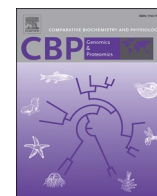




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Research article

Early hepatic protein responses to dietary restriction-refeeding in Japanese quail: A proteomic investigation

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ABSTRACT

Feed intake and refeeding after nutrient scarcity induce rapid metabolic adaptations in the poultry liver; however, hepatic proteomic recovery pathways in the early hours post-refeeding remain poorly defined. This study aimed to characterize early liver protein signatures in Japanese quail (*Coturnix japonica*) recovering from nutritional stress under two refeeding conditions. Eighteen 12-week-old male quails (245.20 ± 0.213 g) were assigned to three groups ($n = 6$): control fed ad libitum (12.13 MJ/kg), 24 h feed deprivation followed by 6 h refeeding, and 24 h low metabolizable energy (6.30 MJ/kg) diet followed by 6 h refeeding. In total, 854 proteins were identified, of which 515 met the filtering criteria. The low metabolizable energy refeeding showed higher abundance of proteins linked to ATP binding and carbohydrate/carboxylic acid metabolism, alongside detoxification-related proteins, while suppressing translation/RNA-binding machinery and antioxidant pathways. Feed-deprived refeeding enriched in oxidative phosphorylation and mitochondrial complex I assembly with reduced cytoplasmic translation, NMD-related components, and sulfur compound metabolism. A direct comparison indicated divergent recovery strategies: low metabolizable energy refeeding mainly reflected oxidoreductase activity and translation initiation, whereas feed-deprived refeeding potentially enriched mitochondrial ATP production and glutathione-based defenses. Our analysis indicate that 6 h of refeeding initiates an early, incomplete recovery toward hepatic homeostasis, with the severity of prior nutritional restriction dictating distinct liver metabolic priorities. Collectively, these findings might provide a preliminary understanding of the hepatic mechanisms involved in recovery from nutrient deprivation and may help in the development of feeding strategies for managing metabolic recovery in poultry. However, these findings should be considered hypothesis-generating pending further validation.

1. Introduction

Energy metabolism, which includes the release, transmission, storage, and use of energy, as well as the material metabolism of proteins,

fats, and carbohydrates, is a fundamental aspect of animal existence (Liu and Chen, 2021). Animals have evolved distinct metabolic response systems to address nutrient deprivation, disease, stress, and other difficulties resulting from alterations in their external environment (Weng

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et al., 2025). In commercial poultry production, periods of reduced feed intake may occur due to feed restriction programs, transportation, heat stress, or disease challenges (Nijdam et al., 2005; Ferket and Gernat, 2006; Lara and Rostagno, 2013).

The nutrient deprivation-refeeding model has been key in uncovering specific genes and metabolic pathways that regulate energy metabolism in liver in chickens (Shen and Mistry, 1979; Saneyasu et al., 2013; Cogburn et al., 2020; Chen et al., 2021; Weng et al., 2025). This model provides a dynamic framework for studying metabolic adaptations by simulating periods of nutrient deprivation followed by nutrient replenishment, which investigate mechanisms underlying metabolic adaptation and recovery.

In poultry, the liver plays an important role in a diverse array of metabolic and homeostatic functions, serving as a biochemical center responsible for synthetic, homeostatic, and detoxification processes (Zaefarian et al., 2019). Moreover, it is important for digestion through bile secretion, regulation of carbohydrate and protein metabolism, and lipid synthesis (Denbow, 2015). Studies have provided critical insights into the modulation of metabolic processes, such as gluconeogenesis, lipid metabolism, and energy homeostasis, in response to nutritional status (Xu et al., 2022; Liu et al., 2024; Liu et al., 2025a). Although some quantitative differences in metabolic traits and detoxification capacity have been reported between quail and chickens, the major physiological functions and metabolic pathways of the liver appear to be highly conserved, making chicken studies a valuable framework for understanding hepatic responses to nutritional challenges in quail (Ishige et al., 2016; Pu et al., 2020; De Lima et al., 2025; Shu et al., 2025).

Proteomics has been widely used in poultry to assess changes in the protein profile under different conditions and to discover new insights and potential biomarkers that can enhance the feed efficiency and energy metabolism understanding (Kong et al., 2016; Zampiga et al., 2018; Kaewsatun et al., 2022). These experiments have examined the protein profiles of chicken skeletal muscle and the duodenum to understand feed metabolism. Some studies have investigated the proteomic profile of the jejunum after short-term refeeding (Simon et al., 2019; Wang et al., 2024); however, limited research has addressed the hepatic proteomic profile after refeeding to detect the recovery pathways used to restore lost energy. Therefore, a comprehensive understanding of the metabolic status of the liver proteomic profile in the first hours after refeeding after nutritional scarcity is important for improving poultry production strategies. We aimed to investigate the early response liver proteins involved in recovery after nutritional stress in quails subjected to different dietary conditions (starvation-refed and low-metabolizable energy-refed) using DIA-NN (library-free) shotgun analysis. We hypothesized that the severity of prior nutritional restriction would induce distinct hepatic proteomic metabolic pathways during the early recovery period.

2. Materials and methods

2.1. Animals and sampling

The study was conducted according to the guidelines of the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes. The experiment was conducted in the animal house of the Institute of Animal Science, Biotechnology, and Nature Conservation, University of Debrecen.

We selected 18 male quails at 12 weeks of age with similar body mass (245.20 ± 0.213 g) and randomly allocated them to individual cages (30 cm × 22 cm × 40 cm, length × width × height) for the acclimatization phase and in order to monitor their feeding behavior and eliminate any abnormal birds that might influence the experiment's results. The birds were acclimatized to the experimental conditions for one week. The birds were housed in the same controlled environment, maintaining a temperature of 23–25 °C, 60–65% relative humidity, and a 12:12 light:dark cycle. During the acclimation period, the animals

Table 1
Ingredient composition and nutrient level of the breeder basal and low metabolizable energy diet.

Ingredients, %	Basal feed	Low metabolic Energy feed
Sunflower hull	0	50
Corn	25	7.48
Wheat	10.1	12.25
Wheat bran	10	5
Pea	13.02	12.5
Soybean meal	24.86	8.69
Oil	9.07	0
Limestone	5.83	2.94
MCP	0.96	0.45
L-Lys	0	0.03
L-Met	0.19	0.1
L-Thr	0.07	0.04
Leucine	0.01	0.04
Isoleucine	0.005	0.02
Valine	0.005	0.02
Salt	0.38	0.19
Vitamin and mineral premix ^a	0.5	0.25
Total	100.00	100.00
Nutrient content, %		
ME, MJ/kg	12.13	6.30
Crude protein	18 (18.2*)	11.60
Lys	1.014	0.67
Met	0.45	0.30
Thr	0.74	0.49
Trp	0.216	0.16
Leu	1.42	0.84
Ile	0.77	0.48
Arg	1.26	0.75
Leu/Ile	1.84	1.75
Ca	2.5	1.44
P	0.608	0.35
Na	0.15	0.08

^a 1 kg premix provided: 1000000 IU/kg vitamin A, 200000 IU/kg vitamin D₃, 4900 IU/kg vitamin E, 200 mg vitamin K₃, 150 mg vitamin B₁, 500 mg vitamin B₂, 1200 mg Ca-d-Pantothenate, 400 mg vitamin B₆, 2 mg vitamin B₁₂, 11 mg biotin, 2502 mg niacin, 60 mg folic acid, 300000 mg choline chloride, 13200 mg Zn, 1920 mg Cu, 9612 mg Fe, 13200 mg Mn, 180 mg I, 42 mg Se, 12 mg Co. MCP: monocalcium phosphate.

* Tested in the department laboratory.

were allowed free access to the feed and water. The study consisted of three experimental groups: a control group fed ad libitum, a group starved for 24 h and refed a basal diet for 6 h (SR), and a group fed a low metabolizable energy diet for 24 h and refed a basal diet for 6 h (LER), with six birds in each group (*n* = 6); they were distributed to achieve a randomized block design, and each bird was considered a biological replicate. The basal (control) breeder feed was prepared according to the National Research Council (Nutrient Requirements of Poultry: Ninth Revised Edition, 1994) recommendations for quails on a corn-wheat-soybean meal basis, containing 18% crude protein and 12.13 MJ/kg metabolizable energy. The low metabolizable energy diet consisted of 50% sunflower hulls and 50% of a designed low-energy diet. The details of the feed composition are presented in Table 1.

In this experiment, only male quails were used to avoid the potential confounding effects of the reproductive cycle and egg production on hepatic lipid and protein metabolism of the quail. In addition, all birds were sampled at the same time of day (2 p.m.) to minimize potential circadian effects. In addition, all cages were subjected to similar routine handling procedures, including cage opening and other measurements, to reduce handling-related variation among groups.

2.2. Tissue sampling and protein extraction

After 6 h of refeeding, the birds were euthanized by cervical dislocation, and the liver was removed, immediately placed in liquid nitrogen, and stored at −80 °C until subsequent analysis.

To extract proteins, the liver was homogenized with a mortar and pestle using liquid nitrogen. The powdered tissue (50 mg) was transferred to a sterile tube, and 500 μ L of lysis buffer (8.5 M urea, 2 M thiourea, 4% (w/v) CHAPS, 30 mM Tris) was added. The mixture was then incubated for 60 min on ice, vortexed every 10 min, and centrifuged at 10,000 $\times$ g for 20 min. The supernatant was collected and stored at -80°C until further analysis. Protein concentration was determined using Bradford reagent (SERVA Electrophoresis GmbH, Heidelberg, Germany).

2.3. Sample preparation for mass spectrometry

Proteins (50 μ g) were denatured with 6 M urea for 30 min; thereafter, reduced with 10 mM dithiothreitol (Bio-Rad, Hercules, CA, USA) at 56°C for 60 min and further alkylated with 20 mM iodoacetamide (Bio-Rad, Hercules, CA, USA) in the dark for 45 min. Before trypsin digestion, the samples were diluted with 25 mM ammonium bicarbonate (Sigma-Aldrich, St. Louis, MO, USA) to decrease the urea concentration to 1 M. Trypsin digestion was performed at 37°C overnight by adding MS-grade modified trypsin (ABSciex, Framingham, MA, USA) at a 1:25 enzyme-to-protein ratio. The digested proteins were dried in a speed-vac and dissolved in 1% formic acid. The samples were desalted with C18 Pierce-Tips (Thermo Scientific, Waltham, MA, USA), dried, and re-dissolved in 1% formic acid before mass spectrometry analyses, and 5 μ g of the digested sample was spiked with iRT peptide mixture according to the manufacturer's instructions (Biognosys, Zurich, Switzerland).

2.4. Mass spectrometry analysis

Prior to mass spectrometric analysis, the peptides were separated in a 50-min water/acetonitrile gradient using an Easy nLC 1200 nano UPLC (Thermo Scientific, Waltham, MA, USA). The peptide mixtures were desalted in an ACQUITY UPLC Symmetry C18 trap column (20 mm $\times$ 180 μ m, 5 μ m particle size, 100 $\text{\AA}$ pore size, Waters, Milford, MA, USA), followed by separation on a bioZen Peptide XB-C18 analytical column (75 μ m $\times$ 150 mm, 2.6 μ m particle size; Phenomenex, Torrance, CA, USA). Solvent A was 0.1% formic acid in LC-MS grade water (Sigma, St. Louis, MO, USA) while solvent B was 95% acetonitrile (Sigma, St. Louis, MO, USA) containing 0.1% formic acid (Sigma, St. Louis, MO, USA). The flow rate was set to 700 nL/min. Mass spectrometry analyses were performed using a Nanospray Flex ion source in positive ion mode. The spray voltage was set to 2300 V, while the sweep gas was 0.2 L/min and the transfer capillary temperature was 300°C . MS1 scans were recorded by the Orbitrap analyzer in a 350–1650 m/z scan range. The resolution was set to 120,000, and the AGC target value was 1×10^6 . MS1 spectra were recorded in profile mode. After MS1 scans, 27% HCD collision energy was used for ion fragmentation after precursor ion isolation in the quadrupole. For MS2 scans, the scan range was between 200 and 2000 m/z , and the MS2 spectra were recorded in centroid mode in the Orbitrap (the resolution was set to 30,000 and the AGC target value was set to 1×10^6). The data was collected using the data-independent acquisition (DIA) method, with 33 scan windows. In each case, six biological replicates, and for each biological replicate, two technical replicates were analyzed in two consecutive technical runs within the same analytical sequence, and technical replicates were treated individually as independent samples.

2.5. Data analysis

For DIA data analysis, we used DIA-NN (v1.8.1) (Demichev et al., 2019); the DIA raw data were aligned against the *Coturnix japonica* subset of the UniProt database (downloaded December 2024). The following settings were applied: FASTA digest for library-free search, missed cleavages set to 2, methionine oxidation and N-terminal acetylation were added as variable modifications, and carbamidomethylation of cysteine as a fixed modification. Proteins were accepted if they were

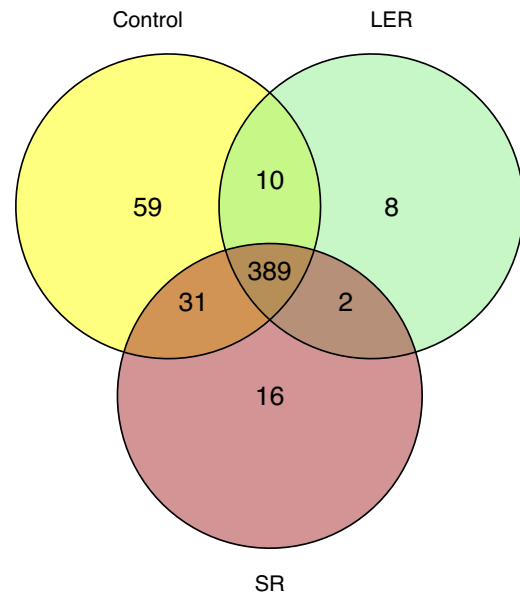


Fig. 1. A Venn diagram illustrating the identified liver proteins that were common or unique to the treatment groups. LER: low metabolizable energy refed group; SR: starved refed group.

identified with at least two peptides. Protein quantification was performed based on the MaxLFQ values, and the data were normalized to iRT peptides. The DIA-NN outputs were filtered for duplicates. In some cases, multiple accession numbers were assigned to a protein (e.g., isoforms, variants); in these cases, the first accession was kept. We then performed another filtration and retained proteins that were present in at least six runs out of 12 per group for further statistical analyses.

2.6. Statistical and network analysis

DIA quantification intensity values were transformed to a logarithmic scale with a base of 2. All statistical analyses were performed using R version 4.5.0 (<http://www.r-project.org/>).

Venn diagrams were generated using the *ggvenn* function provided by the 'ggvenn' package to visualize the common and unique proteins. Heatmaps were visualized using the *pheatmap* function provided by the 'pheatmap' package (version 1.0.13). To obtain heatmaps for proteins uniquely present in one group, missing (NA) values were imputed as zero. These proteins were considered uniquely expressed in a group, where the protein was present in at least six runs in a group and was completely absent from the other group.

We used the Limma package in R to determine differentially abundant proteins (DAPs) (adjusted $p < 0.05$, using the Benjamini-Hochberg (BH) test) between the control, low-energy, and feed-deprived refed groups.

To assess the DAPs, we considered (i) proteins that were present in at least six samples from a group and absent in all samples of the other group and (ii) proteins that showed statistically significant changes between the groups.

To examine the protein-protein interaction networks, the STRING server (version 12.0, <http://string-db.org>) using the Japanese quail (*Coturnix japonica*) database was used, considering a high confidence score of 0.7 for interaction (Minina et al., 2022; Szklarczyk et al., 2023; Yu et al., 2023) and no more than 50 1st shell interactors (Rengaraj et al., 2018). Biological process (Gene ontology), molecular function (Gene ontology), cellular component (Gene ontology), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Reactome pathway enrichment analyses were performed using STRING. Pathways with a similarity of ≥ 0.8 were merged, and those with a minimum count of 10 in the network were considered for further analysis. GO, KEGG, and

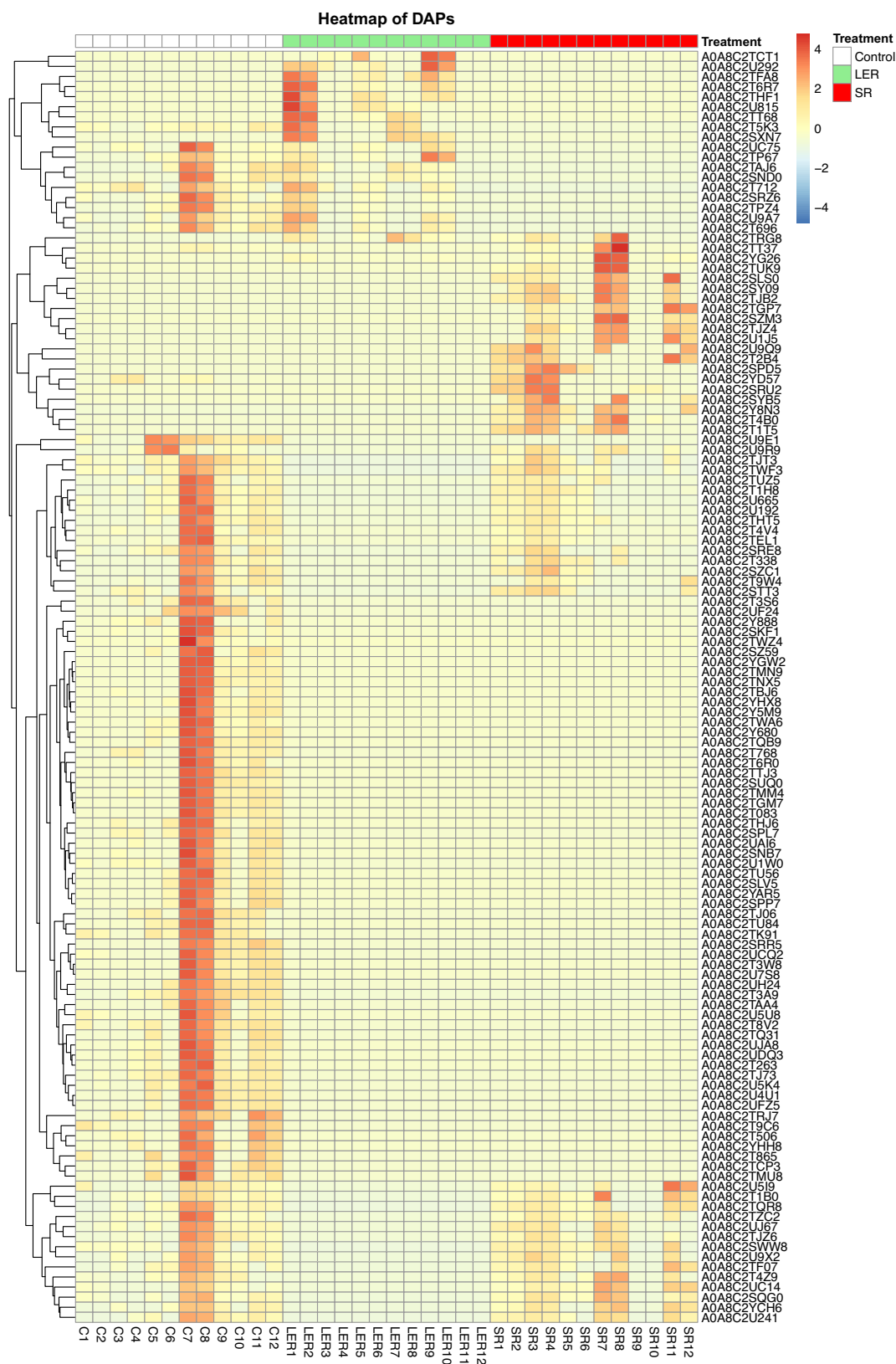


Fig. 2. Heatmap of differentially abundant proteins (DAPs) in the control, LER (low metabolizable energy refed), and SR (starved refed) groups. The expression levels were visualized using gradient colors. Each column represents a sample. Heatmap was generated using duplicate technical runs from 6 biological replicates per group ($n = 12$ technical runs/group).

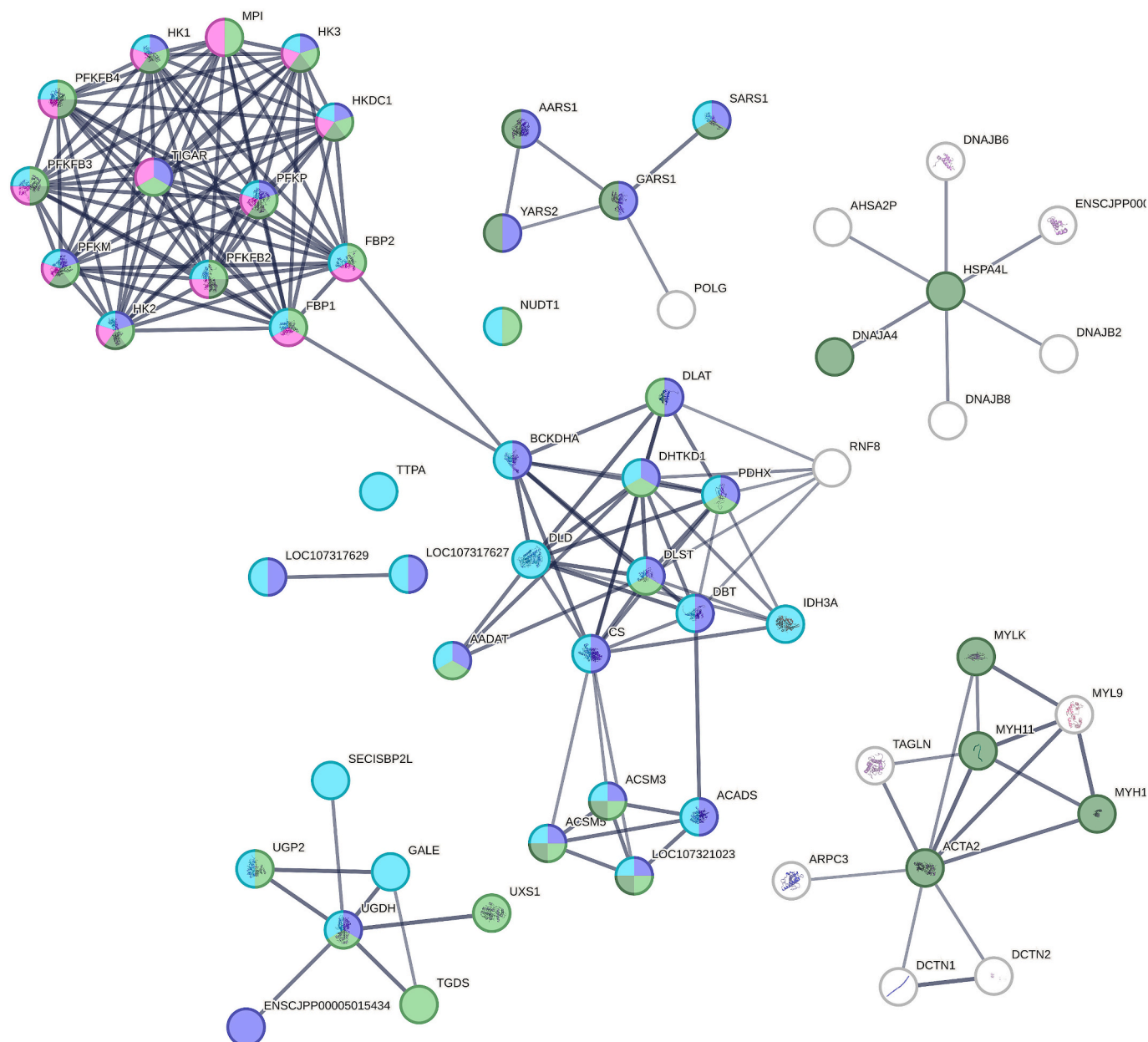


Fig. 3. Protein-protein interaction network of differentially expressed proteins with higher abundance in the low-energy refed group (control vs. low-energy refed comparison). Nodes represent proteins, while the edges represent interactions. Node colors are based on enriched functions: darkgreen: ATP binding, magenta: fructose and mannose metabolism, blue: carboxylic acid metabolic process, lime green: carbohydrate derivative metabolic process, cyan: metabolism. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Reactome pathways with an $FDR \leq 0.05$ were considered enriched.

3. Results

3.1. Overview of the proteomic profile

A total of 854 proteins were identified (Supplementary Table 1). After filtration, 515 proteins were retained out of 854; 389 were common in all three groups (Fig. 1), along with 10 common proteins between the LER and control groups, 31 between the SR and control groups, and 2 between the LER and SR groups.

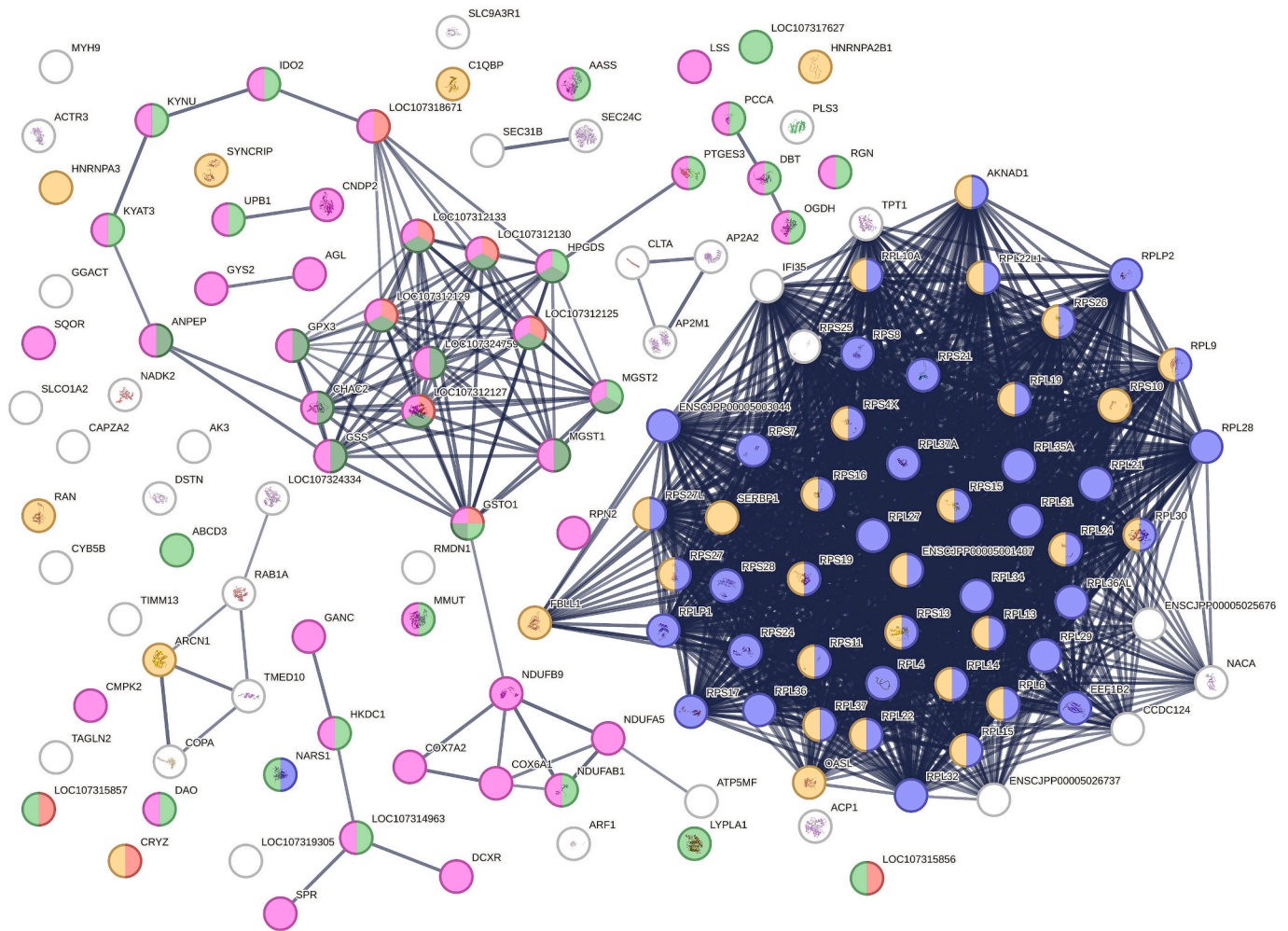
The heatmap shows the unique expression of 59 DAPs in the control group, 16 in the SR group, and 8 in the LER group (Fig. 2, Supplementary Table 2). The statistical analysis indicated no statistically significant difference among the groups; therefore, in further analyses, the proteins

uniquely present in a group and absent in the other were examined as DAPs. PCA and volcano plots for all pairwise comparisons are provided in Supplementary Figs. 1–4.

Protein-protein interaction (PPI) networks of proteins and their first-shell interactors were retrieved from the STRING database, and functional enrichment analysis was conducted to associate the DAPs with their biological functions. Complete GO, KEGG, and Reactome enrichment results for each comparison are presented in Supplementary Tables 3–5.

3.2. Low-energy refed vs control

In the network of DAPs with higher abundance in the LER group than in the control group, ATP binding and metabolism, such as carboxylic acid metabolic process, carbohydrate derivative metabolic process, and



fructose and mannose metabolism, were enriched (Fig. 3).

process, metabolic pathways, metabolism, and eukaryotic translation initiation (Fig. 7).

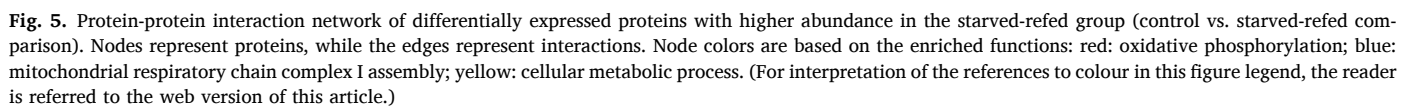
However, in the case of the network of DAPs with higher abundance in the SR group, the enriched pathways were mitochondrial respiratory chain complex I assembly, cellular modified amino acid metabolic process, peptide metabolic process, oxidative phosphorylation, glutathione metabolism, and metabolism (Fig. 8).

4. Discussion

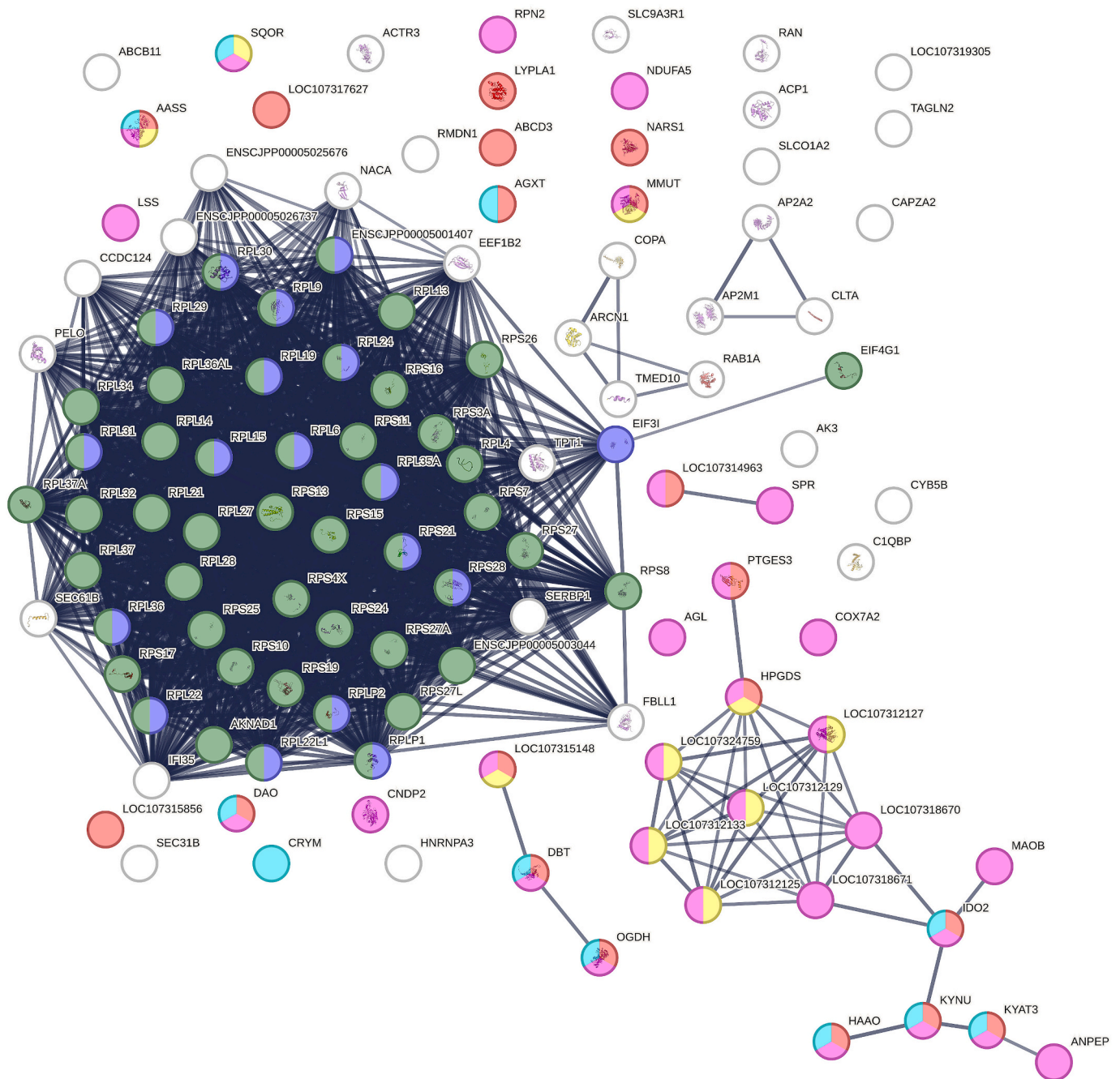
Key regulatory metabolic changes responsible for energy regulation in poultry have commonly been discovered using the nutrient deprivation-refeeding model (Cogburn et al., 2020; Chen et al., 2021). As a metabolic organ, the liver plays an important role in energy metabolism, making it a suitable candidate for monitoring metabolic alterations under different dietary conditions (Zaefarian et al., 2019).

In the first comparison between the LER and control groups, 10 DAPs were for the LER group and 90 DAPs were for the control group. PFKFB2, which is of the LER group, plays a role in the regulation of the glycolysis-gluconeogenesis balance (Ma et al., 2021). The presence of ACSM5, GARS1, HSPA4L, and ACTA2 suggests the concurrent involvement of lipid metabolism, protein synthesis, stress response, and cytoskeletal organization during metabolic recovery (Rockey et al., 2013; Tu et al., 2018; Mizuno et al., 2024; Zhang et al., 2024). In addition, the

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CoA, facilitating their entry into mitochondrial β -oxidation (Winder and Hardie, 1996; He et al., 2011; Cogburn et al., 2018; Mann et al., 2021; Li et al., 2023; Mizuno et al., 2024). Additionally, other proteins, such as UGDH and CYP4A6-like, are involved in the biotransformation of xenobiotics, indicating active detoxification processes (Clarkin et al., 2011; Zhang et al., 2023).



Compared with the LER group, the control group maintained a normal state of metabolic function, including protein synthesis, antioxidant defense, and broad metabolic processes. The high presence of ribosomal proteins (RPL/RPS), elongation factors (EEF1B2), and RNA-processing factors (HNRNP, SYNCRIP) helps maintain normal protein synthesis homeostasis, which usually requires the highest energy expenditure (Liu et al., 2009; Van Riggelen et al., 2010; Fakhri et al., 2023; Liu et al., 2025b). Moreover, multiple mitochondrial respiration proteins (NDUFA5, NDUFAB1, NDUFB9, COX6A1, COX7A2, OGDH) from the electron transport chain (ETC) were present, suggesting the capacity for robust conversion of nutrients to ATP through oxidative

The presence of several proteins involved in fatty acid metabolism (ABCD3, DBT, LYPLA1) and others linked to amino acid metabolism (AASS, DAO, IDO2, KYAT3, and KYNU) in the control group is consistent

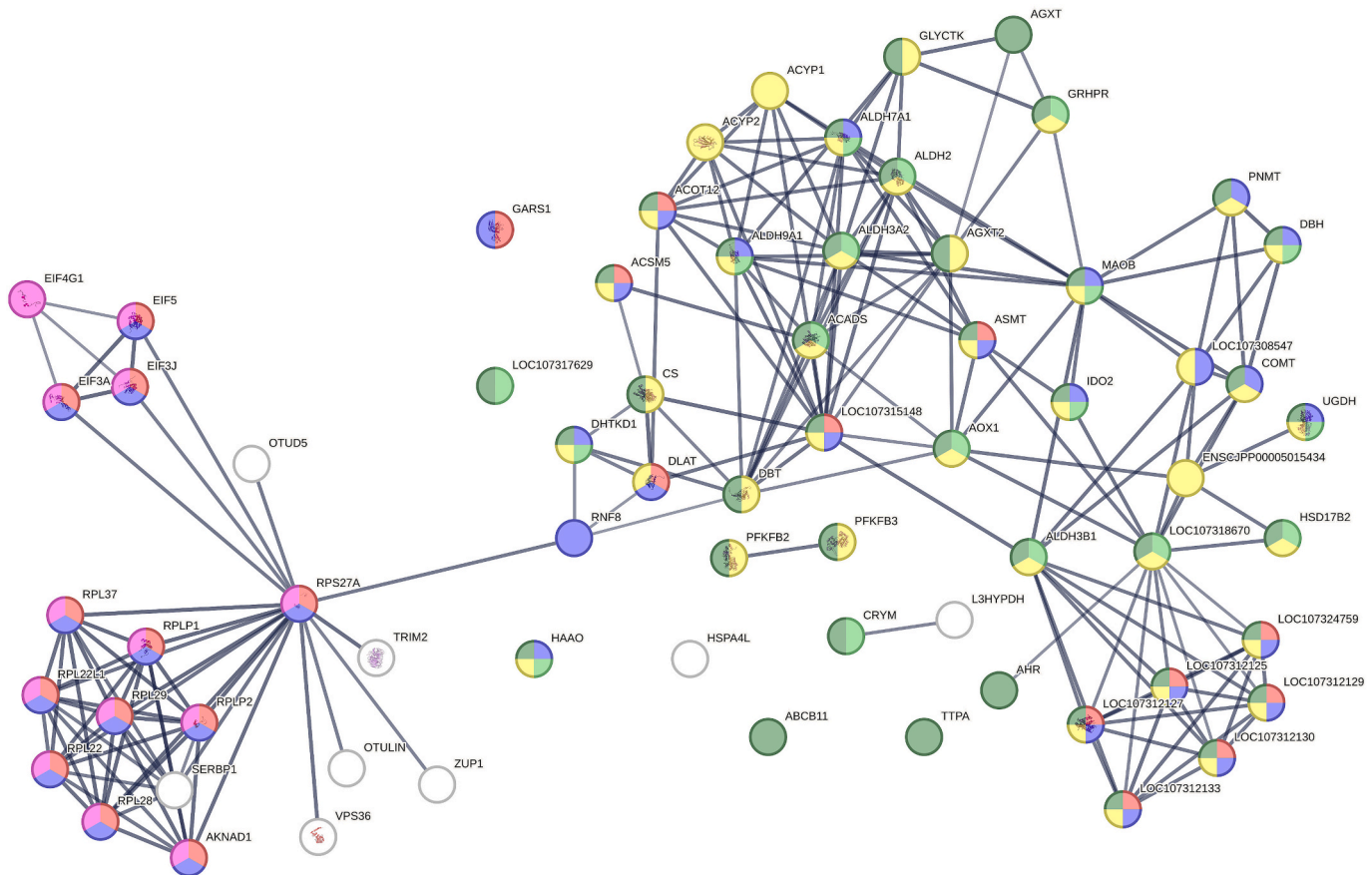


Fig. 7. Protein-protein interaction network of differentially expressed proteins with higher abundance in the low-energy refed group (low-energy refed vs. starved-refed comparison). Nodes represent proteins, while the edges represent interactions. Node colors are based on the enriched functions: dark green: metabolism; blue: cellular nitrogen compound metabolic process; red: cellular amide metabolic process; magenta: eukaryotic translation initiation; yellow: metabolic pathways; lime green: oxidoreductase activity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with active basal metabolic processes, reflecting normal physiological metabolic activity (Mbongue et al., 2015; Fujikawa et al., 2024). In addition, the enrichment of phase I and phase II detoxification proteins (cytochrome P450 (CYPs) and glutathione S-transferase (GST) enzymes) and the glutathione metabolism pathway through proteins such as GPX3, GSTO1, HPGDS, and CRYZ (also known as NQO1), which all have specific roles in oxidative stress and inflammation management, indicates a maintained cellular redox balance and baseline detoxification capacity (Hayes et al., 2005; Chen et al., 2017; Yang et al., 2018; Han et al., 2021; Yang et al., 2021; Li et al., 2024).

While both groups showed enrichment in the carboxylic acid metabolic process, the LER proteins suggest a shift toward substrate utilization pathways, including fatty acid metabolism (ACSM5) and lysine catabolism (DHTKD1). In contrast, the DAPs related to the control were associated with pathways related to the tricarboxylic acid (TCA) cycle and amino acid metabolism (Van Every and Schmidt, 2021; Mizuno et al., 2024).

The control group had a unique presence of ribosomal and redox balance proteins, while the LER group was consistent with increased glycolytic regulation via PFKFB2, along with proteins generating energy from fatty acid and amino acid metabolism (ACSM5, DHTKD1). These proteins may suggest ongoing efforts to compensate for energy reduction.

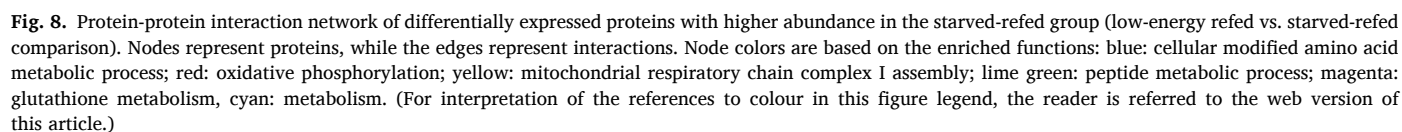
In the second comparison between the control and SR groups, the SR group showed a metabolic profile indicative of ongoing recovery, characterized by strong enrichment of oxidative phosphorylation pathways and the absence of pathways typically associated with stable metabolic homeostasis, indicating that metabolic recovery was still in

progress 6 h after refeeding.

Proteins such as NDUFA2, NDUFB8, and SDHB are involved in mitochondrial oxidative phosphorylation to produce ATP via the electron transport chain (Matsumoto et al., 2012). In addition, other proteins (e.g., NUDT1, RPL11, RPL27A, and STT3A) are associated with cellular metabolic processes that participate in protein synthesis, nucleotide quality control, and post-translational modification (Van Riggelen et al., 2010; Agarwal and de Jesus Perez, 2021; Wen et al., 2022; Zheng et al., 2025).

Similar to the previous comparison of LER-control, the control group proteins were ribosomal proteins. The enriched pathways in the control group were similar in both comparisons. The pathways included metabolic pathways related to lipid and amino acid metabolism, cytoplasmic translation, metabolism of amino acids and derivatives, and carboxylic acid metabolic process. However, we observed new pathways, including nonsense-mediated decay (NMD) independent of the exon junction complex and sulfur compound metabolic processes, which were not observed in the control-LER comparison.

The NMD pathway is a highly conserved cellular process that detects and degrades mRNAs containing premature termination codons (PTCs), thereby preventing the production of potentially harmful truncated proteins (Maquat, 2004). In addition to quality control, NMD regulates the abundance of many normal transcripts, shaping gene expression and impacting development, stress responses, and disease (Hug et al., 2016; Nickless et al., 2017; Karousis et al., 2021). This highlights the high levels of cellular maintenance in the control group, represented by the group of ribosomal proteins, and explains their absence in the SR group. Moreover, sulfur compound metabolic processes are crucial for



structural support, detoxification (HPGDS, GST-like) (Hayes et al., 2005; Han et al., 2021), energy production (AASS, ACSS1-like, MMUT) (Ramon et al., 2023), and cellular redox signaling (SQOR) (Peng et al., 2025), which are essential for normal cellular homeostasis and redox balance.

Functional enrichment analysis revealed distinct metabolic recovery responses after refeeding in the LER and the SR groups. Birds refed after a low-energy diet showed enrichment of pathways associated with eukaryotic translation initiation (EIF4G1, RPL22, RPS27A), cellular amide metabolism (ACSS1-like, ACSM5, GARS1), and nitrogen compound metabolic processes (HAAO, MAOB, UGDH), which may indicate protein synthesis and anabolic processes. MAOB modulates neurotransmitter levels, such as serotonin, dopamine, and norepinephrine, which can influence feeding behavior (Bach et al., 1988; Oyarzún et al., 2025).

In contrast, in quail birds refed after starvation, the enriched pathways included oxidative phosphorylation, mitochondrial respiratory chain complex assembly, and glutathione metabolism, indicating potential increase in mitochondrial activity and restoration of cellular ATP production. This pattern might suggest that the liver points toward the reactivation of aerobic energy metabolism following acute nutrient deprivation, accompanied by antioxidant defenses to mitigate the reactive oxygen species generated during mitochondrial reactivation.

This study has several limitations, including the absence of statistically significant differences after multiple testing correction, potentially influenced by the relatively small sample size and within-group variability, despite the use of moderated statistical approaches. Second, the biological relevance of uniquely detected proteins should be interpreted carefully, as non-detection in one group may result from analytical limitations rather than true biological absence. Therefore, the findings should be interpreted as exploratory and hypothesis-generating, and further validation is required to confirm these observations.

Our findings revealed altered hepatic protein expression in quails after a 6-h refeeding period, highlighting the early recovery phase after nutrient deprivation. Refeeding after a low metabolizable energy diet showed pathways indicative of active substrate utilization and anabolic processes, such as glycolysis, along with the enrichment of carbohydrate derivatives and carboxylic acid metabolic processes. In contrast, refeeding after complete feed deprivation primarily stimulates energy-generating pathways, such as mitochondrial oxidative phosphorylation and glutathione-based redox defense. These distinct responses suggest that the severity of prior nutritional restriction dictates the liver's metabolic priorities during early recovery, with low-energy restriction being consistent with anabolic rebuilding and substrate processing and complete feed deprivation potentially biasing toward mitochondrial ATP production. The persistence of mitochondrial energy metabolism and protein synthesis-related pathways after refeeding suggests that the 6 h refeeding period captures an early, incomplete return to hepatic homeostasis in quails, and further analysis is needed to validate these observations.

CRedit authorship contribution statement

Doha Mohamad Khalifeh: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gabriella Gulyás:** Writing – review & editing, Visualization, Validation, Methodology, Investigation. **Renáta Knop:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Fadella Nur Almira:** Writing – review & editing, Investigation. **Mequanint Gashew:** Writing – review & editing, Investigation. **Eman Moustafa Abdelbary:** Writing – review & editing, Investigation. **Gebreawerika K. Reda:** Writing – review & editing, Validation, Investigation. **Gergő Kalló:** Writing – review & editing, Validation, Investigation, Data curation. **Csaba Szabó:** Writing – review & editing, Validation, Methodology. **Éva Csösz:** Writing – review & editing, Visualization, Validation, Data curation. **Levente**

Czeglédi: Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cbd.2026.101939>.

Data availability

The mass spectrometry proteomics data supporting this study have been deposited in the MassIVE database under accession number MSV000101916.

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