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Proximate compositions evaluation, histology and transcriptome analysis revealed the effects of formulated diets on muscle quality in *Micropterus salmoides*

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ABSTRACT

With the gradually upgrade of formulated feeds, the largemouth bass *Micropterus salmoides* has been domesticated to adapt to formulated diets to reduce the use of bait fish and cut cost. However, whether the formulated diets influence the nutritional content and muscle quality of largemouth bass and related mechanisms remains unclear. In this study, we evaluated the effects of forage fish and formulated diets on growth performance, proximate compositions, muscle histology and fiber character. Largemouth bass with initial weights of 39.37 ± 1.37g and 39.45 ± 1.40g were fed with formulated diets and forage fish for 8 months, respectively. The results showed that formulated diets group (FDG) exhibited higher levels of protein content and free amino acids compared with forage fish group (FFG). Interestingly, the diameter of muscle fiber was larger in FDG than in FFG through microsection observation. A comparative analysis of transcriptome was constructed for muscle tissues of FDG and FFG, respectively. Compared with FFG, 2186 differentially expressed genes (DEGs) were identified in FDG, including 1915 upregulated genes and 271 downregulated genes. Gene ontology (GO) analyses revealed that DEGs were significantly enriched in GO terms regarding mitochondrial metabolism, muscle growth and development. KEGG enrichment indicated that DEGs were involved in the amino acid metabolism. Our results indicated that replacing forage fish with formulated diets affected the muscle quality and fiber character of largemouth bass, which could provide a basis for the regulation of nutrition and accurate selection for meat traits in largemouth bass.

1. Introduction

The largemouth bass *Micropterus salmoides*, a perciform species that is native to the United States, was introduced to China in 1983 [1] and is now widely cultured around the world as a commercially valuable fish, with annual production levels of over 470,000 tons in China [2]. At present, this species is one of the major freshwater cultured fish in Guangdong Province of China due to its fast growth, delicious taste and easy fishing [3].

In traditionally commercial aquaculture, largemouth bass is cultured with forage fish as the main diet, which increases wild fish catches and destroys marine fishery resources. Due to the high costs of frozen raw fish, limited availability, environmental pollution and their potential role as a vector for disease transmission, the use of forage fish for diet has hindered the large-scale development of the largemouth bass aquaculture in China [4]. In recent decades, several pieces of research have been conducted regarding the replacement of fish meal or frozen fish with formulated diets containing plant protein to breed largemouth bass [5–7]. Meanwhile, the implementation of marine fishing bans and environmental protection policies further promotes the popularity of formulated diets. Largemouth bass was gradually domesticated to adapt to formulated diets, but formulated diets could not meet its nutrient requirements. An increasing number of researchers have evaluated the nutritional requirements of largemouth bass, such as alternative proteins, lipid sources or starch levels, which dramatically facilitated the replacement of forage fish with formulated diets for largemouth bass [4, 8]. Meanwhile, the effects of formulated diets and forage fish on growth performance and nutritional components have been revealed in several

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aquatic animals [9–11]. For example, *Nibea albiflora* fed with formulated diets had a lower feed conversion ratio and a significantly higher level of crude protein than those fed with forage fish [10]. The fat content and amino acid contents were significantly higher in *Epinephelus lanceolatus*♂ × *Epinephelus fuscoguttatus*♀ fed with formulated diets than those fed with forage fish [11]. However, little is known about of the effects of formulated diets and forage fish on the nutrition content, such as protein, fat and amino acids, as well as the effects of these diets on muscle growth and histology in largemouth bass.

Transcriptome has emerged as a powerful molecular biological method, which has special advantages in identifying and profiling the expression of coregulating genes, and allows for a wider understanding of related metabolisms affected by formulated feeds. For instance, the dietary carbohydrate in formulated feed changed its key gene expression related to glycometabolism of the largemouth bass liver through transcriptome analysis [8,12]. Another piece of research on the liver of loach *Misgurnus anguillicaudatus* demonstrated that differently expressed genes were enriched in lipid metabolism, which were responsive to dietary oxidized fish oil [13]. Accordingly, transcriptomic analyses provided an effective approach to determine the molecular mechanism of how formulated diets affect the proximate compositions and muscle quality of fish.

Despite our previous working on the effects of formulated diets on the liver damage, it was also interesting to check whether the formulated diets influenced the muscle quality of largemouth bass or not. In this study, meat quality, muscle fiber feature and differentially expressed genes (DEGs) of muscle tissue of largemouth bass between formulated group (FDG) and forage fish group (FFG) were compared by proximate composition analysis, histological section observation and transcriptome analysis. The results helped us comprehensively investigate the potential mechanism that how formulated diets would affect the amino acid metabolism and muscle fiber development of largemouth bass.

2. Materials and methods

2.1. Fish husbandry

One hundred and eighty mixed-sex largemouth bass juveniles of initial weight 39.37 ± 1.37g and 39.45 ± 1.40g were divided into two groups (formulated diets group and forage fish group), and each group, with triplicates, was cultured in three 8 m^3 disinfectant tanks equipped with a circulating filtration system. All three replicates were cultured in different tanks. The pH (7.5–8.0), water temperature (26–30 °C), dissolved oxygen (>5 mg/L), nitrite (<0.1 mg/L) and ammonia nitrogen (<0.2 mg/L) were monitored daily during the feeding trial. FDG and FFG were feed with formulated diets and frozen *Decapterus maruadsi*, respectively, for 8 months. The nutritional compositions of both diets and the dietary formulation of the formulated diet are listed in Table 1 and Table 2. The flow of the formulated diets' procession was in accordance with the previous method [7].

2.2. Growth performance calculation

The survival rate (SR), weight-gain rate (WGR), specific-growth rate (SGR) and feed conversion ratio (FCR) were calculated as follows:

Table 1
Proximate composition of the diets.

Proximate analysis (%)	Formulated diet	The forage fish
Crude protein (%)	46.1	20.0
Crude fat (%)	14.5	4.4
Ash (%)	12.0	4.7
Moisture (%)	7.5	64.3
Crude fiber (%)	0.5	0.6
Carbohydrate (%)	19.4	6.0

Table 2
Ingredients of the formulated compound diets.

Raw materials	%
Fish meal	52
Soybean meal	15
Fish oil	12
Ground wheat	14
Vitamin complex	1
Composite mineral	4
Squid paste (fish food attractant)	2

$$SR\ (\%) = (Nf/Ni) \times 100$$

Ni = initial fish number; Nf = final fish number.

$$WGR\ (\%) = [(wf —ws)/\ ws] \times 100$$

ws = initial average body weight (g); wf = final average body weight (g).

$$SGR\ (\%) = [(\ln Wf — \ln Ws)/d] \times 100$$

Ws = initial total body weight (g); Wf = final total body weight (g); d = days.

FCR = feed consumed (g)/biomass increase (g)

2.3. The proximate compositions and amino acid profile analysis

All experimental procedures in this study were performed in accordance with the guidelines and after the approval of the Animal Care and Use Committee of Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences. For muscle and amino acid content analyses, muscle tissues of 5 samples on both sides of the spine were cut from FDG and FFG, respectively. Fishes were anesthetized using MS-222 (100 mg/kg) before sampling. The proximate compositions, including moisture, crude fat, crude protein and ash, were determined according to the direct drying method (GB 5009.3-2016) [14], Soxhlet extraction method (GB 5009.6-2016) [15], Kjeldahl method (GB 5009.5-2016) [16] and Burning weighing method (GB 5009.4-2016) [17], respectively. The amino acid content in foods (GB 5009.124-2016) [18] was determined to detect the amino acid profile (cystine and tryptophan were not tested in GB Standards Methods) of both groups. Component determination was accomplished in the Institute of Analysis, Guangdong Academy of Science.

2.4. Nutritional quality evaluation of amino acids

According to the amino acid scoring standard model, as recommended by the FAO/WHO, and using egg protein as an ideal protein reference [19], indices including the Chemical Score (CS) Amino Acid Score (AAS), and Essential Amino Acid Index (EAAI) were calculated as follows to measure the amino acid quality.

aa represents the amino acid content of the sample (%); AA_{Egg} represents the content of the same amino acid in whole egg protein (%); $AA_{FAO\&WHO}$ represents the content of the same amino acid recommended by FAO&WHO (%); n represents the number of essential amino acids (n = 8). A, B, …, H is the content of essential amino acids of sample protein (mg/g, prot); AE, BE, …, HE is the content of essential amino acids of whole egg protein (mg/g, prot).

2.5. Histological assessment

Muscle tissues (0.5cm × 0.5cm × 0.5 cm) were collected below the dorsal fin on the left side. Seven fresh tissues of each group were fixed with 4% PFA for more than 24 h. After dehydrating with gradient

alcohol and clearing with xylene, samples were wax-soaked and sliced on the paraffin slicer (Lecia RM2016, German). The 4 μm slices were routinely stained with hematoxylin and eosin (H&E), after dewaxing and dehydrating with xylene and ethanol.

The target area of the tissues was selected for 400-times imaging using the Eclipse CiL microscope (Nikon, Japan). When imaging, the entire field of view was filled with the tissue to ensure the background light of each photo was consistent. The H&E slides were analyzed with Image-Pro Plus 6.0 (Media Cybemetics, America). Five muscle fibers of each slice were selected to measure the diameter and area. The density of muscle fiber (n/mm^2) was calculated by counting the fiber number and measuring the area (mm^2) of three fields for each slide. Ten slides were taken for each sample.

2.6. Sample collection, RNA extraction, sequencing, assembly and functional annotation

Total RNA was extracted from muscle of FDG and FFG, respectively, using TRIzol Reagent (Invitrogen, USA), following the manufacture's instruction. The quality and quantity of RNA were checked with Nanodrop ND-2000 and Technologies 2100 Bioanalyzer (Agilent Tech, USA). The same amount of RNA for three females and three males from the same group were mixed as a sample to remove the bias caused by gender. A total of 5 μg of mixed RNA of each sample was used for library construction and high-throughput sequencing on the Illumina HiSeq™ 2000 sequencing platform. Assemblies were evaluated before and after filtering using a combined suite of metrics, including the N50 values and median contig lengths reported by the TrinityStats.pl script from the Trinity assembler [20], TransRate scores [21] and BUSCO completeness scores [22]. The expression levels of transcripts were calculated with RSEM, differentially expressed genes (DEG) analysis was conducted by edgeR with adjusted P value using the Benjamini–Hochberg procedure $\leq$0.001, fold change $\geq$2. KEGG, and GO pathway enrichment analysis was conducted by R package clusterProfiler 3.10 [23].

2.7. Quantitative realtime PCR (qRT-PCR)

The first-strand cDNA was synthesized from total RNA following the protocols of the PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, China) in a 20 μl reaction volume. Gene-specific primers (Table S1) were designed with Primer premier 5.0. Quantitative real-time PCR (qRT-PCR) was performed using an ABI 7300 Real-Time PCR System with SYBR® Premix Ex Taq™ II (TaKaRa, China). Relative expression levels of target genes were analyzed with three biological replicates in each reaction and were normalized to EF1-α gene and calculated using the 2-ΔΔCT method.

2.8. Statistical analysis

Statistical analyses were performed using SPSS 26.0 (SPSS Inc., Chicago, IL, USA). All the datapoints were indicated using means ± standard deviation (SD). Comparison between FDG and FFG was carried out using Student's t-test, in which $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Growth performance

The largemouth bass of FDG and FFG did not show significant differences in survival rate (Table 3). Fish fed with formulated diets exhibited a significantly lower weight-gain rate, specific-growth rate and feeding conversion ratio (Table 3).

Table 3
Growth performance and somatic indexes (Mean ± SD).

Parameters	Formulated diet group	Forage fish group
Survival rate (%)	96.67 ± 0.05	94.44 ± 0.04
Initial weight (g)	39.37 ± 1.37	39.45 ± 1.40
Final weight (g)	282.73 ± 81.28**	467.25 ± 44.95**
Weight gain rate (WGR) (%)	618.00 ± 34.77**	1084.40 ± 20.46**
Specific growth rate (SGR) (%)	0.82 ± 0.02**	1.03 ± 0.01**
Feed conversion ratio (FCR)	1.62 ± 0.02**	4.25 ± 0.26**

** represents $P < 0.01$.

3.2. Comparison of proximate compositions of muscle tissues between FDG and FFG

Table 4 showed that the crude protein content in muscle tissues of FDG was significantly higher than that of FFG (19.52 ± 0.07 vs 18.76 ± 0.54, P = 4.70E-02). No significant difference was observed in crude fat (2.46 ± 0.89 vs 3.54 ± 1.33), moisture (76.52 ± 0.77 vs 75.76 ± 1.26) or ash content (1.28 ± 0.04 vs 1.24 ± 0.08) between the two groups (P > 0.05).

3.3. Amino acid profiles in muscle tissue

The comparison of free amino acids (FAA) composition between two groups is depicted in Fig. 1a. Compared with FFG, the concentration of all FAA except glutamic acid, methionine and proline were significantly higher in FDG (P < 0.05) (Table S2). The level of total FAAs in FDG increased by approximately 10% in comparison to FFG, in accordance with the elevated protein content in formulated diets. Similarly, the contents of essential amino acid (EAA) and nonessential amino acid (NEAA) were observed to be significantly higher in FDG than in FFG (Fig. 1b. P = 1.47E-02, 1.41E-02, respectively).

3.4. Nutrition evaluation of amino acids

The AAS, CS and EAAI of FDG and FFG were calculated and compared with the amino acid scoring standard pattern suggested by FAO/WHO and the standard amino acid pattern of whole-egg protein. The AAS and CS were consistently lower in FFG than those in FDG, suggesting FDG was richer in essential amino acids and better balanced in its composition (Table 5). Valine was the main limiting amino acid of both groups, as suggested by its content. The EAAI of FDG was higher than that of FFG, indicating a higher protein quality in FDG (98.05 vs 92.63).

3.5. Muscle fiber characteristics analysis

To assess the influence of different diets on myofiber morphology, we compared the fiber parameters of two groups. The muscle fiber features are displayed in Fig. 2a and b. Compared with FFG, the fiber diameter was significantly larger in FDG (0.099 ± 0.016 vs 0.072 ± 0.016, P = 1.59E-02, Fig. 2d). However, FFG exhibited an in-creased density of muscle fibers compared to FDG (120.63 ± 22.97 vs 225.97 ± 80.18, P = 3.06E-02, Fig. 2c).

Table 4
Comparison of proximate compositions between Formulated diet group (FDG, n = 5) and Forage fish group (FFG, n = 5).

	FDG (g/100g)	FFG (g/100g)	P value
Moisture	76.52 ± 0.77	75.76 ± 1.26	0.34
Crude protein	19.52 ± 0.07	18.76 ± 0.54	0.047
Crude fat	2.46 ± 0.89	3.54 ± 1.33	0.22
Ash	1.28 ± 0.04	1.24 ± 0.08	0.41

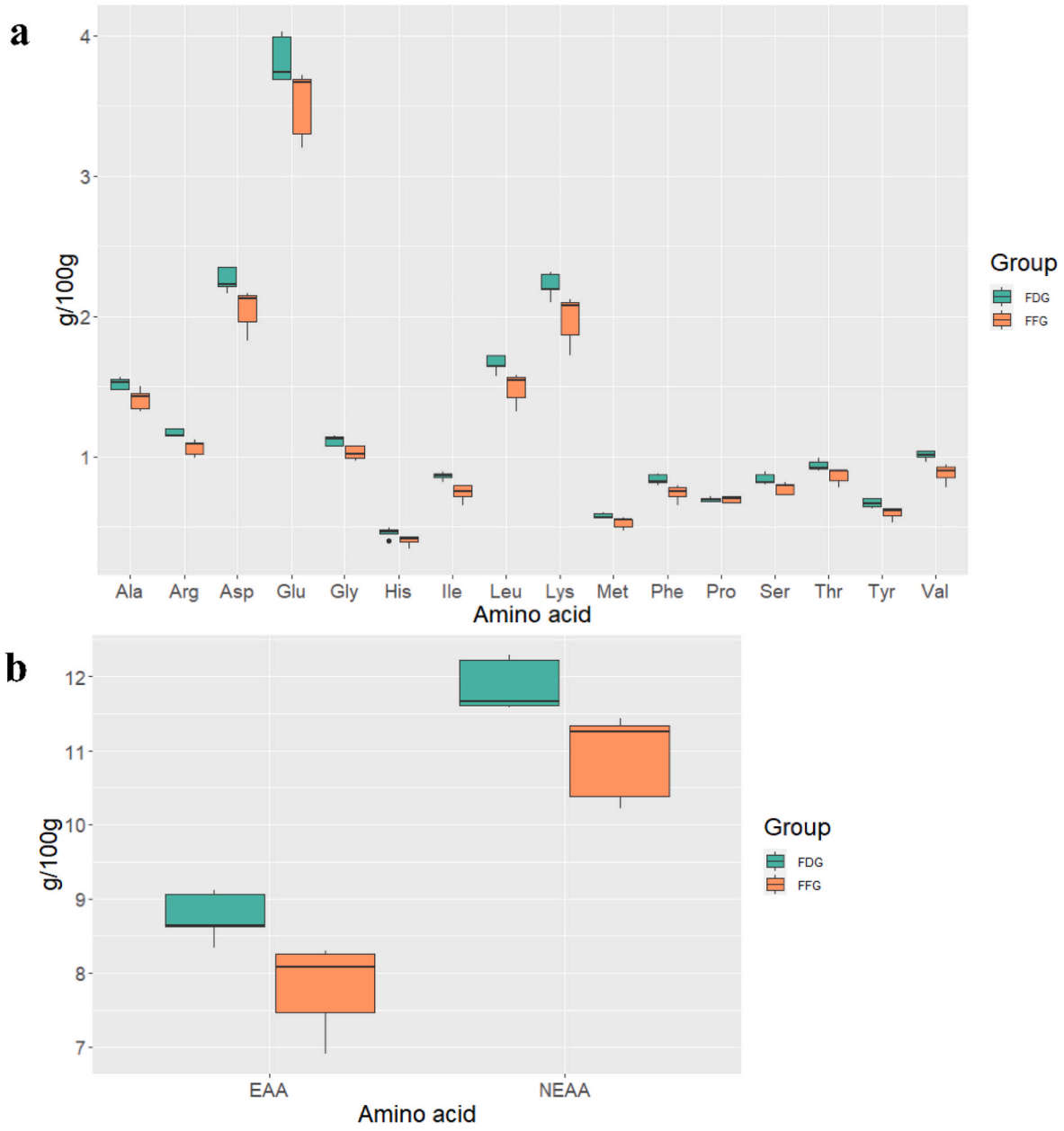

Fig. 1. Comparison of amino acid content between formulated diets group (FDG, n = 5) and forage fish group (FFG, n = 5). (a) The concentration of all free amino acid except glutamic acid, methionine and proline were significantly higher in FDG ($P < 0.05$). (b) The content of essential amino acid (EAA) and nonessential amino acid (NEAA) were observed to be significantly higher in FDG ($P < 0.05$). The green bar and orange bar represents FDG and FFG, respectively.

Table 5
Essential amino acid composition and evaluation of muscles of largemouth bass.

Amino acid	FAO/WHO standard (mg·g^{-1} prot)	Egg protein standard (mg·g^{-1} prot)	FDG			FFG		
			content (mg·g^{-1} prot)	AAS	CS	content (mg·g^{-1} prot)	AAS	CS
Ile	40	54	43.95	1.10	0.81	39.31	0.98	0.73
Leu	70	86	84.94	1.21	0.99	79.05	1.13	0.92
Thr	40	47	47.95	1.20	1.02	45.93	1.15	0.98
Lys	55	70	113.63	2.07	1.62	105.33	1.92	1.50
Val	50	66	51.74	1.03	0.78	46.88	0.94	0.71
Phe + Tyr	60	93	76.36	1.27	0.82	70.96	1.17	0.78
EAAI			98.05			92.63		

FDG: Formulated diet group; FFG: Forage fish group; CS: Chemical Score; AAS: Amino Acid Score, EAAI: Essential Amino Acid Index.

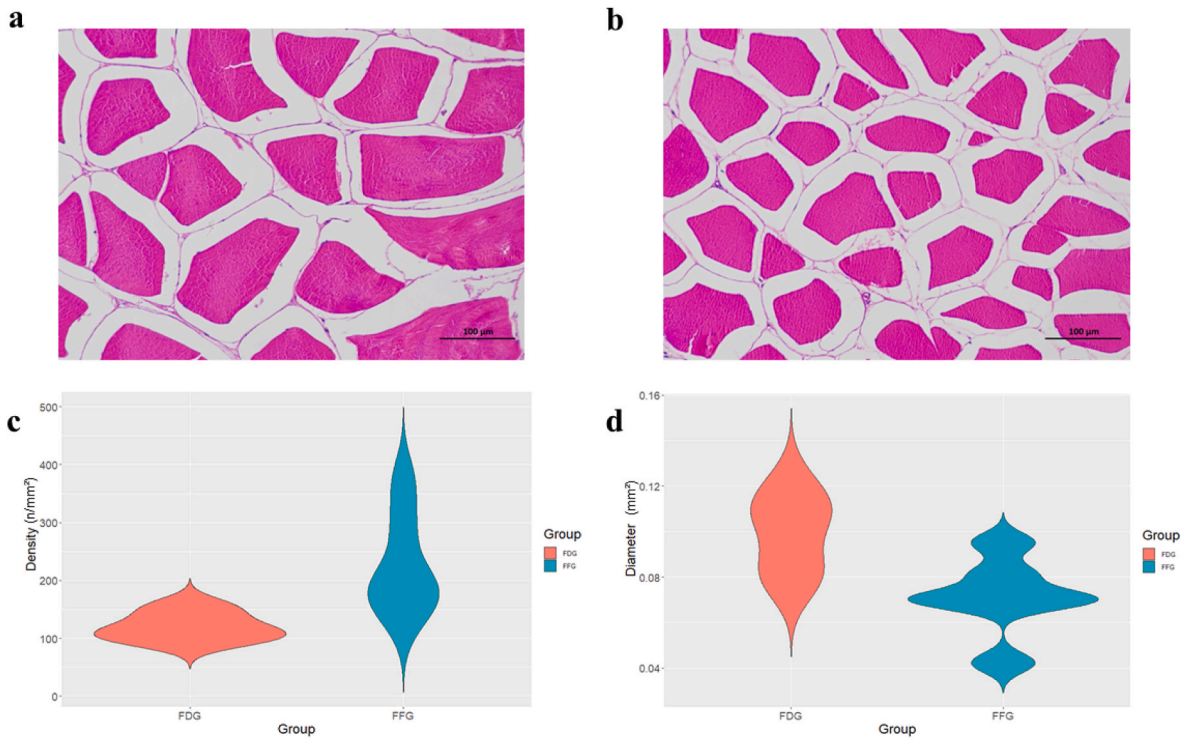

Fig. 2. Comparison of muscle fiber features between formulated diets group (FDG, n = 7) and forage fish group (FFG, n = 7). (a, b) Microstructure observation in the muscle. The black column is scale bar (100 μm). (c) The fiber density was significantly lower in FFG. (d) The muscle fiber diameter was significantly larger in FDG. The blue bar and orange bar represent FFG and FDG, respectively.

3.6. De novo transcriptome assembly of the muscle tissues of Micropterus salmoides

The above-described studies demonstrated that formulated diets may increase the protein content and quality and change the fiber characters of muscle tissues in largemouth bass. To explore the potential molecular regulatory mechanism underlying this biological process, we extracted total RNA from muscle tissues of FDG and FFG and performed transcriptome sequencing. Clean reads were pooled for FFG and FDG, respectively, and assembled de novo to generate reference sequences. The sequencing and assembly statistics are summarized in Table S3.

3.7. Screening and functional annotation of differentially expressed genes

A total of 2186 differentially expressed genes (DEGs) were assigned with adjusted P value ≤ 0.001(FDR) and fold-change ≥2 (Table S4). Compared with FFG, 1915 and 271 genes were upregulated and downregulated in FDG, respectively (Fig. 3a). To validate the different expressions of genes, eight genes were randomly selected for qPCR analysis (Fig. 3b). The expression levels were consistent with transcriptome results. An analysis of the correlation between the RNA-seq and qRT-PCR results yielded a Pearson correlation coefficient of 0.92, which indicated the reliability of the sequencing results.

Through KEGG and GO enrichment analysis, we explored the function of 2186 DEGs. DEGs were significantly enriched into 32 KEGG pathways (Fig. 4). "Glycolysis/Gluconeogenesis" related to glycometabolism was significantly enriched (adjusted P value = 5.48E-03). Notably, three pathways directly associated with amino acid metabolism, including "Glycine, serine and threonine metabolism", "Arginine and proline metabolism", "Cysteine and methionine metabolism", were significantly enriched (adjusted P value = 3.33E-07, 1.1E-03, 9.01E-03, respectively). The pathway analysis of the DEGs identified some key genes that were closely associated with enzyme. Taking "Arginine and proline metabolism" as an example, the expression level

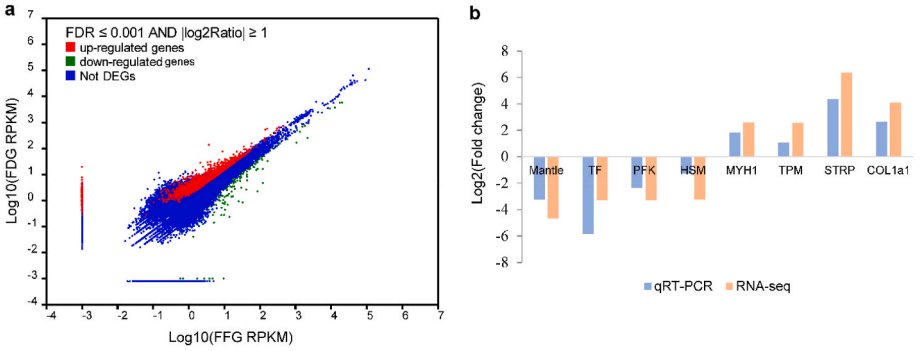

Fig. 3. Identification and verification of differentially expressed genes (DEGs). (a) Distribution and expression levels of DEGs. Red dots indicate the upregulated DEGs, and green dots represent the down regulated DEGs. (b) Comparison of the fold change of FDG/FFG in eight DEGs determined by qPCR and RNA-seq. The x-axis and y-axis represent the Log2(Fold change) and the gene names, respectively.

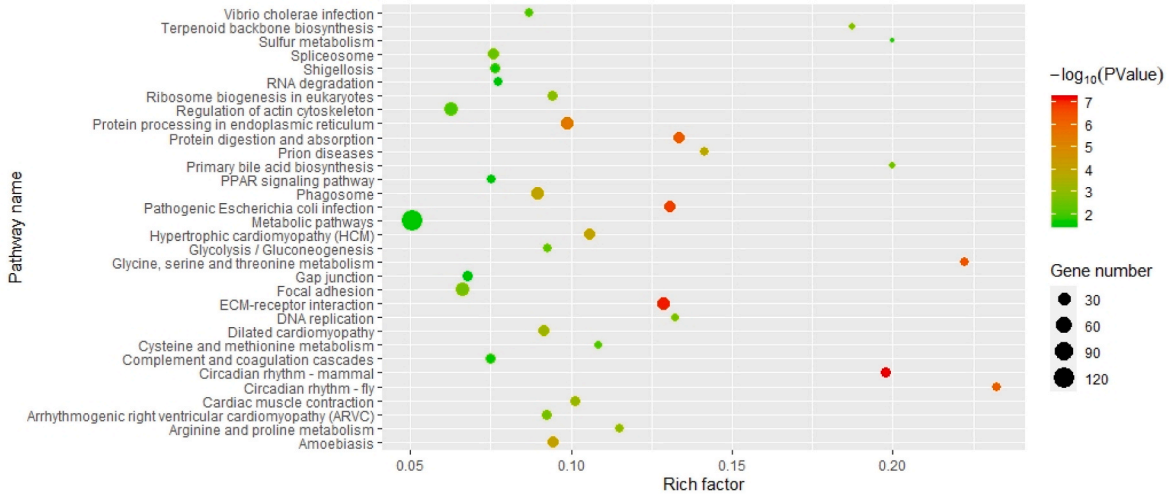

Fig. 4. KEGG pathway enrichment analysis of differentially expressed genes (DEGs) (q-values≤0.05). The x-axis represents the rich factor of each pathway and y-axis shows pathway name. The size of circle indicates the numbers of DEGs assigned to the corresponding pathway.

of pyr-roline-5-carboxylate reductase 2 (*PCRY2*), ornithine decarboxylase (*ODC*), prolyl 4-hydroxylase subunit alpha-1 (*P4HA1*), S-adenosylmethionine decarboxylase proenzyme (*AMD1*), Ornithine aminotransferase (*OAT*) and creatine kinase B (*CKB*) were significantly upregulated. Moreover, aminoacylase-1A-like (*ACY1*) and Arginase II (*ARG2*), which were important enzymes participating in arginine catabolism, were downregulated (Fig. 5a). Besides, "Protein processing in endoplasmic reticulum" which involved in protein metabolism was also significantly enriched (adjusted P value = 4.54E-07,2.92E-06, respectively). Notably, homology genes Sec*61α*, Sec*23A* and Sec*23D*, which were indispensable in protein translocation and transportation, were significantly upregulated (Fig. S1). Core genes encoding fibronectin, collagen and laminin in "ECM-receptor interaction" pathway were significantly upregulated, and involved in muscle development (Fig. 5b).

Several GO terms that were significantly enriched in the three sections (Cellular Component, Molecular Function, Biological Process) are displayed in Fig. 6. Fifteen terms related to muscle development and growth were significantly enriched in molecular function (adjusted P value < 0.05), such as "skeletal muscle contraction", "striated muscle cell differentiation", "myofibril assembly". Cellular Component involved in mitochondria were enriched, for instance, "mitochondrion", "mitochondrial part" and "mitochondrial membrane".

4. Discussion

4.1. Growth performance, influenced by different diets

Diet is an important source of nutrition and energy, which greatly affects the growth and development of fishes. In this study, the largemouth bass of FDG showed a slower growth rate but lower FCR than FFG (Table 3). Likewise, several previous studies have also verified that aquatic animals, such as turbot (*Scophthalmus maximus*), *Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂ and *Epinephelus coioides*, fed with forage fish exhibited a much faster growth rate and a much higher FCR when compared with those fed with a formulated diet [24–26]. For example, the FCR of turbot fed with forage fish and a formulated diet was 3.00 and 1.05, respectively [24]. Therefore, researchers have

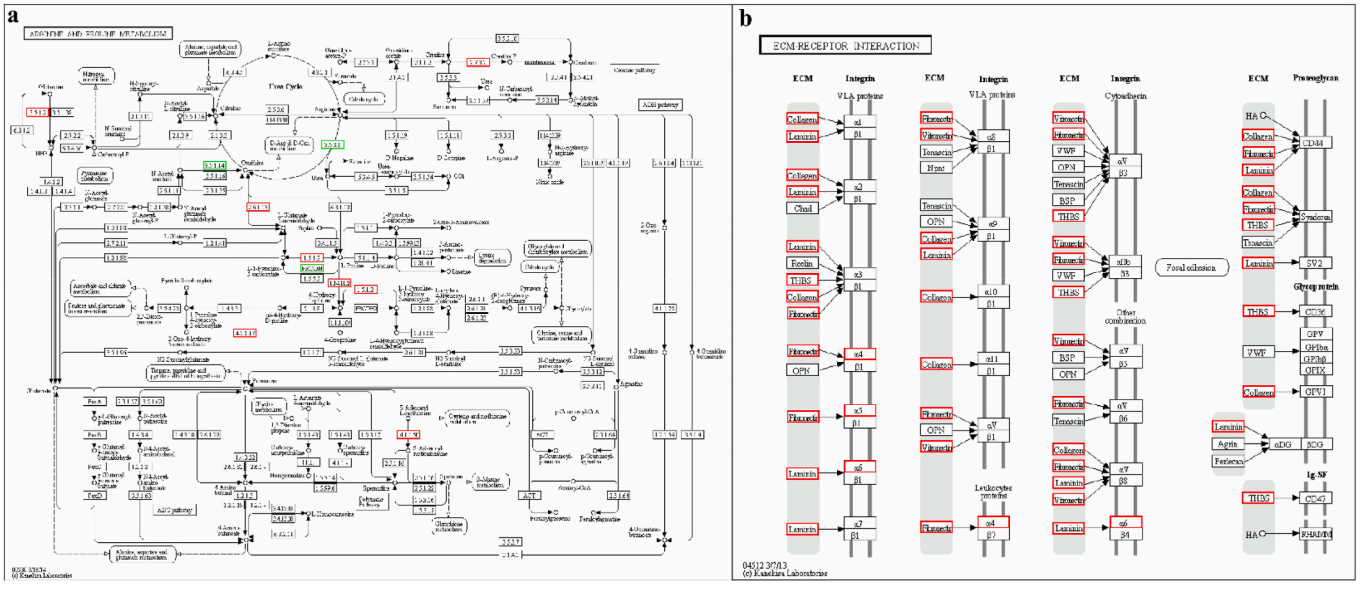

Fig. 5. KEGG pathway analysis of differentially expressed genes (DEGs). (a) Thirteen DEGs in arginine and proline metabolism pathway. (b) Thirty DEGs in ECM-interaction receptors. Up-regulated and downregulated DEGs are shown in red and green block, respectively.

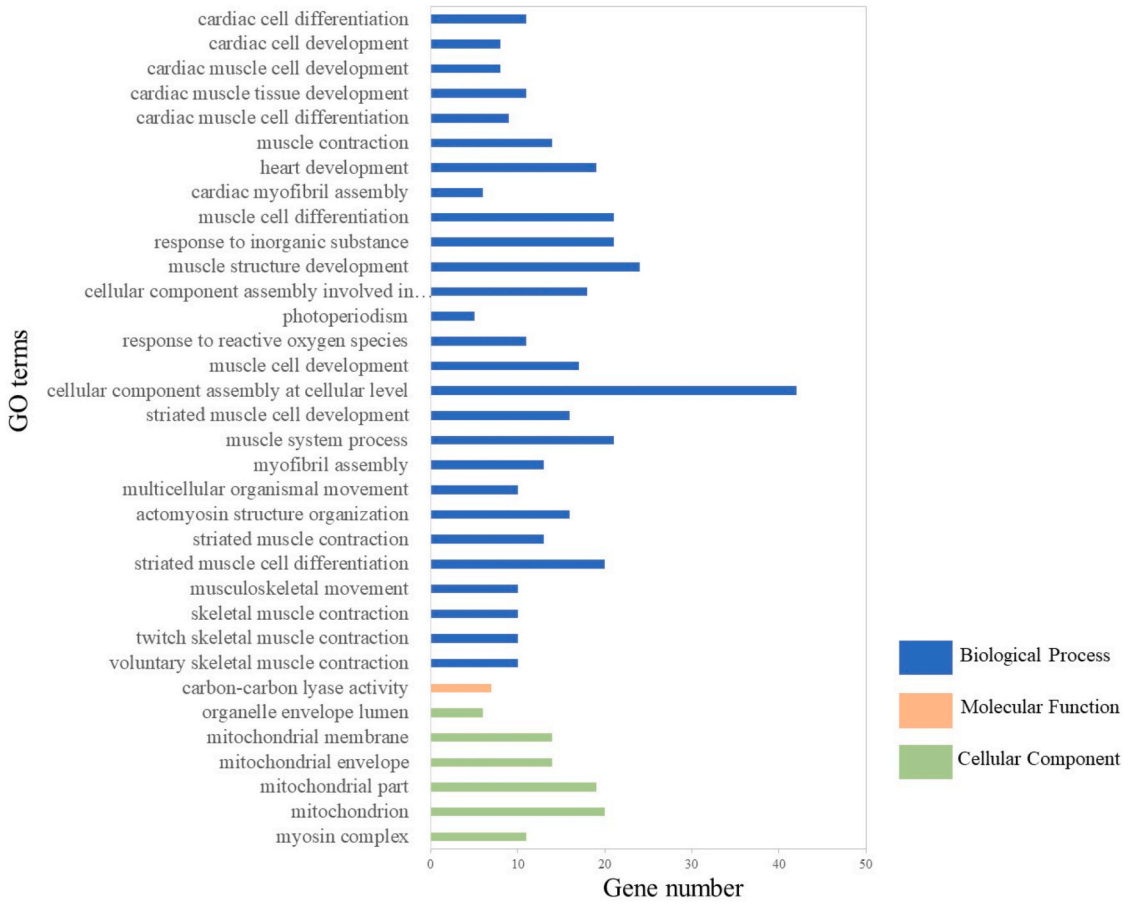

Fig. 6. Gene ontology (GO) analysis of differentially expressed genes (DEGs). The vertical axis shows the secondary nodes of three GO categories. The horizontal axis displays the number of annotated DEGs. The blue, orange and green bars represent biological processes, molecular function and cellular component, respectively.

suggested an urgent need to replace forage fish with a formulated diet, which would reduce the FCR, cut costs and produce greater economic benefits [27,28]. Notably, considering the slower growth rate of aquatic animals fed a formulated diet, it is essential to optimize the nutritional formula of compound feed, referring to the nutritional characteristics of forage fish [29].

4.2. Amino acid metabolism alternated by formulated diets

Muscle is a key nutritional part of fish, and its components are strongly affected by nutrients or special ingredients in diets [30–33]. In this study, we evaluated the nutritional effects of formulated diets on muscle quality compared to a control diet with forage fish. We found that the muscle of largemouth bass fed with formulated diets contained higher level of crude protein, which was consistent with the result of other aquatic animals, such as *Siniperca chuatsi* and *Scylla paramamosain* [34,35]. All FAA except glutamic acid, methionine and proline had a higher content in FDG than in FFG, which may be attributed to the higher crude protein content (46.1% vs 20.0%, Table 5), more reasonable proportion of fish meal and various protein sources found in formulated diets compared to forage fish. Consistent with the result of *Pseudosciaena crocea* fed with a compound diet [36], AAS, CS and EAAI were higher in largemouth bass fed with a formulated diet, indicating a better protein quality in the muscle of FDG.

Furthermore, to investigate the molecular mechanism of diets implicated in regulating the protein metabolism of muscle, we performed comparative transcriptome analyses of muscle tissues. The results demonstrated that some key genes *OAT* and *GLS* were significantly upregulated, while *ARG2* were downregulated, in "Arginine and proline metabolism" pathway (Fig. 5a). On the one hand, *OAT* forms glutamate from ornithine, with the notable exception of the intestine, where arginine (Arg) is the end product [37]. On the other hand, the catabolic enzyme of arginase, *ARG2* plays a role in the regulation of the extra-urea cycle arginine metabolism [38]. Taken together, we speculated the increase in biosynthesis and decrease in catabolism that led to the gradually accumulation of arginine in FDG. As for "Glycine, serine and threonine metabolism", *BHMT* was downregulated and *CBS*, *Mat* and *Mat2* were upregulated. Previous reports found that *BHMT* encoded a cytosolic enzyme that participated in the catabolism of cysteine and catalyzed the conversion of betaine and homocysteine [39,40]. Importantly, the metabolism enhancement of muscle tissue required more energy, leading to the upregulation of *CKB*, which catalyzed the transfer of phosphate between ATP and various phosphagens and played a central role in energy transduction in the skeletal muscle [41].

4.3. Upregulated pathways involved in muscle development

In the present study, muscle fiber was demonstrated to have a larger diameter and decreased density in FDG compared with FFG, which would strongly affect the muscle quality. Several previous studies of the grass carp *Ctenopharyngodon idella* indicated that diet significantly influenced the muscle fiber diameter, firmness and shear force [42,43]. In general, a bigger fiber diameter leads to lower fiber density and vice versa [44,45]. Muscle fibers are composed of myofibrils, comprising actin and myosin filaments called myofilaments, which are the basic functional units of the muscle fiber necessary for muscle contraction [46]. In the current study, "myofibril assembly" was significantly enriched, with several core genes being upregulated, including *Titin*,

MYH1, MYH2, MYH7 and *MYH10*, which encode multiple myosin II molecules that generate force in the skeletal muscle through a power stroke mechanism fueled by the energy released from ATP hydrolysis [47]. Up-regulation of these genes could provide more energy to maintain normal muscle activity. Accordingly, twelve common genes (*TOM40*, *Hspb1*, *ACSL1*, *NDUFS1*, *PPOX*, *RHOT1*, *AKAP1*, *COX7A2L*, *NDUFA2*, *COX6A*, *ACSL6*, *MSTO1*, *ACLSL4*) shared by "mitochondrion", "mitochondrial part", "mitochondrial envelope" and "mitochondrial envelope" were up-regulated suggesting muscle fibers diameter increasing needed multiple mitochondria to meet energy needs.

Interestingly, we also found that the "ECM-receptor interaction" pathway was significantly up-regulated (Fig. 5b). Extracellular matrix material (ECM) is composed of collagens (dominated by collagen IV), fibronectin, laminins, and proteoglycans, which coat the muscle fibers [48]. The current study demonstrated that the expression level of genes encoding ECM proteins, including collagen (*COL1a1, COL1a1b*, *COL5a3*), fibronectin (*FN1a*) and laminin (*LAMB3*, *LAMA4*), dramatically increased in FDG. Fibronectin promotes the proliferation of myoblast and participates in collagen fibrillogenesis [49]. Laminin is indispensable in several processes involving myogenesis, since it enhances myoblast proliferation and migration [48]. High amounts of the fibronectin-binding α5β1 integrin were expressed in proliferating and migrating myoblasts [39]. ECM was initially deemed to provide mechanical support for bearing force transmission [49]. Studies have demonstrated that muscle cells adhere to and connect with the ECM, which provides a permissive environment for muscle development [48]. Previous studies have confirmed that ECM was closely related to differentiation of muscle fibers in Grass carp and Italian Large White pigs [50,51]. According to previous studies and our results, we speculated that the upregulation of the "ECM-receptor interaction" pathway could stimulate the growth muscle fiber in largemouth bass.

5. Conclusions

In summary, this study provided the first demonstration that replacing forage fish with formulated diets could increase the amino acid content and alter the muscle fiber features in the muscle of largemouth bass. Furthermore, we compared the transcriptome profiles of muscles from FDG and FFG to explore the potential mechanism associated with amino acid metabolism and muscle development. We found that the expression levels of several genes that encode anabolic enzymes increased. Moreover, pathways including "myofibril assembly" and "ECM-receptor interaction" were up-regulated, which may be crucial in muscle fiber growth. Our results shed light on the molecular basis of how formulated diets affect muscle characters, and lay the ground-work for future nutritional regulation and genetic improvements in the meat quality of largemouth bass.

Ethic statement

All experimental procedures in this study were performed in accordance with the guidelines and after approval of the Animal Care and Use Committee of Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.repbre.2023.04.002.

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