Complete Replacement of Fishmeal With Plant Protein Ingredients in Gibel Carp (Carassius auratus gibelio) Diets by Supplementation With Essential Amino Acids Without Negative Impact on Growth Performance and Muscle Growth-Related Biomarkers

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This study was conducted to investigate the effects of substitution of dietary fishmeal (FM) by compound plant protein supplemented with essential amino acids on growth performance, plasma physiology, and muscle growth-related genes of gibel carp (Carassius auratus gibelio). Four diets with equal digestible protein were prepared, where 30FM (control feed) contained 30% FM and land animal protein as a protein source, 10FM contained 10% FM, PMAa contained full plant meal (PM) supplemented with crystalline amino acid, and PM contained full PM feed. There was no significant difference in the specific growth rate (SGR) with 30FM, 10FM, and PMAa groups (p > 0.05); however, the SGR of PM group was the lowest with significant difference (p < 0.05). Feed efficiency of the PM group was the lowest with significant difference (p < 0.05). The whole-body crude protein content of fish in PMAa group was significantly higher than that in each group with additional FM (p < 0.05). There were no significant differences in plasma total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) or low-density lipoprotein cholesterol (LDL-C), and free amino acid profile of 30FM, 10FM, and PMAa diets (p > 0.05); however, the SGR of PM group was the lowest with significant difference (p < 0.05). Feed efficiency of the PM group was the lowest with significant difference (p < 0.05). The whole-body crude protein content of fish in PMAa group was significantly higher than that in each group with additional FM (p < 0.05). There were no significant differences in plasma total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) or low-density lipoprotein cholesterol (LDL-C), and free amino acid profile of 30FM, 10FM, and PMAa groups (p > 0.05); however, they were significantly higher than that in PM group (p < 0.05). The expression of key genes in the TOR signaling pathway (tor, s6k1, 4e-bp2, and eif4e), autophagy (ulk1 and atg13), and ubiquitin–proteasome (trim63 and znrf2) system of the PMAa group was similar to that of the FM group (p > 0.05), indicating that the muscle growth-related biomarker genes were positively regulated by the appropriate dietary amino acid composition.
at the transcriptional level. These results suggest that FM in gibel carp diet can be totally replaced by PM without negative impact on growth performance and muscle growth-related biomarkers at the transcriptional level, which provided the requirement of digestible protein and balanced amino acid profile is satisfied.

Keywords: plant protein, fishmeal, digestible protein, amino acid balance, TOR pathway

INTRODUCTION

Over the past decades, aquaculture has been growing rapidly (Verhoeven et al., 2016) and has been a large contributor of animal protein for humans. Fishmeal (FM), which is not only a superior protein source in aquaculture but also a limited resource, plays an important role in aquaculture feed industry (Bu et al., 2018). The long-term sustainability of FM is a serious concern in the aquaculture industry. For sustainable development of aquaculture, more attention has been focused on replacing FM with plant feedstuffs (Benedito-Palos et al., 2007).

Previously, particularly over the past two decades, several studies have been conducted to determine alternative protein sources for substituting FM (Dossou et al., 2018; Inanan and Acar, 2019). It has been confirmed that the content of dietary FM could be reduced using plant ingredients in many cultured species. The addition of FM is low in the commercial feed formulation for omnivorous species. However, studies shown that FM was still the most optimal protein source in the diet and leads to the best growth performance for omnivorous species, such as tilapia (Oreochromis niloticus, Ahmad et al., 2020; Ismail et al., 2020) and carp (Cyprinus carpio, Wang et al., 2020), even herbivorous species, such as grass carp (Ctenopharyngodon idella, Jiang et al., 2016). Few reports are available to guide the total replacement of FM by plant ingredients in Cyprinidae species, which are widely cultured in China. The use of plant protein sources has been reported to induce intestinal inflammation in gibel carp (Liu et al., 2017) and grass carp (Duan et al., 2019), and abnormal cholesterol metabolism in blunt snout bream (Megalobrama amblycephala) (Xu et al., 2017). There are several factors restricting the usage of plant materials in aquatic feeds, such as amino acid imbalances, antinutritional factors (Tibbetts et al., 2017), and low mineral and high cellulose content (Hansen et al., 2007).

Several technologies have been developed to further increase the plant content in feeds, such as the use of mixed plant ingredients, supply of amino acids, steam modulation, and extrusion technology. Multiple usages of several techniques can ensure the supply of the essential amino acid, increase palatability of the diets, and decrease the levels of antinutritional factors (Gao et al., 2019). Albrechtsen et al. (2006) used a complex protein source substitution (a mixture of soybean meal and corn gluten) and found that it has no effect on the growth and feed intake of farmed fish. Some studies have shown that by supplementing feed with exogenous essential amino acids, a few plant protein sources can partially or completely replace dietary FM (Kaushik et al., 1995, 2004; Jiang et al., 2016) and can promote growth performance by improving palatability and protein synthesis (Hansen et al., 2007). Several studies have revealed the importance of feed processing in aquaculture, and that the content of antinutritional factors in feed can be reduced by some processing techniques such as dry, wet heating, and extrusion (Li et al., 2018).

Feed is the main source of amino acids, which are important substrates for protein synthesis and other nitrogenous compounds, which are also considered as signaling molecules that participate in the regulation of major metabolic pathways (Gomes et al., 1993; National Research Council [NRC], 2011). Many studies have demonstrated that the target of rapamycin (TOR) signaling pathway plays a key role in nutrient perception and energy and protein metabolism (Wullschleger et al., 2006; Crovetto, 2010; Sun et al., 2019). Amino acids can activate the TOR complex 1 (tor) (Jobgen et al., 2006; Xia et al., 2016), then upregulate the downstream ribosomal protein S6 kinase 1 (s6k1), and suppress the expression level of eukaryotic translation factor 4E-binding protein 2 (4e-bp2) and eukaryotic translation initiation factor 4E (eif4e) (Zhang et al., 2018).

In addition, imbalanced amino acid concentrations can result in protein degradation (Hansen et al., 2007), which helps to redistribute nutrients from unnecessary processes to critical processes to maintain the quality of proteins (Akiko et al., 2004; Yuan et al., 2019). There are two main protein degradation pathways in living organisms: autophagy and ubiquitin–proteasome system. Autophagy-related protein 13-like (atg13) and serine/threonine-protein kinase ULK1 (ulk1) play important roles in the autophagy pathway (Chan et al., 2009; Uddin et al., 2012). E3 ubiquitin-protein ligase TRIM63 (trim63) and E3 ubiquitin-protein ligase ZNRF2 (znrf2) genes are the important biomarkers in the ubiquitin–proteasome system.

China produced about 2.7 million tons of Carassius spp. each year, about 2 million tons of them are gibel carp (Fisheries Bureau, Department of Agriculture of China, 2020). Gibel carp is an economical freshwater fish species that is known for its high yield, fast growth, and stronger resistibility and is widely cultivated in China (Gu and Zhou, 2010). Although gibel carp is a typical omnivorous fish, Xie et al. (2001) and Liu et al. (2016) have revealed that the best growth performance of gibel carp is obtained with diets containing high FM because of balanced amino acid profile and the absence of terrestrial, plant-derived, antinutritional factors. Thus, the purpose of this study is to reduce the amount of FM in gibel carp diets based on amino acid requirement and investigate the effects of low- or no-FM diets on growth performance, plasma physiology, the content of free amino acids in dorsal muscles and blood, and the expression of some genes involved in muscle protein synthesis and degradation.
MATERIALS AND METHODS

Experimental Diets

Four diets with equal digestible protein and crude lipid were prepared. FM, chicken meal, bone meal, and blood meal were used as the animal protein sources. Seven plant proteins [soybean meal, rapeseed meal, cottonseed meal, distillers dried grains with soluble (DDGS), wheat gluten, soy protein concentrates, and corn gluten meal] were used as the plant protein sources. The chemical compositions and crude protein digestibility of the protein sources are shown in Table 1.

Diet formulations are shown in Table 2. Diet 30FM contained 30% FM and 22% terrestrial animal protein. Diet 10FM contained 10% FM and 45.4% vegetable protein. Diet PMAa and PM both contained 53.9% vegetable protein. Each vegetable protein source was added restrictedly to reduce antinutritional factors. Blood meal was added to low-FM diets to compensate for low amino acid content. Crystalline amino acids (L-lysine, L-methionine, and L-threonine) were added to PMAa to meet the requirements of the fish. Yeast hydrolyzate and spirulina (Arthrospira platensis) were added to 10FM, PMAa, and PM as immunopotentiators.

All ingredients of each diet were mixed, ground through a particle size of 100 mesh, completely mixed again, and then extruded using a twin-screwed extruder (Jinan Dingrun Machinery Co., Ltd., Jinan, Shandong, China). The pellets were stored at 4°C after drying in an oven at 60°C. The amino acid compositions of the diets are shown in Table 3.

Fish and Feeding Trial

All experimental animal care protocols were approved by the Ethics Committee of the Institute of Hydrobiology, Chinese Academy of Sciences. The feeding trial was conducted at Shishou Original Seed Stock Farm of Four Major Carps (Shishou, China). The gibel carp was obtained from Guangqiao hatchery of Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, Hubei, China). The experimental fishes were acclimated for 4 weeks before the feeding trial. All fish were fed with commercial feed two times a day during acclimation. At the beginning of the trial, fish were fasted for 24 h. Fish with apparent healthy and similar size (initial body weight: 14.98 ± 0.04 g) were randomly distributed into 12 floating net cages (2.0 m × 2.0 m × 2.0 m, water depth: 1.7 m) in the center of pond (70 m × 30 m × 3.5 m, water depth: 3 m), and 60 fishes were allocated in each cage. The diets were randomly assigned to triplicate floating net cages. During the feeding experiment, fish were hand-fed to apparent satiation two times a day at 8:30 and 16:30 for 45 days. The water temperature was recorded daily and maintained from 27 to 32°C during the trial. A part of the pond water was renewed every day. The dissolved oxygen was 5.0–7.5 mg/L and PH was 7.2–7.3.
ammonia nitrogen kept under 0.1 mg/L throughout the feeding trial. The photoperiod was subjected to nature.

**Sample Collection**

At the beginning of the trial, three fishes of each net cage were sampled randomly and stored at −20°C for initial body composition analysis. At the end of the experiment, all fish from each cage were weighed after depriving of food for 1 day. A total of two fishes of each cage were sampled randomly and also stored at −20°C for final body composition analysis. Another two individuals were anesthetized with MS-222 (100 mg L−1 tricaine methane sulfonate, Argent Chemical Laboratories Inc., Redmond, WA, United States). The tail venous blood of each fish on ice, and these samples also kept at −80°C for further analysis. After blood sampling, samples of liver and dorsal muscles were dissected from those fish on ice, and these samples also kept at −80°C until analysis.

**Biochemical Composition**

The sample of experimental fish and diets was conducted for proximate composition following the method of the Association of Official Analytical Chemists (AOAC, 2003). The moisture content was determined by oven drying at 105°C until a constant weight. The content of ash was quantified by incineration in a muffle furnace at 550°C for 12 h. A Soxtec system (Soxtec™ 2055, FOSS Tecator, Höganäs, Sweden) was used to analyze the content of crude lipid. Crude protein was tested through a Kjeltec Analyzer Unit (FOSS Tecator 8400, Höganäs, Sweden). Automatic biochemical analyzer (Mindray BS-460, Shenzhen, China) was used to determine the content of plasma total protein (TP), glucose (GLU), total triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), using standard kits according to the instructions [TP (P/N:105-000451-00), GLU (P/N:105-000499-00), TG (P/N:105-000448-00), LDL-C (P/N:105-000464-00), and HDL-C (P/N:105-000463-00)]. The hydrolyzation method of dietary amino acids was according to Liu et al. (2016). The separation method of free amino acids from muscle and plasma was according to Xu et al. (2015). The total amino acid levels of the experimental diets and the free amino acid levels in the dorsal muscle and plasma were with an amino acid analyzer (A300, MembraPure GmbH, Germany).

**Results**

**Chemical Composition of the Fishmeal and Other Protein Sources and the Experimental Diets**

Chemical compositions of the protein sources used in the experimental diet are shown in Table 1. Protein apparent digestibility coefficient of the ingredients of gibel carp was measured in our laboratory, previously, according to the method
TABLE 4 | Primers used for quantitative RT-qPCR.

| Accession no. | Gene name | Primer sequence (5’-3’) | Annealing temperature (°C) | Product length (bp) |
|---------------|-----------|------------------------|---------------------------|-------------------|
| KF772613      | tor1      | F: TGTAGTCAACGCTGTTCTAA R: GCCCTGTCGGTAGGTTAGTA | 60 | 195 |
| KF880601      | stk12     | F: CGAGCTGAGATATTAGGATT R: AGTTGACATGACCATCTATG | 57 | 243 |
| KF900277      | 4e-bp23   | F: CACTTTATCTCTACCAACCC R: GATTTTGATCACTTTCCT | 60 | 175 |
| MF481722      | elf4e8    | F: AAACTATGCTGCTCATCCTCC R: TATTTCTTGCATCTCACCAC | 58 | 150 |
| XM026267678   | atg135    | F: AGTCACCGCTACCTGCTGC R: AGTTCACGCTCTTAAAGACT | 60 | 172 |
| XM026247240.1 | ulk16     | F: TGAGTAAAGCGTCTGAGGTT R: TTCGACAGGTTCGGTGTCC | 60 | 213 |
| XM026290505.1 | trim637   | F: GATCGCGGGTGGTAAAACT R: TGGCTGACAGGTGTAATCAC | 60 | 128 |
| XM026283340.1 | znrf28    | F: TACGCGCTACCTGGATGCG R: TTCAGATGGTGAGGACTTA | 60 | 174 |
| AB039726.2    | β-actin   | F: TTGACACGACGATGGACCGQ R: AGACCCTCAAGGCAACCAGGAA | 60 | 115 |
| AB056104      | elf1a9    | F: GCTTGAAGTCAACAAAGTGAGCACCAC R: CTCCTGATCTGTCAGGCCOAT | 60 | 198 |

1tor: Target of rapamycin. 2stk1: Ribosomal protein S6 kinase. 34e-bp2: Eukaryotic translation factor 4E-binding protein 2. 4elf4e: Eukaryotic translation initiation factor 4E. 5atg13: Autophagy-related protein 13. 6ulk1: Serine/threonine-protein kinase UPRK. 7trim63: E3 ubiquitin-protein ligase trim63. 8znrf2: E3 ubiquitin-protein ligase znrf2. 9elf1a: Translation elongation factor 1-alpha.

described by Dong et al. (2012). The chemical composition and amino acid content of the experimental diets are shown in Tables 3, 5, respectively. The crude protein content of PMAa and PM diets was higher than that of 30FM and 10FM diets. The digestible protein content was almost the same in all diets (Table 5). The limiting amino acids in PM diet were lysine, methionine, and threonine. The essential amino acid requirements of gibel carp were in accordance with our previous work (Zhou et al., 2006; Li, 2009; Li et al., 2010; Ma, 2009; Ma et al., 2010; Jia et al., 2013; Tu et al., 2015). The total contents of essential amino acids were the highest in PMAa and the lowest in PM (Table 3).

Growth Performance and Feed Utilization

The survival rate of fish in all the dietary treatments was 100%. The results of growth performance of gibel carp are shown in Table 6. The final body weight and specific growth rate (SGR) of fish fed 30FM, 10FM, and PMAa diets were not significantly different (p > 0.05), but were significantly higher than those of fish fed PM diet (p < 0.05). Feeding rate in PM group was higher than others though there were no significant differences. However, it was observed that feeding efficiency in PMAa was significantly higher than PM group (p < 0.05) and no significant difference with other groups (p > 0.05). There was no significant difference in protein retention efficiency (PRE) and protein efficiency ratio (PER) among all treatment groups except for PM group (p > 0.05). The PRE and PER of the PM group were significantly lower than those of the other groups (p < 0.05).

Body Composition

Whole-body compositions of gibel carp are presented in Table 7. The moisture and protein contents in the whole body of gibel carp were not significantly different among all groups (p > 0.05). The fish in the PM group had lower whole-body lipid content than that in the other groups (p < 0.05). There were no significant differences in whole-body ash content among all groups (p > 0.05).

Physiological and Biochemical Indices in Plasma

There were no significant differences in TP and GLU in the plasma among all diet groups (Table 8). However, PM group had the lowest TG, TC, LDL-C, and HDL-C levels. Significant differences (p < 0.05) were observed in TG between PM and 30FM groups, in TC and HDL-C between PM and the other three groups, and in LDL-C between PM and the two groups containing dietary FM. The levels of TG, TC, LDL-C, and HDL-C in PMAa group were similar to those in 30FM and 10FM groups;
TC and HDL-C concentrations in PMAa group were higher than in PM group (p < 0.05).

### Free Amino Acid Content in Dorsal Muscle and Blood of Gibel Carp

Free amino acid composition of plasma of gibel carp after the feeding trial is presented in Table 9. The fish from PMAa treatment had similar levels of all free amino acid concentrations with 30FM and 10FM treatments (p > 0.05). The essential free amino acids in the plasma of the PM treatment were also similar to those in other treatments and showed a downward trend (p > 0.05). However, the plasma aspartic acid, serine, alanine, and tyrosine concentrations in the PM group were significantly lower than those in the 30FM group (p < 0.05). The PM group also had the lowest plasma total essential amino acids compared to other groups (p > 0.05).

Results of dorsal muscle-free amino acid concentrations of gibel carp are listed in Table 10. There was a remarkable difference in the levels of free amino acids such as arginine, histidine, and glycine; total essential amino acid; total non-essential amino acid; and sum of flavor amino acids among the dietary groups. Even though there was no significant difference in lysine, methionine, and threonine among the four treatments, the plasma total essential amino acids in the PM group were significantly lower than those in the 30FM group (p < 0.05). The PM group also had the lowest plasma total essential amino acids compared to other groups (p > 0.05).

### Table 9: Plasma free amino acid concentrations (mg/100 ml) in gibel carp fed dietary with different amino acid pattern about 45 days.

|       | 30FM       | 10FM       | PMAa       | PM       |
|-------|------------|------------|------------|----------|
| Lysine| 4.07 ± 0.59 | 4.67 ± 0.52 | 5.14 ± 0.98 | 3.27 ± 0.28 |
| Methionine| 0.73 ± 0.10 | 0.85 ± 0.07 | 0.72 ± 0.04 | 0.55 ± 0.02 |
| Threonine| 11.17 ± 2.17 | 12.30 ± 1.97 | 9.95 ± 2.54 | 7.15 ± 1.08 |
| Arginine| 3.29 ± 0.34 | 4.27 ± 0.24 | 3.77 ± 0.81 | 2.80 ± 0.10 |
| Leucine| 5.05 ± 1.19 | 4.97 ± 0.71 | 4.87 ± 0.99 | 3.45 ± 0.39 |
| Histidine| 4.71 ± 0.67 | 4.67 ± 0.79 | 4.37 ± 0.03 | 3.75 ± 0.08 |
| Isoleucine| 2.74 ± 0.55 | 2.98 ± 0.36 | 2.75 ± 0.57 | 1.89 ± 0.30 |
| Phenylalanine| 2.49 ± 0.45 | 2.86 ± 0.26 | 2.39 ± 0.16 | 1.88 ± 0.28 |
| Valine| 4.01 ± 0.65 | 3.94 ± 0.45 | 4.14 ± 0.89 | 2.85 ± 0.30 |
| Aspartic acid| 1.19 ± 0.09P | 0.95 ± 0.26P | 0.93 ± 0.10P | 0.49 ± 0.07P |
| Serine| 1.56 ± 0.08P | 1.51 ± 0.15P | 1.27 ± 0.20P | 0.93 ± 0.29P |
| Glucose| 1.88 ± 0.29 | 1.78 ± 0.14 | 1.69 ± 0.36 | 1.31 ± 0.24 |
| Glutamic acid| 0.33 ± 0.04 | 0.16 ± 0.03 | 0.43 ± 0.24 | 0.35 ± 0.10 |
| Alanine| 3.41 ± 0.03P | 2.54 ± 0.09P | 2.78 ± 0.06P | 2.47 ± 0.07P |
| Cystine| 0.36 ± 0.05P | 0.39 ± 0.07 | 0.45 ± 0.00 | 0.34 ± 0.02 |
| Tyrosine| 1.41 ± 0.32P | 1.71 ± 0.33P | 1.56 ± 0.52P | 0.65 ± 0.28P |
| Proline| 12.10 ± 3.07 | 9.92 ± 2.39 | 9.81 ± 3.45 | 14.30 ± 2.44 |
| Sigma EAA| 38.28 ± 5.34 | 41.55 ± 3.57 | 38.10 ± 6.87 | 27.80 ± 2.81 |
| Sigma N-EAA| 22.32 ± 2.87 | 18.98 ± 3.13 | 20.19 ± 5.42 | 19.45 ± 4.37 |

Values are mean of three replicates ± SEM (standard error). Means in the same row with different superscripts are significantly different (p < 0.05).
The relative transcriptional expression of genes involved in muscle protein degradation is illustrated in Figure 2. The relative expression of atg13 in PM group was significantly higher than that in 10FM group (p < 0.05). The relative expression level of udk1 in the PM group was also significantly higher than that in 30FM and 10FM groups (p < 0.05). However, the relative expression of trim63 in 10FM group was the highest value among all groups (p < 0.05). There were no significant differences in the expression of znf2 with all treatments (p > 0.05).

**DISCUSSION**

In this study, the possibility of replacing FM with mixed plant protein in the diet of gibel carp was investigated. The results showed that 20% of dietary FM and 22% of terrestrial animal protein could be replaced by mixed plant protein if digestible protein requirement in the diet was satisfied, the antinutritional factors from oil seed meals (soybean meal, rapeseed meal, and cottonseed meal) was low, and single-cell protein (blood meal, yeast hydrolyzate, spirulina) was supplement. All FM and terrestrial animal protein can be replaced by mixed plant protein if essential amino acid requirements are satisfied. Blood meal and spirulina were used as attractants and immunopotentiators. However, without the supplementation of essential amino acids, growth performance decreased significantly.

Many previous studies have revealed that poor growth performance of cultured fishes could result from the high level replacement of FM with single-plant ingredients (Watanabe et al., 1998; Gomez-Requena et al., 2004; Kaushik et al., 2004; Panserat et al., 2008), such as soybean meal (Alexis and Nengas, 2001), rapeseed meal (Burel et al., 2000), and cottonseed meal (Luo et al., 2012), whether in carnivorous (Trachinotus ovatus, Wu et al., 2015; Lates calcarifer, Ma et al., 2019) or in herbivorous (Ctenopharyngodon Idella, Jiang et al., 2016) fish. Therefore, using a combination of protein ingredients with various nutritive properties can improve amino acid profile, dilute or even mitigate antinutrition factors, and consequently allow higher inclusion of alternative protein sources in the diet of cultured fish. However, in this study, PM diet, in which the dietary protein requirement in the diet of cultured fish was investigated. The results showed that 20% of dietary FM and 22% of terrestrial animal protein could be replaced by mixed plant protein if digestible protein requirement in the diet was satisfied, the antinutritional factors from oil seed meals (soybean meal, rapeseed meal, and cottonseed meal) was low, and single-cell protein (blood meal, yeast hydrolyzate, spirulina) was supplement. All FM and terrestrial animal protein can be replaced by mixed plant protein if essential amino acid requirements are satisfied. Blood meal and spirulina were used as attractants and immunopotentiators. However, without the supplementation of essential amino acids, growth performance decreased significantly.

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amino acids without negative effects on growth. There have been several experiments in which the replacement of FM with high proportions or complete plant proteins in diets did not only result in decreased feed intake and growth performance but also caused oxidative stress or subhealth status (Sitjà-Bobadilla et al., 2005; Hansen et al., 2007). Single-cell proteins, such as spirulina and yeast, contain various water-soluble fractions, such as free amino acids, small peptides, nucleotides, vitamins, and minerals. In this study, spirulina and yeast hydrolyzate, which were used as attractants and immunopotentiators to cover for the shortage of FM replacement (Burgents et al., 2004; Sheikhzadeh et al., 2019), were supplied in the low-FM diet. Based on the results, total replacement of FM with compound plant proteins in the diet of gibel carp is a possibility, and it provides a promising approach for the sustainable development of aquaculture.

The whole-body proximate composition of gibel carp did not show considerable variability with the replacement of FM, except for the treatment containing PM only and without amino acid supplement (PM), with which fish had reduced protein and lipid contents. High proportion of dietary FM (200 g kg\(^{-1}\) diet) can be replaced with compound plant proteins without having significant effect on body composition. Similar results have been reported in trout (Santigosa et al., 2008), silvery-black porgy (Sparidentex hasta) (Yaghoubi et al., 2016), and gilthead sea bream (Sitjà-Bobadilla et al., 2005). PRE also decreased in the PM group. This phenomenon could probably be attributed to the unbalanced amino acid composition in the diet, which leads to increased protein degradation (Langar et al., 1993). Besides, when dietary amino acids that are absorbed into the body cannot match the profile needed for protein synthesis, the excess amino acids would result in increased oxidation and catabolism of amino acids (Kim et al., 1983; Fauconneau et al., 1992). The body protein levels and PRE of fish fed the PMAa diet supplemented with limiting amino acids were similar to those of fish fed 30FM diet (Table 7). A similar phenomenon was reported for other cultured fish, such as hybrid grouper (Epinephelus) (Wu et al., 2017) and large yellow croaker (Larimichthys crocea) (Li et al., 2013). Decreased lipid content of fish associated with very high dietary inclusion levels of PM has also been reported for Indian major carp (Cirrhinus mrigala) (Hasan et al., 1997) and Japanese flounder (Paralichthys olivaceus) (Kikuchi, 1999).

Serum parameters reflect the physiological stress response of fish to nutritional changes (Dawood et al., 2015) and have been used to assess the health of fish (Hossain et al., 2016;...
Dawood et al., 2017; Inanan et al., 2021). There are many kinds of literature that have reported the possible physiological effects of dietary plant protein intake on fish (Dias, 1999; Regost, 1999; Kaushik et al., 2004). In this study, plasma TG, TC, LDL-C, and HDL-C concentrations decreased with an increase in dietary PM. A previous study also showed that high level of dietary plant content causes hypocholesterolemic symptoms in rat, which presented low level of plasma cholesterol (de Schrijver, 1990). A significant hypocholesterolemic effect of soybean meal diet was also observed in rainbow trout by Kaushik et al. (1995), who speculated that the problem was in response to the withdrawal of FM rather than the addition of plant protein (Kaushik et al., 2004). However, Sitjà-Bobadilla et al. (2005) inferred that fish hypocholesterolemia may result from increased excretion of bile salts caused by dietary plant protein supply, which inhibits the absorption of cholesterol in the intestine. Plasma TC and HDL-C concentrations of fish fed PMAa diet were slightly lower than those of fish fed 30FM diet ($p > 0.05$) and were significantly higher than those of fish fed PM diet ($p < 0.05$). This phenomenon confirmed that lysine, methionine, and threonine supplement could relieve hypocholesterolemic symptoms (Hirche et al., 2006). The specific mechanism of plant proteins involved in the synthesis and metabolism of cholesterol needs further study (Kaushik et al., 2004; Sitjà-Bobadilla et al., 2005).

The content of amino acids in diets could affect plasma amino acid pool, which could also be used to judge the adequacy of dietary amino acids and reflects nitrogen balance (Mente et al., 2003; Hansen et al., 2007). In this study, we also found that the free amino acid concentrations in the dorsal muscles reflected the dietary amino acid profile. The concentrations of free essential amino acids and non-essential amino acids in the plasma were high in 30FM, 10FM, and PMAa diets but were low in PM diet. Accordingly, in the dorsal muscle, free essential amino acids and flavor amino acids also decreased when a high proportion of dietary FM was replaced with mixed PM. Amino acids in muscles are associated with the synthesis of muscle proteins (Hansen et al., 2007). It is worth mentioning that the concentration of dorsal-free lysine in PMAa group was higher than that in PM group. Lower lysine content in the dietary treatments also resulted in lower free lysine content in the dorsal muscle of the fish; this outcome agrees with that reported by Gomez-Requeni et al. (2004). Carter et al. (2000) suggested that the efficiency of protein synthesis and retention was limited by the lowest relative concentration of an essential amino acid pool. Lysine, methionine, and threonine are recognized as limiting amino acids.

**FIGURE 2** | Relative expression of protein degradation genes ($atg13$, $ulk1$, $trim63$, and $znrf2$) in the muscle of gibel carp β-actin and $ef1\alpha$ was used as reference gene for the assessment of expression. Columns represent the mean ± SEM ($n = 6$). For each index, bars without sharing a common letter indicate significant differences ($p < 0.05$).
for fish (Hansen et al., 2007). In addition to other results of this study, the positive growth performance in the PMAa group was achieved by satisfying the requirement of limiting amino acids.

Target of rapamycin signaling system pathway senses amino acid levels in cells and plays a key role in nutrient perception and energy regulation (Wullschleger et al., 2006). It also plays a key role in protein synthesis both in mammals (Dodd and Tee, 2012; Hernandez-García et al., 2016) and in fishes (Liang et al., 2016). Generally, the replacement of a high proportion of FM with PM may result in TOR signaling system pathway not being effectively activated, and the response of sensing stress pathway being activated (Xu et al., 2015; Song et al., 2016). The suppression effect of antinutritional factors in the TOR pathway was also reported by He and Klionsky (2017), who found that gossypol and soybean saponins suppress tor and promote catabolism, and that concanavalin-A causes inhibited insulin/IGF1/Akt signaling that consequently leads to apoptosis. In this study, compound plant ingredients were used to reduce the content of antinutritional substances. This may be the reason why the expressions of tor and eif4e genes were not significantly different among all treatments. This phenomenon was also reported for juvenile cobia (Rachycentron canadum) and large yellow croaker larvae (Luo et al., 2012; Li et al., 2013). The supplementation of essential amino acids can activate TOR pathway (Chen G. F. et al., 2012; Zhao et al., 2012). In the TOR signaling pathway, there are two downstream sites (4E-BPs and s6k1) for tor. The PMAa treatment had a significantly higher expression level of s6k1 than that in the PM treatment, which gene was positively regulated by the TOR complex. Furthermore, the reverse was found in the 4e-bp2 mRNA levels of the muscle of gibel carp in the PM treatments. These results were justifiable as tor promotes cap-dependent translation initiation through the inactivation of its downstream effector (namely 4E-BPs) or increases the translation of 5'-TOP mRNAs via the activation of S6K1 (Wullschleger et al., 2006). Similar results were reported that dietary supplement of essential amino acids, such as lysine (Cai et al., 2018), threonine (Habte-Tsion et al., 2015), and methionine (Pan et al., 2016), could promote the expression of s6k1 and depress the expression of 4e-bp2 in cultured fish species.

Growth performance can be partly reflected in the dynamic balance of muscle protein synthesis and degradation (Zeitiz et al., 2019). Skeletal muscle protein turnover is the ratio between protein synthesis and protein breakdown rates (Burd et al., 2009). When positive, protein synthesis is higher than breakdown, and results in muscle mass gain. Autophagy and ubiquitin‒proteasome system are the main pathways of protein degradation in the skeletal muscle of vertebrates (Sandri, 2010). This study found that the atg13 and ulk1 genes in PM group were highly expressed and indicated that the dorsal protein degradation rate in the PM treatment was higher than those in the other treatments. Ulk1 is a fundamental protein activated by AMP-activated protein kinase (AMPK) for survival under conditions of reduced nutrient availability (Bach et al., 2011). The high protein breakdown rate in the dorsal muscle of PM treatment explains the low growth performance. TRIM63-encoding muscle ring finger 1 maintains muscle protein homeostasis by tagging the sarcomere proteins with ubiquitin for subsequent degradation by the ubiquitin‒proteasome system (Chen S. N. et al., 2012). In this study, the trim63 gene was highly expressed in 10FM treatment, which diet was not supplied with crystal amino acids. These phenomena suggest that the nutritional requirement of gibel carp could not be satisfied by compound plant ingredients without limiting amino acid supplements.

In summary, this study comprehensively evaluated the possibility of totally replacing FM with PM in the feed of gibel carp. The growth performance, feed utilization, and plasma biochemical composition in PMAa treatments were not different from those of the 30FM treatment. The key genes in the TOR signaling pathway and autophagy and ubiquitin‒proteasome system corresponded with the dietary amino acid profile. We conclude that replacing FM with total plant protein is feasible and provides a reference for cost optimization design of feed formulation for gibel carp.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

ETHICS STATEMENT

The animal study was reviewed and approved by the Ethics Committee of the Institute of Hydrobiology, Chinese Academy of Sciences.

AUTHOR CONTRIBUTIONS

WC and HL made major contributions to this work. WC was analysis of the data and draft of the manuscript. HL reviewed and corrected the manuscript. SX, XZ, YY, DH, and JJ participated in experiment design and sample collection. All authors contributed to the article and approved the submitted version.

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