

Article

Integrated Proteomics and Metabolomics Reveal Molecular Differences and Regulatory Mechanisms Underlying Sturgeon Egg Quality

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Abstract

Integrated proteomics and untargeted metabolomics were employed to systematically characterize molecular differences between *Acipenser schrenckii* embryos with normal versus abnormal 24 h post-fertilization (24 hpf), which were classified as high-quality (HQ) and poor-quality (PQ) groups. Among 1636 proteins and 1102 metabolites, 220 differentially expressed proteins (DEPs) and 365 differential metabolites (DMs) were identified. Functional enrichment demonstrated that HQ samples were predominantly enriched in pathways associated with amino acid biosynthesis, glycolysis/tricarboxylic acid cycle, nucleotide metabolism, and mRNA surveillance, which collectively supported material accumulation, energy supply, and embryonic developmental competence. In contrast, PQ samples were mainly enriched in oxidative phosphorylation, mitochondrial stress response, arachidonic acid metabolism, and immune and inflammatory signaling pathways, indicating severe lipid metabolic disorders and excessive oxidative stress. Spearman correlation analysis identified L-pyroglutamic acid and ascorbic acid as core correlated metabolic hubs associated with high-quality eggs, while natamycin and tetrahydrocorticosterone were correlated characteristic metabolic signatures enriched in deteriorated eggs. Key protein nodes showing strong correlations with 24 hpf developmental phenotypes included GAPDH, glutathione S-transferase, and CYP450 2E1. Collectively, this study reveals divergent correlated molecular profiles linked to normal versus abnormal 24 hpf development and screens correlative multi-omics candidate signatures for distinguishing well-developed and deteriorated oocytes.

Keywords: sturgeon; egg quality; proteomics; metabolomics; molecular mechanism; biomarkers



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Key Contribution: This study provides the first integrated proteomic and metabolomic characterization of sturgeon egg quality. Exploratory analyses indicate that normal embryonic developmental competence is closely associated with robust energy and amino acid metabolism, whereas diminished egg quality is linked to mitochondrial dysfunction and pronounced oxidative stress. Furthermore, it identifies correlated molecular signatures consisting of the ascorbic acid—CYP2E1 pair and the L-pyroglutamic acid—glutathione S-transferase interaction as putative molecular correlates of oocyte viability and redox homeostasis.

1. Introduction

Sturgeon, as a “living fossil” in the aquatic ecosystem, has important economic and ecological value. China, the world’s largest producer and exporter of sturgeon caviar, accounts for 70% of the global caviar output, and sturgeon aquaculture has become an important pillar of the high-value aquatic product industry [1,2]. Nevertheless, alongside the rapid expansion of commercial sturgeon aquaculture, poor egg quality and the consequent scarcity of viable fry have emerged as critical bottlenecks impeding the sustainable development of this sector [3]. Poor-quality eggs are often accompanied by low fertilization and hatching rates, which not only reduce breeding efficiency and economic benefits, but also hinder the conservation of endangered sturgeon species and the standardized development of the industry [4].

Egg quality is a comprehensive trait regulated by multiple factors, including environmental conditions, feeding management, parental physiological status, and genetic background [5]. Currently, the evaluation of sturgeon egg quality mostly relies on traditional morphological and physiological indicators, such as egg size, color, water content, and enzyme activity [3,6]. However, these indicators lack uniformity and specificity, and evaluation standards vary across studies, making it difficult to compare and integrate research results. Furthermore, they fail to accurately reflect the intrinsic quality of eggs at the molecular level. Despite the recognized critical importance of egg quality, the underlying molecular mechanisms governing egg quality remain poorly understood, and standardized evaluation criteria are lacking. This gap severely limits the development of effective technical measures to improve egg quality.

As the main nutrient component of sturgeon eggs (accounting for approximately 24.75% of the egg composition), proteins play a crucial role in the entire process of oocyte maturation, fertilization, and early embryonic development [2,6]. They not only provide essential nutrients and energy for embryonic development, but also participate in signal transduction, cell division, and stress response, directly affecting the hatching rates of eggs [7]. Previous proteomic studies on sturgeon eggs have identified certain proteins (e.g., phosvitin and collagen) that are related to egg quality, finding that the degradation and abnormal expression of these proteins are closely associated with a decline in oocyte viability [2]. Comparative proteomic analysis of Siberian sturgeon ovarian fluid and eggs has also revealed that egg proteins are primarily enriched in developmental metabolic pathways, such as oxidative phosphorylation and fatty acid metabolism [8]. However, these studies have either focused on caviar degradation during storage or analyzed only a single species. Therefore, they lack systematic comparisons between high- and low-quality eggs and fail to clarify the key proteins and regulatory pathways that determine egg quality.

Metabolomic profiling, a core component of systems biology, characterizes changes in small-molecule metabolites that reflect the ultimate outcomes of gene expression and protein regulation. Such an approach has unique advantages in exploring the molecular mechanism of egg quality formation [9]. Indeed, a recent metabolomic study on sturgeon caviar identified palmitic acid and cholesterol as specific molecular markers, and established that pathways such as glycerophospholipid and arachidonic acid metabolism are closely related to sturgeon egg development [10]. However, current sturgeon egg quality studies rely merely on single-omics analysis, which cannot fully elucidate the hierarchical regulatory relationships between upstream protein variation and downstream metabolic reprogramming. Integrated proteomic and metabolomic analysis enables the elucidation of the core regulatory cascade linking protein expression changes to metabolic phenotypic alterations. This multi-layered molecular evidence can further decode the key drivers governing egg developmental competence, thereby providing more systematic and in-depth mechanistic insights into sturgeon egg quality regulation [6,11].

In addition to this methodological gap, existing studies on sturgeon egg quality exhibit other notable deficiencies. Most previous studies have focused on individual biochemical indices or routine compositional detection, failing to correlate molecular profiles with core phenotypic indicators, including fertilization and hatching rates. Furthermore, the molecular regulatory mechanisms underlying sturgeon egg developmental competence have not been fully elucidated, and few studies have integrated the proteomic and metabolomic landscapes to comprehensively explore the key molecules and pathways governing oocyte developmental potential. As documented in the classic monograph on sturgeon developmental biology, the 24 hpf gastrula stage serves as a critical developmental checkpoint that determines subsequent hatching success in acipenserid species including *A. stellatus*, *A. gueldenstaedtii* and *H. huso* [12]. Accordingly, this study performed comparative proteomic and metabolomic analyses of sturgeon eggs with distinct quality differences, strictly stratified by morphological criteria at the 24 hpf gastrula stage into normal development and developmentally arrested groups. The study aims to screen quality-related differential proteins and metabolites, dissecting key biological pathways and regulatory networks governing oocyte developmental competence, and constructing a phenotypic biomarker panel to evaluate sturgeon egg quality. The findings of this study provide a theoretical framework for sturgeon reproductive physiology and critical technical support for the high-quality and sustainable development of the sturgeon breeding industry.

2. Materials and Methods

2.1. Experimental Samples

All experimental fish were sourced from the Heilongjiang Institute of Aquatic Research, Chinese Academy of Fisheries Sciences, located in Harbin, Heilongjiang Province, China. Single female and male sturgeons were artificially fertilized to eliminate genetic variation. Fertilized eggs were incubated at 16 ± 0.5 °C. At 24 h post-fertilization (gastrula stage), eggs were examined microscopically and classified as either high-quality (HQ) and poor-quality (PQ), according to sturgeon embryonic development standards reported in previous studies [6,13]. HQ and PQ samples were classified at 24 hpf based on gastrula-stage morphology (Figure S1, Table S1). In HQ samples, embryos developed normally to the gastrula stage with regular cell division, an intact blastoderm, clear polarization, and high elasticity. Conversely, PQ samples exhibited abnormal or arrested development by the 24 h mark, demonstrating a blurred blastoderm, asynchronous cleavage, opacity, softening, or degeneration. Three full-sibling female Siberian sturgeon with identical parentage, reared in the same tank and synchronously mature, were selected as three independent biological replicates. Each female was artificially inseminated separately with a single mature male Siberian sturgeon to establish three independent spawning batches. At 24 hpf (gastrula stage), oocytes were classified into HQ and PQ groups based on standard morphological criteria. Two HQ subsamples and two PQ subsamples were randomly collected from each maternal batch, resulting in six HQ samples and six PQ samples for subsequent proteomic analysis. Samples were snap-frozen in liquid nitrogen and stored at -80 °C until analysis.

2.2. Label-Free Quantitative Proteomic Analysis

2.2.1. Protein Extraction and Digestion

Frozen egg samples were first ground to a fine powder under liquid nitrogen, and approximately 100 mg of the homogenate was transferred to a centrifuge tube containing lysis buffer (8 M urea, 1% SDS, 50 mM Tris-HCl, pH 8.0, supplemented with $1 \times$ protease inhibitor cocktail and $1 \times$ phosphatase inhibitor cocktail). The mixture was vortexed vigorously for 30 s, followed by sonication on ice to achieve complete cell lysis. Following sonication, the lysate was centrifuged at 12,000 rpm for 20 min at 4 °C to remove insoluble

cell debris. The supernatant was collected, and total protein concentration was quantified using a BCA Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA). Subsequently, the extracted proteins were digested into peptides using trypsin following the standard filter-aided sample preparation (FASP) protocol described by Wiśniewski et al. [14]. The resulting peptides were desalted using Empore C18 SPE cartridges (CDS Analytical, Oxford, PA, USA), with three consecutive rinses using a washing solution composed of 0.1% formic acid and 3% acetonitrile. Peptide concentrations were subsequently measured using a Pierce Quantitative Colorimetric Peptide Assay (Thermo Fisher Scientific, Waltham, MA, USA).

2.2.2. LC-MS/MS Analysis

The peptides were analyzed using an EASY-nLC 1200 UHPLC system (Thermo Fisher, Schwerte, Germany) coupled with a Q Exactive HF-X mass spectrometer (Thermo Fisher, Germany) at Novogene Co., Ltd. (Beijing, China), as previously described [14]. The sample was fractionated using a C18 column (Waters BEHC18, 4.6×250 mm, $5 \mu\text{m}$; Waters Corporation, Milford, MA, USA) on a Rigol L3000 HPLC system. Full MS scans were acquired at m/z 350–1500 (resolution 60,000). The top 40 precursors were fragmented using the HCD for MS/MS (resolution 15,000; Thermo Fisher Scientific, Waltham, MA, USA).

Raw data obtained from mass spectrometry analysis were analyzed using MaxQuant 1.6.14 software for library identification and quantitative analysis. The screening criteria for confident peptides and confident proteins were both the false discovery rate (FDR) ≤ 0.01 . Proteins containing at least two unique peptides were considered confident proteins and were included in subsequent quantitative analysis. The label-free quantification (LFQ) algorithm embedded in MaxQuant software adopted a median centering normalization strategy to correct for systematic biases, including variations in sample loading amount and instrument response drift. Match between runs function in MaxQuant was enabled to reduce missing values at the source. For the remaining missing values of retained proteins, left-truncated normal distribution imputation was performed. For differential abundance analysis, the non-parametric Mann–Whitney U test was performed on a per-protein basis to generate raw p -values. Feature-level multiple-testing correction was then applied across all quantified proteins using the Benjamini–Hochberg (BH) procedure to control FDR. Proteins with an adjusted p -value (FDR) < 0.05 were defined as differentially abundant proteins. Enrichment analysis of differentially expressed proteins (annotated based on KEGG Orthology) was conducted using the R package clusterProfiler (v4.12.0; FDR < 0.1).

2.3. Untargeted Metabolomic Analysis (LC-MS/MS)

2.3.1. Metabolite Extraction

Eighty milligrams of frozen sample were accurately weighed into a sterile EP tube, combined with 400 μL of pre-chilled 80% methanol solution, and thoroughly ground. After high-speed centrifugation, the supernatant was collected and lyophilized into dry powder. The powder was dissolved in acetonitrile for subsequent experiments.

2.3.2. UHPLC-MS/MS Analysis

Chromatographic separation was carried out using a Vanquish Flex ultra-high-performance liquid chromatography (UPLC) system (Thermo Fisher Scientific, Waltham, MA, USA) fitted with a Hypersil Gold reversed-phase column (100×2.1 mm, $1.8 \mu\text{m}$ particle size; Thermo Fisher Scientific, Waltham, MA, USA). Chromatographic parameters were set as follows: flow rate of 0.5 mL/min, injection volume of 2 μL , and a binary mobile phase system comprising solvent A (aqueous solution containing 25 mM ammonium acetate and 25 mM ammonia) and solvent B (acetonitrile). Mass spectrometric detection was performed on a Q Exactive HF mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) operating in both positive and negative ionization modes. Key instrument

parameters were optimized as: spray voltage, 3.5 kV; capillary temperature, 320 °C; sheath gas flow rate, 35 psi; auxiliary gas flow rate, 10 L/min; S-lens RF level, 60; and auxiliary gas heater temperature, 350 °C. Raw mass spectrometry data were processed using Compound Discoverer 3.3 software for peak alignment, relative quantification, and metabolite annotation against the mzCloud, HMDB, LIPIDMaps, and KEGG databases, as described in our previous work [15]. For metabolites with available reference standards, identification was achieved through triple confirmation consisting of retention time matching, precursor ion mass accuracy, and tandem mass spectral matching, corresponding to MSI Level 1. For the remaining metabolites, putative annotation was performed primarily based on tandem mass spectral matching, corresponding to MSI Level 2. A forward match score (dot product) of ≥ 0.7 was set as the confidence threshold for MS/MS spectral matching, and the precursor mass tolerance was strictly controlled within 5 ppm. Orthogonal partial least squares discriminant analysis (OPLS-DA) was constructed to visualize the metabolic profile differences between the two groups. Model evaluation parameters (R^2 , Q^2) were obtained via 7-fold cross-validation, and a permutation test with 200 iterations was performed to verify model reliability and rule out overfitting.

2.4. Multi-Omics Integration Analysis

The global correlation between metabolomic and proteomic datasets was evaluated using Pearson correlation analysis based on Euclidean distance metrics, and the statistical significance of these correlations was assessed via the Mantel test. Weighted correlation network analysis (WGCNA) was employed to construct a co-expression network of integrated proteins and metabolites associated with egg quality. Spearman's rank correlation test was performed between each module eigengene and the egg quality phenotypic trait, and modules with a correlation p -value < 0.16 were retained as phenotype-related modules. Subsequently, integrated pathway enrichment analysis was conducted using MetaboAnalyst 5.0 to identify key biological pathways associated with egg quality, based on the identified differentially expressed proteins and metabolites.

3. Results

3.1. LC-MS/MS Identification of High-Quality and Low-Quality Egg Proteins

Mass spectrometric raw data were searched against the database using the MaxQuant software. A total of 989,019 MS/MS spectra were acquired, of which 69,206 were effectively matched, yielding a spectral matching rate of 6.99% (Table 1). Based on the matched spectra, 7955 non-redundant peptides were identified, corresponding to 1636 annotated proteins. Analysis of the peptide length distribution showed that the identified peptides were primarily distributed between 7 and 20 amino acids, with the highest abundance observed for peptides of 12 amino acids (peak at 8590 sequences, Figure 1). Peptide length distributions for both experimental groups displayed the typical right-skewed profile characteristic of tryptic digestion, with a dominant peak at 12–13 amino acids and the majority of identified peptides falling within the 7–20 amino acid range (Figure S2). The overall distribution was consistent with the typical pattern of trypsin-digested peptides, with no obvious enrichment of extremely short or long peptides.

Table 1. Overview of identified proteins.

Name	Total Spectra	Matched Spectra	Peptides	Identified Proteins
ALL	989,019	69,206	7955	1636

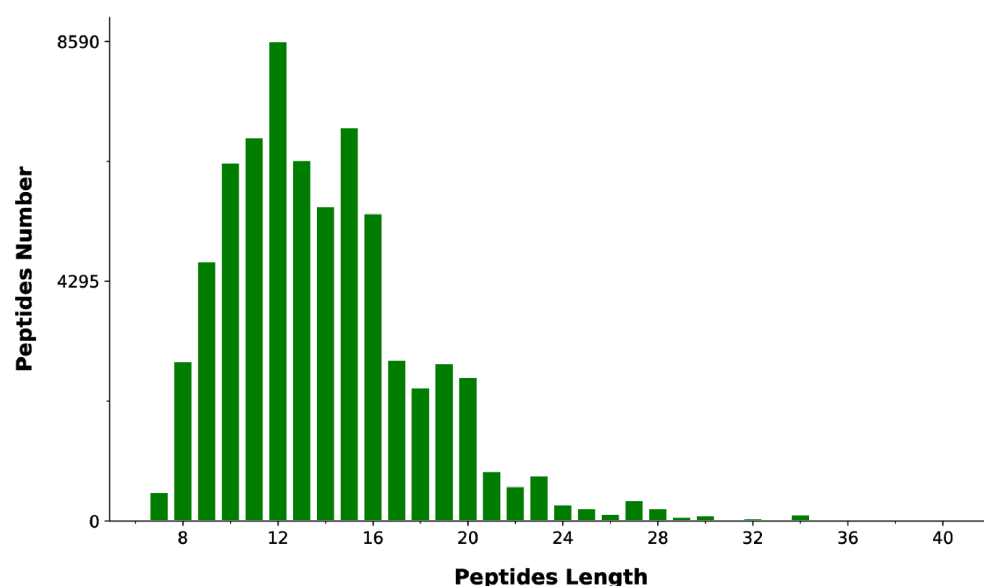


Figure 1. Peptide length distribution of the identified peptides.

Principal component analysis (PCA) was performed based on protein expression levels and KO functional abundance profiles (Figure 2). For the PCA based on protein expression, the first principal component (PC1) and the second principal component (PC2) explained 28.71% and 12.18% of the total variance, respectively. In the PCA based on KO abundance, PC1 and PC2 explained 28.92% and 12.40% of the total variance, respectively. In both analyses, samples from the HQ group were clustered on the negative half-axis of PC1, whereas samples from the PQ group were clustered on the positive half-axis of PC1. The 95% confidence ellipses of the two groups showed no obvious overlap, indicating that the HQ and PQ groups exhibited significantly different protein expression profiles and functional pathway compositions, and that the experimental grouping was biologically meaningful.

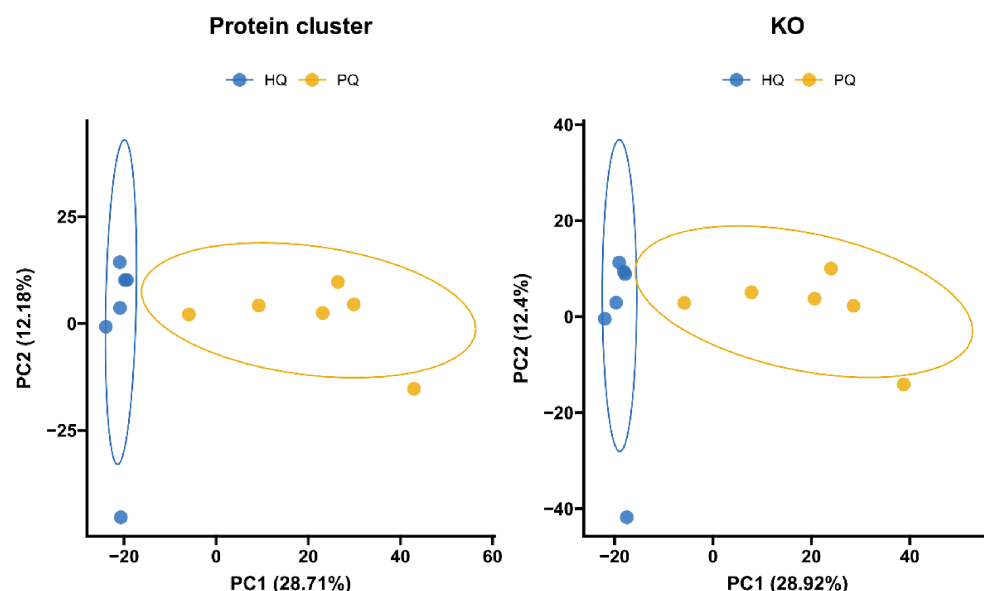


Figure 2. PCA results based on the abundance of protein clusters and KOs. Each point on the PCA graph represents an individual sturgeon egg protein sample. Six subsamples derived from three independent maternal batches ($n = 3$ maternal batches, with 2 subsamples per group per batch). Two data points of HQ subsamples nearly overlap in the plot. Ellipses represent the 95% confidence interval for each experimental group.

3.2. Comparison of HQ and PQ Egg Proteins

Differential protein analysis between the HQ and PQ groups was performed using the nonparametric Mann–Whitney U test. A total of 220 differentially expressed proteins were identified, of which 100 were upregulated in the PQ group and 120 were upregulated in the HQ group (Figure 3). GO functional annotation analysis (Table S2) revealed that the specific upregulated proteins in the HQ group were associated with glycolysis/gluconeogenesis (29 proteins); biosynthesis and metabolism of amino acids (26 proteins); and RNA splicing, transcription, and mRNA surveillance (22 proteins). In contrast, the specific upregulated proteins in the PQ group were related to oxidative phosphorylation and mitochondrial energy metabolism (35 proteins); oxidative stress, reactive oxygen species scavenging, and redox homeostasis (28 proteins); and the synaptic vesicle cycle, exocytosis, secretion, and endocytosis (22 proteins). KEGG pathway analysis (Figure 4) showed that the top 20 pathways in the HQ group were dominated by spliceosomes (10 proteins), glycolysis/gluconeogenesis (6 proteins), biosynthesis of amino acids (6 proteins), and regulation of the actin cytoskeleton (5 proteins). The top 20 pathways in the PQ group were predominantly enriched in oxidative phosphorylation (15 proteins), thermogenesis (12 proteins), and neurodegeneration including multiple diseases (13 proteins).

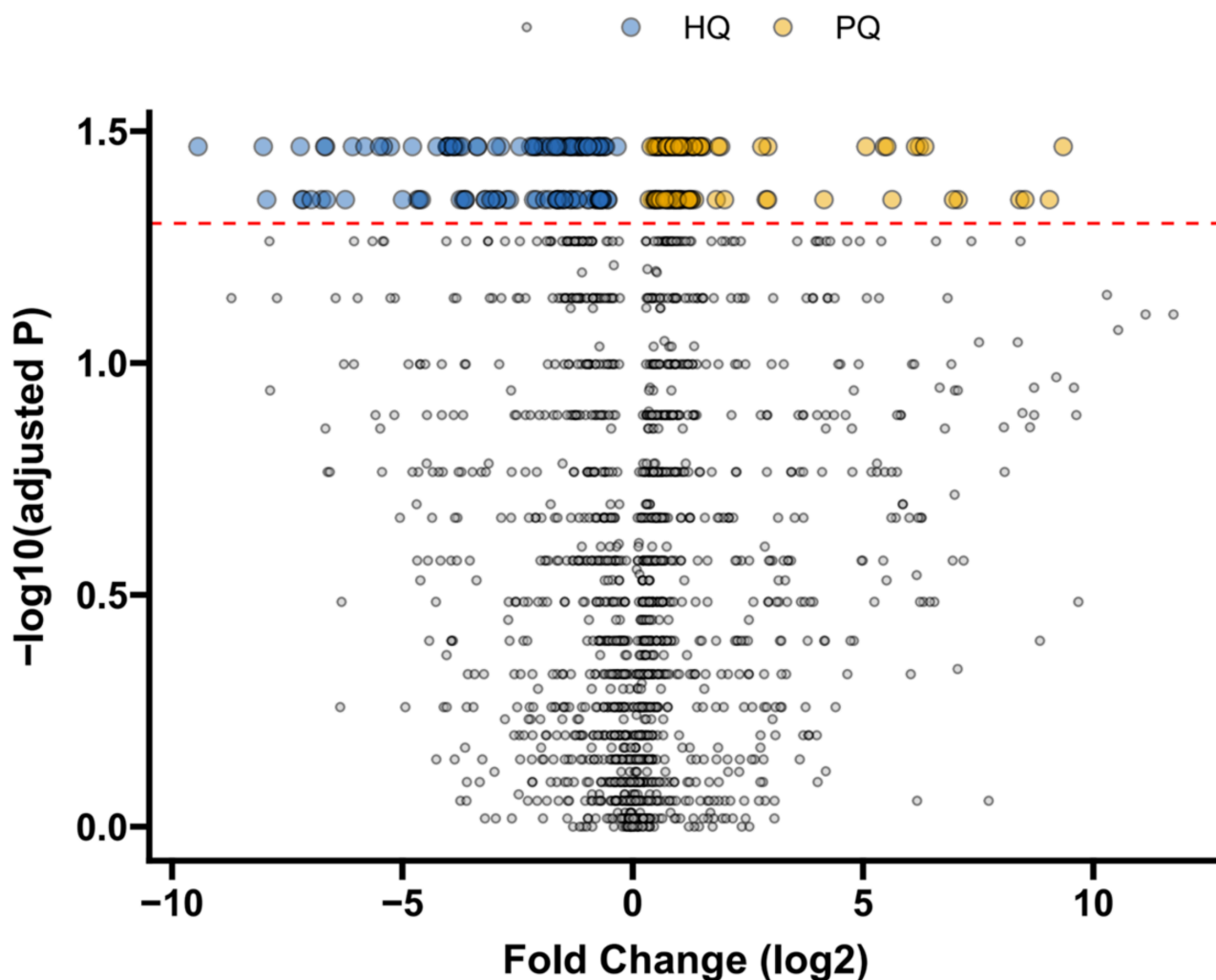


Figure 3. The volcano plot of differentially expressed proteins (DEPs) in the HQ vs. PQ comparisons. The horizontal dashed line represents the statistical significance threshold, corresponding to an adjusted *p*-value of 0.05.

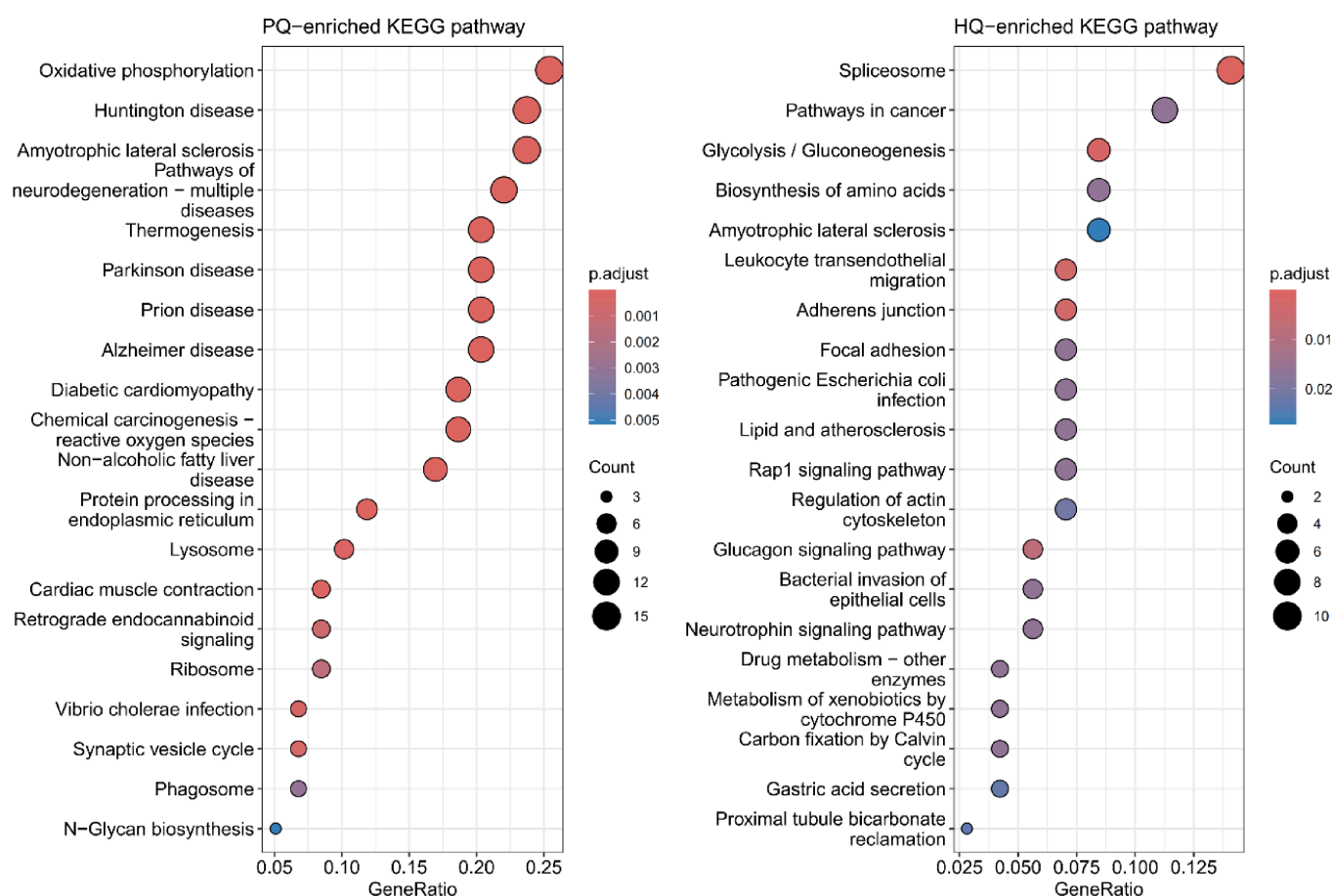


Figure 4. Scatter plot for KEGG enrichment analysis of HQ groups and PQ groups.

3.3. Untargeted Metabolomic Profiling of HQ and PQ Eggs

In this study, 1102 metabolites were identified, with 693 detected in the positive ion mode and 409 in the negative ion mode. Orthogonal partial least squares discriminant analysis (OPLS-DA) revealed a clear separation between the HQ and PQ groups in both the positive ion mode ($R^2Y = 0.96$, $Q^2 = 0.74$) and negative ion mode ($R^2Y = 0.96$, $Q^2 = 0.64$). High R^2Y and Q^2 values indicated excellent model fitness and predictive ability, confirming stable and reproducible differences in metabolic profiles between the two groups (Figure 5).

The non-parametric Mann–Whitney U test combined with OPLS-DA was performed to evaluate the differences in metabolite abundance between the HQ and PQ groups. The variable importance in projection (VIP) of each metabolite was calculated. Metabolites with adjusted $p < 0.05$ and $VIP > 1$ were defined as differentially expressed. In total, 365 differentially expressed metabolites were identified (Figure 6). Among the 236 metabolites enriched in the HQ group, amino acids and their derivatives were most abundant (86 metabolites), followed by nucleotides and nucleoside derivatives (47 metabolites), carbohydrates and organic acids (41 metabolites), lipids and acylcarnitines (32 metabolites), vitamins and coenzymes (12 metabolites), and secondary metabolites (18 metabolites). The 129 metabolites enriched in the PQ group were predominantly lipid-related compounds, including glycerophospholipids and lysophospholipids (58 metabolites), unsaturated fatty acids and eicosanoid derivatives (31 metabolites), sphingolipids (16 metabolites), fatty acid peroxidation products (13 metabolites), and stress-related secondary metabolites (11 metabolites).

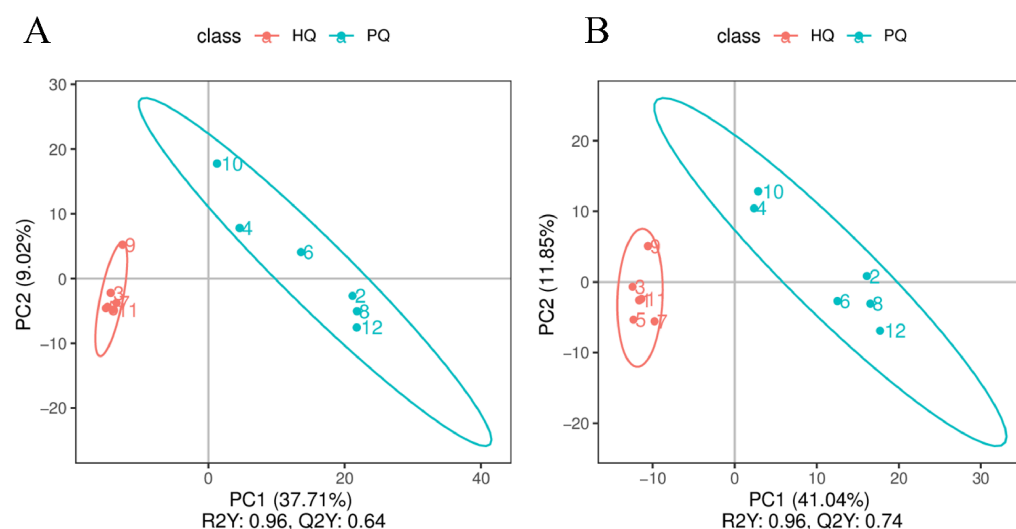


Figure 5. OPLS-DA score scatter plot. (A) Positive ion mode; (B) negative ion mode. X- and Y-axes show sample scores of the first and second principal components, respectively. The numbers marked on each scatter point represent the serial number of subsamples for each group. Specifically, odd-numbered samples (1, 3, 5, 7, 9, 11) belong to the HQ group, and even-numbered samples (2, 4, 6, 8, 10, 12) belong to the PQ group. R2Y stands for model interpretability and Q2Y for predictive capability. The model is well-established if R2Y > Q2Y. Ellipses represent the 95% confidence interval for each experimental group.

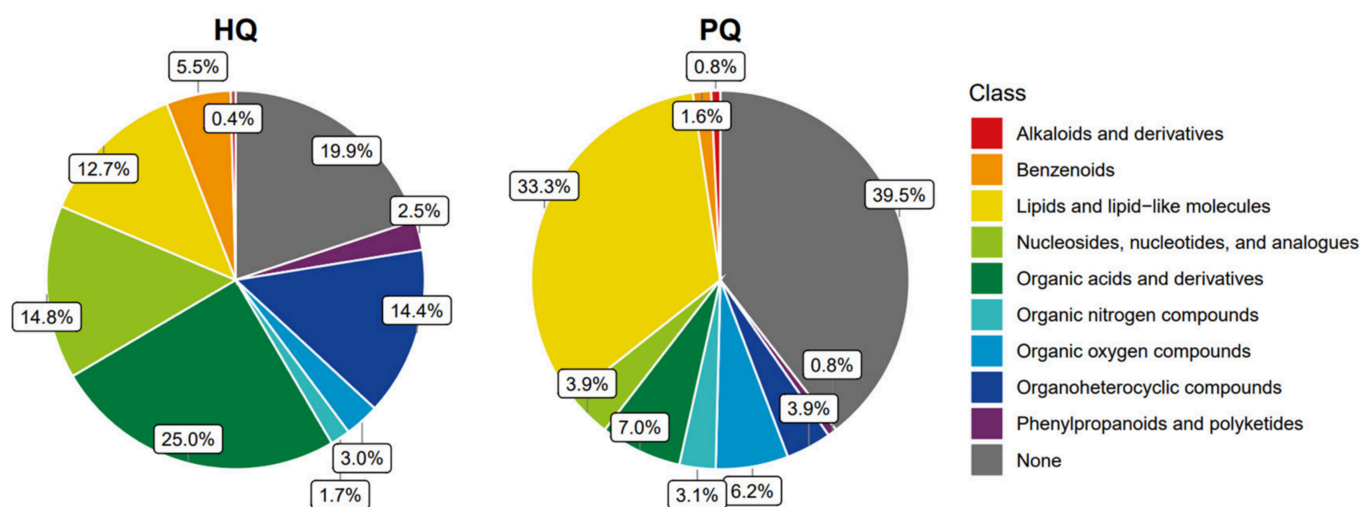


Figure 6. Enrichment distribution of differentially expressed metabolites.

Pathway enrichment analysis was performed on 129 differential metabolites in the PQ group and 236 differential metabolites in the HQ group based on the KEGG database (Figure 7). In the PQ group, 17 enriched metabolic pathways were identified, which were primarily associated with arachidonic acid metabolism, steroidogenesis, amino acid metabolism, retinol metabolism, oxidative stress, and the immune inflammatory pathways. These pathways mainly function in lipid remodeling and environmental stress responses. In contrast, the HQ group exhibited 38 distinct metabolic pathways. Dominated by amino acid biosynthesis, glycolysis/Tricarboxylic Acid (TCA) cycle, nucleotide metabolism, and vitamin and coenzyme metabolism, these pathways are prominently enriched in the regulatory processes of embryonic development, energy supply, and fundamental anabolic metabolism.

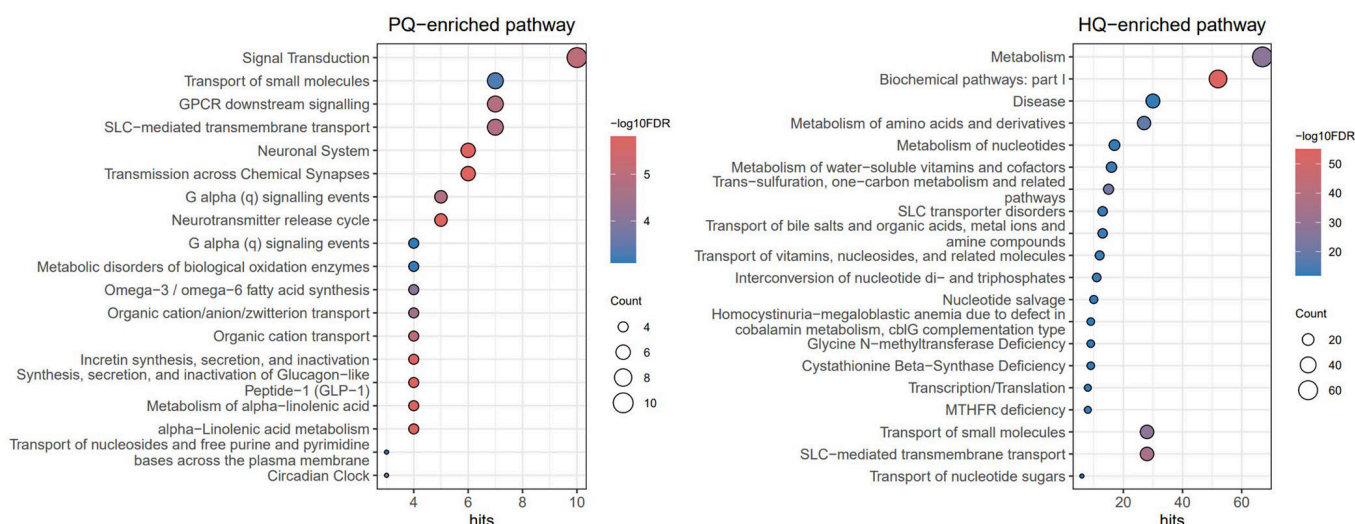


Figure 7. Top 20 enriched pathways of differential metabolites between PQ and HQ groups.

3.4. Combined Analysis Proteomics and Metabolomics Data

To investigate the relationship between proteomics and metabolomics, Procrustes congruence analysis was performed based on the Euclidean distance matrix and the Mantel test was used to verify the statistical significance of the correlation between proteomics and metabolomics (Figure 8). The Procrustes analysis yielded a structural goodness-of-fit value of $m^2 = 0.72$, and the Mantel test presented a correlation coefficient of $r = 0.277$ with $p = 0.041$. The Mantel test showed $p < 0.05$, indicating a significant positive correlation between proteomic and metabolomic profiles.

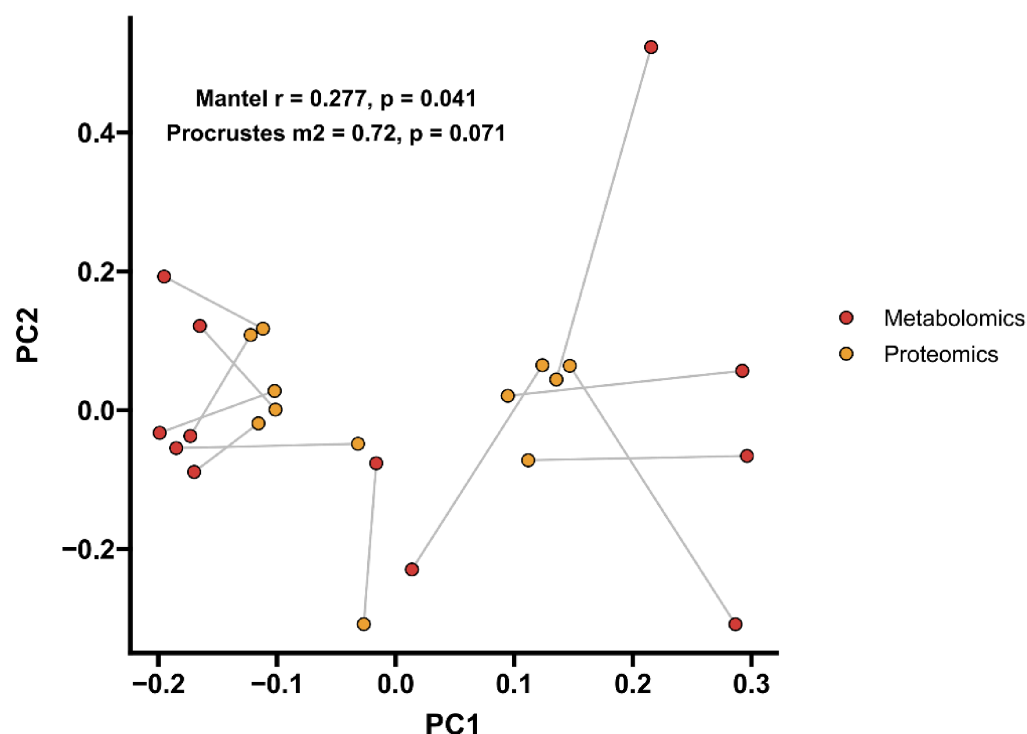


Figure 8. Correlation analysis of proteomics and metabolomics. Each line connects the corresponding position of the same sample in the PCA spaces of the two omics datasets.

Spearman rank correlation analysis was subsequently conducted on 28 KEGG-annotated differential metabolites with $VIP > 1.5$ and 39 differential proteins with

$|\log_2FC| > 2$. Using an adjusted $p < 0.01$ as the screening threshold, a total of 313 significant protein–metabolite correlation pairs were identified. Network hub analysis (Figure 9) revealed that natamycin ranked first in the node degree among the core metabolite hubs, followed by L-pyroglutamic acid, 4-oxoproline, and tetrahydrocorticosterone. Among the protein hub nodes, glyceraldehyde-3-phosphate dehydrogenase (K02503) had the highest degree, followed by glutathione S-transferase (K06691), cytochrome P450 2E1 (K00111), serine/threonine protein kinase (K09276), and lysosome-associated membrane protein (K09561). According to the absolute values of the correlation coefficients, the strongest positive correlations were found between HQ-enriched L-pyroglutamic acid and K06691 and between PQ-enriched natamycin and multidrug resistance-associated protein (K10612, $r = 0.979$). The strongest negative correlations existed between HQ-enriched ascorbic acid and K00111, and between PQ-enriched natamycin and Vitamin C transporter (K17292, $r = -0.979$). The detailed pairwise interaction information available in Supplementary Tables S3–S5. Table S3 contains all node classification and grouping data for Figure 9; Table S4 and Table S5 separately detail the protein–metabolite interactive metabolic pathways enriched in the HQ and PQ groups, respectively.

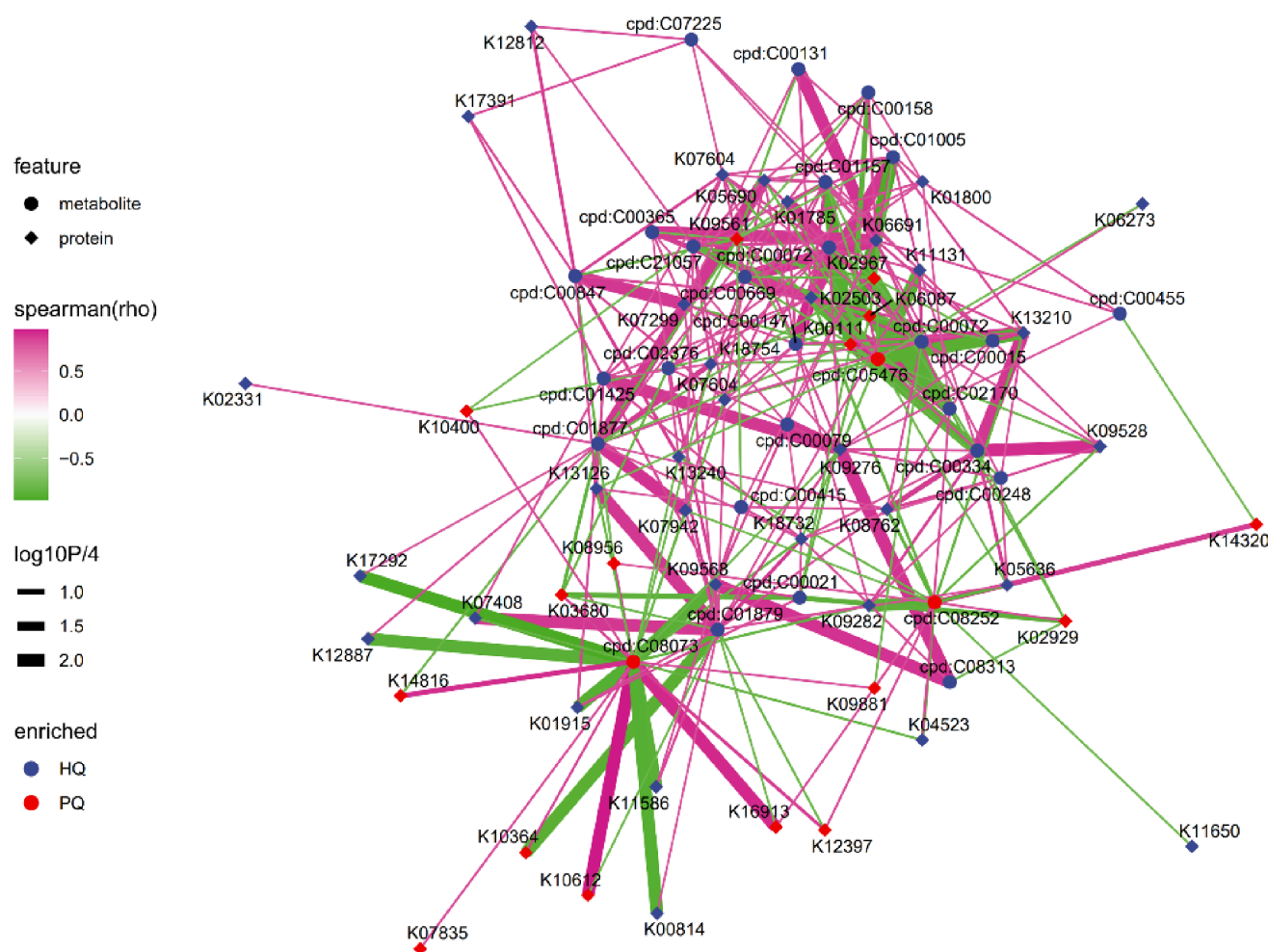


Figure 9. Network diagram of co-regulated proteins and metabolites. Metabolites are depicted as circular nodes and proteins as diamond-shaped nodes. Node color denotes the direction of correlation with stress intensity: red for positive correlations and blue for negative correlations. Node size is proportional to the magnitude of the correlation, with larger nodes representing stronger associations. Edge color transitions from green (negative correlation) to purple (positive correlation), and edge thickness corresponds to the strength of the pairwise correlation.

Subsequently, Joint Pathway Analysis on the MetaboAnalyst.ca (<https://www.metaboanalyst.ca>, accessed 20 March 2026) platform was performed to integrate and analyze the DMs and DEPs. No overlapping significantly enriched pathways were shared between the HQ and PQ groups. The significantly enriched pathways in the HQ group included arginine biosynthesis, alanine/aspartate/glutamate metabolism, and mRNA surveillance. The dominant enriched pathways in the PQ group were tight junctions, cell adhesion molecules, glycerolipid metabolism, and virion–hepatitis virus pathways (Table 2).

Table 2. The significantly enriched pathways in the HQ and PQ groups.

	Pathway	Total	Expected	Hits	FDR	Impact
HQ	Arginine biosynthesis	56	0.407	7	1.10×10^{-5}	0.410
	Alanine, aspartate and glutamate metabolism	76	0.552	8	1.02×10^{-5}	0.298
	mRNA surveillance pathway	99	0.720	5	0.042	0.034
PQ	Tight junction	99	1.920	41	1.88×10^{-47}	0.083
	Cell adhesion molecules	250	1.436	41	6.12×10^{-53}	0.019
	Glycerolipid metabolism	112	0.860	1	1	0.123
	Virion—Hepatitis viruses	75	0.576	41	1.06×10^{-72}	0

4. Discussion

In this study, HQ and PQ sturgeon eggs were selected as research materials. For the first time, we systematically elucidated the molecular basis affecting sturgeon egg quality at both the proteomic and metabolomic levels, providing crucial omics evidence to reveal the molecular mechanism underlying sturgeon egg quality and screening potential biomarkers for egg quality evaluation. Both the proteomic PCA and the metabolomic OPLS-DA model demonstrated that the grouping of the HQ and PQ samples was reliable. Distinct and stable phenotypic differences were observed between the two groups in terms of protein expression profiles and metabolic homeostasis, which laid a solid methodological foundation for subsequent integrated multiomics analyses.

Studies on various fish species, including sturgeons, chum salmon, and eels, have consistently demonstrated a significant positive correlation between the abundance of key enzymes in glycolysis and TCA cycle, the activity of amino acid biosynthesis pathways, and egg hatching potential. Conversely, disruptions in energy metabolism and the down-regulation of protein synthesis are often associated with reduced egg quality and arrested embryonic development [6,16,17]. Our results revealed that the functional pathways of differentially expressed proteins and metabolites in the HQ and PQ groups were distinctly different. In the HQ group, 120 proteins were upregulated and were primarily enriched in pathways such as glycolysis, amino acid biosynthesis, spliceosome, cytoskeleton, and cell junctions. Metabolically, this was reflected by enrichment in fundamental anabolic pathways, including amino acid biosynthesis, glycolysis/TCA cycle, nucleotide metabolism, and vitamin/cofactor metabolism. These findings indicate robust amino acid and nucleotide metabolism in the HQ group eggs, which is consistent with the results of previous studies. Our results are also in agreement with those of Shimizu et al. [18], who reported elevated glucose levels during the development of sterlets (*Acipenser ruthenus*) and bester sturgeons, indicating that gluconeogenesis during embryonic development is a ubiquitous physiological phenomenon among vertebrates. Li et al. [19] employed an iTRAQ-based proteomic approach to analyze the protein expression profiles of spermatozoa from two sturgeon species (*A. baerii* and *A. schrenckii*). They found that the differentially expressed

proteins in high-quality sperm were enriched in the TCA cycle pathway. The striking similarity in the enrichment of the TCA cycle and energy metabolism pathways between high-quality sperm and HQ eggs led us to propose that the activity level of the TCA cycle may be a key factor in determining the energy supply to germ cells.

In contrast, the 100 proteins upregulated in the PQ group were mainly enriched in pathways related to oxidative phosphorylation, mitochondrial stress, redox homeostasis, and lysosomal phagocytosis. The differential metabolites in the PQ group were primarily concentrated in arachidonic acid metabolism, steroidogenesis, amino acid metabolism, oxidative stress, and immune inflammatory pathways, indicating that sturgeon eggs in the PQ group were in a prominent oxidative stress state accompanied by disordered lipid metabolism. These findings are consistent with those of previous studies on fish egg aging and stress-induced egg quality decline, which commonly report mitochondrial structural damage and abnormal activation of the oxidative phosphorylation pathway [20,21]. Proteomic studies of zebrafish egg quality have demonstrated that low-quality samples are significantly enriched in proteins associated with endosome/lysosome function, autophagy, apoptosis, and certain oncogenic products [22]. Jiao et al. [20] confirmed that mitochondrial structural damage and abnormal activation of the oxidative phosphorylation pathway are core characteristics of egg quality deterioration and aging. Ibrahim et al. [23] revealed that environmental stress could markedly induce abnormal upregulation of proteins related to the ovarian mitochondrial respiratory chain and oxidative phosphorylation, leading to mitochondrial dysfunction and subsequent oxidative stress injury. Notably, the TCA cycle pathway was significantly enriched in HQ samples but lacked an enrichment advantage in PQ samples. This result is consistent with the conclusion of Luo et al. [17] in spotted sea perch, who reported that NMN-mediated improvement in egg quality was closely associated with enhanced TCA cycle activity. Collectively, these findings support a correlative link between elevated TCA cycle function and favorable egg quality, with further functional validation required to confirm a definitive regulatory role.

Notably, multiple neurodegenerative disease-associated pathways were significantly enriched in the PQ group. It should be clarified that these KEGG disease pathways are essentially gene sets annotated around conserved core cellular functional modules, rather than specific markers of corresponding disease phenotypes. This interpretation is consistent with previous proteomic studies on fish egg quality, in which enrichment of neurodegenerative pathways in poor-quality eggs was universally attributed to mitochondrial oxidative phosphorylation dysfunction, excessive ROS accumulation, and activation of apoptotic signaling, rather than neural pathological changes [24]. The core mechanisms shared by these pathways are highly consistent with the molecular characteristics of poor-quality sturgeon eggs observed in this study. Similarly, the enrichment of Pathways in cancer in the HQ group does not indicate any neoplastic phenotype. As a comprehensive super pathway in KEGG, it integrates a series of core cellular processes critical for oocyte developmental competence, including cell cycle regulation, cytoskeleton organization, energy metabolism reprogramming, and macromolecule biosynthesis. Such enrichment patterns have been widely reported in studies of fish ovarian development and oocyte maturation, reflecting the well-orchestrated developmental program and robust metabolic capacity of high-quality eggs [25].

Based on the protein–metabolite correlation network constructed in this study, glutathione S-transferase (GST) and L-pyroglutamic acid were identified as key nodes exhibiting a strong positive correlation. The association between glutathione (GSH) metabolism and egg quality has been previously validated in several species. Hou et al. [26] investigated microcystin-LR-induced ovarian damage in zebrafish and found that MC-LR exposure significantly decreased the GSH content, and that GST participated in the detoxification of

MC-LR via a GSH-dependent pathway. Additionally, Kaptaner [27] examined *Alburnus tarichi* in the polluted context of Lake Van, Turkey, and reported significantly higher GSH levels in the ovaries of reproductively arrested females than in those from reference sites. In the present study, L-pyroglutamic acid was significantly enriched in HQ eggs, which may promote the functional activation of GST, thereby maintaining the redox balance and developmental competence of oocytes. Therefore, we propose that these two molecules represent putative paired metabolite–protein molecular correlates associated with sturgeon egg quality.

Studies have confirmed that mature fish eggs generally contain high levels of ascorbic acid, the abundance of which is significantly and positively correlated with oocyte maturation, fertilization capacity, and embryonic hatching potential. Insufficient ascorbic acid readily disrupts redox homeostasis, aggravates lipid damage, and increases the embryonic malformation rate and developmental arrest [28]. In the present study, ascorbic acid was markedly enriched in high-quality eggs of the HQ group and served as a core hub in the metabolite network, which is highly consistent with the findings of previous studies. A significant negative correlation was observed between ascorbic acid and CYP450 2E1 expression. In a zebrafish egg quality study, Miller et al. [29] reported that low-temperature stress simultaneously reduces ovarian ascorbic acid (vitamin C) and glutathione (GSH) levels in zebrafish, thereby leading to oocyte developmental arrest and diminished oocyte quantity. In our study, CYP450 2E1 (K00111) was upregulated in the PQ group and exhibited the strongest negative correlation with ascorbic acid, suggesting a conserved molecular injury pathway: CYP2E1 activation → excessive ROS accumulation, lipid peroxidation, and decline in egg quality. Therefore, ascorbic acid may represent a putative core metabolic correlate associated with the redox buffering capacity of sturgeon eggs, whereas CYP2E1 was identified as a candidate protein indicator linked to low-quality sturgeon eggs. The potential utility of these molecules as bona fide biomarkers requires further independent validation.

L-Pyroglutamic acid, ascorbic acid, natamycin, and tetrahydrocorticosterone were all undetectable in full-process reagent blanks, and their coefficient of variation (CV) values in pooled quality control (QC) samples were below 30%, confirming that they represent authentic biological signals derived from sturgeon oocytes rather than artifacts from sample preparation or mass spectrometry detection. L-Pyroglutamic acid is an endogenous intermediate in glutamate and γ -glutamyl metabolism in fish eggs. Its higher abundance in HQ samples and its high connectivity within the interaction network support its role as a core hub in maintaining amino acid and redox homeostasis during embryonic development [23]. Ascorbic acid cannot be synthesized de novo by Siberian sturgeon (*Acipenser baerii*) and is maternally transferred to oocytes following dietary intake by broodstock. As an essential antioxidant deposited in eggs, it functions as a physiological endogenous metabolite closely linked to developmental competence, rather than an environmental contaminant [30]. Tetrahydrocorticosterone, the primary hepatic metabolite of the teleost glucocorticoid corticosterone, serves as a stable endogenous stress biomarker [31]. Its marked accumulation in PQ samples suggests that maternal stress-induced glucocorticoid deposition may impair oocyte maturation, making it a candidate endogenous marker of compromised egg quality. In contrast to the three compounds above, natamycin is an exogenous polyene antifungal agent produced by *Streptomyces natalensis* and lacks any known biosynthetic pathway in animal tissues. Its pronounced enrichment in PQ samples and strong correlations with multiple proteins and metabolites in the interaction network suggest that the presence of natamycin is associated with intrinsic oocyte metabolic disturbances and diminished egg quality. While natamycin represents a putative exposure-related molecular correlate linked to inferior egg phenotypes, its specific aquaculture-related origins and potential

toxic effects on sturgeon embryos remain to be fully validated via targeted quantification and traceability experiments.

Proteomic investigations of cold-stored processed sturgeon caviar consistently identify degraded structural yolk proteins and secreted microbial metabolic enzymes as the predominant differential molecules underlying storage-associated quality deterioration [32]. In addition, post-ovulatory aging occurs in unprocessed fresh sturgeon oocytes held ex vivo following stripping, a process universally driven by time-dependent intracellular ROS accumulation within 4–24 h after ovulation [33]. Critical multi-omics data from published sturgeon oocyte aging assays further verify that the coordinated covariation of the ascorbic acid/CYP2E1 and L-pyroglutamic acid/glutathione S-transferase molecular pairs is absent during short-term ex vivo oocyte aging. Collectively, these comparative lines of evidence reinforce the specificity and reliability of the molecular signatures identified in the present study.

Joint pathway enrichment analysis revealed that the HQ group was significantly enriched in pathways related to arginine biosynthesis, alanine/aspartate/glutamate metabolism, and mRNA surveillance, which are primarily involved in functions essential for embryonic development, such as amino acid synthesis, energy metabolism, and gene expression regulation. In contrast, the PQ group showed significant enrichment in pathways associated with tight junctions, cell adhesion molecules, oxidative phosphorylation, virion–hepatitis virus interactions, and cardiac muscle contraction, which are mainly linked to environmental stress responses, including cell barrier regulation, immune inflammation, and oxidative stress. No shared, significantly enriched pathways were shared between the two groups, suggesting notable differences in molecular functional profiles associated with the two egg quality phenotypes. These findings reveal the core mechanisms underlying differences in sturgeon egg quality and provide directions for future research. Consistent with our results, Liu et al. [9] observed persistent activation of the MAPK, TNF, IL-17, and Notch signaling pathways in low-quality eggs, along with enrichment of lipid metabolism and energy supply pathways in high-quality eggs in a transcriptomic study of egg quality in the leopard coral grouper (*Plectropomus leopardus*).

Egg quality is a complex and comprehensive trait regulated by multiple factors, including environmental conditions, breeding management, parental physiological status, and genetic background. Although we strictly controlled the consistency of sample sources and the objectivity of grouping criteria in this study, the effects of environmental factors, such as aquaculture water temperature, dissolved oxygen, and feed formulation, on the molecular characteristics of egg quality have not been systematically evaluated. Key differential molecules and pathways were screened based on bioinformatics analysis in the present study. However, core hub proteins and pivotal metabolites have not been validated through qPCR, targeted metabolomics, or in vitro functional experiments, and the underlying molecular regulatory mechanisms require further experimental verification. Subsequent functional validation will be carried out via CRISPR/Cas9-mediated gene knockout in rapidly maturing model organisms, including zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*), to confirm the regulatory roles of these evolutionarily conserved pathways in oocyte development.

5. Conclusions

We conducted the first comprehensive integrated proteomic and untargeted metabolomic investigation to systematically decipher the molecular basis of egg quality divergence in *Acipenser schrenckii*. A total of 220 DEPs and 365 DMs were identified between HQ and PQ samples. HQ samples exhibited significant enrichment in amino acid biosynthesis, TCA cycle, nucleotide metabolism and mRNA surveillance pathways, which collectively

provided the essential macromolecular building blocks, and energy reserves required for embryonic development. In contrast, PQ samples were characterized by profound mitochondrial dysfunction and exacerbated oxidative stress, as evidenced by aberrant upregulation of oxidative phosphorylation, mitochondrial stress response, arachidonic acid metabolism and immune–inflammatory signaling cascades. These differential molecular profiles collectively correlate with severe lipid metabolism shifts and perturbed redox homeostasis that accompany poor sturgeon egg phenotypes. Integrative multi-omics correlation analysis further screened a set of statistically associated molecular signatures linked to divergent oocyte quality statuses. L-pyroglutamic acid and ascorbic acid displayed strong correlative relationships with viable high-quality oocytes and function as candidate central metabolites correlated with favorable oocyte redox status, whereas natamycin and tetrahydrocorticosterone showed significant enrichment correlation with deteriorated eggs and serve as exploratory molecular correlates of poor egg phenotypes. At the proteomic level, GAPDH, glutathione S-transferase and cytochrome P450 2E1 (CYP2E1) were detected as core network nodes exhibiting strong statistical correlations with egg quality traits. The correlative molecular signatures and associated metabolic pathways uncovered herein offer preliminary reference molecular resources for auxiliary evaluation of oocyte quality in farmed sturgeon hatcheries.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/fishes11080449/s1>, Figure S1: HQ and PQ eggs at 24 h post-fertilization; Figure S2: Peptide length distribution of identified peptides in the HQ and PQ groups; Table S1: The comprehensive discrimination criteria for HQ and PQ samples; Table S2: GO functional annotation analysis for differentially expressed proteins; Table S3: Co-regulated proteins and metabolites information of Network diagram; Table S4: The protein–metabolite interactive metabolic pathways enriched in the HQ groups; Table S5: The protein–metabolite interactive metabolic pathways enriched in the PQ groups.

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