Animal Nutrition 13 (2023) 50–63

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Dietary ribose supplementation improves flesh quality through purine metabolism in gibel carp (*Carassius auratus gibelio*)

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ARTICLE INFO

Article history:
Received 31 May 2022
Received in revised form
23 November 2022
Accepted 8 December 2022
Available online 31 December 2022

Keywords:
D-Ribose
Flavour
Flesh quality
Inosine monophosphate
Metabolomic
Purine metabolism

ABSTRACT

Since the aquaculture industry is currently observing a deterioration in the flesh quality of farmed fish, the use of nutrients as additives to improve the flesh quality of farmed fish species is a viable strategy. The aim of this study was to investigate the effect of dietary D-ribose (RI) on the nutritional value, texture and flavour of gibel carp (*Carassius auratus gibelio*). Four diets were formulated containing exogenous RI at 4 gradient levels: 0 (Control), 0.15% (0.15RI), 0.30% (0.30RI) and 0.45% (0.45RI). A total of 240 fish (150 ± 0.31 g) were randomly distributed into 12 fibreglass tanks (150 L per tank). Triplicate tanks were randomly assigned to each diet. The feeding trial was carried out in an indoor recirculating aquaculture system for 60 d. After the feeding trial, the muscle and liver of gibel carp were analysed. The results showed that RI supplementation did not result in any negative impact on the growth performance and 0.30RI supplementation significantly increased the whole-body protein content compared to the control group. The contents of collagen and glycogen in muscle were enhanced by RI supplementation. The alterations in the flesh indicated that RI supplementation improved the texture of the flesh in terms of its water-holding capacity and hardness, therefore improving the taste. Dietary RI facilitated the deposition of amino acids and fatty acids in the muscle that contributed to the meaty taste and nutritional value. Furthermore, a combination of metabolomics and expression of key genes in liver and muscle revealed that 0.30RI activated the purine metabolism pathways by supplementing the substrate for nucleotide synthesis and thereby promoting the deposition of flavour substance in flesh. This study offers a new approach for providing healthy, nutritious and flavourful aquatic products.

1. Introduction

Fish have become an indispensable source of food in daily life due to their delicious taste and because they are rich in nutrients. With the growing emphasis on health and nutrition, the demand for high-quality fish flesh is also increasing (Liu et al., 2020). However, the aquaculture industry is currently observing a deterioration in the quality of farmed fish that receive artificial feed (Wang et al., 2022). The use of artificial feed shortens the growth phase of farmed fish but results in insufficient levels and durations of accumulated flavour compound. Flavour is one of the principal sensory attributes of meat and is an important factor that influences whether consumers make purchases or not (Zang et al., 2020). Inosine monophosphate (IMP) is an important flavour substance in animal tissues and can increase the umami taste of foods by 40-fold compared with that of sodium glutamate (Wang et al., 2020). IMP contributes greatly to the umami of the fish flesh and has been used as an indicator of fish freshness in many countries (Cai et al., 2022). Therefore, the IMP level is an important factor that determines the quality of fish flesh. Improving the quality of fish meat by adding feed additives is an effective strategy that is worth

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2022.12.006

investigating. Wang et al. (2022) found that dietary supplementation with 0.075% glycerol monolaurate could increase the nonessential amino acid and unsaturated fatty acid contents in large yellow croaker (*Larimichthys crocea*) muscles. Dietary supplementation with hydrolysed porcine mucosa improved the muscle quality related to hardness and chewiness in hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) (Yang et al., 2022). Dietary tea polyphenols could alleviate most of the adverse effects caused by a high fat diet on flesh quality of Nile tilapia (*Oreochromis niloticus*) (Wang et al., 2021). The use of nutrients as additives deserves further research in improving the flesh quality and food safety of fish.

D-Ribose (RI) is a naturally occurring pentose with 5 sugars that form a ring structure, and it is a substrate that produces the energy substance adenosine triphosphate (ATP), which is often referred to as molecular currency because of its role in intracellular energy transfer (Mahoney et al., 2018). Ribose is a key component of numerous biomolecules that are involved in many metabolic pathways, especially the pentose phosphate pathway (PPP), nucleotide de novo pathway and glucose metabolism. Ribose participates in the formation of phosphoribosylpyrophosphate (PRPP) in the PPP, leading to the synthesis of adenine nucleotides, including ATP, adenosine monophosphate (AMP) and IMP synthesis (Damanik and Soemantri, 2020). Therefore, RI plays an important role in energy metabolism. Many examples have demonstrated that ribose supplementation improves the recovery of ATP levels and reduces cell damage in humans and animals (Mahoney et al., 2018). Another group of investigators found that ribose could provide a cellular benefit by reducing malondialdehyde levels during hypoxic stress (Seifert et al., 2009). At present, RI is mainly supplied in food and is widely recommended as a supplement for the metabolic treatment of coronary artery disease (Hong et al., 2019). However, research on the effects of RI as a feed additive on livestock, poultry and aquatic animals is limited.

Gibel carp (*Carassius auratus gibelio*) is widely cultivated in China due to its fast growth, high yield and ability to resist diseases. According to recent statistical analyses, the production of gibel carp was close to 2.8 million tonnes in China in 2020 (Fisheries Bureau, 2021). It is favoured by consumers for its tender flesh, delicious taste and high nutritional value (Cai et al., 2020). The flesh in gibel carp has a high content of flavour substances, and the usual way of cooking gibel carp is to make a soup, which releases the flavour substances in the flesh of the fish after it is boiled for a long time.

Metabolomics is a novel methodology that is being increasingly applied in the aquaculture field because it is characterized by a high sensitivity, high throughput and high resolving power (Chen et al., 2017). Metabolomics was used to explore the effects of RI as a feed additive on fish. This endeavour was a relatively novel attempt, and it may also provide in-depth mechanistic insights, which can help to more fully assess the effects of RI on fish. The purpose of the present study was to estimate the positive effects of RI as an alternative feed additive on the quality of muscle flesh. A metabolomics approach based on ultrahigh-performance liquid chromatography coupled with high-resolution mass spectrometry (LC–MS/MS) was used to reveal the potential effects of dietary RI on the molecular mechanism of gibel carp.

2. Materials and methods

2.1. Animal ethics statement

All fish experiments were conducted according to the Guiding Principles for Care and Use of Laboratory Animals and were approved by the Institute of Hydrobiology, Chinese Academy of Sciences (IHB, CAS, Protocol No. 2016-018).

2.2. Experimental diets

In the present study, 4 isonitrogenous (36% crude protein) and isolipidic (8% crude lipid) diets were formulated, and their chemical compositions are shown in Table 1. The ribose supplement levels were 0% (Control), 0.15% (0.15RI), 0.30% (0.30RI) and 0.45% (0.45RI). D-ribose was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Casein and wheat protein concentrate were used as the main protein sources. Fish oil and soybean oil were used as lipid sources. Exogenous limiting amino acids arginine and alanine were supplemented to maintain the balance of amino acids in the diet. All ingredients of each diet were ground through a particle size 100 mesh and then extruded using a win-screwed extruder (Jinan Dingrun Machinery Co., Ltd., Jinan, Shandong, China). The pellets were dried in an oven at 60 °C and then stored at 4 °C.

2.3. Fish and feeding trial

The gibel carp were obtained from Guanqiao hatchery of the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, Hubei, China). Prior to the start of the formal experiment, all fish were reared in fibreglass tanks (water volume 883 L; 150 cm in diameter; 50 cm water depth) and fed up to satiation twice a day at 08:30 and 16:30 with commercial feed. After 4 weeks of acclimatization, a total of 240 fish (mean initial weight 150 ± 0.31 g) were randomly distributed into 12 fibreglass tanks (150 L). Triplicate tanks were randomly assigned to each diet. The experimental fish were hand-fed to apparent satiation three times daily (08:30, 14:00

Table 1
Formulation and compositions of experimental diets (% dry matter).

Ingredients	Con	0.15RI	0.30RI	0.45RI
Casein[1]	20.00	20.00	20.00	20.00
Wheat protein concentrate[2]	20.00	20.00	20.00	20.00
Corn starch[3]	28.30	28.30	28.30	28.30
Fish oil	4.00	4.00	4.00	4.00
Soy oil	4.00	4.00	4.00	4.00
Mineral premix[4]	5.00	5.00	5.00	5.00
Vitamin premix[5]	0.39	0.39	0.39	0.39
Choline chloride[6]	0.11	0.11	0.11	0.11
Carboxymethyl cellulose	3.00	3.00	3.00	3.00
Monocalcium phosphate	2.00	2.00	2.00	2.00
L-Arginine[7]	0.76	0.76	0.76	0.76
L-Alanine[7]	0.60	0.60	0.60	0.60
D-Ribose[7]	0.00	0.15	0.30	0.45
Cellulose	11.84	11.68	11.53	11.38
Proximate composition				
Moisture	12.41	12.43	12.72	13.41
Crude protein	36.67	36.73	36.86	36.57
Crude lipid	7.98	7.61	7.83	7.86
Ash	5.77	5.72	5.72	5.67

[1] Casein was purchased from Gansu Hualing Casein Co., Ltd., Gansu, China.
[2] Wheat protein concentrate was purchased from Qufeng Food Technology Co., Ltd., Shandong, China.
[3] Corn starch was purchased from Yufeng Industrial Group Co., Ltd., Hebei, China.
[4] Mineral premix (mg/kg diet): NaCl, 500.0; $MgSO_4 \cdot 7H_2O$, 8,155.6; $NaH_2PO_4 \cdot 2H_2O$, 12,500.0; KH_2PO_4, 16,000.0; $Ca(H_2PO_4)_2 \cdot H_2O$, 7,650.5; $FeSO_4 \cdot 7H_2O$, 2,286.2; $C_6H_{10}CaO_6 \cdot 5H_2O$, 1,750.0; $ZnSO_4 \cdot 7H_2O$, 178.0; $MnSO_4 \cdot 4H_2O$, 61.4; $CuSO_4 \cdot 5H_2O$, 15.5; $CoSO_4 \cdot 6H_2O$, 0.91; KI, 1.5; $NaSeO_3$, 0.60; Corn starch, 899.7.
[5] Vitamin premix (mg/kg diet): thiamin, 20; riboflavin, 20; pyridoxine, 20; cyanocobalamine, 0.02; folic acid, 5; calcium patotheniate, 50; inositol, 100; niacin, 100; biotin, 5; vitamin A, 11; vitamin D_3, 2; vitamin E, 100; vitamin K_3, 10; starch, 3522.
[6] Choline chloride was composed of 50% choline chloride and 50% silicon dioxide.
[7] L-Arginine, L-Alanine, D-Ribose were purchased from Yuanye Bio-Technology Co., Ltd., Shanghai, China.

and 18:00) for 60 d. During the experiment, the photoperiod was 12 h light:12 h dark with the light period from 8:00 to 20:00. The water temperature was monitored every day and maintained at 29.5 ± 1.5 °C. The ammonia nitrogen content was less than 0.5 mg/L, and the dissolved oxygen was more than 7.5 mg/L. Ammonia nitrogen and dissolved oxygen were monitored once every week. Approximately 20% of the water in each tank was exchanged to purify the water.

2.4. Sample collection

At the termination of the trial, all fish from each tank were weighed after fasting overnight. Two fish were anaesthetized with MS-222 (100 mg/L tricaine methane sulfonate, Argent Chemical Laboratories Inc., Redmond, WA, USA). Blood was obtained from the caudal vein of fish through syringes that were rinsed with heparin sodium and was then centrifuged at 1,160 × *g* for 10 min. Plasma samples were stored at −80 °C for further analysis. After that, liver and dorsal muscle from the fish were quickly sampled and stored at −80 °C. Two fish from each tank were randomly pooled and stored at −20 °C for a final chemical composition analysis. The dorsal muscle samples were dissected from another 3 fish from each tank for a texture analysis.

2.5. Biochemical assays

The moisture, crude protein, crude lipid and ash contents of the experimental fish and diet samples were determined following the methods of Official Analytical Chemists (AOAC, 2005). The moisture content was measured by drying the samples in an oven at 105 °C until a constant weight was obtained. Crude protein content was determined by a Kjeltec Analyzer Unit (FOSS Tecator 8400, Haganas, Sweden). Crude lipids were measured via the petroleum ether extraction method using a Soxtec system (Soxtec, 2055, FOSS Tecator, Haganas, Sweden). Ash content was quantified by incineration in a muffle furnace at 550 °C for 12 h. The glycogen in dorsal muscle was measured with commercial assay kits (A043-1-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China). The total fat content of muscle was determined by the trichloromethane-methanol method, and the procedure was based on that described by Wang et al. (1993) with minor modifications. The free amino acid (FAA) levels of dorsal muscle were measured with an amino acid analyser (A300, membraPure GmbH, Germany), and the method of separating FAA from muscle was performed according to Xu et al. (2016). The determination of the fatty acid compositions in muscle was performed using a gas chromatograph-mass spectrometer (GC–MS, 7890A, Agilent Technologies, USA) equipped with a triple-axis detector and a 30 m × 0.25 mm fused silica capillary column (inert XL EI/CI MSD, 5975C, Agilent Technologies, USA), and the method of separating fatty acids from muscle was performed according to the methods described by Fei et al. (2020). The results are presented as the percentage of total fatty acids. The plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined by an automatic biochemical analyser (Mindray BS-460, Shenzhen, China) using standard kits according to the manufacturer's instructions (AST (P/N:105-000443-00), ALT (P/N:105-000442-00) and ALP (P/N:105-000444-00)).

2.6. Real-time quantitative polymerase chain reaction (qPCR) analysis

RNA extraction, reverse transcription and quantitative real-time PCR were conducted following the methods described in detail by Su et al. (2017). Briefly, total RNA was extracted from the liver and dorsal muscle using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) based on the manufacturer's instructions. The quality of RNA was tested by 1% denaturing agarose gel electrophoresis. An M-MLV First-Strand Synthesis Kit (Invitrogen, Shanghai, China) was used to reverse transcribe RNA into cDNA. Real-time PCR analysis was carried out by a LightCycle 480 II system (Roche, Basel, Switzerland). The parameters were as follows: pre-incubation at 95 °C for 5 min and followed by 40 cycles of 10 s at 95 °C, 20 s at 60 °C, then 10 s at 72 °C. Six biological and 2 technical replicates were performed. *β-actin* and translation elongation factor 1-alpha (*ef1α*) were selected as internal references for normalization. Table S1 shows the primers used for quantitative RT-PCR. The relative expression levels of target genes were calculated according to the method described by Vandesompele et al. (2002).

2.7. Histological analysis

To analyse the content of muscle glycogen and collagen, PAS and Masson staining were performed in dorsal muscle paraffin sections by Servicebio Company (Wuhan, China). Muscle images were obtained from each slide under a fully automatic digital slide scanner (Aperio VERSA 8, Leica, Germany). ImageJ Launcher software was used to identify glycogen and collagen in the dorsal muscle. Glycogen and collagen were coloured purple and blue, respectively. The myofiber diameter and area of glycogen and collagen staining were quantified by this software for semi-quantitative analysis.

2.8. Determination of nucleotide and purine metabolite concentrations

The nucleotide and purine metabolite contents of the flesh and liver were determined according to the methods described by Cheng et al. (2015) with some modifications. Taking flesh as an example, flesh samples (5 g) were minced and homogenized for 15 min in 10 mL of 0.1 M perchloric acid at 4 °C. The homogenate was centrifuged for 10 min at 1,840 × *g* at the same temperature. The residues were centrifuged again approximately 5 min after the addition of 5 mL of perchloric acid. The collected supernatant was neutralized with 1 M potassium hydroxide solution to a pH value of 6.4. The solution was brought to a volume of 50 mL and passed through a 0.45 μm Millipore syringe filter. A 20-μL aliquot of the filtrate was analysed through high-performance liquid chromatography (HPLC, Waters 2695e, USA). The HPLC conditions for the determination of ATP, ADP, AMP and IMP concentrations were as follows: a 5-μm C_{18} column (CNW Athena C18-WP, 4.6 × 250 mm, 5 μm) was utilized. Mobile phase A was 0.01 mol/L potassium (KH_2PO_4). Mobile phase B was methanol. The column temperature was 30 °C. The flow rate was 1.0 mL/min, the injection volume was 20 μL and the UV detector wavelength was 254 nm. The HPLC conditions for the determination of guanosine monophosphate (GMP), inosine, adenosine, hypoxanthine, adenine, guanine and xanthine concentrations were the same as described above except that 0.4753 g of the ion-pairing reagent tetrabutylammonium hydrogen sulfate ($C_{16}H_{37}NO_4S$) was added to each litre of mobile phase A. The eluent compositions, gradient and HPLC program are presented in Tables S2 and S3.

2.9. Texture analysis

The dorsal muscle of the experimental fish was cut into 2 pieces for the texture analysis. Texture parameters, including hardness, toughness, adhesiveness, stringiness, fracturability, stickiness and flexibility, were analysed using a texture analyser (Stable Micro systems, Ltd., UK). A 0.5 diameter spherical probe was used, which

pressed down on the sample at a rate of 30 mm/min until a downwards deformation of 10 mm was achieved, and the probe was held in that position for 32 s. The flesh sample was pressed down to a force of 2 N, the load was maintained for 10 s, and then the flesh was disengaged at a rate of 600 mm/min. The maximum force on the sensing probe from the flesh during the downwards pressure reflected the hardness of the flesh. The area of integration under the downwards pressure curve was used as the toughness, reflecting the energy trend of the downwards pressure. The area of integration under the curve of the detachment process was used as the adhesiveness, reflecting the energy of detachment. The distance of the detachment process was used as the sticky distance, reflecting the stringiness. The slope of the starting and ending points of the downwards pressure process was used as the fracturability, reflecting the rate of increasing the load during the deformation of the flesh. The maximum force value during the detachment of the probe from the flesh was used as the stickiness. The deformation of the downwards pressure up to 2 N load reflected the flexibility of the flesh. The centrifugal water loss of flesh reflected the water-holding capacity of the flesh, which was measured following the methods that were described in detail by Lv et al. (2021). Briefly, 1 g of dorsal muscle was centrifuged at 1,000 × *g* for 30 min at 4 °C after weighing. The surface moisture of sample was dried by using common qualitative filter paper and the sample was weighed again. The centrifugal weight loss was calculated as the difference of the sample weight before and after centrifugation. A high value of centrifugal weight loss indicated a poor water holding capacity.

2.10. LC–MS/MS metabolite profiling analysis

The liver and muscle samples were accurately weighed into a 2-mL EP tube, and 500 μL of precooled extractant (70% methanol in water) was added to the EP tube. The solution was homogenized 4 times at 30 Hz for 30 s. The samples were placed on ice, left for 15 min, and centrifuged at 13,680 × *g* for 10 min at 4 °C. Finally, 200 μL of supernatant was placed into a sample bottle for LC–MS/MS analysis. The data acquisition instrumentation system consisted mainly of ultra-performance liquid chromatography (HPLC) (Shimpack UFLC SHIMADZU CBM30A, https://www.shimadzu.com/) and tandem mass spectrometry (MS/MS) (QTRAP https://sciex.com/) at Wuhan Metcalfe Biotechnology Company (Wuhan, China). All data were processed and normalized by MetaboAnalyst 5.0 and then used to analyse the differences in the levels of metabolites in the gibel carp liver and dorsal muscle between the 0.30RI group and the control group.

2.11. Statistical analysis

All data were statistically analysed with SPSS 20.0 software (IBM, USA). Duncan's multiple range test was used to detect the significance of differences between treatments followed by one-way analysis of variance (ANOVA). Before all analyses, normality of distribution (one-sample Kolmogorov–Smirnov test) and homogeneity of variance (Levene's test) analyses were conducted to check all the values. Differences were regarded as significant at $P < 0.05$, and the results are presented as the means with their standard errors. The calculations of taste active value (TAV) were performed according to methods described by Luo et al. (2021). The TAV was represented by the equation TAV = C/T, where C is the taste compound concentration and T is the threshold value. Generally, compounds with TAV > 1 were considered flavour contributors.

3. Results

3.1. Effect of RI supplementation on growth performance and the whole-body, flesh chemical composition and liver health of gibel carp

After a 60-d breeding trial, the final body weight (FBW), weight gain rate (WGR) and feeding rate (FR) of gibel carp in the 0.45RI group were slightly higher than those in the control group but were not significantly different, and the hepatosomatic index (HSI) and viscerosomatic index (VSI) were also not significantly different, as shown in Table 2. The moisture, crude protein, crude lipid and ash contents in the whole body are also shown in Table 2. Supplementation with 0.30RI significantly increased the crude protein content, but the moisture, crude lipid and ash contents in the RI supplementation groups were not significantly different from those in the control group. In addition, there were also no significant differences in the moisture, crude protein or crude lipids in the dorsal muscle between the experimental groups (Table 2). The plasma levels of AST, ALT and ALP are presented in Table 2. Compared with the control group, no significant differences in plasma levels of AST, ALT and ALP were observed in the experimental groups.

3.2. Effect of RI supplementation on muscular collagen metabolism, glycogen metabolism and textural properties of gibel carp

The results of Masson's trichrome staining with the dorsal muscle are presented in Fig. 1A. By performing semiquantitative analysis of the collagen content in all groups of sections, an increase in dietary ribose levels was observed along with a gradual increase in collagen content in the muscles. The collagen content in the 0.45RI group was elevated compared to that in the control group, although there was no significant difference (Fig. 1B). There was a tendency for muscle fiber diameter to grow with the addition of RI, but there was no significant difference compared to the control group (Fig. 1C). The mRNA expression of genes related to collagen synthesis (prolyl 4-hydroxylase subunit alpha-1 [*p4ha1*] and collagen alpha-1 [*col1a1*]) in muscle was determined (Fig. 1D and E). The results showed that RI supplementation upregulated *p4ha1* and *col1a1* expression. The expression levels of these 2 genes were significantly elevated in the 0.45% RI group compared with the control group.

The PAS-stained sections are shown in Fig. 1F. Semiquantitative analysis was conducted for the glycogen content in muscle, and the glycogen content in the 0.30RI and 0.45RI groups was significantly higher than that in the control group (Fig. 1G). By determining the glycogen content with a commercial kit, we obtained similar results (Fig. 1H). The mRNA expression of genes related to glycogen metabolism (glycogen synthase 2 [*gys*] and glycogen phosphorylase [*gp*]) was determined (Fig. 1I and J). The expression of *gys* mRNA in muscle showed an upregulated trend with increased dietary ribose levels, and the upregulation was significant in the 0.30RI and 0.45RI groups. However, there was no significant difference in the expression of the *gp* gene among all groups.

The muscular texture measurement data are presented in Fig. 1K–R. With increasing dietary ribose levels, the centrifugal water loss of muscle gradually decreased, and the 0.30RI and 0.45RI groups showed significantly lower centrifugal water loss than that of the control group. Conversely, hardness, toughness and adhesiveness tended to increase with increasing concentration of RI in the diet. The hardness in the 0.30RI group was significantly increased compared with that in the control group. The hardness,

Table 2
Effect of different concentrations of ribose (RI) on the growth performance, whole-body composition, flesh composition and liver health of gibel carp.

Parameters	Con	0.15RI	0.30RI	0.45RI
IBW[1], g	150.09 ± 0.31	150.08 ± 0.37	150.13 ± 0.41	150.05 ± 0.37
FBW[2], g	198.09 ± 2.84	196.15 ± 3.98	197.47 ± 2.12	203.43 ± 3.29
WGR[3], %	31.98 ± 2.14	30.69 ± 2.34	31.53 ± 1.21	35.58 ± 2.44
FR[4], %BW/d	1.21 ± 0.02	1.28 ± 0.01	1.24 ± 0.02	1.33 ± 0.01
HSI[5], %	6.48 ± 0.80^{ab}	4.83 ± 0.26^{a}	6.98 ± 0.78^{b}	5.07 ± 0.59^{ab}
VSI[6], %	13.34 ± 0.55	13.34 ± 0.78	14.13 ± 0.89	13.91 ± 1.17
Moisture, %	70.00 ± 1.90	68.63 ± 0.98	68.23 ± 1.25	68.05 ± 1.12
Crude protein, %	14.67 ± 0.46^{a}	15.40 ± 0.27^{ab}	16.00 ± 0.49^{b}	15.27 ± 0.17^{ab}
Crude lipid, %	8.17 ± 0.54	8.24 ± 0.55	8.10 ± 0.30	8.35 ± 0.51
Ash, %	4.57 ± 0.08	4.59 ± 0.08	4.65 ± 0.24	4.42 ± 0.03
Moisture, %	76.88 ± 0.28	77.23 ± 0.28	77.03 ± 0.44	76.73 ± 0.34
Crude protein, %	18.61 ± 0.06	18.52 ± 0.06	18.54 ± 0.08	18.59 ± 0.06
Crude lipid, %	4.98 ± 0.28	4.35 ± 0.31	4.26 ± 0.37	4.84 ± 0.19
AST[7], U/L	128.53 ± 16.72	97.58 ± 15.52	99.15 ± 15.02	114.83 ± 18.02
ALT[8], U/L	11.63 ± 0.87	13.42 ± 0.99	12.77 ± 1.45	12.88 ± 1.68
ALP[9], U/L	25.95 ± 0.86	27.17 ± 1.58	23.00 ± 3.08	25.78 ± 2.11

Data of growth performance, whole-body composition and dorsal muscle composition are presented as the means ± SE ($n = 3$). Data of liver health indictors also presented as the means ± SE ($n = 6$). One-way analysis of variance (ANOVA) was used to compare the means. Values within the same row with different letters are significantly different ($P < 0.05$).

[1] IBW: initial body weight.
[2] FBW: final body weight.
[3] Weight gain rate (WGR, %) = 100 × (final mean weight − initial mean weight) / initial mean weight.
[4] Feeding rate (FR, % BW/d) = 100 × (feed intake in dry matter) / [days × (initial body weight + final body weight)/2].
[5] Hepatosomatic index (HSI, %) = 100 × liver weight (g)/body weight (g).
[6] Viscerasomatic index (VSI, %) = 100 × viscerasomatic weight (g)/body weight (g).
[7] AST: aspartate aminotransferase.
[8] ALT: alanine aminotransferase.
[9] ALP: alkaline phosphatase.

toughness and adhesiveness in the 0.45RI group were significantly increased compared to that of the control group. There were no significant differences in stringiness, fracturability, stickiness or flexibility among all groups.

3.3. Effect of RI supplementation on the taste of flesh in gibel carp

Nucleotides greatly contribute to the taste of fish flesh. TAV is a very useful index to evaluate compounds in flesh regarding their taste impact. Fig. 2A shows the TAV in flavour nucleotides (IMP, AMP and GMP). The TAV of IMP in all groups was greater than 1, and with increasing dietary RI levels, the TAV of IMP showed a trend of increasing and then decreasing, and the highest TAV was obtained in the 0.30RI group. There was no significant difference in the AMP TAV among all groups. The GMP TAV in the control group was significantly higher than that in the 0.15RI group and was not remarkably different from that in the other groups.

The muscle FAA profiles of all groups are shown in Fig. 2B and Table S4. Different FAA result in different tastes. The results showed that the amount of umami FAA consisting of Asp and Glu was significantly higher in the 0.15RI and 0.30RI groups than in the control group. Similarly, the sweet FAA, bitter FAA, total essential amino acids (EAA) and non-essential amino acids (NEAA) contents in the muscle of gibel carp fed 0.15RI and 0.30RI were significantly higher than those in gibel carp fed the control diet. Fig. 2C presents the FAA with TAV > 1 in gibel carp flesh. The contents of Lys, Arg and Ala increased in flesh with increasing levels of dietary RI concentration. His showed a trend of increasing and then decreasing, and the highest content appeared in the 0.15RI group. The Gly and Glu contents in the 0.30RI group were slightly higher than those in the control group but were not significantly different.

The relative contents of muscle fatty acids are shown in Fig. 2D and Table S5. There were no significant differences in Σ saturated fatty acids (ΣSFA), Σ monounsaturated fatty acids (ΣMUFA), Σn-6 polyunsaturated fatty acids (Σn-6 PUFA), Σn-3 polyunsaturated fatty acids (Σn-3 PUFA), Σ highly unsaturated fatty acids (ΣHUFA) and Σn-3/Σn-6 PUFA in all groups. However, the EPA content in the 0.30RI group was significantly increased compared to that in the control group, as shown in Fig. 2E. Other functional fatty acid compositions showed no remarkable difference among all groups.

3.4. Effects of 0.30RI supplementation on the metabolomic features in the muscle of gibel carp

To explore the impacts of RI supplementation on muscle metabolites, metabolomic analysis based on LC–MS/MS was conducted to evaluate the metabolites. A total of 44 metabolites were identified in muscle samples, including nucleotides and derivatives (12), amino acids and derivatives (12), phosphate sugars (7), organic acids and derivatives (6) and others (7) (Fig. 3A). As shown in the principal component analysis (PCA) score scatter plot (Fig. 3B), the metabolic profile of the 0.30RI group was clearly different from that of the control group. This finding indicates a significant change in the muscle metabolites in gibel carp induced by 0.30RI supplementation. The relative peak intensities of all metabolites in the 0.30RI and control groups are visualized as a heatmap in Fig. 3C. A total of 10 metabolites were found to show significant changes (Table S6), with IMP and ATP levels in the 0.30RI group were remarkably increased. Identified metabolites were enriched to specific pathways through the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The results are shown in bubble plots (Fig. 3D). The purine metabolism with the highest value of -log(P) was identified as the most impacted pathway in muscle, followed by the pentose phosphate pathway, amino sugar and nucleotide sugar metabolism, glycolysis/gluconeogenesis and glycerolipid metabolism. Purine metabolism, the pentose phosphate pathway and other associated metabolic pathways are summarized and shown in Fig. 3E, and 10 up-regulated metabolites and 8 down-regulated metabolites were identified.

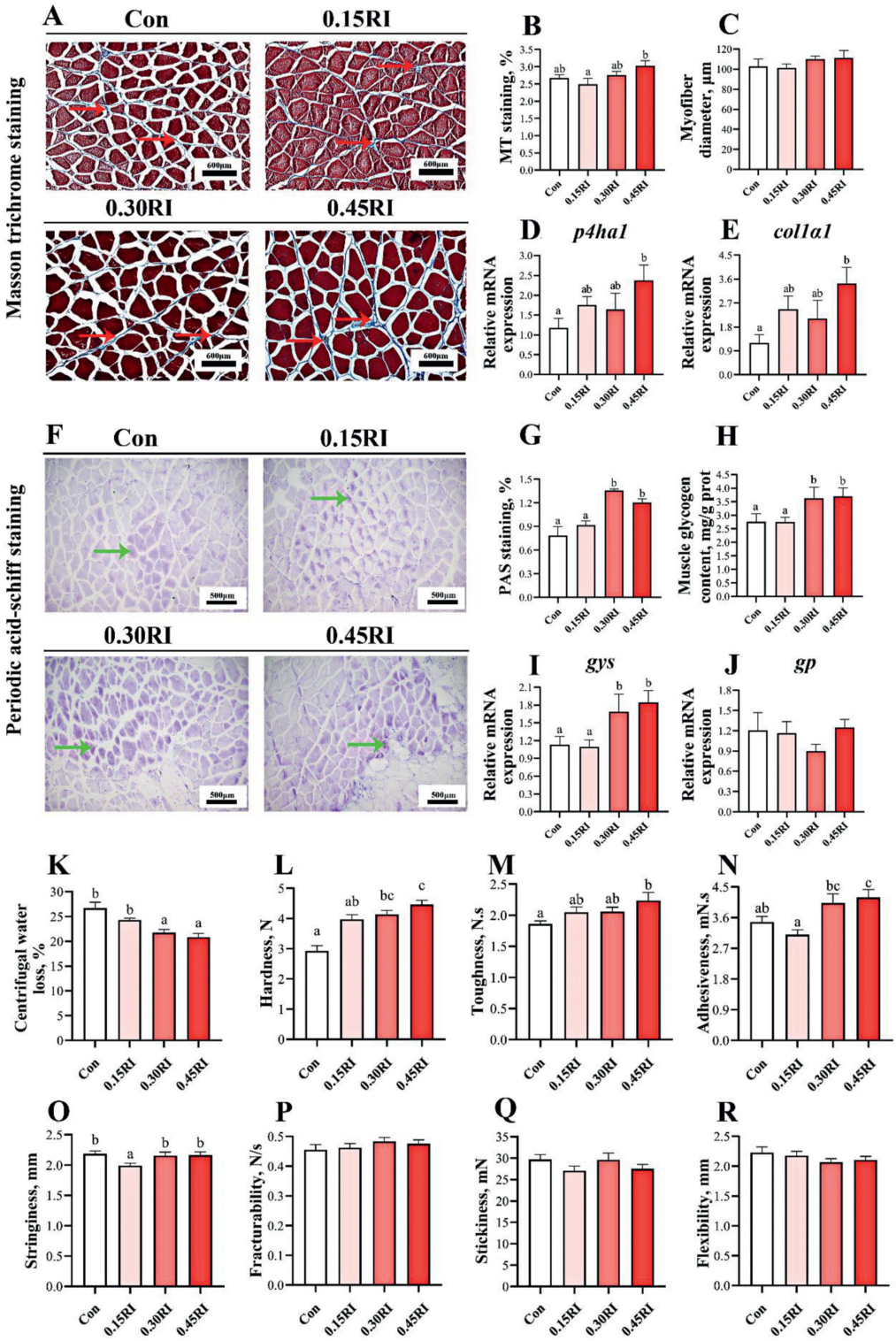

Fig. 1. Effects of different concentrations of D-ribose (RI) on muscular collagen metabolism, myofiber diameter, glycogen metabolism and textural properties of gibel carp. (A to D) Muscular collagen metabolism and myofiber diameter. (A) Histological observations of the dorsal muscle, stained with Masson (magnification 200×). Red arrows point to collagen. (B) The quantitative graph of Masson's trichrome (MT) staining. (C) The quantitative graph of myofiber diameter. (D) The relative mRNA expression of prolyl 4-hydroxylase subunit α-1 isoform X2 (*p4ha1*) in dorsal muscle. (E) The relative mRNA expression of collagen α-1(I) chain-like (*col1a1*) in dorsal muscle. Panels F–J represent muscular glycogen metabolism. (F) Histological observations of the dorsal muscle, stained with periodic acid-Schiff (magnification 200×). Green arrows point to glycogen. (G) The quantitative graph of PAS staining. (H) The glycogen content in muscle measured by commercial assay kit. (I) The relative mRNA expression of glycogen synthase (*gys*) in dorsal muscle. (J) The relative mRNA expression of glycogen phosphorylase (*gp*) in dorsal muscle. Columns represent the mean ± SEM ($n = 6$). (K to R) Muscular textural properties. Columns represent the mean ± SEM ($n = 9$). One-way analysis of variance (ANOVA) was used to compare the means. For each index, bars without sharing a common letter indicate significant differences ($P < 0.05$).

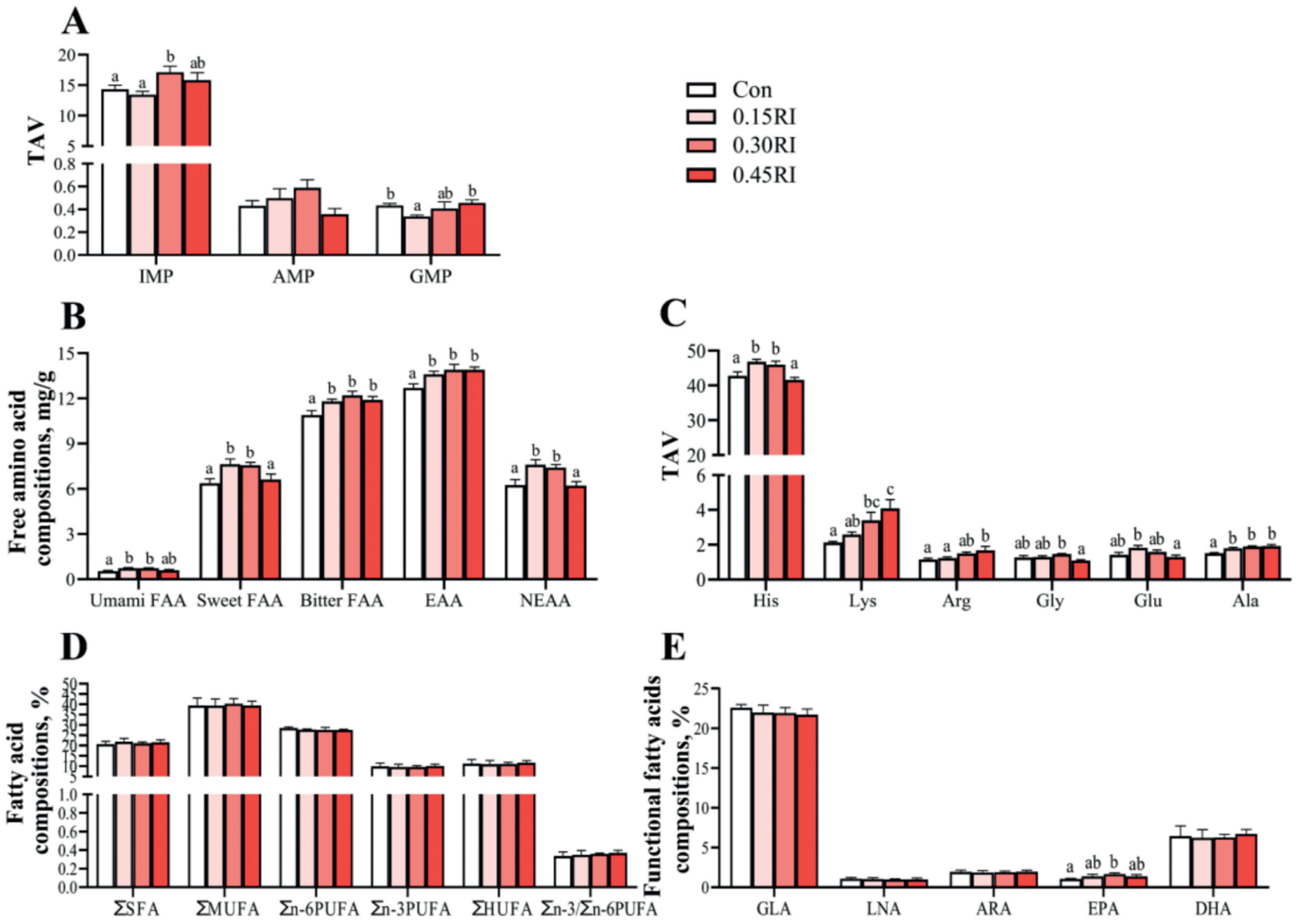

Fig. 2. Effects of different concentrations of D-ribose (RI) on the taste of flesh of gibel carp. (A) Taste active value (TAV) of flavor nucleotide contents of flesh. (B) Free amino acid (FAA) contents of flesh (mg/g dry matter). (C) TAV of FAA of flesh. (D) Fatty acid compositions of flesh (% of total fatty acids). (E) Functional fatty acid compositions of flesh (% of total fatty acids). Columns represent the mean ± SEM ($n = 6$). One-way analysis of variance (ANOVA) was used to compare the means. For each index, bars without sharing a common letter indicate significant differences ($P < 0.05$). Umami FAA: Asp, Glu; Sweet FAA: Gly, Ala, Leu, Met, Asp, Arg and Pro; Bitter FAA: Val, Lys, Leu, Ile, Phe, His and Tyr; EAA: Lys, Met, Thr, Arg, Leu, His, Ile, Phe, and Val; NEAA: Asp, Ser, Glu, Ala, Cys, Gly, Tyr, and Pro. ΣSFA = sum of saturated fatty acids; ΣMUFA = sum of mono-unsaturated fatty acids; Σn-6 PUFA = sum of n-6 polyunsaturated fatty acids; Σn-3 PUFA = sum of n-3 polyunsaturated fatty acids; ΣHUFA: sum of highly polyunsaturated fatty acids. Σn-3/Σn-6 PUFA = sum of n-3 polyunsaturated fatty acids/sum of n-6 polyunsaturated fatty acids. GLA = gamma linolenic acid; LNA = linolenic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

3.5. Effects of 0.30RI supplementation on the metabolomic features of gibel carp liver

To further reveal the effects of RI on the hepatic metabolites of gibel carp, a metabolomics analysis was also conducted to evaluate the metabolites. Sixty-six metabolites of the liver were unambiguously identified, which were dominated by amino acids and derivatives (21), followed by nucleotides and derivatives (15), carbohydrates and derivatives (15), organic acids and derivatives (6) and coenzymes and vitamins (4) (Fig. 4A). As shown in the PCA score scatter plot, a clear differentiation of the samples, with data points of the 0.30RI group, was shifted away from the control group (Fig. 4B). The heatmap displays the 50 metabolites between 0.30RI and the control group (Fig. 4C). A total of 25 metabolites showed significant changes in the 0.30RI group compared to the control group (Table S7). Among them, 20 metabolites showed significant up-regulation and 5 metabolites showed significant down-regulation. Some important metabolic pathways that showed notable changes are identified in Fig. 4D.

The D-glutamine and D-glutamate metabolism pathway with the highest value of $-\log(P)$ was identified, followed by purine metabolism, the pentose phosphate pathway, amino sugar and nucleotide sugar metabolism, arginine biosynthesis and glycolysis/gluconeogenesis. Several significantly changed and interconnected pathways are summarized and shown in Fig. 4E.

3.6. Supplementation and validation of the metabolic effects of different concentrations of RI supplementation on metabolites and the related genes for purine metabolism in gibel carp

The contents of key metabolites in purine metabolism in muscle tissues were determined by HPLC and are shown in Fig. 5A. With the increase in dietary RI levels, the contents of ATP, AMP and IMP showed a trend of increasing and then decreasing, and the highest contents of ATP and IMP were obtained in the 0.30RI group. The highest content of AMP was obtained in the 0.15RI group. Furthermore, the GMP content in the RI supplementation groups decreased to a certain extent compared to that in the control group, especially in the 0.15RI group, which decreased significantly. No significant difference was observed for the inosine, hypoxanthine, adenine, guanosine and guanine contents. The expression of key genes of purine metabolism in muscle tissues is presented in

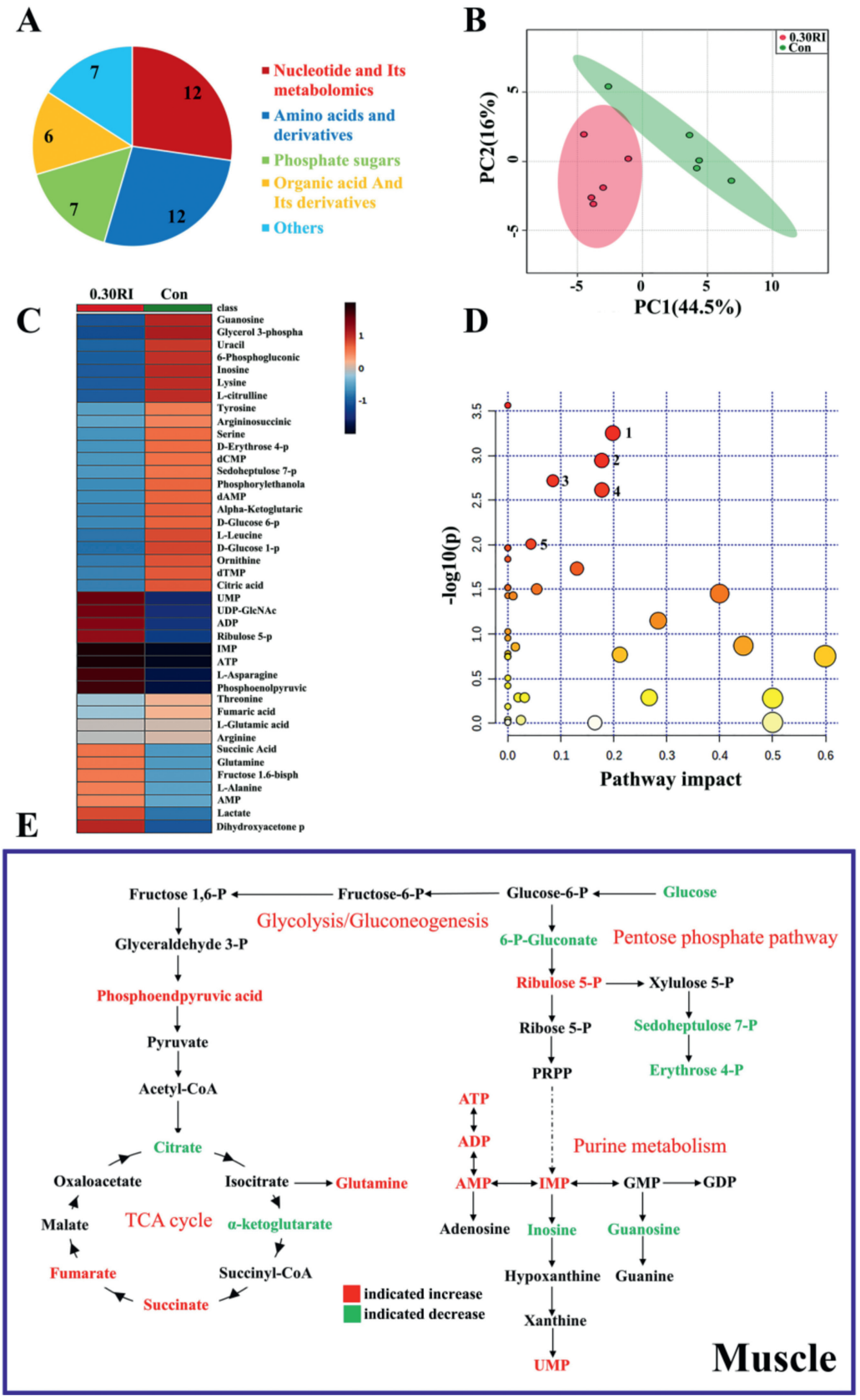

Fig. 3. Effects of 0.3%RI supplementation on the metabolomic features in muscle of gibel carp. (A) The pie chart exhibits the biochemical categories of the metabolites identified in muscle. (B) The principal component analysis score plots for metabolomic profiles of muscle: 0.30RI group in red; Con group in green. (C) Heatmap visualization of differential and overlapping metabolites in 0.30%RI vs Con. Red indicates high abundance, whereas the relatively low-abundance metabolites are shown in blue. (D) Summary of pathway analyses for muscle tissue with MetaboAnalyst 5.0, as visualized by bubble plots. 1. Purine metabolism; 2. Pentose phosphate pathway; 3. Amino sugar and nucleotide sugar metabolism; 4. Glycolysis/Gluconeogenesis; 5. Glycerolipid metabolism. The color and size of each circle is based on the *P* value and the pathways impact value, respectively. (E) Metabolic pathways affected induced by 0.3% RI supplementation in muscle.

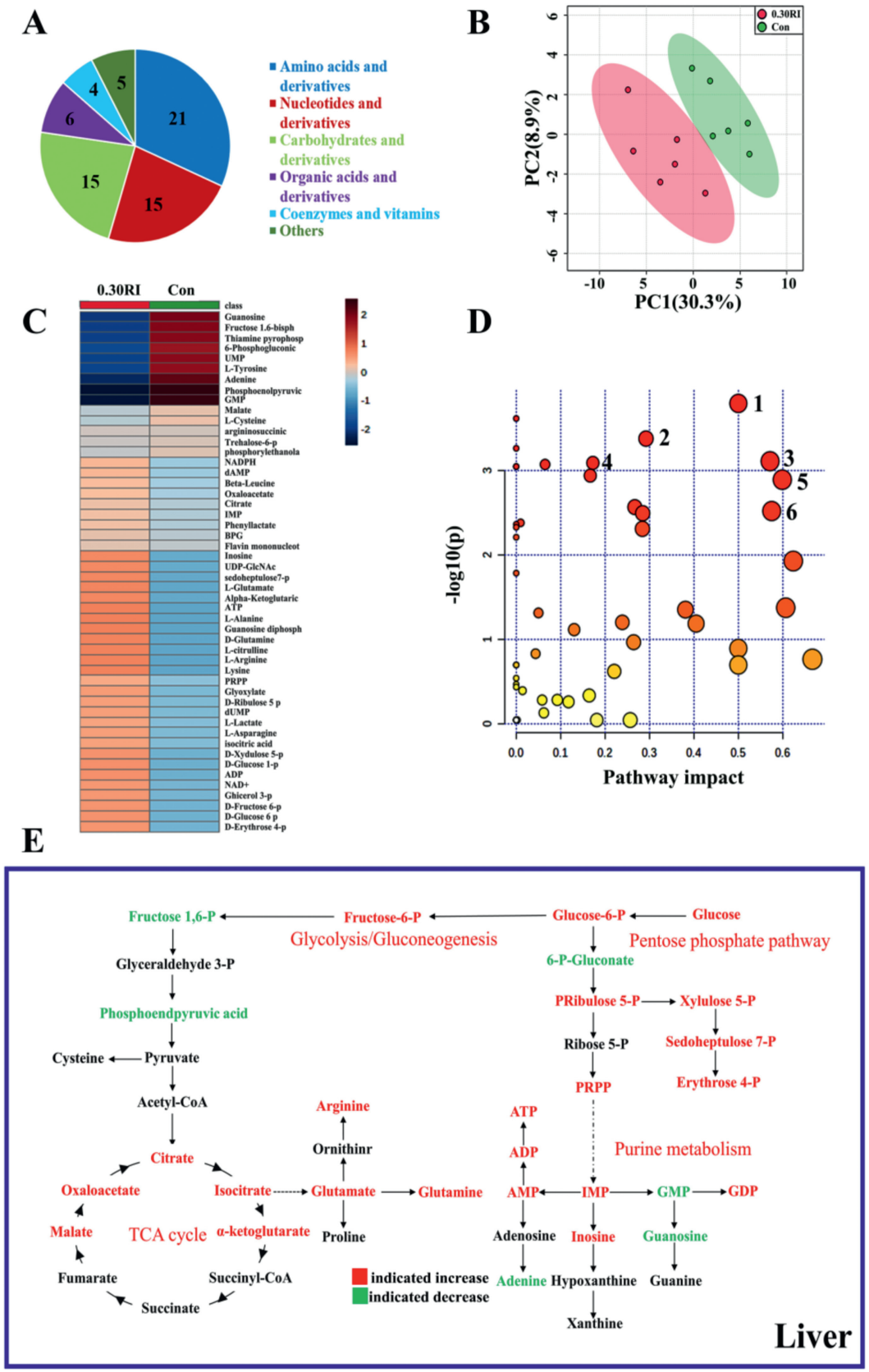

Fig. 4. Effects of 0.3%RI supplementation on the metabolomic features in liver of gibel carp. (A) The pie chart exhibits the biochemical categories of the metabolites identified in liver. (B) The principal component analysis score plots for metabolomics profiles of muscle. 0.30RI group in red; Con group in green. (C) Heatmap visualization of differential and overlapping metabolites in 0.30RI vs Con. Red indicates high abundance, whereas the relatively low-abundance metabolites are shown in blue. (D) Summary of pathway analyses for liver tissue with MetaboAnalyst 5.0, as visualized by bubble plots. 1. D-Glutamine and D-glutamate metabolism; 2. Purine metabolism; 3. Pentose phosphate pathway; 4. Amino sugar and nucleotide sugar metabolism; 5. Arginine biosynthesis; 6. Glycolysis/Gluconeogenesis. The color and size of each circle is based on the *P* value and the pathways impact value, respectively. (E) Metabolic pathways affected induced by 0.3% RI supplementation in liver.

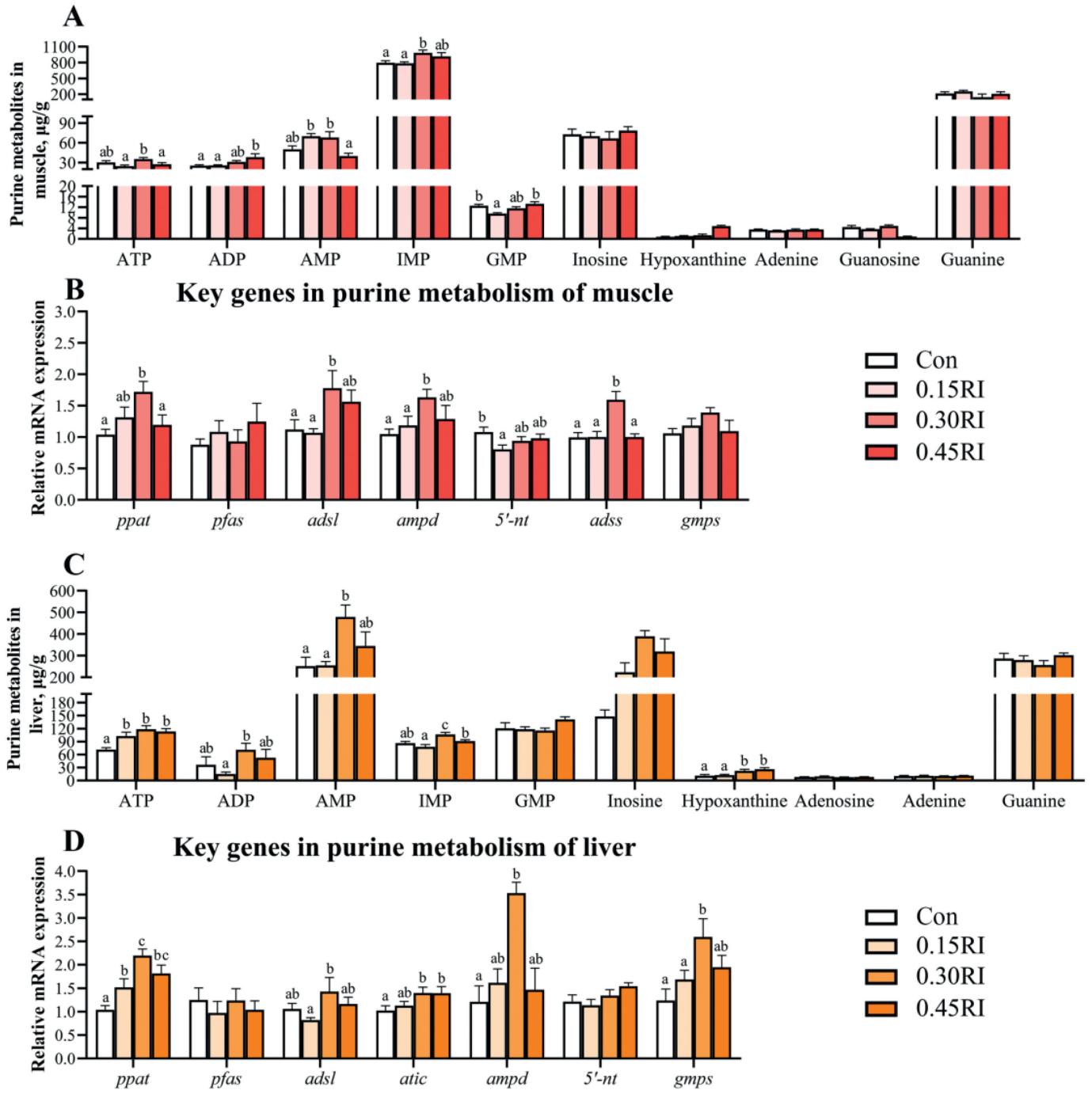

Fig. 5. Supplementation and validation of the metabolomic—effects of different concentrations of RI on liver metabolites and related genes in gibel carp. (A) Determination of purine metabolites content in the muscle. (B) The relative mRNA expression of genes (*ppat, pfas, adsl, ampd, 5′-nt, adss* and *gmps*) involved in muscle purine metabolism of groups. (C) Determination of purine metabolites content in the liver. (D) The relative mRNA expression of genes (*ppat, pfas, adsl, atic, ampd, 5′-nt* and *gmps*) involved in liver purine metabolism of groups. One-way analysis of variance (ANOVA) was used to compare the means. For each index, bars without sharing a common letter indicate significant differences ($P < 0.05$).

Fig. 5B. The increase in the levels of dietary RI increased the expression of phosphoribosyl pyrophosphate amidotransferase (*ppat*), adenylosuccinate lyase (*adsl*), AMP deaminase (*ampd*) and adenylosuccinate synthetase (*adss*), and the highest expression of these genes was found in the 0.30RI group. Compared with the control group, the expression of cytosolic purine 5′-nucleotidase (*5′-nt*) in the experimental group was decreased, and that in the 0.15RI group was decreased significantly. Dietary RI supplementation had no significant effects on the expression of phosphoribosylformylglycinamidine synthase (*pfas*) and GMP synthase (*gmps*).

The changes in metabolites that are involved in purine metabolism induced by RI supplementation in the liver are shown in Fig. 5C. The results were consistent with the observations in muscle tissues, and the increase in RI supplementation and the levels of ATP, ADP, AMP, IMP and inosine gradually increased. The highest level appeared in the 0.30RI group. As displayed in Fig. 5C, it was observed that the contents of hypoxanthine in 0.30RI and 0.45RI

were significantly increased compared with those in the control group. However, there was no significant difference in adenosine, adenine or guanine among all groups. The results mentioned above were consistent with the metabolomic results. The expression of *ppat*, *adsl*, bifunctional purine biosynthesis protein (*atic*), *ampd* and *gmps* in the liver showed an up-regulated and then down-regulated trend with increased dietary RI levels, and the upregulation of *ppat*, *atic*, *ampd* and *gmps* was significant in the 0.30RI group (Fig. 5D). There was no significant difference in *pfas* and *5′-nt* in all groups.

4. Discussion

D-ribose has long been applied to improve cardiovascular diseases and inflammation (Pierce et al., 2022; Ueki et al., 2013). However, the effect of RI as a beneficial feed additive on the meat quality of farmed animals, especially fish, has rarely been reported. Therefore, the main purpose of this study was to identify the possible functions of RI in improving fish flesh quality. This study evaluates the growth performance, nutritional value, texture and taste components of meat. Furthermore, metabolomic analysis of liver and muscle was performed to thoroughly investigate the possible impacts of RI on metabolites in fish.

In this study, it was found that RI supplementation did not result in any negative impact on the growth performance of gibel carp, since the final body weight and weight gain rate was very similar among different levels of RI-added groups. AST, ALT and ALP are a group of haematological indicators which are the main markers of liver health (Yang et al., 2020). In the present study, AST, ALT and ALP in plasma were not significantly different when fish were fed the experimental diets, which suggests that dietary RI supplementation does not negatively affect the liver health of gibel carp. Generally, fish is an important source of protein in food for some countries, and the protein content in fish is a significant indicator for evaluating its nutritional value (Mukhopadhyay and Ray, 2010; Zhao et al., 2010). In the present study, 0.30RI supplementation was effective in increasing the whole-body protein content. It was reported that RI is involved in the glycosylation of protein and leads to protein aggregation (Wei et al., 2012), which may be the reason for the increase in protein content. Lipids are a major source of energy and an important factor affecting the flesh quality of fish as the fatty acid composition of the flesh reflects its nutrition (Cheng et al., 2022). Some studies have shown that feed additives can increase the levels of unsaturated fatty acids in cultured fish species (Yang et al., 2019). Appropriate Br-DMPT increased the flesh EPA content in grass carp (Liu et al., 2020) and glycerol monolaurate supplementation elevated the DHA content in large yellow croaker (Wang et al., 2022). The current study observed that EPA content in muscle was elevated with RI supplementation, which might be partially explained by the fact that the experimental fish produced more ATP for energy when fed a diet with RI, thus conserving the free fatty acids in muscle. However, the specific mechanism needs to be further investigated.

Collagen is a rich connective tissue protein that is a contributing factor to variation in texture and tenderness of meat. In fish, the content of collagen is an important factor affecting flesh quality (Ma et al., 2021). The results of the staining and quantification of collagen in the muscle showed that the addition of 0.45RI increased the collagen content of the muscle, and although the statistical results showed no significant difference between the addition of RI and the control group, the qualitative results demonstrated that RI had a significant effect on the sarcomeres of the dorsal muscle. *P4ha1* and *col1a1* are key genes in collagen anabolism (Zhao et al., 2018). Fu et al. (2022) found that faba bean increased the muscle content of collagen in grass carp by regulating the expression of *col1a1*. A significant increase in the expression of these 2 genes was observed, which suggests that RI supplementation accelerates the rate of collagen synthesis. It has been reported that the level of collagen was positively correlated with firmness of fillets (Yang et al., 2021). We measured the texture of fish flesh and found that the hardness and toughness increased with RI supplementation. In addition, collagen also plays a vital role in maintaining the integrity and cohesiveness of fish fillets (Wei et al., 2017). In the present study, the decrease in centrifugal water loss indicates an increase in the water-holding capacity of flesh. Notably, RI supplementation significantly increased flesh adhesiveness. The texture profile is an important indicator of quality and is closely linked to consumer acceptance (Cheng et al., 2021). Generally, soft fish flesh is less accepted by consumers (Espe, 2008), and current research has demonstrated that the hardness of flesh can be improved by additives.

The correlation between glycogen content and flesh quality has been investigated previously (Matos et al., 2013). Low glycogen levels in muscle at the time of slaughter directly result in a dark-firm-dry syndrome and cause a reduction in meat quality (Pethick et al., 1995). In this experiment, by quantitatively analysing the histological sections and measurement of glycogen content in muscle, we found that fish fed RI-supplemented diets tended to have significantly higher values than those of the control group. The genes *gys* and *gp* play an important role in glycogen synthesis and catabolism, respectively (Guo et al., 2020). We found that the expression of *gys* showed an increasing trend with increasing RI addition, while the expression of *gp* showed no significant change. This suggests that the increase in glycogen content is mainly due to the increased rate of glycogen synthesis by RI. Therefore, the underlying mechanism may be that RI improves the flesh quality by increasing the glycogen content, thus providing good-quality fish flesh.

Metabolomics is an effective analytical method used to identify and quantify small molecule metabolites in organisms, which can more directly and accurately reflect physiological state (Zhang et al., 2022), and it can be used as a powerful strategy to explore changes in fish quality by determining metabolites and metabolic profiles in flesh (Jia et al., 2020). In this study, a total of 44 and 66 metabolites were detected in muscle and liver, respectively. The liver is the metabolic centre of organisms and is therefore rich in metabolites (Carambia and Herkel, 2018). In addition, a significant increase in the content of metabolites such as ATP, IMP and AMP were observed. ATP is a central metabolite that plays a fundamental role as an energy currency within cells, and the supply of ATP is essential for most of the body's vital processes (Tantama and Yellen, 2014). IMP and its decomposition products inosine and hypoxanthine are generally regarded as the main biomarkers for meat quality, and these compounds are helpful for evaluating the differences in fresh meat quality, flavour, nutritive values and functions (Wang et al., 2020; Wen et al., 2020). The accumulation of inosine in muscle results in an unpleasant bitter taste in meats (Liu et al., 2021). The significant decrease in the inosine level in the 0.30RI group may reduce the unpleasant component in flesh induced by RI supplementation. Notably, the changes in metabolites in the liver were in line with those in muscle, in which the levels of ATP, IMP and ADP were increased to varying degrees.

Metabolic pathway analysis reconstructs networks of biochemical reactions, providing internal connections between metabolites and helping to understand the potential mechanisms of the impacts of additives on specific metabolites (Sun et al., 2018). Based on the enrichment of KEGG pathways and the differential metabolites, the pentose phosphate pathway, purine metabolism, glycolysis/gluconeogenesis and TCA cycle were identified as relevant to improving flavour substance in flesh by 0.30RI

supplementation. A previous study reported that medium-chain monoglycerides improved the flavour quality of chickens by increasing the IMP content through the purine pathway (Liu et al., 2021). In addition, Du et al. (2020) demonstrated that microflowing water treatment increased IMP levels in grass carp muscle by affecting purine metabolism and the pentose phosphate pathway. This finding was similar to the phenomenon observed in the present study, but the cause was different. Ribose is one of the building blocks for the biosynthesis of purine nucleotides, and the formation of PRPP is a vital step in the nucleotide de novo synthesis pathway (Zhou et al., 2019; Patkari et al., 2018), which in feed is absorbed by the digestive tract, deposited in the liver and muscle and partially converted to ribose 5-P. The accumulation of ribose 5-P and PRPP accelerated the synthesis of nucleotides, such as ATP, IMP and AMP, in purine metabolism. Therefore, these alterations in purine metabolism induced by 0.30RI supplementation may be the cause of the improvements in the flavour of fish flesh. These findings were supported by metabolomics analysis in the liver, where key metabolites, such as ATP, IMP, AMP, ribose 5-P and PRPP, were increased in the 0.30RI group.

High-performance liquid chromatography and fluorescence quantitative PCR were used to further confirm that purine metabolism was the main pathway affecting flavour substance in flesh induced by RI supplementation. The contents of IMP, ATP, and AMP in both the liver and muscle, which are involved in purine metabolism, were higher in the 0.30RI group, and this result is consistent with the metabolomics analysis. The expression levels of several key genes within the metabolic pathway are the main kinetic indicators that control the metabolic pathway (Alper et al., 2005). Phosphoribosyl pyrophosphate amidotransferase is a key enzyme that catalyses the first rate-limiting step of purine nucleotide synthesis, and phosphoribosylformylglycinamidine synthase and adenylosuccinate lyase play catalytic roles in the fourth and eighth steps of nucleotide synthesis (Bilz et al., 2018). Both in the muscle and liver, the expression of *ppat* was significantly upregulated, and *adsl* was also increased in the 0.30RI group compared with the control group. This result indicated that the reaction rate of nucleotide synthesis was accelerated by 0.30RI supplementation, which explains the increase in flavour substance in the body at a molecular level. AMP deaminase and cytosolic purine 5′-nucleotidase play an important role in purine metabolism for the synthesis and catabolism of IMP. The expression of *ampd* was significantly upregulated, and 5′-nt was downregulated, which suggests that the deposition of IMP increased in muscle. The significant increase in *ampd* expression but not 5′-nt expression in the liver indicated that the higher IMP deposition was due to an increase in the rate of IMP synthesis. Combined metabolomics and expression of key genes in liver and muscle revealed that 0.30RI might promote the deposition of flavour substance in flesh by supplementing the substrates for nucleotide synthesis and thereby activating the purine metabolism pathway. In this way, we have partially revealed, theoretically, the reason RI enhances the content of flavour substance in farmed fish and demonstrated that RI may have the potential to be used as a feed additive to improve the quality of fish flesh.

5. Conclusions

Supplementing gibel carp diets with RI resulted in some improvements mainly associated with texture, nutritional value and flavour. The collagen and glycogen content in muscle were enhanced by RI supplementation. The muscle alterations revealed that RI supplementation increased the water holding-capacity and hardness. Furthermore, the addition of RI facilitated the deposition of amino acids and EPA in the muscle, thus improving the nutritional value of the fish. Furthermore, the combined metabolomics and expression of key genes in liver and muscle revealed that 0.30RI might activate the purine metabolism pathway by supplementing the substrates for nucleotide synthesis, thereby promoting the deposition of flavour substance in flesh. Our study reveals the potential mechanism of RI to enhance the flavour profile in fish flesh and provides a new strategy for improving meat quality. Further studies are necessary to investigate the effects of RI supplementation with practical diets, and optimize inclusion levels, in order to maximize its potential benefits.

Author contributions

Wanjie Cai: writing—original draft, methodology, software, data curation, formal analysis, investigation. **Lele Fu:** methodology, software, formal analysis, investigation. **Cui Liu:** methodology, software, formal analysis, investigation. **Linyue He:** methodology, software, formal analysis, investigation. **Haokun Liu:** conceptualization, writing-review & editing, supervision, funding acquisition. **Dong Han:** resources. **Xiaoming Zhu:** conceptualization, funding acquisition, resources. **Yunxia Yang:** resources. **Junyan Jin:** resources. **Shouqi Xie:** conceptualization, funding acquisition, resources.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgements

This work was financially supported by the National Key R&D Program of China (2018YFD0900400), the earmarked fund for CARS (CARS-45), China and National Natural Science Foundation of China (U21A20266). The authors thank Ms. Jun Men (the Center for Instrumental Analysis and Metrology, Institute of Hydrobiology, Chinese Academy of Science) for technical assistance in the measurement of IMP, ATP, AMP and purine metabolites. The authors also thank Mr. Guanghan Nie for his technical assistance.

Appendix Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2022.12.006.

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