Proteomic analysis reveals change in the lipid metabolism of muscle in beef cattle submitted to early weaning and pasture finished

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I. INTRODUCTION

Early weaning and postnatal nutritional management are crucial strategies in livestock production systems and can affect long-term beef cattle growth [1]. This study aimed to evaluate the effect of early weaning associated with a post-weaning supplementation on the muscle proteome of cattle pasture finished system.

II. MATERIALS AND METHODS

This experiment was approved by the Ethics and Animal Welfare Committee of Sao Paulo University (CEUA 0190/2020). Forty male Nellore calves were submitted into one of two treatments: early weaning (EW) – 120 days; and conventional weaning (CW) 205 days. The EW group was supplemented with 1.5% of BW of commercial concentrate diet until 205 days. On 205 days, both groups were combined in a single group and fed 0.3% protein + energy supplementation for 488 days. During the finishing phase (222 days) all the animals were raised on pasture and supplemented (0.5% BW). All animals were subjected to *Longissimus thoracis* muscle biopsy 24 hours before slaughter. Procedures for proteomics analysis have been previously described by Osorio [2]. Protein identification and quantitation were performed by nanoLC-MS/MS using an Ultimate 3000 liquid chromatography system coupled to a Q-Exactive Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany). Raw data were processed using the Proteome Discoverer software (Thermo Scientific, Bremen, Germany. Protein identification analysis was performed using the UniProt protein sequence database data for the *Bos Taurus* Proteome. The number of unique peptides was set to two (minimum) per protein identified and each protein had to be identified in at least half the samples in each experimental condition. Imputation of missing data was applied to normalized and clean data using KNN method and differentially abundant proteins (DAP) were prospected using Fold Change ($FC < 0.67$ or $FC > 1.5$) and significance levels of Empirical Bayesian Test ($p\text{-value} < 0.05$) using *DEP* [3] package in R. The functional KEGG pathways enrichment was performed by over-representation analysis (ORA) from the list of DAP using *enrichR* [4] and *ClusterProfiler* [5] packages in R.

III. RESULTS AND DISCUSSION

A total of 2784 protein identifications were detected in the muscle *Longissimus thoracis* of Nellore following the LC-MS analysis, and 1546 proteins were selected after filtration. There were 118 differentially abundant proteins (DAP) between EW vs. CW, where 62 proteins were up regulated in the EW treatment. The results are presented in Figure 1A. These include protein such as Short-chain specific acyl-CoA dehydrogenase (ACADS), and Long-chain specific acyl-CoA dehydrogenase (ACADL). On the other hand, other proteins such as Carnitina O-palmitoiltransferase 1 (CPT1B) and, Integrin-linked protein kinase (ILK) were down-regulated in the EW treatment.

Regarding the DAP´s an R analysis was conducted, which found that treatments impacted mainly in the pathways such as Biosynthesis Fatty acid degradation. The interaction network between the DAPs indicated that the proteins CPT1B, ILK as associated with PPAR signaling pathway. The CPT1B is a gene member of the CPT family that is related to β-oxidation [6]. In addition, the ACADS and ACADL were associated with Fatty acid metabolism and Fatty acid degradation. The ACADS is a member of the acyl-CoA dehydrogenase family of enzymes, and it is related to maintenance of energy homeostasis by β-oxidation [7].

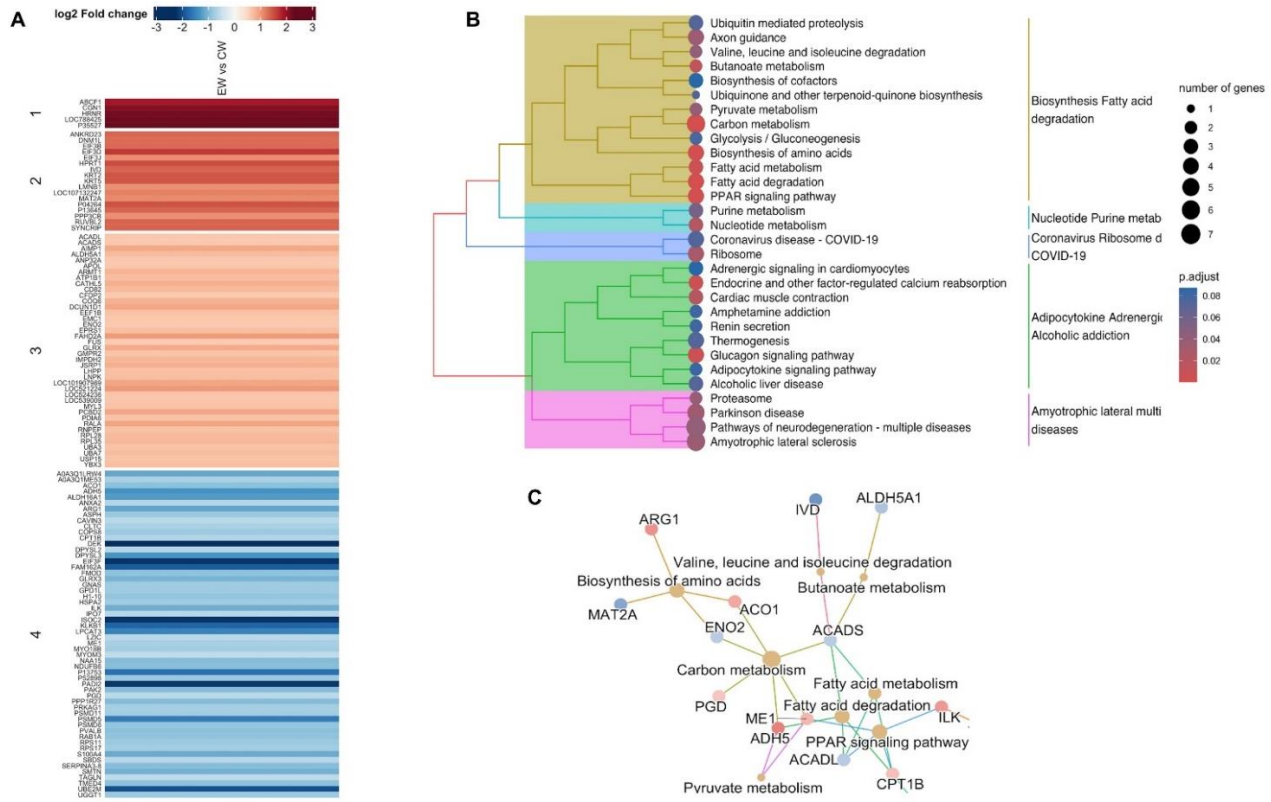

Figure 1. (**A**) Heatmap of differentially abundant proteins. (**B**) Heatmap of enriched terms obtained for the differentially abundant proteins obtained in the comparisons between EW vs. CW. (**C**) Protein interaction network content de most of differentially abundant proteins in the *Longissimus thoracis* muscle related to first pathways cluster (beige) between EW vs. CW.

IV. CONCLUSION

The present study indicates that early weaning alters the *Longissimus thoracis* proteome of cattle pasture finished system. Our data suggest that this strategy acts directly on the abundance of proteins related to the lipid metabolism pathway. The early weaning presented down regulated protein related to β-oxidation but also protein related to fatty acid degradation.

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