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Study on biological characteristics and excellent characters of Baling Tieshan organic bighead carp

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ABSTRACT

Baling Tieshan Reservoir is one of the important artificial inland lakes in Hunan Province, with abundant aquatic resources, including high-quality fish species such as organic bighead carp. In order to further utilize the resource advantages of Baling Tieshan Reservoir and create a high-quality brand, this experiment analysis the biological characteristics and excellent traits of Baling Tieshan organic bighead carp (BTOBC) through techniques such as gene cloning, qRT-PCR, and paraffin section, and provides experimental basis for improving the quality of BOTBC. Pond-cultured bighead carp (PCBC) was used as a control for comparison. The findings reveal that BTOBC exhibits excellent traits in terms of body color, body shape and meat quality, which better meets the market and consumers' demands. Analysis of muscle nutrient composition and observation of muscle fiber histomorphology demonstrate a higher muscle fat proportion, and umami amino acid (AA) content, higher muscle fiber density, enhanced taste in BTOBC. Blood index and liver histology observation indicate that BTOBC has relatively healthier physiological conditions. mRNA expression detection of umami taste receptor genes *T1r1* and *T1r3* in various tissues shows that the meat of BTOBC has a more delicious taste. This study provides a comprehensive understanding of the nutritional characteristics of BTOBC and explores its excellent traits, which can serve as a reference for subsequent processing, comprehensive utilization, and healthy breeding in the reservoir area. Moreover, it offers a scientific basis for the rational use of reservoir resources and the sustainable development of reservoir fisheries.

1. Introduction

Bighead carp (*Aristichthys nobilis*) belongs to the Cyprinidae family and Aristichthys genus. In 2020, its annual production surpassed 3.2 million tons, with China accounting for over 90% of this output. It is one of the important freshwater economic fish in China and is popular among the general public. Bighead carp is mainly distributed in rivers and natural water systems in China, inhabiting the upper layers of water. It has a gentle temperament and grows in the lakes along the rivers during its immature stage [1,2], During the breeding period, when their gonads develop and mature, they spawn in areas with slow water flow in the river. They often return to the lakes along the river for feeding and fattening after spawning [3]. Bighead carp is a filter-feeding fish, primarily consuming cyanobacteria, large rotifers and other plankton [4], earning the nickname "scavenger in the water".

Currently, pond interculture is the primary method of bighead carp aquaculture in China [5,6]. Due to the influence of water quality conditions and the composition of ingested food, numerous studies have shown that bighead carp raised in natural waters possess a more optimal nutritional composition [7], muscle quality [8], flavor and appearance compared to those cultivated in artificial conditions [9]. Consequently, many domestic researchers have conducted studies on the differences

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between wild bighead carp and farmed bighead carp.

The environment of the Yueyang Baling Tieshan Reservoir area is beautiful, with clear mountains and water, and abundant bait organisms, making it very suitable for the growth and reproduction of various fish species. BTOBC, formerly known as "Acacia Lake Fish", is a prime example. As the use of artificial bait is strictly prohibited in Tieshan Reservoir, the bighead carp grows naturally without hormone usage or pollution, resulting in a delicious and sweet taste. It has gained a reputation as the "Tieshan fish water in the world". Consequently, a series of characteristic cuisines featuring "BTOBC" has been developed, generating considerable economic value.

In order to fully understand the nutritional characteristics of the BTOBC and explore excellent traits, this study aims to compare and evaluate the dorsal muscle nutrient composition, blood physiology and immunity, myofiber growth, and expression of fresh taste genes between BTOBC and PCBC. The findings will contribute to improving feeding strategies in bighead carp aquaculture, provide scientific guidance for healthy aquaculture, and provide references for people's health and nutritional diets, and promoting the sustainable development of reservoir green fisheries.

2. Materials and methods

2.1. Ethics statement

All experimental procedures were conducted in accordance with the standards and ethical guidelines established by the Animal Ethical Review Committee of Hunan Normal University, Changsha, China.

2.2. Fish

BTOBC specimens were randomly collected from Baling Tieshan Reservoir in Yueyang with body weights ranging from 1.2 to 7.2 kg and body lengths ranging from 39 to 74 cm. PCBC were randomly purchased from the fresh market of Hunan Normal University, with body weights ranging from 1.27 to 5.6 kg and body lengths ranging from 41 to 71 cm. All samples were healthy, free of injuries and diseases. After measuring the body length and body weight, muscles samples were collected from the back of the head to the front of the tail stalk, following the method described by Huang Feng et al. [10]. The collected samples were cut and mixed, and preserved at low temperature for nutritional detection and analysis. Additionally, eight tissues including liver, kidney, intestine, heart, brain, gill, gonad and muscle of bighead carp were taken for total RNA extraction and qRT-PCR analysis, using the obtained cDNA as a template. The cDNA products were stored in a refrigerator at -20 °C. The specific method was referred to the literature [11].

2.3. Age identification

Take 5 to 10 scales from the upper section of the lateral line and the lower section of the front half of the fish as material for age determination in the bighead carp. Place the scales in warm water and clean them with a soft brush. Observe the growth ring characteristics and determine the age under a microscope [12].

2.4. Determination and analysis of conventional nutrients in bighead carp muscles

Moisture content was determined using the direct drying method, Crude protein content was determined using the Kjeldahl method, Crude fat content was determined using acid hydrolysis, Mineral elements were determined using inductively coupled plasma mass spectrometry, Fatty acid content was determined using the internal standard method, with reference to the national standard GB/T 5009.3-2016, GB/T 5009.5-2016, GB/T 5009.6-2016, GB/T 5009.268-2016 and GB/T 5009.168-2016, respectively.

2.5. Determination and analysis of AA composition and nutritional value in bighead carp muscles

AA determination was measured following Benjama and Masniyom [13]. Protein amino acid quality was evaluated using the amino acid score (AAS) standard [14] and chemical score (CS) model. The evaluation indices included AAS, CS and essential amino acid index (EAAI) [15], amino acid content, and the ratio F value of branched-chain amino acid content to aromatic amino acid content.

2.6. Hematoxylin and eosin (HE) staining and analysis of bighead carp back muscle and liver tissue

The back muscles and liver tissues of BTOBC and PCBC were fixed in Bonn's solution at room temperature for no more than 24h. After fixation, the tissues were dehydrated and embedded in paraffin. Serial sections with a thickness of 5 μm were cut using a Leica RM2015 microtome (Wetzlar, Germany). The sections were stained with HE according to standard procedures and observed histologically, as described in the literature [15,16]. Image J software was used for measuring the longest axis diameter and area and counting the number [17].

2.7. Measurement and assessment of blood indices and immune expression

Blood collection from bighead carp was performed by covering the eyes, head, and tail with a wet cloth and drawing blood from the caudal vein at the lateral line scale. The collected blood samples were placed in 5 mL anticoagulation tubes, labelled, and sent to the Hunan Normal University School Hospital for testing of relevant indicators.

2.8. Molecular cloning and quantitative real-time PCR (qRT-PCR) analysis of T1R1/T1R3

The total RNA was extracted from bighead carp liver tissue as a template, and 5 ′and 3′RACE-ReadycDNA was synthesized using the RACE kit. Specific primers for the coding region were designed based on the cDNA sequences of *T1R1* and *T1R3* genes of *Ctenopharyngodon idella* and *Megalobrama amblycephala* in NCBI. Using the reverse-transcribed cDNA as a template to amplify the intermediate fragments. The specific primers of 5′ and 3′ RACE of the genes were sequenced, and the universal primers UPM and NUP were used for RACE terminal amplification (Table S1).

Total RNA was extracted from major tissues using the protocol described by Zhang et al. [17], RNA was extracted using the RNAfast200 kit and reverse transcribed to cDNA using TAKARA reverse transcription kit. The primers used (Table S2) β-actin selected as an internal reference. Expression differences were analyzed using the $2^{-\triangle\triangle Ct}$ method, as described in Ref. [18].

3. Results and analysis

3.1. Comparative morphological and muscle quality analyses of two kinds of bighead carp

The appearance and morphological indicators of fish, including body length, weight, and plumpness, can be used as indicators to evaluate whether the fish's appearance is beautiful. To screen for excellent characteristics of the BTOBC, we first compared the morphology of BTOBC and PCBC. The age of the bighead carp was determined by examining the scales, and indicators such as weight, body length, and plumpness were measured and recorded for the BTOBC and PCBC.

The comparative chart of the appearance of the BTOBC and PCBC revealed that the former had a darker body surface and a larger head compared to the latter (Fig. 1A and B, Table 1). Fresh muscle comparison demonstrated that the former has firmer muscles, while the latter has

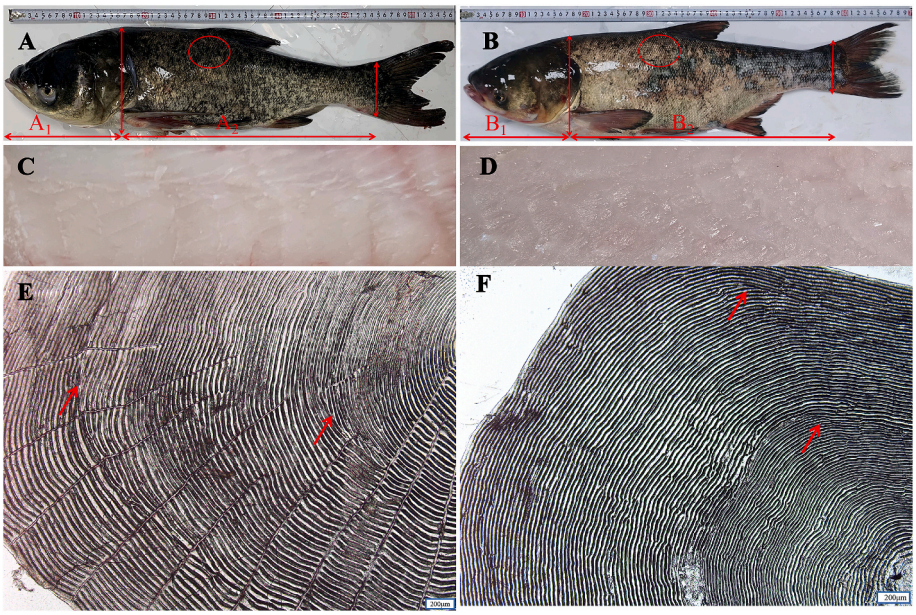

Fig. 1. Sites of scale extraction, muscular (fresh) appearance and scale rings of two species of bighead carp. (A) BTOBC. (B) PCBC. (C)BTOBC fresh muscle. (D)PCBC fresh muscle; (E) BTOBC back scale. (F) PCBC back scale. Scale bar = 200 μm; The red circle indicates the site of scale extraction; A1,B1 represents the length from the head snout to the front of the pectoral fin, A2,B2 indicates the length from the front of the pectoral fin to the front of the caudal fin; E, F red arrow points to the scale ring, the scale shows that the scale bighead carp is 3 years old.

Table 1
Proportions and fullness of two species of bighead carp.

	BTOBC	PCBC
E1 (cm)	172.24	165.49
E2 (cm)	371.90	404.58
E1/(E1+E2)	31.65%	29.03%
E2/(E1+E2)	68.34%	70.97%
Average body weight/g	4074	3490.8
Average body length/cm	60.25	55.3
Fatness degree	1.75 ± 0.20	2.09 ± 0.76*

* It indicated that there was significant difference between BTOBC and PCBC ($P < 0.05$).

relatively looser muscles with higher water content (Fig. 1C and D). These characteristics are more in line with the consumption preferences for eating bighead carp heads. The age range of the bighead carp was determined by identifying the characteristics of the scales' annual rings. It was found that bighead carp weighing 1-2 kg were approximately 2 years old, those weighing 2-3 kg were approximately 3 years old, and those weighing 3 kg or more were 4 years or above (Fig. 1E and F, Table S3).

When comparing BTOBC and PCBC with similar weights, in most cases, the body length of BTOBC is longer than that of PCBC with similar weights (Table 1, S4). Additionally, PCBC displayed significantly higher plump compared to BTOBC (Table 1).

The muscle quality and nutrient content of fish directly impact consumer acceptance and the economic value, as well as human nutrition and health. Therefore, we compared and analyzed the nutritional composition of muscle in two types of bighead carps. The results showed that the crude fat, crude protein, and crude ash content of BTOBC were higher than those of PCBC, while the moisture content was lower in BTOBC (Table 2). The vitamin content in the muscle showed that the vitamin B2, B6, and vitamin E content in BTOBC were much higher than those in PCBC. Among them, the contents of vitamin B2, vitamin B6 and vitamin E in BTOBC were 3 times, 17 times and 2 times higher than those in PCBC respectively, and vitamins B1 and B12 were not detected in either type (Table 2). Furthermore, the content of Ca, Mg, K, Na, Se, and Fe in the muscle of BTOBC was higher than that in PCBC, while the

Table 2
Content of conventional nutrients, vitamins and minerals in the muscle of two species of bighead carp.

	BTOBC	PCBC
Moisture content	78.1	79.3
Crude fat	1	0.7
Crude protein	19.1	18.2
Crude ash content	1.4	1.2
Vitamin A	0.21	0.44
Vitamin B1	<0.10	<0.10
Vitamin B2	0.41	0.15
Vitamin B6	5.33	0.32
Vitamin B12	<5.00	<5.00
Vitamin C	5.49	14.12
Vitamin D	0.29	0.44
Vitamin E	9.53	4.77
Zn	4.18	4.22
Mg	274	249
Fe	4.35	3.3
K	4480	3989
Cu	0.198	0.221
Se	0.286	0.204
Cr	0.025	0.025
Mn	0.56	0.616
Ca	1540	1380
Na	285	270

content of Zn, Cu, and Mn was lower in BTOBC to PCBC. The content of Cr was the same in both (Table 2). The fatty acid composition and content analysis in the muscle of the two bighead carp species are presented in Table 3. BTOBC exhibited higher levels of both saturated and unsaturated fatty acids compared to PCBC, with the content of EPA + DHA being 0.0676g/100g in BTOBC being, and 0.01552g/100g in PCBC.

The AA composition analysis showed that both types of bighead carp had seven essential amino acids (EAAs) and nine non-essential amino acids (NEAAs) in their muscles (Table 4). Except for Gly, the content of the other 15AAs in BTOBC was higher than that in PCBC. The muscle of BTOBC had higher levels of AAs, EAAs and total AAs compared to PCBC. The proportion of umami AAs and EAAs to the total AA content, as well

Table 3
Fatty acids content in muscle tissues of two species of bighead carp.

Fatty acid	BTOBC	PCBC
C14:0	0.0143	<0.0033
C16:0	0.0692	0.0807
C18:0	0.0199	0.025
C23:0	0.011	0.00835
∑SFA	0.1144	0.11405
C16:1	0.0272	0.011
C20:1	0.00343	<0.0033
C18: ln-9c	0.0801	0.0964
∑MUFA	0.11073	0.1074
C18:2n-6c	0.0138	0.0363
n-6PUFA	0.0138	0.0363
C18:3n-3	0.0177	0.0049
C20:5n-3	0.0248	0.00492
C22:6n-3	0.0428	0.0106
n-3PUFA	0.0853	0.02042
∑PUFA	0.0991	0.05672
∑UFA	0.20983	0.16412
∑FA	0.32423	0.27817
DHA + EPA	0.0676	0.01552

Table 4
AA composition and content in muscle tissues of two species of bighead carp.

Classification	AA name	BTOBC	PCBC
EAA	Thr	0.83	0.79
	Val	0.96	0.89
	Met	0.57	0.54
	Leu	0.88	0.82
	Ile	1.58	1.48
	Phe*	0.75	0.68
	Lys	1.79	1.69
	His	0.5	0.45
NEAA	Arg	1.16	1.09
	Asg*	1.91	1.78
	Ser	0.69	0.67
	Glu*	2.88	2.72
	Gly*	0.86	0.88
	Ala*	1.1	1.06
	Tyr*	0.67	0.64
	Pro	0.51	0.49
	∑AA	17.64	16.67
	∑EAA	7.36	6.89
	∑DAA	8.17	7.76
	∑NEAA	10.28	9.78
	∑EAA/∑AA(%)	41.72	41.33
	∑EAA/∑NEAA(%)	71.60	70.45

as the ratio of EAAs to NEAAs, were also higher in BTOBC. The evaluation of the EAAs composition in muscle showed that the EAAI values of BTOBC and PCBC were 84.69 and 83.39, respectively, and the F values were 2.41 and 2.42, respectively. According to AAS and CS scores, it can be seen that Met + Cys was identified as the first limiting AA, followed by Val (Table 5).

3.2. Staining observation on muscle tissue sections of two kinds of bighead carp

Muscle hardness and tenderness are important factors for evaluating the quality and texture of fish flesh [19]. Previous studies have shown that muscle fiber diameter is closely related to muscle hardness and tenderness, with fish muscle hardness being negatively correlated with muscle fiber diameter. Additionally, fish muscle fiber density is positively correlated with subjective indicators of muscle quality such as hardness and cohesion [20,21]. To further assess the quality of the two types of bighead carp muscles, we performed paraffin sectioning and muscle density analysis on both types of bighead carp muscles. We also examined the mRNA expression of growth-related genes in muscle tissue. The results showed that the muscle fiber density of BTOBC was significantly higher than that of PCBC (Fig. 2, Table 5); the expression levels of myostatin (*MSTN*), myogenic regulatory factor family (*MyoD*, *Myf5*, *Myf6*, *MyoG*), and *PRMT5* in BTOBC were significantly higher than those in PCBC; the expression of *MYHC* gene was significantly lower than that in PCBC. There was no significant difference in the expression levels of *Desmin* (Fig. 2).

3.3. Determination of haematological parameters and immune expression of two kinds of bighead carp

Blood is a vital tissue in animals that reflects their nutritional status, physiological metabolism and plays a crucial role in internal transport, defense, immunity, fluid regulation, and maintenance of the internal environment. Determining blood parameters and observing peripheral blood cells in two species of bighead carp will contribute to the knowledge of fish haematology research. Additionally, observing liver tissue sections and studying immune-related genes in bighead carp will provide scientific guidance for healthy breeding. Therefore, we conducted a comparative study on the determination and evaluation of blood parameters and immune expression in PCBC and BTOBC.

The morphological structure of the blood cells did not exhibit significant differences between the two species. Erythrocytes, lymphocytes, thrombocytes, neutrophils and monocytes are all observed in peripheral blood smears (Fig. S1). However, the blood physiological parameters of BTOBC and PCBC differed significantly (Table 6). Levels of Bas, PLT, RDW-C and RDW-S were significantly higher in BTOBC compared to PCBC. On the other hand, levels of RBC, Mon, HGB, HCT, MCV, MCH and MCHC were significantly lower in BTOBC compared to PCBC. The results of serum indicators in Table 7 demonstrated that levels of CHO, HDL-C, LDL-C, Na, Cl, n-Ca and TCa were significantly higher in BTOBC compared to PCBC. Furthermore, levels of IgM, IgG, IgA, AST, CREA, TG, LDH, ALB and A/G were significantly lower in BTOBC compared to PCBC.

The results of the above blood parameters tentatively indicate that BTOBC exhibits superior immune capacity compared to PCBC. Moreover, ALT and AST are mainly present in liver tissue and serve as key indicators of stress and tissue damage in animals [22]. Therefore, based

Table 5
NEAAs profile in muscle of two species of bighead carp.

NEAA	BTOBC	PCBC	WHO/FAO	Egg white	AAS		CS	
					BTOBC	PCBC	BTOBC	PCBC
Thr	271	271	250	292	1.08	1.08	0.93	0.93
Val	314	305	310	411	1.01	0.98	0.76	0.74
Ile	287	281	250	331	1.15	1.12	0.87	0.85
Leu	517	508	440	534	1.18	1.15	0.97	0.95
Met + Cys	186	185	220	386	0.84	0.84	0.48	0.48
Phe + Tyr	464	452	380	565	1.22	1.19	0.82	0.80
Lys	585	580	340	441	1.72	1.71	1.33	1.32
Total	2624	2582	2190	2960				
EAAI	84.69	83.39						
F value	2.41	2.42						

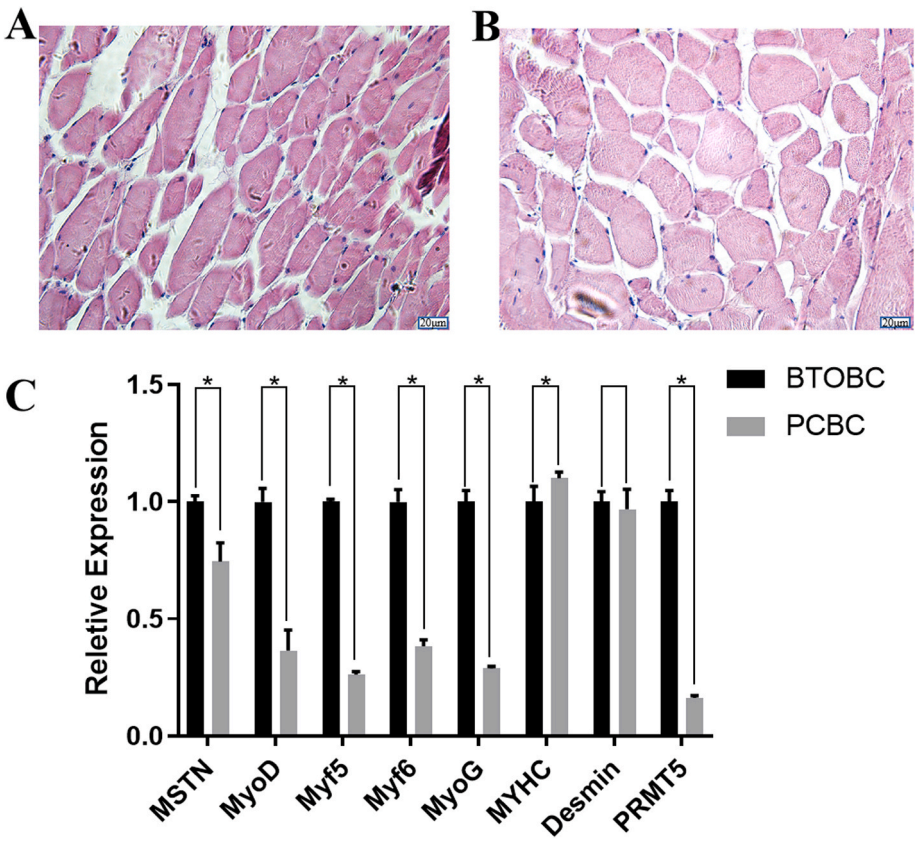

Fig. 2. Muscle paraffin section and mRNA expression of growth-related genes in muscle. **(A)** BTOBC muscle. (B)PCBC muscle. (C)mRNA expression of growth-related genes in muscle tissues of two kinds of bighead carp. Scale bar = 20 μm * represents significant difference.

Table 6
Blood indexes of two species of bighead carp.

Blood index	BTOBC	PCBC
RBC (1012/L) *	1.06 ± 0.72	1.88 ± 0.29
WBC (109/L)	2.11 ± 1.08	3.67 ± 2.99
Neu (109/L)	1.3 ± 1.10	2.07 ± 2.58
Lym (109/L)	0.29 ± 0.29	0.23 ± 0.21
Mon (109/L) *	0.23 ± 0.09	1.28 ± 0.86
Neu (%)	55.54 ± 28.56	46.23 ± 22.57
Lym (%)	7.26 ± 3.55	6.79 ± 1.91
Mon (%) *	12.38 ± 5.96	44.03 ± 22.11
Eos (%)	8.52 ± 11.20	2.89 ± 3.04
Bas (%) *	1.8 ± 2.72	0.09 ± 0.16
HGB (g/L) *	44.22 ± 30.80	116.57 ± 27.53
HCT (%) *	13.19 ± 9.93	31.71 ± 5.29
MCV (fL)*	101 ± 7.75	175.95 ± 10.42
MCH (pg)*	42.59 ± 15.38	61.83 ± 10.80
MCHC (g/L) *	320 ± 5.63	363.86 ± 28.81
PLT (109/L) *	20.56 ± 12.69	7.71 ± 9.62
RDW-C (%) *	25.01 ± 7.57	7.75 ± 0.32
RDW-S (fL) *	109.26 ± 52.57	58 ± 4.30

Table 7
Blood serum indexes of two species of bighead carp.

Serum index	BTOBC	PCBC
IgM (mg/dL) *	0.25 ± 0.08	0.43 ± 0.13
IgG (mg/dL) *	0.21 ± 0.04	0.46 ± 0.15
IgA (mg/dL) *	0.025 ± 0.01927	0.0586 ± 0.03436
ALT (U/L)	39 ± 12.02	47.14 ± 5.15
AST (U/L) *	92.63 ± 19.49	195.86 ± 43.26
TBA (μmol/L)	1.6375 ± 0.91798	1.2286 ± 0.39461
BUN (mmol/L)	0.5075 ± 0.1685	0.9029 ± 0.37779
CREA (μmol/L) *	3.625 ± 1.76777	16 ± 5.35413
UA (μmol/L)	7.295 ± 1.86584	6.7686 ± 3.64521
CHO (mmol/L) *	5.355 ± 1.40288	3.1357 ± 1.09444
TG (mmol/L) *	0.1938 ± 0.09942	0.8471 ± 0.41672
HDL-C (mmol/L) *	1.9188 ± 0.28271	1.3071 ± 0.30407
LDL-C (mmol/L) *	7.0787 ± 2.63159	1.5643 ± 1.43094
GLU (mmol/L)	4.9886 ± 0.44398	4.49 ± 1.41864
K (mmol/L)	1.4063 ± 0.6067	1.4543 ± 1.21953
Na (mmol/L) *	133 ± 4.98569	117.4286 ± 5.34986
Cl (mmol/L) *	102.325 ± 2.61848	77.2571 ± 10.18231
n-Ca (mmol/L) *	1.5575 ± 0.1295	1.1557 ± 0.13986
TCa (mmol/L) *	3.2975 ± 0.26163	2.4557 ± 0.32357
a-AMY (U/L)	92.857 ± 27.65315	107.8571 ± 21.98051
LDH (mg/dL) *	1871.625 ± 493.38827	3307.5714 ± 870.15877
ALP (U/L)	35.5 ± 8.75	48.57 ± 21.92
TP (g/L)	25.45 ± 1.55563	27.8429 ± 2.67884
ALB (g/L) *	12.3875 ± 0.56173	14.5286 ± 1.23655
GLO (g/L)	13.0625 ± 1.04326	13.3143 ± 1.56889
A/G*	0.95 ± 0.04408	1.9043 ± 0.08264

on the comparative analysis of the blood physiological and biochemical parameters of the two types of bighead carp, it is suggested that the liver tissues of PCBC may have an inflammatory response. To further confirm this, liver tissues were observed through paraffin sectioning and HE staining, and expression analysis of immune-related genes was performed.

The number of hepatocytes and vacuoles in the liver tissue of PCBC was significantly higher than that in BTOBC (Fig. 3A and B). Further comparison of mRNA expression levels of immune genes in liver tissue showed that the expression of antioxidant genes *Nrf2*, *Keap-1a* and *Keap-1b* was significantly higher in BTOBC compared to PCBC. Additionally, the expression of anti-inflammatory genes *mTOR*, *CCL4* and *Jun* was significantly higher in BTOBC compared to PCBC. Conversely, the expression of pro-inflammatory genes *IL-1β*, *IL-8*, *TNF-α*, *IFN-γ2* and *IL-6* was significantly lower in BTOBC compared to PCBC (Fig. 3C).

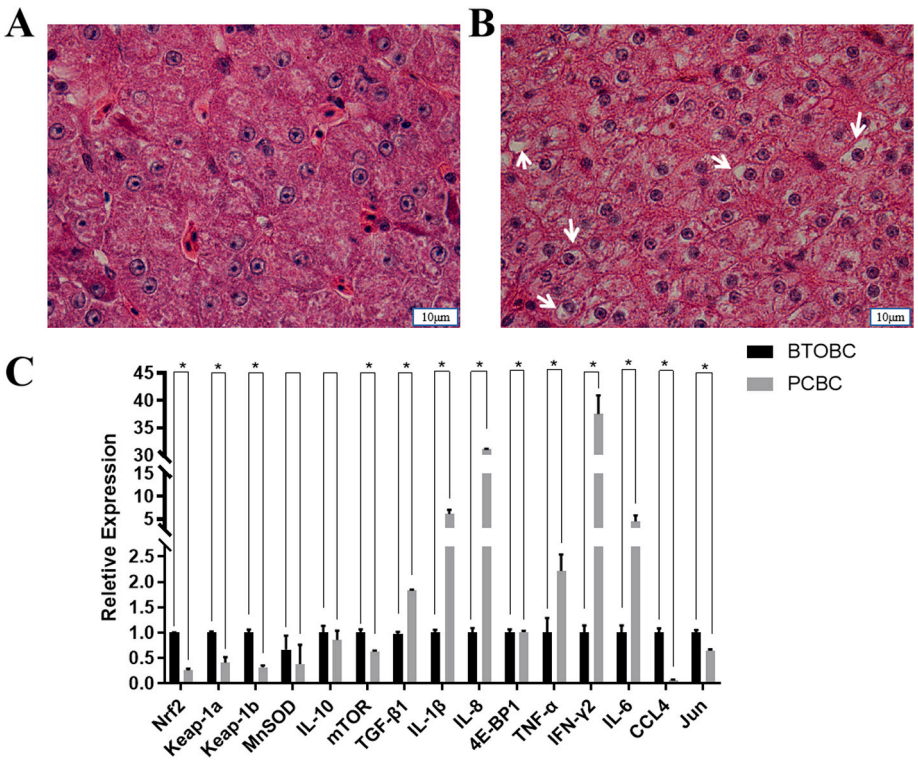

Fig. 3. Paraffin section of liver tissue and mRNA expression of immune-related genes in liver tissue. (A) BTOBC liver tissue. (B) PCBC liver tissue. (C)Immunity-related genes in the two kinds of bighead carp liver tissue mRNA expression. Scale bar = 10 μm * represents a significant difference. The white arrow in the picture indicates hepatocyte vacuoles.

3.4. Molecular cloning of T1R1 and T1R3

The analysis of the nutritional value of BTOBC and PCBC muscles revealed that BTOBC had a relatively higher nutritional value, being richer in umami AAs and unsaturated fatty acids. In order to further explore the molecular mechanism behind the delicious taste of BTOBC, molecular cloning and mRNA expression analysis of the umami taste genes *T1R1* and *T1R3* were conducted.

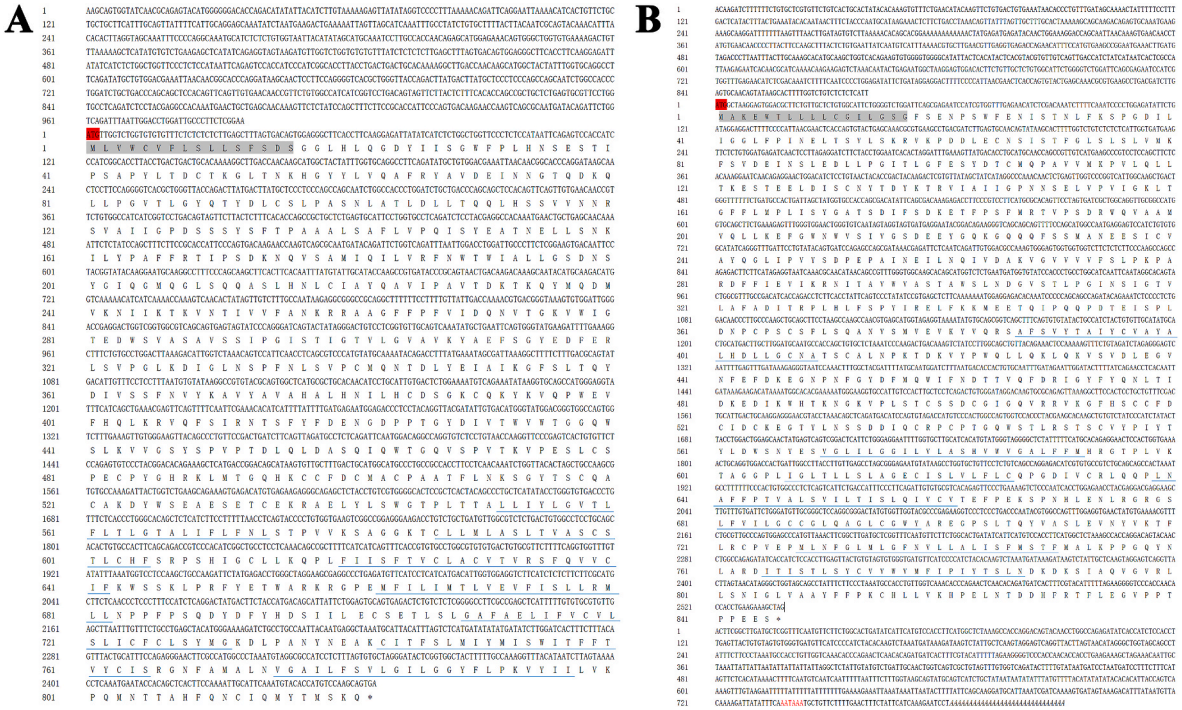

Fig. 4. Gene sequence and deduced AA sequence. (A) *T1R1* Gene sequence and deduced AA sequence. (B)*T1R3* Gene sequence and deduced AA sequence. Red background indicates start codon (ATG). "*" Indicates the termination codon (TAA). The gray background domain is the signal peptide. The blue horizontal line marks the transmembrane area, and the red font "AATAAA" is the polyA trailing signal.

Using RACE technology, the *T1R1* gene was cloned, which includes a 996 bp 5′-UTR and a 2538 bp coding region, encoding 821 AAs (Fig. 4A). Software analysis predicted the protein had a molecular weight of 91,374.02 and contained 55 acidic and 56 basic AAs. The protein had a pI of 7.39, an instability coefficient of 40.87, an aliphatic AA index of 96.05, and hydrophobicity. PSORT II software analysis showed that the protein was localized to the endoplasmic reticulum (44.4%), plasma membrane (33.3%), mitochondria (11.1%) and vesicles (11.1%). Signa lP 5.0 detected a 17-AAs signal peptide at the N-terminus, with the signal peptide cleavage site located between G18 and L19 (Fig. S2). TMHMM Server and SMART4.0 software predicted that there were seven transmembrane regions of the protein (Fig. S3), located at 552-574aa, 587-605aa, 620-642aa, 663-682aa, 709-731aa, 743-765aa and 775-797aa.

The *T1R3* gene was cloned using RACE technology. The cDNA sequence was 4229bp in length, containing an 880bp 5′-UTR, an 811bp 3′-UTR, and a 2538bp intermediate coding region, which could encode 845AAs (Fig. 4 B). ExPASy predicted that the protein contained 83 acidic and 60 basic AAs, with a pI of 4.98. The instability coefficient is 36.32, and it has a hydrophobic nature with a fat amino acid index of 97.99. PSORT II analysis showed that the protein was mainly localized to the plasma membrane (52.2%) and the endoplasmic reticulum (34.8%). Signa lP 5.0 detected a 16-AAs signal peptide at the N-terminal, with the signal peptide cleavage site located between S19 and E20 (Fig. S4). TMHMM Server and SMART4.0 software predicted that there were seven transmembrane regions of the protein (Fig. S5), located at 387-409aa, 570-592-409aa, 607-626aa, 638-661aa, 681-698aa, 728-750aa, 765-787aa.

BLAST homology results showed that the *T1R1* gene of bighead carp exhibited high sequence identity with *Ctenopharyngodon idella* and *Megalobrama amblycephala*, with values of 97.08% and 96.72%, respectively. The *T1R1* gene also exhibited high sequence identity with *Pimephales promelas,* s*Sinocyclocheilus grahami*, *Sinocyclocheilus anshuiensis*, *Sinocyclocheilus rhinocerous*, *Carassius auratus* and*Carassius gibelio,* with values of 93.43%, 91.79%, 91.69%, 91.52%, 91.49%, and 91.16%, respectively. The *T1R1* gene showed sequence identity of 90.35%, 90.01%, 90.51% and 84.48% with *Labeo rohita*, *Puntigrus tetrazona*, *Cyprinus carpio*, *Danio rerio,* respectively. Phylogenetic analysis showed that the *T1R1* gene of bighead carp was most closely related to *Ctenopharyngodon idella*, with bootstrap of 73, followed by *Megalobrama amblycephala.* Additionally, it clustered with *Pimephales promelas*, *Labeo rohita*, and *Puntigrus tetrazona* into one major clade; *Carassius gibelio* and *Carassius auratus* into one major clade; and *Danio rerio* into a separate clade alone (Fig. 5A). The *T1R3* gene of bighead carp exhibited the highest sequence identity with *Ctenopharyngodon idella* and *Megalobrama amblycephala*, with values of 97.64% and 97.02%, respectively. It also showed higher sequence identity with *Pimephales promelas*, *Sinocyclocheilus anshuiensis*, *Sinocyclocheilus rhinocerous*, *Cyprinus carpio*, and *Sinocyclocheilus grahami*, with values of 92.59%, 91.61%, 91.57%, 91.34%, and 91.17%, respectively. The *T1R3* gene exhibited sequence identity of 90.28%, 89.77%, 89.69%, 86.49%, 83.33% and 73.45% with *Labeo rohita*, *Carassius gibelio*, *Carassius auratus*, *Danio rerio*, *Paralichthys olivaceus* and *Oncorhynchus tshawytscha* respectively. Phylogenetic analysis showed that the *T1R3* gene could be divided into three clades. The bighead carp *T1R3* gene first clustered with *Ctenopharyngodon idella* and *Megalobrama amblycephala*, and then with *Pimephales promelas*, *Labeo rohita*, *Carassius gibelio*, and *Cyprinus carpio*. *Danio rerio* formed a separate cluster, while *Oncorhynchus tshawytscha* and *Paralichthys olivaceus* formed another separate cluster (Fig. 5B).

3.5. *T1R1/T1R3 mRNA expression analysis in various tissues of both bigheads*

To compare the mRNA expression levels of *T1R1* in various tissues of the two bighead carps, we found that the expression of *T1R1* was significantly higher in muscle, liver, kidney, heart and gonadal tissues of BTOBC compared to PCBC. However, there was no significant difference in the expression levels in gill, brain, foregut, midgut and hindgut tissues (Fig. 6A). The expression of *T1R3* was significantly higher in muscle, liver, kidney and gonadal tissues of BTOBC compared to PCBC. However, there was no significant difference in the expression levels in gill, heart, brain, foregut, midgut, hindgut and skin tissues (Fig. 6B). In conclusion, the expression of both *T1R1* and *T1R3* genes in muscle tissue, the main site of consumption, was significantly higher in BTOBC compared to PCBC. This indicates that the higher expression of these genes in BTOBC may contribute to its enhanced freshness and taste.

4. Discussion

This study conducted morphological characterization, muscle nutrition component analysis, blood serum index examination, mRNA expression of liver tissue immune genes, and mRNA levels of growth genes in the muscles of BTOBC and PCBC. The umami taste receptor genes *T1R1* and *T1R3* were cloned, and their mRNA expression in various tissues of BTOBC and PCBC were detected. The results showed that BTOBC had better physiological conditions, taste, and nutritional value compared to PCBC, and could better meet the requirements for growth and development, as well as consumer preferences.

The external appearance of a fish plays a crucial role in its quality evaluation, including body shape, color, and flesh characteristics. In our study, BTOBC exhibited a darker body color and a larger head compared to PCBC (Fig. 1A and B). The flesh of BTOBC was denser, with has dense flesh, well-developed muscle fibers, a moist white color with a reddish tinge, and a superior texture. In contrast, the flesh of PCBC was relatively loose, whitish, and had a poor texture (Fig. 1C and D). These observations indicate that BTOBC demonstrates its superior quality and is more favored by consumers. Furthermore, histological analysis revealed that the muscle fibers of BTOBC were smaller in diameter and

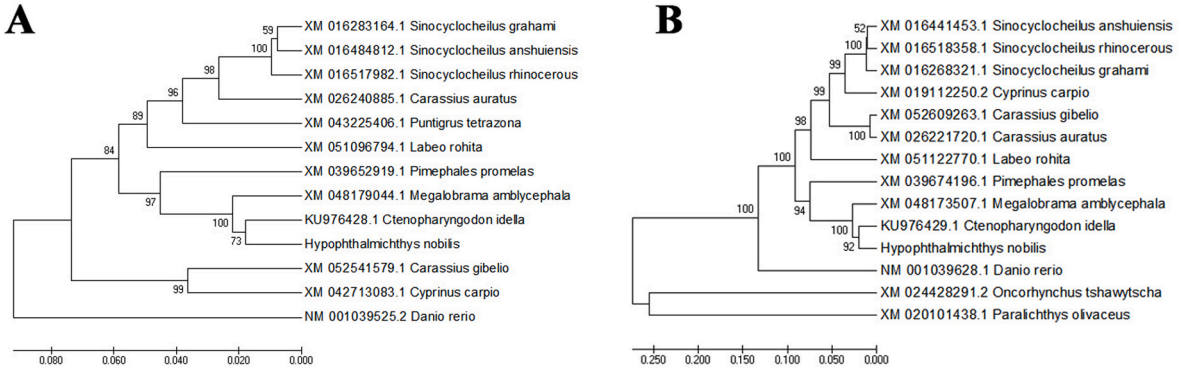

Fig. 5. Phylogenetic analysis of *T1R1* and *TIR3* of bighead carp. (A) Phylogenetic analysis of bighead carp *T1R1*. (B)Phylogenetic analysis of bighead carp *T1R3*.

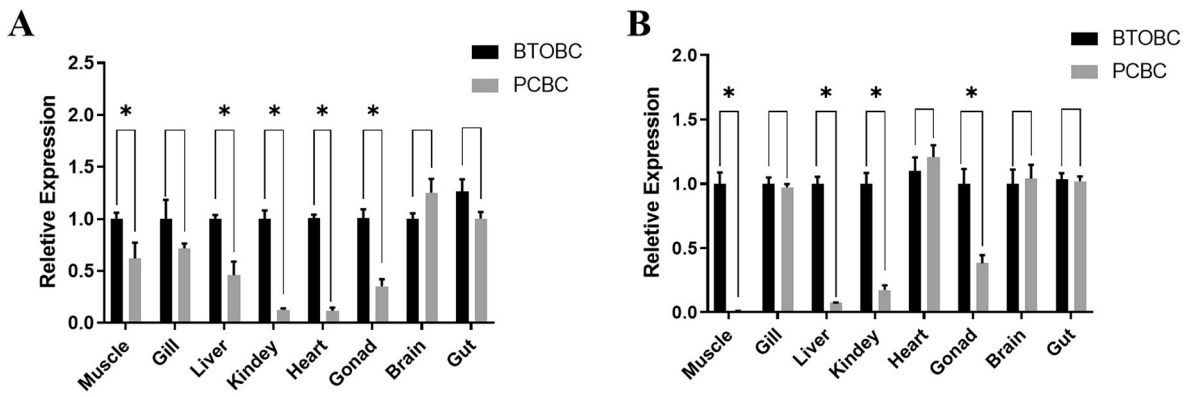

Fig. 6. *T1R1/TIR3* mRNA expression in two kinds of bighead carp tissues. (A)*T1R1* expression in two bighead carp tissues mRNA expression. (B)*T1R3* expression in two bighead carp tissues mRNA expression.

area but had a higher density compared to PCBC. This suggests that BTOBC muscles are firmer and better textured. To explore the molecular mechanism underlying these differences, we examined the mRNA expression levels of muscle growth-related genes, including *MSTN*, *MyoD*, *Myf5*, *Myf6*, *MyoG*. The results showed significantly higher expression of these genes in the muscles of BTOBC compared to PCBC. It is known that *MSTN* and *PRMT5* inhibit muscle growth, *MyoD* and *Mrf5* promotes muscle growth [23]. *Myf5* regulates the growth and development of skeletal muscle cells [24], and *MyoG* is essential for maintaining muscle stability and regulating myoblast fusion. Therefore, the higher expression of these genes may growth-related genes may contribute to the enhanced taste and texture of BTOBC.

Protein content is an important indicator of meat quality, with protein content above 15% considered high-protein meat [25]. The crude protein content of both types of bighead carp is higher than 15%, making them high-protein meat. Fat content up to 3.5-4.5% of the fresh sample will have good palatability and the flavor of the meat continues to change with increasing muscle fat content within a certain range [26]. According to the fat content, <2% is lean fish, 2-4% is low-fat fish, 4-8% is medium-fat fish, and >8% is high-fat fish [27]. BTOBC had a crude fat content of 1%, while PCBC had a content of 0.7%. The slightly higher fat content in BTOBC may contribute to its tender and delicate meat.

Mineral elements and vitamins are essential for normal growth and functioning of an organism. In our study, BTOBC exhibited higher levels of mineral elements such as Ca, Mg, K, Na, Se, Fe and muscle vitamins B2, B6 and vitamin E compared to PCBC. These higher levels indicate that BTOBC provides a richer source of essential minerals and vitamins.

The composition and content of fatty acids play a crucial role in evaluating fish muscle quality. Our analysis revealed that BTOBC contained four saturated fatty acids and seven unsaturated fatty acids, while PCBC contained three saturated fatty acids and six unsaturated fatty acids. The former exhibited higher levels of both saturated and unsaturated fatty acids compared to the latter. The difference may be attributed to the variations in feed between the two types of bighead carp [28]. Studies have shown that n-3 series fatty acids enhance immune function, while n-6 series fatty acids have the opposite effect [29]. The n-3PUFA/n-6PUFA ratio is an important indicator of nutritional balance and human health benefits. According to the standards published by FAO/WHO, a ratio of 0.2 is considered nutritionally balanced, and a higher ratio is more beneficial to human health [30]. In our study, the n-3PUFA/n-6PUFA ratio of BTOBC was 6.2, while PCBC had a ratio of only 0.7. The former exhibited a ratio 8.86 times higher than the latter. These findings suggest that BTOBC may have a better immune effect and more desirable flavor. Furthermore, linolenic acid is a precursor substance for synthesizing EPA, DHA and prostaglandins [31]. The content of linolenic acid in the muscle of BTOBC is 3.6 times that of PCBC. EPA and DHA have various functions, such as promoting cardiovascular health and possessing anti-thrombotic properties [32]. BTOBC exhibited a higher content of EPA + DHA (0.0676g/100) compared to PCBC (0.01552g/100g), with a 4.4-fold difference. Thus, in terms of fatty acid composition, BTOBC demonstrates better quality and provides a healthier option for consumers.

Flavor is a crucial factor in meat quality evaluation and consumer preference [33]. Free amino acids, such as Asp, Ala, His, Glu and Gly, contribute directly to the development of fish flavor [34]. The content and proportion of various AA in fish muscle are important factors in determining its nutritional value. Our study revealed that BTOBC had higher the content of all 15 AAs, except for Gly, compared to PCBC. According to the FAO/WHO recommended standards, a $\sum$EAA/$\sum$AA> 40% and $\sum$EAA/$\sum$NEAA> 60% indicate good quality proteins [35]. In our study, the $\sum$EAA/$\sum$AA ratios were 41.72% and 41.33%, while the $\sum$EAA/$\sum$NEAA ratios were 71.60% and 70.45% in the muscle of BTOBC and PCBC, respectively. This indicates that the AA proportions in both types of muscles are balanced, and they have good nutritional value as high-quality proteins required by the human body. BTOBC exhibited a higher proportion than PCBC, indicating better quality. The AAS and CS scores show that Lys as the highest scoring AA, approximately twice the FAO/WHO standard. This indicates that both PCBC and BTOBC can contribute to Lys supplementation. Additionally, the slightly higher content of Glu in BTOBC (2.88g/100g) muscles contribute to a fresher taste compared to PCBC (2.72g/100g) muscles. The EAAI value of 84.69 for BTOBC muscle was higher than the EAAI value of 83.39 for PCBC muscle, indicating a more reasonable EAA composition in BTOBC.

The biochemical indicators of animal blood serum can directly reflect their nutrition, metabolism, and immune status [36]. MCHC can be used to evaluate anemia in animals [22], and an MCHC value <289 g/L indicates anemia. The MCHC values of all groups were within the normal range, indicating that none of the experimental fish were anemia. However, RBC and HGB levels in blood, as well as serum triglyceride concentrations, were significantly higher in PCBC compared to BTOBC. These findings suggest that PCBC are at a better nutritional level and have a higher metabolic rate due to access to a constant supply of energy resources. ALT and AST in liver tissue are key indicators of animal stress and tissue damage [37]. Our results showed that AST levels were significantly higher in PCBC compared to BTOBC, suggesting that liver tissues in PCBC may be more damaged. Clinical studies have confirmed that oxidative stress and inflammation are the main pathological and physiological factors of acute liver injury [38], which were confirmed by the observation of HE staining in liver tissue paraffin sections. Furthermore, mRNA expression analysis of immune-related genes in liver tissues revealed that the expression of antioxidant factors *Nrf2*, *Keap-1a*, *Keap-1b* and anti-inflammatory genes *mTOR*, *CCL4, Jun* was significantly higher in BTOBC in compared to PCBC. Conversely, the expression of pro-inflammatory genes *TGF-β1, IL-1β*, *IL-8*, *TNF-α*, *IFN-γ2*, *IL-6* was significantly lower in BTOBC. These results demonstrate the superior immune capacity of BTOBC, indicating better

overall health and resistance to oxidative stress and inflammation.

Umami is a complex and desirable taste sensation, the recognition and transduction of freshness in food is accomplished by umami receptors [39].Taste receptor heterodimers *T1R1/T1R3* are considered the major taste receptors responsible for umami perception [40]. In our experiment, we cloned the gene sequences of *T1R1* and *T1R3* and performed a homology comparison, which revealed the highest homology and closest affinity of the *T1R1* and *T1R3* genes in bighead carp to *Ctenopharyngodon idella* and *Megalobrama amblycephala*. Furthermore, we examined the mRNA expression of *T1R1* and *T1R3* genes was examined in different tissues of PCBC and BTOBC, which is consistent with the study that found *T1R1* and *T1R3* genes in different tissues of *Oncorhynchus mykiss*, and moreover confirmed the existence of taste receptors in fish [41]. The expression of both *T1R1* and *T1R3* in muscle were significantly higher in BTOBC compared to PCBC. This finding suggests that the enhanced expression of taste receptors in muscle tissue may contribute to the more delicious taste of BTOBC.

In summary, this study provides comprehensive insights into the differences between BTOBC and PCBC. Compared with PCBC, BTOBC exhibits superior morphological characteristics, higher nutritional value, healthier physiological conditions, and stronger taste attributes. These findings help us understand the factors that affect the quality and taste of bighead carp, and highlight the advantages of BTOBC as a high-quality and highly preferred fish. Additionally, our research provides a scientific basis for the sustainable development of reservoir fisheries.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.repbre.2023.11.002.

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