**Table S1. Feed formulation and proximate analysis of experimental diets.**

|  |  |  |
| --- | --- | --- |
| Ingredients (g kg −1) | Adequate choline | Choline deficiency |
| Casein | 360 | 360 |
| Gelatin | 80 | 80 |
| Fish oil | 30 | 30 |
| Soybean oil | 30 | 30 |
| Wheat flour | 250 | 250 |
| Ascorbyl-2-polyphosphate | 10 | 10 |
| NaCl | 10 | 10 |
| Ca (H2PO4)2·H2O | 10 | 10 |
| Vitamin premix1 | 5 | 5 |
| Mineral premix2 | 5 | 5 |
| Cellulose | 208.5 | 209.5 |
| Choline chloride3 | 1.5 | 0.5 |
| *Proximate analysis (%, dry weight)* |  |  |
| Moisture | 7.29 | 7.22 |
| Crude protein | 40.54 | 39.99 |
| Crude ash | 5.36 | 5.37  |
| Crude lipid | 10.49 | 10.15 |
| Choline (g kg-1) |  1.61 | 0.59 |

1Vitamin premix (mg or IU per kg diet): retinylacetate, 10000IU; cholecalciferol, 1000IU; all-rac-a- tocopheryl acetate, 30IU; menadione nicotinamide bisulfite, 7; thiamine hydrochloride, 6; riboflavin, 3; pyridoxine hydrochloride, 12; D-calcium pantothenate, 30; niacin, 50; biotin, 1; folic acid, 6; cyanocobalamine, 0.03.

2Mineral mixture (mg per kg diet): Ca(H2PO3)2·H2O, 1000; FeSO4·7H2O, 40; ZnSO4·7H2O, 40; MnSO4·H2O, 40; CuSO4·5H2O, 2; CaIO3·6H2O, 3; Na2SeO3, 0.05; CoSO4, 0.05.

3Choline chloride: active choline percentage >98%; Macklin Biochemical Technology Co., Ltd. (Shanghai. China).

**Table S2. Effects of dietary choline supplementation on growth performance and morphological parameters yellow catfish *(Pelteobagrus fulvidraco)* after 10 weeks.**

|  |  |  |
| --- | --- | --- |
| **Diet** | Adequate choline | Choline deficiency |
| **IBW, g/fish** | 3.80±0.06 | 3.78±0.05 |
| **FBW, g/fish** | 30.03±0.63 | 31.38±0.91 |
| **WG2, %** | 690.16±0.10 | 728.60±0.15\* |
| **FCR3** | 1.17±0.02 | 1.15±0.03 |
| **HSI4, %** | 1.30±0.25 | 1.37±0.27 |
| **CF5, %** | 1.69±0.34 | 1.70±0.06 |
| **Survival6, %** | 96.00±4.00 | 98.66±2.30 |

1Values are means ± SEM; n = 3 tanks (30 fish/tank). asterisks indicate significant differences between adequate choline and choline deficiency groups (\* *p* < 0.05).

2WG = (FBW-IBW)/IBW×100.

3FCR = dry feed fed (g)/wet weight gain (g).

4HSI = 100×(liver weight)/(body weight);

5CF = 100×(live weight, g)/(body length, cm)3.

6Survival = 100 ×final fish number/initial fish number.

**Table S3. Primers used for plasmid construction and RT-qPCR analysis**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Gene | Accession No. | Forward primer (5'-3') | Reverse primer (5'-3') | PE(%) | AT (°C) |
| **Bisulfite sequencing for methylation validation** |  |  |
| *ire-1α* | MH078576.1 | TTTTTGGAATTTTGTTTTTATTAGTTG | ATTAACATCTTTTACAAAACCCTCAC | 98 | 54 |
| **Plasmid construction** |  |  |
| *ire-1α* | MH078576.1 | ctagcgtttaaacttaagcttTGGCTAGAGGGCAGTTCTGT | aacgggccctctagactcgagATGGAGTCCACAGGTGCTTC | 97 | 56 |
| **RT-qPCR analysis** |  |  |
| *gapdh* | KP938521 | TTTCAGCGAGAGAGACCCAG | ATGACTCTCTTGGCACCTCC | 99 | 55 |
| *β-actin* | DQ211096.1 | ACCCTGAAGTACCCCATCGA | CAGAGGCATACAGGGACAGC | 102 | 60 |
| *accα* | GU908474.1 | GCTTGCGGCGGTTATTACTG | AGCTGCCTCTCCAACCATTC | 101 | 57 |
| *srebp-1c* | JX992742.1 | TGCAGGAGCTACAACACAGG | GGACTTTCGTCTGCCTGAAG | 105 | 58 |
| *pparγ* | JX992741.1 | CCGTCATCGAGGTTCTCATT | CATCGAGCTCCAGCATGTTA | 96 | 59 |
| *me* | XM\_047818508.1 | AACCCGACTGCTAAAGCTGA | AGCCAGAGCTACACCAGGAA | 95 | 55 |
| *fas* | GU433188 | TCATCCAGCAGTTCACTGGCATT | TGATTAGGTCCACGGCCACA | 94 | 56 |
| *6pgd* | [XM003444904.4](https://www.ncbi.nlm.nih.gov/nucleotide/XM_003444904.4?report=genbank&log$=nucltop&blast_rank=1&RID=HW48H8D6013) | GAAGGGCCTGCTGTTTGTTG | CCCAGTCACAACAAGGCTCT | 101 | 60 |
| *g6pd* | XM005478106 | GAGAAGCCCTTTGGTCGTGA | ATCAAAGTACCCTCCACGGC | 103 | 58 |
| *hsla* | KJ588764 | GTTATCGGAATGCCGTGACT | CAGAATCGTCCAACAGAGCA | 102 | 58 |
| *atgl* | KF614123 | GCGGAAATGTGATTGAGGTT | CTTTGGAGCCGAAGTCTGAG | 98 | 58 |
| *mgl* | KX980491.1 | TATGGCTCCCAGCCTCTCTA | CCGTGCAGAAGAAGAAAAGG | 96 | 59 |
| *pparα* | JX992740 | CGAGGATGGGATGCTGGTG | CGTCTGGGTGGTTCGTCTGC | 98 | 57 |
| *cpt 1α* | JQ074177 | CTGGCAAAGGACTTCCAGAG | TCTGGCAACTTGCAAATGAG | 98 | 63 |
| *echs1* | MG599820.1 | TTTTTGGCACACTGGAACAA | GCACCTGGAATGGTTCCTAA | 95 | 62 |
| *acadm* | MG599804.1 | TGGTGTACAGACGGGTTTGA | TGAGCGTTCATCAGACCAAG | 99 | 61 |
| *hadhb* | MG599812 | GAACAAGGCCAAGTCTCTCG | AGAGAGTGAGAGCGCAAAGC | 103 | 65 |
| *grp78* | FJ436356.1 | ATTTGTTCCGCTCCACCATG | AACTCTTTCACCAGCTGCTG | 102 | 66 |
| *grp94* | KR231692.1 | AGGGTGTGGTGGACTCTGAC | CTGTTGGAATGGTCCTCGAT | 103 | 65 |
| *crt* | KM114875.1 | ATGCCAAAAAGCCTGATGAC | CAGACTTCACCTGCCACAGA | 101 | 66 |
| *perk* | KP687344.1 | GGGAAACTGTGGAGGGATGG | TGCAGCCTTGACCACTTTCT | 96 | 58 |
| *eif2α* | JN195739.1 | TCGGCCCCAGTCTCATTCTA | ATACACCACTCGCCTCTCCT | 96 | 60 |
| *ire1α* | KY081668.1 | TTCTGCGGGAAACGTTTCAC | ACTACGCATGAACCGTTTGG | 98 | 62 |
| *xbp1* | MN701647 | CTCCTGAACAGAAGCAGCCA | CTCGAAGTGCTCTGCCATGA | 97 | 63 |
| *atf6* | XM005471382 | TCCCCGGATCATCGTATGGA | TCCTGCAGTGACTCCTAACG | 94 | 61 |
| *chop* | NC062531.1 | GCAGTAGGGGTGGAGTACCA | GATGCTGCACGATAGGGATT | 99 | 60 |
| *chdh* | XM047800082.1 | CTCCCAGGTGTGGGAAGTAA | CCAGATGAGCTGTAGCACCA | 98 | 65 |
| *badh* | NC062531.1 | TCATCAATCCGTCCACTGAA | TCAATGTCCAGCACTGCTTC | 98 | 66 |
| *mat1α* | XM\_047812984.1 | GCCAAGTCTCTGGTCAAAGC | ACAATAACACCAGGCCGAAG | 101 | 62 |
| *mat2α* | XM\_027145751 | GTGGTGCCTTGCATTTACCT | CCTTCCCAGAGAAAGCTCCT | 98 | 65 |
| *dnmt1* | MH263731.1 | ATAACCACTGGGCTGGACTG | GTCCAGGATATTGCCGAAGA | 99 | 65 |
| *dnmt3α* | MH263732 | CTCTGGTGCACTGAGATGGA | GGCTTCAGTAACAGGCGAAG | 100 | 61 |
| *dnmt3β* | MH263733.1 | ACATTGAAAAATGGGGTCCA | GCTTTGTCCCTGGTGTTCAT | 101 | 61 |
| **siRNA sequences** |  |  |
| siRNA*- ire1a-*839 |  | UAUCAUGAUUGCUGAGAAAUU | UUUCUCAGCAAUCAUGAUAUU |  |  |
| NC-siRNA |  | GCAUGUUUCUAGAGGCAAAUU | UUUGCCUCUAGAAACAUGCUU |  |  |
| **Ire1α** **Δ836-963** |  |  |  |  |  |
| First-round |  | AAACCTAGCGGCATGGAC | CTAATGGTGATGGTGATGATGTAGTGCGTTGTCTTCTT |  |  |
| Second-round |  | ctagcgtttaaacttaagcttATGGGCTGGGGTGTTTGTAGC | aacgggccctctagactcgagCTAATGGTGATGGTGATGATGTAGTG |  |  |
| Mutation |  | TTTCTGGAGCCTTTCCGAGCTGCCTTCACGTAC | TCGGAAAGGCTCCAGAAAAAGGGATGTTTGAGC |  |  |

PE: primer efficiency; AT: Annealing temperature.



**Fig. S1. MTT assay for the** **viability of** **primary hepatocyte of yellow catfish incubated with choline for 48 h.** Values are means ± SEM.



**Fig. S2. Ramachandran plots for structure validation of Fas and Ire-1α.**

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**Fig. S3. The interacting amino acids between Fas and Ire-1α interaction.**



**Fig. S4.** **The structural protein prediction model for Fas, Ire-1α, the interaction prediction score and energy values for Fas/Ire-1α.**