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Camelina sativa as an emerging sustainable feedstuff for broiler quails (*Coturnix japonica*): In-depth exploration of the impacts on early post-mortem muscle using shotgun proteomics and bioinformatics

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I. INTRODUCTION

Camelina sativa (CS) is an oilseed crop of the *Brassicaceae* family native to Europe and Southwest Asia. The potentiality of CS relies on its nutritional composition, being rich in proteins (24.5-30%) and lipids (36.5-40.2%) including beneficial omega-3 fatty acids, and on its environmental sustainability. For these reasons, the CS cake is considered as a promising by-product for poultry diets [1]. However, current knowledge on its impact on the muscle and meat traits is unknown. Therefore, this study aimed to explore for the first time the effects of a CS cake incorporated in the diet of broiler quails (*Coturnix japonica*) on their muscle proteome using a shotgun proteomics approach and bioinformatics.

II. MATERIALS AND METHODS

The *in vivo* trial involved 180 of 15-day old broiler quails. The experiment consisted of three dietary treatments (6 replicated cages/treatment and 10 quails/cage): a control diet, consisting in standard growing-fattening diet (0%), and two diets formulated to include 5% and 10% of the CS cake from the ALAN line, genetically improved for a reduced content of glucosinolates [2]. Diets were provided *ad libitum* for 20 days in mash form. After slaughter and within 20 min post-mortem, breast (*Pectoralis major*) meat samples from n=6 male quails/treatment (n=1/replicated cage) were sampled and frozen at -70 °C. For proteomics, total proteins from 150 mg of frozen tissue were extracted as previously described [3]. The protein extracts were used to prepare protein bands using one-dimensional SDS-PAGE for shotgun proteomics using LC-MS/MS [4]. The proteome database (filtering criteria of 2 unique peptides, 10% coverage score and an FDR of 1%) was analyzed using several approaches. For statistical analyses: 1) Partial Least Square-discriminant analysis (PLS-DA), 2) heatmap hierarchical analyses and 3) pairwise comparisons using volcano plot (1.2-fold change and *p*-value 0.05) to identify the differentially abundant proteins (DAPs). For bioinformatics: pathway enrichment analysis (Gene Ontology - GO) using Metascape® as described by Gagaoua *et al.* [5].

III. RESULTS AND DISCUSSION

The comparison of the muscle proteome of quails fed with different inclusion levels (0%, 5%, 10%) of CS cake are given in Figure 1. The PLS-DA discriminated the three treatment groups (Figure 1A). This indicates that the dietary treatment had a remarkable effect on the quail muscle proteome, with the inclusion level being a key factor in explaining the observed differences. The clear discrimination of the treatments was further evidenced at the individual level as depicted in the statistical heatmap (Figure 1B). The overlap analysis in terms of number of proteins that were changed across the groups revealed higher number in the 10% inclusion level than 5%, both higher compared to 0% (Figure 1C). A significant number of proteins were commonly changing among the treatments. This allowed to explore and compare the molecular pathways to which the proteins belong (Figure 1D) for each comparison, focusing on the top 20 GO enriched terms. Briefly, ten GO terms were common to the three comparisons, but more importantly enriched in the control-ALAN 10% comparison. This suggests a link with the CS inclusion in the feed. Interestingly, CS showed a remarkable up-regulatory effect of pathways encoding for endomembrane system organization, lipid biosynthetic process, mRNA metabolic process, Golgi vesicle transport, ribonucleoprotein complex biogenesis, peptidyl-amino acid modification, amide biosynthetic process, response to wounding and protein catabolic process. The results depicted dynamic changes in the muscle proteins, from which certain pathways such as lipid biosynthetic are in line with the chemical properties of CS cake. The changes in the muscle proteome of quail would have consequences on the nutritional and meat quality properties, which need to be investigated though the correlation of the DAPs with intrinsic meat quality traits.

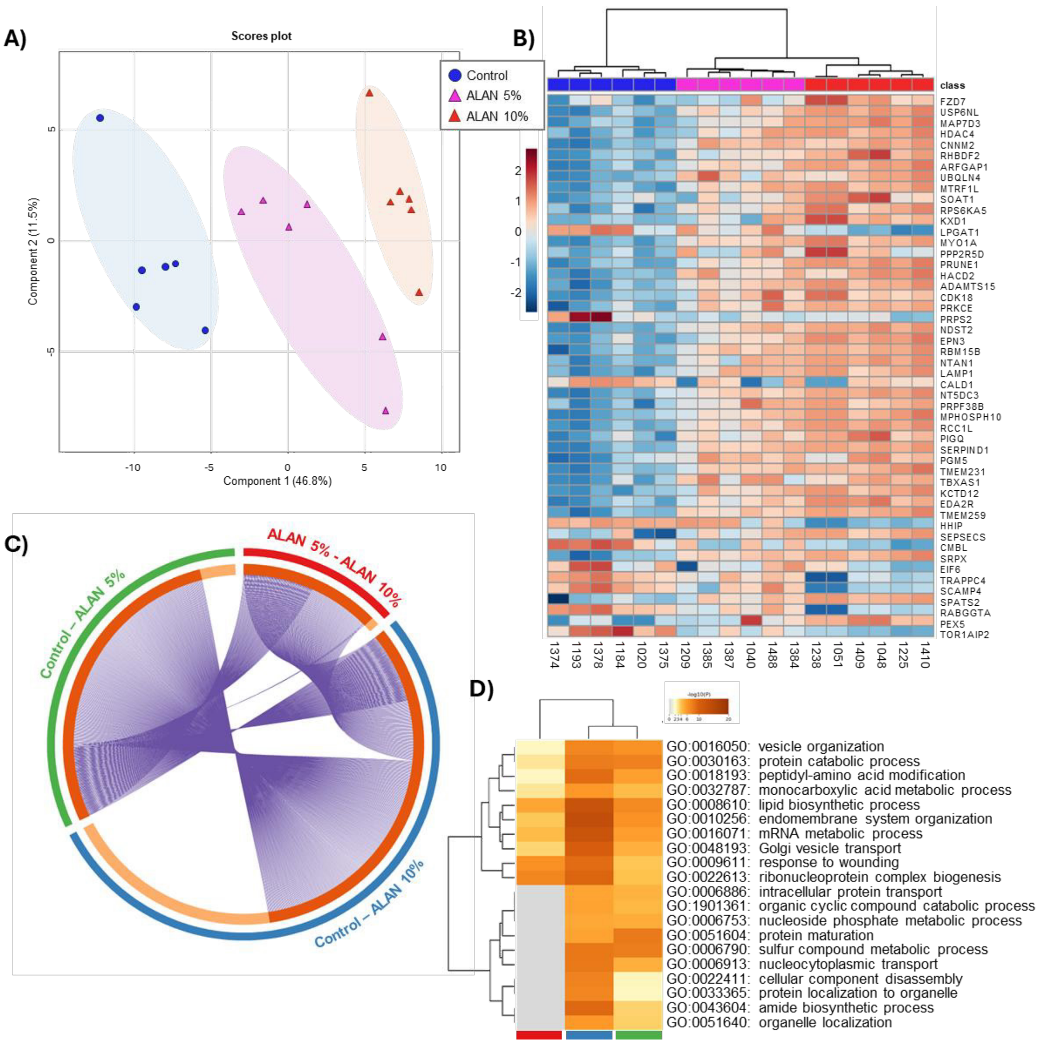

Figure 1. Comparison of quail muscle proteome between the control and after 5% and 10% inclusion of CS cake. **A)** PLS-DA score plot; **B)** Heatmap visualization and dendrogram; **C)** Circos plot depicting the degree of overlap in the proteins across the three comparisons; **D)** Hierarchical Heatmap clustering comparing the degree of enrichment in the molecular pathways using the top 20 significantly enriched GO terms.

IV. CONCLUSION

The findings evidenced that dietary inclusion of CS cake into quail's diet induced dynamic changes in the muscle proteome, which seemed to be directly depended on the inclusion level of the feedstuff.

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