Animal Nutrition 11 (2022) 369–380

Contents lists available at ScienceDirect

Animal Nutrition

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

Original Research Article

Dietary isoleucine affects muscle fatty acid and amino acid profiles through regulating lipid metabolism and autophagy in hybrid catfish (*Pelteobagrus vachelli* ♀ × *Leiocassis longirostris* ♂)

Ye Zhao [a, 1], Xia Dong [a, 1], Chang-Rui Guo [a, 1], Lin Feng [b, c], Yang Liu [b, c], Wei-Dan Jiang [b, c], Pei Wu [b, c], Wei Luo [a], Xiao-Li Huang [a], De-Fang Chen [a], Xiao-Qiu Zhou [b, c, *], Jun Jiang [a, c, *]

[a] College of Animal Science and Technology, Sichuan Agricultural University, Chengdu 611130, China
[b] Animal Nutrition Institute, Sichuan Agricultural University, Chengdu 611130, China
[c] Fish Nutrition and Safety Production University Key Laboratory of Sichuan Province, Sichuan Agricultural University, Ya'an 625014, China

ARTICLE INFO

Article history:
Received 29 July 2021
Received in revised form
7 June 2022
Accepted 20 July 2022
Available online 2 August 2022

Keywords:
Autophagy
Hybrid catfish
Isoleucine
Lipid metabolism
Muscle amino acid profiles
Muscle fatty acid profiles

ABSTRACT

The present study explored the impacts of Ile on muscle fatty acid and amino acid profiles, lipid metabolism, and autophagy in hybrid catfish. Seven isonitrogenous (387.8 g/kg protein) semi-purified diets were formulated to contain 5.0 (control), 7.5, 10.0, 12.5, 15.0, 17.5, and 20.0 g Ile/kg diet respectively. The fish (initial weight of 33.11 ± 0.09 g) were randomly assigned to 7 groups for a 56-day trial. Each group has 3 replicates with 30 fish per replicate, fed at 08:00 and 18:00 each day. Results showed that muscle protein and lipid, C14:0, C18:0, C22:0, C14:1, C18:1n-9, polyunsaturated fatty acid (PUFA), Arg, Ile, Ala, Cys, Gly, Tyr, essential amino acid (EAA), and total amino acid (TAA) contents and flavor amino acid (FAA)/TAA in muscle had positive linear and/or quadratic responses to dietary Ile levels ($P < 0.05$). Fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD), acetyl-CoA carboxylase (ACC), and lipoprotein lipase (LPL) activities had positive linear and/or quadratic responses, but carnitine palmitoyl transferase 1 (CPT1) activity had a negative response with increasing dietary Ile levels ($P < 0.05$). The mRNA expressions of *FAS*, *SCD*, *ACC*, *LPL*, fatty acid binding protein 4 (*FABP4*), *FATP1*, sterol response element-binding protein 1c (*SREBP-1c*), sequestosome 1 (*SQSTM1*), and adenosine 5′-monophosphate-activated protein kinase (*AMPK*) had positive linear and/or quadratic responses to dietary Ile levels ($P < 0.05$). The mRNA expressions of hormone-sensitive lipase (*HSL*), *CPT1*, peroxisome proliferator-activated receptor α (*PPARα*), *PPARγ*, uncoordinated 51-like kinase 1 (*ULK1*), beclin1 (*Becn1*), autophagy-related protein 9α (*Atg9α*), *Atg4b*, *Atg7*, autophagy marker light chain 3 B (*LC3B*), and *SQSTM1* in muscle had negative linear and/or quadratic responses to dietary Ile levels ($P < 0.05$). The p-AMPK and ULK1 protein levels, and p-AMPK/AMPK were decreased by 12.5 g Ile/kg in the diet ($P < 0.05$). Finally, SQSTM1 protein level had the opposite effect ($P < 0.05$). The above results indicate that dietary Ile improves fish muscle fatty acid and amino acid profiles potentially via respectively regulating lipid metabolism and autophagy. The Ile requirement of hybrid catfish (33 to 72 g) were estimated to be 12.63, 13.77, 13.75, 11.45, 10.50, 12.53 and 12.21 g/kg diet based on the regression analysis of protein, lipid, SFA, PUFA, FAA, EAA, and TAA muscle contents, respectively.

1. Introduction

As one of the fastest-growing food production sectors globally during the past 50 years, aquaculture provides over 50% of fish available globally for human consumption since 2016 (Garlock et al., 2020). Muscle tissue is the primary edible and most commercially valued component of the fish. The tissue comprises 40% to 60% of body mass in fish including hybrid catfish

* Corresponding authors.
E-mail addresses: zhouxq@sicau.edu.cn (X.-Q. Zhou), jjun@sicau.edu.cn (J. Jiang).
[1] These authors contributed equally.
Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2022.07.006

(*Pelteobagrus vachelli* ♀ × *Leiocassis longirostris* ♂) (Rescan, 2019; Valente et al., 2013; Zhao et al., 2020a). With increased consumer health awareness, people have recently begun to pay attention to muscle quality (Ballco et al., 2020). Flesh quality largely depends on muscle characteristics, such as texture, color, and nutritional composition (Fuentes et al., 2010). The lipid and fatty acid contents of fish muscle are the important decisive factors to affect the nutritional and organoleptic properties of the flesh (Lefevre et al., 2015; Robb et al., 2002). Protein and amino acid (AA) contents may directly reflect the flesh nutritional quality (Pawar and Sonawane, 2013). Accumulating evidence demonstrated that muscle fatty acid and AA contents are strongly influenced by dietary AA (Wu et al., 2020; Zhao et al., 2019). Ile is 1 of 3 branched-chain AA, and the Ile requirement has been determined in some omnivorous fish species, such as hybrid catfish (Zhao et al., 2020c), Nile tilapia (*Oreochromis niloticus*) (Araújo et al., 2021), Jian carp (*Cyprinus carpio* L.) (Zhao et al., 2012), and blunt snout bream (*Megalobrama amblycephala*) (Ren et al., 2017). In general, Ile requirement ranges from 12.9 to 15.2 g/kg diet based on the growth of fish. Ile deficiency results in poor growth and low feed efficiency in fish (Araújo et al., 2021; Zhao et al., 2020c). Recent studies indicate Ile improves muscle AA profiles in Japanese flounder (*Paralichthys olivaceus*) and fatty acid profiles in finishing pigs (Wang et al., 2017; Luo et al., 2019). However, the roles of Ile in regulating muscle fatty acid and AA profiles are not elucidated in fish.

As the largest tissue in fish, skeletal muscle is a major site of lipid deposition in catfish (Zheng et al., 2014). Lipid accumulation is a complex process including lipogenesis, lipid transport, and lipolysis (Guo et al., 2021; Ren et al., 2016). Fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) are 2 marker genes responsible for fatty acid synthesis. As an important decisive factor, FAS is involved in de novo biosynthesis of long-chain fatty acids from acetyl-CoA and malonyl-CoA (Ganguly et al., 2018; Vestergren et al., 2013). The ACC catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, and stearoyl-CoA desaturase (SCD) catalyzes the conversion of palmitic acid and stearic acid into the corresponding unsaturated fatty acid. They are rate-limiting enzymes in the de novo lipogenesis pathway (Lopes et al., 2021) and monounsaturated fatty acids (MUFA) biosynthesis (Zhou et al., 2021), respectively. Lipoprotein lipase (LPL) can hydrolyze TG-rich lipoprotein to produce free fatty acids (Kaneko et al., 2016). Hormone-sensitive lipase (HSL) is the rate-limiting lipase of hydrolyzing triglycerides and diglycerides (Chen et al., 2014). The adenosine 5′-monophosphate-activated protein kinase (AMPK) and sterol response element-binding protein 1 (SREBP-1) are 2 key regulators in lipid metabolic processes (Lounis et al., 2017; O'Neill et al., 2013). A previous study reports that dietary Ile increases muscle fat deposition in grass carp *Ctenopharyngodon idellus* (Gan et al., 2014). Ile deficiency dramatically increases fat mobilization in white adipose tissue and reduces fat quality in mice (Du et al., 2012). Surplus Ile in pig diets may enhance the synthesis of MUFA and fat accumulation in skeletal muscle by regulating lipid metabolism (Luo et al., 2019). The above data suggests that Ile might have a potential role in regulating lipid metabolism. However, whether dietary Ile can regulate fish lipid metabolism remains largely unclear, especially in fish muscle, which is deeply related to flesh quality (Guan et al., 2021; Zhao et al., 2019).

Protein content and AA profiles affect the nutritional value and flavor of fish muscle (Bermúdez et al., 2014), which are the result of protein synthesis and degradation. The ubiquitin-proteasome and autophagy–lysosome pathways are 2 important signaling pathways involving in protein degradation (Matias et al., 2020; Wang et al., 2018; Zhao et al., 2020). Previous studies mostly focus on the ubiquitin-proteasome pathway (Todgham et al., 2016; Zhang et al., 2022). Newer evidence from Nile tilapia indicates that the autophagy-lysosome pathway exerts an important role in protein degradation (Han et al., 2019). Autophagy involves autophagosome initiation, elongation, nucleation, cargo absorption, closure/maturation, and lysosome fusion (Nakamura and Yoshimori, 2017; Parzych and Klionsky, 2014). Aminl acid deficiency induces autophagy in eukaryotic cells (Lahiri et al., 2019; Shen et al., 2021). In mice, Ile deficiency induces autophagy leading to proteasomal protein degradation in the rectus femoris muscle (Wang et al., 2017). Leu negatively regulates autophagosome biogenesis via the impact of its metabolite acetyl-CoA on the target of rapamycin complex 1 (Son et al., 2020). Optimal dietary Ile may increase muscle AA contents in grass carp (Gan et al., 2014). However, whether Ile improves fish muscle AA profiles by regulating autophagy requires further study.

In recent years, the hybrid catfish has been largely cultured in China due to its advantage of flesh quality (Zhao et al., 2019; Zhao et al., 2020). Our previous studies reported that Ile improved growth, immunity, and physical barrier function of the intestine and skin in this hybrid catfish (Yin et al., 2020; Zhao et al., 2020c). However, less attention is paid to the relationship between dietary Ile and lipid metabolism, and protein degradation in fish. Hence, using the same experimental methodology (Zhao et al., 2020), the current study explores the possible influence of dietary Ile on muscle fatty acid and AA profiles, lipid metabolism, and autophagy for the first time. The results may improve our understanding of the potential regulation mechanisms of flesh growth by Ile in fish.

2. Materials and methods

2.1. Animal ethics statement

Experimental design and procedures in this study were reviewed and approved by the Animal Ethical Committee of Sichuan Agricultural University (Approval number: DKY-2018202027).

2.2. Experimental design and diets

Feed ingredients and proximate composition are shown in Table 1. The diet contains 3 protein sources (fish meal, casein, and gelatin), 2 lipid sources (soybean oil and fish oil), and one carbohydrate source (wheat flour). The crude protein and crude fat contents were 387.8 and 71.0 g/kg respectively. Dietary AA and fatty acid contents are shown in Tables 2 and 3. Seven experimental diets were supplemented with crystalline AA to simulate whole body AA compositions of hybrid catfish, except for Ile, as previously described (Zhao et al., 2020c). The diets were supplemented with crystalline Ile to provide Ile at the levels of 5.0 (control), 7.5, 10.0, 12.5, 15.0, 17.5, and 20.0 g/kg diet (Table 2), and balance nitrogen via L-Gly inclusion. Feed ingredients were crushed and sieved through a 60-mesh sieve and weighed according to the formulation. Then fish oil, soybean oil, and water were added and mixed well. Each diet was extruded and pelletized through a 3.0-mm die using a MY-165 twin-screw extruder (EXT50A, Yang gong Machine, China) according to Zhao et al. (2020c). The extruded particles were dried at 40 °C and sealed in plastic bags. The diets were kept at −20 °C but stored at 4 °C 1 day before use.

Table 1
Composition and nutrient content of basal diet (air-dry basis, g/kg).

Item	Content
Ingredients	
Peru fish meal [1]	170.0
Casein	10.0
Gelatin	10.0
Corn starch	168.3
Alpha-starch	30.0
Menhaden oil [1]	26.0
Soybean oil	210.0
Monocalcium phosphate	40.0
Vitamin premix [2]	10.0
Ile premix [3]	120.0
Mineral element premix [4]	20.0
Crystal amino acid premix [5]	170.0
Choline chloride (50%)	10.0
Ethoxy quinoline (30%)	0.5
Cellulose	5.0
Xanthophy II	0.2
Total	1000.0
Nutrient contents [6]	
Moisture	92.38
Crude protein	387.8
Crude lipid	71.0
Crude ash	145.2

[1] Peru fish meal and menhaden oil were provided by Guangzhou Fishtech Fisheries Science & Technology Co. Ltd (Guangzhou).

[2] One kilogram of premix provides the following vitamins: DL-α-tocopherol acetate (500 g/kg), 53.600 g; menadione (230 g/kg), 0.217 g; retinyl acetate (50,0000 IU/g), 8.063 g; cholecalciferol (50,0000 IU/g), 0.100 g; thiamin nitrate (900 g/kg), 0.111 g; riboflavine (800 g/kg), 1.125 g; pyridoxine hydrochloride (810 g/kg), 0.370 g; cyanocobalamin (10 g/kg), 0.100 g; niacin (990 g/kg), 3.143 g; folic acid (960 g/kg), 0.521 g; meso-inositol (990 g/kg), 52.323 g; D-biotin (20 g/kg), 5.0 g; calcium-D-pantothenate (900 g/kg), 1.667 g; ascorhyl acetate (930 g/kg), 86.022 g. All ingredients were diluted with corn starch to 1 kg.

[3] The Ile premix respectively provides Ile, Gly, and corn starch for a kilogram of diet 1 to 7: L-Ile 0.000, 20.833, 41.667, 62.500, 83.333, 104.167, 125.000 g; Gly 89.777, 74.814, 59.851, 44.888, 29.926, 14.963, 0.000 g; corn starch 910.223, 904.353, 898.482, 892.612, 886.741, 880.871, 875.000 g.

[4] One kilogram of mineral element premix provides the following minerals: $FeSO_4 \cdot 7H_2O$ (300 g/kg Fe), 13.333 g; $CuSO_4 \cdot 5H_2O$ (250 g/kg Cu), 1.300 g; $ZnSO_4 \cdot 7H_2O$ (345 g/kg Zn), 13.043 g; $MnSO_4 \cdot H_2O$ (318 g/kg Mn), 4.717 g; KI (38 g/kg I), 1.447 g; $NaSeO_3$ (10 g/kg Se), 1.000 g. All ingredients were diluted with $CaCO_3$ to 1 kg.

[5] One kilogram of crystal amino acid premix provides the following amino acids: Lys (780 g/kg), 125.204 g; Met (990 g/kg), 60.071 g; Trp (980 g/kg), 11.723 g; Arg (997 g/kg), 116.491 g; His (997 g/kg), 27.508 g; Thr (980 g/kg), 163.764 g; Leu (985 g/kg), 20.824 g; Phe (996 g/kg), 17.364 g; Val (992 g/kg), 11.302 g; Cys (999 g/kg), 11.432 g. All ingredients were diluted with corn starch to 1 kg.

[6] Crude protein, crude fat, and crude ash, and Ile contents were measured values.

Table 2
Amino acid composition of experimental diets (DM basis, g/kg).

Item	Dietary Ile levels, g/kg						
	5.0 (Control)	7.5	10.0	12.5	15.0	17.5	20.0
Thr	31.9	32.8	31.7	32.4	33.2	32.2	32.5
Val	8.3	7.5	8.0	8.5	8.4	7.7	7.9
Met	13.7	12.8	13.6	13.1	14.1	13.3	13.0
Ile	4.7	7.7	9.7	12.9	14.3	17.8	20.6
Leu	13.8	12.7	13.0	13.3	13.1	12.9	13.4
Phe	8.2	7.6	8.5	7.9	8.3	7.7	8.1
His	8.2	7.8	8.5	8.4	8.7	7.9	8.3
Lys	26.1	25.5	26.4	26.6	25.8	26.3	26.7
Arg	26.8	27.3	27.7	28.1	27.1	28.5	28.3
Trp	3.7	3.4	2.8	3.6	4.2	2.9	3.7
Cys	2.8	2.7	3.3	3.4	3.0	3.5	3.2
Tyr	1.8	2.4	2.1	1.9	2.0	2.5	2.3

Table 3
Fatty acid composition of experimental diets (% of total FA methyl esters)[1].

Item	Dietary Ile levels, g/kg						
	5.0	7.5	10.0	12.5	15.0	17.5	20.0
SFA	31.16	31.07	31.91	31.04	32.63	29.79	32.44
C14:0	3.97	3.54	3.77	3.82	3.65	3.68	3.79
C15:0	0.34	0.29	0.31	0.36	0.28	0.39	0.40
C16:0	20.53	19.88	21.23	20.47	21.85	19.53	22.17
C18:0	4.89	5.17	5.28	4.95	5.37	5.02	4.77
C20:0	1.25	1.02	1.18	1.32	1.25	0.99	1.14
C22:0	0.11	0.14	0.09	0.06	0.16	0.10	0.07
C24:0	0.07	0.03	0.05	0.06	0.07	0.08	0.10
MUFA	45.79	45.21	44.20	45.85	45.61	45.85	46.35
C16:1	3.55	3.27	3.36	3.33	3.58	3.24	3.66
C18:1	2.94	3.31	3.12	3.05	3.47	3.19	3.03
C18:1n-7	5.18	4.85	4.98	5.02	5.14	4.86	5.27
C18:1n-9	28.42	27.97	26.85	28.63	27.69	29.11	28.56
C20:1n-9	2.63	2.96	2.75	2.84	2.92	2.69	2.88
C22:1n-9	3.07	2.85	3.14	2.98	2.81	2.76	2.95
PUFA	26.89	25.44	26.43	26.57	26.26	24.84	27.59
C18:2n-6	12.32	10.72	11.95	10.84	11.11	9.83	12.15
C18:3n-3	3.28	3.35	3.19	3.67	3.75	3.18	3.62
C20:3n-3	0.45	0.54	0.39	0.48	0.55	0.46	0.57
C20:3n-6	0.06	0.07	0.04	0.08	0.03	0.05	0.08
C20:4n-6	0.31	0.28	0.23	0.27	0.19	0.32	0.26
C20:5n-3	4.41	4.73	4.85	4.91	4.62	4.54	4.79
C22:5n-3	0.83	0.77	0.62	0.75	0.68	0.81	0.74
C22:6n-3	5.23	4.98	5.16	5.57	5.33	5.65	5.38
n-3PUFA	14.20	14.37	14.21	15.38	14.93	14.64	15.10
n-6PUFA	12.69	11.07	12.22	11.19	11.33	10.20	12.49

FA = fatty acid; SFA = saturated FA; MUFA = monounsaturated FA; PUFA = polyunsaturated FA; n-3 PUFA = sum of n-3 polyunsaturated FA; n-6 PUFA = sum of n-6 poly-unsaturated FA.

[1] The following FA were utilized for calculating the FA classes but they are not listed because blow 0.03% of the total FAME: C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C17:0, C21:0, C23:0, C14:1, C15:1, C17:1, C24:1n-9, C20:2, and C22:2.

2.3. Fish feeding and management

The feeding trial was executed at the Ya'an Aquaculture Station of Sichuan Agricultural University (Ya'an, Sichuan). Hybrid catfish from a local commercial farm were fed twice daily (08:00 and 18:00) with a basal diet of about 3% of body weight and acclimatized for 1-week at laboratory conditions. A total of 630 hybrid catfish (33.11 ± 0.09 g) were randomly and equally assigned into 21 outdoor concrete tanks giving 30 fish per tank. Each tank had a water volume of 2100 L (200 cm × 100 cm × 105 cm). Water was continuously aerated using air stones. One-third of the total water volume of each tank was replaced with aerated tap water every day. Each diet was randomly assigned and fed to 3 replicates for 8 weeks. During the feeding trial period, fish were hand-fed daily to apparent satiation at 08:00 and 18:00 under the natural photoperiod. Forty minutes after feeding, uneaten feed was removed, dried, and weighed to calculate feed intake. The feeding trial was conducted in summer (3rd June to 28th July) and water temperature was suitable for the growth of this hybrid catfish. Water parameters were monitored daily using YSI Professional Plus Multiparameter Instrument (YSI Incorporated, Yellow Springs, OH, USA). The water temperature (ranged from 23 to 27 °C), dissolved oxygen (above 6.0 mg/L), pH (ranged from 6.9 to 7.5), nitrite (low; 0.05 mg/L), and NH_4^+-N (less than 0.5 mg/L) contents were recorded throughout the experiment.

2.4. Sample collection

At the end of the feeding trial, fish were deprived of feed for 24 h and then captured from each replicate, counted, and bulk weighed. Then, 12 fish from each replicate were anesthetized with benzocaine solution (50 mg/L). The left side muscle of 6 fish were quickly

sampled and frozen in liquid nitrogen from each replicate. The samples were then stored at −80 °C for detecting gene expression and protein levels. The right-side muscle of the same 6 fish were sampled for muscle composition and AA analysis. Another 6 fish from each replicate were randomly selected to sample the left and right muscles. The samples were stored at −80 °C for fatty acid and biochemical analysis.

2.5. Muscle amino acid and fatty acid analysis

The AA concentrations were detected by an AA auto-analyzer (L-8080, Hitachi, Japan). The lyophilized samples were hydrolyzed in 6 mol/L HCl (110 °C, 24 h) under a nitrogen rich atmosphere. The fatty acid composition of diet and muscle was measured using gas chromatography (GC-2010, Shimadzu, Japan) according to Zhao et al. (2019). Briefly, lipids were extracted from the diet and muscle samples by homogenization with a mixture of chloroform and methanol (2:1, vol:vol), respectively. The lipid extracts were hydrolyzed by 2 mL KOH-ethanol at 80 °C saponification for 60 min. Then, they were esterified with 7% BF_3-methanol solution at 80 °C for 20 min. Fatty acid methyl esters (FAME) were separated by 20 mg/mL hexane and saturated NaCl solution. The StarGC Workstation (version 6.30) was used for determination of the percentage content of fatty acids in the sample. By comparing the FAME profiles of the samples with those of FAME standards (Supelco, 37 Component FAME mix C4–C14, Sigma No. CRM47885, Sigma–Aldrich, St. Louis. MO, USA), the fatty acid species were identified and expressed as a percentage of all the fatty acids detected in the analysis.

2.6. Biochemical and gene expression analysis

The sample (100 mg) was weighed and homogenized in ice-cold 0.9% sterile saline (wt:vol, 1:10). The homogenate was centrifuged at 6,000 × *g* at 4 °C for 20 min and the supernatant was used to assay FAS, ACC, LPL, HSL, SCD, and carnitine palmitoyl transferase 1 (CPT1) activities by the commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu). Total RNA was extracted from the sample by TRIzol reagent (TaKaRa, Dalian, Liaoning). The integrity and purity of RNA samples were evaluated respectively by electrophoresing on a 1% agarose gel and the ratio of absorbance at 260/280 nm. The reverse transcription of RNA was conducted using RT Reagents (TaKaRa, Dalian, Liaoning). The mRNA expressions of target genes including *FAS*, *ACC*, *SCD*, *HSL*, *CPT1*, *LPL*, *AMPK*, *SREBP-1c*, peroxisome proliferator-activated receptor α (*PPARα*), *PPARγ*, uncoordinated 51-like kinase 1 (*ULK1*), autophagy-related protein 9α (*Atg9α*), autophagy marker light chain 3 B (*LC3B*), beclin1 (*Becn1*), *Atg5*, *Atg7*, sequestosome 1 (*SQSTM1*), and target of rapamycin (*TOR*) were analyzed by real-time quantitative PCR (RT-qPCR) using CFX96 RT PCR Detection System (Bio-Rad, Hercules, CA, USA). The specific primer sequences of RT-qPCR are provided in Table 4. Each sample was subjected to RT-qPCR according to the following steps: 2 min at 95 °C, followed by 39 cycles of 5 s at 95 °C, and 30 s at an optimal annealing temperature (Table 4). The gene expression was computed using the $2^{-\Delta\Delta CT}$ method and the housekeeping genes (β-actin and 18 S rRNA) were used as the reference genes to normalize the target gene expression.

2.7. Protein extraction and Western blot analysis

The extraction of each sample protein was conducted in RIPA buffer and protease phosphatase inhibitor mixture after homogenization (Beyotime, Shanghai). Protein concentration was quantified by a protein quantification kit (Beyotime, Shanghai). Then, protein extractions were mixed with 12% SDS-polyacrylamide gel and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Inc., Bedford, USA). After treatment with blocking buffer, the membranes were exposed to primary antibodies of AMPK (Proteintech, catalogue no. 66536-1-Ig), p-AMPK (phospho Thr172-AMPK, ABclonal, catalogue no. AP0432), ULK1 (ABclonal, catalogue no. A8529), SQSTM1 (ABclonal, catalogue no. A11250) and β-actin (Cell Signaling, catalogue no. D6A8), respectively, at 4 °C for 12 h. Next, the membranes were washed with TBS/T 5 times for 5 min each and incubated with second antibodies at room temperature for 1 h. Finally, membranes were visualized by enhanced chemiluminescence (ECL). Band density was quantified by Gel-Pro Analyzer (Media Cybernetics Bethesda, MD, USA) after scanning. The results were normalized to β-actin.

Table 4
The primers and AT (°C) used for real-time quantitative PCR.

Name	Sequences (5′ to 3′)	AT	GenBank ID
FAS-QF	AAAGCAGTAAGAAGACCAAC	57.0	MH253823.1
FAS-QR	GCTATGACGATTTCCTCCATG		
ACC-QF	ATACGACCCTCGGTGGAT	59.4	MH253822.1
ACC-QR	CATAAACGAGCCGAAGTCAA		
SCD-QF	AATACTCCGAGACAGACGCA	63.3	KJ818303.1
SCD-QR	GGATCAGGAAGCACATCACTAC		
HSL-QF	CCTGCTAATGGATACCGCT	59.4	XM_027172606
HSL-QR	TGCTGGAATGATACCTCTTGT		
CPT1-QF	ATTTGAAGAAGCACCCAGAGTATGT	64.2	JN579122.1
CPT1-QR	CCCTTTTATGGACGGAGACAGA		
FABP4-QF	TAGGAAACCGAACCAAGCC	63.3	XM_027175558.1
FABP4-QR	CCTCACCGCCACCACAT		
FATP1-QF	TCTGCCGCTACCTGCTGTCT	65.0	XM_027162220.1
FATP1-QR	ATCCCACCTTGCCGTCCAT		
LPL-QF	TCATTGTGGTGGACTGGCTT	63.4	XM_027159331.1
LPL-QR	CTGTGATCCTGCTGACTTTGTTT		
PPARα-QF	GAATAAGGCTAAAGCACGAGG	60.3	MH570217.1
PPARα-QR	CATTTGGGTGACCAGAACAG		
PPARγ-QF	GTCGCTTTCAGAAGTGCCT	61.3	XM_027140763.1
PPARγ-QR	GCTTTGGTCAGGGGGAA		
SREBP-1c-QF	CTGGGTCATCGCTTCTTTGTG	64.5	KR020013.1
SREBP-1c-QR	TCCTTCGTTGGAGCTTTTGTCT		
AMPK-QF	CTCACCACTTTGCCAAGCCTCAC	65.0	KX852428
AMPK-QR	GCCTGTAACTGGGTTCTTCCTCC		
ULK1-QF	GGAGGGTGTTGTGATGTTTGAGGC	59.0	XM_027176183.1
ULK1-QR	CTTGCTGGTGTAGGCAGGTTGTG		
Becn1-QF	CTCAACTGGACCGCCTGAAGAAA	59.0	XM_027177667.1
Becn1-QR	CACTCCACAGGAACGCTGGGTAAT		
Atg9a-QF	CTGCCCATCCGTTTCCGTTTACC	59.0	XM_027160018.1
Atg9a-QR	TTCGGACTTCAGACTCCATTCGTTT		
Atg4b-QF	GCTGCGATGTGGACAGATGATTC	65.0	XM_027147236.1
Atg4b-QR	TCTTGTCTATGAAGGCATTGAGGATGT		
Atg5-QF	CAGAACCGTTTTATCTTCTCCTACCG	59.0	XM_027142793.1
Atg5-QR	CGTCTACATCTTCAGCTTTCACGACTT		
Atg7-QF	CTCCTATTCAAACCCAGTGCGTCAG	65.0	XM_027140729.1
Atg7-QR	ATCAGTGCCTCCAACTGCTCCAC		
LC3B-QF	CCTGACCACGTCAACATGAGCGAACT	65.0	XM_027140826.1
LC3B-QR	GGAAATGGCGGCAGACACGGAGA		
SQSTM1-QF	TTTCTCAAACCTCCAAAATGTCGC	63.3	KY062772
SQSTM1-QR	GGGAAGTCACGCTTGTGCTCCTT		
18 S-QF	CCTGAGAAACGGCTACCACATCC	57.1	KP938527
18 S-QR	AGCAACTTTAATATACGCTATTGGAG		
β-actin-QF	CCTAAAGCCAACAGGGAAAA	59.0	EU161066
β-actin-QR	ATGGGGCAGAGCATAACC		

AT = annealing temperature; *FAS* = Fatty acid synthase; *ACC* = acetyl-CoA carboxylase; *SCD* = stearoyl-CoA desaturase; *HSL* = hormone sensitive lipase; *CPT1* = carnitine palmitoyl transferase 1; *FABP4* = fatty acid binding protein 4; *FATP1* = fatty acid transport protein 1; *LPL* = lipoprotein lipase; *PPARα* = peroxisome proliferator-activated receptor α; *PPARγ* = peroxisome proliferator-activated receptor γ; *SREBP-1c* = sterol regulating element binding protein-1c; *AMPK* = adenosine 5′-monophosphate-activated protein kinase; *ULK1* = uncoordinated 51-like kinase 1; *Becn1* = beclin 1; *Atg9a* = autophagy-related protein 9a; *Atg4b* = autophagy-related protein 4 b; *Atg5* = autophagy-related protein 5; *Atg7* = autophagy-related protein 7; *LC3B* = autophagy marker light chain 3 B; *SQSTM1* = sequestosome 1.

2.8. Statistical analysis

Data expressed as percentages or ratios were subjected to data transformation before statistical analysis. The Shapiro–Wilk and Levine's tests were used to evaluate the distribution normality and variance homogeneity of data. The one-way ANOVA was used to determine any significant differences according to dietary Ile levels. Duncan's multiple range test was used to analyze differences between the means. In addition, orthogonal polynomial contrasts were used to test linear and quadratic effects of dietary Ile level as described by Wei et al. (2019). All analyses were performed using statistical software SPSS 20.0 and the $P < 0.05$ was used to show statistical significance. The results were expressed as means ± SEM.

3. Results

3.1. Muscle fatty acid and amino acid profiles

The present study continues to explore the effects of Ile on muscle fatty acid and AA profiles. According to Table 5, the contents of C14:0, C17:0, C18:0, C22:0, C14:1, C18:1n-9, C18:2n-6, C20:5n-3, saturated fatty acid (SFA), MUFA, polyunsaturated fatty acid (PUFA), and essential fatty acid (EFA) were significantly affected by dietary Ile level ($P < 0.05$). With increasing dietary Ile level, the contents of C14:0, C18:0, C14:1, C18:1n-9, and PUFA increased quadratically ($P < 0.05$). The C22:0 content increased linearly and quadratically ($P < 0.05$). No significant linear or quadratic trends were found between the increasing levels of Ile and the responses of C17:0, C18:2n-6, C20:5n-3, SFA, MUFA, and EFA. A positive quadratic trend was found between dietary Ile levels and the contents of C15:0 and n-6 PUFA.

The AA contents are shown in Table 6. The contents of Met, Arg, Ile, Phe, Ala, Cys, Gly, Tyr, essential amino acids (EAA), flavor amino acid (FAA), total amino acids (TAA), EAA/non-essential amino acid (NEAA), EAA/TAA, and FAA/TAA were affected by dietary Ile level ($P < 0.05$). The Ala, Cys, and Gly contents increased and decreased linearly with increasing dietary Ile level ($P < 0.05$). The contents of Ile, Tyr, EAA, FAA, and TAA increased quadratically ($P < 0.05$). The Arg content increased linearly and quadratically ($P < 0.05$). The FAA/TAA increased linearly ($P < 0.05$). No significant linear or quadratic trends were found between the increasing levels of Ile and the responses of Met, Phe, EAA/NEAA, and EAA/TAA. The positive quadratic trend was found between dietary Ile levels and the contents of Thr, Leu, Val, Ser, and NEAA.

3.2. Enzymes activities and gene expressions related to lipid metabolism in muscle

The SCD, ACC, LPL, and CPT1 activities increased quadratically with increasing dietary Ile level (Table 7, $P < 0.05$). The FAS activity significantly increased linearly and quadratically (Table 7, $P < 0.05$). No significant difference in HSL activity was detected among treatments (Table 7). With increasing dietary Ile level, the *PPARγ* mRNA expression decreased linearly (Table 8, $P < 0.05$). The *SCD*, *ACC*, and *LPL* mRNA expression increased quadratically (Table 8, $P < 0.05$). The *HSL* and *PPARα* mRNA expression decreased quadratically (Table 8, $P < 0.05$). The *FAS*, fatty acid binding protein 4 (*FABP4*), *FATP1*, and *SREBP-1c* mRNA expression increased linearly and quadratically (Table 8, $P < 0.05$). The *CPT1* (Table 8) and *AMPK* (Fig. 2) mRNA expressions decreased linearly and quadratically ($P < 0.05$). The p-AMPK level decreased quadratically (Fig. 2, $P < 0.05$).

3.3. Autophagy-related gene mRNA expression and protein level in muscle

There was a linear effect of dietary Ile level on *ULK1* mRNA expression (Table 9, $P < 0.05$). Dietary Ile level had quadratic effects on *Becn1* and *Atg9a* mRNA expressions (Table 9, $P < 0.05$). There was also a linear and quadratic effect of dietary Ile levels on *Atg4b*, *Atg7*, *LC3B*, and *SQSTM1* mRNA expression (Table 9, $P < 0.05$). With increasing dietary Ile level, ULK1 protein level decreased linearly (Fig. 3, $P < 0.05$), SQSTM1 protein level increased quadratically (Fig. 3, $P < 0.05$).

3.4. Optimal dietary ile levels for hybrid catfish

As shown in Table 10 and Table S1, based on the quadratic regression analysis for muscle protein, lipid, SFA, PUFA, FAA, EAA, and TAA contents, Ile requirement of hybrid catfish (33 to 72 g) were estimated to be 12.63, 13.77, 13.75, 11.45, 10.50, 12.53, and 12.21 g/kg diet, respectively. Table 11.

4. Discussion

The present study builds on the growth experiment reported in our previous study, which revealed that dietary optimal Ile improved the growth of hybrid catfish (Zhao et al., 2020c). Our previous report demonstrated that the final body weight and percent weight gain are improved with increasing dietary Ile levels up to 12.5 g/kg diet in hybrid catfish (Zhao et al., 2020c). The results showed that muscle lipid (linear and quadratic) and protein (quadratic) content significantly increased with increasing dietary Ile levels (Jiang et al., 2021). Fish growth primarily depends on the depositions of lipid and protein in muscle (Tie et al., 2019). The present study showed dietary 12.5 g/kg Ile resulted in higher lipid and protein depositions in the muscle compared to the other diets, which was consistent with the growth of hybrid catfish. These findings suggested that appropriate Ile level in diets may improve utilization of dietary lipid and protein for hybrid catfish growth. This outcome also agreed with previous results, which showed that dietary Ile observably increased muscle protein and lipid contents in grass carp (Gan et al., 2014) and Nile tilapia (Neu et al., 2017). As far as we know, fish muscle fatty acid and AA contents are 2 important factors affecting flesh quality (Cai et al., 2021). Thus, the effects of dietary Ile on muscle fatty acid and AA profiles and its potential mechanisms were explored in the present study.

4.1. Dietary Ile changes muscle fatty acid profiles by regulating lipid metabolism

As one of the major tissues of lipid deposition, fish muscle is closely related to fish growth (Nanton et al., 2007). The content of muscle lipid is an important factor in determining flesh quality because it not only contains EFA, but also influences taste and sensory quality (Lefevre et al., 2015; Robb et al., 2002; Zhao et al., 2019). The present study found dietary Ile could improve muscle lipid, SFA, and MUFA contents, and modify muscle fatty acid profiles. The result was consistent with the improved growth performance of the hybrid catfish (Zhao et al., 2020c). Thus, it might be inferred that the dietary optimal Ile level could allow a better lipid deposition for muscle growth and change muscle fatty acid composition. In addition, the finding was in line with reports in pigs demonstrating that excess dietary Ile intake improved fat accumulation and modified fatty acid profiles in skeletal muscle (Luo et al., 2019) and high Ile and Val intake improves intramuscular fat content (Duan et al., 2016). The SFA and MUFA contents have a positive relationship with muscle flavor (Cameron et al., 2000). As one of several n-3

Table 5
Effect of dietary Ile on muscle FA profiles (% of total FA methyl esters)[1].

Item	Dietary Ile levels, g/kg							*Pr* > *F*[2]		
	5.0	7.5	10.0	12.5	15.0	17.5	20.0	ANOVA	Linear	Quadratic
SFA	24.82 ± 0.50^{ab}	24.58 ± 0.28^{a}	25.25 ± 0.16^{ab}	25.63 ± 0.74^{ab}	25.72 ± 0.11^{b}	25.11 ± 0.50^{ab}	25.02 ± 0.21^{ab}	0.02	0.21	0.05
C12:0	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.08 ± 0.00	0.07 ± 0.00	0.40	0.17	0.40
C14:0	1.31 ± 0.05^{bcd}	1.33 ± 0.03^{cd}	1.22 ± 0.11^{abc}	1.14 ± 0.06^{a}	1.16 ± 0.01^{ab}	1.23 ± 0.06^{abc}	1.42 ± 0.06^{d}	0.03	0.73	0.00
C15:0	0.22 ± 0.02	0.19 ± 0.03	0.19 ± 0.02	0.20 ± 0.01	0.20 ± 0.00	0.22 ± 0.02	0.23 ± 0.03	0.17	0.20	0.02
C16:0	17.7 ± 0.58	17.71 ± 0.00	18.06 ± 0.48	18.5 ± 0.58	18.63 ± 0.19	18.23 ± 0.48	18.33 ± 0.39	0.34	0.07	0.21
C17:0	0.44 ± 0.03^{ab}	0.36 ± 0.02^{a}	0.33 ± 0.04^{a}	0.38 ± 0.05^{a}	0.35 ± 0.02^{a}	0.53 ± 0.11^{b}	0.4 ± 0.03^{ab}	0.01	0.30	0.13
C18:0	4.12 ± 0.02^{a}	3.95 ± 0.27^{a}	4.01 ± 0.06^{a}	4.56 ± 0.01^{c}	4.49 ± 0.16^{bc}	4.15 ± 0.15^{a}	4.19 ± 0.04^{ab}	0.02	0.07	0.04
C20:0	0.08 ± 0.01	0.07 ± 0.01	0.04 ± 0	0.06 ± 0.01	0.06 ± 0.02	0.07 ± 0.01	0.07 ± 0.02	0.53	0.60	0.14
C21:0	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.04 ± 0.01	0.49	0.32	0.34
C22:0	0.68 ± 0.06^{b}	0.71 ± 0.10^{b}	1.09 ± 0.24^{c}	0.57 ± 0.14^{b}	0.57 ± 0.15^{b}	0.39 ± 0.05^{ab}	0.11 ± 0.02^{a}	0.00	0.00	0.01
C23:0	0.07 ± 0.01	0.07 ± 0.00	0.13 ± 0.09	0.06 ± 0.00	0.08 ± 0.02	0.12 ± 0.00	0.09 ± 0.07	0.70	0.53	0.89
C24:0	0.04 ± 0.03	0.03 ± 0.02	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.73	0.13	0.85
MUFA	37.87 ± 1.92^{ab}	37.96 ± 1.08^{ab}	37.74 ± 0.36^{ab}	39.26 ± 1.45^{b}	37.57 ± 0.52^{ab}	37.47 ± 0.16^{ab}	35.82 ± 2.10^{a}	0.04	0.18	0.13
C14:1	0.03 ± 0.00^{a}	0.02 ± 0.00^{a}	0.06 ± 0.00^{b}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.07 ± 0.01^{b}	0.02 ± 0.00^{a}	0.00	0.17	0.02
C16:1	3.94 ± 0.1	3.83 ± 0.16	4.23 ± 0.00	4.09 ± 0.18	4.05 ± 0.17	4.00 ± 0.07	4.07 ± 0.25	0.37	0.39	0.30
C17:1	0.27 ± 0.02	0.19 ± 0.03	0.19 ± 0.06	0.20 ± 0.07	0.18 ± 0.03	0.24 ± 0.02	0.20 ± 0.00	0.50	0.55	0.21
C18:1n-9	28.97 ± 1.25^{ab}	29.48 ± 1.77^{ab}	29.53 ± 0.43^{ab}	31.71 ± 0.62^{b}	29.73 ± 1.84^{ab}	28.82 ± 0.61^{ab}	27.22 ± 1.45^{a}	0.03	0.22	0.02
C20:1n-9	2.69 ± 0.22	2.68 ± 0.21	2.34 ± 0.51	2.22 ± 0.24	2.15 ± 0.15	2.35 ± 0.26	2.63 ± 0.42	0.47	0.42	0.07
C22:1n-9	0.34 ± 0.07	0.29 ± 0.09	0.34 ± 0.05	0.23 ± 0.03	0.28 ± 0.03	0.45 ± 0.12	0.27 ± 0.22	0.51	0.94	0.71
C24:1n-9	1.59 ± 0.44	1.45 ± 0.27	1.02 ± 0.5	0.75 ± 0.36	1.14 ± 0.91	1.52 ± 0.06	1.39 ± 0.2	0.57	0.85	0.12
PUFA	37.30 ± 1.42^{ab}	37.44 ± 0.79^{ab}	37 ± 0.19^{ab}	35.09 ± 0.71^{a}	36.7 ± 0.64^{ab}	37.4 ± 0.33^{ab}	39.15 ± 2.31^{b}	0.02	0.27	0.03
C18:2n-6	21.53 ± 1.76^{ab}	21.93 ± 0.47^{b}	20.15 ± 0.46^{ab}	20.55 ± 0.17^{ab}	20.47 ± 0.06^{ab}	19.53 ± 0.5^{a}	21.59 ± 0.94^{ab}	0.03	0.21	0.07
C18:3n-3	2.62 ± 0.06	3.17 ± 0.79	2.54 ± 0.57	3.14 ± 0.09	3.52 ± 0.48	2.51 ± 0.56	3.10 ± 0.29	0.36	0.57	0.50
C18:3n-6	1.53 ± 0.01	1.60 ± 0.06	1.82 ± 0.21	1.51 ± 0.27	1.84 ± 0.20	2.21 ± 0.12	2.12 ± 0.95	0.50	0.08	0.65
C20:3n-3	0.37 ± 0.02	0.42 ± 0.02	0.38 ± 0.02	0.36 ± 0.01	0.41 ± 0.08	0.47 ± 0.02	0.48 ± 0.15	0.59	0.13	0.45
C20:3n-6	0.62 ± 0.00	0.65 ± 0.02	0.74 ± 0.08	0.62 ± 0.11	0.81 ± 0.06	0.9 ± 0.05	0.87 ± 0.39	0.46	0.07	0.76
C20:4n-6	0.05 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.01	0.08 ± 0.00	0.06 ± 0.04	0.56	0.15	0.67
C20:5n-3	0.57 ± 0.05^{ab}	0.60 ± 0.07^{ab}	0.48 ± 0.11^{a}	0.53 ± 0.05^{ab}	0.65 ± 0.12^{ab}	0.74 ± 0.07^{b}	0.68 ± 0.06^{ab}	0.04	0.05	0.24
C22:6n-3	9.97 ± 0.39	8.99 ± 2.04	10.80 ± 1.68	8.31 ± 0.01	8.91 ± 0.2	10.93 ± 0.56	10.23 ± 2.08	0.40	0.59	0.34
n-3 PUFA	13.55 ± 0.3	13.2 ± 1.18	14.22 ± 0.97	12.35 ± 0.14	13.51 ± 0.48	14.66 ± 1.03	14.49 ± 1.88	0.38	0.23	0.29
n-6 PUFA	23.75 ± 1.73	24.24 ± 0.39	22.78 ± 0.77	22.73 ± 0.56	23.18 ± 0.15	22.73 ± 0.69	24.65 ± 0.43	0.23	0.98	0.04
n-3/n-6	0.57 ± 0.05	0.54 ± 0.05	0.62 ± 0.06	0.54 ± 0.00	0.58 ± 0.01	0.64 ± 0.06	0.58 ± 0.06	0.48	0.35	0.96
EFA	24.16 ± 1.82^{ab}	25.1 ± 1.27^{b}	22.7 ± 1.03^{ab}	23.69 ± 0.26^{ab}	24 ± 0.41^{ab}	22.04 ± 0.06^{a}	24.69 ± 1.24^{ab}	0.02	0.44	0.21

FA = fatty acid; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; n-3 PUFA = sum of n-3 polyunsaturated fatty acids; n-6 PUFA = sum of n-6 poly-unsaturated fatty acids; EFA = essential fatty acid.

[1] The following FA were utilized for calculating the FA classes but they are not listed because blow 0.03% of the total FAME: C4:0, C6:0, C8:0, C10:0, C11:0, C13:0, C15:1, C20:2, and C22:2. Values are means ± SEM (n = 18). Values within the same rows having different superscripts are significantly different (P < 0.05).

[2] Significance probability associated with the *F*-statistic.

PUFA, eicosapentaenoic acid (EPA, C20:5n-3) exerts important physicial functions in preventing or attenuating cardiovascular problems and inflammatory responses (Yang et al., 2019). Excitingly, the present study reported for the first time that dietary Ile increased SFA, MUFA, and EPA proportions in fish muscle. The change of lipid content is a result of lipid accumulation, which primarily depends on the metabolism of lipogenesis and lipolysis. The FAS and ACC are key regulatory enzymes for fatty acid biosynthesis. The SCD (Paton and Ntambi, 2009) introduces the first double bond and converts SFA (C16:0 and C18:0) to MUFAs (C18:1n-9). The present study indicated that dietary 12.5 g/kg Ile level remarkably improved FAS, ACC, as well as SCD activities, indicating that dietary Ile enhanced the lipogenesis in fish muscle. These results coincide with the report on pig skeletal muscle (Luo et al., 2019). The activities of these enzymes are tightly related to their transcriptional levels. The present study therefore further detected the effect of Ile on gene transcription of enzymes related to lipogenesis in hybrid catfish muscle. The present results demonstrated that the mRNA expression of *FAS*, *ACC*, and *SCD* responded to the dietary Ile level in a positive linear and/or quadratic manner. These results might be linked to the increased lipid, SFA, and MUFA deposition in fish muscle. The SREBP-1c activates downstream FAS including *FAS*, *ACC*, and *SCD* gene expressions (DeBose-Boyd and Ye, 2018). The AMPK is a key protein to phosphorylate SREBP-1c for inhibiting the nuclear translocation of these transcription factors responsible for the de novo synthesis of fatty acid and cholesterol (Bordoloi et al., 2019). In the present study, *SREBP-1c* mRNA expression had a positive linear and quadratic response, whereas the *AMPK* mRNA expression had a negative linear and quadratic response with increasing dietary Ile level. Western blot result demonstrated that the p-AMPK level was dramatically decreased by dietary Ile. The correlative analysis also showed that the SREBP-1c was negatively linked with AMPK (r = −0.876, P = 0.01), whereas positively associated with *FAS* (r = 0.869, P < 0.05), *ACC* (r = 0.767, P = 0.095), and *SCD* (r = 0.706, P < 0.05) mRNA expression (Table 10). The results indicated the increased muscle lipid content by dietary Ile might be partly ascribed to elevated muscle lipid synthesis via AMPK-mediated SREBP-1c signaling pathway. The AMPK signaling can be regulated by AA (Dalle Pezze et al., 2016). The study on pigs demonstrated that dietary branched-chain AA deficiency activated AMPK of the skeletal muscle (Duan et al., 2017). The activation of AMPK can induce phosphorylation and activation of peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α), which enhances fatty acid oxidation in murine myotubes (Jäger et al., 2007). Therefore, a decreased fatty acid oxidation by AMPK might have triggered an enhanced lipid accumulation of fish muscle in the present study. The underlying mechanism however needs a further investigation.

The LPL exerts an important role in the removal of lipoprotein triglyceride from the circulation (Chen et al., 2021) and CPT1 is a rate-limiting enzyme of lipolysis and regulates fatty acid β-oxidation (Schlaepfer and Joshi, 2020). In the present study, muscle CPT1 activity and mRNA expression decreased in response to increasing

Table 6
Amino acid profiles of muscle in hybrid catfish fed diets supplemented with graded levels of Ile for 8 weeks (g/100 g dry weight)[1].

Item	Dietary Ile levels, g/kg							*Pr* > *F*[2]		
	5.0	7.5	10.0	12.5	15.0	17.5	20.0	ANOVA	Linear	Quadratic
EAA										
Lys	6.15 ± 0.17	6.09 ± 0.39	6.33 ± 0.17	6.39 ± 0.17	6.31 ± 0.02	6.18 ± 0.25	6.24 ± 0.13	0.80	0.63	0.32
Met	1.38 ± 0.16^{ab}	0.92 ± 0.06^{a}	1.36 ± 0.37^{ab}	1.71 ± 0.34^{b}	1.07 ± 0.04^{a}	1.14 ± 0.08^{a}	1.05 ± 0.03^{a}	0.03	0.31	0.18
Thr	3.22 ± 0.06	3.34 ± 0.04	3.41 ± 0.12	3.36 ± 0.02	3.31 ± 0.05	3.21 ± 0.11	3.21 ± 0.09	0.19	0.26	0.03
Arg	4.23 ± 0.00^{a}	4.23 ± 0.26^{a}	4.73 ± 0.29^{b}	4.88 ± 0.14^{b}	4.76 ± 0.09^{b}	4.82 ± 0.27^{b}	4.63 ± 0.11^{ab}	0.02	0.01	0.03
Leu	5.48 ± 0.16	5.54 ± 0.06	5.74 ± 0.16	5.73 ± 0.06	5.61 ± 0.06	5.51 ± 0.13	5.55 ± 0.07	0.26	0.91	0.04
His	1.54 ± 0.06	1.55 ± 0.12	1.61 ± 0.10	1.63 ± 0.05	1.63 ± 0.02	1.62 ± 0.08	1.61 ± 0.04	0.78	0.23	0.32
Ile	2.98 ± 0.03^{a}	3.06 ± 0.03^{ab}	3.07 ± 0.08^{ab}	3.17 ± 0.01^{b}	3.12 ± 0.04^{ab}	3.00 ± 0.11^{ab}	2.98 ± 0.10^{a}	0.03	0.84	0.02
Phe	2.78 ± 0.14^{ab}	2.70 ± 0.11^{a}	2.87 ± 0.01^{ab}	2.94 ± 0.11^{b}	2.83 ± 0.05^{ab}	2.78 ± 0.01^{ab}	2.88 ± 0.00^{ab}	0.04	0.23	0.36
Val	3.12 ± 0.06	3.20 ± 0.03	3.24 ± 0.15	3.23 ± 0.02	3.30 ± 0.02	3.23 ± 0.06	3.13 ± 0.05	0.33	0.59	0.03
NEAA										
Ala	3.96 ± 0.11^{a}	4.15 ± 0.00^{b}	4.07 ± 0.07^{ab}	4.08 ± 0.01^{ab}	3.98 ± 0.03^{ab}	3.91 ± 0.08^{a}	3.92 ± 0.09^{a}	0.02	0.04	0.06
Asp	6.96 ± 0.13	7.21 ± 0.11	7.27 ± 0.19	7.24 ± 0.05	7.13 ± 0.00	6.91 ± 0.27	7.04 ± 0.18	0.27	0.42	0.07
Cys	0.47 ± 0.02^{a}	0.54 ± 0.10^{ab}	0.64 ± 0.05^{ab}	0.65 ± 0.08^{b}	0.63 ± 0.05^{ab}	0.61 ± 0.09^{ab}	0.65 ± 0.00^{b}	0.03	0.03	0.12
Glu	10.68 ± 0.31	11.24 ± 0.10	11.25 ± 0.33	11.11 ± 0.01	10.99 ± 0.16	10.77 ± 0.45	10.7 ± 0.28	0.28	0.31	0.05
Gly	3.70 ± 0.18^{bc}	4.00 ± 0.10^{c}	3.70 ± 0.05^{bc}	3.66 ± 0.16^{b}	3.46 ± 0.15^{ab}	3.50 ± 0.07^{ab}	3.33 ± 0.09^{a}	0.02	0.00	0.29
Pro	2.34 ± 0.11	2.41 ± 0.22	2.49 ± 0.16	2.42 ± 0.14	2.34 ± 0.22	2.34 ± 0.01	2.19 ± 0.05	0.62	0.24	0.17
Ser	2.92 ± 0.07	3.05 ± 0.01	3.09 ± 0.09	3.06 ± 0.00	3.02 ± 0.07	2.92 ± 0.13	2.90 ± 0.07	0.20	0.25	0.03
Tyr	2.36 ± 0.09^{a}	2.33 ± 0.04^{a}	2.49 ± 0.06^{b}	2.54 ± 0.03^{b}	2.42 ± 0.04^{ab}	2.42 ± 0.00^{ab}	2.44 ± 0.00^{ab}	0.03	0.12	0.03
EAA	25.14 ± 0.47^{a}	24.87 ± 0.66^{a}	26.06 ± 0.17^{ab}	26.57 ± 0.70^{b}	25.57 ± 0.01^{ab}	25.08 ± 0.56^{a}	25.08 ± 0.22^{a}	0.04	0.89	0.01
NEAA	39.21 ± 1.08	40.76 ± 0.62	41.41 ± 1.44	41.32 ± 0.22	40.4 ± 0.86	39.85 ± 1.13	39.47 ± 0.93	0.27	0.59	0.03
FAA	25.32 ± 0.75^{ab}	26.62 ± 0.12^{b}	26.32 ± 0.66^{ab}	26.10 ± 0.10^{ab}	25.58 ± 0.35^{ab}	25.10 ± 0.74^{a}	25.01 ± 0.66^{a}	0.04	0.06	0.05
TAA	64.35 ± 1.56^{a}	65.64 ± 1.28^{ab}	67.48 ± 1.61^{ab}	67.89 ± 0.48^{b}	65.97 ± 0.87^{ab}	64.94 ± 1.69^{ab}	64.55 ± 1.15^{a}	0.01	0.65	0.01
EAA/NEAA, %	64.12 ± 0.56^{ab}	61.01 ± 0.69^{a}	62.96 ± 1.77^{ab}	64.31 ± 2.05^{b}	63.31 ± 1.31^{ab}	62.95 ± 0.37^{ab}	63.54 ± 0.94^{ab}	0.02	0.61	0.79
EAA/TAA, %	40.95 ± 0.33^{ab}	41.00 ± 0.66^{b}	40.93 ± 0.24^{ab}	41.12 ± 0.22^{ab}	40.97 ± 0.10^{ab}	41.57 ± 0.40^{ab}	40.56 ± 0.57^{ab}	0.04	0.99	0.35
FAA/TAA , %	39.34 ± 0.20^{a}	40.56 ± 0.97^{b}	39.00 ± 0.05^{a}	38.45 ± 0.43^{a}	38.77 ± 0.02^{a}	38.64 ± 0.14^{a}	38.74 ± 0.33^{a}	0.02	0.01	0.28

EAA = essential amino acids; NEAA = non-essential amino acids; FAA = flavor amino acid, include Ala, Asp, Glu, Gly and Ser; TAA = total amino acid, include EAA, and NEAA.
[1] Values are means ± SEM (n = 18). Values within the same rows having different superscripts are significantly different (P < 0.05).
[2] Significance probability associated with the *F*-statistic.

Table 7
The FAS, SCD, ACC, LPL, HSL, and CPT1 activities (U/L) in the muscle of hybrid catfish fed graded levels of Ile diets for 8 weeks[1].

Item	Dietary Ile levels (g/kg)							*Pr* > *F*[2]		
	5.0	7.5	10.0	12.5	15.0	17.5	20.0	ANOVA	Linear	Quadratic
FAS	51.52 ± 4.21^{a}	59.47 ± 3.4^{ab}	65.15 ± 5.88^{bc}	104.92 ± 3.35^{e}	226.52 ± 1.7^{f}	78.41 ± 3.16^{d}	73.86 ± 5.63^{cd}	0.00	0.00	0.00
SCD	18.87 ± 1.47^{a}	21.62 ± 1.47^{abc}	24.33 ± 0.73^{bc}	24.76 ± 0.54^{c}	23.59 ± 0.81^{bc}	21.27 ± 1.43^{ab}	20.23 ± 0.48^{a}	0.00	0.65	0.00
ACC	24.65 ± 1.02^{a}	24.61 ± 0.81^{a}	27.59 ± 0.75^{b}	33.98 ± 0.7^{d}	30.87 ± 0.96^{c}	27.46 ± 0.85^{b}	23.75 ± 0.95^{a}	0.00	0.18	0.00
LPL	17.52 ± 0.42^{a}	17.92 ± 0.62^{ab}	18.01 ± 0.49^{ab}	19.44 ± 0.29^{bc}	20.21 ± 0.39^{c}	18.64 ± 0.88^{abc}	17.29 ± 0.34^{a}	0.00	0.29	0.00
HSL	55.99 ± 4.04	54.69 ± 2.39	53.91 ± 3.32	52.34 ± 1.44	54.17 ± 3.69	55.21 ± 3.06	58.59 ± 2.64	0.87	0.58	0.17
CPT1	116.16 ± 2.66^{b}	87.17 ± 2.16^{a}	81.74 ± 2.86^{a}	80.65 ± 2.43^{a}	81.38 ± 2.2^{a}	86.45 ± 1.72^{a}	113.26 ± 3.16^{b}	0.00	0.43	0.00

FAS = fatty acid synthase; SCD = stearoyl-CoA desaturase; ACC = acetyl-CoA carboxylase; LPL = lipoprotein lipase; HSL = hormone sensitive lipase; CPT1 = carnitine palmitoyl transferase 1.
[1] Values are means ± SEM (n = 18). Values within the same rows having different superscripts are significantly different (P < 0.05).
[2] Significance probability associated with the *F*-statistic.

dietary Ile level, whereas muscle LPL activity and mRNA expression increased. These results were consistent with the pig's statement (Luo et al., 2019). The FATP1 and FABP4 regulate long-chain fatty acid uptake and transport (Blanchard et al., 2016). The PPARα binds the promoter region of CPT1 (Song et al., 2010; Wang et al., 2016; Zheng et al., 2014) and PPARγ has emerged as an important metabolic transcription regulator that promotes lipid accumulation via direct binding to the promoter region of adipogenic genes (Gavrilova et al., 2003; Oku and Umino, 2008; Zheng et al., 2014). The increased uptake of derived lipids during PPARγ activation is accompanied by a consistent increase in LPL activity (Blanchard et al., 2016). The present study found PPARγ exhibited a similar pattern to *LPL* and *FATP1* mRNA expression. Correlation analysis also showed that the *PPARα* was positively related with *CPT1* mRNA expression (Table 10, r = 0.916, P < 0.01). The above results indicated that dietary Ile might improve the lipid accumulation of fish muscle by regulating lipid metabolism via AMPK-mediated SREBP-1c, PPARγ and PPARα signaling pathway.

4.2. Dietary Ile improved muscle protein accumulation by down-regulating autophagy

Protein content is a key indicator of muscle quality (He et al., 2020). Fish muscle AA profiles could directly reflect the quality of protein (Yang et al., 2019). The present results showed muscle protein and total AA contents responded to the dietary Ile level in a positive quadratic manner. These findings paralleled those of hybrid catfish growth (Zhao et al., 2020c). These results suggest that improved dietary protein utilization by optimal Ile level may be one of the factors to improve AA profiles in fish muscle. The Ala, Asp, Gly, and Glu are regarded as flavor precursors and can react with soluble reducing sugars to form flavoring substances (Yang et al., 2019). The present results showed that dietary 12.5 g/kg Ile markedly improved total flavor AA, Ala, and Gly content and tended to increase Asp and Glu content in fish muscle. Similar observations have been reported in grass carp (Zhang et al., 2019). The EAA exert a crucial role on maintaining human health. The EAA content and ratio of EAA to

Table 8
Effects of dietary Ile on *FAS*, *SCD*, *ACC*, *LPL*, *HSL*, *CPT1*, *FABP4*, *FATP1*, *PPARα*, *PPARγ*, and *SREBP-1c* mRNA expressions in muscle of hybrid catfish fed graded levels of Ile diets for 8 weeks[1].

Item	Dietary Ile levels, g/kg							*Pr* > *F*[2]		
	5.0	7.5	10.0	12.5	15.0	17.5	20.0	ANOVA	Linear	Quadratic
FAS	1.00 ± 0.05^{a}	1.22 ± 0.11^{b}	2.35 ± 0.36^{d}	2.64 ± 0.08^{e}	2.42 ± 0.35^{e}	2.05 ± 0.19^{c}	1.90 ± 0.05^{c}	0.00	0.00	0.00
SCD	1.01 ± 0.13^{a}	1.42 ± 0.36^{ab}	3.25 ± 0.22^{c}	2.83 ± 0.39^{c}	1.71 ± 0.25^{b}	1.48 ± 0.06^{ab}	1.30 ± 0.21^{ab}	0.00	0.60	0.00
ACC	1.09 ± 0.14^{a}	1.18 ± 0.21^{a}	1.46 ± 0.08^{ab}	2.30 ± 0.10^{c}	1.99 ± 0.29^{bc}	1.52 ± 0.24^{ab}	1.32 ± 0.08^{a}	0.00	0.05	0.00
LPL	1.01 ± 0.06^{a}	1.24 ± 0.12^{ab}	1.51 ± 0.01^{c}	1.60 ± 0.08^{c}	1.36 ± 0.12^{bc}	1.11 ± 0.02^{a}	1.04 ± 0.09^{a}	0.00	0.46	0.00
HSL	1.20 ± 0.27^{b}	1.02 ± 0.12^{ab}	1.09 ± 0.04^{b}	0.63 ± 0.10^{a}	0.67 ± 0.08^{a}	1.02 ± 0.12^{ab}	1.34 ± 0.07^{b}	0.00	0.99	0.00
CPT1	1.01 ± 0.10^{b}	0.92 ± 0.14^{b}	0.83 ± 0.05^{a}	0.79 ± 0.08^{a}	0.94 ± 0.04^{b}	0.98 ± 0.10^{b}	1.12 ± 0.12^{c}	0.00	0.00	0.00
FABP4	1.01 ± 0.17^{a}	1.13 ± 0.11^{a}	2.02 ± 0.23^{e}	1.89 ± 0.13^{e}	1.59 ± 0.21^{d}	1.33 ± 0.05^{c}	1.25 ± 0.19^{bc}	0.00	0.02	0.00
FATP1	1.00 ± 0.07^{a}	1.20 ± 0.13^{b}	1.48 ± 0.11^{d}	2.10 ± 0.12^{e}	1.53 ± 0.18^{d}	1.36 ± 0.06^{c}	1.34 ± 0.04^{c}	0.00	0.00	0.00
PPARα	1.06 ± 0.13^{b}	0.96 ± 0.05^{b}	0.73 ± 0.07^{a}	0.56 ± 0.04^{a}	0.70 ± 0.04^{a}	1.07 ± 0.12^{b}	1.16 ± 0.13^{b}	0.00	0.24	0.00
PPARγ	1.03 ± 0.09^{d}	0.88 ± 0.10^{c}	0.83 ± 0.04^{c}	0.61 ± 0.05^{b}	0.58 ± 0.03^{b}	0.48 ± 0.02^{ab}	0.38 ± 0.04^{a}	0.00	0.00	0.50
SREBP-1c	1.00 ± 0.02^{a}	1.28 ± 0.13^{b}	2.47 ± 0.24^{d}	2.54 ± 0.21^{d}	1.85 ± 0.22^{c}	1.99 ± 0.19^{c}	1.85 ± 0.15^{c}	0.00	0.00	0.00

FAS = fatty acid synthase; *SCD* = stearoyl-CoA desaturase; *ACC* = acetyl-CoA carboxylase; *LPL* = lipoprotein lipase; *HSL* = hormone sensitive lipase; *CPT1* = carnitine palmitoyl transferase 1; *FABP4* = fatty acid binding protein 4; *FATP1* = fatty acid transport protein 1; *PPARα* = peroxisome proliferator-activated receptor α; *PPARγ* = peroxisome proliferator-activated receptor γ; *SREBP-1c* = sterol regulating element binding protein-1c.

[1] The relative mRNA expression levels were calculated using the arithmetic formula $2^{-\Delta\Delta CT}$. The β-actin and 18 S rRNA were used as housekeeping genes. Values are means ± SEM (n = 18). Values within the same rows having different superscripts are significantly different (P < 0.05).

[2] Significance probability associated with the F-statistic.

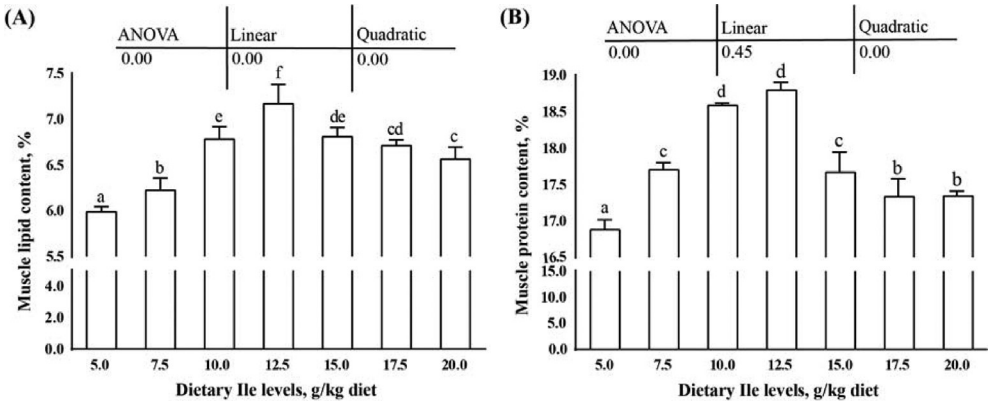

Fig. 1. Effects of dietary Ile on crude lipid (A) and protein (B) contents of hybrid catfish muscle. Values are means ± SEM (n = 18) and different letter denotes significant difference (P < 0.05).

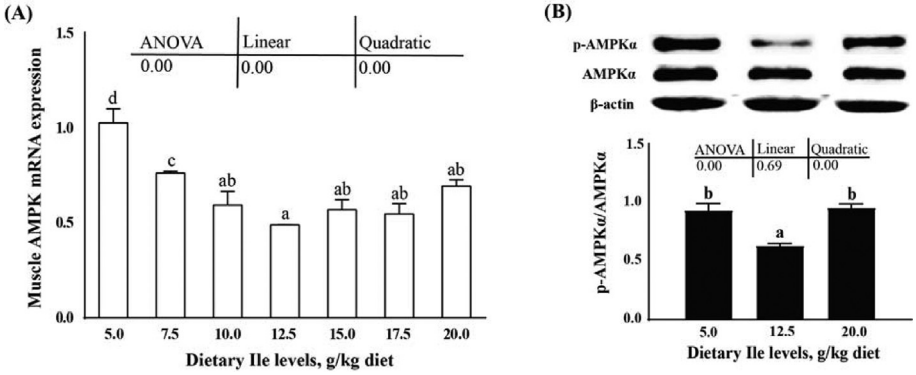

Fig. 2. Effects of dietary Ile on *AMPK* mRNA expression (A), and AMPK and p-AMPK protein levels (B) of hybrid catfish muscle. Values are means ± SEM (n = 18) and different letter denotes significant difference (P < 0.05). AMPK = adenosine 5′-monophosphate-activated protein kinase; ANOVA = the variance analyzed by one-way ANOVA; Linear = linear trend analyzed by orthogonal polynomial contrasts; Quadratic = quadratic trend analyzed by orthogonal polynomial contrasts.

NEAA reflect nutritional value of muscle protein. The present study showed that EAA content and ratio of EAA to NEAA were increased by dietary Ile. These results suggested that dietary Ile could improve fish muscle protein nutritional value. Muscle protein deposition is determined by protein synthesis and degradation rates. Autophagy-lysosome system is an important proteolytic system involved in intracellular protein degradation in fish (Seiliez et al., 2010; Wang et al., 2018). During macro autophagy, a part of the cytoplasm is firstly surrounded by an isolation membrane called phagophore to form autophagosome, then fused with lysosomes to form autolysosomes, and the isolated internal substances are finally degraded. The ULK1 can sense the level of intracellular nutrients to initiate early

Table 9
Effects of dietary Ile on *ULK1*, *Becn1*, *Atg9a*, *Atg4b*, *Atg5*, *Atg7*, *LC3B*, and *SQSTM1* mRNA expressions in muscle of hybrid catfish fed graded levels of Ile diets for 8 weeks[1].

Item	Dietary Ile levels (g/kg)							$Pr > F$[2]		
	5.0	7.5	10.0	12.5	15.0	17.5	20.0	ANOVA	Linear	Quadratic
ULK1	1.15 ± 0.22^{b}	0.81 ± 0.13^{a}	0.73 ± 0.04^{a}	0.71 ± 0.07^{a}	0.74 ± 0.14^{a}	0.72 ± 0.03^{a}	0.62 ± 0.06^{a}	0.04	0.01	0.14
Becn1	1.20 ± 0.24^{b}	0.80 ± 0.10^{a}	0.89 ± 0.04^{a}	0.84 ± 0.06^{a}	0.77 ± 0.08^{a}	0.76 ± 0.03^{a}	1.05 ± 0.10^{ab}	0.03	0.28	0.01
Atg9a	1.17 ± 0.23^{d}	0.65 ± 0.27^{bc}	0.14 ± 0.01^{a}	0.30 ± 0.10^{ab}	0.52 ± 0.05^{abc}	0.40 ± 0.04^{ab}	0.75 ± 0.04^{c}	0.00	0.07	0.00
Atg4b	1.00 ± 0.01^{e}	0.62 ± 0.08^{d}	0.38 ± 0.07^{ab}	0.31 ± 0.07^{a}	0.45 ± 0.11^{bc}	0.50 ± 0.12^{c}	0.69 ± 0.19^{d}	0.00	0.00	0.00
Atg5	1.25 ± 0.29^{c}	0.53 ± 0.09^{a}	0.58 ± 0.06^{ab}	0.99 ± 0.15^{bc}	0.89 ± 0.13^{abc}	0.88 ± 0.02^{abc}	0.92 ± 0.07^{abc}	0.01	0.97	0.06
Atg7	1.09 ± 0.17^{b}	0.48 ± 0.10^{a}	0.58 ± 0.06^{a}	0.60 ± 0.08^{a}	0.47 ± 0.09^{a}	0.39 ± 0.02^{a}	0.54 ± 0.04^{a}	0.00	0.00	0.00
LC3B	1.01 ± 0.04^{d}	0.65 ± 0.04^{c}	0.52 ± 0.03^{b}	0.33 ± 0.01^{a}	0.48 ± 0.01^{b}	0.49 ± 0.02^{b}	0.69 ± 0.01^{c}	0.00	0.00	0.00
SQSTM1	1.00 ± 0.04^{a}	1.13 ± 0.11^{ab}	1.27 ± 0.06^{bc}	1.49 ± 0.08^{cd}	1.54 ± 0.07^{d}	1.27 ± 0.09^{bc}	1.16 ± 0.08^{ab}	0.00	0.02	0.00

ULK1 = uncoordinated 51-like kinase 1; *Becn1* = beclin 1; *Atg9a* = autophagy-related protein 9α; *Atg4b* = autophagy-related protein 4 b; *Atg5* = autophagy-related protein 5; *Atg7* = autophagy-related protein 7; *LC3B* = autophagy marker light chain 3 B; *SQSTM1* = sequestosome 1.
[1] The relative mRNA expression levels were calculated using the arithmetic formula $2^{-\Delta\Delta CT}$. The β-actin and 18 S rRNA were used as housekeeping genes. Values are means ± SEM ($n = 18$). Values within the same rows having different superscripts are significantly different ($P < 0.05$).
[2] Significance probability associated with the *F*-statistic.

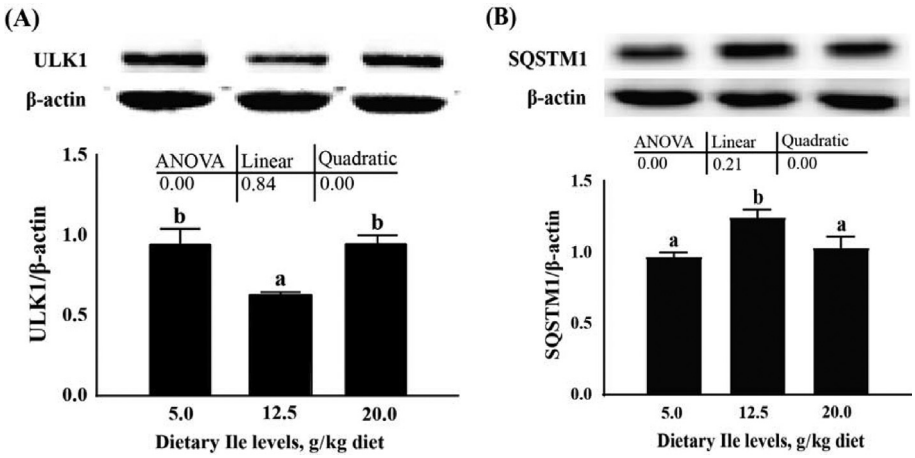

Fig. 3. The ULK1 (A) and SQSTM1 (B) protein levels were determined by Western blot analysis. Equal loading was monitored with anti-β-actin antibody. Values are means ± SEM ($n = 18$) and different letter denotes significant difference ($P < 0.05$). ULK1 = uncoordinated 51-like kinase 1; SQSTM1 = sequestosome 1; ANOVA = the variance analyzed by one-way ANOVA; Linear = linear trend analyzed by orthogonal polynomial contrasts; Quadratic = quadratic trend analyzed by orthogonal polynomial contrasts.

Table 10
Regressions of FBW, PWG, and muscle protein, lipid, SFA, PUFA, FAA, EAA, and TAA contents in hybrid catfish fed graded levels of Ile diets for 8 weeks.

Item	Equation	R^2	*P*-value	Optimal dietary Ile levels, g/kg
FBW[1]	$Y = -0.258x^2 + 6.283x + 28.97$	0.75	$P = 0.06$	12.20
PWG[1]	$Y = -0.007x^2 + 0.169x - 0.0414$	0.83	$P < 0.05$	12.43
Protein	$Y = -0.024x^2 + 0.606x + 14.611$	0.69	$P < 0.05$	12.63
Lipid	$Y = -0.013x^2 + 0.358x + 4.449$	0.86	$P < 0.05$	13.77
SFA	$Y = -0.012x^2 + 0.330x + 23.214$	0.62	$P = 0.05$	13.75
PUFA	$Y = 0.040x^2$ - $0.916x + 41.436$	0.71	$P < 0.05$	11.45
FAA	$Y = -0.016x^2 + 0.336x + 24.448$	0.69	$P = 0.05$	10.50
EAA	$Y = -0.019x^2 + 0.476x + 23.013$	0.52	$P < 0.05$	12.53
TAA	$Y = -0.052x^2 + 1.270x + 59.406$	0.79	$P < 0.05$	12.21

FBW = final body weight; PWG = percent weight gain; SFA = saturated fatty acid; PUFA = polyunsaturated fatty acid; FAA = flavor amino acid; EAA = essential amino acids; TAA = total amino acid.
[1] Regressions of FBW and PWG according to the published results in Zhao et al. (2021).

Table 11
Correlation analysis of parameters in the muscle of hybrid catfish.

Independent parameters	Dependent parameters	Correlation coefficients	*P*-value
SREBP-1c	AMPK	−0.876	$P = 0.01$
	FAS	0.869	$P < 0.05$
	ACC	0.767	$P = 0.10$
	SCD	0.706	$P < 0.05$
PPARα	CPT1	0.916	$P < 0.01$

SREBP-1c = sterol regulating element binding protein-1c; PPARα = peroxisome proliferator-activated receptor α; AMPK = adenosine 5′-monophosphate-activated protein kinase; FAS = fatty acid synthase; ACC = acetyl-CoA carboxylase; SCD = stearoyl-CoA desaturase; CPT1 = carnitine palmitoyl transferase 1.

events of autophagy process (King et al., 2021). Becn1, an important autophagy-promoting protein, regulates the early stages of autophagosome formation (Kaur and Changotra, 2020). The other autophagy-related proteins (Atg9a, Atg4b, Atg5, and Atg7) are known to engage in autophagosome formation (Hocking et al., 2012). The LC3-II is closely related to the membrane of autophagosomes (Rouschop et al., 2016). The SQSTM1, an important LC3 interactor under autophagy stimulation, degrades normally during autophagy and accumulated during autophagy damage (Poon et al., 2021). The present study found that dietary Ile deficiency up-regulated *ULK1*, *Becn1*, *Atg9a*, *Atg4b*, *Atg5*, *Atg7*, and *LC3B* mRNA expressions, indicating that autophagy was induced. Western blot results also demonstrated Ile markedly decreased ULK1 protein level and increased SQSTM1 protein level. TOR activity is also one of the most important autophagy regulators (Yina et al., 2020). An active TOR promotes phosphorylation of ULK1 on Ser 757 to inhibit autophagy (Cao et al., 2021). A previous study also demonstrated dietary 12.5 g/kg Ile elevated the TOR and p-TOR protein levels (Jiang et al., 2021). These results suggest that dietary Ile could activate the TOR/ULK1 signaling pathway to suppress autophagy in fish muscle. Together, findings of the present study indicated that dietary Ile could suppress protein degradation via inhibiting intracellular autophagy-lysosome in fish muscle. Based on the quadratic regression analysis for muscle protein, lipid, SFA, PUFA, FAA, EAA, and TAA contents, Ile requirement of hybrid catfish (33 to 72 g) was respectively estimated to be 12.63, 13.77, 13.75, 11.45, 10.50, 12.53 and 12.21 g/kg diet. These levels – except PUFA, FAA, and TAA – are in majority higher than 12.43 g/kg diet base on percent weight gain (Zhou et al., 2021).

5. Conclusion

The present study demonstrated that dietary Ile modified fish muscle fatty acid and AA profiles, by regulating lipid metabolism via AMPK-mediated SREBP-1c and PPARα signaling pathway, and protein degradation via the autophagy-lysosome system, respectively. Based on the quadratic regression analysis for muscle protein, lipid, SFA, PUFA, FAA, EAA, and TAA contents, the Ile requirement of hybrid catfish (33 to 72 g) was estimated to be 12.63, 13.77, 13.75, 11.45, 10.50, 12.53 and 12.21 g/kg diet, respectively. These results provided evidence that fish muscle nutritional value was effectively improved by dietary supplementation of Ile.

Author contributions

Ye Zhao, **Xia Dong**, and **Chang-Rui Guo**: Formal analysis, Investigation, Writing-original draft, Writing-review & editing. **Lin Feng**, **Yang Liu**, and **Wei-Dan Jiang**: Formal analysis, Investigation. **Pei Wu**, **Xiao-Li Huang**, and **De-Fang Chen**: Methodology, Resources. **Wei Luo**: Resources. **Xiao-Qiu Zhou** and **Jun Jiang**: Conceptualization, Formal analysis, Investigation, Writing-review & editing.

Declaration of competing interest

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

Acknowledgements

This research has received funding from National Natural Science Foundation of China (32172987) and National Key R&D Program of China (2019YFD0900200).

Appendix supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2022.07.006.

References

Araújo F, Michelato M, Schemberger MO, Salaro AL, Vidal LVO, Da Cruz TP, et al. Assessment of isoleucine requirement of fast-growing Nile tilapia fingerlings based on growth performance, amino acid retention, and expression of muscle growth-related and mTOR genes. Aquaculture 2021;539:736645. https://doi.org/10.1016/j.aquaculture.2021.736645.

Ballco P, Caputo V, De-Magistris T. Consumer valuation of European nutritional and health claims: do taste and attention matter? Food Qual Prefer 2020;79:103793. https://doi.org/10.1016/j.foodqual.2019.103793.

Bermúdez R, Franco D, Carballo J, Sentandreu MÁ, Lorenzo JM. Influence of muscle type on the evolution of free amino acids and sarcoplasmic and myofibrillar proteins through the manufacturing process of celta dry-cured ham. Food Res Int 2014;56:226–35. https://doi.org/10.1016/j.foodres.2013.12.023.

Blanchard PG, Turcotte V, Té MC, Gélinas Y, Nilsson S, Olivecrona G, et al. Peroxisome proliferator-activated receptor γ activation favours selective subcutaneous lipid deposition by coordinately regulating lipoprotein lipase modulators, fatty acid transporters and lipogenic enzymes. Acta Physiol 2016;217:227–39. https://doi.org/10.1111/apha.12665.

Bordoloi J, Ozah D, Bora T, Kalita J, Manna P. Gamma-glutamyl carboxylated gas6 mediates the beneficial effect of vitamin k on lowering hyperlipidemia via regulating the AMPK/AREBP1/PPARα signaling cascade of lipid metabolism. J Nutr Biochem 2019;70:174–84. https://doi.org/10.1016/j.jnutbio.2019.05.006.

Cai L, Tong F, Tang T, Ao Z, Wei Z, Yang F, et al. Comparative evaluation of nutritional value and flavor quality of muscle in triploid and diploid common carp: application of genetic improvement in fish quality. Aquaculture 2021;541:736780. https://doi.org/10.1016/j.aquaculture.2021.736780.

Cameron ND, Ensero M, Nuten GR. Genotype with nutrition interaction on fatty acid composition of intramuscular fat and the relationship with flavour of pig meat. Meat Sci 2000;55:187–95. https://doi.org/10.1016/S0309-1740(99)00142-4.

Cao W, Li J, Yang K, Cao D. An overview of autophagy: mechanism, regulation and research progress. B Cancer 2021;108:304–22. https://doi.org/10.1016/j.bulcan.2020.11.004.

Chen Q, Luo Z, Song Y, Wu K, Huang C, Pan Y, et al. Hormone-sensitive lipase in yellow catfish *Pelteobagrus fulvidraco*: molecular characterization, mRNA tissue expression and transcriptional regulation by leptin in vivo and in vitro. Gen Comp Endocr 2014;206:130–8. https://doi.org/10.1016/j.ygcen.2014.06.031.

Chen Y, Wu X, Lai J, Liu Y, Song M, Li F, et al. Characterization of two lipid metabolism-associated genes and their expression profiles under different feeding conditions in *Acipenser dabryanus*. Aquaculture Reports 2021;21:100780. https://doi.org/10.1016/j.aqrep.2021.100780.

Dalle Pezze P, Ruf S, Sonntag AG, Langelaar-Makkinje M, Hall P, Heberle AM, et al. A systems study reveals concurrent activation of AMPK and mTOR by amino acids. Nat Commun 2016;7:13254. https://doi.org/10.1038/ncomms13254.

DeBose-Boyd RA, Ye J. SREBPs in lipid metabolism, insulin signaling, and beyond. Trends Biochem Sci 2018;43:358–68. https://doi.org/10.1016/j.tibs.2018.01.005.

Du Y, Meng Q, Zhang Q, Guo F. Isoleucine or valine deprivation stimulates fat loss via increasing energy expenditure and regulating lipid metabolism in wat. Amino Acids 2012;43:725–34. https://doi.org/10.1007/s00726-011-1123-8.

Duan Y, Li F, Wang W, Guo Q, Wen C, Yin Y. Alteration of muscle fiber characteristics and the AMPK-SIRT1-PGC-1α axis in skeletal muscle of growing pigs fed low-protein diets with varying branched-chain amino acid ratios. Oncotarget 2017;8:107011–21. https://doi.org/10.18632/oncotarget.22205.

Duan YH, Duan YM, Li F, Li Y, Guo Q, Ji Y, et al. Effects of supplementation with branched-chain amino acids to low-protein diets on expression of genes related to lipid metabolism in skeletal muscle of growing pigs. Amino Acids 2016;48:2131–44. https://doi.org/10.1007/s00726-016-2223-2.

Fuentes A, Fernández-Segovia I, Serra JA, Barat JM. Comparison of wild and cultured sea bass (*Dicentrarchus labrax*) quality. Food Chem 2010;119:1514–8. https://doi.org/10.1016/j.foodchem.2005.09.074.

Gan L, Jiang W, Wu P, Liu Y, Jiang J, Li S, et al. Flesh quality loss in response to dietary isoleucine deficiency and excess in fish: a link to impaired Nrf2-dependent antioxidant defense in muscle. PLoS One 2014;9:e115129–52. https://doi.org/10.1371/journal.pone.0115129.

Ganguly S, Mahanty A, Mitra T, Mohanty S, Das BK, Mohanty BP. Nutrigenomic studies on hilsa to evaluate flesh quality attributes and genes associated with fatty acid metabolism from the rivers hooghly and padma. Food Res Int 2018;103:21–9. https://doi.org/10.1016/j.foodres.2017.10.017.

Garlock T, Asche F, Anderson J, Bjørndal T, Kumar G, Lorenzen K, et al. A global blue revolution: aquaculture growth across regions, species, and countries. Rev Fish Sci Aquac 2020;28:107–16. https://doi.org/10.1080/23308249.2019.1678111.

Gavrilova O, Haluzik M, Matsusue K, Cutson JJ, Johnson L, Dietz KR, et al. Liver peroxisome proliferator-activated receptor γ contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass. J Biol Chem 2003;278:34268–76. https://doi.org/10.1074/jbc.M300043200.

Guan W, Nong W, Wei X, Zhu M, Mao L. Impacts of a novel live shrimp (*Litopenaeus vannamei*) water-free transportation strategy on flesh quality: insights through

stress response and oxidation in lipids and proteins. Aquaculture 2021;533:736168. https://doi.org/10.1016/j.aquaculture.2020.736168.
Guo Y, Huang D, Chen F, Ma S, Zhou W, Zhang W, et al. Lipid deposition in abalone haliotis discus hannai affected by dietary lipid levels through AMPKα2/PPARα and JNK/MTOR/STEBP1c pathway. Aquaculture 2021;532:736040–53. https://doi.org/10.1016/j.aquaculture.2020.736040.
Han S, Wang J, Zhang Y, Qiao F, Chen L, Zhang M, et al. Inhibited autophagy impairs systemic nutrient metabolism in Nile tilapia. Comp Biochem Physiol A Mol Integr Physiol 2019;236:110521. https://doi.org/10.1016/j.cbpa.2019.06.021.
He J, Feng P, Lv C, Lv M, Ruan Z, Yang H, et al. Effect of a fish-rice co-culture system on the growth performance and muscle quality of tilapia (*Oreochromis niloticus*). Aquac Rep 2020;17:100367–74. https://doi.org/10.1016/j.aqrep.2020.100367.
Hocking LJ, Whitehouse C, Helfrich MH. Autophagy: a new player in skeletal maintenance? J Bone Miner Res 2012;27:1439–47. https://doi.org/10.1002/jbmr.1668.
Jäger S, Handschin C, St-Pierre J, Spiegelman BM. Amp-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1α. P Natl Acad Sci Usa 2007;104:12017–22. https://doi.org/10.1073/pnas.0705070104.
Jiang Q, Yan M, Zhao Y, Zhou X, Yin L, Feng L, et al. Dietary isoleucine improved flesh quality, muscle antioxidant capacity, and muscle growth associated with AKT/TOR/S6K1 and AKT/FOXO3a signaling in hybrid bagrid catfish (*Pelteobagrus vachelli*♀ × *Leiocassis longirostris*♂). J Anim Sci Biotechno 2021;12:53. https://doi.org/10.1186/s40104-021-00572-4.
Kaneko G, Shirakami H, Yamada T, Ide S, Haga Y, Satoh S, et al. Short-term fasting increases skeletal muscle lipid content in association with enhanced mrna levels of lipoprotein lipase 1 in lean juvenile red seabream (*Pagrus major*). Aquaculture 2016;452:160–8. https://doi.org/10.1016/j.aquaculture.2015.10.030.
Kaur S, Changotra H. The beclin 1 interactome: modification and roles in the pathology of autophagy-related disorders. Biochimie 2020;175:34–49. https://doi.org/10.1016/j.biochi.2020.04.025.
King KE, Losier TT, Russell RC. Regulation of autophagy enzymes by nutrient signaling. Trends Biochem Sci 2021;46:687–700. https://doi.org/10.1016/j.tibs.2021.01.006.
Lahiri V, Hawkins WD, Klionsky DJ. Watch what you (self-) eat: autophagic mechanisms that modulate metabolism. Cell Metab 2019;29:803–26. https://doi.org/10.1016/j.cmet.2019.03.003.
Lefevre F, Cardinal M, Bugeon J, Labbe L, Medale F, Quillet E. Selection for muscle fat content and triploidy affect flesh quality in pan-size rainbow trout, *Oncorhynchus mykiss*. Aquaculture 2015;448:569–77. https://doi.org/10.1016/j.aquaculture.2015.06.029.
Lopes C, Rocha E, Pereira IL, Madureira TV. Deciphering influences of testosterone and dihydrotestosterone on lipid metabolism genes using brown trout primary hepatocytes. Aquat Toxicol 2021;235:105819. https://doi.org/10.1016/j.aquatox.2021.105819.
Lounis MA, Bergeron KF, Burhans MS, Ntambi JM, Mounier C. Oleate increases SREBP-1 signaling activity in SCD1 deficient hepatocytes. Am J Physiol Endocrinol Metab 2017;313:E710–20. https://doi.org/10.1152/ajpendo.00151.2017.
Luo YH, Zhang X, Zhu ZP, Jiao N, Qiu K, Yin JD. Surplus dietary isoleucine intake enhanced monounsaturated fatty acid synthesis and fat accumulation in skeletal muscle of finishing pigs. J Anim Sci Biotechnol 2019;9:88. https://doi.org/10.1186/s40104-018-0306-5.
Matias AC, Dias J, Barata M, Araujo RL, Bragança J, Pousão-Ferreira P. Taurine modulates protein turnover in several tissues of meagre juveniles. Aquaculture 2020;528:735478. https://doi.org/10.1016/j.aquaculture.2020.735478.
Nakamura S, Yoshimori T. New insights into autophagosome-lysosome fusion. J Cell Sci 2017;130:1209–16. https://doi.org/10.1242/jcs.196352.
Nanton DA, Vegusdal A, Rørå AMB, Ruyter B, Baeverfjord G, Torstensen BE. Muscle lipid storage pattern, composition, and adipocyte distribution in different parts of atlantic salmon (*Salmo salar*) fed fish oil and vegetable oil. Aquaculture 2007;265:230–43. https://doi.org/10.1016/j.aquaculture.2006.03.053.
Neu DH, Boscol WR, Almeida FLAD, Zaminhan HM, Dallagnol JM, Furuya WM. Growth performance, hematology, and muscle growth in isoleucine fed nile tilapia. Bol Inst Pesca 2017;43:231–42. https://doi.org/10.20950/1678-2305.2017v43n2p231.
O'Neill HM, Holloway GP, Steinberg GR. AMPK regulation of fatty acid metabolism and mitochondrial biogenesis: implications for obesity. Mol Cell Endocrinol 2013;366:135–51. https://doi.org/10.1016/j.mce.2012.06.019.
Oku H, Umino T. Molecular characterization of peroxisome proliferator-activated receptors (PPARs) and their gene expression in the differentiating adipocytes of red sea bream *Pagrus major*. Comp Biochem Physiol B Biochem Mol Biol 2008;151:268–77. https://doi.org/10.1016/j.cbpb.2008.07.007.
Parzych KR, Klionsky DJ. An overview of autophagy: morphology, mechanism, and regulation. Antioxid Redox Sign 2014;20:460–73. https://doi.org/10.1089/ars.2013.5371.
Paton CM, Ntambi JM. Biochemical and physiological function of stearoyl-COA desaturase. Am J Physiol Endocrinol Metab 2009;297:E28–37. https://doi.org/10.1152/ajpendo.90897.2008.
Pawar HM, Sonawane SR. Fish muscle protein highest source of energy. Int J Biodivers Conserv 2013;5:433–5. https://doi.org/10.5897/IJBC12.043.
Poon A, Saini H, Sethi S, O'Sullivan GA, Plun-Favreau H, Wray S, et al. The role of sqstm1 (p62) in mitochondrial function and clearance in human cortical neurons. Stem Cell Rep 2021;16:1276–89. https://doi.org/10.1016/j.stemcr.2021.03.030.
Ren M, Zhang S, Liu X, Li S, Mao X, Zeng X, et al. Different lipopolysaccharide branched-chain amino acids modulate porcine intestinal endogenous β-defensin expression through the SIRT1/ERK/90RSK pathway. J Agric Food Chem 2016;64:3371–9. https://doi.org/10.1021/acs.jafc.6b00968.
Ren M, Habte-Tsion HM, Liu B, Miao L, Ge X, Xie J, et al. Dietary isoleucine requirement of juvenile blunt snout bream, *Megalobrama amblycephala*. Aquacult Nutr 2017;23:322–30. https://doi.org/10.1111/anu.12396.
Rescan P. Development of myofibres and associated connective tissues in fish axial muscle: recent insights and future perspectives. Differentiation 2019;106:35–41.
Robb DHF, Kestin SC, Warriss PD, Nute GR. Muscle lipid content determines the eating quality of smoked and cooked atlantic salmon (*Salmo salar*). Aquaculture 2002;205:345–58. https://doi.org/10.1016/S0044-8486(01)00710-4.
Schlaepfer IR, Joshi M. CPT1a-mediated fat oxidation, mechanisms, and therapeutic potential. Endocrinology 2020;161:bqz046. https://doi.org/10.1210/endocr/bqz046.
Seiliez I, Gutierrez J, Salmerón C, Skiba-Cassy S, Chauvin C, Dias K, et al. An in vivo and in vitro assessment of autophagy-related gene expression in muscle of rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol B Biochem Mol Biol 2010;157:258–66. https://doi.org/10.1016/j.cbpb.2010.06.011.
Shen JZ, Wu G, Guo S. Amino acids in autophagy: regulation and function. Adv Exp Med Biol 2021;1332:51–66. https://doi.org/10.1007/978-3-030-74180-8_4.
Son SM, Park SJ, Stamatakou E, Vicinanza M, Menzies FM, Rubinsztein DC. Leucine regulates autophagy via acetylation of the mTORC1 component raptor. Nat Commun 2020;11:3148. https://doi.org/10.1038/s41467-020-16886-2.
Song S, Attia RR, Connaughton S, Niesen MI, Ness GC, Elam MB, et al. Peroxisome proliferator activated receptor α (PPARα) and PPAR gamma coactivator (PGC-1α) induce carnitine palmitoyltransferase ia (CPT-1a) via independent gene elements. Mol Cell Endocrinol 2010;325:54–63. https://doi.org/10.1016/j.mce.2010.05.019.
Tie HM, Wu P, Jiang WD, Liu Y, Kuang SY, Zeng YY, et al. Dietary nucleotides supplementation affect the physicochemical properties, amino acid and fatty acid constituents, apoptosis and antioxidant mechanisms in grass carp (*Ctenopharyngodon idellus*) muscle. Aquaculture 2019;502:312–25. https://doi.org/10.1016/j.aquaculture.2018.12.045.
Todgham A, Crombie T, Hofmann G. The effect of temperature adaptation on the ubiquitin-proteasome pathway in notothenioid fishes. J Exp Biol 2016;220:369–78. https://doi.org/10.1242/jeb.145946.
Valente LMP, Moutou KA, Conceição LEC, Engrola S, Fernandes JMO, Johnston IA. What determines growth potential and juvenile quality of farmed fish species? Rev Aquacult 2013;5:S168–93. https://doi.org/10.1111/raq.12020.
Vestergren AS, Trattner S, Pan J, Johnsson P, Kamal-Eldin A, Brännäs E, et al. The effect of combining linseed oil and sesamin on the fatty acid composition in white muscle and on expression of lipid-related genes in white muscle and liver of rainbow trout (*Oncorhynchus mykiss*). Aquac Int 2013;21:843–59. https://doi.org/10.1007/s10499-012-9511-y.
Wang B, Feng L, Chen G, Jiang W, Liu Y, Kuang S, et al. Jian carp (*Cyprinus carpio* var. Jian) intestinal immune responses, antioxidant status and tight junction protein mRNA expression are modulated via Nrf2 and PKC in response to dietary arginine deficiency. Fish Shellfish Immunol 2016;51:116–24. https://doi.org/10.1016/j.fsi.2015.10.032.
Wang G, Xu Q, Jin X, Hang F, Liu Z, Zhao J, et al. Effects of lactobacilli with different regulatory behaviours on tight junctions in mice with dextran sodium sulphate-induced colitis. J Funct Foods 2018;47:107–15. https://doi.org/10.1016/j.jff.2018.05.044.
Wang L, Han Y, Jiang Z, Sun M, Si B, Chen F, et al. Interactive effects of dietary leucine and isoleucine on growth, blood parameters, and amino acid profile of Japanese flounder *Paralichthys olivaceus*. Fish Physiol Biochem 2017;43:1265–78. https://doi.org/10.1007/s10695-017-0370-3.
Wang L, Wu L, Liu Q, Dianfu Z, Yin J, Xu Z, et al. Improvement of flesh quality in rainbow trout (*Oncorhynchus mykiss*) fed supranutritional dietary selenium yeast is associated with the inhibited muscle protein degradation. Aquac Nutr 2018;24:1351–60. https://doi.org/10.1111/anu.12672.
Wei Y, Shen H, Xu W, Pan Y, Chen J, Zhang W, et al. Replacement of dietary fishmeal by antarctic krill meal on growth performance, intestinal morphology, body composition and organoleptic quality of large yellow croaker *Larimichthys crocea*. Aquaculture 2019;512:734281. https://doi.org/10.1016/j.aquaculture.2019.734281.
Wu P, Qu B, Feng L, Jiang W, Kuang S, Jiang J, et al. Dietary histidine deficiency induced flesh quality loss associated with changes in muscle nutritive composition, antioxidant capacity, Nrf2 and TOR signaling molecules in on-growing grass carp (*Ctenopharyngodon idella*). Aquaculture 2020;526:735399–409. https://doi.org/10.1016/j.aquaculture.2020.735399.
Yang B, Jiang WD, Wu P, Liu Y, Zeng YY, Jiang J, et al. Soybean isoflavones improve the health benefits, flavour quality indicators and physical properties of grass carp (*Ctenopharygodon idella*). PLoS One 2019;14:e0209570–91. https://doi.org/10.1371/journal.pone.0209570.
Yin L, Zhao Y, Zhou XQ, Yang C, Jiang J. Effect of dietary isoleucine on skin mucus barrier and epithelial physical barrier functions of hybrid bagrid catfish *Pelteobagrus vachelli* × *Leiocassis longirostris*. Fish Physiol Biochem 2020;46:1759–74. https://doi.org/10.1007/s10695-020-00826-4.
Yina S, Zhongjie C, Kaiyu C, Chenghua L, Xiaodong Z. Target of rapamycin signaling inhibits autophagy in sea cucumber *Apostichopus japonicus*. Fish Shellfish Immun 2020;102:480–8. https://doi.org/10.1016/j.fsi.2020.05.013.

Zhang F, Teng Z, Wang L, Wang L, Huang T, Zhang X. Dietary selenium deficiency and excess accelerate ubiquitin-mediated protein degradation in the muscle of rainbow trout (*Oncorhynchus mykiss*) via AKT/FOXO3a and NF-κB signaling pathways. Biol Trace Elem Res 2022;200:1361–75. https://doi.org/10.1007/s12011-021-02726-x.

Zhang YL, Duan XD, Jiang WD, Feng L, Zhou XQ. Soybean glycinin decreased growth performance, impaired intestinal health, and amino acid absorption capacity of juvenile grass carp (*Ctenopharyngodon idella*). Fish Physiol Biochem 2019;45:1589–602. https://doi.org/10.1007/s10695-019-00648-z.

Zhao J, Liu Y, Jiang J, Wu P, Chen G, Jiang W, et al. Effects of dietary isoleucine on growth, the digestion and absorption capacity and gene expression in hepatopancreas and intestine of juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquaculture 2012;368–369:117–28. https://doi.org/10.1016/j.aquaculture.2012.09.019.

Zhao Y, Jiang Q, Zhou X, Xu S, Feng L, Liu Y, et al. Effect of dietary threonine on growth performance, and muscle growth, protein synthesis and antioxidant related signaling pathways of hybrid catfish *Pelteobagrus vachelli*♀ × *Leiocassis longirostris*. Br J Nutr 2020a;123:121–34. https://doi.org/10.1017/S0007114519002599.

Zhao Y, Li J, Yin L, Feng L, Liu Y, Jiang W, et al. Effects of dietary glutamate supplementation on flesh quality, antioxidant defense and gene expression related to lipid metabolism and myogenic regulation in Jian carp (*Cyprinus carpio* var. Jian). Aquaculture 2019;502:212–22. https://doi.org/10.1016/j.aquaculture.2018.12.050.

Zhao Y, Li J, Jiang Q, Zhou X, Feng L, Liu Y, et al. Leucine improved growth performance, muscle growth, and muscle protein deposition through AKT/TOR and AKT/FOXO3a signaling pathways in hybrid catfish *Pelteobagrus vachelli* × *Leiocassis longirostris*. Cells 2020b;9:327–48. https://doi.org/10.3390/cells9020327.

Zhao Y, Yan M, Jiang Q, Yin L, Zhou X, Feng L, et al. Isoleucine improved growth performance, and intestinal immunological and physical barrier function of hybrid catfish *Pelteobagrus vachelli* × *Leiocassis longirostris*. Fish Shellfish Immunol 2020c;109:20–33. https://doi.org/10.1016/j.fsi.2020.09.035.

Zheng JL, Luo Z, Zhuo MQ, Pan YX, Song YF, Hu W, et al. Dietary L-carnitine supplementation increases lipid deposition in the liver and muscle of yellow catfish (*Pelteobagrus fulvidraco*) through changes in lipid metabolism. Brit J Nutr 2014;112:698–708. https://doi.org/10.1017/S0007114514001378.

Zhou X, Zhu X, Li C, Li Y, Ye Z, Shapiro VS, et al. Stearoyl-COA desaturase-mediated monounsaturated fatty acid availability supports humoral immunity. Cell Rep 2021;34:108601. https://doi.org/10.1016/j.celrep.2020.108601.