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A comparative study of muscle nutrition and intermuscular bone number in improved diploid carp

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ABSTRACT

The common carp (*Cyprinus carpio* L., COC, 2n = 100) is one of the most widely consumed and distributed freshwater fish in the world. It ranks fourth in total freshwater aquaculture volume in various regions of China, behind *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, and *Aristichthys nobilis*. However, in recent years, environmental degradation, inbreeding, disordered breeding, and other adverse effects have caused problems such as low seed quality and poor disease and stress resistance of the carp. In our laboratory, we developed two types of improved diploid carp the hybrid F_1 of common carp (♀) × blunt snout bream (*Megalobrama amblycephala*, BSB, 2n = 48) (♂). To investigate the differences between IDC and IDMC compared to COC, in this study we compared the relevant characteristics of these two types of improved carp with those of COC in terms of muscle nutrient composition, intermuscular bone type, and number. The results showed that among the muscle nutrients, IDC had a higher protein content (18.50%) and lower carbohydrate content (0.70%). In addition, the unsaturated fatty acid content of IDC (3.45%) and IDMC (1.25%) were significantly higher than that of COC ($P < 0.05$) (0.50%). For monounsaturated fatty acids such as oleic acid (OA, C18:1 n-9) and linoleic acid (PA, C16:1), the content of IDC and IDMC were also abundant. In terms of intermuscular bone morphology, the morphology and type of intermuscular bone in IDC and IDMC medullary arch ossicles were consistent with those of COC. However, the number of intermuscular bones was different, and the average number of intermuscular bones of IDMC decreased by 14.77% ($P < 0.05$) compared with COC. The advantages of IDC and IDMC are different, with IDC having a higher value of muscle nutrients and IDMC having a lower intermuscular bone content. The distant hybridization of common carp (♀) × blunt snout bream (♂) developed two improved diploid carp varieties with excellent traits, which added carp germplasm resources with high muscle nutrition and lower intermuscular bone, and provided theoretical support for their production application.

1. Introduction

China is the world's largest producer, exporter and consumer of aquatic products, accounting for about one-third of the global market share of aquatic products [1]. In our country, fisheries have always been an important part of our national economy [2]. According to the data from the 2021 China Fishery Statistics Yearbook, the total output value of the fishery economy in 2020 was 2754.35 billion yuan, accounting for 2.72% of the 2020 domestic GDP. Among them, the value of fishery output was 1351.72 billion yuan, accounting for 1.33%. China's total output of freshwater aquatic products was 32.35 million tons, including 30.89 million tons of freshwater aquaculture products, which accounts for 95.49% of China's total freshwater aquatic product output. Products farmed in China not only meet the domestic demand, but also make an important contribution to the global food security and nutritional supply [3].

Aquatic products can provide human with high-quality proteins, unsaturated fatty acids, vitamins, minerals and other nutrients [4]. According to the 2021 China Fisheries Statistics Yearbook, fish aquaculture production accounts for 52.86% of the total output of aquatic products,

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with fish being a key component. The essential amino acid (EAA) pattern of fish protein is very close to human's need, and its digestion and absorption rate is as high as 95%, which is an ideal source of animal protein [5]. Intermuscular bones (intermuscular spines or intermuscular ossicles) are an essential trait in fish genetic breeding [6]. It is a kind of bone unique to teleost fish, located between a muscle ganglion [7], which has the functions of supporting muscles [8] and transmitting mechanical forces between muscles and bones [9]. However, the presence of intermuscular bone can also negatively affect fish's edibility and economic value [10]. For example, deep processing of fish fillets, fish balls, consumption of fresh fish, and etc. Many fish with more intermuscular bones are not favored by consumers [11]. Therefore, the development of improved fish varieties with high nutritional value and reduced intermuscular bone is crucial in fish genetics and breeding.

COC is the main economic fish in China and one of the main freshwater fish species [12]. It is widely distributed in China, especially in the Yellow River Basin Provinces, northern Jiangsu and Liaoning Provinces of important breeding and eating objects [13]. COC has a long history of breeding in China. After continuous development, it has formed many types of carp lineages (varieties), such as the scattered mirror carp [14], Furong carp [15], Amur carp [16], Xingguo red carp [17,18] and so on. According to the 2021 China Fisheries Statistics Yearbook, the 2020 carp aquaculture production was 2.90 million tons, ranking fourth behind *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, and *Aristichthys nobilis*. In recent years, however, some carp germplasm resource reserves have been occupied or reduced, and their genetic diversity has declined due to overfishing and the ongoing deterioration of the aquatic ecological environment. Carp germplasm resources have also shown signs of degradation [2]. Nowadays, the germplasm quality of carp is uneven, the disease resistance of fry is poor, the quality of seedlings is not high [19], and its genetic characteristics need improvement [20]. Hybridization is one of the most commonly used breeding techniques for fish genetic improvement [21]. To solve the problem of germplasm degradation, the genetic material of two individuals or groups with different genetic compositions can be recombined through hybrid breeding technology to form offspring with hybrid advantages [22]. In this study, two types of improved diploid carp (IDC-F_1 2n = 100 and IDMC-F_1 2n = 100) were developed in the hybrid F_1 of common carp (*Cyprinus carpio* L, COC, 2n = 100) (♀) × blunt snout bream (*Megalobrama amblycephala*, BSB, 2n = 48) (♂) [23]. This study will compare the characteristics of these two types of improved carp with those of common carp in terms of muscle nutrient composition, intermuscular bone type, and number.

2. Materials and methods

2.1. Ethics statement

Guidelines developed by the Animal Experiment Administration stipulate that approval from the China Science and Technology Administration and the Wildlife Administration is not required when the experimental fish in question is neither rare nor close to extinction (first-class or second-class protection). Therefore, the experiments performed in this study do not require approval.

2.2. Experimental materials

COC, IDC and IDMC each have three samples, of which common carp was purchased from the vegetable market of Hunan Normal University. IDC and IDMC were taken from the Engineering Research Center of Polyploid Fish Breeding and Breeding Technology of the Ministry of Education of Hunan Normal University. The specifications for several fish species are listed in Table 1.

2.3. Nutrient content determination

Moisture determination: Samples were treated at −55 °C for 72 h using a freeze dryer to weigh the change in weight [24].

Fat determination: From frozen muscle tissue, fat content was determined using the cable extraction method, referring to the national standard GB/T 5009.6-2016.

Protein determination: Lyophilized tissue protein content determined by the Kjeldahl method [5] refers to the national standard GB/T 5009.5-2016.

Ash determination: After powdering the lyophilized tissue of known weight, it was determined by the high-temperature ashing method [25], referring to the national standard GB/T 5009.4-2016.

Carbohydrate: That is the sum of the mass fractions of carbohydrates minus fat (F), protein (CP), and ash (A). Formula: Total carbohydrates (%) = 100 (F + CP + A).

Estimation of total energy value (calorific value): energy = protein (CP) × 17 + fat (F) × 37 + carbohydrate (C) × 17 [24,26].

Amino acid content: The fish muscle was hydrolyzed using acid hydrolysis and analyzed using Hitachi Amino Acid Automatic Analyzer 8900.

Unsaturated fatty acid content: The free fatty acids in the sample extract were neutralized by acid-base titration with a potassium hydroxide-ethanol standard solution, and the endpoint of the titration was indicated using phenolphthalein as an indicator. The fatty acid content was calculated [24].

2.4. Counting of intermuscular bones and morphological observation

The external morphology of all experimental fish was measured using conventional methods. Three fresh samples of 22-month-old females were taken for each species of fish and measured the measurable shapes such as full length, body length, head length, and weight (shown in Table 1). After measuring and counting, the experimental fish were taken to the radiology department of Hunan Normal University Hospital for X-ray pictures.

After the morphological measurement of the experimental fish, the fish body was completely wrapped in a large piece of gauze, steamed in a pot for 10–15 min, cooled for 3–5 min, and then the gauze was removed. Carefully peel the skin of the fish with a scalpel, scissors, and forceps. The morphological distribution of the intermuscular bone is related to the division of the muscle tissue part, with the vertebrae as the boundary, while the anterior and posterior sections are bounded by the posterior edge of the abdominal cavity. The body of fish is divided into four major regions: the inferior caudal axil, the superior caudal axius muscle, the inferior axial muscle of the trunk, and the supraaxial muscle of the trunk. The intermuscular bone is peeled according to the four parts, arranged in order on black paper, and photographed.

Table 1
The morphological features of COC, IDC, IDMC.

Species	Weight (g)			Body Length (cm)			Number of intermuscular bones (gen)		
	Range	Mean	Standard deviation	Range	Mean	Standard deviation	Range	Mean	Standard deviation
COC	1010.00–1480.00	1261.70	193.32	35.00–41.00	38.80	2.72	83~93	88	4.08
IDMC	380.00–625.00	475.00	107.32	24.50–30.00	24.50	2.48	74~77	75	1.41
IDC	790.00–1060.00	895.00	118.11	32.00–36.00	32.00	1.70	83~89	85	2.83

2.5. Data statistics and analysis

SPSS version 21.0 was used for statistical analysis of the data.

3. Results

3.1. Muscle nutrient composition analysis

From Table 2, it can be seen that the moisture value of IDC (74.60%) is significantly lower than that of COC (79.10%) and IDMC (75.40%) ($P < 0.05$). In terms of fat content, IDC (5.00%) was significantly higher than IDMC (3.70%) ($P < 0.05$), while the fat content of IDMC (3.70%) was significantly higher than that of COC (1.90%) ($P < 0.05$). The protein content of IDC (18.50%) was significantly higher than that of COC (16.90%) and IDMC (16.50%) ($P < 0.05$). In the ash determination, the ash content of IDC (1.20%) was similar to that of COC (1.20%), which was slightly higher than that of IDMC (1.10%). Among the total carbohydrates, the content of IDMC (3.30%) was the highest, and the content of IDC (0.70%) was slightly lower than that of COC (0.90%). In terms of total energy value, IDC (511.40 kJ/100g) was higher than IDMC (473.50 kJ/100g) and COC (372.90 kJ/100g). As a result, IDC has a lower water content, higher protein, fatter content, and lower carbohydrate content than the other two types of carps.

3.2. Analysis of amino acid composition and content

In addition to the very low cystine content, 17 amino acids were measured and the results were presented in Table 3. All three types of carp differ only slightly in terms of individual amino acid content. In terms of total amino acid content, the content of IDC (19.08 g/100g) was higher than that of COC (18.95 g/100g) and IDMC (18.44 g/100g) (Fig. 1). The total content of 8 essential amino acids in IDC (8.06 g/100g) and COC (8.05 g/100g) were similar and significantly higher than that of IDMC (7.45 g/100g) ($P < 0.05$).

The umami of carp muscle is mainly associated with four flavored amino acids, such as aspartic acid, glutamic acid, glycine, and alanine. Among them, the total flavoring amino acids, glutamic acid content and alanine content were the highest in COC, followed by IDC and the lowest in IDMC. However, the content of glycine was the highest in IDMC (0.92 g/100g), followed by common COC (0.88 g/100g), and the lowest in IDC (0.87 g/100g). The content of aspartic acid in the three types of carp was the highest in IDC (2.05 g/100g).

3.3. Contents and types of fatty acids

Unsaturated fatty acids can prevent cardiovascular diseases and reduce the risk of heart disease [27]. Unsaturated fatty acids, in turn, include Monounsaturated fatty acids (MUFA) and Polyunsaturated fatty acids (PUFA). It can be seen from Table 4, the content of total unsaturated fatty acids of IDC (3.54 g/100g) was the highest, followed by IDMC (1.25 g/100g), and COC (0.50 g/100g) was the lowest (Fig. 2). The contents of oleic acid (OA, C18:1 n-9) and linoleic acid (PA, C16:1) of IDC were significantly higher than those of IDMC ($P < 0.05$), while those of IDMC were significantly higher than those of COC ($P < 0.05$). In addition, IDC also had great contents of gamma-linolenic acid (GLA, C18:3 n-6) (0.02 g/100g), α-linolenic acid (ALA, C18:3 n-3) (0.16 g/100g), arachidonic acid (ARA, C20:4 n-6) (0.04 g/100g) and EPA (0.02 g/100g). The content of DHA [28], which has the effect of activating brain cells, delaying vision decline and reducing cancer occurrence, was also the highest in IDC (0.06 g/100g), followed by IDMC (0.05 g/100g) and COC (0.04 g/100g) (Fig. 3).

Table 3
Amino acid content in the muscles of COC, IDC, and IDMC.

Amino acid	COC	IDC	IDMC
Aspartic acid (Asp)[c], g/100g	2.03	2.05	2.01
Glutamic acid (Glu)[c], g/100g	2.95	2.91	2.86
Alanine (Ala)[c], g/100g	0.99	0.99	0.96
Glycine (Gly)[c], g/100g	0.88	0.87	0.92
Tyrosine (Tyr), g/100g	0.65	0.64	0.66
Serine (Ser), g/100g	0.78	0.77	0.80
Proline (Pro), g/100g	0.56	0.56	0.64
Histidine (His)[b], g/100g	0.65	0.72	0.64
Arginine (Arg)[b], g/100g	1.41	1.51	1.50
Threonine (Thr)[a], g/100g	0.86	0.87	0.83
Valine (Val)[a], g/100g	0.89	0.89	0.83
Methionine (Met)[a], g/100g	0.56	0.55	0.54
Isoleucine (Ile)[a], g/100g	0.93	0.92	0.82
Leucine (Leu)[a], g/100g	1.63	1.63	1.56
Phenylalanine (Phe)[a], g/100g	0.85	0.87	0.81
Lysine (Lys)[a], g/100g	2.19	2.19	1.92
Tryptophan (Trp)[a], g/100g	0.14	0.14	0.14
DAA	6.85	6.82	6.75
EAA	8.05	8.06	7.45
NEAA	8.84	8.79	8.85
TAA	18.95	19.08	18.44
DAA/TAA (%)	36.15	35.74	36.61
EAA/TAA (%)	42.48	42.24	40.40
EAA/NEAA (%)	91.06	91.70	84.18

DAA: delicious amino acids.
EAA: essential amino acids.
NEAA: non-essential amino acids.
TAA: total amino acids.
[a] Indicated essential amino acids.
[b] Indicated semi-essential amino acids.
[c] Indicated delicious amino acids.

3.4. Comparison of total intermuscular bone number in different fish

As shown in Table 1, the number of intermuscular bones of COC ranged from 83 to 93 with an average of 88, that of IDMC ranged from 74 to 77 with an average of 75, and that of IDC ranged from 83 to 89 with an average of 86 (shown in Table 1). Among the three carp species, COC had the most intermuscular bones, followed by IDC, and IDMC had the least.

3.5. Comparison of bone morphology between muscles

Fishes have a variety of forms of intermuscular bones. According to their complexity, Lv Yaoping et al. [29] summarized them into seven types: "I" shape (without any forks), "卜" shape (two forks at one end obviously unequal length), "Y" shape (two forks at one end equal length), one end is multi-forked (three or more forks at one end), two forks at both ends (two or more forks at both ends), multiple forks at both ends (two or more forks at both ends) and branch shape (more than four forks at both ends, and bifurcating on top of bifurcating). In this study, almost all three carp species contained the above-mentioned seven types of intermuscular bones, but IDC did not have one end of the multiple fork type.

Cyprinid fishes contain small bones of the medullary arch connected

Table 2
Muscle nutrient content of COC, IDC, and IDMC.

Ingredients	Moisture content (g/100g)	Fat (g/100g)	Protein (g/100g)	Ash content (g/100g)	Carbohydrates (g/100g)	Total energy value (kJ/100g)
COC	79.10	1.90	16.90	1.20	0.90	372.90
IDC	74.60	5.00	18.50	1.20	0.70	511.40
IDMC	75.40	3.70	16.50	1.10	3.30	473.50

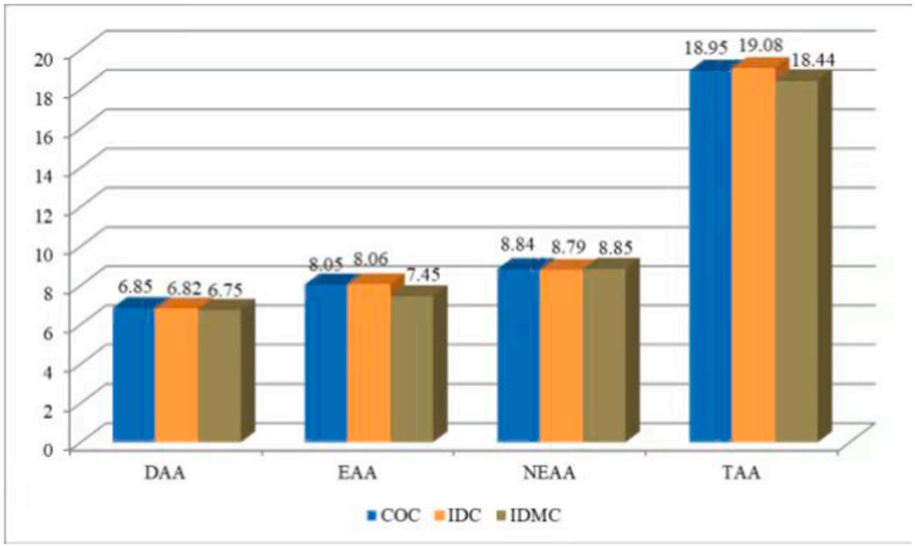

Fig. 1. Comparison of DAA, EAA, NEAA and TAA of COC, IDC, and IDMC.

Table 4
Types and contents of fatty acids in muscle of COC, IDC, and IDMC (g/100g).

Fatty acid	COC	IDC	IDMC
C14:0	–	0.04	0.02
C15:0	–	0.01	–
C16:0	0.08	0.76	0.27
C18:0	0.04	0.19	0.14
∑SFAs	0.12	1.00	0.44
C16:1	0.01	0.25	0.13
C18:1 n-9c	0.16	1.78	0.55
C20:1	0.02	0.11	0.05
C22:1 n-9	0.05	0.07	0.08
C24:1	–	0.10	0.00
∑MUFAs	0.24	2.31	0.81
C18:2 n-6c (LA)	0.15	0.87	0.28
C18:3 n-6(GLA)	–	0.02	0.01
C18:3 n-3(ALA)	0.01	0.16	0.04
C20:2	0.01	0.02	0.01
C20:3 n-6	0.01	0.04	0.02
C20:4 n-6(ARA)	0.01	0.04	0.02
C20:5 n-3(EPA)	0.02	0.02	0.01
C22:6 n-3(DHA)	0.04	0.06	0.05
∑PUFAs	0.26	1.23	0.44
∑n-3 PUFA	0.07	0.24	0.10
∑n-6 PUFA	0.17	0.97	0.33
∑n-9 MUFA	0.21	1.85	0.63
∑(EPA + DHA)	0.06	0.08	0.06
∑(EPA + DHA)/∑PUFA	0.23	0.07	0.14
∑n-3 PUFA/∑n-6 PUFA	0.41	0.25	0.30
∑PUFA/∑SFA	2.17	1.23	1.00
∑UFAs	0.50	3.54	1.25

∑n-3 PUFA: n-3 polyunsaturated fatty acids.
∑n-6 PUFA: n-6 polyunsaturated fatty acids.
∑n-9 MUFA: n-9 monounsaturated fatty acids.
∑(EPA + DHA): Eicosapentaenoic acid and docosahexaenoic acid.
∑(EPA + DHA)/PUFA: the ratio of eicosapentaenoic acid and docosahexenoic acid to polyunsaturated fatty acids.
∑n-3 PUFA/∑n-6 PUFA: the ratio of n-3 polyunsaturated fatty acids to n-6 polyunsaturated fatty acids.

to the medullary arch (epineural bone) and hypaxial muscle segments connected to the pulse arch or ventral ribs (epipleural bone), but not vertebral bones connected to the vertebral body (epicentral bone) existing in the horizontal diaphragm. It does not contain the epicentral bone attached to the vertebrae in the horizontal septum. In this study, all three carp species had epineural bone and epipleural bone, and epineural bone had seven types of intermuscular bone morphology. The epineural bone of COC was "I" shape, "卜" shape, "Y" shape, multi-forked at one end, two forked at both ends, multi-forked at both ends and dendritic shape (Fig. 4C). The epineural bone of IDC has five types of intermuscular bone morphology: "I" shape, "卜" shape, "Y" shape, multi-forked at one end and multi-forked at both ends (Fig. 4F). The epineural bone of IDMC also has seven intermuscular bone morphology: "I" shape, "卜" shape, "Y" shape, multi-forked at one end, two-forked at both ends, multi-forked at both ends and dendritic shape (Fig. 4I). The epipleural bones are simpler than the epineural bones. COC had three types of intermuscular ossicles: "I" shape, "卜" shape and "Y" shape. IDMC has "I" shape and "Y" shape intermuscular bone morphology. The epipleural bone of IDC has four types of intermuscular bone morphology: "I" shape, "卜" shape, "Y" shape and two forked shapes at both ends.

In conclusion, the intermuscular bone types of epineural bone of COC were consistent with those of IDC and IDMC (both contain seven intermuscular bone morphologic types), but the number of intermuscular bone morphologic types was more. The morphology of the three types of epipleural bone is simpler than that of epineural bone. The number statistics of intermuscular bones of 7 different morphological types in the four regions [30] of the fish body of different hybrid carp, above and below the left axis, above and below the right axis, are shown in Table 5.

3.6. Distribution of intermuscular bone in different parts

The three types of intermuscular bones described in this study have a similar morphological distribution, with the epineural and epipleural bones arranged from front to back in the living parts of the fish. The arrangement of the intermuscular bones on either side of the fish is not exactly the same, but slightly different. The number of epineural and epipleural bones on both sides of the fish is also inconsistent. Fig. 4 shows the arrangement of the three species of carp. According to the arrangement of X-rays and intermuscular ossicles' in Fig. 4, the number of epineural and epipleural bones on the left and right sides of the body are not equal in different carp species, but the overall difference is not significant. The morphological distribution of the intermuscular bone is connected with the division of the muscular parts. The upper and lower parts (dorsoventral) are bounded by vertebrae, and the posterior margin of the abdominal cavity is bounded in front and back. The body of the fish is divided into four parts: the supra-axial muscle, the subaxial muscle, the supra-axial muscle, and the subaxial muscle.

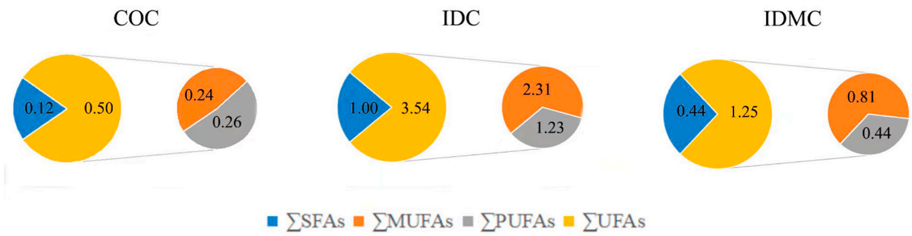

Fig. 2. Fatty acid composition of COC, IDC, and IDMC.
∑ SFAs: Saturated fatty acids content in muscle tissue. ∑ MUFAs: Monounsaturated fatty acids content in muscle tissue. ∑ PUFAs: Polyunsaturated fatty acids content in muscle tissue. ∑ UFAs: Unsaturated fatty acids content in muscle tissue.

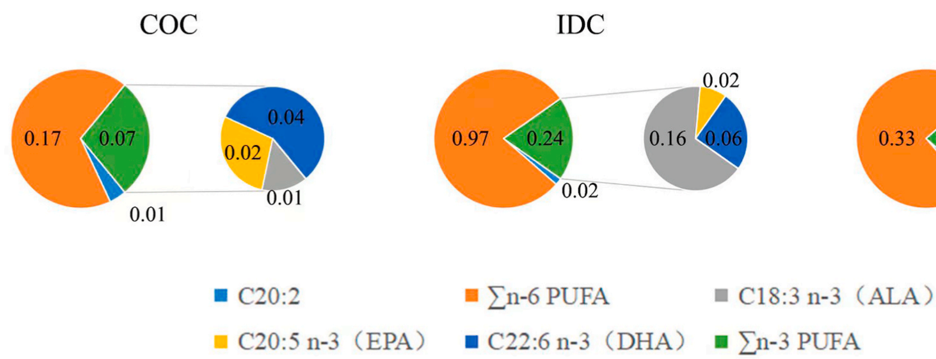

Fig. 3. Polyunsaturated fatty acid composition of COC, IDC, and IDMC. C20:2: Arachidonic acid. ∑n-6 PUFA: n-6 polyunsaturated fatty acids. C18:3 n-3 (ALA): linolenic acid. C20:5 n-3 (EPA): EPA. C22:6 n-3 (DHA): DHA. ∑n-3 PUFA: n-3 polyunsaturated fatty acids.

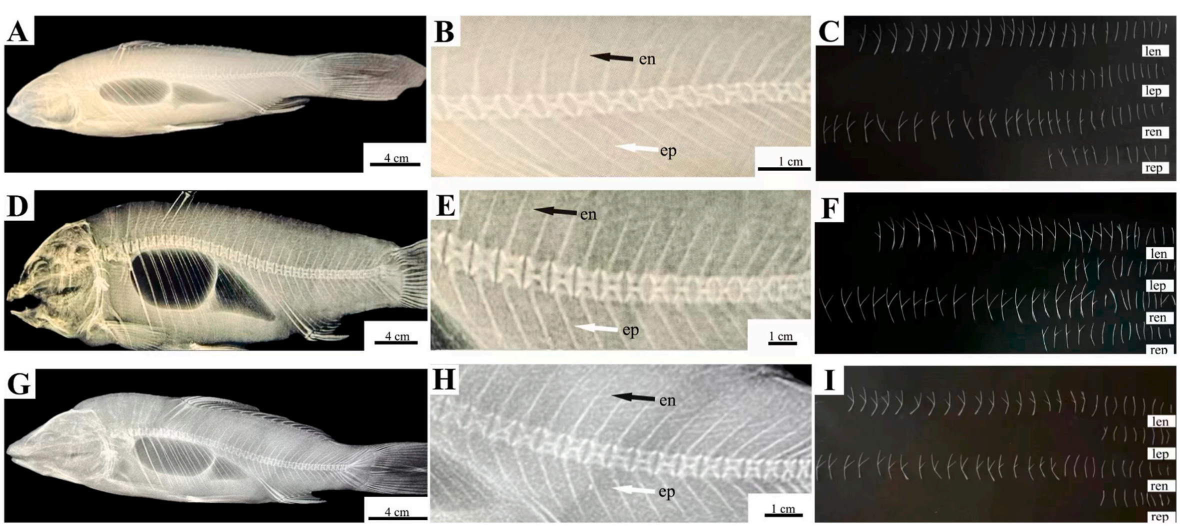

Fig. 4. Morphological observation of small bones and the arrangement of small bones between COC, IDC, and IDMC.
A, D, and G represented the overall morphology of COC, IDC, and IDMC under X-ray, respectively. B, E, and H represented local images of intermuscular ossicles of COC, IDC, and IDMC under X-ray, respectively. C, F, and I represented the arrangement of muscle ossicles of COC, IDC, and IDMC, respectively. The intermuscular ossicles were arranged in order. Len: Left epineural bone; lep: Left epipleural bone; ren: Right epineural bone; rep: Right epipleural bone.

4. Discussion

In this study, the water content of IDC (74.60%) was significantly lower than that of COC (79.10%) ($P < 0.05$) and IDMC (75.40%) ($P < 0.05$). The protein content of IDC (18.50%) was significantly higher than that of COC (16.90%) ($P < 0.05$) and IDMC (16.50%) ($P < 0.05$). However, the carbohydrate content of IDC (0.70%) was lower than that of COC (0.90%) and IDMC (3.30%). The muscle of IDC has higher protein content and lower carbohydrate components, indicating that IDC is a new type of high-quality carp with excellent nutritional structure in terms of a balanced diet [31].

For the muscle nutritional index analysis, the essential amino acid pattern of fish protein is very close to human needs and the nutritional value of protein depends on its amino acid composition and content. In terms of total amino acid content, the content of IDC (19.08 g/100g) was higher than that of COC (18.95 g/100g) and IDMC (18.44 g/100g) (Fig. 1). Flavorful amino acid content is one of the important indicators to measure the flavor of meat [24]. The umami taste of carp muscle is mainly related to aspartic acid, glutamic acid, glycine, and alanine. Experimental results showed that IDC had a high level of

Table 5
The number of intermuscular bones in different morphologic types of COC, IDC, and IDMC (intermuscular bones in the four regions of the fish body).

Fish type	Intermyobone numbers in various morphological types								
	Distribution position	“I” shape	“ト” shape	“Y” shape	one end is multi-forked	two forks at both ends	multiple forks at both ends	branch shape	total
COC	on the left axis	8	6	8	2	2	4	1	31
	On the right axis	10	6	8	3	1	5	1	34
	epineural bone	18	12	16	5	3	9	2	65
	Below the left axis	9	0	1	1	0	0	0	11
	Below the right axis	8	2	2	1	0	0	0	13
	epipleural bone	17	2	3	2	0	0	0	24
IDMC	on the left axis	4	2	3	4	2	10	3	28
	On the right axis	4	2	2	5	2	8	4	27
	epineural bone	8	4	5	9	4	18	7	55
	Below the left axis	10	0	0	1	0	0	0	11
	Below the right axis	9	0	0	0	0	0	0	9
	epipleural bone	19	0	0	1	0	0	0	20
IDC	on the left axis	5	2	11	0	3	5	3	29
	On the right axis	7	1	12	0	2	4	3	29
	epineural bone	12	3	23	0	5	9	6	58
	Below the left axis	9	1	3	0	0	0	0	13
	Below the right axis	10	1	3	0	0	0	0	14
	epipleural bone	19	2	6	0	0	0	0	27

flavor-enhancing amino acids, proving that IDC was an economical fish with good taste. In addition, IDC had higher unsaturated fatty acid content than COC and IDMC. Monounsaturated fatty acids have physiological functions such as protecting the heart, lowering blood sugar, regulating blood lipids, lowering cholesterol and preventing memory decline [32]. In this study, IDC had the highest total unsaturated fatty acid content, followed by IDMC, and COC had the lowest. Among the fatty acids that have been proven to play an important role, such as oleic acid (OA, C18:1 n-9), linoleic acid (PA, C16:1), gamma-linolenic acid (GLA, C18:3 n-6), α-linolenic acid (ALA, C18:3 n-3), arachidonic acid (ARA, C20:4 n-6), DHA, etc. IDC also has the most significant advantages. In summary, IDC has obvious advantages in unsaturated fatty acids compared to COC and IDMC.

In terms of the number of intermuscular bones, IDMC had the fewest intermuscular bones, IDC had slightly more intermuscular bones, and COC had the most intermuscular bones. The intermuscular bones of fish have various forms. According to their complexity, Lv Yaoping et al. [29] summarized them into seven types: “I” shape, “ト” shape, “Y” shape, multi-forked shape at one end, two-forked shape at both ends, multi-forked shape at both ends and dendritic shape. Bing Zhi reported the morphological observation of the intermuscular bone of young carp [33]. Ossification was found in the carp’s greater lateral muscular septum. The intermuscular bone was membranous hard bone, which did not go through the cartilaginous stage in the process of morphological development, but first differentiated from the precursor connective tissue, and then ossified from the tail to the front of the body in turn [34]. All three species of carp mentioned in this study have seven forms of intermuscular bone. The species of intermuscular bone of IDC and IDMC were not significantly reduced compared with COC. But compared with COC, IDMC’s average number of intermuscular bones was decreased by 14.77% ($P < 0.05$), which also proved that IDMC was a high-quality improved carp with fewer intermuscular bones.

Hybridization is a widely used and important breeding technique, which effectively prevents the degradation of varieties and creates excellent varieties [21]. Distant hybridization can produce phenotypic and genotypic changes, leading to new hybrid lineages with genetic variation [35]. The unsaturated fatty acid content and the proportion of flavorable amino acids in IDC muscles were higher than that in common carp, and the number of intermuscular bones in IDMC was lower than that in common carp. Fusion of genomes from different parents (common carp and blunt snout bream) could lead to changes in the phenotype of improved diploid carp, including changes in the proportion of flavorable amino acids and the number of intermuscular bones. According to the experimental results, the advantages of IDC and IDMC are different. IDC has a higher muscular nutritional value, while IDMC has a lower intermuscular bone content. Two types of improved diploid carp with good characteristics were developed by the distant hybridization of common carp (♀) × blunt snout bream (♂), which not only added higher muscle nutrition and fewer intermuscular bones in the muscle but also provided more high-quality aquatic products for the aquatic product processing industry.

Compliance and ethics

The authors declare no competing financial interests.

DAA: delicious amino acids. EAA: essential amino acids. NEAA: non-essential amino acids. TAA: total amino acids.

Declaration of competing interest

The authors declare that they have no competing interests.

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References

[1] W. Zhang, X. Ma, China’s aquaculture development trends since 2000 and future directions, J.Shanghai Ocean Univ. 29 (5) (2020) 661–674.
[2] F. Hu, H. Zhong, C. Wu, S. Wang, Z. Guo, M. Tao, C. Zhang, D. Gong, X. Gao, C. Tang, Development of fisheries in China, Reprod. Breed. 1 (1) (2021) 64–79.
[3] S. Bagheri, A. Gholamhosseini, M. Banaee, Investigation of different nutritional effects of dietary chromium in fish: a literature review, Biol. Trace Elem. Res. 201 (5) (2023) 2546–2554.
[4] Z. Yong, L. Jing, W. Qian, C. Huan, O. Jie, P. Yingjie, Nutritional and health efficacy of aquatic products for humans with the replenishment of terrestrial resources, J. Fish. China 45 (7) (2021) 1235–1247.
[5] S. Li, X. Yang, S. Fan, Z. Zhou, R. Zhou, C. Wu, D. Gong, M. Wen, Y. Wang, M. Tao, Comparative analysis of muscle nutrient in two types of hybrid bream and native bream, Reprod. Breed. 2 (3) (2022) 71–77.

[6] X. Yang, G. Tong, T. Yan, L. Dong, Z. Sun, H. Xu, G. Tang, Y. Kuang, Effects of the CILP gene on the development of the intermuscular bone and spine in zebrafish, J. Shanghai Ocean Univ. (2022) 1–17 (in Chinese).
[7] J. Yang, G. Tong, X. Zheng, Z. Sun, W. Lv, X. Sun, Comparative analysis of skeletal development between wildtype zebrafish and intermuscular bone-deficient mutants, Acta Hydrobiol. Sin. 44 (3) (2020) 546–553.
[8] Z. Dong, D. Huang, L. Li, X. Yuan, W. Miu, Q. Chen, Z. Lu, B. Zhang, Preliminary Study on Intermuscular Bones of Several Cultured Cyprinids. Ournal of Shanghai Fisheries University, vol. 4, 2006, pp. 425–429.
[9] I.A.K. Fiedler, S. Zeveleva, A. Duarte, X. Zhao, B. Depalle, L. Cardoso, S. Jin, J. P. Berteau, Microstructure, mineral and mechanical properties of teleost intermuscular bones, J. Biomech. 94 (2019) 59–66.
[10] J. Chen, X. Chen, X. Huang, G. Huang, Z. Gao, W. Wang, H. Liu, Genome-wide Analysis of Intermuscular Bone Development Reveals Changes of Key Genes Expression and Signaling Pathways in Blunt Snout Bream (Megalobrama amblycephala), Genomics, 2020 (prepublish).
[11] L. Ma, Z. Dong, S. Su, J. Zhang, W. Liu, L. Li, X. Yuan, Research progress on intermuscular spines in fish, Jiangsu Agric. Sci. 40 (4) (2012) 234–235 (in Chinese).
[12] Y. Lu, Discussion on the status quo and germplasm of carp culture, Agric. Technol. 34 (5) (2014) 176 (in Chinese).
[13] Q. Zhu, Y. Qian, Introduction of the main edible carp aquaculture species in China, Fish. Guide Rich (22) (2017) 37–38 (in Chinese).
[14] T. Liu, W.-Y. Wei, K.-Y. Wang, Q. Yang, E.-L. Wang, Pathological and immunological analyses of Thelohanellus kitauei (Myxozoa: myxosporea) infection in the scattered mirror carp, Cyprinus carpio, Sci. Rep. 9 (1) (2019) 1–12.
[15] H. Cheng, Pan Qiansheng, G. Ma, D. Ren, C. Yang, Advance on common carp breeding, J. Inner Mongolia Univ. Nationalities (Nat. Sci. Ed.) (2) (2001) 155–158.
[16] A.V. Nedoluzhko, M.V. Gladysheva-Azgari, G.M. Shalgimbayeva, A.A. Volkov, N. V. Slobodova, S.V. Tsygankova, E.S. Boulygina, V.Q. Nguyen, T.T. Pham, D. T. Nguyen, Genetic contribution of domestic European common carp (Cyprinus carpio carpio) and Amur carp (Cyprinus carpio haematopterus) to the wild Vietnamese carp population as revealed by ddRAD sequencing, Aquaculture 544 (2021), 737049.
[17] G. Hu, X. Liu, L. Zhong, H. Liang, S. Hu, G. Zou, Complete mitochondrial genome of Xingguo red carp (Cyprinus carpio var. singuonensis) and purse red carp (Cyprinus carpio var. wuyuanensis), Mitochondrial DNA Part A, DNA mapping, sequencing, and analysis 27 (1) (2016).
[18] C. Wang, A Study on the Genetic Diversity of Chinese Red Carp, Shanghai Fisheries University, 2002 (in Chinese).
[19] J. Tang, A brief analysis of the current situation and countermeasures of carp culture, Mod. Animal Husband. Sci.Technol. (1) (2018) 36 (in Chinese).
[20] J. Zhu, Discussion on the status quo and germplasm of carp culture, China Fish. 3 (2001) 79–80 (in Chinese).
[21] S. Wang, C. Tang, M. Tao, Q. Qin, C. Zhang, K. Luo, R. Zhao, J. Wang, L. Ren, J. Xiao, et al., Establishment and application of distant hybridization technology in fish, Sci. China Life Sci. 62 (1) (2019).
[22] Z. Zhang, J. Chen, L. Li, M. Tao, C. Zhang, Q. Qin, J. Xiao, Y. Liu, S. Liu, Research advances in animal distant hybridization, Sci.China(Life Sci.) 57 (9) (2014) 889–902.
[23] Y. Fu, Study of Genetic Variation in Modified Diploid Carp Strains, Hunan Normal University, 2020 (in Chinese).
[24] Z. He, S. Liu, J. Xiao, F. Hu, M. Wen, L. Ye, C. Zhang, K. Xu, M. Tao, K. Luo, et al., Muscle nutrients of the backcross progeny of female diploid F_1 hybrid(blunt snout bream × topmouth culter) × male blunt snout bream and its parents, J. Fish. China 38 (10) (2014) 1786–1792.
[25] J. Yan, Y. Li, X. Liang, Y. Zhang, M.A. Dawood, D. Matuli'c, J. Gao, Effects of dietary protein and lipid levels on growth performance, fatty acid composition and antioxidant-related gene expressions in juvenile loach Misgurnus anguillicaudatus, Aquacult. Res. 48 (10) (2017) 5385–5393.
[26] D. Gong, L. Xu, Q. Liu, S. Wang, Y. Wang, F. Hu, C. Wu, K. Luo, C. Tang, R. Zhou, A new type of hybrid bream derived from a hybrid lineage of Megalobrama amblycephala (♀)× Culter alburnus (♂), Aquaculture 534 (2021), 736194.
[27] N. Du, X. Huang, Q. Hao, C. Li, S. Wu, Z. Chen, Analysis of nutritional components and evaluation of nutritive quality in muscle of eight members in salmonidae, Chin. J. Fish. 35 (4) (2022) 9–18.
[28] L. Zhou, D. Shen, H. Zhan, Studies on the nutritional components of fish muscles and human health, Animal Husband. Feed Sci. 34 (5) (2013) 69–71.
[29] Y. Lv, B. Bao, Y. Jiang, L. Yang, J. Li, Comparative analysis of intermuscular bones in lower teleosts, J. Fish. China (5) (2007) 661–668.
[30] Z. Zhong, A Comparative Analysis of the Intermuscular Bone of the Bream and its Parent, Hunan Normal University, 2014 (in Chinese).
[31] Y. Chen, Chinese dietary reference intakes:A historical overview, Acta Nutr. Sin. 43 (2) (2021) 105–110.
[32] W. Zhang, G. Zhong, W. Wang, Study survey of nutrition and biological function of MUFA, J. Cereals Oilseeds (3) (2005) 13–15.
[33] Z. Bing, Observation of the septum needle of the large side of the juvenile carp, Acta Zool. Sin. (2) (1962) 175–179 (in Chinese).
[34] G. Karsenty, E.F. Wagner, Reaching a genetic and molecular understanding of skeletal development, Dev. Cell 2 (4) (2002).
[35] K. Luo, S. Wang, Y. Fu, P. Zhou, X. Huang, Q. Gu, W. Li, Y. Wang, F. Hu, S. Liu, Rapid genomic DNA variation in newly hybridized carp lineages derived from Cyprinus carpio (♀)× Megalobrama amblycephala (♂), BMC Genet. 20 (1) (2019) 1–19.