University of São Paulo
"Luiz de Queiroz" College of Agriculture

Systems biology approach to identify clusters of genes associated with the pro and anti-inflammatory cytokines abundance in the liver and skeletal muscle of pigs

Fernanda Nery Ciconello

Dissertation presented to obtain the degree of Master in Science. Area: Animal Sciences and Pastures

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Fernanda Nery Ciconello
Veterinarian

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Advisor:

Prof[a]. Dr[a]. **ALINE SILVA MELLO CESAR**

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RESUMO

Abordagem de biologia de sistemas para identificar conjuntos de genes associados com abundância de citocinas pró e anti-inflamatórias no fígado e músculo esquelético de suínos

Os suínos são utilizados como modelos animais para estudos em saúde nutricional, oferecendo insights valiosos sobre doenças metabólicas e respostas imunológicas. Pesquisas envolvendo diferentes fontes de ácidos graxos (AGs) em suas dietas destacam o papel desses nutrientes nos sistemas metabólico e imunológico desses animais. Os AGs são classificados com base no número e posição das duplas ligações em sua cadeia, sendo divididos em saturados (AGS), monoinsaturados (AGMI) e poliinsaturados (AGPI). AGMI e AGPI têm efeitos menos pró-inflamatórios que o AGS. Estudos recentes investigaram os efeitos de dietas contendo óleo de canola (OC), óleo de soja (OS) e óleo de peixe (OP) em suínos. Os resultados mostraram que o OC, rico em AGMI, diminuiu os níveis de citocinas inflamatórias, enquanto o OS, rico em AGPI, reduziu o fator de necrose tumoral alfa (TNF alfa) e a interleucina-6 (IL-6). O OP também mostrou redução nos níveis de TNF alfa. O estudo teve como objetivo avaliar a expressão gênica e os níveis de citocinas em diferentes tecidos utilizando a técnica de ensaio de imunoabsorbância associado à enzima (ELISA), RNA-Seq e WGCNA. Suínos foram alimentados com dietas contendo 3% de cada tipo de óleo por 98 dias. Os resultados indicaram diferenças na expressão de genes e níveis de citocinas entre os tecidos e dietas, com destaque para a elevada quantidade de interferon-gama (IFN-gama) e a menor quantidade de interleucina-10 (IL-10) no fígado com o OC, e uma menor quantidade de TNF alfa no músculo esquelético com o OC em comparação com OS e OP. A análise de rede de coexpressão de genes identificou genes relevantes para processos imunológicos e metabólicos. Esses resultados fornecem insights importantes sobre os efeitos dos AGs na saúde dos suínos e seres humanos, contribuindo para uma melhor compreensão dos mecanismos envolvidos em doenças metabólicas e respostas imunológicas associadas com ácidos graxos.

Palavras-chave: Ácidos graxos, ELISA, Inflamação, Modelo animal, WGCNA

ABSTRACT

Systems biology approach to identify clusters of genes associated with the pro and anti-inflammatory cytokines abundance in the liver and skeletal muscle of pigs

Pigs are used as animal models for studies in nutritional health, providing valuable insights into metabolic diseases and immune responses. Research involving different sources of fatty acids (FAs) in their diets highlights the role of these nutrients in the metabolic and immune systems of these animals. FAs are classified based on the number and position of double bonds in their chain, being divided into saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA). MUFA and PUFA have less pro-inflammatory effects than SFA. Recent studies have investigated the effects of diets containing canola oil (CO), soybean oil (SO), and fish oil (FO) in pigs. The results showed that CO, rich in MUFA, decreased inflammatory cytokine levels, while SO, rich in PUFA, reduced tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6). FO also showed a reduction in TNF-alpha levels. The study aimed to evaluate gene expression and cytokine levels in different tissues using enzyme-linked immunosorbent assay (ELISA), RNA-Seq, and weighted gene co-expression network analysis (WGCNA). Pigs were fed diets containing 3% of each type of oil for 98 days. The results indicated differences in gene expression and cytokine levels among tissues and diets, with a high amount of interferon-gamma (IFN-gamma) and a lower amount of interleukin-10 (IL-10) in the liver with CO, and a lower amount of TNF-alpha in skeletal muscle with CO compared to SO and FO. Gene co-expression network analysis identified genes relevant to immune and metabolic processes. These results provide important insights into the effects of FAs on the health of pigs and humans, contributing to a better understanding of the mechanisms involved in metabolic diseases and immune responses associated with fatty acids.

Keywords: Fatty acids, ELISA, Inflammation, Model animal, WGCNA

1. GENERAL INTRODUCTION

The metabolism of lipids is intricately influenced by fatty acids. Incorporating fatty acids into the diet of pigs can significantly impact lipid metabolism. Cellular membranes, comprising predominantly lipids composed of fatty acids, play crucial roles in metabolic diseases and inflammation (Fanalli et al., 2023). Moreover, lipids serve as energy reserves and aid in gene transcription regulation (De Pablo Martinez and Álvarez De Cienfuegos, 2000; Palmquist, 2009; Fanalli et al., 2022). Essential dietary fatty acids, such as arachidonic acid (C20:4 n-6, AA), eicosapentaenoic acid (C20:5 n-3, EPA), and docosahexaenoic acid (C22:6 n-3, DHA), are fundamental components of lipids and are involved in various physiological processes (Duan et al., 2014; Sakomura et al., 2014).

Fatty acids exist in three main chemical forms: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (Calder, 2002). Oleic acid is a type of MUFA, while linoleic acid, alpha-linolenic acid, DHA, EPA, and AA are examples of PUFA. Fatty acids exert regulatory effects on gene transcription in muscle, adipocytes, and blood cells, impacting processes such as chemotaxis, phagocytosis, and cytokine synthesis (De Pablo Martinez and Álvarez De Cienfuegos, 2000; Duan et al., 2014; Fanalli et al., 2022). Different types of fats or oils influence meat composition, animal growth performance, and intramuscular fat content (Park et al., 2012).

Fatty acids provide high energy density to animal feed, promoting rapid growth and early slaughter of animals (Verussa, 2015). Factors such as administration time, type of dietary fatty acids, sex, and species of animals can modify cellular membrane composition in mononuclear and polymorphonuclear cells (De Pablo Martinez and Álvarez De Cienfuegos, 2000; Ramayo-Caldas et al., 2012). Fatty acids, particularly AA, contribute to the regulation of eicosanoid production, which includes leukotrienes, prostaglandins, and thromboxane, controlling inflammatory reactions. Consequently, fatty acids play roles in inflammatory reactions, platelet aggregation, cellular membrane composition, vitamin D synthesis, bile acid synthesis, steroid hormone synthesis, and atherosclerotic plaque formation (Calder, 2002; Sakomura et al., 2014; Xing et al., 2019).

Metabolism of fatty acids is regulated by the liver, skeletal muscle, and adipose tissues. Liver and adipose tissues collaborate in de novo cholesterol synthesis, fatty acid oxidation, and lipogenesis (Ramayo-Caldas et al., 2012). Fatty acids are absorbed in the intestine through apolipoprotein A4, enhancing chylomicron absorption and lipoprotein secretion (Nelson and Cox, 2011). Following absorption, fatty acids are converted into triacylglycerols in the liver and transported via very-low-density lipoprotein (VLDL) into the bloodstream (Palmquist, 2009). Subsequently, cells absorb fatty acids, with SFAs being conjugated to coenzyme A for beta-oxidation, while MUFAs and PUFAs require hydrogenation before utilization in beta-oxidation (Nelson and Cox, 2011; Sakomura et al., 2014).

Cellular membranes are susceptible to oxidation by reactive oxygen species (ROS), necessitating dietary fatty acids for membrane replacement. Additionally, fatty acids contribute to lipid metabolism and cholesterol synthesis, serving as precursors for steroid hormones (Duan et al., 2014). Previously, it was believed that fatty acid consumption increased fat deposition, lipidemia, and cholesterol levels (Hasler-Rapacz et al., 1994; Palmquist, 2009).

Increased ingestion of fatty acids can lead to greater fat density in adipose tissue and muscle fibers in animals, impacting human nutrition indirectly. However, excessive intake of SFAs can contribute to metabolic diseases in humans. In contrast, consumption of oleic and alpha-linolenic acids offers benefits (De Pablo Martinez and Álvarez De Cienfuegos, 2000; Palmquist, 2009). While oleic acid synthesis occurs via hepatic desaturase enzymes, there are no enzymes for unsaturation on carbons three (n-3) and six (n-6), emphasizing the importance of n-3 and n-6 supplementation in the diet (Duan et al., 2014; Sakomura et al., 2014). Increasing the dosage of n-3 fatty acids progressively may reduce enzymatic activity involved in lipogenesis, particularly PUFA, in the liver (Tonnac et al., 2016). Imbalance in the ratio of n-6 to n-3 fatty acids can lead to metabolic diseases or, conversely, improved weight gain and food efficiency in pigs (Duan et al., 2014).

Elevated consumption of n-6 fatty acids and insufficient intake of n-3 can result in heightened secretion of inflammatory cytokines, including interleukin (IL)-6, IL-1 beta, and tumor necrosis factor (TNF) alpha, in serum, skeletal muscle, and adipose tissues (Duan et al., 2014). Incorporating fish oil into diets has shown benefits in conditions such as rheumatoid arthritis, reducing leukotriene B4 and lipopolysaccharide-induced (LPS) IL-1 production by blood mononuclear cells. Fish oil has also been beneficial in multiple sclerosis, diabetes, autoimmune glomerulonephritis, colitis, lupus, atopic dermatitis, asthma, and psoriasis (Chen and Yeh, 2003).

Moreover, fish oil in parenteral diets reduces acetiltransferasis diacylglycerol in the liver, decreases carboxilasis acetil-CoA activity, inhibits de novo synthesis of fatty acids, and decreases levels of enzymes such as superoxide dismutase and glutathione peroxidase, reducing free radicals and lipid peroxidation in tissues. Additionally, fish oil reduces the production of TNF alpha and IL-10 (Chen and Yeh, 2003).

In summary, fatty acids play crucial roles in modulating cytokine production, influencing immune responses, inflammation, and lipid metabolism. Understanding the intricate interplay between fatty acids and cytokines is essential for elucidating their roles in health and disease.

2. FINAL CONSIDERATIONS

As future steps of this study, it is important to verify if these hub genes and nodes, identified in both tissues: liver and skeletal muscle, are in accessible regions of DNA, and if these regions will be transcribed, processed, and translated on pigs's DNA sequence. One way available to test this future hypothesis is to realize the Assay for Transposase-Accessible Chromatin using Sequencing (ATAC-Seq). Another future study is to make an annotation of possible isoforms through a serious study of splicing of these hub genes and remarkable nodes, which were identified by functional enrichment analysis, to analyze if these pigs are producing useful isoforms in both tissues. For muscle metabolism, canola oil seemed to be a better diet compared to o soybean and fish oils. However, for liver metabolism, soybean and fish oils seemed to be more suitable than canola oil. So, the fatty acids metabolism is completely different for the liver and skeletal muscle, that's why one source of fatty acids is more acceptable for one tissue than another in pigs and humans.

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