Brain fatty acid and transcriptome profiles of pig fed diets with different levels of soybean oil

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Research Article

Keywords: fatty acid, immune response, calcium transport, metabolic pathways, oxidative stress, pigs

Posted Date: May 19th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1654172/v1

Abstract

Background

The high similarity in anatomical and neurophysiological processes between pigs and humans make pigs an excellent model for metabolic diseases and neurological disorders. Lipids are essential for brain structure and function. Polyunsaturated fatty acids (PUFA) have anti-inflammatory and positive effects against cognitive dysfunction in neurodegenerative diseases. Thus, our main goal was to evaluate the effect of different levels of dietary soybean oil on the lipid profile and brain tissue transcriptome in pigs. For this, thirty-six male pigs were used in a 98-day study. Treatments consisted of corn-soybean meal diets containing either 1.5% soybean oil (SOY1.5) or 3.0% soybean oil (SOY3.0). After slaughter, the brain samples were collected for total lipid content and fatty acid profile determination. Total mRNA extraction was performed for brain transcriptome sequencing.

Results

There were no differences for total lipid content and fatty acid profile between the two treatment groups. For differential expression analysis, a total of 34 differentially expressed genes (DEG, log2 fold change ≥ 1; ≤−1; FDR-corrected *p*-value < 0.05) between the SOY1.5 and SOY3.0 diets were identified by the DESeq2 statistical package. These DEG 25 were annotated, of which 11 were up-regulated and 14 were down-regulated for the SOY1.5 group compared to SOY3.0. The functional enrichment analysis performed by MetaCore with the 34 DEG (FDR < 0.05), identified four pathway maps (*p*-value < 0.05), which are related to the genes *ALOX15B*, *CALB1* and *CAST*. The network calcium transport was also identified (*p*-value = 2.303e-2), with the *CAST* and *CALB1* genes.

Conclusion

The results found in this study contribute to understanding the pathways and networks associated with processes involved intracellular calcium, lipid metabolism, and oxidative processes in brain tissue. Moreover, these results may aid in better understanding the modulating effects of soybean oil and its fatty acids (FA) composition on processes and diseases affecting brain tissue.

1. Introduction

The pigs (*Sus scrofa*) have global economic impact as it is the second most consumed meat-based protein source [1, 2]. Additionally, pigs are considered an animal model and have been used in research in the area of nutrigenomics and human metabolic diseases. Moreover, pigs can be used to understand neurodegenerative diseases due to similar of the brain anatomy, development, function and neurophysiological process compared to the brains of small laboratory animals and humans [3–6].

The brain contains high lipid content, making up approximately 50% of the brain's dry weight, only lower than to adipose tissue [7]. Lipids are essential for brain structure and function, and the central nervous system is fundamental for the regulation of metabolism and lipid balance [8, 9]. In addition, some regions of the brain are capable to detect nutrients and hormones that regulate energy balance and feeding [8, 9].

A noteworthy factor is that the diet fed to the pigs can alter the lipid and fatty acids (FA) profiles of the tissues [10, 11]. Thus, soybean oil has been commonly used as feed for growing and finishing pigs because it results in improved growth performance and beneficial effects to consumers [12]. In addition, soybean oil is high in polyunsaturated fatty acids (PUFA), being rich in linolenic acid (LA, C18:2 n-6), which is associated with the reduction of cardiovascular diseases and serum cholesterol [13].

Dietary derived FA, such as LA and alpha-linolenic acid (ALA, C18:3 n-3), act as precursors of PUFA like docosahexaenoic acid (DHA, C22:6 n-3) and arachidonic acid (AA, C20:4 n-6). Dietary supplementation of DHA, may have potential neuroprotection effects against chronic and acute inflammation in the central nervous system, as well as slowing cognitive decline in Alzheimer's disease [14]. The PUFA and their metabolites act in the brain by activating receptors and cell signaling pathways. Additionally, they are responsible for modulating the system related to signaling lipids, present in phospholipids of the neuronal cell membrane, and are responsible for regulating synaptic function [15, 16].

While the roles of specific classes of FA in brain function are being elucidated, the understanding of the genes involved in the dietary modulation of FA in the brain is unclear and limited. Thus, the objective of this work was to determine if different levels of dietary soybean oil fed to male pigs would modify the lipid and transcriptome profile of the brain.

2. Material And Methods

2.1 Ethics Statement

All procedures involving animals were evaluated and approved by the Ethics Committee for the Use of Animals (CEUA) of the School of Agriculture "Luiz de Queiroz" (ESALQ/USP), receiving protocol number 2018.5.1787.11.6 and CEUA number 2018-28. In addition, all procedures followed the guidelines established by the Brazilian Council of Animal Experimentation and the ethical principles in animal research, according to the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching [17].

2.2 Animals, experimental design, and diets

Thirty-six homozygous halothane-negative (NN) immunocastrated male pigs, offspring of Large White breed sires, were used in the study. The pigs had an average body weight of 28.44 ± 2.95 kg and an average age of 71 ± 1.8 days. The pigs were randomly distributed to the treatments during the experimental period of 98 days. There were two treatments, six replicate pens per treatment, and three pigs per pen, totalizing 18 pigs per treatment. Each pen was equipped with a three-hole dry feeder and a nipple drinker, allowing the pigs 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) [18, 19], according to the manufacturer's recommendations.

The experimental diet consisted of a six-phase diet: Grower I - day 0 to 21; Grower II - day 21 to 42; Finisher I - day 42 to 56; Finisher II - day 56 to 63; Finisher III - day 63 to 70; and, Finisher IV - day 70 to 98. Dietary treatments consisted of corn-soybean meal diets either 1.5% soybean oil (SOY1.5), a commercial diet used in pig production, or containing 3.0% soybean oil (SOY3.0). The diets were formulated to meet or exceed the nutritional requirements according to Rostagno et. al. [20] and were provided as a meal form, without antibiotic growth promoters. The diets were formulated to have a similar level of digestible energy. All detailed procedures were described in Almeida et. al. [18].

The pigs were slaughtered with a final body weight of 133.9 ± 9.4 kg on day 98 of the experiment. The frontal lobe of brain samples were collected, immediately frozen in liquid nitrogen, and then stored at -80°C until analyses. Complete procedures were described in Silva et. al. [21].

2.3 Total lipid content and FA profile analyses

For the analysis of total lipid content, 5 g of frozen brain samples were used (in duplicate), which were ground, packed in plastic bags and stored under refrigeration. The ground samples were dried in an oven with air circulation at 105°C for 12 hours. After drying, the samples were packed in filter paper cartridges and placed in a Soxhlet type extraction system. The extraction was conducted with hexane and occurred during six hours, according to the method described by AOAC [22]. The percentage of total lipid in the samples was obtained by the difference between the weight of the flask containing the extracted lipid and the empty flask (previously weighed, the flask was left in an oven at 105°C for 2 hours before each weighing) multiplied by 100.

The FA profile was determined from the total lipid content using 10 g samples of brain tissue. The lipids were cold extracted using the method proposed by Bligh Dyer [23] and the methylation of the samples was performed according to Hartman e Lago [24], with adaptations based on AOCS [25] (method AM 5 – 04). The complete procedures were describe by Silva et al. [21] and Almeida et al. [18].

Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), with pen was considered as the experimental unit. The model included the random effects of pen and block and the fixed effects of soybean oil levels. Outliers were removed from the data sets and residuals were tested for a normal distribution using the Shapiro-Wilk test (UNIVARIATE procedure). Means were adjusted by using the LSMEANS statement. Differences were declared significant when p-value ≤ 0.05 based on the F-test.

2.4 RNA extraction, library preparation and sequencing

For the total RNA extraction from the brain samples, we used the commercial kit for RNA extraction (RNeasy® Mini Kit, Qiagen) together with the Trizol reagent (Invitrogen). The inclusion of a first step using

the Trizol reagent, adapting the manufacturer's recommendations, allowed for better phase separation and thus lipid removal as brain tissue has a large amount of lipids (~ 10%).

Quality and concentration of total RNA was obtained by the NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific) and verified by the Qubit® 2.0 Fluorometer. The integrity was evaluated by the Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). All samples presented an RNA Integrity Number (RIN) greater than or equal to 7.5 (Additional file 1).

After verifying the quality and integrity of the samples, the preparation and sequencing of the libraries was initiated. For library preparation, 2 µL of total RNA from each sample was used, according to the protocol described in the TruSeq RNA Sample Preparation kit v2 manual (Illumina, San Diego, CA). The average library size, was estimated using the Agilent Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA) and the libraries were quantified using quantitative PCR with the quantification kit, from the KAPA library (KAPA Biosystems, Foster City, CA, USA).

TruSeq PE Cluster kit v3-cBot-HS (Illumina, San Diego, CA, USA) was used for the sequencing. The samples were pooled (five lanes, with a pool of all 36 samples in each lane) and sequenced using the HiSeq 2500 equipment (Illumina, San Diego, CA, USA) with a TruSeq SBS Kit v3-HS (200 cycles), according to the manufacturer's instructions. All sequencing steps were performed at the ESALQ/USP Animal Genomics Center, located in the Animal Biotechnology Laboratory of ESALQ/USP, Piracicaba, São Paulo, Brazil.

2.5 Quality control and alignment of the reads

Low complexity reads and adapters were removed using Trim Galore software (v.0.6.5). The minimum length of reads after removal was 70 bases, with Phred Score lower than 33. Quality control was done by FastQC software (v.0.11.8) [http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/]. The reference genome used was the *Sus Scrofa 11.1*, available from Ensembl [http://www.ensembl.org/Sus_scrofa/Info/Index]. Alignment, mapping and abundance (read counts) of mRNAs for all annotated genes was performed using STAR software (v.2.7.6a) [26].

2.6 Identification of differentially expressed genes

The differentially expressed genes (DEG) between the SOY1.5 and SOY3.0 groups were identified by the DESeq2 statistical package (R/Bioconductor) [http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html], using a multi-factor design [27]. Before the statistical analysis, some data filtering criteria were used: i) removal of genes with zero counts for all samples, that is, unexpressed genes, ii) removal of genes with less than one read per sample on average were removed (very lowly expressed); iii) removal of genes that were not present in at least 50% of the samples were removed (rarely expressed). The model used in the DEG analysis, included treatments as the variable of interest and father as a fixed effect. Correction for multiple testing was performed, according to the False Discovery Rate (FDR) method [28], and the threshold value used for significance was FDR < 0.05.

2.7 Functional enrichment analysis

The enrichment analysis was performed using the MetaCore software (Clarivate Analytics, v.21.4, build 70700) [https://clarivate.com/products/metacore/, Clarivate Analytics, London, UK]. The pathway maps were identified from the list of 34 DEG of SOY1.5*vs*SOY3.0 (FDR < 0.05) comparison. For annotation and functional enrichment, the *Homo sapiens* genome was used as background reference and a standard parameter. Functional enrichment analysis to obtain comparative pathways and networks was performed with the "Analyze Single Experiment" option, using the standard parameter of MetaCore. The following filters of interest were used in pathway maps: 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. To understand the behavior of genes and their interactions, networks were created using the Process Networks tool.

3. Results

3.1 Total lipid content and FA profile

The total lipid content and FA profile from brain tissue of pigs fed diets with different levels of soybean oil (SOY1.5*vs*SOY3.0) are demonstrated in Table 1. No changes (p-value ≤ 0.05) were identified in the total lipid content and the FA profile between the treatments.

Table 1. Total lipid and FA profile from brain of pigs fed with different levels soybean oil

Fatty acid, %	Dietary treatment[1]		Pooled SEM[2]	*p*-value
	SOY1.5	SOY3.0		
Total lipids	9.928	10.292	0.113	0.199
Saturated fatty acid (SFA)				
Myristic acid (C14:0)	0.522	0.521	0.006	0.927
Palmitic acid (C16:0)	26.848	27.037	0.189	0.709
Stearic acid (C18:0)	29.131	28.371	0.208	0.110
Monounsaturated fatty acid (MUFA)				
Palmitoleic acid (C16:1)	0.494	0.462	0.015	0.387
Oleic acid (C18:1 n-9)	30.071	29.955	0.143	0.678
Eicosenoic acid (C20:1 n-9)	1.897	1.898	0.024	0.967
Polyunsaturated fatty acid (PUFA)				
Linoleic acid (C18:2 n-6)	2.321	2.309	0.262	0.984
Alpha-linolenic acid (C18:3 n-3)	ND[3]	ND	-	-
Eicosapentaenoic acid (C20:5 n-3, EPA)	0.141	0.135	0.006	0.759
Docosahexaenoic acid (C22:6 n-3, DHA)	8.781	8.926	0.151	0.620
Total SFA	56.584	55.925	0.277	0.396
Total MUFA	32.494	32.501	0.192	0.987
Total PUFA	10.852	11.685	0.240	0.062
Total n-3 PUFA[4]	8.705	9.014	0.125	0.136
Total n-6 PUFA[5]	1.806	1.768	0.097	0.901
PUFA:SFA ratio[6]	0.192	0.207	0.005	0.134
n-6:n-3 PUFA ratio[7]	0.210	0.231	0.018	0.607
Atherogenic index[8]	0.664	0.661	0.008	0.921

[1]Pigs (n = 36; 18 pigs/treatment) were fed either a corn-soybean meal diet containing 1.5% soybean oil (SOY1.5) or diets containing with 3.0% soybean oil (SOY3.0). 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 [30].

3.2 RNA-Seq and DEG Data

An average number of total reads per sample of 33.4 M and 32.9 M, before and after quality control, respectively, was obtained for the SOY1.5 group. For the SOY3.0 group, the average number of sequenced reads, before and after quality control, were 34.3 M and 33.9 M, respectively. Of the total reads obtained for both groups, after quality control, 95.02% of the reads were mapped against the reference genome *SScrofa11.1* (Additional file 2).

Differential analysis of gene expression was performed comparing the level of gene expression between the groups and a total of 22,931 genes were expressed in the brain (Additional file 3). Of the total, 34 genes (25 annotated) were DEG (log2 fold change ≥1; ≤−1; FDR-corrected *p*-value < 0.05) between the SOY1.5 and SOY3.0 diets. Of these annotated genes, 11 were up-regulated (log2 fold change ranging from +0.25 to +2.93) and 14 were down-regulated (log2 fold change ranging from -3.43 to -0.36) in the SOY1.5 compared to the SOY3.0 (Additional file 4). The most altered genes were *CALB1* (down-regulated) and *VMO1* (up-regulated).

3.3 Functional enrichment analysis

The MetaCore software was used to identify pathway maps from the list of 34 DEG (FDR < 0.05). Four pathway maps were identified (*p*-value < 0.05), related to the following genes: arachidonate 15-lipoxygenase type B (*ALOX15B*), calbidin-1 (*CALB1*), and calpastatin (*CAST*), which are demonstrated in Table 2.

Table 2. Pathway maps SOY1.5[1] *vs* SOY3.0[2] from brain of pigs fed different levels of soybean

Pathway map	*p*-value	DEG[3]	log2 fold change
Linoleic acid metabolism	1.970e-02	*ALOX15B*	-1.489
Prostaglandin 1 biosynthesis and metabolism	3.597e-02	*ALOX15B*	-1.489
Renal secretion of inorganic electrolytes	3.721e-02	*CALB1*	-3.431
Immune response_IL-5 signaling via PI3K, MAPK, and NF-kB	4.77e-02	*CAST*	+0.421

[1]SOY1.5: corn-soybean meal diet containing 1.5% soybean oil. [2]SOY1.5: corn-soybean meal diet containing 3.0% soybean oil. [3]DEG: Differentially expressed genes.

The *ALOX15B* gene, was identified as a DEG in brain tissue of pigs fed different levels of soybean oil, showing a down-regulation in the SOY1.5 group compared to SOY3.0 (log2 fold change -1.489). The *ALOX15B*, participate in two of the four significant enrichment pathway maps identified: “Linoleic acid metabolism” (p-value = 1.970e-2), which is represented in Figure 1, and “Prostaglandin 1 biosynthesis and metabolism” (*p*-value = 3.597e-2), which is represented in Figure 2.

The *CALB1* gene was identified as a DEG in brain tissue of pigs fed different levels of soybean oil, showing a down-regulation in the SOY1.5 group compared to SOY3.0 (log2 fold change -3.431). The *CALB1*, participates in the enriched pathway map “Renal secretion of inorganic electrolytes” (*p*-value = 3.721e-2), which is represented in Figure 3.

The *CAST* gene was identified as a DEG in brain tissue of pigs fed different levels of soybean oil, showing an up-regulation in the SOY1.5 group compared to SOY3.0 (log2 fold change +0.421). The *CAST* participates in the enriched pathway map “Immune response_IL-5 signaling via PI3K, MAPK, and NF-kB” (*p*-value = 4.770e-2), which is represented in Figure 4.

To better understand the behavior of the genes and their interactions, process networks were additionally generated by using the MetaCore software. The “Calcium transport” process network (*p*-value = 2.303e-2), was the only network detected herein, containing the DEG *CALB1* (log2 fold change -3.431) and *CAST* (log2 fold change +0.421) (Figure 5).

4. Discussion

No changes were identified in the total lipid content and the FA profile between the treatments, thus demonstrating that brain tissue is not affected by diet in the short-term. The results found in the functional enrichment, demonstrated that the use of different levels of soybean oil alters the transcriptomic profile of pig brain, affecting key processes for the well-functioning of this tissue. For the enriched pathways in Figs. 1 and 2, the DEG *ALOX15B* participates in lipid oxidation and peroxidation reactions. According to Stelzer et al. [31], among the pathways associated with this gene there are “eicosanoid synthesis” and “arachidonic acid metabolism” and the related Gene Ontology (GO) annotations include “calcium ion binding” and “lipid binding”. In our study we observed that *ALOX15B* has a lower expression in the SOY1.5 group compared to SOY3.0.

Lipoxygenases (LOX) are a family of enzymes responsible for the oxidation of lipids and the generation of a range of metabolites such as eicosanoids and PUFA-related compounds. These metabolites play diverse physiological and pathological roles in inflammatory, neurodegenerative, and cardiovascular diseases, as well as, in defense mechanisms [32, 33]. The LOX have also been reported in cell differentiation [34, 35], apoptosis [36], and play an important role in the immune response by helping to regulate cytokine secretion [37].

Among the LOX reported in mammals, the *ALOX15* isoform my oxygenate complex lipid-protein assemblies found in biomembranes and lipoproteins [38]. The *ALOX15* also binds to membranes, with intracellular calcium as a main cofactor for this interaction [39, 40]. It is reported that *ALOX15* is expressed at higher levels in in human airway epithelial cells, in eosinophils and immature red blood cells [41]. Furthermore, according to van Leyen et al. [42] and Han et al. [43], expression and regulation of *ALOX15* transcription also occurs in various areas of the brain, but at lower levels. In the study of Shalini et al. [44], a higher expression of *ALOX15* mRNA was found in the prefrontal cortex.

The main product of AA oxygenation by *ALOX15* and *ALOX15B* is 15-hydroxyeicosatetraenoic (*15-HETE*) [45]. The *15-HETE* is considered an important precursor of specialized pro-resolving lipid mediators (SPM) and is associated with pro- and anti-inflammatory effects [46, 47]. It has also been reported that *15-HETE* is a ligand and activator of peroxisome proliferator-activated receptor gamma (*PPAR-γ*), which at high

concentrations may generate reactive oxygen species (ROS) in cells [48, 49] and may induce the production of the pro-inflammatory cytokine Interleukin-12 (IL-12) [47, 50].

Among the results of DHA oxidation by *ALOX15*, are the SPM resolvin D5, a mediator that may be associated in the resolution of inflammation and in regulating the immune response [51]. Another important mediator related to the resolution of inflammation, reduction of leukocyte trafficking, and negative regulation of cytokine expression is neuroprotectin D1 (NPD1) [52, 53]. NPD1 is reported as an anti-inflammatory molecule, which acts in neuroplasticity and brain signaling and when in altered conditions, may be found in neuroinflammatory disorders and chronic neurodegeneration [44].

The *ALOX15* was found to have increased expression in the brains of Alzheimer's patients [38, 54, 55]. Praticò et al. [54], reported higher levels of *12/15-LOX* and its metabolites *12/15(S)-HETE* in the temporal and frontal brain regions of Alzheimer's patients. It was further found in in vitro studies using neuronal cells with Alzheimer's mutation that *12/15-LOX* is associated with regulation of tau phosphorylation and Aβ plaque production, and regulates synaptic pathology associated with behavioral deficiencies [56, 57].

In addition, *12/15-LOX* has been shown to play an important role in Parkinson's disease. In the study of Li et al. [58] and Canals et al. [59], the authors showed that the activation of these isoforms was associated with a decrease in glutathione concentration (a marker of Parkinson's disease) in neurons, which may lead to nitric oxide neurotoxicity and damage dopaminergic neurons. In the study of Zhang et al. [60], the inhibition of *12/15-LOX* assisted in reducing the generation of ROS induced neuronal cell death. Thus, it is commonly found in the literature that *12/15-LOX* and its metabolites possess both pro-inflammatory and anti-inflammatory effects. This controversial nature is dependent on the metabolites generated, the site of inflammation, as well as, the levels generated of these metabolites [47]. Thus, with a down-regulation of *ALOX15B* in the SOY1.5 group, our study demonstrates that diet may have positively or negatively influenced various metabolic and oxidative processes in brain tissue. Further studies are needed to verify this action.

For the enriched pathway in Fig. 3, the *CALB1* gene binds to intracellular calcium transported via the epithelial calcium channel and transports it across the cytosol toward the basolateral membrane [61]. The *CALB1* is a protein-encoding gene that acts in calcium transport. The GO annotations related to this gene include “calcium ion binding” and “vitamin D binding” [31]. In our study we observed that *CALB1* also showed lower expression for the SOY1.5 group compared to SOY3.0.

The *CALB1* is highly conserved in evolution and belongs to a family of high-affinity calcium-binding proteins [62, 63]. The *CALB1* is found highly expressed in brain tissue, is present in most neuronal cell groups, and is not vitamin D dependent [62–64].

Calcium is one of the most important signaling factors and acts to regulate several important cellular functions such as growth, differentiation, proliferation, cell survival and apoptosis, membrane excitability, and gene transcription [65]. Calcium is also essential for maintaining normal brain function [65]. Thus, the dysregulation of calcium homeostasis and endoplasmic reticulum stress is associated with several

pathological conditions such as Parkinson's, Huntington's, and Alzheimer's diseases, and affects numerous signaling pathways [65, 66]. This pathogenic event may also cause amyloidogenesis, energy deficits in neurons, protein aggregation and oxidative stress, and changes in mitochondrial dysfunction, plasticity and synaptic transmission [67].

Disturbed mitochondrial calcium regulation may also be associated with the link between neuronal dysfunction and disruption of the mitochondria-associated membrane (MAM) contact site of the endoplasmic reticulum and mitochondria, since calcium acts to modulate neurotransmitter release during the synapse [68]. This dysregulation of the MAM-mitochondria linkage dysfunction may also be associated with neurodegenerative diseases such as Alzheimer's disease [68]. The MAMs are regions of the endoplasmic reticulum that mediate communication between the reticulum and the mitochondria [68, 69]. They are regions that are involved in calcium transport, are responsible for several lipid biosynthetic enzymatic activities, and are also a strategic site for lipid metabolism [68, 70, 71]. According to Vance [68], defects associated with these regions have been identified in neurodegenerative diseases and insulin resistance/type 2 diabetes.

The *CALB1* helps maintain calcium homeostasis, helps regulate intracellular calcium responses to physiological stimuli, and assists in modulating synaptic transmission [62]. Another important role of *CALB1*, is its action in the prevention of neuronal death [62, 72]. The *CALB1* also plays an important role in buffering cytosolic calcium and helps prevent lipid peroxidation, through its expression in pancreatic-β cells, by eliminating the production of lipid hydroperoxide, which is induced by proinflammatory cytokines [73]. There is evidence that *CALB1* acts to protect neurons against calcium-mediated neurotoxicity and may be considered a cytochemical marker for neuronal plasticity [64].

Decreases in *CALB1* expression/concentration in brain tissue has been associated with neurodegeneration in Alzheimer's, Parkinson's, and Huntington's diseases [31, 74] and in ischemic injury studies [75, 76]. Lower *CALB1* expression has also been associated with a higher rate of neuronal death [77]. Increased expression of *CALB1*, on the other hand, has been reported to induce neurite growth in dopaminergic neuronal cells, demonstrating its protective role, especially in neurological diseases, such as Parkinson's disease [72, 78].

For Alzheimer's disease, it has been reported that *CALB1* has protective effects against the pro-apoptotic action of mutant presenilin 1 (PS-1), attenuating the increase in intracellular calcium and aiding in the prevention of impaired mitochondrial function [79]. PS-1 acts by sensitizing cells to apoptosis induced by Aβ peptide, which damages neurons through a mechanism involving disruption of calcium homeostasis and generation of oxidative stress [79]. Thus, with a down-expression of *CALB1*, we observed that with a lower percentage of soybean oil, the *CALB1* gene was less expressed, showing a negative relationship with this diet.

For the enriched pathway in Fig. 4, IL-5 activates and elevates the expression of *CAST*. The DEG *CAST* binds to and inhibits calpain 1 (mu) in the presence of calcium, which activates and cleaves the apoptosis regulatory protein Bax. The Bax will act by preventing or reducing the frequency, rate, or extent of cell

death by apoptotic process [80, 81]. In our study, *CAST* had a higher expression (up-regulation) in the SOY1.5 group compared to SOY3.0. The protein encoded by *CAST* is an endogenous calpain inhibitor and is also related to the proteolysis of amyloid precursor protein. Moreover, this protein likewise is thought to affect the expression levels of genes that are responsible for encoding structural or regulatory proteins [31]. Among the related pathways associated with this gene are “neuroscience” and “neurodegenerative diseases”. Related GO annotations include “RNA binding” and “cysteine-type endopeptidase inhibitor activity” [31].

The *CAST* is a cell-permeable peptide that acts as an endogenous inhibitor of calpain in the central nervous system [82, 83]. Calpains are cysteine proteases that are activated by calcium, that is, they are positively regulated by calcium and negatively regulated by *CAST* [84, 85]. These proteases, when in dysregulation of calcium homeostasis, have been implicated in neuronal cell dysfunction and death [84], as well as, neurodegenerative diseases [86–88].

Calpains have several important roles such as differentiation, cell attachment motility, signal transduction covering cell signaling pathways, regulation of gene expression and membrane fusion [82, 85]. Furthermore, calpains are reported to play important roles in neuronal functions, implying that the activation of this protease needs to be under a rigid control, which is performed by *CAST*. Thus, the well-known calpain-calpastatin system may be an important target for therapeutic approaches related to neurodegenerative diseases [84].

According to Goll et al. [85], *CAST* is further associated in the regulation of kinases, receptors and transcription factors. Increased expression of *CAST* has been reported to have a neuroprotective effect in cerebral ischemia [89]. In the study of Rao et al. [90], higher expression of *CAST* in JNPL3 (mutant tau P301L) mouse models for aided in the attenuation of calpain, which has been reported in the development of tauopathy (neurotoxicity caused by tau protein) and neurodegeneration reported in Alzheimer's disease. Higher expression of *CAST* was also associated with neuroprotective results in an Amyotrophic lateral sclerosis (ALS) mouse model. According to Rao et al. [91], the *CAST* gene acts by reducing calpain activation, decreasing abnormal breakdown of cytoskeletal proteins, increasing survival time, inhibiting tau production and *CDK5* activation, and reducing *SOD1* [91].

The calpain-calpastatin system is also reported in excitotoxicity, a pathological or neurodegenerative process that is initiated by overactivation of neurotransmitters such as glutamate and it is like. Excitotoxicity, leads to increased cellular calcium levels, which causes activation of various proteases, including calpains [92]. Furthermore, missing *CAST* may impair early stages of neurogenesis [93]. Thus, we observed a higher expression of *CAST* in SOY1.5, that suggests a positive relationship between the gene and the metabolic and oxidative processes found for this group.

The identified network together with the illustrated genes corroborate the results found in the pathway maps, and thus, shows us that altering the level of soybean oil in the diet of immunocastrated male pigs has an effect on gene expression in brain tissue. Moreover, it is noteworthy the importance of the detected DEG and their association with intracellular calcium.

Thereby, the network of processes "Calcium transport" identified and the genes present, corroborate the results found in the pathway maps, and thus, shows us that altering the level of soybean oil in the diet of pigs have an effect on gene expression in brain tissue. Moreover, it is noteworthy the importance of the detected DEG and their association with intracellular calcium. Thus, the results found in our study represent an important direction for the understanding of pathways and networks associated with calcium-dependent metabolic processes involved in lipid metabolism and oxidative processes in brain tissue. Further, more studies are needed to better understand the mechanisms by which dietary factors such as FA may influence important physiological processes and gene expression in the brain tissue. In addition, understanding the mechanisms involved in calcium homeostasis and energy metabolism involved in the initiation and progression of neurodegenerative diseases and oxidative/inflammatory processes is also quite relevant.

5. Conclusion

This study, identified that different dietary levels of soybean oil in the diet of male pigs affects the transcriptomic profile, but not total lipid content or FA profile of brain tissue. The genes, pathways and networks identified herein participate in important processes associated with lipid metabolism, immune response and calcium transport. Furthermore, because pigs are model animals for metabolic diseases in humans, the DEG identified, as well as, their action in brain tissue demonstrate the importance of FA in metabolic and oxidative processes. Thus, the current study may help direct future research in the area of nutrigenomics and help to better understand how the diet, with the inclusion of soybean oil, may influence and modulate biological processes important for brain tissue.

Abbreviations

FA: fatty acids; PUFA: polyunsaturated fatty acids; LA: linolenic acid; ALA: alpha-linolenic acid; DHA: docosahexaenoic acid; AA: arachidonic acid; DEG: differentially expressed genes; FDR: false discovery rate; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; EPA: eicosapentaenoic acid; ALOX15B: arachidonate 15-lipoxygenase type B; CALB1: calbidin-1; CAST: calpastatin; GO: c gene ontology; LOX: lipoxygenases; 15-HETE: 15-hydroxyeicosatetraenoic; SPM: specialized pro-resolving lipid mediators; PPAR-γ: peroxisome proliferator-activated receptor gamma; ROS: reactive oxygen species; NPD1: neuroprotectin D1; MAM: mitochondria-associated membrane; PS-1: presenilin 1; ALS: amyotrophic lateral sclerosis.

Declarations

Acknowledgements: We acknowledge the collaborative efforts among University of São Paulo and Iowa State University. Appreciation is extended to DB Genética Suína and gratefully acknowledge the support of Crista Indústria e Comércio Ltda and Patense Indústria de Rendering.

Authors' contributions: All authors contributed to this study. B.P.M.S, H.F, G.C.M.M, J.M.R, J.E.K, D.K, J.C.C.B, L.L.C, G.B.M, A.L.F and A.S.M.C contributed to the conception and design. B.P.M.S performed formal and data analysis. V.V.A performed statistical analysis. B.P.M.S prepared the original draft. B.P.M.S discussed the obtained data. S.L.F, J.D.G, V.V.A, F.A.O.F, G.C.M.M, B.S.V, J.M.R, J.E.K, D.K, S.M.A, J.P.M.S, L.L.C, J.A., L.C.A.R, and A.S.M.C critically reviewed and provided feedback on the original manuscript. A.S.M.C acquisition of financial support. All authors read and approved the final manuscript.

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Funding: This study was supported by the São Paulo Research Foundation (FAPESP, Grant numbers: 2021/01694-2, 2020/10042-6, 2020/14148-3, 2018/26797-6, 2018/26816-0, and 2017/25180-2, the Brazilian National Council for Scientific and Technological Development (CNPq) that provided a researcher fellowship to A. S. M. Cesar, L. L. Coutinho and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001.

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

Ethics approval and consent to participate: All animal procedures were approved by the Animal Care and Use Committee of Luiz de Queiroz College of Agriculture (University of São Paulo, Piracicaba, Brazil, protocol number: 2018.5.1787.11.6 and number CEUA 2018-28) and followed ethical principles in animal research, according to the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching.

Consent for publication: Not applicable.

Competing interests: The authors declare that they have no competing interests.

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Supplementary Information: Additional file 1 - Table A1: Quality and integrity of brain samples from pigs fed different levels of soybean oil; Additional file 2 - Table A2: Mapped reads (*Sscrofa11.1*) of brain samples from pigs fed different levels of soybean oil; Additional file 3 - Table A3. Expressed genes of brain samples from pigs fed different levels of soybean oil; Additional file 4 - Table A4. DEG of brain samples from pigs fed different levels of soybean oil.

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Figures

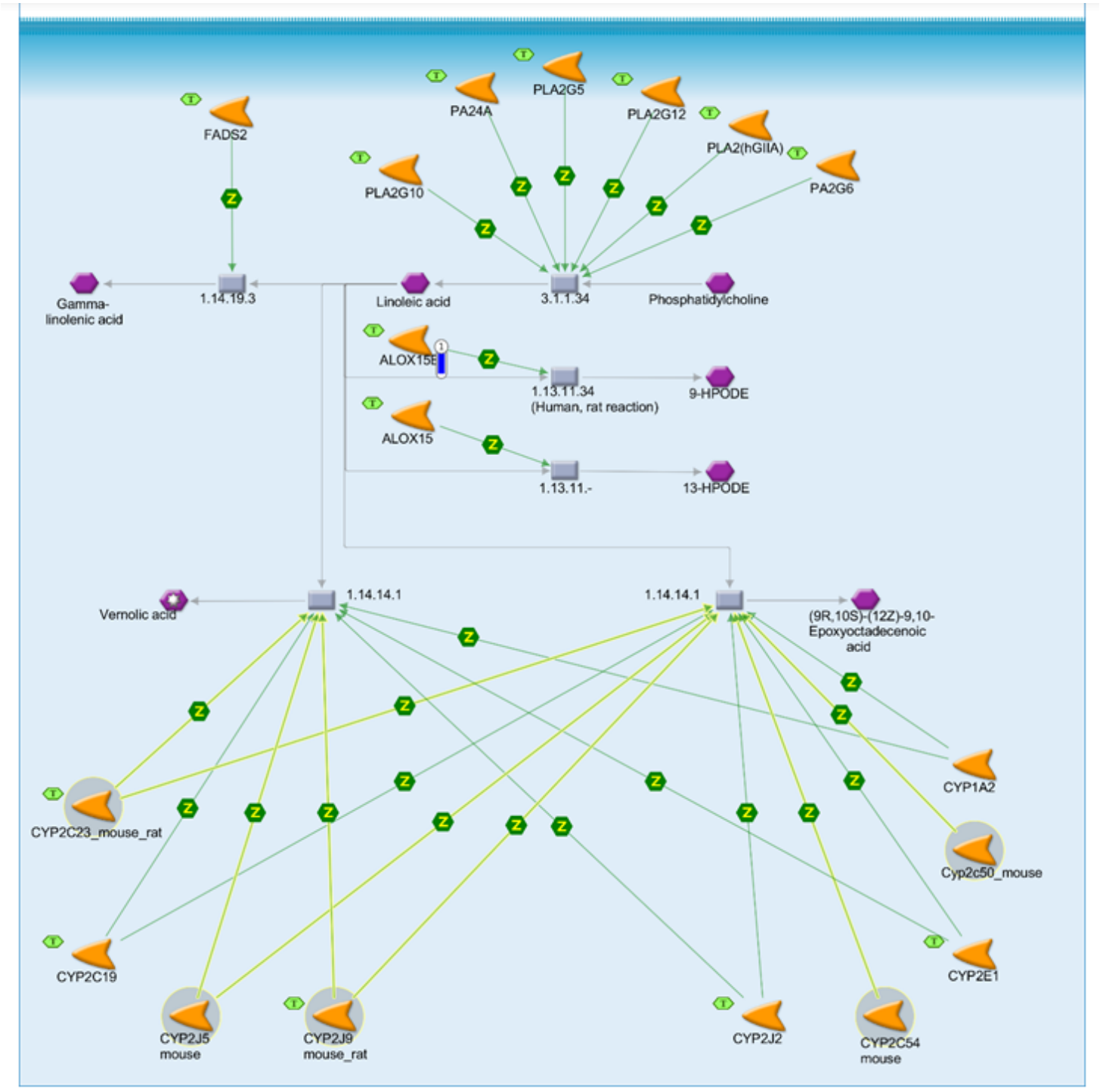

Figure 1

Linoleic acid metabolism from brain of pigs fed different levels of soybean (SOY1.5[1] *vs* SOY3.0[2]). [1]SOY1.5: corn-soybean meal diet containing 1.5% soybean oil. [2]SOY1.5: corn-soybean meal diet containing 3.0% soybean oil. The experimental data is represented by the thermometer-like figure on the map. The downward thermometer (blue) indicates down-regulation of the *ALOX15B* DEG (log2 fold change -1.489) in the SOY1.5 group compared to SOY3.0. Network objects are represented by individual symbols. The green "T" flashing icon shows which object is associated with the brain tissue. Interactions between objects are represented by arrows, mechanisms, and logical relationships. Further explanations are provided at https://portal.genego.com/legends/MetaCoreQuickReferenceGuide.pdf.

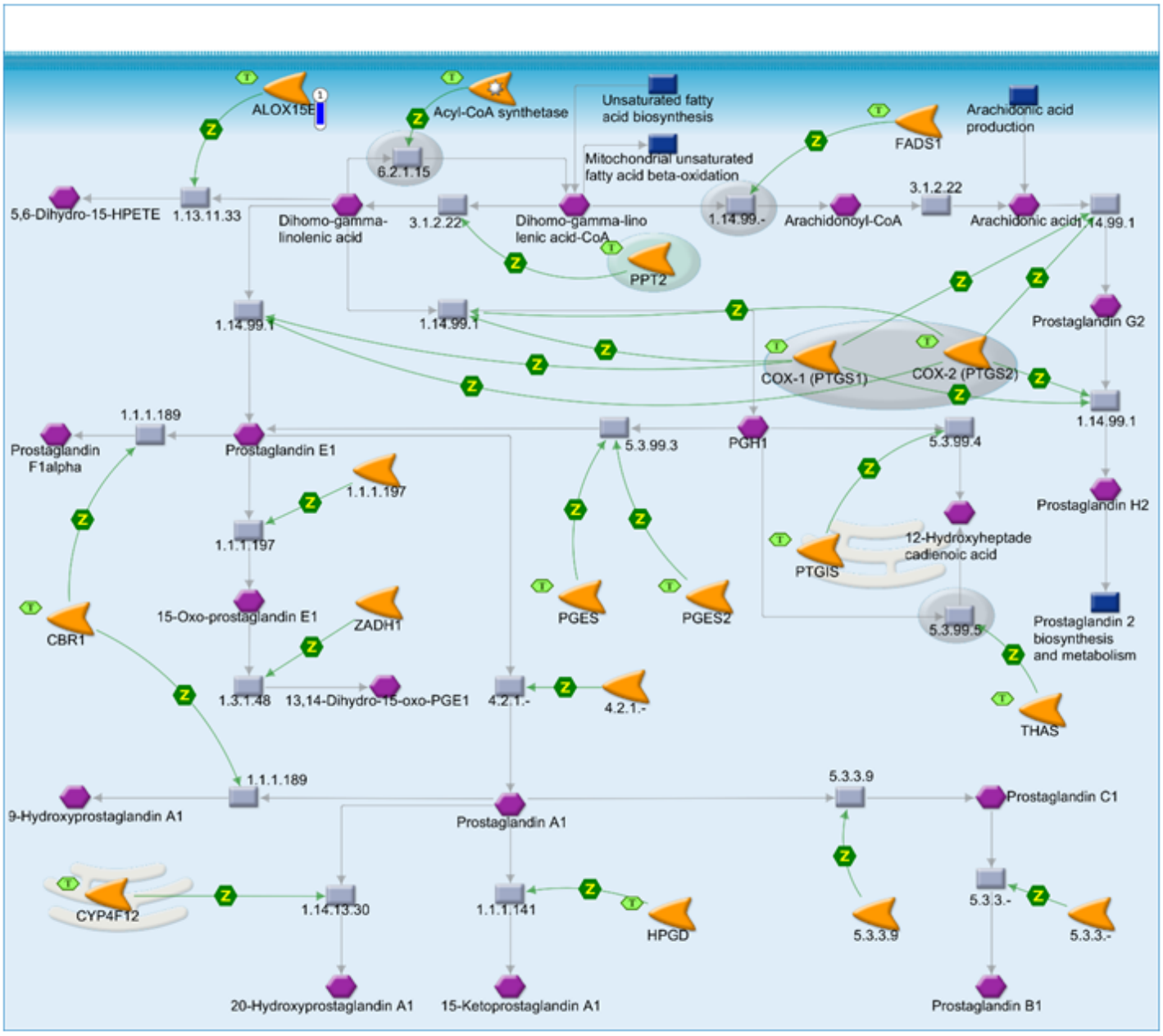

Figure 2

Prostaglandin 1 biosynthesis and metabolism from brain of pigs fed different levels of soybean (SOY1.5[1] *vs*SOY3.0[2]). [1]SOY1.5: corn-soybean meal diet containing 1.5% soybean oil. [2]SOY1.5: corn-soybean meal diet containing 3.0% soybean oil. The experimental data is represented by the thermometer-like figure on the map. The downward thermometer (blue) indicates down-regulation of the *ALOX15B* DEG (log2 fold change -1.489) in the SOY1.5 group compared to SOY3.0. Network objects are represented by individual symbols. The green "T" flashing icon shows which object is associated with the brain tissue. Interactions between objects are represented by arrows, mechanisms, and logical relationships. Further explanations are provided at https://portal.genego.com/legends/MetaCoreQuickReferenceGuide.pdf.

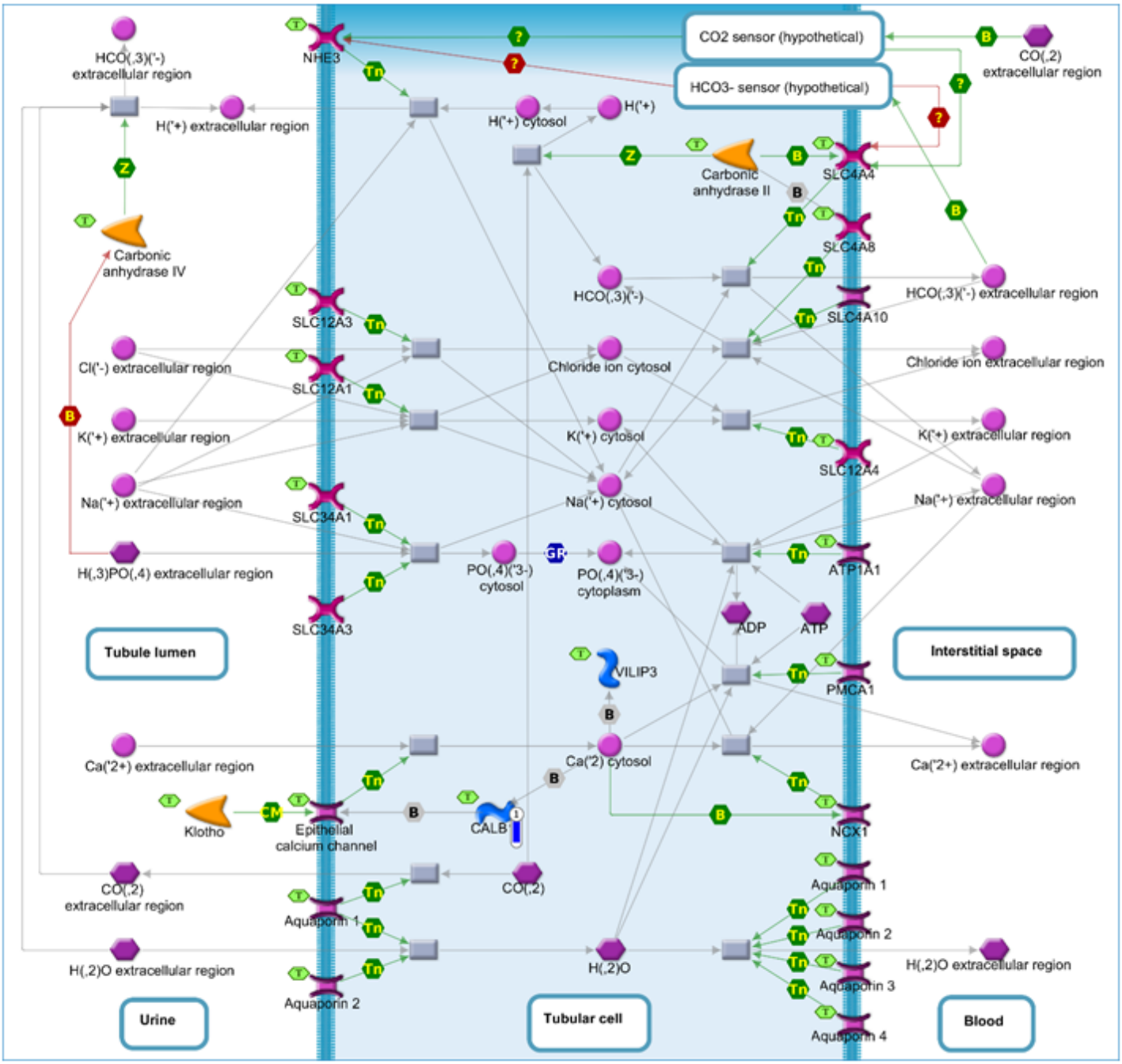

Figure 3

Renal secretion of inorganic electrolytes from brain of pigs fed different levels of soybean (SOY1.5[1] *vs*SOY3.0[2]). [1]SOY1.5: corn-soybean meal diet containing 1.5% soybean oil. [2]SOY1.5: corn-soybean meal diet containing 3.0% soybean oil. The experimental data is represented by the thermometer-like figure on the map. The downward thermometer (blue) indicates down-regulation of the *CALB1* DEG (log2 fold change -3.431) in the SOY1.5 group compared to SOY3.0. Network objects are represented by individual symbols. The green "T" flashing icon shows which object is associated with the brain tissue. Interactions between objects are represented by arrows, mechanisms, and logical relationships. Further explanations are provided at https://portal.genego.com/legends/MetaCoreQuickReferenceGuide.pdf.

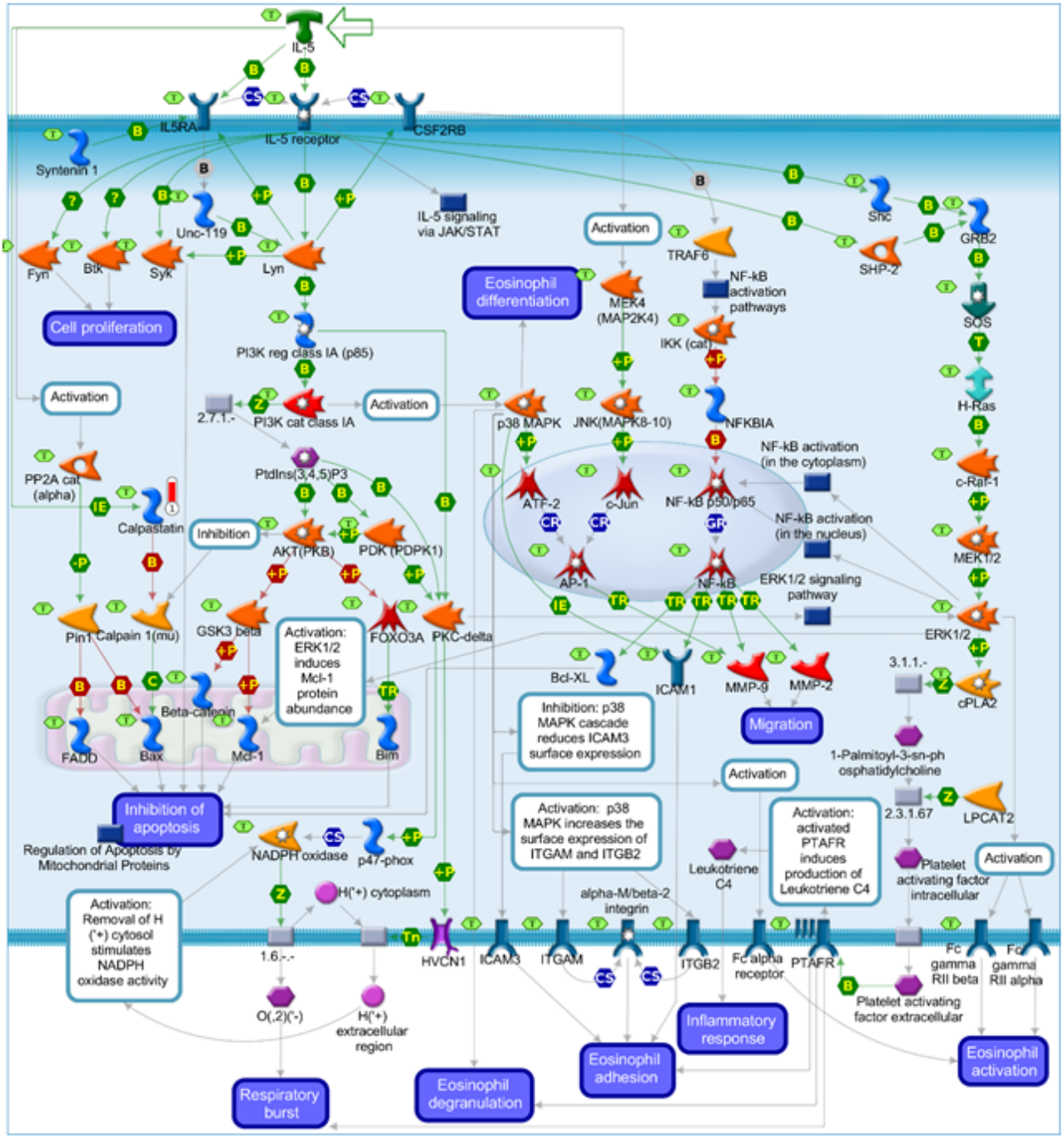

Figure 4

Immune response_IL-5 signaling from brain of pigs fed with fed different levels of soybean (SOY1.5[1] *vs* SOY3.0[2]). [1]SOY1.5: corn-soybean meal diet containing 1.5% soybean oil. [2]SOY1.5: corn-soybean meal diet containing 3.0% soybean oil. The experimental data is represented by the thermometer-like figure on the map. The upward thermometer (red) indicates up-regulation of the *CAST* DEG (log2 fold change +0.421) in the SOY1.5 group compared to SOY3.0. Network objects are represented by individual symbols. The green "T" flashing icon shows which object is associated with the brain tissue. Interactions between objects are represented by arrows, mechanisms, and logical relationships. Further explanations are provided at https://portal.genego.com/legends/MetaCoreQuickReferenceGuide.pdf.

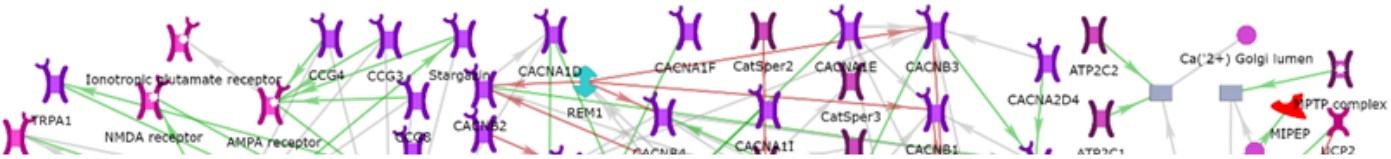

Figure 5

Calcium transport network from brain of pigs fed with fed different levels of soybean (SOY1.5[1] *vs* SOY3.0[2]). [1]SOY1.5: corn-soybean meal diet containing 1.5% soybean oil. [2]SOY1.5: corn-soybean meal diet containing 3.0% soybean oil. The experimental data are represented by circles on the network. The blue circle indicates down-regulation of the *CALB1* DEG, and the red circle indicates up-regulation of the *CAST* DEG SOY1.5 group compared to SOY3.0. Green arrows indicate positive interactions, red arrows indicate negative interactions, and gray arrows indicate unspecified interactions. Further explanations are provided at https://portal.genego.com/legends/MetaCoreQuickReferenceGuide.pdf.

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