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ORIGINAL ARTICLE

Transcriptome profile of skeletal muscle using different sources of dietary fatty acids in male pigs

Simara Larissa Fanalli[1] · Bruna Pereira Martins da Silva[1] · Julia Dezen Gomes[2] · Vivian Vezzoni de Almeida[3] · Gabriel Costa Monteiro Moreira[4] · Bárbara Silva-Vignato[2] · Juliana Afonso[5] · Felipe André Oliveira Freitas[2] · James Mark Reecy[6] · James Eugene Koltes[6] · Dawn Koltes[6] · Dorian Garrick[7] · Luciana Correia de Almeida Regitano[5] · Júlio César de Carvalho Balieiro[8] · Gerson Barreto Mourão[2] · Luiz Lehmann Coutinho[2] · Heidge Fukumasu[1] · Mirele Daiana Poleti[1] · Severino Matias de Alencar[2] · Albino Luchiari Filho[2] · Aline Silva Mello Cesar[1,2]

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Abstract

Pork is of great importance in world trade and represents the largest source of fatty acids in the human diet. Lipid sources such as soybean oil (SOY), canola (CO), and fish oil (FO) are used in pig diets and influence blood parameters and the ratio of deposited fatty acids. In this study, the main objective was to evaluate changes in gene expression in porcine skeletal muscle tissue resulting from the dietary oil sources and to identify metabolic pathways and biological process networks through RNA-Seq. The addition of FO in the diet of pigs led to intramuscular lipid with a higher FA profile composition of C20:5 n-3, C22:6 n-3, and SFA (C16:0 and C18:0). Blood parameters for the FO group showed lower cholesterol and HDL content compared with CO and SOY groups. Skeletal muscle transcriptome analyses revealed 65 differentially expressed genes (DEG, FDR 10%) between CO *vs* SOY, and 32 DEG for CO *vs* FO, and 531 DEG for SOY *vs* FO comparison. Several genes, including *AZGP1, PDE3B*, *APOE, PLIN1,* and *LIPS*, were found to be down-regulated in the diet of the SOY group compared to the FO group. The enrichment analysis revealed DEG involved in lipid metabolism, metabolic diseases, and inflammation between the oil groups, with specific gene functions in each group and altered blood parameters. The results provide mechanisms to help us understand the behavior of genes according to fatty acids.

Keywords Pig model · RNA-Seq · Metabolic diseases · Skeletal muscle · Differentially expressed genes · Soybean oil

✉ Aline Silva Mello Cesar
alinecesar@usp.br

1 Faculty of Animal Science and Food Engineering, University of São Paulo, São Paulo, FZEAPirassununga, Brazil

2 Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo, Piracicaba, São Paulo, Brazil

3 College of Veterinary Medicine and Animal Science, Federal University of Goiás, Goiânia, Goiás, Brazil

4 GIGA Medical Genomics, Unit of Animal Genomics, University of Liège, Liège, Belgium

5 Brazilian Agricultural Research Corporation, Embrapa Pecuária Sudeste, São Paulo, São Carlos, Brazil

6 Animal Science Department, Iowa State University, Ames, IA, USA

7 AL Rae Centre for Genetics and Breeding, Massey University, Hamilton, New Zealand

8 School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Pirassununga, Brazil

Introduction

Pigs (*Sus scrofa*) are monogastric animals, widely used as scientific animal model, and one of the most globally important species in meat production (Pan et al. 2021). Pigs produce the most consumed meat in the world (USDA 2022). Dietary lipids influence the quantity and quality of fatty acids (FAs) present in pork products (Martins et al. 2018). Soybean oil (SOY) is a primary source of lipids in pig diets and contains unsaturated fatty acids (UFA) (Burnett et al. 2020; Gomes et al. 2021; Fanalli et al. 2022a). However, other lipid sources such as canola (CO) and fish oil (FO) are also used in pig diets. Consumption of SOY compared to CO leads to significant differences between FA profiles, mainly for linoleic acid (AL), alpha-linolenic acid (ALA), and oleic acid (OA). Canola oil has approximately 7% of saturated fat (SFA) content and a high ALA content (11%),

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which is reflected in the ratio of UFA and SFA in pork carcass fat (Myer et al. 1992; Peñuela-Sierra et al. 2015). Fish oils contain a beneficial ratio of omega 3 (n-3) polyunsaturated fatty acids (PUFAs) providing benefits in reduced inflammation, lipid metabolism, and reduced oxidative stress processes (Zhang et al. 2019). Feeding pigs different mixed lipid sources, such as SOY, FO, or CO, results in changes in lipid profiles that can lead to high levels of UFA (Mitchaothai et al. 2007; Benz et al. 2011; Martins et al. 2015). Although the use of different dietary lipid sources has been well known to result in changes in pig carcasses, the optimal lipid sources for pig growth and for support of human health is unclear.

The FA composition of foods affects human nutrition by altering risks for obesity, high plasma cholesterol, and cardiovascular disease (Shimizu et al. 2015; Park and Han 2018). Monounsaturated (MUFA) and PUFA have appealing functional properties, providing beneficial effects on health, such as the prevention of chronic non-communicable diseases, regulation of the immune response, reduction of the risk of atherosclerosis, and reduced occurrence of type 2 diabetes (Salgado 2017). Some studies have demonstrated beneficial effects on the inflammatory system caused by the inclusion of lipids in the diet (Ramayo-Caldas et al. 2012; Duan et al. 2014).

Therefore, scientific studies that allow us to improve our nutrigenomic knowledge about pork products, and human health through their consumption, are of great importance. In this study, we evaluated changes in blood parameters, fatty acid profile in *Longissimus lumborum* intramuscular fat, and gene expression in the skeletal muscle tissue of immunecastrated male pigs fed diets enriched with different FA profiles, and identify affected metabolic pathways and biological process networks in which the identified genes are involved.

Methods

All animal procedures were conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Fass 2010) and were approved by the Animal Care and Use Committee of Luiz de Queiroz College of Agriculture (University of São Paulo, Piracicaba, Brazil, protocol: 2018.5.1787.11.6 and number CEUA 2018–28). This study was carried out in compliance with the ARRIVE guidelines.

Animals and diets

Fifty-four genetically purebred male pigs (offspring of large white sires × large white dams) with 71 ± 1.8 days of age and initial body weight (BW) of 28.44 ± 2.95 kg were allocated into one of three dietary treatments in a randomized complete block design with six replicate pens per treatment and three pigs per pen. Pigs were housed in an all-in/all-out double-curtain-sided building. Each pen was equipped with a three-hole dry self-feeder and a nipple drinker which allowed ad libitum access to feed and water throughout the experimental period (98 days). All pigs were halothane *(RYR1* gene) homozygous-free (NN) by molecular test and immunecastrated through administration of two 2-mL dose of Vivax® (Pfizer Animal Health, Parkville, Australia) on fatting day 56 (127 days of age) and fatting day 70 (141 days of age), in accordance with the manufacturer's recommendations.

The experimental diets were modified according to growing and finishing phases, where day 0 represents the start of the trial when pigs averaged 71 days of age: day 0 to 21 for grower I diets, day 21 to 42 for grower II, day 42 to 56 for finisher I, day 56 to 63 for finisher II, day 63 to 70 for finisher III, and day 70 to 98 for finisher IV diets (Almeida et al. 2021). Dietary treatments consisted of corn-soybean meal growing-finishing diets I, II, III and IV, supplemented with 3% canola oil (CO) or 3% fish oil (FO) or 3% soybean oil (SOY). Diets were formulated to meet or exceed Rostagno (Rostagno 2011) recommendations for growing-finishing pigs. No antibiotics were used, and all diets were provided in a mash meal form; details of animals and diets of this study are described in Supplementary Tables 1–4, which were adapted from our previous study (Almeida et al. 2021).

Fatty acid profile of samples

Sample collection and FA profile

After 98 days on trial, all pigs were slaughtered (average final body weight of 133.9 ± 9.4 kg, and 155 days of age on average), and *Longissimus lumborum* muscle samples were collected. The tissue samples were quickly excised, snap-frozen in liquid nitrogen, and then stored at − 80 °C until further analyses.

The FA profile determination was performed from the total lipid isolated from 100 g of the *Longissimus lumborum* samples using the cold extraction method by Bligh and Dyer (1959) and methylated according to the procedure outlined by AOCS (2004; Method AM 5–04). A full description of the analyses can be found in our previous study (Almeida et al. 2021).

Blood parameters

Four days before slaughter, blood was sampled from the jugular vein and immediately transferred into serum-separating vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) for the blood parameters analysis.

Then, the samples were stored at room temperature for 2 h to allow for coagulation then centrifuged at 3000 × g for 10 min to obtain serum that was stored in duplicated 1.5–mL tubes at − 80 °C. Serum lipid and other parameters were analyzed by using the Mindray BS-120 chemistry analyzer (Guangdong, China) in the Pathology Laboratory at the University of São Paulo, Pirassununga, SP, Brazil. Blood serum glucose content was quantified by the colorimetric enzymatic method according to Trinder (Trinder 1969) using the commercial kits (VIDA Biotecnologia S/A, Minas Gerais, Brazil), following the manufacturer's protocol. The quantification of total cholesterol and fractions was performed by enzymatic-colorimetric method, by selective precipitation using commercial kits (Gold Analisa Diagnóstica Ltda, Belo Horizonte, Minas Gerais, Brazil), according to the manufacturer's instructions. The analysis for the determination of total proteins was performed using commercial kits (VIDA Biotecnologia S/A, Lagoa Santa, Minas Gerais, Brazil), following manufacturer's protocol with modifications described by Gornall; Bardawill, and David (Gornall et al. 1949).

Statistical analyses

Statistical analyses to verify the differences in the FA profile of skeletal muscle between the diets were performed using the MIXED procedure of the SAS statistical software (SAS Inst. Inc., Cary, NC, v. 9.4), where a mixed model was fitted using restricted maximum likelihood (REML) methodology. In the model, the block effects were assumed as random effects and the dietary treatments as fixed effects. The UNIVARIATE procedure (v. 9.4) was used to test for divergence from a normal distribution with homogeneity of residuals for each of the variables. Diagnostics of the density distribution of the Studentized Residual of the model were made with the Shapiro–Wilk test (SAS v.9.4). Means were adjusted by using the LSMEANS statement. Differences were declared significant when p value ≤ 0.05 based on the Tukey test.

RNA extraction, library preparation, and sequencing

Total RNA was extracted from muscle samples using the RNeasy® Mini Kit (Qiagen Hilden, Germany), according to the manufacturer's instructions. Total RNA quantification, purity, and integrity were evaluated by Nanodrop 1000 and Bioanalyzer. All samples presented an RNA Integrity Number (RIN) greater or equal to seven. From the total RNA of each sample, 2 µg was used for library preparation according to the protocol described in the TruSeq RNA Sample Preparation kit v2 guide (Illumina, San Diego, CA). The Agilent Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA) was used to calculate libraries average size, and the libraries were quantified using quantitative PCR with the KAPA Library Quantification kit (KAPA Biosystems, Foster City, CA, USA). Quantified samples were diluted and labeled by barcoding and pooled to be run in different lanes (five pools of all 36 samples each), using the TruSeq DNA CD Index Plate (96 indexes, 96 samples, Illumina, San Diego, CA, USA). All samples were sequenced across five lanes of a sequencing flow cell, using the TruSeq PE Cluster kit v4-cBot-HS kit (Illumina, San Diego, CA, USA), and were clustered and sequenced using the HiSeq2500 equipment (Illumina, San Diego, CA, USA) with a TruSeq SBS Kit v4-HS (200 cycles), according to manufacturer's instructions. All the sequencing analyses were performed at the Genomics Center at ESALQ, localized in the Animal Biotechnology Laboratory at ESALQ – USP, Piracicaba, São Paulo, Brazil.

Quality control, and alignment

Sequencing adaptors and low complexity reads were removed in an initial data filtering step by Trim Galore 0.6.5 software. Reads with Phred score higher than 33 and a length higher than 70 nucleotides were kept after trimming. Quality control and reads statistics were estimated with FASTQC version 0.11.8 software [http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/]. *Sus Scrofa* 11.1 available at Ensembl [http://www.ensembl.org/Sus_scrofa/Info/Index] was used as the reference assembly. The abundance (*read counts*) of mRNAs for all annotated genes was calculated using STAR-2.7.6a (Dobin and Gingeras 2015). The gene expression levels were normalized using the counts scaled by total number of reads or counts per million (CPM) (Fanalli et al. 2022b).

Differentially expressed genes

Differentially expressed genes (DEGs) between the pairwise comparisons of different diets (CO *vs* SOY, SOY *vs* FO and CO *vs* FO) were identified using DESeq2, available at Bioconductor open-source software for bioinformatics, using a multi-factor design (Love et al. 2014). Prior to statistical analysis, the read count data was filtered as follows: (i) unexpressed genes were genes with zero counts for all samples, (ii) very lowly expressed were genes with less than one read per sample on average; (iii) rarely expressed genes that were not present in at least 50% of the samples. Unexpressed, very lowly expressed and rarely expressed genes were all removed from the analysis. Sire was fit as a factor in the multi-factor model. The cut-off approach performed to identify the DEG was Benjamini; Hochberg, (Benjamini and Hochberg 1995) methodology, used to control false discovery rate (FDR) at 10% (Cesar et al. 2016) according to previous studies and DESeq2 recommendations (Love et al. 2014; Cesar et al. 2016).

Functional enrichment analysis

Between CO *vs* SOY the functional enrichment analysis by MetaCore software (Clarivate analytics) (Clarivate analytics 2022) was applied to identify the pathway maps from the list of 65 DEG, and SOY vs FO to identify the pathway maps from the list of 531 DEG (FDR 10%), and for CO *vs* FO the pathway maps from the list of 32 DEG.

The functional enrichment analysis of DEG (FDR < 0.10) was performed to obtain comparative networks by "analysis of a single experiment" using *Homo sapiens* genome annotation as background reference and a standard parameter of MetaCore software v.21.4 build 70,700, filtering for the metabolic maps, energy metabolism, lipid metabolism, steroid metabolism; cardiovascular diseases, atherosclerosis; regulation of metabolism; nutritional and metabolic diseases; and nervous system diseases.

Protein–protein interaction (PPI) network analysis

To identify potential interactions and enrichment analysis among DEG across comparisons, we employed the STRING tool [https://string-db.org]. We used the species "*Sus scrofa*" to construct protein–protein interaction (PPI) networks, with a confidence (score) cutoff > 0.4 for CO *vs* SOY comparisons. Additionally, for the CO vs FO comparison, it should be noted that the maximum additional interactors were limited to 10. An interaction score > 0.7 was used for the SOY *vs* FO comparison. Due to the larger number of genes in the network, we used the Cytohubba software (Chin et al. 2014) to identify highly connected regions, and selected the top 10 MCC for the SOY *vs* FO comparison. Finally, the PPI network was visualized using Cytoscape software version 3.9.1 (Shannon et al. 2003).

Results

In previous work of our group, Almeida et al. (Almeida et al. 2021) observed that the inclusion of SOY or CO to pig diets reduced loin shear force, but the diets did not alter growth performance from day 0 to 21, day 21 to 42, day 42 to 56, and day 70 to 98. Consumer overall liking score in loins were greater from pigs fed SOY. Thus, in the current study, we performed blood parameters, FA composition of intramuscular fat, and differential expression analysis of the animals fed with diets containing different types of oils (SOY, CO, FO).

Blood parameters and fatty acid profile

The three different oil dietary sources altered serum concentrations of total protein, albumin (p value < 0.01), cholesterol (p value = 0.04), and HDL (p value = 0.04). Total protein and albumin levels were lower in pigs fed SOY compared with CO or FO (p value < 0.01) (Table 1).

The FA composition was different (p value < 0.05) in the *Longissimus lumborum* (LL) intramuscular fat. Concentrations of palmitic acid (p value < 0.01), stearic acid (p value < 0.01), oleic acid (OA) (p value < 0.01), ALA (p value = 0.76), eicosapentaenoic acid (EPA) (p value = 0.52), docosahexaenoic acid (DHA) (p value < 0.01), SFA (p value < 0.01), MUFA (p value < 0.01), total n-3 PUFA (p value < 0.01), and n-6:n-3 PUFA ratio (p value < 0.01) were different across dietary treatments. Considering all FA, there were differences in the FO treatment compared to either SOY or CO. For palmitic acid (p value < 0.01), stearic acid (p value < 0.01), EPA (p value < 0.01), DHA (p value < 0.01), SFA (p value < 0.01), and total n-3 PUFA (p

Table 1 Effects of dietary treatments on blood parameters of pigs

Variable	Dietary treatment			Pooled SEM[2]	p value
	CO	FO	SOY		
Glucose (mg/dL)	86.11	89.54	83.40	5.07	0.48
Aspartate aminotransferase (U/L)	42.72	42.88	38.13	3.01	0.21
Total proteins (g/dL)	6.84[a]	6.82[a]	6.46[b]	0.14	0.01
Albumin (g/dL)	3.80[a]	3.87[a]	3.46[b]	0.09	< 0.01
Globulin (g/dL)	3.04	2.94	3.00	0.13	0.73
Triglycerides (mg/dL)	45.67	39.78	35.70	4.89	0.13
Cholesterol (mg/dL)	99.60[a]	90.34[b]	96.49[a]	3.72	0.04
HDL (mg/dL)	45.59[a]	40.11[b]	43.66[a]	2.21	0.04
LDL (mg/dL)	44.89	42.28	45.71	2.53	0.38
VLDL (mg/dL)	9.11	7.94	7.13	1.00	0.14

[1]Pigs (n = 54) fed a corn-soybean meal diet enriched with 3% soybean oil (SOY), canola oil (CO), or fish oil (FO). Values represent the least square means from 18 pigs/treatment

[2]SEM = standard error of the least square means

[a–b]Within a row, values without a common superscript differ ($p \leq 0.05$) using Tukey's method

value = < 0.01), they were elevated in LL according to the FO dietary treatment. The FO dietary treatment decreased oleic acid (p value < 0.01), MUFA (p value = < 0.01), and n-6:n-3 PUFA ratio (p value < 0.01). The PUFA and the individual FA, myristic acid, palmitoleic acid, eicosenoic acid, LA, and ALA were similar among all diets. The highest atherogenic index was identified in FO group compared to other diets; between the CO and SOY diets, the index was lower (p value = 0.02) (see Table 2).

Sequencing data and differential expression analysis

The average numbers of sequenced reads (paired end) before and after filtering for samples from the skeletal muscle for the CO group were 33,568,010 and 33,085,594; for the SOY group were 31,955,613 and 31,491,236; and, for the FO group were 33,895,987 and 33,393,094 (Supplementary Table 5). A total of 65 DEG (FDR < 0.10) were identified between CO *vs* SOY groups, where 47 were down-regulated (log2-fold change ranging from − 5.57 to − 0.29) and 18 up-regulated (log2-fold change ranging 0.22 from to 3.07) in the CO group. A total of 32 DEG (FDR < 0.10) were identified between CO *vs* FO groups, where 21 were down-regulated (log2-fold change ranging from − 4.84 to − 0.47) and 11 up-regulated (log2-fold change ranging 0.50 from to 2.92) in the CO group. Finally, for the SOY *vs* FO groups, there were 406 down-regulated genes (log2-fold change ranging from − 4.99 to − 0.23) and 125 up-regulated genes (log2-fold change ranging 0.21 from to 3.52) in the SOY group (Supplementary Table 6). Table 3 displays a summary of the genes and the log2-fold changes between diet groups.

Table 2 Effects of dietary treatments on the *Longissimus lumborum* intramuscular FA profile of pigs[1]

Fatty acid (%)	Dietary treatment			Pooled SEM[2]	p value
	CO	FO	SOY		
Saturated fatty acid (SFA)					
Myristic acid (C14:0)	1.21	1.24	1.20	0.03	0.64
Palmitic acid (C16:0)	24.95[a]	26.43[b]	25.05[a]	0.35	< 0.01
Stearic acid (C18:0)	11.28[a]	12.63[b]	11.83[ab]	0.30	< 0.01
Monounsaturated fatty acid (MUFA)					
Palmitoleic acid (C16:1)	3.05	3.22	3.18	0.11	0.48
Oleic acid (C18:1 n-9)	44.95[a]	40.33[b]	44.28[a]	1.04	< 0.01
Eicosenoic acid (C20:1 n-9)	0.58	0.56	0.55	0.02	0.52
Polyunsaturated fatty acid (PUFA)					
Linoleic acid (C18:2 n-6)	13.33	14.21	13.14	0.70	0.42
Alpha-linolenic acid (C18:3 n-3)	0.53	0.56	0.57	0.04	0.76
Eicosapentaenoic acid (C20:5 n-3)	0.09[a]	0.46[b]	0.15[a]	0.05	< 0.01
Docosahexaenoic acid (C22:6 n-3)	0.11[a]	0.61[b]	0.15[a]	0.05	< 0.01
Total SFA	37.44[a]	40.29[b]	38.08[a]	0.59	< 0.01
Total MUFA	48.59[a]	44.11[b]	47.84[a]	1.11	< 0.01
Total PUFA	14.24	16.61	14.63	1.05	0.15
Total n-3 PUFA[3]	0.68[a]	1.70[b]	0.83[a]	0.12	< 0.01
Total n-6 PUFA[4]	13.33	14.21	13.14	0.70	0.42
PUFA:SFA ratio[5]	0.39	0.41	0.38	0.03	0.73
n-6:n-3 PUFA ratio[6]	22.48[a]	8.96[b]	17.19[c]	1.14	< 0.01
Atherogenic index[7]	0.48[a]	0.53[b]	0.48[a]	0.01	0.02

[1]Pigs (n = 54) fed a corn-soybean meal diet enriched with 3% canola oil or 3% fish oil or 3% soybean oil (SOY). Values represent the least square means from 18 pigs/treatment

[2]SEM = standard error of the least square means

[3]Total n-3 PUFA = {[C18:3 n-3] + [C20:5 n-3] + [C22:6 n-3]}

[4]Total n-6 PUFA = C18:2 n-6

[5]PUFA:SFA ratio = total PUFA/total SFA

[6]Σ n-6/Σ n-3 PUFA ratio

[7]Atherogenic index = (4 × [C14:0]) + (C16:0)/(total MUFA] + [total PUFA]), where brackets indicate concentrations (Ulbricht and Southgate 1991)

[a,b,c]Within a row, values without a common superscript differ ($P \leq 0.05$) using Tukey's method (Adapted from ALMEIDA et al. 2021

Table 3 Differentially expressed genes in the skeletal muscle of pigs

Diet group	DEG[1] Down-regulated	log2FC[2]	DEG[1] Up-regulated	log2FC[2]	Total DEG[1]
CO *vs* SOY	47	−5.57 to −0.29	18	0.22 to 3.07	65
CO *vs* FO	21	−4.84 to −0.47	11	0.50 to 2.92	32
SOY *vs* FO	406	−4.99 to −0.23	125	0.21 to 3.53	531

[1]Differentially expressed genes; [2]log2-fold change. Comparisons between canola oil (CO); soybean oil (SOY), and fish oil (FO)

Supplementary Figure S1 shows the Volcano plot of log2-fold change (x-axis) *vs* -log10FDR-corrected *p*-value (y-axis) from the differential gene expression analysis for the skeletal muscle (A) CO *vs* SOY, (B) SOY *vs* FO, and (C) CO *vs* FO

Functional enrichment analysis for skeletal muscle differential expression (CO vs SOY)

Ten different pathway maps (Fig. 1) were detected (*p* value < 0.10), which are linked to the following DEG, alcohol dehydrogenase 7 (class IV) (*ADH7*), "Fatty acid omega oxidation" pathway (Fig. 2); aldehyde dehydrogenase 3 family member A2 (*AL3A2, ALDH3A2*), "FA omega oxidation" (Fig. 2), "leukotriene 4 biosynthesis and metabolism," "triacylglycerol metabolism p.1," and "oxidative stress in adipocyte dysfunction in type 2 diabetes and metabolic syndrome X" pathways (Supplementary Fig. 2, 3, 4); angiopoietin 1 (*ANGPT1*), "role of adipose tissue hypoxia in obesity and type 2 diabetes" pathway (Supplementary Fig. 5); nuclear receptor subfamily 0 group B member 2 (*SHP, NR0B2*), "regulation of lipid metabolism RXR-dependent regulation of lipid metabolism via PPAR, RAR, and VDR," "regulation of lipid metabolism FXR-dependent negative-feedback regulation of bile acids concentration," "selective Insulin resistance in type 2 diabetes in liver," "regulation

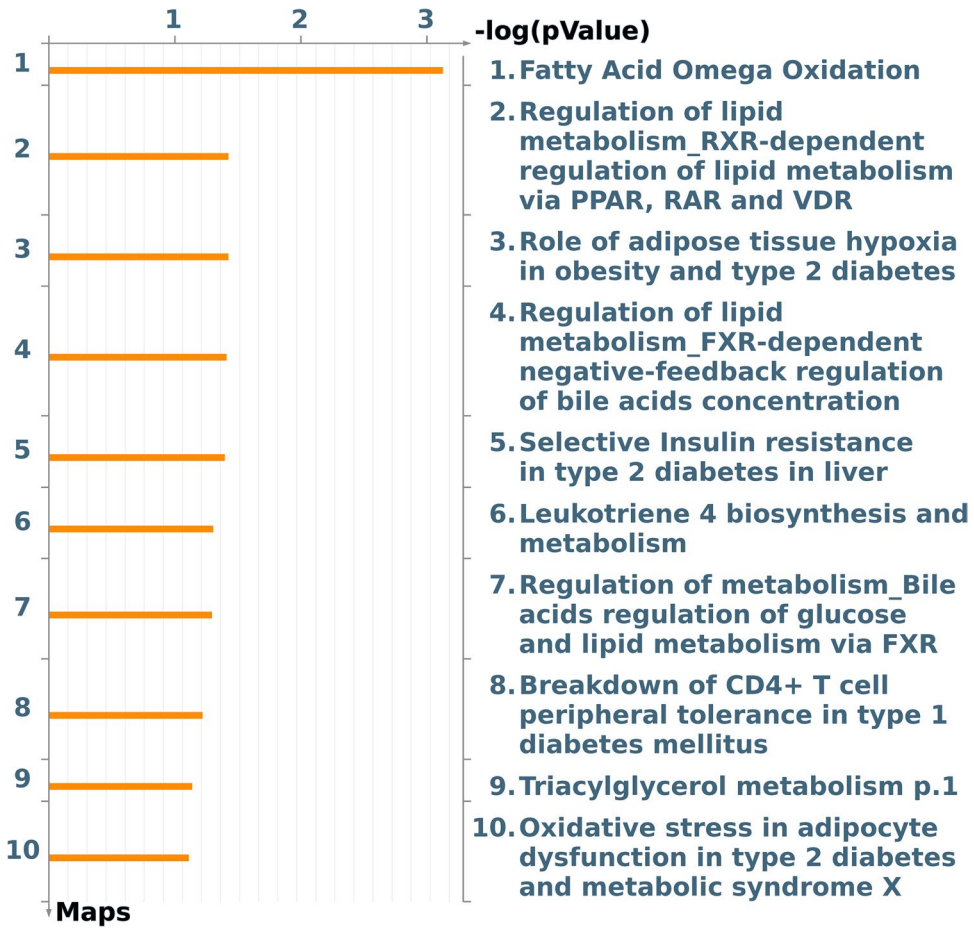

Fig. 1 Pathway maps by MetaCore software (*p* value < 0.10) from the list of differentially expressed genes (FDR 10%) in the skeletal muscle of pigs fed with different oils (3.0% canola oil *vs* 3.0% soybean oil)

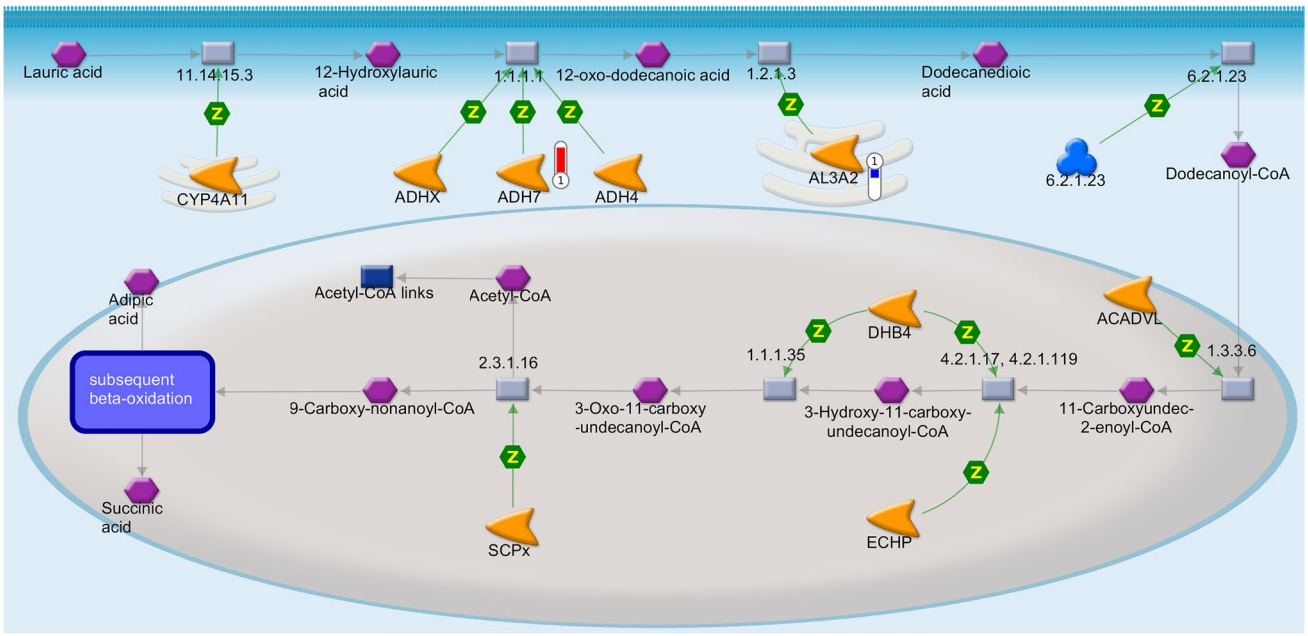

Fig. 2 Fatty acid omega oxidation pathway map by MetaCore software (p value < 0.10) from the list of differentially expressed genes (FDR 10%) in the skeletal muscle of pigs fed different oil sources in the diet (3.0% canola oil and 3.0% soybean oil). The blue thermometer indicates down-regulation of DEG (log2-fold change − 0.79), and the red thermometer indicates up-regulation of DEG (log2-fold change + 2.37) in the diet with 3.0% of canola oil (CO). Green arrows indicate positive interaction and gray arrows indicate unspecified interaction. For a detailed definition, see https://portal.genego.com/legends/MetaCoreQuickReferenceGuide.pdf

of metabolism: Bile acids regulation of glucose and lipid metabolism via FXR" pathways (Supplementary Fig. 6, 7, 8, 9); and T cell surface glycoprotein (*CD4*) DEG related to "breakdown of CD4 + T cell peripheral tolerance in type 1 diabetes mellitus" (Supplementary Fig. 10).

In our study, several DEG were identified, including the *ADH7* in skeletal muscle of pigs fed with different oils (CO *vs* SOY), which was more expressed in the CO group (log2-fold change + 2.34). Moreover, *AL3A2* (log2-fold change − 0.79) DEG, which participates in the "fatty acid omega oxidation" (p value < 0.10) (Fig. 4) and "leukotriene 4 biosynthesis and metabolism" pathways (Supplementary Fig. 2), was less expressed in the CO group. The *AL3A2* DEG was further enriched (p value < 0.10) for the "triacylglycerol metabolism" pathway (Supplementary Fig. 3) and identified in the "oxidative stress in adipocyte dysfunction in type 2 diabetes and metabolic syndrome X" pathway (Supplementary Fig. 4), which catalyzes the oxidation of 4-Hydroxy-2(E)-nonenal to the nontoxic 1,4-dihydroxynonene.

The DEG angiopoietin-1 *(Ang-1, ANGPT1)* was identified in our study as less expressed (log2-fold change − 1.22) in the group of animals fed 3.0% CO. The enrichment analysis demonstrated its relation with the "role of adipose tissue hypoxia in obesity and type 2 diabetes" pathway (Supplementary Fig. 5). Another DEG was the nuclear receptor subfamily gene 0 group B member 2 (*NR0B2*). Nuclear receptors (*NRs*) are a family of TF that play a critical role in different aspects in mammals as can be seen in the pathways enriched by MetaCore analysis (Supplementary Fig. 7, 8, and 9). Lastly, the *SHP* DEG was more expressed in the CO group (log2-fold change + 2.15).

The *CD4*, an essential gene in the immune response processes, was identified as DEG when comparing CO *vs* SOY diets, with lower expression (log2-fold change − 1.55) in the CO group. This gene is involved in the "breakdown of *CD4* + T cell peripheral tolerance in type 1 diabetes mellitus" (Supplementary Fig. 10) pathway map, enriched by MetaCore.

To observe the interactions of DEG in gene networks, the process networks analysis was performed using MetaCore software (Table 4) and allowed us to identify networks related (p value < 0.1) to immune response, metabolism regulation, and signal transduction. The identified immune response networks "antigen presentation" was enriched for the *CD4* DEG (log2-fold change − 1.55); the "transport bile acids transport and its regulation," "regulation of bile acid metabolism and negative *FXR*-dependent regulation of bile acids concentration," and "signal transduction_ESR2" pathways were enriched for the nuclear receptor subfamily 0 group B member 2 (*SHP, NR0B2*; log2-fold change + 2.15) DEG.

The PPI network generated by STRING for the CO *vs* SOY comparison identified the DEG sarcosine

Table 4 Processes networks by MetaCore software (p value < 0.10) from the list of differentially expressed genes (FDR 10%) in the skeletal muscle of pigs fed with different oil sources (canola oil and soybean oil CO *vs* SOY)

Process networks	p value	DEG[1]
Immune response_Antigen presentation	0.01	*CD4*
Transport_Bile acids transport and its regulation	0.07	*SHP*
Regulation of metabolism_Bile acid regulation of lipid metabolism and negative FXR-dependent regulation of bile acids concentration	0.07	*SHP*
Signal transduction_ESR2 pathway	0.08	*SHP*

[1]Differentially expressed genes

dehydrogenase (*SARDH),* ADAM metallopeptidase with thrombospondin type 1 motif 16 *(ADAMTS16),* aldehyde dehydrogenase 3 family member A2 *(ALDH3A2),* solute carrier family 47 member 2 *(SLC47A2),* ADAM metallopeptidase with thrombospondin type 1 motif 20 *(ADAMTS20),* aldehyde dehydrogenase 3 family member A1 *(ALDH3A1),* and solute carrier family 16 member 9 *(SLC16A9)*, in addition to alcohol dehydrogenase 1C (class I), beta polypeptide (*ADH1C)* (Fig. 3). The enrichment analysis using STRING resulted in KEGG Pathway terms such as ssc00010, ssc00340, ssc00350, ssc00410, ssc00071, ssc00980, ssc00982, ssc05204, and ssc01100 with FDR < 0.05.

Functional enrichment analysis for skeletal muscle differential expression (CO vs FO)

Different pathway maps were detected using the gene list the CO *vs* FO comparison (p value < 0.10) (Fig. 4), most of them presented the sterols-CoA desaturase (*SCD*) DEG, such as "adiponectin in pathogenesis of type 2 diabetes" (Fig. 5); "regulation of lipid metabolism RXR-dependent regulation of lipid metabolism via PPAR, RAR, and VDR" (Supplementary Fig. 11); "putative pathways for stimulation of fat cell differentiation by bisphenol A" (Supplementary Fig. 12); "regulation of lipid metabolism via LXR, NF-Y, and SREBP" (Supplementary Fig. 13); and, "regulation of metabolism: Bile acids regulation of glucose and lipid metabolism via FXR and Role of ER stress in obesity and type 2 diabetes" (Supplementary Fig. 14). We also detected pathways presenting the *AL3A2* DEG, like the "fatty acid omega oxidation" (Supplementary Fig. 15); "leukotriene 4 biosynthesis and metabolism" (Supplementary Fig. 16); and "triacylglycerol metabolism" (Supplementary Fig. 17). As well as, the "breakdown of CD4 + T cell peripheral tolerance in type 1 diabetes mellitus" pathway with the *CD4* DEG (Supplementary Fig. 18).

In our study, *SCD* expression was lower (log2-fold change − 1.62) in the group of pigs fed CO diet. The *SCD*-enriched pathways were "adiponectin in pathogenesis of type 2 diabetes"; "regulation of lipid metabolism *RXR*-dependent regulation of lipid metabolism via PPAR, RAR, and VDR"; "putative pathways for stimulation of fat cell differentiation by bisphenol A"; "regulation of lipid metabolism regulation of lipid metabolism via LXR, NF-Y and SREBP"; and, "regulation pathway of metabolism bile acids regulation of glucose and lipid metabolism via *FXR* and Role of ER stress in obesity and type 2 diabetes."

The *AL3A2* gene was identified as DEG between CO *vs* FO, the expression of *AL3A2* was lower (log2-fold change − 0.60) in the CO group, as observed between CO *vs* FO in which *AL3A2* was less expressed (log2-fold change − 0.79) in the CO group. This gene was enriched

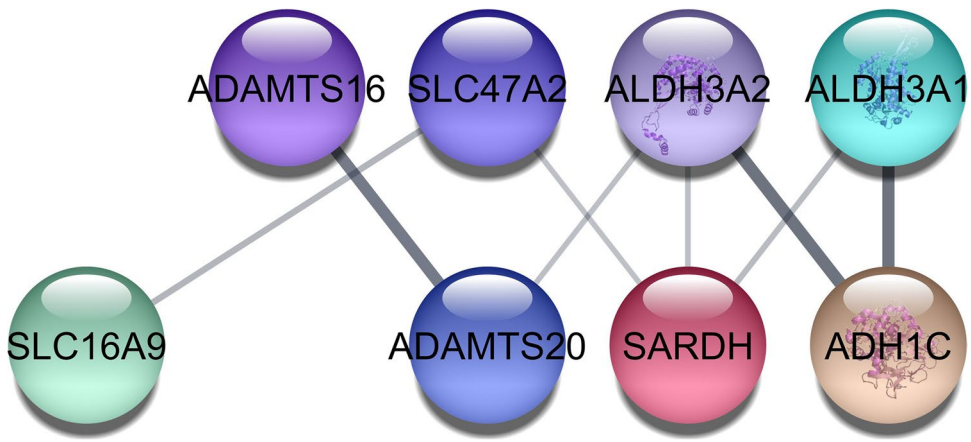

Fig. 3 Protein–protein interaction networks were constructed by STRING followed by Cytoscape and Cytohubba programs. The network includes 8 edges (interactions) between 8 nodes. All differentially expressed genes (DEG) that presented connections between the comparisons are presented

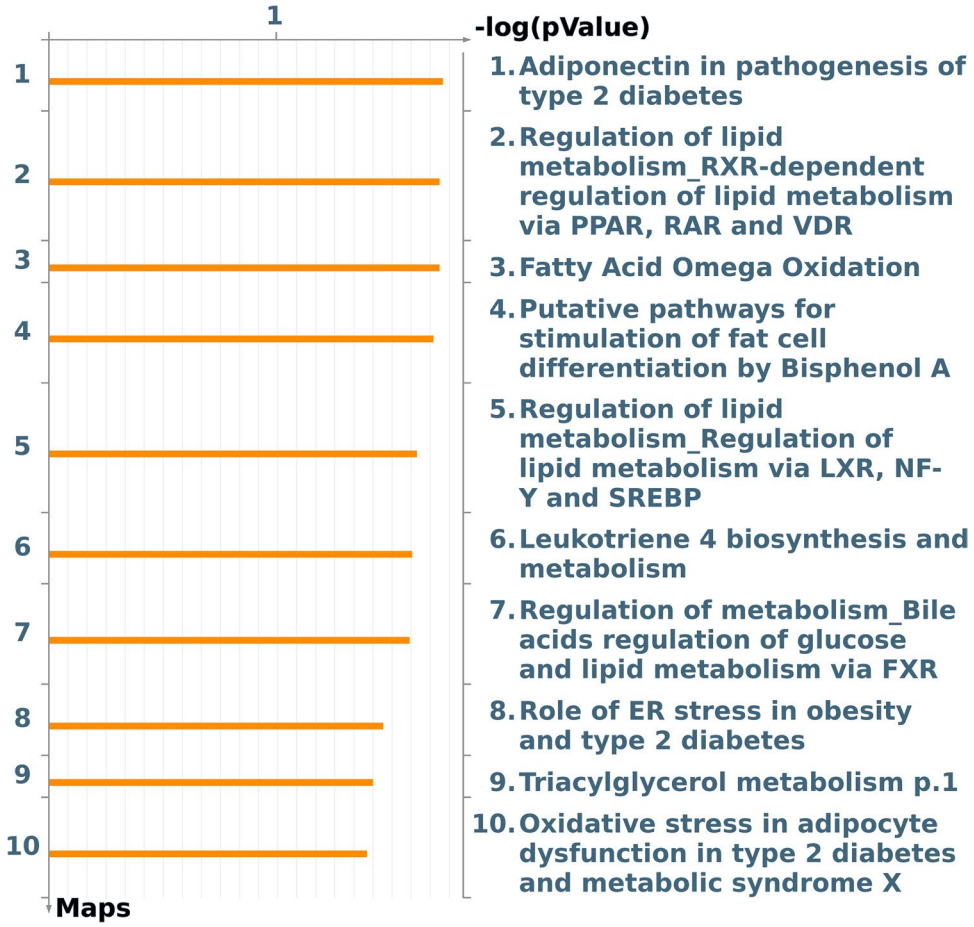

Fig. 4 Pathway maps by MetaCore software (p value < 0.10) from the list of differentially expressed genes (FDR 10%) in the skeletal muscle of pigs fed with different oil (3.0% canola oil *vs* 3.0% fish oil)

for "fatty acid omega oxidation," "leukotriene 4 biosynthesis and metabolism," and "triacylglycerol metabolism" pathways. Lastly, T cell surface glycoprotein *CD4 (CD4)* was enriched in pathways related to the "breakdown of CD4 + T cell peripheral tolerance in type 1 diabetes mellitus."

A process networks analysis was performed for better understanding of the enriched pathways through interactions by gene networks, which are related to dietary oil manipulation. Emphasizing the pathways regulation of bile acid metabolism and negative *FXR* dependent regulation of bile acid concentration, signal transduction, inflammation amphoterin signaling, chemotaxis, development skeletal muscle with the genes *SCD, AKAP3, Myosin heavy chain (MyHC),* and *CD4* (Fig. 6).

In the CO *vs* FO comparison, the PPI network generated by STRING identified key genes associated with DEG (Fig. 7). Furthermore, the PPI networks were enriched for the KEGG pathways ssc01100, ssc00410, ssc00360, ssc00350, ssc00250, ssc00330, ssc00630, ssc00260, ssc00010, ssc01200, ssc00340, ssc00640, ssc00620, and ssc00280.

Functional enrichment analysis for skeletal muscle differential expression (SOY vs FO)

Some pathway maps detected (p value < 0.10) are shown in Table 5 with the corresponding p value and DEG. The complete list of enriched pathway maps is shown in Supplementary Table 7.

The genes zinc α-2-glycoprotein 1 (*AZGP1*), phosphodiesterase 3B (*PDE3B*), apolipoprotein E (*APOE*), Perilipin 1 (*PLIN1*), and lipase E hormone sensitive type (*LIPS*) were identified as DEG. The *AZGP1* gene, a lipid-mobilizing adipokine, was identified with lower expression (log2-fold change − 3.79) in pigs from the SOY group. The cited genes were enriched in the "*TNF-α* pathway, *IL-1* β induces dyslipidemia and inflammation in obesity and type 2 diabetes in adipocytes" pathway (Fig. 8). In addition, *APOE* was enriched in "regulation of metabolism: Bile acids regulation of glucose and lipid metabolism via FXR" (Supplementary Fig. 19) and "transport HDL-mediated reverse cholesterol transport" (Supplementary Fig. 20) pathways.

Another DEG that may be inhibited by *TNF*-α is *Perilipin 1*, which presented lower expression (log2-fold

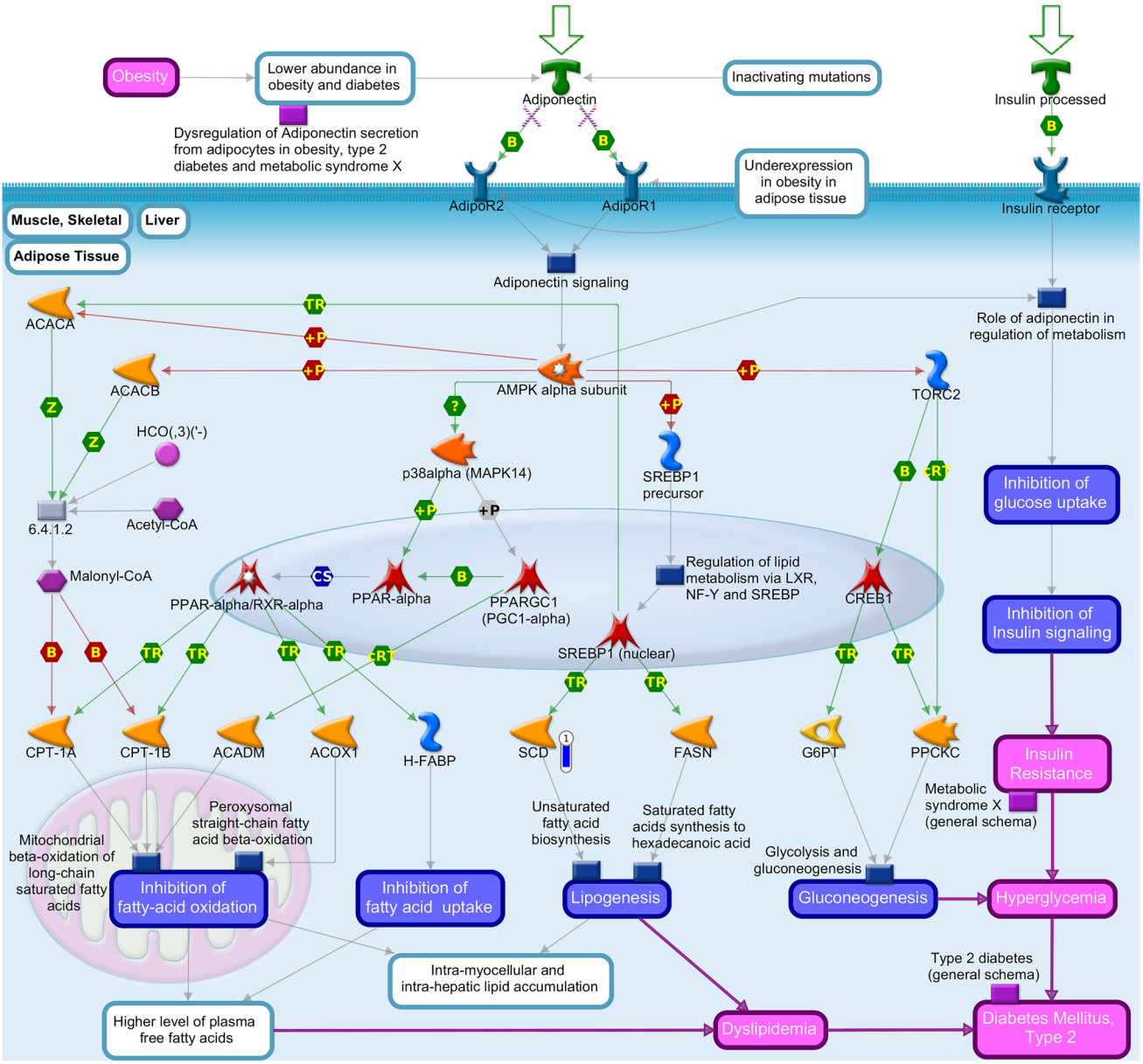

Fig. 5 Adiponectin in pathogenesis of type 2 diabetes pathway map by MetaCore software (p value < 0.10) from the list of differentially expressed genes (FDR 10%) in the skeletal muscle of pigs fed different oil sources in the diet (3.0% canola oil and 3.0% fish oil). The large green arrows indicate path to start, blue thermometer indicates down-regulation of DEG in the diet with 3.0% of canola oil (CO). Green arrows indicate positive interaction, red arrows indicate negative interaction (inhibition), gray arrows indicate unspecified interaction, and smaller blue boxes indicate normal process. For a detailed definition, see https://portal.genego.com/legends/MetaCoreQuickReferenceGuide.pdf

change – 2.99) in the SOY group. *APOE* showed lower expression (log2-fold change – 2.10) in groups fed a diet containing SOY. Such as *Perilipin 1*, *LIPS* (log2-fold change – 1.30), and *PPARG* (log2-fold change – 1.92) were identified with lower expression in the SOY group. The second one, *PPARG* was enriched for the "putative pathways for stimulation of fat cell differentiation by bisphenol A" (Supplementary Fig. 21); the "role of IL-6 in obesity and type 2 diabetes in adipocytes" (Supplementary Fig. 22); and the "dysregulation of adiponectin secretion from adipocytes in obesity, type 2 diabetes and metabolic syndrome X" (Supplementary Fig. 23) pathways, the last two pathways together with the CCAAT enhancer binding protein alpha (*C/EBP-alpha,* log2-fold change – 2.39).

The *SCD* gene was identified as a DEG with lower expression (log2-fold change – 1.6) in the SOY group and was enriched in the "putative pathways for stimulation of fat cell differentiation by bisphenol A," "adiponectin in

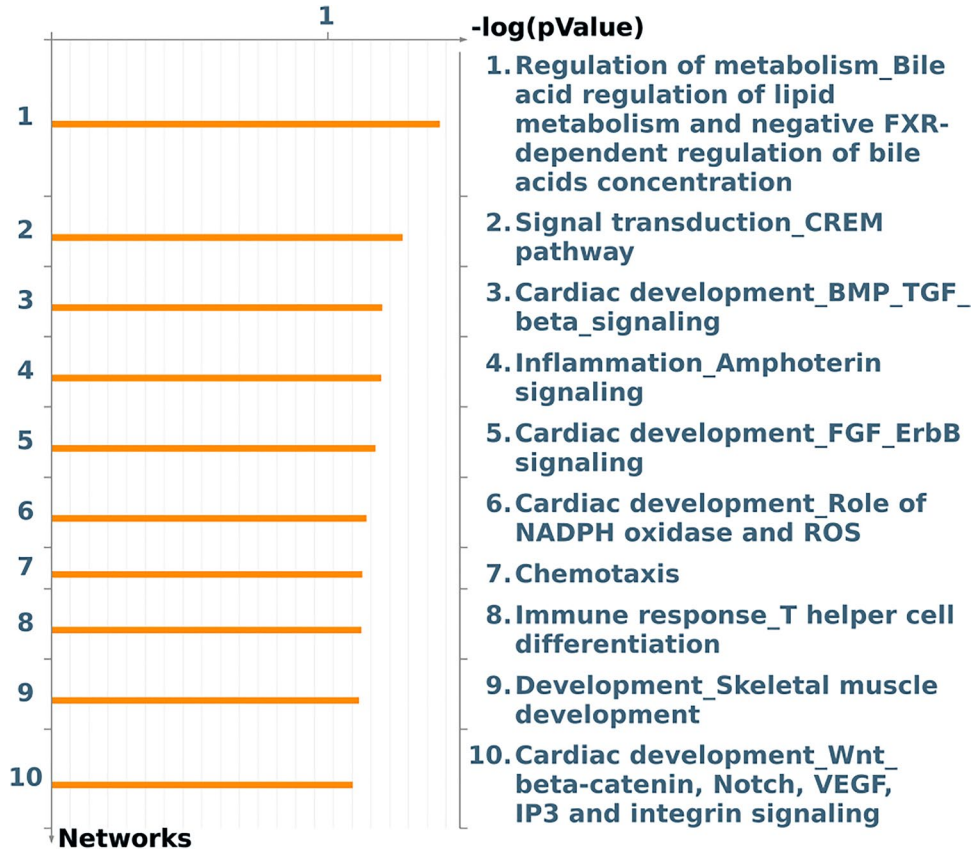

Fig. 6 Top 10 enriched networks identified by MetaCore software from the list of differentially expressed genes (FDR 10%) in the skeletal muscle of pigs fed with different oils (3.0% canola oil and 3.0% of fish oil)

pathogenesis of type 2 diabetes," and "regulation of metabolism: Bile acids regulation of glucose and lipid metabolism via FXR" pathways. The transcription factor 7 like 2 (*TCF7L2*) was identified as the DEG with the lowest expression (log2-fold change − 0.96) in the SOY group. In addition, *TCF7L2* participates in the "signal transduction_*WNT/Beta-catenin* signaling in tissue homeostasis" pathway (Supplementary Fig. 24). *C/EBPalpha* was identified as DEG in our study with the smallest expression (log2-fold change − 2.4) related to the SOY group. *C/EBPalpha* was enriched for the "putative pathways for stimulation of fat cell differentiation by Bisphenol A" pathway.

The analysis of process networks was performed using MetaCore software (Fig. 9) to better understand the observed interactions of DEG in gene networks. Networks related (p value < 0.1) to muscle contraction (p value 2.429E-06), "Regulation of angiogenesis" (p value 7.863E-03), and "immune response" (p value 1.878E-02) were identified herein. Some DEG were identified enriched in the networks like the *GPCR* in the "chemostaxis" network; *PPARG, APOE, MELC,* and *PACAP* receptor 1 in the "development neurogenesis axonal guidance" network; *APOE, MELC, Actin, C/EBP,* and *SR-BI,* among others, in the "immune response phagocytosis" network.

In the comparison between SOY *vs* FO, in addition to a higher number of DEG, a greater number of interactions were identified in the PPI network (Fig. 10). The enriched KEGG Pathway terms were ssc01100, ssc04934, ssc00340, ssc00561, ssc00830, ssc01040, ssc01212, ssc00410, ssc03320, ssc04146, ssc00280, ssc00350, ssc04152, ssc00620, ssc04310, ssc05224, ssc00071, ssc04916, ssc00330, ssc05217, ssc00010, ssc04614, ssc04060, ssc04927, ssc05200, ssc00020, ssc04925, ssc04975, ssc00380, ssc04913, ssc00140, ssc04931, ssc05205, ssc00120, ssc00230, ssc04910, ssc05321, ssc00053, ssc04151, ssc00592, ssc04080, ssc04390, ssc05226, ssc00360, ssc05225, ssc05010, and ssc04630.

Discussion

Fatty acid profile and blood parameters: We observed with the analysis of the intramuscular lipid composition, that the addition of FO in the diet of growing and finishing pigs changed the FA profile composition mainly for C20:5 n-3, C22:6 n-3, and SFA content, as C16:0 and C18:0. The total SFA also showed a higher content of pigs that were fed with FO. According to Jeromson et al. (2015), diets rich in SFA may be associated with the onset of obesity and type 2 diabetes. In the other hand, pigs fed FO demonstrated the lowest n-6:n-3 PUFA ratio, thus showing the potential of the diet in modifying pork composition through FA modulation in

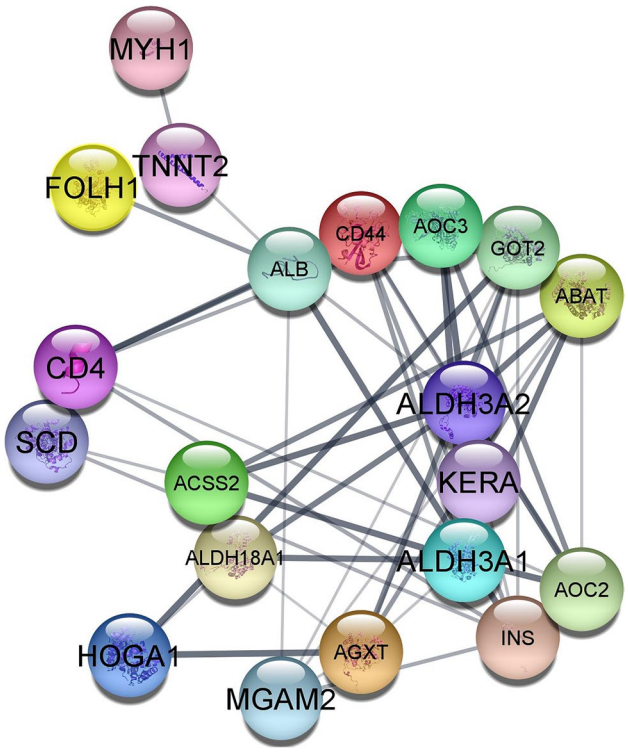

Fig. 7 Protein–protein interaction networks were constructed by STRING followed by Cytoscape and Cytohubba programs. The network includes 55 edges (interactions) between 20 nodes. Among the key genes, the node with the largest letter represents the differentially expressed gene (DEG) between the comparisons (3.0% canola oil and 3.0% of fish oil)

the pig diet (Ma et al. 2016). Our findings corroborate with Jeromson et al. (2015), which reported that skeletal muscle may be manipulated according to changes in the animal diet.

In our study, we observed higher content of n-3 PUFA in the FO group. The n-3 PUFA has been linked to protection against chronic and metabolic diseases, whereas omega 6 (n-6) PUFA may be related to inflammation, blood vessel constriction, and aggregation. Therefore, the proportion of these PUFA and the dietary LA and ALA content may be relevant to the regulation of bodily homeostasis inflammation and anti-inflammatory effect (Saini and Keum 2018). The modification of pork FA can show us pathways and networks related to both inflammation and relevant diseases and metabolic processes. Moreover, the amount and type of fat play a key role in regulating the metabolic health of the whole body, FA are components of cell membranes, act as signaling molecules, and can change related to the muscle lipid pool, thus can modify both metabolic and physical function of the skeletal muscle (Jeromson et al. 2015).

According to Saini and Keum (Saini and Keum 2018), n-3 and n-6 PUFA have opposite effects on the metabolic functions of the body, so they are important to be analyzed. Myristic acid, involved in the increase in plasma cholesterol concentration, has a harmful cardiovascular effect in humans (Kasprzyk et al. 2015).

According to Corominas et al. (Corominas et al. 2013), modifications related to the replacement of SFA by MUFA or PUFA decrease serum LDL-cholesterol and total cholesterol; in our study, we did not observe a difference in serum LDL-cholesterol and total cholesterol in the groups fed with FO and SOY compared to the CO group. For FO diet, we observed higher total PUFA composition in the liver and in LL intramuscular FA, with higher deposition of total n-3 PUFA. The analysis of blood parameters is essential, mainly referring to serum lipids that are related to cardiovascular diseases and obesity-related metabolic diseases (Uddin et al. 2011).

The CO diet showed the highest concentration of HDL, involved in the elimination of excess cholesterol, despite the greater deposition of total cholesterol and no statistical difference between LDL and the other oils used (SOY and FO). Evidence supporting the benefits of CO is related to the positive effects of MUFA compared to SFA effects. Moreover, in addition, this oil influences biological functions that

Table 5 Pathway maps enriched by MetaCore software (p value < 0.10) from the list of differentially expressed genes (FDR 10%) in the skeletal muscle of pigs fed with different oil sources

Pathway Maps	p-value	DEG
TNF-α, IL-1 β induce dyslipidemia and inflammation in obesity and type 2 diabetes in adipocytes	1.165E-04	*AZGP1, PDE3B, APOE, Perilipin, LIPS*
Putative pathways for stimulation of fat cell differentiation by Bisphenol A	4.803E-04	*PPAR-gamma, TCF7L2 (TCF4), SCD, C/EBPalpha*
Signal transduction_WNT/Beta-catenin signaling in tissue homeostasis	1.367E-03	*TCF7L2 (TCF4), Tcf(Lef), WNT, PPCKC*
Adiponectin in pathogenesis of type 2 diabetes	4.446E-03	*SCD, PPCKC, ACOX1*
Role of IL-6 in obesity and type 2 diabetes in adipocytes	5.884E-03	*PPAR-gamma, Perilipin, LIPS*
Dysregulation of Adiponectin secretion from adipocytes in obesity, type 2 diabetes and metabolic syndrome X	9.516E-03	*PPAR-gamma, IL-18, C/EBPalpha*
Regulation of metabolism_Bile acids regulation of glucose and lipid metabolism via FXR	1.173E-02	*APOE, SCD, PPCKC*
Transport_HDL-mediated reverse cholesterol transport	1.252E-02	*APOE, CES1, SR-BI*

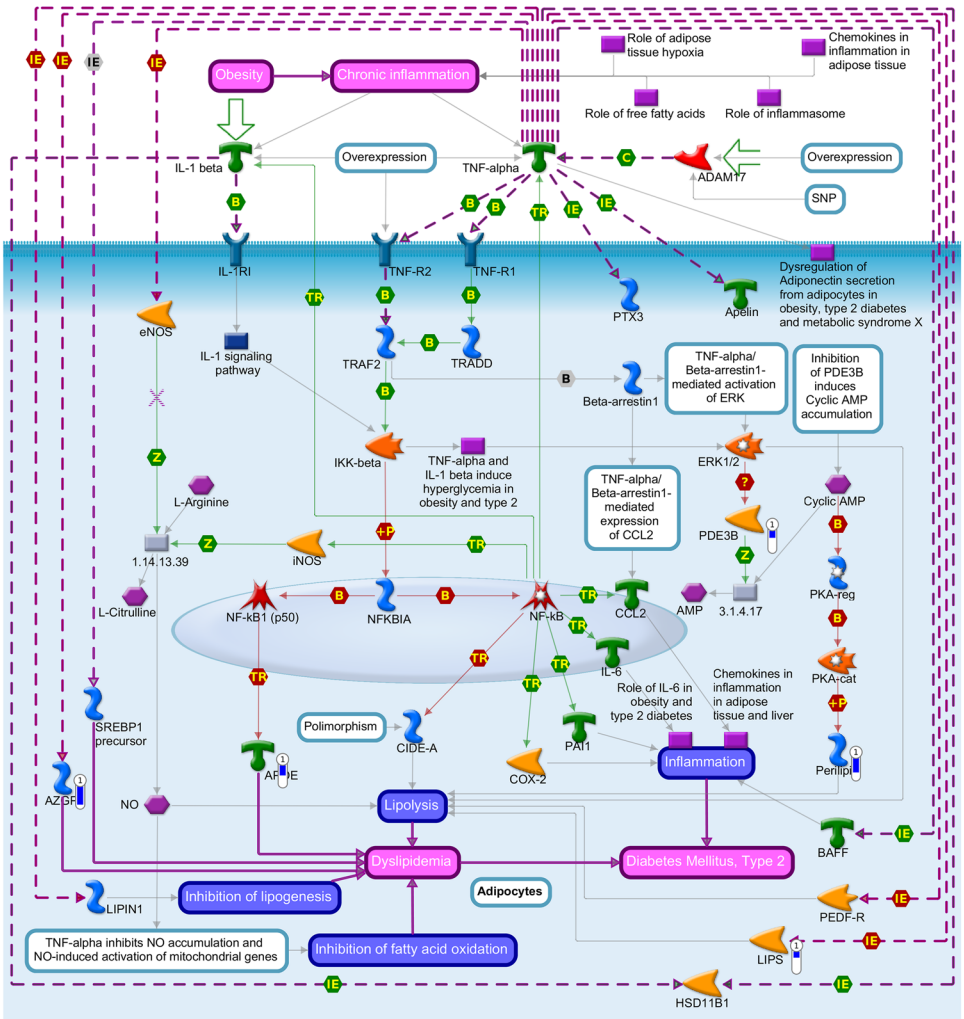

Fig. 8 TNF-α, IL-1 β induces dyslipidemia and inflammation in obesity and type 2 diabetes in adipocytes pathway map by MetaCore software (p value < 0.10) from the list of differentially expressed genes (FDR 10%) in the skeletal muscle of pigs fed different oil sources in the diet (3.0% soybean oil and 3.0% fish oil). The large green arrows indicate path to start, blue thermometer indicates down-regulation of DEG in the diet with 3.0% of soybean oil (SOY). Purple lines indicate enhancement in diseases and purple dotted line emerges in diseases. Green arrows indicate positive interaction, red arrows indicate negative interaction (inhibition), and gray arrows indicate unspecified interaction. For a detailed definition, see https://portal.genego.com/legends/MetaCoreQuickReferenceGuide.pdf

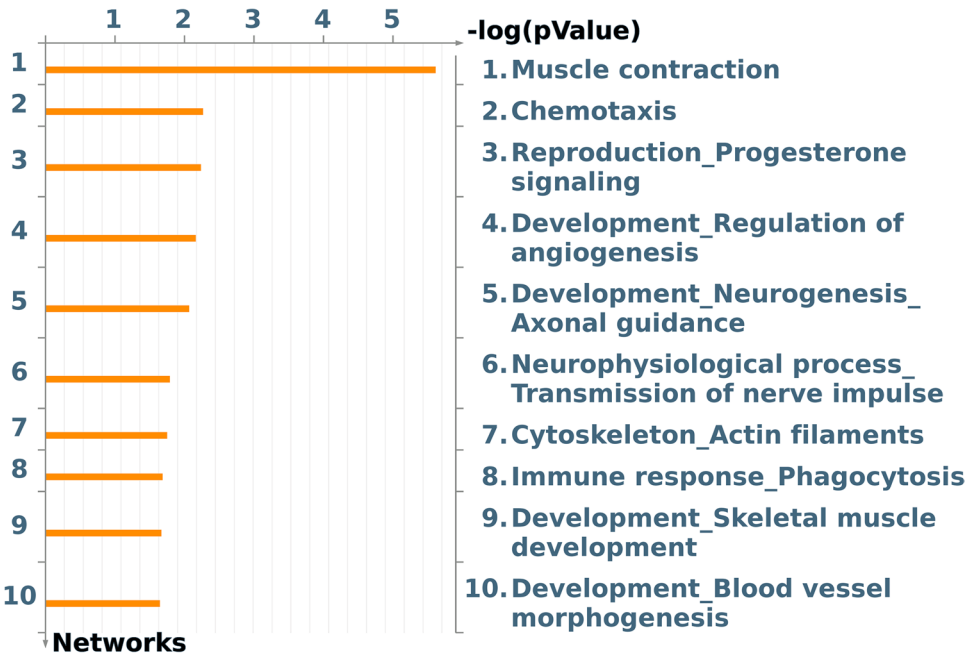

Fig. 9 Top 10 enriched networks identified by MetaCore software from the list of differentially expressed genes (FDR 10%) in the skeletal muscle tissue of pigs fed with different oils (3.0% soybean oil and 3.0% of fish oil)

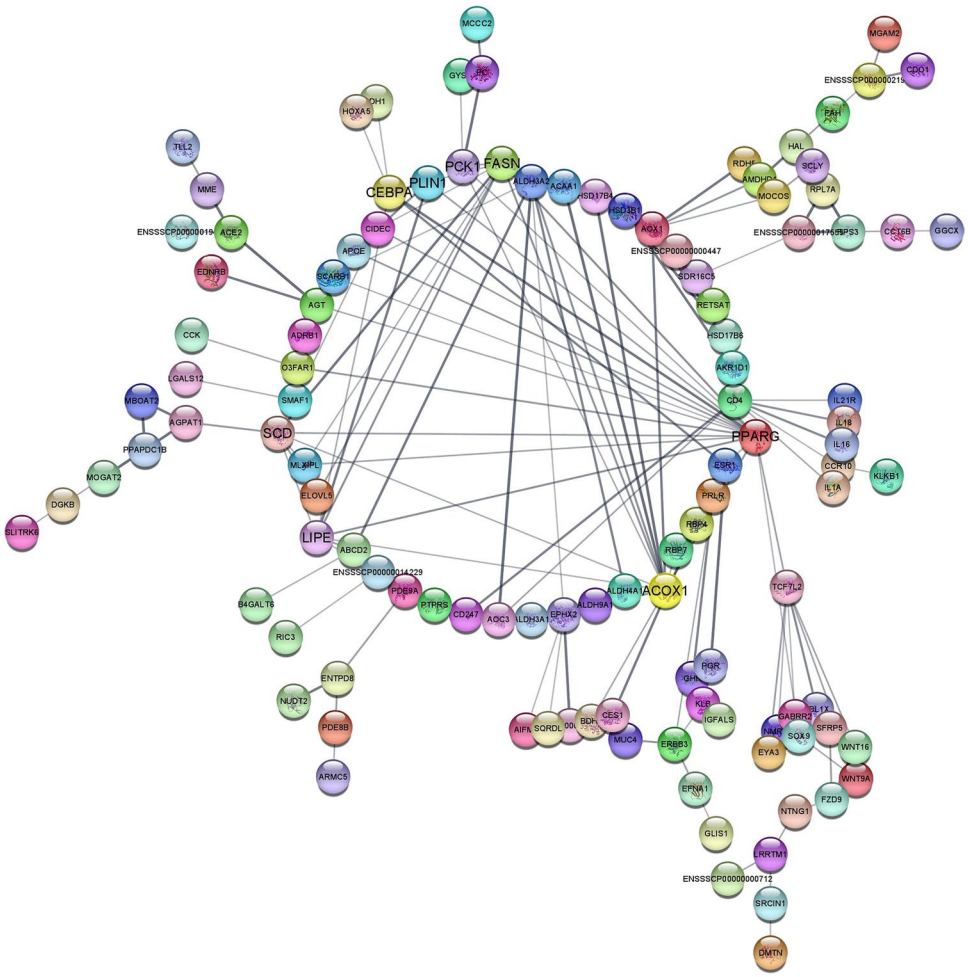

Fig. 10 Protein–protein interaction networks were constructed by STRING followed by Cytoscape and Cytohubba programs. Network includes 159 edges (interaction) between 114 nodes, respectively. Among key genes, the node with the largest letter represents the superhub gene with the highest betweenness centrality and node degree

affect several other disease risk biomarkers (Lin et al. 2013). In our study, we identified a greater amount of OA and consequently MUFA in animals of the CO group. The results of a systematic review and meta-analysis (Ghobadi et al. 2019) on the effect of CO in humans showed that CO consumption could decrease serum LDL compared to sunflower oil and saturated fat, although it did not change significantly HDL, different from that the results obtained in our study. Despite these findings, the exact mechanisms of CO on serum lipids are still not well understood (Ghobadi et al. 2019).

Canola oil has a high OA content, which leads to greater beneficial health effects compared to other oils, where a previous study showed that diets with higher oleic acid content increase HDL blood level (Saedi et al. 2017). Herein, we observed that our results corroborate with the previous study, the CO and SOY groups presented higher OA content in the skeletal muscle and HDL blood level compared with FO group. The HDL regulates that the level of transcription initiation by transcription factors is related to post-transcriptional regulation through microRNAs and other non-coding RNAs, among other mechanisms in various processes (Kardassis et al. 2015). Despite the CO group presenting a higher total blood cholesterol, an atherogenic factor, most of this was represented by HDL, which has an antiatherogenic effect and is involved in the decreased occurrence of inflammatory injury and oxidative stress (White et al. 2017).

The atherogenic index is related to predicting the risk of developing atherosclerosis and coronary heart disease. In addition, it is related to dyslipidemia, which is characterized by high plasma concentration of triglycerides and total cholesterol, HDL, LDL (Niroumand et al. 2015). In our results, the highest serum concentration of cholesterol was found in the CO group and the atherogenic index showed a low concentration in CO and SOY groups compared to FO. Previous studies have already linked CO to interference in cholesterol metabolism due to phytosterols that can inhibit cholesterol esterase enzymes reducing intracellular cholesterol esterification (Ghobadi et al. 2019).

Transcriptome modulation in response to FA: The transcriptome analysis showed that the modification of FA source impacted gene expression between the comparisons. The CO and SOY diets led to similar deposited FA profiles, and both diets presented higher levels of cholesterol and HDL when compared to the FO group, in the transcriptomic

comparison resulted in several DEG and important pathways related to lipid metabolism. Within the identified pathways there were "fatty acid omega oxidation," "leukotriene 4 biosynthesis and metabolism," and "triacylglycerol metabolism," with *AL3A2* DEG down-regulated in the CO group.

Among the DEG identified in the comparisons CO *vs* SOY and CO *vs* FO, the *AL3A2* gene was identified down-regulated in the CO group for both diets. The encoded enzyme AL3A2 participates in the oxidation of long-chain aliphatic aldehydes to FA (Stelzer et al. 2016), possibly being related to the oxidation of 12-oxo-dodecanoic acid to dodecanedioic acid. Furthermore, the aldehyde dehydrogenase family may play a relevant role in the function and induction of regulatory T cells, cellular detoxification, and amino acid metabolism (Bazewicz et al. 2019). Moreover, it is important to the detoxifying role in the ethanol-caused accumulation of free FA and triacylglycerol, ethanol inhibition of lipoprotein export, increasing FA uptake, and lipid peroxidation (Galli et al. 1999). Thus, the consumption of SOY and FO may be related to the improvement of lipid oxidation rate when compared to the CO group. In our previous study, similar results were observed in the liver with comparison of different proportions of SOY, in which the increase of SOY in the pig diet (3.0% of SOY) resulted in a possible improvement of the lipid oxidation rate (Fanalli et al. 2022c). Studies are still needed to understand the specific functions of *AL3A2* (Udhaya Kumar et al. 2020). Also, in the CO *vs* SOY comparison, the *AL3A2* was enriched in the "leukotriene 4 biosynthesis and metabolism" pathway. This pathway is important because leukotrienes are pro-inflammatory mediators. Leukotriene B4 (LBT4) is a lipid mediator derived from arachidonic acid and plays a relevant role in chronic inflammatory diseases such as arthritis, cardiovascular diseases, cancer, and metabolic disorders. In studies using fibroblasts, the LBT4 was increased in obese adipose tissue, contributing to obesity (Chakrabarti et al. 2011; Mothe-Satney et al. 2012; Wan et al. 2017). Leukotriene B4 (20-Carboxy-LTB4) can undergo more beta oxidation, which can be directly impacted by the enzyme *AL3A2,* which in the CO group has lower expression. In addition, *AL3A2* was enriched in the "triacylglycerol metabolism" pathway. In muscle, FA are a substrate for oxidation, producing triacylglycerol that will later be secreted as very low-density lipoprotein. There is a cooperation among different tissues, mainly the adipose, liver, and skeletal muscle tissues, so if there is an accumulation of triacylglycerol in skeletal muscle and liver it will probably result in insulin resistance (Frayn et al. 2006). Furthermore, *AL3A2* has been linked to Sjögren-Larsson syndrome, as an interruption of *AL3A2* function causes fat accumulation in cells, underscoring the importance of this enzyme in detoxification in various lipid degradation pathways (Udhaya Kumar et al. 2020). The *AL3A2* was also identified in the "oxidative stress in adipocyte dysfunction in type 2 diabetes and metabolic syndrome X" pathway. Lower expression levels of *AL3A2* and *GSTA4* may result in attenuation of the lipid peroxide elimination system in obese and insulin-resistant humans, increasing the levels of 4-Hydroxy-2(E)-nonenal, which, when binding xanthine oxidase, increases oxidative activity, and consequently, the additional generation of intracellular reactive oxygen species (ROS) (Grimsrud et al. 2007; Unoki et al. 2007; Curtis et al. 2010). When pigs were fed SOY, there was a higher expression of *AL3A2*, which may be involved in the increase of the lipid peroxide elimination system. *AL3A2* have cell-specific functions associated with inflammation, differentiation, or oxidative stress responses in which the CO group may be targeting a lower incidence of the inflammatory process (Bazewicz et al. 2019; Chu et al. 2019).

In this study, we observed that the enriched pathways in the CO *vs* SOY and CO *vs* FO comparisons corroborate the relationship of SHP transcription factor (CO *vs* SOY) and DEG *SCD* (CO *vs* FO) to HDL in "regulation of lipid metabolism: RXR-dependent regulation of lipid metabolism via PPAR, RAR, and VDR" pathway. As well as between the comparison SOY *vs* FO with the enrichment of the pathways "regulation of metabolism: Bile acids regulation of glucose and lipid metabolism via FXR", and "transport_HDL-mediated reverse cholesterol transport." Due to the negative regulation of some identified DEG that participate in these pathways and are directly related to HDL functions, they may have caused an increase in cholesterol as observed.

In the study performed by Puig-Oliveras et al. (2014), *SCD* was enriched in FA divergent pigs related to oxidation, accumulation, concentration, and lipid homeostasis. In this study, SCD was identified as down-regulated in the adipose tissue of animals with higher PUFA content in Iberian pigs x Landrace, and with greater expression in animals that showed greater accumulation of intramuscular fat. In addition, *SCD* affected meat quality traits in the population of pigs analyzed by Piórkowska (Piórkowska et al. 2020), in a work evaluating mutations in candidate genes. The *SCD* DEG is also present in the "putative pathways for stimulation of fat cell differentiation by Bisphenol A" pathway (SOY *vs* FO) was down-regulated in the SOY group. We hypothesize that it is related to the biosynthesis of FA, mainly the synthesis of oleic acid, fundamental in the regulation of the expression of genes that are involved in lipogenesis (Stelzer et al. 2016). In a study evaluating some candidate genes from commercial crossbred pigs (Shanzhu × Duroc), *SCD* expression levels showed a correlation with intramuscular fat content (Wang et al. 2016). Our findings indicate that even with a higher expression of *SCD*, the diet with the addition of FO did not present a higher amount of OA when compared to the other diets analyzed.

In the "putative pathways for stimulation of fat cell differentiation by Bisphenol A" other DEG (*TCF7L2 and C/EBPalpha*) were enriched for SOY *vs* FO and may be related to a direct effect or mechanism on *PPARG*. In addition, *PPARG* (down-regulated in SOY group) was enriched in "role of IL-6 in obesity and type 2 diabetes in adipocytes," and "dysregulation of adiponectin secretion from adipocytes in obesity, type 2 diabetes and metabolic syndrome X." Studies have identified the relationship of *PPAR* to nutrition and involvement in skeletal muscle, such as the study from Yu et al. (Yu et al. 2011) evaluating a diet high in saturated fat (beef tallow) or high in unsaturated fat (fish oil) in transgenic mice, which resulted in the decreased deposition of lipids in the liver by PUFA, and the ability to stimulate the expression of adipogenic genes and glucose metabolism genes in *PPARG* transgenic mice when fed FO. Another study in mice identified that *PPARG* knockout in muscle affected insulin sensitivity in skeletal muscle (Hevener et al. 2003). Furthermore, there is an important involvement of *PPARG* in the direct regulation of lipid metabolism in immune cells (Welch et al. 2003) and may be related to adipogenesis (Lazar 2005). The *PPAR* form heterodimers with retinoid X receptors (RXRs) and these heterodimers regulate the transcription of several genes (Stelzer et al. 2016). Thus, in the FO group, there seems to be a direction to be implicated in inflammatory processes due to the PPARG functions related to the pathology of diseases, such as obesity, diabetes, and also atherosclerosis and cancer.

Another group of enriched DEG were related to pathways involved in obesity-associated metabolic diseases, in which a higher expression of these genes was identified in the FO group. *AZGP1, APOE, Perilipin, LIPS*, and *PDE3B* (down-regulated in SOY group) were enriched in the "TNF-α pathway, IL-1 β induce dyslipidemia and inflammation in obesity and type 2 diabetes in adipocytes." This signaling pathway is related to the overexpression of TNF-α and IL-1 β, which in obese adipose tissue activate *NF-KB* and *EKR*, impairing genes with important functions. Obesity predisposes to insulin resistance, type 2 diabetes, and cardiovascular problems (Mracek et al. 2010). The cytokines TNF-α and IL1 β are considered the main regulators of inflammation involved in the pathogenesis of type 2 diabetes mellitus (Alexandraki et al. 2006). TNF-α inhibits *PDE3B* leading to the accumulation of Cyclic AMP (Zhang et al. 2002). In this some pathway, TNF-α also inhibits both *LIPS* inducing lipolysis, as well as *AZGP1* and *APOE* that are underexpressed in obese tissue and may promote the development of dyslipidemia (Zhi et al. 2007; Mracek et al. 2010). Thus, in the TNF-α pathway, the diet with FO leads to a greater expression of important genes in normal functions in the body that, if inhibited by TNF-α, can be relate to inflammation.

The DEG *AZGP1* (SOY *vs* FO) was identified as DEG and is related to the stimulation of lipid degradation in adipocytes, in addition to be involved in extensive fat loss in some advanced cancers (Stelzer et al. 2016). The *PLIN1* belongs to the *Perilipin* protein family and was differentially expressed in *Longissimus* muscle regulating the deposition of the intramuscular fat content of commercial hybrids (Pietrain × Duroc) × (Landrace × Yorkshire) (Li et al. 2018). In the study performed by Li et al. (Li et al. 2018), the authors suggested that a *PLIN1* knockdown can decrease the level of triglycerides and lipid droplet size in adipocytes. In Gandolfi et al. (Gandolfi et al. 2011) study, *Perilipin1* and *Perilipin2* were expressed in the semimembranosus muscle of commercial crossbred male castrated pigs and the specificity of the antibodies was confirmed by Western blot analysis (Gandolfi et al. 2011).

Another DEG identified was *APOE* (SOY *vs* FO), a protein associated with lipid particles that is essential for the normal catabolism of the constituents of triglyceride-rich lipoproteins, and is involved in the immune innate adaptive responses. In addition, it participates in the biosynthesis of VLDL by the liver (Stelzer et al. 2016). Dysfunctions in APOE protein can result in familial dysbetalipoproteinemia (type III hyperlipoproteinemia), and consequently, atherosclerosis (Fang et al. 2018). Using the CRISPR/Cas9 technique, Fang et al. (Fang et al. 2018) interrupted *APOE* expression in miniature pigs fed an induction diet high in fat and cholesterol, revealing that the animals had severe hypercholesterolemia and developed progressive atherosclerotic lesions. Additionally, *APOE* may be involved in the "transport HDL-mediated reverse cholesterol transport" pathway, in which *APOE* increases HDL binding to SRBI and selective cholesteryl ester uptake (van der Velde 2010). In FO group it was up-regulated; *APOE* helps in the normal function of parameters such as VLDL even though no statistical difference was observed in our results. Finally, in a study performed by Song et al. (Song et al. 2020), in which the animals were fed a diet with an n-6:n-3 PUFA ratio of 4:1 or 2:1, *LIPS/HSL* showed a higher relative gene expression compared to the control group with an 18:1 ratio. In our study, the pattern obtained was similar, in which the FO diet showed a lower n-6:n-3 PUFA ratio and consecutively higher expression of *LIPS/HSL* when compared to SOY diet.

The importance of enriched DEG that are involved in regulatory pathways and networks related to bile acids that regulate glucose and lipid metabolism is noted. In relation to the processes networks enriched, we identified networks related to muscle contraction with DEG down-regulated from the SOY group, demonstrating that SOY negatively regulated muscle contraction genes compared to animals from the FO group.

The results obtained from the constructed PPI networks suggest that these genes may be involved in distinct biological processes between the comparisons and may be potential targets for future studies on the relationship between diet and

health. All comparisons presented KEGG pathways related to metabolic pathways. Specifically, in the CO *vs* SOY comparison, genes involved in fatty acid degradation and metabolism of xenobiotics by cytochrome P450 were identified; in the CO *vs* FO comparison, beta-alanine metabolism and glycolysis/gluconeogenesis were identified; and finally, in the SOY *vs* FO comparison, biosynthesis of unsaturated fatty acids, fatty acid metabolism, PPAR signaling pathway, fat digestion and absorption, and Alzheimer's disease were identified.

We identified (FDR 10%) 65 DEG between CO *vs* SOY, with genes involved in the improving of lipid peroxide elimination system in SOY group; 531 DEG in the comparison SOY *vs* FO; and 32 DEG for CO *vs* FO with genes related to lipogenesis regulation in FO. In general, the main processes involved are related to lipid metabolism, metabolic diseases, and inflammation. In addition, the different oils modified the cholesterol and HDL levels, with a lower amount in the FO group. The CO and SOY have a high OA content, which leads to a greater beneficial health effect, in addition to having a higher amount of HDL when compared to FO group. More studies are needed to understand the genes directly related to each of the fatty acids and their association with blood parameters.

Conclusion

The enrichment of the basal diet with oil containing different FA profiles influenced FA profile, blood parameters, and gene expression in the skeletal muscle of pigs. Diets containing CO and SOY showed higher OA content, and higher amount of HDL when compared to FO group. Conversely, they also had a higher amount of cholesterol. The FO showed a higher amount of EPA, DHA, total n-3 PUFA, and genes related to the regulation of lipogenesis. The current study provided important details and relationships between relevant genes in lipid metabolism, contributing to human health as the pig is an excellent animal model. The results provide mechanisms to help us understand the behavior of concerning different FA profile.

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Authors contribution All authors contributed to this study. Conceptualization, S. L. F., G. C. M. M., J. M. R., J. E. K., D. K., H. F., L. C. A. R., J. C. d. C. B., L. L. C., and A. S. M. C.; writing (original draft preparation), S. L. F. J. D. G., B. P. M. d. S., and A. S. M. C.; performed data analysis, S. L. F., V. V. d. A., F. A. O. F., and A. S. M. C.; interpretation and discussion of the results, S. L. F., G. C. M. M., V. V. d. A., L. L. C., H. F., L. C. A. R., A. L. F., A. S. M. C.; critically reviewed and editing, V. V. d. A., B. S. V, J. A., G. C. M. M., J. M. R., J. E. K., D. K., D. G., H. F., L. C. A. R., G. B. M., L. L. C., M. D. P., S. M. d. A., A. L. F., and A. S. M. C.; supervision, A. S. M. C.; funding acquisition, A. S. M. C.

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Data availability The dataset supporting the conclusions of this article is available in the in the European Nucleotide Archive (ENA) repository (EMBL-EBI), under accession PRJEB52629 [http://www.ebi.ac.uk/ena/data/view/PRJEB52629]. The original contributions presented in the study are included in the article/"Supplementary information"; further inquiries can be directed to the corresponding author.

Declarations

Ethics approval All animal procedures were conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching and were approved by the Animal Care and Use Committee of Luiz de Queiroz College of Agriculture (University of São Paulo, Piracicaba, Brazil, protocol: 2018.5.1787.11.6 and number CEUA 2018–28).

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Competing interests The authors declare no competing interests.

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