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Regulatory networks involved in modulating fat deposition in pigs identified by gene co-expression analysis

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Abstract

The regulatory mechanisms underlying the interaction between fatty acid (FA) profiles and gene expression are highly complex, involving signaling pathways and transcription factors that control lipid metabolism. Using the gene co-expression approach, we can identify key gene regulators and gain a better understanding of gene interactions that may play an important role in regulatory mechanisms. Therefore, this study aims to identify gene-expression regulatory mechanisms associated with FA deposition profiles in skeletal muscle across different diets. We used basal diets with different levels of soybean oil (1.5% soybean oil [SOY1.5], reference diet; or 3% soybean oil [SOY3.0], enriched diet) added during the growth and finishing phases in a 98-day study. Total RNA was extracted, and mRNA was sequenced (Illumina). Bioinformatics analysis was performed with quality control, preprocessing, and alignment using *Sus scrofa*11.1. Gene abundance was normalized to transcripts per million. To identify co-expressed modules, we used weighted gene co-expression network analysis (WGCNA) with RNA-Seq data and the deposited FA profile. After filtering, data from 33 immunocastrated male pigs were used in this study. To identify pathways and Gene Ontology (GO) terms affected by the enriched diet (with 3% soybean oil), DAVID and REVIGO were used. Diets with varying levels of soybean oil affect metabolic processes differently. In general, we identified co-expression networks mainly involved in lipid metabolism, diseases and general response involved in inflammatory processes, glucose homeostasis. We constructed co-expression networks and identified the hub genes, including *TPM1* and *SLC38A10*, as well as *CSRNP1*, *TRIP10*, *ZFP30*, and *MYBPH*. Co-expression analysis using WGCNA provides new insights into fatty acid deposition by identifying candidate genes potentially involved in lipid regulation and influenced by dietary differences.

Keywords Pig model, Skeletal muscle, IMF, Systems biology, Soybean oil, Lipid metabolism, Diseases

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Background

Fatty acid (FA) profile and intramuscular fat (IMF) composition are key indicators of pork quality [1] and IMF is an important trait for studying gene-diet interactions [2]. The FA profile is a determining factor for both the quality of the meat and implications for diseases in humans, such as obesity and diabetes [1, 3]. In addition, FA can affect gene expression and, consequently, the genes that encode enzymes that influence elongases and desaturases in lipogenic tissues, as well as various cellular processes, including membrane fluidity [3–6]. The regulatory mechanisms involved in FA profile and gene expression are complex, with multiple genes involved and containing several transcription factors (TF). These TF participate in regulatory networks, signaling pathways, and signal transduction, affecting various biological processes [7].

Molecular signaling pathways, such as leptin, mTOR, and Wnt, regulate fat characteristics through genetic, nutritional, and environmental factors [8]. Nutrients interact through signaling pathways, epigenetic mechanisms, nutrient-responsive pathways, or genes on phenotypes. However, the relationship between muscle gene expression and the FA composition of muscle is not completely elucidated [4, 9–11]. In pigs, lipid deposition has economic relevance and is a complex characteristic involved in parameters such as meat quality and immunity [12]. Pork meat plays an important role in the human diet, where muscle tissue has the highest economic value in pig production [13, 14]. Along with the muscle, the liver has great importance in lipid metabolism [3]. The identification of regulatory genes involved in the composition of IMF in pigs will be important for the development of breeding strategies using functional enrichment tools and for advancing our understanding of gene regulation in muscle [9].

The FA present in pork carcasses and meat are saturated FA (SFA), and unsaturated FA, such as monounsaturated FA (MUFA) with oleic acid (OA; C18:1) and polyunsaturated FA (PUFA). The main PUFA are omega 3 (n-3), omega 6 (n-6), linoleic acid (LA; C18:2) and α-Linolenic acid (ALA; C18:3), which are important for the industry and consumers [15–17]. Oleic acid is one of the most common free FA (FFA) important in cellular metabolism [18]. Higher intake of LA has been associated with improved cardiometabolic health; however, the effects of LA, SFA, and n-6 and n-3 FA on health remain a topic of debate and controversy [19].

Animal models are essential tools for research, and pigs are suitable for studying human lipid-related metabolic diseases. Their similarities to humans in size, genome, anatomical structure, physiology, and immunology make them valuable models [14, 20–22]. In addition, pigs are important livestock species, underscoring their importance in both biomedical and agricultural research [14, 20–22]. Pigs are important nutritionally, and biomedical models require an in-depth exploration of their genomics and functional genomics [23].

Several network analysis strategies are available to understand molecular-level interactions associated with complex phenotypes. One of these approaches is the application of the weighted gene co-expression network analysis (WGCNA), which is based on correlation patterns of expression and traits [24, 25]. WGCNA provides a more robust bio-signature for phenotypic traits and better candidate biomarkers for future studies by enabling high-resolution analysis to identify key functional genes [26]. The WGCNA has been employed to identify putative genes involved in the composition of the IMF and biological and molecular processes across different species, as well as to integrate the data in pigs [5, 11, 27].

Identification of genes at the systems level is a valuable tool to improve our understanding of gene interaction mechanisms between different diets. Through the analysis of modules that have correlations with specific traits, we can identify key genes and the location of regulatory centers associated with these traits [28]. Therefore, the identification of gene expression changes based on deposited FA is essential to understand how diet influences key co-expressed genes associated with different FA depositions. By studying the behavior of gene co-expression, we can interpret the variability of molecular mechanisms associated with traits of interest [11]. Thus, we hypothesize that different levels (1.5 or 3%) of soybean oil in the diet al.ter the regulatory mechanisms by influencing the co-expression patterns of the FA profile. Therefore, the aim of this study was to identify gene expression regulatory mechanisms correlated with FA deposition profiles in skeletal muscle under different diets.

Methods

Ethics statement

The animal experiments were approved under protocol 2018.5.1787.11.6 and CEUA number 2018-28 by the Animal Care and Use Committee of ESALQ (Luiz de Queiroz College of Agriculture), Brazil.

Animals, diet, and sampling procedures

All details of animal, experimental design, housing, and diet treatments were described previously [29, 30]. Male pigs homozygous negative for the halothane gene (NN) (Large White × Large White offspring) were used in this study. They were blocked for initial body weight and randomly assigned to diets with different levels of soybean oil. Animals were housed in an all-in/all-out, double-curtained building with six replicate pens per treatment, each containing four pigs (n = 24 per treatment). Supplementary Figure S1 summarizes all the analyses

performed. Each pen had a three-hole dry self-feeder and a nipple drinker to ensure *ad libitum* access to food and water. All pigs were immunocastrated with two 2-mL doses of Vivax® (Pfizer Animal Health, Australia).

The diets were adjusted according to the phases, with day 0 marking the beginning of the trial when the pigs averaged 71 days of age. Phases included grower I (days 0–21), grower II (days 21–42), finisher I (days 42–56), finisher II (days 56–63), finisher III (days 63–70), and finisher IV (days 70–98). Diets were corn and soybean meal-based and formulated to meet or exceed all nutritional requirements [31]. During the growing and finishing phases, pigs were fed either 1.5% soybean oil (SOY1.5, reference diet) or 3% soybean oil (SOY3.0, enriched diet). Details of the diets in this study are described in Supplementary Tables S1A-C [29, 30, 32]. The experimental population originated from a private breeding program involving three sires and 32 dams, with the inbreeding level controlled below 14% [29]. The pigs had an average initial body weight (BW) of 28.44 ± 2.95 kg and an average age of 71 ± 1.8 days. At slaughter, the animals averaged 133.9 ± 9.4 kg body weight and were 155 days old [33]. After the 98-day experimental period, three pigs per pen (n = 18 per treatment) were euthanized by electrical stunning followed by exsanguination according to standard industry procedures. Samples of skeletal muscle (*Longissimus lumborum*) between the 10th and 11th ribs were collected within 30 min. The samples were immediately frozen in liquid nitrogen and stored at -80 °C in an ultra-freezer [34].

RNA-Seq data

Total RNA was extracted from muscle samples using the RNeasy® Mini Kit (Qiagen) and analyzed for quantification, purity, and integrity (RIN ≥ 7) (Supplementary Table S2). Library preparation was performed using 2 µg of RNA per sample according to the TruSeq RNA Sample Preparation Protocol (Illumina). The estimation of libraries' average size was made with the Agilent Bioanalyzer 2100, and the libraries were quantified using quantitative PCR with the KAPA Library Quantification kit [35]. Sequencing was performed on a HiSeq2500 (Illumina) using the TruSeq SBS Kit v4-HS (200 cycles). The complete details of the procedure are provided in Fanalli et al. [35]. All sequencing analyses were performed at the Genomics Center of ESALQ - USP, Piracicaba, São Paulo, Brazil.

mRNA data are available from PRJEB52629 [https://www.ebi.ac.uk/ena/browser/view/PRJEB52629]. FastQC, v. 0.11.8 software, was used to check the quality of the RNA-Seq data [http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc] before and after trimming. After trimming (Trim Galore v. 0.6.5), sequencing adapters and low complexity reads were removed [https://www.bioinformatics.babraham.ac.uk/projects/trim_galore]. Phred score and length greater than 33 and 70 nucleotides, respectively, were retained. Pig reference genome *Sus Scrofa* 11.1 [http://www.ensembl.org/Sus_scrofa/Info/Index], using the assembly available at Ensembl (Release 102), was used for alignment analysis. The abundance (read counts) of mRNAs for all genes was aligned and quantified using Bowtie2 v. 2.4.3 [36] and RSEM (RNA-Seq by Expectation Maximization) v. 1.3.1 software, respectively [37]. Expression quantification was performed with *rsem-calculate-expression.*

Fatty acid profile and data adjustment

To better understand the genes involved in diet modulation, we used FA profile data [29, 38] (Supplementary Table S3). For FA analysis, total lipids were isolated from 100 g of *Longissimus lumborum* muscle following the cold extraction [39] method and methylated (AOCS 2004; Method AM 5 – 04). Fatty acid methyl esters were quantified using gas chromatography (Shimadzu GC-2010 plus AF, Canby, OR, USA). Total monounsaturated FA (MUFA), palmitoleic acid (PA), α-linolenic acid (ALA), oleic acid (OA), total saturated FA (SFA), and total unsaturated FA (PUFA), PUFA: SFA ratio, atherogenic index (AI), omega 6: omega 3 (n-6: n-3) ratio, and phenotypes were used for co-expression analysis.

To analyze the phenotypic data using the FA profile, the data were centered and scaled to ensure comparability. A linear model was fitted in R. To examine the co-expression networks, the data was adjusted using the following equation: $y^* = \mu + y - X\hat{\beta} + \epsilon$, considering μ as the overall mean vector, y^* as the phenotype vector, X as the incidence matrix for fixed effects, $\hat{\beta}$ as the fixed effects vector, and ϵ as the residual vector. Fixed effects, including block and sire, were used; the block was defined by the body weight at nursery exit and at the beginning of the experimental period [40]. In the SOY3.0 group, one animal was identified as an outlier based on the three standard deviations criterion and was removed from further analysis. Complete data used is presented in Supplementary Table 4a-b.

Weighted gene co-expression network analysis (WGCNA)

To identify candidate genes potentially involved in regulatory mechanisms under different diets, gene co-expression network analysis was performed using RNA-Seq data and FA profile. Based on the filtered transcripts per million (TPM) value of all expressed coding genes, hierarchical clustering was performed separately for each of the sample groups (SOY1.5 and SOY3.0) to identify changes that occurred and the behavior of the clustered genes.

Co-expression analyses are based on constructing an adjacency matrix by calculating Pearson correlation

coefficients for all genes in TPM after filtering with WGCNA v. 1.73. A filtering step was applied to remove low-expression genes, retaining only those expressed in at least 50% of the samples. We use the β exponent, adhering to the free-scale topology ($R^2 > 0.81$) [40–42]. The networks were constructed using the signed function in WGCNA to classify co-expressed gene modules [43]. Topological Overlap Matrix (TOM) modules were defined based on dissimilarity (1-TOM), with TOMType set to "*signed*. We used the *cutreeDynamic* function from the WGCNA package, based on the gene dendrogram and the TOM dissimilarity matrix, with the following parameters: *deepSplit = 2, pamRespectsDendro = FALSE, and minModuleSize = 100* [44]. Modules were then merged based on eigengene dissimilarity, where eigengenes represent the first principal component of each module and summarize its overall gene expression profile. The module eigengene (ME) dissimilarity threshold (*MEDissThres*) was set to 0.25, corresponding to an eigengene similarity of 0.75 (1 – 0.25). Genes that did not belong to any module were assigned to the grey module. We considered all MEs that were significantly correlated with FA, with a significant criterion of $p < 0.05$. Module–trait relationships were evaluated using gene significance (GS) and module membership (MM) [43] with *p*-value < 0.05 [40].

Network visualization and enrichment analysis

The network generated in this study was visualized using the Cytoscape software v. 3.10.1 [45] after being extracted using the "*exportNetworkToCytoscape*" function (*threshold = 0.02, weighted = TRUE*) to generate node and edge files, which played a key role in rendering the network. We applied a threshold to the weight values to ensure the network maintained at least 3,000 interactions, preventing disruptions to its structure. Additionally, nodes with connectivity counts below 10 were removed after filtering [44]. In this context, the term "weight" in the Cytoscape input files refers to the strength of connections (edges) between nodes (genes) as indicated by correlation values derived from the topological overlap matrices (TOM).

For better biological interpretation of the identified clustering genes co-expressed with deposited FA, we conducted a functional enrichment analysis using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) 2021 (Dec. 2021), DAVID Knowlegebase (v2023q4) [46]. Gene Ontology (GO) terms [47] and Kyoto Encyclopedia of Genes and Genomics (KEGG) pathways [48, 49] were set at a *p*-value < 0.05. In REVIGO v. 1.8.1 [50], we performed a comparative analysis to better understand the overall effects of the diets using the default parameters. GO terms enriched (*p*-value < 0.05) in all modules for each diet were used.

Detection of candidate genes – hub genes of modules that co-expressed with FA deposition

To identify candidate hub genes, we used the Maximal Clique Centrality (MCC) algorithm from the CytoHubba v.0.1 plugin [51]. This algorithm measures the importance of nodes in a biological network. For annotation of genes in the module, we used the stringApp v. 2.1.1 plugin – STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) [52], with the STRINGFy function, in Cytoscape. The ggplot2 v. 3.5.1 package R [53] was used to visualize the information from the enriched data.

Results

Here, we used a robust systems biology approach to identify pathway and network modifications between different diets using skeletal muscle transcriptome data. Gene read counts were normalized using TPM (Transcripts Per Million) to enable comparative analysis of expression levels across genes. Initially, we identified 24,358 genes. Following quality control, 15,497 and 15,527 genes were used for SOY1.5 and SOY3.0, respectively. For this study, we obtained the β value from the scale-free topology analysis: 14 for SOY1.5 and 20 for SOY3.0 (Supplementary Figures S4a-b). The modules that meet the criteria are identified as such: $|r| > 0.48$, $p < 0.05$. The results obtained show the modification of the clusters according to the level of soybean oil for the SOY1.5 and SOY3.0 groups. This suggests a different regulation of gene co-expression according to the amount of soybean oil added to the diet.

Gene modules in response to different diets

As expected, our results show that the co-expression patterns change depending on the diet used. Firstly, for the SOY1.5 group, no outliers were detected. As shown in Fig. 1 and Table 1, in the SOY1.5 group (p-value < 0.05), we identified that total SFA was associated with the *Yellow* module ($r = +0.49$) and the *Cyan* module (r = -0.51). For Total polyunsaturated fatty acids (PUFA), the *Turquoise* module had a correlation of r = -0.47, and the same module was related to the PUFA: SFA ratio (r = -0.48). Regarding the Atherogenic Index (AI), the *Yellow* module exhibited a correlation of $r = +0.5$, while the *Cyan* module showed r = -0.63. The co-expressed module with the highest correlation was the *Cyan* module with r = -0.63 for the AI, and – 0.51 for the total SFA.

Samples 11 and 46 were identified as outliers when analyzing data from the SOY3.0 group sample clustering analysis and were subsequently removed (Supplementary Figures S3a-b). Following these removals, the SOY3.0 group consisted of 15 samples. We identified modules co-expressed with certain FA that were different from those found in the SOY1.5 group. This suggests a change in co-expression when a higher level of soybean

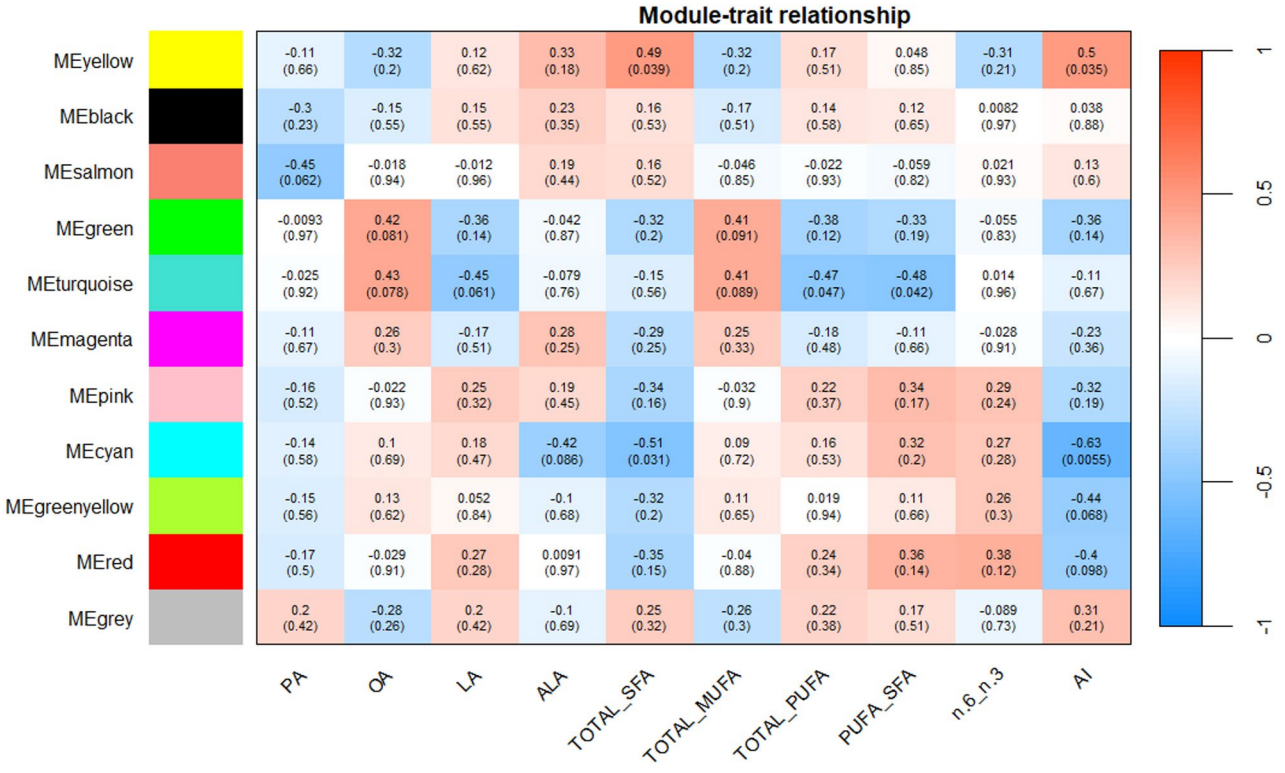

Fig. 1 The data used to construct the modules for the 1.5% soybean oil group (SOY1.5) illustrates the associations between the module eigengenes (ME) listed in the rows and the fatty acids (FA) listed in the columns. The numbers outside the parentheses are Pearson's correlation coefficients, while those inside represent p-values. Red indicates a positive correlation, and blue indicates a negative correlation. FA: palmitoleic acid (PA), oleic acid (OA), α-linolenic acid (ALA), total saturated FA (SFA), total unsaturated FA (PUFA), total monounsaturated FA (MUFA), PUFA: SFA ratio, omega 6: omega 3 (n-6: n-3) ratio, and atherogenic index (AI). Subsequent analyses used a P-value of less than 0.05

Table 1 Data extracted from modules (p-value < 0.05) with co-expressed fatty acids, module, hub gene and regulation identified for the SOY1.5 and SOY3.0 groups

Fatty acids	**Modules (SOY1.5)**	**Hub gene**	**Regulation SOY1.5vs.SOY3.0**
Total SFA and Atherogenic index	*Yellow* (974 nodes)	Tropomyosin 1 (*TPM1*)	Up-regulated
	Cyan (110 nodes)	Regulator of calcineurin 1 *(RCAN1)*	Up-regulated
PUFA: SFA and Total PUFA	*Turquoise* (5881 nodes)	Solute carrier family 38 member 10 gene (*SLC38A10*)	Down-regulated
Fatty acids	**Modules (SOY3.0)**	**Hub gene**	**SOY1.5vsSOY3.0**
Palmitoleic acid	*Darkgreen* (191 nodes)	Cysteine-serine-rich nuclear protein 1 (*CSRNP1*)	Up-regulated
	Darkred (204 nodes)	Thyroid hormone receptor interactor 10 (*TRIP10*)	Up-regulated
	Black (1289 nodes)	Zinc Finger Protein 30 (*ZFP30*)	Down-regulated
Oleic acid and total MUFA	*Lightcyan* (295 nodes)	Fatty acid binding protein 7 (*FABP7*)	Up-regulated
Linoleic acid and total PUFA	*Royalblue* (222 nodes)	Myosin binding protein H (*MYBPH*)	Up-regulated

*The "Regulation" column indicates whether the log2FC is higher (Up-regulated) or lower (Down-regulated) compared to SOY1.5vs.SOY3.0 based on Counts per Million (CPM) previously reported by our group [30]. In this work, these values are included solely for contextual interpretation and were not used in downstream analyses. Since these hub genes are not classified as differentially expressed genes (DEGs), only their regulatory direction is reported

oil is included in the diet of pigs. For the SOY3.0 group (Fig. 2 and Table 1), five FA presented co-expressed modules (p-value < 0.05), comprising palmitoleic acid with 3 ME - *Darkgreen* r = -0.65; *Darkred* r = -0.68, and *Black* r = + 0.68; OA with *Lightcyan* r = + 0.59; LA with ME *Royalblue* r = + 0.55; total MUFA *Lightcyan* r = + 0.58, and total PUFA with *Royalblue* module r = + 0.52.

Functional analysis and identification of hub genes between the groups

The *Yellow* module demonstrated a correlation with both total SFA and AI (positive correlation). The *Yellow* module was identified as related to the lipid and atherosclerosis pathway. Furthermore, it presented genes involved with FA biosynthesis and metabolism. The enriched pathways identified in the *Yellow* module were

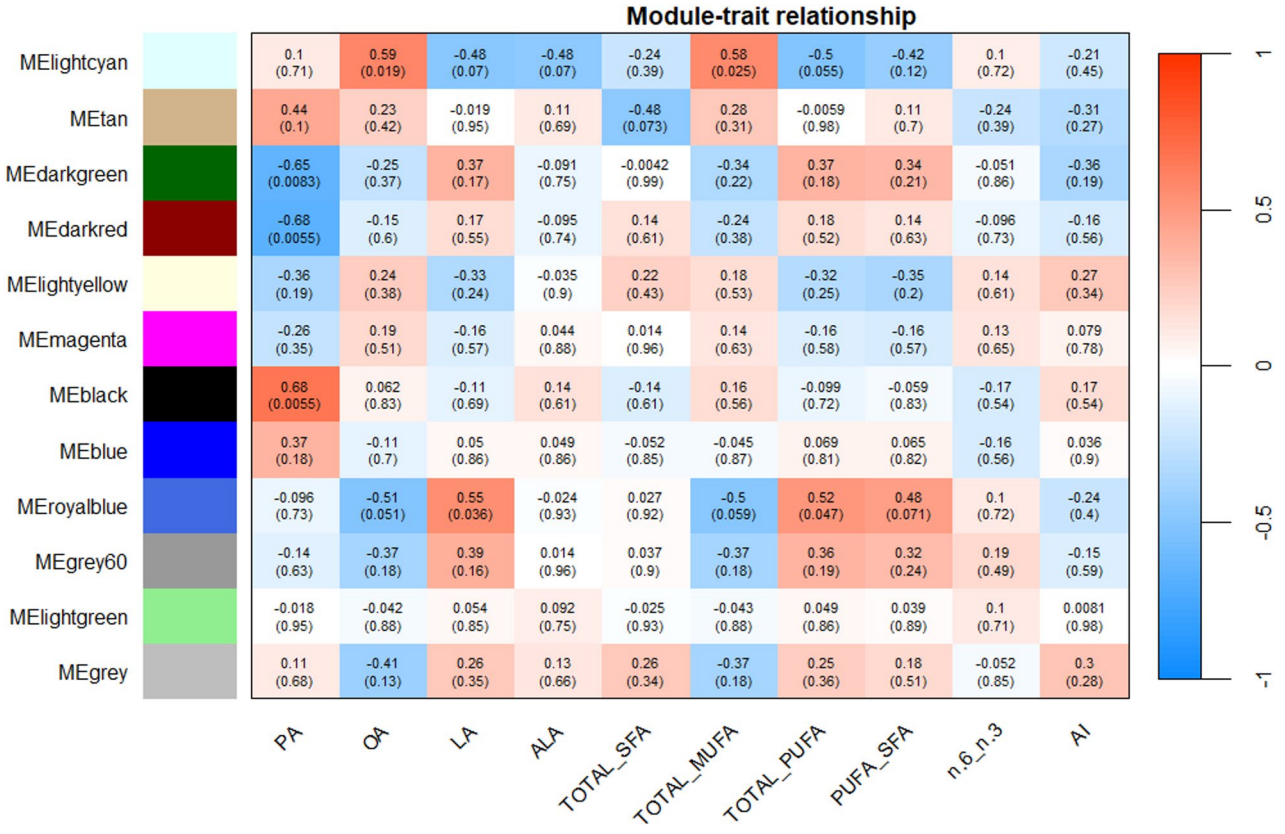

Fig. 2 The data used to construct the modules for the 3% soybean oil group (SOY3.0) illustrate the associations between the module eigengenes (ME) listed in the rows and the fatty acids (FA) listed in the columns. The numbers outside the parentheses are Pearson's correlation coefficients, while those inside represent p-values. Red indicates a positive correlation, and blue indicates a negative correlation. FA: palmitoleic acid (PA), oleic acid (OA), α-linolenic acid (ALA), total saturated FA (SFA), total unsaturated FA (PUFA), total monounsaturated FA (MUFA), PUFA: SFA ratio, omega 6: omega 3 (n-6: n-3) ratio, and atherogenic index (AI). Subsequent analyses used a P-value of less than 0.05

the glucagon signaling pathway (ssc04922); glycolysis/gluconeogenesis (ssc00010); insulin signaling pathway (ssc04910); Hypoxia-inducible factor 1 (HIF-1) signaling pathway (ssc04066), and metabolic pathways (ssc01100) (Supplementary Table S5a). The hub gene identified was tropomyosin 1 (*TPM1*) (Fig. 3). This gene is known to bind actin filaments in both muscle and non-muscle cells. It is also linked to the troponin complex, which plays a key role in the calcium-dependent regulation of striated muscle contraction [54].

The *Turquoise* module was identified as co-expressed (negative correlation) with total PUFA and the PUFA: SFA ratio in the SOY1.5 group. The KEGG pathways and GO terms identified (Supplementary Figure S3c) include lipid metabolic process, angiogenesis, lipid binding, inflammatory response, AGE-RAGE signaling pathway in diabetic complications, and the PPAR signaling pathway featuring genes such as peroxisome-proliferator activated receptor gamma (*PPARγ*); perilipin 4 (*PLIN4*); acyl-CoA oxidase 1 (*ACOX1*); fatty acid binding protein 4 (*FABP4*); perilipin 1 (*PLIN1*), stearoyl-CoA desaturase (*SCD*), lipoprotein lipase (*LPL*), and adiponectin *(ADIPOQ)*. This module presented GO terms related to cholesterol; lipid metabolic process *(AOX1*, *PLIN1*, *LEP*, *LPL)*, transport, homeostasis, and binding; fatty acid elongase activity and elongation of PUFA, SFA, and MUFA. The hub gene of this module is the solute carrier family 38-member 10 gene (*SLC38A10*) (Supplementary Figure S5). SLC38A10 is involved in glutamate homeostasis, response to nutrient detection, and may play a role in fatty acid regulation [55].

In contrast to the reference diet, analysis of data from pigs fed a diet supplemented with 3% soybean oil revealed a distinct co-expression pattern. Only for the total PUFA co-expression was identified, as in the SOY1.5 group, but it differed in the functional analysis. This represents a difference between the diets that we may be regulated by the amount of oil deposited, for the *Royalblue* module (positive co-expression with LA and total PUFA). The enrichment analyses that were revealed in this module were as follows: positive regulation of interleukin-1 beta production (GO:0032731); development of skeletal muscle fibers (GO:0048741); and organization of actin filaments (GO:0007015) (Supplementary Table S6e). The hub gene

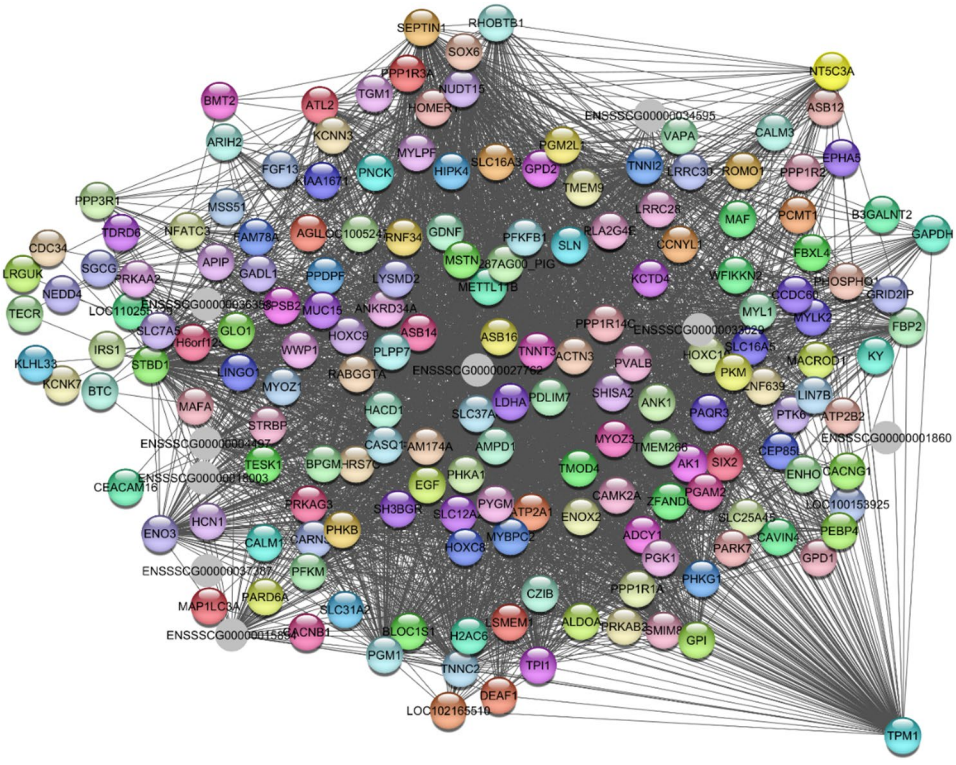

Fig. 3 Network constructed using Cytoscape to highlight interactions of the hub gene tropomyosin 1 (*TPM1*). Interaction networks in skeletal muscle tissue from pigs fed a diet containing 1.5% soybean oil (SOY1.5). The *Yellow* module was co-expressed with the total saturated fatty acid (SFA) and atherogenic index (AI) phenotypes. Colored nodes represent genes/proteins included in the query, and grey lines represent all interactions between nodes

identified was myosin binding protein H (*MYBPH*) (Fig. 4). This gene is related to the structural constituent of muscle [54].

Another module identified in the SOY1.5 group was *Cyan*. This module showed negative correlations with total SFA and AI and was enriched with KEGG pathways and GO terms, including chaperone binding (GO:0051087). It also included terms like myosin complex (GO:0016459) and insulin receptor signaling pathway (GO:0008286) (Supplementary Table S5b). The hub gene identified for the *Cyan* module was regulator of calcineurin 1 *(RCAN1)* (Supplementary Figure S6). RCAN1 has previously been implicated in diabetic cardiomyopathy and associated with metabolic disorders such as mitochondrial fission and lipid accumulation [56]. We identified that SFA and AI present a relationship of enriched modules related to the insulin pathway in the SOY1.5 group.

Black, *Darkgreen*, *Darkred*, and *Lightcyan* were the other modules identified as co-expressed only with FA in the SOY3.0 group (Supplementary Table S6a-e). In the *Black* module, we identified positive co-expression with palmitoleic acid. The pathways and GO terms identified in the *Black* module were metabolic pathways (ssc01100); insulin signaling pathway and secretion (ssc04910; ssc04911); MAPK signaling pathway (ssc04010); glucagon signaling pathway (ssc04922); alpha-linolenic acid metabolism (ssc00592); lipid metabolic process (GO: 0006629); fatty acid degradation and metabolism (ssc00071; ssc01212); cholesterol binding (GO:0015485). The hub gene found in this module was Zinc Finger Protein 30 (*ZFP30*) (Supplementary Figure S7).

The *Darkgreen* module with negative co-expression with palmitoleic acid was also identified. The *Darkgreen* module was enriched for pathways and GO terms related to non-alcoholic fatty liver disease (ssc04932); interleukin (IL)-17 signaling pathway (ssc04657); positive regulation of IL-1 beta production (GO:0032731); positive regulation of angiogenesis (GO:0045766); regulation of neurogenesis (GO:0050767); Tumor necrosis factor (TNF) signaling pathway (ssc04668); nuclear factor (NF)-kappa B signaling pathway (ssc04064) and others (Supplementary Table S5). The hub gene for this module was cysteine-serine-rich nuclear protein 1 (*CSRNP1*) (Supplementary Figure S8). The *CSRNP1* gene was previously identified as being under the influence of nutrient supply in porcine skeletal muscle and associated with energy homeostasis [57].

The enrichment analysis results for the *Darkred* module (negative correlation with palmitoleic acid) include genes involved to diseases, signaling, and transport such as: type II diabetes mellitus (ssc04930); insulin response

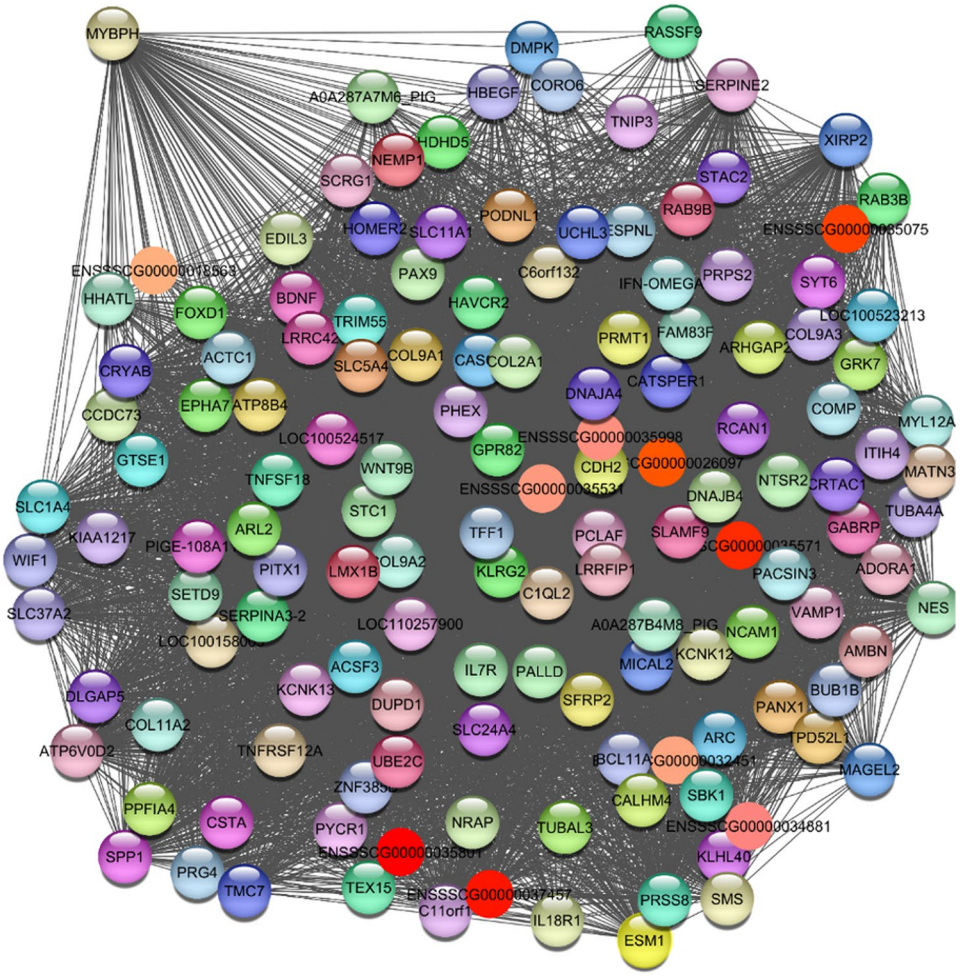

Fig. 4 Network view highlighting interactions of the hub gene myosin binding protein H (*MYBPH*). Interaction networks within the module represent the nodes and edges identified after filtering in relation to the group that received diet with 3.0% soybean oil inclusion (SOY3.0) were constructed using Cytoscape, followed by the stringApp plugin. The network associated with the *Royalblue* module is co-expressed with the Linoleic acid and Total PUFA phenotypes. Colored nodes represent genes/proteins included in the query

(GO:0032868); transport of medium-chain fatty acids (GO:0001579); chromatin (GO:0000785); gluconeogenesis (GO:0006094); and the glucagon signaling pathway (ssc04922). The hub gene of the *Darkred* module was thyroid hormone receptor interactor 10 (*TRIP10*) (Supplementary Figure S9), identified as a biomarker for Huntington's disease, related to signal transduction and lipid binding [54].

Functional analysis and identification related to the *Lightcyan* module showed positive co-expression with OA, and total MUFA present some genes in disease-related pathways. Such as non-alcoholic fatty liver disease (ssc04932); Huntington disease (ssc05016); cardiac muscle contraction (ssc04260); diabetic cardiomyopathy (ssc05415); Parkinson disease (ssc05012); regulation of response to food (GO:0032095); cardiac muscle contraction (ssc04260), and others (Fig. 5). For this module correlated with OA and total MUFA, the hub gene was fatty acid binding protein 7 (*FABP7*), which was identified as related to oxidative stress [58] (Supplementary Figure S10).

We observed that the identified modules and pathways show how gene co-expression varies with different dietary oil levels. These changes influence processes related to insulin signaling, glucose metabolism, immunology, and diseases, possibly reflecting metabolic and regulatory adaptations to lipid availability. In addition, the identified hub genes are involved in lipid metabolism, fatty acid regulation, and dietary modification. This underscores the significance of these genes, which were influenced by different diets and co-expressed with FA.

A summary of the pathways identified in each group and the FA involved is shown in Fig. 6. In this study these results provide a more detailed understanding of the gene expression co-expressed with specific FA. In addition, the identified gene clusters provide new insight into the molecular mechanisms that coordinate and regulate skeletal muscle deposition in response to diet. Figure 6

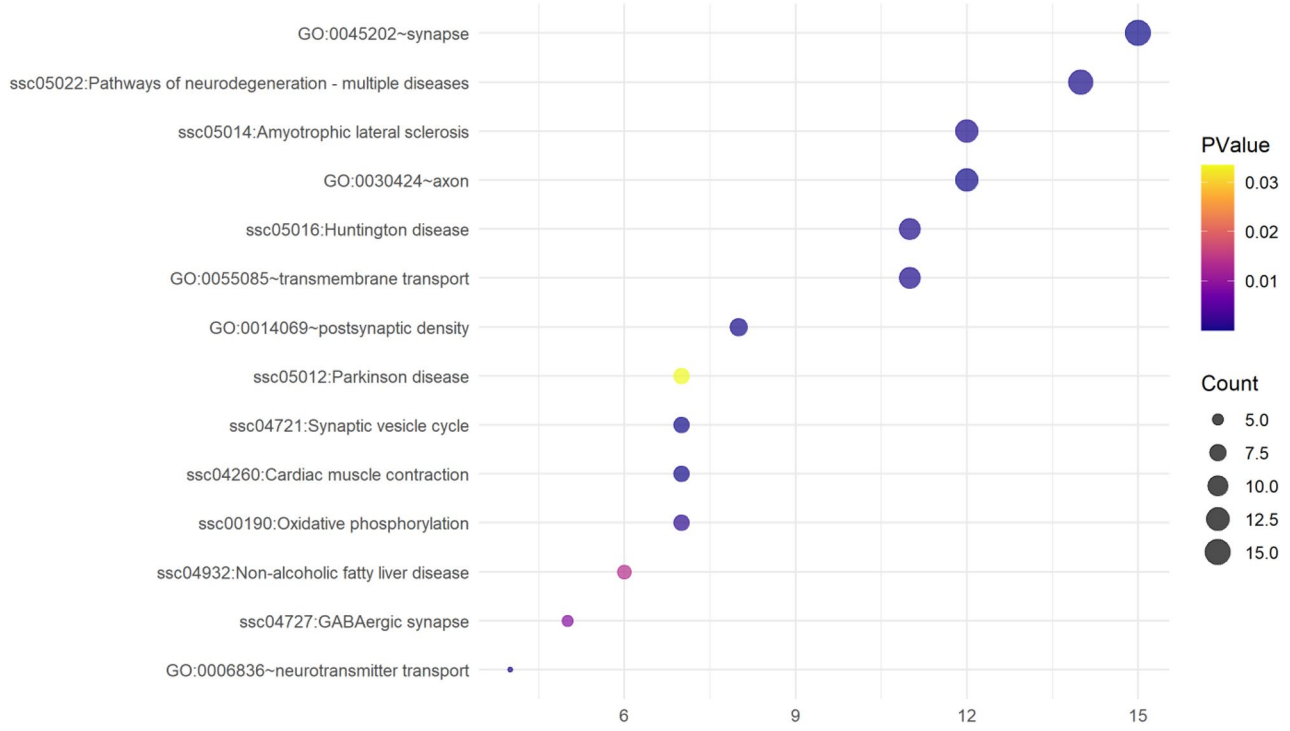

Fig. 5 Functional enrichment analysis of genes in the *Lightcyan* module in the SOY3.0 group. The figure displays selected KEGG pathways and GO terms along with the number of enriched genes (counts) with *p*-value < 0.05

Fig. 6 Summary of identified modules, hub genes, KEGG pathways, and GO terms involved in the regulation of fatty acid deposition. The colors of the enrichment KEGG pathways and GO terms represent negative (blue) and positive (red) co-expression with the module. The SOY1.5 group: basal diet supplemented with 1.5% soybean oil; SOY3.0: basal diet supplemented with 3.0% soybean oil. Fatty acids (FA): total saturated FA (SFA), total PUFA, total monounsaturated FA (MUFA), total polyunsaturated FA (PUFA): SFA ratio, n-6: n-3 ratio, and Atherogenic Index (AI)

Table 2 Top 5 GO terms related to biological processes and molecular function identified by REVIGO with a dispensability score < 0.05 for the SOY1.5 and SOY3.0 group

Biological Process (BP) for the SOY1.5 group	
GO:0003009	skeletal muscle contraction
GO:0006096	glycolytic process
GO:0006869	lipid transport
GO:0009408	response to heat
GO:0050729	positive regulation of inflammatory response
Biological Process (BP) for the SOY3.0 group	
GO:0006357	regulation of transcription by RNA polymerase II
GO:0006836	neurotransmitter transport
GO:0035914	skeletal muscle cell differentiation
GO:0042593	glucose homeostasis
GO:0002309	T cell proliferation involved in immune response
Molecular Function (MF) for the SOY1.5 group	
GO:0000146	microfilament motor activity
GO:0003774	cytoskeletal motor activity
GO:0016829	lyase activity
GO:0017147	Wnt-protein binding
GO:0022857	transmembrane transporter activity
Molecular Function (MF) for the SOY3.0 group	
GO:0000978	RNA polymerase II cis-regulatory region sequence-specific DNA binding
GO:0000981	DNA-binding transcription factor activity, RNA polymerase II-specific
GO:0005245	voltage-gated calcium channel activity
GO:0030020	extracellular matrix structural constituent conferring tensile strength
GO:0030545	signaling receptor regulator activity

represents the two groups of pigs fed diets with different levels of soybean oil. The figure highlights the identified co-expressed FA, the individual modules, their KEGG pathways, GO terms, and hub genes for each group. The colors indicate correlation values, with blue representing negative correlations and red indicating positive module-trait co-expression. The hub gene is not directly involved in the identified pathways; however, it has been identified as a central gene within the module, suggesting potential influence on genes related to these pathways.

After the analysis, we summarized all enriched GO terms (p-value < 0.05) within the modules using REVIGO, removing redundant GO terms to identify the most representative subset for each group (SOY1.5 and SOY3.0). Overall, the results indicate changes in gene expression regulation and cell signaling in the SOY3.0 group as evidenced by the molecular functions identified (Table 2 and Supplementary Figure S11). These findings highlight the role of dietary fat in modulating molecular pathways related to muscle function, metabolic process, and immune response.

Discussion

In this study, we extended our previous research on fatty acid (FA) composition by applying WGCNA to investigate the effects of increasing levels of dietary soybean oil on muscle FA metabolism in immunocastrated pigs. This approach allowed us to identify co-expression modules associated with FA phenotypic traits in a soybean oil-enriched diet. We identified novel key genes associated with the modulation of specific FA in response to the dietary soybean oil levels. These findings highlight how dietary differences can influence gene regulation and contribute to a better understanding of the underlying regulatory mechanisms involved in lipid metabolism. Our goal is to provide data for understanding differences in gene co-expression within a systems biology context and to evaluate how increasing dietary soybean oil levels affects FA co-expression in pigs. Previously, we found that increased oil supplementation led to differences between both groups [30], including the identification of 45 differentially expressed genes (DEG) in skeletal muscle when comparing diets with added soybean oil. These DEG were associated with signaling pathways related to inflammation, immune processes, oxidative stress, type II diabetes, and metabolism. Now, we build upon these findings by identifying the relationships between specific FA and gene expression modulation, as well as key genes and pathways involved.

The impact of dietary fat on gene expression represents an adaptive mechanism in response to changes in fat intake [59]. The SOY1.5 group identified clusters of co-expressed genes related to total PUFA, total SFA, PUFA: SFA ratio, and AI. Conversely, in the SOY3.0 group, co-expressed gene clusters were linked to palmitoleic acid, OA, LA, total MUFA, and total PUFA, highlighting their regulatory significance across different fat intake levels. It is noteworthy that total PUFA was associated with the modules in both diets, demonstrating the complexity of the interactions and how it may induce different regulatory mechanisms due to the influence of other components, signaling, regulatory pathways, and metabolic responses. For the SOY1.5 group, we identified three modules of co-expressed genes. Our analysis showed a positive relationship between the AI and the *Yellow* module, and a negative relationship with the *Cyan* module, in addition to a correlation with total SFA. However, there were changes in gene co-expression that highlighted the involvement of the SOY1.5 group, reinforcing our hypothesis of differentiation in the co-expression pattern.

We identified an altered co-expression with total SFA and AI, with a positive correlation to the *Yellow* module. The pathways involved are mainly related to glycogen, gluconeogenesis, and insulin signaling, suggesting regulatory mechanisms involved in glycogen metabolism and homeostasis. In addition to the relationship

between inflammation and HIF-1 signaling. HIF-1 is also relevant to glucose metabolism because it may regulate genes involved in atherosclerosis, and may be related to cholesterol accumulation and effects on lipid homeostasis in macrophages [60, 61]. A human population study evaluating the overall inflammatory potential of dietary components through a food frequency questionnaire of 10,047 adults aged between 35 and 65 years has shown a positive relationship between dietary inflammation and atherogenic indices [62]. Considering numerous factors related to the Dietary Inflammatory Index (DII), such as some FA in addition to calcium. Emphasizing the importance of understanding the omics profile involved and the response to diet, which may lead to specific regulatory pathways and be important when the organism is affected by specific diseases. The hub gene *TPM1*, identified in the *Yellow* module, has previously been linked to the positive regulation of cytoskeleton organization pathways in type II diabetes mellitus, when compared to non-diabetic controls in mice [63]. In the *Yellow* module, we also identified the gene *HACD1*, which is expressed in both skeletal muscle and the heart, and interacts with ELOVL enzymes [6], highlighting the significance of the findings in this module. HACD1 is involved in FA elongation and exhibits activity related to 3-OH acyl-CoAs saturated, monounsaturated, and polyunsaturated FA, as demonstrated in a lipidomic study using HACD1 knockout mice [64].

Another module identified in SOY1.5 is *Cyan*. The role of insulin in glucose homeostasis is defined by its effects on skeletal muscle, white adipocytes, and the liver. In skeletal muscle, insulin promotes glucose uptake and facilitates glycogen synthesis [65]. In addition, insulin is important for glycolysis, synthesis of FA and glycogen, and possibly involves the metabolism of glycose and lipids [66]. *RCAN1* was identified as a hub gene in the *Cyan* module for the SOY1.5 group. *RCAN1* is highly expressed in tissues such as brain, heart and skeletal muscle and is involved in mitochondrial regulation and is sensitive to oxidative stress. Its primary function is to inhibit the phosphatase calcineurin, which helps maintain normal β-cell function and regulate plasma insulin levels. In a study using RCAN1 transgenic mice (RCAN1^{ox}), overexpression of RCAN1 was associated with the development of hyperglycemia, metabolic dysfunction, and diabetes [67]. In the SOY1.5 group, hub genes are supported by previous studies that have shown their expression in muscle tissue and involvement in relevant biological and metabolic processes. These findings offer new insights into these genes in the context of a 1.5% soybean oil diet.

We identified more co-expressed modules co-expressed with the SOY3.0 group. Specifically, in relation to palmitoleic acid, which was deposited at a higher percentage in this group (Supplementary Table S1), the *Darkgreen* and *Darkred* modules exhibited a negative correlation with palmitoleic acid. In the *Darkgreen* co-expressed module, *CSRNP1* was identified as a hub gene. In a bioinformatics study using publicly available transcriptome data from diabetic and non-diabetic patients, the gene *CSRNP1* was identified as a potential transcription factor [68]. In pigs, *CSRNP1* was associated with the metabolic response to feed intake in Duroc breed pigs [13]. CSRNP1 was identified in swine through the integration of overlapping differentially expressed genes (DEGs), gene set enrichment analysis (GSEA), and WGCNA. CSRNP1 was enriched for GO:0003700 (DNA-binding transcription factor activity) and was considered a candidate gene associated with intramuscular fat content [69]. Moreover, attenuation of CSRNP1 expression has been shown to reduce lipid accumulation in a study investigating human adipogenic transcription factors [70].

In the *Darkred* module, key pathways and GO terms identified included type II diabetes mellitus, response to insulin, medium-chain fatty acid transport, and chromatin organization. Type II diabetes mellitus develops with a decrease in the ability of insulin to stimulate muscle to remove glucose from the blood. Muscle "insulin resistance" is associated with metabolic syndrome, and insulin secretion is increased to maintain adequate glucose concentration. When it progresses to diabetes, there is inefficiency in insulin balance and muscle glucose uptake. In the case of diabetes, the pancreas does not secrete insulin in response to hyperglycemia [71]. These results highlight the role of lipids as key modulators of glucose metabolism and whole-body lipid [59], aligning with previous findings and reinforcing their regulatory influence in metabolic processes.

In contrast to the negative correlations with palmitoleic acid of *Darkgreen* and *Darkred*, the *Black* module is positively co-expressed. The *Black* module is enriched for pathways and GO terms, including metabolic pathways, the insulin signaling pathway, alpha-linolenic acid metabolism, and lipid metabolism. In a study of male C57BL/6 mice divided into low fat (control) and HFD (obese) diets, biochemical analyses of liver and serum samples showed that palmitoleic acid was involved in reducing aspects of hepatic steatosis and in regulating lipogenesis and FA oxidation [72]. The hub gene detected in *the Black* module was *ZFP30*. The *ZFP30* was also identified by Chen et al. [73] as an adipogenesis candidate involved in directly increasing *PPARγ2* expression during adipogenesis in a study using 3T3-L1 cells (ATCC), 293T cells (ATCC), and parental IBA cells. In pigs, ZFP30 has been identified in crossbred piglets as a gene associated with the longissimus dorsi muscle, participating in biological processes such as GO:0006350 (transcription) and GO:0045449 (regulation of transcription) [74]. Additionally, an epigenome-wide analysis of skeletal muscle DNA revealed

that CpG sites within ZFP30 were more highly methylated in homozygous Pietrain pigs susceptible to malignant hyperthermia (MHS) [75]. Importantly, ZFP30 has also been reported as a positive regulatory component of the adipogenic regulatory network, acting in coordination with the expression of *KAP1* and *Pparg2* [73].

Another pathway identified related to the *Black* module was insulin resistance, which is common in metabolic diseases, diabetes, metabolic syndrome, and obesity [76]. There is also evidence that food intake influences the expression of transcription factors that are important for the coordination of metabolic response and nutrient availability. Muscle metabolism is altered in response to nutrient influx, highlighting the need to better understand these interactions and the genetic basis of energy homeostasis [57].

Palmitoleic acid exhibits anti-inflammatory and insulin-sensitizing properties. These properties have been linked to improved insulin sensitivity and reduced diabetes incidence in humans. Palmitoleic acid serves as an indicator of de novo lipogenesis, exerting lipokine effects that improve insulin sensitivity in muscle and reduce hepatosteatosis in mice [77]. Palmitoleic acid is already considered a lipokine with beneficial effects in preventing and treating metabolic diseases [78].

Furthermore, palmitoleic acid increases glucose uptake in adipocytes through activation of AMP-activated protein kinase (AMPK) [79]. The AMPK signaling pathway was also identified in the *Darkred* module. An association between circulating and dietary palmitoleate and decreased incidence of diabetes, lower risk of cardiovascular disease and inflammation status was detected by Frigolet and Gutiérrez-Aguilar [79]. In obesity, palmitoleic acid showed beneficial effects on liver inflammation and metabolism in wild male C57BL/6J mice fed with a diet with 9% calories from fat or a high-fat diet (59% calories) [80]. Results in humans are controversial, so further studies are needed to better understand its effects on skeletal muscle and its relationship to disease [79]. We identified modules with a negative co-expression (*Darkgreen* and *Darkred* module) of palmitoleic acid in diseases such as NAFLD and type II diabetes. This suggests that palmitoleic acid may play an anti-inflammatory role, potentially influencing complex regulatory mechanisms involved in lipid metabolism and inflammation using SOY3.0. In contrast, the *Black* module (positive correlation) related to metabolic pathways, lipid, and cholesterol metabolism reflects the complexity of FA in metabolic regulation. The SOY3.0 group is interesting for in-depth studies related to these regulatory mechanisms, observing the correlation with inflammatory markers and serum cholesterol levels to better identify its role.

Oleic acid and total MUFA were identified (positive correlation) with the *Lightcyan* module. OA is an important FA in muscle; it is related to the capture of complex associations. In the study of Valdés-Hernández et al. [9] OA presented common or specific genes associated with FA metabolism. Furthermore, this study performed with pigs identified genes enriched in KEGG pathways and GO terms related to lipid metabolism, NAFLD, and the insulin signaling pathway, confirming a complex and bipartite relationship between IMF composition and gene expression [9]. Furthermore, studies evaluating the relationship between OA and palmitic acid, triglyceride (TG), and cholesterol may help to better understand this regulatory relationship and the effect of FFA. Oleic acid, a key component of the Mediterranean diet, has been associated with several health benefits. These include modulation of the OA/linoleic acid (LA) ratio in adipocyte membranes and improved vasodilation through endothelium-dependent flow, which collectively link an OA-rich diet to potential antiatherogenic effects, particularly in diabetic patients [81].

In our study, interesting pathways were enriched in relation to the SOY3.0 group, correlated with OA, such as NAFLD, Huntington's disease (HD), cardiac muscle contraction, diabetic cardiomyopathy, Parkinson's disease, and regulation of response to food. The pathway of NAFLD can be justified in this study due to the relationship between the muscle and liver, which are target organs for insulin. HD has increasingly been described as a systemic condition, with reported associations with alterations in whole-body energy balance and glucose regulation [82–84]. Accordingly, these enrichments represent conserved biological processes rather than disease manifestation. In muscle tissue, OA has been shown to correlate with genes associated with the Parkinson's disease pathway in other species, particularly under conditions of extreme FA content [85].

In the *Lightcyan* module the hub gene identified was *FABP7*, which correlates with OA and total MUFA. Although *FABP7* is known to be expressed in the adult human brain and skeletal muscle, its exact role remains unclear. OA has been identified in a study with mice as an endogenous ligand of the orphan nuclear receptor TLX/NR2E1, which plays a key role in the proliferation of stem cells. This highlights its potential role in metabolic regulation [86, 87]. In a study with mice, FABP7 demonstrated a protective effect against oxidative stress caused by reactive oxygen species in primary organic astrocytes [58]. Oxidative stress has been related as a potential etiology for various neurodegenerative diseases [58]. FABP7 is part of the FA binding protein (FABP) family, which is involved in the regulation of FA uptake, transport, and gene expression, controlling the lipid dynamic [58, 88, 89]. FABP7 may influence cell growth and differentiation, and its function could be modulated by the proportions of n-3 and n-6 [88, 89]. The FABPs are chaperones that

facilitate the transport, intracellular binding, and targeting of FA. The importance of these binding proteins is mainly related to lipid signaling functions in pathways that impact diseases and that can help us understand how some diets with added oil may be correlated with gene expression. For *FABP7*, the binding preference is directed towards PUFA and MUFA due to tail conformations adopted by the bound ligand. One of the results obtained by Lenz et al. [90] through simulations with a combined biophysical-computational approach demonstrates that *FABP7* can bind to micelles of OA and docosahexaenoic acid (DHA). In the nucleus, FABP7 can affect the expression of peroxisome proliferator-activated receptors [90]. In another study, the *FABP7* gene was correlated with MUFA in pigs [91]. FABP7 has a high affinity for EPA, DHA, and OA. A study by Umaru et al. [92] has already shown that OA is a potential ligand of *FABP7* in glioma cells, increasing their proliferation and potentially altering the epigenetic status of genes that regulate proliferation through histone acetylation.

The *Turquoise* module was identified as co-expressed negatively related to total PUFA and the PUFA: SFA ratio in the SOY1.5 group. Among the enriched pathways, we identified the PPAR signaling pathway, which has already been reported in other studies in pigs [4, 27, 93]. Among the genes identified, the *PPARγ* gene encodes a protein that is related to the regulation of lipid metabolism and glucose homeostasis, and is also involved in the regulation of adipocyte differentiation and is implicated in the pathology of diseases such as obesity and diabetes [54]. This gene is an important gene in the lipid metabolism pathway. In addition, polymorphisms detected in this *PPARγ* gene are related to gene expression and IMF deposition in pigs [27]. Furthermore, the *PPARγ* gene has also been reported as a coordinator of glucoregulatory responses in skeletal muscle in a study in *PPARγ* knockout mice of all ages, in which postprandial hyperglycemia, hyperinsulinemia, and insulin resistance were identified. Insulin-stimulated glucose elimination also occurs in muscle cells [94].

Another identified gene present in this pathway is *PLIN1*, which encodes a protein previously reported to play a fundamental role in regulating lipid storage and determining IMF composition, and is a putative candidate gene for IMF deposition [9, 95]. This lipolytic gene was identified as correlated with FA composition, with negative correlations with PUFA in studies using *Longissimus Dorsi* RNA-Seq and IMF composition profiles from Iberian × Duroc backcross pigs [4, 9] and Duroc pigs [27]. Similarly, our findings reinforce the biological significance of these pathways. PLIN1 has also been identified in previous studies as a key determinant of differences in FA composition in muscle, playing a role in specific molecular functions related to lipid metabolism and bile acids, including oxidation, accumulation, synthesis, concentration, homeostasis, and obesity [14]. Furthermore, the PPARγ complex can recruit other transcription factors and activate the transcription of adipogenic genes through PPAR-responsive elements, including *FABP4*. These genes have already been identified as candidates involved in pathways influencing IMF levels in pigs [27]. *ADIPOQ*, along with *PLIN1*, *PPARγ*, SCD, and *FABP4*, has been previously identified as co-expressed in the same module using WGCNA with graphical Gaussian models in a study of the 129 backcrossed pigs, highlighting their relationship with muscle gene expression and FA profile [95]. In our study, we identified a negative correlation between the module and PUFA content, similar to findings from a previous study, suggesting that the regulation of these genes influenced the FA composition of the IMF [95]. However, the absence of co-expression with MUFA and palmitoleic acid in this module reflects our specific experimental conditions on gene regulatory networks.

The *SLC38A10* hub gene of the *Turquoise* module is a bidirectional transporter of glutamine aspartic acid and others, from the family of solute transporters (SLCs) that are involved in the metabolic and cellular control of cells, relevant in monitoring the flow of substances across the membrane cell [55, 96]. The role of the *SLC38A10* gene is related to mTOR-dependent protein and FA regulation [55]. The *SLC38A10* gene's activity is related to the transmembrane transport of amino acids. Amino acid transporters are important for their dual roles in nutrient sensing and transport. They influence protein synthesis by regulating the availability of amino acids, which can adjust the rate of synthesis depending on nutrient levels. This regulation plays a role in cellular response to nutrient availability, thereby impacting metabolic and physiological processes [96]. In mice, *SLC38A10* is expressed primarily in mouse excitatory and inhibitory neurons, and silencing expression in PC12 cells has a transceiver function involved in reducing nascent protein synthesis [97, 98]. Identified genes in the module demonstrate that PUFA can act as ligands for transcription factors as well as metabolic regulators, influencing different cellular functions involved in permeability, transport functions, formation of ion channels, and enzymatic functions associated with the membrane. Additionally, these genes are involved in receptors that control the degradation of metabolites and cell signaling [99].

The *Royalblue* module was positively correlated with total PUFA in the SOY3.0 group and showed a correlation with LA. Notably, LA deposition was lower in the SOY3.0 group compared to SOY1.5. The *Royalblue* module was enriched for positive regulation of interferon-gamma production, interleukin-1 beta production, skeletal muscle fiber development, and the hub gene identified was

MYBPH. Studies have observed a stimulatory effect of LA on T-cell proliferation, suggesting that LA may decrease immune cell function [100]. The PUFA play a role in fat deposition, muscle development, and glycolipid metabolism [101]. The effects of diet, due to the presence of specific dietary compounds and differences in deposition, show expressive changes in genetic co-expression and regulation mechanisms. MYBPH protein has been previously identified in an omics integration study focused on lipid metabolism and oxidative stress in finishing pigs. It has been implicated in dietary incorporation effects, particularly in response to *Chlorella vulgaris* supplementation and exogenous enzymes [102].

Other studies have reported changes in co-expression patterns between diets that may result from processes related to adaptation to dietary response, changes in nutrient availability, and changes in regulators that may be target genes [103, 104]. We identified interesting changes between the modules obtained in both groups. Of note are the potential new roles of the hub genes *ZFP30* and *CSRNP1* in lipid deposition in pig muscle. These findings suggest novel pathways and key genes that warrant further exploration to better understand their functional significance related to skeletal muscle. These observations reflect FA deposition and how gene expression behaves in each diet, which may help us better understand the processes involved when the diet was changed to a diet enriched (3%) with soybean oil. Co-expression and functional analysis revealed differential regulation of KEGG pathways and GO terms in the SOY1.5 and SOY3.0 groups. We observed that the SOY1.5 group was associated with pathways related to glucagon signaling, insulin receptor, PPAR signaling, angiogenesis, positive and negative regulation of the inflammatory response, cholesterol metabolism, and fatty acid elongation. The FA, total SFA, AI, total PUFA, and the PUFA: SFA ratio were correlated with genes such as *RCAN1*, *TPM1*, and *SLC38A10.* In contrast, modules co-expressed in the SOY3.0 group were associated with OA, LA, total MUFA, and PUFA with hub genes such as *CSRNP1*, *TRIP10*, *ZFP30*, *FABP7*, *and MYBPH*. These genes may regulate lipid deposition and are influenced by dietary differences. The co-expression analysis reveals novel candidate genes and provides new insights into the effects of different diets on metabolic pathways and disease-related processes. Our results highlight how FA may be involved in specific pathways through gene modulation, which could support dietary strategies.

Conclusion

Differences in co-expression suggest that variation in dietary soybean oil levels can modulate gene expression in distinct ways, highlighting the interplay among nutrition, gene expression, and fatty acids. The pathways involved in this SOY3.0 group included TNF signaling, lipid metabolism, chromatin, and diseases such as Huntington's disease. Key genes identified for the SOY3.0 group included *MYBPH* and *TRIP10*. In general, processes related to lipid metabolism (both groups) and energy metabolism (glycolysis, lipid transport) are found in the SOY1.5 group. In the 3% group, GO terms indicate a more general response involving metabolism and physiology, such as inflammatory processes and glucose homeostasis. The results provide mechanisms to enhance our understanding of how enriched pig diets may contribute to nutrigenomics research and human health, given that the pig is an important animal model.

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

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Authors' contributions

S.L.F., R.P.M.A.C. and A.S.M.C. conceptualized. S.L.F. and A.S.M.C., manuscript writing. S.L.F., J.D.G., I.C.G., and A.S.M.C., performed data analysis. S.L.F., R.P.M.A.C., A.S.M.C., interpretation and discussion of the results. R.P.M.A.C., I.C.G., J.D.G., V.V.A., G.C.M.M., S.M.A., and A.S.M.C., critical revision and editing. R.P.M.A.C., A.S.M.C., supervision. A.S.M.C., provided funding. All authors have reviewed the manuscript.

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Data availability

The dataset analyzed during the current study is available in the European Nucleotide Archive (ENA) repository (EMBL-EBI), under accession PRJEB52629 [http://www.ebi.ac.uk/ena/data/view/PRJEB52629]. All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

The animal experiments were approved under protocol 2018.5.1787.11.6 and CEUA number 2018-28 by the Animal Care and Use Committee of ESALQ (Luiz de Queiroz College of Agriculture), Brazil.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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