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Different oil sources impacting brain lipid and transcriptome profiles of pigs

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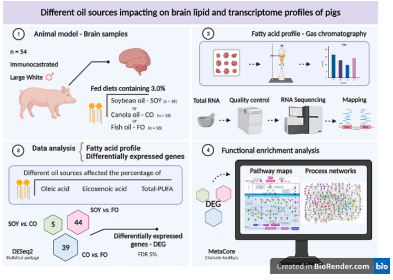
HIGHLIGHTS

- Fatty acids are important functional components for brain tissue and play a significant role in maintaining various biological and physiological processes.
- Lipids altered the transcriptomic profile of the pig brain, affecting essential genes and biological processes.
- The *CALB1* and *TRPC3* modulated by a diet containing 3 % soybean oil may contribute to calcium homeostasis and assist in reducing the association with neurodegenerative diseases.
- Differentially expressed genes modulated by lipid sources participate in essential processes for the maintenance of brain tissue.

GRAPHICAL ABSTRACT



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ABSTRACT

Lipids are fundamental to the structure and function of the brain, and their fatty acids (FA) composition is rich in polyunsaturated fatty acids (PUFA), which have protective effects and modulate gene transcription. For nutrigenomics studies, pigs (*Sus scrofa*) have been widely used as a biomedical model. Thus, the aimed to investigate whether different dietary oil sources modify the pig brain's lipid and transcriptomic profile. A 98-day study was

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Lipid homeostasis
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Pig model

performed using fifty-four male pigs. Treatments consisted of corn-soybean meal diets containing 3 % of soybean oil (SOY), canola oil (CO), or fish oil (FO). Total mRNA was extracted for sequencing. As a result, feeding diets with different oil sources affected the percentage of some FA. Palmitic acid showed a greater concentration in diets containing SOY with 27.037 %. Oleic acid and eicosenoic acid, showed a greater concentration in diets containing SOY, with 30.968 % and 2.096 %, respectively; and, total PUFA showed a better concentration in diets containing SOY and FO, with 11.685 % and 12.150 %, respectively. After quality control, considering the total reads obtained for the three groups, 94.87% were mapped against the reference genome *SScrofa11.1*. A comparison of gene expression between the groups of pigs was carried out by using the DESeq2 statistical package (R/Bioconductor). From SOY *vs* CO comparison, five differentially expressed genes (DEG, FDR < 0.05) were identified, from SOY *vs* FO forty-four DEG were verified, and from CO *vs* FO thirty-nine DEG were found. The functional enrichment analysis resulted in pathway maps ($P < 0.05$) related to apoptosis and cell proliferation, obesity and type 2 diabetes, neurophysiological process, and inflammation. The networks were associated with signal transduction, calcium transport, and oxidative stress. Overall, the results showed that diets with different oil sources could affect some brain tissue parameters and may help guide future research on the availability of dietary FA in the brain.

1. Introduction

Lipids are a class of molecules necessary for brain structure and function, responsible for a wide range of physiological processes (Bruce et al., 2017; Chanted et al., 2021). Besides being an energy source, fatty acids (FA) have important biological, structural, and functional purposes in the human organism. Moreover, in the central nervous system, lipid balance and regulation is essential (Bruce et al., 2017; Taghibiglou and Khalaj, 2017).

Brain tissue is generally rich in long-chain polyunsaturated fatty acids (PUFA), particularly arachidonic acid (AA; C20:4 n-6), eicosapentaenoic acid (EPA; C20:5 n-3), and docosahexaenoic acid (DHA; C22:6 n-3) (Bruce et al., 2017; Chanted et al., 2021). This organ has the highest amounts of lipids in terms of content and diversity. The brain FA exert several beneficial impacts on health and are involved many vital functions, such as cognitive function (Huguet et al., 2023; Taghibiglou and Khalaj, 2017). The PUFA are essential for cell proliferation, integrity maintenance and differentiation of neuronal cell membranes (Borsonelo and Galduróz, 2008; Nagy and Tiuca, 2017). Nevertheless, the human body cannot synthesize essential FA, which are extremely important for the proper functioning of the brain, so it is necessary to consume them through the diet (Ellulu et al., 2015; Kulkarni et al., 2022). The shortage of these FA in the brain tissue may result in alterations in neurotransmission systems, leading to various neurological disorders (Borsonelo and Galduróz, 2008; Nagy and Tiuca, 2017).

Soybean, canola, and seafood oil can be a source of PUFA in higher concentrations (Ellulu et al., 2015). Soybean oil has high concentrations of PUFA, such as linoleic acid (LA; C18:2 n-6), which is considered, along with alpha-linolenic acid (ALA; C18:3 n-3), an essential precursor of DHA and AA, important FA for the proper functioning of the brain (Fan and Eskin, 2015; Sun et al., 2018). Canola oil has high concentrations of oleic acid (OA; C18:1 n-9), LA and ALA, which, if adequately consumed may have a cardioprotective effect through reducing plasma cholesterol levels (Lin et al., 2013). Fish oil has high levels of omega-3 series (n-3) PUFA, including EPA and DHA, which benefit brain function and protect against oxidative stress and inflammatory processes, helping prevent neurological disorders (Farooqui et al., 2007).

The brain is capable of obtaining PUFA through diet or by the conversion of essential FA (Yehuda et al., 2002). PUFA display protective effects and modulates gene transcription, topics widely studied in nutrigenomics. In this area, as technological advances accelerate, "omics" have played a key role in better understanding molecular mechanisms (Afman and Müller, 2012; Taghibiglou and Khalaj, 2017). Omics studies have widely used pigs (*Sus scrofa*) as a biomedical model (Hoffe and Holahan, 2019; Taghibiglou and Khalaj, 2017) due to their similarity to humans, reflected in anatomy, pathophysiology, physiology, and neurophysiological processes (Pan et al., 2021; Walters et al., 2012). This similarity allows pigs to be a valuable tool in recent research demonstrating the effects of dietary interventions on brain function, digestive and metabolic processes (Maj et al., 2022; Valent et al., 2020), offering new insights into relevant biological processes and their associations with diseases affecting human health (Lunney, 2007; Meurens et al., 2012). Pigs are also of great economic and agricultural importance; consequently, there is a wealth of information about these animals (Walters et al., 2012). Therefore, the hypothesis of this study was that diets with different FA composition alter lipid composition and gene expression in the brain of pigs. This study aimed to investigate the effects of different dietary oil sources on lipid and transcriptome profiles in pig brain tissue.

2. Methods

2.1. Ethics statement, animals, and diets

The Ethics Committee for the Use of Animals (CEUA) approved all procedures involving animals (number 2018–28), of the Luiz de Queiroz College of Agriculture (ESALQ, protocol number 2018.5.1787.11.6). The procedures followed the ethical principles in animal research according to FASS (2010), with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. This study complies with the ARRIVE guidelines.

Fifty-four homozygous halothane-negative (NN) males were selected for the study, offspring of three sires and thirty-two females of the Large White breed (inbreeding below 14 %), with an average initial body weight of 28.44 ± 2.95 kg. The pigs had an average age of 71 ± 1.8 days and were randomly assigned to one of three dietary treatments during the experimental period of 98 days. A total of 18 pigs were used for each treatment. These 18 animals were distributed in six pens (replicate), with three pigs each (one animal representative of each sire). Each pen was equipped with a three-hole dry feeder and a nipple drinker, allowing *ad libitum* access to feed and water throughout the experimental period. The immunocastration was performed by administering two 2 mL doses of Vivax® (Pfizer Animal Health, Parkville, Australia) on day 56 (127 days of age) and day 70 (141 days of age) (Almeida et al., 2021), according to the manufacturer's recommendations.

The experimental treatments consisted of corn-soybean meal diets containing 3 % soybean oil (SOY), 3 % canola oil (CO), or 3 % fish oil (FO). The experimental diet was divided into six phases: Grower I - days 0 to 21; Grower II - days 21 to 42; Finisher I - days 42 to 56; Finisher II - days 56 to 63; Finisher III - days 63 to 70; and, Finisher IV - days 70 to 98. The diets were formulated to meet or exceed the nutritional requirements according to Rostagno et. al. (2011) and were provided as a meal form without antibiotic growth promoters. Within each pen, the animals were subjected to the same diet. The diets were formulated to have a similar metabolizable energy content (3.36 Mcal/kg). Details of the diets used in this study are described in Supplementary Table 1, which was adapted from previous study (Almeida et al., 2021). On day 98 of the experiment, pigs with a final body weight of 133.9 ± 9.4 kg

were slaughtered at a commercial abattoir, by electrical stunning followed by exsanguination, according to the industry standards and Brazilian legislation, after a 16 h rest period. After slaughter, the brains were collected, frozen in liquid nitrogen, and stored at -80 °C until analyses. Samples from the animals' frontal lobes of the brain were used for the analyses.

2.2. Total lipid content, fatty acid profile and blood biochemical parameters

To analyze total lipid content, 5 g of brain samples were used (in duplicate), which were ground, packed in plastic bags and stored at -20 °C until analyses. The ground samples were dried and packaged in filter paper cartridges, and inserted into a Soxhlet extraction system according to the method described by AOAC (1995). The FA profile was determined from the total lipid content using 10 g brain tissue samples. The lipids were cold extracted using the method proposed by Bligh Dyer (1959). Briefly, the samples were homogenized with methanol: chloroform (2:1) and the content was filtered, placed in a proper separating funnel, and added KCl solution (0.88 %). The lower phase obtained was collected, and a Rotary Evaporator (Tecnal TE-210) coupled to a Tecnal TE-0581 vacuum pump was used. The extracted fat was collected and stored at -20 °C until analyses. Methylation of the samples was performed according to Hartman e Lago (1973), with adaptations based on AOCS (2005) (method AM 5–04). After methylating, the brain samples were inserted in a gas chromatograph to obtain tissue fatty acid profile. Then, the methyl esters went to the stage of analysis by high-performance gas chromatography, using a Shimadzu GC-2010 plus AF chromatograph, equipped with an RTX-Wax column. The complete procedures were described by Almeida et al. (2021), Silva et al. (2021) and Da Silva et al. (2023).

As described in Almeida et al. (2021), the total of Saturated fatty acid (SFA) content was calculated from the sum of the percentages of myristic, palmitic and stearic acids. The total percentage of Monounsaturated fatty acid (MUFA) was calculated by summing the percentages of palmitoleic acid, eicosenoic acid and OA. The total proportion of PUFA included AL, ALA, EPA and DHA. The total of the omega-6 series (n-6) PUFA included only AL in this study, and was divided by the total of all the n-3 (ALA, EPA and DHA) to calculate the n-6:n-3 PUFA ratio. The PUFA:SFA ratio was calculated by dividing the total proportion of PUFA by the total proportion of SFA. The atherogenic index was calculated by using the formula proposed by Ulbricht and Southgate (1991): (4 × [Myristic acid]) + [Palmitic acid]/[total MUFA] + [total PUFA], where brackets also indicate the concentration (percentage) of the FA.

Four days before the slaughter, the blood was sampled from the jugular vein and immediately transferred into non-anticoagulant vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). The samples were stored at room temperature for 2 h, centrifuged at 3000 × *g* for 10 min to obtain serum, and stored in duplicated 1.5 mL tubes at -80 °C until analyses. All serum biochemical measurements were analyzed using the Mindray BS120 Spectrophotometer (Guangdong, China) with diagnostic kits (Labtest, Brazil), following the manufacturer's guidelines. The analyses were performed at the Pathology Laboratory at the University of São Paulo, Pirassununga, SP, Brazil. Blood serum glucose content was quantified by the colorimetric enzymatic method (Trinder, 1969). Total protein was performed using the Biuret method with some modifications (Gornall et al., 1949). The biochemical parameters measured were: glucose, aspartate aminotransferase, total proteins, albumin, globulin, triglycerides, cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL), and very low-density lipoproteins (VLDL).

2.3. RNA extraction, sequencing, quality control and alignment of the reads

Total RNA was isolated from brain samples (RNeasy® Mini Kit, Qiagen) using Trizol reagent (Invitrogen), according to the manufacture's guidelines. The quality and concentration of total RNA were obtained using the NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific) and the Qubit® 2.0 Fluorometer, respectively. The RNA integrity was evaluated with Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). All samples presented an RNA Integrity Number (RIN) greater than or equal to 7.4 (see Supplementary Table 2). For library preparation, 2 μL of total RNA was used according to the manual TruSeq RNA Sample Preparation kit v2 (Illumina, San Diego, CA). The average library size was estimated using the Agilent Bioanalyzer 2100 (Agilent, Santa Clara, CA). The libraries were quantified by quantitative PCR with a quantification kit from KAPA library (KAPA Biosystems, Foster City, CA). Sequencing was performed using the TruSeq PE Cluster kit v3-cBot-HS (Illumina, San Diego, CA). Briefly, samples were pooled (five lanes, with a pool of all 54 samples) and sequenced using the HiSeq 2500 equipment (Illumina, San Diego, CA) with a TruSeq SBS Kit v3-HS (200 cycles), according to the manufacturer's instructions. All sequencing steps were performed at the Animal Genomics Center at the Animal Biotechnology Laboratory of ESALQ, Piracicaba, São Paulo, Brazil.

Quality control and alignment steps were described elsewhere (Fanalli et al., 2022). Low-complexity reads and adapters were removed using Trim Galore software (v.0.6.5). After removal, the minimum length of reads was 70 bases, with Phred Score lower than 33. FastQC software (v.0.11.8) [http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/] was used for visualization. The reference genome used was the *Sus Scrofa 11.1*, available in Ensembl database [http://www.ensembl.org/Sus_scrofa/Info/Index]. STAR software (v.2.7.6a) was used for alignment, mapping, and abundance (read counts) of mRNA for all known genes (Dobin and Gingeras, 2015). The gene expression levels were normalized using counts scaled by the total number of reads or counts per million (CPM).

2.4. Statistical analysis

Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), with pen as the experimental unit. The model included random effects of pen and block, and the fixed effects of oil sources. Outliers were removed and residuals were tested for a normal distribution using the Shapiro–Wilk test (UNIVARIATE procedure). Treatment means were separated using the LSMEANS statement, and comparisons were made using the PDIFF option based on Student's *t*-test. Differences were declared significant when $P < 0.05$.

Differential expression analysis was performed for SOY *vs* CO, SOY *vs* FO and CO *vs* FO diets comparisons, and differentially expressed genes (DEG) were identified with a multi-factor design by using the DESeq2 statistical package (R/Bioconductor) [http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html] (Love et al., 2014). Before statistical analysis, filtering criteria were used for all samples: (i) removal of genes with zero counts, that is, unexpressed genes; (ii) removal of genes with less than one read per sample on average (very lowly expressed); (iii) removal of genes that were not present in at least 50 % of the samples (rarely expressed). The model included treatments as the variable of interest and fathers as a fixed effect. Correction for multiple testing was performed according to the False Discovery Rate (FDR) method (Benjamini and Hochberg, 1995), and the threshold value used for significance was FDR < 0.05.

The enrichment analysis was performed using MetaCore software (Clarivate Analytics, v.22.1, build 70800) [https://clarivate.com/products/metacore/, Clarivate Analytics, London, UK]. Pathway maps were identified from the lists of known DEG from the SOY *vs* CO, SOY *vs* FO, and CO *vs* FO comparisons (FDR < 0.05). For annotation and

functional enrichment, the *Homo sapiens* genome was used as background reference, and the analysis was performed with the "Analyze Single Experiment" option from each list. Filters for metabolic maps of interest were used: energy metabolism, lipid metabolism, steroid metabolism, regulation of cellular processes (immune response, neurophysiological process, and oxidative stress), regulation of metabolism, mental disorders, nutritional and metabolic diseases, nervous system diseases, and tox processes. Networks were created to understand the behavior of genes and their interactions.

3. Results

3.1. Total lipid content, FA profile and blood biochemical parameters

The total lipid content and FA profile in the brain tissue of pigs fed diets with different FA profiles (SOY, CO and FO) are displayed in Table 1, and the FA profile of the oils used to supplement the pigs' diet is shown in Table 2. Pigs-fed diets containing SOY showed a greater concentration of palmitic acid ($P< 0.05$) in brain tissue compared to those containing CO and FO. Feeding pig diets containing CO resulted in greater proportions of OA ($P< 0.05$) than SOY-fed pigs, but similar results were observed compared to FO-fed pigs. The CO-fed pigs also showed greater proportions of eicosenoic acid ($P< 0.05$) than SOY- and FO-fed pigs. The percentage of total PUFA was greatest ($P< 0.01$) in brain tissue from SOY- and FO-fed pigs. The dietary treatments altered no additional FA.

Table 1
Total lipids and fatty acid (FA) profile (%) in brain tissue of pigs fed diets containing different oil sources.

Fatty acid,%	Dietary treatment[1]			Pooled SEM[2]	P
	SOY	CO	FO		
Total lipids	10.237	10.177	10.195	0.019	0.585
Saturated fatty acid (SFA)					
Myristic acid (C14:0)	0.522	0.542	0.536	0.007	0.568
Palmitic acid (C16:0)	27.037[a]	26.366[b]	26.382[b]	0.121	0.044
Stearic acid (C18:0)	28.371	28.386	28.044	0.140	0.453
Monounsaturated fatty acid (MUFA)					
Palmitoleic acid (C16:1)	0.462	0.471	0.528	0.014	0.089
Oleic acid (C18:1 n-9, OA)	29.955[b]	30.968[a]	30.399[ab]	0.169	0.037
Eicosenoic acid (C20:1 n-9)	1.898[b]	2.096[a]	1.858[b]	0.036	0.011
Polyunsaturated fatty acid (PUFA)					
Linoleic acid (C18:2 n-6, AL)	2.309	1.919	2.435	0.143	0.214
Alpha-linolenic acid (C18:3 n-3, ALA)	ND[3]	ND	ND	–	–
Eicosapentaenoic acid (C20:5 n-3, EPA)	0.131	0.143	0.146	0.005	0.612
Docosahexaenoic acid (C22:6 n-3, DHA)	9.205	8.895	9.686	0.178	0.092
Total SFA	55.925	55.585	55.035	0.209	0.127
Total MUFA	32.501	33.531	32.815	0.195	0.092
Total PUFA	11.685[a]	11.085[b]	12.150[a]	0.208	< 0.01
Total n-3 PUFA[4]	9.014	8.829	9.715	0.174	0.094
Total n-6 PUFA[5]	2.309	1.919	2.435	0.143	0.214
PUFA:SFA ratio[6]	0.207	0.203	0.221	0.004	0.052
n-6:n-3 PUFA ratio[7]	0.231	0.214	0.232	0.015	0.805
Atherogenic index[8]	0.661	0.638	0.637	0.006	0.085

[1] Pigs ($n = 54$; 18 pigs/treatment) were fed either a corn-soybean meal diet containing 3% soybean oil (SOY), canola oil (CO), or fish oil (FO). Values represent the least square means. [2]SEM = standard error of the least square means. [3]ND = not detected. [4]Total n-3 PUFA = {[C18:3 n-3] + [C20:5 n-3] + [C22:6 n-3]}. [5]Total n-6 PUFA = C18:2 n-6. [6]PUFA:SFA ratio = total PUFA/total SFA. [7]Σ n-6/Σ n-3 PUFA ratio. [8]Atherogenic index = (4 × [C14:0]) + (C16:0)/(total MUFA] + [total PUFA]), where brackets indicate concentrations (Ulbricht and Southgate, 1991). [a–c]Within a row, values without a common superscript differ ($P < 0.05$) using Student's *t*-test.

Table 2
Fatty acid (FA) profile (%) of the oils used to supplement the pigs' diet.

Fatty acid,%		Soybean oil	Canola oil	Fish oil
Myristic acid	C14:0	ND[a]	ND	4.00
Palmitic acid	C16:0	10.96	4.61	26.35
Palmitoleic acid	C16:1	ND	ND	7.98
Stearic acid	C18:0	4.48	2.23	5.90
Oleic acid	OA, C18:1 n-9	24.77	64.19	35.48
Linoleic acid	AL, C18:2 n-6	52.14	19.50	9.95
Alpha linolenic acid	ALA, C18:3 n-3	6.51	7.55	1.09
Arachidonic acid	C20:0	0.41	0.92	0.21
Eicosenoic acid	C20:1	0.22	0.32	1.62
Eicosapentaenoic acid	EPA, C20:5 n-3	ND	ND	2.51
Behemic acid	C22:0	0.47	0.32	0.08
Docosahexaenoic acid	DHA, C22:6 n-3	ND	ND	4.24

[a] ND = not detected.

The blood biochemical parameters were adapted from previous study (Fanalli et al., 2023), and were shown in Table 3. Pigs-fed diets containing CO or FO showed a greater concentration of total proteins ($P= 0.01$) and albumin ($P< 0.01$) when compared to a diet containing SOY. For cholesterol ($P< 0.05$) and HDL ($P< 0.05$), the diet containing CO resulted in higher concentrations when compared to those diets containing SOY and FO. Concentrations of glucose, aspartate aminotransferase, globulin, triglycerides, LDL, and VLDL were not statistically different ($P< 0.05$) among all diets.

3.2. Sequencing and differentially expressed genes data

The average number of total reads per sample for the SOY group was 34.3 M before quality control and 33.9 M after that; for the CO group, was 33.5 M and 33.1 M before and after quality control, respectively; and, for the FO group, it was 34.4 M and 34.0 M, respectively. After quality control, considering the total reads obtained for the three groups, 94.87% were mapped against the reference genome *SScrofa11.1* (see Supplementary Table 3)

The gene expression comparison between groups of pigs fed diets

Table 3
Blood biochemical parameters from male pigs fed diets containing different sources of oil.

Parameters	Unit	Diets[1]			Pooled SEM[2]	P
		SOY	CO	FO		
Aspartate aminotransferase	U/L	38.13	42.72	42.88	3.01	0.21
Total proteins	g/dL	6.46[b]	6.84[a]	6.82[a]	0.14	0.01
Albumin	g/dL	3.46[b]	3.80[a]	3.87[a]	0.09	< 0.01
Globulin	g/dL	3.00	3.04	2.94	0.13	0.73
Glucose	mg/dL	83.40	86.11	89.54	5.07	0.48
Triglycerides	mg/dL	35.70	45.67	39.78	4.89	0.13
Cholesterol	mg/dL	96.50[b]	99.60[a]	90.34[b]	3.72	0.04
HDL[3]	mg/dL	43.66[b]	45.59[a]	40.11[b]	2.21	0.04
LDL[4]	mg/dL	45.71	44.89	42.28	2.53	0.38
VLDL[5]	mg/dL	7.13	9.11	7.94	1.00	0.14

[1] The added oil diets consisted of 3% soybean oil (SOY), or canola oil (CO), or fish oil (FO). Values represent the least square means from 18 pigs/treatment ($n = 54$). [2]SEM = standard error of the least square means. [3]Low-density lipoprotein. [4]High-density lipoprotein. [5]Very-low-density lipoproteins. [a-b]Within a row, values without a common superscript differ ($P < 0.05$) using Tukey's test. Adapted from Fanalli et al. (2023).

enriched with different sources of oils (SOY *vs* CO, SOY *vs* FO, and CO *vs* FO) were performed. For the SOY *vs* CO comparison, 22,938 genes were expressed. Of these, five were DEG (FDR < 0.05). Within the five DEG, four were known genes, two being up-regulated (log2 fold change ranging from: +0.44 to +0.52) and two being down-regulated (log2 fold change ranging from: -1.98 to -0.77) in the SOY compared to the CO. The genes with the most altered expression were *aldehyde dehydrogenase 3 family member A1* (*ALDH3A1*, log2 fold change -1.98; FDR < 0.01) and *prolyl carboxypeptidase* (*PRCP*, log2 fold change +0.52; FDR < 0.01).

For the SOY *vs* FO comparison, 22,974 genes were expressed, and 44 were DEG (FDR < 0.05). Considering the 44 DEG, 28 were known genes, 14 being up-regulated (log2 fold change ranging from: +0.37 to +2.12) and 14 being down-regulated (log2 fold change ranging from: -2.49 to -0.36) in the SOY compared to the FO. The genes with the most altered expression were *cytochrome P450 family 3 subfamily A member 29* (*CYP3A29*, log2 fold change -2.49; FDR < 0.05) and *vitelline Membrane Outer Layer 1 Homolog* (*VMO1*, log2 fold change +2.12; FDR < 0.01).

For the CO *vs* FO comparison, 22,947 genes were expressed, with 39 DEG (FDR < 0.05). Within the 39 DEG, 21 were known genes, 16 being up-regulated (log2 fold change ranging from: +0.42 to +3.06), and five being down-regulated (log2 fold change ranging from: -1.62 to -0.33) in the CO compared to the FO. The genes with the most altered expression were *ALDH3A1* (log2 fold change -1.62; FDR < 0.05) and *solute carrier family 10 member 4* (*SLC10A4*, log2 fold change +3.06; FDR < 0.05). The complete data for SOY *vs* CO, SOY *vs* FO, CO *vs* FO comparisons and DEG are shown in Supplementary Table 4.

The "Compare experiments" tool of MetaCore software was used for analyzing the commonly known DEG related to all comparisons (SOY *vs* CO, SOY *vs* FO, and CO *vs* FO). The three comparisons' DEG lists (FDR < 0.05) were used together. *ALDH3A1* and *PRCP* were identified as common DEG in all comparisons (Table 4). The distribution of known genes among diet comparisons was visualized using a Venn diagram (Fig. 1) and data is shown in Supplementary Table 5.

Table 4
Commonly known DEG[1] among dietary comparisons SOY *vs* CO, SOY *vs* FO, and CO *vs* FO, in brain tissue of pigs fed diets containing different oil sources.

Gene	Description	log2 fold change
ENSSSCG00000018044 Aldehyde dehydrogenase 3 family member A1 *ALDH3A1*	Responsible for the oxidation of aldehydes. Involved in detoxification of alcohol-derived acetaldehyde and in neurotransmitter metabolism and lipid peroxidation. The gene is located in the Smith-Magenis syndrome region (crh 17). Associated diseases include Sjogren-Larsson syndrome and Paranoid schizophrenia. Gene Ontology (GO) annotations include oxidoreductase activity and aldehyde dehydrogenase (NAD^+) activity (Stelzer et al., 2016)	SOY[2] *vs* CO[3] -1.98 SOY *vs* FO[4] 1.56 CO *vs* FO -1.62
ENSSSCG00000014899 Prolylcarboxypeptidase *PRCP*	Encodes a member of the S28 peptidase family of serine exopeptidases. The protein encoded is processed to generate a mature lysosomal prolylcarboxypeptidase. This enzyme cleaves the C-terminal amino acids bound to proline into peptides. The enzyme has been shown to be an activator of cell matrix-associated precalicrein. GO processes include: energy homeostasis, glucose homeostasis, proteolysis, and regulation of reactive oxygen species metabolic processes (Stelzer et al., 2016)	SOY *vs* CO +0.52 SOY *vs* FO +0.58 CO *vs* FO +0.45

[1] DEG: differentially expressed genes. [2]SOY: corn-soybean meal diet containing 3 % soybean oil. [3]CO: corn-soybean meal diet containing 3 % canola oil. [4]FO: corn-soybean meal diet containing 3 % fish oil.

3.3. Functional enrichment analysis

The MetaCore software was used to identify pathway maps from the lists of five, 44 and 39 DEG from SOY *vs* CO, SOY *vs* FO, and CO *vs* FO (FDR < 0.05), respectively.

3.3.1. Comparison of SOY vs CO

For the SOY *vs* CO comparison, the amount of DEG (FDR < 0.05) observed in the differential analysis step was low, so no significant pathway maps ($P< 0.05$) were found by using MetaCore software.

3.3.2. Comparison of SOY vs FO

One pathway ($P< 0.05$) related to the cholinergic receptor nicotinic alpha 6 subunit (*CHRNA6* or *nAChR alpha-6*) has been identified. The gene was identified as a DEG, showing up-regulation in the SOY group compared to FO (log2 fold change +1.950). *CHRNA6* participates in the enriched pathway map "Role of prenatal nicotine exposure in apoptosis and proliferation of pancreatic beta cells" ($P< 0.05$, see Supplementary Figure 1).

The process networks were also generated using MetaCore software to understand the genes' behavior and interactions (Table 5). The process networks highlighted in this study were "Calcium transport" ($P< 0.01$, see Supplementary Figure 2), "Signal Transduction-Neuropeptide" ($P< 0.05$, see Supplementary Figure 3) and "Response to hypoxia and oxidative stress" ($P< 0.05$, see Supplementary Figure 4).

3.3.3. Comparison of CO vs FO

Four pathway maps ($P< 0.05$) were identified (Table 6), related to the following genes: *CHRNA6*, perilipin (*PLIN1*) and netrin-1 (*NTN1*).

The DEG *CHRNA6*, found previously in the SOY *vs* FO comparison, was also identified, showing an up-regulation in CO group compared to FO (log2 fold change +2.292). The *CHRNA6* participates in the enriched pathway map "Role of prenatal nicotine exposure in apoptosis and proliferation of pancreatic beta cells" ($P< 0.05$, see Supplementary Figure 1).

For this comparison, the *PLIN1* gene was identified as a DEG, showing an up-regulation in the CO group compared to FO (log2 fold change +1.558). The *PLIN1* participates in two of the four significant identified enriched pathway maps: "Role of IL-6 in obesity and type 2 diabetes in adipocytes" ($P< 0.05$, see Supplementary Figure 5), and "TNF-alpha and IL-1 beta induce dyslipidemia and inflammation in obesity and type 2 diabetes in adipocytes" ($P< 0.05$, see Supplementary Figure 6).

For this comparison, the *NTN1* gene was identified as a DEG, showing an up-regulation in the CO group compared to FO (log2 fold change +1.058). The *NTN1* participates in the enriched pathway map "Neurophysiological Process-Netrin-1 in the regulation of axon guidance" ($P< 0.05$, see Supplementary Figure 7).

Finally, the process network found for this comparison was "Calcium transport" ($P< 0.05$). The DEG enriched were *Calbindin 1* (*CALB1,* log2 fold change= +2.614), *CHRNA6* (log2 fold change= +2.292), and *Transient Receptor Potential Cation Channel Subfamily C Member 3* (*TRPC3,* log2 fold change= +1.627, see Supplementary Figure 8).

4. Discussion

The main goal of this study is to understand how diets differing in FA sources can affect the endogenous FA and transcriptome profile of pigs' brains. Knowing how the diet can affect pigs could help to figure out possible changes occurring in the brains of humans submitted to different eating conditions since these animals are excellent models for human studies (Moghadasian and Shahidi, 2017).

The frontal lobe, the part of the brain used in this study, plays a crucial role in many cognitive processes, motor control and neuropsychiatric functions. In humans, cognitive processes are fundamental in maintaining memory, executive function, attention and language, and

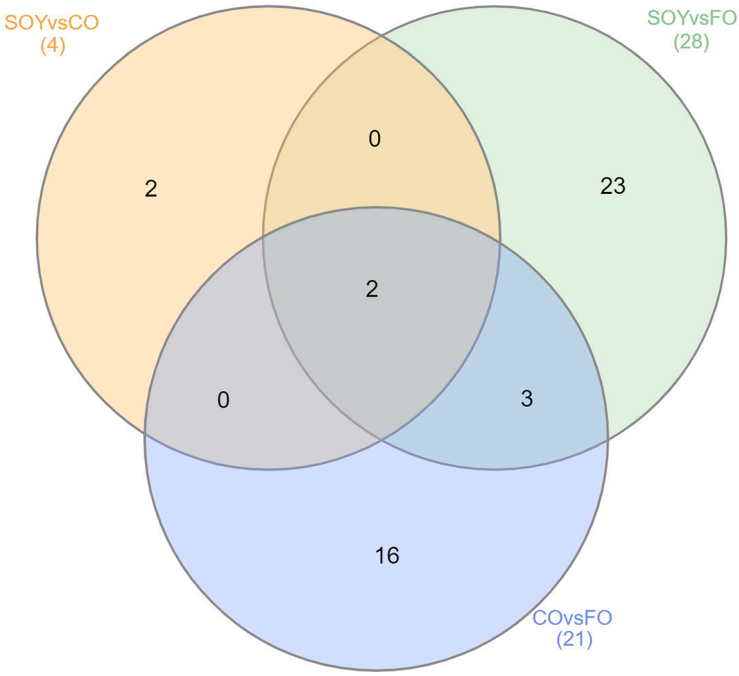

Fig. 1. Venn diagram of the distribution of known DEG[1] between comparisons of diet groups SOY[2], CO[3] and FO[4], in brain tissue of pigs fed diets containing different oil sources. [1]DEG: differentially expressed genes. [2]SOY: corn-soybean meal diet containing 3 % soybean oil. [3]CO: corn-soybean meal diet containing 3 % canola oil. [4]FO: corn-soybean meal diet containing 3% fish oil.

Table 5
Process networks based on Metacore software ($P < 0.05$) from the list of differentially expressed genes (DEG, FDR < 0.05) in the brain of pig fed diets containing different oil sources[1].

Process network	DEG	P
Chemotaxis	*ACKR2/GPCRs*	< 0.01
Muscle contraction	*GPCRs/GPCRs/CHRNA*	< 0.01
Cell adhesion-Leucocyte chemotaxis	*ACKR2/GPCRs*	< 0.01
Calcium transport	*CAST/ CATSPER3/ CHRNA6*	< 0.01
Signal Transduction-Cholecystokinin signaling	*GPCRs*	< 0.05
Development-Neuromuscular junction	*CHRNA/CHRNA6*	< 0.05
Signal transduction-Neuropeptide	*GPCRs*	< 0.05
Response to hypoxia and oxidative stress	*GSTM2/GSTM3*	< 0.05

[1] SO: corn-soybean meal diet containing 3 % soybean oil, and FO: corn-soybean meal diet containing 3 % fish oil.

Table 6
Pathway maps based on Metacore software ($P < 0.05$) from the list of differentially expressed genes (DEG, FDR < 0.05) in the brain of pig fed diets containing different oil sources[1].

Pathway map	DEG	log2 fold change	P
Role of prenatal nicotine exposure in apoptosis and proliferation of pancreatic beta cells	*CHRNA6*	+2.292	< 0.05
Role of IL-6 in obesity and type 2 diabetes in adipocytes	*PLIN1*	+1.558	< 0.05
Neurophysiological process-Netrin-1 in regulation of axon guidance	*NTN1*	+1.058	< 0.05
TNF-alpha and IL-1 beta induce dyslipidemia and inflammation in obesity and type 2 diabetes in adipocytes	*PLIN1*	+1.558	< 0.05

[1] CO: corn-soybean meal diet containing 3 % canola oil, and FO: corn-soybean meal diet containing 3 % fish oil.

underlying processes such as affection, personality, self-awareness, and social and moral reasoning (Chen et al., 2019). Nutrients such as FA (especially n-3), glucose, vitamins (B, A, E, and C), and minerals (zinc, iron, magnesium, and calcium) play key roles in brain tissue health and cognitive functions (Gómez-Pinilla, 2008). Moreover, studies have shown that besides being related to animal health, using fatty acids in pig diets can help production systems by improving diet energy value and animal welfare (Andersen et al., 2005). Thus, dietary inclusion of different oil sources with diverse FA profiles may be a good approach for studying and understanding the mechanisms involved in lipid signaling and modulation in normal and pathological conditions. Also, the structure and lipid composition of brain membranes and barriers may be affected by the availability of dietary FA (Pifferi et al., 2021; Yehuda et al., 2002).

4.1. Fatty acid profile and blood biochemical parameters and its effects on the brain tissue

In the present study, the SOY group showed a higher percentage of palmitic acid compared to the CO and FO groups. Palmitic acid may act on several adaptor proteins when in excess, activating the nuclear factor kappa B (NF-κB). This activation may be associated with the induction of hyperproliferation of pro-inflammatory cytokines and with the brain's high metabolic demand, inducing oxidative stress and increasing neuroinflammation (Camandola and Mattson, 2017; Ugbaja et al., 2021). Increased palmitic acid concentrations have been identified in Parkinson's and Alzheimer's disease (Fabelo et al., 2011; Schommer et al., 2018). Although studies have shown that excess palmitic acid has been associated with adverse features in the brain, and the present results showed that the SOY group may have modulated the availability of this FA, effects of this FA in the frontal lobe remain unknown. Further studies are needed to elucidate the impact of dietary palmitic acid on different brain tissue regions.

The CO group showed a greater percentage of OA compared to the SOY group, but similar results were observed for the FO group. According to Song et al. (2019), OA is a major constituent of membrane phospholipids and is critical for proper brain development and function.

It has been reported that OA acts on modulating gene transcription and is an important anti-inflammatory and neuroprotective factor. It works by activating peroxisome proliferator-activated receptor gamma (PPAR-γ) and inhibiting the activation of NF-kB signaling pathways (Silva-Santi et al., 2018). In addition, OA has also shown the potential for reducing β-amyloid and 7-ketocholesterol, which was reported in patients with some neurodegeneration (Debbabi et al., 2017, 2016).

Canola oil has a higher percentage of OA (Table 2), which may be associated with greater health benefits when compared to other oils. OA has also been associated with lower levels of LDL and higher levels of HDL. Saedi et al. (2017), demonstrated that diets involving a higher OA content improve HDL levels, corroborating the results demonstrated (Table 3), in which the CO group had a higher OA content and a greater HDL blood level when compared to the SOY and FO groups. This association may help prevent cardiovascular disease, diabetes, inflammation, and cognitive decline (Lauretti et al., 2017; Moghadasian and Shahidi, 2017).

Pastor et al. (2021), demonstrated in their study that current evidence points to a protective effect of OA consumption on the lipid profile. In addition, it has been, observed that replacing some FA with OA can improve the lipid profile, resulting in a reduction in total cholesterol and LDL. When SFA are replaced by OA, it is possible to observe an increase in HDL and a decrease in triglycerides. Although the results showed herein for the CO group were consistent with an increase in HDL, they also showed an increase in cholesterol, without observing a reduction in LDL. A higher intake of MUFA or PUFA has also been associated with lower total- and LDL-cholesterol (Corominas et al., 2013). In this study, the CO group presented the lowest percentage of total PUFA showing an increase in cholesterol, without modifying LDL levels. Regarding the blood parameters, the present study also showed that, despite having the highest levels of cholesterol, the CO group was also the diet group with the highest levels of HDL, albumin, and total protein.

The CO group showed a greater percentage of eicosenoic acid compared to the SOY and FO. It is well known that eicosenoic acid (or gondoic acid) is an elongase product of OA. Even though eicosenoic acid has not been widely studied, an increased level of this FA was detected in red blood cell membranes of children with regressive autism (Farag and Gad, 2022). In a study of neuroleptic-naïve UHR individuals, higher levels of eicosenoic acid were identified in individuals with impaired cognitive function compared to intact individuals. These results demonstrate that imbalances in the availability of FA, such as eicosenoic acid, may be associated with neurodevelopmental disturbances related with cognitive impairment (Kim et al., 2014). Herein, CO may act by modulating greater amounts of eicosenoic acid by altering the FA profile. However, further studies are needed to investigate the relevance of eicosenoic acid in brain tissue and its relationship to metabolic processes linked to lipid metabolism and disease progression.

In the current study, SOY and FO groups had the highest PUFA percentage compared with the CO group. PUFA are widely involved in normal brain development and function, and neuronal membrane phospholipids (McNamara et al., 2015). Among the most important FA for the brain are the long-chain PUFA, such as AA and DHA (Silva-Santi et al., 2018). These FA have a key role activating receptors and signaling pathways, which play a fundamental function in membrane fluidity (Bazinet and Layé, 2014). In mouse models for Parkinson's disease, n-3 PUFA, have demonstrated neuroprotective effects (Bousquet et al., 2011; Chen et al., 2018). It was observed that mice fed a diet enriched with n-3 showed decreased Aβ peptide and less accumulation of amyloid plaques in brain tissue. In contrast, n-6 has been reported in Alzheimer's disease contributing to the increased synthesis of the Aβ peptide (Amtul et al., 2011).

Thus, analyzing the results of the FA profile of brain tissue (Table 1), the FA profile of oils used in pig diets (Table 2), and the results of biochemical blood parameters (Table 3), CO provided low amounts of palmitic acid and eicosenoic acid, higher percentages of OA and modulated the level of blood HDL. So, the hypothesis was that the lower availability of these FA, and the higher content of OA and HDL may have contributed to the reduction of inflammatory and neurodegenerative processes. Moreover, despite CO having the lowest percentage of total PUFA in this study, canola oil has an interesting PUFA profile, with higher ALA and intermediate LA contents. This profile may have contributed to the higher availability of total PUFA, and could, consequently, increase anti-inflammatory processes, leading to better conditions for proper brain function.

4.2. *Dietary effects on differentially expressed genes and functional enrichment*

Differential expression and functional enrichment analysis revealed that using different oil sources altered the transcriptomic profile of pig's brain, affecting essential genes and biological processes. The *ALDH3A1* was found in this study with a down-regulated expression in the SOY and CO groups compared to FO (showed most altered expression), and also as a common DEG in all comparisons. This gene is part of a family of enzymes, which through the metabolism of exogenous and endogenous reactive compounds, play important roles in metabolic pathways of lipids, amino acids, drugs, and neurotransmitters (Jackson et al., 2011). The ALDH3 family are enzymes capable of oxidizing aliphatic and aromatic aldehydes, such as peroxides and fatty aldehydes, and have antioxidant properties. Mutations in ALDH genes are the molecular basis of several complex pathological processes, including Alzheimer's disease and cancer (Jackson et al., 2011; Muzio et al., 2012). The *ALDH3A1* is the key candidate gene in further studies linked to the lipid metabolism since it is involved in pathological process and, a common DEG for all diet comparisons.

The *PRCP* (up-regulated) showed the most altered expression for the SOY *vs* CO comparison, and was found to be a common DEG for the three comparisons studied herein. *PRCP* is part of the carboxypeptidase family that is essential for catalytic activity and is expressed in various tissues such as the kidney, liver, lung and brain (hippocampus, brainstem nuclei and cortex) (Jeong and Diano, 2014). In the brain, PRCP acts in inactivating alpha-melanocyte stimulating hormone (α-MSH), modulating melanocortin signaling acting to control energy metabolism and food intake (Jeong et al., 2012). Its activity can impact blood flow and pressure, act on inflammatory processes, and has been associated with a new target for studying and treating obesity and related disorders (Shariat-Madar et al., 2010).

Another important DEG for brain tissue and their functions found in this study was *CALB1*, which was more expressed in the CO group when compared to FO, and was also found in the "Calcium transport" process network reported in Supplementary Figure 8. *CALB1* is a member of the calcium-binding protein superfamily, and is highly expressed in brain tissue. In the brain tissue, it acts in the maintenance of intracellular calcium homeostasis and cellular response, and assists in modulating synaptic transmission (Stelzer et al., 2016). Dysregulation of calcium homeostasis has been associated with Parkinson's, Huntington's, and Alzheimer's diseases. It has also been reported that low expression of *CALB1* has been associated with neurodegenerative diseases (Genovese et al., 2020; Iacopino and Christakos, 1990). In this study, *TRPC3* was up-regulated in the CO group when compared to FO and was found in the "Calcium transport" process network reported in Supplementary Figure 8. *TRPC3* is part of a family of non-selective cation channels that control the influx of Ca^{2+} and other cations, and are widely found in the brain. *TRPC3* channels are critical in the neutrophil factor signaling cascade, contributing to Alzheimer's disease (Bathina and Das, 2015). Genetic mutations in the channels are associated with memory loss, neurodegenerative diseases and hypertension (Sierra-Valdez et al., 2018). In this study, *CALB1* and *TRPC3* showed a positive association with CO diet, which may have contributed to the maintenance of calcium and a lower association with neurodegeneration.

The *CHRNA6* was up-regulated in the SOY and CO groups compared

to FO. It was found in the "Role of prenatal nicotine exposure in apoptosis and proliferation of pancreatic beta cells" pathway map (Supplementary Figure 1), and the process network "Calcium transport" reported in Supplementary Figures 2 and 8. This DEG is responsible for encoding an alpha subunit of neuronal nicotinic acetylcholine receptors (nAChRs), which function as ion channels and are responsible for mediating dopaminergic neurotransmission (Stelzer et al., 2016). The *CHRNA6* is widely distributed throughout the peripheral and central nervous system, such as the frontal cortex and hippocampus (Paterson and Nordberg, 2000). The nAChR receptors are also significantly modulated by lipids, in which lipid-protein interactions may alter the activation, inhibition, and function of ion channels (Barrantes, 2004; Domville and Baenziger, 2018). As reviewed by Paterson and Nordberg (2000), a significant loss of nAChR sites has been reported in Parkinson's and Alzheimer's disease. In this study, *CHRNA6* showed a positive association with SOY and CO diets and may have been modulated by lipids, altering gene expression in brain tissue.

The *PLIN1* was more expressed in the CO group than the FO, and was found in the "Role of IL-6 in obesity and type 2 diabetes in adipocytes" and "TNF-alpha and IL-1 beta induce dyslipidemia and inflammation in obesity and type 2 diabetes in adipocytes" pathways maps illustrated in Supplementary Figures 5 and 6, respectively. This gene belongs to a family of five conserved proteins (PLIN1-PLIN5), essential for lipid metabolism (Conte et al., 2021). *PLIN1* restricts lipolysis and is a key regulator against insulin resistance and inflammatory processes (Sohn et al., 2018), as well as, a major lipid droplet (LD) coat protein that helps regulate lipid metabolism, acting as a barrier and recruitment site for lipases (Hansen et al., 2017; Sohn et al., 2018). The LD is a dynamic and specialized intracellular lipid storage organelle containing neutral lipid metabolites that play important roles in energy homeostasis, protection against oxidative stress, and the control of cellular metabolism (Ralhan et al., 2021; Sohn et al., 2018). Although adipose tissue is the most enriched in LD (Missaglia et al., 2019), they may also affect other systems, such as the central nervous system (Farmer et al., 2020). For the brain, lipid homeostasis is critical to maintaining neuronal function and normal synaptic plasticity. The interaction between perilipins and lipases in LD is primarily responsible for lipid storage, metabolism, and degradation (Olzmann and Carvalho, 2019). In this study, *PLIN1* may have been modulated by FA available in the CO diet, showing a positive association with this diet group, which may be associated with lower oxidative stress and a lower rate of inflammation. Thus, understanding the role of perilipins, may provide clues to their role in the brain-adipose tissue system, and help to clarify how perilipins act in the prevention or progression of metabolic and neurodegenerative diseases.

The *NTN1* presented an up-regulated expression in the CO group compared to FO, and was found in the "Neurophysiological Process-Netrin-1 in regulation of axon guidance" pathway (Supplementary Figure 7). This gene is responsible for activating the Deleted in Colorectal Cancer (DCC) and unc-5 netrin receptor B (UNC5B) receptors, which regulate axon guidance. The netrins are part of a secreted protein family that is related to laminin (Stelzer et al., 2016), which play an important role in the regulation of blood-brain barrier, responsible for maintaining the homeostasis of the central nervous system (Gautam et al., 2016). Disruption or impairment of the blood-brain barrier contributes to the development and progression of several neurological disorders, such as stroke and Alzheimer's disease (Gautam et al., 2016). According to He et al. (2018), *NTN1* acted on astrocyte activation and neuronal inflammation regulation in cerebral ischemia, attenuating brain damage in mice. As recently reviewed by Glasgow et al. (2021), human polymorphisms in *NTN1* have been correlated with neurodevelopmental disorders, such as autism, schizophrenia, and neurodegenerative disorders. Also, the *NTN1*/DCC system has been reported as a key factor in developing and maintaining the central nervous system (Livesey and Hunt, 1997). In the study of Jasmin et al. (2021), *NTN1* depletion in the gray matter of mouse brain was responsible for inducing cleavage of DCC, resulting in a significant loss of dopamine neurons, causing motor deficits. Herein, the *NTN1* demonstrated a positive association with the CO group, hypothesizing that the expression of this gene may assist the maintenance of dopaminergic neurons, the homeostasis of the blood-brain barrier, synapses, and cognitive function.

Finally, the differential expression between CO and FO treatments showed a different proportion of up-and down-regulated genes, compared to the other two comparisons studied. This differential expression could corroborate with the difference in the FA profile of brain tissue (Table 1), where eicosenoic acid was less abundant and total PUFA was more abundant for the CO group (Raatz et al., 2018). Moreover, the identified genes were related to calcium homeostasis, lipid peroxidation, oxidative stress control, and maintenance of cognitive function, among others, showing some advantages in feeding the animals with CO compared to the other diets.

5. Conclusion

This study elucidated the impact of dietary oil sources on the transcriptomic and FA profiles of pig's brain tissue, as well as its impact on blood biochemical parameters. The CO group was associated with better outcomes regarding FA availability and expression of neuroprotection-related genes. The DEG identified in this study, participate in essential processes related to lipid metabolism, calcium homeostasis, cell signaling, synaptic transmission, and inflammation. Considering the relevance of pigs as models for metabolic and neurodegenerative diseases in humans, these results can be the initial step to direct future research, highlighting the importance of FA availability in human diet. In addition, these results contribute to understanding the mechanisms that link FA to essential physiological processes in the brain.

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Availability of supporting data

The dataset supporting this study is available in the European Nucleotide Archive (ENA) repository (EMBL-EBI), under accession PRJEB52665 [http://www.ebi.ac.uk/ena/data/view/PRJEB52665].

CRediT authorship contribution statement

Bruna Pereira Martins da Silva: Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Simara Larissa Fanalli:** Writing – review & editing, Writing – original draft. **Julia Dezen Gomes:** Writing – review & editing, Writing – original draft. **Vivian Vezzoni de Almeida:** Writing – review & editing, Formal analysis. **Heidge Fukumasu:** Writing – review & editing, Formal analysis. **Gabriel Costa Monteiro Moreira:** Writing – original draft, Conceptualization. **Bárbara Silva-Vignato:** Writing – review & editing. **Juliana Afonso:** Writing – review & editing. **James Mark Reecy:** Writing – review & editing, Conceptualization. **James Eugene Koltes:** Writing – review & editing, Conceptualization. **Dawn Koltes:** Writing – review & editing, Conceptualization. **Júlio Cesar de Carvalho Balieiro:** Writing – review & editing, Conceptualization. **Luciana Correia de Almeida Regitano:** Writing – review & editing. **Severino Matias de Alencar:** Writing – review & editing. **Gerson Barreto Mourão:** Writing – review & editing. **Luiz Lehmann Coutinho:** Writing – review & editing, Conceptualization. **Albino Luchiari Filho:** Writing – review & editing, Conceptualization. **Aline Silva Mello Cesar:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition,

Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

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Supplementary materials

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