The PI3K-Akt signaling pathway plays an important role in the differentiation of adipocytes promoting the adipogenesis in crossbred calves supplemented with vitamin A

Chardulo, L. A. L.[1*], Baldassini, W. A.[1], Losi Filho, A. [1], Costa, C. E. [1], Curi, R. A.[1], Machado-Neto, O. R.[1], Ladeira, M. M.[2], Pereira, G. L.[1]

[1] São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science, Botucatu, Brazil

[2] Federal University of Lavras (UFLA), Lavras, Minas Gerais, Brazil

*Corresponding author email: luis.artur@unesp.br

I. INTRODUCTION

In Brazil, with the increased use of crossbreeding with *Bos taurus* in meat production, the amount of intramuscular fat (IMF) has received greater attention from consumers and meat industry, since zebu or *Bos indicus* animals (predominant in the country) produce meat with little or even no marbling [1]. With higher levels of IMF content (or marbling), the tenderness, juiciness, and flavor of the meat can be improved [2]. The vitamin A supplementation in young cattle has drawn interest due to its capacity to modulate adipogenesis, as evidenced [3]. This nutritional intervention holds potential for enhancing IMF content. The objective of this study is to identify the signaling pathways of adipogenesis in crossbred *Bos taurus* × *Bos indicus* calves supplemented with vitamin A and finished in feedlot.

II. MATERIALS AND METHODS

Thirty-four F1 Montana × Nellore male calves were used (17 without vitamin A [Control] and 17 with vitamin A [VitA]). At birth, VitA calves received a intramuscular dose of 300,000 IU of Vitamin A, while Control calves received a placebo. At 270 days all animals were weaned and feedlot finished for 180 days. Experimental groups were slaughtered with final body weights of 398 and 415 kg ± 11 kg - Control and VitA, respectively. The intramuscular fat (IMF) content of meat was assessed by infrared spectroscopy in a FoodScan™ equipment (FOSS, Denmark). Total RNA from the *Longissimus thoracis* (LT) samples (n=6/ group) was extracted individually from 100 mg of LT using TRIzol® (Life Technologies, USA), according to the manufacturer's instructions, and analyzed on the Bioanalyzer 2100® (Agilent, USA). The RNA libraries for each sample were prepared using the TruSeq RNA Sample Preparation Kit (Illumina, USA) from 2 µg of total RNA, according to manufacturer's instructions. Finally, sequencing was carried out on the Illumina NextSeq550® (Illumina, USA) in order to produce paired-end reads of 100 bp. The genes with differential expression as a function of VitA treatment were identified according to their biological function and subsequently categorized into functional groups using *enrichR* and *ClusterProfiler* packages in R.

III. RESULTS AND DISCUSSION

Differences ($P < 0.05$) were found in IMF content of meat, with the Control group exhibiting 2.57% while the VitA group showed 4.10% (SEM = 0.28). Such differences in IMF content can be explained by adipogenic and lipogenic pathways upregulated in response to vitamin A (Table 1; and Figure 1). Adipogenesis involves a cascade of transcription factors that regulate the expression of genes involved in adipocyte development. For example, the phosphatidylinositol 3-kinase (PI3K)-Akt pathway plays an important role in adipocyte differentiation, promoting adipogenesis through the phosphorylation of certain substrates [4]. Studies have investigated the association of the FoxO, PI3K-Akt and cAMP pathways as regulators of glycolytic [5] and lipid metabolism [6], which help to explain greater IMF observed in animals from VitA treatment in the current study. However, the regulation mechanisms of some genes expressed in the PI3-Akt pathway for IMF deposition in cattle are still poorly understood.

Table 1. Absolute and relative numbers of skeletal muscle genes of Montana × Nellore male calves.

Contrast[1]	Total of DEGs[2]	Expression[3]	Absolute	Relative (%)
VitA vs. Control	165	Down	106	0.75
		NS	13.911	98.82
		Up	59	0.42

[1] VitA = calves suplemented with 300,000 IU of Vitamin A; Control = non vitamin A suplemented;

[2] DEGs = diferencially expressed genes obtained by the likelihood ratio test; A $\log_2$ fold change of 0.5 and significance adjusted to false discovery rate [FDR] < 0.05 were adopted to identify DEGs.

[3] Down = down-regulated; Up = up-regulated; NS = non-significant

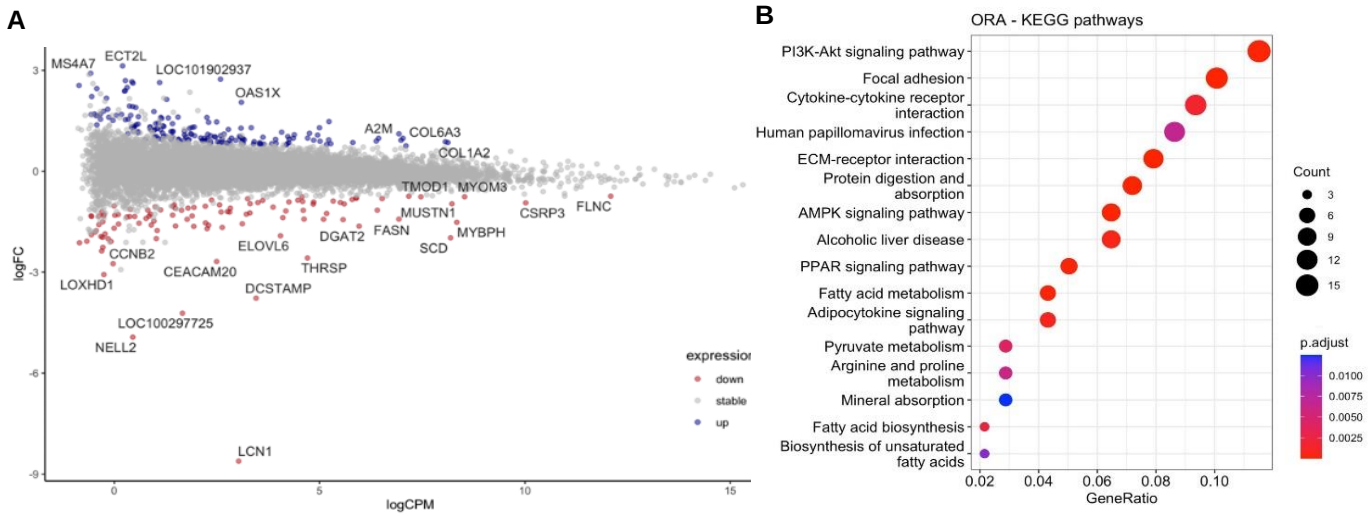

Figure 1. Differentially expressed genes (DEG; *P*-value > 0.05 and logFC > 0.5). Up-regulated in blue and down-regulated in red (**A**). Enriched metabolic pathways (KEEG) in VitA versus Control treatment (**B**).

In agreement with previous studies [3, 7, 8], upregulating *PCK2* and *PIK3R3* genes in lipid metabolism pathways increased IMF content. Notably, the present study reveals changes in these genes associated with the PI3-Akt pathway following VitA treatment. Specifically, the upregulation of *PCK2* potentially disrupts the balance of energy metabolism pathways, thus impacting lipid metabolism dynamics. Furthermore, greater expression of *PIK3R3* likely enhances PI3K activity, thereby modulating downstream signaling cascades involved in lipid metabolism and cellular growth. These findings shed light on the molecular mechanisms driving changes in IMF content and suggest a potential regulatory role for VitA in cattle lipid metabolism.

IV. CONCLUSION

The differences observed in the deposition of IMF in the LT muscle of Montana × Nellore crossbred calves were related to genes and pathways of lipid metabolism. Our study presents novel evidence suggesting that the PI3K-Akt signaling pathway may serve as a crucial regulator in adipocyte differentiation, thereby promoting adipogenesis and lipogenesis in crossbred calves receiving vitamin A supplementation (intramuscular dose) at birth.

ACKNOWLEDGEMENTS

FAPESP - Grant Nos. 2019/14572-2 and 2020/11300-9, CAPES and CNPq.

REFERENCES

[1] Chardulo, L.A.L.; Silveira, A.C.; Vianello, F. (2013) Analytical aspects for tropical meat quality assessment. In Lima, J.P., Vianelo, F., Food Quality, Safety and Technology pp. 53-62. Springer-Verlag Wien.

[2] Frank D, Kaczmarska K, Paterson J, Piyasiri U, Warner R. Meat Sci. 2017;133:61-68. doi.org/10.1016/j.meatsci.2017.06.006

[3] Wang W, Xue W, Xu X, Jin B, Zhang X. Czech J. of Anim. Sci. 2016; 61:333-339. doi.org/ 10.17221/85/2015-CJAS

[4] Wang L, Zhang S, Cheng G, Mei C, Li S, Zhang W, Zan L. Genomics. 2020. 112:2688-2694. doi.org/10.1016/j.ygeno.2020.02.020

[5] Xiaoyun w, Zhou X, Chu M, Guo X, Pei J, Xiong L et al. Meat Sci. 194:108948. doi.org/10.1016/j.meatsci.2022.108948

[6] Junjvlieke Z, Khan R, Mei C, Cheng G, Wang S, Raza S H A, Zan L. Genomics. 2020. 112:2282-2290. doi.org/10.1016/j.ygeno.2019.12.024

[7] Fassah D M, Jjeong J Y, Baik M. Asian-Australasian J. of Anim. Sci. 2018. 31:537-547. doi.org/10.5713/ajas.17.0875

[8] Raza S H A, Khan R, Cheng G, Long F, Bling S, Easa A A, Zan L. Int. J. of Biological Macromolecules. 2022. 195:198-206. doi.org/10.1016/j.ijbiomac.2021.11.202