Santana, M., Brito, L., Carvalho, F., Polizel, G., Fernandes, A., Furlan, E., Antonelo, D., Rocha, A., Ferraz, J. (2026) Longissimus muscle lipidomics profile of Nellore cattle submitted to fetal programming, *Proceedings 13th World Congress on Genetics Applied to Livestock Production* 24398: https://doi.org/10.31274/wcgalp.23498
©2026 *Santana, et al.* CC BY-NC-ND

Longissimus muscle lipidomics profile of Nellore cattle submitted to fetal programming

M.H.A. Santana[1*], L.F. Brito[2], F.E. Carvalho[1], G.H.G. Polizel[1], A.C. Fernandes[1], É. Furlan[1], D.S. Antonelo[3], A.O. Rocha[2], J.B.S. Ferraz[1]

[1]University of São Paulo, Pirassununga, SP, Brazil; [2] Purdue University, West Lafayette, IN, USA; [3]Lipid Marker-Omics Science, Leme, SP, Brazil; [*]Corresponding author: mhasantana@usp.br

Introduction

Maternal nutrition during critical windows of developmental plasticity can exert lasting effects on skeletal muscle growth and maturation, hallmarks of fetal programming within the Developmental Origins of Health and Disease (DOHaD) framework (Caton et al., 2025). Early-life cues influence myofiber number and type, mitochondrial biogenesis, intramuscular adipogenesis, connective-tissue deposition, and postnatal growth in ruminants, with downstream consequences for carcass composition and meat quality traits (Du et al., 2010).
Accordingly, prenatal-nutrition effects should be amenable to capture by lipidomics, with fetal programming leaving specific signatures in membrane classes and bioactive species that serve as sensitive readouts of nutritional status (Safain et al., 2025). These mechanisms imply that subtle changes in mitochondrial membranes and bioactive lipids can reverberate through skeletal muscle physiology and postmortem stability (Decker & Funai, 2024). Additionally, during early gestation, maternal hepatic lipid classes exhibited robust alterations whereas fetal programming related effects in the fetus were minimal, supporting the hypothesis that fetal signatures are often small or modest and difficult to detect in isolation (Safain et al., 2025).
Therefore, because the effects are often small and distributed, adopting approaches that quantify lipid species and classes and provide functional interpretation, with an explicit focus on membrane lipids and bioactive mediators, and comparing different nutritional levels in prenatal nutrition may enable detection of small, coordinated changes in the multivariate signature (Idkowiak et al., 2023). Likewise, maternal manipulations early in gestation can remodel tissue-specific windows in adipose tissue that are sensitive to physiological variation (Du et al., 2022). Thus, the main objective of this study was to characterize the skeletal muscle lipidome in Nellore progeny subjected to different gestational nutritional levels.

Materials & Methods

A total of 28 young Nellore bulls (*Bos taurus indicus*), born to nulliparous heifers that underwent fixed-time artificial insemination using sex-sorted semen from a single Nellore sire, were produced under two gestational nutritional treatments. Heifers were randomly assigned to treatments after stratification by body weight (BW) and body condition score. Treatments consisted of either mineral supplementation (0.3 g/kg BW; Control, NP; n = 14) or protein–energy supplementation (5 g/kg BW; Supplemented, FP; n = 14). Throughout gestation, heifers were managed in treatment-specific paddocks with *Urochloa brizantha cv*. Marandu pasture and received the assigned mineral or protein–energy supplement once daily.
After parturition, cows and their offspring (regardless of the prenatal nutrition group), were managed together on a single Marandu pasture (*Urochloa brizantha cv.* Marandu) with *ad libitum* access to a mineral supplement until weaning at 8 months of age. Following weaning, calves were raised on pasture for 12 months and finished in a feedlot, with the bulls housed in individual pens 12 m² for 97 days. After feedlot finishing, bulls were slaughtered at the Campus Fernando Costa Slaughterhouse (Pirassununga, SP, Brazil), located ~500 m from the feedlot.

Animals weighed 566±55.7 kg BW in slaughter. Stunning was performed with a percussive captive bolt, followed by jugular vein incision for exsanguination, in accordance with humane slaughter practices set forth in the Regulation for Health Inspection and Industrialization of Products of Animal Origin.

Approximately 50 mg of each skeletal muscle sample collected at 24 h postmortem was cryo-ground in liquid nitrogen for lipid extraction using the method proposed by Bligh & Dyer (1959). Targeted lipid profiling was performed using discovery MRM-profiling strategies and instrumentation as reviewed by Xie et al. (2021) and was used to interrogate 3,437 MRMs spanning 12 lipid classes. The transition set comprised 1,752 triacylglycerols (TG), 482 diacylglycerols (DG), 179 free fatty acids (FFA), 178 phosphatidylcholines (PC), 145 phosphatidylethanolamines (PE), 141 phosphatidylinositols (PI), 141 phosphatidylglycerols (PG), 139 phosphatidylserines (PS), 120 ceramides (CER), 84 acylcarnitines (CAR), 42 sphingomyelins (SM), and 34 cholesteryl esters (CE). TGs were monitored using precursor ions and product ions diagnostic of specific fatty-acyl residues (C16:0, C16:1, C18:0, C18:1, C18:2, and C20:4).

Lipidome data were uploaded to MetaboAnalyst 6.0 (www.metaboanalyst.ca/; Pang et al. (2024) and data were Pareto-scaled prior to statistical and bioinformatics analyses. Volcano plot analysis was performed and lipids with a fold change (FC) ≥ 1.5 or ≤ 0.66 and P-value ≤ 0.05 were considered as differently abundant (DAL) between treatments at 24 h postmortem. Differences were considered statistically significant when P-value ≤ 0.05. Unsupervised (principal component analysis [PCA]) and supervised (partial least square discriminant analysis [PLS-DA]) analyses were also performed to assess potential clustering between the groups. The statistical significances of the group patterns in the PCA were evaluated using PERMANOVA. The PLS-DA validation was performed using a LOOCV cross validation method, and the values of R^2 (cumulative interpretation ability of model), Q^2 (predictive ability of model) and accuracy were employed as initial indicators for evaluating the goodness of fit of the models. In the PLS-DA, the variable importance in projection (VIP) was used to rank the lipids based on their importance in discriminating groups. Based on VIP scores, compounds that contributed most (VIP > 1.0) to the overall model separation and the classification accuracy in the PLS-DA plot between treatments were highlighted.

A pathway-based enrichment analysis from relational database of metabolomics pathway (RaMP-DB), which integrates 3,694 metabolite and lipid pathways from KEGG via Reactome and WikiPathways, was performed by using the DAL identified in the volcano plot (FC ≥ 1.5 or ≤ 0.66 and P ≤ 0.05) and the lipids with VIP > 1.0 in the PLS-DA to identify the altered enriched pathways (P < 0.05) related to fetal programming effects.

Results

The targeted lipidome profiling initially scanned 3,437 ion transitions (MRMs) in a pooled sample for identifying the detectable lipids, in which 408 had intensities of at least 1.3-fold higher than the control sample and were used for interrogating individual samples (n = 7 per treatment). These compounds were related mainly to TG (192), PC (112), PE (41), and SM (36) lipids. The statistical comparison of the lipid profiles in the LT muscle lipidome from NP or FP animals revealed 10 DALs, in which 9 were up-regulated and 1 was down-regulated in NP animals (Figure 1). Moreover, there were no differences for carbon chain length and degree of unsaturation of the TG between NP and FP carcasses.

The PCA showed no segregation between the treatments (P = 0.41). The PLS-DA (Figure 2A) also segregated the treatments, in which presented a modest predictive ability of the model (Accuracy = 0.50; $Q^2 = 0.76$; $R^2 = 0.16$). Based on VIP scores (Figure 2B), compounds (n = 46) that contributed most (VIP > 1.0) to the overall model separation and the classification accuracy in the OPLS-DA plot between treatments were highlighted.

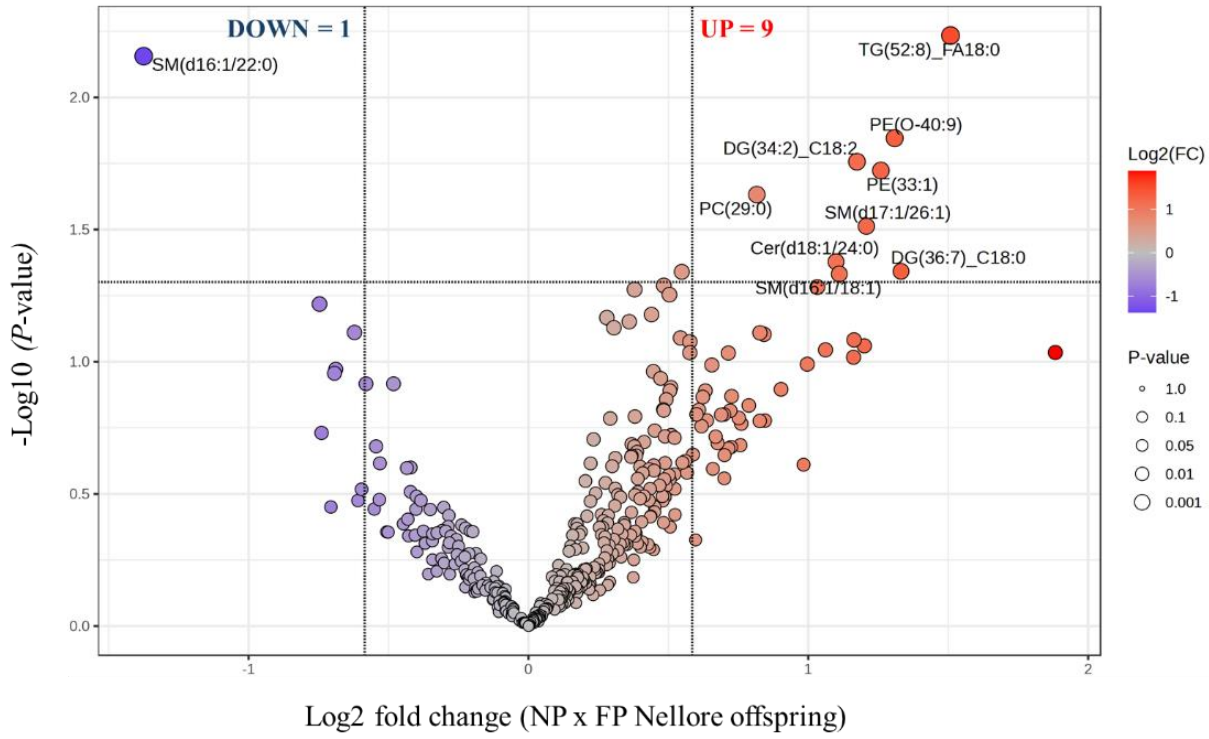

Figure 1. Volcano plot analysis (P-value ≤ 0.05; fold change ≥ 1.5 or ≤ 0.66) of lipidome profile from non-programmed (NP) and fetal-programmed (FP) Nellore offspring.

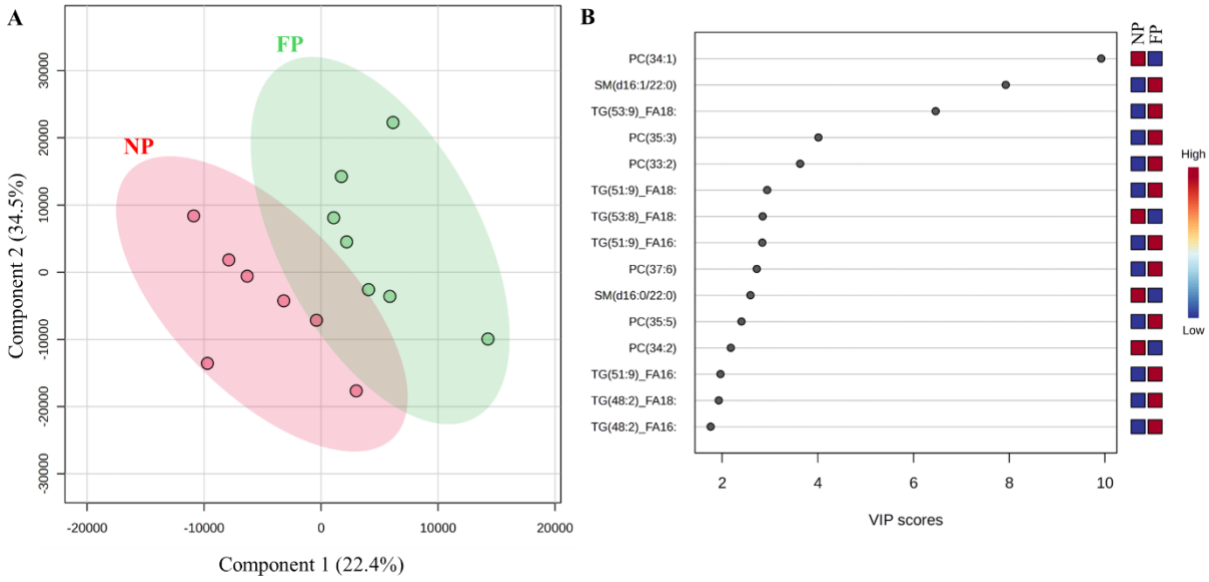

Figure 2. Partial least square discriminant analysis (PLS-DA) score plot (A) and top 15 variable importance of projection (VIP) scores in the PLS-DA (B) of lipidome from Nellore offspring.

The pathway-based enrichment analysis revealed twenty significant pathways between NP and FP Nellore offspring, which included hydrolysis of LPC (P-value = 0.004), biosynthesis of unsaturated fatty acids (P-value = 0.007), beta-oxidation of pristanoyl-CoA (P-value = 0.009), toll-like receptor cascades (P-value = 0.017), and phospholipid metabolism (P = 0.017). Interestingly, most of the enriched pathways were affected by LPC(18:1) (P-value = 0.129; FC

= 1.21; VIP score = 1.36) or CAR(2:0) (P-value = 0.167; FC = 1.80; VIP score = 1.02), even though their individual concentrations were not significantly different between treatments.

Discussion

Cattle that were FP through maternal supplementation during gestation exhibited lower abundances of some PE, PC, DG, TG, SM and CE lipids, while showing higher levels of SM(d16:1/22:0) compared with NP animals. These lipidomic alterations suggest that prenatal nutritional supplementation induced persistent metabolic adaptations that optimized lipid utilization, membrane composition, and mitochondrial function. Maternal nutrition during critical stages of gestation has been shown to influence epigenetic regulation and expression of genes related to lipid metabolism, mitochondrial activity, and myogenesis (Du et al., 2010). Such early-life programming can lead to long-term modifications in postnatal metabolism, tissue composition, and muscle lipid architecture (Gionbelli et al., 2018).

In this study, the lower concentrations of PE(O-40:9), PE(33:1) and PC(29:0) observed in FP animals may indicate more efficient mitochondrial function and reduced oxidative membrane damage postnatally. These phospholipids are major constituents of biological membranes and are dynamically remodeled in response to oxidative stress and mitochondrial turnover (Wang & Tontonoz, 2019). This interpretation is supported by evidence that improved maternal nutrition enhances fetal antioxidant capacity and mitochondrial development, mitigating oxidative stress in skeletal muscle (Zou et al., 2016).

Therefore, NP animals appear to rely on phospholipid degradation and carnitine-mediated acetyl flux as adaptive responses, whereas FP animals promote stable lipid homeostasis, efficient β-oxidation, and improved redox balance. Thus, these results reinforce the concept that prenatal nutrition can reprogram lipid metabolism and mitochondrial dynamics, influencing postnatal muscle bioenergetics and potentially beef quality.

Conclusions

We established a mechanism-aware framework to characterize the skeletal-muscle lipidome of Nellore offspring exposed to prenatal nutrition. This characterization provides a coherent basis for subsequent targeted validation and for translating lipidomic readouts into actionable metrics for herd management and product quality, thereby positioning lipidomics as a practical tool to monitor and understand FP-related variation in beef production.

Acknowledgements

This research was funded by the São Paulo Research Foundation (FAPESP), grant number 17/12105-2, and the National Council for Scientific and Technological Development (CNPq), grant number 304968/2024-2.

References

Bligh E.G. and Dyer W.J. (1959). Can. J. Biochem. Physiol. 37:911–917. https://doi.org/10.1139/o59-099
Caton J.S., et al. (2025). J. Dairy Sci. 108: 7696–7709. https://doi.org/10.3168/jds.2024-25788
Decker S.T. and Funai K. (2024). Cell Metabolism 36, 1963–1978. https://doi.org/10.1016/j.cmet.2024.07.024
Du L., et al. (2022). Genes 14: 37. https://doi.org/10.3390/genes14010037
Du M. et al. (2010). J. Animal Sci., 88(13 elect suppl), E51–E60. https://doi.org/10.2527/jas.2009-2311
Idkowiak J. et al. (2023). Analyt. Bioanalyt. Chem. 415: 935–951. https://doi.org/10.1007/s00216-022-04490-w
Pang Z. et al. (2024). Nucleic Acids Research, 52(W1), W398–W406. https://doi.org/10.1093/nar/gkae253
Safain K.S. et al. (2025). Metabolites, 15(5), 302. https://doi.org/10.3390/metabo15050302
Xie Z., Ferreira C.R. Virequ A.A., Cooks R.G. (2021). Chemistry and Physics of Lipids 235, 105048. https://doi.org/10.1016/j.chemphyslip.2021.105048