UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ZOOTECNIA E ENGENHARIA DE ALIMENTOS

BRUNA PEREIRA MARTINS DA SILVA

Lipid and transcriptome profile of the brain of pigs fed with different oil sources

Pirassununga
2022

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Versão corrigida

Dissertação apresentada à Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo, como parte dos requisitos para a obtenção do título de Mestre em Ciências do Programa de Mestrado em Biociência Animal.

Área de Concentração: Biociência Animal

Orientadora: Prof[a]. Dr[a]. Aline Silva Mello Cesar

Pirassununga
2022

Ficha catalográfica elaborada pelo
Serviço de Biblioteca e Informação, FZEA/USP,
com os dados fornecidos pelo(a) autor(a)

Silva, Bruna Pereira Martins da
S586l Lipid and transcriptome profile of the brain of pigs fed with different oil sources / Bruna PereiraMartins da Silva ; orientadora Aline Silva Mello Cesar. -- Pirassununga, 2022.
98 f.

Dissertação (Mestrado - Programa de Pós-Graduaçãoem Biociência Animal) -- Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo.

1. RNA-seq. 2. gene expression. 3. fatty acids. 4. brain function. 5. neuroprotection. I. Cesar,Aline Silva Mello , orient. II. Título.

CERTIFICADO CEUA

UNIVERSIDADE DE SÃO PAULO
ESCOLA SUPERIOR DE AGRICULTURA "LUIZ DE QUEIROZ"

Comissão de Ética no Uso de Animais – CEUA
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CERTIFICADO

Certificamos que a proposta intitulada "Efeito da adição de ácido oleico na dieta de suínos sobre o perfil da resposta imunológica e de ácidos graxos de diferentes tecidos", registrada com o número de protocolo 2018.5.1787.11.6, n° CEUA: 2018-28, sob a responsabilidade de Aline Silva Mello Cesar, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino), encontra-se de acordo com os preceitos da Lei nº 11.794, de 08 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal-CONCEA, e foi aprovada pela Comissão de Ética no Uso de Animais (CEUA) da Escola de Agricultura Luiz de Queiroz-ESALQ/USP, em reunião ordinária no dia 18 de setembro de 2018.

Finalidade	**( ) Ensino (X) Pesquisa Científica**
Vigência da autorização	22/11/2018 a 31/07/2022
Espécie/Linhagem/raça	Suíno/Comercial
Nº de animais	72
Peso/Idade	125 dias / 70Kg
Sexo	M
Origem	Estação Experimental Fazenda São Gabriel – DB Genética Suína – Presidente Olegário - MG

CERTIFICATE

This is to certify that study "Effect off dietary oleic acid on fatty acid composition and inflammatory response profile in different tissues of pigs", protocol number 2018.5.1787.11.6, under the responsibility of Aline Silva Mello Cesar, has been approved by the Institutional Animal Care and Use Committee, College of Agriculture "Luiz de Queiroz", Piracicaba, SP, Brazil, University of São Paulo.

Piracicaba, 25 de setembro de 2018.

Prof. Dr. Urbano dos Santos Ruiz
Coordenador da CEUA/ESALQ/USP

Folha de Aprovação

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Data de Aprovação: 23/06/2022

Banca examinadora:

_____

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Embrapa Pecuária Sudeste

AGRADECIMENTOS

Agradeço à Profa. Dra. Aline Silva Mello Cesar, pela oportunidade, orientação, paciência, profissionalismo, ensinamentos e amizade. Sou grata por fazer parte do seu grupo de pesquisa!

À Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos, especialmente ao Programa de Pós Graduação em Biociência Animal e a Escola Superior de Agricultura "Luiz de Queiroz", pela oportunidade e por toda infraestrutura disponibilizada e por toda e equipe de docentes, técnicos e funcionários.

À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) pela bolsa de Mestrado (Processo 2020/10042-6) e por possibilitar o desenvolvimento desta pesquisa.

À DB Genética Suína e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), que foram fundamentais para a realização desta pesquisa.

A todos os professores e pesquisadores pelo suporte, ensinamentos e sugestões, em especial ao Dr. Luiz Lehmann Coutinho, Dr. Heidge Fukumasu, Dra. Vivian Vezzoni de Almeida, Dra. Juliana Afonso, Dra. Bárbara Silva-Vignato, Dra. Dawn Koltes, Dra. Luciana Correia Almeida Regitano, Dr. Gabriel Costa Monteiro Moreira, Dr. Júlio Cesar de Carvalho Balieiro e Dr. Albino Luchiari Filho.

Aos membros da banca examinadora pelo aceite, pelas sugestões e apoio.

À Me. Simara Larissa Fanalli e a Julia Dezen Gomes pelo companheirismo e amizade.

A todos os integrantes do Laboratório de Biologia Molecular e Micotoxinas (LABMIC) da ESALQ/USP, por todo apoio e ajuda ao longo do mestrado, em especial a Milena, Tota, Ivani, Felipe e Fernanda. Ao Laboratório de Qualidade e Processamento de Carnes e ao Laboratório de Biotecnologia Animal (LBA), pelo apoio e análises.

Às pessoas mais importantes da minha vida, Pedro, Edna, Julia e Stefano, pelo amor, carinho, suporte, incentivo e paciência. Obrigada por estarem ao meu lado em todos os momentos e possibilitarem mais essa conquista.

Bruna Martins

Pelo amor e incentivo, dedico este trabalho à minha família!

FONTE FINANCIADORA E INSTITUIÇÃO

Este projeto foi desenvolvido com o apoio financeiro da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), com processo de número 2020/10042-6, vinculado ao projeto Jovem Pesquisador (JP), de processo número 2017/25180-2. A realização do projeto, ocorreu com a colaboração dos laboratórios: Biologia Molecular e Micotoxinas (LABMIC) – Departamento de Agroindústria, Alimentos e Nutrição (LAN) – ESALQ/USP; Laboratório de Qualidade e Processamento de Carnes – Departamento de Agroindústria, Alimentos e Nutrição (LAN) – ESALQ/USP; Microbiologia de Alimentos – Departamento de Agroindústria, Alimentos e Nutrição (LAN) – ESALQ/USP; Laboratório de Biotecnologia Animal (LBA)/Centro de Genômica Animal – Departamento de Zootecnia (LZT) – ESALQ/USP; Piracicaba, São Paulo, Brasil; Laboratório de Oncologia Comparada e Translacional (LOCT) – Departamento de Medicina Veterinária (ZMV) – FZEA/USP; Pirassununga, São Paulo, Brasil.

RESUMO

SILVA, B. P. M. **Perfil lipídico e do transcriptoma do cérebro de suínos alimentados com diferentes fontes de óleo.** 2022. 98 f. Dissertação (Mestrado) - Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2022.

Os suínos (*Sus scrofa*) são animais de produção de grande importância para a economia devido à produção de proteína animal de qualidade e para a ciência animal e humana, como animais modelo biomédicos. O cérebro de suínos possui grande similaridade com o dos humanos e pode ter seu conteúdo e perfil de ácidos graxos (AG) modulados pela dieta. Os lipídios atuam como sinalizadores que regulam diversos processos biológicos importantes e podem influenciar na expressão gênica. Assim, o estudo dos efeitos de diferentes fontes de lipídios sobre o perfil do transcriptoma do cérebro, é de grande importância para novos *insights* associados à saúde animal e humana. O objetivo geral desse estudo foi avaliar o efeito de diferentes fontes e níveis de óleo sobre o perfil lipídico e transcricional do tecido cerebral. As dietas experimentais consistiam em dietas de milho e farelo de soja contendo 1,5% de óleo de soja (SOY1.5), ou 3% de óleo de soja (SOY3.0), ou 3% de óleo de canola (CO), ou 3% de óleo de peixe (FO). O período experimental foi de 98 dias, as dietas foram oferecidas nas fases de crescimento e terminação de machos imunocastrados e foram utilizados 18 animais por tratamento/dieta. Após o abate, as amostras de cérebro foram coletadas para a determinação do conteúdo total de lipídios e para o perfil de AG. A extração de RNAm total foi realizada para o sequenciamento do transcriptoma do cérebro dos suínos. Para a análise estatística da expressão diferencial foi utilizado o pacote DESeq2, em que foram identificados os GDE (FDR < 0,05) entre as amostras de cérebro. Após a identificação dos GDE, foi realizada a análise de enriquecimento funcional pelo software MetaCore, a partir do qual foram identificadas vias e redes metabólicas (p-valor < 0,05). Para a análise dos diferentes níveis de óleo de soja (SOY1.5 ou SOY3.0), não foram observadas mudanças no conteúdo total de lipídios e no perfil de AG. As vias e redes metabólicas foram associadas ao metabolismo lipídico, a resposta imune e ao transporte de cálcio. Para a análise das diferentes fontes de óleo não houve mudanças no conteúdo total de lipídios e alguns AG apresentaram diferenças estatísticas. As principais vias e redes identificadas foram associadas a sinalização celular, ao metabolismo lipídico, a transmissão sináptica e a processos inflamatórios. Os resultados aqui encontrados podem nos orientar para uma melhor compreensão dos efeitos e mecanismos dos AG dietéticos no transcriptoma e no perfil de AG do tecido cerebral. Além disso, esses resultados contribuem para os avanços na área da nutrigenômica e para melhorias na saúde animal e humana. Os resultados propiciaram a escrita de dois manuscritos, já submetidos em revistas científicas internacionais, disponibilizados nos capítulos 1 e 2 desta dissertação.

Palavras-chave: Ácidos graxos. Expressão gênica. Função cerebral. Homeostase lipídica. Neuroproteção. RNA-seq. Sinalização celular. Suínos. Vias metabólicas.

ABSTRACT

SILVA, B. P. M. **Lipid and transcriptome profile of the brain of pigs fed with different oil sources.** 2022. 98 f. M.Sc. Dissertation - Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2022.

Pigs (*Sus scrofa*) are production animals of great importance for the economy due to the production of quality animal protein and for animal and human science as biomedical model animals. The brain of pigs has great similarity to that of humans and may have its fatty acid (FA) content and profile modulated by diet. Lipids act as signals that regulate several important biological processes and may influence gene expression. Thus, the study of the effects of different lipid sources on the brain transcriptome profile, is of great importance for new insights associated with animal and human health. The general aim of this study was to evaluate the effect of different sources and levels of oil on the lipid and transcriptional profile of brain tissue. The experimental diets consisted of corn and soybean meal diets containing 1.5% soybean oil (SOY1.5), or 3% soybean oil (SOY3.0), or 3% canola oil (CO), or 3% fish oil (FO). The experimental period was 98 days, the diets were offered in the growth and finishing phases of immunocastrated males, and 18 animals per treatment/diet were used. After slaughter, brain samples were collected for determination of total lipid content and for FA profiling. Total mRNA extraction was performed for the sequencing of the pig brain transcriptome. For statistical analysis of differential expression, the DESeq2 package was used, in which DEG were identified (FDR < 0.05) among brain samples. After the DEG identification, functional enrichment analysis was performed by using MetaCore software, from which metabolic pathways and networks were identified (p-value < 0.05). For the analysis of different levels of soybean oil (SOY1.5 or SOY3.0), no changes in total lipid content and FA profile were observed. The metabolic pathways and networks were associated with lipid metabolism, immune response, and calcium transport. For the analysis of the different oil sources there were no changes in total lipid content and some FA showed statistical differences. The main pathways and networks identified were associated with cell signaling, lipid metabolism, synaptic transmission, and inflammatory processes. The results found herein could guide us for a better understanding of the effects and mechanisms of dietary FA on the transcriptome and the FA profile of the brain tissue. Furthermore, these results contribute to advances in the nutrigenomics field and to improvements in animal and human health. The results allowed the writing of two manuscripts, already submitted to international scientific journals, available in chapters 1 and 2 of this dissertation.

Keywords: Fatty acids. Gene expression. Brain function. Lipid homeostasis. Neuroprotection. RNA-seq. Cell signaling. Pigs. Metabolic pathways.

CONTENTS

INTRODUCTION

Pigs (*Sus scrofa*) have an impact on agriculture and the global economy. They are one of the most important livestock species for meat production and are considered the second most consumed source of animal protein in the world (WALTERS et al., 2012; OECD-FAO, 2021). In addition to their nutritional and economic importance, pigs are widely used as biomedical model animals to study metabolic and neurodegenerative diseases that affect humans (DAWSON et al., 2019; HOFFE; HOLAHAN, 2019). Pigs have anatomical, physiological, functional, neurophysiological and disease progression similarities to humans (LUNNEY, 2007; PAN et al., 2021). Larger model animals, such as pigs, are gaining in research, and with the large availability of data and in conjunction with "omics" tools, the use of these animals is helping to provide new insights into complex molecular mechanisms associated with human disease (WALTERS et al., 2012; PAN et al., 2021).

Another important factor is that the pig brain has a high similarity in anatomy, growth, and development to that of humans. Furthermore, because these animals are monogastric, the diet fed to them may alter the lipid and fatty acid (FA) profile of various tissues (LU et al., 2008; ALENCAR et al., 2021), among them the brain tissue (WEISINGER; VINGRYS; SINCLAIR, 1995; GOUSTARD-LANGELIER et al., 1999). This dietary influence may help to understand the progression of neurodegenerative diseases such as Parkinson's and Alzheimer's, which are associated with dysregulation of lipid metabolism (ALECU; BENNETT, 2019; ESTES et al., 2021; YIN, 2022).

The proper functioning of the nervous system is closely linked to lipids and their intermediates (RALHAN et al., 2021). Lipids are vital to all organisms and represent a diverse class of structural and functional biomolecules (YIN, 2022). Lipids are also fundamental components of neuronal membranes and are essential for brain development and maintenance (GNONI et al., 2021). The brain contains a high content of lipids, about 50% of the brain's dry weight (BARBER; RABEN, 2019; GNONI et al., 2021; RALHAN et al., 2021), the main class of FA present are the long-chain polyunsaturated fatty acids (PUFA), such as arachidonic acid (AA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (BRUCE; ZSOMBOK; ECKEL, 2017; BARBER; RABEN, 2019). These PUFA participate in various cellular functions that affect enzyme activities, eicosanoid synthesis, membrane fluidity, and gene expression (YOUDIM; MARTIN; JOSEPH, 2000; KITAJKA et al., 2004; AFMAN; MÜLLER, 2012).

Recent studies have shown that FA can cross the blood-brain barrier and be taken up by neurons (BRUCE; ZSOMBOK; ECKEL, 2017; BARBER; RABEN, 2019). Neurons and

glia, as well as the entire central nervous system, require tight regulation, being dependent on adequate lipid transport for lipid exchange and for the elimination of lipotoxic metabolites (BREKK et al., 2020). In addition, brain tissue lipid metabolism is reported to be a means of signaling nutritional status, acting in modulating the metabolism of important peripheral tissues. The detection of FA by the brain, may act in decreasing hepatic production of glucose, modifying lipogenesis and secretion, acting in the regulation of the whole systemic metabolism (BRUCE; ZSOMBOK; ECKEL, 2017).

Lipids are also key components of cell membranes (synapses and myelin sheaths) and act as signaling components that regulate several biological processes (YIN, 2022). Thus, alterations in lipid metabolism and homeostasis, such as the accumulation of toxic lipids and a reduced availability of omega-3 (n-3) PUFA, may induce neuroinflammatory processes and consequently neurodegenerative and metabolic diseases (LUKIW et al., 2005; ROMANO et al., 2017; CONTE et al., 2021). Lower levels of DHA have been reported in the brains of patients with Alzheimer's disease, since DHA availability is positively correlated with cognitive function (BELKOUCH et al., 2016; FONTEH et al., 2020). On the other hand, higher levels of AA, an FA associated with inflammatory processes, were found in Alzheimer's patients (YIN, 2022).

The decline in the integrity of brain structure and function is mainly caused by a decline in n-3 PUFA, an increase in saturated fatty acids (SFA), n-6 PUFA, and reactive oxygen species (ROS) (YOUDIM; MARTIN; JOSEPH, 2000). The balance of n-6:n-3, besides being very important for the good functioning of the brain, is fundamental for several biological processes and also for the maintenance of the lipid and metabolic homeostasis in organisms (YEHUDA et al., 2002; SZOSTAK et al., 2016). Thus, maintaining the n-6:n-3 ratio in brain tissue may be a means of reducing the adverse effects of various pathologies (YOUDIM; MARTIN; JOSEPH, 2000; SZOSTAK et al., 2016).

The understanding of important metabolic pathways and networks in the brain associated with lipid metabolism is still quite limited. Thus, understanding the effects and mechanisms of dietary FA on gene expression and the involvement of biological pathways associated with metabolic and neurodegenerative diseases in pigs is of great importance for animal and human health. Thus, the hypothesis of this work is that the addition of oils with different fatty acid profiles in the diet of pigs, in the phases of growth and termination, alters the fatty acid profile and transcriptome of brain tissue. Thus, the aims of our research were: (1) to evaluate alterations in the lipid profile, gene expression profile, and to identify metabolic pathways and gene networks altered in the brain of pigs fed different levels of

soybean oil (1.5% or 3.0%); and (2) to evaluate the effect of adding different sources of oil (soybean, canola or fish) on the lipid and transcriptomic profile of the pig brain, as well as identify metabolic pathways and gene networks impacted. The two following chapters are dedicated to discussing the results and implications linked to each one of our aims.

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CHAPTER 1: BRAIN FATTY ACID AND TRANSCRIPTOME PROFILES OF PIG FED DIETS WITH DIFFERENT LEVELS OF SOYBEAN OIL

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ABSTRACT

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. 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 using the DESeq2 statistical package. From 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 using 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 *CAST* and *CALB1* genes. The results found in this study contribute to understanding the pathways and networks associated with processes involved in intracellular calcium, lipid metabolism, and oxidative processes in the brain tissue. Moreover, these results may help a better comprehension of the modulating effects of soybean oil and its fatty acids (FA) composition on processes and diseases affecting the brain tissue.

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

1.INTRODUCTION

The pigs (*Sus scrofa*) have global economic impact as it is the second most consumed meat-based protein source worldwide (OECD-FAO, 2021; PAN et al., 2021). 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 (LIND et al., 2007; LUNNEY, 2007; DAWSON et al., 2019; HOFFE; HOLAHAN, 2019).

The brain contains high lipid content, making up approximately 50% of the brain's dry weight, only lower than the adipose tissue (HSU; HUANG; OUYANG, 2020). Lipids are essential for brain structure and function, and the central nervous system is fundamental for the regulation of metabolism and lipid balance (HAMILTON et al., 2007; BRUCE; ZSOMBOK; ECKEL, 2017). In addition, some regions of the brain are capable to detect nutrients and hormones that regulate energy balance and feeding (HAMILTON et al., 2007; BRUCE; ZSOMBOK; ECKEL, 2017).

A noteworthy factor is that the diet fed to the pigs can alter the lipid and fatty acids (FA) profiles of the tissues (LU et al., 2008; ALENCAR et al., 2021). Thus, soybean oil has been commonly used as part of the feed composition for growing and finishing pigs because it results in improved growth performance and beneficial effects to consumers (STEIN et al., 2008). 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 (FAN; ESKIN, 2015).

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 (SUN et al., 2018). 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 (RAPOPORT, 2013; BAZINET; LAYÉ, 2014).

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 (FASS, 2010).

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 doses of 2 ml 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; FANALLI et al., 2022a, 2022b), 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 containing 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. (2011), 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. (2021).

The pigs were slaughtered with a final body weight of 133.9 ± 9.4 kg on day 98 of the experiment. Samples of the brain frontal lobe were collected, immediately frozen in liquid nitrogen, and then stored at -80°C until analyses. Complete procedures were described in Silva et. al. (2021).

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 (1995). 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 (1959) and the methylation of the samples was performed according to Hartman e Lago (1973), with adaptations based on AOCS (2005) (method AM 5-04). The complete procedures were described by Silva et al. (2021) and Almeida et al. (2021).

Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), with pen being 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 of total RNA was obtained by using the NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific) and the concentration by using the Qubit® 2.0 Fluorometer. The RNA integrity was evaluated by using the Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). All samples presented an RNA Integrity Number (RIN) greater than or equal to 7.5 (APPENDIX A – Table S1).

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 by 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 using 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) (DOBIN; GINGERAS, 2015).

2.6 Identification of differentially expressed genes

The differentially expressed genes (DEG) between the SOY1.5 and SOY3.0 groups were identified by using the DESeq2 statistical package (R/Bioconductor)

[http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html], using a multi-factor design (LOVE; HUBER; ANDERS, 2014). 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 (BENJAMINI; HOCHBERG, 1995), 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 annotated DEG obtained from 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, using the standard parameter of MetaCore. The filters for the 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. To understand the behavior of genes and their interactions, networks were created using the Process Networks tool from MetaCore.

3.RESULTS

3.1 Total lipid content and FA profile

The total lipid content and FA profile from the 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	*p*-value
	SOY1.5	SOY3.0	SEM[2]	
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 data and differentially expressed genes

An average number of total reads per sample of 33.4 M and 32.9 M, was obtained for the SOY1.5 group, before and after quality control, respectively. 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 them reads were mapped against the reference genome *SScrofa11.1* (APPENDIX A – Table S2).

Differential analysis was performed comparing the level of gene expression between the groups, and a total of 22,931 genes were identified in the brain tissue. Of this 34 were DEG (log2 fold change ≥1; ≤−1; FDR-corrected *p*-value < 0.05). Within the 34 DEG, 25 were annotated, 11 being up-regulated (log2 fold change ranging from +0.25 to +2.93) and 14 being down-regulated (log2 fold change ranging from -3.43 to -0.36) in the SOY1.5 compared to the SOY3.0 (APPENDIX A – Table S3). The genes with the most altered expression were *CALB1* (log2 fold change -3.43; FDR = 0.03) and *VMO1* (log2 fold change +2.93; FDR < 0.01).

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 oil

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]SOY3.0: corn-soybean meal diet containing 3.0% soybean oil. [3]DEG: Differentially expressed genes.

The *ALOX15B* DEG, 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 enriched pathway maps identified: “Linoleic acid metabolism” (p-value = 1.970e-2, Figure 1), and “Prostaglandin 1 biosynthesis and metabolism” (*p*-value = 3.597e-2, Figure 2).

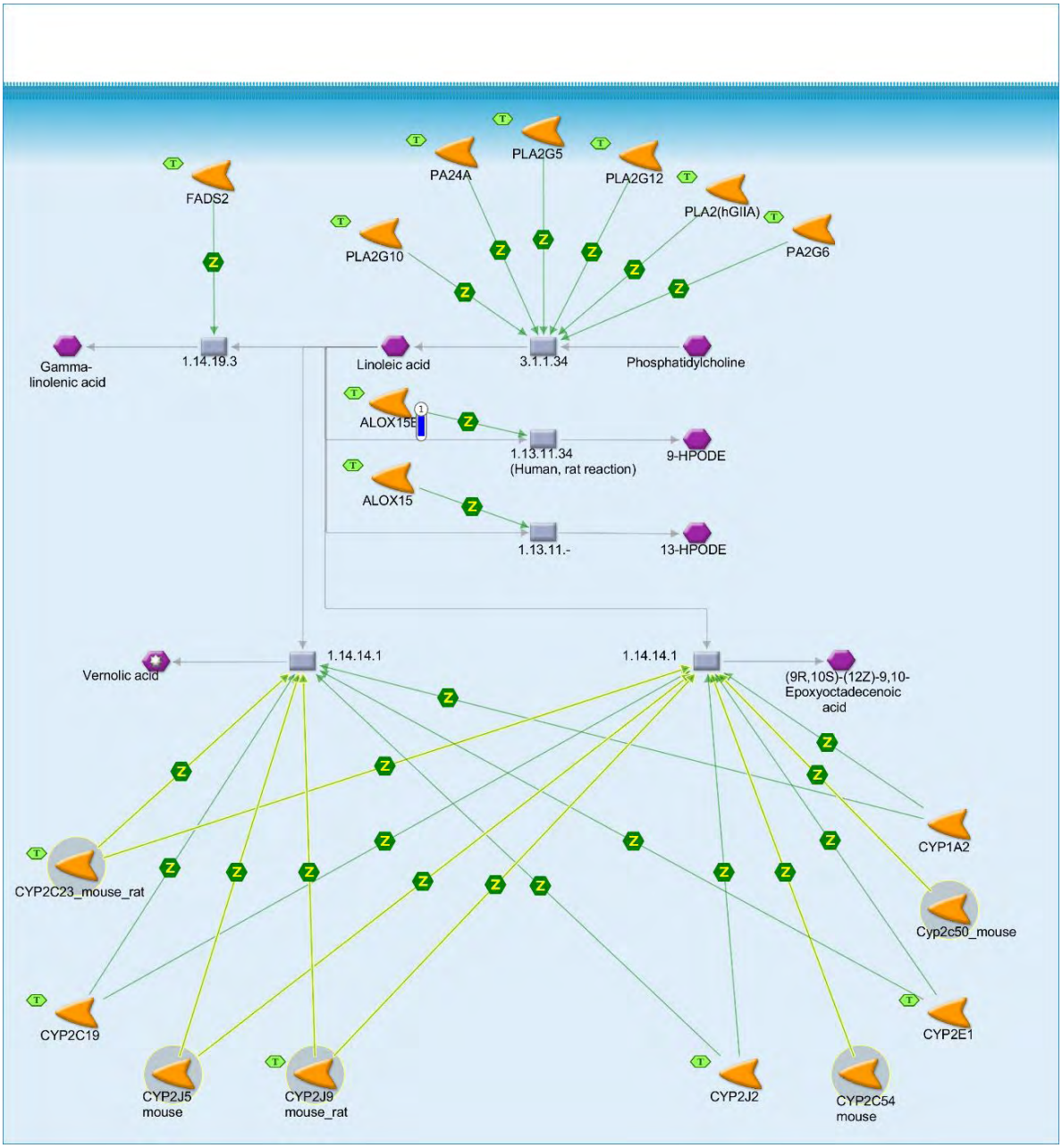

Figure 1. Linoleic acid metabolism from brain of pigs fed different levels of soybean oil (SOY1.5[1]*vs*SOY3.0[2]). [1]SOY1.5: corn-soybean meal diet containing 1.5% soybean oil. [2]SOY1.5: corn-soybean meal diet containing 3.0% soybean oil. The experimental data is represented by the thermometer-like 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.

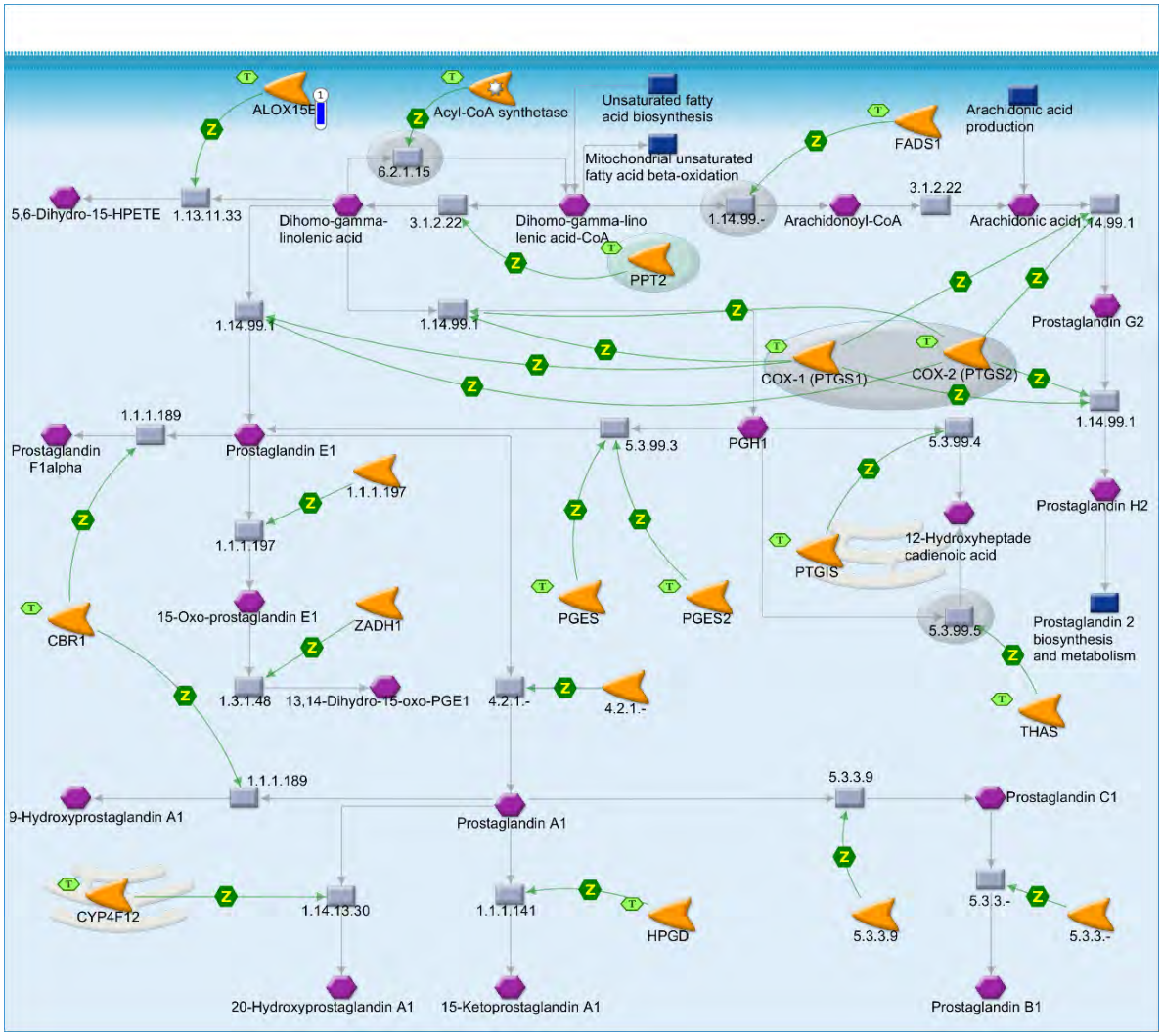

Figure 2. Prostaglandin 1 biosynthesis and metabolism from brain of pigs fed different levels of soybean oil (SOY1.5[1]*vs*SOY3.0[2]). [1]SOY1.5: corn-soybean meal diet containing 1.5% soybean oil. [2]SOY1.5: corn-soybean meal diet containing 3.0% soybean oil. The experimental data is represented by 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.

The *CALB1* DEG, 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, Figure 3).

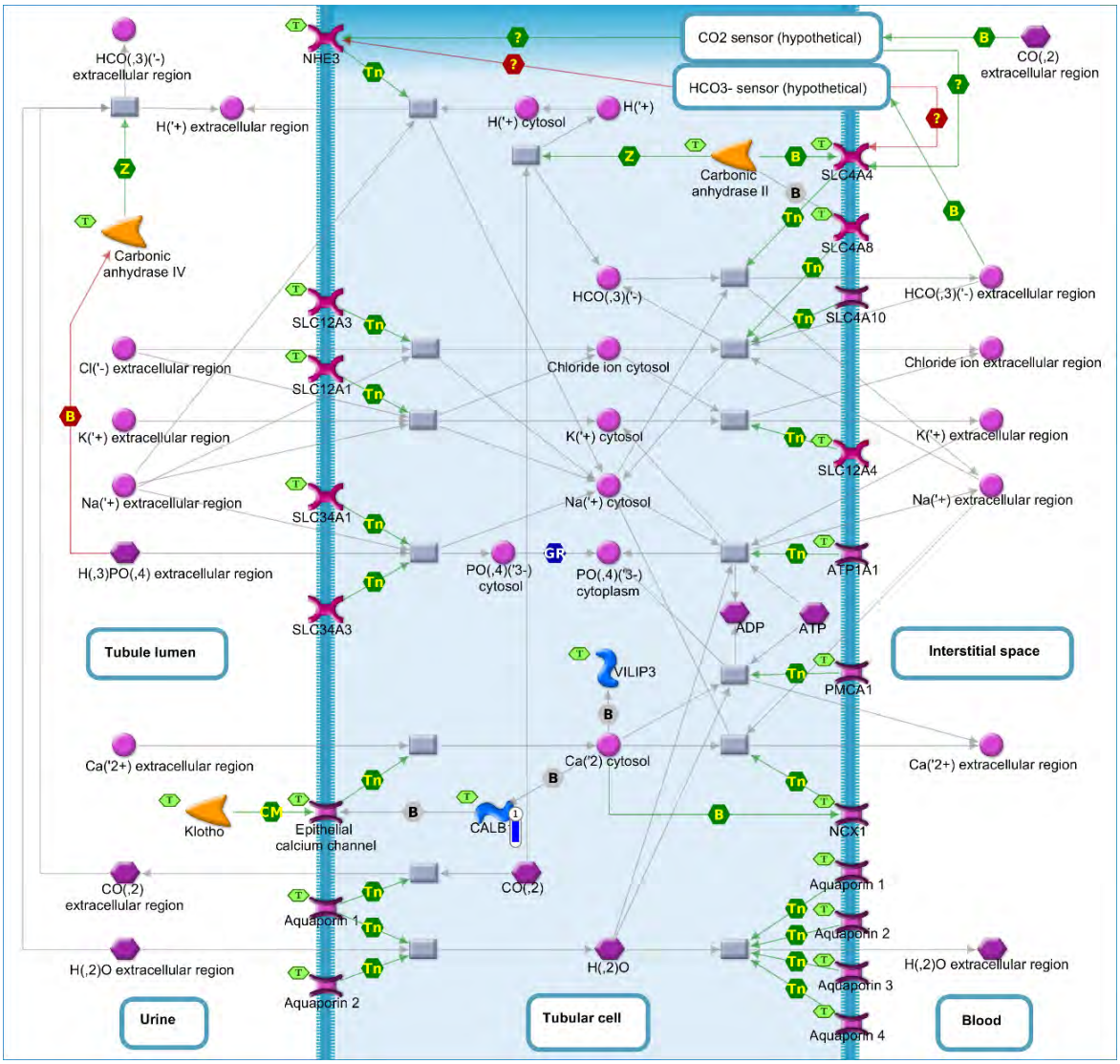

Figure 3. Renal secretion of inorganic electrolytes from brain of pigs fed different levels of soybean oil (SOY1.5[1]*vs*SOY3.0[2]). [1]SOY1.5: corn-soybean meal diet containing 1.5% soybean oil. [2]SOY1.5: corn-soybean meal diet containing 3.0% soybean oil. The experimental data is represented by the 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.

The *CAST* DEG, 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, Figure 4).

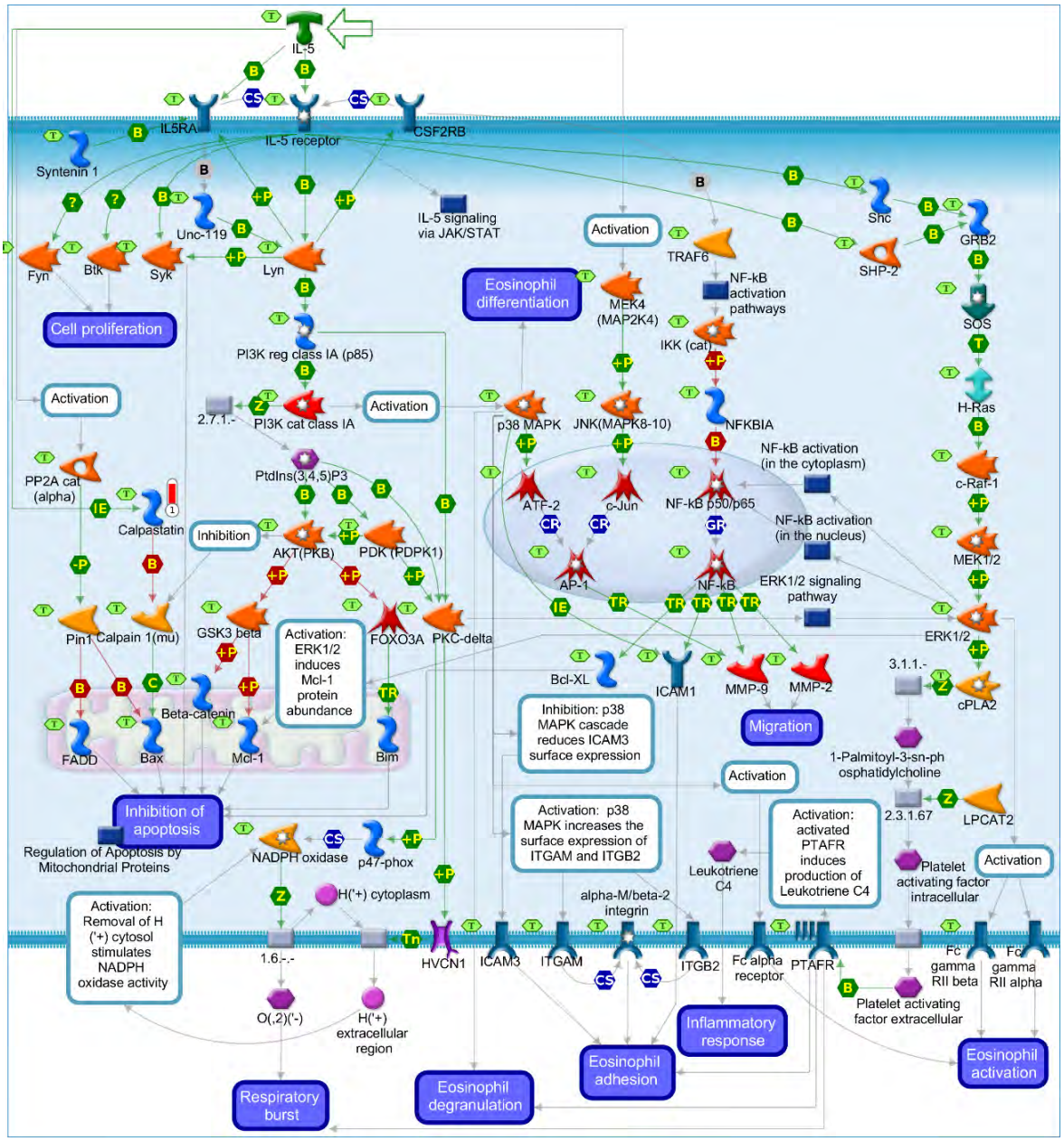

Figure 4. Immune response_IL-5 signaling from brain of pigs fed with fed different levels of soybean oil (SOY1.5[1]*vs*SOY3.0[2]). [1]SOY1.5: corn-soybean meal diet containing 1.5% soybean oil. [2]SOY1.5: corn-soybean meal diet containing 3.0% soybean oil. The experimental data is represented by the 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.

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).

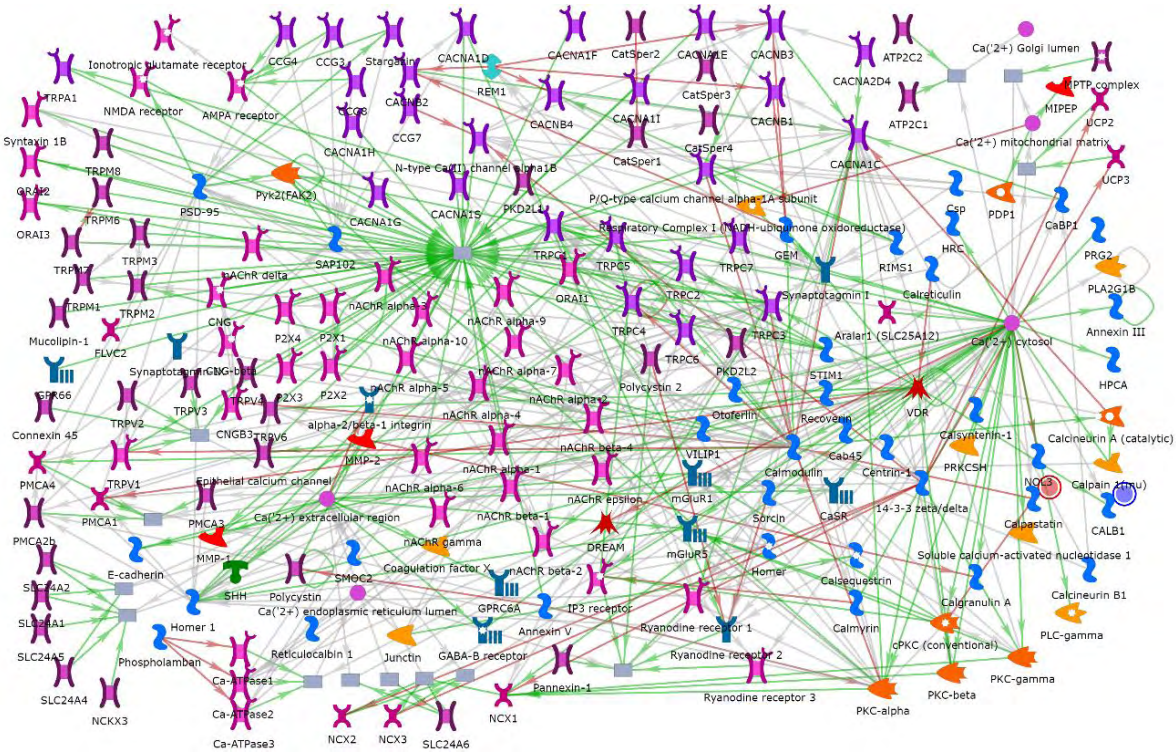

Figure 5. Calcium transport network from brain of pigs fed with fed different levels of soybean oil (SOY1.5[1]*vs*SOY3.0[2]). [1]SOY1.5: corn-soybean meal diet containing 1.5% soybean oil. [2]SOY1.5: corn-soybean meal diet containing 3.0% soybean oil. The experimental data are represented by the intensity of the blue and red 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.

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 period. The results found in the functional enrichment analysis, 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 illustrated in Figures 1 and 2, the *ALOX15B* participates in lipid oxidation and peroxidation reactions. According to Stelzer et al. (2016), among the pathways associated with this gene there were “eicosanoid synthesis” and “arachidonic acid metabolism” and the related Gene Ontology (GO) annotations include “calcium ion binding” and “lipid binding”.

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 defence mechanisms (GERTOW et al., 2011; KARATAS; CAKIR-AKTAS, 2019). Lipoxygenases have also been reported in cell differentiation (VAN LEYEN et al., 1998; ADEL et al., 2016), apoptosis (CLARIA, 2006), and play an important role in the immune response by helping to regulate cytokine secretion (DANIELSSON et al., 2008).

Among the LOX reported in mammals, the *ALOX15* isoform may oxygenate complex lipid-protein assemblies found in biomembranes and lipoproteins (IVANOV; KUHN; HEYDECK, 2015). The *ALOX15* also binds to membranes, with intracellular calcium as a main cofactor for this interaction (WATSON; DOHERTY, 1994; BRINCKMANN et al., 1998). It has been reported that *ALOX15* is expressed at higher levels in human airway epithelial cells, in eosinophils and immature red blood cells (NADEL et al., 1991). Furthermore, according to van Leyen et al. (2006) and Han et al. (2015), 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. (2018), 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*) (SNODGRASS; BRÜNE, 2019). The *15-HETE* is considered an important precursor of specialized pro-resolving lipid mediators (SPM) and is associated with pro- and anti-inflammatory effects (KUTZNER et al., 2017; SINGH; RAO, 2019). It has also been reported that *15-HETE* is a ligand and activator of the peroxisome proliferator-activated receptor gamma (*PPAR-γ*), which at high concentrations may generate reactive oxygen species (ROS) in cells (HUANG et al., 1999; SUN et al., 2015), and may induce the production of the pro-inflammatory cytokine Interleukin-12 (IL-12) (LI et al., 2013; SINGH; RAO, 2019).

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 the regulation of immune response (PERRY et al., 2020). Another important mediator related to the resolution of inflammation, reduction of leukocyte trafficking, and negative regulation of cytokine expression is neuroprotectin D1 (NPD1) (HONG et al., 2003; KOHLI; LEVY, 2009). 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 (SHALINI et al., 2018).

The *ALOX15* was found to have increased expression in the brains of Alzheimer's patients (PRATICÒ et al., 2004; YANG et al., 2010; IVANOV; KUHN; HEYDECK, 2015). Praticò et al. (2004), 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. In addition, regulates synaptic pathology associated with behavioral deficiencies (SUCCOL; PRATICÒ, 2007; JOSHI; GIANNOPOULOS; PRATICÒ, 2015).

In addition, *12/15-LOX* has been shown to play an important role in Parkinson's disease. In the study of Li et al. (1997) and Canals et al. (2003), 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. (2004), 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 (SINGH; RAO, 2019). 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 Figure 3, the *CALB1* gene binds to intracellular calcium transported via the epithelial calcium channel and transports it across the cytosol toward the basolateral membrane (LAMBERS et al., 2006). 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” (STELZER et al., 2016).

The *CALB1* is highly conserved in evolution and belongs to a family of high-affinity calcium-binding proteins (CHRISTAKOS; GABRIELIDES; RHOTEN, 1989; CHRISTAKOS; MADY; DHAWAN, 2018). The *CALB1* is found highly expressed in brain tissue, present in most neuronal cell groups, and is not vitamin D dependent (CHRISTAKOS; GABRIELIDES; RHOTEN, 1989; FERRANTE; KOWALL; RICHARDSON, 1994; CHRISTAKOS; MADY; DHAWAN, 2018).

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 (GENOVESE et al., 2020). Calcium is also essential for maintaining normal brain function (GENOVESE et al., 2020). 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 (SUPNET; BEZPROZVANNY, 2010; GENOVESE et al., 2020). 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 (LINDHOLM; WOOTZ; KORHONEN, 2006).

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 (VANCE, 2014). This dysregulation of the MAM-mitochondria linkage dysfunction may also be associated with neurodegenerative diseases such as Alzheimer's disease (VANCE, 2014). The MAMs are regions of the endoplasmic reticulum that mediate communication between the reticulum and the mitochondria (VANCE, 2014; YANG et al., 2020). 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 (VANCE, 2014; ARRUDA; HOTAMISLIGIL, 2015; LEE; MIN, 2018). According to Vance (2014), defects associated with these regions have been identified in neurodegenerative diseases and insulin resistance/type 2 diabetes.

The *CALB1* helps maintain calcium homeostasis, regulate intracellular calcium responses to physiological stimuli, and modulating synaptic transmission (CHRISTAKOS; MADY; DHAWAN, 2018). Another important role of *CALB1*, is its action in the prevention of neuronal death (CHRISTAKOS; MADY; DHAWAN, 2018; CASTROGIOVANNI et al., 2021). 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 (RABINOVITCH et al., 2001). There is evidence that *CALB1* acts to protect neurons against calcium-mediated neurotoxicity and may be considered a cytochemical marker for neuronal plasticity (FERRANTE; KOWALL; RICHARDSON, 1994).

Decreases in *CALB1* expression/concentration in brain tissue has been associated with neurodegeneration in Alzheimer's, Parkinson's, and Huntington's diseases (IACOPINO;

CHRISTAKOS, 1990; STELZER et al., 2016) and in ischemic injury studies (BURKE; BAIMBRIDGE, 1993; AHMADIAN et al., 2015). Lower *CALB1* expression has also been associated with a higher rate of neuronal death (KOOK et al., 2014). 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 (YUAN et al., 2013; CASTROGIOVANNI et al., 2021).

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 (GUO et al., 1998). 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 (GUO et al., 1998). Thus, with a down-expression of *CALB1* in the SOY1.5 group, we observed that with a lower percentage of soybean oil, the *CALB1* was less expressed, showing a negative relationship with this diet, and a positive association with neurodegenerative processes.

For the enriched pathway in Figure 4, IL-5 activates and elevates the expression of *CAST*. The *CAST* binds to and inhibits calpain 1 (mu) in the presence of calcium, which activates and cleaves the apoptosis regulatory protein Bax. Bax will act by preventing or reducing the frequency, rate, or extent of cell death by apoptotic process (SHEN et al., 2009; ILMARINEN; MOILANEN; KANKAANRANTA, 2014). 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 (STELZER et al., 2016). 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” (STELZER et al., 2016).

The *CAST* is a cell-permeable peptide that acts as an endogenous inhibitor of calpain in the central nervous system (YANZHANG et al., 2009; TENG et al., 2020). Calpains are cysteine proteases that are activated by calcium, that is, they are positively regulated by calcium and negatively regulated by *CAST* (GOLL et al., 2003; STIFANESE et al., 2010). These proteases, when in dysregulation of calcium homeostasis, have been implicated in neuronal cell dysfunction and death (STIFANESE et al., 2010), as well as neurodegenerative diseases (RAY, 2006; VOSLER; BRENNAN; CHEN, 2008; JOURDI et al., 2009).

Calpains have several important roles such as differentiation, cell attachment motility, signal transduction covering cell signaling pathways, regulation of gene expression and membrane fusion (GOLL et al., 2003; YANZHANG et al., 2009). 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 (STIFANESE et al., 2010).

According to Goll et al. (2003), *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 (RAMI et al., 2003). In the study of Rao et al. (2014), higher expression of *CAST* in JNPL3 (mutant tau P301L) mouse models, was used to attenuates calpain expression, 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. (2016), 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* (RAO et al., 2016).

The calpain-calpastatin system is also reported in excitotoxicity, a pathological or neurodegenerative process that is initiated by overactivation of neurotransmitters such as glutamate. Excitotoxicity leads to increased cellular calcium levels, which causes activation of various proteases, including calpains (NEUMAR et al., 2001). Furthermore, missing *CAST* may impair early stages of neurogenesis (MACHADO et al., 2015). Thus, we observed a higher expression of *CAST* in the SOY1.5 group, 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 influences on gene expression in brain tissue. Moreover, it is noteworthy the importance of the detected DEG and their association with intracellular calcium.

Thereby, the processes network "Calcium transport" identified and the genes enriched in this network, corroborate the results found in the pathway maps, and thus, shows us that altering the level of soybean oil in the diet of pigs impacts on gene expression. 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. 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.

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CHAPTER 2: DIFFERENT OIL SOURCES IMPACTING ON BRAIN LIPID AND TRANSCRIPTOME PROFILE OF MALE PIGS

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ABSTRACT

Lipids are a class of molecules fundamental to the structure and function of the brain. The fatty acid (FA) composition of the brain is generally rich in polyunsaturated fatty acids (PUFA). In general, PUFA can be obtained from vegetable oils, such as soybean and canola, as well as from seafood oils. PUFA have protective effects and can modulate gene transcription. For nutrigenomics studies, pigs have been widely used as biomedical model, due to the similarities in anatomy and neurophysiological processes with humans. Thus, we aimed to determine if different sources of dietary oil fed to male pigs would modify the lipid and transcriptomic profile of the brain. Fifty-four male pigs were used in the experimental period of 98 days. Treatments consisted of corn-soybean meal diets containing either 3% soybean oil (SOY), or 3% canola oil (CO), or 3% fish oil (FO). At the end of the experiment, all pigs were slaughtered, and brain samples were collected for total lipid content and FA profile analyses determination. Additionally, total mRNA was extracted for brain transcriptome sequencing. Significant differences were identified in the amount of palmitic acid, oleic acid, eicosenoic acid and total PUFA. For the differential expression analysis, performed by using the DESeq2 statistical package, differentially expressed genes (DEG, log2 fold change ≥1; ≤−1; FDR-corrected p-value < 0.05) were found among comparisons, being five of the SOY*vs*CO, 44 of the SOY*vs*FO, and 39 of the CO*vs*FO. In the functional enrichment performed in MetaCore software, we identified pathway maps (*p*-value < 0.05) related to apoptosis and cell proliferation, interleukin signaling in obesity and type 2 diabetes, neurophysiological process, and inflammation. The networks identified, were associated with signal transduction, calcium transport and oxidative stress. The results found in our study demonstrate the importance of dietary FA availability for brain tissue and help direct future investigations.

Keywords: brain function, blood-brain barrier; cell signaling; cognitive function; dopaminergic neuron; lipid droplets; lipid homeostasis; neuroprotection; pigs.

1.INTRODUCTION

Lipids are a class of molecules important for brain structure and function and are responsible for a wide range of physiological functions (BRUCE; ZSOMBOK; ECKEL, 2017; CHANTED et al., 2021). The fatty acids (FA), besides being a source of energy, have important biological, structural, and functional purposes in the human organism (TAGHIBIGLOU; KHALAJ, 2017). An increasing number of studies highlight the contribution of the central nervous system in lipid balance and regulation (BRUCE; ZSOMBOK; ECKEL, 2017; TAGHIBIGLOU; KHALAJ, 2017). The brain is the organ with the highest amounts of lipids in terms of content and diversity, and FA are involved in many important functions, such as cognitive function (TAGHIBIGLOU; KHALAJ, 2017).

The composition of FA in the brain is distinct and is generally rich in long-chain polyunsaturated fatty acids (LC-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; ZSOMBOK; ECKEL, 2017; CHANTED et al., 2021). The PUFA are also essential for cell proliferation, maintenance of the integrity and differentiation of neuronal cell membrane (BORSONELO; GALDURÓZ, 2008; NAGY; TIUCA, 2017). However, the human body does not have the capacity to synthesize essential FA, which are fundamental for the maintenance of health, so it is necessary to consume them through diet (ELLULU et al., 2015). Thus, the shortage of these FA in the brain tissue, can cause alterations in neurotransmission systems, leading to various neurological disorders (BORSONELO; GALDURÓZ, 2008; NAGY; TIUCA, 2017).

The PUFA may be obtained in higher concentrations from vegetable oils, such as soybean and canola, as well as from seafood oils (ELLULU et al., 2015). Canola oil has high concentrations of oleic acid (OA; C18:1 n-9), linoleic acid (LA; C18:2 n-6) and alpha-linolenic acid (ALA; C18:3 n-3), which if adequately consumed may have a cardioprotective effect by reducing plasma cholesterol levels (LIN et al., 2013). Fish oil, on the other hand, has high levels of n-3 PUFA (omega-3 series), including EPA and DHA, which confer beneficial effects on brain function, neurological disorders, protection against oxidative stress and inflammatory processes (FAROOQUI et al., 2007).

The brain is capable to obtain PUFA through diet or by the conversion of essential FA (YEHUDA et al., 2002). Thus, PUFA may have protective effects and modulate gene transcription, facts that have been widely studied in the nutrigenomics area (AFMAN; MÜLLER, 2012). With the technological advances, "omics" has played a relevant role for a better understanding of the molecular mechanisms of diseases (AFMAN; MÜLLER, 2012;

TAGHIBIGLOU; KHALAJ, 2017). Moreover, in omics studies pigs have been widely used as a biomedical model (TAGHIBIGLOU; KHALAJ, 2017; HOFFE; HOLAHAN, 2019).

Pigs (*Sus scrofa*) have similarities to human anatomy, pathophysiology, and physiology (DOUGLAS, 1972; WALTERS et al., 2012; PAN et al., 2021), and have neurophysiological processes similar to humans (TAGHIBIGLOU; KHALAJ, 2017; HOFFE; HOLAHAN, 2019). Pigs are also of great economic and agricultural importance, and consequently there is a wealth of information related to these animals (WALTERS et al., 2012). In this way, the use of these animals resulting in scientific advances may help in the understanding and new insights into relevant biological processes and associations to pathologies that affect humans (LUNNEY, 2007; MEURENS et al., 2012). Thus, our hypothesis is that the use of different oil sources (SOY, CO, and FO) in the diet of Large White pigs may alter the lipid and transcriptomic profile of brain tissue and modulate important processes related to lipid metabolism and the progression of neurodegenerative diseases. The objective of this work was to determine if different sources of dietary oil fed to male pigs would modify the lipid and transcriptome profile of the brain tissue.

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 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 (FASS, 2010).

2.2 Animals, experimental design, and diets

Fifty-four 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) (ALMEIDA et al., 2021; FANALLI et al., 2022a, 2022b), 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 3% soybean oil (SOY), or 3% canola oil (CO), or 3% fish oil (FO). 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. The diets were formulated to have a similar level of digestible energy. All detailed procedures were described in Almeida et. al. (2021).

The pigs were slaughtered with a final body weight of 133.9 ± 9.4 kg on day 98 of the experiment. 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. (2021).

2.3 Total lipid content and FA profile analyses

For the analysis of total lipid content, 5 g of 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 (1995). 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 (1959) and the methylation of the samples was performed according to Hartman e Lago (1973), with adaptations based on AOCS (2005) (method AM 5-04). The complete procedures were described by Silva et al. (2021) and Almeida et al. (2021).

Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), with pen considered as the experimental unit. The model included the random effects of pen and block and the fixed effects of oil sources.

Outliers were removed from the data sets and residuals were tested for a normal distribution using the Shapiro-Wilk test (UNIVARIATE procedure). Treatment means were separated by using the LSMEANS statement and comparisons were made using the PDIFF option based on Student's t-test. Differences were declared significant when p-value ≤ 0.05.

2.4 RNA extraction, library preparation and sequencing

For the total RNA extraction from the brain samples, we used a commercial kit for RNA extraction (RNeasy® Mini Kit, Qiagen) together with the Trizol reagent (Invitrogen). Quality of total RNA was obtained by using the NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific) and the concentration by using the Qubit® 2.0 Fluorometer. The RNA integrity was evaluated by using the Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). All samples presented an RNA Integrity Number (RIN) greater than or equal to 7.4 (APPENDIX B – Table SF1).

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 (SILVA et al., 2022).

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 using 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]. The STAR software (v.2.7.6a) (DOBIN; GINGERAS, 2015) was used for alignment,

mapping, and abundance (read counts) of mRNA for all annotated genes (SILVA et al., 2022).

2.6 Identification of differentially expressed genes

The differentially expressed genes (DEG) of SOY*vs*CO, SOY*vs*FO and CO*vs*FO comparisons were identified by using the DESeq2 statistical package (R/Bioconductor) [http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html], using a multi-factor design (LOVE; HUBER; ANDERS, 2014). 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, 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 (BENJAMINI; HOCHBERG, 1995), and the threshold value used for significance was FDR < 0.05 (SILVA et al., 2022).

2.7 Functional enrichment analysis

The enrichment analysis was performed using the software MetaCore (Clarivate Analytics, v.22.1, build 70800) [https://clarivate.com/products/metacore/, Clarivate Analytics, London, UK]. The pathway maps were identified from the lists of annotated 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, a standard parameter and the analysis was performed with the "Analyze Single Experiment" option from each list. The filters for the 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. To understand the behavior of genes and their interactions, networks were created using MetaCore (SILVA et al., 2022).

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 FA profiles (SOY, CO and FO) are demonstrated in Table 1. Significant differences were

identified in the amount of palmitic acid (C16:0, *p*-value = 0.044), oleic acid (OA, C18:1, *p*-value = 0.037), and eicosenoic acid (C20: 1, *p*-value = 0.011), in addition to total PUFA (*p*-value < 0.01) (marked in bold in Table 1).

Table 1. Total lipids and FA profile from brain tissue of pigs fed with different oil sources

Fatty acid, %	Dietary treatment[2]			Pooled SEM[2]	*p*-value
	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)	**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)	2.309	1.919	2.435	0.143	0.214
Alpha-linolenic acid (C18:3 n-3)	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; SOUTHGATE, 1991). [a–c]Within a row, values without a common superscript differ (*p*-value ≤ 0.05) using Student's t-test.

3.2 Sequencing and Differentially Expressed Genes Data

The average number of total reads per sample for the SOY group was 34.3 M and 33.9 M, before and after quality control, respectively; for the CO group was 33.5 M and 33.1 M, respectively; and, for the FO group was 34.4 M and 34.0 M, respectively. Of the total reads

obtained for the three groups, after quality control, 94.87% of the reads were mapped against the reference genome *SScrofa11.1* (APPENDIX B – Table SF2)

Differential analysis of gene expression was performed comparing the level of gene expression between the groups of animals that were fed diets enriched with different types of oils (SOY*vs*CO; SOY*vs*FO; and CO*vs*FO). For the SOY*vs*CO comparison, a total of 22,938 genes were expressed, of this five were DEG (log2 fold change ≥1; ≤−1; FDR-corrected *p*-value < 0.05). Within the five DEG, four were annotated, 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 *ALDH3A1* (log2 fold change -1.98; FDR < 0.01) and *PRCP* (log2 fold change +0.52; FDR < 0.01).

For the SOY*vs*FO comparison, a total of 22,974 genes were expressed, of this 44 were DEG (log2 fold change ≥1; ≤−1; FDR-corrected *p*-value < 0.05). Within the 44 DEG, 28 were annotated, 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 *CYP3A29* (log2 fold change -2.49; FDR = 0.03) and *VMO1* (log2 fold change +2.12; FDR < 0.01).

For the CO*vs*FO comparison, a total of 22,9747 genes were expressed, of this 39 were DEG (log2 fold change ≥1; ≤−1; FDR-corrected *p*-value < 0.05). Within the 39 DEG, 21 were annotated, 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 = 0.02) and *SLC10A4* (log2 fold change +3.06; FDR = 0.02). The complete DEG data can be seen in APPENDIX B – Table SF3.

To analyze the common annotated DEG to all comparisons (SOY*vs*CO, SOY*vs*FO, and CO*vs*FO), the "Compare experiments" tool of the MetaCore software was used. For this analysis, the DEG lists (FDR < 0.05) of the three comparisons were used together. The DEG *ALDH3A1* and the *PRCP* were identified as common DEG to all comparisons (Table 2). The distribution of annotated DEG among diet comparisons was done using a Venn diagram (HEBERLE et al., 2015), and the result is illustrated in Figure 1. The complete data can be seen in APPENDIX B – Table SF4.

Table 2. Common annotated DEG[1] among dietary comparisons SOY*vs*CO, SOY*vs*FO, and CO*vs*FO, from brain tissue of pigs fed with 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.

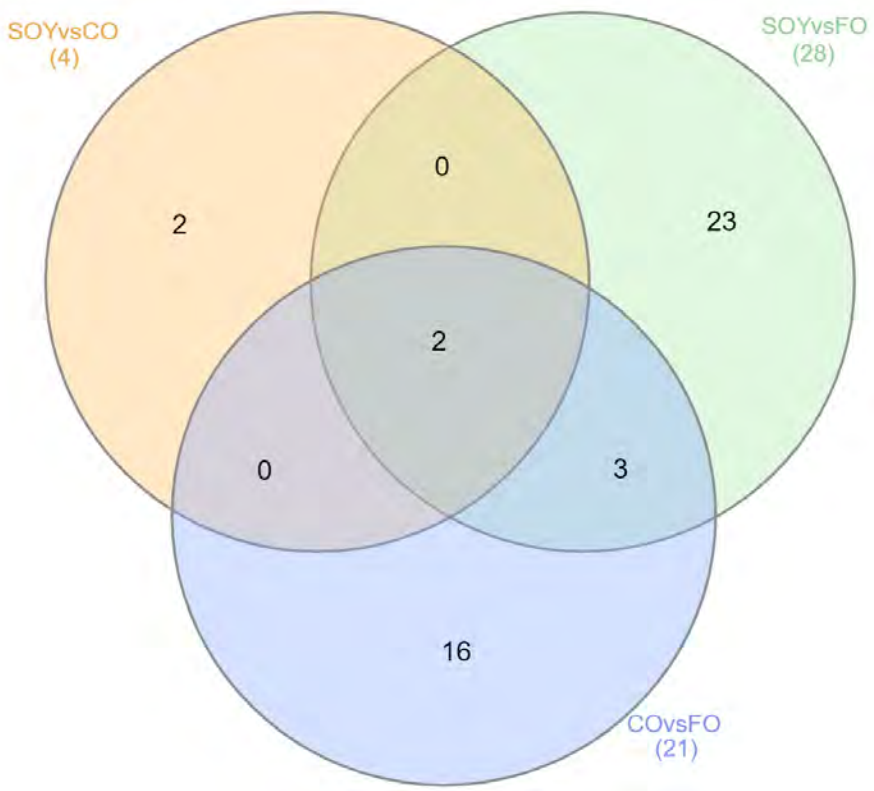

Figure 1. Venn diagram of the distribution of annotated DEG between comparisons of diet groups SOY[1], CO[2] and FO[3], from brain tissue of pigs fed with different oil sources. [1]SOY: corn-soybean meal diet containing 3% soybean oil. [2]CO: corn-soybean meal diet containing 3% canola oil. [3]FO: corn-soybean 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 comparison of the expression level between the SOY*vs*CO, the amount of DEG (FDR < 0.05) found in the differential analysis step was low, so no significant pathway maps (*p*-value < 0.05) were found by using the MetaCore software.

3.3.2 Comparison SOY*vs*FO

For this comparison the cholinergic receptor nicotinic alpha 6 subunit (*CHRNA6* or *nAChR alpha-6*) gene was identified as a DEG, showing up-regulation in the SOY group compared to FO (log2 fold change +1.950). The *CHRNA6* gene, participates in the enriched pathway map "Role of prenatal nicotine exposure in apoptosis and proliferation of pancreatic beta cells" (*p*-value = 3.315e-02), represented in Figure 2.

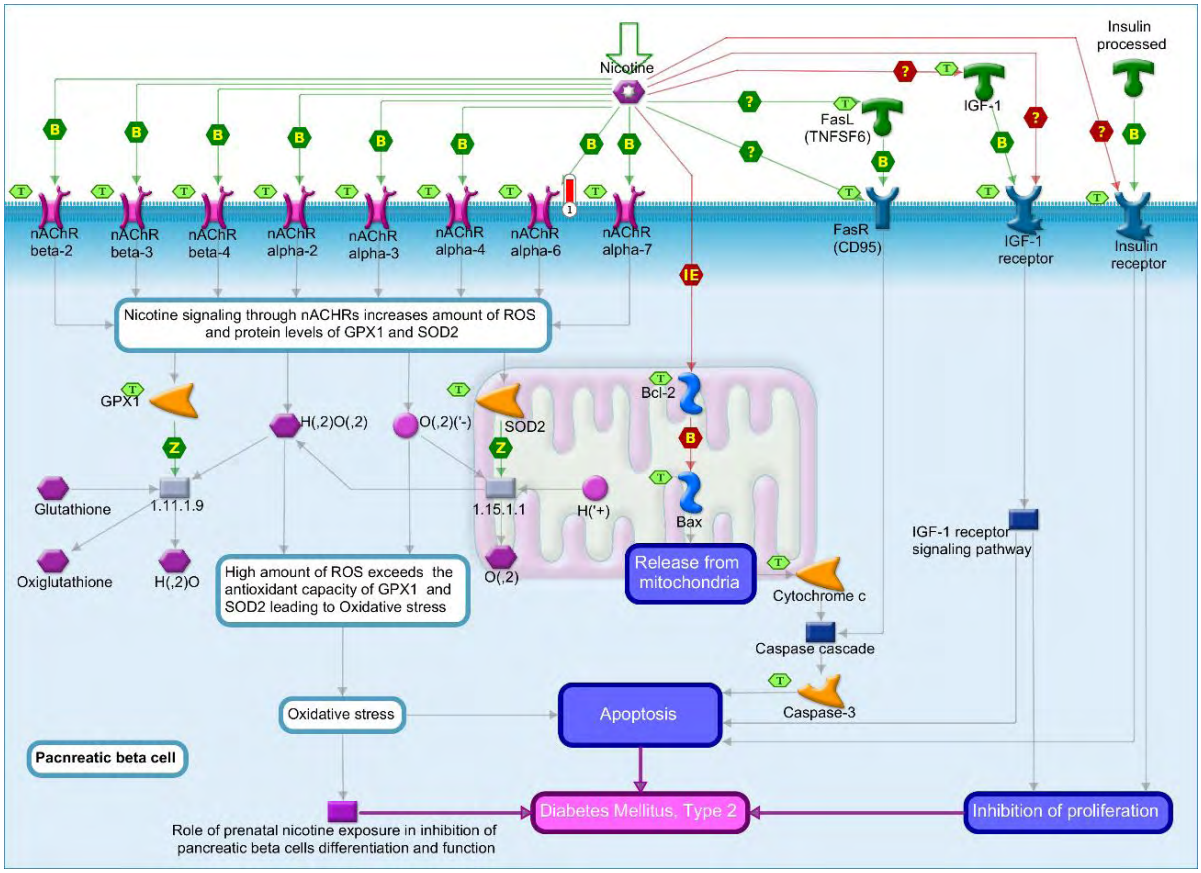

Figure 2. Role of prenatal nicotine exposure in apoptosis and proliferation of pancreatic beta cells pathway map, from brain of pigs fed with different oil sources (SOY[1]*vs*FO[2]). [1]SOY: corn-soybean meal diet containing 3% soybean oil. [2]FO: corn-soybean meal diet containing 3% fish oil. The experimental data is represented by the thermometer-like figure on the map. The upward thermometer (red) indicates up-regulation of the *CHRNA6* DEG (log2 fold change +1.950) in the SOY group compared to FO. 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.

To better understand the behavior of the genes and their interactions, process networks were additionally generated using MetaCore software. The results obtained are presented in Table 3. The process networks highlighted in this study are “Calcium transport” (*p*-value = 6.933e-3, Figure 3), “Signal transduction_Neuropeptide” (*p*-value = 4.114e-2, Figure 4) and “Response to hypoxia and oxidative stress” (*p*-value = 4.408e-2, Figure 5).

Table 3. Process networks in the SOY[1]*vs*FO[2] comparison from brain tissue

Process Network	*p*-value	DEG[3]
Chemotaxis	2.797e-3	*ACKR2/ GPCRs*
Muscle contraction	5.021e-3	*GPCRs/ GPCRs/ CHRNA*
Cell adhesion_Leucocyte chemotaxis	5.794e-3	*ACKR2/ GPCRs*
Calcium transport	6.933e-3	*CAST/ CATSPER3/ CHRNA6*
Signal Transduction_Cholecystokinin signaling	2.036e-2	*GPCRs*
Development_Neuromuscular junction	3.735e-2	*CHRNA/ CHRNA6*
Signal transduction_Neuropeptide	4.114e-2	*GPCRs*
Response to hypoxia and oxidative stress	4.408e-2	*GSTM2/ GSTM3*

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

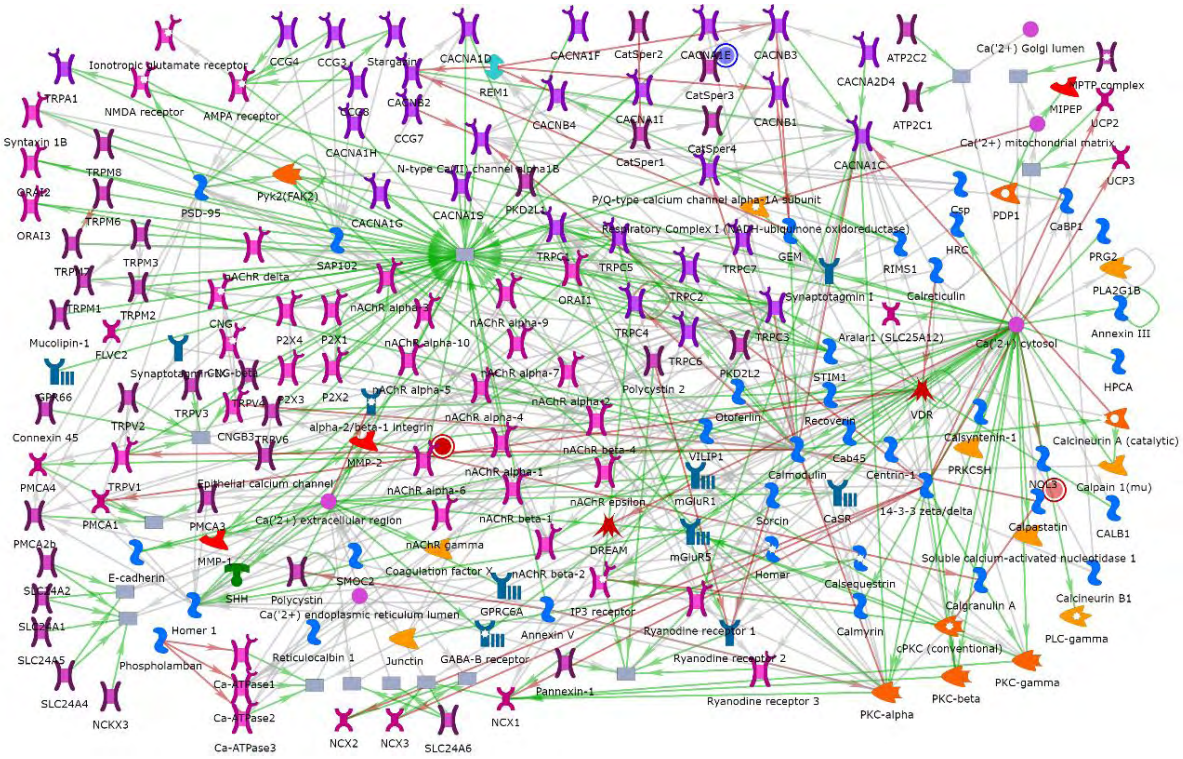

Figure 3. Calcium transport network from brain tissue of pigs fed with different oil sources (SOY[1]*vs*FO[2]). [1]SOY: corn-soybean meal diet containing 3% soybean oil. [2]FO: corn-soybean meal diet containing 3% fish oil. The experimental data are represented by the intensity of the blue and red circles on the network. The red circles indicate up-regulation of the *CAST* and *CHRNA6* DEG, and the blue circle indicates down-regulation of the *CATSPER3* DEG in the SOY group compared to FO. 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.

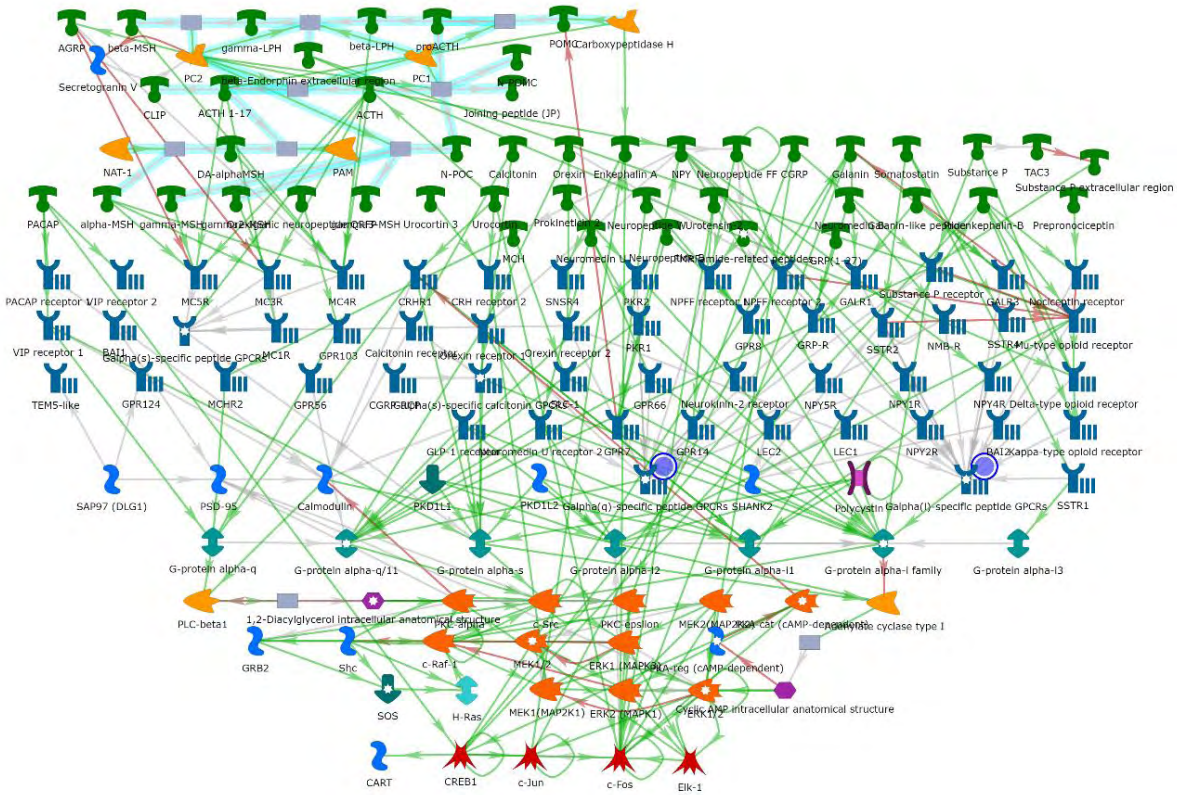

Figure 4. Signal transduction_Neuropeptide network from brain tissue of pigs fed with different oil sources (SOY[1]*vs*FO[2]). [1]SOY: corn-soybean meal diet containing 3% soybean oil. [2]FO: corn-soybean meal diet containing 3% fish oil. The experimental data are represented by the intensity of the blue and red circles on the network. The blue circles indicate down-regulation of the *Galpha(i)-specific peptide GPCRs* and *Galpha(q)-specific peptide GPCRs* DEG in the SOY group compared to FO. 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.

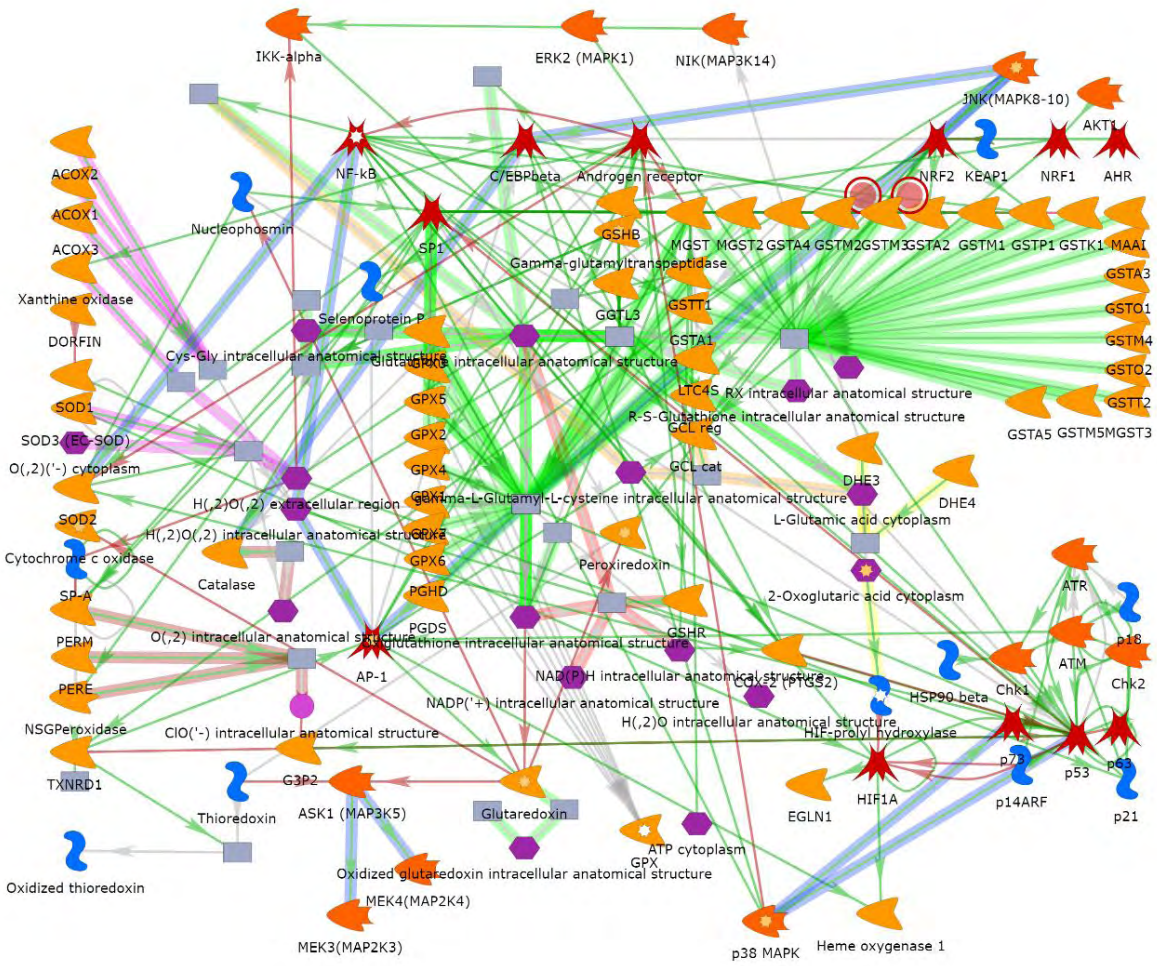

Figure 5. Response to hypoxia and oxidative stress network from brain tissue of pigs fed with different oil sources (SOY[1]*vs*FO[2]). [1]SOY: corn-soybean meal diet containing 3% soybean oil. [2]FO: corn-soybean meal diet containing 3% fish oil. The experimental data are represented by the intensity of the blue and red circles on the network. The red circles indicate up-regulation of the *GSTM2* and *GSTM3* DEG in the SOY group compared to FO. 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.

3.1.1 Comparison CO*vs*FO

For this comparison, four pathway maps were identified (p-value < 0.05), related to the following genes: *CHRNA6*, perilipin (*PLIN1*) and netrin-1 (*NTN1*) which are demonstrated in Table 4.

Table 4. Pathway maps CO[1]vsFO[2] from brain tissue of pigs fed with different oil sources

Pathway map	*p*-value	DEG[3]	log2 fold change
Role of prenatal nicotine exposure in apoptosis and proliferation of pancreatic beta cells	3.082e-2	*CHRNA6*	+2.292
Role of IL-6 in obesity and type 2 diabetes in adipocytes	3.284e-2	*PLIN1*	+1.558
Neurophysiological process_Netrin-1 in regulation of axon guidance	4.089e-2	*NTN1*	+1.058
TNF-alpha and IL-1 beta induce dyslipidemia and inflammation in obesity and type 2 diabetes in adipocytes	4.289e-2	*PLIN1*	+1.558

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

The DEG *CHRNA6*, found earlier in the SOY*vs*FO comparison, was also identified as a DEG in this comparison, showing an up-regulation in the CO group compared to FO (log2 fold change +1.950). The *CHRNA6*, participates in the enriched pathway map "Role of prenatal nicotine exposure in apoptosis and proliferation of pancreatic beta cells" (*p*-value = 3.315e-02), represented in Figure 6.

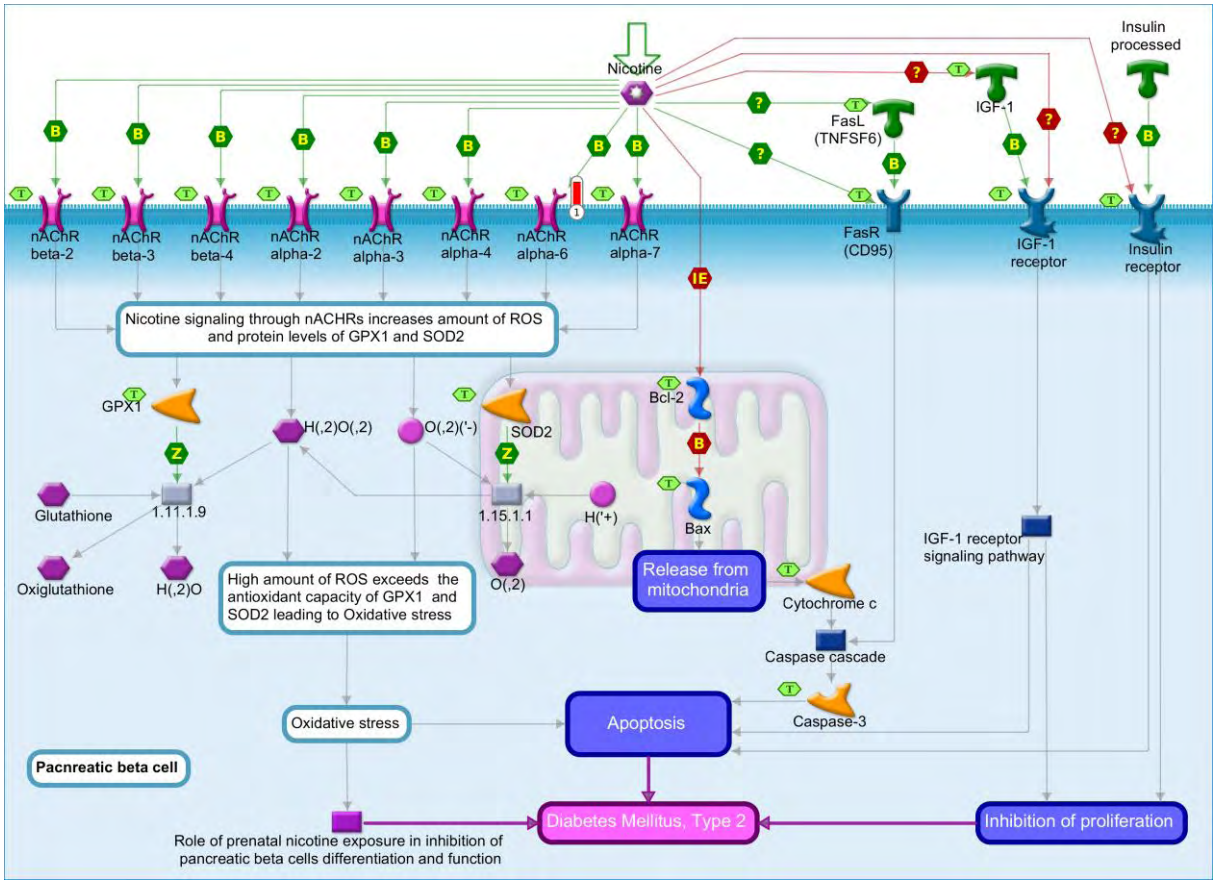

Figure 6. Role of prenatal nicotine exposure in apoptosis and proliferation of pancreatic beta cells pathway map from brain tissue of pigs fed with different oil sources (CO^{1}*vs*FO^{2}). ^{1}CO: corn-soybean meal diet containing 3% canola oil. ^{2}FO: corn-soybean meal diet containing 3% fish oil. The experimental data is represented by the thermometer-like figure on the map. The upward thermometer (red) indicates up-regulation of the *CHRNA6* DEG (log2 fold change +2.292) in the CO group compared to FO. 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.

For this comparison the perilipin (*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*, participate in two of the four significant enriched pathway maps identified: “Role of IL-6 in obesity and type 2 diabetes in adipocytes” (*p*-value = 3.284e-2, Figure 7), and “TNF-alpha and IL-1 beta induce dyslipidemia and inflammation in obesity and type 2 diabetes in adipocytes” (*p*-value = 4.289e-2, Figure 8).

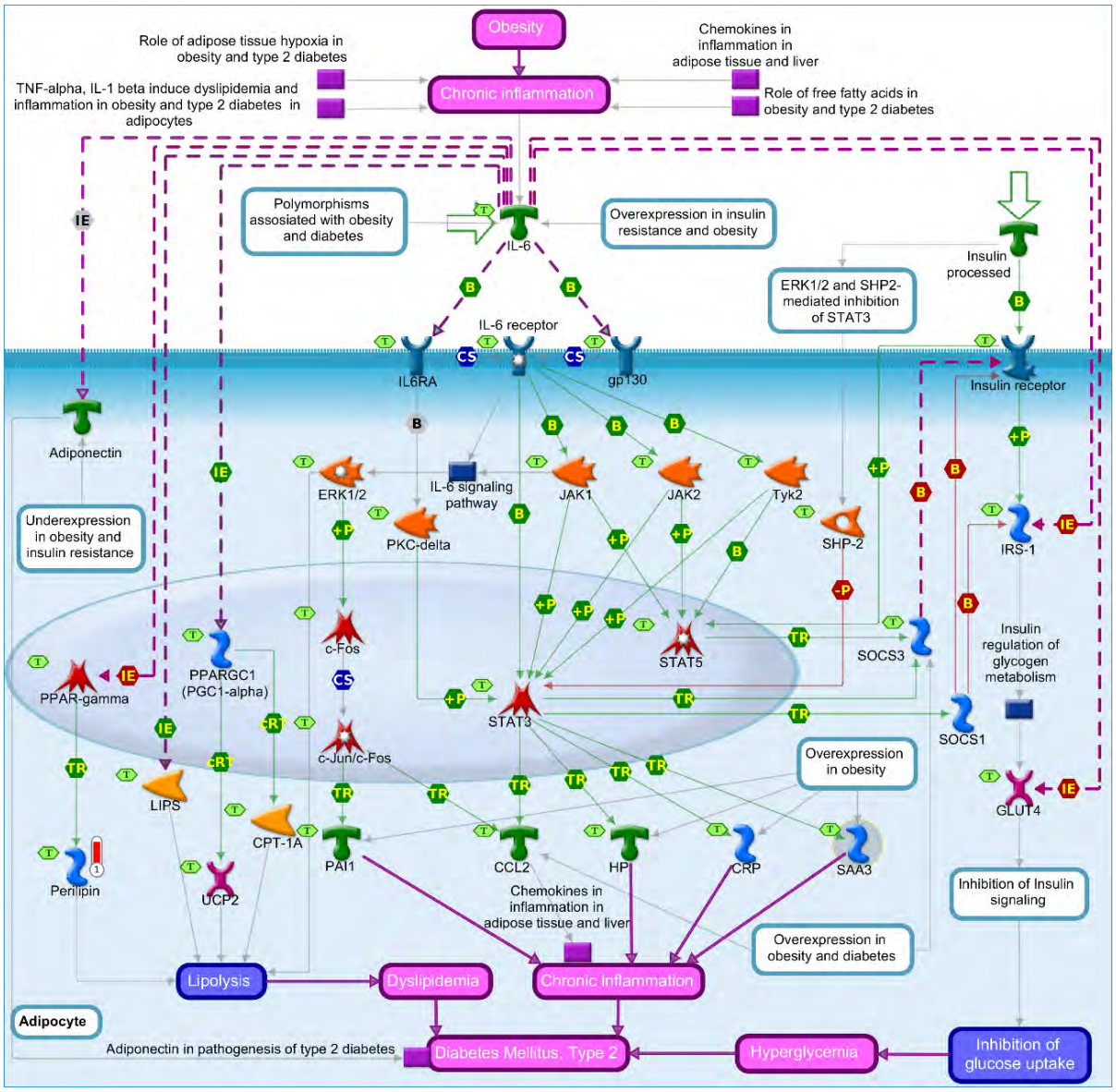

Figure 7. Role of IL-6 in obesity and type 2 diabetes in adipocytes pathway map from brain tissue of pigs fed with different oil sources (CO[1]*vs*FO[2]). [1]CO: corn-soybean meal diet containing 3% canola oil. [2]FO: corn-soybean meal diet containing 3% fish oil. The experimental data is represented by the thermometer-like figure on the map. The upward thermometer (red) indicates down-regulation of the *PLIN1* DEG (log2 fold change +1.558) in the CO group compared to FO. 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.

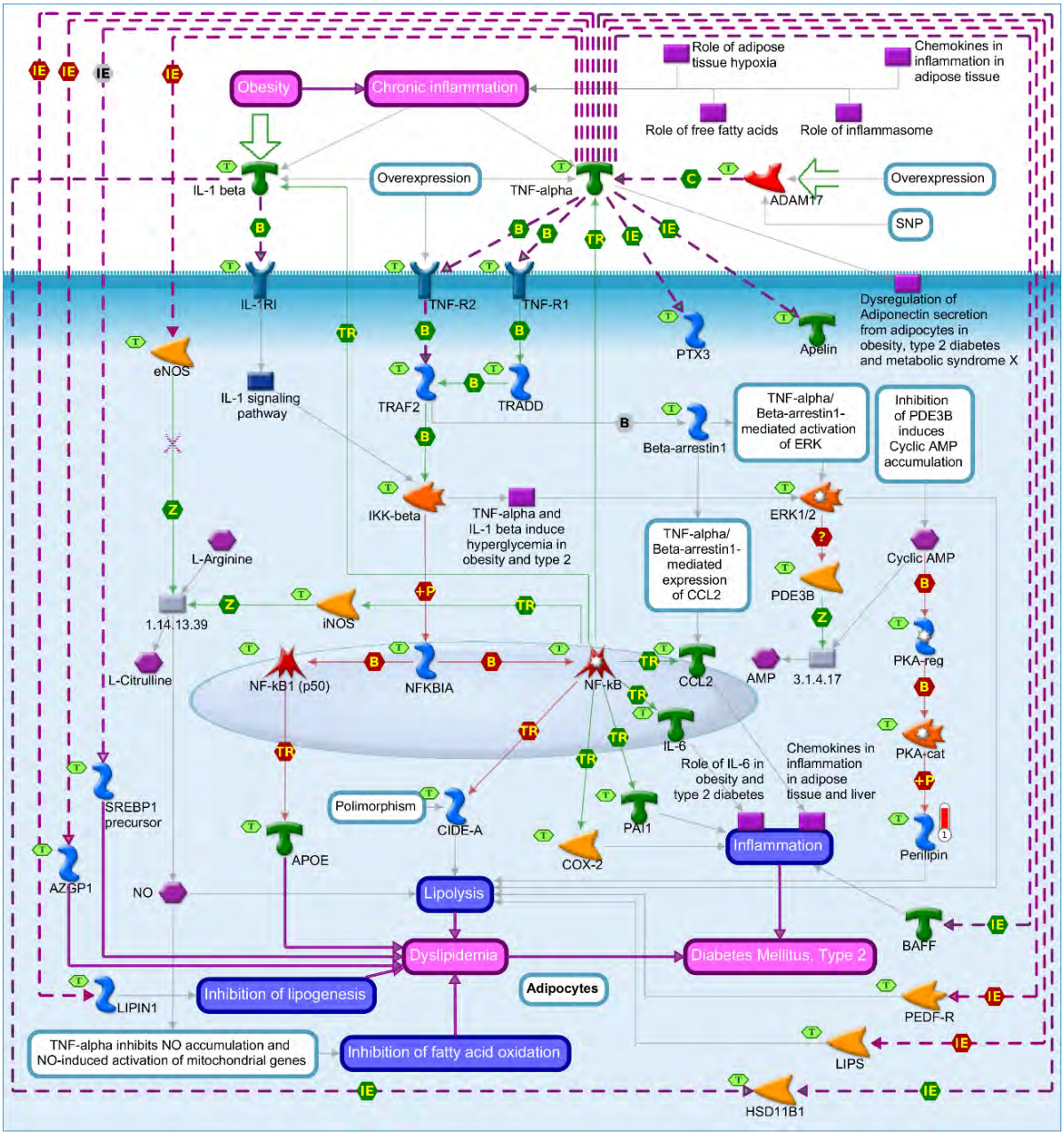

Figure 8. TNF-alpha and IL-1 beta induce dyslipidemia and inflammation in obesity and type 2 diabetes in adipocytes pathway map from brain tissue of pigs fed with different oil sources (CO^{1}*vs*FO^{2}). ^{1}CO: corn-soybean meal diet containing 3% canola oil. ^{2}FO: corn-soybean meal diet containing 3% fish oil. The experimental data is represented by the thermometer-like figure on the map. The upward thermometer (red) indicates up-regulation of the *PLIN1* DEG (log2 fold change +1.558) in the CO group compared to FO. 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.

For this comparison the netrin-1 (*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 regulation of axon guidance” (*p*-value = 4.089e-2), illustrated in Figure 9.

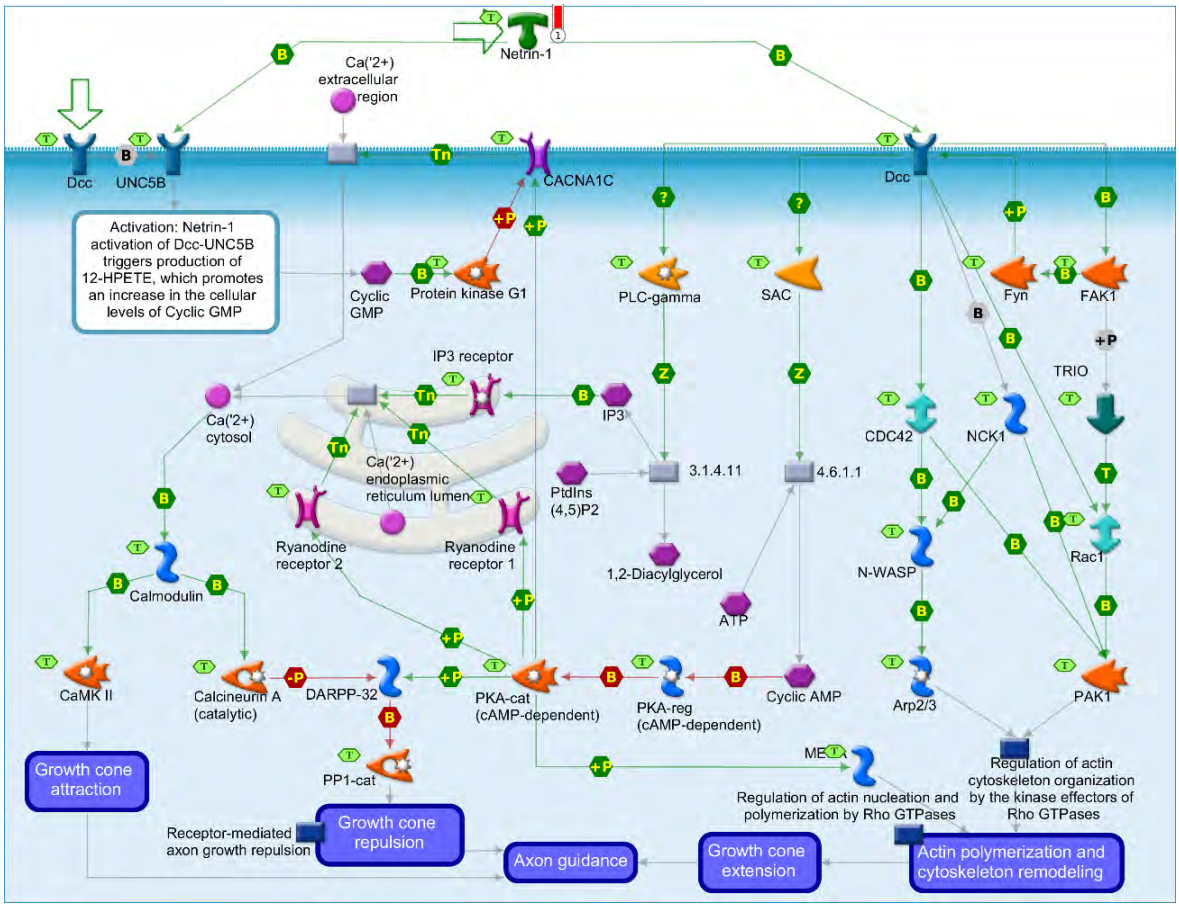

Figure 9. Neurophysiological process_Netrin-1 in regulation of axon guidance pathway map from brain tissue of pigs fed with different oil sources (CO[1]*vs*FO[2]). [1]CO: corn-soybean meal diet containing 3% canola oil. [2]FO: corn-soybean meal diet containing 3% fish oil. The experimental data is represented by the thermometer-like figure on the map. The upward thermometer (red) indicates up-regulation of the *NTN1* DEG (log2 fold change +1.058) in the CO group. Network objects are represented by individual symbols. 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.

Additionally, process networks were generated using MetaCore software. The Transport_Calcium transport (*p*-value = 9.810e-4) process network was identified in this analysis, with the DEG *CALB1* (log2 fold change = +2.614), *CHRNA6* (log2 fold change = +2.292), and *TRPC3* (log2 fold change = +1.627), as showed in Figure 10.

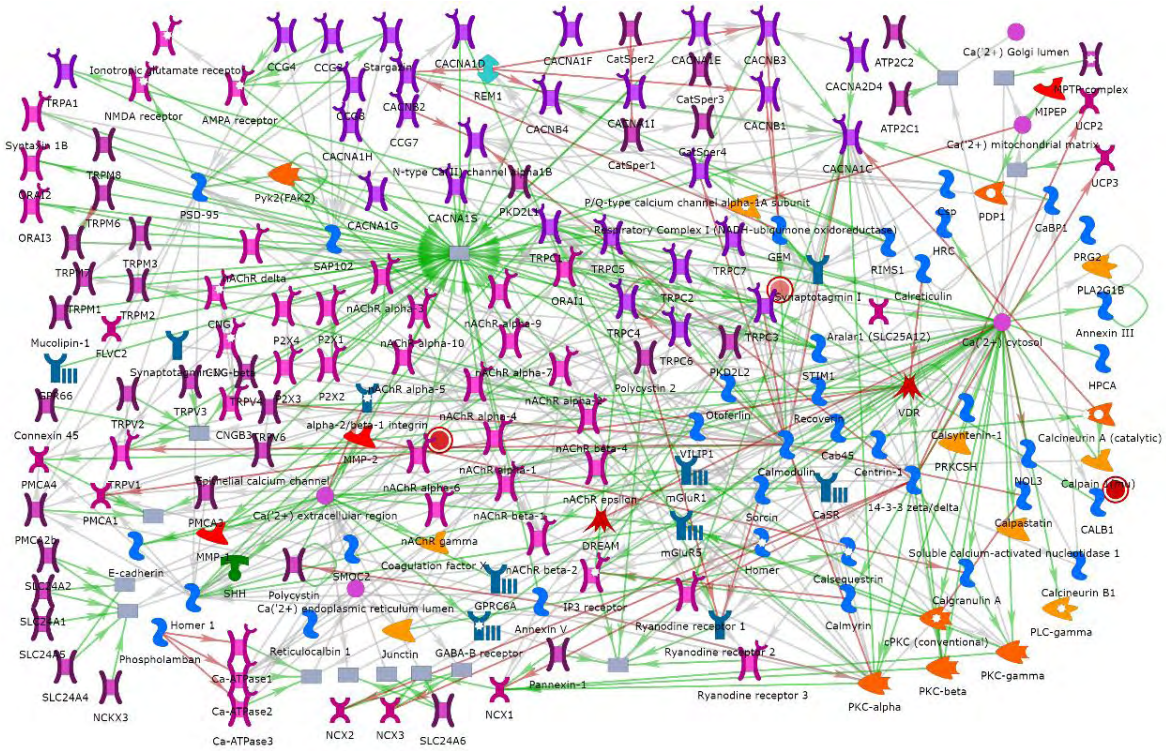

Figure 10. Calcium transport network from brain tissue of pigs fed with different oil sources (CO[1]*vs*FO[2]). [1]CO: corn-soybean meal diet containing 3% canola oil. [2]FO: corn-soybean meal diet containing 3% fish oil. The experimental data are represented by the intensity of the blue and red circles on the network. The red circles indicate up-regulation of the *CALB1*, *CHRNA6* and *TRPC3* DEG in the CO group compared to FO. 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.

4.DISCUSSION

The brain is an important regulator of systemic metabolism and lipid homeostasis (BRUCE; ZSOMBOK; ECKEL, 2017). Increasing evidence shows that the regulation of lipid metabolism in the brain has been associated with nutritional status and the modulation of lipids in peripheral tissues such as the liver (MORTON et al., 2006; BRUCE; ZSOMBOK; ECKEL, 2017; TAGHIBIGLOU; KHALAJ, 2017). Thus, the inclusion of different oil sources in the diet, with diverse FA profiles, helps to understand the mechanisms involved in lipid signaling and modulation, since the structure and lipid composition of brain membranes and barriers can be affected by the availability of dietary FA (YEHUDA et al., 2002; PIFFERI; LAURENT; PLOURDE, 2021).

4.1 FA profile and the effects on the brain tissue

In this study, the SOY group showed a higher percentage of palmitic acid compared to CO and FO. According to Carta et al. (2015, 2017), palmitic acid is the most common SFA in the human body, accounting for 20-30% of the total FA from membrane phospholipids and triacylglycerols. This FA may be obtained by diet or synthesized by de *novo* lipogenesis. The synthesis of palmitic acid occurs in the cytosol of hepatocytes and adipocytes and in intramuscular fat deposits (BERGEN; MERSMANN, 2005; SMITH et al., 2021), however it has been characterized that lipogenesis also occurs in some regions of brain tissue (LACOMBE et al., 2018; SMITH et al., 2021).

In pathophysiological conditions, the control of palmitic acid homeostasis can be altered, affecting biophysical membrane properties and various biological functions (WILKE et al., 2009; CARTA et al., 2017). This FA, when in excess, may act on several adaptor proteins, activating nuclear factor kappa B (NF-κB) (GABER; STREHL; BUTTGEREIT, 2017; UGBAJA et al., 2021). The activation of NF-κB may be associated with the induction of hyperproliferation of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and IL-1β, which, in conjunction with the high metabolic demand of the brain, induces oxidative stress and increases neuroinflammation (DALI-YOUCEF; RICCI, 2015; CAMANDOLA; MATTSON, 2017).

High levels of palmitic acid have been identified in neurodegenerative conditions, such as Parkinson's and Alzheimer's disease (FRASER; TAYLER; LOVE, 2010; FABELO et al., 2011). In the study performed by Schommer et al. (2018), the application of a diet enriched with palmitic acid in m-Thy1 transgenic mice, was associated with an increase in α-synuclein, a pathogenic protein that may cause dopaminergic neuronal death in Parkinson's disease. Furthermore, an increase in SFA, mainly palmitic acid, may be associated with deterioration of cognitive function through apoptosis of neuronal cells, accelerating the accumulation of β-amyloid, a pathological condition in Alzheimer's disease (HSIAO et al., 2014).

The CO group showed a higher percentage of MUFA OA and eicosenoic acid. According to Song et al. (2019), OA is a major constituent of membrane phospholipids and is critical for proper brain development and function. OA is reported to modulate insulin sensitivity, aid in the reduction of triacylglycerol, aid in glucose control, and act in modulating gene transcription (PETROVIC; ARSIC, 2016; FARAG; GAD, 2022). OA is also reported to be an important anti-inflammatory and neuroprotective factor, by activating peroxisome proliferator-activated receptor gamma (PPAR-γ) and inhibiting the activation of

NF-kB signaling pathways (SILVA-SANTI et al., 2018; SONG et al., 2019). This FA also showed potential for reducing β-amyloid and 7-ketocholesterol, reported in patient with some type of neurodegeneration (DEBBABI et al., 2016, 2017).

Diets enriched with MUFA, such as Mediterranean diets, are associated with reduced cognitive decline dysfunction in Alzheimer's disease in experimental models (SCARMEAS et al., 2009; LAURETTI; IULIANO; PRATICÒ, 2017). Eicosenoic acid, also known as gondoic acid, although not widely studied, is reported in red blood cell membranes, and when in increased levels, has been reported in children with regressive autism (FARAG; GAD, 2022). For eicosenoic acid, there is still not enough evidence in the literature to demonstrate its effects on brain tissue, so we cannot infer whether its greater availability in the CO diet positively or negatively affected our study. Thus, more studies are needed to verify its importance in this tissue and also its relationship with metabolic processes associated with lipid metabolism and disease progression.

For total PUFA, the SOY and FO groups had the highest percentages. PUFA are widely implicated in normal brain development and function (MCNAMARA; VANNEST; VALENTINE, 2015) and are abundant in neuronal membrane phospholipids (MARTÍNEZ; MOUGAN, 1998). Among the most important FA for the brain are the long-chain PUFA such as arachidonic acid (AA, C20:4 n-6) and docosahexaenoic acid (DHA, C22:6 n- 3) (KITAJKA et al., 2004; SILVA-SANTI et al., 2018). These FA have an important role in the activation of receptors and signaling pathways, playing a fundamental role in membrane fluidity, and being reported in neurogenesis and in the resolution of inflammatory processes (BAZINET; LAYÉ, 2014).

Joffre et al. (2016), in a literature revision showed several studies, with mice, mentioning that a decrease in DHA may cause alterations in spatial memory, depression-related symptoms, and changes in neuronal plasticity in the hippocampus and prefrontal cortex. In mouse models for Parkinson's disease, n-3 PUFA, such as DHA, have demonstrated neuroprotective effects (BOUSQUET; CALON; CICCHETTI, 2011; DYALL, 2015; CHEN et al., 2018). Amtul et al. (2011), observed in the brains of mice fed a diet enriched in n-3 PUFA, a decrease in Aβ peptide (a pathological marker of Alzheimer's disease) and less accumulation of amyloid plaques. In contrast, n-6 PUFA, such as AA, which aggravated the neuropathology of Alzheimer's disease by increasing the synthesis of Aβ peptide (AMTUL et al., 2012).

Thus, analyzing the results of the FA profile of brain tissue (Table 1) and the FA profile of the oils used in the pig diets (APPENDIX B - Table SF5), the canola oil used

showed low amounts of palmitic acid and eicosenoic acid, demonstrating that a lower availability of these FA may have contributed to the reduction of inflammatory and neurodegenerative processes. In addition, canola oil also showed an interesting PUFA profile, with higher amounts of ALA and intermediate amounts of LA. This may have contributed to a higher availability of total PUFA and consequently increased anti-inflammatory processes and resulting in better conditions for proper brain function.

4.2 Effects of comparisons SOY*vs*FO and CO*vs*FO on functional enrichment

The results found in the functional enrichment analysis, demonstrated that the use of different oil sources alters the transcriptome profile of pig brain, affecting important biological processes. For the enriched pathways showed in Figures 2 and 3, *CHRNA6* DEG and other genes of the nicotinic acetylcholine receptor (nAChR) family that are expressed in pancreatic beta cells are activated by binding. These genes are responsible for increasing the amount of reactive oxygen species (ROS) and the expression of glutathione peroxidase 1 (GPX1) and superoxide dismutase 2 (SOD2) proteins. The high amount of ROS exceeds the endogenous antioxidant capacity of GPX1 and SOD2, resulting in oxidative stress that may cause the apoptosis of pancreatic beta cells and thus the downregulation of the insulin hormone, resulting in the pathology of type 2 diabetes mellitus (BRUIN et al., 2008; STELZER et al., 2016).

In the brain, the *CHRNA6* gene is responsible for encoding an alpha subunit of neuronal 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 (PATERSON; NORDBERG, 2000; HOGG; RAGGENBASS; BERTRAND, 2003), as frontal cortex and hippocampus. Also, this gene is involved in various aspects of cognitive function such as learning, attention and memory (LEVIN; SIMON, 1998). Acetylcholine (ACh) is the orthosteric nAChR ligand, but several other endogenous and exogenous ligands may modulate brain tissue nAChR activity. These receptors may be activated by nicotine, phospholipids, and cholesterol-derived hormones (GREENBAUM; LERER, 2009; TALY et al., 2009). nAChR receptors are also significantly modulated by lipids, in which lipid-protein interactions may alter the activation or inhibition of these receptors and the function of ion channels (BARRANTES, 2004; DOMVILLE; BAENZIGER, 2018; SHARP; BRANNIGAN, 2021). Thus, in our study, *CHRNA6* may have been modulated by lipids and thereby altered gene expression in brain tissue.

The ACh receptors play important roles in information processing and synaptic transmission, they are associated with several important physiological processes and have been implicated in several neurological pathological conditions (LEVIN; SIMON, 1998; PATERSON; NORDBERG, 2000; MUFSON et al., 2008). Paterson and Nordberg (2000), in a literature revision showed several studies mentioning that a significant loss of nAChR sites is reported in Parkinson's and Alzheimer's disease. Presynaptic nAChRs mediate the release of several neurotransmitters, such as glutamate, dopamine, and gamma-aminobutyric acid (ZEIGER et al., 2007). This mediation occurs through mechanisms including, transmembrane calcium permeability or intracellular calcium signaling and the modification of pre-terminal membrane excitability (DAJAS-BAILADOR; WONNACOTT, 2004; DANI; BERTRAND, 2007). This may have been evidenced in the current study by the functional enrichment of the process network "Calcium transport" found in the SOY*vs*FO and CO*vs*FO comparisons.

Herein the *CHRNA6* DEG (up-regulated), present in two of the three diet comparisons, showed a positive association with the SOY and CO diets relative to FO. Thus, a greater availability of FA present in these oils may have assisted in greater *CHRNA6* expression, contributing to the maintenance of neurotransmission mediation and intracellular calcium signaling, synaptic transmission, and may also have assisted in less cognitive decline. With this, it is critical to understand the mechanism responsible for modulating these receptors to understand how and where lipids interact. In addition, it is also important to understand how modifications in lipid environments affect these processes and how this may alter pathological conditions in brain tissue.

For the enriched pathways illustrated in Figures 7 and 8, in obese adipose tissue, overexpression of TNF-alpha and IL-1β and IL-6 leads to activation of pathways that result in impaired expression of genes involved in inflammation, lipogenesis, lipolysis, FA oxidation, and oxidative stress, affecting multiple systems. Perilipins belong to a family of five conserved proteins (PLIN1-PLIN5), which are essential for lipid metabolism (CONTE et al., 2021). The PLIN1 is a major lipid droplets (LD) coat protein that helps regulate lipid metabolism, especially of adipocytes, acting as a barrier and recruitment site for lipases (HANSEN et al., 2017; SOHN et al., 2018). In the study of Sohn et al. (2018), it has been suggested that *PLIN1* acts by restricting lipolysis, and as a key regulator against insulin resistance and inflammatory processes.

The LD are dynamic and specialized intracellular lipid storage organelles. These organelles contain neutral lipid metabolites that play important roles in energy homeostasis by responding to the physiological state of cells (SOHN et al., 2018; RALHAN et al., 2021). In

addition to helping control cellular metabolism, LD may play a role in protecting against oxidative stress (RALHAN et al., 2021). The storage and release of these metabolites is essential for the lipid synthesis, cell signaling, and energy production to occur in an adequate manner. The storage of metabolites also guarantees a protection against the accumulation of FA in the cytosol, which may be toxic (UNGER; ORCI, 2002; WALTHER; CHUNG; FARESE, 2017; OLZMANN; CARVALHO, 2019). According to Sohn et al. (2018), *PLIN1* in its basal state, acts by engaging LD and inhibits lipolysis in adipocytes.

Although adipose tissue is the most enriched in LD (MISSAGLIA et al., 2019), other systems may also be affected by them, such as the central nervous system (FARMER et al., 2020). It has been reported that LD are present in all cell types of brain tissue (RALHAN et al., 2021). The LD formation may also play important roles in cell health during the progression of several metabolic and inflammatory diseases (FARMER et al., 2020; RALHAN et al., 2021). Farmer et al. (2020) and Ralhan et al. (2021), reported in their studies that LD are also associated with neurodegenerative diseases, such as Amyotrophic Lateral Sclerosis, Huntington's, Parkinson's and Alzheimer's diseases, and Hereditary Spastic Paraplegia (HSP).

For brain, lipid homeostasis is critical to maintain neuronal function and normal synaptic plasticity, furthermore, the interaction between perilipins and lipases in LD are primarily responsible for lipid storage, metabolism, and degradation (OLZMANN; CARVALHO, 2019; MONTESINOS; GUARDIA-LAGUARTA; AREA-GOMEZ, 2020). Thus, understanding the role of perilipins, especially *PLIN1*, which may act in the brain-adipose tissue system, is of high importance.

The adipose tissue has several bioactive derivatives that circulate and transmit information to other metabolically active organs such as the liver, muscle, brain, and pancreas (SCHERER, 2006; LUO; LIU, 2016; PARIMISETTY et al., 2016). In metabolic states the adipose tissue acts by secreting adipokines that impair lipid and energy homeostasis, which may affect the brain and other organs (SOHN et al., 2018). Furthermore, according to Roh et al. (2016), the brain is also responsible for modulating various aspects of metabolism such as energy expenditure, glucose and FA metabolism in adipose tissue and skeletal muscle, and insulin secretion.

Thus, according to Farmer et al. (2020), protein components external to LD, such as perilipins, are responsible for a variety of unique interactions, which may help in understanding these organelles in energy homeostasis, cell communication, and disease progression in various tissues. Moreover, understanding brain-adipose tissue effects may help

to clarify how perilipins act, for example, in the prevention or progression of metabolic and neurodegenerative diseases (LUO; LIU, 2016). In the present study *PLIN1* (up-regulated), showed a positive association with the CO group compared to FO. Thus, the expression of this gene associated with lipid metabolism and LD formation, may have contributed to lower oxidative stress and a lower rate of inflammation.

For the enriched pathway illustrated in Figure 9, the *NTN1* DEG acts by binding activation of the DCC and UNC5B receptors. These receptors are responsible for acting in the regulation of axon guidance. Netrin is part of a secreted proteins family that are related to laminin (STELZER et al., 2016), which are important basal membrane glycoproteins (AUMAILLEY, 2013). Laminins may influence cell differentiation, migration, and adhesion of various tissues, and also play an important role in the regulation of the blood-brain barrier, which is responsible for maintaining the homeostasis of the central nervous system (AUMAILLEY, 2013; GAUTAM; ZHANG; YAO, 2016). The blood-brain barrier is a dynamic structure responsible for regulating the accessibility of circulating lipids to brain cells (PIFFERI; LAURENT; PLOURDE, 2021). Disruption or impairment of this barrier homeostasis contributes to the development and progression of several neurological disorders, such as stroke and Alzheimer's disease (GAUTAM; ZHANG; YAO, 2016).

The *NTN1* acts as a survival factor through its association with receptors that prevent the initiation of apoptosis (MAZELIN et al., 2004). Netrins also exert angiogenic and anti-inflammatory activity (WILSON et al., 2006; ROSENBERGER et al., 2009). The *NTN1* may also act in regulating the migration of immune cells such as lymphocytes, monocytes, and macrophages (LY et al., 2005; TAYLOR et al., 2016). In the study of He et al. (2018), the *NTN1* acted on the regulation of astrocyte activation and neuronal inflammation in cerebral ischemia, attenuating brain damage in mice.

In the study of Zheng et al. (2018), *NTN1* promoted functional recovery of neurons after brain ischemia, demonstrating that binding of this gene to the netrin receptor protein DCC (deleted in colorectal cancer), aids axonal regeneration and synaptic formation (WONG et al., 2019). The DCC is considered the canonical receptor of the *NTN1* gene, being a single-pass transmembrane protein (GLASGOW; RUTHAZER; KENNEDY, 2021), which acts by triggering an increase in intracellular calcium, regulating local protein synthesis, and activating GTPases of the Rho family, key components in signaling networks (SUN; CORREIA; KENNEDY, 2011; SADOK; MARSHALL, 2014; KIM; MARTIN, 2015). Glasgow et al. (2021), in a literature revision showed several studies mentioning that in humans, polymorphisms in *NTN1* and other netrins have been correlated with

neurodevelopmental disorders, such as autism and schizophrenia, and neurodegenerative disorders, such as Alzheimer's and Parkinson's disease, and Amyotrophic Lateral Sclerosis.

The *NTN1*/DCC system is reported to be a key factor in the development and maintenance of the central nervous system, and *NTN1* and its receptor have high expression in the gray matter of the adult brain (LIVESEY; 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, which led to a significant loss of dopamine neurons causing motor deficits. In other words, *NTN1* was shown to be critical for the maintenance of dopaminergic neurons, also suggesting a neuroprotective role in Parkinson's disease.

Another important factor related to the *NTN1*/DCC system, is that axon guidance receptors, such as DCC, together with surface receptors and signaling enzymes, act through lipid rafts in cell signaling (SIMONS; TOOMRE, 2000; GUIRLAND et al., 2004). Hérincs et al. (2005), demonstrated that the canonical *NTN1* receptor, is a constitutive part of small lipid raft domains, furthermore, this localization is critical for DCC to act as a *NTN1* receptor. Another important point is that lipid raft integrity is required, so that NTN1-mediated axon growth can occur properly.

In our study the *NTN1* (up-regulated), demonstrated a positive association with the CO group compared to FO. Thus, the expression of this gene may have assisted in the maintenance of dopaminergic neurons (neuroprotection) and in the homeostasis of the blood-brain barrier. In addition, the *NTN1*/DCC system may be related to the maintenance of synapses and cognitive function. Thus, despite the advances, further studies are needed to understand the role of *NTN1* in pathological conditions in brain tissue, as well as to understand the pathways that regulate its expression.

5.CONCLUSION

In this study, we found that different sources of oils administered in the diet of pigs affected the transcriptomic and FA profile of brain tissue, but not the total lipid content. Among the diets provided, the CO group demonstrated to be associated with better outcomes related to FA availability and expression of neuroprotection-related genes. The pathways, networks and DEG found, participate in important processes related to lipid metabolism, cell signaling, synaptic transmission and inflammation. Thus, considering pigs as an animal model for metabolic and neurodegenerative diseases in humans, our results demonstrate the importance of FA availability and help to direct future investigations. This will help the

understanding of the mechanisms involved between FA and physiological processes important for the brain, directing more efficient solutions for animal and human health.

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CONCLUSION AND FINAL CONSIDERATIONS

As general conclusions, no changes in total lipid content were identified between treatments, demonstrating that the brain is not affected by diet in the short term. For the different levels of soybean oil (chapter one) no differences in FA profile were identified, but differences in brain tissue gene expression were identified. For the different oil sources (different FA profiles - chapter two), differences in FA profile and gene expression were identified. Furthermore, the canola diet, which contained higher percentages of OA, LA and ALA, showed a better association with anti-inflammatory and neuron protective processes. Thus, this study demonstrated the potential of including different FA in the diet of pigs and their association with biological processes relevant to brain tissue. Furthermore, a better understanding of the mechanisms involved between different levels of soybean oil or FA profiles and physiological processes important for the brain, may help to target more efficient solutions for animal and human health.

APPENDIX A: SUPPLEMENTARY FILE – CHAPTER 1

Table S1. Quality, concentration and RNA integrity number of brain samples from pigs fed different levels of soybean oil

Sample	NanoDrop	Qubit	Wavelength (nm)		Ratio absorbances		RIN
	------ (ng/µl) ------		260	280	260/280	260/230	
B3	147.70	185.00	3.69	1.82	2.03	1.71	9.30
B4	287.70	280.00	7.19	3.45	2.08	1.41	8.20
B7	285.40	308.00	7.14	3.41	2.09	1.93	9.00
B10	315.30	246.00	7.88	3.79	2.08	2.13	7.90
B11	378.90	398.00	9.47	4.51	2.10	2.20	7.90
B12	161.80	130.00	4.05	1.98	2.04	2.01	8.40
B13	316.30	380.00	7.91	3.79	2.09	2.13	8.30
B14	160.30	127.00	4.01	1.97	2.03	1.97	9.00
B16	144.40	99.60	3.61	1.85	1.95	1.58	8.50
B18	474.40	474.00	11.86	5.67	2.09	2.02	8.40
B19	1183.80	994.00	29.59	14.16	2.09	1.13	8.60
B26	238.50	222.00	5.96	2.87	2.08	0.90	8.80
B28	258.70	324.00	6.47	3.07	2.11	2.09	7.90
B32	502.60	656.00	12.57	6.09	2.06	2.18	8.60
B33	349.20	258.00	8.73	4.21	2.08	2.04	9.40
B34	109.70	84.40	2.74	1.37	2.01	1.58	8.60
B35	154.80	124.00	3.87	1.88	2.06	2.01	8.30
B37	138.40	102.00	3.46	1.74	1.99	1.66	8.00
B41	243.40	173.00	6.09	2.94	2.07	2.05	8.80
B43	139.10	112.00	3.48	1.68	2.07	2.20	8.30
B46	207.60	190.00	5.19	2.50	2.08	1.91	8.20
B47	190.60	161.00	4.77	2.28	2.09	1.96	8.20
B49	402.50	528.00	10.06	4.82	2.09	2.15	8.10
B53	760.30	692.00	19.01	9.18	2.07	2.20	8.00
B54	210.40	178.00	5.26	2.54	2.07	0.82	7.80
B55	476.10	572.00	11.90	5.70	2.09	2.16	8.00
B56	447.90	502.00	11.20	5.32	2.10	2.19	8.00

B59	429.50	470.00	10.74	5.11	2.10	2.18	8.00
B61	220.20	163.00	5.51	2.82	1.96	1.48	7.90
B62	277.90	177.00	6.95	3.40	2.05	1.84	8.30
B63	246.70	204.00	6.17	3.02	2.04	1.32	8.30
B66	130.80	114.00	3.27	1.61	2.03	1.74	8.30
B68	576.00	548.00	14.40	7.00	2.06	2.23	8.60
B69	450.00	388.00	11.25	5.44	2.07	2.16	8.10
B70	200.70	197.00	5.02	2.41	2.08	2.12	7.50
B72	67.50	60.60	1.69	0.82	2.05	1.57	10.00

Table S2 - Mapped reads (*Sscrofa11.1*) of brain samples from pigs fed different levels of soybean oil

Sample	Group	reads strandR1	reads strandR2	Total_reads	R1_trimmed	R2_trimmed	Total_final	uniquely mapped (%)	unmapped (%)	multimapping (%)	noFeature (%)	ambiguous (%)
B3	SOY3.0	17601495	17601495	35202990	17332444	17332444	34664888	90.93	5.81	3.26	13.13	5.17
B4	SOY1.5	17117523	17117523	34235046	16877780	16877780	33755560	92.49	5.35	2.15	14.19	5.22
B7	SOY1.5	16547196	16547196	33094392	16364152	16364152	32728304	93.77	4.24	1.99	14.84	5.10
B10	SOY3.0	16072772	16072772	32145544	15836940	15836940	31673880	91.88	5.94	2.18	14.93	5.39
B11	SOY3.0	17953371	17953371	35906742	17705414	17705414	35410828	92.82	5.01	2.18	14.59	5.08
B12	SOY3.0	20082967	20082967	40165934	19805222	19805222	39610444	92.02	5.48	2.50	12.64	5.45
B13	SOY1.5	17766014	17766014	35532028	17512425	17512425	35024850	92.46	5.44	2.10	14.84	4.99
B14	SOY1.5	19202586	19202586	38405172	18939187	18939187	37878374	90.27	6.42	3.31	16.70	4.84
B16	SOY1.5	17408051	17408051	34816102	17182452	17182452	34364904	92.31	5.30	2.38	12.30	5.64
B18	SOY3.0	17327254	17327254	34654508	17068514	17068514	34137028	93.39	4.50	2.10	18.82	4.88
B19	SOY3.0	16914885	16914885	33829770	16709885	16709885	33419770	92.55	5.26	2.19	13.90	5.11
B26	SOY3.0	17349376	17349376	34698752	17136861	17136861	34273722	91.69	4.84	3.47	16.09	4.84
B28	SOY1.5	17144000	17144000	34288000	16908192	16908192	33816384	91.76	5.57	2.67	14.05	5.26
B32	SOY1.5	17451764	17451764	34903528	17196369	17196369	34392738	91.07	5.93	3.00	14.68	5.29
B33	SOY1.5	17343401	17343401	34686802	17145831	17145831	34291662	92.74	5.29	1.97	13.27	5.33
B34	SOY1.5	15761509	15761509	31523018	15548988	15548988	31097976	86.31	3.71	9.99	9.47	5.87
B35	SOY1.5	17197746	17197746	34395492	17016885	17016885	34033770	95.25	2.52	2.23	14.05	5.37
B37	SOY1.5	19449390	19449390	38898780	19179187	19179187	38358374	93.28	4.48	2.25	15.86	5.22
B41	SOY1.5	10686257	10686257	21372514	10565162	10565162	21130324	93.58	4.11	2.31	15.08	5.30
B43	SOY1.5	14585064	14585064	29170128	14401126	14401126	28802252	93.48	3.89	2.63	15.41	5.27
B46	SOY3.0	14759998	14759998	29519996	14593462	14593462	29186924	96.13	1.34	2.52	12.16	5.59
B47	SOY1.5	14266238	14266238	28532476	14087373	14087373	28174746	93.83	3.96	2.21	14.02	5.27
B49	SOY1.5	18150233	18150233	36300466	17898669	17898669	35797338	92.93	4.47	2.60	14.32	5.15
B53	SOY3.0	16978283	16978283	33956566	16778279	16778279	33556558	90.84	5.97	3.19	15.63	5.11
B54	SOY3.0	16207227	16207227	32414454	16018594	16018594	32037188	92.44	5.21	2.35	14.48	5.12
B55	SOY3.0	16300470	16300470	32600940	16086128	16086128	32172256	90.81	6.43	2.77	14.48	5.01
B56	SOY1.5	16806959	16806959	33613918	16583154	16583154	33166308	91.54	5.74	2.72	14.52	4.95
B59	SOY3.0	19113710	19113710	38227420	18860382	18860382	37720764	92.90	4.84	2.27	14.45	5.18
B61	SOY3.0	19532678	19532678	39065356	19256541	19256541	38513082	92.65	5.02	2.33	14.60	5.09

B62	SOY1.5	16501318	16501318	33002636	16278946	16278946	32557892	92.03	5.13	2.84	14.95	5.23
B63	SOY3.0	17021291	17021291	34042582	16786108	16786108	33572216	92.09	5.56	2.35	12.91	5.66
B66	SOY1.5	17186785	17186785	34373570	16964040	16964040	33928080	93.48	4.51	2.01	14.00	5.27
B68	SOY3.0	17077873	17077873	34155746	16862359	16862359	33724718	92.49	5.45	2.06	14.23	5.03
B69	SOY3.0	16448318	16448318	32896636	16233190	16233190	32466380	92.10	5.77	2.13	11.74	5.50
B70	SOY3.0	15547760	15547760	31095520	15344621	15344621	30689242	92.41	5.21	2.38	14.88	5.28
B72	SOY3.0	16907211	16907211	33814422	16727130	16727130	33454260	91.64	4.33	4.03	11.67	5.10

Table S3 - DEG of brain samples from pigs fed different levels of soybean oil

Comparision	Gene_stable_id	Gene_name	Gene_description	log2 fold change	*p*-value	FDR
SOY1.5*vs*SOY3.0	ENSSSCG00000014899	PRCP	prolylcarboxypeptidase [Source:VGNC Symbol;Acc:VGNC:91771]	0.599936086	1.03E-11	1.97E-07
SOY1.5*vs*SOY3.0	ENSSSCG00000017915	VMO1	vitelline membrane outer layer 1 homolog [Source:VGNC Symbol;Acc:VGNC:94833]	2.926880299	1.92E-09	1.85E-05
SOY1.5*vs*SOY3.0	ENSSSCG00000048787	Novel	0	0.713396861	3.38E-09	2.16E-05
SOY1.5*vs*SOY3.0	ENSSSCG00000051166	Novel	0	-4.770152851	5.95E-09	2.86E-05
SOY1.5*vs*SOY3.0	ENSSSCG00000046723	Novel	0	1.876190111	1.82E-07	0.000700298
SOY1.5*vs*SOY3.0	ENSSSCG00000000063	POLR3H	RNA polymerase III subunit H [Source:VGNC Symbol;Acc:VGNC:91658]	-0.490438684	3.55E-07	0.000852215
SOY1.5*vs*SOY3.0	ENSSSCG00000050056	CCDC201	coiled-coil domain containing 201 [Source:HGNC Symbol;Acc:HGNC:54081]	0.9460129	2.96E-07	0.000852215
SOY1.5*vs*SOY3.0	ENSSSCG00000008731	OTOP1	otopetrin 1 [Source:VGNC Symbol;Acc:VGNC:91093]	2.088715707	3.16E-07	0.000852215
SOY1.5*vs*SOY3.0	ENSSSCG00000009667	Novel	gulonolactone (L-) oxidase [Source:NCBI gene (formerly Entrezgene);Acc:396759]	-1.659653167	5.40E-07	0.001151686
SOY1.5*vs*SOY3.0	ENSSSCG00000014168	ELL2	elongation factor for RNA polymerase II 2 [Source:VGNC Symbol;Acc:VGNC:87649]	0.659336842	1.24E-06	0.002381141
SOY1.5*vs*SOY3.0	ENSSSCG00000015742	TMEM177	transmembrane protein 177 [Source:VGNC Symbol;Acc:VGNC:96166]	-0.512662235	2.24E-06	0.003914874
SOY1.5*vs*SOY3.0	ENSSSCG00000017963	Novel	dynein axonemal heavy chain 2 [Source:VGNC Symbol;Acc:VGNC:87370]	-2.317572738	4.11E-06	0.006574502
SOY1.5*vs*SOY3.0	ENSSSCG00000014170	CAST	calpastatin [Source:VGNC Symbol;Acc:VGNC:99603]	0.421185164	7.50E-06	0.011087414
SOY1.5*vs*SOY3.0	ENSSSCG00000029805	RHOBTB3	Rho related BTB domain containing 3 [Source:VGNC Symbol;Acc:VGNC:92286]	0.621033982	8.40E-06	0.01152517
SOY1.5*vs*SOY3.0	ENSSSCG00000045438	Novel	0	-1.298973001	1.12E-05	0.013474961
SOY1.5*vs*SOY3.0	ENSSSCG00000017278	TEX2	testis expressed 2 [Source:VGNC Symbol;Acc:VGNC:93894]	0.257682826	1.07E-05	0.013474961
SOY1.5*vs*SOY3.0	ENSSSCG00000008700	HGFAC	HGF activator [Source:VGNC Symbol;Acc:VGNC:88870]	-1.579995415	2.32E-05	0.024795253
SOY1.5*vs*SOY3.0	ENSSSCG00000023436	Novel	0	0.8158259	2.26E-05	0.024795253
SOY1.5*vs*SOY3.0	ENSSSCG00000018044	ALDH3A1	aldehyde dehydrogenase 3 family member A1 [Source:VGNC Symbol;Acc:VGNC:85238]	-1.527379009	2.58E-05	0.025847707
SOY1.5*vs*SOY3.0	ENSSSCG00000049532	Novel	0	0.523682618	2.69E-05	0.025847707
SOY1.5*vs*SOY3.0	ENSSSCG00000008725	CYTL1	cytokine like 1 [Source:VGNC Symbol;Acc:VGNC:87138]	-1.264931303	3.01E-05	0.026295731
SOY1.5*vs*SOY3.0	ENSSSCG00000031105	C9orf85	chromosome 1 C9orf85 homolog [Source:VGNC Symbol;Acc:VGNC:85976]	0.465323244	2.98E-05	0.026295731
SOY1.5*vs*SOY3.0	ENSSSCG00000019064	SNORD113-9	small nucleolar RNA, C/D box 113-9 [Source:HGNC Symbol;Acc:HGNC:32988]	-3.318530876	3.46E-05	0.026552916
SOY1.5*vs*SOY3.0	ENSSSCG00000041251	Novel	0	-1.424085582	3.45E-05	0.026552916
SOY1.5*vs*SOY3.0	ENSSSCG00000017446	Novel	0	0.738490825	3.22E-05	0.026552916
SOY1.5*vs*SOY3.0	ENSSSCG00000006125	CALB1	calbindin 1 [Source:VGNC Symbol;Acc:VGNC:86138]	-3.431092962	3.61E-05	0.026666334
SOY1.5*vs*SOY3.0	ENSSSCG00000007816	IL21R	interleukin 21 receptor [Source:VGNC Symbol;Acc:VGNC:89096]	1.431765203	3.93E-05	0.027975279
SOY1.5*vs*SOY3.0	ENSSSCG00000017978	ALOX15B	arachidonate 15-lipoxygenase type B [Source:VGNC Symbol;Acc:VGNC:98972]	-1.488631631	5.40E-05	0.0370442

SOY1.5*vs*SOY3.0	ENSSSCG00000051421	Novel	0	-2.961309665	6.40E-05	0.040258281
SOY1.5*vs*SOY3.0	ENSSSCG00000035991	CENPM	centromere protein M [Source:VGNC Symbol;Acc:VGNC:86553]	-0.581454369	6.50E-05	0.040258281
SOY1.5*vs*SOY3.0	ENSSSCG00000002645	GALNS	galactosamine (N-acetyl)-6-sulfatase [Source:VGNC Symbol;Acc:VGNC:97061]	-0.462477671	6.18E-05	0.040258281
SOY1.5*vs*SOY3.0	ENSSSCG00000011692	ZIC1	Zic family member 1 [Source:VGNC Symbol;Acc:VGNC:95166]	-2.50586115	8.53E-05	0.049585239
SOY1.5*vs*SOY3.0	ENSSSCG00000022842	Novel	0	-1.805445139	8.78E-05	0.049585239
SOY1.5*vs*SOY3.0	ENSSSCG00000030358	SARM1	sterile alpha and TIR motif containing 1 [Source:VGNC Symbol;Acc:VGNC:99038]	-0.364527877	8.28E-05	0.049585239

APPENDIX B: SUPPLEMENTARY FILE – CHAPTER 2

Table SF1. Quality, concentration and RNA integrity number of brain samples from pigs fed different oil sources

Sample	NanoDrop	Qubit	Wavelength (nm)		Ratio absorbances		RIN
	------ (ng/µl) ------		260	280	260/280	260/230	
B1	330.40	504.00	8.26	3.94	2.10	2.23	8.20
B2	265.30	456.00	6.63	3.16	2.10	2.13	7.60
B3	147.70	185.00	3.69	1.82	2.03	1.71	9.30
B5	409.70	424.00	10.24	4.86	2.11	2.07	8.00
B6	447.20	500.00	11.18	5.33	2.10	2.21	8.30
B8	466.70	518.00	11.67	5.65	2.06	2.04	8.70
B9	379.10	418.00	9.48	4.68	2.03	1.78	8.20
B10	315.30	246.00	7.88	3.79	2.08	2.13	7.90
B11	378.90	398.00	9.47	4.51	2.10	2.20	7.90
B12	161.80	130.00	4.05	1.98	2.04	2.01	8.40
B15	335.60	598.00	8.39	4.01	2.10	2.18	8.10
B17	1149.00	838.00	28.73	13.52	2.13	2.30	8.50
B18	474.40	474.00	11.86	5.67	2.09	2.02	8.40
B19	1183.80	994.00	29.59	14.16	2.09	1.13	8.60
B20	617.10	818.00	15.43	7.35	2.10	2.28	8.50
B21	217.90	200.00	5.45	2.63	2.07	1.82	8.70
B22	62.50	89.80	1.56	0.77	2.03	1.65	9.70
B23	345.90	312.00	8.65	4.14	2.09	2.00	7.40
B24	236.60	210.00	5.91	2.86	2.06	1.92	8.90
B25	395.60	362.00	9.89	4.73	2.09	2.07	7.90
B26	238.50	222.00	5.96	2.87	2.08	0.90	8.80
B27	105.60	97.40	2.64	1.31	2.02	1.96	7.90
B29	133.40	137.00	3.34	1.68	1.99	0.74	8.10
B30	292.40	304.00	7.31	3.55	2.06	1.73	7.90
B31	835.10	860.00	20.88	9.81	2.13	2.18	8.50
B36	785.20	928.00	19.63	9.32	2.11	2.32	8.70

B38	1435.60	972.00	35.89	17.07	2.10	2.11	8.30
B39	138.80	113.00	3.47	1.72	2.01	0.97	8.80
B40	453.70	472.00	11.34	5.38	2.11	1.84	9.10
B42	168.10	129.00	4.20	2.09	2.01	1.68	9.10
B44	85.40	71.40	2.14	1.07	2.00	2.01	9.50
B45	70.30	60.20	1.76	0.88	2.00	1.88	9.50
B46	207.60	190.00	5.19	2.50	2.08	1.91	8.20
B48	99.30	69.80	2.48	1.29	1.93	1.55	8.60
B50	306.90	266.00	7.67	3.74	2.05	1.93	8.40
B51	131.00	102.00	3.27	1.62	2.02	1.64	9.80
B52	632.10	704.00	15.80	7.52	2.10	2.24	8.20
B53	760.30	692.00	19.01	9.18	2.07	2.20	8.00
B54	210.40	178.00	5.26	2.54	2.07	0.82	7.80
B55	476.10	572.00	11.90	5.70	2.09	2.16	8.00
B57	264.60	244.00	6.62	3.18	2.08	2.07	8.30
B58	379.80	322.00	9.50	4.54	2.09	1.98	8.30
B59	429.50	470.00	10.74	5.11	2.10	2.18	8.00
B60	519.10	478.00	12.98	6.22	2.09	2.20	8.60
B61	220.20	163.00	5.51	2.82	1.96	1.48	7.90
B63	246.70	204.00	6.17	3.02	2.04	1.32	8.30
B64	437.30	408.00	10.93	5.19	2.11	2.21	7.60
B65	109.40	122.00	2.74	1.36	2.02	1.88	8.10
B67	392.90	376.00	9.82	4.71	2.09	1.80	8.40
B68	576.00	548.00	14.40	7.00	2.06	2.23	8.60
B69	450.00	388.00	11.25	5.44	2.07	2.16	8.10
B70	200.70	197.00	5.02	2.41	2.08	2.12	7.50
B71	1237.10	1020.00	30.93	14.60	2.12	2.27	7.40
B72	67.50	60.60	1.69	0.82	2.05	1.57	10.00

Table SF2 - Mapped reads (*Sscrofa11.1*) of brain samples from pigs fed different oil sources

Sample	Group	reads_strandR1	reads_strandR2	Total_reads	R1_trimmed	R2_trimmed	Total_final	uniquely mapped (%)	unmapped (%)	multimapping (%)	noFeature (%)	ambiguous (%)
B1	FO	18001448	18001448	36002896	17703949	17703949	35407898	92.23	5.15	2.62	14.75	5.02
B2	CO	17864464	17864464	35728928	17565422	17565422	35130844	88.64	5.83	5.53	17.35	4.76
B3	SOY	17601495	17601495	35202990	17332444	17332444	34664888	90.93	5.81	3.26	13.13	5.17
B5	FO	16639561	16639561	33279122	16398484	16398484	32796968	91.10	6.47	2.43	14.70	5.07
B6	FO	16129347	16129347	32258694	15900921	15900921	31801842	92.90	4.69	2.41	14.04	5.27
B8	CO	16776436	16776436	33552872	16536906	16536906	33073812	92.24	4.96	2.80	13.78	5.16
B9	CO	17926053	17926053	35852106	17584512	17584512	35169024	91.68	6.36	1.96	11.91	5.26
B10	SOY	16072772	16072772	32145544	15836940	15836940	31673880	91.88	5.94	2.18	14.93	5.39
B11	SOY	17953371	17953371	35906742	17705414	17705414	35410828	92.82	5.01	2.18	14.59	5.08
B12	SOY	20082967	20082967	40165934	19805222	19805222	39610444	92.02	5.48	2.50	12.64	5.45
B15	FO	18301808	18301808	36603616	18037486	18037486	36074972	92.29	5.21	2.50	14.46	5.21
B17	FO	18102172	18102172	36204344	17917883	17917883	35835766	93.36	4.35	2.29	11.89	5.35
B18	SOY	17327254	17327254	34654508	17068514	17068514	34137028	93.39	4.50	2.10	18.82	4.88
B19	SOY	16914885	16914885	33829770	16709885	16709885	33419770	92.55	5.26	2.19	13.90	5.11
B20	FO	17270705	17270705	34541410	17031967	17031967	34063934	91.95	5.48	2.57	15.01	4.96
B21	FO	17406635	17406635	34813270	17165388	17165388	34330776	91.61	6.47	1.92	14.12	5.12
B22	CO	17363940	17363940	34727880	17124299	17124299	34248598	88.23	5.31	6.46	10.71	5.19
B23	FO	15251081	15251081	30502162	15022834	15022834	30045668	87.70	5.06	7.23	14.59	5.07
B24	CO	17135468	17135468	34270936	16921878	16921878	33843756	92.41	5.08	2.51	14.01	5.09
B25	CO	19136511	19136511	38273022	18865143	18865143	37730286	92.25	5.36	2.39	14.32	5.15
B26	SOY	17349376	17349376	34698752	17136861	17136861	34273722	91.69	4.84	3.47	16.09	4.84
B27	FO	17565119	17565119	35130238	17315786	17315786	34631572	92.72	4.76	2.52	15.17	5.22
B29	FO	17405629	17405629	34811258	17203298	17203298	34406596	91.71	5.82	2.47	15.84	4.98
B30	FO	17606717	17606717	35213434	17357758	17357758	34715516	91.83	5.47	2.70	15.79	5.07
B31	FO	17592378	17592378	35184756	17366918	17366918	34733836	91.60	5.79	2.61	13.49	5.12
B36	FO	18034282	18034282	36068564	17829010	17829010	35658020	94.77	3.03	2.20	15.25	5.21
B38	FO	17012709	17012709	34025418	16795381	16795381	33590762	92.10	4.38	3.52	16.03	4.98
B39	CO	16295027	16295027	32590054	16071312	16071312	32142624	92.48	5.32	2.20	13.99	5.39
B40	FO	16704823	16704823	33409646	16484149	16484149	32968298	93.43	4.31	2.26	15.44	5.00

B42	CO	14825066	14825066	29650132	14638565	14638565	29277130	93.67	3.82	2.51	19.51	4.89
B44	CO	15565107	15565107	31130214	15354453	15354453	30708906	88.88	4.83	6.29	11.44	5.10
B45	CO	15415616	15415616	30831232	15220862	15220862	30441724	88.34	4.33	7.33	10.60	5.43
B46	SOY	14759998	14759998	29519996	14593462	14593462	29186924	96.13	1.34	2.52	12.16	5.59
B48	FO	16699654	16699654	33399308	16498103	16498103	32996206	94.72	3.29	1.99	13.91	5.24
B50	CO	17325520	17325520	34651040	17112019	17112019	34224038	91.62	5.28	3.09	15.33	4.86
B51	FO	16678347	16678347	33356694	16445673	16445673	32891346	88.60	6.43	4.97	11.09	4.83
B52	CO	16776789	16776789	33553578	16544368	16544368	33088736	92.66	4.97	2.37	14.72	4.95
B53	SOY	16978283	16978283	33956566	16778279	16778279	33556558	90.84	5.97	3.19	15.63	5.11
B54	SOY	16207227	16207227	32414454	16018594	16018594	32037188	92.44	5.21	2.35	14.48	5.12
B55	SOY	16300470	16300470	32600940	16086128	16086128	32172256	90.81	6.43	2.77	14.48	5.01
B57	FO	17697551	17697551	35395102	17416291	17416291	34832582	91.92	5.75	2.33	12.73	5.57
B58	CO	16371810	16371810	32743620	16161131	16161131	32322262	92.00	5.96	2.04	13.98	5.11
B59	SOY	19113710	19113710	38227420	18860382	18860382	37720764	92.90	4.84	2.27	14.45	5.18
B60	CO	16025169	16025169	32050338	15812937	15812937	31625874	92.69	5.29	2.02	12.58	5.23
B61	SOY	19532678	19532678	39065356	19256541	19256541	38513082	92.65	5.02	2.33	14.60	5.09
B63	SOY	17021291	17021291	34042582	16786108	16786108	33572216	92.09	5.56	2.35	12.91	5.66
B64	CO	17774840	17774840	35549680	17518165	17518165	35036330	92.00	5.51	2.49	13.95	5.08
B65	CO	17062209	17062209	34124418	16867471	16867471	33734942	93.06	4.62	2.32	15.57	5.30
B67	CO	17134028	17134028	34268056	16922744	16922744	33845488	92.46	5.45	2.09	14.95	5.21
B68	SOY	17077873	17077873	34155746	16862359	16862359	33724718	92.49	5.45	2.06	14.23	5.03
B69	SOY	16448318	16448318	32896636	16233190	16233190	32466380	92.10	5.77	2.13	11.74	5.50
B70	SOY	15547760	15547760	31095520	15344621	15344621	30689242	92.41	5.21	2.38	14.88	5.28
B71	CO	15280939	15280939	30561878	15074764	15074764	30149528	93.70	3.95	2.35	11.61	5.68
B72	SOY	16907211	16907211	33814422	16727130	16727130	33454260	91.64	4.33	4.03	11.67	5.10

Table SF3 - DEG of brain samples from pigs fed different oil sources

Comparision	Gene_stable_id	Gene_name	Gene_description	log2 fold change	*p*-value	FDR
CO*vs*FO	ENSSSCG00000051166	Novel	0	-5.231841503	1.54E-09	3.03E-05
CO*vs*FO	ENSSSCG00000041461	Novel	0	0.972687171	4.60E-09	4.52E-05
CO*vs*FO	ENSSSCG00000006793	Novel	0	-1.569089079	8.08E-09	5.29E-05
CO*vs*FO	ENSSSCG00000041596	Novel	0	3.983033766	2.63E-08	0.000129141
CO*vs*FO	ENSSSCG00000023338	CHRNA6	cholinergic receptor nicotinic alpha 6 subunit [Source:VGNC Symbol;Acc:VGNC:95723]	2.291985007	1.05E-07	0.000412764
CO*vs*FO	ENSSSCG00000037563	5_8S_rRNA	5.8S ribosomal RNA [Source:RFAM;Acc:RF00002]	1.466380941	1.66E-07	0.000543366
CO*vs*FO	ENSSSCG00000007237	PDRG1	p53 and DNA damage regulated 1 [Source:VGNC Symbol;Acc:VGNC:95745]	0.418085193	2.15E-07	0.000604777
CO*vs*FO	ENSSSCG00000009942	DAO	D-amino acid oxidase [Source:VGNC Symbol;Acc:VGNC:87155]	2.804635985	4.88E-07	0.001198518
CO*vs*FO	ENSSSCG00000010981	NUDT2	nudix hydrolase 2 [Source:VGNC Symbol;Acc:VGNC:96457]	0.428959368	1.41E-06	0.003083123
CO*vs*FO	ENSSSCG00000034990	Novel	0	-0.739734801	1.92E-06	0.003763269
CO*vs*FO	ENSSSCG00000045913	Novel	0	1.268505951	3.92E-06	0.00641146
CO*vs*FO	ENSSSCG00000001844	PLIN1	perilipin 1 [Source:VGNC Symbol;Acc:VGNC:91557]	1.55780543	3.79E-06	0.00641146
CO*vs*FO	ENSSSCG00000014899	PRCP	prolylcarboxypeptidase [Source:VGNC Symbol;Acc:VGNC:91771]	0.446646764	7.32E-06	0.011060523
CO*vs*FO	ENSSSCG00000039977	Novel	0	-1.722449934	9.56E-06	0.01342109
CO*vs*FO	ENSSSCG00000041875	Novel	0	2.964306609	1.03E-05	0.013512532
CO*vs*FO	ENSSSCG00000014943	DEUP1	deuterosome assembly protein 1 [Source:VGNC Symbol;Acc:VGNC:87264]	-0.477195478	1.28E-05	0.015776877
CO*vs*FO	ENSSSCG00000017993	NTN1	netrin 1 [Source:VGNC Symbol;Acc:VGNC:90931]	1.057899496	1.43E-05	0.016501153
CO*vs*FO	ENSSSCG00000018044	ALDH3A1	aldehyde dehydrogenase 3 family member A1 [Source:VGNC Symbol;Acc:VGNC:85238]	-1.620829048	2.00E-05	0.021864808
CO*vs*FO	ENSSSCG00000045477	Novel	0	-0.889610126	2.36E-05	0.022926492
CO*vs*FO	ENSSSCG00000001750	PAQR8	progestin and adipoQ receptor family member 8 [Source:VGNC Symbol;Acc:VGNC:91178]	0.423920888	2.62E-05	0.022926492
CO*vs*FO	ENSSSCG00000037070	GRID2IP	Grid2 interacting protein [Source:VGNC Symbol;Acc:VGNC:88675]	1.716174141	2.37E-05	0.022926492
CO*vs*FO	ENSSSCG00000006125	CALB1	calbindin 1 [Source:VGNC Symbol;Acc:VGNC:86138]	2.613911687	2.62E-05	0.022926492
CO*vs*FO	ENSSSCG00000046775	Novel	0	2.695974667	2.70E-05	0.022926492
CO*vs*FO	ENSSSCG00000008823	SLC10A4	solute carrier family 10 member 4 [Source:VGNC Symbol;Acc:VGNC:92917]	3.05771148	2.80E-05	0.022926492
CO*vs*FO	ENSSSCG00000003768	NEXN	nexilin F-actin binding protein [Source:VGNC Symbol;Acc:VGNC:98154]	1.971407232	4.35E-05	0.034220878
CO*vs*FO	ENSSSCG00000009092	TRPC3	transient receptor potential cation channel subfamily C member 3 [Source:VGNC Symbol;Acc:VGNC:94461]	1.626726778	4.56E-05	0.034485573
CO*vs*FO	ENSSSCG00000001764	SH2D7	SH2 domain containing 7 [Source:VGNC Symbol;Acc:VGNC:92814]	1.255687911	4.83E-05	0.03513422

CO*vs*FO	ENSSSCG00000034360	CELSR2	cadherin EGF LAG seven-pass G-type receptor 2 [Source:VGNC Symbol;Acc:VGNC:98748]	-0.688082321	5.88E-05	0.041250187
CO*vs*FO	ENSSSCG00000032113	Novel	0	-1.006058651	6.26E-05	0.042195777
CO*vs*FO	ENSSSCG00000016808	C5orf22	chromosome 16 C5orf22 homolog [Source:VGNC Symbol;Acc:VGNC:85951]	-0.330151465	6.56E-05	0.042195777
CO*vs*FO	ENSSSCG00000035583	Novel	0	0.890224693	6.66E-05	0.042195777
CO*vs*FO	ENSSSCG00000032599	Novel	0	1.964571472	6.90E-05	0.042387751
CO*vs*FO	ENSSSCG00000027052	PEX11A	peroxisomal biosis factor 11 alpha [Source:VGNC Symbol;Acc:VGNC:91321]	0.511052367	7.26E-05	0.04324725
CO*vs*FO	ENSSSCG00000016733	Novel	NAC alpha domain containing [Source:VGNC Symbol;Acc:VGNC:90562]	0.454843293	7.56E-05	0.043694502
CO*vs*FO	ENSSSCG00000012832	Novel	0	-0.720281859	8.41E-05	0.04719434
CO*vs*FO	ENSSSCG00000048831	Novel	0	-0.645976106	9.26E-05	0.047863163
CO*vs*FO	ENSSSCG00000026663	DTD2	D-aminoacyl-tRNA deacylase 2 [Source:VGNC Symbol;Acc:VGNC:97970]	-0.509695144	9.50E-05	0.047863163
CO*vs*FO	ENSSSCG00000007003	Novel	0	0.559446585	9.47E-05	0.047863163
CO*vs*FO	ENSSSCG00000035669	Novel	0	1.896051161	9.00E-05	0.047863163
SOY*vs*CO	ENSSSCG00000051166	Novel	0	-5.509834138	7.16E-11	1.37E-06
SOY*vs*CO	ENSSSCG00000018044	ALDH3A1	aldehyde dehydrogenase 3 family member A1 [Source:VGNC Symbol;Acc:VGNC:85238]	-1.978069351	4.47E-07	0.004284225
SOY*vs*CO	ENSSSCG00000014899	PRCP	prolylcarboxypeptidase [Source:VGNC Symbol;Acc:VGNC:91771]	0.518130921	8.03E-07	0.005133238
SOY*vs*CO	ENSSSCG00000012448	ITM2A	integral membrane protein 2A [Source:VGNC Symbol;Acc:VGNC:89250]	0.445623011	1.27E-06	0.00609592
SOY*vs*CO	ENSSSCG00000015592	TATDN3	TatD DNase domain containing 3 [Source:VGNC Symbol;Acc:VGNC:93753]	-0.772567771	2.56E-06	0.009841753
SOY*vs*FO	ENSSSCG00000014899	PRCP	prolylcarboxypeptidase [Source:VGNC Symbol;Acc:VGNC:91771]	0.580042314	1.32E-14	2.48E-10
SOY*vs*FO	ENSSSCG00000051166	Novel	0	-5.329652898	1.96E-09	1.84E-05
SOY*vs*FO	ENSSSCG00000050056	CCDC201	coiled-coil domain containing 201 [Source:HGNC Symbol;Acc:HGNC:54081]	0.8475696	1.40E-08	8.74E-05
SOY*vs*FO	ENSSSCG00000041596	Novel	0	3.21094389	2.98E-08	0.000140093
SOY*vs*FO	ENSSSCG00000031154	ACKR2	atypical chemokine receptor 2 [Source:VGNC Symbol;Acc:VGNC:85012]	-1.25953119	8.45E-08	0.000317298
SOY*vs*FO	ENSSSCG00000022842	Novel	0	-2.606873419	1.99E-07	0.000551483
SOY*vs*FO	ENSSSCG00000008725	CYTL1	cytokine like 1 [Source:VGNC Symbol;Acc:VGNC:87138]	-1.209825919	2.06E-07	0.000551483
SOY*vs*FO	ENSSSCG00000049992	Novel	0	-0.59218671	5.24E-07	0.001229983
SOY*vs*FO	ENSSSCG00000041875	Novel	0	2.39680335	6.73E-07	0.00126437
SOY*vs*FO	ENSSSCG00000008731	OTOP1	otopetrin 1 [Source:VGNC Symbol;Acc:VGNC:91093]	1.974362961	6.71E-07	0.00126437
SOY*vs*FO	ENSSSCG00000048787	Novel	0	0.682458536	1.28E-06	0.002192313
SOY*vs*FO	ENSSSCG00000007816	IL21R	interleukin 21 receptor [Source:VGNC Symbol;Acc:VGNC:89096]	1.947426094	2.21E-06	0.003461065
SOY*vs*FO	ENSSSCG00000049532	Novel	0	0.507374405	2.61E-06	0.003763819

SOY*vs*FO	ENSSSCG00000034360	CELSR2	cadherin EGF LAG seven-pass G-type receptor 2 [Source:VGNC Symbol;Acc:VGNC:98748]	-0.771437332	3.00E-06	0.004026128
SOY*vs*FO	ENSSSCG00000017915	VMO1	vitelline membrane outer layer 1 homolog [Source:VGNC Symbol;Acc:VGNC:94833]	2.117646331	6.39E-06	0.008002504
SOY*vs*FO	ENSSSCG00000041734	Novel	0	3.318406435	7.91E-06	0.009286113
SOY*vs*FO	ENSSSCG00000045891	Novel	0	-2.867347779	1.37E-05	0.015162187
SOY*vs*FO	ENSSSCG00000014168	ELL2	elongation factor for RNA polymerase II 2 [Source:VGNC Symbol;Acc:VGNC:87649]	0.560861515	1.49E-05	0.015580754
SOY*vs*FO	ENSSSCG00000006821	GSTM3	glutathione S-transferase mu 3 [Source:VGNC Symbol;Acc:VGNC:88726]	1.26753455	1.63E-05	0.016066443
SOY*vs*FO	ENSSSCG00000029805	RHOBTB3	Rho related BTB domain containing 3 [Source:VGNC Symbol;Acc:VGNC:92286]	0.523878745	1.82E-05	0.017133771
SOY*vs*FO	ENSSSCG00000028724	N6AMT1	N-6 adenine-specific DNA methyltransferase 1 [Source:VGNC Symbol;Acc:VGNC:104001]	-0.454023549	1.95E-05	0.017460995
SOY*vs*FO	ENSSSCG00000018044	ALDH3A1	aldehyde dehydrogenase 3 family member A1 [Source:VGNC Symbol;Acc:VGNC:85238]	-1.557228126	2.30E-05	0.017985445
SOY*vs*FO	ENSSSCG00000020522	Novel	0	-3.139055205	2.30E-05	0.017985445
SOY*vs*FO	ENSSSCG00000010505	DNTT	DNA nucleotidylexotransferase [Source:VGNC Symbol;Acc:VGNC:87386]	1.372857284	2.28E-05	0.017985445
SOY*vs*FO	ENSSSCG00000041251	Novel	0	-1.397413063	2.60E-05	0.019498269
SOY*vs*FO	ENSSSCG00000016318	ASB18	ankyrin repeat and SOCS box containing 18 [Source:VGNC Symbol;Acc:VGNC:95922]	-0.914151779	2.75E-05	0.019548332
SOY*vs*FO	ENSSSCG00000027178	MAN2B2	mannosidase alpha class 2B member 2 [Source:VGNC Symbol;Acc:VGNC:98100]	1.209520085	2.81E-05	0.019548332
SOY*vs*FO	ENSSSCG00000011157	PITRM1	pitrilysin metallopeptidase 1 [Source:VGNC Symbol;Acc:VGNC:95855]	-0.38776794	3.34E-05	0.021930024
SOY*vs*FO	ENSSSCG00000017022	HMMR	hyaluronan mediated motility receptor [Source:VGNC Symbol;Acc:VGNC:88911]	0.907585795	3.39E-05	0.021930024
SOY*vs*FO	ENSSSCG00000000063	POLR3H	RNA polymerase III subunit H [Source:VGNC Symbol;Acc:VGNC:91658]	-0.440191967	4.04E-05	0.025138597
SOY*vs*FO	ENSSSCG00000032599	Novel	0	1.535908471	4.15E-05	0.025138597
SOY*vs*FO	ENSSSCG00000023338	CHRNA6	cholinergic receptor nicotinic alpha 6 subunit [Source:VGNC Symbol;Acc:VGNC:95723]	1.949879798	4.59E-05	0.026940079
SOY*vs*FO	ENSSSCG00000046723	Novel	0	1.376264409	4.93E-05	0.028049153
SOY*vs*FO	ENSSSCG00000048799	Novel	0	1.980649729	5.32E-05	0.029393721
SOY*vs*FO	ENSSSCG00000023320	CYP3A29	cytochrome P450 family 3 subfamily A member 22 [Source:NCBI gene (formerly Entrezgene);Acc:100144468]	-2.492002133	5.66E-05	0.030364501
SOY*vs*FO	ENSSSCG00000028294	CATSPER3	cation channel sperm associated 3 [Source:VGNC Symbol;Acc:VGNC:86211]	-1.520907827	6.11E-05	0.03188154
SOY*vs*FO	ENSSSCG00000014170	CAST	calpastatin [Source:VGNC Symbol;Acc:VGNC:99603]	0.393910667	6.39E-05	0.032448633
SOY*vs*FO	ENSSSCG00000007099	SCP2D1	SCP2 sterol binding domain containing 1 [Source:VGNC Symbol;Acc:VGNC:105249]	-1.855259332	7.02E-05	0.033294839
SOY*vs*FO	ENSSSCG00000011493	ATXN7	ataxin 7 [Source:VGNC Symbol;Acc:VGNC:99704]	-0.378254566	7.09E-05	0.033294839
SOY*vs*FO	ENSSSCG00000041461	Novel	0	0.842274438	6.98E-05	0.033294839

SOY*vs*FO	ENSSSCG00000000333	Novel	0	2.360837509	8.25E-05	0.037791284
SOY*vs*FO	ENSSSCG00000010981	NUDT2	nudix hydrolase 2 [Source:VGNC Symbol;Acc:VGNC:96457]	0.369822558	8.46E-05	0.037841121
SOY*vs*FO	ENSSSCG00000003814	EFCAB7	EF-hand calcium binding domain 7 [Source:VGNC Symbol;Acc:VGNC:87566]	-0.372587121	8.83E-05	0.03854647
SOY*vs*FO	ENSSSCG00000017521	SCRN2	secernin 2 [Source:VGNC Symbol;Acc:VGNC:92648]	-0.361068565	9.62E-05	0.041040563

Table SF4 – Annotated DEG of brain samples from pigs fed different oil sources

Venn diagram distribution (by comparison)	Gene stable id				
[SOY*vs*CO]	ENSSSCG00000012448	ENSSSCG00000015592			
[SOY*vs*FO]	ENSSSCG00000050056	ENSSSCG00000031154	ENSSSCG00000008725	ENSSSCG00000008731	ENSSSCG00000007816
	ENSSSCG00000017915	ENSSSCG00000014168	ENSSSCG00000006821	ENSSSCG00000029805	ENSSSCG00000028724
	ENSSSCG00000010505	ENSSSCG00000016318	ENSSSCG00000027178	ENSSSCG00000011157	ENSSSCG00000017022
	ENSSSCG00000000063	ENSSSCG00000023320	ENSSSCG00000028294	ENSSSCG00000014170	ENSSSCG00000007099
	ENSSSCG00000011493	ENSSSCG00000003814	ENSSSCG00000017521		
[CO*vs*FO]	ENSSSCG00000037563	ENSSSCG00000007237	ENSSSCG00000009942	ENSSSCG00000001844	ENSSSCG00000014943
	ENSSSCG00000017993	ENSSSCG00000001750	ENSSSCG00000037070	ENSSSCG00000006125	ENSSSCG00000008823
	ENSSSCG00000003768	ENSSSCG00000009092	ENSSSCG00000001764	ENSSSCG00000016808	ENSSSCG00000027052
	ENSSSCG00000026663				
[SOY*vs*FO] and [CO*vs*FO]	ENSSSCG00000034360	ENSSSCG00000023338	ENSSSCG00000010981		
[SOYvsCO] and [SOY*vs*FO] and [CO*vs*FO]	ENSSSCG00000018044	ENSSSCG00000014899			

Table SF5 - Fatty acid profile (%) of the oils used to supplement the animal's diet

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

[1]ND = Not detected.