**Table S1. The gene primers sequences for quantitative real-time reverse-transcription PCR.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **Accession Number** | **Primers Sequences（5’-3’）** | **Primer Length** |
| *β-Actin* | ENSGALG00000009621 | F: GAACCCCAAAGCCAACAG | 182 bp |
| R: GGGCGTAGCCTTCATAGA |
| *MGST1* | ENSGALG00000013098 | F: GGCATTTGCCAACCCAGAAG | 199 bp |
| R: CCTAAAATGCAGCAAGGCCG |
| *MGST2* | ENSGALG00000009803 | F:CGTGTCTCTGCTTTCTGTCCT | 197 bp |
| R:GATTGCAAAACCATCCTGCGA |
| *NQO1* | ENSGALG00000023437 | F:CGCCGAGCAGAAGAAGATTG | 190 bp |
| R:GGTGGTGAGTGACAGCATGG |
| *ABCG5* | ENSGALG00000009955 | F:CCTCGCTCAGAACTCTTCAGG | 196 bp |
| R:CGAGTTCACGTTCCTTGCTTC |
| *ABCG8* | ENSGALG00000009958 | F:GGTAAAAGAGCCCAGGACAGTA | 182 bp |
| R:CCTGAGTTCCAGGGCAATAG |
| *F2R* | ENSGALG00000014983 | F:GACCCGTTTTGTTCCTTCGG | 217 bp |
| R:CCGAGGTCCAAATACCCAGTT |
| *FA2H* | ENSGALG00000002775 | F:AAGTACGACGAGTGGGTGC | 214 bp |
| R:AGTATTTGTGCACGGGGATG |

**Table S2.** **Egg quality** **parameters, eggshell calcium, phosphorus, ash content and the chicken plasma biochemical indexes.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Index** | **Items** | **Control C** | **Control T** | ***P*-value** |
| Egg quality | Egg weight/g | 44.97±3.73 | 45.71±3.56 | 0.12 |
| Eggshell index | 1.42±1.10 | 1.31±0.05 | 0.31 |
| Eggshell color | 9.25±1.96 | 8.61±1.94 | 0.51 |
| Egg yolk ratio/% | 34.42±2.27 | 34.42±2.47 | 0.98 |
| Albumen height/mm | 4.13±0.71 | 4.10±0.92 | 0.80 |
| Harrington unit | 67.71±6.09 | 66.85±7.65 | 0.34 |
| Eggshell strength/(N/cm2) | 43.61±9.06 | 43.28±10.35 | 0.80 |
| Calcium, Phosphorus and  ash content in eggshell | Calcium/% | 33.78±1.23 | 34.86±0.90 | 0.50 |
| Phosphorus/% | 0.14±0.007 | 0.13±0.006 | 0.40 |
| Ash content | 90.44±2.51 | 87.82±1.47 | 0.39 |
| Plasma biochemical indexes | T-AOC/(U/ml) | 10.34±2.43 | 9.98±1.04 | 0.77 |
| CAT（U/ml） | 9.96±0.65 | 7.82±0.64 | 0.07 |
| GSH-PX | 884.23±27.36 | 836.39±38.58 | 0.39 |
| SOD（U/ml） | 56.49±1.24 | 53.79±2.30 | 0.37 |
| TG（mmol/L） | 26.57±2.19 | 28.50±1.44 | 0.50 |

**Note:** T-AOC: total antioxidant capacity; CAT: catalase; GSH-PX: glutathione peroxidase; SOD: superoxide dismutase; TG: triglyceride; Ca: Calcium

**Table S3. Small intestinal morphology and structure, enzyme activity and antioxidant capacity index.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Tissue** | **Intestinal** | **Items** | **Group C (n = 10)** | **Group T (n = 10)** | ***P*-value** |
| Duodenum | Morphometry | Villus length | 951.67±79.36 | 765.65±39.87 | 0.07 |
| Crypt depth | 202.90±18.80 | 301.45±26.41 | 0.16 |
| Digestive enzymes | Diastase (U/gprot) | 545.91±107.18 | 533.09±82.34 | 0.79 |
| Lipase (U/gprot) | 5.84±1.31 | 5.79±1.15 | 0.93 |
| Energy metabolism index | Na+K+-ATPase (U/mgprot) | 1.61±0.72 | 1.26±0.37 | 0.22 |
| Ca2+Mg2+-ATPase (U/mgprot) | 1.20±0.22 | 1.11±0.15 | 0.37 |
| Antioxidant capacity index | T-AOC (mmol/gprot) | 21.38±3.29 | 20.22±2.27 | 0.47 |
| CAT (U/mgprot) | 10.59±0.63 | 10.31±2.70 | 0.82 |
| SOD (U/mgprot) | 65.62±6.55 | 62.46±7.41 | 0.38 |
| Jejunum | Morphometry | Villus length | 1051.83±89.04 | 1058.08±62.40 | 0.96 |
| Crypt depth | 175.12±7.03 | 229.96±30.42 | 0.12 |
| Villus length/Crypt depth | 6.00±0.45 | 4.97±0.80 | 0.29 |
| Digestive enzymes | Diastase (U/gprot) | 546.05±84.69 | 543.41±96.11 | 0.95 |
| Chymotrypsin (U/mgprot) | 7.28±1.18 | 7.23±1.14 | 0.94 |
| Energy metabolism index | Ca2+Mg2+-ATPase (U/mgprot) | 1.12±0.09 | 1.05±0.17 | 0.26 |
| T-ATPase (U/mgprot) | 10.23±1.04 | 10.57±0.94 | 0.54 |
| AKP (U/mgprot) | 5.47±0.72 | 5.55±0.69 | 0.84 |
| SDH (U/mgprot) | 7.84±2.12 | 7.44±1.36 | 0.66 |
| Antioxidant capacity index | CAT (U/mgprot) | 9.96±1.88 | 8.78±1.20 | 0.19 |
| SOD (U/mgprot) | 56.17±4.28 | 56.40±3.78 | 0.91 |
| MDA (nmol/mgprot) | 2.31±0.17 | 2.30±0.35 | 0.93 |
| Ileum | Morphometry | Villus length | 820.72±105.64 | 706.51±68.50 | 0.42 |
| Crypt depth | 158.29±12.85 | 207.19±36.47 | 0.21 |
| Villus length/Crypt depth | 5.27±0.78 | 3.63±0.54 | 0.15 |
| Digestive enzymes | Chymotrypsin (U/mgprot) | 7.08±1.23 | 7.05±1.00 | 0.94 |
| Lipase (U/gprot) | 5.54±1.04 | 5.43±1.02 | 0.83 |
| Energy metabolism index | Na+K+-ATPase (U/mgprot) | 1.02±0.05 | 1.04±0.24 | 0.51 |
| T-ATPase (U/mgprot) | 10.23±1.90 | 9.85±1.22 | 0.62 |
| AKP (U/mgprot) | 5.43±0.68 | 5.23±0.80 | 0.59 |
| SDH (U/mgprot) | 7.18±0.70 | 6.86±0.98 | 0.46 |
| Antioxidant capacity index | T-AOC (mmol/gprot) | 21.13±2.78 | 19.69±2.34 | 0.32 |
| CAT (U/mgprot) | 10.28±1.99 | 10.09±3.09 | 0.88 |
| GSH-Px (umol/gprot) | 52.23±6.07 | 52.16±2.95 | 0.97 |
| SOD (U/mgprot) | 54.19±6.11 | 54.27±4.73 | 0.98 |
| MDA (nmol/mgprot) | 1.93±0.48 | 2.08±0.26 | 0.43 |

**Note:** AKP: alkaline phosphatase; Ca2+Mg2+-ATPase: calcium and magnesium ATPase; CAT, catalase; GSH-Px: glutathione peroxidase; MDA: malondialdehyde; Na+K+-ATPase: natrium potassium ATPase; SDH: succinate dehydrogenase; SOD, superoxide dismutase; T-ATPase: total antioxidant capacity; T-AOC: total antioxidant capacity.

**Table S4. Overview of sequencing data quality control**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **Raw reads** | **Base** | **Clean reads** | **Base** | **Ratio%** | **Q20%** | **Q30%** | **GC%** |
| N\_1 | 49847702 | 7.48G | 46829816 | 7.02G | 93.95 | 99.88 | 97.55 | 52.00 |
| N\_2 | 49817198 | 7.47G | 45640464 | 6.85G | 91.62 | 99.88 | 97.69 | 50.50 |
| N\_3 | 54963066 | 8.24G | 52062936 | 7.81G | 94.72 | 99.84 | 96.75 | 50.00 |
| N\_4 | 53877008 | 8.08G | 48664492 | 7.30G | 90.33 | 99.88 | 97.69 | 51.00 |
| N\_5 | 55070252 | 8.26G | 47844996 | 7.18G | 86.88 | 99.85 | 97.54 | 51.00 |
| N\_6 | 50712066 | 7.61G | 41549494 | 6.23G | 81.93 | 99.86 | 97.58 | 51.00 |
| N\_7 | 52804110 | 7.92G | 46274100 | 6.94G | 87.63 | 99.88 | 97.70 | 51.00 |
| T\_1 | 45971970 | 6.90G | 42863576 | 6.43G | 93.24 | 99.87 | 97.47 | 51.50 |
| T\_2 | 55668976 | 8.35G | 52293940 | 7.84G | 93.94 | 99.87 | 97.73 | 51.00 |
| T\_3 | 41669192 | 6.25G | 39217052 | 5.88G | 94.12 | 99.89 | 97.66 | 51.00 |
| T\_4 | 44604480 | 6.69G | 41117192 | 6.17G | 92.18 | 99.87 | 97.71 | 51.00 |
| T\_5 | 42107860 | 6.32G | 39271816 | 5.89G | 93.26 | 99.86 | 97.54 | 51.50 |
| T\_6 | 40786044 | 6.12G | 38508396 | 5.78G | 94.42 | 99.88 | 97.63 | 51.00 |
| T\_7 | 53127114 | 7.97G | 50224626 | 7.53G | 94.54 | 99.85 | 97.65 | 51.50 |
| T\_8 | 53419078 | 8.01G | 45604422 | 6.84G | 85.37 | 99.85 | 97.45 | 52.00 |
| T\_9 | 52967082 | 7.95G | 47086888 | 7.06G | 88.90 | 99.85 | 97.57 | 51.00 |

**Table S5. Overview of sequencing data mapping rate to the reference genome**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | VR | MR | UMR | MMR | PEMR | RMS | RMA | NSR | SR |
| **N\_1** | 46829816(100%) | 37684595(80.47%) | 28855369(61.62%) | 8829226(18.85%) | 34567214(73.81%) | 18341508(39.17%) | 18597193(39.71%) | 26970470(57.59%) | 9968231(21.29%) |
| **N\_2** | 45640464(100%) | 41162836(90.19%) | 30921063(67.75%) | 10241773(22.44%) | 37975384(83.21%) | 20039273(43.91%) | 20118271(44.08%) | 31118346(68.18%) | 9039198(19.81%) |
| **N\_3** | 52062936(100%) | 44869210(86.18%) | 34050093(65.40%) | 10819117(20.78%) | 41328168(79.38%) | 21797052(41.87%) | 21897401(42.06%) | 34045594(65.39%) | 9648859(18.53%) |
| **N\_4** | 48664492(100%) | 43137008(88.64%) | 32541593(66.87%) | 10595415(21.77%) | 39790856(81.77%) | 21011916(43.18%) | 21129303(43.42%) | 32702674(67.20%) | 9438545(19.40%) |
| **N\_5** | 47844996(100%) | 40919172(85.52%) | 31551378(65.94%) | 9367794(19.58%) | 37523254(78.43%) | 19885238(41.56%) | 20030911(41.87%) | 30028077(62.76%) | 9888072(20.67%) |
| **N\_6** | 41549494(100%) | 37400906(90.02%) | 28784165(69.28%) | 8616741(20.74%) | 34404280(82.80%) | 18290135(44.02%) | 18373201(44.22%) | 28934141(69.64%) | 7729195(18.60%) |
| **N\_7** | 46274100(100%) | 40431635(87.37%) | 30556391(66.03%) | 9875244(21.34%) | 37338652(80.69%) | 19717636(42.61%) | 19834265(42.86%) | 29715607(64.22%) | 9836294(21.26%) |
| **T\_1** | 42863576(100%) | 35448064(82.70%) | 27207021(63.47%) | 8241043(19.23%) | 32629922(76.13%) | 17272881(40.30%) | 17352191(40.48%) | 25668030(59.88%) | 8957042(20.90%) |
| **T\_2** | 52293940(100%) | 45427057(86.87%) | 34184434(65.37%) | 11242623(21.50%) | 41974444(80.27%) | 22155841(42.37%) | 22239519(42.53%) | 33646889(64.34%) | 10748471(20.55%) |
| **T\_3** | 39217052(100%) | 34466843(87.89%) | 26094925(66.54%) | 8371918(21.35%) | 32071250(81.78%) | 16923480(43.15%) | 16951146(43.22%) | 25463257(64.93%) | 8411369(21.45%) |
| **T\_4** | 41117192(100%) | 35330798(85.93%) | 26717367(64.98%) | 8613431(20.95%) | 32426970(78.86%) | 17212291(41.86%) | 17308546(42.10%) | 27048775(65.78%) | 7472062(18.17%) |
| **T\_5** | 39271816(100%) | 33343076(84.90%) | 24854473(63.29%) | 8488603(21.61%) | 30887646(78.65%) | 16257624(41.40%) | 16337901(41.60%) | 24331899(61.96%) | 8263626(21.04%) |
| **T\_6** | 38508396(100%) | 32268609(83.80%) | 24715368(64.18%) | 7553241(19.61%) | 29812084(77.42%) | 15775380(40.97%) | 15831327(41.11%) | 22104273(57.40%) | 9502434(24.68%) |
| **T\_7** | 50224626(100%) | 43165945(85.95%) | 31557937(62.83%) | 11608008(23.11%) | 39900622(79.44%) | 20867050(41.55%) | 21024382(41.86%) | 31510881(62.74%) | 10380551(20.67%) |
| **T\_8** | 45604422(100%) | 38646459(84.74%) | 29536570(64.77%) | 9109889(19.98%) | 35513042(77.87%) | 18860600(41.36%) | 18932458(41.51%) | 28406751(62.29%) | 9386307(20.58%) |
| **T\_9** | 47086888(100%) | 41680721(88.52%) | 31239775(66.34%) | 10440946(22.17%) | 38068820(80.85%) | 20148115(42.79%) | 20297174(43.11%) | 31348685(66.58%) | 9096604(19.32%) |

**Note**: VR: Valid reads; MR: Mapped reads; UMR: Unique Mapped reads; MMR: Multi Mapped reads; PEMR: PE Mapped reads; RMSS: Reads map to sense strand; RMA: Reads map to antisense strand; NSR: Non-splice reads; SR: Splice reads

**Table S6 Statistical interval distribution of FPKM value in samples.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **0-0.1 FI** | **0.1-0.3 FI** | **0.3-3.57 FI** | **3.57-15 FI** | **15-60 FI** | **>60 FI** |
| N\_1 | 8083(33.81%) | 1309(5.47%) | 5346(22.36%) | 5723(23.94%) | 2757(11.53%) | 692(2.89%) |
| N\_2 | 8547(35.75%) | 1337(5.59%) | 5580(23.34%) | 5179(21.66%) | 2587(10.82%) | 680(2.84%) |
| N\_3 | 8170(34.17%) | 1304(5.45%) | 5139(21.49%) | 5570(23.30%) | 3041(12.72%) | 686(2.87%) |
| N\_4 | 8356(34.95%) | 1317(5.51%) | 6034(25.24%) | 5242(21.92%) | 2309(9.66%) | 652(2.73%) |
| N\_5 | 8570(35.84%) | 1306(5.46%) | 5279(22.08%) | 5299(22.16%) | 2710(11.33%) | 746(3.12%) |
| N\_6 | 8617(36.04%) | 1331(5.57%) | 5746(24.03%) | 5107(21.36%) | 2453(10.26%) | 656(2.74%) |
| N\_7 | 8505(35.57%) | 1271(5.32%) | 5324(22.27%) | 5334(22.31%) | 2742(11.47%) | 734(3.07%) |
| T\_1 | 8773(36.69%) | 1361(5.69%) | 5348(22.37%) | 4933(20.63%) | 2659(11.12%) | 836(3.50%) |
| T\_2 | 8255(34.53%) | 1354(5.66%) | 5487(22.95%) | 5310(22.21%) | 2696(11.28%) | 808(3.38%) |
| T\_3 | 8345(34.90%) | 1249(5.22%) | 5068(21.20%) | 5520(23.09%) | 2940(12.30%) | 788(3.30%) |
| T\_4 | 8537(35.70%) | 1374(5.75%) | 5689(23.79%) | 5257(21.99%) | 2432(10.17%) | 621(2.60%) |
| T\_5 | 8348(34.91%) | 1259(5.27%) | 5105(21.35%) | 5374(22.48%) | 3021(12.63%) | 803(3.36%) |
| T\_6 | 8563(35.81%) | 1253(5.24%) | 4902(20.50%) | 5087(21.28%) | 3074(12.86%) | 1031(4.31%) |
| T\_7 | 8350(34.92%) | 1315(5.50%) | 5058(21.15%) | 5396(22.57%) | 3032(12.68%) | 759(3.17%) |
| T\_8 | 8601(35.97%) | 1262(5.28%) | 5200(21.75%) | 5191(21.71%) | 2854(11.94%) | 802(3.35%) |
| T\_9 | 8612(36.02%) | 1370(5.73%) | 5460(22.84%) | 5204(21.76%) | 2591(10.84%) | 673(2.81%) |