UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ZOOTECNIA E ENGENHARIA DE ALIMENTOS

LERIANA GARCIA REIS

Evaluation of gene expression and immune system of swine after consumption of biofortified milk with n-3 and n-6

Corrected Version

Pirassununga
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Evaluation of gene expression and immune system of swine after consumption of biofortified milk with n-3 and n-6

Versão Corrigida

Thesis submitted to the Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo as part of the requirements for obtaining the title of Doctor of Science in the postgraduate program in Animal Science.

Concentration Area: Animal Science

Advisor: Prof. Dr. Arlindo Saran Netto

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UNIVERSIDADE DE SÃO PAULO
Faculdade de Zootecnia e Engenharia de Alimentos
Comitê de Ética em Pesquisa da FZEA

CERTIFICADO

Certificamos que a proposta intitulada "Suplementação com fontes de w3 e w6: seus efeitos sobre eficiência produtiva e reprodutiva em vacas lactantes e avaliação nutracêutica do leite utilizando suínos como modelo para aplicabilidade em humanos", protocolada sob o CEUA nº 4939070317 (ID 000736), sob a responsabilidade de **Arlindo Saran Netto** - 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 - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo - FZEA/USP (CEUA/FZEA) na reunião de 08/11/2017.

We certify that the proposal "Supplementation with omega-6 and omega-3 fatty acids sources: effects on productive and reproductive efficiency in lactating cows and milk nutraceutical evaluation by using pigs as a model for applicability in humans", utilizing 24 Bovines (24 females), 60 Swines (60 females), protocol number CEUA 4939070317 (ID 000736), under the responsibility of **Arlindo Saran Netto** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Animal Science and Food Engineering - (São Paulo University) (CEUA/FZEA) in the meeting of 11/08/2017.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 03/2017 a 11/2017 Área: Nutrição Animal/zaz

Origem:	Faculdade de Zootecnia e Engenharia de Alimentos (FZEA/USP)						
Espécie:	Bovinos	sexo:	Fêmeas	idade:	3 a 7 anos	N:	24
Linhagem:	Holandês			Peso:	550 a 750 kg		
Origem:	Faculdade de Zootecnia e Engenharia de Alimentos (FZEA/USP)						
Espécie:	Suínos	sexo:	Fêmeas	idade:	21 a 23 dias	N:	60
Linhagem:	Landrace x Large White			Peso:	5 a 7 kg		

Local do experimento: O experimento 1 será conduzido no setor de bovinocultura leiteira no Campus de Pirassununga, pertencente à Universidade de São Paulo, O experimento 2 e 3 serão conduzidos no setor de suinocultura da Prefeitura do Campus e no Laboratório de Pesquisa em Suínos (LPS), do Departamento de Nutrição e Produção Animal, da Faculdade de Medicina Veterinária e Zootecnia (VNP/FMVZ), da Universidade de São Paulo, campus de Pirassununga-SP, concomitante com o experimento da produção de leite pelas vacas, Experimento 01.

Pirassununga, 16 de agosto de 2019

Profa. Dra. Daniele dos Santos Martins
Coordenadora da Comissão de Ética no Uso de Animais
Faculdade de Zootecnia e Engenharia de Alimentos da
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UNIVERSIDADE DE SÃO PAULO
Faculdade de Zootecnia e Engenharia de Alimentos
Comitê de Ética em Pesquisa da FZEA

CERTIFICADO

Certificamos que a proposta intitulada "Impacto do leite enriquecido naturalmente com ômegas em leitões", protocolada sob o CEUA nº 6983020920 (ID 001567), sob a responsabilidade de **Arlindo Saran Netto** - 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 - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo - FZEA/USP (CEUA/FZEA) na reunião de 01/10/2020.

We certify that the proposal "Impact of milk naturally enriched with omegas in piglets", utilizing 360 Swines (males and females), protocol number CEUA 6983020920 (ID 001567), under the responsibility of **Arlindo Saran Netto** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Animal Science and Food Engineering - (São Paulo University) (CEUA/FZEA) in the meeting of 10/01/2020.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 07/2020 a 07/2021 Área: Nutrição Animal/zaz

Origem:	Faculdade de Zootecnia e Engenharia de Alimentos (FZEA/USP)						
Espécie:	Suínos	sexo:	Machos e Fêmeas	idade:	12 a 16 dias	N:	360
Linhagem:	Landrace x Large White			Peso:	3 a 6 kg		

Local do experimento: O experimento será conduzido no setor de suinocultura da Prefeitura do Campus e no Laboratório de Pesquisa em Suínos (LPS), do Departamento de Nutrição e Produção Animal, da Faculdade de Medicina Veterinária e Zootecnia (VNP/FMVZ), da Universidade de São Paulo, campus de Pirassununga-SP.

Pirassununga, 02 de outubro de 2020

Profa. Dra. Cristiane Gonçalves Titto
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Dedication

To my examples of determination, strength, inspiration and love, my beloved mother EDNA NEVES GARCIA, my sister PRISCILA REIS ZANDONÁ, my lovely nephews OLÍVIA REIS ZANDONÁ and BENJAMIM REIS ZANDONÁ, the happiness of my life, and to my great friends who have enriched my life day by day.

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I thank God for the strength and courage he has given me in times of challenge and difficulty. His constant presence strengthened me, renewed my hopes, and gave me the determination needed to overcome the obstacles that came my way.

To my dear mother, Edna Neves Garcia, a woman of unmatched strength and generous heart. Her constant presence, unconditional love and tireless support were essential pillars throughout my academic journey. I thank her for being my inspiration, my greatest encourager, and for teaching me never to give up on my dreams.

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To my lovely sister Priscila Reis Zandoná, whose presence in my life is a priceless gift. Your support has always been important for my progress. I thank her for being my source of inspiration and for always believing in me.

To my dear nephews Olívia Reis Zandoná and Benjamim Reis Zandoná, who have brought light and happiness into my life. Their endless curiosity and brilliant spirit are a constant inspiration in my journey. I thank them for being my motivation and for reminding me of the importance of never stopping to learn and explore the world. I wish you to always be inspired to pursue your own dreams and achieve great realizations.

To my maternal and paternal grandparents (*in memoriam*), Delcídia Pimenta Neves Garcia, José Pedro Garcia, Maria José dos Santos Reis, and Arlindo Reis, who have guided me with love and wisdom throughout my life. Even in their heavenly places, they continue to enlighten me. This thesis is a tribute to their legacy and constant inspiration.

To my esteemed advisor, Dr. Arlindo Saran Netto, for his tireless dedication and valuable guidance, which contributed to making my academic journey an extraordinary achievement. Thank you for sharing your knowledge and inspiring my passion for research. This thesis is the result of our collaboration and mutual devotion.

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Lastly, I dedicate this doctoral thesis to all the people who have accompanied me throughout this journey, leaving their indelible marks on my academic and personal trajectory.

ABSTRACT

REIS, L. G. **Evaluation of gene expression and immune system of swine after consumption of biofortified milk with n-3 and n-6**. 2023. 127 f. PhD Thesis – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2023.

Dietary supplementation with low ratio of n-6 and n-3 polyunsaturated fatty acids (PUFA) can decrease the risk of many of the chronic disease, the cardiovascular disease, cancer, inflammation, obesity, and autoimmune disease consequently benefiting health. This study aimed to investigate the potential health benefits of supplementing sows' diets with cow's milk biofortified with n-3 or n-6 polyunsaturated fatty acids, utilizing 8.26, 7.92, and 2.72 n-6/n-3 ratios, and on sow and their offspring. Furthermore, the study placed emphasis on several crucial elements, such as the regulation of gene expression in the liver to control lipolysis, lipogenesis, and the regulation of appetite in the hypothalamus. Also, alterations in the production of immunoglobulins, interleukins, and eicosanoids that influence the immune system were examined. Additionally, changes in the hemolytic activity of the alternative pathway of the complement system were investigated. Another objective of the study was to examine the impact of dietary changes in circulating fatty acids in the organism, colostrum and milk. Sows fed milk biofortified with n-6 and n-3 PUFA had an increased total number of embryos, greater backfat thickness in earlier gestation, altered plasma fatty acids profile, reduced IgG in colostrum, and the piglets had higher concentration of IgA, IgM and TNF-α, with lower IL-10. Sows supplemented with n-6 PUFA compared to n-3, had lower very low density lipoproteins (VLDL) and TNF-α in circulation. Whereas offspring of n-6 versus n-3 supplemented sows had lower IL-10 in circulation and lower levels of SREPB-1 mRNA in plasma. N-6 and n-3, also, enhanced sow backfat thickness from D40 to D107 of gestation, the arachidonic acid (ARA) levels in milk, viability 1 of piglets, and IgA in piglets' plasma. The findings provide valuable insights into the potential health benefits of dietary supplementation with biofortified milk in swine, which can be used as a model study for humans, contributing to a better understanding of the relationship between nutrition, gene expression, immune function, and trans-generational effects, potentially leading to improved dietary recommendations and interventions for both human and animal populations.

Keywords: Biofortified milk. Genes. Human health. Immunity. Lipids. Obesity.

RESUMO

REIS, L. G. **Avaliação da expressão gênica e do sistema imunológico de suínos após o consumo de leite biofortificado com n-3 e n-6**. 2023. 127 f. Tese (Doutorado) – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2023.

A suplementação dietética com baixa proporção de ácidos graxos poli-insaturados (AGPI) n-6 e n-3 pode diminuir o risco de muitas doenças crônicas, como doenças cardiovasculares, câncer, inflamação, obesidade e doenças autoimunes, beneficiando assim a saúde. O objetivo deste estudo foi investigar os possíveis efeitos benéficos para a saúde a partir da suplementação na dieta das porcas com leite de vaca enriquecido com ácidos graxos poliinsaturados n-6 ou n-3, utilizando as proporções n-6/n-3 de 8,26, 7,92 e 2,72, sobre as porcas e seus descendentes. Além disso, o estudo enfatizou vários elementos cruciais, como a regulação da expressão gênica no fígado para controlar a lipólise, a lipogênese e a regulação do apetite no hipotálamo. Além disso, foram examinadas as alterações na produção de imunoglobulinas, interleucinas e eicosanoides que influenciam o sistema imunológico. Além disso, foram investigadas as mudanças na atividade hemolítica da via alternativa do sistema de complemento. Outro objetivo do estudo foi examinar o impacto das mudanças na dieta sobre os ácidos graxos circulantes no organismo, no colostro e no leite. As porcas alimentadas com leite enriquecido com PUFA n-6 e n-3 apresentaram maior número total de embriões, maior espessura de toucinho no início da gestação, perfil de ácidos graxos plasmáticos alterado, redução de IgG no colostro, e os leitões apresentaram maior concentração de IgA, IgM e TNF-α, com menor IL-10. As porcas suplementadas com PUFA n-6, em comparação com n-3, apresentaram menor nível de lipoproteínas de densidade muito baixa (VLDL) e TNF-α na circulação. Por outro lado, a prole de porcas suplementadas com n-6 em comparação com n-3 apresentou menos IL-10 na circulação e níveis mais baixos de mRNA de SREPB-1 no plasma. O n-6 e o n-3 também aumentaram a espessura de toucinho das porcas no D40 a D107 da gestação, os níveis de ácido araquidônico (ARA) no leite, a viabilidade 1 dos leitões e a IgA no plasma dos leitões. As descobertas fornecem informações valiosas sobre os possíveis benefícios para a saúde da suplementação dietética com leite enriquecido em suínos, os quais servem como um modelo de estudo para humanos, contribuindo para uma melhor compreensão da relação entre nutrição, expressão gênica, função imunológica e efeitos transgeracionais, potencialmente levando a melhores recomendações e intervenções dietéticas para populações humanas e animais.

Palavras-chave: Leite enriquecido. Genes. Saúde humana. Imunidade. Lipídios. Obesidade.

LIST OF TABLES

ABBREVIATIONS AND ACRONYMS

ACC	Acetyl-CoA carboxylase
ACTB	β-Actin
AFR	Adjusted Fertilization Ratio
AGRP	Agouti Related Neuropeptide
AI	Artificial Insemination
ALA	α-linolenic acid
apo B	Apolipoprotein B
ARA	Arachidonic acid
ARH	Arcuate Nucleus of the Hypothalamus
BT	Backfat Thickness
BW	Body Weight
BWB	Body Weight at Birth
C1	Contrast Control vs n-6+n-3
C2	Contrast n-6 vs n-3
CART	Cocaine and Amphetamine-Related Transcript
CH50	50% Haemolytic Complement
CI	Colostrum Intake
D40G	Day 40 of gestation
D75G	Day 75 of gestation
D107G	Day 107 of gestation
D14L	Day 14 of lactation
D1L	Day 1 of lactation
D21L	Day 21 of lactation
D6D	Delta-6 Desaturase
DHA	Docosahexaenoic acid
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	Eicosapentaenoic acid
FA	Fatty Acid
FAMEs	Fatty Acid Methyl Esters
FAS	Fatty Acid Synthase
FR	Fertilization Ratio
HDL	High-Density Lipoprotein
HPRT1	Hypoxanthine Phosphoribosyltransferase 1
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-10	Interleukin-10
IL-6	Interleukin-6
INSR	Insulin receptor
IS	Internal Standards

LC-MS/MS	Liquid Chromatography coupled with Tandem Mass Spectrometry
LA	Linoleic acid
LC-PUFA	Long Chain Polyunsaturated Fatty Acids
LDL	Low-Density Lipoprotein
LEPR	Leptin receptor
LTB4	Leukotriene B4
LXRs	Liver X Receptors
MAC	Membrane Attack Complex
MHC	Major Histocompatibility Complex
mRNA	Messenger RNA
mTORC1	mTOR complex-1
MUFA	Monounsaturated Fatty Acids
n-3	Omega-3 family
n-6	Omega-6 family
n-6/n-3	Σ Omega-6/Σ Omega-3
NEFA	Non-Esterified Fatty Acids
NPY	Neuropeptide-Y
Ob-Rb	Leptin receptor
OD	Optical Density
PGE2	Prostaglandin E2
PGF2α	Prostaglandin F2α
POMC	Pro-Opiomelanocortin
PPAR-α	Peroxisome Proliferator-Activated Receptor α
PPAR-β	Peroxisome Proliferator-Activated Receptor β
PPAR-γ	Peroxisome Proliferator-Activated Receptor γ
PUFA	Polyunsaturated Fatty Acids
RBP4	Retinol Binding Protein 4
RNA	Ribonucleic Acid
RXRs	Retinoid X Receptors
SCD	Stearoyl-CoA Desaturase or Δ9-Desaturase
SEM	Standard Error of the Mean
SFA	Saturated Fatty Acids
SFA/USFA	Σ Saturated/Σ Unsaturated
SPE	Solid Phase Extraction
SPMs	Pro-Resolving Mediators
SREBP-1	Sterol Regulatory Element-Binding Protein 1
T1/2	Capacity of serum proteins to promote 50% of lysis
TAP	Thapsigargin
TG	Triglycerides
TNF-α	Tumor necrosis factor α
TXB2	Thromboxane B2
USFA	Unsaturated Fatty Acids
VLDL	Very Low-Density Lipoprotein

WG	Weight Gain
Σ n-3	Σ Omega-3 Fatty Acids
Σ n-6	Σ Omega-6 Fatty Acids
ΣMUFA	Σ Monounsaturated Fatty Acids
ΣPUFA	Σ Polyunsaturated Fatty Acids
ΣSFA	Σ Saturated Fatty Acids
ΣUSFA	Σ Unsaturated Fatty Acids

SUMMARY

1 INTRODUCTION

Pigs are a relevant model for studying the human immune system and gene expression considering the potential of functional foods to improve health. The porcine immune system has been shown to be very similar to its human counterpart, making it a valuable model for studying human health priorities such as obesity, cardiovascular disease, and nutritional studies. Therefore, porcine models can be used to enhance our comprehension of immune system function and response in humans. In the long-term, studies regarding immune system functionality in pigs can aid in the development of novel food products that possess enhanced nutritional value and promote human health, as well as the development of novel treatments for human diseases.

Overall, there is an imbalance in the ratio of omega-6 (n-6) to omega-3 (n-3) polyunsaturated fatty acids (PUFA) in the Western diet, with a higher concentration of n-6 PUFAs compared to n-3 PUFAs at a ratio of 20:1 to 50:1, when compared to the diet composition of human ancestors was about 1:1. However, the synthesis of PUFAs from linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3) varies among different populations and can be attributed to adaptation to diverse nutrient environments during the evolution of *Homo sapiens*. Thus, some studies suggest different ratios that benefit lipid metabolism, inflammation and human health such as, 1:1, 5:1 and even 10:1 (DUAN et al., 2014; SIMOPOULOS, 2006, 2011; WIJENDRAN; HAYES, 2004). This imbalance has been associated with various effects on the immune system and inflammation in the body. Dietary interventions aimed at lowering the ratio need to reduce the consumption of n-6 PUFAs and increase the consumption of n-3 PUFAs. Therefore, it is of interest to have a greater availability of foods that meet this ideal ratio. Dairy products are rich in various nutrients serve as an excellent source of a relatively inexpensive protein. In addition, a complex assembly of lipids are available in milk, many of which are integral components of cell membranes and have significant influences on metabolism, including hormonal synthesis, vitamin absorption, modulation of gene expression, and synthesis of inflammatory mediators. Despite the potential health benefits of milk, the ratio of n-6/n-3 PUFA is relatively high. In this regard, healthcare professionals have recommended removing milk from the diet or consuming skim milk. However, the supplementation of vegetable oils or seeds in the diets of dairy cows has shown positive changes in the fatty acid (FA) profile of milk, making it healthier for human consumption.

Lipids have been extensively studied for their role in the immune system and gene expression, as membrane phospholipids are known to trigger the release of cytokines involved in the inflammatory process. However, when they are replaced by another type of lipid, the series of cytokines produced is altered, resulting in a different metabolic response. Considering the global epidemic of obesity, which is recognized as a low-grade inflammatory state, there is a significant concern about the increased susceptibility of obese individuals to infections, which correlates with immune system alterations. A preventive approach may be more successful than the current general approach, as gene expression can be influenced by dietary factors.

Increased intake of saturated fatty acids (SFA) and omega-6 polyunsaturated fatty acids (n-6 PUFA) is associated with a more pro-inflammatory profile, while omega-3 polyunsaturated fatty acids (n-3 PUFA) exhibit anti-inflammatory properties. Therefore, it is essential to not only consider the individual actions of lipids but also understand their interplay within the body to define the functionalities of biofortified foods.

Furthermore, the assessment of gene expression after the ingestion of biofortified milk with n-3 and n-6 polyunsaturated fatty acids can facilitate comprehension of the impact of the dietary n-6/n-3 fatty acid ratio on lipid metabolism by modulating genes associated with this process. Additionally, such evaluation aids in elucidating the influence of dietary and microbial fatty acids in the regulation of inflammation.

This overall goal of this study was to determine if there is any potential to long-term maternal consumption of biofortified milk with n-6 and n-3 FA on sows and her offspring. In particular, the studies in the literature describe the effect of supplementing n-3 and n-6 PUFA using other sources of supplementation to sows on their fertility, body composition and colostrum quality and quantity. Moreover, we used analyzed the effect of maternal diets on gene expression and immune system functionality in the context of biological processes, bring new insights into the regulation of lipid metabolism, modulation of the immune cell activity, and the cytokine production. These types of studies are necessary as they can provide information for designing interventions or dietary strategies to improve body function in both humans and animals' health or encourage the development and utilization of biofortified feed or other strategies to enhance the nutritional value of livestock products, such as milk.

Since the effect of balance n-6/n-3 is not consolidated due to different source and species, this research outcomes would contribute to the existing scientific literature, that may provide a basis for further studies and open new research avenues in the field of nutritional immunology and gene expression.

2 BIBLIOGRAPHIC REVIEW

Lipids can be classified according to their saturation into saturated and unsaturated. The proportions between them in the human diet have been a cause for concern since saturated fatty acids (SFA) have been identified as the target of research into their relationship with different diseases (XAVIER et al., 2020). For this reason, there is a recommendation to reduce the intake of SFA and increase the intake of unsaturated fatty acids (UFA), which, in turn, are divided into monounsaturated (MUFA) and polyunsaturated (PUFA).

Within the PUFA classification, it is possible to emphasise polyunsaturated fatty acids from the n-3 family (PUFA n-3) and the n-6 family (PUFA n-6), which are called essential fatty acids (EFA), i.e. those that are not synthesized by mammals and are thus so important. Of each family, those that receive the most attention is linolenic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3), precisely because they are precursors of long-chain polyunsaturated fatty acids (LC-PUFA), the 20-carbon PUFAs, that are involved in metabolic functions of higher relevance in health.

Much has been discussed about the change in the consumption of the FA profile, mainly in the Western population, which started to ingest a greater amount of n-6 than n-3, achieving a ratio of 20:1 to 50:1 while the recommended would be around 1:1, 5:1 and even 10:1, thus altering the n-6/n-3 ratio in the organism. A higher concentration of n-6 PUFA combined with a reduction in the intake of n-3 PUFA has been associated with increasing cases of obesity, inflammatory status and other health problems as cancer, atherosclerosis and diabetes (SIMOPOULOS, 2016).

Since eicosanoid synthesis is regulated by LC-PUFA, the serie produced has an influence on the anti- or pro-inflammatory role in the system and associated with health problems. Eicosanoids are divided into the following subfamilies: prostaglandins (PG), leukotrienes (LT), thromboxanes (TX) and lipoxins. Reactions via the enzymatic pathway give rise to TX and PG by cyclooxygenase; via the non-enzymatic pathway, utilization occurs from arachidonic acid (ARA) (the main precursor as it is present in large quantities in the membrane of most cells), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and dihomo-γ-linolenic acid (DGL). ARA is mainly responsible for the synthesis of series 2 eicosanoids, which have a more pro-inflammatory profile, and the others for series 1 and 3, with more anti-inflammatory responses (FOLCO, 2006; TSIKAS and ZOERNER, 2014; WANG et al., 2014; ZHANG et al., 2015).

Animal studies show the regulation of the expression of some genes determining lipid metabolism, inflammatory process, and appetite regulation, by saturated (SFA) and unsaturated fatty acids (USFA). Many effects of supplementation with different lipid profiles have been associated with brain development, obesity, type 2 diabetes, and insulin resistance (MORRIS; CHEN, 2008; XAVIER et al., 2020).

Obesity represents a pervasive global health concern that is influenced by factors such as physical activity and dietary patterns. Nevertheless, it is crucial to acknowledge the contributions of genetics and epigenetics. The consumption of food has a profound impact on the physiological processes of the organism, and furthermore, the prenatal environment plays a significant role in the development of the fetus and the determination of the susceptibility to obesity and related metabolic disorders, including glucose intolerance and hypertension, later in life (CHEN et al., 2008). Nonetheless, given the widespread prevalence of the obesity epidemic worldwide, it is imperative to comprehend the specific implications of maternal nutrition both independently and in relation to the developing embryo, as maternal obesity and its associated intrauterine nutrition have been correlated with the occurrence of childhood obesity (SAMUELSSON et al., 2008).

Considering nutrition supplemented with LC-PUFA during pregnancy, it is possible to modulate gene expression and alter fetal metabolic programming to decrease the risk of cardiovascular and metabolic diseases, such as obesity (GUTIERREZ-GOMEZ et al., 2016).

Polyunsaturated fatty acids (PUFAs), particularly those from the n-3 series, have been found to play a role in preventing various human diseases. They achieve this by affecting membrane composition, cellular metabolism, signaling, and gene expression. PUFAs regulate gene expression in different tissues, including the liver, heart, adipose tissue, and brain, with the involvement of transcription factors like SREBP-1c (Sterol Regulatory Element-Binding Protein 1c) and nuclear receptors including PPAR-α (Peroxisome Proliferator-Activated Receptor α), which mediate the nuclear effects of PUFAs (SAMPATH; NTAMBI, 2004).

Although studies have demonstrated that the expression of lipogenic genes can change shortly after animals consume diets high in PUFAs, in vivo has various factors, such as basal Non-Esterified Fatty Acids (NEFA) levels and the rates of fatty acid elongation, desaturation, or oxidation, enable PUFAs to regulate gene expression differentially (JUMP; CLARKE, 1999). The identification of lipogenic genes is crucial, since it is possible to modulate them through diet (JIANG; PAPPU; ROTHSCHILD, 2007).

FAs activate the three different classes of PPAR (α, β, and γ), but they have distinct functions. PPAR α and β activate the oxidation of FAs, controlling genes related to ketogenesis

and FA catabolism. PPAR-γ, on the other hand, is a key inducer for the lipogenesis process. In the liver the α class is expressed at higher levels than β and γ. In general, PPAR regulates the expression of genes involved in fatty acid uptake, oxidation and synthesis (POULSEN; SIERSBÆK; MANDRUP, 2012).

FA oxidation is regulated by the liver function through the activation of PPARα, whose expression will be higher the higher the PUFA concentration (RAMAYO-CALDAS et al., 2012; THEIL; LAURIDSEN, 2007). In divergence from the mechanism of peroxisome proliferator-activated receptor (PPAR) activation, polyunsaturated fatty acids (PUFA) modulate the nuclear presence of sterol regulatory element-binding proteins (SREBPs) through two distinct modes: regulation of proteolytic processing of SREBP precursors and modulation of transcriptional activity of the SREBP-1c gene, as well as mRNA turnover. PUFA exhibit a feed-forward activation effect on PPARs, whereas paradoxically acting as feedback inhibitors of liver X receptors (LXRs) and SREBPs (JUMP, 2002; KITAJKA et al., 2004).

PUFAs are also able to modify the rate of fatty acid biosynthesis by suppressing acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), thus inhibiting hepatic lipogenesis (DENTIN et al., 2006; JUMP, 2002).

Duran-Montgé et al. (2009) investigated the effect of several lipid sources in the diet of 56 sows on hepatic expression of lipogenic genes and SREBF. The seven dietary treatments were: NF: diet without addition of oil in the formulation; T: diet with addition of tallow (high content of SFA); HOSF: diet with sunflower oil rich in oleic acid (high content of oleic acid); SFO: diet with sunflower oil (high content of linoleic acid, component of the ω-6 series); LO: linseed oil diet (high content of linolenic acid, component of the ω-3 series); FB: mixture of 55% T, 35% SFO and 10% LO (similar content regarding the main essential GAs, palmitic, stearic, oleic, linoleic and linolenic) and FO: fish oil (40% fish oil and 60% LO - high content of PUFA). The n-6/n-3 ratio of the treatments were respectively: 7.0; 7.5; 16.5; 56.4; 0.6; 1.4 and 0.5. The proteins ACC, SREBP-1 and stearoyl-CoA desaturase or Δ9-desaturase (SCD), were highly expressed in the liver for the HOSF treatment, suggesting that the stimulus corresponds to the high oleic acid content. On the other hand, the FO composition caused low expression of SREBP-1, which possibly inhibited lipogenic genes and resulted in lower expression of ACC and SCD. The lipid composition of the diet directly influenced the expression of some genes, as, the higher concentration of MUFA (monounsaturated fatty acids) was positively correlated to ACC, SREBP-1 and SCD, while n-3 was negatively correlated to SCD and the ratio n-6/n-3 positively to FAS. Diets rich in n-6 (SFO) and n-3 (LO) did not

reduce the expression of lipogenic genes when compared to tallow diet (T) (DURAN-MONTGÉ et al., 2009).

Over the past decade the genes hypothalamic appetite regulators have been studied, and revealed that supplementing mothers with n-3 PUFAs during pregnancy and achieving a lower ratio of n-6 to n-3 PUFAs generally leads to a reduction in leptin expression (LEPR) and circulating levels. This effect is attributed to epigenetic modifications of the gene promoter in both animal and human offspring, offering potential health benefits later in life. However, it should be noted that there has been inconsistency in the conclusions drawn from various studies (COLEMAN; MURPHY; RELLING, 2018; LI et al., 2021; MASLOVA et al., 2018; SHI et al., 2022; XAVIER et al., 2020).

A reduction of level of leptin through n-3 PUFA or low n-6/n-3 ratio usually suppress the hypothalamic orexigenic peptides as neuropeptide-Y (NPY) and stimulating anorexigenic peptides as pro-opiomelanocortin (POMC) (SCHWARTZ et al., 2000). Furthermore, recent research indicates that n-3 PUFA offer a diverse array of advantageous biological impacts, including the mitigation of insulin resistance. Notably, a significant change in the n-6/n-3 ratio in favor of n-3 PUFA plays a crucial role in the prevention and management of metabolic disorders, such as metabolic syndrome (OPPEDISANO et al., 2020; ROŠKARIĆ et al., 2021).

In cases of dietary obesity, it is commonly observed that leptin, a hormone produced by adipose tissue, acts in conjunction with insulin to directly influence the hypothalamus via the insulin receptor (INSR). This interaction resulting in the anorexigenic actions of insulin signaling, which is to suppress NPY and Agouti Related Neuropeptide (AGRP) and promote POMC expression, leading to the inhibition of feeding and an increase in energy expenditure, mediated through the long form of the leptin receptor (Ob-Rb). However, central resistance to leptin and insulin is frequently observed in individuals with dietary obesity (LEVIN; DUNN-MEYNELL; BANKS, 2004).

Omega-3 and omega-6-derived metabolites also have important immune-regulatory functions since the immune system is divided into two pathways, innate and adaptative system, the second one with highly specificity cells, B cells, and T cells. Each one has its way to influence the pro-resolving mediators (SPMs), leading to different responses at the organism (GUTIÉRREZ; SVAHN; JOHANSSON, 2019).

The innate immune system comprises a diverse array of components that collectively serve as the body's first line of defense against pathogens. These components encompass physical barriers, such as tight junctions in the skin and the epithelial and mucous membrane surfaces, as well as the mucus layer itself. Additionally, anatomical barriers play a crucial role

in this defense mechanism. Within this system, various enzymes found in epithelial and phagocytic cells, such as lysozyme, contribute to the innate immunity. Furthermore, a cadre of phagocytes, including neutrophils, monocytes, and macrophages, are central players in this defense mechanism. Complement proteins, C-reactive protein, lectins like mannose-binding lectin, and ficolins, collectively categorized as inflammation-related serum proteins, further fortify the innate immune response. Additionally, certain cells, such as macrophages, mast cells, and natural killer cells, release cytokines and inflammatory mediators to orchestrate the immune response. Upon encountering a pathogen, a signaling cascade is initiated, intensifying the immune response and activating specific mechanisms. This innate immune response is strategically designed to achieve three primary objectives: prevent infection, eradicate invading pathogens, and stimulate the acquired immune response (ARISTIZÁBAL; GONZÁLEZ, 2013).

B cells are responsible for production of immunoglobulins, and n-3 PUFA, by itself, appears to have a dampening effect on B cell, however, the EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) can increase IgM in humans and mouse, although the activation through the FA present some contradictions (RAMON et al., 2012; TEAGUE et al., 2014). On the surface of B cell an expression of several molecules can occur according to stimulus causing a positive or negative effect, moreover it can interact with other immune cells initiating different secretions (HARWOOD; BATISTA, 2010), which turns it into a complex and often unpredictable system.

Furthermore, it is essential to consider the variations in fatty acid metabolism across different animal models when interpreting the findings of studies investigating the effects of dietary omega-3 and omega-6 fatty acids in vivo, even because the transfer of antibodies across the placenta relies on the structure of the placenta and the specific pathway through which the antibodies are transported. In humans, the placenta is hemochorial, and the transfer of IgG antibodies is facilitated by FcRn (neonatal Fc receptor). On the other hand, pigs have an epitheliochorial placenta, which prevents the passage of antibodies from the mother to the fetus within the uterus. This type of placenta consists of six cell layers that effectively hinder the transmission of immunoglobulins and other immunological factors to the fetus during pregnancy. As a result, in pigs, the transfer of IgG antibodies occurs solely through colostrum (BOURNE; CURTIS, 1973; CERVENAK; KACSKOVICS, 2009; HURLEY; THEIL, 2011; MATÍAS et al., 2017).

Immune system also is involved in production of cytokines, as macrophages and T cells are some of those responsible for this (OH et al., 2010). It has been suggested n-3 PUFA has a

potential effect on the production and secretion of cytokines and chemokines, mainly by macrophages, with the ability to reduce inflammation, besides the ability to phagocytose, and the polarization into classically activated or alternatively activated macrophages (DJURICIC; CALDER, 2021; ROESSLER et al., 2017). Studies have shown a reduction in pro-inflammatory cytokines as TNF-α, IL-6 and an increase in the anti-inflammatory cytokine IL-10 by the treatment with n-3 PUFA (JIN et al., 2018b; SCHOENIGER et al., 2011).

However, the modifications observed in macrophage biology are directly attributed to the treatment with omega-3 fatty acids, rather than the interaction with other cell types that may also be affected by omega-3 fatty acids, as observed in vivo studies (GUTIÉRREZ; SVAHN; JOHANSSON, 2019). Despite of divergence among the studies, a lower n-6/n-3 ratio is attributed to higher immune cell function as well as the usually effect of EPA and DHA, since the higher levels of n-6 PUFA and arachidonic acid (ARA) contributing to inflammatory responses, which is important, however acute inflammatory responses are meant to be quickly suppressed. Therefore, maintaining a proper n-6/n-3 ratio in one's diet is crucial to prevent the occurrence of an excessive and prolonged inflammatory reaction. Such a response has the potential to cause harm to tissues and increase the risk of developing autoimmune diseases (DINICOLANTONIO; O'KEEFE, 2020).

The complement system consists of more than 30 inactive proteins present in the plasma, which can be activated by three different pathways (classical, alternative, and lectin) and form the membrane attack complex (MAC), represented only by C5b-9, resulting in pores in the cell membrane that allow an osmotic imbalance and consequently, the destruction of target cells. The main pro-inflammatory mediators formed are: C3a and C5a, which recruit inflammatory immune cells and regulate T cell function. Complement activation can also eliminate dead cells reducing inflammation, meaning that it can affect the inflammatory mechanisms in the body in various ways (CRAVEDI; VAN DER TOUW; HEEGER, 2013; MARTIN; BLOM, 2016; RICKLIN et al., 2010; WALPORT, 2001).

According to Vlaicu et al. (2016) the complement system is an important associate in insulin resistance, atherosclerosis and obesity, since adipose tissue synthesizes the proteins (factor B, C3, factor D, factor H and properdine) that regulate it, as well as being a target. Obesity has been related to an exaggerated activation of the complement system, precisely by increasing inflammation signaling (WÄRNBERG et al., 2006).

In individuals who lose weight, a reduction of C3 is seen, suggesting its role in obesity, also because it is strongly associated with insulin resistance (MUSCARI et al., 2007), in the same way that in obese individuals, the levels of C3 and C4 are extremely high, with positive

correlation of C3 changes and body mass index, weight, coagulation parameters, inflammatory parameters and leptin, shown in the study of Nestvold et al. (2015). In this context, n-6 SFA and PUFA act as pro-inflammatory factors interacting with the complement system, and promote chronic low-grade inflammation, consequently insulin resistance, increasing the risk of obesity (KREMER, 2017; POREBA et al., 2017).

Jin et al. (2018a) through their study with rats, had as one of the objectives, to determine the effect of a diet rich in fish oil (source of n-3 PUFA) on the activation of the complement system, since this action still remains unknown. In these animals, a higher concentration of MAC, CH_{50} (50% Haemolytic Complement) and C3 (the latter is present in all pathways of the complement system) was found in serum, increased expression of mRNA of factor B (component of the alternative pathway) and a reduction of factor H (inhibitor of the alternative pathway), indicating that this diet can promote an activation of the complement system. It is believed that part of the results obtained was due to the high concentration of n-6 SFA and n-6 PUFA present in the fish oil diet compared to the control diet, which may have masked the effect of n-3 (JIN et al., 2018a). All lipid ratios within the diet, should be taken into consideration, as the effects found may be influenced by the ratios of other fatty acid groups present.

The complement system will act in defense of the organism, favoring at first pro-inflammation to finish off the pathogen and then reduce this process. However, any imbalance can cause the immune system to act against healthy cells, as occurs with autoimmune diseases. Furthermore, activation of the complement system along with complement components in adipose tissue, may contribute to the development of obesity (PHIELER et al., 2013; RICHARDSON; SMITH; CARTER, 2013; RICKLIN et al., 2010). Therefore, it is of interest to verify the potential action of n-3 or lower n-6/n-3 ratio as a controlling agent of inflammatory activities.

By incorporating different ingredients in the diet of cows it is possible to change the composition of some nutrients, even the profile of fatty acids of the milk, become healthier for human consumption. Biofortification is a procedure aimed at augmenting the nutritive profile of micronutrients, encompassing particular vitamins and minerals, is facilitated via the application of biotechnological interventions, agronomic methodologies as elucidated by Bouis and Saltzman (2017), or alternatively, via manipulation of animal feed components. The enhancement of milk's nutritional and sensory attributes can be achieved through dietary modifications administered to livestock. Consequently, the influence of animal feed constituents on the composition of milk has attracted substantial attention, as it improves

nutritional quality and has a positive impact on human and livestock health, as underscored by the investigations conducted by Kennelly (1996), Oliveira et al. (2021), Pfrimer et al. (2018) and Salles et al. (2022).

In our previous studies (OLIVEIRA et al., 2021; REIS et al., 2021, 2022) we have shown how different ratios of n-6/n-3 PUFA can cause different responses in the organism. OLIVEIRA et al., 2021 supplemented Holstein cows with soybean oil and linseed oil. Supplementing cows with vegetable oil reduced milk fat percentage. Soybean and linseed oils lowered saturated fatty acids, increased unsaturated fatty acids, and improved the ratio of saturated to unsaturated fatty acids in milk. Both oils also raised monounsaturated fatty acids, with linseed oil increasing omega-3 content. Blood cholesterol levels were higher with vegetable oil supplementation. Linseed oil produced milk with more omega-3 and a better omega-6/omega-3 ratio than soybean oil making a healthier milk fat profile for human consumption.

This milk fed to sows was also able to alter the FA profile of plasma, colostrum, and milk, as well as the FA profile of the plasma of their piglets (REIS et al., 2021). Study of REIS et al. (2021) examined the effects of supplementing gilts with cow's milk rich in n-3 and n-6 polyunsaturated fatty acids (PUFA) on reproductive outcomes and the blood profiles of swine females and their offspring. The gilts were divided into three groups: Control, fed a basal diet + milk from cows without oil; n-3, fed a basal diet + milk from cows fed a diet enriched with linseed oil; n-6, fed a basal diet + milk from cows fed a diet enriched with soybean oil. The gilts receiving the diets containing PUFA had higher serum urea and very-low-density lipoprotein levels and lower serum total protein and low-density lipoprotein levels compared to the Control group. Females supplemented with n-3 presented higher serum palmitic acid and γ-linolenic acid levels than those fed n-6. Piglets from the Control group were heavier at birth than those from females supplemented with enriched milk. Piglets from enriched milk-fed females were lighter at birth but gained more weight from 1 to 21 days old. They also had higher levels of eicosapentaenoic acid in their serum, leading to a reduced AA/EPA ratio. Gilts supplemented with PUFA-enriched cow’s milk showed changes in their serum palmitic and γ-linolenic acid levels, in addition to improved performance, EPA concentration and consequently reduced AA/EPA ratio in their piglets, demonstrating beneficial results for their progeny.

Another part of the same study showed that in grower gilts up to 190 days of age n-3 and n-6 milk reduced the concentration of myristic acid in the blood and increased the leukocytes. Milk enriched with n-3 compared to n-6 reduced the stearic acid, concluding that milk with a better PUFA profile can reduce saturated fatty acids in the blood and alter the concentration of cells in the defense system (REIS et al., 2022). These results indicate that milk with a better FA

profile can impact health and performance in a variety of forms. A decision to change the diet of dairy cows has a high potential influence on the consumer and even the offspring. Therefore, we need to rethink how we are eating, not only for our health, but also considering our progeny, or even as benefit of the livestock health, which directly influence us.

The importance of balancing n-6/n-3 in the maternal diet has been studied and its impact of progeny as shown for several researchers (MUKHAMETOV et al., 2022; SIMOPOULOS, 2002, 2016). Lauridsen, Stagsted and Jensen (2007) investigated the effects of different dietary fats and vitamin E supplementation on lactating sows and their piglets, the results showed a decreased the activity of glutathione peroxidase (an antioxidant enzyme) in liver cytosol using sunflower oil in the diets. In addition, the composition of fatty acids in the alveolar macrophages of piglets was influenced by the dietary fat sources provided to the sows, since the highest ratio n-6/n3 and increased of ex vivo synthesis of prostaglandin E2 and thromboxane B2 were found in the piglets suckling sows on the sunflower oil treatments, while it was lowest in piglets suckling sows fed fish oil.

A recent study questioned the optimal n-6/n-3 ratio in the maternal diet and evaluate the effects on reproduction, milk fatty acid profile, and plasma leptin concentration in pigs through treatments with soybean oil and linseed oil as a source of n-6 and n-3 respectively. They found that a diet with a low ratio of n-6/n-3 alone resulted in improved weaning survival rate, weight gain in suckling piglets, and increased levels of total n-3 fatty acids in colostrum and milk (NGUYEN et al., 2020).

Due to limited research in this area, the study of Manaig et al. (2022) aimed to investigate the direct impact of sow nutrition on the deposition of protein and fat in suckling piglets. To accomplish this, sows were fed with different ratios of n-6/n-3 polyunsaturated fatty acids (PUFA), specifically 13:1 (SOY) and 4:1 (LIN), throughout the gestation and lactation periods. The study assessed the content and deposition of polyunsaturated fatty acids in muscle and adipose tissues, as well as the relative abundance of proteins. Thus, the results revealed that the low n-6/n-3 PUFA maternal diet influenced the early growth performance and fat deposition in suckling piglets. Furthermore, utilizing a proteome approach on muscle and fat tissue provided evidence of positive changes in the immune status of piglets when sows were fed low n-6/n-3 PUFA diets.

Pigs serve as valuable animal models for human research due to their anatomical and physiological similarities to humans, such as size, immunology, genome, and physiology (LUNNEY et. al., 2021). These similarities make pigs preferable to rodents for translational and clinical research, given their short generation times, large litters, and editable genomes

(SEOK et al., 2013). Pigs are used in drug development studies due to their similar size and physiology, aiding in defining safe dosage ranges. Customized pigs can be generated using surgical, drug, or genome editing methods, making them important large animal models (CAMACHO et al., 2016).

Their respiratory tract anatomy shares similarities with humans, making them relevant for respiratory disease and vaccine research. Pigs are extensively used to study embryonic development and immune responses comparable to humans, facilitating disease and vaccine research. The pig genome annotation is a valuable resource for studying immunity (WILSON; OBRADOVIC, 2015). While there are differences between pigs and humans, such as muscle locations, liver anatomy, and placenta barrier type, these distinctions offer unique advantages for specific research areas. With continued scientific advancements and technology development, pigs, especially gene-edited models, will remain vital in translational medical research (LUNNEY et. al., 2021).

In addition, the advances discovered not only contribute to human health, but also to pig breeding and production themselves, since there is plenty still to be discovered about the metabolism of this species, in addition to the fast changes that are being brought about by their genetic evolution.

3 OBJECTIVE

Establish whether dietary supplementation of sows with milk biofortified with n-3 or n-6 PUFA, using different n-6/n-3 ratios of milk, provide health benefits to swine or their offspring, used as a model for humans:

- ✓ Alter the expression of genes that control lipid oxidation and lipogenesis in the liver, and appetite regulators in the hypothalamus;
- ✓ Modify the production of immunoglobulins, interleukins and eicosanoids that affect the immune system;
- ✓ Promote changes in the hemolytic activity of the alternative pathway of the complement system;
- ✓ Replace circulating fatty acids in the organism, in colostrum and milk;
- ✓ Drive positive trans-generational effects.

4 HYPOTHESIS

The supplementation of cow's milk with lower n-6/n-3 ratio, or higher amount of n-3 PUFA in the diet of sows, used as a model for human, alters the profile of unsaturated fatty acids, in both the sow and her offspring. Alterations in fatty acids profiles will be related to a suppression of lipogenic genes and activation of lipolytic genes.

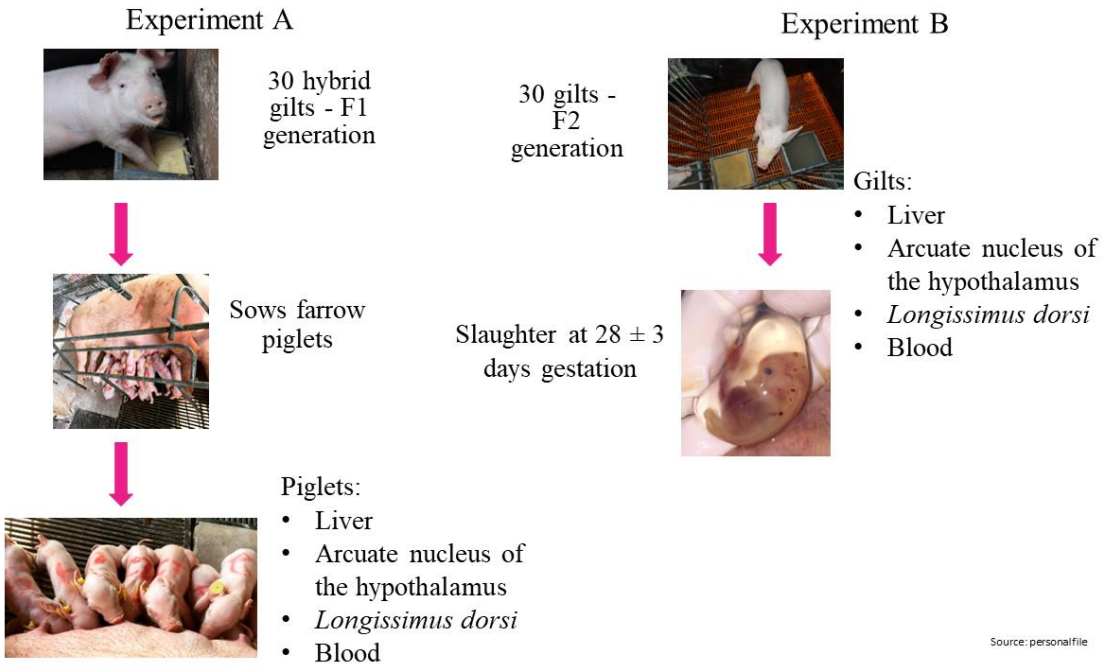
5 MATERIAL AND METHODS

The procedures and experimental use of animals were approved by the Animal Care and Use Committee of the School of Animal Science and Food Engineering at the University of São Paulo (Protocol #4939070317 and #6983020920).

5.1 EXPERIMENTAL DESIGN

The study involved 30 hybrid gilts - F1 generation (Landrace x Large White), 34 days old and initial body weight (BW) of 9.59 ± 1.28 kg (experiment A), and 30 gilts - F2 generation (Landrace x Large White), 30 ± 2 days and initial BW of 8.49 ± 1.81 kg (experiment B), were housed individually and used in a completely randomised experimental design. Gilts were randomly allocated into one of three groups: (1) Control - basal diet + milk from cows without oil supplementation; (2) n-3 - basal diet + milk from cows fed a diet enriched with linseed oil; and (3) n-6 - basal diet + milk from cows fed a diet enriched with soybean oil. All the gilts were supplemented with the treatments until their offspring were 21 days old (experiment A) or until day 28 ± 3 of pregnancy (experiment B) (Figure 1). More details can be found in the study of Oliveira et al. (2021), about the biofortified milk, which was obtained from Holstein cows from the University of São Paulo herd. The cows were supplemented or not with 2.5% (on a dry matter (DM) basis) of linseed or soybean oil, sources of n-3 and n-6, respectively.

Figure 1 - General outline of the animals



Source: Own authorship.

Supplementation with milk was performed daily at 8:00 a.m., after individual feeding. From weaning to artificial insemination (AI), the females were supplemented with 200 mL (days 34-76), 300 mL (days 77-128), 400 mL (days 129-174), 500 mL (days 175-331) and 1 L until weaning (Experiment A) or day 28 ± 3 of pregnancy (experiment B) of cows' milk per animal per day (Figure 2). The effects of vegetable oil on the quality of the lipid fraction of the dairy cows' milk provided for gilts are presented in Table 1. The milk dose was based on the recommendations of the Brasil (2008) for an average weight Brazilian man (IBGE, 2008), at 5 mL/kg body weight, which was adjusted according to the weight of the animals.

Figure 2 - Scheme of the physiological phases of the swine females, and consumption of enriched milk in each phase of the experiment.

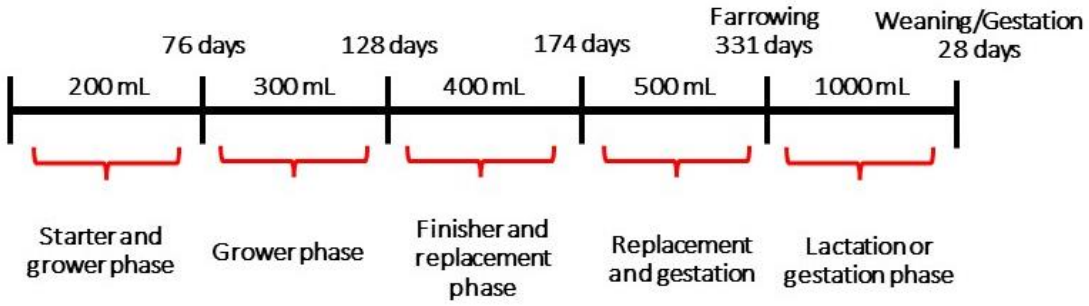


Table 1. The lipid fraction of milk from Holstein dairy cows fed diets supplemented linseed and soybean oil

Fatty Acid Profile [a], g/100 g	Diets [b]			SEM [c]	*p*-Value [d]		
	CON	LIN	SOY		Treatment	C1	C2
ΣSFA	66.89	56.60	56.52	1.441	<0.01	<0.01	0.969
ΣUSFA	33.05	43.35	43.39	1.438	<0.01	<0.01	0.986
SFA/USFA	2.12	1.36	1.34	0.107	<0.01	<0.01	<0.01
ΣMUFA	29.58	39.47	39.55	1.301	<0.01	<0.01	0.966
ΣPUFA	3.57	3.98	3.93	0.275	0.199	0.076	0.860
Σn-3	0.32	1.02	0.36	0.029	<0.01	<0.01	<0.01
Σn-6	2.48	2.25	2.88	0.208	0.004	0.589	0.001
n-6/n-3	7.92	2.72	8.26	0.504	<0.01	<0.01	<0.01
Cholesterol, g/100 mL	9.94	8.74	10.05	1.649	0.174	0.403	0.097

Source: Own authorship.

[a] ΣSFA = Σ saturated fatty acids; ΣUSFA = Σ unsaturated fatty acids; SFA/USFA = Σ saturated/Σ unsaturated; ΣMUFA = Σ monounsaturated fatty acids; ΣPUFA = Σ polyunsaturated fatty acids; Σn-3 = sum of n-3 fatty acids; Σn-6 = n-6: sum of n-6 fatty acids; n-6/n-3 = Σ omega-6/Σ omega-3.

[b] Cows fed with a Control diet (CON), supplemented with linseed oil (LIN) or soybean oil (SOY).

[c] SEM, standard error of the mean.

[d] C1, contrast CON vs. LIN+SOY; C2, contrast LIN vs. SOY.

5.2 DIETS

The feed was offered according to the phases described below: pre-starter phase, from weaning to 43 days old; starter phase, from 44-72 days old; grower phase, from 73-130 days old; finisher phase, from 131-152 days old; replacement phase, from 155-AI; gestation phase; and lactation. The gilts were fed 2.0 kg/d of gestation diet from d 21 of gestation until the day of farrowing. On the day of farrowing, sows were transferred onto the lactation diet with *ad libitum* feeding (experiment A). Gilts from experiment B were fed with 2 kg/d of gestation diet from d 21 of gestation until the day of slaughter (experiment B). All diets were based on corn and soybean feedstuffs and did not contain any oil as an ingredient (Table 2) and were formulated to meet the nutritional requirements of females for each physiological phase. The descriptive analysis of the FA profile and lipid fraction of diets is shown in Table 3. Diet analysis showed that the most abundant fatty acids were linolenic acids (44.64%), oleic (25.52%) and palmitic (18.49%).

Table 2. Composition of pre-initial, initial, grower and finisher diets, specific to each physiological phase of swine females

Item	Pre-Initial	Initial	Grower	Finisher	Replacement	Gestation	Lactation
Ingredients, g/kg							
Ground corn	399.0	649.0	699.0	739.0	644.4	594.0	587.8
Soybean meal	200.0	300.0	280.0	240.0	240.8	140.0	265.1
Wheat bran	-	-	-	-	86.5	240.0	-
UNIMIX[a]	400.0	50.0	20.0	20.0	25.0	25.0	30.0
L-lysine	-	-	-	-	-	-	10.0
DL-methionine	-	-	-	-	-	-	2.0
Sugar	-	-	-	-	-	-	100.0
Calcitic limestone	-	-	-	-	2.3	-	4.1
Mycofix	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Chemical composition							
Dry matter, %	83.41	87.92	87.07	88.93	88.12	89.42	88.25
Ashes, %	6.69	5.43	4.80	3.70	4.54	5.23	5.62
Crude energy, cal/g	4347.0	4393.0	4419.0	4465.0	4411.5	4360.0	4376.0
Ether extract, %	4.14	1.71	2.41	1.71	1.37	1.40	1.56
Crude fiber, %	4.54	4.32	4.30	4.07	3.01	5.23	5.28
Crude protein, %	18.04	21.21	18.07	20.46	19.04	17.59	19.99
Calcium, %	0.90	0.80	0.63	0.51	0.82	0.81	0.94
Phosphorus, %	0.69	0.53	0.41	0.37	0.38	0.46	0.43

Source: Own authorship.

[a]The premix used in each phase was based on Rostagno et al. (2017).

Table 3. Fatty acid composition and lipid fraction of diets offered to swine females

Fatty acids [a], g/100 g	Pre-Initial	Initial	Grower	Finisher	Replacement	Gestation	Lactation
Lauric, C12:0	10.63	0.17	0.01	0.07	0.01	0.03	0.01
Myristic, C14:0	4.17	0.20	0.07	0.14	0.09	0.18	0.14
Palmitic, C16:0	18.24	21.31	16.19	20.52	17.92	17.72	17.47
Stearic, C18:0	5.04	3.58	2.70	2.62	2.62	2.48	2.68
Palmitoleic, C16:1c9	0.13	0.08	0.07	0.09	0.07	0.18	0.15
Elaidic, C18:1t	0.21	n.d. [c]	n.d.[c]	n.d.[c]	n.d.[c]	0.06	0.05
Oleic, C18:1c9	23.75	25.72	32.63	25.46	25.52	22.11	23.39
Vaccenic, C18:1c11	1.44	1.56	1.76	1.53	1.50	1.55	1.39
Linoleic, C18:2 n-6	28.94	43.40	43.36	46.01	48.70	51.52	50.41
α-Linolenic, C18:3 n-3	1.84	1.78	1.59	1.71	2.11	2.26	2.35
DHA, C22:06 n-3	n.d.[c]	0.06	0.04	0.03	n.d.[c]	0.07	0.06
Others [b]	5.55	2.06	1.57	1.82	1.48	1.81	1.81
ΣSFA	42.96	26.73	19.86	24.43	21.45	21.33	21.32
ΣUSFA	56.30	72.55	79.41	74.80	77.89	77.67	77.74
ΣMUFA	26.19	27.97	35.14	27.82	27.74	24.75	25.74
ΣPUFA	30.77	45.24	44.99	47.75	50.81	53.87	52.84
Σn-3	1.84	1.84	1.63	1.74	2.11	2.32	2.41
Σn-6	28.94	43.40	43.36	46.01	48.70	51.52	50.41
SFA/USFA	0.76	0.37	0.25	0.33	0.28	0.27	0.27
n-6/n-3	15.76	23.58	26.57	26.38	23.10	22.19	20.92
Total	99.92	99.93	99.99	100.00	100.00	99.95	99.90

Source: Own authorship.

[a]DHA: docosahexaenoic acid; ΣSFA = Σ saturated fatty acids; ΣUSFA = Σ unsaturated fatty acids; ΣMUFA = Σ monounsaturated fatty acids; ΣPUFA = Σ polyunsaturated fatty acids; Σ n-3 = Σ omega-3 fatty acids; Σ n-6 = Σ omega-6 fatty acids; SFA/USFA = Σ saturated/Σ unsaturated; n-6/n-3 = Σ omega-6/Σ omega-3.

[b] Composed of the following fatty acids: C4:0, C6:0, C8:0, C10:0, C10:1, C11:0, C12:1, C13:0 iso, C13:0, C14:0 iso, C14:1c9, C15:0 iso, C15:0 anteiso, C15:0, C17:0 iso, C17:0, C17:1, C18:1 c12, C18:1 c13, C20:0, C20:1, C20:2, C22:0, C 22:1n9, C23:0, C24:0, C24:1.

[c] Not detectable.

5.3 PERFORMANCE PARAMETERS

Individual gilts were weighed and scanned for backfat thickness (BF) on gestation day 40 (D40G) and day 107 (D107G) to determine BW loss and BF changes, in experiment A. The BF of the gilts was measured at the P2 position (5 cm off the midline at the 10th rib) with ultrasound instrument (Pie-Medical Scanner 100, 3.5 MHz convex probe, Philipsweg, Belgium). The same evaluations were recorded throughout lactation (REIS et al., 2021).

5.4 REPRODUCTIVE MANAGEMENT

All gilts were synchronized using an orally synthetic progesterone (Altrenogest, Regumate®, Intervet Inc., MSD Animal Health, São Paulo, Brazil), for 18 days at a rate of 5 ml/day. Gilts were paired by AI at 198 ± 9 days of age using refrigerated semen from hybrid boars (DB LM6200, Patos de Minas, Brazil), at 24-h intervals until the end of estrus. All seminal doses were used for up to 3 days, stored at 17°C and, the final concentration of 30 × 10^6 sperm/ml (seminal dose was 100 ml). Estrus was checked twice daily in the presence of a mature boar. At days 21, pregnancy was confirmed by ultrasound instrument (Pie-Medical Scanner 100, 3.5 MHz convex probe, Philipsweg, Belgium).

5.5 SAMPLE COLLECTION

On day 40 and 107 of gestation, day 1 and 21 of lactation, blood samples were collected from sows from jugular vein into nonheparinized tubes. Blood was centrifuged at 2000g at ~25 °C for 10 min to isolate serum for fatty acids profile and biochemical parameters. On day 40, 75 and 107 of gestation, day 1 and 21 of lactation for sows, and day 1 and 14 of lactation for piglets blood samples were collected to analyze immunoglobulins and interleukins. Serum samples were stored at -80ºC for subsequent assay.

Samples of colostrum (20 mL) were collected at the moment of ejection started during farrowing, without any induction. Milk samples (50 mL) were obtained on day 14 of lactation by manually milking all functional mammary glands of each sow. To facilitate milk collection, 1 mL of oxytocin (Ocitovet, Ceva, Brazil) was administered through the sows' ear vein. All samples were stored at −20°C until they were analyzed.

To identify the eicosanoids and analyze the complement system, the whole blood (1 mL) was collected at day 13 ± 4 days of lactation (experiment A) for sows and piglets and 24 ± 6 days of gestation (experiment B) for gilts using plastic tubes containing heparin as an anticoagulant. Plasma samples were stored at -80ºC until analysis.

At day 14 day of lactation were collected from 120 piglets, 2 males and 2 females per sow (experiment A) and 28±3 of pregnancy for gilts (experiment B), *Longissimus dorsi*, liver and arcuate nucleus of the hypothalamus were collected.

5.6 DETERMINATION OF FATTY ACIDS PROFILE AND BIOCHEMICAL PARAMETERS IN SERUM – EXPERIMENT A

The serum FA profile was analysed by gas chromatography (HUANG; WANG; CRENSHAW, 2006). Serum levels of urea, total protein, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), glucose, and triglycerides (TG) were determined in the Clinical Analysis Diagnostics laboratory (DAC, Pirassununga, SP, Brazil) using commercially available kits (VIDA Biotecnologia® and LABTEST®, Minas Gerais, Brazil) according to the manufacturer's instructions.

5.7 COLOSTRUM AND MILK FATTY ACID ANALYSIS – EXPERIMENT A

The FA profile of diets, colostrum and sow milk was determined by the Animal Nutrition and Growth Laboratory of the Luiz de Queiroz School of Agriculture (ESALQ/USP). Fat in colostrum and milk samples (400 mg) was separated by centrifuging samples at 4700 rpm for 45 min, according to Hara and Radin (1978), and then methylated using a methanolic sodium methoxide solution, according to Christie (1982). The resulting fatty acid methyl esters (FAMEs) were determined by gas chromatography (ThermoFinnigan®, model Trace 2000), using a fused silica capillary column, CP-Sil 88 (100 mm x 0.25 mm x 0.2 mm; Agilent Technologies, USA). Hydrogen was used as carrier gas. The program followed with protocol: 70 °C for 4 min; increased by 13°C/min until 175°C, constant for 27 min; increased by 4°C/min to 215°C, constant for 9 min; increased by 7°C/min to 230°C, constant for 5 min. The identification of FAMEs was performed by comparing the retention times and the percentages of FAMEs using the software Chromquest 4.1 (Thermo Electron, Italy). Standards used were Supelco 37 Component FAME Mix (CRM47885, Supelco) and linoleic acid (conjugated methyl ester, O5632, Sigma-Aldrich).

5.8 MEASURE OF COLOSTRUM PRODUCTION AND INTAKE – EXPERIMENT A

Colostrum intake (CI) of piglets alive was estimated using the model developed by Theil et al. (2014) 24 h after the first-born piglet, which CI and weight gain (WG) were expressed in grams, body weight at birth (BWB) in kilograms and duration of CI in minutes (D):

$$CI = -106 + 2.26WG + 200BWB + 0.111D - 1{,}414WG/D + 0.0182WG/BWB$$

According to Devillers et al. (2007) a negative CI was assumed to be 0. The sum of the individual CI of each piglet within the litter was used to calculate colostrum production of the sow (THEIL et al., 2014).

5.9 APGAR SCORE AND UMBILICAL CORD EVALUATION – Experiment A

Neonates were evaluated at birth for vitality by three persons previously trained using the Apgar score described by Randall (1971), adapted by Zaleski and Hacker (1993) and modified by Mota-Rojas et al. (2005). In order to avoid interference with vitality criteria, no aid was given to newborn piglets at the time of farrowing and during the vitality evaluation.

The following variables were quantified: time from birth to breathing: >1 min, between 16 s and 1 min and <15 s; heart rate: bradycardia (<120 bpm), normal (between 121 and 160 bpm) and tachycardia (>161 bpm); meconium stain: severe, mild or absent (Mota-Rojas et al., 2006); color of the skin on the snout: cyanotic, pale or pink; and attempts to stand on all four legs: >5 min, between 1 and 5 min and <1 min. The score for each variable was from 0 (the least favorable) to 2 (the most favorable) and the sum of them in an Apgar score ranging from 1 to 10 for each neonatal piglet (Figure 3). All piglets were classified into three groups of Apgar scores, within each litter: low vitality (scores ≤5); medium vitality (scores between 6 and 7); and high vitality (scores ≥8) (Trujillo-Ortega et al., 2011). Since scores ≥6 means medium to high vitality, lower scores are of concern regarding neonate survival. Thus, piglets with Apgar vitality scores between 1 and 5 were classified in the statistical analysis as viability 1, i.e., representing low survival, in view of the interest in verifying the potential of biofortified milk to reduce low survival at birth. Umbilical cords were evaluated at birth and classified as adhered (normal) or broken (abnormal) according to the criteria from Mota-Rojas et al. (2002).

Heart rates (beats/min) were measured by use of a stethoscope, and first inspiratory efforts were considered when a movement was observed in the thoracic area accompanied by exhalation of air. The time until each newborn was able to stand on all four feet was measured with a chronometer. In addition, the first mammary contact was recorded through individual piglet observation, independently if there was colostrum consumption or not, from the moment the newborn was relocated close to the mother's vulva. At the end, all procedures were carried out within 5 min, to shorten the handling duration. All the piglets were individually identified with numbers on their backs and subsequently with ear tags.

Figure 3 - Assessment form for the Apgar score

Female n°:		Treatment:																				
Evaluated parameters		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Birth time																							
Blood colection (2F e 2M) – 2 mL																							
Time from birth to breathing	> 1 min (0)																						
	16 s– 1 m (1)																						
	<15 s (2)																						
Umbilical cord	1. Adhered																						
	2. Broken																						
Meconium stain	severe – > 40% body (0)																						
	mild – < 40% body (1)																						
	absent (2)																						
Color of the skin on the snout	cyanotic (1)																						
	pale (0)																						
	pink (2)																						
Rectal temperature																							
Heart rate (bpm)	<120 – Bradycardia (0)																						
	121–160 – Normal (1)																						
	>161 – Tachycardia (2)																						
Attempts to stand on all four legs	>5 mim (0)																						
	1–5 min (1)																						
	< 1 min (2)																						
First contact with teat (minutes)																							
Stillbirth	Prepartum																						
	Intrapartum																						

5.10 HEMOLYTIC ACTIVITY OF THE COMPLEMENT SYSTEM

The hemolytic activity of complement system was measured on serum sows and their piglets (experiment A) and gilts (experiment B) (FERRIANI; BARBOSA; DE CARVALHO, 1999) in Department of Clinical, Toxicological and Bromatological Analysis, Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Brazil. The rabbit erythrocytes eluted in Alsever's solution was chelated by TEA-EDTA in 0.1 % gelatin solution, incubate at 37 °C for 15 min, centrifuged at 480 x g at 4 °C for 10 minutes, suspended in TEA-$MgSO_4$ and washed 3 times before use. These erythrocytes were suspended in TEA-EGTA-$MgSO_4$ in 0.1% gelatin solution, OD between 0.7 and 0.8 at 700 nm adjusted to pig serum methodology. The serum diluted was added to the erythrocyte suspension and read at 700 nm for 15 min at 37ºC for performed of the hemolytic activity of complement (Epoch 2; BioTek Instruments, Inc., Winooski, VT, USA). Serum pre-heated at 56 °C for 30 min was used as a negative control. The calculation of 50% hemolysis was derived from the hemolysis curves obtained by diluting serum from female swine. This involved determining the value of T1/2, which represents the time required for the complement to decrease the initial optical density of erythrocytes from rabbits in suspension by 50%. Conversely, for piglets, the ΔOD was utilized, which is the difference between the final and initial optical densities.

5.11 QUANTIFICATION OF IMMUNOGLOBULINS AND INTERLEUKINS – EXPERIMENT A

Enzyme-linked immunosorbent assay (ELISA) kits were used to measure levels of IgA, IgM, IgG (Bethyl Laboratories, Montgomery, TX, USA), IL-10, TNF-alpha and IL-6 (R&D Systems, Minneapolis, MN). All colostrum samples were defrosted in ambient temperature and centrifuged (10,000 g for 5 min) to remove fat. Samples were diluted according to manufacturer's instructions. The plates were read on a microplate reader at 540 nm and the results were corrected for dilution factor.

5.12 EMBRYO SURVIVAL IN GILT SUPPLEMENTED WITH BIOFORTIFIED MILK – Experiment B

At day 28±3 of pregnancy, gilts were humanely slaughtered, at a local abattoir (University of São Paulo) through electrical stunning and exsanguination. The entire reproductive tract was taken and evaluated immediately. The number of embryos was counted and categorized as viable or nonviable. The number of corpora lutea was counted and the fertilization rate was determined by dividing the number of total embryos by the number of corpora lutea. The adjusted fertilization rate was calculated using number of viable embryo compared with the total number of corpora lutea counted. Pregnant uterus weight was taken and recorded.

5.13 ANALYSIS OF EICOSANOIDS BY LIQUID CHROMATOGRAPHY TANDEM-MASS SPECTOMETRY (LC-MS/MS)

According to Galvão et al. (2016) samples were placed in a warm (37ºC) water bath and stimulated for 20 min with thapsigargin (TAP) at the concentration 1.3 μM. The reactions were stopped by placing the samples in an ice bath. Samples were then centrifuged at 300 x g at ~4ºC for 20 min and 300 μL of plasma was stored at -80ºC until extraction and analysis. Dimethylsulfoxide (DMSO) served as a negative control. The analysis was carried out in collaboration with CEQIL – Center of Excellence in Quantification and Identification of Lipids, Faculty of Pharmaceutical Sciences of Ribeirão Preto (FCFRP), University of São Paulo. The methodologies used in this project were developed by the CEQIL technical team and referenced below (https://fcfrp.usp.br/en/pesquisa-extensao/pesquisa/centrais-multiusuarios/ceqil/).

The methodology was used according to Sorgi et al. (2018). The lipids were purified by Solid Phase Extraction (SPE) using HPLC-grade solvents (Merck, Kenilworth, NJ, USA),

added of deuterated internal standards (IS) were purchased from Cayman Chemical Co (Ann Arbor, MI, USA) and for the step columns Hypersep C18 (500 mg, 3 mL, Thermo Scientific-Bellefonte, PA, USA) were used. All the target analytes were quantified using high-resolution MRM (MRM^{HR}), which are: LTB_4, 6-trans-LTB_4, 20-OH-LTB_4, LTC_4, LTD_4, 11-trans-LTD_4, LTE_4, LXA_4, RvD_1, RvD_2, TXB_2, PGB_2, PGE_2, PGD_2, 15-keto-PGE_2, 20-OH-PGE_2, PGD_2, PGJ_2,15-deoxi-δ-12,14-PGJ_2, 6-keto-$PGF_1\alpha$, $PGF_2\alpha$, 19-OH-PGB_2, PGG_2, MaR-1, 5-HETE, AA, 5-oxo-ETE, 20-HETE, 5,6-DiHETE, 12-HETE, 8-HETE, 11-HETE, 12-oxo-ETE, 12-oxo-LTB_4, 15-oxo-ETE, 11,12-DiHETrE, 14,15-DiHETrE, EPA, DHA, 15-HETE, 5,6-DiHETrE.

The liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) system (Nexera X2, Shimadzu-Kyoto, HO, Japan) and interfaced with a $TripleTOF5600^+$ Mass Spectrometer (Sciex-Foster, CA, USA) which was conducted in the negative-ion mode, equipped with a Turbo-V IonSpray. Additional instrumental parameters were as follows: nebulizer gas (GS1), 50 psi; turbo-gas (GS2), 50 psi; curtain gas (CUR), 25 psi; electrospray voltage (ISVF), −4.0 kV; and turbo ion spray source temperature, 550°C. The mass range of the product ion experiments was from m/z 50 to 700 and the dwell time was 10 ms. The lipid species were identified using PeakView 2.1 (Sciex-Foster, CA, USA) and for quantitative analysis MultiQuant was used.

5.14 HISTOLOGYCAL ANALYSIS

Longissimus dorsi muscle samples were collected from piglets slaughtered, 2 males and 2 females per sow, at 14 days postnatal for experiment A, and from gilts (28±3 days of gestation) from the experiment B. Samples were collected and fixed in 10% formalin for 24 h and stored in 70% alcohol until embedding in parafin. Sections (4 μm) were obtained and stained with hematoxylin and eosin, as previously described (PROPHET et al., 1992).

Three random areas (40x) of each slide were captured as image for each location (muscle and adipose), using a microscope Leica DM500 connected a camera Leica ICC50-HD (Leica Microsystems, Heerbrugg, Suíça). The images were evaluated by an image analysis software (Image-Pro Plus®, Media Cybernetics, Rockville, USA), to determine through Manual Tag tool: area, maximum diameter, minimum diameter, and average diameter of muscle and adipose cells. The per-area is a percentage of occupied area by delimited object inside to the total image. This is a way to quantify the area of relative form. The morphometric analysis was performed blindly, and cells were chosen with good boundaries cytoplasmic. The aim was to verify if

differences in physical structure of lipids on diet in the long-term could modulate the development of muscle and adipose tissue.

5.15 EVALUATION GENE EXPRESSION BY qRT-PCR ANALYSIS

Liver samples were collected from piglets, 2 males and 2 females per sow, on d14 postnatal (experiment A) and liver and arcuate nucleus of the hypothalamus (ARH) from gilts of 28±3 days of gestation (experiment B) and immediately placed in cryotubes with RNA*later*™ Stabilization Solution (Invitrogen™, AM7024, Carlsbad, CA, USA) and snap frozen in liquid nitrogen. Samples were stored at -80ºC until analysis.

The primers were designed using FASTA into the National Center for Biotechnology Information (NCBI) GenBank database to determinate sequence identity, based on *Sus scrofa* plublished nucleotides sequences (Table 4 and 5). In addition, was used the NetPrimer Software (PREMIER Biosoft International, Palo Alto, CA, USA).

Total RNA was extracted using RNeasy Mini Kit (Qiagen, #74104, Venlo, Limburg, Netherlands) according to the manufacturer's instructions. The quantity of the RNA was determined using NanoDropOneC spectrophotometer (Thermo Scientific, Carlsbad, CA, USA), and the integrity was assessed by visualization of the 28S/18S rRNA band pattern in a 1% agarose gel. Isolated RNA was treated with DNAse I (Thermo Scientific, Carlsbad, CA, USA) to eliminate possible genomic DNA contamination. The synthesis of cDNA was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied BiosystemsTM, #4368814, Foster City, CA, USA), according to the manufacturer's protocol. Relative quantitative analyses of mRNAs were carried out in 10 µL reactions containing 2x qPCRBIO SyGreen Mix (PCRBiosystems, London, UK), 1 µl cDNA, and forward and reverse primers in optimized concentrations determined for each gene with the following cycling parameters: 2 min at 95ºC followed by 40 repeats of 5 s at 95ºC and 30 s at 60ºC followed by melt curve analysis to confirm amplification of single cDNA products. Relative quantitative gene expression was calculated to the transcript level of hypoxanthine phosphoribosyltransferase 1 (HPRT1) and β-actin (ACTB) (housekeeping genes), as previously described (HELLEMANS et al., 2007; VANDESOMPELE et al., 2002). Raw Ct values were normalized to the geometric average of HPRT1 and ACTB, to identify the differences among competence groups, as previously described for similar tissues (DURAN-MONTGÉ et al., 2009; PIERZCHAŁA et al., 2011). Normalized results of duplicate samples were used to calculate the relative expression using 2^{-}

$^{\Delta Ct}$ transformation and the relative expression values were calculated using the ΔCt method (SCHMITTGEN; LIVAK, 2008).

Table 4. Gene names and primer sequences of liver

Gene abbreviation[a]	Gene name	Primer sequences (5'-3')	GenBank sequence	References
ACC	Acetyl-CoA Carboxylase	F: ATGTTTCGGCAGTCCCTGAT R: TGTGGACCAGCTGACCTTGA	NM_001114269.1	Chen et al. (2017)
SREBP-1	Sterol Regulatory Element-Binding Protein 1	F: CTACCACAAGCTGCACCAG R: GGGAGACTGGTCTTGACTCG	NM_214157.1	Li et al. (2016)
SCD	Stearoyl-CoA Desaturase	F: TTGATCCCCACCTGCAAGAT R: CGTGTTGGCAATGATCAGGA	NM_213781.1	Cirera et al. (2020)
PPAR-α	Peroxisome Proliferator Activated Receptor Alpha	F: TTTCCCTCTTTGTGGCTGCT R: GGGGTGGTTGGTCTGCAAG	NM_001044526.1	Mentzel et al. (2018)
D6D	Delta-6 Desaturase	F: ACATGAACCTGTTCGAGAGC R: TGATGAACTTGTGGACGATG	NM_001171750.1	Meadus, et al. (2011)
FAS	Fatty Acid Synthase	F: GTGGGCTACAGCATGATAGG R: GAATTGCAGCGAGGAGTTAG	NM_001099930.1	Meadus, et al. (2011)
HPRT1	Hypoxanthine Phosphoribosyltransferase 1	F: AAGGACCCCTCGAAGTGTTG R: CACAAACATGATTCAAGTCCCTG	NM_001032376.2	Svobodová, Bílek and Knoll (2008)
ACTB	β-Actin	F: TCTGGCACCACACCTTCT R: TGATCTGGGTCATCTTCTCAC	NM_021086047.1	Jiao, et al. (2020)

Source: Own authorship.
[a]Housekeeping genes: HPRT1 and ACTB.

Table 5. Gene names and primer sequences of ARH

Gene abbreviation[a]	Gene name	Primer sequences (5'-3')	GenBank sequence	References
CART	Cocaine and Amphetamine-Related Transcript	F: CCGCCCTGCTGCTGCTGCTAC R: AGGGACTTGGCCATACTTCTTCTC	NM_001099925.1	Óvilo et al. (2010)
INSR	Insulin Receptor	F: AAACGCCAGGGACATCGTCAAGG R: CCGCAGGGAACGCAGGTAACTCT	XM_021083940.1	Óvilo et al. (ÓVILO et al., 2014)
LEPR	Leptin Receptor	F: GAAAAACACCGGAATGATGC R: AAAAGAAGAGGGCCAAATGTC	NM_001024587.1	Óvilo et al. (2014)
NPY	Neuropeptide Y	F: TCGGCGTTGAGACATTACATCA R: GTCTCGGGACTAGATCGTTTTCC	NM_001256367.1	Óvilo et al. (2010)
HPRT1	Hypoxanthine Phosphoribosyltransferase 1	F: AAGGACCCCTCGAAGTGTTG R: CACAAACATGATTCAAGTCCCTG	NM_001032376.2	Svobodová, Bílek and Knoll (2008)
ACTB	β-Actin	F: TCTGGCACCACACCTTCT R: TGATCTGGGTCATCTTCTCAC	NM_021086047.1	Jiao, et al. (2020)

Source: Own authorship.
[a]Housekeeping genes: HPRT1 and ACTB.

5.16 STATISTICAL ANALYSIS

All data were analyzed using SAS software (Version 9.4, SAS Institute Inc., Cary, NC). The experimental model followed a completely randomized design, the animal was considered as an experimental unit. Animals in pre-initial period were randomly assigned to 1 of 3 treatments. The normality of the residuals was verified by the Shapiro-Wilk test (PROC UNIVARIATE of SAS) and information with studentized residuals greater than +3 or less than −3 were excluded from the analysis. The homogeneity of variances was compared using the Levene test.

Variables with continuous distribution (size of muscle and fat cells, FA profile of diet, colostrum, milk, FA profile and blood biochemistry, gene expression) were analyzed using the MIXED procedure of SAS (SAS Institue Inc.), and GLIMMIX when the non-normality of the residuals was founded. When the time factor was not present, the statistical model included: 'treatment' and 'sex' (when present) as a fixed effect, 'animal', 'gestation period', 'lactation period' and 'residual' as random effects. When the time factor was present, repeated measures in time were performed, in which the statistical model included: fixed effects of 'treatment', 'time', and 'treatment-by-time interaction'. The condition of the umbilical cord was evaluated by the Chi-Square Test. Piglet weight at 1 and 14 days of life, the effect of piglet per sow, the weight of sows, order and time of birth, sex, were used as covariates, when appropriate.

The covariance structure for each parameter was determined based on the lowest value of the Akaike information criteria (AIC). Orthogonal contrasts were declared as: C1 (Control group vs. n-6+n-3) and C2 (n-6 vs. n-3). The data of hemolytic activity of the complement system was analyzed using ANOVA followed by Dunnett's test for multiple comparisons. Effects were considered significant when $P \leq 0.05$.

6 RESULTS AND DISCUSSION

6.1 MILK BIOFORTIFIED WITH PUFA N-3 LOWER BACKFAT THICKNESS GILTS

In order to investigate the effect of milk biofortified with PUFA n-3 and PUFA n-6 on fat mobilization, we measured the BF of gilts at gestation period. On D40G, the BF of gilts supplemented with n-6 and n-3 milk was greater ($P = 0.014$) than CON. We observed that comparing to n-6 milk, gilts of the high n-3 PUFA milk had lower BF difference ($P < 0.029$) (Table 6).

BF depth changed differentially ($P = 0.010$) by treatments across the days of gestation, with a significant interaction between treatment and time (Table 7 and Figure 4). There was no difference in BF depth between D40G and D107G in the CON group. The sows fed n-6 biofortified milk increased BF depth from 15.76 to 16.41 mm whereas sows fed n-3 milk decreased BF depth from 17.29 to 15.44 mm between 40 to 107 days of gestation, which can be inferred that the higher ratio of n-6/n-3 in the milk led to an increase in BF, and the lower ratio led to a decrease.

Table 6. Effect of supplementation of milk biofortified with PUFA n-3 and n-6 on the backfat thickness of gilts from d 40 to d 107 of gestation

	Treatment[b]			***P-value*[d]**				
Item[a]	**CON (± SEM[c])**	**n-6 (± SEM[c])**	**n-3 (± SEM[c])**	**Treatment**	**Time**	**Treat*Time**	**C1**	**C2**
Body weight average, kg	185.32 ± 3.55	181.06 ± 3.98	188.38 ± 3.050	0.389	**<0.001**	0.270	0.196	0.433
Weight D40G, kg	162.44 ± 3.35	162.50 ± 3.35	166.44 ± 3.16	0.610	-	-	0.325	0.990
Weight D107G, kg	208.57 ± 4.92	202.79 ± 4.34	205.67 ± 4.34	0.682	-	-	0.998	0.388
Weight difference, D107G - D40G, kg	46.35 ± 1.08	43.91 ± 1.08	41.20 ± 1.08	0.562	-	-	0.360	0.562
Backfat thickness average, mm	15.08 ± 0.42	16.07 ± 0.37	16.36 ± 0.34	0.086	0.920	**0.010**	0.091	0.102
Backfat thickness D40G, mm	14.30 ± 0.66	14.73 ± 0.61	16.42 ± 0.54	**0.043**	-	-	**0.014**	0.640
Backfat thickness D107G, mm	16.09 ± 0.37	17.01 ± 0.39	16.10 ± 0.39	0.169	-	-	0.346	0.096
Backfat thickness difference, D107G - D40G, kg	1.44 ± 0.74	2.38 ± 0.78	-0.26 ± 0.83	0.084	-	-	0.686	**0.029**

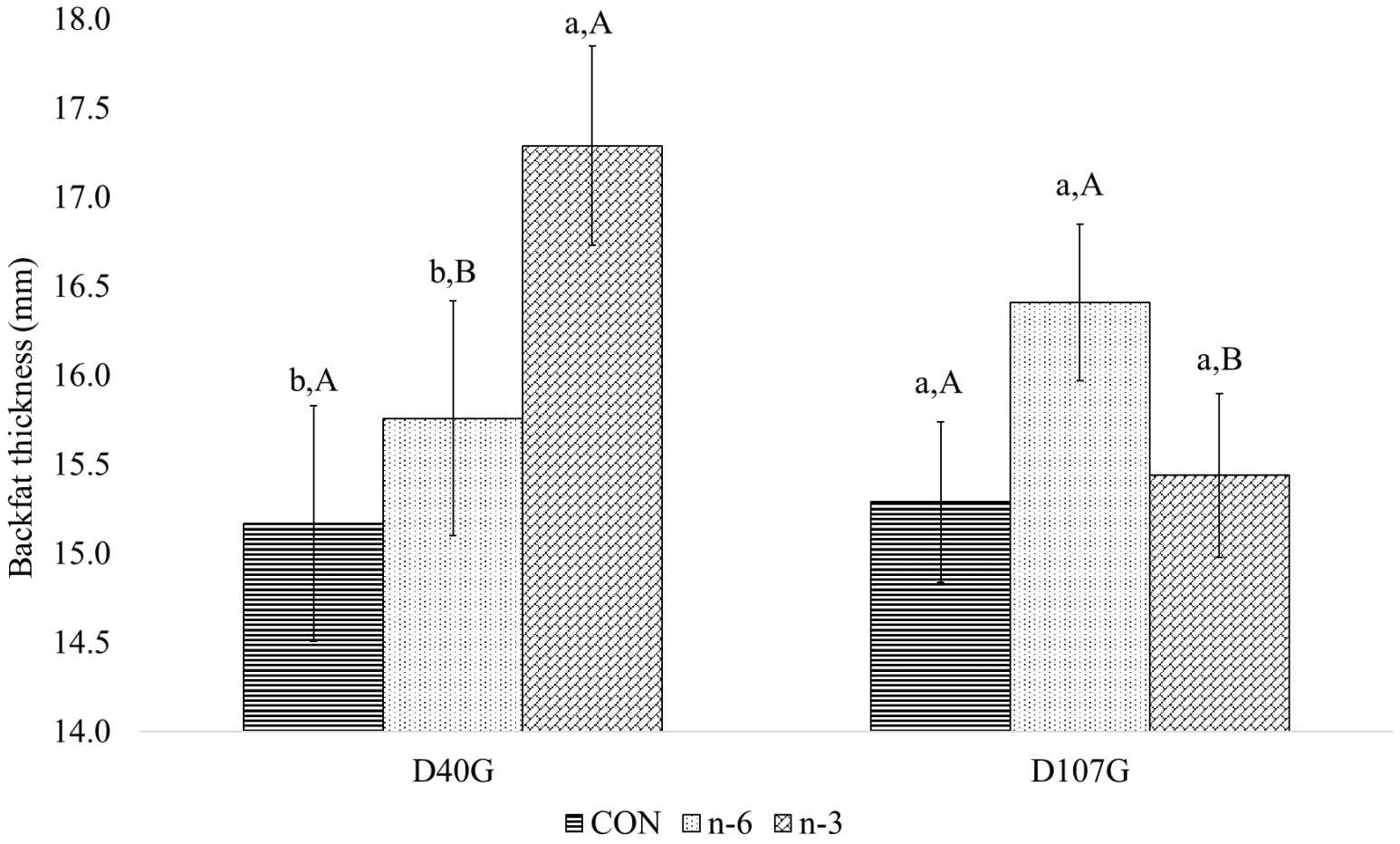
Source: Own authorship.
[a]D40G: 40 days of gestation; D107G: 107 days of gestation.
[b]Gilts fed with a Control milk (CON), supplemented with cow's milk biofortified with n-3 or n-6.
[c]SEM, standard error of the mean.
[d]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

Table 7. Backfat thickness average (mm) from 40 to 107 days of gestation of swine females

	Day	
Treatment	**D40G[a] (± SEM[b])**	**D107G[a] (± SEM[b])**
CON	15.17 ± 0.66 [b,A]	15.29 ± 0.45[a,A]
n-6	15.76 ± 0.66 [b,B]	16.41 ± 0.44 [a,A]
n-3	17.29 ± 0.56 [a,A]	15.44 ± 0.46 [a,B]

Source: Own authorship. Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed by the same lowercase letter, do not differ.
[a]D40G: 40 days of gestation; D107G: 107 days of gestation.
[b]SEM, standard error of the mean.

Figure 4 - Effect of treatment and time on backfat thickness average of sows on early and late gestation



Source: Own authorship.
Average followed by the lowercase letter means the comparison among the treatments at one point in time. Average followed by the capital letter means the comparison of one treatment at different times.

The period between D40G and D107G is characterized by catabolism of body mobilizing protein and fat as a source of maintenance energy and milk production requirements (HOLEN et al., 2022; NRC, 2012). Our study demonstrated that PUFA supplementation through cow's milk leads to an increase ($P = 0.014$) in 8.92% of the backfat thickness at D40G, also an increase in loss of backfat thickness for sow's n-3 group ($P = 0.029$) from D40 to D107 of gestation, while the n-6 group had an increment of 15.5%. Observing D40G specifically in the interaction between treatment and time, shows the n-3 group had a higher backfat thickness compared to other groups. Consequently, it can be suggested that cow's milk biofortified with n-3 PUFA supported the body's condition at the beginning of gestation while cow's milk biofortified with n-6 PUFA drives the same response at the end of this period. In the present study, the colostrum yield among treatments was not significant ($P > 0.05$) even though Decaluwé et al. (2013) reported changes in backfat thickness during late gestation were associated with colostrum yield.

The effect of body condition can affect fertility, productivity, survival and even the incidence of leg problems. Some authors have recommended achieving a backfat thickness (BF) between 16 and 19 mm (TARRÉS et al., 2006) or between 18 and 20 mm (YANG et al., 1989) so that this can be avoided, as obesity (BF above 36 mm) shows a negative impact on breast development (HEAD AND WILLIAMS, 1991; FARMER et al., 2016 a,b), which may mean reduced milk production (HEAD et al., 1991) and affect piglet weight gain. In the present study, despite the differences observed, the sows were not considered obese (BF between 14 and 17 mm), in addition, in late gestation, there was no difference between the treatments.

Sows in groups n-6 and n-3 had greater backfat thickness on D40 of gestation, and group n-6 increased BF from D40G to D107G, which reflected in greater body weight of piglets on days 1 and 21 of lactation, while group n-3 lost BF from D40G to D107G (REIS et al., 2021). Despite this, the average body weight and daily gain of the piglets were higher for the groups that received biofortified milk (REIS et al., 2021).

Thus, biofortified milk, under these conditions, can promote the stability of the sows' body condition, without apparently damaging the development of the mammary gland, and also improve the weight condition of the piglets, both at birth and until weaning.

6.2 EFFECTS OF MILK WITH DIFFERENT FA PROFILE ON SERUM BIOCHEMICAL OF SWINE FEMALES – EXPERIMENT A

Serum was collected to investigate the effect of treatment diets on circulating levels of urea, total protein, total cholesterol, HDL, LDL, VLDL, glucose, and TG as well as free fatty

acid after supplementation with changed fat profile milk. The concentration of VLDL was higher in sows supplemented with n-3 milk compared to n-6 group. Compared to CON, both groups that received biofortified milk (n-3 and n-6) had lower levels of TG (Table 8) probably due to lower SFA/USFA ratios in those milk. A significant treatment by time interaction for TG level (P = 0.040) was also found (Table 9 and Figure 5). Day had a significant impact on circulating levels of TG for CON and n-6 group. Within each treatment levels of TG were lowest on D1L compared to all other days sampled (D40G, D107G and D21L).

The reduction of TG level at D40G and D21L by the sows of the n-3 group, is probably due to the consumption of milk with lower SFA/USFA and n-6/n-3. In addition, at D21L, the females of the n-6 group also showed the reduction of TG.

Table 8. Effects of biofortified cow's milk on the biochemical parameters of blood of swine females during pregnancy and lactation

	Treatments[a]			***P-value*[c]**				
Parameters	**CON (± SEM[b])**	**n-6 (± SEM[b])**	**n-3 (± SEM[b])**	**Treatment**	**Time**	**Treat*Time**	**C1**	**C2**
Urea, mg/dL	24.36 ± 0.06	24.89 ± 0.06	28.25 ± 0.06	0.189	0.0097	0.753	0.263	0.149
Total protein, g/dL	7.31 ± 1.75	8.12 ± 1.75	8.46 ± 1.75	0.589	<0.001	0.132	0.940	0.309
Total cholesterol, mg/dL	66.85 ± 2.82	62.86 ± 2.75	63.78 ± 2.92	0.578	<0.001	0.725	0.314	0.820
HDL, mg/dL	27.71 ± 1.77	26.54 ± 1.77	25.84 ± 1.77	0.723	<0.001	0.634	0.451	0.761
LDL, mg/dL	26.97 ± 2.79	24.15 ± 2.79	22.77 ± 2.79	0.332	<0.001	0.357	0.163	0.601
VLDL, mg/dL	6.46 ± 2.99	4.26 ± 2.99	7.84 ± 2.99	**0.030**	<0.001	0.061	0.500	**0.011**
Glucose, mg/dL	101.08 ± 2.74	97.20 ± 2.74	104.31 ± 2.74	0.313	<0.001	0.083	0.920	0.131
Triglycerides, mg/dL	88.38 ± 6.51	69.88 ± 5.10	68.03 ± 4.57	**0.039**	<0.001	**0.040**	**0.012**	0.788

Source: Own authorship.

[a]Swine females fed with a Control milk (CON), supplemented with cow’s milk biofortified with n-6 or n-3.

[b]SEM, standard error of the mean.

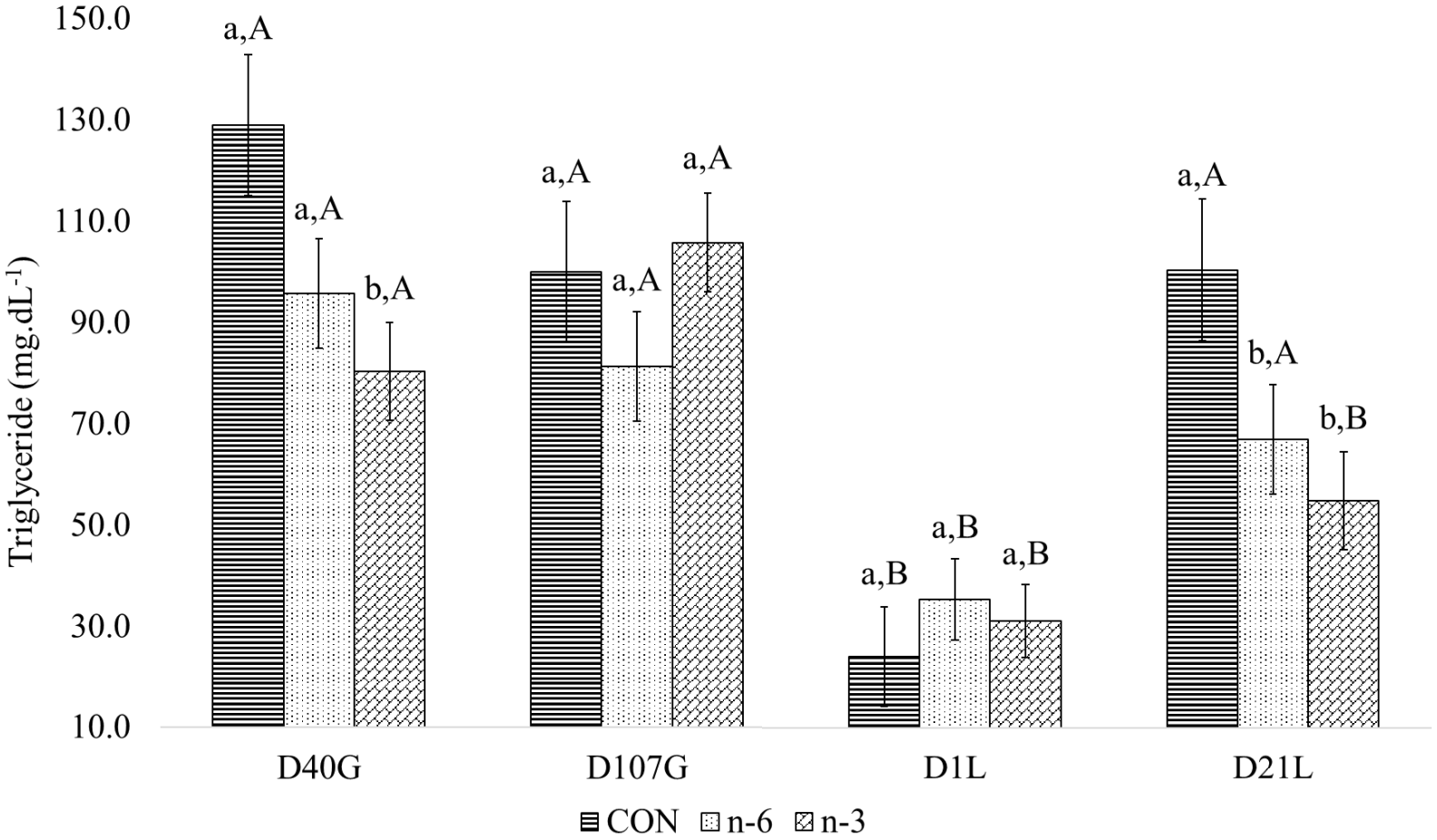
[c]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

Table 9. Triglyceride's concentration (mg.dL-1) at 40 and 107 days of gestation and at 1 and 21 days of lactation of swine females

	Gestation		Lactation	
Treatment	**D40G[a] (± SEM[b])**	**D107G[a] (± SEM[b])**	**D1L[a] (± SEM[b])**	**D21L[a] (± SEM[b])**
CON	129.00 ± 13.92[a,A]	100.00 ± 13.92[a,A]	24.10 ± 9.84[a,B]	100.40 ± 13.92[a,A]
n-6	95.80 ± 10.81[a,A]	81.40 ± 10.81[a,A]	35.33 ± 8.06[a,B]	67.00 ± 10.81[b,A]
n-3	80.40 ± 9.69[b,A]	105.80 ± 9.69[a,A]	31.11 ± 7.22[a,B]	54.80 ± 9.69[b,B]

Source: Own authorship.
Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed by the same lowercase letter, do not differ.
[a]D40G: 40 days of gestation; D107G: 107 days of gestation; D1L: first day of lactation; D21L: 21 days of lactation.
[b]SEM, standard error of the mean.

Figure 5 - Effect between treatments and time for triglyceride of swine females



Source: Own authorship.
Average followed by the lowercase letter means the comparison among the treatments at one point in time. Average followed by the capital letter means the comparison of one treatment at different times.

Our results demonstrate the lower concentration of triglycerides in sow's plasma due to supplementation using cow's milk biofortified with n-6 and n-3 PUFA during gestation and lactation period, also in the corresponding period, the blood showed a higher level of palmitoleic acid (C16:1c9). According to Yang, Miyahara and Hatanaka (2011) this fatty acid can reduce hypertriglyceridemia and improve the insulin sensitivity in mouse model, just as down-regulated mRNA expression of lipogenic genes as SREBP-1, FAS and SCD-1 in liver.

During the initial two-thirds of gestation, there is a notable increase in adipose fatty acid synthesis and lipid accumulation, but as we approach the last third of gestation, there is a discontinuity in the increase in the accumulation of maternal fat deposits, together with a decrease in fatty acid synthesis in adipose tissue and in lipoprotein lipase (LPL) activity (HERRERA; ORTEGA-SENOVILLA, 2014).

Additionally, with increased BF in late gestation, an increase in cholesterol and triglyceride fractions is expected according to Zhou et al. (2013). Interestingly, even when the n-3 group had a higher BF at D40G or when the n-6 group increased its BF from D40G to D107G, cholesterol levels remained unchanged, and triglycerides, contrary to expectations, were reduced. These results indicate that biofortified milk has a remarkable regulatory potential in the deposition and use of adipose fat, regulating blood triglyceride levels.

An intake of n-3 PUFA or reduced n-6/n-3 ratio by supplements is known to decrease VLDL cholesterol levels in humans (BARBOSA-CORTES et al., 2023; GRIFFIN et al., 2006; XYDA et al., 2020). Furthermore, the liver's secretion of very low-density lipoprotein (VLDL) is subject to the influence of several factors, among them are the synthesis of apolipoprotein (apo) B, insulin, the availability of triglycerides, free fatty acids (FFA), cholesteryl ester and microsomal triglyceride transfer protein (FERNANDEZ; WEST, 2005; JULIUS, 2003; JUMP, 2002). However, we found higher concentration of VLDL, for n-3 group, in plasma of sows during gestation and lactation period whereas stearic, elaidic, palmitoleic and oleic acids increased at plasma.

6.3 MILK n-6 AND n-3 IMPROVE SERUM FA PROFILE OF SWINE FEMALES – EXPERIMENT A

The modification in blood FA profile after supplementation with biofortified milk during gestation and lactation of sows can be seen in Table 10. Analysis of FA profiles found a significant increase in palmitoleic acid ($P = 0.0032$) for n-3 and n-6 group compared with CON. Relative to n-3 the MUFA elaidic ($P = 0.0354$), palmitoleic ($P = 0.0028$) and oleic acid ($P = 0.0398$) were higher in the n-6 compared with n-3 group. Whereas stearic acid, a SFA, was

lower (P = 0.0038) in sows that received milk biofortified with PUFA n-3 compared with the n-6 group. Together resulting in a higher concentration of MUFA for n-6 group compared to n-3 (P = 0.040).

Considering the interactions of treatment by time, we also found for the FA profile, the n-6 and n-3 group showing a lower ARA/EPA ratio from 107 days of gestation until 21 days of lactation (Table 11 and Figure 6). At D107G only the sows in the n-3 group showed a reduction and at D1L both n-6 and n-3 PUFA biofortified milks caused this reduction in ARA/EPA levels. Concerning the level of γ-linolenic acid, was possible to observe that the behavior of n-6 PUFA and n-3 PUFA treatment were similar, showing a lower level in D40G and D1L, and higher levels in D107G and D21L. Considering D1L, milk biofortified with n-6 PUFA increased and at D21L n-3 PUFA decreased the level of γ-linolenic acid in sow serum (Table 12 and Figure 7). Only at D1L the level of elaidic acid was reduced in the sows' serum after supplementation with milk biofortified with n-6 PUFA. Furthermore, the behavior across time for the different treatments showed that for the n-6 and n-3 groups, there was a drop in concentration at D1L more accentuated than for CON (Table 13 and Figure 8).

The changes in the proportions of fatty acids in the blood are probably due to the contribution of the FA present in the control milk or milk biofortified with n-3 or n-6, interacting with the requirements of the organism at each stage the sows were in.

Table 10. Effects of biofortified cow's milk on the fatty acid profile (mg/mL) of sow serum during gestation and lactation

	Treatment[b]			***P-value*[d]**				
Items[a]	**CON (± SEM[c])**	**n-6 (± SEM[c])**	**n-3 (± SEM[c])**	**Treatment**	**Time**	**Treat*Time**	**C1**	**C2**
Myristic, C14:0	10.459 ± 0.10	9.489 ± 0.10	8.305 ± 0.10	0.324	<0.001	0.146	0.221	0.381
Palmitic, C16:0	199.160 ± 9.64	197.660 ± 9.48	184.640 ± 9.47	0.493	<0.001	0.170	0.497	0.336
Stearic, C18:0	138.212 ± 0.05	162.580 ± 0.05	134.328 ± 0.05	**0.008**	<0.001	0.147	0.201	**0.004**
Elaidic, C18:1t n-9	2.995 ± 0.07	3.497 ± 0.06	2.688 ± 0.07	0.102	<0.001	**0.007**	0.810	**0.035**
Palmitoleic, C16:1c9	10.334 ± 0.46	13.091 ± 0.42	11.136 ± 0.43	**<0.001**	<0.001	0.151	**0.003**	**0.003**
Oleic, C18:1c9	244.250 ± 11.34	267.420 ± 11.00	233.680 ± 11.00	0.106	<0.001	0.069	0.651	**0.040**
Linoleic, C18:2 n-6	330.580 ± 15.17	315.550 ± 14.83	298.290 ± 14.83	0.321	<0.001	0.454	0.207	0.415
Dihomo-γ-linolenic, C20:3 n-6	4.901 ± 0.45	4.975 ± 0.45	5.619 ± 0.45	0.448	<0.001	0.653	0.467	0.308
ARA, C20:4 n-6	106.910 ± 8.45	109.150 ± 8.45	93.225 ± 8.41	0.335	0.020	0.358	0.570	0.180
γ-Linolenic, C18:3 n-6	4.464 ± 0.05	4.527 ± 0.05	4.822 ± 0.05	0.598	<0.001	**0.008**	0.502	0.443
α-Linolenic, C18:3 n-3	6.511 ± 0.06	5.932 ± 0.05	6.362 ± 0.05	0.548	<0.001	0.091	0.476	0.425
EPA, C20:5 n-3	2.500 ± 0.06	2.881 ± 0.07	2.484 ± 0.06	0.391	<0.001	0.606	0.498	0.222
DHA, C22:6 n-3	2.644 ± 0.10	2.525 ± 0.10	1.900 ± 0.10	0.161	0.094	0.157	0.242	0.134
ΣSFA	354.640 ± 12.01	375.320 ± 13.24	345.600 ± 12.05	0.259	<0.001	0.058	0.695	0.109
ΣUSFA	717.600 ± 28.51	736.160 ± 27.66	659.930 ± 27.66	0.149	<0.001	0.168	0.577	0.063
SFA/USFA	0.490 ± 0.01	0.520 ± 0.01	0.510 ± 0.01	0.100	0.010	0.547	0.036	0.695
ΣMUFA	258.630 ± 12.02	284.260 ± 22.72	248.370 ± 11.72	0.104	<0.001	0.077	0.604	**0.040**
ΣPUFA	458.970 ± 19.20	451.900 ± 18.62	411.560 ± 18.62	0.177	<0.001	0.425	0.253	0.138
Σn-3	12.990 ± 0.59	13.000 ± 0.87	11.500 ± 0.63	0.124	<0.001	0.130	0.573	0.070
Σn-6	446.480 ± 18.69	435.700 ± 18.18	339.960 ± 18.18	0.189	<0.001	0.461	0.217	0.176
n-6/n-3	35.267 ± 0.03	34.075 ± 0.03	36.122 ± 0.03	0.436	<0.001	0.067	0.892	0.205
ARA/EPA	47.586 ± 3.47	37.536 ± 3.58	45.252 ± 3.67	0.118	<0.001	**0.045**	0.144	0.135

Source: Own authorship.

[a]ARA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; ΣSFA = Σ saturated fatty acids; ΣUSFA = Σ unsaturated fatty acids; SFA/USFA = Σ saturated/Σ unsaturated; ΣMUFA = Σ monounsaturated fatty acids; ΣPUFA = Σ polyunsaturated fatty acids; Σ n-3 = Σ omega-3 fatty acids; Σ n-6 = Σ omega-6 fatty acids; n-6/n-3 = Σ omega-6/Σ omega-3; ARA/EPA: arachidonic acid/eicosapentaenoic acid.

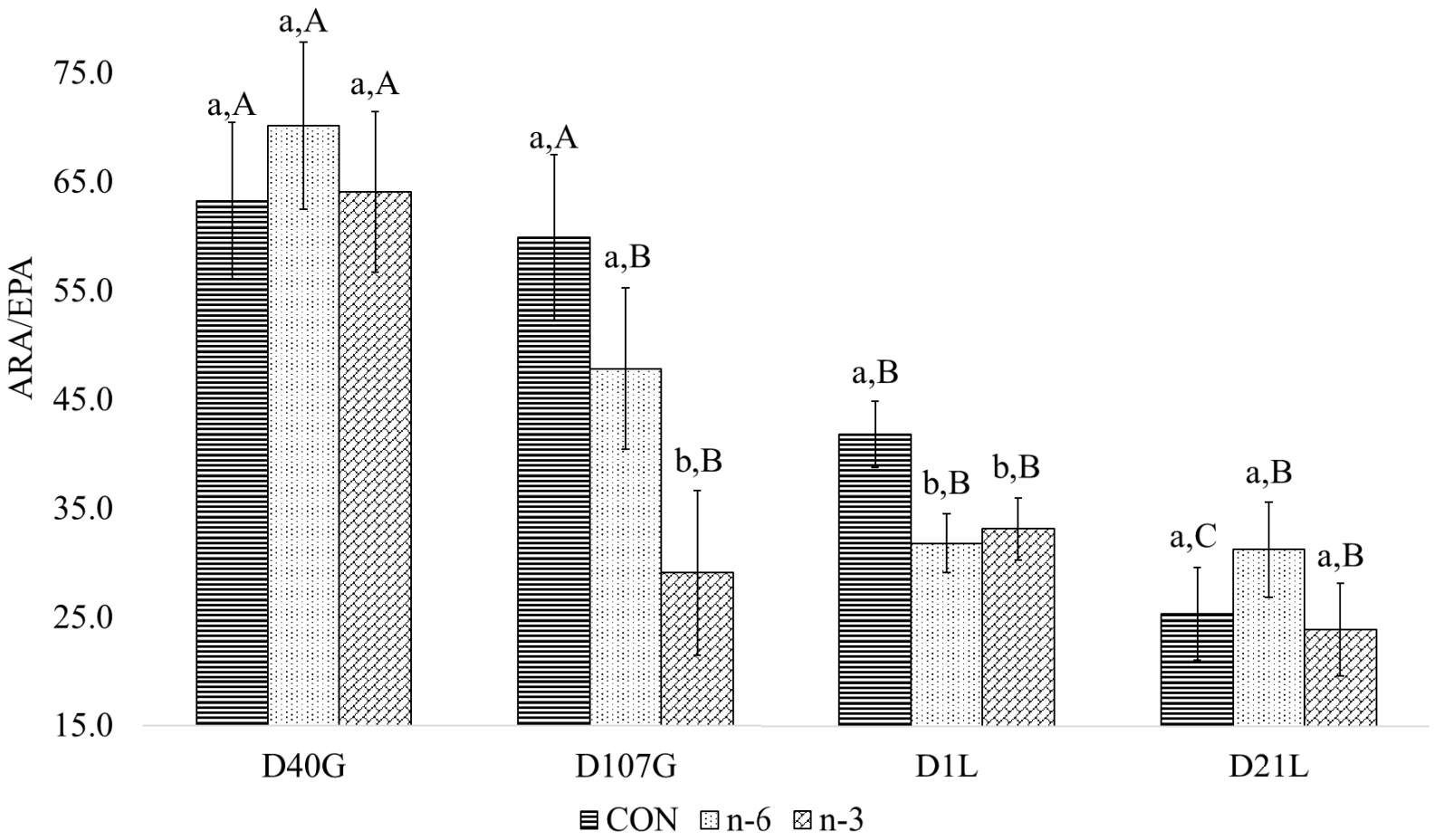
[b]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.
[c]SEM,standard error of the mean.
[d]C1, contrast Control vs n-3+n-6; C2, contrast n-3 vs n-6.

Table 11. ARA/EPA ratio at 40 and 107 days of gestation and at 1 and 21 days of lactation of swine females

	Gestation		Lactation	
Treatment	**D40G[a] (± SEM[b])**	**D107G[a] (± SEM[b])**	**D1L[a] (± SEM[b])**	**D21L[a] (± SEM[b])**
CON	63.264 ± 7.16[a,A]	59.917 ± 7.59[a,A]	41.825 ± 3.04[a,B]	25.339 ± 4.26[a,C]
n-6	70.146 ± 7.67[a,A]	47.843 ± 7.40[a,B]	31.797 ± 2.69[b,B]	31.222 ± 4.39[a,B]
n-3	64.069 ± 7.40[a,A]	29.090 ± 7.59[b,B]	33.114 ± 2.86[b,B]	23.872 ± 4.26[a,B]

Source: Own authorship.
Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed by the same lowercase letter, do not differ.
[a]D40G: 40 days of gestation; D107G: 107 days of gestation; D1L: first day of lactation; D21L: 21 days of lactation.
[b]SEM, standard error of the mean.

Figure 6 - Effect of interaction treatments and time for ARA/EPA ratio of swine females



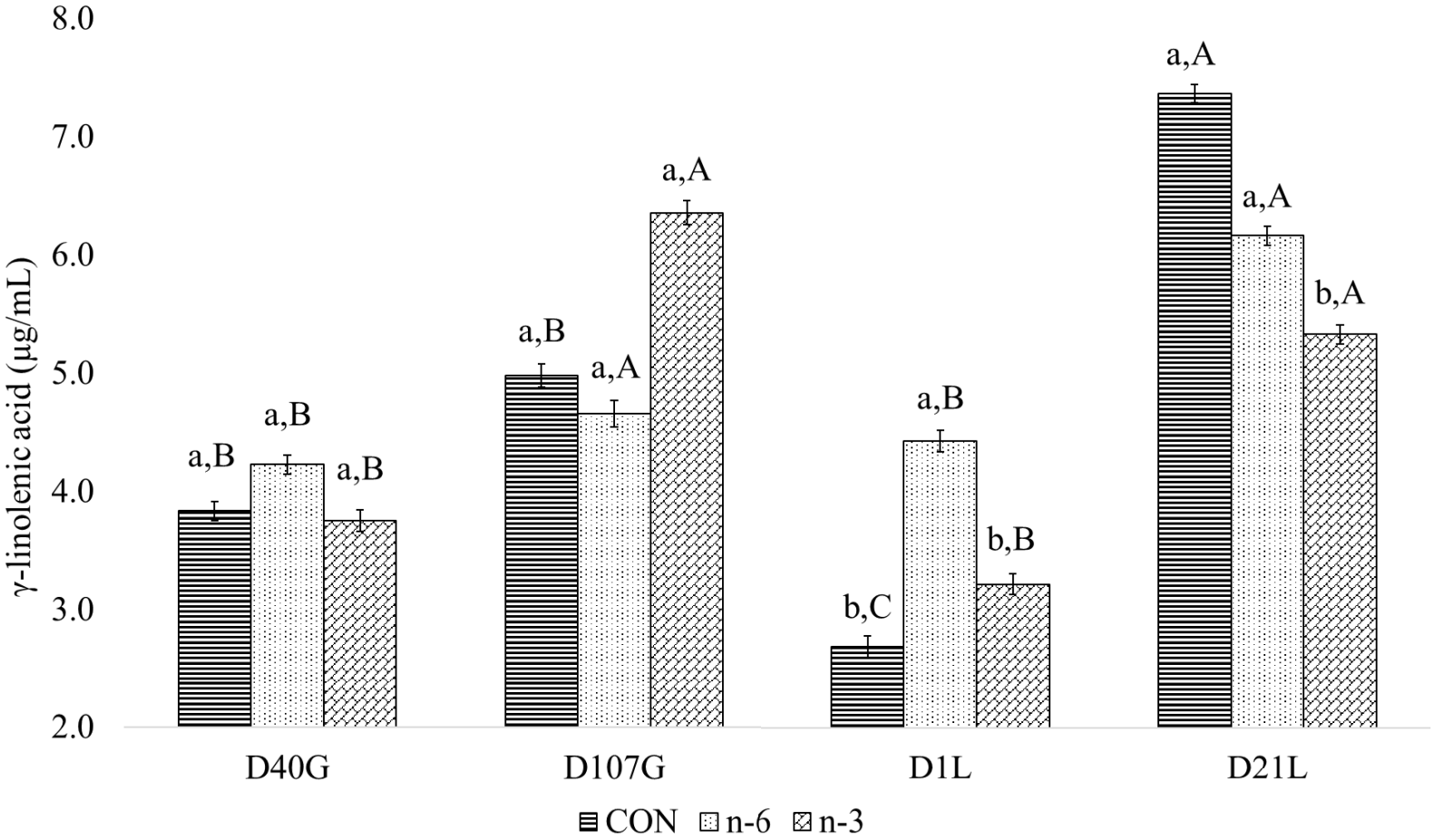
Source: Own authorship.
Average followed by the lowercase letter means the comparison among the treatments at one point in time. Average followed by the capital letter means the comparison of one treatment at different times.

Table 12. Concentration of γ-linolenic acid (µg/mL) at 40 and 107 days of gestation and at 1 and 21 days of lactation of swine females

	Gestation		Lactation	
Treatment	**D40G[a] (± SEM[b])**	**D107G[a] (± SEM[b])**	**D1L[a] (± SEM[b])**	**D21L[a] (± SEM[b])**
CON	3.835 ± 0.08^{a,B}	4.979 ± 0.10^{a,B}	2.686 ± 0.09^{b,C}	7.365 ± 0.08^{a,A}
n-6	4.228 ± 0.08^{a,B}	4.658 ± 0.11^{a,A}	4.425 ± 0.09^{a,B}	6.162 ± 0.08^{a,A}
n-3	3.754 ± 0.09^{a,B}	6.358 ± 0.10^{a,A}	3.216 ± 0.09^{b,B}	5.329 ± 0.08^{b,A}

Source: Own authorship.
Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed by the same lowercase letter, do not differ.
[a]D40G: 40 days of gestation; D107G: 107 days of gestation; D1L: first day of lactation; D21L: 21 days of lactation.
[b]SEM, standard error of the mean.

Figure 7 - Effect of interaction treatments and time for γ-linolenic acid of swine females



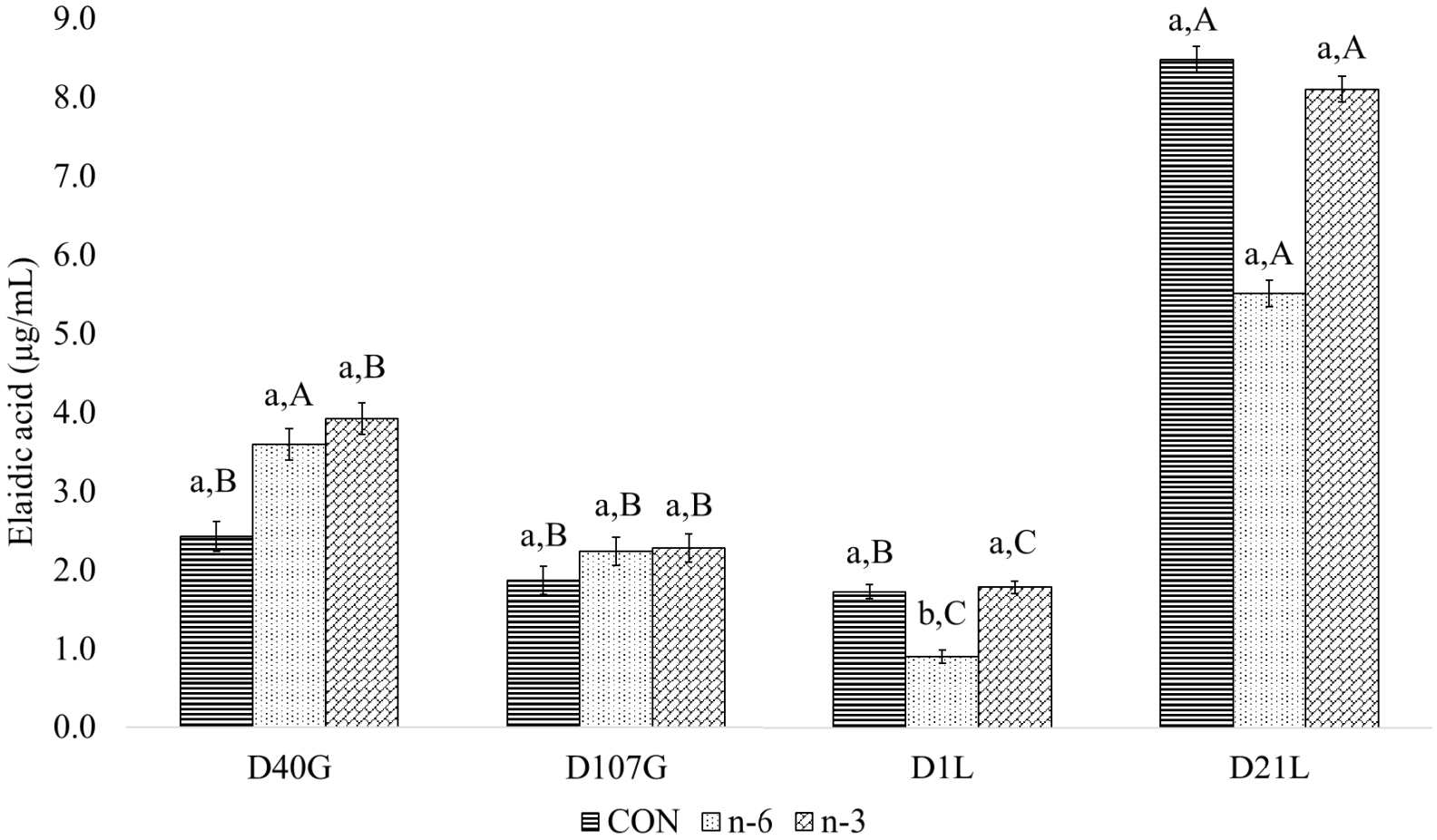
Source: Own authorship.
Average followed by the lowercase letter means the comparison among the treatments at one point in time. Average followed by the capital letter means the comparison of one treatment at different times.

Table 13. Concentration of elaidic acid (µg/ mL) at 40 and 107 days of gestation and at 1 and 21 days of lactation of swine females

	Gestation		Lactation	
Treatment	**D40G[a] (± SEM[b])**	**D107G[a] (± SEM[b])**	**D1L[a] (± SEM[b])**	**D21L[a] (± SEM[b])**
CON	2.430 ± 0.19^{a,B}	1.875 ± 0.18^{a,B}	1.725 ± 0.09^{a,B}	8.482 ± 0.16^{a,A}
n-6	3.593 ± 0.20^{a,A}	2.237 ± 0.18^{a,B}	0.903 ± 0.08^{b,C}	5.514 ± 0.17^{a,A}
n-3	3.922 ± 0.20^{a,B}	2.279 ± 0.18^{a,B}	1.782 ± 0.08^{a,C}	8.105 ± 0.16^{a,A}

Source: Own authorship.
Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed by the same lowercase letter, do not differ.
[a]D40G: 40 days of gestation; D107G: 107 days of gestation; D1L: first day of lactation; D21L: 21 days of lactation.
[b]SEM, standard error of the mean.

Figure 8 - Effect of interaction treatments and time for elaidic acid of swine females



Source: Own authorship.
Average followed by the lowercase letter means the comparison among the treatments at one point in time. Average followed by the capital letter means the comparison of one treatment at different times.

6.4 EXPERIMENT A: n-3 AND n-6 COW MILK IMPROVE FATTY ACIDS PROFILE IN COLOSTRUM AND MILK OF SWINE FEMALES – EXPERIMENT A

Since our data suggested the alteration of fatty acids profile on serum, we expected modifications on lipid levels colostrum as much as milk. To verify this, we conducted analysis of FA profile by gas chromatography, which revealed (Table 14) a reduction ($P < 0.05$) of three SFA (anteisotridecylic, isomyristic and isomargaric acids), an increase to MUFA (cis-10-heptadecenoic, elaidic and cis-15-octadecenoic acids) and increase of α-linolenic (PUFA n-3) on sow' colostrum n-3 and n-6 groups compared to CON. Consequently, a higher n-3 PUFA and lower n-6/n-3 ratio ($P < 0.05$) was found in the n-6 and n-3 groups compared to CON (Table 15). The n-3 group relative to the n-6 group had lower isopentadecylic acid (SFA) and higher cis-15-octadecenoic (MUFA), α-linolenic and EPA (PUFA n-3) concentration ($P < 0.05$) on day 1 of lactation (Table 14).

Analysis of mature milk samples collected on lactation d14 found the n-3 group had more the cis-15-octadecenoic (MUFA) and less ARA (PUFA n-6) than n-6 group (Table 16). Milk from n-6 and n-3 sows also had less n-6/n-3 ratio than CON (Table 17) on d 14 of lactation.

Table 14. Effect of supplementation of milk biofortified with n-3 and n-6 on the fatty acid profile (g/100 g) of sows' colostrum

Continue

	Treatments[b]			*P-value*[d]		
Items[a]	**CON (± SEM[c])**	**n-6 (± SEM[c])**	**n-3 ± (SEM[c])**	**Treatment**	**C1**	**C2**
Caproic, C6:0	0.011 ± 0.007	0.000* ± 0.000	0.000* ± 0.000	0.309	0.133	1.000
Caprylic, C8:0	0.000* ± 0.000	0.000* ± 0.000	0.000* ± 0.000	0.619	0.337	1.000
Capric, C11:0	0.010 ± 0.002	0.008 ± 0.003	0.009 ± 0.001	0.834	0.623	0.743
Hendecanoic, C11:0	0.001 ± 0.001	0.000 ± 0.000	0.001 ± 0.000	0.844	0.617	0.781
Lauric, C12:0	0.062 ± 0.009	0.047 ± 0.010	0.053 ± 0.004	0.453	0.265	0.579
Isotridecyl, C13:0 iso	0.005 ± 0.005	0.000* ± 0.000	0.000* ± 0.000	0.400	0.341	0.342
Anteisotridecylic, C13:0 anteiso	0.018 ± 0.007	0.000* ± 0.000	0.000* ± 0.000	0.092	**0.032**	1.000
Tridecylic, C13:0	0.004 ± 0.001	0.001 ± 0.001	0.003 ± 0.001	0.085	0.131	0.086
Isomyristic, C14:0 iso	0.012 ± 0.002	0.008 ± 0.001	0.009 ± 0.000	0.124	**0.047**	0.802
Myristic, C14:0	2.424 ± 0.243	2.216 ± 0.190	2.516 ± 0.141	0.556	0.813	0.300
Isopentadecylic, C15:0 iso	0.047 ± 0.003	0.082 ± 0.009	0.051 ± 0.006	**0.004**	**0.024**	**0.005**
Anteisopentadecylic, C15:0 anteiso	0.076 ± 0.009	0.066 ± 0.004	0.072 ± 0.004	0.498	0.336	0.504
Pentadecylic, C15:0	0.249 ± 0.018	0.235 ± 0.012	0.268 ± 0.010	0.271	0.870	0.115
Isopalmitic, C16:0 iso	0.033 ± 0.005	0.031 ± 0.004	0.025 ± 0.004	0.351	0.334	0.281
Palmitic, C16:0	25.550 ± 0.662	25.360 ± 0.418	24.526 ± 0.394	0.346	0.346	0.266
Isomargaric, C17:0 iso	0.033 ± 0.003	0.015 ± 0.002	0.022 ± 0.002	**0.001**	**0.001**	0.084
Margaric, C17:0	0.385 ± 0.021	0.404 ± 0.033	0.421 ± 0.013	0.573	0.365	0.607
Stearic, C18:0	5.347 ± 0.219	5.529 ± 0.361	5.160 ± 0.207	0.641	0.995	0.356
Arachidic, C20:0	0.094 ± 0.006	0.091 ± 0.007	0.082 ± 0.004	0.344	0.300	0.303
Behenic, C22:0	0.057 ± 0.006	0.054 ± 0.003	0.054 ± 0.007	0.906	0.664	0.981
Tricosilic, C23:0	0.015 ± 0.002	0.011 ± 0.002	0.016 ± 0.002	0.234	0.615	0.108
Lignoceric, C24:0	0.069 ± 0.013	0.078 ± 0.008	0.061 ± 0.008	0.536	0.994	0.274
Lauroleic, C12:1	0.000* ± 0.000	0.001 ± 0.001	0.001 ± 0.001	0.397	0.187	0.834
Myristoleic, C14:1c9	0.050 ± 0.007	0.038 ± 0.008	0.050 ± 0.002	0.357	0.468	0.219
Palmitoleic, C16:1c9	2.656 ± 0.117	2.502 ± 0.251	2.377 ± 0.134	0.420	0.275	0.631
cis-10-Heptadecenoic, C17:1c10	0.263 ± 0.016	0.314 ± 0.012	0.305 ± 0.019	0.091	**0.034**	0.683

Table 14. Effect of supplementation of milk biofortified with n-3 and n-6 on the fatty acid profile (g/100 g) of sows' colostrum

Conclusion

	Treatments[b]			*P-value*[d]		
Items[a]	**CON (± SEM[c])**	**n-6 (± SEM[c])**	**n-3 ± (SEM[c])**	**Treatment**	**C1**	**C2**
Elaidic, C18:1t	0.494 ± 0.042	0.713 ± 0.071	0.704 ± 0.029	**0.016**	**0.005**	0.900
Oleic, C18:1c9	32.282 ± 1.353	32.843 ± 0.604	31.780 ± 0.900	0.759	0.981	0.467
Vaccenic, C18:1c11	3.859 ± 0.224	3.882 ± 0.128	3.722 ± 0.130	0.770	0.785	0.513
cis-12-Octadecenoic, C18:1c12	0.380 ± 0.015	0.356 ± 0.019	0.379 ± 0.017	0.562	0.570	0.370
cis-13-Octadecenoic, C18:1c13	0.210 ± 0.008	0.201 ± 0.018	0.175 ± 0.007	0.130	0.145	0.146
trans-16-Octadecenoic, C18:1t16	0.035 ± 0.004	0.040 ± 0.003	0.037 ± 0.006	0.696	0.521	0.587
cis-15-Octadecenoic, C18:1c15	0.008 ± 0.002	0.010 ± 0.002	0.068 ± 0.008	**<0.001**	**<0.001**	**<0.001**
Gondoic, C20:1	0.293 ± 0.018	0.271 ± 0.013	0.273 ± 0.011	0.486	0.241	0.921
Erucic, C22:1 n-9	0.098 ± 0.008	0.121 ± 0.007	0.127 ± 0.013	0.127	**0.050**	0.663
Nervonic, C24:1	0.119 ± 0.019	0.151 ± 0.015	0.139 ± 0.011	0.364	0.196	0.576
Linoleic, C18:2 n-6	21.612 ± 0.890	21.260 ± 0.736	23.177 ± 1.072	0.319	0.596	0.162
γ-Linolenic, 18:3 n-6	0.206 ± 0.024	0.208 ± 0.019	0.198 ± 0.020	0.938	0.918	0.737
α-Linolenic, 18:3 n-3	0.783 ± 0.043	0.827 ± 0.017	1.081 ± 0.057	**0.001**	**0.007**	**0.001**
Octadeca-9,11-dienoic, C18:2c9t11	0.073 ± 0.010	0.073 ± 0.007	0.092 ± 0.012	0.321	0.418	0.205
11cis-14cis-Eicosadienoic, C20:2	0.327 ± 0.021	0.288 ± 0.026	0.282 ± 0.027	0.400	0.188	0.863
Dihomo-γ-linolenic, 20:3 n-6	0.252 ± 0.023	0.238 ± 0.012	0.217 ± 0.019	0.431	0.299	0.442
Dihomo-α-linolenic, 20:3 n-3	0.043 ± 0.007	0.035 ± 0.004	0.038 ± 0.005	0.564	0.330	0.686
ARA, C20:4 n-6	1.048 ± 0.074	0.976 ± 0.062	0.998 ± 0.074	0.762	0.489	0.833
Docosa-13,16-dienoic, C22:2c13c16	0.031 ± 0.002	0.029 ± 0.004	0.030 ± 0.003	0.973	0.757	0.901
EPA, C20:5 n-3	0.028 ± 0.002	0.019 ± 0.003	0.032 ± 0.004	0.052	0.531	**0.020**
Clupadonic, C22:5	0.296 ± 0.046	0.308 ± 0.033	0.308 ± 0.018	0.960	0.780	1.000
DHA, C22:6 n-3	0.074 ± 0.016	0.063 ± 0.016	0.056 ± 0.009	0.605	0.348	0.896

Source: Own authorship.

[a]ARA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

[b]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-3 or n-6; 0.000*: Values lower than 10^{-6} g/100g.

[c]SEM: standard error of the mean.

[d]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

Table 15. Effect of supplementation of milk biofortified with n-3 and n-6 on the quality of the lipid fraction of sows' colostrum

	Treatment[b]			***P-value*[d]**		
Item[a]	**CON (± SEM[c])**	**n-6 (± SEM[c])**	**n-3 ± (SEM[c])**	**Treatment**	**C1**	**C2**
ΣSFA	34.50 ± 0.86	34.24 ± 0.32	33.35 ± 0.44	0.3799	0.3452	0.3076
ΣUSFA	65.52 ± 0.85	65.77 ± 0.32	66.64 ± 0.44	0.3898	0.3575	0.3098
ΣMUFA	40.75 ± 1.60	41.44 ± 0.80	40.14 ± 1.00	0.7419	0.9770	0.4494
ΣPUFA	24.77 ± 1.05	24.32 ± 0.86	26.51 ± 1.19	0.3279	0.6236	0.1641
Σn-3	0.93 ± 0.07	0.94 ± 0.02	1.21 ± 0.06	**0.0050**	**0.0451**	**0.0046**
Σn-6	23.12 ± 0.94	22.68 ± 0.80	24.59 ± 1.10	0.3672	0.6668	0.1842
SFA/USFA	0.53 ± 0.02	0.52 ± 0.01	0.50 ± 0.01	0.2940	0.3440	0.1936
n-6/n-3	25.18 ± 1.04	24.10 ± 1.02	20.40 ± 0.30	**0.0006**	**0.0156**	**0.0034**

Source: Own authorship.

[a]ΣSFA = Σ saturated fatty acids; ΣUSFA = Σ unsaturated fatty acids; ΣMUFA = Σ monounsaturated fatty acids; ΣPUFA = Σ polyunsaturated fatty acids; Σ n-3 = Σ omega-3 fatty acids; Σ n-6 = Σ omega-6 fatty acids; SFA/USFA = Σ saturated/Σ unsaturated; n-6/n-3 = Σ omega-6/Σ omega-3.

[b]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-3 or n-6.

[c]SEM: standard error of the mean.

[d]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

Table 16. Effect of supplementation of milk biofortified with n-3 and n-6 on the fatty acid profile (g/100 g) of sows' milk

Continue

	Treatment[b]			*P-value*[d]		
Item[a]	**CON (± SEM[c])**	**n-6 (± SEM[c])**	**n-3 ± (SEM[c])**	**Treatment**	**C1**	**C2**
Butiric, C4:0	0.025 ± 0.005	0.032 ± 0.005	0.027 ± 0.005	0.627	0.448	0.561
Caproic, C6:0	0.019 ± 0.005	0.037 ± 0.007	0.022 ± 0.006	0.120	0.196	0.098
Caprylic, C8:0	0.031 ± 0.007	0.041 ± 0.006	0.040 ± 0.008	0.561	0.293	0.956
Capric, C11:0	0.189 ± 0.048	0.238 ± 0.040	0.250 ± 0.059	0.659	0.379	0.863
Caproleic, C10:1c9	0.016 ± 0.005	0.028 ± 0.006	0.031 ± 0.011	0.312	0.135	0.775
Hendecanoic, C11:0	0.002 ± 0.001	0.001 ± 0.000	0.001 ± 0.000	0.384	0.181	0.817
Lauric, C12:0	0.307 ± 0.054	0.320 ± 0.022	0.334 ± 0.043	0.906	0.710	0.818
Isotridecyl, C13:0 iso	0.003 ± 0.001	0.002 ± 0.001	0.001 ± 0.001	0.656	0.493	0.552
Anteisotridecylic, C13:0 anteiso	0.002 ± 0.002	0.000* ± 0.000	0.000* ± 0.000	0.397	0.183	1.000
Lauroleic, C12:1	0.004 ± 0.001	0.005 ± 0.001	0.006 ± 0.002	0.762	0.513	0.605
Tridecylic, C13:0	0.004 ± 0.001	0.003 ± 0.001	0.004 ± 0.001	0.833	0.680	0.668
Isomyristic, C14:0 isso	0.004 ± 0.000	0.003 ± 0.000	0.005 ± 0.002	0.235	0.262	0.230
Myristic, C14:0	3.218 ± 0.415	3.570 ± 0.133	3.650 ± 0.430	0.666	0.384	0.876
Isopentadecylic, C15:0 iso	0.076 ± 0.024	0.048 ± 0.017	0.031 ± 0.011	0.253	0.129	0.523
Anteisopentadecylic, C15:0 anteiso	0.041 ± 0.004	0.041 ± 0.004	0.038 ± 0.004	0.874	0.777	0.672
Myristoleic, C14:1c9	0.203 ± 0.052	0.262 ± 0.025	0.304 ± 0.085	0.495	0.249	0.595
Pentadecylic, C15:0	0.134 ± 0.017	0.120 ± 0.009	0.122 ± 0.010	0.722	0.431	0.939
Isopalmitic, C16:0 iso	0.015 ± 0.003	0.017 ± 0.001	0.017 ± 0.005	0.798	0.751	0.563
Palmitic, C16:0	27.363 ± 1.974	28.129 ± 0.816	28.150 ± 1.848	0.928	0.704	0.993
Isomargaric, C17:0 iso	0.112 ± 0.098	0.020 ± 0.001	0.017 ± 0.003	0.540	0.739	0.281
Palmitoleic, C16:1c9	7.722 ± 1.348	9.709 ± 1.223	9.438 ± 2.063	0.642	0.360	0.906
Margaric, C17:0	0.279 ± 0.028	0.229 ± 0.033	0.223 ± 0.013	0.292	0.126	0.879
cis-10-Heptadecenoic, C17:1c10	0.380 ± 0.031	0.350 ± 0.029	0.404 ± 0.013	0.355	0.913	0.159
Stearic, C18:0	4.761 ± 0.786	3.935 ± 0.241	3.727 ± 0.493	0.405	0.195	0.795
Elaidic, C18:1t	0.382 ± 0.037	0.450 ± 0.054	0.365 ± 0.014	0.292	0.603	0.144
Oleic, C18:1c9	36.788 ± 2.503	35.053 ± 1.315	36.110 ± 3.059	0.878	0.690	0.761

Table 16. Effect of supplementation of milk biofortified with n-3 and n-6 on the fatty acid profile (g/100 g) of sows' milk

Conclusion

	Treatment[b]			***P-value*[d]**		
Item[a]	**CON (± SEM[c])**	**n-6 (± SEM[c])**	**n-3 ± (SEM[c])**	**Treatment**	**C1**	**C2**
Vaccenic, C18:1c11	3.637 ± 0.318	3.724 ± 0.165	3.633 ± 0.250	0.959	0.896	0.803
cis-12-Octadecenoic, C18:1c12	0.362 ± 0.028	0.375 ± 0.067	0.386 ± 0.074	0.960	0.802	0.897
cis-13-Octadecenoic, C18:1c13	0.199 ± 0.026	0.196 ± 0.018	0.173 ± 0.010	0.480	0.570	0.325
trans-16-Octadecenoic, C18:1t16	0.032 ± 0.003	0.025 ± 0.005	0.029 ± 0.003	0.510	0.352	0.498
cis-15-Octadecenoic, C18:1c15	0.009 ± 0.001	0.006 ± 0.002	0.021 ± 0.003	**0.004**	0.075	**0.002**
Linoleic, C18:2 n-6	11.602 ± 0.405	11.062 ± 0.406	10.571 ± 0.433	0.254	0.149	0.420
Arachidic, C20:0	0.067 ± 0.004	0.064 ± 0.003	0.056 ± 0.006	0.275	0.195	0.230
γ-Linolenic, 18:3 n-6	0.102 ± 0.014	0.094 ± 0.019	0.071 ± 0.017	0.260	0.138	0.265
α-Linolenic, 18:3 n-3	0.470 ± 0.020	0.475 ± 0.015	0.485 ± 0.018	0.849	0.667	0.714
Gondoic, C20:1	0.312 ± 0.058	0.266 ± 0.024	0.284 ± 0.055	0.805	0.549	0.806
Octadeca-9,11-dienoic, C18:2c9t11	0.058 ± 0.012	0.063 ± 0.011	0.057 ± 0.010	0.914	0.870	0.702
11cis-14cis-Eicosadienoic, C20:2	0.178 ± 0.038	0.155 ± 0.011	0.156 ± 0.042	0.855	0.584	0.983
Dihomo-γ-linolenic, 20:3 n-6	0.088 ± 0.010	0.068 ± 0.007	0.070 ± 0.008	0.226	0.093	0.845
Behenic, C22:0	0.022 ± 0.003	0.025 ± 0.003	0.025 ± 0.003	0.769	0.478	1.000
Dihomo-α-linolenic, 20:3 n-3	0.021 ± 0.005	0.015 ± 0.001	0.019 ± 0.006	0.574	0.521	0.558
ARA, C20:4 n-6	0.420 ± 0.041	0.459 ± 0.028	0.366 ± 0.017	**0.036**	0.900	**0.012**
Erucic, C22:1 n-9	0.087 ± 0.007	0.091 ± 0.011	0.079 ± 0.004	0.603	0.859	0.332
Tricosylic, C23:0	0.007 ± 0.001	0.004 ± 0.001	0.006 ± 0.002	0.542	0.435	0.440
Docosa-13,16-dienoic, C22:2c13c16	0.012 ± 0.002	0.014 ± 0.002	0.011 ± 0.003	0.716	0.810	0.444
EPA, C20:5 n-3	0.015 ± 0.003	0.015 ± 0.003	0.016 ± 0.003	0.965	0.896	0.821
Lignoceric, C24:0	0.022 ± 0.009	0.016 ± 0.004	0.020 ± 0.005	0.783	0.628	0.647
Nervonic, C24:1	0.079 ± 0.019	0.063 ± 0.017	0.054 ± 0.011	0.552	0.318	0.694
Clupadonic, C22:5	0.107 ± 0.009	0.089 ± 0.009	0.091 ± 0.005	0.268	0.118	0.759
DHA, C22:6 n-3	0.017 ± 0.003	0.037 ± 0.019	0.007 ± 0.003	0.073	0.498	0.134

Source: Own authorship.

[a]ARA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

[b]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-3 or n-6; 0.000*: Values lower than 10^{-6} g/100g.
[c]SEM: standard error of the mean.
[d]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

Table 17. Effect of supplementation of milk biofortified with n-3 and n-6 on the quality of the lipid fraction of sows' milk

	Treatment[b]			*P-value*[d]		
Item[a]	**CON (± SEM[c])**	**n-6 (± SEM[c])**	**n-3 ± (SEM[c])**	**Treatment**	**C1**	**C2**
ΣSFA	36.70 ± 2.12	36.90 ± 0.78	36.77 ± 2.10	0.997	0.954	0.960
ΣUSFA	63.30 ± 2.11	63.15 ± 0.79	63.23 ± 2.10	0.998	0.961	0.973
ΣMUFA	50.21 ± 1.96	50.60 ± 0.46	51.32 ± 1.92	0.887	0.711	0.759
ΣPUFA	13.09 ± 0.50	12.55 ± 0.46	11.92 ± 0.48	0.267	0.172	0.376
Σn-3	0.52 ± 0.02	0.54 ± 0.02	0.53 ± 0.02	0.779	0.665	0.586
Σn-6	12.21 ± 0.46	11.68 ± 0.41	11.08 ± 0.43	0.226	0.146	0.347
SFA/USFA	0.59 ± 0.05	0.59 ± 0.02	0.59 ± 0.05	0.999	0.992	0.968
n-6/n-3	23.41 ± 0.98	21.57 ± 0.40	21.04 ± 0.49	0.069	**0.026**	0.594

Source: Own authorship.

[a]ΣSFA = Σ saturated fatty acids; ΣUSFA = Σ unsaturated fatty acids; ΣMUFA = Σ monounsaturated fatty acids; ΣPUFA = Σ polyunsaturated fatty acids; Σ n-3 = Σ omega-3 fatty acids; Σ n-6 = Σ omega-6 fatty acids; SFA/USFA = Σ saturated/Σ unsaturated; n-6/n-3 = Σ omega-6/Σ omega-3.

[b]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.

[c]SEM: standard error of the mean.

[d]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

Colostrum and milk quality, can be affected readily by nutrition among several others components, mainly immunoglobulins and fat which are sensitive to nutritional changes (HASAN et al., 2019; POLIDORI et al., 2022; QUESNEL; FARMER, 2019). Indeed, significant changes was noticed in fatty acid profile of colostrum (D1L) and milk (D14L) among the CON, n-6 and n-3 group of sows. According to Kensingert, Collier and Bazer (1986), during the latter gestation, the production of milk components occurs, and it is at 105 days of gestation that the initial lipid droplets become apparent, considering the gestational period of a sow lasts 115 days.

The composition of fatty acids (FA) in the colostrum and milk of sows can be significantly influenced by the feed supplementation received by these animals (LAURIDSEN; DANIELSEN, 2004; LAVERY et al., 2019). In addition, it is important to note that variation in the results of other studies can be attributed to a number of factors, including differences in experimental design, litter size, duration of feeding, amount of supplementation provided and initial survival rates in specific herds, as observed by Seerley in 1984.

Another factor that deserves attention is the source of fatty acids used in supplementation, as this variable can contribute to the heterogeneity of the results. There is currently a lack of reports available that address the influence of the diverse composition of fatty acids on the performance of sows, the health of their offspring and the composition of the colostrum and milk produced by these animals.

6.5 COLOSTRUM PRODUCTION AND INTAKE – EXPERIMENT A

Following the observation of changes in fatty acid profile and piglet performance in our previous study (REIS et al., 2021) we decided to further investigate sow colostrum production and piglet IC on the first day of lactation. However, our current findings indicated no significant differences in the quantities of colostrum produced by the sows or the colostrum intake (CI) of the piglets ($P > 0.05$) (Table 18).

As colostrum production was not affected, and assuming the relationship among BF, mammary gland development and milk production (HEAD AND WILLIAMS, 1991; FARMER et al., 2016 a,b), it is possible to infer that biofortified milk does not cause any detrimental effects on mammary tissue, and may even protect it from potential problems. Interestingly, despite the lack of variations in the chemical composition of the colostrum (REIS et al., 2021), it appears that the changes observed can be attributed to the arrangement of fatty acids present in the biofortified milk.

Table 18. Effects of supplementing biofortified milk in estimated colostrum production of sows and intake of their piglets in the first 24 hours

	Treatment[b]			***P-value*[d]**		
Item[a]	**CON (± SEM[c])**	**n-6 (± SEM[c])**	**n-3 ± (SEM[c])**	**Treatment**	**C1**	**C2**
Production, kg	4.867 ± 0.364	4.835 ± 0.393	4.864 ± 0.556	0.998	0.984	0.954
Intake, g	422.943 ± 0.066	409.797 ± 0.070	397.298 ± 0.072	0.827	0.581	0.756

Source: Own authorship.

[a]Colostrum intake of piglets alive was estimated by the model to predict of Theil et al. (2014) 24 h after the first-born piglet. The sum of the individual colostrum intake of each piglet within the litter was used to calculate colostrum production of the sow.

[b]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.

[c]SEM: standard error of the mean.

[d]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

6.6 VIABILITY: APGAR AND UMBILICAL CORD AFTER SUPPLEMENTATION WITH BIOFORTIFIED MILK – EXPERIMENT A

In order to further explore the effect of lipids on clinical status immediately after birth, we assessed the Apgar score which is a marker of a newborn's vitality right after birth it allows prompt identification of any problems or necessity for immediate intervention, thus the higher score indicates a better state of health. As the umbilical cord blood flow interruption can be one of the reasons for asphyxia, which happens during labor or shortly after birth, were evaluated at birth. Interestingly, the analysis of the Apgar score variables of the neonates indicated a difference in viability score 1, representing low survival, which was higher for the n-6 group compared to n-3 and CON (Figure 9), although there was no difference ($P > 0.05$) in the percentage of adhered or ruptured umbilical cords (Figure 10). The percentages born with vitality 1 were CON 1.01%, n-6 19.28% and n-3 0.95%.

The supplementation of the mother with LC-PUFA and the effects on the offspring do not yet provide evidence on cognitive development, but they do provide evidence of changes in blood, head size and body composition in human babies (BROŚ-KONOPIELKO et al., 2023; LAURITZEN et al., 2005). We can consider that in the literature there are no results on the effects of the n-6/n-3 ratio on cognitive development, meaning that this study is a strong indication for further research into this area. This could also provide us with information on neural development with a view to treating or preventing certain diseases.

Figure 9 - Effects of supplementing biofortified milk of pregnant sows on proportion of newborn piglets viability 0 or 1 classified according to the Apgar score

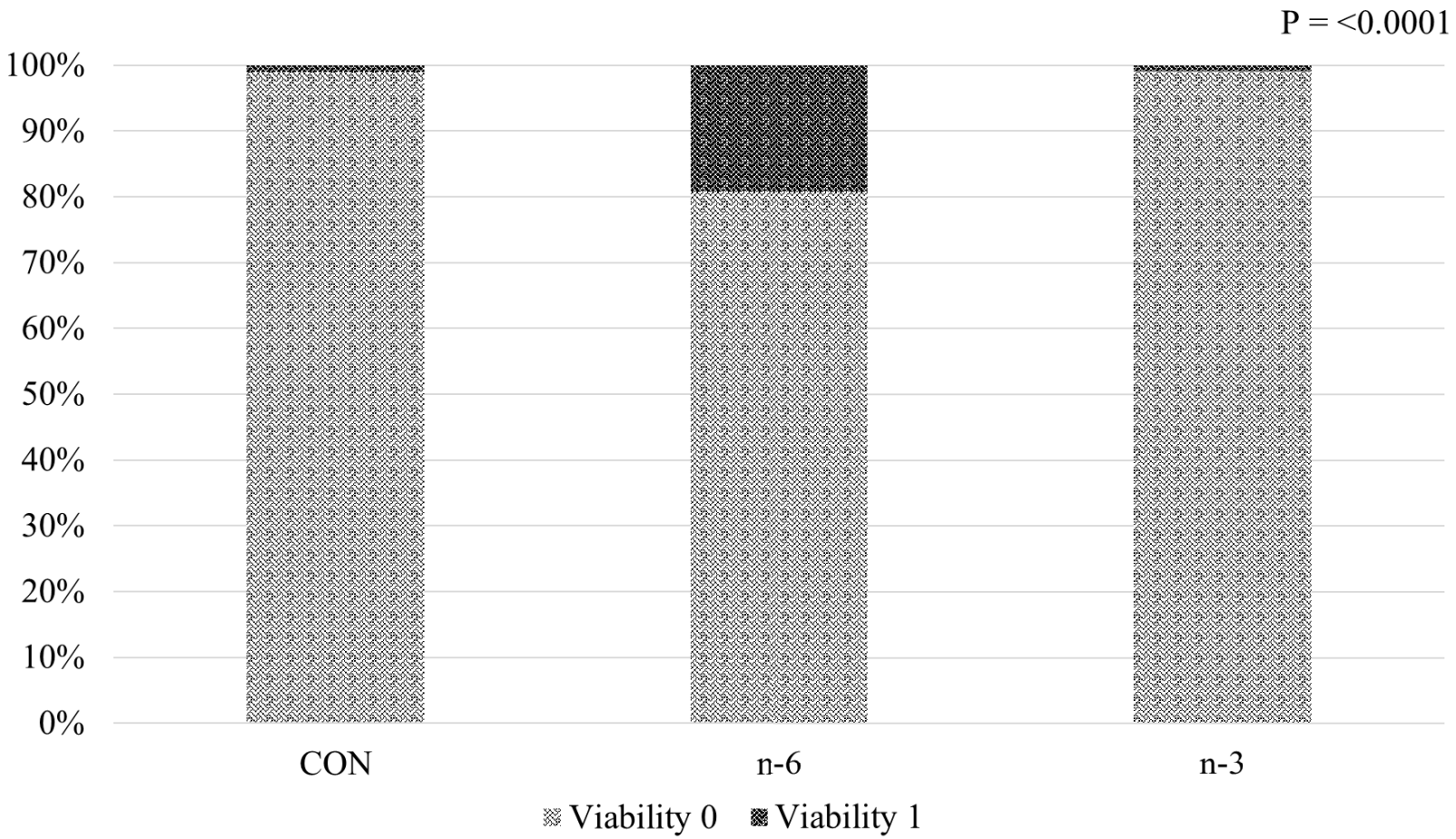
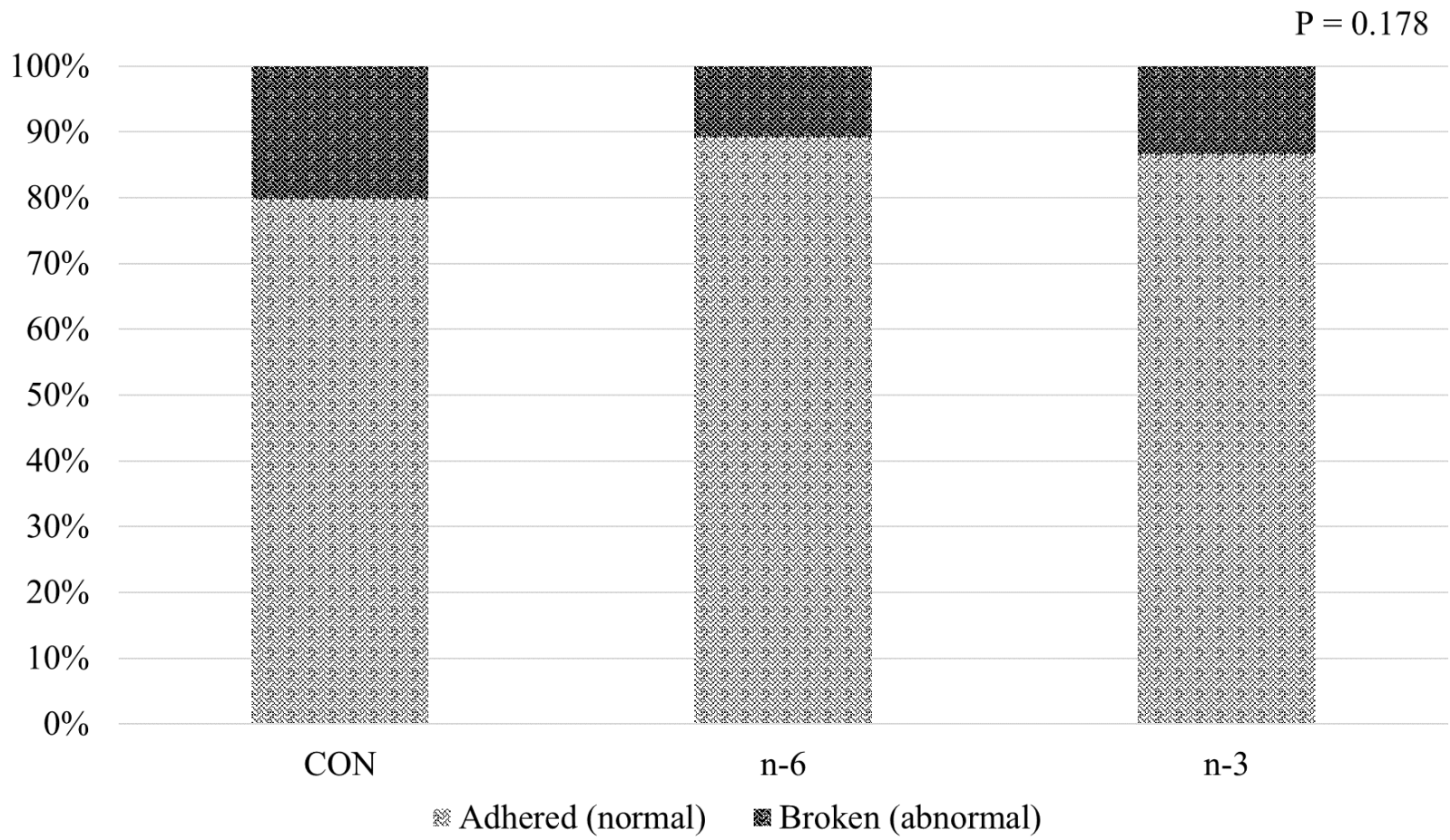


Figure 10 - Condition of the umbilical cord of newborn piglets classified according to the treatment CON, n-6 or n-3



Source: Own authorship.

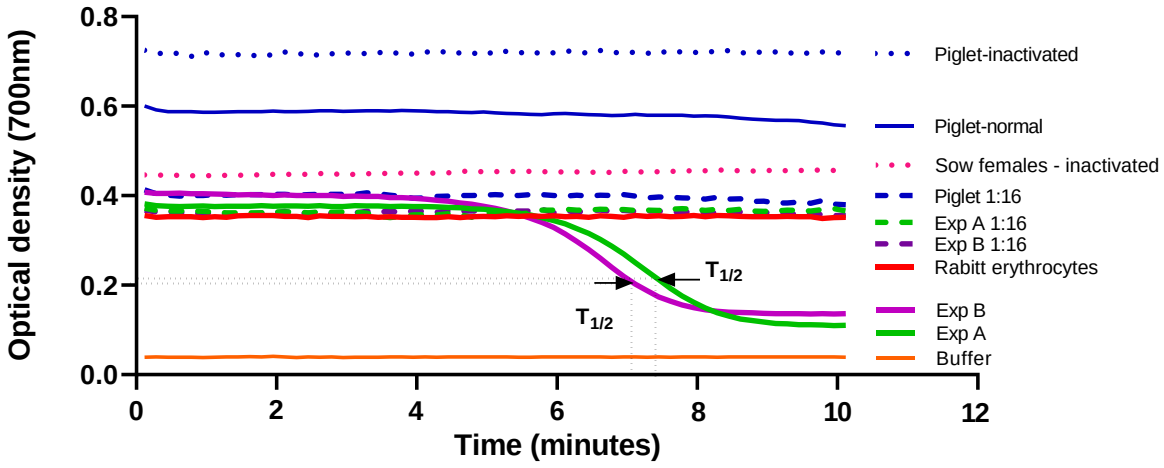
Apgar scoring system has been used to evaluate neonatal vitality by assessing non-invasive physiological traits of newborns within one minute after birth (MOTA-ROJAS et al., 2006). Nonetheless, there is a lack of scientific evidence regarding the impact of the n-6/n-3 ratio to pregnant sows on piglets' vitality or blood parameters. The intriguing aspect of these findings is that the n-6 group had a poor viability tendency than the n-3 group, suggesting that the offspring' Apgar is more influenced by mother's supplementation with cow's milk n-6 PUFA biofortified.

6.7 HIGHER HEMOLYTIC ACTIVITY OF COMPLEMENT SYSTEM FROM SOWS SUPPLEMENTED WITH BIOFORTIFIED MILK

The hemolytic activity of the complement system was determined by a kinetic test, through the capacity of complement proteins to promote 50% of lysis (T1/2) of the standard suspension of erythrocytes from rabbits according to methodology described before. The figure 11 represents the lysis curve achieved for standardization of trial. The serum samples of experimental groups: control, experiment A, experiment B and piglets were used in this standardization. Each experimental group, pooled of serum was prepared and submitted to test undiluted or diluted 1:16. Additionally, a share of each serum pools was submitted to 56ºC for 30 minutes to inactivate of proteins of complement system, used as negative control. Thus, from the lysis curves the value of T1/2 was calculated as the necessary time to the complement lower 50% of initial optical density of erythrocytes from rabbits in suspension. These values account for the hemolytic activity per sample, in other words, the lower T1/2, the higher hemolytic activity of complement.

The outcomes presented in figure 11 are T1/2 values to hemolytic activity of complement in serum samples of experiments A and B were determined undiluted, also indicated by the arrow. However, were not observed hemolytic activity to dilutions 1:16. As to the pooled of serum piglet samples the T1/2 values were not determined to samples undiluted or 1:16. In this case, the calculations will be based on variation between initial and final optic density (ΔDO).

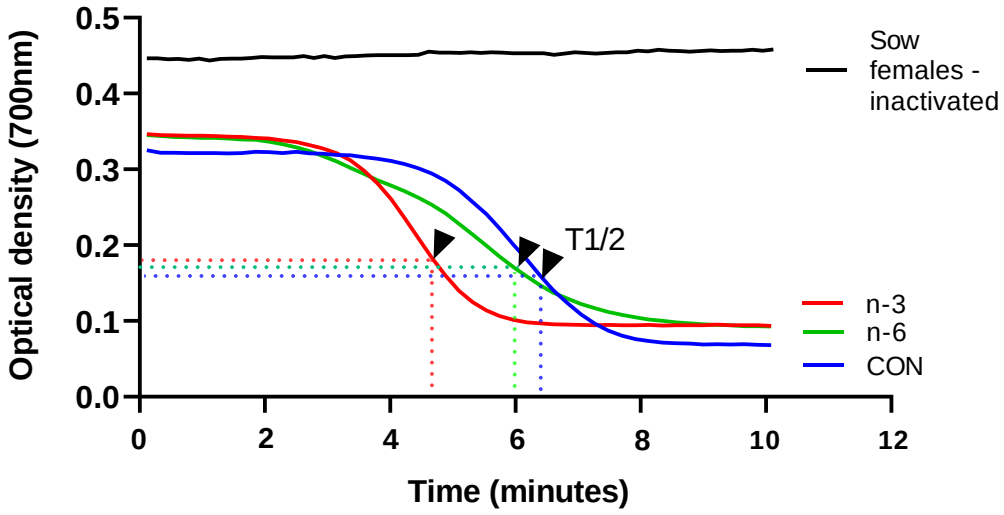
Figure 11 - Representation of the lysis curves obtained to evaluate the hemolytic activity of the alternative pathway of the complement system.



Source: Own authorship. Pooled samples of serum from the experimental groups were tested undiluted and diluted 1:16. The experimental controls were pooled inactivated serum from the respective experimental groups, buffer in the absence of serum and erythrocytes, and rabbit erythrocytes in the absence of serum. Arrows indicate T1/2 values for serum pools from experiments A and B. Exp, experiment; T1/2, time required for complement to reduce 50% of the initial optical density of the rabbit erythrocyte suspension.

The results of hemolytic activity of complement system from experiment A are represented in Figure 12. In this assessment, the T1/2 values were calculated from samples undiluting, per animal in each group: control, n-6, and n-3. Black arrows indicate the value of T1/2 in minutes for each group.

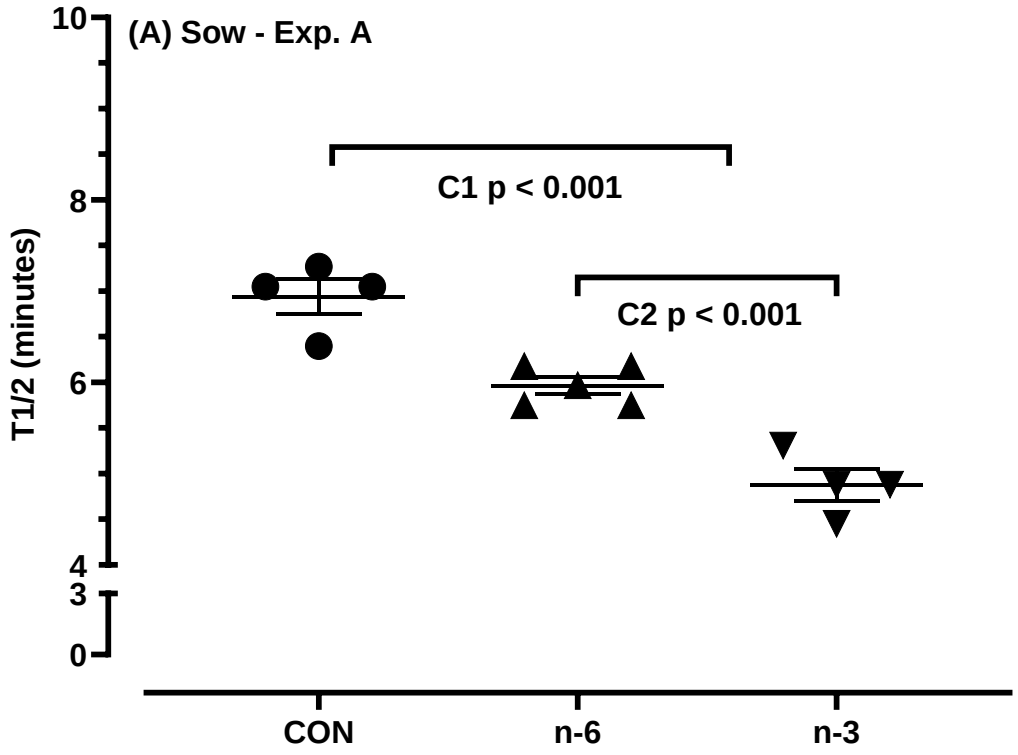
Figure 12 - Hemolytic activity of the alternative pathway of the complement system - experiment A by kinetic assay

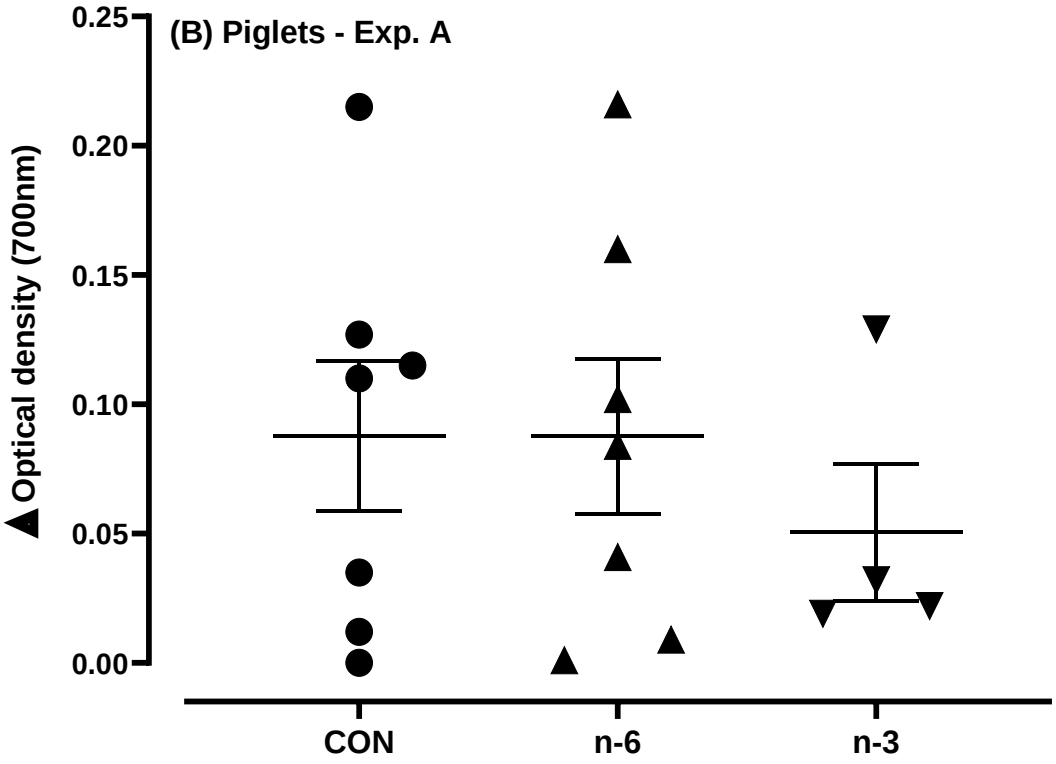


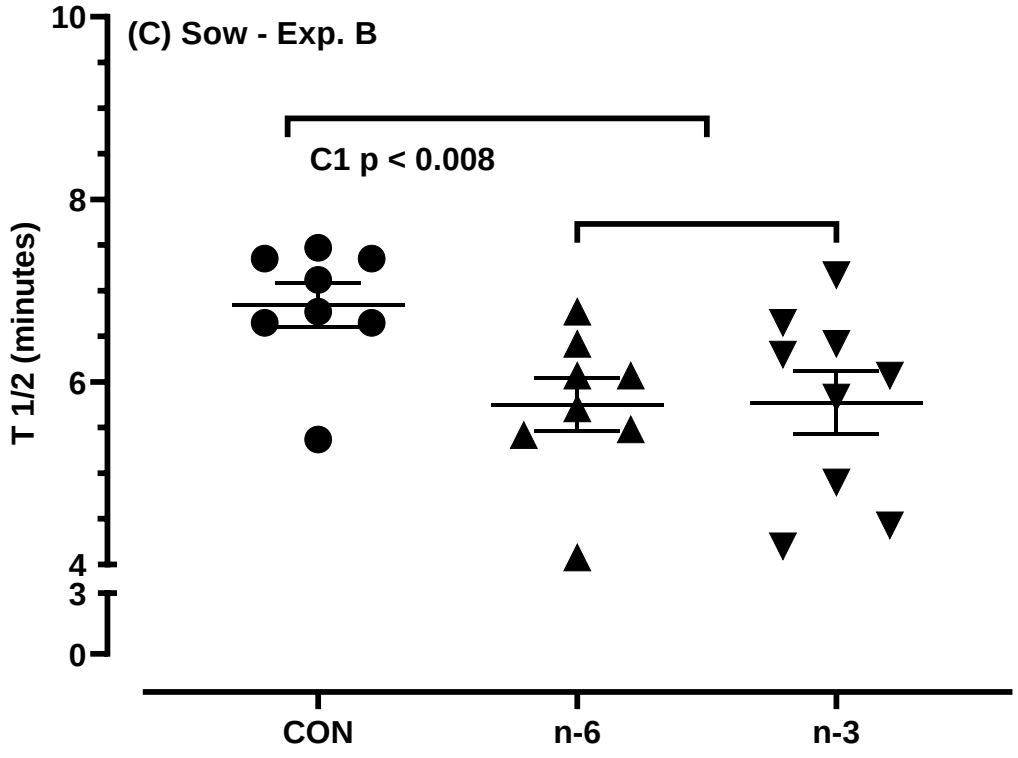
Source: Own authorship. Serum samples from the animals in the control, n-6 and n-3 groups were tested without dilution. A: lytic curves of the mean of each experimental group and the negative control of complement system activity (inactivated sow serum pool).

Comparatively, the hemolytic activity of complement for sows was much higher with PUFA biofortified milk than CON milk (experiment A and B). Another difference could be observed between sows (experiment A) as the n-3 group had a quick response in their organism compared to n-6 group. However, the treatments did not influence the complement in their piglets (Figure 13 and Table 19).

Figure 13 - T1/2 values (minutes) determined as the hemolytic activity of the alternative pathway of the complement system of the serums from experiment A, sows (A) and piglets (B), and experiment B, sows (C)







Source: Own authorship. Sow fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3. T1/2 values in minutes for samples from the respective experimental groups analyzed in duplicates. T1/2, time required for complement to reduce 50% of the initial optical density of the rabbit erythrocytes suspension. The T1/2 values for each sample in the groups correspond to the average of duplicate trials. Serum samples from piglets T1/2 values were based on the variation

between initial and final optical density (ΔOD). The bars indicate the means ± standard error of the mean. C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs. n-3.

Table 19 - Hemolytic activity of the alternative pathway of the complement system for swine females and piglets

Item	Treatment[a]			P-value[c]		
	CON (± SEM[b])	n-6 (± SEM[b])	n-3 (± SEM[b])	Treat	C1	C2
T1/2 Sow Exp. A	6.942 ± 0.156	5.966 ± 0.140	4.880 ± 0.156	**<0.001**	**<0.001**	**<0.001**
ΔOD Piglet Exp. A	0.088 ± 0.028	0.088 ± 0.028	0.050 ± 0.037	0.680	0.613	0.4343
T1/2 Sow Exp. B	6.841 ± 0.31	5.752 ± 0.306	5.774 ± 0.288	**0.028**	**0.008**	0.959

Source: Own authorship.
[a]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.
[b]SEM: standard error of the mean.
[c]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

Activation of the complement system is an essential response to help eliminate the pathogen, i.e. in the first instance it is important to increase the inflammatory status. However, this action should not be too prolonged, and this time can vary according to the pathogen present in the system, and obesity has been positively related to inflammatory status. Depending on the source and composition, high fat diets, especially SFA and n-6 PUFA, may contribute to inflammation, leading to a more active complement system (KREMER, 2017; POREBA et al., 2017; WÄRNBERG et al., 2006). In a study using fish oil as a source of n-3 PUFA for mice showed an activation of the complement system (JIN et al., 2018a). The contradictory results in the literature are strongly influenced by different combinations of sources, levels, forms of supplementation, and species. This is difficult to compare the results across divergent research.

However, in the present study, we observed a higher activation capacity of the complement system by milk biofortified with PUFA, and even higher activation by the n-3 group milk, which contains lower n-6/-3, SFA/USFA ratio and richer in n-3 PUFA.

The lactation period presents several challenges, as it increases the demand for energy and nutrients by the organism, consequently increasing food intake and mobilization of corporal fat. Thus, lactation phase induces an inflammatory state in sows as shown by Rosenbaum et al. (2012a, 2012b) through liver analysis. Another critical phase is the early stages of pregnancy, as the conceptus needs to signal its presence strongly for pregnancy to be stabilized. Among the signaling molecules are pro-inflammatory cytokines that mediate the communication between the conceptus and endometrium, leaving the organism in a more inflammatory overall condition (MATHEW; LUCY; GEISERT, 2016).

Considering in this study, the evaluation period of 13 ± 4 days of lactation for the sows in experiment A and 24 ± 6 days of gestation for the sows in experiment B, it is possible to suggest that both times present a challenge for the sows, either by the lactation phase, or by the

recognition of gestation. Therefore, milk biofortified with n-3 PUFA may provide a prompt response from the complement system to the organism, which is necessary and beneficial for both processes to complete. However, because these are so specific points, it is not possible to measure the duration of this condition, which if elongated, can bring damage to the organism.

As the first line of defense against pathogens, innate immunity plays an essential role, and the complement system, being part of this structure, is able to involve adaptive immunity, enhancing the response action. In the present study, it was possible to observe that biofortified milk has the capacity to modulate the innate and adaptive systems, as we will show in the immunoglobulin results. In any case, this fast initial response is of great advantage for the body's defense.

The effect of treatments indicating an increase in the hemolytic activity of the complement system can have important implications in the area of immunology and health, because complement destroys invading microorganisms and damaged cells by opsonization, resulting in the release of their intracellular contents. Normally, the hemolytic activity of the complement system is carefully regulated to prevent damage to the organism's own cells. However, under certain conditions, such as autoimmune diseases, infections, or other immune dysfunctions, an uncontrolled increase in the hemolytic activity of complement can occur.

Thus, a treatment that results in increased hemolytic activity of the complement system, can have a variety of implications. In the case of autoimmune diseases, inflammation, or even an uncontrolled response, high activity can worsen symptoms, lead to an uncontrolled and non-specific response, resulting in indiscriminate damage and widespread inflammation. In contrast, an experimental or therapeutic treatment that results in an intentional increase in hemolytic complement activity may be desirable in certain situations, such as fighting certain types of cancer or killing specific pathogens.

Overall, the present study shows that biofortified milk with a change in the FA profile can alter the activity of the complement system, but the real benefits depend on the clinical context and the specific disease involved. It is essential that the results be reviewed by appropriate healthcare professionals to determine the clinical significance and appropriate therapeutic implications.

6.8 CONCENTRATION OF IMMUNOGLOBULIN AND INTERLEUKIN IN COLOSTRUM, MILK, SERUM OF SOW AND PIGLET

The milk biofortified with n-6 and n-3 PUFA had effect on milk IgA, IgM, and IgG content on the time ($P < 0.05$; Table 20). Plasma levels of TNF-α were significantly greater in

CON compared to n-6+n-3 group (P = 0.042), and in n-3 versus the n-6 group (P = 0.001; Table 23). There was also a treatment by time effect for plasma IgG, IgM and TNF-α levels (P < 0.05; Table 20). The CON group had higher concentration of IgM level on D1L, and the n-3 group on D21L, while the n-6 group remains constant throughout the analyzed period. Between treatments, it is not possible to identify any difference between the specific days evaluated (Table 21 and Figure 14). The IgG level during the gestation period showed a decrease D107G for CON and n-6 treatments, while n-3 remained stable during the whole period. Considering the beginning of gestation, at D40G, the sows that received the milk biofortified with n-3 PUFA had a lower level of IgG in Plasma (Table 22 and Figure 15). The n-3 group showed the highest level of TNF-α concentration at D1L, while for the other treated groups the concentration was maintained throughout gestation and lactation (Table 23 and Figure 16) The treatments were different from each other only at D1L, in which the CON and n-6 PUFA group showed lower TNF-α levels when compared to the n-3 PUFA group (56.5314 $pg.mL^{-1}$).

Table 20. Concentration of the immunoglobulins and interleukins in plasma of sows as the response to supplementation during the gestation and lactation period with biofortified cow's milk

	Treatment[b]			***P-value*[d]**				
Item[a]	**CON (± SEM[c])**	**n-6 (± SEM[c])**	**n-3 (± SEM[c])**	**Treatment**	**Time**	**Treat*Time**	**C1**	**C2**
IgA, $mg.mL^{-1}$	4.346 ± 0.781	3.911 ± 0.731	4.161 ± 0.781	0.852	<0.001	0.599	0.583	0.860
IgG, $mg.mL^{-1}$	224.31 ± 25.882	187.310 ± 25.079	181.260 ± 25.882	0.172	<0.001	**0.025**	0.066	0.856
IgM, $mg.mL^{-1}$	20.111 ± 3.451	20.751 ± 3.261	19.155 ± 3.451	0.839	0.001	**0.011**	0.983	0.555
TNF-α, $pg.mL^{-1}$	29.030 ± 2.845	31.639 ± 2.506	37.265 ± 2.908	0.261	0.120	**0.009**	**0.042**	**0.001**
IL-6, $pg.mL^{-1}$	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
IL-10, $pg.mL^{-1}$	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

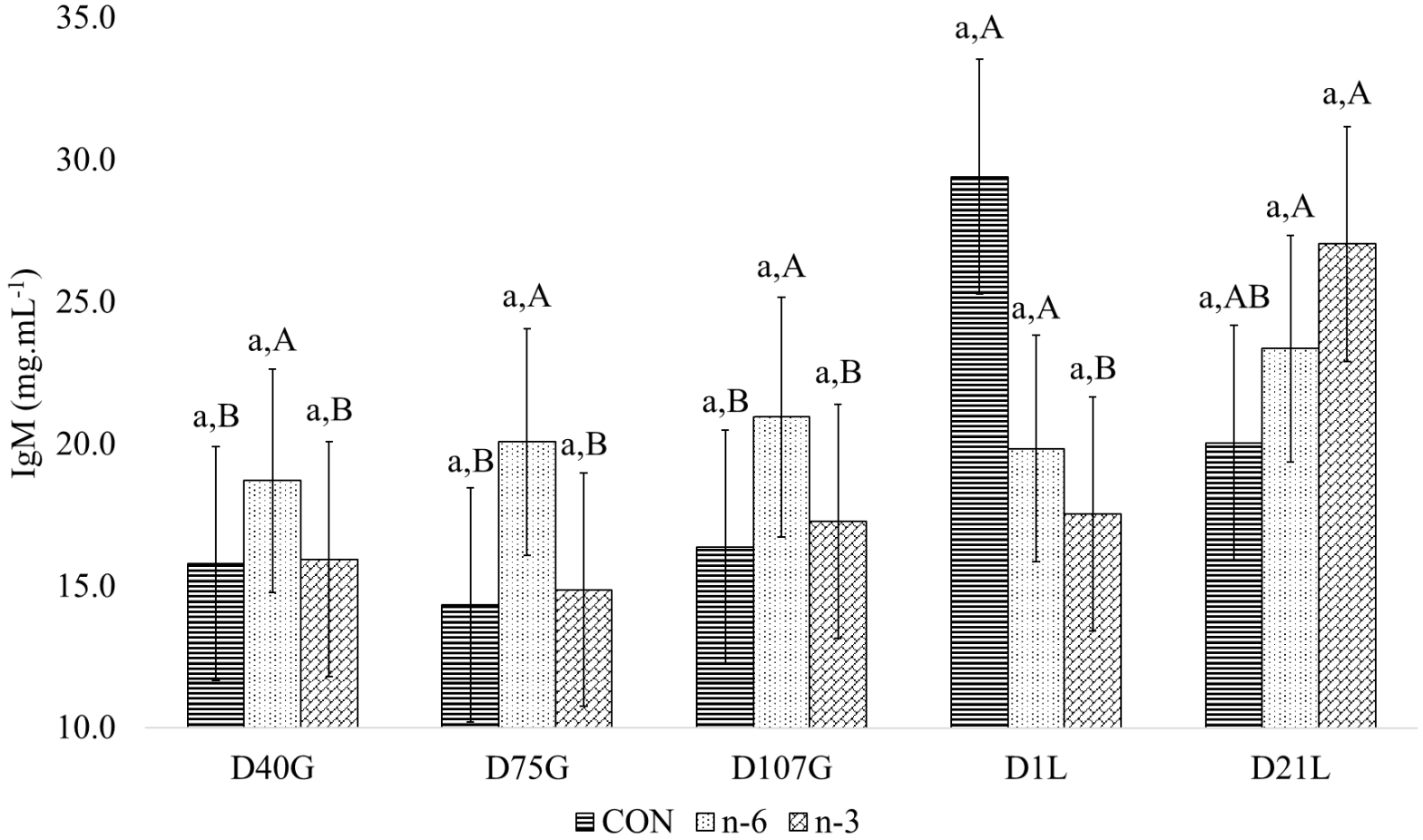
Source: Own authorship.
[a]The variable IgG had only the period for gestation included on the statistical analysis as a consequence of the limitations of the method to measure the concentration in the sample based on the standard curve.
[b]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.
[c]SEM: standard error of the mean.
[d]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.
n.d: not detectable. The values were not estimated due to missing, as a consequence of the limitations of the method to measure the concentration in the sample base on the standard curve.

Table 21. Concentration of IgM (mg.mL-1) at 40, 75 and 107 days of gestation and at 1 and 21 days of lactation of swine females

	Gestation			**Lactation**	
Treatment	**D40G[a] (± SEM[b])**	**D75G[a] (± SEM[b])**	**D107G[a] (± SEM[b])**	**D1L[a] (± SEM[b])**	**D21L[a] (± SEM[b])**
CON	15.793 ± 4.123 a,B	14.332 ± 4.123 a,B	16.373 ± 4.123 a,B	29.405 ± 4.123 a,A	20.038 ± 4.123 a,AB
n-6	18.711 ± 3.934 a,A	20.083 ± 3.987 a,A	20.942 ± 4.225 a,A	19.838 ± 3.987 a,A	23.351 ± 3.987 a,A
n-3	15.952 ± 4.123 a,B	14.863 ± 4.123 a,B	17.271 ± 4.123 a,B	17.529 ± 4.123 a,B	27.032 ± 4.123 a,A

Source: Own authorship.
Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed by the same lowercase letter, do not differ.
[a]D40G: 40 days of gestation; D75G: 75 days of gestation; D107G: 107 days of gestation; D1L: first day of lactation; D21L: 21 days of lactation.
[b]SEM, standard error of the mean.

Figure 14 - Increased plasma IgM for the CON group in D1L of swine females



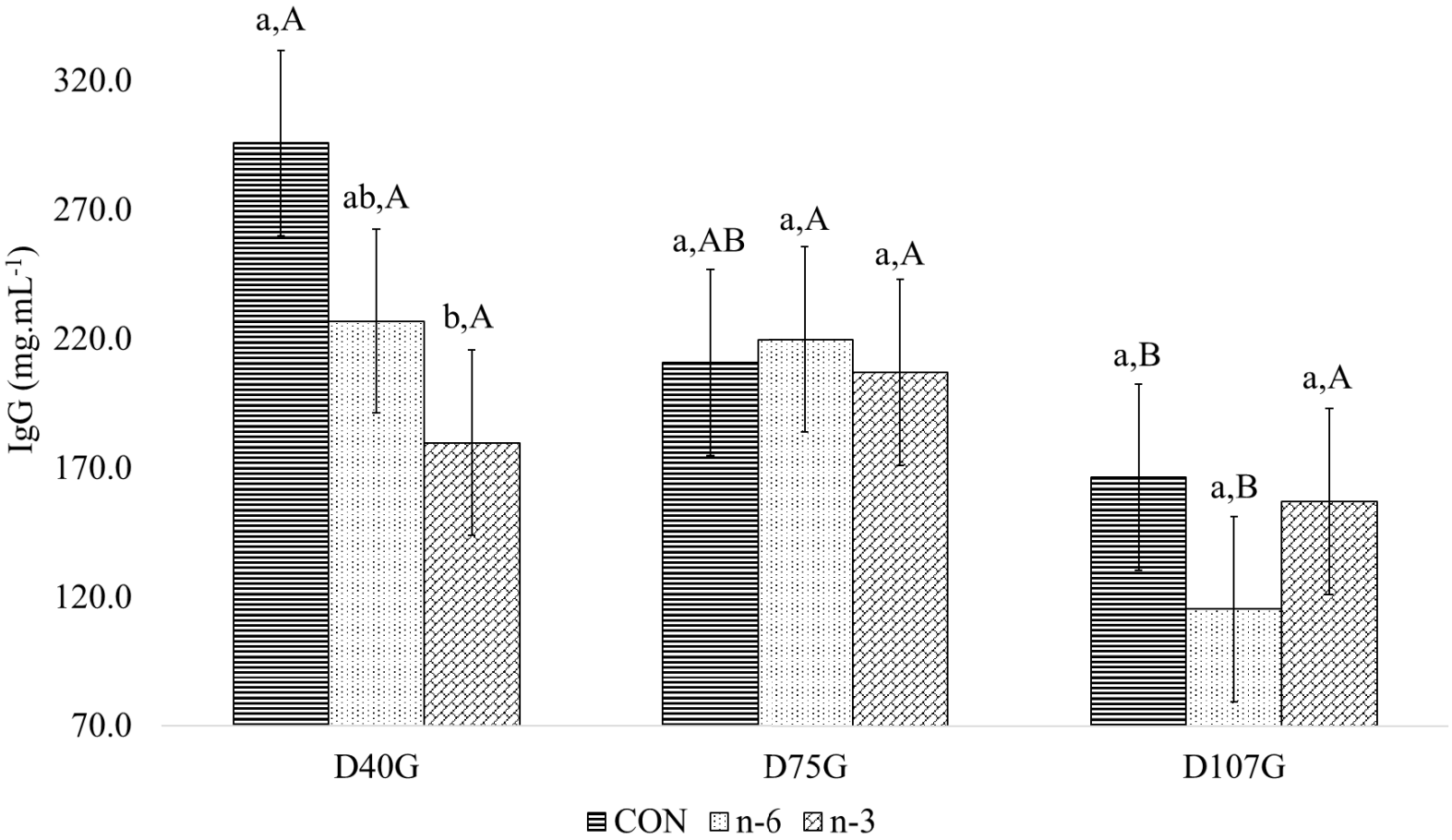
Source: Own authorship. Average followed by the lowercase letter means the comparison among the treatments at one point in time. Average followed by the capital letter means the comparison of one treatment at different times.

Table 22. Concentration of IgG (mg.mL-1) at 40, 75 and 107 days of gestation of swine females

	Gestation		
Treatment	**D40G[a] (± SEM[b])**	**D75G[a] (± SEM[b])**	**D107G[a] (± SEM[b])**
CON	295.77 ± 35.9982 a,A	210.77 ± 35.9982 a,AB	166.40 ± 35.9982 a,B
n-6	226.92 ± 35.6524 ab,A	219.73 ± 35.7781 a,A	115.28 ± 35.7781 a,B
n-3	179.84 ± 35.9982 b,A	206.93 ± 35.9982 a,A	157.00 ± 35.9982 a,A

Source: Own authorship.
Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed by the same lowercase letter, do not differ.
[a]D40G: 40 days of gestation; D75G: 75 days of gestation; D107G: 107 days of gestation.
[b]SEM, standard error of the mean.

Figure 15 - Effect between treatments and time in plasma for IgG of swine females



Source: Own authorship. Average followed by the lowercase letter means the comparison among the treatments at one point in time. Average followed by the capital letter means the comparison of one treatment at different times.

Table 23. Concentration of TNF-α (pg.mL-1) at 40, 75 and 107 days of gestation and at 1 and 21 days of lactation of swine females

	Gestation			Lactation	
Treatment	**D40G[a] (± SEM[b])**	**D75G[a] (± SEM[b])**	**D107G[a] (± SEM[b])**	**D1L[a] (± SEM[b])**	**D21L[a] (± SEM[b])**
CON	32.927 ± 5.376 a,A	30.057 ± 5.376 a,A	37.482 ± 5.376 a,A	22.580 ± 6.191 b,A	22.102 ± 5.376 a,A
n-6	38.544 ± 4.802 a,A	35.048 ± 4.806 a,A	37.403 ± 5.363 a,A	23.122 ± 4.806 b,A	24.078 ± 4.806 a,A
n-3	26.040 ± 5.376 a,B	40.827 ± 5.376 a,AB	29.369 ± 6.191 a,AB	56.531 ± 6.191 a,A	33.560 ± 5.376 a,AB

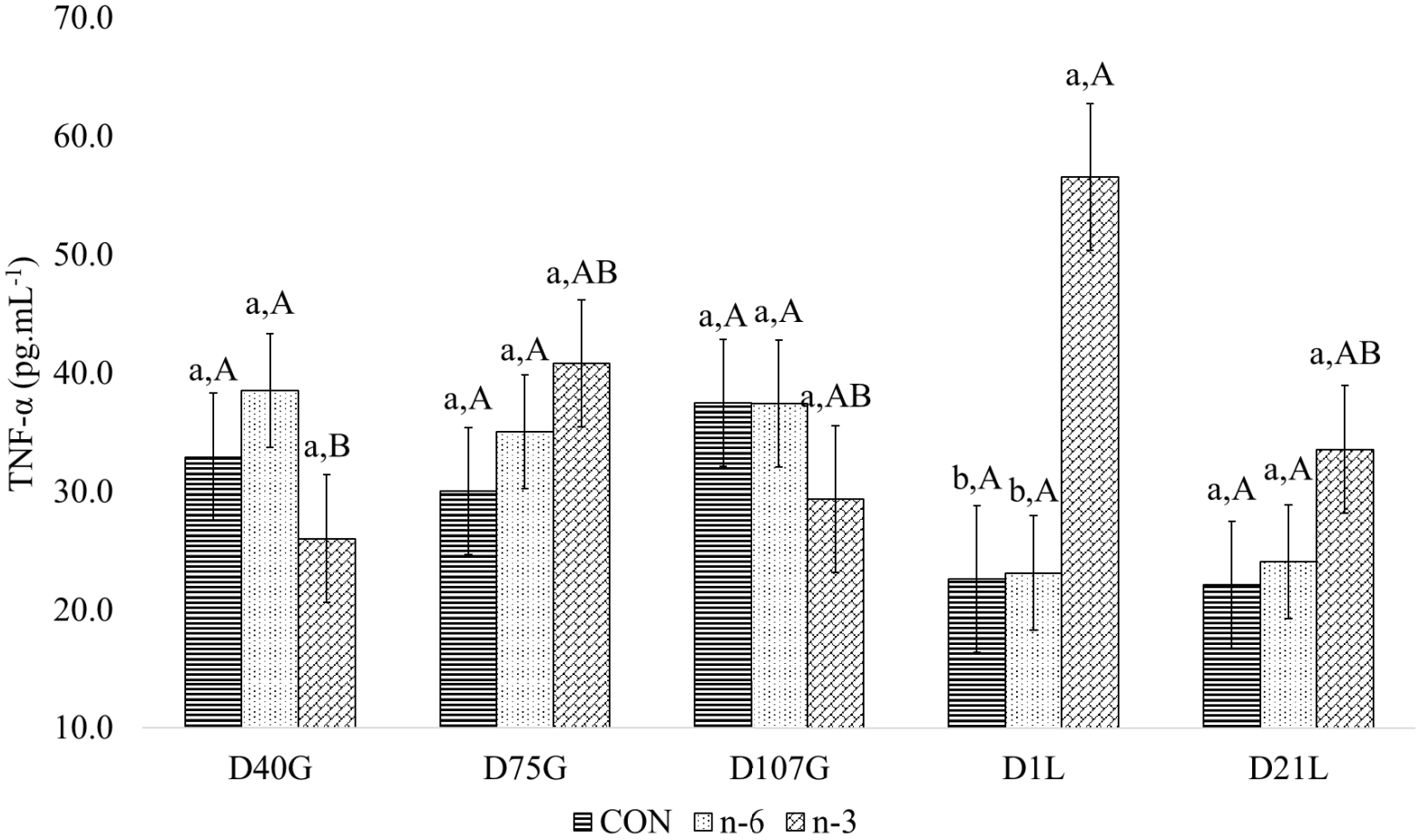
Source: Own authorship.

Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed by the same lowercase letter, do not differ.

[a]D40G: 40 days of gestation; D75G: 75 days of gestation; D107G: 107 days of gestation; D1L: first day of lactation; D21L: 21 days of lactation.

[b]SEM, standard error of the mean.

Figure 16 - Increased plasma TNF-α for the n-3 group in D1L of swine females



Source: Own authorship. Average followed by the lowercase letter means the comparison among the treatments at one point in time. Average followed by the capital letter means the comparison of one treatment at different times.

The concentration of immunoglobulin and interleukin in colostrum are presented in Table 24. On average, the concentration of IgG was 67.862 mg.mL^{-1} in n-6 and n-3 groups, implying a reduction by approximately 51% from control colostrum.

Table 24. Total immunoglobulin and interleukin concentration in the colostrum of sows

	Treatment[a]			***P-value*[d]**		
Item	**CON (± SEM[b])**	**n-6 (± SEM[b])**	**n-3 (± SEM[b])**	**Treatment**	**C1**	**C2**
IgA, mg.mL^{-1}	16.460 ± 4.577	15.920 ± 2.895	20.182 ± 2.895	0.573	0.758	0.325
IgG, mg.mL^{-1}	137.601 ± 1.599	57.862 ± 1.520	77.862 ± 1.731	**0.050**	**0.022**	0.378
IgM, mg.mL^{-1}	9.294 ± 0.218	7.497 ± 0.165	6.723 ± 0.165	0.535	0.312	0.668
TNF-α, pg.mL^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
IL-6, pg.mL^{-1}	35.810 ± 47.211	26.593 ± 29.859	115.960 ± 29.859	0.139	0.510	**0.063**
IL-10, pg.mL^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Source: Own authorship.
[a]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.
[b]SEM: standard error of the mean.
[c]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.
n.d: not detectable. The values were not estimated due to missing, as a consequence of the limitations of the method to measure the concentration in the sample base on the standard curve.

Table 25 illustrates the impact of supplementation with cow's milk biofortified in sow milk. The concentration of IgG to n-6 and n-3 groups had a decrease of 0,079 and 0,277 mg.mL^{-1}, respectively, compared to the control group.

Table 25. Total immunoglobulin and interleukin concentration in the milk of sows on day 14 of lactation

	Treatment[a]			***P-value*[d]**		
Item	**CON (± SEM[b])**	**n-6 (± SEM[b])**	**n-3 (± SEM[b])**	**Treatment**	**C1**	**C2**
IgA, mg.mL^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
IgG, mg.mL^{-1}	0.781 ± 0.069	0.702 ± 0.077	0.504 ± 0.069	**0.043**	**0.064**	**0.082**
IgM, mg.mL^{-1}	1.905 ± 0.445	1.732 ± 0.445	1.607 ± 0.445	0.929	0.722	0.905
TNF-α, pg.mL^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
IL-6, pg.mL^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
IL-10, pg.mL^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Source: Own authorship.
[a]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.
[b]SEM: standard error of the mean.
[c]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.
n.d: not detectable. The values were not estimated due to missing, as a consequence of the limitations of the method to measure the concentration in the sample base on the standard curve.

Circulating levels of antibodies and cytokines in piglets on day 1 or 14 postnatal were significantly affected by sow supplementation (Table 26). IgA, IgG, TNF-α and IL-10 concentrations were higher ($P < 0.05$) in CON offspring compared to those of n-3 and n-6

supplemented sows. Time also had a significant impact on IgA, IgG and IgM levels ($P < 0.001$). In addition, the milk biofortified with n-6 or n-3 PUFA increased IgA, IgM and TNF-α, and reduced IL-10 concentration in plasma of piglets compared to piglets from control group ($P < 0.05$). IgA and IgG in n-6 group were found, respectively, 79.2% (3.36 $mg.mL^{-1}$) and 78.2% (83.742 $mg.mL^{-1}$) higher than n-3 group. In the case of IL-10, the n-3 group showed an increase in 135.5% compared to n-6 group (10.657 *vs.* 4.526 $pg.mL^{-1}$).

Table 26. Concentration of the immunoglobulins and interleukins in plasma of piglets at day 1 and 14 of lactation

	Treatment[b]			***P-value*[d]**				
Item[a]	**CON (± SEM[c])**	**n-6 (± SEM[c])**	**n-3 (± SEM[c])**	**Treatment**	**Time**	**Treat*Time**	**C1**	**C2**
IgA, mg.mL^{-1}	1.131 ± 0.342	3.360 ± 0.361	1.875 ± 0.362	**<0.001**	<0.001	0.629	**0.011**	**0.001**
IgG, mg.mL^{-1}	75.418 ± 14.845	83.742 ± 29.391	46.983 ± 15.127	**0.024**	<0.001	**0.002**	0.211	0.073
IgM, mg.mL^{-1}	4.898 ± 0.742	6.283 ± 1.181	4.445 ± 0.757	0.058	<0.001	**<0.001**	**0.047**	0.272
TNF-α, pg.mL^{-1}	45.643 ± 18.278	86.595 ± 18.287	88.594 ± 18.278	**0.007**	-	-	**0.002**	0.781
IL-6, pg.mL^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
IL-10, pg.mL^{-1}	14.816 ± 3.126	4.526 ± 3.297	10.657 ± 2.699	**0.021**	0.796	0.126	**0.027**	**0.044**

Source: Own authorship.

[a]The variable TNF-α had only the day 14 of lactation included on the statistical analysis as a consequence of the limitations of the method to measure the concentration in the sample based on the standard curve.

[b]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.

[c]SEM: standard error of the mean.

[d]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

n.d: not detectable. The values were not estimated due to missing, as a consequence of the limitations of the method to measure the concentration in the sample base on the standard curve.

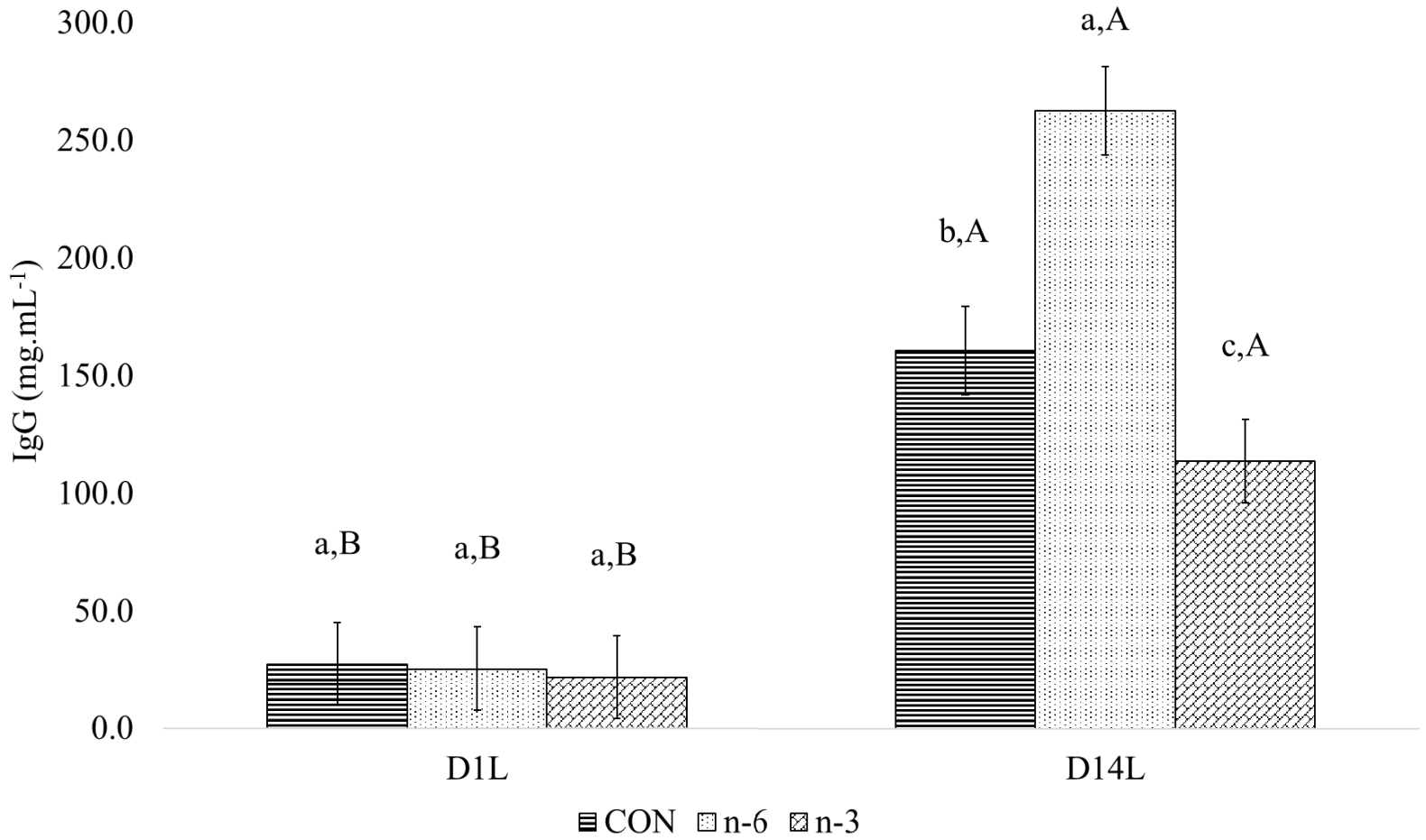
The significance of interaction was observed to IgG (Table 27 and Figure 17) and IgM (Table 28 and Figure 18). The increase of concentration from D1L to D14L (day 14 of lactation) was similar for both, however on D1L the n-6 and n-3 groups had lower abundance of IgM (in average 0.900 $mg.mL^{-1}$) compared to CON group (2.988 $mg.mL^{-1}$), while in the D14L the concentration was 10.295 $mg.mL^{-1}$ for n-6 group, so 39.21% and 61.92% higher than n-3 and CON group, respectively. Whereas, the outcomes for IgG in D1L there is no differences, and D14L showed that n-3 treatment reduced the concentrations by 29.27% and 56.73%, respectively, compared to CON (160.69 $mg.mL^{-1}$) and n-6 group (262.69 $mg.mL^{-1}$).

Table 27. Concentration of IgG (mg.mL-1) at 1 and 14 days of lactation in plasma of piglets

	Day	
Treatment	**D1L[a] (± SEM[b])**	**D14L[a] (± SEM[b])**
CON	27.375 ± 17.831 a,B	160.69 ± 18.813 b,A
n-6	25.441 ± 17.831 a,B	262.69 ± 18.813 a,A
n-3	21.867 ± 17.831 a,B	113.65 ± 17.831 c,A

Source: Own authorship.
Average on the same line followed by the same capital letter, do not differ.
Averages in the same column followed by the same lowercase letter, do not differ.
[a]D1L: 1 day of lactartion; D14L: 14 days of lactation.
[b]SEM, standard error of the mean.

Figure 17 - Effect between treatments and time in plasma for IgG of piglets



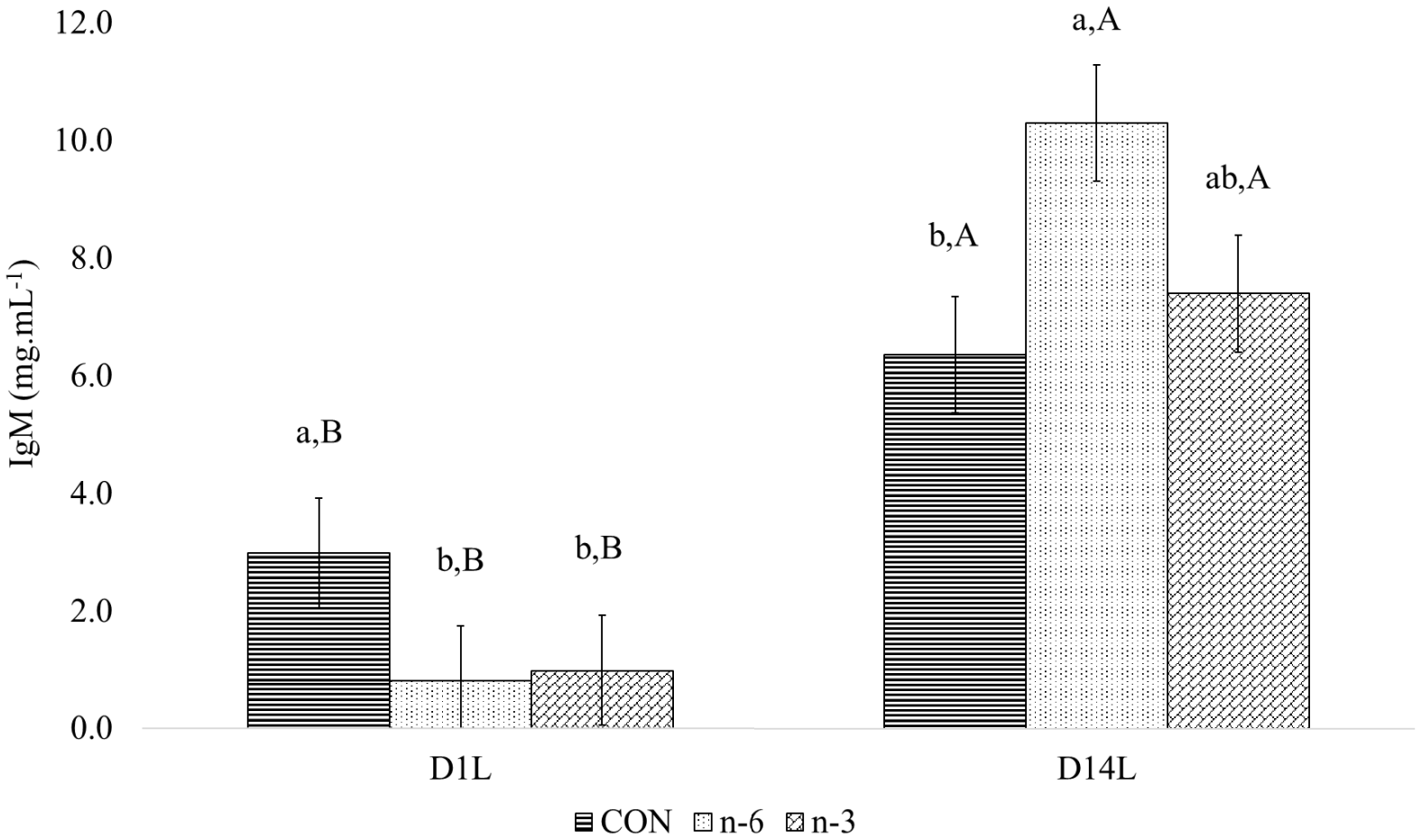
Source: Own authorship. Average followed by the lowercase letter means the comparison among the treatments at one point in time. Average followed by the capital letter means the comparison of one treatment at different times.

Table 28. Concentration of IgM (mg.mL-1) at 1 and 14 days of lactation in plasma of piglets

	Day	
Treatment	**D1L[a] (± SEM[b])**	**D14L[a] (± SEM[b])**
CON	2.988 ± 0.940 a,B	6.358 ± 0.992 b,A
n-6	0.812 ± 0.940 b,B	10.295 ± 0.992 a,A
n-3	0.989 ± 0.940 b,B	7.395 ± 0.992 ab,A

Source: Own authorship.
Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed by the same lowercase letter, do not differ.
[a]D1L: 1 day of lactartion; D14L: 14 days of lactation.
[b]SEM, standard error of the mean

Figure 18 - Effect between treatments and time in plasma for IgM of piglets



Source: Own authorship. Average followed by the lowercase letter means the comparison among the treatments at one point in time. Average followed by the capital letter means the comparison of one treatment at different times.

As has been reported previously, the acquisition of passive immunity by piglets through colostrum and milk from sows is crucial owing to the epitheliochorial nature of the pig placenta, which precludes the transfer of immunoglobulins across the placenta. Milk, especially colostrum, is rich in immune cells that confer temporary immunity until the piglets develop their own active immunity. Usually, this process can be facilitated by administering vaccines to the sow during gestation (MATÍAS et al., 2017).

Although, in this study was not possible to observe the IgA in milk due to higher values above the standard curve (assay range 1.37 - 1000 ng/ml), there is no doubt that IgA and IgM dominates the mature milk, just as the IgG is the predominant immunoglobulin in sow colostrum, supported by research (CURTIS; BOURNE, 1971; KLOBASA; WERHAHN; BUTLER, 1987). In addition, IL-6 was not detected in the mature milk, which is the opposite of colostrum. Antigenic specificity of IgG and IgA in piglets mirrors the maternal exposure to environmental and vaccine antigens, that the immunity conferred by colostrum within 24 to 36 h of life and milk, provides protection to piglets against these antigens (ROOKE; BLAND, 2002).

Recent evidence shows that the acquisition of passive immunity by colostrum and the development of active immunity by the piglet are positively correlated (ROOKE; BLAND, 2002). In addition, genotype, parity, age, vaccination stage and endocrine status of the sow, nutrition, and herd management are influential factors of colostrum yield and composition (MACIAG et al., 2022; MILLER et al., 2013).

Due to colostrum from sows in the CON group showed higher IgG concentration compared to n-6 and n-3, a higher concentration of IgG was expected in the plasma of piglets of CON at D14L. However, the n-6 group showed the highest IgG concentration, followed by CON and n-3 group. Currently, the mechanism by which n-6 and n-3 PUFA alter the concentrations of IgG, IgA, and IgM is not clear, also the pattern between the ratio n-6/n-3 and the change of immunoglobulin concentrations in plasma.

A modest decrease in the stimulation of B cells can occur by the action of n-3 PUFA. These cells are involved in inflammatory processes and humoral immune responses, through the major histocompatibility complex (MHC) class II pathway, by suppressing antigen presentation and thus reducing antibody production. In addition, n-3 PUFA decrease the secretion of pro-inflammatory cytokines (i.e. IL-6 and TNF-α), which in turn may also exert an influence on B-cell autoregulation (CALDER, 1998; WHELAN; GOWDY; SHAIKH, 2016). IL-6, via the helper T cell, can stimulate the B cell to synthesize more antibodies (JONES, 2005), so since n-3 PUFA can be performing these rules, a decreased amount of antibodies and

pro-inflammatory interleukins were expected in the sows and piglets of the n-3 group at this study. However, there is a disparity in the literature that reports the opposite effect of the stimulation of n-3 PUFA (CHE et al., 2019; CHEN et al., 2017; WHELAN; GOWDY; SHAIKH, 2016).

In this study, we show that milk biofortified with PUFA increased IgA, IgM and TNF-α, and decreased IL-10 compared to CON group in plasma of piglets. Also, n-3 PUFA decreased IgA and increased IL-10 compared to n-6 PUFA, which was expected probably due to activity on B cell. Considering the interactions presented, the highest concentration of IgG and IgM at D14L compared to D1L is possible to attribute the development of active immunity of piglets. Our results showed in IgG that the effect of treatments on progeny only occurred through the lactation phase, disregarding the level of placenta, but not for IgM which shown differences even before the first sucking, as well as was higher concentration in n-6 group at D14L.

At the literature has not studies establishing the relation between n-6 PUFA and B cells or spleen function, which hinder the understanding to describe the potential pathways considering this treatment.

Maternal supplementation with n-3 and n-6 PUFA increased the interleukin pro-inflammatory TNF-α and reduced the anti-inflammatory IL-10 compared to CON, however the increase of IL-10 in the plasma of piglets indicates that the sows whose received milk biofortified with linseed oil (n-3 group) might improve the anti-inflammatory capability in the suckling piglets compared to n-6 group thought the impact of cytokines since they can affect T cells and are produced by different T helpers. Even the precise definition of these cytokines remains unclear, studying the transfer of diverse cytokines in the colostrum, milk and plasma of gilts and the link between their offspring, is a promising research area that can enhance our understanding of the involved mechanisms and their advantages (FORNER et al., 2021).

Quite unexpectedly, the milk biofortified with n-3 PUFA increased the levels of TNF-α in the sow's plasma compared to n-6 group, also both compared to CON. This result is inconsistent with the reports of Lee and Kang (2019), who reported that providing flaxseed oil to miniature pigs can reduce the TNF-α production in blood serum. In addition, evidence leads to believe that maybe the inclusion of low-ratio n-6/n-3 PUFA supplementation in the diet resulted in a significant reduction of TNF-α levels in sick individuals, whereas it did not have the same effect in healthy individuals (WEI et al., 2021).

IgM is well-known for being the first antibody produced in immune response, effective in activating the complement system and controlling bloodstream infections (JANEWAY et al., 2001). The parturition presents several challenges for the body, including the immune response

and inflammation in the right dose, which apparently was controlled for n-6 and n-3 treatments at the D1L in sow's plasma.

In fact, only in earlier gestation (D40G) the treatments were significant since the n-3 group presented the lower concentration of IgG in sow's plasma, and this type of antibody is the principal neutralizer for toxins found in tissues. Earlier gestation period is highly sensitive by involving the establishment of gestation, so at this point, the milk biofortified with n-3 PUFA mitigated the level of this antibody in the blood, which can be an outstanding result since the organism should recognize the fetuses to maintain the pregnancy. Immunoglobulin G had a higher concentration in colostrum (137.6 $mg.mL^{-1}$, P =0.022) and tendency (0.781 $mg.mL^{-1}$, P = 0.064) in CON sow' milk, which can be explained by the higher amount in plasma for the group CON (224.31 $mg.mL^{-1}$, P = 0.066), whereas the transport occurs from serum into colostrum and milk (HURLEY; THEIL, 2011).

In the present study, biofortified milk showed its power to modulate the adaptive system. For a better understanding, future studies need to investigate supplementation in situations that challenge the organism.

6.9 EFFECT OF SUPPLEMENTATION WITH PUFA ON EICOSANOID PLASMA OF SOWS AND PIGLETS

In plasma, 3 metabolites were detected and quantified (LTB4, PGF2α and TXB2). There was no difference between treatments for any of the groups ($P > 0.05$) or contrasts applied ($P > 0.05$). The stimulation with TAP or DMSO had a significant effect only for TXB2 (sows from experiment A, B and piglets, $P = < 0.0001$) and LTB4 (sows from experiment B, $P < 0.0001$) (Table 29, 30 and 31). TAP is calcium ionophore that we throw on the cell, so that the cell drops out whatever it produces as a lipid mediator. Other stimuli could be used, such as microorganisms and LPS (lipopolysaccharide). In the present study, we did not have a specific stimulus as a treatment, and TAP was chosen due to it being the cleanest and safest method. DMSO is used as a solvent for TAP, and we tested the samples using only DMSO to verify if it could be harmful to the cell.

Table 29. Plasma concentrations of LTB4, PGF2α and TXB2 in sows at 13 ± 4 days of lactation

Item[a]	Treatment[b]					*P-value*[d]				
	CON (± SEM[c])	n-6 (± SEM[c])	n-3 (± SEM[c])	TAP	DMSO	Treat	Stimulus	Treat*Sti	C1	C2
LTB4, pg.mL^{-1}	580.281 ± 213.378	647.393 ± 197.550	448.515 ± 261.334	558.729 ± 130.321	n.e.	0.879	n.e.	n.e.	0.715	0.723
PGF2α, pg.mL^{-1}	46.911 ± 36.291	57.158 ± 28.111	41.504 ± 38.936	72.363 ± 19.754	n.e.	0.390	0.063	0.302	n.e.	0.463
TXB2, pg.mL^{-1}	20.266 ± 24.119	38.421 ± 26.554	58.128 ± 43.023	74.793 ± 18.777	3.084 ± 32.280	0.847	0.0001	0.922	0.908	0.580

Source: Own authorship.
[a]Analytes present in greater evidence in the sample
[b]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.
[c]SEM: standard error of the mean.
[d]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.
n.e: not estimable. The values were not estimable due to missing, as a consequence of the limitations of the method to measure the concentration of analytes in the sample.

Table 30. Plasma concentrations of LTB4, PGF2α and TXB2 in piglets at 13 ± 4 days of age

Item[a]	Treatment[b]					*P-value*[d]				
	CON (± SEM[c])	n-6 (± SEM[c])	n-3 (± SEM[c])	TAP	DMSO	Treat	Stimulus	Treat*Sti	C1	C2
LTB4, pg.mL^{-1}	257.400 ± 205.240	498.349 ± 229.466	568.989 ± 458.931	441.579 ± 184.209	n.e.	0.625	n.e.	n.e.	0.472	0.551
PGF2α, pg.mL^{-1}	48.433 ± 126.229	278.644 ± 105.937	75.158 ± 180.405	147.565 ± 86.231	n.e.	0.913	0.176	n.e.	n.e.	n.e.
TXB2, pg.mL^{-1}	45.245 ± 6.095	31.884 ± 5.220	76.663 ± 7.869	88.2901 ± 5.502	14.238 ± 5.086	0.608	<0.0001	0.094	0.347	0.796

Source: Own authorship.
[a]Analytes present in greater evidence in the sample
[b]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.
[c]SEM: standard error of the mean.
[d]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.
n.e: not estimable. The values were not estimated due to missing, as a consequence of the limitations of the method to measure the concentration of analytes in the sample.

Table 31. Plasma concentrations of LTB4, PGF2α and TXB2 in sows at 24 ± 6 days of gestation

Item[a]	Treatment[b]					*P-value*[d]				
	CON (± SEM[c])	n-6 (± SEM[c])	n-3 (± SEM[c])	TAP	DMSO	Trat	Stimulus	Treat*Sti	C1	C2
LTB4, pg.mL^{-1}	240.085 ± 324.069	330.581 ± 330.016	129.152 ± 322.062	403.904 ± 129.271	62.641 ± 352.802	0.465	0.037	0.784	0.359	0.414

PGF2α, pg.mL^{-1}	26.425 ± 18.191	72.973 ± 18.191	22.171 ± 24.255	31.180 ± 9.902	49.866 ± 21.391	0.403	0.890	0.110	0.960	0.183
TXB2, pg.mL^{-1}	11.924 ± 4.755	7.042 ± 4.842	16.615 ± 3.381	19.852 ± 2.606	3.8685 ± 4.331	0.139	<0.0001	0.550	0.114	0.212

Source: Own authorship.
[a]Analytes present in greater evidence in the sample.
[b]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.
[c]SEM: standard error of the mean.
[d]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

6.10 EFFECT OF BIOFORTIFIED PUFA MILK SUPPLEMENTATION ON EMBRYO DEVELOPMENT – EXPERIMENT B

In Experiment B, sows in each treatment group were euthanized on gestation d28 to determine effect of treatment on fertility. The total number of embryos was 14.81% higher for n-3 and n-6 than CON sows ($P = 0.048$; Table 32), resulting in a tendency for 2.28 more viable embryos ($P = 0.060$). Unviable embryos, number of corpora lutea, fertilization rate, adjusted fertilization rate, and pregnant uterus were not statistically different among groups ($P > 0.05$).

Table 32. Survival of porcine embryos after sow' supplementation with control, omega-3 and omega-6 milk at 28 days of gestation

	Treatment[b]			***P-value*[d]**		
Item[a]	**CON (± SEM[c])**	**n-6 (± SEM[c])**	**n-3 ± (SEM[c])**	**Treatment**	**C1**	**C2**
Number of embryo's, n						
Viable	15.00 ± 1.27	17.57 ± 1.11	16.67 ± 0.94	0.099	**0.060**	0.185
Unviable	0.38 ± 0.18	0.75 ± 0.41	0.33 ± 0.24	0.297	0.738	0.154
Total	15.43 ± 1.32	18.43 ± 1.07	17.00 ± 0.94	**0.060**	**0.048**	0.101
Corpora lutea, n	18.75 ± 1.06	20.25 ± 1.05	18.44 ± 1.30	0.301	0.712	0.132
FR[2], %	80.78 ± 6.61	83.38 ± 5.11	95.15 ± 7.40	0.266	0.321	0.206
AFR[3], %	78.70 ± 6.71	79.90 ± 5.12	93.43 ± 7.63	0.233	0.360	0.156
Pregnant uterus, kg	4.850 ± 0.544	4.749 ± 0.518	5.293 ± 0.473	0.695	0.916	0.408

Source: Own authorship.

[a]FR: Fertilization ratio = (Number of embryo's total/Corpora lutea) x 100; AFR: Adjusted Fertilization ratio = (Number of embryo's viable/Corpora lutea) x 100.

[b]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.

[c]SEM: standard error of the mean.

[d]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

The daily intake of omega-3 fatty acids can effective reducing early and any preterm birth, also, have a longer gestational length decreasing the synthesis of prostaglandins, which emphasize the possibility of negative impact to consume a high dosage of n-3 PUFA (AKERELE; CHEEMA, 2016; ROSZKOS; TÓTH; MÉZES, 2020). However, the supplementation and mechanisms of how the process at body is has to be clearly elucidated. In another hand, several benefits are shown such as healthier brain and eyes development (SINCLAIR, 2019), fetal growth during pregnancy (HERAS-MOLINA et al., 2022), improvement of cognitive and physiological functions (FANG et al., 2020; GÁZQUEZ; LARQUÉ, 2021).

The hormonal synthesis and recognition of pregnancy by maternal body through the growth embryo signal are the key to survival of embryo. N-3 PUFA is reported as a contribution to decrease prostaglandins as PGF2α and PGE2 when linseed oil was supplemented, due to reduced ARA and/or ARA substrate in the eicosanoid's metabolism (CHARTRAND et al., 2003). Besides that, the impact of n-3 PUFA on the maturation of follicles and the synthesis of progesterone through corpus luteum size may contribute to sustaining pregnancy and lowering the occurrence of early embryo loss, particularly in pigs. On the other hand, ARA arises from n-6 PUFA, which has a role in increasing the rate of embryo growth (ROSZKOS; TÓTH; MÉZES, 2020).

Therefore, in our study, considering a tendency ($P = 0.06$) to improve in 14,1% the viable and an increase ($P = 0.048$) of 14,8% in the total number of embryos for n-6 and n-3 sow's groups, is acceptable to attribute each result to the play of each source of PUFA. In other words, n-3 PUFA could reduce metabolites dangerous for pregnancy recognition, and n-6 PUFA improved the embryo's growth and helped to signal to the maternal body their presence, following the statement of researchers, which is the LC-PUFAs play a crucial role in enhancing the dam's ability to recognize pregnancy, potentially leading to a decrease in early embryo mortality (ROSZKOS; TÓTH; MÉZES, 2020).

Moreover, we have shown in the previous study (REIS et al., 2021) that the total born for experiment A had no difference among the treatments, nonetheless, this current outcome leads us to question whether the generation of sows (F1 or F2) has a strong influence since we prove this enhancement in the total number of embryos in experiment B, as long as fed sows with fish oil as a source of n-3 PUFA increases the litter size in the subsequent parity (SMITS et al., 2011).

These results are an indication that supplementation with biofortified milk can bring great benefits both to women who have difficulty getting pregnant, and even to animal production, and therefore more studies are needed to understand the mechanisms involved.

6.11 DETERMINATION OF THE MICROSTRUCTURE OF *LONGISSIMUS DORSI* – EXPERIMENT A AND B

To determine the effect of treatment on body composition of sows and their offspring the area, minimum, medium, and maximum diameters of muscle fibers and adipose cells in *Longissimus dorsi* were evaluated. There was no difference in any of these variables between dietary treatments ($P > 0.05$) in gilts from Experiment B and offspring from experiment A (Table 33 and 34). Despite no difference in muscle histomorphology of offspring by treatment, weight at 14 days was significant ($P < 0.05$ non-publish data) for all variables of adipose cells and muscle fibers, also piglet sex significantly impacted the area, minimum and medium diameter of adipose cells with males having greater measures than females ($P < 0.05$ non-publish data). Supplementation with biofortified milk did not cause any impact on muscle fibers or amount of fat in the muscle, indicating that it would not affect pig production.

Table 33. Histological characteristics of the longissimus dorsi muscle from gilts at 28 days of gestation – experiment B

	Treatment[b]			***P- value[d]***		
Item[a]	**CON (± SEM[c])**	**n-6 (± SEM[c])**	**n-3 (± SEM[c])**	**Treatment**	**C1**	**C2**
Adipose cells						
Area, µm	1806.480 ± 226.140	2250.370 ± 225.620	2195.670 ± 214.750	0.321	0.135	0.862
Maximum diameter, µm	63.266 ± 2.897	67.845 ± 2.909	67.819 ± 2.753	0.436	0.200	0.995
Minimum diameter, µm	35.097 ± 2.238	38.107 ± 2.247	38.648 ± 2.127	0.477	0.233	0.863
Medium diameter, µm	47.816 ± 2.475	51.605 ± 2.486	51.763 ± 2.352	0.441	0.204	0.964
Per-area, %	0.220 ± 0.024	0.270 ± 0.024	0.255 ± 0.023	0.327	0.152	0.648
Muscle fiber						
Area, µm	4396.880 ± 737.560	5265.260 ± 755.280	4542.460 ± 780.220	0.413	0.364	0.329
Maximum diameter, µm	88.693 ± 6.062	104.200 ± 6.124	92.680 ± 6.169	0.187	0.197	0.197
Minimum diameter, µm	57.750 ± 4.732	62.934 ± 4.842	58.452 ± 5.001	0.466	0.418	0.352
Medium diameter, µm	72.201 ± 6.200	80.194 ± 6.342	74.028 ± 6.559	0.358	0.297	0.324
Per-area, %	0.345 ± 0.028	0.381 ± 0.028	0.396 ± 0.028	0.429	0.217	0.698

Source: Own authorship.

[a]Per-area: percentage of the area filled by the object delimited within the total image, as a way of quantifying the relative area.

[b]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.

[c]SEM: standard error of the mean.

[d]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

Table 34. Histological characteristics of the longissimus dorsi muscle from 14 day-old piglets – experiment A

	Treatment[b]			*P-value*[d]		
Item[a]	**CON (± SEM[c])**	**n-6 (± SEM[c])**	**n-3 (± SEM[c])**	**Treatment**	**C1**	**C2**
Adipose cells						
Area, µm	841.240 ± 2.760	883.790 ± 2.760	1076.050 ± 2.770	0.210	0.245	0.182
Maximum diameter, µm	43.633 ± 2.087	44.638 ± 2.222	48.222 ± 2.494	0.265	0.260	0.227
Minimum diameter, µm	25.108 ± 1.432	25.797 ± 1.510	28.841 ± 1.717	0.152	0.196	0.134
Medium diameter, µm	33.701 ± 1.722	34.555 ± 1.823	37.808 ± 2.054	0.206	0.225	0.182
Per-area, %	0.098 ± 0.029	0.098 ± 0.029	0.114 ± 0.029	0.522	0.580	0.318
Muscle fiber						
Area, µm	250.61 ± 0.10	240.51 ± 0.10	284.72 ± 0.12	0.405	0.688	0.192
Maximum diameter, µm	21.606 ± 0.048	21.276 ± 0.049	23.257 ± 0.060	0.414	0.637	0.208
Minimum diameter, µm	13.980 ± 0.038	13.776 ± 0.040	14.765 ± 0.048	0.448	0.679	0.221
Medium diameter, µm	17.653 ± 0.045	17.262 ± 0.048	18.769 ± 0.056	0.427	0.722	0.203
Per-area, %	0.030 ± 0.001	0.029 ± 0.001	0.034 ± 0.001	0.444	0.626	0.232

Source: Own authorship.

[a]Per-area: percentage of the area filled by the object delimited within the total image, as a way of quantifying the relative area.

[b]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.

[c]SEM: standard error of the mean.

[d]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

6.12 RELATIVE EXPRESSION LEVELS OF GENES RELATED TO REGULATING LIPID HOMEOSTASIS AND METABOLISM, IN LIVER OF GILTS AND PIGLETS

The relative expression of several genes that control lipolysis and lipogenesis was measured in liver tissue collected from sows in Experiment B and piglets in Experiment A using real-time-quantitative PCR (RT-qPCR). There was no effect of n-3 or n-6 supplementation on hepatic expression of these genes in gilts ($P > 0.05$) (Table 35). However, hepatic expression of these genes in offspring was affected by maternal diet with SREBP-1 expression was increased 75.8% in n-3 group compared to n-6 group ($P = 0.032$) (Table 36). Also, the SCD gene was affected by sex ($P = 0.038$), with higher relative expression in males (1.241 ± 0.182) then females (0.944 ± 0.170).

Table 35. Liver expression levels of genes involved in lipid metabolism in gilts from control, n-6 and n-3 groups – experiment B

Item	**Treatment[a]**			***P-value*[c]**		
	TC (± SEM[b])	**n-6 (± SEM[b])**	**n-3 (± SEM[b])**	**Treat**	**C1**	**C2**
ACC	1.084 ± 0.212	1.049 ± 0.268	1.143 ± 0.212	0.959	0.963	0.786
D6D	1.325 ± 0.326	1.674 ± 0.412	0.966 ± 0.326	0.414	0.991	0.195
FAS	1.308 ± 0.454	1.994 ± 0.525	1.786 ± 0.428	0.589	0.317	0.762
SCD	1.487 ± 0.461	1.114 ± 0.532	1.170 ± 0.435	0.475	0.495	0.358
PPAR-α	1.028 ± 0.123	0.933 ± 0.155	0.889 ± 0.116	0.710	0.462	0.822
SREBP-1	1.131 ± 0.681	2.594 ± 0.786	0.893 ± 0.681	0.235	0.868	0.093

Source: Own authorship.
[a]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.
[b]SEM: standard error of the mean.
[c]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

Table 36. Liver expression levels of genes involved in lipid metabolism in piglets 14-day-old from treated sows – experiment A

Item	**Treatment[b]**			***P-value*[d]**				
	TC (± SEM[c])	**n-6 (± SEM[c])**	**n-3 (± SEM[c])**	**Treat**	**Sex**	**Treat*Sex**	**C1**	**C2**
ACC	1.054 ± 0.095	0.830 ± 0.103	0.879 ± 0.125	0.286	0.449	0.594	0.137	0.768
D6D	1.589 ± 0.381	0.847 ± 0.284	1.275 ± 0.217	0.295	0.248	0.305	0.234	0.254
FAS	0.980 ± 0.215	0.946 ± 0.242	1.572 ± 0.548	0.665	0.442	0.752	0.564	0.457
SCD	1.310 ± 0.278	0.671 ± 0.131	1.297 ± 0.249	0.226	**0.038**	0.825	0.555	0.113
PPAR-α	0.983 ± 0.124	0.945 ± 0.129	0.922 ± 0.140	0.946	0.319	0.599	0.754	0.909
SREBP-1	0.951 ± 0.163	0.909 ± 0.162	1.596 ± 0.240	0.074	0.489	0.376	0.189	**0.033**

Source: Own authorship.
[a]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.
[b]SEM: standard error of the mean.
[c]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

Studies have reported beneficial actions of PUFA suppression the SREBP-1, decreasing the expression of lipogenic genes such as ACC, fatty acid synthase (FAS) and stearoyl-CoA

desaturase (SCD) (NAKAMURA et al., 2004; NAKATANI et al., 2003; TAI; DING, 2010; XU et al., 1999). Also, occurred upregulation of peroxisome proliferator-activated receptor alpha (PPAR-α) (CLARKE, 2004; GOTO et al., 2013; VALENZUELA; VIDELA, 2011). On the other hand, PPAR-α and SREBP are regulators of transcription level of D6D (Delta-6 Desaturase) (MATSUZAKA et al., 2002).

In the present study, the piglets which suckling their mothers whose received the cow's milk biofortified with n-3 PUFA exhibited a significantly up-regulated expression of SREBP-1 gene. This result are not supported by literature, which attribute as a role of n-3 PUFA a negative regulation of hepatic lipogenesis and have an inhibitory effect on the SREBP pathway (DAEMEN; KUTMON; EVELO, 2013; JUMP, 2008). Since the sow's colostrum of n-3 group had a higher concentration of α-linolenic, EPA, so more Σn-3 and lower n-6/n-3 ratio, also the milk had a decrease of ARA at D14L, and the sow's plasma decreased certain of saturated and monounsaturated fatty acids, we expected the same consistent with previous studies.

On the other hand, ARA also is a PUFA with potential to reduce the SREBP-1 (CHEN et al., 2004), and as sows' milk presented reduction of this fatty acid at 14 days of lactation in n-3 group, the reason to found lower expression of SREBP-1 in n-3 group compared to n-6 group in piglets at 14 days-old, can be explained by that.

Hepatic mRNA expressions of SREBP-1 are affected by several conditions, cellular functions and levels e.g. cholesterol, insulin, glucose, PPAR-α, mTOR complex-1 (mTORC1), fibroblast growth factor 21 (FGF21), retinol binding protein 4 (RBP4) and relation between liver X receptors (LXRs) and retinoid X receptors (RXRs) (DAEMEN; KUTMON; EVELO, 2013). Considering our findings are not enough to explain the expression of SREBP-1, thus a further and wide investigation is necessary to understand how this composition of fatty acids regulated the distinct mechanisms involving the SREBP-1, as a several factors can influence.

Instead, we found differences related to SCD expression, which males had higher SCD than females in the liver, further studies have shown that there are differences between sex in mouse and cattle, perhaps attributed to the hormones and fatty acids composition in their bodies (BARTON et al., 2011; GAMARRA et al., 2018; LEE; PARIZA; NTAMBI, 1996), however, the mechanisms behind of mRNA expression, is currently unknown. Furthermore, the adipocytes presented higher area, minimum and medium diameter for males, considering the relation between SCD expression and muscle effect (STACHOWIAK; FLISIKOWSKI, 2019; YANG et al., 2016), is reasonable to suggest the possibility of SCD had an influence on *L. dorsi* adipocytes from this study, also its potential as molecular target to modulate porcine fatness, according to Stachowiak and Flisikowski (2019).

6.13 ARCUATE NUCLEUS OF THE HYPOTHALAMUS (ARH) RELATIVE EXPRESSION LEVELS OF GENES IN GILTS – EXPERIMENT B

Lipids cross the blood-brain barrier and, according to their function, some studies have reported their ability to alter the response of some key molecules that regulate energy homeostasis and food intake. Understanding the effect that PUFAs can have on these regulators is essential to mediate these signals and their impact on an individual's health (DAMIANI; DAMIANI, 2011; SCHWINKENDORF et al., 2010). There was no significant effect (P > 0.05) of treatment on expression of ARH genes in gilts from experiment B (Table 37).

Table 37. Relative expression of genes in the gilt hypothalamus supplemented with milk biofortified with PUFA n-6 and n-3

Item	Treatment[a]			*P-value*[c]		
	TC (± SEM[b])	n-6 (± SEM[b])	n-3 (± SEM[b])	Treat	C1	C2
CART	6.198 ± 2.445	3.439 ± 2.445	3.010 ± 2.305	0.986	0.895	0.926
INSR	1.134 ± 0.181	0.637 ± 0.169	1.008 ± 0.159	0.130	0.163	0.125
LEPR	1.842 ± 0.787	1.188 ± 0.736	1.316 ± 0.787	0.794	0.631	0.652
NPY	2.409 ± 0.873	2.211 ± 0.873	2.025 ± 0.823	0.920	0.739	0.812

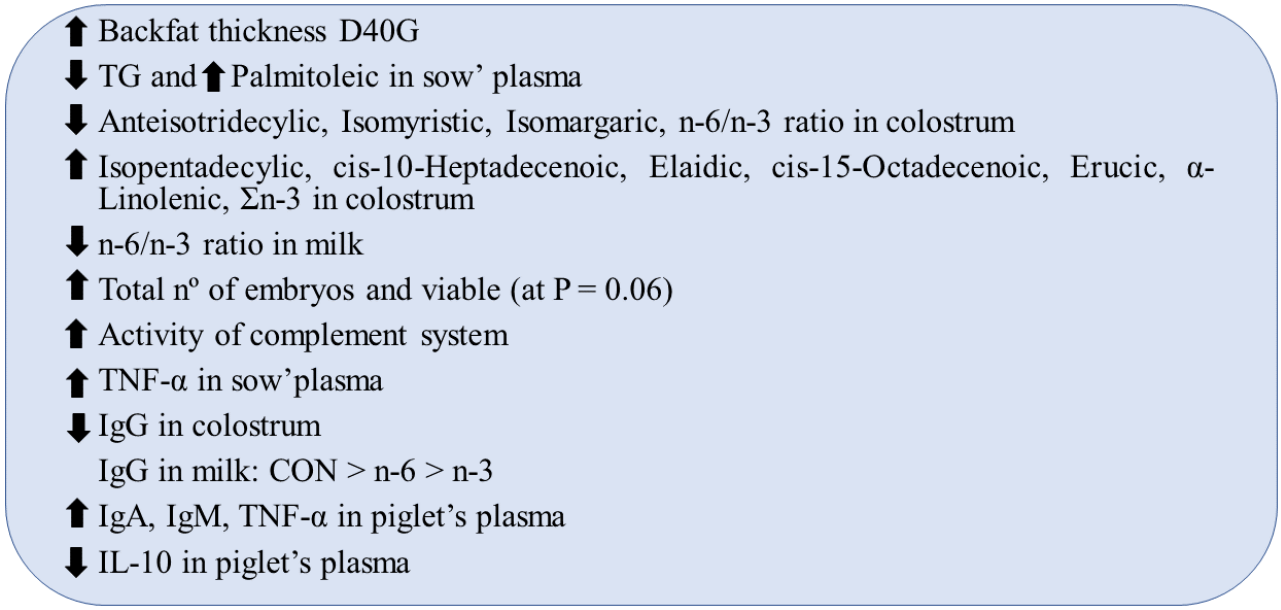
Source: Own authorship.
[a]Swine females fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.
[b]SEM: standard error of the mean.
[c]C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

The dissimilarities discerned in the literature are due to the extensive array of investigations encompassing disparate species, divergent dosages, variable durations of interventions, and heterogeneous sources of lipids. Based on these findings, it can be inferred that in certain cases, a lower dosage of n-3 PUFA and/or an intermediate n-6/n-3 ratio would be more favorable.

In addition, observing the significant results in general and considering the first contrast Control vs n-6+n-3, it is necessary to recognize that the effects may come not only from milk richer in PUFA, but also with more USFA, MUFA and Σn-3 PUFA, less SFA and lower SFA/USFA and n-6/n-3 ratio. Furthermore, the results coming from the n-6 vs n-3 contrast it is possible to attribute the difference shown by milk from cows that received linseed oil (n-3 group) with higher Σn-3 and lower n-6/n-3 ratio, as well as milk from cows that received soybean oil in the diet (n-6 group) with higher Σn-6 concentration and higher n-6/n-3 ratio, and a tendency (P = 0.097) for higher cholesterol concentration. Furthermore, the combination of these factors can lead to present results, without considering the interaction of these lipid fractions with other compounds present in the body or from the diet.

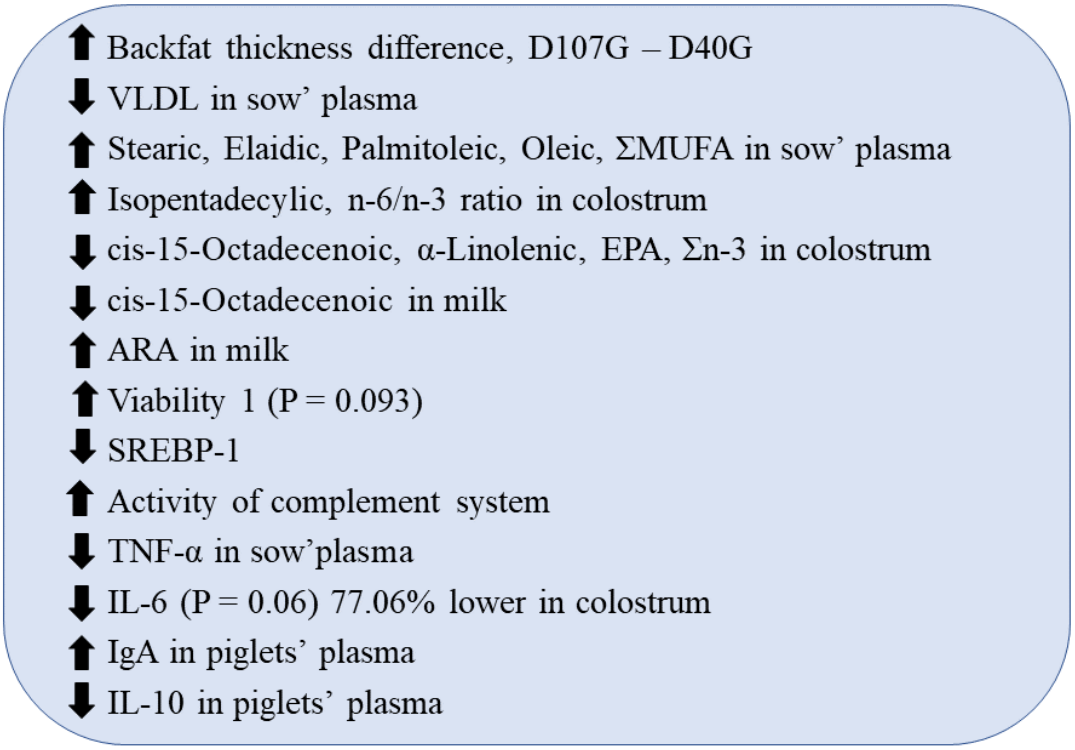
In summary, the contrast results (C1 and C2) are shown in figures 19 and 20 below, as the treatment*time interaction effects may be observed in the graphs and figures previously presented.

Figure 19 - Contrast 1: treatments effect n-6 + n-3 in comparison with CON



Source: Own authorship.

Figure 20 - Contrast 2: treatment effect of n-6 in comparison with n-3



Source: Own authorship.

7 CONCLUSIONS

In conclusion, these results indicated that the consumption of biofortified milk with altered n-6/n-3 ratios or rich in n-3 PUFA reduced backfat thickness and triglycerides, improved the blood FA profile, reducing MUFA and lower ARA/EPA ratio. In colostrum the profile became richer in n-3 PUFA, changing the n-6/n-3 ratio, as well as in milk this ratio was also modified. The worst viability of piglets was observed in group n-6. Biofortified milk showed immunomodulatory potential by making the response time of the complement system faster, and altering immunoglobulins and interleukins in the colostrum, milk and blood of sows and their litter. Furthermore, the expression of genes involved in lipid metabolism needs to be better studied, not for their expression but for their action and release, suggesting a regulatory potential, which can be further investigated as benefits for obesity conditions, for sows or their offspring. The effect of biofortified milk also benefited the total number of embryos, showing the effect on the reproductive system. While some results were in agreement with the hypothesis, others diverged from the expected effects, however, these results provide valuable insights into the effects of biofortified milk consumption. Further studies are warranted to elucidate the underlying mechanisms and determine the long-term health implications of these dietary interventions, with potential applications in both animal production and human health promotion.

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