EXPLORING THE IMPACT OF SILKWORM-SUPPLEMENTED DIETS ON GENE EXPRESSION IN JAPANESE QUAIL

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I. INTRODUCTION

RNA-seq is a method utilized to investigate the transcriptome of organisms, enabling a deeper understanding of gene expression. By analysing sequences from organisms subjected to varying experimental conditions, RNA-seq studies can identify whether genes have been upregulated or downregulated as a result of the experiment. These analyses can detect alterations in gene expression and ascertain which genes were active or expressed in specific cell or tissue samples [1, 2]. The Japanese quail (*Coturnix japonica*) has become an intriguing choice for poultry species to meet the increasing demand for animal proteins [3]. While some studies have focused on RNA-seq of quail embryos, research on laying quails remains limited. The present study aims to conduct RNA-seq analysis on caecum tissue samples from Japanese quails fed with a diet supplemented with silkworm (*Bombyx mori L.)* pupa meal [4], in an effort to identify changes in gene expression attributable to the diet.

II. MATERIALS AND METHODS

Initially, 15 fastq files containing quail RNA sequences from caecum tissue samples were obtained and processed using Ion Torrent sequencing. These files were biological triplicates corresponding to three groups of laying quails differing in age. There was approximately a one-month age difference between the birds in Group 1 (3 samples) and Group 2 (6 samples), as well as between Group 2 and Group 3 (6 samples). Group 1 birds were fed commercial feed and served as the baseline. The other two groups had two conditions each: 'fed with the experimental diet' (control) and 'fed with the experimental diet containing 12% silkworm meal' (treated). The first step in the analysis involved quality control of the fastq files, during which one baseline sample was removed due to its low quality. Next, the sequences were aligned with the Japanese quail reference genome. The index was generated using the Coturnix japonica RefSeq genome (version 2.1). The sequences were subsequently aligned using bowtie2 (version 2.3.5.1) and sorted with samtool (version 1.10). The number of reads mapped to each gene was then quantified using HTSeq (version 2.0.2). These counts were utilized for gene expression analysis in R-studio (version 4.2.2) using the DeSeq2 package (version 1.38.3). Finally, the most significantly expressed genes (P-value < 0.05) were selected from each analysis, and KEGG Pathway analysis was conducted using the functional annotation tool DAVID (version 2021).

III. RESULTS AND DISCUSSION

The most significantly upregulated genes (Table 1) of the analysis are associated with metabolism and cell adhesion, while the downregulated genes are predominantly related to muscle contraction. This trend was more pronounced in Group 2, whereas Group 3 appears to have reached a more stable state, with an equilibrium in gene expression, potentially due to the transition of the animals to

adulthood and adaptation in response to the dietary changes. This observation suggests that the addition of silkworm meal to the diet may have a more significant impact on younger quails leading to noticeable alterations in gene expression.

Table 1 Significantly enriched molecular pathways of upregulated genes

KEGG Pathway Term	Genes involved	Expression
Cell adhesion molecules	OCLN, CLDN3, CLDN7, CLDN23, F11R, PTPRF	Upregulated
Tight junction	OCLN, CLDN3, CLDN7, CLDN23, ERBB2, CGN, EZR, F11R	Upregulated
Biosynthesis of nucleotide sugars	MPI, UAP1	Upregulated
Metabolic pathways	CA9, CAD, CANT1, CHAC1, DGAT2, DGKA, DGKD, DNMT3B, ELOVL6, ELOVL7, FDPS, FLAD1, FTCD, GALNT6, GBA2, GCDH, GPX2, MARS1, MPI, PDE4C, SHMT1, UAP1	Upregulated
Ribosome biogenesis in eukaryotes	NOB1, IMP3, UTP18	Upregulated
Fructose and mannose metabolism	FCSK, ALDOC	Upregulated

Moreover, it is important to note that, among the significantly upregulated genes, GBA2, MPI, FCSK, and ALDOC are involved in the metabolism of sugars. This increase could potentially be attributed to the presence of the molecule 1-Deoxynojirimycin (1-DNJ) in the silkworm, which originates from their alkaloid-rich diet [4,5]. 1-DNJ acts as a competitive inhibitor of alpha-glucosidase enzymes which are involved in the breakdown of disaccharides and monosaccharides [4,6], and the observed upregulation is likely a response to the reduced glucose absorption in quails. To ensure the robustness and validity of the conclusions drawn in this study, it is essential to conduct further investigations incorporating a larger number of RNA-seq samples collected from diverse experimental conditions.

IV. CONCLUSION

The findings of this study provide valuable insights into the gene expression changes that occur in quails when fed a silkworm-supplemented diet. The upregulation of genes related to metabolism and cell adhesion, and the downregulation of genes involved in muscle contraction, highlight the potential physiological and biochemical adaptations that quails undergo in response to their diet. The significantly upregulated genes involved in sugar metabolism can be potentially due to the presence of DNJ in the silkworm. Further research is necessary to confirm these findings and to explore the long-term effects of silkworm supplementation on quail health and development.

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