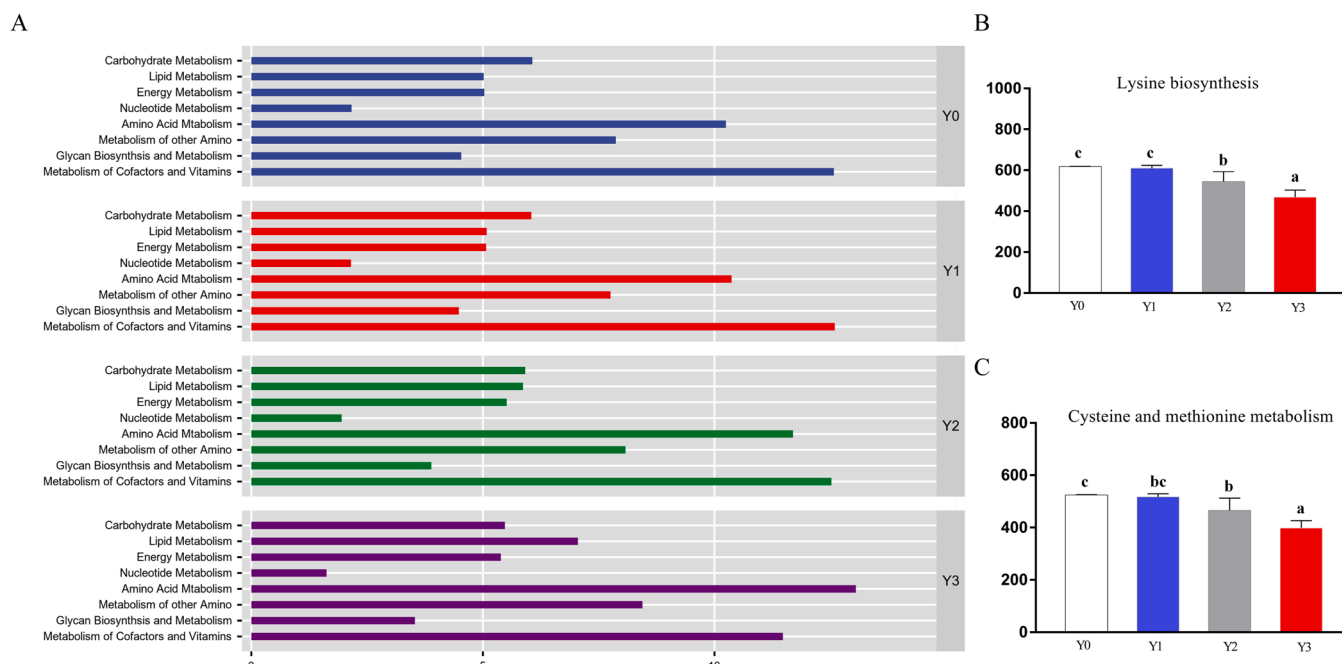


Fig. 7. Functional prediction of related pathways at level 2 (A) and level 3 (B) in fish fed different levels of YC. Values with different superscript letters significantly different ( $P < 0.05$ ).

level, the apparent digestibility coefficient showed a downward trend, which is consistent with the changes of digestive enzymes activities and intestinal morphology. Therefore, the decrease in digestive enzyme activities and the VH, VM and MT of intestine may be responsible for the reduction of apparent digestibility of dry matter, crude protein and crude lipid in fish in Y3 group. Many studies have reported the beneficial effects of YC on growth performance and health of aquatic animals (Bu et al., 2019; Ayiku et al., 2020). In this study, our results also showed significant improvement of growth performance in fish fed 10 g/kg YC as demonstrated by increasing SGR, WGR, FD and decreasing FCR compared with the control group, simultaneously, the decrement of growth performance was observed in fish fed 30 g/kg YC. Similar results were reported by Gong et al. (2019) who found that the addition of 15 g/kg YC to the diet promoted the growth performance of largemouth bass (*Micropterus salmoides*), but the growth performance decreased when YC was added at the level of 30 g/kg. The reason may be that dietary 30 g/kg YC supplementation increased the relative abundance of Proteobacteria in the intestine, which may lead to disorders of intestinal metabolism, destruction of intestinal structure and reduction of digestive enzyme activities, eventually reducing growth performance. These results revealed that dietary 10 g/kg YC could improve the growth performance of common carp whereas excessive levels of YC might weaken the positive effect on growth. On the basis of this trial, dietary 10 g/kg YC supplementation for carp diet is recommended.

## 5. Conclusions

In summary, the present study suggests that dietary 10 g/kg YC has a positive effect on growth performance by improving feed digestibility and feed efficiency. The positive effect of dietary YC is dependent on the improvement of intestinal digestive enzyme activity and the intestinal morphology. Additionally, YC can alter the structure of the intestinal flora and intestinal metabolic function, and high levels of YC increase the relative abundance of *Aeromonas* and *Burkholderia* of Proteobacteria, further leading to intestinal ecological imbalances and metabolic disturbances, ultimately affecting nutrient uptake and utilization and growth performance for common carp. The results of this study provide a theoretical basis for the applicability of YC as a growth promoter for common carp.

## CRedit authorship contribution statement

**Jiahao Wang:** Methodology. **Shengqiang Tao:** Writing – original draft. **Wenhao Zhou:** Project administration. **Yi Wang:** Methodology. **Yuhong Yang:** Writing – review & editing. **Tingyin Liang:** Software. **Yichen Bai:** Data curation. **Zhiyuan Xue:** Software.

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## 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.

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## Data availability

Data will be made available on request.

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