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Research Article

Methionine-Mediated Regulation of Intestinal Structure and Lipid Transport in the Rice Field Eel (*Monopterus albus*)

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An eight-week feeding trial discusses how methionine affects intestinal barrier and lipid transport on rice field eel (*Monopterus albus*). Six isoenergetic and isonitrogenous feeds contained different levels of methionine (0, 2 g/kg, 4 g/kg, 6 g/kg, 8 g/kg, or 10 g/kg). Compared with M0 (0 g/kg), gastric amylase, lipase, and trypsin were remarkably increased as dietary methionine ($P < 0.05$); intestinal amylase, lipase, and trypsin were remarkably increased in M8 (8 g/kg) ($P < 0.05$). Compared with M0, gastric fovea was remarkably increased ($P < 0.001$), gastric epithelium is neater in M8 than that in M0, intestinal villus height and muscular thickness are remarkably increased in M8 ($P < 0.001$), and amounts of goblet cells per root in M8 were increased ($P > 0.05$), while intestinal crypt depth was markedly decreased ($P < 0.001$). Lipid droplets in the intestinal villus and mucosal layer in the M8 (8 g/kg) group were more than that in M0 (0 g/kg). Compared with M0 (0 g/kg), the intestinal *gcn2* and *eif2α* were downregulated in M8 (8 g/kg) ($P < 0.01$ and $P < 0.05$, respectively), while *occ*, *cl12*, *cl15*, *zo-1*, *zo-2*, *hdlbp*, *ldlrap*, *npc1l1*, *cd36*, *fatp1*, *fatp2*, *fatp6*, *apo*, *apoa*, *apob*, *apoc*, *apoe*, *mct1*, *mct2*, *mct8*, *lpl*, *mttp*, *moat2*, and *dgat2* were upregulated markedly in M8 (8 g/kg). Intestinal *eif2α* expression was positively correlated with *gcn2*, and intestinal *zo-1*, *cl15*, *fatp6*, *ldlrap*, *mct2*, *mct8*, *apo*, *apob*, *mct1*, *apoc*, *fatp1*, *mttp*, *cd36*, *occ*, *npc1l1*, *hdlbp*, *fatp2*, *apoe*, *lpl*, and *moat2* gene expression was negatively correlated with *gcn2*. In conclusion, methionine deficiency affected the gastric and intestinal structures, damaged the intestinal barrier, and decreased lipid and fatty acid transport. Besides, *gcn2* could be activated when *M. albus* was fed methionine-deficient feed.

1. Introduction

With the continuous growth of the aquatic industry, the requirement for fish meals has quickly grown. However, the production of fish meal has continuously decreased, and prices were increased, so the study of replacement of fish meal in aquatic feed has become particularly important [1]. Soybean protein is widely used for replacing fish meal in aquatic feed with high grade of protein [2]. However, methionine is the most deficient amino acid of soybean, which is also an important essential amino acid for aquatic animals ([3]) and must be obtained from feed [4]. Various studies showed that methionine restriction limits protein synthesis, disturbs various metabolism, inhibits growth performance, and damages the healthy state of fish [3, 5, 6], also decreasing intestinal immunomodulatory, digestive, and antioxidant enzymes in rohu (*Labeo rohita*) [7].

In the process of evolution, animals have gradually evolved the ability to adapt to the lack of essential nutrients, such as essential amino acids. A previous study in primary muscular cells of turbot (*Scophthalmus maximus* L.) demonstrated that methionine restriction reduces cellular lipogenesis while stimulating lipolysis, decreases the content of intracellular lipid, promotes energy expenditure by accelerating progress of tricarboxylic acid cycle and oxidative phosphorylation, and activates the general controlled nonderepressible 2 (*gcn2*, also encoded by *eif2ak4*) expression [8]. *gcn2* plays a role in vertebrates in response to essential

Table 1: Composition of the diets and level of nutrition (g/kg).

Ingredients	M0	M2	M4	M6	M8	M10
Fish meal	110	110	110	110	110	110
Soy protein concentrate	400	400	400	400	400	400
Fish oil	40	40	40	40	40	40
DL-Methionine[1]	0	2	4	6	8	10
Lysine	3.6	3.6	3.6	3.6	3.6	3.6
Glycine	16	14	12	10	8	6
Glutamate	4	4	4	4	4	4
Food attractant[2]	1	1	1	1	1	1
Wheat meal	138.4	138.4	138.4	138.4	138.4	138.4
α-Starch	200	200	200	200	200	200
Brewer yeast	50	50	50	50	50	50
Choline chloride	5	5	5	5	5	5
$Ca(H_2PO_4)_2$	20	20	20	20	20	20
Vitamin and mineral premix[3]	12	12	12	12	12	12
Total	1000	1000	1000	1000	1000	1000
Proximate analysis						
Dry matter (g/kg)	922.66	925.27	928.12	928.43	923.63	924.78
Crude protein (g/kg)	445.92	443.41	458.73	447.40	451.84	450.77
Crude lipid (g/kg)	67.86	67.11	68.69	67.70	67.92	68.07
Crude ash (g/kg)	102.60	101.90	100.60	102.60	101.90	100.60
Gross energy (kJ/g)	19.10	18.86	18.74	19.17	19.25	19.10

[1]DL-Methionine (BR, 99%): Shanghai Yuanye Biotechnology Co., Ltd (Shanghai, China). [2]Attractants: 40% betaine, 20% DMPT, 20% threonine, 10% glycine, and 10% inosine-5′-diphosphate trisodium salt. [3]Vitamin and mineral premix: MGOTer Bio-Tech Co. Ltd (Qingdao, Shandong, China); premix composition (mg/kg diet): KCl, 200 mg; KI (1%), 60 mg; $CoCl_2{\cdot}6H_2O$ (1%), 50 mg; $CuSO_4{\cdot}5H_2O$, 30 mg; $FeSO_4{\cdot}H_2O$, 400 mg; $ZnSO_4{\cdot}H_2O$, 400 mg; $MnSO_4{\cdot}H_2O$, 150 mg; $Na_2SeO_3{\cdot}5H_2O$ (1%), 65 mg; $MgSO_4{\cdot}H_2O$, 2000 mg; zeolite power, 3645.85 mg; VB_1, 12 mg; riboflavin, 12 mg; VB_6, 8 mg; VB_{12}, 0.05 mg; VK_3, 8 mg; inositol, 100 mg; pantothenic acid, 40 mg; niacin acid, 50 mg; folic acid, 5 mg; biotin, 0.8 mg; VA, 25 mg; VCP_1, 5 mg; VE, 50 mg; VC, 100 mg; ethoxyquin, 150 mg; wheat meal, 2434.15 mg.

amino acid sensing and metabolism as part of adaptation to nutrient deprivation by regulating its downstream gene expression [9].

The gastrointestinal tract is the main position for animals' digestion and absorption. Gut health has important implications for an animal's whole healthy statement and utilization of nutrients, because various gastrointestinal functions include digestion and absorption of nutrients by epithelial cells and goblet cells, secretion of mucins and immunoglobulins, and formation of barrier against harmful antigens and pathogens [10]. A previous study showed that different branched-chain amino acids could improve intestinal morphology and cell proliferation, promote intestinal amino acid absorption by regulating intestinal amino acid transporter expression, and increase intestinal protein metabolic efficiency [11]. Methionine is also beneficial for the maintenance of gut morphology and balance of gut bacteria; this system responds to the extensive catabolism of dietary methionine in the gut [12]. A study on nursery pigs showed that supplemented methionine improved small intestinal morphology by increasing villous height and reducing the bacteria fermentation via promoting nutrient digestion and absorption [13]. Dietary methionine could produce glutathione and improve the morphology of the duodenum in nursery pigs [14]. However, there has been little study reporting methionine regulating intestinal health and lipid digestion and absorption, especially for fatty acid transport and absorption in fish.

The rice field eel (*Monopterus albus*, *M. albus*) is a subtropical freshwater benthic fish and a very economical fish and is in central and southern China, widely raised in cages [15]. *M. albus* can prey on insects, frog eggs, earthworms, and water earthworms in nature [11] and has a straight tubular gastrointestinal system; we can easily distinguish between stomach and intestine by evident segmentation from anatomy; it also requires better quality and higher levels of protein and also optimum protein/lipid ratio in its diet, as referenced in our previous studies ([16], Hu et al., 2021), which provide an ideal experimental object for our study. Our previous study showed that fish meal was replaced by soybean meal [17] and soy protein concentrate [18] inhibiting the growth performance of *M. albus*. Our previous study also found that dietary deficiency of methionine in feed decreased the growth performance of *M. albus*, induced lipid metabolism disorder, and decreased whole-body lipid content accumulation [19]. In this study, we used soy protein concentrate to replace fish meal, designed more serious methionine deficiency diets as we described in a previous study [19], and explored the mechanism of how methionine regulates the intestinal structure and barrier, digestion, lipid transport, and absorption in *M. albus*.

Table 2: The contents of amino acids in experimental feed (g/kg).

Amino acids	M0	M2	M4	M6	M8	M10
His☆	9.787	9.629	9.926	9.727	9.996	9.768
Ser	18.942	18.519	19.070	18.690	18.904	18.570
Arg☆	23.417	23.854	23.425	23.199	23.535	23.118
Gly	32.731	30.514	28.362	26.275	24.132	22.012
Asp	42.245	42.158	42.106	42.711	42.535	42.631
Glu	75.484	75.673	75.215	75.742	75.918	75.681
Thr☆	15.514	15.230	15.556	15.925	15.412	15.881
Ala	19.718	19.301	19.759	19.447	19.697	19.424
Pro	20.227	19.697	20.153	20.330	20.575	20.228
Cys	1.084	1.029	1.088	1.091	1.084	1.094
Lys☆	36.887	36.186	36.894	36.382	36.818	36.248
Tyr	9.802	9.759	9.852	9.040	9.397	9.634
Met☆	1.860	3.781	5.920	7.739	9.609	11.525
Val☆	18.640	18.211	18.637	18.379	18.590	18.323
Ile☆	17.478	17.136	17.618	17.638	17.890	17.465
Leu☆	29.125	29.612	29.267	29.666	29.493	29.420
Phe☆	18.457	18.104	18.558	18.220	18.565	18.100
Trp	—	—	—	—	—	—

*Note: ☆ for essential amino acids; Trp not detected.

2. Materials and Methods

2.1. Experimental Diets. Different levels of methionine (0, 2 g/kg, 4 g/kg, 6 g/kg, 8 g/kg, or 10 g/kg) were supplemented to the basic feed (110 g/kg fish meal; 400 g/kg soy protein concentrate) obeying equal nitrogen and energy based on our previous studies [20]. The composition and nutrition level of the diets are shown in Table 1.

Proximate analysis (moisture, crude lipid, crude protein, ash, and gross energy) of experimental feed and *M. albus* was determined referencing our previous papers (Hu et al., 2021). Amino acids were analyzed by an automatic amino acid analyzer (Agilent-1100, Agilent Technologies Co., Ltd., Santa Clara, CA, USA) referencing the method reported by ([21]); fatty acids were analyzed by GC-MS (Agilent 7890B-5977A, Agilent Technologies Co., Ltd., Santa Clara, CA, USA) referencing the method reported by [22], showed in Tables 2 and 3.

2.2. Fish Rearing. M. albus was obtained from Changde, China. We chose a uniform size of *M. albus* (25.08 ± 0.31 g) randomly distributed into 18 float cages ($2.0 \times 1.5 \times 1.5$ m), every group including triplicates, 60 fish per cage. For more details, view our previous manuscript [20].

2.3. Ethics Statement. This study was supported by the Animal Care Committee of Hunan Agricultural University (Changsha, Hunan Province, China). All experimental fish were anesthetized with eugenol (1 : 12,000) (Shanghai Reagent Corporation, Shanghai, China) before sampling to minimize suffering according to the guidelines established by the National Institutes of Health.

Table 3: The contents of fatty acids in experimental feed (mg/100 g).

Fatty acids	M0	M2	M4	M6	M8	M10
C4:0	13.21	13.72	14.49	13.53	13.15	14.16
C8:0	5.07	5.08	4.91	5.05	5.04	5.00
C12:0	3.13	3.64	4.34	3.35	3.35	4.37
C13:0	11.13	10.39	9.71	11.29	10.32	10.14
C14:0	181.39	183.69	182.55	182.37	183.62	182.57
C14:1	2.19	2.62	2.81	2.88	2.70	2.83
C15:0	19.90	20.22	20.52	19.93	20.21	20.51
C16:0	609.04	608.96	606.58	609.36	608.55	606.84
C16:1	6.46	7.59	6.88	6.56	7.58	6.88
C17:0	12.58	13.74	13.65	12.80	13.42	13.52
C17:1	6.27	6.91	7.33	6.73	6.97	7.38
C18:0	120.68	121.92	121.78	121.68	121.97	121.80
18:1-T	16.16	16.09	17.89	16.10	16.02	17.86
C18:1N9C	415.27	410.17	418.66	413.30	410.15	418.53
18:2-T	2.74	3.35	2.45	2.73	3.34	2.46
C18:2N6C	17.35	16.63	18.71	18.34	16.86	18.12
C20:0	11.13	10.45	10.49	10.30	10.40	10.42
C20:1	25.44	27.37	27.27	23.43	27.34	27.22
C18:3N3	235.71	235.00	236.16	235.11	236.65	235.11
C20:2	10.35	10.88	10.31	10.36	10.85	10.34
C22:0	5.84	5.85	5.95	5.39	5.88	5.91
C22:1N9	197.83	197.62	194.40	197.33	197.65	196.49
C20:3N3	32.37	31.17	34.19	32.74	33.13	34.16
C20:4N6	25.20	25.82	25.45	25.57	25.18	25.40
C24:0	248.36	249.92	237.64	248.40	249.18	237.43
C20:5N3	101.77	100.98	101.88	101.17	101.90	101.89
C24:1	21.19	21.36	22.29	21.39	21.32	23.23
C22:6N3	575.88	571.14	571.93	575.90	571.16	570.93

2.4. Sample Collection and Analyses. After fasting 24 h, stomach and intestine were obtained from five fish each cage and stored at -80°C until use. Gastric and intestinal digestive enzymes (amylase, lipase and trypsin) were determined by the kit of Nanjing Jiancheng Bioengineering (Nanjing, China).

The stomach and intestine from five fish in each cage were taken for histometric evaluation. The method of making slides and observing the intestinal sections stained with H&E referenced our previous manuscript [23]. The intestine was sectioned (8 μm) using a cryostat microtome, stained with Oil red O [24]. The slides were observed by CaseViewer.

Total intestinal RNA was obtained from 5 fish in each cage by the Monzol™ reagent (Monad, Shanghai, China). Smart cDNA was synthesized by a SMART cDNA Synthesis kit (Clontech Laboratories, Palo Alto, CA). Primers obtained from Biosune Biotechnology, Inc. (Shanghai, China) are showed in Table 4. The operation steps of quantitative real-time PCR (q-PCR) referenced our previous manuscript [25]. The amplification efficiency was between 0.95 and 1.10, which is calculated by the formula $E = 10 * (-1/\text{slope}) - 1$;

Table 4: Primer sequence for q-PCR.

Gene	Forward (5′-3′)	Reverse (5′-3′)	Accession no.*	Size (bp)
gcn2[1]	GGAACTCGTCCTGAACTG	TGGTGAAGAACTTGCCTAT	XM_020586241.1	298
eif2a[2]	CCCCTTCCTTTGTTCGTC	GCTGAGGCTTTCTTGTTCC	XM_020621840.1	121
lpl[3]	CGTTGACATCGGAGACCTGA	CAAAGACCACCTTGGACTGAG	XM_020613041.1	146
moat2[4]	TCTCCCTGCCTCTCTTTCA	TGTCCACTCCATAGTTGCCT	XM_020622089.1	213
dgat2[5]	ACTTCCGCTTTCCCTTG	ATTCCCTGTCTCGTTATGTG	XM_020622054.1	104
mttp[6]	AAGATGCTCCAGGCTTTGTT	TGTCAGGACCCTCTAAAATCAG	XM_020602163.1	172
hdlbp[7]	CCACCCCAGACGACAAAGAC	GGCGAGCAACAAAATAACGA	XM_020609988.1	165
ldlrap[8]	CAGGAAGACAAAAGCAAGAAGG	CGAGTGGGGTTACTATGAGGC	XM_020617284.1	194
vldlr[9]	ACATCCGTCGTTTGGGTCTA	GTGGTAGTGTCCCCTCGTTT	XM_020601062.1	169
npc1l1[10]	TTGGAGTCCCAGTTTATTT	TACACTTGCGTCCACATT	XM_020590431.1	297
mct1[11]	TCCTATGCCTTCCCTAAAT	AAGTTGAATGCCAGTCCC	XM_020586598.1	287
mct2[12]	TGGGCTTGTCACCATTAT	CTCCTCGTCCAGTTTCTT	XM_020589687.1	181
mct4[13]	GAGGAGCAGTGGTGGATG	GGGAAGGCGTAGGAGAAA	XM_020608921.1	112
mct7[14]	GTTGTCATTGGCACCCTT	ACCTGAGTCCTCCGAACC	XM_020608232.1	210
mct8[15]	CAGCAGGACCTTCCAAAT	AAAGTAGCCCAGGACAGC	XM_020592011.1	271
mct12[16]	GTTGGCGTATGGGATTGC	TTTGGCGAGATTTGGATGT	XM_020616669.1	223
cd36[17]	TTGAAAGGGATTGAGGTG	TCTCGCAAGGATGGACTA	XM_020616796.1	212
fatp1[18]	GCGAGCCAGGTATGTTAG	CAGCAAGGCACTGAGGAC	XM_020587461.1	263
fatp2[19]	CTTTGATTACAGCCTTGC	CTTTCCGTTGTCCTTTCT	XM_020602138.1	100
fatp6[20]	CAGTAGGACTTTGGGCATTT	GTCGCACTTTGTGAACTTTATC	XM_020618747.1	267
fatp7[21]	ACTGTAATCATCAGCCAAGA	GGTTTCGTCAAACTCCTC	XM_020588511.1	105
apo[22]	GGGCTGCTCTGGATGTCT	CCCGCAAAGCACTAATCT	HQ603782.1	147
apoa[23]	CAAGAAGGTCCAGGTTGA	TTAGTAAGGGATTGGTAGAGG	XM_020590134.1	147
apob[24]	TGCCAATAACTATCCGCTAC	TCTTCCTGACATCATCCC	XM_020615697.1	247
apoc[25]	GCTGCTGGTCGTTACTGT	AGTCCCTAATGGTTTCTATG	XM_020590135.1	176
apoe[26]	CGCTGCGTGGAAGGAAAC	CTGCCAGAGCAAGGATGAGA	XM_020590131.1	220
apof[27]	AGGTGGTAAGCCTGATAGA	CCAACCCTCATAGTGTCC	XM_020592847.1	185
apoo[28]	GCTCAGGTTCGGTTTGTT	GGTGGCAACTCTGGGTAT	XM_020595548.1	207
occ[29]	TGTCGGGGAGTGGGTAAA	TCCAGGCAAATAAAGAGGCT	XM_020616177.1	130
zo-1[30]	GGCATCATCCCCAACAAA	GCGAAGACCACGGAACCT	XM_020621576.1	111
zo-2[31]	AGCCGAGGTCGCACTTTA	GCTTTGCTTCTGTGGTTGAT	XM_020615114.1	246
cl-12[32]	TCACCTTCAATCGCAACG	ATGTCTGGCTCAGGCTTATCT	XM_020607277.1	250
cl-15[33]	CTCGCTGCTTGCTTTGACT	TTGAAGGCGTACCAGGACA	XM_020611334.1	225
rpL17[34]	CGAGAACCCGACTAAATCA	GTTGTAGCGACGGAAAGG	XM_020587712.1	169

[1]*gcn2*: general control nonderepressible; [2]*eif2a*: eukaryotic translation initiation factor 2; [3]*lpl*: lipoprotein lipase; [4]*moat2*: monoacylglycerol O-acyltransferase 2; [5]*dgat2*: diacylglycerol acyltransferase 2; [6]*mttp*: microsomal triglyceride transfer protein; [7]*hdlbp*: high-density lipoprotein binding protein; [8]*ldlrap*: low-density lipoprotein receptor adapter protein; [9]*vldlr*: very low-density lipoprotein receptor; [10]*npc1l1*: NPC1 like intracellular cholesterol transporter 1; [11]*mct1*: monocarboxylate transporter 1-like; [12]*mct2*: monocarboxylate transporter 2-like; [13]*mct4*: monocarboxylate transporter 4-like; [14]*mct7*: monocarboxylate transporter 7-like; [15]*mct8*: monocarboxylate transporter 8-like; [16]*mct12*: monocarboxylate transporter 12-B-like; [17]*cd36*: CD36 molecule; [18]*fatp1*: fatty acid transport protein 1; [19]*fatp2*: fatty acid binding protein 2; [20]*fatp6*: fatty acid transport protein 6; [21]*fatp7*: fatty acid binding protein 7; [22]*apo*: apolipoprotein; [23]*apoa*: apolipoprotein A; [24]*apob*: apolipoprotein B; [25]*apoc*: apolipoprotein C; [26]*apoe*: apolipoprotein E. [27]*apof*: apolipoprotein F; [28]*apoo*: apolipoprotein O; [29]*occ*: occludin-like; [30]*zo-1*: tight junction protein ZO-1-like; [31]*zo-2*: tight junction protein ZO-2-like; [32]*cl-12*: claudin 12; [33]*cl-15*: claudin 15; [34]*rpL17*: ribosomal protein L17; it is reference gene. *NCBI Reference Sequence.

5-fold serial dilutions of cDNA (triplicate) were used to generate the standard curve. $2^{-\Delta\Delta Ct}$ was used to calculate the relative mRNA expression [26].

2.5. Statistical Analysis. Data were analyzed by one-way analysis of variance (ANOVA), and significant differences among all groups were assessed by Duncan's multiple-range test; the data of two groups (M0 and M8) was calculated by an independent T-test; ANOVA and independent T-test were performed by SPSS 22 software. The results were presented as the means ± SEM (standard error of the mean), and differences were considered significant at $P < 0.05$.

Table 5: Effects of different levels of methionine on gastric and intestinal digestive enzymes of *M. albus* after 8 weeks (U/g protein).

Item	M0	M2	M4	M6	M8	M10	*P* value
Stomach							
Amylase	231.94 ± 5.14^{a}	257.95 ± 4.87^{b}	289.7 ± 3.72^{c}	299.84 ± 3.78^{c}	358.05 ± 4.46^{d}	357.61 ± 4.07^{d}	<0.001
Lipase	427.83 ± 11.73^{a}	474.53 ± 5.54^{b}	485.74 ± 4.73^{b}	513.76 ± 11.36^{c}	566.08 ± 5.61^{d}	586.63 ± 3.74^{d}	<0.001
Trypsin	2156.23 ± 22.47^{a}	2244.78 ± 23.86^{b}	2438.06 ± 43.50^{c}	3081.08 ± 20.33^{d}	3423.03 ± 14.14^{e}	3467.2 ± 43.26^{e}	<0.001
Intestine							
Amylase	270.7 ± 3.84^{a}	275.38 ± 3.55^{a}	278.51 ± 4.37^{a}	281.63 ± 3.99^{ab}	294.12 ± 3.17^{c}	291 ± 3.55^{bc}	0.001
Lipase	335.53 ± 3.79^{a}	347.1 ± 3.99^{a}	363.93 ± 9.45^{b}	411.26 ± 3.79^{c}	429.15 ± 2.30^{d}	439.67 ± 2.66^{d}	<0.001
Trypsin	2161.05 ± 32.27^{a}	2249.94 ± 36.82^{b}	2273.27 ± 14.48^{b}	2445.51 ± 18.24^{c}	2457.73 ± 15.94^{c}	2472.73 ± 14.26^{c}	<0.001

Values showed as means ± SEM ($n = 3$). Values in the same row with the same superscript or absence of superscripts are not significantly different ($P > 0.05$).

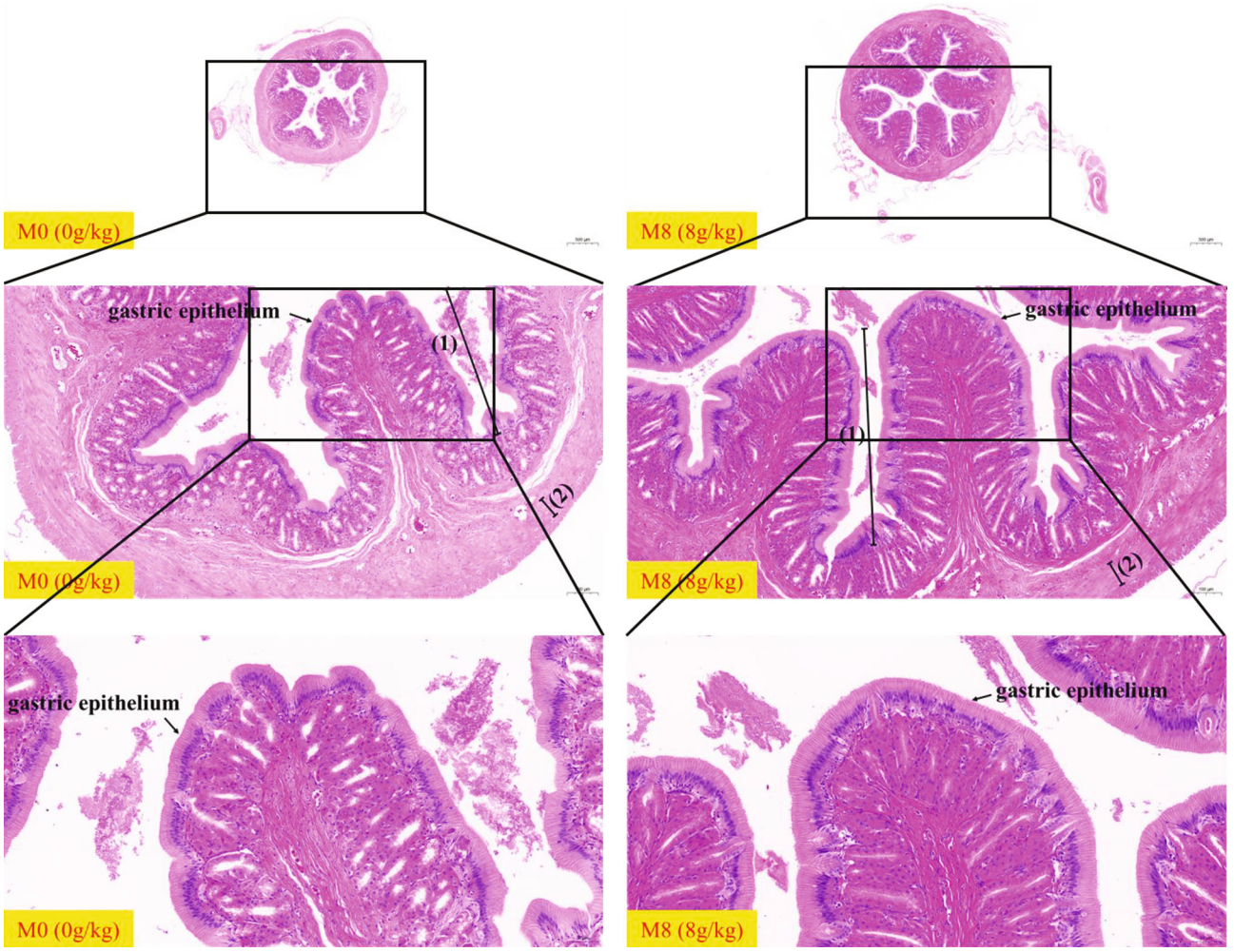

Figure 1: Effects of deficient and optimum methionine diet on the gastric H&E stain of *M. albus* after 8 weeks. (1) and (2) were expressed as gastric fovea and gastric muscular thickness, respectively.

3. Result

3.1. Gastric and Intestinal Digestive Enzymes. Compared with M0 (0 g/kg), gastric amylase, lipase, and trypsin activities were remarkably increased as dietary methionine ($P < 0.05$); intestinal amylase was remarkably increased as added 8 g/kg methionine ($P < 0.05$), intestinal lipase was remarkably increased as supplemented methionine higher than 2 g/kg ($P < 0.05$), and intestinal trypsin was remarkably increased as dietary methionine ($P < 0.05$) (Table 5).

3.2. Gastric and Intestinal Sections Stained with H&E. The results of gastric and intestinal sections stained with H&E are presented in Figures 1 and 2. Compared with M0 (0 g/kg), gastric fovea increased remarkably in M8 (8 g/kg) ($P < 0.001$); gastric epithelium is neater in M8 than that in M0 (Table 6).

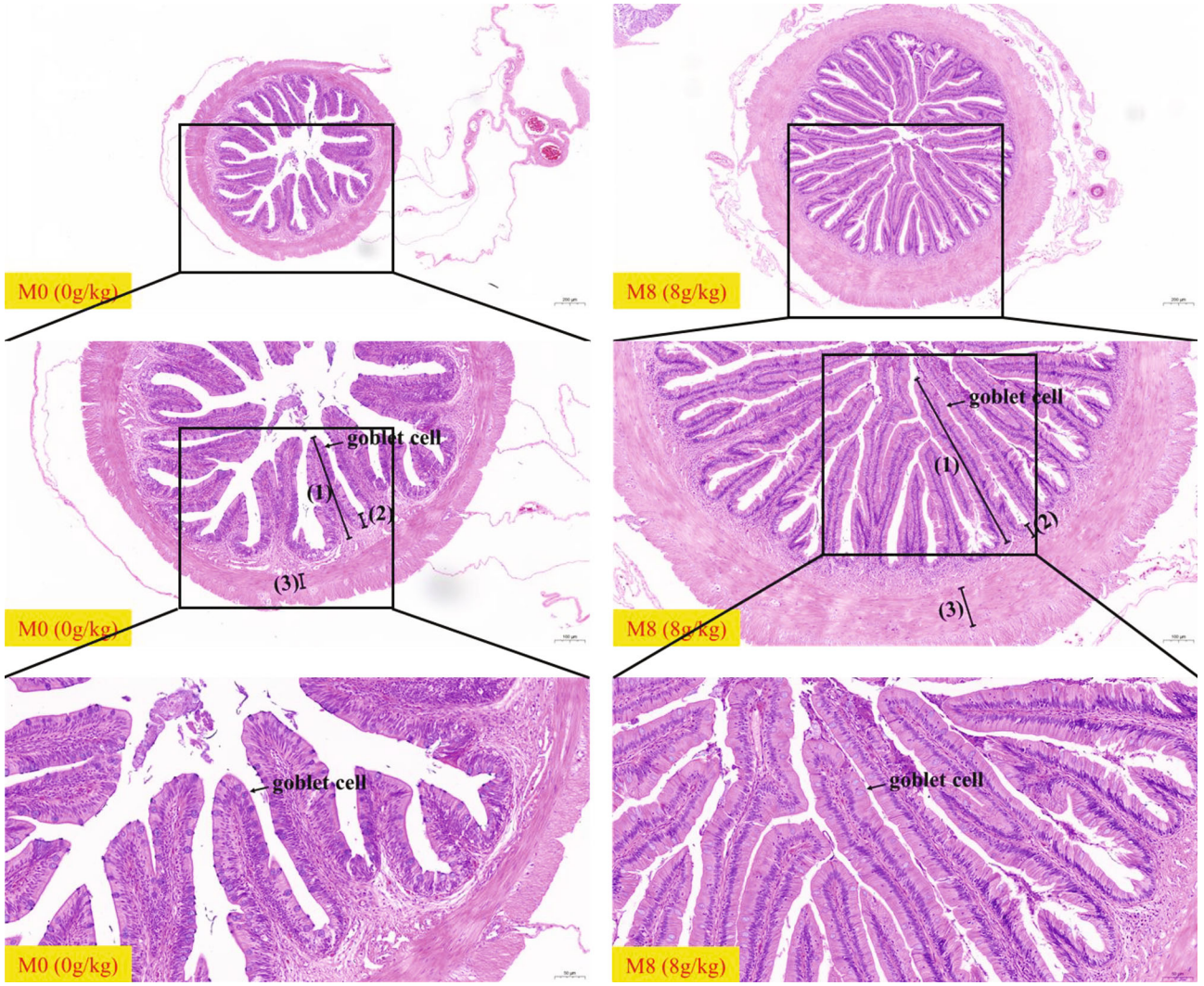

Figure 2: Effects of deficient and optimum methionine diet on the intestinal H&E stain of *M. albus* after 8 weeks. (1), (2), (3), and (4) were expressed as intestinal villus height, crypt depth, muscular thickness, and goblet cell, respectively.

Table 6: Effects of deficient and optimum methionine diet on the gastric H&E stain (×100 and ×400) of *M. albus* after 8 weeks ($n = 3$).

Item	M0	M8	*P* value
GF[1]	440.11 ± 27.08	648.31 ± 24.30	<0.001
GMT[2]	94.48 ± 0.98	97.31 ± 1.77	0.193

Values are presented as means ± SEM ($n = 3$). Values were considered not significantly different at $P > 0.05$. [1]GF: gastric fovea (μm); [2]GMT: gastric muscular thickness (μm).

Table 7: Effects of deficient and optimum methionine diet on the intestinal sections stained with H&E (×100 and ×400) of *M. albus* after 8 weeks ($n = 3$).

Item	M0	M8	*P* value
IVH[1]	380.71 ± 12.14	693.54 ± 21.81	<0.001
CD[2]	47.12 ± 1.63	32.82 ± 0.91	<0.001
IMT[3]	53.11 ± 3.40	94.53 ± 2.00	<0.001
AIGC[4]	27 ± 3	33 ± 2	0.084

Values showed as means ± SEM ($n = 3$). Values were considered not significantly different at $P > 0.05$. [1]IVH: intestinal villus height (μm); [2]CD: crypt depth (μm); [3]IMT: intestinal muscular thickness (μm); [4]AIGC: amounts of intestinal goblet cells per root.

Compared with M0, intestinal villus height and intestinal muscular thickness remarkably increased in M8 ($P < 0.001$), and amounts of goblet cells per root in M8 increased ($P > 0.05$), while intestinal crypt depth markedly decreased ($P < 0.001$) (Table 7). In addition, lipid droplets in intestinal villus and mucosal layer in the M8 (8 g/kg) group were more than that in M0 (0 g/kg) (Figure 3).

3.3. Intestinal Regulatory mRNA Expression. Compared with M0 (0 g/kg), the intestinal *gcn2* and *eif2α* are downregulated in M8 (8 g/kg) ($P < 0.01$ and $P < 0.05$, respectively), while *occ*, *cl12*, *cl15*, *zo-1*, *zo-2*, *hdlbp*, *ldlrap*, *npc1l1*, *cd36*, *fatp1*, *fatp2*, *fatp6*, *apo*, *apoa*, *apob*, *apoc*, *apoe*, *mct1*, *mct2*, *mct8*,

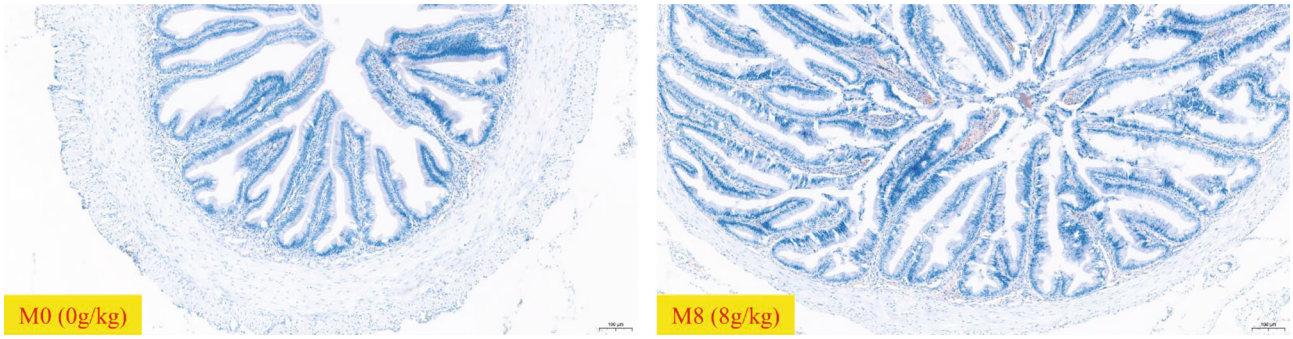

Figure 3: Effects of deficient and optimum methionine diet on the intestinal Oil red O stain (×100) of *M. albus* after 8 weeks.

lpl, *mttp*, *moat2*, and *dgat2* were upregulated markedly in M8 (8 g/kg) (Figure 4).

3.4. Intestinal Regulatory mRNA Expression. We observed that intestinal *eif2α* expression was positively correlated with *gcn2*, and intestinal *zo-1*, *cl15*, *fatp6*, *ldlrap*, *mct2*, *mct8*, *apo*, *apob*, *mct1*, *apoc*, *fatp1*, *mttp*, *cd36*, *occ*, *npc1l1*, *hdlbp*, *fatp2*, *apoe*, *lpl*, and *moat2* gene expression was negatively correlated with *gcn2* ($P < 0.05$, $P < 0.05$, $P < 0.05$, $P < 0.05$, $P < 0.01$, $P < 0.01$, $P < 0.05$, $P < 0.01$, $P < 0.01$, $P < 0.01$, $P < 0.01$, $P < 0.01$, $P < 0.01$, $P < 0.05$, $P < 0.05$, $P < 0.05$, $P < 0.01$, $P < 0.05$, $P < 0.05$, and $P < 0.05$) (Figure 5).

4. Discussions

Methionine is the most deficient essential amino acid of most plant proteins, especially for soybean protein [27]. Our previous study showed that dietary methionine restriction not only restricted muscle fiber growth, muscular development, and differentiation of *M. albus* and inhibited growth performance of *M. albus* [20] but also induced lipid metabolism disorder and decreased lipid content of *M. albus* (Hu et al., 2021). Our previous study showed that crude lipid and crude protein of *M. albus* were significantly promoted as supplemented with 8 g/kg methionine. In this study, compared with M0 (0 g/kg), gastric amylase, lipase, and trypsin were remarkably increased as dietary methionine; intestinal amylase, lipase, and trypsin were remarkably increased as dietary high than the level of 8 g/kg (M8) methionine. Our finding was similar to the study in grass carp (*Ctenopharyngodon idella*) [28]. We hold that methionine deficiency decreased gastric-intestinal main digestive enzymes (amylase, lipase, and trypsin) of *M. albus* and mainly affected the stomach.

Based on the photos of intestinal H&E staining, the intestinal lumen in M8 (8 g/kg) was bigger than that in M0 (0 g/kg); the reason was that the fish were smaller in M0 than that in M8 because methionine restriction inhibited the growth performance of *M. Albus* as we early reported [20]. Here, compared with M0 (0 g/kg), gastric fovea increased remarkably in M8 (8 g/kg); gastric epithelium is neater in M8 than that in M0, which meant that the capacity of the gastric digestive system became weak; this phenomenon explained that amylase, lipase, and trypsin were remarkably decreased as methionine restriction. In addition, in this paper, compared with M0, we also observed that intestinal villus height and muscular thickness were remarkably increased in M8, and amounts of goblet cells per root in M8 were increased, while intestinal crypt depth remarkably decreased; besides, lipid droplet in intestinal villus and mucosal layer in the M8 (8 g/kg) group were more than that in M0 (0 g/kg) in this study, which meant that the function of intestinal absorption was declined and the intestinal barrier was damaged [29], also including lipid. Our previous study showed a similar result as dietary soy isoflavone and soy saponin damage the intestinal barrier and decrease intestinal function [23].

To further explain the reasons how methionine restriction influences gastrointestinal lipid digestion and absorption of *M. albus*, the M0 (0 g/kg) and M8 (8 g/kg) groups were selected to explore the molecular mechanism. *gcn2* and *eif2a* are a response to essential amino acid deprivation and regulate its downstream lipid metabolism relative genes [30]. In this study, compared with M0 (0 g/kg), the intestinal *gcn2* and *eif2α* are downregulated in M8 (8 g/kg); this meant that amino acid deficiency can be sensed by *M. albus*. Intestinal tight junction protein includes occludens, claudin, and zonula, could form the epithelial barrier and prevent infiltration, and is indispensable in protecting barrier integrity and function [31]. We observed that *occ*, *cl12*, *cl15*, *zo-1*, and *zo-2* genes are upregulated markedly in M8 (8 g/kg), which explained that methionine deficiency affected gastric and intestinal structures and damaged the intestinal barrier. As we know, lipid can be digested into fatty acids and alcohols in the intestine; then, fatty acids and alcohols were absorbed, and fatty acids and alcohols assemble into lipid; eventually, this lipid transports into the whole body by blood circulation. Microsomal triglyceride transfer protein (*mttp*) facilitates the transport of fat by assisting in the assembly and secretion of triglyceride-rich lipoproteins [32]. Lipoprotein lipase (*lpl*) is involved in lipolysis [33], while mogat2 and dgat2 participate in lipogenesis [34, 35]. In this study, intestinal *mttp*, *lpl*, *moat2*, and *dgat2* were upregulated markedly in M8 (8 g/kg), which indicated that lipid metabolism is more active as a dietary appropriate level of methionine. This phenomenon indirectly explained why supplementation with methionine enhanced gastric and intestinal digestive enzymes and promoted the ability of digestion in our study. High-density lipoprotein-binding protein (*hdlbp*) regulates the endocrine of both lipids and cholesterol ([36]); the

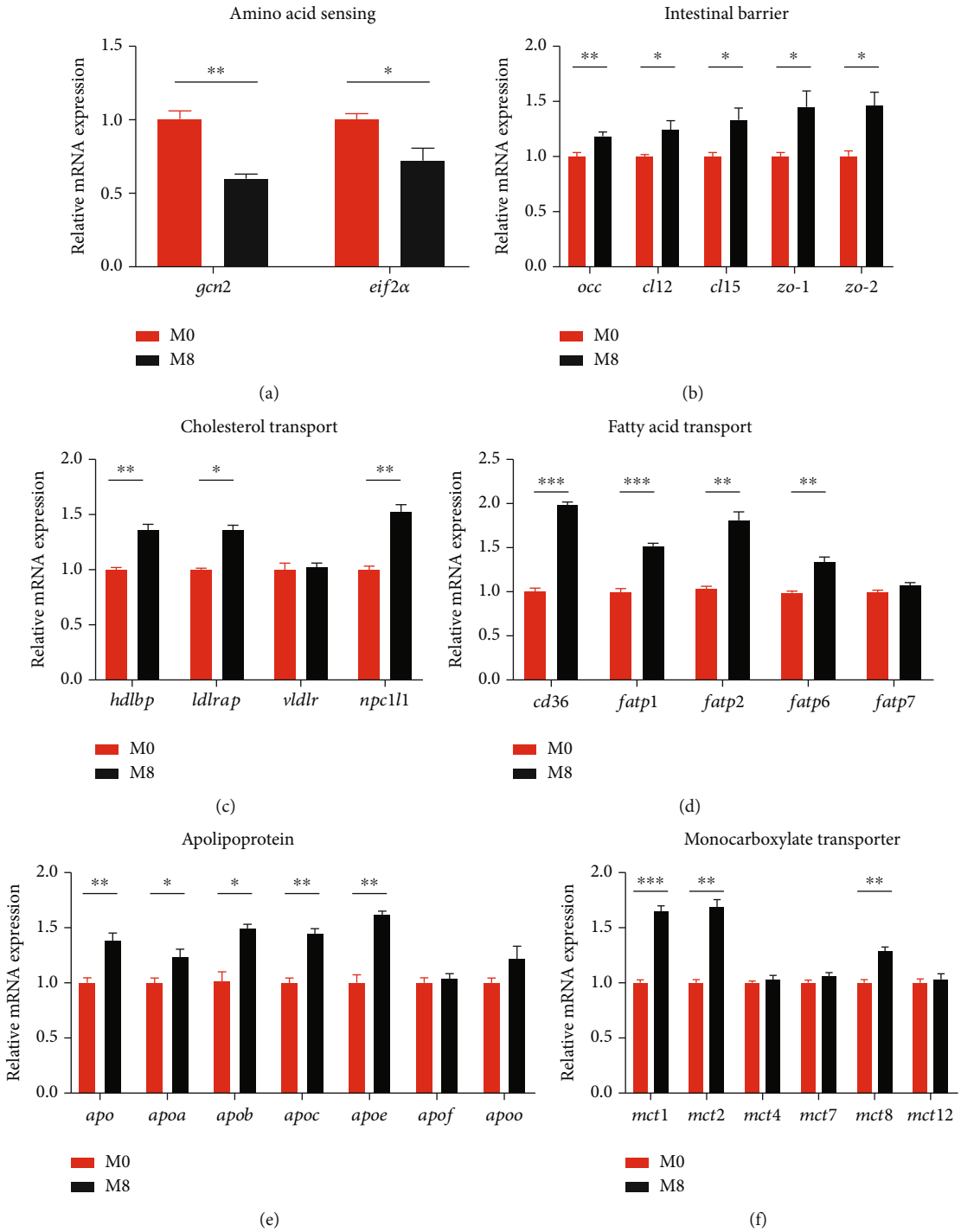

Figure 4: Continued.

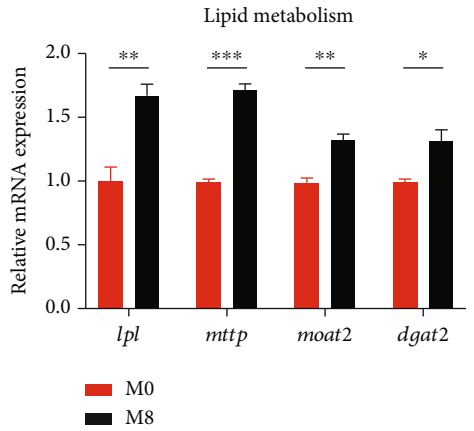

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Figure 4: Effects of dietary methionine on intestinal mRNA expression of *M. albus* after 8 weeks ($n = 3$). Single, double, or triple numbers of asterisks were significantly different at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

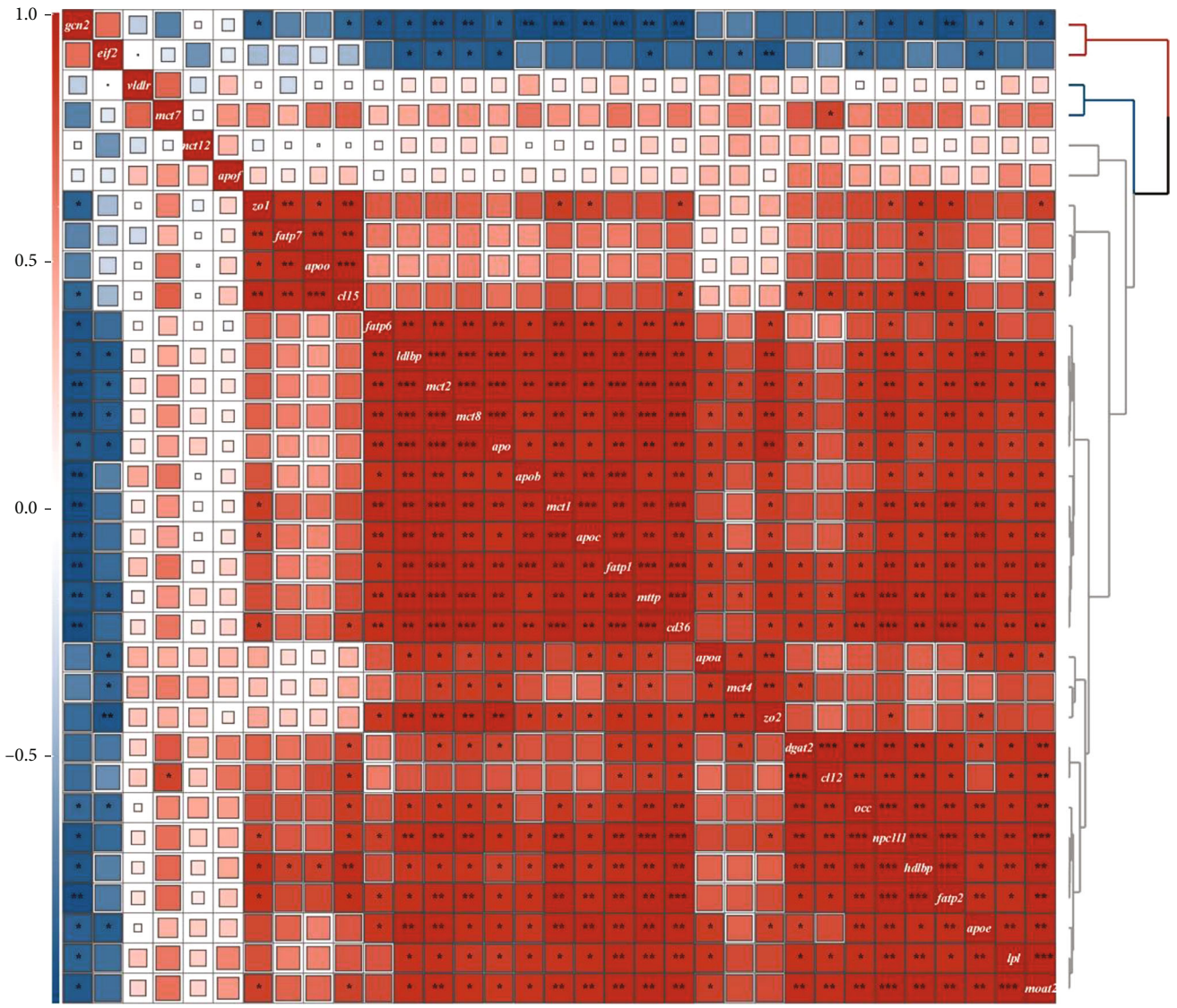

Figure 5: Correlative analysis of intestinal regulatory mRNA expression used by R Programming Language. Single, double, or triple numbers of asterisks were significantly different at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

low-density lipoprotein receptor adapter protein (*ldlra*) pathway has emerged as a target to reduce circulating cholesterol [37]. NPC1 like intracellular cholesterol transporter 1 (*npc1l1*) is mainly charged in cholesterol absorption [38]. CD36 molecule (*cd36*) plays a role in fatty acid absorption and transport [39, 40]. Apolipoprotein can bind and transport lipoproteins [41]. Monocarboxylate transporter mainly transports short-chain monocarboxylates, including lactate, pyruvate, and ketone bodies [42]. In this study, intestinal *hdlbp*, *ldlrap*, *npc1l1*, *cd36*, *fatp1*, *fatp2*, *fatp6*, *apo*, *apoa*, *apob*, *apoc*, *apoe*, *mct1*, *mct2*, and *mct8* were upregulated in M8 (8 g/kg) than that in M0 (0 g/kg). We inferred that methionine deficiency suppressed intestinal mucosal growth and inhibits intestinal epithelial cell proliferation, damaged the intestinal barrier [29, 43], and then declined intestinal lipid and fatty acid transport.

We observed that intestinal *eif2α* expression was positively correlated with *gcn2*, and intestinal *zo-1*, *cl15*, *fatp6*, *ldlrap*, *mct2*, *mct8*, *apo*, *apob*, *mct1*, *apoc*, *fatp1*, *mttp*, *cd36*, *occ*, *npc1l1*, *hdlbp*, *fatp2*, *apoe*, *lpl*, and *moat2* gene expression was negatively correlated with *gcn2*. We inferred that *M. albus* could sense methionine deficiency by *gcn2* and regulated the lipid and fatty acid transport.

5. Conclusion

Methionine deficiency mainly affected gastric and intestinal structures, damaged the intestinal barrier, and decreased the lipid and fatty acid transport of *M. albus*. Besides, *gcn2* could be activated when *M. albus* was fed methionine-deficient feed.

Data Availability

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

Conflicts of Interest

There are no conflicts of interest to this manuscript.

Authors' Contributions

Yajun Hu was in charge of methodology, data curation, and writing (original draft). Minglang Cai was in charge of data curation and software. Huan Zhong was in charge of formal analysis and writing (review and editing). Wuying Chu was in charge of formal analysis, software, and writing (review and editing). Yi Hu was in charge of funding acquisition, writing (review and editing), and validation.

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