Supplemental Table S1. Composition of riboflavin-deficient basal diet (as-fed basis).

|  |  |
| --- | --- |
| Item | Basal diet (from hatch to day 21) |
| Ingredient, g/kg |  |
| Corn | 622.4 |
| Soybean | 120.0 |
| Corn gluten meal | 210.0 |
| Limestone | 10.0 |
| Dicalcium phosphate | 16.0 |
| Vitamin and trace mineral premix\* | 10.0 |
| Sodium chloride | 3.0 |
| DL-Methionine  | 0.7 |
| L-Lysine·HCl | 5.3 |
| L-Threonine | 0.6 |
| L-Tryptophan | 1.0 |
| L-Arginine | 1.0 |
| Calculated composition |  |
| Metabolizable energy†, MJ/kg | 12.64 |
| Crude protein, g/kg | 200.0 |
| Calcium, g/kg | 9.9 |
| Nonphytate phosphorus, g/kg | 4.2 |
| Lysine, g/kg  | 11.0 |
| Methionine, g/kg | 4.7 |
| Methionine + cysteine, g/kg  | 8.2 |
| Threonine, g/kg  | 7.6 |
| Tryptophan, g/kg  | 2.2 |
| Arginine, g/kg  | 10.0 |
| Riboflavin‡, mg/kg | 1.38 |

\* Supplied per kilogram of total diet: Cu (CuSO4•5H2O), 10 mg; Fe (FeSO4•7H2O), 60 mg; Zn (ZnO), 60 mg; Mn (MnSO4•H2O), 80 mg; Se (NaSeO3), 0.3 mg; I (KI), 0.2 mg; choline chloride, 1000 mg; vitamin A (retinyl acetate), 10000 IU; vitamin D3 (Cholcalciferol), 3000 IU; vitamin E (DL-α-tocopheryl acetate), 20 IU; vitamin K3 (menadione sodium bisulfate), 2 mg; thiamin (thiamin mononitrate), 2 mg; pyridoxine hydrochloride, 4 mg; cobalamin, 0.02 mg; calcium-D-pantothenate, 20 mg; nicotinic acid, 50 mg; folic acid, 1 mg; biotin, 0.2 mg.

† The values are calculated according to the AME of chickens (Ministry of Agriculture of China, 2004).

‡ The amount was determined using high performance liquid chromatography.

Supplemental Table S2. Growth performance on day 21 of ducks in the riboflavin-deficient (RD), pair-fed (CPF), and *ad. libitum* control (CAL) groups from hatch to 21 days of age

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Variable  | RD | CPF | CAL | SEM | *P*-value |
| Mortality (%) | 9.38a | 0.00b  | 1.04b | - | 0.002 |
| ADG (g/d) | 15.1c | 17.2b | 60.7a | 3.57 | <0.001 |
| ADFI (g/d) | 29.4b | 29.1b | 100.3a | 5.71 | <0.001 |
| G:F / (g:g) | 0.51b | 0.59a | 0.61a | 0.008 | <0.001 |
| Relative liver weight \* | 4.47a | 3.51b | 3.36b | 0.15 | <0.001 |

ADG, average daily weight gain; ADFI, average daily feed intake; G:F, gain to feed ratio; and SEM, standard error of the mean.

a, b, c Mean values with unlike superscript letters were significantly different (*P* < 0.05). Data were analyzed by one-way ANOVA followed by Tukey’s multiple comparison. Data was expressed as mean and SEM (n=12).

\* Relative liver weight were calculated as liver weight:body weight (g/100 g).

Supplemental Table S3.Liver fatty acid composition (% of the total fatty acid) on day 21 of ducks in the riboflavin-deficient (RD), pair-fed (CPF), and *ad. libitum* control (CAL) groups

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| % of the total fatty acid | RD  | CPF | CAL | SEM | *P*-value |
| C6:0 | 0.133a | 0.090b | 0.048c | 0.012 | 0.001 |
| C8:0 | 0.013a | 0.013a | 0.010b | 0.001 | 0.026 |
| C10:0 | 0.020 | 0.021 | 0.019 | 0.001 | 0.755 |
| C12:0 | 0.041a | 0.032b | 0.029b | 0.002 | 0.016 |
| C14:0 | 0.372b | 0.472a | 0.403b | 0.017 | 0.025 |
| C14:1 | 0.044b | 0.069a | 0.049b | 0.004 | 0.001 |
| C15:0 | 0.041 | 0.038 | 0.022 | 0.004 | 0.052 |
| C16:0 | 25.2a | 23.9b | 21.5c | 0.472 | <0.001 |
| C16:1 | 1.14b | 1.07b | 1.40a | 0.059 | 0.037 |
| C17:0 | 0.116a | 0.125a | 0.094b | 0.005 | 0.011 |
| C18:0 | 24.7a | 21.2b | 17.3c | 1.01 | 0.001 |
| C18:1n9c | 22.3b | 19.2b | 29.7a | 1.64 | 0.008 |
| C18:2n6c | 6.90b | 9.98a | 7.31b | 0.431 | <0.001 |
| C18:3n3 | 0.139 | 0.136 | 0.099 | 0.021 | 0.732 |
| C20:0 | 0.222a | 0.227a | 0.175b | 0.008 | 0.003 |
| C20:1 | 0.384b | 0.310c | 0.453a | 0.021 | 0.002 |
| C21:0 | 1.08b | 1.33b | 2.19a | 0.151 | <0.001 |
| C20:2 | 0.031a | 0.030a | 0.021b | 0.002 | 0.029 |
| C20:3n6 | 1.00b | 1.51a | 1.40a | 0.083 | 0.012 |
| C20:4n6 | 13.7 | 17.3 | 15.2 | 0.679 | 0.082 |
| C20:3n3 | 0.052 | 0.039 | 0.047 | 0.005 | 0.653 |
| C20:5n3 | 0.076b | 0.121a | 0.135a | 0.009 | 0.002 |
| C22:0 | 0.376b | 0.514a | 0.345b | 0.024 | 0.0003 |
| C22:1n9 | 0.040 | 0.044 | 0.051 | 0.003 | 0.2492 |
| C23:0 | 0.243b | 0.392a | 0.263b | 0.023 | 0.001 |
| C24:0 | 0.490b | 0.765a | 0.512b | 0.041 | <0.001 |
| C22:6n3 | 0.742 | 0.659 | 0.664 | 0.040 | 0.679 |
| C24:1 | 0.484 | 0.484 | 0.473 | 0.018 | 0.964 |
| Total SFA | 53.0a | 49.1b | 43.0c | 1.35 | <0.001 |
| Total MUFA | 24.4b | 21.2b | 32.1a | 1.69 | 0.007 |
| Total PUFA | 22.7b | 29.8a | 24.9b | 1.14 | 0.013 |
| Total n-3 | 1.01 | 0.95 | 0.95 | 0.05 | 0.891 |
| Total n-6 | 21.6b | 28.8a | 23.9b | 1.12 | 0.008 |
| n-6:n-3 | 22.3b | 30.5a | 25.5ab | 1.30 | 0.016 |

SFA, saturated fatty acid; MUFA, mono-unsaturated fatty acid; PUFA, poly-unsaturated fatty acid.

a, b, c Mean values with unlike superscript letters were significantly different (P < 0.05). Data were analyzed by one-way ANOVA followed by Tukey’s multiple comparison. Data was expressed as mean and SEM (n=12).

Supplemental Table S4.Differentially expressed proteins in liver on day 21 of ducks caused by riboflavin deficiency (RD)

|  |  |  |  |
| --- | --- | --- | --- |
| Accession no. | Protein name | Gene name | Fold change\* |
| RD vs. CAL | RD vs. CPF | CPF vs. CAL |
| tr|R7VUZ3|R7VUZ3\_COLLI | Frataxin | FXN | -90.9 | -90.9 | -1.05 |
| tr|Q9DG15|Q9DG15\_CHICK | P95-APP1 | none | -90.9 | -90.9 | -1.10 |
| tr|R7VXW2|R7VXW2\_COLLI | Inositol polyphosphate 1-phosphatase | INPP1 | -83.3 | -90.9 | 2.33 |
| tr|Q38HW8|Q38HW8\_ANAPL | Thyroid hormone responsive Spot 14 alpha | THRSP | -62.5 | -71.4 | 1.24 |
| tr|U3JV22|U3JV22\_FICAL | Flotillin-2 | FLOT2 | -62.5 | -71.4 | 1.27 |
| tr|F1NAM7|F1NAM7\_CHICK | 3-hydroxybutyrate dehydrogenase type 2 | BDH2 | -23.8 | -23.3 | 1.43 |
| tr|U3I9X5|U3I9X5\_ANAPL | Annexin | ANXA7 | -16.4 | -76.9 | 4.66 |
| tr|U3J4D8|U3J4D8\_ANAPL | Prefoldin subunit 3 | VBP1 | -14.1 | -14.7 | -1.41 |
| tr|U3I0A3|U3I0A3\_ANAPL | Keratin, type II cytoskeletal 7 | KRT7 | -11.6 | -8.85 | -1.47 |
| tr|R0K082|R0K082\_ANAPL | NADH-ubiquinone oxidoreductase 75 kDa subunit | NDUFS1 | -11.4 | -11.1 | 1.15 |
| RRRRRtr|F1NSA7|F1NSA7\_CHICK | None  | LOC100857563 | -9.62 | -9.62 | -1.16 |
| tr|Q8AYP9|Q8AYP9\_CHICK | 6-phosphofructokinase | PFK | -8.00 | -9.26 | 1.13 |
| tr|U3I7F8|U3I7F8\_ANAPL | 39S ribosomal protein L21 | MRPL21 | -7.58 | -6.37 | 1.21 |
| tr|U3J1J0|U3J1J0\_ANAPL | Acyl-CoA dehydrogenase family member 9 | ACAD9 | -6.41 | -8.40 | 1.41 |
| tr|U3IL38|U3IL38\_ANAPL | Malic enzyme | ME1 | -6.02 | -8.70 | 1.36 |
| tr|U3IR48|U3IR48\_ANAPL | Dihydrolipoyl dehydrogenase | DLD | -5.71 | -8.26 | 1.47 |
| tr|U3J8W0|U3J8W0\_ANAPL | Short-chain specific acyl-CoA dehydrogenase | ACADS | -4.74 | -6.80 | 1.17 |
| tr|Q5ZJZ9|Q5ZJZ9\_CHICK | Protein arginine N-methyltransferase 5 | PRMT5 | -4.69 | -21.3 | 4.83 |
| tr|U3IHZ7|U3IHZ7\_ANAPL | Delta-aminolevulinic acid dehydratase | ALAD | -3.83 | -4.17 | 1.09 |
| tr|U3ITA9|U3ITA9\_ANAPL | Medium-chain specific acyl-CoA dehydrogenase | ACADM | -2.99 | -3.25 | 1.08 |
| tr|U3J3V1|U3J3V1\_ANAPL | Electron transfer flavoprotein-ubiquinone oxidoreductase | ETFDH | -2.88 | -3.19 | 1.06 |
| tr|U3JW36|U3JW36\_FICAL | Coactosin-like F-actin binding protein 1 | COTL1 | -2.83 | -7.81 | 3.08 |
| tr|U3J680|U3J680\_ANAPL | Caldesmon | CALD1 | -2.75 | -4.37 | 1.33 |
| tr|G1N071|G1N071\_MELGA | Malate dehydrogenase | MDH1 | -1.98 | -2.27 | -1.01 |
| tr|E1C958|E1C958\_CHICK | Legumain | LGMN | -1.98 | -1.75 | -1.13 |
| tr|U3IQB4|U3IQB4\_ANAPL | Hydroxyacid oxidase 2 | HAO2 | -1.92 | -1.60 | -1.21 |
| tr|U3J788|U3J788\_ANAPL | Alveolar soft part sarcoma chromosome region, candidate 1 | ASPSCR1 | -1.68 | -2.03 | 1.20 |
| sp|Q5ZML3|SRSF1\_CHICK | Serine/arginine-rich splicing factor 1 | SRSF1 | -1.68 | -1.82 | 1.08 |
| tr|U3ID88|U3ID88\_ANAPL | Collagen alpha-3(VI) chain | COL6A3 | -1.66 | -2.13 | 1.25 |
| tr|U3J5K2|U3J5K2\_ANAPL | NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8 | NDUFA8 | -1.64 | -3.40 | 2.03 |
| tr|U3IAF7|U3IAF7\_ANAPL | Copine-1 | CPNE1 | -1.63 | -1.71 | 1.12 |
| tr|U3INW1|U3INW1\_ANAPL | Sideroflexin-1 | SFXN1 | 1.51 | 1.94 | -1.32 |
| tr|U3IJZ6|U3IJZ6\_ANAPL | Fatty acid amide hydrolase | FAAH | 1.59 | 2.17 | -1.36 |
| tr|U3IL01|U3IL01\_ANAPL | Heme oxygenase 1 | HMOX1 | 1.68 | 1.94 | -1.22 |
| tr|U3J6P0|U3J6P0\_ANAPL | Complement component 3 | C3 | 1.79 | 6.86 | -3.83 |
| tr|U3IXZ9|U3IXZ9\_ANAPL | 4-aminobutyrate aminotransferase | ABAT | 1.79 | 2.54 | -1.46 |
| tr|U3J8L3|U3J8L3\_ANAPL | Solute carrier family 25 (aspartate/glutamate carrier), member 13 | SLC25A13 | 1.84 | 4.88 | -2.81 |
| tr|U3IKU2|U3IKU2\_ANAPL | Non-specific lipid-transfer protein | SCP2 | 1.92 | 2.23 | -1.13 |
| tr|U3IEH8|U3IEH8\_ANAPL | Acetyl-CoA acetyltransferase | ACAT2 | 2.00 | 1.60 | 1.26 |
| tr|U3IWA4|U3IWA4\_ANAPL | Apolipoprotein B-100 | APOB | 2.01 | 2.97 | -1.45 |
| tr|U3IZY1|U3IZY1\_ANAPL | Aldehyde dehydrogenase 7 family, member A1 | ALDH7A1 | 2.13 | 3.95 | -1.94 |
| tr|U3I3S5|U3I3S5\_ANAPL | Nicotinamide nucleotide transhydrogenase | NNT | 2.21 | 4.41 | -2.17 |
| tr|U3IU70|U3IU70\_ANAPL | Xanthine dehydrogenase/oxidase | XDH | 2.27 | 2.07 | 1.10 |
| tr|R0K9V0|R0K9V0\_ANAPL | Acetyl-coenzyme A synthetase | ACS | 2.31 | 4.61 | -2.13 |
| tr|R0JGG2|R0JGG2\_ANAPL | Hydroxymethylglutaryl-CoA synthase, cytoplasmic | HMGCS1 | 2.42 | 4.21 | -1.82 |
| tr|R0JWK3|R0JWK3\_ANAPL | Lipoamide acyltransferase component of branched-chain alpha-keto acid dehydrogenase complex | DBT | 2.42 | 1.60 | 1.41 |
| tr|F1NFC6|F1NFC6\_CHICK | 40S ribosomal protein S4, X isoform | RPS4X | 2.47 | 3.73 | -1.54 |
| tr|U3III1|U3III1\_ANAPL | Acyl-coenzyme A thioesterase 1 | ACOT1 | 2.61 | 4.17 | -1.66 |
| tr|U3I2B5|U3I2B5\_ANAPL | Solute carrier family 27 (fatty acid transporter), member 5 | SLC27A5 | 2.75 | 5.45 | -2.29 |
| tr|U3IE74|U3IE74\_ANAPL | L-lactate dehydrogenase | LDHA | 2.81 | 11.7 | -5.85 |
| tr|U3J3G1|U3J3G1\_ANAPL | Enoyl-CoA hydratase | ECHS1 | 2.83 | 2.78 | 1.05 |
| tr|R0JLG5|R0JLG5\_ANAPL | Calnexin | CANX | 3.13 | 6.61 | -2.15 |
| tr|U3I559|U3I559\_ANAPL | Microsomal triglyceride transfer protein | MTTP | 3.22 | 9.20 | -3.40 |
| tr|U3IU30|U3IU30\_ANAPL | Long-chain-fatty-acid--CoA ligase 1 | ACSL1 | 3.37 | 4.17 | -1.20 |
| tr|U3J4Z9|U3J4Z9\_ANAPL | Long-chain-fatty-acid--CoA ligase 5 | ACSL5 | 3.57 | 9.73 | -3.25 |
| tr|U3J7S6|U3J7S6\_ANAPL | None  | LOC101796596 | 4.17 | 3.60 | 1.09 |
| tr|U3J4X1|U3J4X1\_ANAPL | Heat shock protein 90kDa beta (Grp94), member 1 | HSP90B1 | 4.66 | 3.63 | 1.21 |
| tr|R0LYJ5|R0LYJ5\_ANAPL | Trifunctional purine biosynthetic protein adenosine-3 | GART | 5.06 | 4.37 | 1.13 |
| tr|R0KBX6|R0KBX6\_ANAPL | Protein disulfide-isomerase | PDIA4 | 8.24 | 10.1 | -1.02 |
| tr|H0ZD22|H0ZD22\_TAEGU | 39S ribosomal protein L38 | MRPL38 | 71.8 | 71.8 | -1.01 |
| RRRRRtr|R4GKG1|R4GKG1\_CHICK | TANK-binding kinase 1-binding protein 1 | TBKBP1 | 80.9 | 80.9 | -1.01 |
| tr|H0ZF88|H0ZF88\_TAEGU | Acyl-CoA synthetase family member 2 | ACSF2 | 86.3 | 86.3 | -1.01 |
| RRRRRtr|U3IQK5|U3IQK5\_ANAPL | Ski oncogene | SKI | 87.9 | 87.9 | -1.01 |

CPF: pair-fed; CAL: *ad. libitum* control

\* Fold change are expressed as the ratio between two of three groups. For diminished proteins, the fold change was transformed to the corresponding negative value.

Supplemental Table S5. Enriched GO (biological processes)-based sets of differentially expressed proteins in the liver of starter Pekin ducks in response to riboflavin deficiency

|  |  |  |  |
| --- | --- | --- | --- |
| GO term | GO accession | *P* value | Count |
| **Lipid metabolism**  |  |  |  |
| fatty acid metabolic process | GO:0006631 | 17.0E-15 | 17 |
| fatty acid oxidation | GO:0019395 | 2.7E-9 | 8 |
| acylglycerol metabolic process | GO:0006639 | 8.3E-9 | 9 |
| cellular lipid catabolic process | GO:0044242 | 28.0E-9 | 9 |
| lipid modification | GO:0030258 | 49.0E-9 | 9 |
| acylglycerol biosynthetic process | GO:0046463 | 750.0E-9 | 6 |
| long-chain fatty acid metabolic process | GO:0001676 | 1.8E-6 | 6 |
| fatty-acyl-CoA metabolic process | GO:0035337 | 31.0E-6 | 4 |
| **Cellular respiration** |  |  |  |
| cellular respiration | GO:0045333 | 62.0E-9 | 9 |
| respiratory electron transport chain | GO:0022904 | 9.8E-6 | 6 |
| aerobic respiration | GO:0009060 | 110.0E-6 | 4 |
| oxidative phosphorylation | GO:0006119 | 360.0E-6 | 4 |
| mitochondrial electron transport, NADH to ubiquinone | GO:0006120 | 610.0E-6 | 3 |
| mitochondrial respiratory chain complex I assembly | GO:0032981 | 1.0E-3 | 3 |
| **Other processes** |  |  |  |
| carboxylic acid catabolic process | GO:0046395 | 69.0E-12 | 12 |
| cofactor biosynthetic process | GO:0051188 | 360.0E-12 | 10 |
| coenzyme biosynthetic process | GO:0009108 | 630.0E-9 | 7 |
| heme metabolic process | GO:0042168 | 4.7E-6 | 4 |

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Supplemental Fig. S1.Plasma riboflavin concentrations (A) and liver riboflavin, flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD) concentrations (B) in 21-day-old ducks from the riboflavin-deficient (RD), pair-fed (CPF), and *ad. libitum* control (CAL) groups, respectively.Data are means ± SEM (n=12).Mean values with unlike superscript letters were significantly different (*P* < 0.05). Data were analyzed by one-way ANOVA followed by Tukey’s multiple comparison.