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## Duodenum transcriptome profile of laying hens submitted to different levels of calcium and phosphorus in the diet

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### ABSTRACT

Calcium (Ca) and phosphorus (P) are essential minerals required to animal's health, especially in laying hens, since they are important components for bone structuring and eggshell formation. Diets with inadequate levels tend to interfere with their absorption and digestibility, resulting in eggshell quality losses and reduced productive life, affecting production and welfare. Therefore, this study evaluated the intestinal gene expression profile through transcriptome analysis of laving hens that received different levels of Ca and P in the diet for 50 weeks. Out of nine groups studied, two were chosen for RNA-Seq analysis based on performance traits, such as egg quality and production: 1) with highest (4.71% Ca and 0.21% P) and 2) with lowest performance (3.29% Ca and 0.49% P), totaling 18 duodenum samples (9 samples/group). Total RNA was extracted, and libraries prepared using the Illumina Stranded mRNA Prep kit with a paired-end sequencing. Reads were submitted to quality control using Trimmomatic, mapped against the reference genome (GRCg6a) using STAR and statistical analysis performed using the limma package in R. A total of 105 genes were differentially expressed (DE, FDR < 0.05) in the duodenum in response to the two dietary Ca:P ratios. Using the MSigDB database for functional annotation, 25 genes were enriched in the biological processes: epithelial mesenchymal transition, myogenesis and delayed response to estrogen. Out of DE genes, we highlight the WIPF1, PTHLH, MYLK, TAGLN, EMP2, CAV1, ADIPOQ and CAB39L that are related to the regulation and absorption of minerals. None of these genes have been previously associated with Ca:P regulation and their identification could help understanding the complex mechanisms involved with the dietary modulation and efficient use of these minerals in the duodenum of laying hens.

KEYWORDS: RNA sequencing; chicken; differentially expressed genes

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