



FATTY ACID PROFILE IN BRAIN AND HEPATIC TISSUES FROM PIGS SUPPLEMENTED WITH CANOLA OIL

Perfil de ácidos graxos nos tecidos cerebral e hepático de suínos suplementados com óleo de canola

Julia P. M. SILVA, Karine A. COSTA², Vivian V. ALMEIDA³, Luiz L. COUTINHO⁴, Bruna P. M. Silva⁵, Aline S. M. CESAR^{6*}

ABSTRACT: Canola oil is an important source of oleic acid, in addition to being an accessible source for its use in the production of pigs' diets. Oleic acid in turn is a type of unsaturated fatty acid that in pork is beneficial for human health. Therefore, this study was conducted to describe the fatty acid profile in brain and liver tissues from pigs supplemented with canola oil for 98 days, during the growth and finishing phases. For the analysis was used eighteen male pigs that had free access to feed and water throughout the experimental period. Dietary treatment consisted of corn-soybean meal growing-finishing diets supplemented with 3% fat from canola oil (CO). To obtain the fatty acid profile, the lipids from each tissue were cold extracted using the adapted method from Bligh and Dyer, methylated, and posteriorly injected in a gas chromatograph to obtain the fatty acid profile of the tissue. In both tissues there was a greater abundance of saturated fatty acids (stearic acid). The most abundant monounsaturated fatty acid was the oleic acid. Regarding polyunsaturated fatty acids, in the liver the most abundant was linoleic acid and in the brain docosahexaenoic acid. In summary, animals' diet influences the fatty acid profile in different tissues. Such modifications can increase unsaturated fatty acids concentration in relation to saturated, making pork healthier for human consumption.

Key words: human health, liver, healthy pork, oleic acid

RESUMO: O óleo de canola é uma fonte importante de ácido oleico, além de ser uma fonte acessível para utilização nas dietas de suínos. O ácido oleico, por sua vez, é um tipo de ácido graxo insaturado que, na carne suína, é benéfico para a saúde humana. Portanto, este estudo foi realizado com o objetivo de descrever o perfil de ácidos graxos nos tecidos cerebral e hepático de suínos suplementados com óleo de canola por 98 dias, nas fases de crescimento e terminação. Para análise foram utilizados dezoito machos que tiveram livre acesso a ração e água durante todo o período experimental. O tratamento dietético consistiu em dietas de crescimento e terminação de farelo de milho/soja suplementadas com 3% de óleo de canola (CO). Para obtenção do perfil de ácidos graxos, os lipídios de cada tecido foram extraídos a frio pelo método adaptado de Bligh e Dyer, e posteriormente, as amostras foram inseridas em um cromatógrafo a gás para obtenção do perfil de ácidos graxos do tecido. Em ambos os tecidos houve maior abundância de ácidos graxos saturados (ácido esteárico). O ácido graxo monoinsaturado mais abundante foi o ácido oleico. Em relação aos ácidos graxos poli-insaturados, no fígado o mais abundante foi o ácido linoléico e no cérebro o ácido docosahexaenóico. Em resumo, a dieta dos animais influencia no perfil dos ácidos graxos em diferentes tecidos. Tais modificações podem aumentar a concentração de ácidos graxos insaturados em relação aos saturados, tornando a carne suína mais saudável para o consumo humano.

Palavras-chave: saúde humana, fígado, carne suína saudável, ácido oleico

*Autor para correspondência

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¹Undergraduate Student, Department of AgriFood Industry, Food and Nutrition, University of São Paulo, Piracicaba, SP, 13418-900, Brazil, juliamartins@usp.br.

²Post-Doctor, Department of AgriFood Industry, Food and Nutrition, University of São Paulo, Piracicaba, SP, 13418-900, Brazil, kryneacosta@yahoo.com.br.

³Professor, Department of Animal Science, Federal University of Goiás, Goiânia, GO, 74690-900, Brazil, vivian.almeida@ufg.br.

⁴Professor, Department of Animal Science, University of São Paulo, Piracicaba, SP, 13418-900, Brazil, llcouthino@usp.br.

⁵Master's Student, Department of AgriFood Industry, Food and Nutrition, University of São Paulo, Piracicaba, SP, 13418-900, Brazil, brunamartins@usp.br.

⁶Professor, Department of AgriFood Industry, Food and Nutrition, University of São Paulo, Piracicaba, SP, 13418-900, Brazil, alinecesar@usp.br.

INTRODUCTION

Pork is an important source of animal protein, being the most consumed meat in the world. In addition to genetics, numerous factors influence the meat characteristics, one of these factors is the animals' nutrition. In pigs, nutritional management influences the fatty acid profile in the meat (WOOD et al., 2008; LU et al., 2020).

Fatty acid composition contributes significantly to several aspects of meat quality and nutritional value of meat for human consumption (WOOD et al., 2008). Saturated fatty acids (SFA) play important roles in cellular and tissue function and metabolism, but they also influence factors involved in increased risk of cardiovascular disease, type 2 diabetes and other metabolic diseases when over-consumption (CALDER, 2015). However, the unsaturated fatty acids in the meat are beneficial to human health (CAMERON et al., 2000). It is known that pork is an important source of unsaturated fatty acids such as oleic and linoleic acid (KRITCHEVSKY, 2000; TERÉS et al., 2008; PAUWELS, 2011; SCHMID, 2011), these in turn are important for human health (LAAKSONEN et al., 2015) by reducing blood cholesterol levels, the occurrence of diabetes type 2, and neurodegenerative diseases such as Parkinson and Alzheimer. Studies in humans have evaluated the effect of replacing saturated fatty acids in the diet with monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) (CALDER, 2015).

There are several plant oilseeds with high concentration of unsaturated fatty acids such as linoleic and oleic acids. In this context, the supplementation of pigs' diets with oilseeds have been a strategy to enhance the content of these acids in the meat, leading to the production of healthier meat (HUANG et al., 2020). Besides that, there is interest in adding fats and oils to swine diets to improve growth rate and feed efficiency in different phases of the swine production (THACKER, 1998). Canola oil is an important source of oleic acid and according to Carrapiso et al. (2020) pigs fed on the high oleic acid-content diet tended to have higher monounsaturated fatty acids and lean brightness.

Studies that show the effects of lipids supplementation of pigs diets in tissues fatty acids profile are important, since it is evident that fatty acids have a range of general and specific biological activities and influence health, well-being and disease risk (CALDER, 2015). In this sense, our aim was to evaluate the fatty acid profile in liver and brain tissues from pigs supplemented with canola oil at the growing-finishing phase.

MATERIAL AND METHODS

Ethical procedures, animals, experimental design and diet

Experimental protocol was approved by the "Luiz de Queiroz" College of Agriculture Animal Care and Use Committee (University of São Paulo, Piracicaba, Brazil, number CEUA 2018-28) and followed ethical principles in animal research according to the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

A total of 96 immunocastrated male pigs genetically lean of Large White breed and halothane homozygous-negative (NN) were used in a 98-day study. The animals were blocked by initial body weight (28.44 ± 2.95 kg) and allotted to a randomized complete block design with four treatments, six replicate pens per treatment, and four pigs per pen. Pigs were housed in an all-in/all-out double-curtain-sided building with partially slatted concrete floor pens. All pigs had *ad libitum* access to feed and water throughout the experimental period. Intact male pigs were injected with 2-mL primer dose (Vivax®, Pfizer Animal Health, Parkville, Australia) on day 56 and second 2-mL dose on day 71.

Dietary treatment consisted of corn-soybean meal growing-finishing diets supplemented with 3% fat from canola oil (CO). The canola-based oil treatment used in this study was high in OA content (64.2%) and low in alpha-linolenic acid content (C18:3 n-3; 7.6%) compared to traditional CO (56.1% OA and 9.3% C18:3 n-3) available on the market as described in NRC (2012).

Pig slaughter and fatty acid profile analysis

At the completion of the trial, three pigs from each pen, totaling 18 pigs per treatment ($n = 72$), were randomly chosen and slaughtered according to the industry standards after a 16-hour rest period in the lairage pens. Animals were slaughtered by electrical stunning followed by exsanguination.

After slaughtering the animals, samples from the brain and liver of the animals were collected. For the collection of the brain, the carcass of the animal was split in the two longitudinal parts including the head and the tissue were collected, then the samples were identified and immediately stored in liquid nitrogen. For the hepatic tissue sample, the animals were eviscerated, and part of the liver was cut and collected, packed in appropriate plastic bags, identified and stored in refrigerated transport boxes to be sent to the laboratory where the analyzes would be carried out.

To obtain the fatty acid profile, the lipids from each tissue were cold extracted first using the method adapted from Bligh and Dyer (1959). Initially, the sample was weighed (liver 25g; brain 10g), methanol: chloroform (2: 1 in relation to the amount of sample) was added and homogenized for 4 minutes, an additional part of chloroform was added and homogenized by one minute. The contents were filtered using filter paper and one part of chloroform was poured over the sample retained on the filter paper to remove any lipid residues.

The content obtained by filtration was poured into an appropriate separating funnel and added with one part of 0.88% KCl solution, stirring to separate the phases. The lower phase obtained in the separating funnel was collected through a glass funnel with filter paper and anhydrous sodium sulfate at the bottom, being collected in a flat-bottomed amber flask. The flask was coupled to a Rotary Evaporator (TE-210 Tecnal brand) coupled to a Tecnal TE-0581 vacuum pump to eliminate the solvent. The extracted fat was collected from the bottom of the balloon and stored under freezing. Methylation of the extracted lipid samples was performed according to Hartman (1973), with adaptations based on the AOCS method (2003).

After methylating the liver and brain samples, they were inserted in a gas chromatograph to obtain tissue fatty

acid profile. After this stage, the methyl esters went to the stage of analysis by high-performance gas chromatography, using a Shimadzu GC-2010 plus AF chromatograph, equipped with an RTX-Wax column (30m; 0.32mm; 0.25 μ m) – Crossbond Carbowax of polyethylene glycol, to a flame ionization detector, with auto injector and Split injection. An internal standard solution of 0.1mg / ml concentration was prepared in hexane, and 100 μ l of this solution was added directly to 1mL of the methylated sample. The injector and detector temperatures were set at 250 ° C. The carrier gas was nitrogen with an average linear velocity of 1.2 mL / min. The sample was injected at a ratio of 1:10 to the liver and 1: 3 to the brain in Split mode. The column oven was programmed as follows: 60 ° C (maintained for 0 minutes), 20 ° C / minute until 210 ° C (maintained for 7 minutes), 30 ° C / minute until 240 ° C (maintained for 18 minutes).

The saturated and unsaturated methyl esters produced were identified by comparing the retention time with the fatty acid methyl esters of the Fatty Acid Methyl Esters (FAME) standard C8 - C22 (Supelco Analytical). For the identification of EPA and DHA fatty acids, CG-MS analysis was performed at Laboratory of Biochemistry and Instrumental Analysis of Escola Superior de Agricultura “Luiz de Queiroz” (ESALQ) of University of São Paulo (USP).

Statistical analyses

A descriptive analysis of the data sets was performed using the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC).

RESULTS AND DISCUSSION

The descriptive analysis of hepatic tissue fatty acid composition of growing-finishing pigs supplemented with canola oil are shown in Table 1. We observed that the liver tissue mainly presents SFA, followed by MUFA and PUFA (means = 45.66%, 27.24% and 26.46%, respectively). Among the SFA the most prevalent is stearic acid with an average of 24.95%, followed by palmitic acid (20.59%) and myristic acid (0.79%). In the human body, palmitic and stearic acids are also the most common SFA (STAWARSKA; JELI; BOBROWSKA-KORCZAK, 2020). The most abundant of the MUFA is the oleic acid (Mean = 26.61%), the palmitoleic acid was observed in the 0.63%. Oleic acid has beneficial impacts in many processes such as cardiovascular diseases, rheumatoid arthritis (BERBERT et al., 2005), organ dysfunction, plasma non-esterified fatty acid concentration, the reactive oxygen species synthesis, production of pro-inflammatory cytokines (GONÇALVES-DE-ALBUQUERQUE; MEDEIROS-DE-MORAES, 2016; MEDEIROS-DE-MORAES et al., 2018) and on the risk of some cancer (ESCRICH; MORAL, 2011). Among PUFA the most prevalent is linoleic acid (Mean = 24.02%) followed by docosahexaenoic acid (1.02%), alpha-linoleic acid (1.00%) and eicosapentaenoic acid (0.36%).

Our results show that the ratio of PUFA to SFA has an average of 0.58 in the liver. Besides the fatty acid composition, the ratios between SFA, MUFA, and PUFA play an important role in cellular processes and metabolism (STAWARSKA; JELI; BOBROWSKA-KORCZAK, 2020), being important to maintain the balance between SFA and

unsaturated fatty acids (STAWARSKA; JELI; BOBROWSKA-KORCZAK, 2020). The disruption of fatty acid ratios may lead to pathological states such as cardiovascular and neurodegenerative diseases and cancer (CARTA et al., 2017). In this context, the replacement of SFA with MUFA, administered as vegetable oil results in the reduction of metabolic diseases (SCHWINGSHACKL; HOFFMANN, 2014). Stawarska et al. (2020) observed that dietary supplementation with vegetable oils caused a decrease in the content of SFA in serum from rats, and the type of supplementation influenced the MUFA and PUFA amount.

The intake ratio of n-6 / n-3 fatty acids is important since they have different body functions and influences in the body metabolism (PAWLOSKY et al., 2003). A high n-6 content in the diet may increase the synthesis of inflammatory compounds that contribute to the formation of thrombi and atheroma in the body, while the n-3 obtained by the diet act as partial substitutes for n-6 favoring the formation of anti-inflammatory compounds (SURETTE, 2008). Thus, alternatives that are capable of altering the n-6 / n-3 ratio are being increasingly used. The n-3 ingested by humans in the diet partially replaces the n-6 fatty acids mainly in the membranes and liver cells, modifying their fatty acid composition, favoring the anti-inflammatory compounds formation (SURETTE, 2008).

According to Trumbo et al., (2002), the amount of 10:1 to 5:1 was considered to be a satisfactory n-6 / n-3 ratio. In another study, the optimal dietary omega-6 to omega-3 PUFA ratio was determined in 2:1 or lesser and the Western diet is usually established in the range of 10:1 to 25:1 (WEISER; BUTT; MOHAJERI, 2016). The n-6: n-3 PUFA ratio average in this study was 10.30 PUFA belonging to n-6 and n-3 families, are thought to participate in regulation of inflammation, glycemic control, lipid metabolism, oxidative stress, cardiovascular diseases (CVD), skin changes, asthma, nervous system disturbances, or cancer, summarizing in many physiological and pathological processes (KRIS-ETHERTON; FLEMING; HARRIS, 2010; CALDER, 2012; LI et al., 2018). Table 1 presents more information about fatty acid composition of hepatic tissue of growing-finishing pigs fed a corn-soybean meal diet containing 3% canola oil.

Table 1. Descriptive analysis for fatty acid composition (%) of hepatic tissue of immunocastrated males pigs fed a corn-soybean meal diet containing 3% canola oil

Item	Minimum	Mean	Maximum	SD ¹	CV, % ²
Ether extract, %	1.31	1.75	2.12	0.31	17.40
Saturated fatty acid (SFA)					
Myristic acid (C14:0)	0.64	0.79	0.93	0.10	12.91
Palmitic acid (C16:0)	19.58	20.59	21.55	0.91	4.41
Stearic acid (C18:0)	22.17	24.95	28.03	2.35	9.40
Monounsaturated fatty acid (MUFA)					
Palmitoleic acid (C16:1)	0.61	0.63	0.67	0.02	3.39
Oleic acid (C18:1 n-9)	25.35	26.61	28.93	1.43	5.37
Polyunsaturated fatty acid (PUFA)					
Linoleic acid (C18:2 n-6)	22.79	24.02	24.94	0.90	3.76
Alpha-linolenic acid (C18:3 n-3)	0.87	1.00	1.15	0.12	11.79
Eicosapentaenoic acid (C20:5 n-3, EPA)	0.26	0.36	0.63	0.14	38.08
Docosahexaenoic acid (C22:6 n-3, DHA)	0.72	1.02	1.23	0.23	22.49
Total SFA	44.47	45.66	47.64	1.33	2.91
Total MUFA	25.87	27.24	29.57	1.47	5.40
Total PUFA	25.06	26.46	28.13	1.19	4.50
Total n-3 PUFA ³	1.97	2.32	2.69	0.23	10.04
Total n-6 PUFA ⁴	22.79	24.02	24.94	0.90	3.76
PUFA:SFA ratio ⁵	0.53	0.58	0.61	0.04	6.20
n-6:n-3 PUFA ratio ⁶	7.45	10.30	12.06	1.56	15.19
Atherogenic index ⁷	0.41	0.44	0.47	0.02	4.78

¹Standard deviation²Coefficient variation³Total n-3 PUFA = {[C18:3 n-3] + [C20:5 n-3] + [C22:6 n-3]}.⁴Total n-6 PUFA = C18:2 n-6.⁵PUFA:SFA ratio = total PUFA/total SFA.⁶ Σ n-6/ Σ n-3 PUFA ratio.⁷Atherogenic index = $(4 \times [C14:0]) + (C16:0) / ([total\ MUFA] + [total\ PUFA])$, where brackets indicate concentrations (Ulbricht and Southgate, 1991).

The results of fatty acids descriptive analysis of the brain tissue are shown in table 2. The brain tissue of growing-finishing pigs has mainly SFA (Mean=55.59%), among the SFA the following averages were observed for stearic acid (28.39%), palmitic acid (26.37%) and myristic acid (0.54%). The circulating concentrations of myristic, palmitic, and stearic acids in humans influenced by the diet were all positively associated with metabolic diseases such as type 2 diabetes (CALDER, 2015). 33.53% of the brain tissue is composed of MUFA mainly by oleic acid (30.97%). The average of the total PUFA in brain tissue is 11.08%, and the most abundant PUFA was docosahexaenoic acid (8.90%), followed by linoleic acid (1.92%) and eicosapentaenoic acid (0.20%). In human brain, docosahexaenoic acid and arachidonic acid are the most important PUFA (CHEVALIER et al., 2019) and docosahexaenoic acid constitutes 10–20% of total lipids in the brain (BRENNNA; DIAU, 2007). Docosahexaenoic acid can modulate many cellular and physiological processes such as membrane fluidity, release of neurotransmitters, gene expression, myelination, neuroinflammation and neuronal growth (UAUY; DANGOUR, 2006; BUS et al., 2019). According to Avallone

et al. (2019), the docosahexaenoic acid in the diet necessary to induce positive results in humans still requires further research. The ratio of PUFA to SFA in brains was lower than

that observed in the liver, with an average of 0.20. In both tissues, liver and brain, the most abundant MUFA is oleic acid. Regarding PUFA, in the liver the most abundant was linoleic acid and in the brain docosahexaenoic acid. According to Calder (2015), different cells and tissues have different fatty acid compositions, and these may be influenced by diet. In this context, Carrapiso et al. (2020) found significant effects of diet composition (Canola oil supplementation) in subcutaneous fatty acids profiles of pigs.

The interest in fatty acid content modification of meat arises since the intramuscular fat cannot be removed before consumption, as in the case of visible fats, increasing the search for meat that has health-beneficial acids in its composition (RAES; SMET; DEMEYER, 2004). MUFA and PUFA fatty acids have been described as compounds with beneficial effects on human health, especially long-chain n-3 and n-6 fatty acids, making them recommended for consumption (KOUBA et al., 2003; NIETO; ROS, 2012). In addition, fatty acids composition directly influences meat

quality considering sensory attributes and nutritional value (NIETO; ROS, 2012).

In monogastric species such as pigs the change in the fatty acid profile is relatively simple because the nutrients are absorbed practically unchanged through the intestines of the animals reaching the bloodstream and deposited in the muscle and fat tissues (ENSER et al., 2000). Thus, pork becomes an excellent option for delivering meat with a healthier composition fat for human consumption (MOREL et al., 2013).

In the last years, the adoption of a nutritional approach have been highly recommendable to reduce diseases in humans such as cardiovascular and neurodegenerative diseases (Parkinson and Alzheimer) (AVALLONE; VITALE; BERTOLOTTI, 2019). In this context, fatty acids are thought to play a crucial role in human's health, since the disturbances of their profile and metabolites may be the basis of various

disorders (AVALLONE; VITALE; BERTOLOTTI, 2019). Pigs are an important source of protein and the nutritional management such as fatty acids supplementation can influence in fatty acids profile producing a healthier meat to human consumption.

We show in this study the effect of canola oil supplementation of pigs during the growing and finishing phase in the fatty acids profile in the liver and brain. Studies like this are necessary for animal nutrition, to determine the optimal diet that contain functional components (GRELA; FLOREK; WOJTASZEWSKA, 2020). These functional components in the animals diet provide not only the basal nutrients but also various biologically active substances that lead to production of a better and healthier meat (LIU et al., 2018).

Table 2. Descriptive analysis for fatty acid composition (%) of brain tissue of immunocastrated male pigs fed a corn-soybean meal diet containing 3% canola oil

Item	Minimum	Mean	Maximum	SD ¹	CV, % ²
Ether extract, %	9.73	10.02	10.29	0.22	2.17
Saturated fatty acid (SFA)					
Myristic acid (C14:0)	0.52	0.54	0.60	0.03	5.34
Palmitic acid (C16:0)	25.76	26.37	27.09	0.52	1.95
Stearic acid (C18:0)	27.96	28.39	28.99	0.37	1.30
Monounsaturated fatty acid (MUFA)					
Palmitoleic acid (C16:1)	0.39	0.47	0.52	0.04	9.45
Oleic acid (C18:1 n-9)	29.97	30.97	32.03	0.75	2.42
Eicosenoic acid (C20:1 n-9)	1.95	2.10	2.22	0.10	4.79
Polyunsaturated fatty acid (PUFA)					
Linoleic acid (C18:2 n-6)	1.48	1.92	2.58	0.39	20.36
Eicosapentaenoic acid (C20:5 n-3, EPA)	0.12	0.20	0.46	0.14	70.01
Docosahexaenoic acid (C22:6 n-3, DHA)	8.32	8.90	9.91	0.55	6.23
Total SFA	54.78	55.59	56.17	0.56	1.01
Total MUFA	32.46	33.53	34.67	0.82	2.43
Total PUFA	10.14	11.08	12.10	0.78	7.00
Total n-3 PUFA ³	7.95	8.83	9.97	0.71	8.07
Total n-6 PUFA ⁴	1.48	1.92	2.58	0.39	20.36
PUFA:SFA ratio ⁵	0.18	0.20	0.22	0.01	6.67
n-6:n-3 PUFA ratio ⁶	0.16	0.27	0.60	0.17	62.37
Atherogenic index ⁷	0.60	0.64	0.67	0.03	4.50

¹Standard deviation

²Coefficient variation

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

⁴Total n-6 PUFA = C18:2 n-6.

⁵PUFA:SFA ratio = total PUFA/total SFA.

⁶ Σ n-6/ Σ n-3 PUFA ratio.

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

CONCLUSIONS

The animals' diet influences the fatty acid profile in different tissues. Such modifications can influence the concentration of unsaturated fatty acids in relation to saturated, making pork healthier for human consumption.

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