INTERNATIONAL DOCTORAL
SCHOOL OF THE USC

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PhD Thesis

Fatty acids contribution of bovine and maternal milk to humans: assessment and influential factors

Lugo, 2022

Doctoral Programme in Innovation in Food Safety and Technology

TESE DE DOUTORAMENTO

FATTY ACIDS CONTRIBUTION OF BOVINE AND MATERNAL MILK TO HUMANS: ASSESSMENT AND INFLUENTIAL FACTORS

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ESCOLA DE DOUTORAMENTO INTERNACIONAL DA UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

PROGRAMA DE DOUTORAMENTO EN INNOVACIÓN EN SEGURIDADE E TECNOLOXÍAS ALIMENTARIAS

LUGO

2022

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Fatty acids contribution of bovine and maternal milk to humans: assessment and influential factors

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RESUMO

A importancia dos lípidos na nutrición e no desenvolvemento humano é recoñecida e estudada dende hai décadas. Estes compostos cumpren diversas funcións no organismo, tanto enerxéticas como estruturais e reguladoras, sendo a principal fonte de reserva enerxética, ademais de compoñentes esenciais das membranas celulares, participantes activos na dixestión, absorción e transporte de vitaminas liposolubles, reguladores da temperatura corporal, e mediadores hormonais. Na nutrición humana, o consumo de lípidos está asociado cun crecemento axeitado e cun desenvolvemento da actividade normal dos tecidos. Algúns lípidos son esenciais para o ser humano e deben ser achegados pola dieta xa que os humanos carecen de sistema encimático para a súa síntese. Os exemplos máis destacables son as vitaminas liposolubles e provitaminas, ou algúns ácidos graxos de cadea longa. Pero, ademais do seu papel beneficioso no metabolismo humano e na saúde, tamén hai outros lípidos que poderían estar relacionados con diversas doenzas como a obesidade, enfermidades cardíacas ou algúns tipos de cancro. Por outra banda, os lípidos confírenlles ós alimentos certas características organolépticas coma o sabor, o aroma ou a textura. Ademais, os lípidos teñen un papel importante nas propiedades tecnolóxicas durante o procesamento dos alimentos. Dentro da gran diversidade estrutural que caracteriza ós lípidos, os ácidos graxos (FAs) son probablemente as estruturas máis relevantes e de maior interese nutricional por formar parte dos triglicéridos, dos lípidos complexos e dos ésteres de colesterol.

Os ácidos graxos están compostos por cadeas hidrocarbonadas, de maior ou menor lonxitude, variadas na súa estrutura podendo ser lineais, ramificadas ou alicíclicas. Xeralmente, están formados por una cadea cun número par de átomos de carbono, aínda que tamén hai FAs con número impar de carbonos no leite e graxa dos ruminantes, con átomos de hidróxeno ao longo da cadea. Nun extremo da cadea sitúase un grupo hidroxilo (CH3-) e un grupo carboxilo (—COOH) no outro extremo. A coexistencia de ambos os grupos nunha mesma molécula converte os ácidos graxos en moléculas debilmente anfipáticas e confírelles a súa natureza insoluble en auga. O número de FAs naturais coñecidos de diversas fontes de lípidos (animais, vexetais e microbianos) supera os 1000, aínda que só un número relativamente pequeno son os que se atopan habitualmente na dieta humana, cunha lonxitude de cadea entre 2 e 24 átomos de carbono. Os FAs poden clasificarse en diferentes categorías segundo diferentes criterios como a lonxitude da cadea hidrocarbonada, ou o grao de saturación e a localización do primeiro dobre enlace nela. A maneira máis común de dividir os ácidos graxos da dieta é segundo o grao de insaturación que presenten. Así os ácidos graxos saturados (SFAs) non presentan dobres enlaces na súa estrutura, os ácidos graxos monoinsaturados (MUFAs) presentan un dobre enlace, e os ácidos graxos poliinsaturados (PUFAs) presentan dous ou máis dobres enlaces. Os PUFAs a súa vez poden clasificarse en omega-3 (*n*-3) e omega-6 (*n*-6) dependendo da posición do primeiro dobre enlace dende o extremo do grupo hidroxilo. É importante subliñar que certos ácidos graxos son clasificados como esenciais (EFAs) xa que o ser humano non é capaz de sintetizalos e teñen que ser inxeridos na dieta. Os dous ácidos graxos esenciais para os humanos son o ácido α-linolénico (ALA) e o ácido linoleico (LA), a partir dos cales o corpo humano é capaz de sintetizar outros ácidos graxos necesarios da familias omega-3 e omega-6, respectivamente.

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Os SFAs inxeridos na dieta proveñen principalmente de fontes animais: leite, carne, manteiga e ovos. Os alimentos procesados que conteñan aceites vexetais naturais como o aceite de palma ou de coco tamén son unha fonte deste tipo de ácidos graxos. O consumo de alimentos ricos en SFAs está asociado cun maior risco de enfermidades crónicas e coronarias ou con problemas de colesterol sanguíneo elevado. Pola contra, algúns ácidos graxos de cadea curta e media (entre 3 e 12 átomos de carbono) foron estudados polos seus posibles efectos beneficiosos para a saúde. Estes ácidos graxos, como o propiónico (3:0), o butírico (4:0), o caproico (6:0) ou o cáprico (10:0), son relativamente raros na dieta dos humanos e atópanse principalmente nos produtos lácteos. Foron propostos en diversos estudos como protectores do colon e redutores da inflamación intestinal, e foron vinculados con funcións antivirais e antibacterianas e incluso como axentes anticarie. Os MUFAs máis comúns na dieta humana conteñen entre 10 e 32 carbonos pero a maioría están formados por 18 átomos. En xeral, estes ácidos graxos pódense atopar en diversos alimentos como carnes, produtos lácteos e maiormente en fontes vexetais como aceites de varias sementes. Un maior consumo de MUFAs na dieta fronte a SFAs ou carbohidratos vinculouse cunha redución do colesterol LDL e dos triglicéridos sanguíneos. O ácido oleico (OA) é o monoinsaturado máis común e está presente en cantidades considerables en fontes tanto de orixe animal como vexetal. O OA é moi estudado pola súa ligazón coa prevención de enfermidades cardíacas e certos tipos de cáncer. Cabe destacar tamén o ácido vaccénico que está naturalmente presente no leite e na carne dos ruminantes.

Os PUFAs poden dividirse en 12 familias diferentes podendo conter os dobres enlaces entre a posición n-1 e a n-12 da cadea hidrocarbonada. As familias máis importante para a saúde humana son as n-6 e n-3, sendo o LA o precursor da familia n-6 e o ALA da familia n-3. A partires destes EFAs sintetízanse outros ácidos graxos con importantes funcións para o organismo. Destacan polo seu papel na saúde o ácido docosahexaenoico (DHA) e do ácido eicosapentaenoico (EPA), derivados do ALA, particularmente polos seus efectos sobre a saúde perinatal, pois están asociados co desenvolvemento visual e cognitivo do feto e do neno. Tamén se estudou a súa implicación na prevención da depresión materna e na menor incidencia de nacemento de nenos prematuros. As maiores fontes de PUFAs na dieta son os aceites vexetais, ricos en ALA, e os lípidos mariños (principalmente peixe azul) que provén ó organismo de EPA e DHA. Outros alimentos como froitos secos, sementes, ovos e carne contribúen aínda que, en menor proporción, á cantidade de PUFAs da dieta. Ademais destes tipos de ácidos graxos, SFAs, MUFAs e PUFAs, a dieta humana tamén inclúe ácidos graxos trans, que proveñen principalmente de ruminantes e de graxas lácteas (trans naturais), ou de alimentos preparados a partir de aceites parcialmente hidroxenados. Nos últimos anos as investigacións centraron a súa atención noutros ácidos graxos pouco comúns como pode ser o ácido linoleico conxugado (CLA) que se atopa principalmente nos produtos lácteos e na carne de vacún, e está ligado coa redución da graxa corporal e posibles efectos antidiabéticos.

O leite materno descríbese a miúdo como o "estándar de ouro" da nutrición infantil xa que na súa composición contén todos os macronutrientes e vitaminas necesarios para o desenvolvemento óptimo do lactante. Ademais, o leite humano é capaz de liberar anticorpos que inmunizan o bebé contra enfermidades. Os lípidos son a principal fonte de enerxía para o lactante e por tanto son necesarios para un normal crecemento. O leite maduro proporciona o 50% da inxesta diaria de enerxía en forma de graxa. Tamén é unha fonte importante de LA e ALA, que se converten en poliinsaturados de cadea longa como o DHA, vitais no desenvolvemento estrutural e funcional dos sistemas

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visual, sensorial, perceptivo e cognitivo do bebé. Estes ácidos graxos son tan importantes para o bebé que a FAO estableceu unhas doses alimentarias recomendadas para lactantes (0 a 24 meses) e para os nenos (2-18 anos) dos principais ácidos graxos esenciais que se atopan no leite materno, ademais de recomendar inxestas dietéticas de graxa e ácidos graxos a adultos. O leite materno proporciona principalmente ácidos graxos saturados e monoinsaturados (OA) e é unha boa fonte de PUFAs de cadea longa e ácidos graxos esenciais. Varios factores, como o período de lactación, o sexo do bebé, a composición corporal da nai, etc., poden influír na composición do perfil lipídico do leite materno. A orixe e variedade dos ácidos graxos que a nai consome durante o embarazo e a lactación inflúen na transferencia destes ácidos graxos antes (a través da placenta) e despois (a través do leite humano) do parto, con efectos beneficiosos e adversos sobre o desenvolvemento do lactante.

Cando a práctica da lactación materna non é posíbel, sexa por problemas médicos da nai que a levan a tomar medicamentos que poden pasar ao leite materno, por necesidades específicas do bebé que requiran unha fórmula especializada (alerxias ou intolerancias), ou por unha razón social ou cultural, ou porque a nai simplemente decide non aleitar a peito, as fórmulas infantís (IF) están dispoñibles e deseñadas para proporcionar unha nutrición completa ao lactante, imitando o máis cerca posible a composición do leite materno e satisfacendo as necesidades nutricionais normais do lactante. As fórmulas infantís pódense clasificar segundo a súa natureza e orixe da proteína. Así, atópanse fórmulas a base de leite de vaca (caseína), a base de concentrado proteico (fórmula a base de soia), e baseadas en hidrolizados de proteínas (fórmulas especializadas). O leite de vaca é a base da maioría das fórmulas infantís. Para facelo o máis parecido posible ó humano, desnátase, dilúese, engádenselle aceites vexetais, minerais, vitaminas e ácidos graxos esenciais. Estes cambios son necesarios xa que o leite de vaca contén unha porcentaxe máis elevada de SFAs, proteínas, e minerais como o calcio, e pola contra, é deficitario en ácidos graxos esenciais comparado co leite materno. A diferenza doutros animais, os humanos consomen leite e produtos lácteos doutras especies de mamíferos ó longo da súa vida. Ó leite de vaca non só se modifica para os leites infantís, senón que a industria láctea foi adaptando o leite envasado segundo as necesidades do ser humano ó longo do tempo. Así pódese atopar no mercado leite desnatado, sen lactosa, leites enriquecidos, leites con perfís de ácidos graxos mellorados mediante a alimentación animal, ou o recentemente comercializado “leite A2”.

O leite bovino e os produtos lácteos derivados teñen unha longa tradición na nutrición humana e, ademais, son alimentos cun alto valor nutricional grazas á súa rica composición. O leite achega proteínas altamente dixeribles e de alto valor biolóxico imprescindibles para o crecemento e desenvolvemento humano. A lactosa é o principal carbohidrato e a súa función é principalmente enerxética; ten un efecto beneficioso sobre a absorción intestinal de calcio e magnesio e pode actuar como prebiótico. O leite contén unha variedade de minerais (calcio, potasio, fósforo, magnesio, selenio e cinc) que xogan un papel multifuncional na saúde humana: participan na estrutura ósea e dental e tamén son necesarios para numerosos procesos metabólicos. O perfil vitamínico do leite contén vitaminas hidrosolubles (grupo B e vitamina C) e liposolubles (A, D, E). Pero considérase pobre nalgúns nutrientes como o ferro e a vitamina C e D. A fracción graxa do leite é variábel porque certos factores poden afectar tanto a súa produción como a súa composición. Estes factores levan sendo estudados ó longo da historia da gandaría láctea para poder obter mellores rendementos nas explotacións leiteiras, destacando a selección xenética e a mellora nas razas leiteiras, os factores climáticos, as boas prácticas nas explotacións, o tempo de lactación, os tratamentos veterinarios e a

máis importante e eficaz, as modificacións na dieta dos animais. Porén, nos últimos anos tanto en España como noutros países desenvolvidos, produciuse unha diminución do consumo de leite UHT. Moitas veces, a fracción saturada deste alimento foi relacionada con diversas patoloxías pero esta imaxe negativa non está apoiada por outras evidencias científicas que sosteñen que o consumo de leite de vaca e produtos lácteos forman parte dunha dieta equilibrada.

En base a todo o apuntado anteriormente, para poder realizar este proxecto de tese de doutoramento, partiuse da seguinte hipótese principal: o leite humano é o alimento personalizado definitivo para o lactante, xa que proporciona un perfil de ácidos graxos moi particular que non se asemella á composición do leite bovino nin á das fórmulas infantís. A partir desta hipótese, establecéronse 4 obxectivos principais, cos seus correspondentes obxectivos secundarios, de ser o caso. O obxectivo 1 foi estudar a evolución dos ácidos graxos do leite materno ao longo da lactación, así como as posibles modificacións derivadas da dieta da nai. O obxectivo 2 foi estudar o perfil dos ácidos graxos das fórmulas infantís, así como valorar as súas aptitudes como substituto e/ou complemento da graxa do leite humano. O obxectivo 3 foi estudar o perfil de ácidos graxos do leite bovino baixo distintas situacións produtivas, máis concretamente, as súas modificacións debido á preñez da vaca ou despois dun tratamento con somatotropina bovina recombinante (rbST). En conxunto, nos obxectivos 1 e 3 estudáronse factores que puideran modificar o perfil de ácidos graxos en leite humano e bovino. Finalmente, un cuarto obxectivo foi comparar a achega de ácidos graxos de cada un dos anteriores tipos de leite (humano, bovino, fórmulas) e valorar o seu axuste aos requirimentos do ser humano nas distintas etapas da vida. Para a medición en laboratorio dos FAs presentes nas distintas mostras de leite, empregouse un método de dixestión ácida con metilación posterior dos analitos, para ser determinados mediante cromatografía de gases e detector de ionización de chama (GC-FID). As nais lactantes que participaron nos estudos incluídos nesta tese, doaron a súa mostra de leite desinteresadamente, e cubriron distintos cuestionarios sobre os seus hábitos dietéticos, características antropométricas, e sociodemográficas, así como un consentimento informado. O proxecto orixinal foi aprobado polo Comité Ético de Investigación Clínica de Galicia (código 2016/280), e rexistrado no ClinicalTrials.gov con número de identificación NCT03245697. Respectouse a Declaración de Helsinki de principios éticos para investigación médica en seres humanos. En canto aos estudos levados a cabo con animais, todos eles foron autorizados polo organismo correspondente e seguiron as directrices reunidas na Directiva 2010/63/EU, relativa á protección dos animais utilizados para fins científicos. O estudo sobre os efectos da rbST en vacas de leite forma parte dun proxecto de ámbito nacional, con código FASL2014-58881-R, financiado polo Ministerio de Ciencia e Innovación.

Os traballos de investigación relativos ao primeiro obxectivo abordaron o estudo dos ácidos graxos no leite materno como o primeiro alimento para o lactante, nos primeiros meses de vida, e a súa evolución ata e durante a lactación prolongada (máis dun ano de lactación). A calidade da dieta materna é fundamental tanto para a nai como para o neno xa que debe achegarlle ácidos graxos esenciais ó lactante. Para determinar como a dieta materna pode influír no perfil lipídico do leite humano, foron analizadas mostras de leite dun grupo de mulleres residentes en Galicia e comparadas con datos bibliográficos de ácidos graxos de 27 países diferentes situados nos 5 continentes, sabendo que os alimentos básicos e as dietas de cada localización xeográfica son moi diferentes entre eles. Os resultados obtidos na mostra de mulleres locais mostraron a existencia de perfís específicos de ácidos graxos para cada muller, así como cambios significativos sobre a

lactación debidos á evolución natural nalgúns ácidos graxos. O leite materno galego foi semellante ao doutros países de Europa, con excepción daqueles que seguen unha Dieta Mediterránea, e moi distinto ao de mulleres residentes en países con dietas moi diferentes ás europeas, como son os países asiáticos. Tamén se puido comprobar que certos alimentos ricos en graxas, como os aceites e determinados peixes e carnes, son os que máis inflúen no perfil de ácidos graxos do leite materno, deixando unha pegada distinguible na graxa deste fluído. Para avaliar como inflúe o período de lactación no perfil lipídico materno ao longo dos meses, analizáronse mostras de leite materno recollidas en distintos puntos da lactación. Primeiramente estudáronse os cambios nos 5 primeiros meses de lactación exclusiva onde as mesmas nais doaron mostras de leite materno, e nun segundo traballo comparáronse mostras de dous grupos de mulleres, un deles con mostras de máis de un ano de lactación. Con estes traballos evidenciouse a existencia dunha evolución dos FAs do leite materno ó longo do tempo e como estes cambios se producen para adecuarse ás necesidades que o neno vai tendo ó longo de todo o proceso. Cando a lactación vai máis alá dun ano, denomínase lactación prolongada. En sociedades occidentais esta práctica non é moi usual xa que o período de lactación depende dun conxunto de factores que van dende o estado socioeconómico da nai, ambiente laboral, opinión social, e ata un descoñecemento por parte dos profesionais da saúde que as asesoran. Co estudo de mostras de leite de lactación prolongada, viuse que o leite de máis dun ano de lactación continúa a ser unha boa fonte de ácidos graxos esenciais e de cadea longa, considerándose un alimento completo e nutritivo para o neno.

Cando por calquera motivo non sexa posíbel a lactación materna, a alternativa máis frecuente é alimentar o lactante con fórmulas infantís adecuadas a cada período de lactación. No segundo obxectivo desta tese de doutoramento, estudáronse as diferenzas entre o leite materno e as fórmulas infantís (a nivel de perfil de FAs) adecuadas a cada etapa de lactación. Para iso analizáronse, comparáronse e discutíronse as diferenzas do leite materno de mulleres galegas en distintas fases de lactación e de fórmulas infantís que se poden atopar no mercado español. Unha vez máis comprobouse como os ácidos graxos do leite materno son variables: aumentan, diminúen ou permanecen constantes ó longo do tempo. Pola súa contra, as fórmulas permanecen máis estables, variando unicamente neses ácidos graxos que son importantes para o desenvolvemento do neno e que os fabricantes engaden deliberadamente a estes alimentos en po. As fórmulas de primeira etapa (para nenos de 0 a 6 meses) foron as máis parecidas ó leite materno. Xa que son o único alimento que o neno pode tomar neses primeiros seis meses, é recomendábel que isto suceda así. Máis alá dos 6 meses, notouse un incremento na concentración de ALA das fórmulas desta etapa, sendo moito máis alta que no leite materno, e suxerindo un sobreenriquecemento deste ácido graxo nelas.

Os factores que poden alterar a produción (cuantitativa e cualitativa) no gando de leite son variados. Estes factores poden dividirse en endóxenos (aqueles que son propios do animal como a raza, o xenotipo, o estado de lactación ou o nivel hormonal) e esóxenos (que dependen de factores externos ó animal como as condicións climáticas, as modificacións na dieta, os aspectos zootécnicos ou a administración de certas substancias que poden afectar a produción de leite). Os traballos de investigación relativos ao terceiro obxectivo centráronse en dous factores pouco estudados na bibliografía para determinar se realmente se producen cambios na composición dos ácidos graxos do leite bovino. O primeiro estudo focalizouse no impacto que o estado

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natural de preñez na vaca pode ter no perfil de ácidos graxos lácteos, xa que as vacas leiteiras están preñadas e producindo leite ó mesmo tempo durante a meirande parte do seu período de lactación. Para este propósito, leite de vacas preñadas e non preñadas foi analizado para determinar o perfil de ácidos graxos e avaliar se se atopaban diferenzas. O leite de animais preñados deu como resultado un perfil de ácidos graxos máis adecuado dende o punto de vista nutricional para o ser humano, obténdose un menor perfil de SFAs e maior en MUFAs, sobre todo ácido oleico. Nun segundo traballo estudouse como a administración da somatotropina recombinante bovina (rbST) pode variar o perfil de ácidos graxos do leite da vaca. Esta substancia é unha versión sintética da hormona natural do animal (somatotropina ou hormona do crecemento) con efectos galactopoéticos, aumentando a produción de leite. A rbST está prohibida na Unión Europea e nalgúns países máis como Australia, Canadá ou Arxentina, pero está permitida noutros como os Estados Unidos. Para este estudo contouse cun grupo de animais de experimentación, un total de nove vacas, seleccionadas e separadas para a administración de rbST. Seis vacas foron tratadas con esta substancia e as tres restantes foron o grupo control. A administración da rbST nos animais tratados, causou un descenso na porcentaxe de SFAs do leite, e un aumento nos MUFAs, principalmente no oleico.

En base ao último obxectivo, os resultados obtidos das publicacións recompiláronse para ser discutidos en conxunto e ver as diferenzas entre os tres tipos de leite estudados, especialmente importantes nas primeiras etapas da vida. En comparación co leite materno, o leite de vaca ten unha porcentaxe maior de SFAs mentres que no leite humano e nas fórmulas infantís o contido entre SFAs e MUFAs está mais igualado. A porcentaxe de PUFAs, onde se atopan os ácidos graxos esenciais, é maior no leite humano que no bovino, e é por iso que o seu perfil é copiado nas fórmulas infantís facéndoas un alimento acorde coas necesidades nutricionais do neno ó longo do período de lactación. Centrándose nestes PUFAs esenciais e nas recomendacións de inxestas adecuadas da OMS, observouse como os leites infantís teñen un maior contido en ALA e inferior en AA e DHA, tanto en comparación co leite materno como coas recomendacións indicadas. Así, ponse de manifesto porque o leite bovino non é recomendábel na alimentación do lactante nos primeiros meses de vida e ten que ser incluído como alimento posteriormente. No entanto, o leite bovino é unha boa base para as fórmulas infantís, xa que a adición de certos FAs permite achegar a fórmula o máis posible ó perfil de ácidos graxos do leite humano.

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ABSTRACT

The importance of lipids in nutrition and human development has been recognized and studied for decades. These compounds fulfil diverse functions in the organism, both energetic and structural and regulatory, being the main source of energy reserve. They are also essential components of cell membranes, active participants in the digestion, absorption and transport of fat-soluble vitamins, regulators of body temperature and hormonal mediators. In human nutrition, lipid intake is associated with adequate growth and development of normal tissue activity. Some lipids are essential for humans and must be provided by diet because humans lack an enzymatic system for their synthesis. The most notable examples are fat-soluble vitamins and provitamins, or some long chain fatty acids. But, in addition to their beneficial role in metabolism and human health, there are also other lipids that could be related to various diseases such as obesity, heart disease or some types of cancer. On the other hand, lipids give foods certain organoleptic characteristics such as flavour, aroma or texture. In addition, lipids play an important role in the technological properties during food processing. Within the great structural diversity that characterizes lipids, fatty acids (FAs) are probably the most relevant structures and of greatest nutritional interest as they are part of triglycerides, complex lipids and cholesterol esters.

Fatty acids are composed of hydrocarbon chains, of longer or shorter length, varied in structure and can be linear, branched or alicyclic. They generally consist of a chain with an even number of carbon atoms, although there are also FAs with an odd number of carbon atoms in ruminant milk and fat, with hydrogen atoms along the chain. At one end of the chain there is a hydroxyl group (CH3-) and a carboxyl group (-COOH) at the other end. The coexistence of both groups in the same molecule makes fatty acids weakly amphipathic molecules and gives them their water-insoluble character. The number of known natural FAs from various lipid sources (animal, plant and microbial) exceeds 1000, although only a relatively small number are commonly found in the human diet, with chain lengths between 2 and 24 carbon atoms. FAs can be classified into different categories according to different criteria, such as the length of the hydrocarbon chain, the degree of saturation and the location of the first double bond in it. The most common way of classifying dietary fatty acids is according to their degree of unsaturation. Thus, saturated fatty acids (SFAs) have no double bonds in their structure, monounsaturated fatty acids (MUFAs) have one double bond and polyunsaturated fatty acids (PUFAs) have two or more double bonds. PUFAs in turn can be classified into omega-3 (n-3) and omega-6 (n-6) according to the position of the first double bond from the end of the hydroxyl group. It is important to emphasize that certain fatty acids are classified as essential (EFAs) because humans are not able to synthesize them and they have to be ingested in the diet. The two essential fatty acids for humans are α-linolenic acid (ALA) and linoleic acid (LA), from which the human body is able to synthesize other necessary fatty acids of the omega-3 and omega-6 families.

The SFAs consumed in the diet come mainly from animal sources: milk, meat, butter and eggs. Processed foods containing natural vegetable oils such as palm oil or coconut oil are also a source of this type of FAs. The consumption of foods rich in SFAs is associated with an increased risk of chronic and coronary heart disease or high blood cholesterol. In contrast, some short- and medium-chain fatty acids (between 3 and 12

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carbon atoms) have been studied for their potential health benefits. These fatty acids, such as propionic (3:0), butyric (4:0), caproic (6:0) or capric (10:0), are relatively rare in the human diet and are found mainly in dairy products. They have been proposed in several studies as colon protectors and reducers of intestinal inflammation, and have been linked to antiviral, antibacterial and even anticaries functions. The most common MUFA in the human diet contain between 10 and 32 carbons, but most are composed of 18 atoms. In general, these fatty acids can be found in various foods such as meats, dairy products and mainly in vegetable sources such as oils from various seeds. A higher intake of MUFAs in the diet versus SFAs or carbohydrates has been associated with a reduction in LDL cholesterol and triglycerides in the blood. Oleic acid (OA) is the most common monounsaturated acid and is present in considerable amounts in both animal and vegetable sources. OA is widely studied for its link to the prevention of heart disease and certain types of cancer. Also of note is the naturally occurring vaccenic acid present in the milk and ruminants meat.

PUFAs can be divided into 12 different families and may contain double bonds between the n-1 and n-12 position of the hydrocarbon chain. The most important families for human health are n-6 and n-3, with LA being the precursor of the n-6 family and ALA of the n-3 family. Other fatty acids with important functions in the body are synthesized from these EFAs. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), derivatives of ALA, stand out for their role in health, particularly for their effects on perinatal health, since they are associated with the visual and cognitive development of the fetus and child. They have also been studied for their involvement in the prevention of maternal depression and lower incidence of preterm birth. The major sources of PUFAs in the diet are vegetable oils, rich in ALA, and marine lipids (mainly oily fish) that provide EPA and DHA to the body. Other foods such as nuts, seeds, eggs and meat contribute, albeit to a lesser extent, to the amount of PUFA in the diet. In addition to these types of fatty acids, SFAs, MUFAs and PUFAs the human diet also includes trans fatty acids, which come mainly from ruminant and dairy fats (natural trans), or foods prepared from partially hydrogenated oils. In recent years, research has focused on other rare fatty acids, such as conjugated linoleic acid (CLA), which is found mainly in dairy products and beef and is associated with reduced body fat and possible antidiabetic effects.

Human milk is often described as the "gold standard" of infant nutrition, as it contains all the macronutrients and vitamins necessary for optimal infant development. In addition, human milk is capable of releasing antibodies that immunize the infant against disease. Lipids are the infant's main source of energy and are therefore necessary for normal growth. Mature milk provides 50% of the daily energy intake in the form of fat. It is also an important source of LA and ALA, which are converted into long-chain polyunsaturated such as DHA, vital in the structural and functional development of the infant's visual, sensory, perceptual, and cognitive systems. These fatty acids are so important for the infant that the FAO has established recommended dietary allowances for infants (0-24 months) and children (2-18 years) of the major essential fatty acids found in breast milk, in addition to recommending dietary intakes of fats and fatty acids in adults. Breast milk provides mainly saturated and monounsaturated fatty acids (MUFAs) and is a good source of long-chain PUFAs and essential fatty acids. Several factors, such as lactation period, sex of the infant, body composition of the mother, etc., can influence the composition of the lipid profile of breast milk. The origin and variety of fatty acids consumed by the mother during pregnancy and lactation influence the

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transference of these fatty acids before (through the placenta) and after (through human milk) delivery, with beneficial and adverse effects on the infant's development.

When breastfeeding practice is not possible, either because of medical problems of the mother that lead her to take medications that can pass into breast milk, because of specific needs of the baby that require a specialized formula (allergies or intolerances), or for social or cultural reasons, or because the mother simply chooses not to breastfeed, infant formulas (IF) are available and designed to provide complete nutrition to the infant, mimicking as closely as possible the composition of breast milk and meeting the infant's normal nutritional needs . Infant formulas can be classified according to their nature and protein source. Thus, there are formulas based on cow's milk (casein), based on protein concentrate (soy-based formula), and based on protein hydrolysates (specialized formulas). Cow's milk is the basis of most infant formulas. To make it as similar as possible to human milk, it is skimmed, diluted, vegetable oils, minerals, vitamins and essential fatty acids are added. These changes are necessary because cow's milk contains a higher percentage of SFAs, proteins and minerals such as calcium, but is deficient in essential fatty acids compared to breast milk. Unlike other animals, humans consume milk and milk products from other mammalian species throughout their lives. Cow's milk is not only modified for infant formula, but the dairy industry has been adapting packaged milk according to human needs over time. Thus, on the market you can find skimmed milks, lactose-free milks, enriched milks, milks with fatty acid profiles improved through animal feed, or the recently commercialized "A2 milk".

Bovine milk and dairy products have a long tradition in human nutrition and, in addition, are foods with high nutritional value due to their rich composition. Milk provides highly digestible proteins of high biological value essential for human growth and development. Lactose is the main carbohydrate and its function is mainly energetic; it has a beneficial effect on intestinal absorption of calcium and magnesium and can act as a prebiotic. Milk contains a variety of minerals (calcium, potassium, phosphorus, magnesium, selenium and zinc) that play a multifunctional role in human health: they are involved in bone and dental structure and are also necessary for numerous metabolic processes. The vitamin profile of milk contains water soluble vitamins (group B and vitamin C) and fat-soluble vitamins (A, D, E). But it is considered poor in some nutrients such as iron and vitamins C and D. The milk fat fraction is variable because certain factors can affect both its production and composition. These factors have been studied throughout the history of dairy farming in order to obtain better yields in dairy farms, highlighting the selection and genetic improvement in dairy breeds, climatic factors, good practices in farms, lactation time, veterinary treatments and most important and effective, changes in animals' diet. However, in recent years both in Spain and in other developed countries there has been a reduction in the UHT milk intake. Often, the saturated fraction of this food has been linked to various pathologies, but this negative image is not backed up by other scientific evidence that supports the consumption of cow's milk and dairy products as part of a balanced diet.

Based on the above, the main hypothesis for this doctoral thesis project was the following: human milk is the personalized food par excellence for the infant, since it provides a very particular fatty acid profile that does not resemble the composition of bovine milk or infant formula. Based on this hypothesis, 4 main objectives were established, with their corresponding secondary objectives. Objective 1 was to study the evolution of fatty acids in breast milk throughout lactation, as well as possible

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modifications derived from the mother's diet. Objective 2 was to study the fatty acid profile of infant formulas, as well as to evaluate their suitability as a substitute and/or complement to human milk fat. Objective 3 was to study the fatty acid profile of bovine milk under different production situations, more specifically, its changes due to cow gestation or after treatment with recombinant bovine somatotropin (rbST). Overall, objectives 1 and 3 studied the factors that could modify the fatty acid profile in human and bovine milk. Finally, a fourth objective was to compare the fatty acid contribution of each of the above types of milk (human, bovine, formulas) and to evaluate their adjustment to human requirements at different stages of life. For the laboratory analysis of the FAs present in the different milk samples, an acid digestion method was used with subsequent methylation of the analytes, to be determined by gas chromatography and flame ionization detector (GC-FID). The lactating mothers who participated in the studies included in this thesis, selflessly donated their milk sample and filled out different questionnaires about their dietary habits, anthropometric and sociodemographic characteristics, as well as an informed consent. The original project was approved by the Clinical Research Ethics Committee of Galicia (code 2016/280), and registered in ClinicalTrials.gov with identification number NCT03245697. The Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects was respected. As for the studies performed on animals, all of them were authorized by the corresponding body and followed the guidelines established in Directive 2010/63/EU on the protection of animals used for scientific purposes. The study on the effects of rbST in dairy cows is part of a national project; code FASL2014-58881-R, funded by the Ministry of Science and Innovation.

The research studies related to the first objective addressed the study of the fatty acids in breast milk as the infant's first food, in the first months of life, and its evolution up to and during prolonged lactation (more than one year of breastfeeding). The quality of the mother's diet is critical for both mother and child, as it must provide the infant with essential fatty acids. To determine how the maternal diet can influence the lipid profile of human milk, milk samples from a group of women living in Galicia were analyzed and compared with bibliographic data on fatty acids from 27 different countries located in the 5 continents, knowing that the basic foods and diets of each geographical location are very different from each other. The results obtained in the samples of local women showed the existence of specific fatty acid profiles for each woman, as well as significant changes throughout lactation due to the natural evolution of some fatty acids. Galician breast milk was similar to that of other European countries, with the exception of those following a Mediterranean diet, and very different from that from women that living in countries with diets very different from European ones, such as Asian countries. It has also been found that certain foods rich in fats, such as oils and some fish and meats, have the greatest influence on the fatty acid profile of breast milk, leaving a distinguishable trace in the fat of this fluid. To evaluate how the lactation period influences the maternal lipid profile over the months, samples of breast milk collected at different times during lactation were analysed. First, changes in the first 5 months of exclusive breastfeeding where the mothers themselves donated breast milk samples were studied, and in a second work, samples from two groups of women were compared, one of them with samples over one year of lactation. These studies showed the existence of a FAs evolution in breast milk over time and how these changes occur in order to adapt to the needs of the child throughout the process. When breastfeeding exceeds one year, it is called prolonged breastfeeding. In Western societies this practice is not very common since the breastfeeding period depends on a series of factors

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ranging from the socioeconomic level of the mother, the work environment, social opinion, and even the lack of knowledge on the part of the health professionals who advise her. The study of milk samples from prolonged breastfeeding showed that milk from more than one year of breastfeeding continues to be a good source of essential fatty acids and long chain fatty acids, and it is considered a complete and nutritious food for the child.

When breastfeeding is not possible for any reason, the most common alternative is to feed the infant with infant formulas suitable for each breastfeeding stage. In the second objective of this doctoral thesis, the differences between breast milk and infant formulas (at the FAs profile level) suitable for each stage of breastfeeding were studied. For this purpose, the differences between the breast milk of Galician women at different lactation stages and infant formulas that can be found in the Spanish market were analysed, compared and discussed. Once again, it was shown that the fatty acids in breast milk are variable: they increase, decrease or remain constant over time. In contrast, formulas remain more stable, varying only in those fatty acids that are important for infant development and those manufacturers deliberately adds to these powdered foods. The first-stage formulas (for infants from 0 to 6 months) were the most similar to breast milk. Since they are the only food the child can take in those first six months, it is recommended that this be the case. Beyond 6 months, an increase in the concentration of ALA was noted in the formulas of this stage, being much higher than in breast milk, and suggesting an over-enrichment of this fatty acid in the formulas

The factors that can alter production (quantitative and qualitative) in dairy cattle are varied. These factors can be divided into endogenous (those that are specific to the animal such as breed, genotype, lactation status or hormonal level) and exogenous (which depend of animal external factors such as climatic conditions, changes in diet, zootechnical aspects or the administration of certain substances that can affect milk production). The research work related to the third objective focused on two factors poorly studied in the literature to determine changes in the fatty acid composition of bovine milk. The first study focused on the impact that the natural state of pregnancy in the cow may have on the fatty acid profile of milk, since dairy cows are pregnant and produce milk at the same time during most of their lactation period. For this purpose, milk from pregnant and non-pregnant cows was analyzed to determine the fatty acid profile and to evaluate if differences were found. Milk from pregnant animals resulted in a fatty acid profile that was more nutritionally adequate for humans, with a lower profile of SFAs and a higher profile of MUFAs, especially oleic acid. In a second article, we studied how the administration of recombinant bovine somatotropin (rbST) can vary the fatty acid profile of cow's milk. This substance is a synthetic version of the animal's natural hormone (somatotropin or growth hormone) with galactopoietic effects, increasing milk production. rbST is banned in the European Union and some other countries such as Australia, Canada or Argentina, but is allowed in others such as the United States. For this study we had a group of dairy animals, a total of nine cows, selected and separated for the administration of rbST. Six cows were treated with this substance and the remaining three were the control group. The administration of rbST in treated animals caused a decrease in the percentage of SFAs in milk and an increase in MUFAs, mainly oleic acid.

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Based on the last objective, the results obtained from the publications were compiled to be discussed as a whole and to see the differences between the three types of milk

studied, especially important in the first stages of life. Compared to breast milk, cow's milk has a higher percentage of SFAs, while in human milk and infant formulas the content between SFAs and MUFAs is more similar. The percentage of PUFAs, where essential fatty acids are found, is higher in human milk than in bovine milk, so their profile is copied in infant formulas, making them a food in line with the nutritional needs of the child throughout its life. Focusing on these essential PUFAs and the WHO recommendations for adequate intake, it was observed that infant formulas have a higher content of ALA and a lower content of AA and DHA, both in comparison with breast milk and with the recommendations indicated. Therefore, it is clear why cow's milk is not recommended in the infant's diet in the first months of life and should be included as a food later in life. However, bovine milk is a good basis for infant formulas, since the addition of certain FAs allows the formula to come as close as possible to the fatty acid profile of human milk.

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LIST OF PUBLICATIONS AND QUALITY INDICATORS

Barreiro, R., Díaz-Bao, M., Cepeda, A., Regal, P., Fente, C.A. Fatty acid composition of breast milk in Galicia (NW Spain): A cross-country comparison (2018) *Prostaglandins Leukotrienes and Essential Fatty Acids*, 135, pp. 102-114. DOI: 10.1016/j.plefa.2018.06.002.

Quality indicators:

- Cited by 20 documents (Scopus 30/09/2022)
- Document type: Article
- Journal impact factor (IF) in JCR, category in the year of publication (2018): 2.86, T2, Q3 (Biochemistry & Molecular Biology).
- ISSN: 09523278

Barreiro, R., Regal, P., López-Racamonde, O., Cepeda, A., & Fente, C. (2020). Evolution of breast milk fatty acids in Spanish mothers after one year of uninterrupted lactation. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 159, pp. 102-141. DOI: 10.1016/j.plefa.2020.102141

Quality indicators:

- Cited by 2 documents (Scopus 30/09/2022)
- Document type: Article
- Journal impact factor (IF) in JCR, category in the year of publication (2020): 4.0, T2, Q3 (Biochemistry & Molecular Biology).
- ISSN: 09523278

Barreiro, R., Regal, P., López-Racamonde, O., Cepeda, A., & Fente, C. A. (2018). Comparison of the fatty acid profile of Spanish infant formulas and Galician women breast milk. *Journal of Physiology and Biochemistry*, *74*(1), 127-138. DOI: 10.1007/s13105-017-0580-2.

Quality indicators:

- Cited by 12 documents (Scopus 30/09/2022)
- Document type: Article
- Journal impact factor (IF) in JCR, category in the year of publication (2018): 2.5, T2, Q2 (Physiology)
- ISSN: 11387548

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Barreiro, R., Regal, P., Díaz-Bao, M., Vázquez, B. I., & Cepeda, A. (2017). Effects of bovine pregnancy on the fatty acid composition of milk: the significance for humans needs. *Food Additives & Contaminants: Part A*, *34*(4), 608-616. DOI: 10.1080/19440049.2016.1277270

Quality indicators:

- Cited by 2 documents (Scopus 30/09/2022)
- Document type: Article
- Journal impact factor (IF) in JCR, category in the year of publication (2017): 1.88, T2, Q2 (Food Science and Technology)
- ISSN: 19440049

Barreiro, R., Lamas, A., Miranda, J.M., Franco, C.M., Cepeda, A., Regal, P. (2022). Impact of recombinant bovine somatotropin on bovine milk composition and *fatty acidome*: a multidose longitudinal study. *Foods*, *11*(21), 3477.

Quality indicators:

- Cited by 0 documents (Scopus 29/11/2022)
- Document type: Article
- Journal Impact factor (IF) JCR, category in 2021: 4.35, T1, Q2 (Food Science and Technology)
- ISSN: 23048158

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ABBREVIATIONS AND ACRONYMS

AA: arachidonic acid

ALA: α-linolenic acid

BF: breasfeeding

BH: biohydrogenation

CHD: coronary heart disease

CLA: conjugated linoleic acid

DHA: docosahexaenoic acid

EFAs: essential fatty acids

EFSA: European Food Safety Authority

EPA: eiocosapentaenoic acid

FAMEs: fatty acids methyl esters

FAO: Food and Agriculture Organization

FAs: fatty acids

FDA: Food and Drug Administration

FOF: follow-on formulas

FSF: first-stage formulas

GC: gas chromatography

GC-FID: gas chromatography-flame-ionization detection

GUF: growing-up formulas

IF: infant formulas

LA: linoleic acid

LC-FAs: long-chain fatty acids

MC-FAs: medium-chain fatty acids

MUFAs: monounsaturated fatty acids

OA: oleic acid

OPLS-DA: orthogonal partial least square discriminant analysis

PCA: principal components analysis

PUFAs: polyunsaturated fatty acids

RA: rumenic acid

rbST: recombinant bovine somatotropin

SEAD: Southern European Atlantic Diet

SC-FAs: short-chain fatty acids

SFAs: saturated fatty acids

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tFAs: trans fatty acids

UFAs: unsaturated fatty acids

VA: vaccenic acid

WHO: World Health Organization

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1 INTRODUCTION

1.1 LIPIDS IN HUMAN NUTRITION

The importance of lipids in nutrition and human development has been recognized for decades. These compounds serve several functions in organism such as: energy storage, structural components of biomembranes, basic structures of lipoproteins, hormonal mediators, and thermal regulation (Mataix & Gil, 2002). For human nutrition lipid consumption are associated with adequate growth, development and normal tissue activity (Lorenzo, Munekata, Moure, Barba, & Dominguez, 2022). Some lipids are essential for humans and they must be provided by diet since humans lack enzymatic system for their synthesis, the most important examples are fat-soluble vitamins and provitamins or some fatty acids (Pateiro, Domínguez, Munekata, Barba, & Lorenzo, 2019). But, in addition to their roles in human metabolism and health, there are also other lipids that could be relation with various diseases such as: obesity, cardiac disease, or some types of cancers (Lorenzo et al., 2022). On the other hand, lipids are associated with the quality characteristics of food as flavours, aroma and texture involving the consumer satisfaction. Finally, lipids have important role in technological properties during food processing.

1.1.1 Lipids: definition and classifications

No exact and unique definition of lipids exists; they could be defined as heterogeneous group insoluble in water and soluble in non-polar organic solvents such as diethyl ether, hexane, benzene, chloroform or methanol. They also contain a long-chain hydrocarbon groups in their molecules and the lipids are found ubiquitously in plants, animals, and microorganisms, as well as the human body. But relying on their solubility has some problems, for example, very short-chain fatty acids (1:0-4:0) are lipids completely miscible with water and insoluble in non-polar solvents (Akoh, 2017). Additionally, some compounds that are considered as lipids, such as some *trans* fatty acids as 18:1 *trans*-9, 18:1 *trans*-11 (those not from bacterial hydrogenation), are not derived directly from living organisms.

Lipids are basically formed by carbon, hydrogen and, to a lesser extent, oxygen; although sometimes also may contain phosphorus, sulphur and nitrogen. Lipids can be classified, according to their physical properties at room temperature, liquids like oils or solid like fats. Depending on their polarity, lipids may be polar (glycerophospholipids and glycerolipids) or neutral (fatty acids, alcohols, glycerides and sterols). But, the separation into polarity classes is rather arbitrary, as some short-chain fatty acids are highly polar. A classification based on structure is, therefore, preferable. Thus, based on their molecular structure, lipids can be non-saponifiable, when they do not possess such property or fatty acids in the structure ("fat-soluble" vitamins A and E, cholesterol, prostagrandins…) or can be saponifiable, form soaps when reacting with NaOH or KOH and contain fatty acids in their structure (triacylglycerides, glycerophospholipids, and the sphingolipids). Additionally, lipids have been broadly subdivided into “simple” and “complex” groups. The first group (simple lipids) is composed of fatty acids and alcohol components, include sterols and their esters, acylglycerols, ether acylglycerols, and wax esters. The group of complex lipids includes glyceroglycolipids (glycolipids),

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glycerophospholipids (phospholipids), and sphingolipids (Akoh, 2017). In 2005, Fahy *et al.* (Fahy et al., 2005) proposed a new definition for lipids on a chemical basis and also a new classification of them more exhaustive, cataloguing them by their physical-chemical properties and by their molecular structure. According to this classification, the lipids were divided into eight different categories as shown in Table 1, each category presenting different types and subtypes of molecules facilitating international communication.

Table 1. Lipid categories according to their structure and physical-chemical properties and examples, as classified by Fahy *et al.* (2005).

Category	Example	Foods sources
Fatty acids	Oleic acid	Olive oil, nuts…
Glycerolipids	Triadylglycerol	Butter, oils…
Glycerophospholipids	Fotidilcolin	Marine fish, mammalian brain, eggs…
Sphingolipids	Sfingosin	Dairy products, eggs, soybeans…
Sterol lipids	Cholesterol Phytosterols	Meat, eggs, fish, dairy products… Oils, seeds, nuts, legumes…
Prenol lipids	Vitamin K	Plants sources, citrus fruits, wine…
Saccharolipids (glycolipid)	UDP-3-O-(3R-hydroxy-tetradecanoy)-acetylglucosamine	Shellfish shells from shrimp, lobster and crabs.
Polyketides	Aflatoxin	Bacteria, fungi…

From a nutritional point of view, the qualitatively and quantitatively most important and characteristic lipid components of food are triglycerides (triacliglycerols) (Mataix & Gil, 2002). They are compounds formed by three fatty acids attached to a molecule of glycerol and are the main form of energy storage in food of animal and plant origin, as well as in the human organism. They also have a high energy content providing 9 kcal/g compared to 4 kcal/g originated by carbohydrates and proteins.

1.1.2 Fatty acids: structure, nomenclature and classification

Within the great structural diversity that characterizes lipids, fatty acids (FAs) are perhaps the most relevant structures because being part of triglycerides, complex lipids and cholesterol esters; make them the lipid molecules of greatest nutritional interest. Thus, they are necessary in human nutrition as a source of energy (storage in adipose tissue and skeletal muscle and transport of energy), fulfil structural functions (component of cell membranes), metabolic functions (gene regulators) and are also important for thermal and electrical insulation, and for mechanical protection (Rustan & Drevon, 2001).

Fatty acids are comprised of hydrocarbon chains, greater or lesser in length and varied in its structure can be lineal, branched or alicyclic. Commonly, a fatty acid consists of a straight chain of an even number of carbon atoms, with hydrogen atoms along its length and at one end of the chain (CH_3-), and a carboxyl group (—COOH) at the other end. The coexistence of both groups in the same molecule converts fatty acids into weakly amphipathic molecules (the -COOH group is hydrophilic and the hydrocarbon chain is hydrophobic) and gives them their water insoluble nature. Water solubility decreases as the length of the chain increases. Thus, the fatty acid formula could be: CH_3- $(CH_2)_n$-COOH where *n*, determines the number of carbons. The number of know natural FAs from various lipid sources: animal, vegetable and microbial;

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exceeds 1000 although only a relative small number (perhaps 20-50) are of common concern and as for the FAs that are commonly found in food, their number is considerably reduced (Gunstone, 1996).

Conforming to the strictest rules of nomenclature, a chemical name must identify and describe its chemical structure unambiguously using a systematic nomenclature without possibility of mistaken when this system is used (Lobb & Chow, 2007). The most frequently used systematic nomenclature for carboxylic acids is that used by the International Union of Pure and Applied Chemistry (IUPAC). This system names acids only based on the number of carbon atoms and the number and position of the double bonds related to the carboxyl group. This nomenclature uses a Greek prefix to indicate the number of carbons it contains and in the case of fatty acids, the suffix "-ic" is added and the word "acid". Geometric configuration and optical activity at double bonds are also identified. Systematic nomenclature will be usually described for the saturated fatty acids and then for other types of FAs as necessary thereafter (Lobb & Chow, 2007). LIPID MAPS® Lipidomics Gateway is a free, comprehensive website for researchers interested in lipid biology. It provides a lipid classification system with access to lipid nomenclature, shorthand annotation, tools, protocols, standards, publications, and other resources for a correct standardized reporting of lipid species data and construction of data resources (The LIPID MAPS®; Liebisch et al., 2020).

There are several abbreviated annotations to refer to FAs; one of the first proposals was to designate the length of the chain with a C- followed by the number of carbons containing the fatty acid and the unsaturated double bonds be serialized (C14:0, C16:0, C18:0...) (Morris, 1961) although it is also common to appear named without C- (14:0, 16:0, 18:0...). The Greek letters omega (ω) and delta (Δ) are sometimes used in naming FAs. The omega system indicates how far double bond is from the terminal methyl carbon irrespective of the chain length. There is another nomenclature system analogous to the omega system, it is called n-x notation system, is used in natural *cis* unsaturated and polyunsaturated fatty acids whose double bonds are separated by a methylene. This system indicates the position of the first double bond in the carbon chain, counting from the end opposite to the carboxyl group or closest to the methyl end of the molecule: n-9 (or ω-9), n-6 (or ω-6) and n-3 (or ω-3), etc… The other delta system, followed by a numeral or numerals, is used to designate the presence and position of one or more double or triple bonds in the hydrocarbon chain. This system specifies the position of all double bonds as well as their *cis/trans* configuration. Table 2 shows examples of the different nomenclatures that can be found in the literature.

Table 2. Different nomenclatures for some common fatty acids found in food.

Systematic name (IUPAC)	Trivial name	Short name	
		n or ω system	Δ system
Hexadecanoic acid	Palmitic acid	16:0	16:0
Octadecanoic acid	Stearic acid	18:0	18:0
cis9-Octadecenoic acid	Oleic acid (OA)	18:1 (n-9)	18:1 Δ^{9}
cis9,cis12-Octadecadienoic acid	Linoleic acid (LA)	18:2 (n-6)	18:2 $\Delta^{9,12}$
cis5,cis8,cis11,cis14,cis17- Eicosapentaenoic acid	Eicosapentaenoic acid (EPA)	22:5 (n-3)	22:5 $\Delta^{5,8,11,14,17}$

Adapted from Lobb K., 2007.

Thus, for example, in the fatty acid *cis* 20:4n-6 (or *cis* 20:4 ω6) the first number, after the C, indicates the carbon numbers that compound the molecule, the second number indicates to the number of double bonds, and the n-6 designate the position of the last terminal double bond. Actually, trivial names are used much more frequently than systematic names, because most researchers in the field of lipidomics or lipid research are more familiar with these names (Züllig, Trötzmüller, & Köfeler, 2020).

Fatty acids can be classified into different categories according to different criteria such as chain length, degree of saturation and location of the first double bond in length. Nutritionally, FAs can be separated as essential (if not synthesized by the organism, it must be ingested in the diet) and non-essential (if they can be synthesized). The classification with respect to the length of the chain is determined by the number of carbon atoms that the FAs form. Among the authors, there is no single criterion to define how many groups there are within this classification and the number of carbon atoms belonging to each group. Mataix J. (Mataix & Gil, 2002) and Agostoni *et al.* (Agostoni & Bruzzese, 1992) classified FAs into four groups according to chain length: short-chain, medium-chain, long-chain and very-long-chain FAs but, the carbons number of each group do not necessary coincide between the authors. Other article (Gómez-Cortés, Juárez, & de la Fuente, Miguel Angel, 2018) classified FAs in three groups:

- Short-chain fatty acids (SC-FAs) up to six carbons
- Medium-chain fatty acids (MC-FAs) from 8 to 12 carbons
- Long-chain fatty acids (LC-FAs) with more than 12 carbons

Depending on the degree of saturation of the fatty acids, the presence or absence of double bonds (-CH=CH-) in the chain, fatty acids are divided into two large groups:

- Saturated fatty acids (SFAs) when they do not have double bonds
- Unsaturated fatty acids (UFAs) when they have at least a double bond

Unsaturated fatty acids, depending on their degree of unsaturation, can be classified as monounsaturated or monoenoic fatty acids (MUFAs), when they present only a single double bond and polyunsaturated fatty acids (PUFAs), when they present two or more double bonds in the carbon chain. When talking about lipids in the diet, this last classification is the most used. The Expert Consultation FAO/WHO recognized that there is a need for universal definitions and recommend a classification for the SFAs and UFAs sub-classes (FAO, 2010). MUFAs and PUFAs can be classified into three groups depending on the position of the first double bond in the length. The position of the double bonds is indicated with respect to the last carbon in the chain (the CH3- end), i.e. the ω carbon:

- ω9 (or n-9 when first double bond in carbon 9)
- ω6 (or n-6 when first double bond in carbon 6)
- ω3 (or n-3 when first double bond in carbon 3)

The length of the chain and the degree of unsaturation give FAs two of their most important physical properties: melting point and solubility. Thus, the melting point of FAs increases with the length of the chain, the longer the chain the greater the number of Van der Wall's bonds. In terms of solubility, FAs are amphipathic. The carboxyl group (-COOH) has an affinity for water as it is polar hydrophilic, and the carbonate chain is insoluble in water as it is hydrophobic non-polar. FAs also have important chemical properties to take into account both in industrial processes and for the samples lipids and FAs extraction and analysis as: esterification reaction when FA binds to an alcohol by a covalent bond forming; saponification reaction where FA formed a soap when binds with a strong base (NaOH, KOH) and hydrogenation reaction that consisting of the addition of hydrogen at double bonds of unsaturated acyl groups that can converted of liquid oils into plastic fats and formed *trans* isomers.

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1.1.2.1 Saturated fatty acids

Saturated fatty acids have a general structure CH3-$(CH2)_n$-COOH, with "*n*" corresponding to the number of carbon atoms. Most of the SFAs occurring in nature have unbranched structures with an even number of carbon atoms. They are referred to as normal alkanoic acid and under the systematic rules of nomenclature, in SFAs, the final "e" of the corresponding hydrocarbon is replaced by the suffix "-oic". These simple carbon-carbon bonds give the SFAs a rectilinear zig-zag structure (Figure 1). These acids range from short-chain-length volatile liquids to waxy solids having chain lengths of ten or more carbon atoms. The most common and important fatty acids usually contain between 12 and 22 carbons and are found in many different plant and animal fats but, however, fatty acids appear in nature with up to 30 carbon atoms in their structure and some with an odd number of carbon atoms.

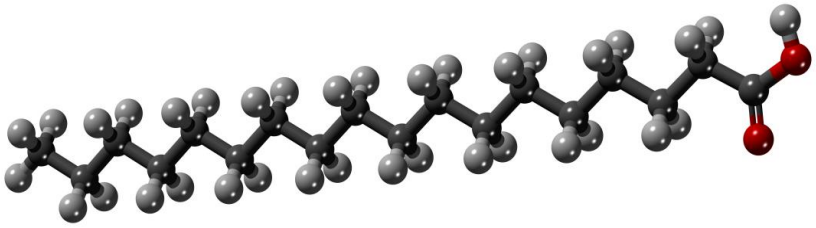


Figure 1. Stearic (or octadecanoic acid) fatty acid structure (18:0). This image is made available under the Creative Commons CC0 1.0 Universal Public Domain Dedication.

The main sources of SFAs in food are animal products, such as butter, bovine milk, meat, salmon, and egg yolk. Ruminant dairy fats provide some odd-chain SFAs (15:0, 17:0) and mainly fatty acids from 4:0 to18:0. Recent studies have highlighted effects of SC-SFAs at cellular and molecular levels in the organism and its antimicrobial activity and anti-inflammatory effects in the gut (Gómez-Cortés et al., 2018). Appreciable levels of SFAs are also present in some plant products such as chocolate and cocoa butter and in some tropical oils: palm oil, kernel oil, peanut oil and coconut oil. These foods mainly provide LC-SFAs (20:0-24:0) (FAO, 2010). SFAs play important roles in normal cellular and tissue metabolism and function but, they also have been considered a crucial dietary factor, associated to several human health problems such as coronary, heart disease, stroke, weight gain and obesity and cancer risk (Calder, 2015). For this reason, The European Food Safety Authority (EFSA) proposed that SFAs intake should be "as low as possible", while the World Health Organisation (WHO) were published in May 2018 a draft guidelines on dietary saturated and *trans* fatty acids for adults and children, where recommend reducing intake of total SFAs to less than 10% of total energy consumption (at the 2010 expert consultation (FAO, 2010), they set the quantity of SFAs at 10%) and replacing with polyunsaturated fat and monounsaturated fat to reduce incidence of cardiovascular disease and related mortality (WHO, 2018).

1.1.2.2 Unsaturated fatty acids

UFAs contain one or more double or triple bonds for example oleic, arachidonic, or linoleic acid. Alkenoic are those unsaturated fatty acids that contain a single double bond in its structure, if it has a single triple bond is called alkynoic. For its nomenclature the prefix di-, tri-, tetra- (and so forth) is inserted before "-enoic" to indicate the number of such double bonds. According to these double bonds UFAs are

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separated into mono or polyunsaturated. UFAs with a double bond can have two possible configurations, either *cis* or *trans*, depending on the relative position of the alkyl groups.

1.1.2.2.1 Monounsaturated fatty acids

MUFAs have one carbon-carbon double bond, which can occur in different positions. The presence of a double bond in the hydrocarbon chain gives rise to geometrical isomerism, which is due to restricted rotation around carbon–carbon double bonds. The most common MUFAs have chain length of 16-22 and a double bond with the *cis* configuration (the hydrogen atoms on either side of the double bond are oriented in the same direction) (Rustan & Drevon, 2001). MUFAs are present in oils of vegetable (macadamia, canola, sunflower, olive), marine and animal origin. Oleic acid (OA) is the most common MUFA and it is present in considerable quantities in both animal and plant sources (Figure 2-A) (FAO, 2010). It is also one of the most studied fatty acid thanks of olive oil consumption; it has health benefits for humans its antioxidant activity, gut microbiota benefits, and its ability in preventing cardiovascular disease (Gavahian et al., 2019).

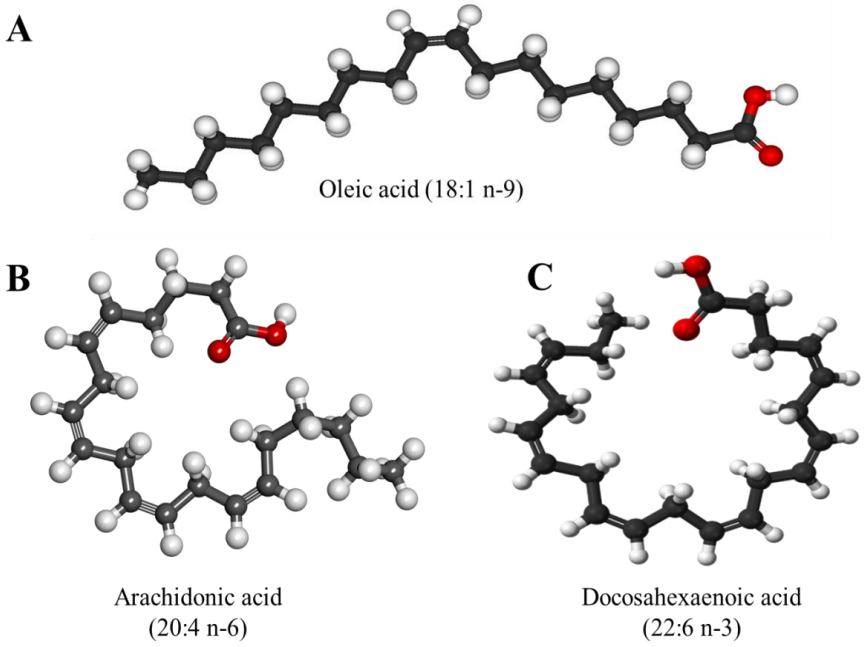


Figure 2. (A) oleic acid structure; (B) arachidonic acid structure; (C) docosahexaenoic acid structure. These images are made available under the Creative Commons CC0 1.0 Universal Public Domain Dedication.

Trans fatty acids (tFAs) have long been used in food industry due to their melting point at room temperature. tFAs are manufactured by partial hydrogenation and deodorization of vegetable oils, and heating oil at very high temperature, between saturated and unsaturated fats. These fats are widely studied due to their excessive consumption in the diet, are a significant risk factor for cardiovascular events as well as a risk factor for cancer and diabetes and even constitute a risk factor for infertility in both sexes. (M. A. Islam et al., 2019; Shah & Thadani, 2019; Çekici & Akdevelioglu, 2019). Although not a majority, there are also FAs with double bonds in the *trans* position, they proceed naturally in milk fat and ruminant meat thanks to the gastric compartment ruminal flora. But there can also appear artificially in certain technological processes

such as hydrogenation or oil refining, transforming the double *cis* bonds in double *trans* bonds.

1.1.2.2.2 Polyunsaturated fatty acids

Natural PUFAs with *cis* configuration are characterized by having more than one double bound in their structure located at the n-1 to the n-12 position and separated from each other by a methylene group. PUFAs, which are produced only by plants and phytoplankton, are essential to all higher organisms as mammals. As the number of double bonds increases, an important curvature in the fatty acid molecule occurs with relevant repercussions on the conformation of the molecules, triglycerides and phospholipids that contain these FAs (Mataix & Gil, 2002). Omega-6 PUFAs are found in evening primrose, borage, blackcurrant seed oils and in the case of linoleic acid (LA) is found in most vegetable oils. Also, some omega-6 fatty acids are found as minor components in animal tissues and in the case of arachidonic acid (AA, Figure 2-B) it found in animal fats, liver, egg lipids and fish. If the first double bond may be found between the third and the fourth carbon atom; these are called omega-3 fatty acids. Omega-3 PUFAs are found in different vegetable and animal fats. The essential α-linolenic fatty acid (ALA) is found in flaxseed oil, perilla oil, canola oil and soybean oil. Eiocosapentaenoic acid (EPA) and docosahexaenoic acid (DHA, Figure 2-C) are the most important n-3 fatty acids in human nutrition and they are found in fish, especially oily fish (salmon, herring, anchovy, smelt and mackerel) (FAO, 2010).

Even though these omega-6 to omega-3 FAs are essential to the human body, is necessary consuming a balance between these two families. In the past decades, in societies that follow westernized diet styles, this ratio(omega-6/omega-3) was increased from 1-2/1 during human evolution to 20-50/1 today when, the highly recommended ratio is 4-5/1 (Mariamenatu & Abdu, 2021). This elevated proportion of omega-6, promote the pathogenesis of many diseases, including inflammatory and autoimmune diseases, cardiovascular disease and cancer (Simopoulos, 2002).

1.1.3 Analytical methods for fatty acids determination in foods

The determination of fatty acid composition is very complex because presents an enormous variety of compounds, chain length (from 4 to 26 carbon atoms), and positional and geometric isomers of mono and polyunsaturated fatty acids. The standard analysis of fatty acids is generally carried out in several steps including: extraction of the lipids from the sample, conversion of the extracted lipids in fatty acids derivatives with lower boiling points, such as alcoholic esters denominated fatty acid methyl esters (FAMEs). And finally these FAMEs are separated, identified and quantified, usually using gas chromatography. The most moderns' techniques for lipid identification focus on lipidomics. Lipidomics is a branch of metabolomics oriented on the full characterization of the molecular species of lipids ant it plays an essential role in exploring biomolecular interactions and metabolic pathways (Han, 2017). It is a powerful tool that can be used in different areas as medicine, for search disease biomarkers and discuss their mechanism in diverse medical disciplines and nutrition and health (Anjos et al., 2019; Dasilva & Medina, 2019; Hirakata, Yokomizo, & Matsuda, 2019; Jiang et al., 2019; Perttula et al., 2018; Picó, Serra, Rodríguez, Keijer, & Palou, 2019; Stark, 2019). In agriculture and food chemistry, lipidomics has been used to analyse lipid profiles of different foods, evaluate the nutritional value and its authenticity (Napolitano, Cerulli, Pizza, & Piacente, 2018; Rocchetti & O'Callaghan, 2021; Rubert, Hurkova, Stranska, & Hajslova, 2018; Song et al., 2019; X. Wang et al., 2019).

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1.1.3.1 Methods for extraction total lipids in foods

For the quantitative recovery of the complex lipid mixture found in the different animal matrices, it is the use of procedures that apply a mixture of polar solvents as chloroform/methanol. This mixture is used in the most commonly lipid extraction procedures such as Folch *et al.* (Folch, Lees, & Sloane Stanley, 1957) who utilized this for the isolation and purification of total lipid content from animal tissues (brain, muscle, plasma and liver), Bligh & Dyer (Bligh & Dyer, 1959) that described a method where the total lipids of biological materials (muscle and meal from different fish) were extracted and purified in a single operation. These methods are still used today because the cold extraction is applicable to almost any foodstuffs and, in addition, the extract can be used for fatty acids, sterols, etc. determination. There are also official methods for different food matrices as ISO-IDF 14156 (International Organization for Standardization- International Dairy Federation) (ISO-IDF, 2001) which specifies methods for the extraction or separation of a representative part of the fat (containing lipids and liposoluble compounds) from milk and milk products. This official procedure previously adds an ammonium hydroxide solution to the milk and then extracts the lipid fraction with n-pentane and diethyl. The Association of Official Analytical Chemists 996.06 (AOAC, 2005) is other official method applicable to determination of fat in foods where fat is extracted into ether.

1.1.3.2 Methods for preparation fatty acids methyl ester

After extraction, derivatization is necessary to convert the polar, non-volatile long chain fatty acids into methyl ester derivatives that are less polar and relatively volatile and thermally stable, resulting in a more efficient separation. FAMEs are usually prepared by transesterification in an acid-catalysed as boron trifluoride or sulphuric acid in methanol as proposed by the International Organization for Standardization-International Dairy Federation (ISO-IDF) (ISO-IDF, 2001; Metcalfe, Schmitz, & Pelka, 1966; Van Wijngaarden, 1967) or base- catalysed transesterification with sodium methoxide or potassium hydroxide in methanol (ISO-IDF, 2002; Bannon et al., 1982) and using temperature or not. The extracted FAMEs are non-polar, which is why they are extracted with alkanes such as n-hexane before being injected into the gas chromatograph. But the management of several steps in the extraction may be responsible for loss of a portion of the lipid phase and contamination. For these reasons, fast, low cost (using fewer amounts of solvents), simple (with fewer steps) and accurate methods for determining fatty acids concentrations in foods have been development. To reduce time and costs, some authors have been changing the multistep methods (saponification-methylation) for one-step methods with a direct transesterification of lipids. That way, the methods will be more rapidly, and it will only one reagent is necessary for extraction (Eder, 1995; Juárez et al., 2008). In addition, these methods seek that methylation was complete and that artefacts or strange components which, can be wrongly identified as FAs or overlap with FAME peaks in the gas chromatography (GC) analysis, were not formed (Luna, Juárez, & de la Fuente, 2008).

1.1.3.3 Analysis of fatty acids methyl ester

The most widely used technique for measuring fatty composition in foods are by gas chromatography with the methyl esters and normally, performed with a flame ionization detector (GC-FID). This technique is very sensitive and has good linearity over a wide range of concentrations (Ackman & Ratnayake, 1989). For the detection of FAMEs it is important to choose a column with good separation and resolution abilities of UFAs taking into account the double bonds they may have. Polar capillary columns

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with lengths of 30, 60 and 100 meters, and hydrogen as carrier gas are commonly used. These columns combine the advantages of a relatively high-resolution capability with those of a relatively high thermal stability (it can withstand temperatures up to 260ºC) (Eder, 1995). In order to quantify the FAMEs it is necessary to perform a calibration of the system with standards containing know amounts of FAMEs using commercial standars. Numerous commercially available FAME standards make it possible to perform absolute quantification for both high- and low-abundance FAs. A high percentage of FAs analyses in different foods matrices (milk, flaxseed oil, fish and meat samples or fruits) are determinate with this method (Figueiredo et al., 2016; R. Islam, Islam, Shoeb, & Nahar, 2018; Lieb et al., 2019; Martínez, Miranda, Franco, Cepeda, & Rodríguez, 2012; Yurchenko et al., 2018). Other technique used for measuring fatty acid composition in different and multiples food matrices is gas chromatography–mass spectrometry. This procedure allows profiling of FAs synthesis and metabolism using stable isotopes being a fast and sensitive method for FAMEs determination (de Falco, Fiore, Bochicchio, Amato, & Lanzotti, 2018; Liu, Ezernieks, Rochfort, & Cocks, 2018; Mihai, Negoiță, Adascălului, Ionescu, & Belc, 2018; R. Z. Wang, Lu, Cao, & Tang, 2018).

1.2 Significance of bovine milk in the evolution of humans

1.2.1 Milk composition

The discovery of agriculture and livestock in the Neolithic period was crucial for the human being. Human civilizations were able to select as dairy animals an array of species (e.g. cows, sheep, goats, buffaloes, yaks, camels, horses, and asses) characterized by relatively dilute milks containing modest levels of fat, caseins, and whey proteins, and relatively high levels of lactose (Oftedal, 2012) (Figure 3). In addition, this milk has been used for the obtaining of products like cheese, yogurt, and butter among others. On the other hand, the populations stabilized as they had livestock to look after and they stopped being nomads, they began to cultivate plants and their life expectancy increased.

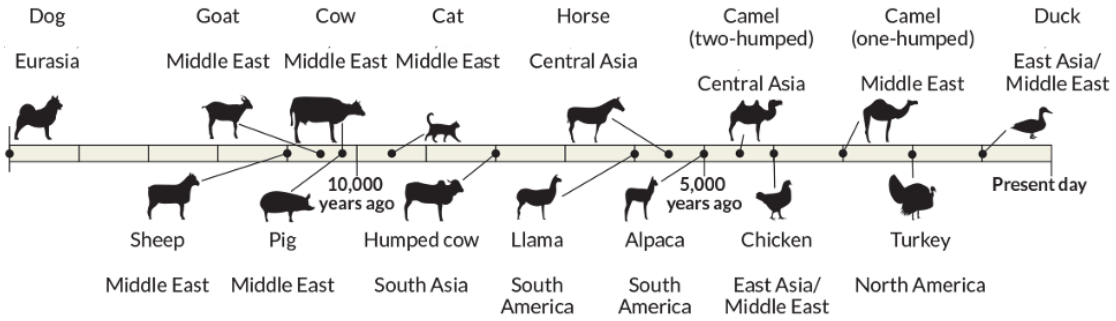


Figure 3. An estimated timeline of animal domestication. Reproduced from DNA Evidence Is Rewriting Domestication Origin Stories (https://www.sciencenews.org/article/dna-evidence-rewriting-domestication-origin-stories), under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License.

Milk is defined as the biological fluid secreted by females from mammals whose role is to provide the energy and nutrients necessary for the growth and development of the newborn of the corresponding species during the first months of life (Llano, Alonso, Krug, Torres, & Moreiras, 2017). But unlike other animals, humans consume milk from other mammalian species either freshly expressed or as dairy products. For this reason, the Codex Alimentarius defines milk as "normal mammary secretion from dairy animals obtained from one or more milking without any kind of addition or extraction, intended for consumption in liquid milk form or for further processing" (FAO/WHO, 2022).

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Milk is a balanced and effective source of lipids, essential amino acids and fatty acids, proteins (caseins and whey proteins), carbohydrates (mainly lactose), minerals (e.g. calcium and phosphate), enzymes, vitamins and its energy value depends primarily on the fat content (Tamime, 2009). Mammals milk is constituted mainly by water being the dispersing phase in which the proteins are organized in colloidal dispersion as micelles; the fats emulsified globules coated with a membrane, and most minerals and all lactose in true solution. Milk protein has a high biological value since supply young mammals: vitamin-binding and metal-binding proteins, immunoglobulins, various protein hormones and essential amino acids, important for the development of muscular and other protein-containing tissues (Fox, Uniacke-Lowe, McSweeney, & O'Mahony, 2015).

1.2.2 Bovine milk and human health

The term "milk" is reserved only for milk from the mammary gland of healthy bovine animals, any milk from a different female must be accompanied by the species of animal from which it is derived (goat's milk, sheep's milk, human milk) (Patton, 2004). Thus, bovine milk and dairy products have long tradition in human nutrition, and, in addition, are foods with a high nutritional value thanks to their rich composition as they are part of many important nutrients in relatively high quantities. Milk provides highly digestible proteins with high biological value essential for growth and development with casein being the main protein (approximately 80%), important because is not found in other foods. Lactose is the main carbohydrate; its function is mainly energetic, has a beneficial effect on the intestinal absorption of calcium and magnesium and can act as a prebiotic (Michaelsen, Nielsen, Roos, Friis, & Molgaard, 2011). Milk contains a variety of minerals (calcium, potassium, phosphorus, magnesium, selenium and zinc) that play a multifunctional role in human health: participate in the bone and dental structure and are also necessary for numerous metabolic processes (FEN/FINUT,2015). Milk vitamin profile contains hydrosoluble (B- group and vitamin C) and liposoluble (A, D, E) vitamins. But it is considered poor in some nutrients such as iron and vitamin C and D (Haug, Hostmark, & Harstad, 2007).

In Spain, the consumption of milk and milk products has progressively increased since the 1950s in parallel with the increase in education level and purchasing power of the population (FEN/FINUT, 2015). The central government gave diffusion to the milk consumption when from 1958 it was established the obligatory of the production and commercialization of the sanitized milk, pursuit of fraud, a system to supervise the sector and, new technologies to prolong the milk shelf life (Hernández Adell & Pujol, 2017). However, in recent years, both in Spain and in other developed countries, there has been a decrease in milk consumption. By the end of 2021 (according to the Spanish Ministry of Agriculture), UHT milk lost importance in Spanish households, both in volume and value, with declines of 4.7% and 4.1%, compared to previous year. Although there is an increase in purchases in 2020 due to the coronavirus crisis, the long-term trend from 2008 to 2021 data is downward (Ministerio de Agricultura, Pesca y Alimentación, 2021). The consumption of plant-based drinks (soy, rice, almond or oat) has growth in recent years. Lactose intolerances and allergy to cows' milk protein are the major factors that lead to this consumption, but many people choose this type of products when they relate to healthy lifestyles (Zandona, & Lannes, 2020). European Union regulated the name of this type of products of vegetal as origin "beverages" according to their origin (soy beverage, almond beverage) and not as milks (European Union Council., 2007).The biggest problem that was attributable to milk is, on the one hand, the management of dairy cattle on large farms, animal welfare and the use of certain substances that can leave residues in milk and meat and, nutritionally, the

content of a high fraction of saturated fatty acids, assumed to contribute to the pathologies mentioned above. However, this negative image is not supported by others scientific evidences which sustains that the consumption of cow´s milk and dairy products are a part of a balanced diet when these foods are consumed according to appropriate national guidelines (especially in infancy and childhood) (Scholz-Ahrens, Ahrens, & Barth, 2019; Singhal, Baker, & Baker, 2017). Recently, a new type of milk denominated "A2 milk" has been released to the market. This type of milk is characterized by being free of the A1 variant of β-casein providing the reduction in digestive intolerance associated with the consumption of A1 milk (Fernández-Rico et al., 2022).

1.2.2.1 Bovine milk fatty acids and human health

Lipids are the most important constituents of bovine milk because they are a good source of energy and provide numerous organoleptic properties to dairy products. The main lipids in dairy fat are the triacylglycerides and their composition is extremely complex. Cow's milk contains more than 400 different fatty acids of which the majority fraction is saturated. Many of these SFAs have a long-chain but there are also short- and medium-chain fatty acids that are easily absorbed and are an immediate source of energy, so they have a low tendency to accumulate in adipose tissue. An example is butyric acid (4:0), which is found exclusively in milk fat and is attributed important roles in health and disease: reduction of inflammatory processes, promoter of gut health and integrity, promotion of satiety, inhibition of colonic carcinogenesis, primary energy source for cells and antimicrobial activity (Gómez-Cortés et al., 2018). Just over a third of the fatty acids present in milk correspond to lauric acid (12:0), myristic acid (14:0) and palmitic acid (16:0), which are responsible for attributing hypercholesterolemia properties to milk, as well as increasing the risk of coronary heart disease (Iggman & Risérus, 2011). However, recent research has shown that dairy matrix components , mainly peptides, phosphorus, calcium and the milk fat globule membrane, modify blood lipid responses to saturated fatty acids intake. So, these SFAs with unhealthy properties would have no impact on cardiovascular health parameters when supplied in the dairy matrix (Thorning et al., 2017). Among saturated fatty acids, odd chain fatty acids (15:0, 17:0) are used as biomarkers of milk fat intake (Riserus & Marklund, 2017).

OA is the UFA most abundant in milk; its multiple benefits for human health have been studied for years. Linked to olive oil and the Mediterranean diet as its food par excellence, cow's milk contains around 20% of this fatty acid (R. Barreiro, Regal, Díaz-Bao, Vázquez, & Cepeda, 2017). Among its benefits are the prevention of different disorders as cardiovascular or autoimmune, immunomodulation, prevention of metabolic disturbances, skin injury, cancer and prominent role in drug absorption (Sales-Campos, Reis de Souza, Crema Peghini, Santana da Silva, & Ribeiro Cardoso, 2013). The principal PUFAs in milk are LA and ALA, the so-called essential fatty acids (EFAs) because they cannot be synthesized in the human body. For this reason, EFAs must be ingested in the diet. In the human body, these fatty acids give rise to other important LC-PUFAs: AA, EPA and DHA that play key roles in regulating body homeostasis (Saini & Keum, 2018). The ruminant's milk is considered an important source of n-3 fatty acids in the human diet, and this is important because of the benefits they bring to the organism. Evidence of both independent and shared effects in neuroprotection and in the treatment for a variety of neurological and neurodegenerative disorders is patent in the literature. Furthermore, DHA is an important constituent of the retina and the nervous system (Altomonte, Salari, & Martini, 2018; Mansson, 2008).

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*t*FAs occur naturally in ruminant feeds as a result of the partial biohydrogenation of dietary PUFAs by rumen microorganisms. The main *trans* fatty acid in bovine milk is vaccenic acid (VA; 18:1 n-7), a positional and geometric isomer of oleic acid, denominated the principal natural *trans*. Field CJ (Field, Blewett, Proctor, & Vine, 2009) has highlighted the benefits of VA on human health, suggesting a beneficial effect of VA in reducing tumor growth. Even when it has not been convincingly demonstrated a relationship between VA intake and the risk of myocardial infarction, plasma levels of VA may affect the risk of CHD (coronary heart disease) in a beneficial manner. Finally, VA is the only dietary precursor to the conjugated linoleic acid (CLA; c9,t11 or rumenic acid), other important fatty acid produced by incomplete biohydrogenation of linoleic acid in the rumen. CLA has been studied extensively in recent years for its great health benefits describing it as anti-carcinogenic and anti-obesogenic fatty acid and possess an anti-atherosclerotic properties (den Hartigh, 2019).

The FAs in milk have a double origin, on the one hand the FAs are captured from the blood circulation from the diet, from the ruminal biohydrogenation process or from body reserves and, on the other hand, *de novo* synthesis of FAs in the mammary gland. Forty to sixty per cent of milk FAs are LC-FAs derived from the diet, mostly FAs with 18 carbon atoms. Fatty acids from 4:0 to 14:0 carbon atoms are synthesized *de novo* in mammary gland and palmitic acid (16:0) arises from both diet and *de novo* synthesis (Palmquist, 2006).

1.2.2.1.1 Fatty acids in cow´s diet

Dairy cattle feed on green grass, silage (grass or maize), hay, feed and supplements such as oilseeds. The diet depends on the season and the housing system (grazing, semi-intensive, intensive). These feeds provide mostly fiber, essential to sustain ruminal functionality, stimulate chewing and rumination, and maintain an adequate ruminal pH to allow good health and digestion of the animal. In addition, it contains protein, energy, water, vitamins and minerals. Natural ruminants diet is not rich in lipids, for this reason, their diets are supplemented with fat because it increases energy concentration and enhances animal performance since it also improves production, growth and reproduction (Santos, Bilby, Thatcher, Staples, & Silvestre, 2008), always coupled to proper management of these supplements.

Dairy cattle diets, without any supplemental fat, contain approximately 2-3% of fat and the predominant FAs are PUFAs with a chain of 18 carbon atoms and vegetable origin as LA and ALA. This last PUFA is the main fatty acid and represents between 55% and 65% of fresh grass fatty acids (Doreau & Poncet, 2000). The raw materials used in the manufacture of animal feed are mainly cereals (corn, wheat, barley…), legumes (soya), oilseeds (sunflower, rapeseed…) and additional components (molasses, fishmeal…) (Hospido, Moreira, & Feijoo, 2003). The FA content of cereals, oilseeds and free fats is variable, higher and in the form of triglycerides. They are mostly rich in PUFAs, especially LA and a low percentage of SFAs. The type of fats consumed in animal diet, will be reflected in fatty acid composition of bovine milk.

1.2.2.1.2 Fatty acids metabolism in rumen and mammary gland

Lipids consumed by ruminants are generally in esterified form, either as triglycerides in concentrated feeds, in the form of galactolipids and phospholipids in green fodder, or in a specific form such as FA soaps in some commercial products. The digestive tract of ruminant animals is characterized by four well differentiated compartments functionally and anatomically (Van Soest, 1994). Rumen is the largest of the four compartments (80% of total volume), and is where most microbial activity is

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concentrated. Two main processes take place in the rumen which is intrinsically bound together, lipolysis of the dietary lipids and biohydrogenation (BH) of their UFAs constituents with the involvement of rumen microflora. Lipolysis process is carried out mainly by rumen bacterial and it is a necessary step before BH. In this last process, the responsible anaerobic bacteria reduce the number of double bonds of UFAs by adding hydrogen (Jenkins, Wallace, Moate, & Mosley, 2008). In addition, BH is a protection mechanism that ruminal bacteria put in place to prevent the toxic effects of UFAs.

As mentioned above, the fatty acids present in forages, cereals and oilseeds mainly contain: LA, ALA and OA. Thus, the most studied and best known biohydrogenation is that of these PUFAs and MUFA. The main pathway for LA begins with an isomerization reaction of the double bond *cis*12 to *trans*11 leading to the formation of one of the major isomers of conjugated linoleic acid, rumenic acid (RA). The second reaction is the reduction of VA (Jayanegara, 2013). Similarly, the main biohydrogenation pathway of ALA, this PUFA is isomerizated in the double bond *cis*12 to *trans*11 followed by a reaction of the double bonds cis until the formation of 18:1 t11. Finally, OA is isomerizated to 18:1 *t*11 (Jayanegara, 2013). The final product of these 3 biohydrogenations is always the same: stearic saturated fatty acid (18:0) (Figure 4). For this reason, milk and meat from ruminants is characterized by the presence of a higher concentration of SFAs than UFAs.

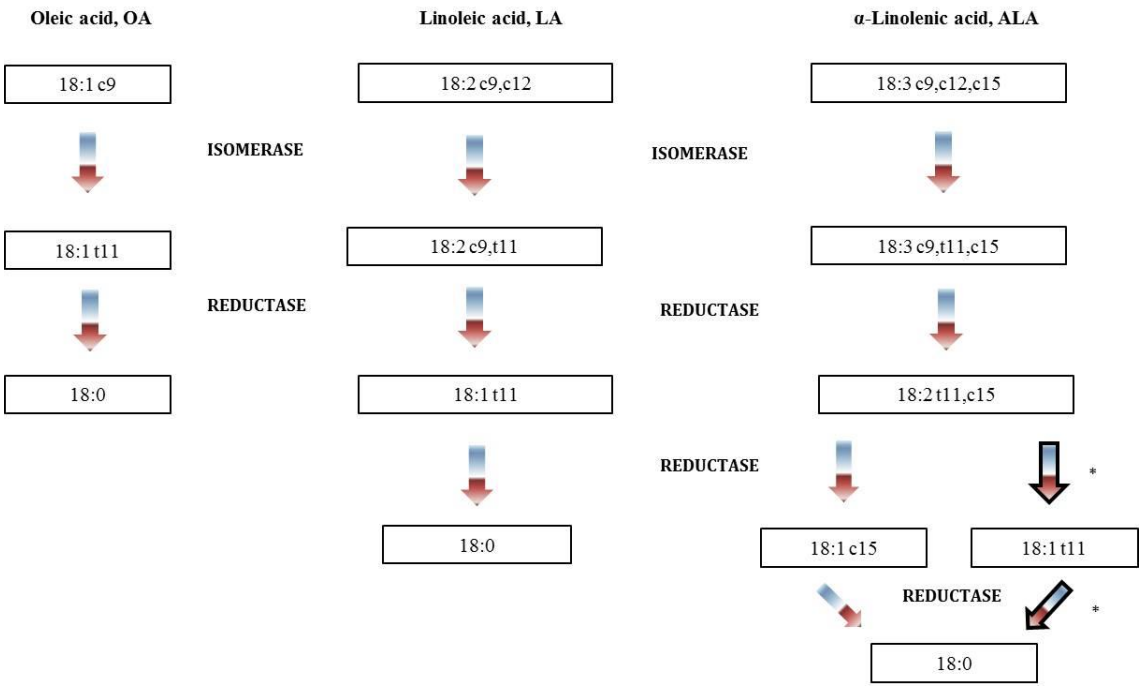


Figure 4. Biohydrogenation pathways in the rumen of OA, LA and ALA to steraidonic acid (* main route). Adapted from Jenkins T.C., 2008.

The *de novo* synthesis of fatty acids in ruminant mammary gland takes place in the cytoplasm of the mammary cells consists of two steps: forming malonyl CoA catalyzed by the enzyme acetyl-CoA alpha carboxylase and converting acetyl-CoA and malonyl CoA to short and medium chain fatty acids and palmitate fatty acid by adding acetyl-CoA molecules to the lipid chain (Dils, 1986). The use of acetate (2:0) and ß-hydroxybutyrate by elongated enzymes explains why most milk FAs has an even

number of carbon atoms. However, odd chain FAs can also be found in milk and their origin, although largely ruminal, can also be *de novo* synthesis in the mammary gland using propionic acid (3:0) as precursor (Vlaeminck *et al.*, 2006).

There is another process in the mammary gland that would explain the endogenous origin of certain bovine milk FA, the desaturation. The stearonyl-CoA desaturase enzyme is responsible for increasing the degree of unsaturation of fatty acids. The activity of this enzime performs the formation of myristoleic (14:1), palmitoleic (16:1), and OA, using as substrates myristic (14:0), palmitic (16:0), and stearic (18:0) acids, respectively (Angulo, Mahecha, & Olivera, 2009). As explained above, in the bovine mammary gland desaturation is possible, but not the elongation of fatty acids of more than 16 carbon atoms because the necessary enzymes do not exist, for this reason, FAs of more than 18 carbon atoms (except those originating in desaturation) come from circulating lipids in the blood. Figure 5 shows an overview of the different origins of bovine milk fatty acids.

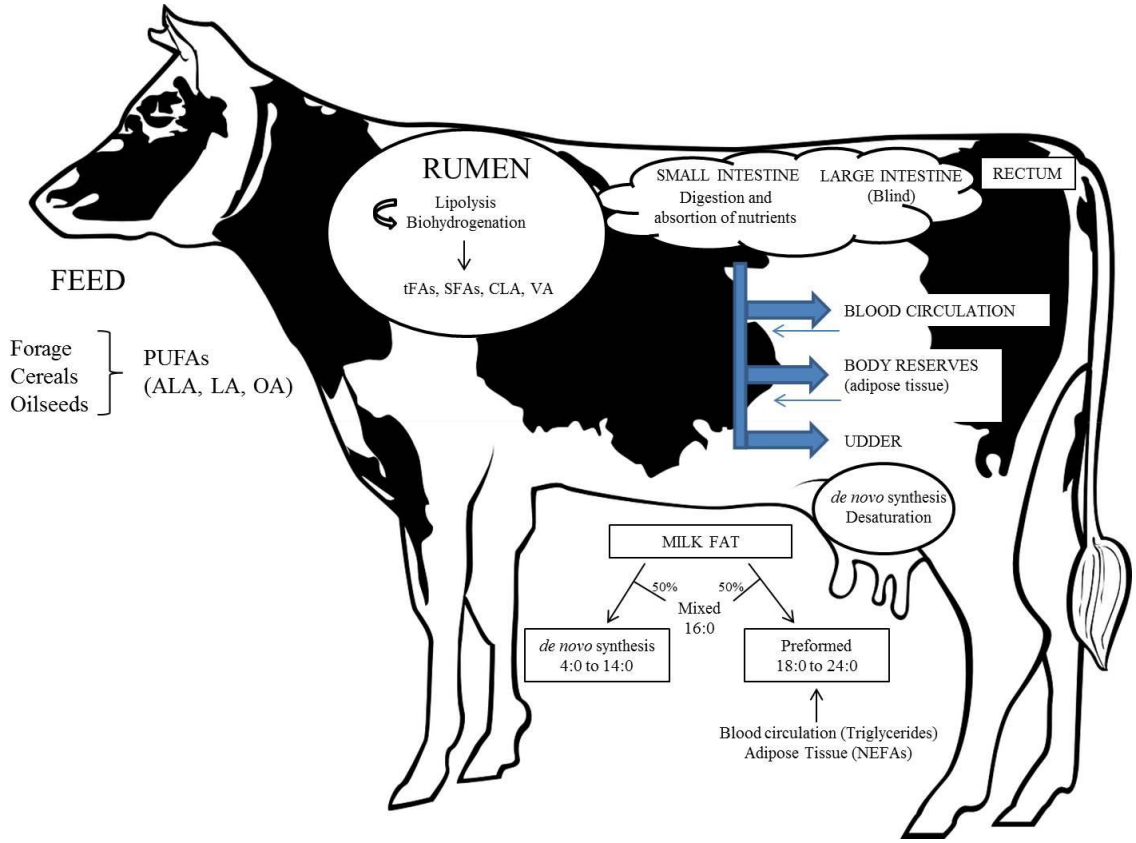


Figure 5. Bovine milk fatty acids provided by diet, formed in rumen and de novo synthesis in mammary gland (created by Barreiro, Rocío).

1.2.2.2 Factors influencing milk production and composition

The current world trend is to produce milk with increased quality (more concentrated in the protein and mineral fraction and less concentrated in the lipid fraction) and increasing in turn the volume (Manterola, 2014). There is a wide variety of factors that can affect milk production and composition in dairy herds. These factors may be divided into two groups (Figure 6), the endogenous factors that can be defined as those that are intrinsic to the animal itself (breeds and genotypes, stage of lactation, hormonal levels, foetus sex…) and exogenous factors that are highly dependent on the environment where the animal is found (season, climatic factors…) or human behaviour

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(feeding and handling). Humans can control some of these factors by improving the bovine animals' life quality and increase the dairy farm profitability.

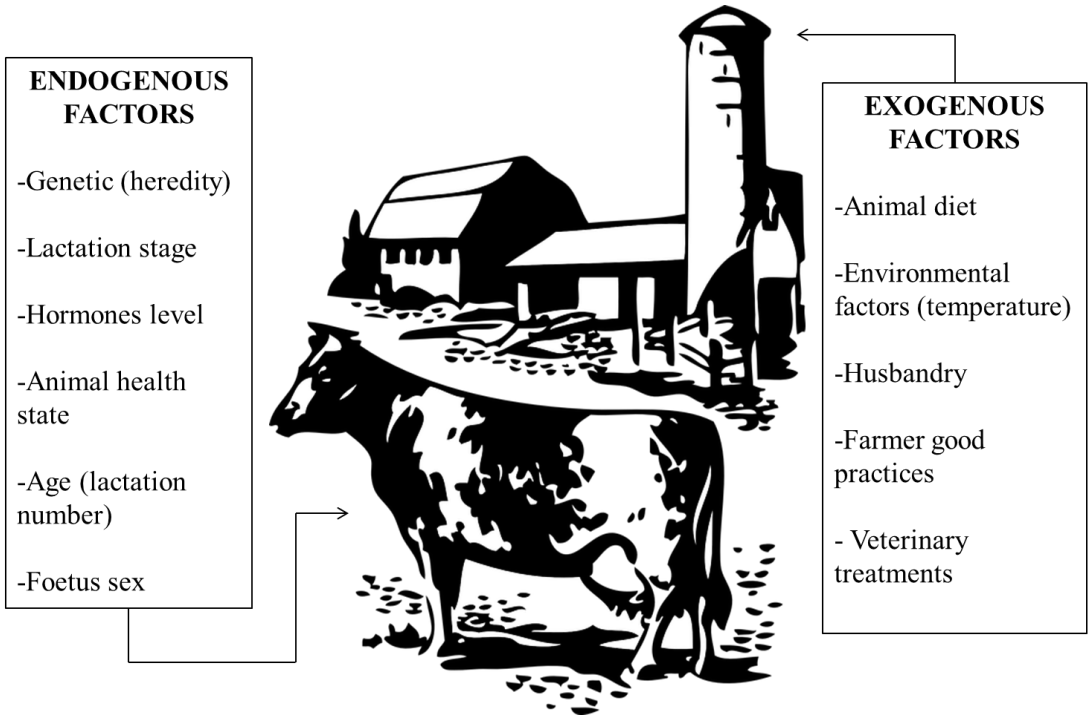


Figure 6. Endogenous and exogenous factors that may influence bovine milk composition (created by Barreiro, Rocío).

The genetic (heredity) of the animal determines its potential milk production. The human being has taken advantage of genomic information for livestock improvement selecting bovine dairy breeds with high production volumes, higher grazing aptitudes, variations in fat or protein as well as improved reproductive capabilities (fertility, calving). Lactation stage altering milk fat content because is closely associate with energy balance, thus, milk produced in early lactation is richer in LC-FAs that produced in lactation (Mesilati-Stahy & Argov-Argaman, 2014). Bovine milk contains considerable quantities of hormones since they are part of animal metabolism (P. Regal, Cepeda, & Fente, 2012), pregnancy is an important physiological status in the cow because can produce variations in the hormonal level, in fact produce variations in the lipid profile (R. Barreiro et al., 2017; P. Regal et al., 2012). The health state of animal can influence total milk solids: the mastitis in the mammary gland decreasing the lactose content, the ruminal acidosis provokes the syndrome of "fall of fat", the ketosis that produces a decrease in the milk protein and febrile states that can reduce both the flow and the concentration of total solids (Manterola, 2014). With increasing age or number of lactations, fat and lactose decrease while protein remains constant (Campabadal, 1999). The sex of the foetus influences the capacity of mammary gland to synthesize milk (Hinde, Carpenter, Clay, & Bradford, 2014), cows' favor females, producing significantly more milk for female than for male across lactation but, this diminish for males was not compensated by higher protein and fat production.

Animal's diet is the factor most studied because its modifications offer a means of enhancing milk production and making rapid changes in its composition. Of all the components of milk, the percentage of fat is the most variable and the one that suffers the most changes with the diet (Campabadal, 1999). For this reason and considering the stage of lactation of the animal the use of forages, animal fats or marine oils have

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important effects on bovine milk fat (and fatty acids) secretion and composition (Chilliard, Ferlay, & Doreau, 2001). Supplement the diets with oilseed and vegetable oils that provide unsaturated lipids, among which linseed, soybeans, rapeseed, or sunflower are the most common ones. Oilseeds provide mostly OA, LA and ALA. Linseeds stand out for their high percentage of ALA (54%), rapeseeds for their OA content (60%) and sunflower seeds with 67% of LA (Glasser, Ferlay, & Chilliard, 2008). Temperature is one of the environmental factors that can alter the composition of milk: high temperatures provoke that food consumption decreases due to heat stress causing a reduction in the volume of milk, the concentration of fat and protein (Sargeant, Shoukri, Martin, Leslie, & Lissemore, 1998). Good husbandry during milking and lactation has important effects on the volume and concentration of total milk solids. The health of the livestock also depends on the good practices of the farmer, veterinary medicines and not administering illegal substances that alter milk production (e.g., clembuterol, thyrostatics, growth hormone etc.).

1.3 HUMAN MILK AND BREASTFEEDING

1.3.1 History and evolution of breastfeeding

Breastfeeding (BF) is where milk is obtained from the breast and provides babies with the nutrients necessary for optimal development, growth and health in the first months of life. Human milk is specifically adapted to the unique digestive and metabolic characteristics of the human infant. But BF is a human biological trait subject to modification by social, economic and cultural influences. Thus, the way of understanding BF has undergone several changes throughout history. One of the most important facts was the appearance of shared BF (breastfeeding a non-biological child) by mercenary (wet nurses). References were found in Egypt, Mesopotamia, Babylon, classical Greece and noble women of the Roman Empire (Wickes, 1953). At this time, the reference treatise of Gynecology and Obstetrician consulted for more than 1500 years (Gynecia, Sorano of Ephesus 98-138 A.D.) was written the conditions for choosing a good wet nurse, her diet, lifestyle and ways of BF (Wickes, 1953).

By the later medieval period it was well-established for European women of the upper classes to employ, wet nurses become in a paid job and a symbol of purchasing power, social and aesthetic position. In Spain, the system of wet nurses reached enormous proportions, being the only one in Europe that regulated mercenary breastfeeding (Asociación Española de Pediatría, 2008). The popularity of feeding by wet nurses began to decline when infant mortality rates increased due to various diseases that were transmitted to the infant through breast milk (syphilis or gonorrhoea). The role of the wet nurse fell completely to the detriment of her total disappearance in the 1980s, coinciding with the emergence of HIV and becoming aware that human milk can transmit deadly diseases to the baby (Gamboa, 2008). The frequency and duration of breastfeeding and maternity decreased dramatically throughout the 20th century. The incorporation of women into the wage-earning world, schooling of girls, the equality of civil rights for women and the possibility of using contraceptive methods, was brought about a reduction in the birth rate (Asociación Española de Pediatría, 2008). In addition, the initial diet based on maternal milk is replaced by modified cow's milk to bring its composition closer to human milk.

Breast milk provides not only nutritional benefits but also psychological, immune, and biological benefits in comparison to breast milk substitutes (formulas) (Espósito & Córdoba, 2018). In recent years, many cohort and case-control studies were demonstrated a positive dose-response relationship contributing to a sound evidence

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base. Breast milk is so complete that it offers benefits not only for the baby but also for mother, family and for society (Table 3). The WHO, according to scientific evidence, recommends exclusively breastfeeding for the first six months of life. From this date, complementary feeding is introduced, along with human lactation, up to two years and beyond (WHO, 2014). In Spain, according the last document published by the Spanish Association of Paediatrics on breastfeeding habits extracted by national health surveys, the breastfeeding rate (including exclusive and partial breastfeeding) at 6 weeks has remained fairly stable with global figures around 71%, while there has been, in the last 15 years, a progressive increase in BF at 3 (66.5% in 2012) and 6 months of life (46.9%) (Comité de Lactancia Materna, Asociación Española de Pediatría, 2015.). Seeing the high rates of abandonment at six months or earlier we understand why BF beyond this period is rare in Western societies. Mothers who decide to continue BF find little professional support and information (especially at weaning). Prolonged lactation is understood as the BF of the small child after the first year of life. There is no scientific evidence or consensus to show that from a certain moment on breastfeeding is an inconvenience or that milk loses its nutritional or immunological properties. Studies reflecting the advantages or disadvantages of prolonged lactation are few but it has been observed that a longer duration of lactation can only bring benefits for the mother and the child (Filipiak-Pittroff et al., 2018; B. L. Horta, 2019; B. L. Horta & de Lima, 2019; Mohammadi, Naderi, Ansari Moghaddam, Mahdavifar, & Mohammadian, 2018; Rito et al., 2019; Victora et al., 2016).

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Table 3. Breastfeeding benefits for infant, mother, family, and society.

Infant	Mother	Family	Society
•Continued breastfeeding reduce mortality rates (Li et al., 2022; Sankar et al., 2015).	•Favors postpartum recovery as it causes the uterus to contract and diminishes lochia (Zamora & Cañamero, 2008).	•Lower incidence of diseases at home (Zamora & Cañamero, 2008).	•Health costs: less visits to the pediatrician and fewer medications (Zamora & Cañamero, Abril 2008).
•Lower incidence of infectious diseases (Sankar et al., 2015) and obesity protection against childhood (Rito et al., 2019).	•Delays the menstrual period. This amenorrhea causes the loss of iron decreasing the anemia risk (Del Ciampo & Del Ciampo, Ieda Regina Lopes, 2018).	•Considerable economic savings on the use of infant formulas and bottles (Zamora & Cañamero, 2008).	•Reduced parental absenteeism (Asociación Española de Pediatría, 2015).
•Protection from gastrointestinal infections and influence the development of the gut microbiota (Güngör et al., 2019; Hennet & Borsig, 2016).	•Recuperation of body weight from calorie consumption decreasing their risk of obesity, diabetes or other chronic disorders (Bartick et al., 2017; Del Ciampo & Del Ciampo, Ieda Regina Lopes, 2018).	•Generational union link (Asociación Española de Pediatría, 2015).	•Environmentally friendly: it does not leave an ecological footprint in its consumption and production (Brahm & Valdes, 2017).
•Low risk of eczema and asthma (Güngör et al., 2019; Oddy, 2017).	•Possible protective effect against breast and ovarian cancer (González-Jiménez, 2018; Modugno et al., 2019) .		
•Prevention of chronic and recurrent otitis media and chronic respiratory disease (Brennan-Jones et al., 2017; Cunningham, 1995).	•Reduces the risk of type 2 diabetes (Chowdhury et al., 2015).		
•Protection from sudden infant death syndrome (Boyer & Walton, 2019; Buñuel Álvarez & Cuervo Valdés, 2011).	•Lower risk of bone fractures and osteoporosis in the postmenopausal period (Saei Ghare Naz, Ghasemi, Kiani, Rashidi Fakari, & Ozgoli, 2019).		
• Important role in neurodevelopment and cognitive function (especially in preterm infants) (Schwarzenberg & Georgieff, 2018).	•Exclusive breastfeeding provides protection against postpartum depression (Webber & Benedict, 2019).		
• Comfort, affection, companionship and security for the baby (Zamora & Cañamero, Abril 2008).	•Encourage the formation of a constant and lasting affective bond with the child (Sara & Elena, 2018).		

1.3.2 Human milk composition

Lactation is an essential part of the total reproductive process in mammals thus, it is important that the onset and duration of lactation is timed to coincide with the demands of the progeny. BF is regulated by neuroendocrine phenomena; many of the hormones controlling pregnancy and parturition also play an important role in the establishment and maintenance of lactation as shown in Figure 7 (Hooley & Findlay, 1981). There are three important phases in lactation: mammogenesis, lactogenesis and galactopoieses. The transition from one lactational phase to another coincides with a percentage alteration in various hormones. Mammogenesis is the development and proliferation of the mammary glands in preparation for milk secretion. It covers from the pre-pubertal stage to the post-gestational stage and development occurs by the effect of estrogen and progesterone (Asociación Española de Pediatría, 2015). Lactogenesis is divided into two stages. Stage I start at the beginning of pregnancy; the placenta secretes estrogen and progesterone which initiate the physical development of the breasts. Stage II is the beginning of milk secretion and occurs immediately after delivery. Estrogens and progesterone drop sharply, and prolactin levels increase. Milk secretion requires the stimulation of other hormones such as growth hormone, cortisol, parathyroid hormone and insulin (Pascual, 2017). Last, galactopoiesis is the maintenance of milk secretion. The mechanism begins with the sectioning of the baby by stimulating the sensitive receptors of the nipple, triggering the sectioning of prolactin and oxytocin (Palafox & Ortega, 2007). Prolactin stimulates milk synthesis and production. The amount secreted is proportional to the number of times the nipple is stimulated

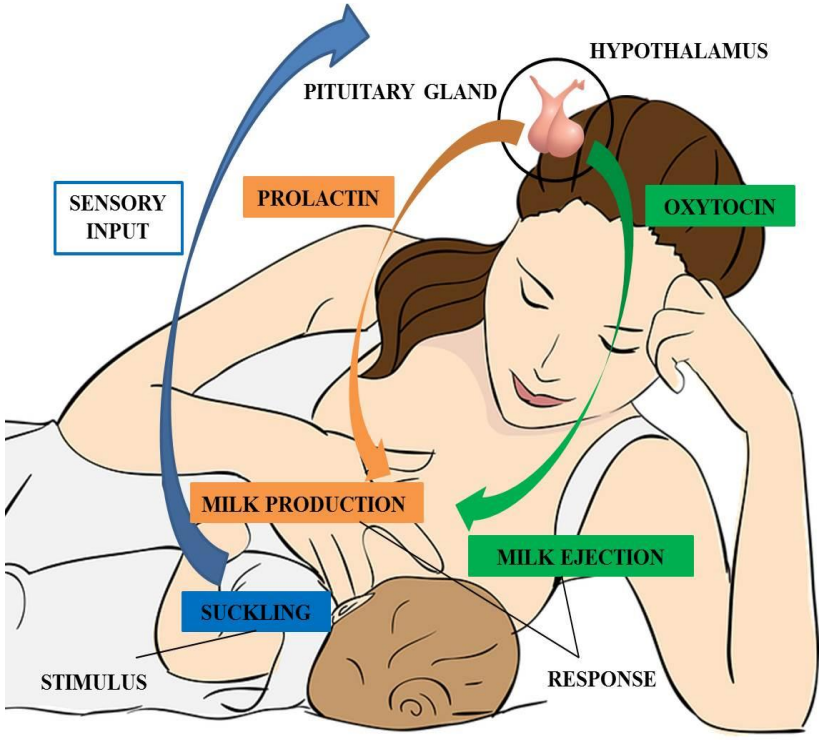


Figure 7. Physiologic control of lactation (created by Barreiro, Rocío).

Breast milk is often described as the "gold standard" of infant nutrition since in its composition it contains all the macronutrients and vitamins required for the optimal development of the suckling infant. In addition, human milk is capable of releasing antibodies

immunizing the baby against diseases being not only as a nutrition source but also as a manner of adapting infants to their extra-uterine environment. Breast milk is a system that is structured in three phases: emulsion phase (oils, fats, free fatty acids, vitamins), suspended phase (casein, calcium, phosphorus) and solution fraction (carbohydrates, proteins, enzymes, hormones some vitamins and minerals) (Lozano de la Torre, 2010). Depending on the lactation stage, the milk produced is different and varies in its composition. The colostrum is the first and most important food for the baby with which it feeds around the first five days, adapts to the needs of the newborn and facilitates the elimination of meconium avoiding neonatal jaundice (Zamora & Cañamero, 2008). It is a thick yellow liquid due to its carotene content and its composition is very different from mature milk. The 87.2% of its volume is water and has a pH of 5.45 favoring gastric emptying and it is considered as an anti-infective agent and destructive to bacteria and viruses (Zamora & Cañamero, 2008). The milk that is produced from the fourth day until approximately the 14th day is known as transitional milk. It is characterized by an increase in the content of lactose, fat, cholesterol, phospholipids and hydrosoluble vitamins while the content of proteins, immunoglobulins and liposoluble vitamins decrease due to the fact that they are diluted due to the increase in production volume (Zamora & Cañamero, 2008). At this point, milk begins to have its white color due to the emulsification of fats and the presence of calcium caseinate. From the third week of lactation and up to more than two years of breast milk is known as mature milk. Its composition is very different from colostrum with a change in pH from ~6 to 7.

Breast milk is characterized by a low level of protein and are divided into: 70% of whey proteins (alpha-lactoalbumin , lactoferrin , immunoglobulin A, lysozyme) and 30% of caseins. The level of carbohydrates is high with lactose being the main component providing 40% of the caloric value (Palafox & Ortega, 2007). It also has more than 200 oligosaccharides (the synthesis of which are determined by the maternal genotype) that function as growth factors of Bifidobacterias. Women's milk contains higher concentrations of β-carotene and vitamin E, but it is deficient in Vitamin D and K. The concentration of most minerals in human milk is not significantly affected by the maternal diet (Sánchez et al., 2020). The iron that is absorbed by 50% (much more than cow's milk) can be found attached to seroproteins. The calcium-phosphorus ratio is 2:1, so 75% of ingested calcium is absorbed. Human milk also contains zinc, fluoride (although not very high in breast milk) is useful in preventing cavities and magnesium that maintains a very stable balance with calcium to prevent hypocalcemia in the newborn (García-López, 2011). Nitrogen compounds include amino acids such as taurine, carnitine, glutamic acid, cystine and glutamine.

Lipids are the main energy source for the infant; mature milk provides for 50% of the energy intake in the fat form. Lipids are transported within the fat globule whose membrane is composed mainly of proteins, phospholipids and cholesterol. Inside of the fat globule is composed mainly of triacylglycerols of which LC-PUFAs account for up to 88 %. The major fatty acids in breast milk are palmitic acid and OA the former heavily concentrated in the 2-position and the latter in the 1- and 3-positions of the triglyceride (Ballard & Morrow, 2013; R. Barreiro, Regal, López-Racamonde, Cepeda, & Fente, 2018). It is also an important source of EFAs (LA and ALA); these fatty acids are converted in LC-PUFAs such as DHA, vital in the structural and functional development of the infant's visual-sensory, perceptual and cognitive systems; and AA useful as a substrate for the synthesis of eicosanoids such as prostaglandins, leukotrienes and thromboexanes, which modulate inflammatory and immune responses by activating the proliferation of lymphocytes, the production of cytokines and IgE in non-inflammatory cells (García-López, 2011). In addition to these nutrients, breast milk also contains other substances that can exert subtle control over metabolism, from cell division to

the child's behavior: hormones (oxytocin, prolactin, prostaglandins, insulin...), numerous nucleotides and multiple enzymes with different functions (Grosvenor, Picciano, & Baumrucker, 1993; Millán Jiménez, 2005).

1.3.2.1 Metabolism of breast milk fatty acid

FAs in human milk are derived from, maternal body reserve, endogenous synthesis in the mammary gland and from maternal plasma. FAs up to 14:0 originate from de novo synthesis in the breast, whereas FAs with a chain length greater than 14:0 originate from the maternal diet or body store (Sosa-Castillo, Rodríguez-Cruz, & Moltó-Puigmartí, 2017). *De novo* synthesis is the metabolic pathway that converts dietary carbohydrates into FAs that, once esterified, are stored in adipose tissue as an energy reservoir in the form of triacylglycerides. The final product of this reaction is palmitic acid (16:0) and these reactions take place in the multienzymatic complex of FAs synthetase. Once 16:0 is released from the complex, it can be elongated to stearic acid (18:0) (FAO, 2010). In animal tissues the desaturation of SFAs synthesized *de novo* is stopped by the formation of n-9 MUFA by Δ9-desaturase (very active in mammals) with OA being the main product (Figure 8).

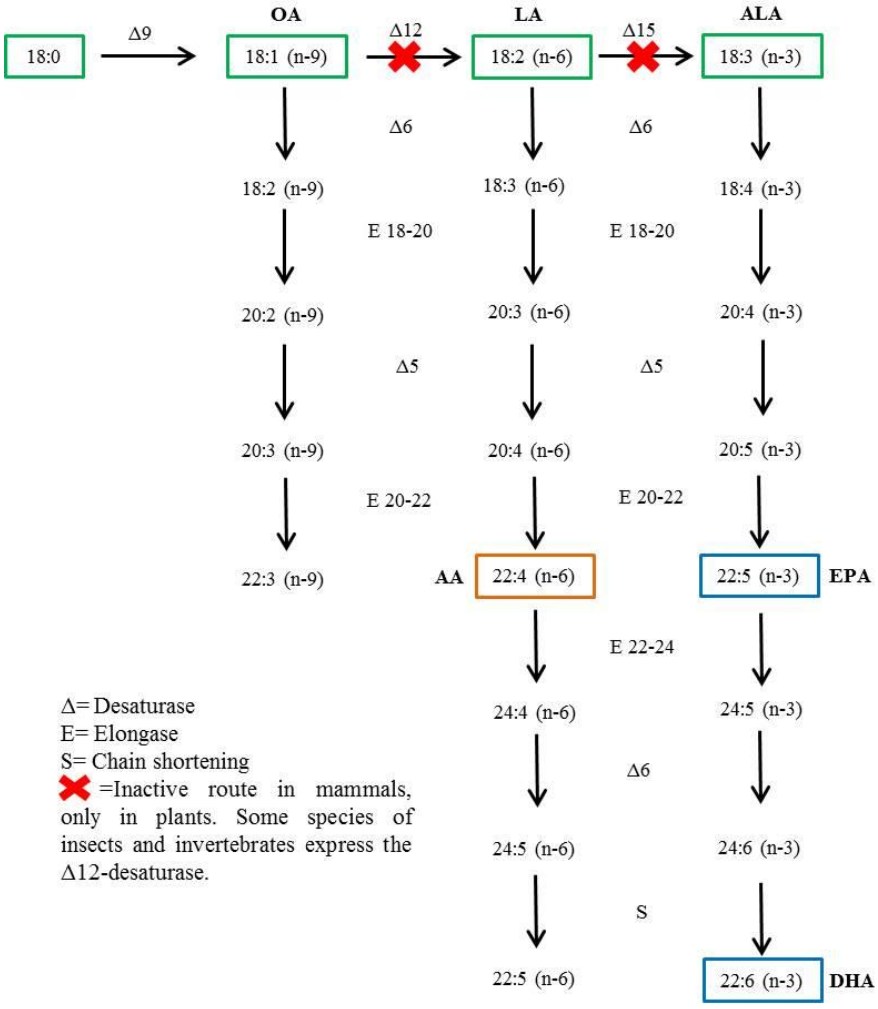


Figure 8. Metabolic pathways for the transformation of dietary OA, LA and ALA into long-chain PUFAs. Chain shortening is produced by a ß-oxidation. Created by Barreiro, Rocío.

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Although mammals can easily introduce double bonds at position Δ9, they cannot introduce additional double links at position Δ10 because mammalian cells lack Δ12 and Δ15 desaturase enzymes. For this reason, the n-6 and n-3 PUFAs family are considered as EFAs and depend on diet. Once obtained EFAs (LA and ALA) from the diet, they can be converted into LC-PUFA through a series of alternate reactions of desaturation and elongation. The routes for LA and ALA are independent and there is no cross-reaction even though and they use the same enzymes. Although humans and animals have the ability to transform ALA into EPA and DHA, the yield of this transformation is low, so only high levels of these FAs are reached in blood or breast milk when incorporated as such into the diet.

1.3.2.2 Factors affecting breast milk fatty acid composition

Various factors such as lactation period, sex of the baby, body composition of the mother, etc., can influence the fatty acid composition of breast milk (Figure 9). Mother´s dietary fatty acid consumption during pregnancy and lactation time, influence maternal-to-infant FAs transfer, before (via placenta) and after (via human milk) birth with both beneficial and adverse effects on infant development. FAs are important source of metabolic and storage energy; during pregnancy, placental transfer of fatty acids underpins the growth and development of the foetus facilitated by membrane transporters (Lewis, Childs, & Calder, 2018). The most important FAs that should not be missing in the diet are EFAs and LC-PUFAs. These FAs play important roles during gestation, lactation and infancy as these periods are associated with rapid growth and tissue development. The needs of EFAs and especially AA and DHA are especially high in the last trimester of pregnancy since human brain growth is at peak velocity in this period and first few months after birth (Innis, 2007). The retina also has an exceptionally high content of DHA; most of the LC-PUFAs present in this tissue are deposited between the 24th week of gestation and birth (Gil & Gil, 2004).

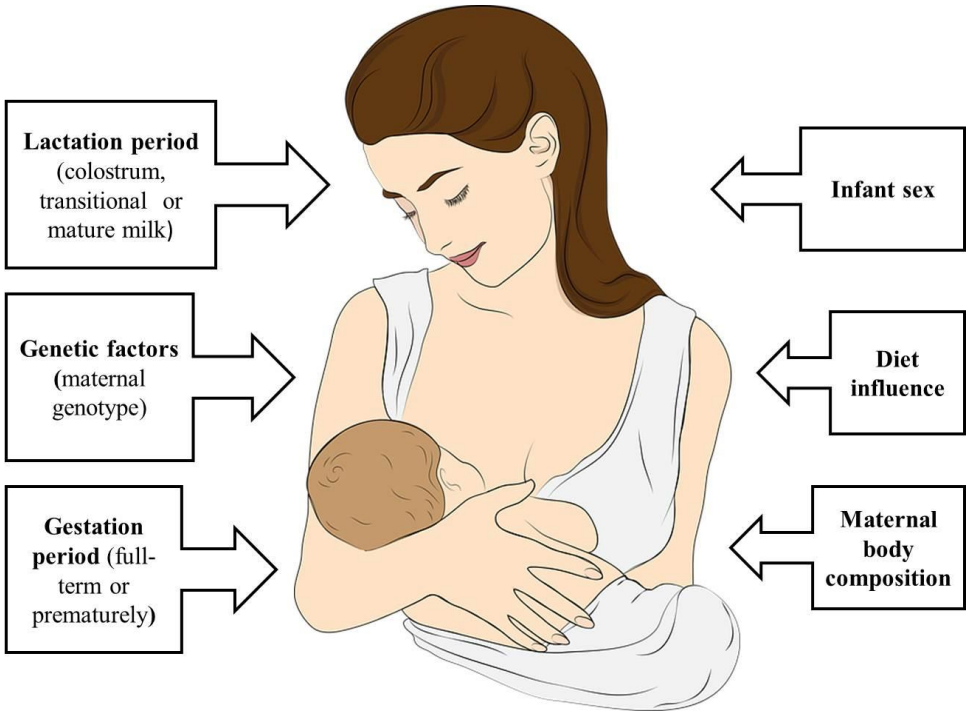


Figure 9. Maternal factors that influence human milk fatty acid composition (created by Barreiro, Rocío).

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The breastfeeding mother must take care of her diet, which will be varied and balanced. Blue fish provide LC-PUFAs such as EPA and DHA. AA can be found in the egg yolk and fat of seed-fed animals. It is advisable to increase the intake of carbohydrate-rich foods such as potatoes, legumes and cereals. The fat intake should be rich in EFAs and vitamin E, so many nuts and seed oils are rich in LA and ALA. It is recommended to increase the intake of vitamins, meat and dairy products are rich in vitamins B1 and B2, vegetables and fresh fruit that provide beta-carotene, folic acids and vitamin C and fiber. Milk and dairy products is the main source of vitamins A and D and calcium, this mineral is an essential component of breast milk while avoiding the demineralization of the mother (Zamora & Cañamero, Abril 2008). Human milk FAs change with the lactation period, breast milk does not remain static over time but matures to meet the baby's needs (R. Barreiro, Díaz-Bao, Cepeda, Regal, & Fente, 2018). Breast milk always provides energy and EFAs even beyond one year of lactation (R. Barreiro, Regal, López-Racamonde, Cepeda, & Fente, 2020). Certain genetic variations (genes mutations) were recently reported to affect human milk composition or human milk supply. These variations were associated with changes in human milk components as: zinc-deficient, higher levels of unmetabolized folic acid, significantly lower levels of 14:0, AA and EPA (Golan & Assaraf, 2020). The fatty acid profile of human milk from mothers delivering preterm babies is not specifically adapted to meet their infants' long chain PUFAs needs (Genzel-Boroviczényy, Wahle, & Koletzko, 1997). Breast milk FAs composition differed between overweight and normal weight women by a higher level of SFAs and lower level of n-3 PUFAs (Mäkelä, Linderborg, Niinikoski, Yang, & Lagström, 2013). The sex of the infant is one of the important factors affecting the composition of mother's milk. Breast milk for female infants is richer in fat than for males (Hosseini, Valizadeh, Hosseini, Khatibshahidi, & Raeisi, 2020).

1.3.3 Bottle feeding: infant formulas

Despite all the benefits of BF, there may be certain cases where this practice is not possible: medical problems of the mother leading her to take medication that can pass into breast milk, specific needs of the baby that require an specialized formula (allergies or intolerances), social or cultural reason or the mother simply decides not to breastfeed the baby (Green Corkins & Shurley, 2016). In this context, infant formulas (IF) are designed to supply a complete nutrition for the baby, mimic as closely as possible breast milk composition. Thus, IF is defined as a food for infants that is suitable to replace all or part of human milk, satisfying the normal nutritional needs of the infant (R. Barreiro et al., 2018b).

1.3.3.1 Legislation and composition of infant formulas

In the 19th century, the composition of one of the first IF was made from a mixture of wheat flour, barley, cow's milk and potassium bicarbonate. At the same time Von Bunge published the first analysis of mother's milk and cow's milk composition, knowing then the differences between the two milks. With this information it was possible to begin to modify the formulas to bring them as close as possible to breast milk: dilute cow's milk to reduce the content of proteins and electrolytes or add sugar to increase the content of carbohydrates (Hernández, 2016). Already in the 20th century, the industry began to make qualitative and quantitative changes to cow's milk thanks to the knowledge that was acquired on the composition of breast milk, so it was necessary the emergence of regulatory standards for the manufacture of milk formulas. Thus, between 1977 and 1991, the Nutrition Committee of European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN), developed the characteristics of artificial formulas. The different regulations governing the nutritional composition of the different dairy preparations have been established taking as a

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reference the average nutrient content of breast milk. The diverse international organizations draw up their own reference tables for the infant formula formulation: American Paediatric Association (APA), Food and Drug Administration (FDA), FAO/WHO and the European Commission. All these recommendations are modified and updated periodically in the light of scientific progress about the nutritional needs of the infant.

In European countries, the Commission Delegated Regulation (EU) 2016/127, supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council, regulates the essential composition of infant formula and follow-on formula must satisfy the nutritional requirements of infants in good health as established by generally accepted scientific data (EU, 2015). It defines processing standards and criteria for composition (including, where necessary, minimum, and maximum levels), labelling, advertising, information, donations, distribution and marketing. It also fixes precise terms, prohibitions, limitations, health registration conditions, penalty system, composition criteria and reference values for the labelling of nutritional properties. It is important to highlight the addition of nucleotides in 1999 and LC-PUFAs in 2002 opening a new era for the development of infant formulas (Martinez & Ballew, 2011). In the United States, the FDA provides as well nutritional and safety standards for infant formulas (FDA, 2014).

Table 4. Recommendations for the composition of a general infant formula and specifically follow-on formula (information gathered from Koletzko *et al.*, 2005, and Koletzko *et al.*, 2013).

Component	Unit	Infant formula	Follow-on formula
Energy	kcal/100ml	60-70	60-70
Proteins			
Cows´ milk protein	g/100kcal	1.8-3	1.7-2.5
Soy protein isolates	g/100kcal	2.25-3	2.1-2.5
Total carbohydrates	g/100kcal	9.0-14.0	9.0-14.0
Lipids			
Total fat	g/100kcal	4.4-6.0	4.4-6.0
Linoleic acid (LA)	g/100kcal	0.3-1.2	0.3-1.4
α-linolenic acid (ALA)	mg/100kcal	50-NS	50-NS
Ratio linoleic/ α-linolenic acids		5:1-15:1	5:1-15:1
Lauric + Myristic acids	% of fat	NS-20	NS-20
Trans fatty acids	% of fat	NS-3	NS-3
Erucic acid	% of fat	NS-1	NS-1

In 2005, at the request of the FAO Codex Alimentarius Commission, the Federation of International Societies of Pediatric, Gastroentrology, Hepatology and Nutrition (FISPGHAN) coordinated an International Expert Group (IEG) in nutrition that defined the new recommendations for formula composition standards (Koletzko et al., 2005; Koletzko et al., 2013). Decrease the caloric density of formulas (the energy expenditure of children is lower than previously thought and would lead to greater weight gain) and it sets the ranges for proteins, carbohydrates and lipids. A review carried out by a new group of experts belonging to FISPGHAN stipulates the recommendations for the composition of follow-on formulas, intended for infants over six months of age and which can also be used in infants over one year of age (Koletzko et al., 2013). These recommendations for infant formula and specifically follow-on alternatives are presented in Table 4.

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1.3.3.2 Classification and types of infant formulas

Infant formulas can be classified according to their nature and origin of the protein. Thus, three major classes can be found: formulas based on cow's milk (casein), based on protein concentrate (soy-based formula), when the infant suffers from allergy to cow's milk protein mediated by IgE, galactosemia, primary or secondary lactose intolerance, or animal protein-free diets for religious or ethical reasons and based on protein hydrolysates (specialized formula) for babies and infants with a cow and soy protein allergy (Martin, Ling, & Blackburn, 2016). Cow's milk is the basis for most infant formulas. This milk is skimmed and diluted to make it more like human because, the composition is very different between there; cow's milk contains higher levels of SFAs, protein and minerals as calcium. In addition, vegetable oils, minerals, vitamins, essential fatty acids, and iron are added for consumption by most healthy full-term infants. IF should only contain components and amounts necessary for a nutritional purpose or another benefit (Koletzko et al., 2005).

IF can be found on the market in three different ways: ready to take, it does not have to be mixed or measured; it can be given to the infant without any previous manipulation. Concentrated liquid formula, it requires mixing equal parts of water and formula and powdered formula, it is the most economical as it lasts nine months once a package is opened and the exact amount can be prepared when needed. With powdered formulas a series of precautions must be taken, first of all hygiene in the parts of the baby bottle and by the user who reconstitutes the formula. Second, the water used to reconstitute the formula must be boiled and finally, it is important to feed the baby in the shortest possible time and in case of leftovers, discard any unused formula. Despite these precautions, in recent years, IF have been the subject of controversy especially since 2004 where the Chinese market suffered from string events: counterfeit IF in 2004, melamine adulteration (that caused about 300,000 clinical cases, including six deaths) in 2008, aflatoxin (a human carcinogen) and mercury contamination in 2012 (Adelman & Schmeiser, 2018). IF are also subject to chemical contaminations either during powder processing or of the cow's milk from which it is derived (Boué et al., 2018b). The addition of tap water (microbiological and chemicals exposure) (Boué et al., 2018a) it also a source of contamination as is the unappropriated use of bottles containing bisphenols or phthalates (Boué et al., 2018b). Although Community legislation determines the composition of foods for infants, it does not detail in depth all aspects of their composition. As in all general nutrition, scientific advances and commercial pressure have led to many novelties and changes in IF.

In general, infant milks are divided into three categories according to infant age. Food products intended for infant feeding from birth to 4 or 6 months of age was denominated first-stage formulas (FSF). This milk will meet the nutritional needs of the new-born on its own as it is the only food it will receive so it should resemble breast milk as much as possible. FSF are characterized by an energy capacity of 65-75 kcal/100 ml, with a proportion of 50-55% fat, 5% protein and 30-35% carbohydrates (García Barrionuevo, 2009). Other components, such as taurine, prebiotics, nucleotides and LC-PUFAs should also be added. Follow-on formulas (FOF) are used from 4-6 months to 1 year of age. These formulas are not going to be the only food for the infant, but they will be the indispensable complement to the complementary food. It composition are less complex than those of FSF since the physiological maturity of the infant is now greater and do not require as many modifications as a starting milk (Salinas, 2002). FOF have higher energy density through the addition of carbohydrates, higher protein content, more carbohydrates variety and higher content of some minerals. Some trademarks continue to add probiotics and prebiotics, nucleotides and LC-PUFAs to their composition. Finally, the growing-up formulas (GUF) are partially modified

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FOF intended for children from 12 to 36 months of age and are an option as an intermediate step between these FOF and the introduction of cow's milk. Just as FSF and FOF have regulations on their composition and labelling, the growing milks do not have a specific guideline to regulate them (García Barrionuevo, 2009). Normally are thus supplemented with vitamins, trace elements and iron (an essential nutrient in this step to maintain haemoglobin values in the blood and to increase the body's reserves). GUF have a higher energy intake with optimized protein content and provide a more balanced lipid profile compared to cow´s milk. Some formulas incorporate LC-PUFAs on their composition.

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2 OBJECTIVES

Lipids are important nutritional components of mammalian milk due to their fundamental role in the development and evolution of the progeny. They provide energy and many essential nutrients for the newborn animal, as it is the case of fatty acids. Milk fat it is also the easily and more rapidly modifiable fraction of milk, as it can vary upon many endogenous and exogenous of the lactating female. In humans, maternal diet plays an important role in varying the fatty acid lipid profile nourishing the foetus and the newborn baby with OA, EFAs and LC-PUFAs, the latter being very important FAs in brain and retina development of the baby. In this context, humans are the only species who consume milk from other species. Bovine milk is the most consumed alternative in western populations so it's important to know what factors can vary its fatty acid profile. Besides, cow´s milk is also used as the main component for infant formulas and, although it is part of a balanced diet in children and adults, it is necessary to add certain fatty acids to its composition to mimic breast milk.
The leading hypothesis of this Doctoral Thesis is that human milk is the ultimate personalized food for infant humans, providing a very particular fatty acid profile that does not match that of composition of bovine milk and/or formulas.
Based on all the above, the objectives of this Doctoral Thesis are described below.

Objective 1
Assessment of breast milk fatty acids over lactation and dietary modifications.

1.1 Determination of the influence of maternal diet on breast milk fatty acid profiles.
1.2 Determination of the influence of lactation time on breast milk fatty acid profiles.

Objective 2
Assessment of fatty acids in infant formulas manufactured from bovine milk.

2.1 Evaluation of infant formulas as alternative or complement to breastfeeding, in terms of fatty acid composition.

Objective 3
Assessment of bovine milk as source of fatty acids for human nutrition under different endogenous and exogenous factors.
3.1 Determination of the impact of bovine pregnancy on the fatty acid profile of milk.
3.2 Determination of the impact of recombinant bovine somatotropin (rbST) on the fatty acid profile of milk.

Objective 4
Comparison of the fatty acid contribution of bovine and maternal milk to humans and assessment of their adaptation to specific dietary requirements.

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3 METHODOLOGY

A brief description of the methodology used to develop the Doctoral Research Project that resulted in this Doctoral Thesis, entitled "Fatty acids contribution of bovine and maternal milk to humans: assessment and influential factors", is presented below.

3.1 ANALYTICAL METHOD FOR FATTY ACID ANALYSIS OF MILK

Methanol, sulfuric acid, isooctane, water, *n*-hexane, and sodium sulfate anhydrous were purchased from Merck (Darmstadt, Germany). Standard mixtures of fatty acid methyl esters "F.A.M.E. mix, C4:0 to C24:0" and "PUFA No. 1, marine source", "Linoleic acid methyl ester, cis/trans-isomers" mixture, individual fatty acids (cis-9,trans-11 CLA and trans-10,cis-12 CLA isomers), and internal standard tricosanoic acid (C23:0) were obtained from Sigma Aldrich (Madrid, Spain). All samples were analysed either freshly, or after no more than four weeks stored under −25 °C. Briefly, 10 µL of milk (bovine or human) was mixed with 2 mL of H_2SO_4 (2.5%) in methanol for digestion overnight and incubated at 60 ºC for two hours for fatty acid methylation. Fatty acid methyl esters (FAMEs) in *n*-hexane were analysed using gas chromatography with a flame ionization detector (6850 GC system from Agilent Technologies, Palo Alto, CA, USA). The capillary column was a DB-Was from Agilent Technologies, Inc. (Santa Clara, CA, USA). Data were collected by integrator Software GC ChemStation version B.03.02 (Agilent Technologies). Individual fatty acids were identified, and percentages of fatty acids by weight were calculated by dividing the peak area for a particular fatty acid by the total sum of the peak areas for all identified fatty acids.

The total of saturated fatty acids (SFAs) resulted from the sum of the individual saturated fatty acids: C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, and C24:0. For the total monounsaturated fatty acids (MUFAs), the included fatty acids were C14:1n-5, C16:1n-9, C16:1n-7, C16:1n-5, C16:1n-13t, C17:1n-9, C18:1n-9, C18:1n-7, C20:1n-11, C20:1n-9, C22:1n-11, and C22:1n9 and for the total of polyunsaturated fatty acids (PUFAs) were included C18:2n-6, C18:2n-6 9-12t, C18:2n-6 9t-12, CLA18:2n-7 9t-11t, CLA18:2n-6 10t-12, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C18:3n-3, C18:4n-3, C20:3n-3, C20:4n-3, C20:5n-3, C22:5n-3, and C22:6n-3.

3.2 STATISTICS

Univariate statistics were performed using PASW Statistics 18.0 (SPSS Ibérica, Madrid, Spain) and/or GraphPad Prism 9 (San Diego, CA, USA). Multivariate analysis was performed by means of SIMCA-P+12.0 (Umetrics AB, Sweden) software, or GraphPad Prism 9 (San Diego, CA, USA).

3.3 HUMAN DATA COLLECTION TOOLS

Participants were recruited in collaboration with a local midwifery service and a Spanish breastfeeding association, between April 2016 and May 2018. Each volunteer completed a questionnaire providing information on age and weight at the point of sample collection, height, gestational weigh gain, infant birth weight, delivery, and lactation details (occurrence of mastitis, delivery/gestational problems, tandem breastfeeding), number of children and gender, current medication, life habits (smoke, alcohol consumption), and socio-demographic

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factors (nationality, residency, employment). A food frequency questionnaire with more than 60 items adapted to the target population was used to collect dietary data.

3.4 ETHICAL CONSIDERATIONS AND FINANCIAL SUPPORT

3.4.1 Breast milk samples

This work belongs to a broader cross-sectional study aimed at evaluating the breast milk composition of lactating mothers living in the northwest of Spain. The study protocol was approved by the Galician Clinical Research Ethics Committee (approval code 2016/280), and it is registered in ClinicalTrials.gov with identification number NCT03245697. It adhered to the principles of the Helsinki Declaration of 1975, as revised in 1983. Written informed consent was obtained from all participants.

3.4.1 Bovine milk samples

All procedures with dairy cattle were performed respecting animal welfare and causing no more pain, suffering, distress, or lasting harm than the equivalent to that caused by the introduction of a needle in accordance with good veterinary practice. As stated in EU guidelines and national laws on animal experiments, in particular, Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, article 1, point 5, the bovine animals involved in this thesis cannot be considered experimental animals as they were subject to non-experimental common clinical veterinary practices.

Financial support was received from the Spanish Innovation Program “Programa Estatal de Investigación, Desarrollo e Innovación Orientada a los Retos de la Sociedad” (project AGL2014-58881-R).

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4 RESULTS

The Doctoral Thesis entitled “Fatty acids contribution of bovine and maternal milk to humans: assessment and influential factors” is structured in four main objectives, with their results presented below.

4.1 Results of Objective 1.

Assessment of breast milk fatty acids over lactation and dietary modifications: 2 original articles.

4.2 Results of Objective 2.

Assessment of fatty acids in infant formulas manufactured from bovine milk: 1 original article.

4.3 Results of Objective 3.

Assessment of bovine milk as source of fatty acids for human nutrition under different endogenous and exogenous factors: 2 original articles.

4.4 Results of Objective 4.

Comparison of the fatty acid contribution of bovine and maternal milk to humans and assessment of their adaptation to specific dietary requirements.

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4.1 RESULTS OF OBJECTIVE 1: ASSESSMENT OF BREAST MILK FATTY ACIDS OVER LACTATION AND DIETARY MODIFICATIONS.

4.1.1 Determination of the influence of maternal diet on breast milk fatty acid profiles.

4.1.2 Determination of the influence of lactation time on breast milk fatty acid profiles.

Results of objective 1 are presented in two original articles, as presented hereafter.

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Fatty acid composition of breast milk in Galicia (NW Spain): A cross-country comparison.

Reference: Barreiro, R., Díaz-Bao, M., Cepeda, A., Regal, P., Fente, C.A. (2018). Fatty acid composition of breast milk in Galicia (NW Spain): A cross-country comparison. *Prostaglandins Leukotrienes and Essential Fatty Acids*, 135, 102-114.

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Author Contributions: AC, PR and CAF designed the study; RB and PR conducted experimental part; RB, PR and MDB analyzed data and managed literature searches; RB and PR wrote the paper and PR had primary responsibility for final content. All authors read and approved the final manuscript.

Quality scores:
Cited by 20 documents (Scopus 30/09/2022)
Corresponding author: Regal, P.
Document type: Article
Impact factor (IF) JCR, category in the year of publication (2018): 2.86, T2, Q3 (Biochemistry & Molecular Biology)
DOI: 10.1016/j.plefa.2018.06.002
ISSN: 09523278

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Conflicts of Interest Statement: The authors declare no conflicts of interest.

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Abstract

Based on the premise that the fatty acid composition of human milk can be substantially altered by diet, the current study investigated the fatty acid profile (fattyacidome) of breast milk in Galicia, a small region located in the north-west of Spain and characterized by the Southern European Atlantic Diet (SEAD). A cross-country comparison was also performed to assess worldwide variety and diet impact, reviewing the profiles reported various European, North and South American, Asian and African countries and Australia. Galician human milk appeared similar to the rest of Europe, with some particular features related to the SEAD (dairy, pork, beef and sunflower and olive oils), such as relatively high levels of linoleic acid and lower α-linolenic acid. The results also showed the existence of woman-specific profiles and significant changes over lactation in some fatty acids. Worldwide, the fatty acid profiles were similar, with the clear exception of Asiatic breast milk. The impact of fatty acids on infant health warrants further investigation.

Access to full article through its Digital Object Identifier:
https://doi.org/10.1016/j.plefa.2018.06.002

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Evolution of breast milk fatty acids in Spanish mothers after one year of uninterrupted lactation.

Reference: Barreiro, R., Regal, P., López-Racamonde, O., Cepeda, A., & Fente, C. (2020). Evolution of breast milk fatty acids in Spanish mothers after one year of uninterrupted lactation. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 159, 102-141.

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Author Contributions: AC, PR and CAF designed the study; RB and PR conducted experimental part. OLR sample collection and data analysis. RB and PR wrote the paper and PR had primary responsibility for final content. All authors read and approved the final manuscript.

Quality scores:
Cited by 2 documents (Scopus 30/09/2022)
Corresponding author: Regal, P.
Document type: Article
Impact factor (IF) JCR, category in the year of publication (2020): 4.0, T2, Q3 (Biochemistry & Molecular Biology)
DOI: 10.1016/j.plefa.2020.102141
ISSN: 09523278

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Conflicts of Interest Statement: The authors declare no conflicts of interest.

Abstract

The WHO recommends exclusive breastfeeding of infants for the first sixth months of life and advises that it shall continue for up to two years of age or beyond in combination with complementary foods. However, the image of a woman breastfeeding a toddler or a preschooler is unusual in western societies. Exploring the nutritional properties of milk during prolonged lactation can help normalizing prolonged breastfeeding. Human milk fatty acid composition was determined in sixteen lactating mothers practicing prolonged lactation (≥12 months) and sixteen women on their first twelve months of lactation. Breast milk after one year is richer in saturated fatty acids, particularly lauric and myristic, showing a tendency towards lower levels of oleic acid, and higher of arachidonic, α-linolenic and docosahexaenoic acids, in comparison to early milk (< 1 year). The age and body condition of the mother, parity, sex of the baby, and diet influence also the *fattyacidome* of milk.

Access to full article through its Digital Object Identifier:
https://doi.org/10.1016/j.plefa.2020.102141

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4.2 Results of Objective 2: Assessment of fatty acids in infant formulas manufactured from bovine milk

4.2.1 Evaluation of infant formulas as alternative or complement to breastfeeding, in terms of fatty acid composition.

Results of objective 2 are presented in one original article, as presented hereafter.

Comparison of the fatty acid profile of Spanish infant formulas and Galicia women breast milk

Reference: Barreiro, R., Regal, P., López-Racamonde, O., Cepeda, A., & Fente, C. A. (2018). Comparison of the fatty acid profile of Spanish infant formulas and Galician women breast milk. *Journal of Physiology and Biochemistry*, *74*(1), 127-138.

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Author Contributions: AC, PR and CAF designed the study; RB and PR conducted experimental part and formulas collection. OLR volunteer recruitment. RB and PR wrote the paper and PR had primary responsibility for final content. All authors read and approved the final manuscript.

Quality scores:
Cited by 12 documents (Scopus 30/09/2022)
Corresponding author: Barreiro, R.
Document type: Article
Impact factor (IF) JCR, category in the year of publication (2018): 2.5, T2, Q2 (Physiology)
DOI: 10.1007/s13105-017-0580-2.
ISSN: 11387548

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STROBE Statement: This is a STROBE-compliant national survey-based cross-sectional observational study.

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Conflicts of Interest Statement: The authors declare no conflicts of interest.

Abstract

The importance of dietary lipids during childhood is evident, as they are necessary for correct growth and development of the newborn. When breastfeeding is not possible, infant formulas are designed to mimic human milk as much as possible to fulfill infant's requirements. However, the composition of these dairy products is relatively constant, while human milk is not a uniform bio-fluid and changes according to the requirements of the baby. In this study, breast milk samples were donated by 24 Spanish mothers in different lactation stages and different infant formulas were purchased in supermarkets and pharmacies. Gas chromatography coupled to flame ionization detection was used for the fatty acid determination. Compared to breast milk, first-stage formulas are apparently very similar in composition; however, no major differences were observed in the fatty acid profiles between formulas of different lactation stages. The Galician women breast milk has a fatty acid profile rich in oleic acid, linoleic acid, arachidonic acid, and docosahexaenoic acid. When comparing human milk with formulas, it becomes evident that the manufacturers tend to enrich the formulas with essential fatty acids (especially with α-linolenic acid), but arachidonic and docosahexaenoic acid levels are lower than in breast milk. Additionally, the obtained results demonstrated that after 1 year of lactation, human milk is still a good source of energy, essential fatty acids, and long-chain polyunsaturated fatty acids for the baby.

Access to full article through its Digital Object Identifier:
https://doi.org/10.1007/s13105-017-0580-2

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4.3 Results of Objective 3: Assessment of bovine milk as source of fatty acids for human nutrition under different endogenous and exogenous factors.

Results of objective 3 are presented in two original articles, as presented hereafter.

4.3.1 Determination of the impact of bovine pregnancy on the fatty acid profile of milk.

Results are presented in one original article.

Effects of bovine pregnancy on the fatty acid composition of milk: the significance for humans needs.

Reference: Barreiro, R., Regal, P., Díaz-Bao, M., Vázquez, B. I., & Cepeda, A. (2017). Effects of bovine pregnancy on the fatty acid composition of milk: the significance for humans needs. *Food Additives & Contaminants: Part A*, *34*(4), 608-616.

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Article contribution: AC, PR and BIV designed the study; RB and PR conducted experimental part; RB, PR and MDB analyzed data and managed literatura searches; RB and PR wrote the paper and PR had primary responsibility for final content. All authors read and approved the final manuscript.

Quality scores:
Cited by 2 documents (Scopus 30/09/2022)
Corresponding author: Barreiro, R.
Document type: Article
Impact factor (IF) JCR, category in the year of publication (2017): 1.88, T2, Q2 (Food Science and Technology)
DOI: 10.1080/19440049.2016.1277270
ISSN: 19440049

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Conflicts of Interest Statement: The authors declare no conflicts of interest.

Abstract

Milk from 40 Holstein dairy cows was collected from two different farms in Galicia (Spain). The differences in the fatty acid composition of two groups of cows, 20 pregnant and 20 non-pregnant, was studied to determine whether pregnancy status is a determinant factor that can alter the fatty acid profile of milk. Gas-chromatography (GC) coupled to flame ionisation detection (FID) was used for the determination of the fatty acids. Differences in the milk fatty acids between pregnant and non-pregnant cows were pronounced showing statistically significant differences for some fatty acids and the total saturated and monounsaturated fatty acids. Milk from non-pregnant cows was lower in saturated fatty acids and higher in monounsaturated fatty acids (unlike milk from pregnant cows). The effects of the consumption of bovine milk, particularly milk fat, on human health have been studied in depth and sometimes are associated with negative effects, but milk has also several beneficial characteristics linked to some fatty acids.

Access to full article through its Digital Object Identifier:
https://doi.org/10.1080/19440049.2016.1277270

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4.3.2 Determination of the impact of recombinant bovine somatotropin (rbST) on the fatty acid profile of milk.

Results are presented in one original article, as presented hereafter.

Impact of recombinant bovine somatotropin on bovine milk composition and *fatty acidome*: a multidose longitudinal study.

Reference: Barreiro, R., Lamas, A., Miranda, J.M., Franco, C.M., Cepeda, A., Regal, P. (2022). Impact of recombinant bovine somatotropin on bovine milk composition and *fatty acidome*: a multidose longitudinal study. *Foods*, *11*(21), 3477.

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Article contribution: Conceptualization, C.M.F. and A.C.; methodology, R.B., J.M.M. and P.R.; software, A.L. and P.R.; formal analysis, R.B. and P.R.; resources, C.M.F. and A.C.; data curation, A.L. and P.R.; writing—original draft preparation, P.R. and R.B.; writing—review and editing, J.M.M., A.C. and P.R.; supervision, A.C.; project administration, A.C.; funding acquisition, A.C. and C.M.F. All authors have read and agreed to the published version of the manuscript.

Quality scores:
Cited by 0 documents (Scopus 29/11/2022)
Corresponding author: Regal, P.
Document type: Article
Impact factor (IF) JCR, category in 2021: 4.35, T1, Q2 (Food Science and Technology)
DOI: 10.3390/foods11213477
ISSN: 23048158

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Conflicts of Interest Statement: The authors declare no conflicts of interest.

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Abstract

Somatotropin is a species-specific polypeptide hormone produced in the pituitary gland of vertebrates. When administered exogenously to cattle, it can increase milk yield. However, the trade and administration of recombinant bovine somatotropin (rbST) to farm animals has been banned in the European Union (EU). Aside from food safety issues, very little is known about the effects of this hormone on milk composition and quality. In this work, a wide profile of fatty acids (the so-called *fatty acidome*) was determined by GC-FID in raw milk collected from control and rbST-treated lactating cows in a multidose longitudinal study. Milk composition (lactose, protein, fat, dry matter), including minerals (Ca, K, Mg, Na, P), was also determined, and milk yield was recorded. A tendency toward a less saturated profile was observed in the milk collected from animals treated with rbST, with higher concentrations of monounsaturated fatty acids. In addition, less calcium and potassium and more lactose and protein content were observed in milk from treated animals than in regular milk. As a result of this multicomponent profiling of milk, a clear impact of somatotropin treatment on milk quality was observed. The obtained results should be particularly interesting for those countries that permit the use of this hormone in dairy production.

Access to full article through its Digital Object Identifier:
https://doi.org/10.3390/foods11213477

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5 GENERAL DISCUSSION

Milk is part of the human diet at all stages of life particularly in Western societies. It is the first food ingested at birth from the mother's breast and the one exclusively recommended by WHO during the first six months of the child's life. After exclusive breastfeeding, complementary feeding (beikost) is included when new foods are introduced into the diet along with breast milk. This stage represents an opportunity in the acquisition of healthy habits that often remain for the rest of life (Jiménez Ortega, Martínez García, Velasco Rodríguez-Belvis, & Ruiz Herrero, 2017). It is at this stage, when humans begin to consume milk from other mammals, mainly cow's milk. Although it should not be given as a main food in children under one year, it can begin to be introduced in small amounts in the diet. According to scientific literature and following the available nutritional guidelines, an appropriate consumption of bovine milk and its derivatives may be beneficial across all age groups (except for specific medical conditions) (Marangoni et al., 2018).

Cattle have become the most numerous and economically important domestic species in the world due to their ease of milking (udder size) and high production capacity. Of the world's milk production, 81% is from cows, 15% from buffaloes and 4% from goats, sheep and camels combined. Over the next few years, world milk production is forecast to grow 1.6% per year to reach 997 million tons by 2029, indicating a faster pace than any of the other major agricultural commodities (OECD & Food and Agriculture Organization of the United Nations, 2020). Despite data and studies on milk benefits, the consumption of liquid milk has undergone a decline in recent years associated with negative health effects, mainly due to its saturated fraction and certain fatty acids (FAs) considered pro-atherogenic (lauric acid 12:0, miristic acid 14:0, palmitic acid 16:0). Thus, milk fat has been the most demonized component of dairy for its health implications but also the most studied for the benefits provided by numerous FAs (butyric acid 4:0, oleic acid OA and polyunsaturated fatty acids PUFAs). In addition, milk FAs fraction is the easiest and quickest part to modify through changes in cattle diet, specific animal situations or substances administered by human that alter its composition. The foods diet ingested, lactation period, maternal body composition among others, are also susceptible to change human milk FAs.

For these reasons, this Thesis project focused on the study of the lipid profile of the different types of milk that humans can consume throughout their live: human milk and/or formula and bovine milk. Modifications in the FAs profile that may arise due to endogenous or exogenous factors of cattle and their implications for human health have been investigated. Also, the influence of maternal diet and lactation period in the FAs profile of human milk, have been evaluated. Figure 10 shows a scheme relating different research studies presented in this Doctoral Thesis, and the factors studied that can modify the fatty acids profile of human and bovine milk.

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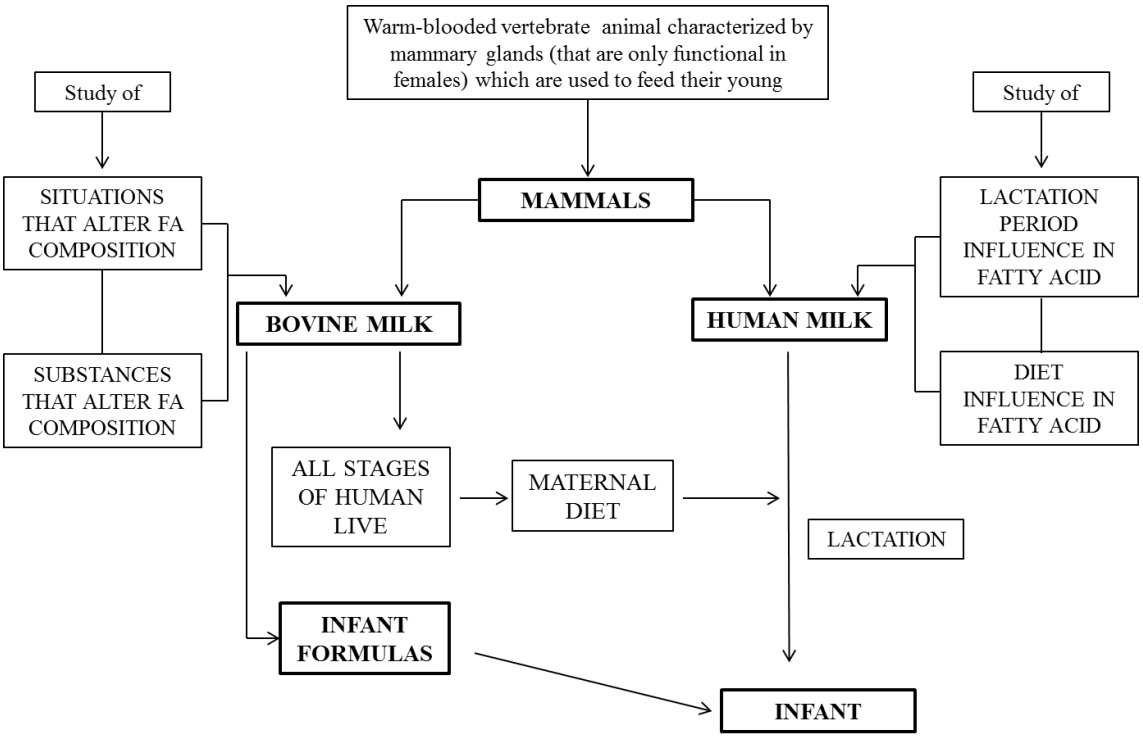


Figure 10. Diagram of the Thesis project, design, and development.

5.1 IMPORTANCE OF BREAST MILK IN HUMAN NUTRITION: FACTORS AFFECTING ITS FATTY ACID PROFILE

For all mammals, the milk secreted by females of each species is the result of 150 million years of evolution and it supplies nutrition and immunological protection to the young. Human milk is the only natural source of nutrition for a child in the first few months of life whose composition is adapted to the state of health and needs of the infant (Bobinski & Bobinska, 2022). After exclusive lactation, continuous breastfeeding is recommended until the primary two years of life due to its beneficial effects (Comité de Lactancia Materna, Asociación Española de Pediatría (AEP), 2015.). Breast milk fat supplies the infant with more than 50% of their daily energy and includes more than 200 different FAs (Robert G. Jensen, 1999). Breast milk FAs are one of the most important substances ensure the correct development of child in all of these life stages. FAs present in high concentration in human milk are OA, 16:0 and LA. Also, it an important source of LC-PUFAs as ALA, ARA and DHA related with the full development of neurological functions, beside they are a precursor to the "series 2″ eicosanoids, important metabolic biomediators, and constituting cellular structures. (Del Prado et al., 2001; Demmelmair & Koletzko, 2018). In addition, LC-PUFAs are important markers for the ratio DHA and AA in infant formula (Bakry et al., 2021).

The proportion of FAs in human milk is not constant and its composition can vary with the lactation period, mother´s diet and the health of the newborn among other factors. Two of these factors, maternal diet and lactation stages, have been studied in detail in this Thesis project to observe how they influence breast milk fatty acid profile. For this purpose, breast milk donations from Galician lactating women were collected from 2016 to 2020 at different lactation stages. These studies were reviewed and approved by the Regional Research Ethics Committee of Galicia (*Comité Autonómico de Ética da Investigación de Galicia*) and adhered

to the principles of the Helsinki Declaration of 1975 as revised in 1983. Written informed consents, different questionnaires and dietary data were obtained from all participants. In addition, breastfeeding association "Bico de Leite" from Lugo, has provided support, cooperation, and advice.

5.1.1 Variations of human milk fatty acid profile with maternal diet: a cross-country comparison

The link between fatty acid composition of maternal diet and FAs in milk is strong (Bakry et al., 2021). Recent reviews demonstrate the diverse range of implications for maternal diet on offspring health, as the combination of pre-pregnancy through to lactation dietary modifications may reduce the future potential infant health risks (Jouanne, Oddoux, Noël, & Voisin-Chiret, 2021; Marshall et al., 2021; Reynolds & Vickers, 2022). LC-PUFAs are accumulating in the brain of the foetus during the last trimester of pregnancy and first and second year of infant life. Guesnet *et al.* (Guesnet, Marmonier, Boyer, & Delplanque, 2018) conclude in their review that specific deficit intake in n-3 PUFAs during pregnancy was associated with a reduction in visual acuity in 2 months old infants, low DHA content was associated with the degree of cognitive development in 6-years-old children, and excessive dietary intakes of n-6 PUFAs promote the development of adipose tissue and obesity (Innis, 2014; Innis & Friesen, 2008). In addition, a correct n-3 LC-PUFAs intake during pregnancy may prevent the incidence of allergic diseases in children (Zhang et al., 2017).

The Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) elaborated in 2020 a report where they reviewed and updated the dietary recommendations for the Spanish population. They recommend adopting a healthy and sustainable diet characterized by a prevalence of plant-based foods and moderate animal-based foods consumption. In addition is important promoting the intake of seasonal and local products. The classic diet to follow in Spain is the Mediterranean diet, one of the most studied and well-known dietary patterns worldwide (Guasch-Ferré & Willett, 2021; Sgroi, Moscato, & Moscato, 2022). However, in the northwest of Spain, the diet followed follows an Atlantic diet model (Guallar-Castillón, Oliveira, Lopes, López-García, & Rodríguez-Artalejo, 2013). Galicia is part of this diet characterized by an abundance of seasonal foods from plants (vegetables, fruits, potatoes, cereals and bread, legumes, nuts and chestnuts), honey, high consumption of fish and shellfish and veal meat fed exclusively with breast milk and pastures. The consumption of milk is moderate and the main oil consumption is olive oil. Besides, the use of sauces with low energy load and high-quality fat and homemade desserts composed mainly of eggs, flour and nuts are common in this diet. (Leis Trabazo, R., de Lamas Pérez, C., Castro Pérez, X., & Solla, P., 2019). Atlantic diet should be considered as an excellent dietary pattern and lifestyle (Tejera-Perez, Sanchez-Bao, Bellido-Guerrero, & Casanueva, 2021).

To determine how maternal diet may influence the lipid profile of milk, we compared bibliographic data on FAs profiles from 27 different countries located in Europe, North and South America, Asia, Africa and Australia, knowing that the basic foods and diets are very different among them (Barreiro et al., 2018a). Since the number of FAs present in milk is high, multivariate statistic was used to visualize the data. To perform multiple sample comparison by multivariate modelling, all variables were log transformed and scaled (Pareto) to become uniform and suitable for the analysis. A principal components analysis (PCA) was run to obtain a general vision of the variance in the fatty acid profile of human milk.

A multivariate analysis was performed with FAs of the Galician mothers participating in the study and with the fatty acids human milk data obtained in the literature from 27 countries to observe possible differences. In a PCA plot (Figure 11 A and B) two clear groups could be

distinguished: Asian countries (west and east) grouped on one side and the rest of the countries to the other side.

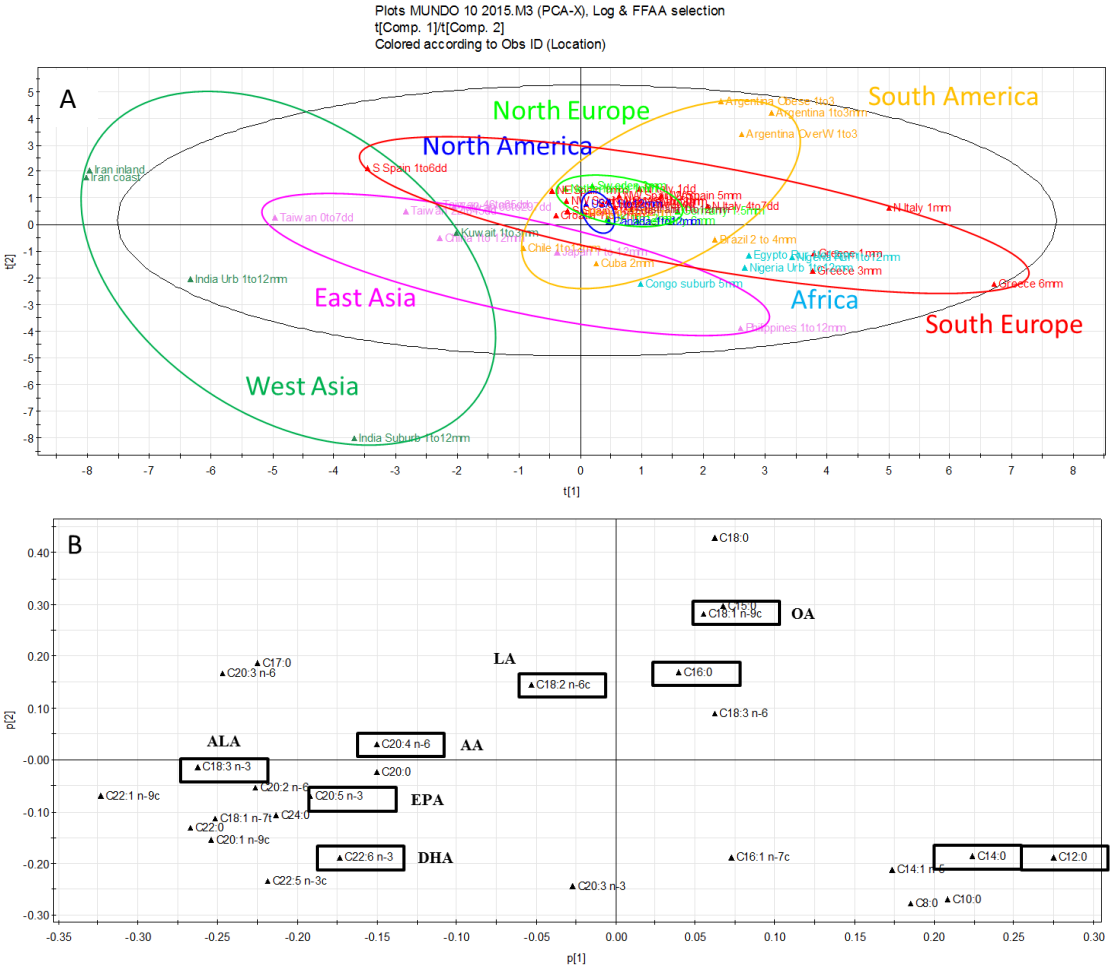


Figure 11. (A) PCA score scatter plot of fatty acid profiles of breast milk reported in this study and in the literature for different countries (B) Loading plot, the most important fatty acids were highlighted.

The FAs responsible for these differences between countries were analysed in the PCA loading plot (Figure 11-B). The influence or relative contribution of each fatty acid to the separation of the classes for the discriminant analysis was represented by this loading S-plot. The Asian countries were related to high levels of n-3 PUFAs, some particular MUFAs and were low in determinate SFAs. Countries with the highest values of saturated and n-6 PUFAs were situated on the right side of the PCA plot. This difference also coincided with the separation between countries with very different diets such as: Asian countries (India, Japan, China, and Taiwan), countries with a Mediterranean diet (Italy and Greece) and the grouping of countries that fall into the Atlantic diet (Northern Spain, Portugal, Sweden, and Netherlands). Figure 11-B the FAs present in the different worldwide diets can be related. To define why FAs are separated in this way it is necessary to compare the values of FAs between countries. A summary of some of the important FAs highlighted in Figure 11 can be seen in Table 5.

Table 5. Summary of the most distinctive FAs of breast milk profiles across different countries with characteristic diets.

Country and lactation stage	SFAs	12:0	14:0	MUFAs	OA	LA	ALA	EPA	DHA
Greece 6m	45.62	8.91	8.22	36.73	33.87	14.68	0.06	0.11	0.52
Sweden 3m	40.72	3.91	6.12	45.15	41.40	10.93	1.60	0.06	0.25
Spain NW 1-5m	40.81	6.55	6.51	40.55	34.49	14.60	0.50	0.11	0.32
Portugal 4m	43.08	5.80	6.81	31.48	26.36	16.02	0.61	0.14	0.43
Canada 1 to 12m	-	5.25	5.84	-	35.18	11.48	1.22	0.08	0.17
Argentina 1-3m	42.70	4.67	6.02	36.94	33.36	16.61	0.47	0.09	0.13
Cuba 2m	41.29	7.73	8.30	34.12	29.08	18.86	0.88	0.09	0.33
China 1.5m	35.13	4.44	4.05	36.88	33.53	22.00	2.81	0.41	0.38
Japan 1 to 12m	41.53	5.86	6.11	38.31	31.43	12.66	1.33	0.26	0.99
Taiwan 66 to 297d	-	4.03	4.44	-	27.76	23.62	1.83	0.17	0.79
Philippines 1 to 12mo	-	13.82	12.12	-	21.85	7.90	0.43	1.15	0.74
Indian Urban 1 to 12m	35.13	2.43	3.67	34.03	28.35	10.58	1.68	0.39	0.72
Egypt 1 to 12m	47.70	9.10	9.10	27.70	24.60	23.8	-	-	-
Congo 5m	52.86	10.53	13.24	28.66	24.76	13.62	1.19	0.17	0.55

Data gathered from Barreiro *et al*. (2018a); m: month, d: day, NW: north-west

The reviewed studies indicated in Table 5 reflected fine examples of the diverse dietary situations around the world and even various point of the lactation period. Asian countries showed the lowest levels of 12:0 and 14:0, but Philippines are located far from the other Asian countries. Filipino women had higher percentages of 12:0 and 14:0. The most commonly fat for cooking in Philippines is coconut fat, and for this fact might be reflected in human milk (Feranil, Duazo, Kuzawa, & Adair, 2011). Indian women were the only ones who showed large amounts of long-chain saturated fatty acids (20:0, 22:0, 24:0) although their content of total SFAs is one of the lowest. In Philippines the consumption of peanut oil is high, and particularly his oil is rich in these three SFAs this is the most likely explanation for the high values of these FAs. The highest value of SFAs corresponds to the Congo women who, as can be seen in plot 11-B, are close to 14:0 and 12:0. One possible reason is the high consumption of palm oil in this country (Thaker & Barton, 2012). High levels of OA can be considered a key feature of European breast milks; this fact is reflected in the position of these countries closely to OA in loading plot (11-B). The most consumed oil in India is mustard oil rich in erucic acid (22:1 n-9); in the plot this fatty acid is closer to this country (Roy, Dhar, & Ghosh, 2012). LA is the most relevant PUFA in Western diets for this reason all European countries are closely of this fatty acid. The highest values of LA in human milk were those of China, Taiwan and Egypt related with the consumption of different vegetable oils as soybean, sunflower and unhydrogenated cottosed oil in Egypt (Borschel et al., 1986). The highest ALA values were for China, Taiwan and India related to the consumption of oils such as mustard oil, soybean and sunflower seeds, peanut or sesame oil (Roy et al., 2012; Wu, Lau, Chen, Wu, & Tang, 2010). Linolenic acid in breast milk have been positively associated with infants' growth in the first year of life (Calvo-Lerma et al., 2022). Dietary intake of EPA and DHA is supplied by fish, especially blue oily fish, for this reason the majority of DHA was obtained in Taiwan and Japan. Highlight the higher level of EPA in India maybe related with the consumption of eggs and sources plant-based omega-3 (Roy et al., 2012).

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Table 6. Summary of dietary characteristics of Galician mothers included in the study of Barreiro *et al*. (2020), presented as frequency of food consumption (percentage of participants).

	Frequency of consumption (%)			
Food items	Seldom or never	Monthly	Weekly	Daily
Cow´s milk	81	-	11	7
Semi-skimmed milk	61	4	11	25
Skimmed milk	68	4	7	21
Vegetable drinks: soybean, oat, rice, almonds	89	1	7	3
Yoghourt (125g)	29	18	25	29
Hard and semi-firm cheeses (50g)	35	16	38	10
Chicken eggs	4	4	93	-
Chicken or turkey (skinless)	16	13	68	4
Beef, veal	11	25	64	-
Pork	14	14	71	-
Serrano ham	4	25	68	4
Cooked ham (turkey, chicken)	14	7	68	11
White fish (hake)	18	22	60	-
Oily fish (sardine/mackerel/horse mackerel)	43	39	18	-
Lettuce, escarole, raw tomato (salad)	4	7	70	20
Zucchini, cucumbers, eggplant, carrot, pumpkin	22	29	45	4
Roasted or cooked potatoes	4	18	79	-
Oranges, grapefruits, mandarins	4	11	41	44
Bananas	4	7	71	18
Apples, pears	5	18	63	14
Nuts (30g)	25	43	21	11
Lentils	11	36	54	-
White bread	19	13	43	26
Rice	11	18	71	-
Pasta	4	11	86	-
Extra virgin olive oil	-	4	33	63
Sunflower oil	54	25	21	-
Butter (20g)	43	32	14	11
Homemade cakes	18	36	43	4

Portions: milk and vegetable drinks 200ml, protein 100-150g, vegetables 100-150g, fruits 1 piece, legumes and cereals 100-150g, oils 1 tablespoon, bakery 1 unit.

With this compilation of bibliographic data and those provided in the article for Galician women, it was possible to ensure that the FAs ingested in the different foods of the diet can confer a distinguishable *fingerprint* in breast milk. Consumption of oils and different animal fats, meat and fish were strongly reflected in the fatty acid composition of human milk. Bravi *et al*. (Bravi et al., 2021) also related in their study that the FAs, in particular omega-3, are influenced by maternal dietary habits during breastfeeding. Siziba *et al.* (Siziba et al., 2021) studied changes in the lipid profile of breast milk in a cohort of lactating mothers compared with another cohort 10 years later. After that time, they were able to conclude how changes in maternal dietary habits caused changes in breast milk. Galician woman breast milk was

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characterized by an equilibrium between SFAs and MUFAs where the majority fatty acid was OA. Odd chain SFAs are a good marker of dairy (Smedman, Gustafsson, Berglund, & Vessby, 1999) and in Galicia breast milk these average levels were higher than other studies with Spanish samples. The dietary questionnaires provided by the nursing mothers participating in the study revealed a low consumption of liquid milk and vegetable drinks and moderate intake of dairy products (Table 6). Weekly the mothers consumed abundant carbohydrates (potatoes, rice, pasta, homemade cakes) and fruits (banana, apple, pear). As protein they consumed eggs, chicken or turkey and pork weekly, and they opted for white fish more than blue fish. The most consumed oil by the mothers was extra virgin olive oil. Breast milk in Galicia has a profile similar to the rest of the European countries but more different of those considered purely Mediterranean as Greece and Italy. Zhang *et al.* (Z. Zhang et al., 2022) in his recent review, found a clear difference in the human milk fatty acid profile around the world. Breast milk from Western countries had high concentration of MUFAs and OA but low concentration of PUFAs and EFAs compared with non-western countries. This difference is clearly related to the mother's diet.

5.1.2 Variations of human milk fatty acid profile with lactation period: from the first month to prolonged lactation

Human milk is not and cannot be a static fluid and as such, the fatty acid composition of breast milk varies with lactating period and geographical regions according to the requirements of the baby (Barrera et al., 2018). Despite the many benefits that breastfeeding has to children and mother during lactation period, the duration of breastfeeding in Western societies does not normally go beyond the first 6 months postpartum (Victora et al., 2016). For this reason, it is necessary the study of human milk fatty acid profile beyond this period and determine if its composition is appropriated for infant requirements.

In order to study how the lactation period influences the lipid profile over the months, in article Barreiro *et al.* (Barreiro et al., 2018a) 16 women donated breast milk samples during the first 5 months of lactation for FAs analysis by GC-FID. In the univariate statistics, no significant differences were found for the most important fatty acids: 16:0, OA, ALA, LA, AA, EPA or DHA between the first five months of lactation. Totals values for SFAs, MUFAs and PUFAs were similar over five months of the study. In the work of Giuffrida *et al.* (Giuffrida et al., 2022) 223 European women who delivered at term, provided HM samples over first four months of lactation for lipid profile analysis. Although the samples were from women from different countries and dietary factors were not taken into account, short-, medium- and odd-chain saturated fatty acids as well as MUFAs and PUFAs were similar to the present study and did not vary significantly over the four months. The same occurs in several studies consulted for the bibliography of the paper in studies of 1 to 6 months (Antonakou et al., 2013; Lopez-Lopez, Lopez-Sabater, Campoy-Folgoso, Rivero-Urgell, & Castellote-Bargallo, 2002; Ribeiro et al., 2008; Scopesi et al., 2001; Szabo et al., 2010). However, in a study carried out with Egyptian women (Bakry et al., 2021), the difference between transitional milk (7-15 days) and mature milk (more than 15 days) was very marked in SFAs and PUFAs, the authors attributed these differences in percentages to the maternal diet. These results not coincide with the studies carried out in Italy (Scopesi et al., 2001) and Spain (Lopez-Lopez et al., 2002) that compared human milk in the same lactation period, transitional and mature milk.

A multivariate analysis was performed for search other different vision of FAs profiles of human milk in addition to the numerical results of the univariate statistics. PCA plot (Figure 12A) was performed with all data regardless of the lactation period. From sixteen lactating mothers enrolled in the study (Barreiro et al., 2018a), a total of 622 human milk samples were

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analysed and identified fatty acid profile between 1 and 5 months postpartum. Milk samples of each woman were grouped relatively close together. This fact was manifested in the existence of a specific lipid profile of each woman for her baby, this view agrees with the review of Selma-Royo M. *et al.* (Selma-Royo et al., 2021) where he indicated that human milk is unique beyond nutritional aspects. But focusing on the nutritional aspects it can also be observed that it is personalized for each baby consuming breast milk. This remark is on the way to answering many of the questions still raised by breast milk as exposed by Christian P. et al (Christian et al., 2021) in their review as: "could human milk be an exemplar of precision nutrition and specifically produced by the mother for her infant?". In loading plot Figure 12-B it is observed how the FAs were distributed in the plot by having the breast milk samples placed near the representative FAs in each lipid profile. Women with a fatty acid profile higher in n-6 and ALA were placed in the second quadrant (upper right) of the plot and those with a profile richer in SFAs in the fourth quadrant (bottom right) of the plot. Women who increased their MUFAs and OA content are located to the right of the plot.

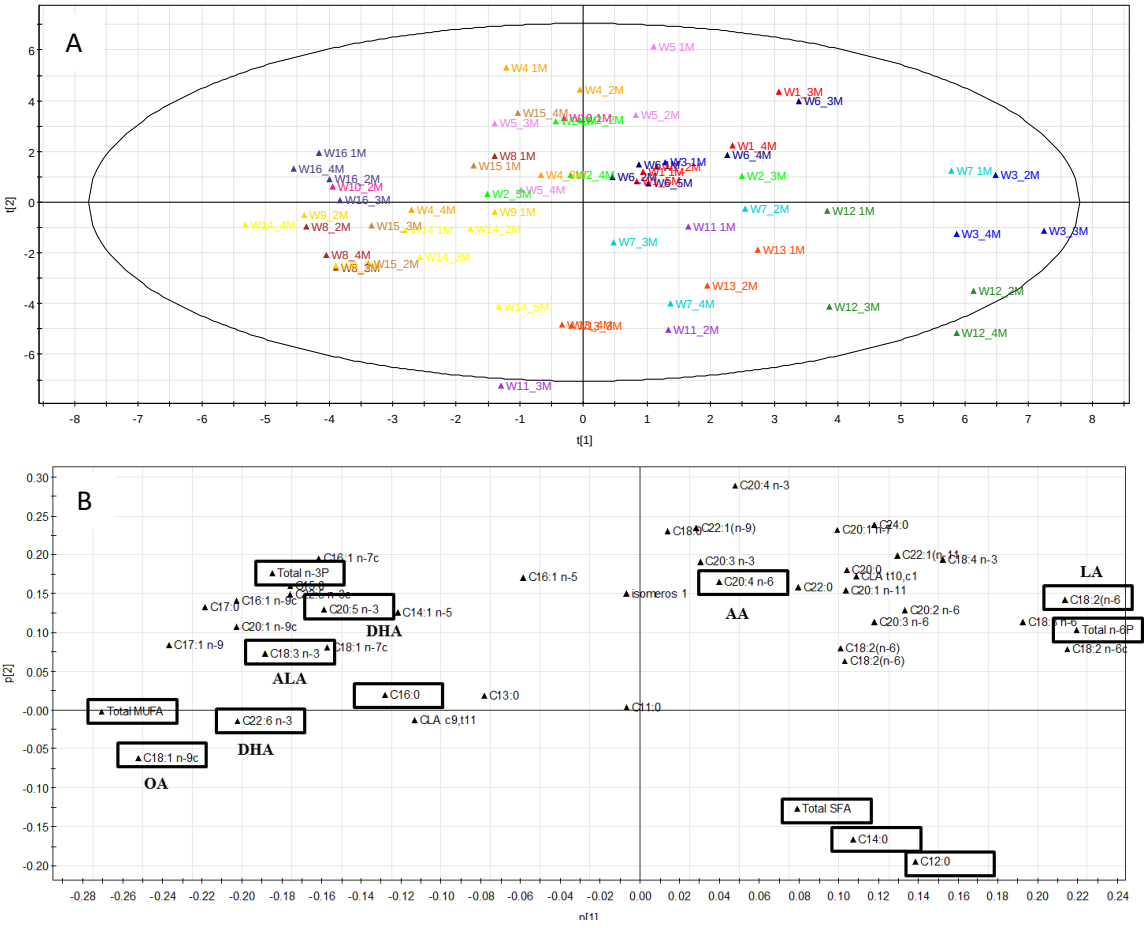


Figure 12. (A) PCA score scatter plot of the fatty acid profile in Galician women breast milk. (B) Loading plot, the most important fatty acids were highlighted. The "W" plus number correspond to woman participant and "M" corresponds to month of lactation; samples from the same woman and at different time points are coloured with the same colour.

In a second plot (Figure 13) orthogonal partial least square discriminant analysis (OPLS-DA) was used to highlight class discriminating variables in the quantified milk profiles (X matrix) related to a response (y) called dummy vector. This vector is often the study variant as

lactation stage. The same data were analysed taking into account the lactation period. In this case, the samples from the different females were grouped along the plot from the first to the fifth month of lactation, showing an evolution in the fatty acid profile depending on the lactation period. This discriminant analysis confirms the results obtained in the univariate statistics by placing the FAs that showed statistically significant modifications on opposite sides of the loading plot. Those FAs that increasing their levels were situated on the right side and those whose values decreased over lactation were located on the left side of the plot. This plot illustrates the existence of a time-dependent evolution for a short lactation period such as the first five months. There are variations in the lipid profile which, although not statistically significant differences in all FAs, develop according to lactation time. These differences are not easily observed in the statistical analysis but they can corroborate how human milk is a "live fluid" whose composition varies between women and changes over the course of lactation as describe in its article Bode *et al.* (Bode, Raman, Murch, Rollins, & Gordon, 2020).

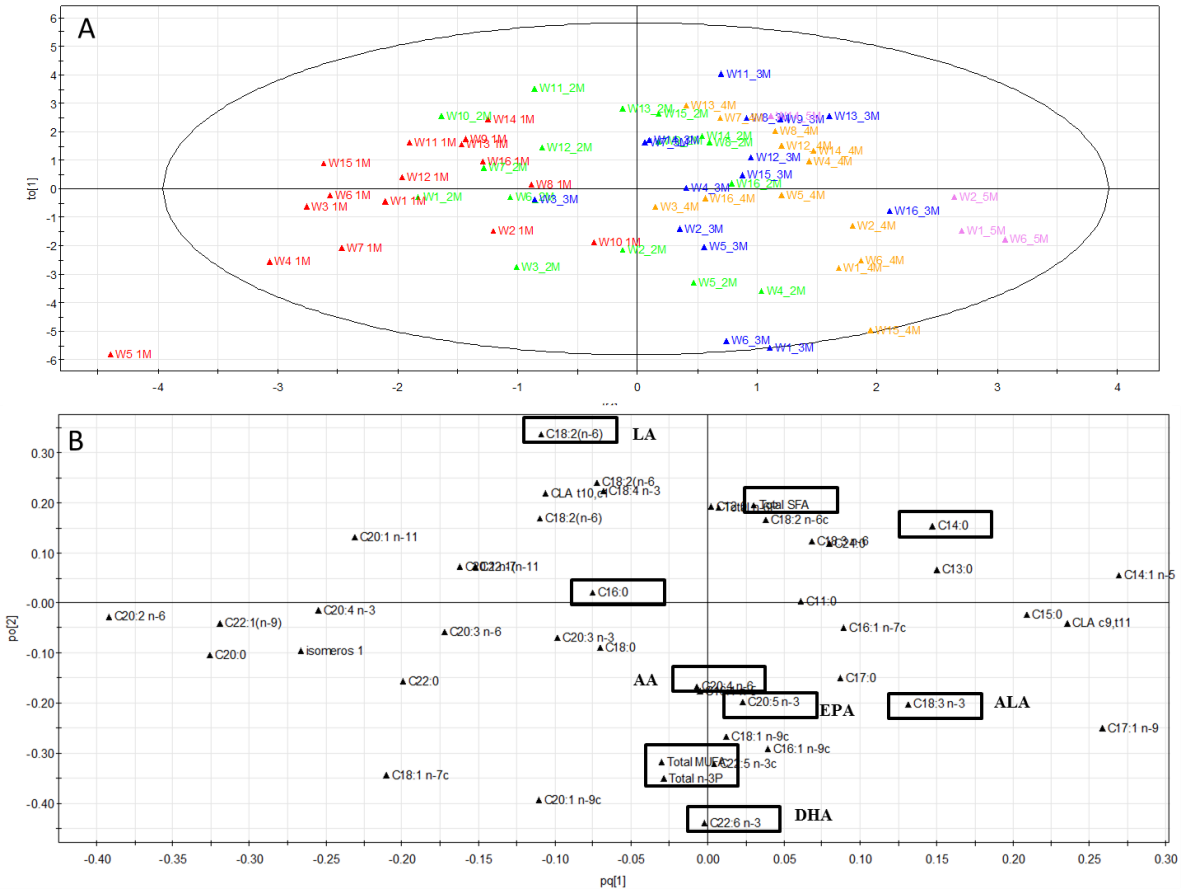


Figure 13. (A) OPLS-DA score scatter plot of the fatty acid profile in Galician breast milk classified and coloured according to the month of lactation. (B) Loading plot, the most important fatty acids were highlighted.

Breast milk composition has been studied exhaustively during the first twelve months of lactation as the duration of human lactation does not normally go beyond the first 3-6 months postpartum (Victora et al., 2016). The causes of breastfeeding abandonment are diverse, sometimes influenced by society and sometimes by erroneous medical beliefs (Díaz-Gómez, Ruzafa-Martínez, Ares, Espiga, & De Alba, 2016). Published data of breast milk composition

beyond one year postpartum is scarce, Mandel *et al.* (Mandel, Lubetzky, Dollberg, Barak, & Mimouni, 2005) concludes in their article that breast milk produced in prolonged lactation is rich in fat and has higher energy content in comparison to mature milk. To determine in what way FAs change with prolonged lactation, a cross-sectional survey study of breast milk fatty acid profile was designed in this Thesis project. All milk samples were extracted the FAMEs and analysed by GC-FID. Breast milk from mothers who had been lactating for more than one year (without interruption) was compared with mothers in others lactation stages (< 12 months) (Barreiro et al., 2020). Table 7 shows the values of the most important fatty acids in Galician breastmilk, divided into 3 stages of lactation: 0-6 months, 6-12 months and from 12 months to 8 years of lactation. This study has been approved by the corresponding Clinical Research Ethics Committee (*Comité Ético de Investigación Clínica de Galicia*) and it has been adhered to the principles of the Helsinki Declaration of 1975 as revised in 1983. This research is also part of the study registered in ClinicalTrials.gov with the identification number NCT03245697. The women for this study were selected with similar anthropometric and sociodemographic characteristics to make the two groups as homogeneous as possible.

Table 7. Main fatty acids (% wt/wt of total fatty acids) and fatty acid groups at different points of lactation in Galician women. Data extracted from Barreiro *et al.* (2020).

Fatty Acids	HM 0 - 6m n=10			HM 6 - 12m n=6			HM >12m - 8y n=16		
	Mean	±	SD	Mean	±	SD	Mean	±	SD
SFAs	47.85	±	3.37	40.12	±	3.02	47.06	±	4.30
12:0*	9.99	±	1.61	8.77	±	1.36	10.68	±	2.15
14:0*	6.69	±	1.27	7.06	±	1.76	9.46	±	2.63
16:0	20.95	±	2.03	16.22	±	1.93	18.84	±	2.12
MUFAs*	31.83	±	4.36	41.37	±	4.34	31.02	±	5.24
OA*	26.48	±	4.23	36.12	±	4.41	25.39	±	5.56
PUFAs	20.24	±	3.36	18.46	±	3.71	21.86	±	4.38
n-6	17.54	±	3.19	16.13	±	3.76	18.68	±	3.63
LA	15.62	±	3.03	14.77	±	3.88	16.74	±	3.30
AA*	0.61	±	0.11	0.45	±	0.10	0.69	±	0.16
n-3*	1.77	±	0.70	1.59	±	0.65	2.27	±	1.10
ALA*	0.70	±	0.31	0.69	±	0.19	1.12	±	0.85
EPA	0.14	±	0.06	0.12	±	0.09	0.13	±	0.08
DHA	0.49	±	0.30	0.46	±	0.31	0.60	±	0.28

Note: statistical significance is indicated with an asterisk (*) when the median in in prolonged human milk is statistically different from the median in conventional milk. HM: human milk, m: month, y: year, n: number of women

The SFAs, EFAs and PUFAs increase with the lactation period in particular, 12:0, 14:0, ALA and AA concentrations were higher during prolonged lactation and these fatty acids were statistically different. As a consequence MUFAs and OA decrease over 12 months of lactation. In the previous article of Barreiro R. (Barreiro et al., 2018a), the same trend could be observed for these fatty acids but the differences were not significant for any of them. In other study (R. Barreiro et al., 2018b) of breast milk from Galician mothers in the same lactation stages (0-6m, 6-12m and >12m) the percentages in FAs profile it was not the same. In this article the percentages of 12:0 and 14:0 also increased with lactation time as did MUFAs especially OA. However, EFAs and PUFAs decreased with time. It should be noted that this

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study was conducted with only 4 women in prolonged lactation and the samples were always between 1 year and 1 year and 9 months. In the prolonged lactation study, breast milk from 16 women with lactation periods of 3, 5, 6 and 8 years was analyzed. In the study by Siziba et al (Siziba et al., 2019) analyzed breast milk samples at 6 weeks, 6 months and 12 months of lactation, they also observed a marked increase in the total lipid content of human milk, and changes in the fatty acid composition at 12 months of lactation making it clear that the fatty acid profile of human milk during prolonged lactation is not identical to that of human milk during short-term lactation. The results of Hewelt-Belka.et al (Hewelt-Belka, Garwolińska, Młynarczyk, & Kot-Wasik, 2020) in their study based in lipidomics analysis of human milk over one year of lactation, also proved that human milk after one year postpartum is still rich in lipid species and LC-PUFAs knows for their great nutritional value. Other studies also observed this tendency to increase the saturated fraction with lactation time (Lubetzky et al., 2012; Mitoulas, Gurrin, Doherty, Sherriff, & Hartmann, 2003) specifically in these works they describe the increase of 12:0 and 14:0 as occurs in this study. These FAs are formed *de novo* in the mammary gland (Ameer, Scandiuzzi, Hasnain, Kalbacher, & Zaidi, 2014) and are related to the consumption of carbohydrates in the diet.

5.2 FATTY ACIDS IN INFANT FORMULAS

Despite all benefits for mother and baby, breastfeeding is not always possible for different reasons. Mother´s health problems or medications than can pass into breast milk, social or cultural reasons, or special situations of the baby, may result in a breastfeeding termination. In this context, infant formulas (IF) are the option for substituting breast milk because are designed to mimic, as closely as possible, its properties. The composition of formula is of vital importance especially in the first six months of life since this artificial food is the only one that the baby can digest. Mothers, who have or want to appeal to these artificial formulas, must be completely sure of the correct formulation of these preparations to supply all the nutritional needs of the baby.

Cow's milk is the main ingredient of most infant formulas but, at the beginning of the 20th century, the negative perception of SFAs and cholesterol caused bovine milk fat to be replaced by vegetable oil mixtures (palm, coconut, sunflower). However, since the 1970s, it was found that the structure of fatty acids in the triglyceride of vegetable oils was not the most appropriate since most of the LC-SATs are in the sn-1 and sn-3 position of the triglyceride. This fact caused attention to be paid to the triglyceride structure of milk fat. In cow's milk (and breast milk) LC-SATs such as 16:0 are in the *sn*-2 position. In this way, the structure of triglycerides in milk fat allows a greater absorption of fat by the baby and, therefore, energy, as well as a lower formation of calcium soaps resulting in softer stools and, consequently, a greater absorption of calcium (Orta, 2018). Studies indicated that, blending bovine milk fat with vegetable fat in combination with the development of more gentle processing techniques might be a future direction to improve infant formulas (Hageman et al., 2019).

As it has been corroborated in previous works, (Barreiro et al., 2018a; Barreiro et al., 2020) breast milk is not a homogeneous and static fluid but varies with various factors such as lactation time. To determine whether the fatty acid profile of the formulas was close to that of breast milk, a study was carried out to analyse and compare fatty acid profile of 23 infant formulas on the Spanish market, separated into: 5 first-stage formulas (0-6 months of age), 13 follow-on formulas (for babies from 6 months) and 5 growing-up milks (young children), with breast milk from Galician women in the same lactation period. All formulas samples and human milk samples were extracted and analysed the FAMEs by GC-FID. As in previous studies (Barreiro et al., 2018a; Barreiro et al., 2020), it was observed that breast milk fatty acid profile

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evolves with the lactation period while IF remain more static. The labelling of the different IF was variable among different brands, with the most complete nutritional information on the 0-6 months formulas, where it indicated the values in mg/100ml for all EFAs. This fact can be partially explained because this lactation period is the most studied and hence, more information can be found in the literature on milk composition. In this age range (0-6 months), and where formula milk is the only food, the lipid profile is the most similar to breast milk in all fatty acid summations as can be seen in the summary in Table 8.

Table 8. Summary of the main fatty acids and groups (% wt/wt of total fatty acids) in infant formulas and human milk. Data from Barreiro R. *et al.* (2017).

	FSF	HM (0-6m)	FOF	HM (6m-1y)	GUF	HM (1-2y)
Fatty Acids	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
SFAs	39.67 ± 2.14	39.40 ± 2.42	40.73 ± 2.29	38.67 ± 2.50	38.70 ± 2.90	42.41 ± 0.51
MUFAs	40.51 ± 1.77	40.92 ± 2.05	40.73 ± 3.38	44.84 ± 44.84	41.30 ± 1.10	44.10 ± 1.86
PUFAs	19.61 ± 0.64	19.60 ± 3.21	18.71 ± 4.05	16.42 ± 4.35	20.60 ± 2.42	13.43 ± 1.92
n-6	17.02 ± 0.52	17.32 ± 3.27	16.08 ± 3.93	14.76 ± 4.41	17.30 ± 2.28	11.37 ± 1.84
LA	16.26 ± 0.52	15.78 ± 3.31	15.63 ± 3.91	13.60 ± 4.46	16.5 ± 2.33	10.30 ± 1.69
AA	0.28 ± 0.17	0.47 ± 0.09	0.26 ± 0.07	0.39 ± 0.10	0.11 ± 0.09	0.37 ± 0.10
n-3	2.02 ± 0.45	1.59 ± 0.54	2.13 ± 0.39	1.04 ± 0.13	2.19 ± 0.42	1.50 ± 0.65
ALA	1.69 ± 0.48	0.84 ±0.48	1.88 ± 0.28	0.52 ± 0.13	1.96 ± 0.36	0.77 ± 0.61
EPA	0.07 ± 0.05	0.11 ± 0.05	0.07 ± 0.05	0.07 ± 0.07	0.07 ± 0.03	0.08 ± 0.04
DHA	0.21 ± 0.04	0.33 ± 0.03	0.22 ± 0.08	0.21 ± 0.21	0.09 ± 0.08	0.33 ± 0.05

SFS: firs-stage infant formula, FOF: follow-on infant formulas, GUF: growing-up infant formulas, HM: human milk, m: month, y: year

Since 2002, infant formulas have been fortified with LC-PUFAs due to the recommendations proposed by the WHO for these FAs, considering them essential for body and brain growth as well as optimal neural function (Salem Jr & Van Dael, 2020). As it can be observed in Table 8, fatty acid profiles for FOF and GUF, were more variable compared to breast milk. The values for ALA are higher in IF than in breast milk, while the values for AA and DHA are lower in IF than human milk. These data coincide with those reported by Sanchez-Hernandez *et al.* (Sánchez-Hernández et al., 2019) who compared the fatty acid profile of human milk samples (colostrum, transitional, and mature milk group) and IF. However, the data obtained in the study of Sanchez-Hernandez *et al.* show a similar value of LA between IF and breast milk. But, in Barreiro *et al.* article, this fatty acid was always higher in IF than in human milk. The labelling of FOF and GUF does not show the value of EFAs in all commercial brands. Many of the formulas indicate that they are enriched in LC-PUFAs but in this study it was observed that ALA is much higher in artificial formulas than in breast milk, suggesting that formulas are over-enriched with this fatty acid. It was also observed that the balance between AA and DHA in IF is similar while in breast milk, the value of AA is always higher than that of DHA in the three lactation periods. This fact is important because the new EU regulation indicates that AA is not needed to be included in IF (EU, 2015). However, intervention studies suggest that a DHA/AA balance is vital in the contribution to cognitive and behavioural development of infants because AA has different biological functions compared to DHA (Ruiz-Ojeda et al., 2022). For this reason, IF with DHA and no AA runs the risk of cardio and cerebrovascular morbidity and even mortality through suppression of the favourable oxylipin derivatives of

AA (Crawford, Wang, Forsyth, & Brenna, 2015). Lastly, a multivariate analysis was performed on the fatty acid profile of breast milk and infant formulas at all stages of lactation to visualize possible differences between them. Figure 14-A represents the PCA scatter plot of the model build up with the fatty acid profiles of all mothers in three lactation periods (0-6 months, 6-12 months, and 1-2 years *postpartum*) and the profiles of the three types of infant formulas available in the Spanish market (first-stage, follow-on and growing-up). In part B of Figure 14, the loading plot is presented, showing which variables or fatty acids have the largest effect on each component of the PCA.

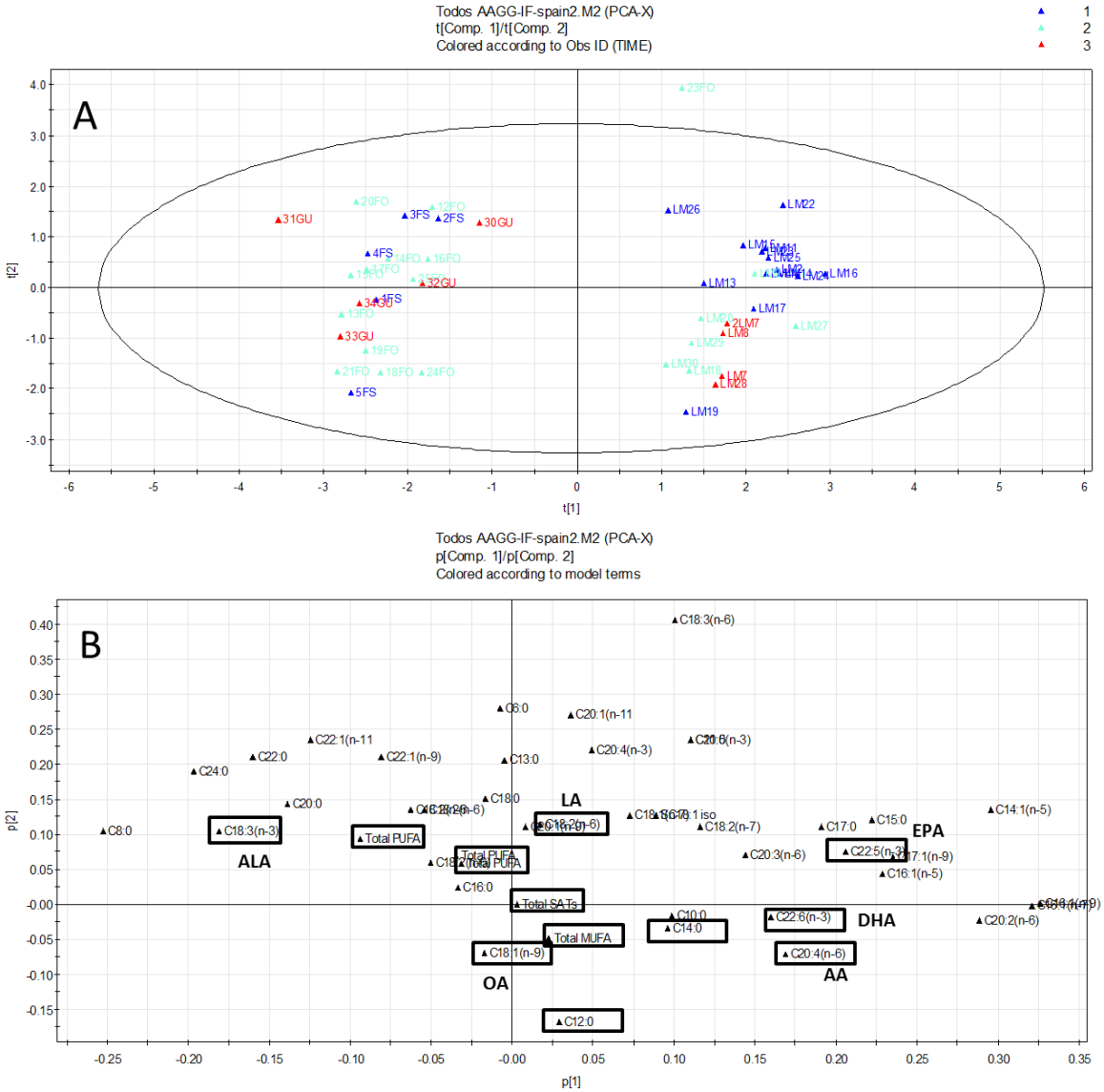


Figure 14. (A) PCA score scatter plot of human milk and infant formulas fatty acids profile collected from three lactation periods, coloured as follows: blue for human milk from 0-6 months and firs-stage infant formulas, green for human milk from 6-12 months and follow-on infant formulas, and red for human milk from 1-2 years and growing-up infant formulas. (B) Loading plot. LM: human milk.

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In this scatter plot, two distinct groups or clusters can be observed. On the right side of the plot, breast milk samples are grouped, while the formulas were at the opposite side. In human

milk a difference in lactation periods continued to be observed as previous studies (Barreiro et al., 2018a; Barreiro et al., 2020). The breast milk samples from 0-6 months, being placed in the upper right side of the plot (blue colour) and samples with more lactation time at the bottom right of the plot (green and red colour). The infant formulas were placed on the left side of the plot with no clear difference between the three states indicated by the producers: first-stage, follow-on and growing-up. This separation indicated the differentiation of the fatty acid profile between human milk and artificial formulas. In loading plot (Figure 14-B) shows the relative contribution of each fatty acid to the separation of breast milk and infant formulas. Thus, on the left side of the plot is placed ALA, a fatty acid that is present in considerable quantities in infant milks rather than in human milk as can be seen in Table 7. Total MUFAs and SFAs and OA are placed in the middle of the plot as they are the major FAs in breast milk and formulas. On the right side of the plot was placed the rest of the EFAs: AA, EPA and DHA and most of the n-6 and n-3. These fatty acids are the ones that characterize human milk. LM19 was a sample of 0-6 months of lactation but it was located near the samples of breast milk with more lactation time.

5.3 BOVINE MILK IN HUMAN NUTRITION: FACTORS AFFECTING ITS FATTY ACID PROFILE

Cow's milk is the most consumed milk as part of the traditional diet and being a staple from infancy to old age. Milk components have been studied in detail for its multiple benefits for human health. Of all of them, fat (and fatty acids) is the most variable fraction and his role in human nutrition has been largely discussed. More than 60% of the FAs that constitute the milk lipid profile are SFAs which have been related to several human health problems such as weight gain, obesity and heart disease. But, recent reviews based on epidemiological studies, have reported that the metabolic impact of full-fat dairy products is more beneficial than predictable upon their SFAs richness (Badshah, Bharti, & JHA, 2021; Mohan, O'Callaghan, Kelly, & Hogan, 2020; Unger, Torres-Gonzalez, & Kraft, 2019; Vors, Le Barz, Bourlieu, & Michalski, 2020).

The fat fraction is variable because certain factors can affect both its production and composition (quantity and quality). Many of these factors have been studied to obtain better yields on dairy farms: genetic selection and improvements in bovine dairy breeds, stage of lactation, climatic factors, husbandry, milking practices, and the most important and effective factor, diet modifications. However, the relation between milk fat and physiological conditions of the animal (hormonal level, pregnancy, etc…) or exogenous substances administration, have not been studied in detail. But, these factors may also have an impact on the bovine milk composition because it can modify fatty acid profile and other nutrients that are present in milk. Regal *et al.* (P. Regal et al., 2012) studied in their article the existence of variation in bovine milk hormonal levels depending on animal pregnancy, finding significant differences in some hormones. This research was controversial considering that in current dairy farms; most female bovines are milking and pregnant at the same time during most of their lactation period. This article has also been a first step to study more deeply pregnancy as an endogenous factor. Thus, in (Barreiro et al., 2017) study that, the impact of pregnancy on bovine milk lipid profile has been evaluated and the changes in bovine milk fatty acid composition obtained from pregnant animals and how can influence in human health have been discussed. In the final experiment of this Doctoral Thesis (Barreiro et al., 2022), the effects of the administration of recombinant growth hormone (rbST) in cattle was studied, as it is used in some countries to increase milk production and it can modify the lipid profile of milk.

When multiple physiological situations occur simultaneous as: pregnancy, lactation and exogenous hormone administration in addition to the diet influence and housing conditions of

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the animal, it is difficult to guess which one orchestrates or coordinates the milk synthesis and changes in composition. But, in both articles, differences in FAs profile of pregnant or treated animals and control animals were calculated for univariate statistic and several FAs gave significant differences. Moreover, thanks to the study of fattyacidomics profiling and multivariate statistics, the discriminations of control and pregnant/treated groups of animals are more easily visible thanks to the scatter plots constructed with the milk FAs profiles.

5.3.1 Variations of bovine milk fatty acids profile by an endogenous animal factor: study of bovine pregnancy

During bovine pregnancy and lactation, changes in the milk composition can occur due to natural causes. These situations are special physiological states in any mammal where a change in the priorities of nutrient and energy utilization arises because they are needed for a correct growth of the foetus and foetal membranes and gravid uterus, as well as development of the mammary gland. Subsequently, some changes in milk composition (quantity and quality) obtained from pregnant animals may be expected. As pregnancy affects the composition of milk, it is a rarely studied factor since, whether the animal is pregnant or not, the milk is stored in the same tank and goes together to dairy plant.

To evaluate the impact of pregnancy on the bovine milk lipid profile, milk from 20 pregnant and 20 non-pregnant cows was analysed by GC-FID to determine the differences in terms of FAs profile (Barreiro et al., 2017). Two farms different in number of dairy cows and farm conditions but with the same breed (Holstein-Friesians) and similar basal diet of the animals were chosen. Farm A had 300 Holstein dairy cows in high-production capabilities and under a confined feeding system. Farm B had 100 Holstein cows with less modern facilities and also under a confined intensive system. The basal diets were composed of concentrate, corn, soybean meal, and pasture silages, all corrected with mineral supplements, plus hay and water *ad libitum* and zero-grazing; what is known as intensive dairy farming. But, the farm with more animals in production adjusted the feed to the needs of cows and in the smaller and more traditional farm, the diet was not so strictly controlled. This fact could influence the lipid profile for example in MUFAs level, but OA percentage was lower in milk from pregnant cows in both farms. The lipid profile of pregnant cows was more saturated than non-pregnant cows, and the main fatty acids considered pro-atherogenic (12:0, 14:0 and 16:0) were higher in pregnant animals (Table 9). As a result, the total MUFAs was lower; especially the OA in pregnant animals and the polyunsaturated fraction was slightly richer in n-6 and n-3 components than non-pregnant animals even though the differences were not statistically significant.

No studies have been found in the literature discussing pregnancy as a factor that can modify milk fatty acids. Bauman & Currie (Bauman & Currie, 1980) explained in their paper how pregnancy and lactation are important physiological states in any female that requires metabolic control to regulate nutrient distribution and develop the mammary gland. These two types of regulation are defined as homeostasis and homeorhesis which, together with possible endocrine control, may be responsible for the modification of the lipid profile of milk.

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Table 9. Fatty acid composition (% w/w of total fatty acids) of milk collected from pregnant and non-pregnant animals of the two farms (R. Barreiro et al., 2017).

	Endogenous factor	
	Pregnant	Non-pregnant
Fatty Acids (%)	Mean ± SD	Mean ± SD
SFAs*	69.58 ± 3.85	65.74 ± 4.81
12:0*	6.08 ± 1.71	5.08 ± 1.06
14:0*	13.38 ± 1.94	12.13 ± 1.43
16:0	32.55 ± 3.49	30.99 ± 3.18
MUFAs*	24.04 ± 3.59	28.26 ± 4.66
OA*	17.75 ± 3.93	22.14 ± 4.60
PUFAs	6.31 ± 0.28	5.96 ± 0.32
Σn-6	4.02 ± 0.95	3.80 ± 0.64
LA	3.14 ± 0.82	3.09 ± 0.58
Σn-3	0.84 ± 0.21	0.79 ± 0.15
ALA	0.50 ± 0.13	0.51 ± 0.12
EPA	0.07 ± 0.04	0.07 ± 0.03
DHA*	0.05 ± 0.02	0.03 ± 0.01
CLA (RA)	1.31 ± 0.06	1.25 ± 0.03

Note: Significance is indicated when the median in pregnant animals was statistically different from the median in non-pregnant animals.

Toledo-Alvarado *et al.* (Toledo-Alvarado et al., 2018) investigated the effects of various estrous phases on milk and FAs composition. The variation in milk FAs profiles of cows could potentially be taken as a marker of the estrous cycle phases for correct detection insemination timing. Thus, they schematized the variation in milk hormones throughout the days of the estrous cycle from the time of heat and insemination, the cow's pregnancy status, and the start of the cycle again if pregnancy is not carried out (Figure 15-A). If the cow is pregnant, progesterone is maintained at the highest value corresponding to the Diestrus-HP (high-progesterone) state. In this cycle state 14:0 and 16:0 increases while 18:0 and OA are lower than in the non-pregnancy stage (Figure 15-B). Total fatty acids also vary: SFAs content increases and MUFAs and PUFAs decrease from estrus to Diestrus-HP stage (Figure 15-C). Although in our study the average number of days pregnant was higher (82 ± 58), the same result for FAs, SFAs and MUFAs was obtained (R. Barreiro et al., 2017). Only differences were found in the PUFAs, while in the Toledo-Alvarado *et al.* study their content decreased, in our case, the PUFAs increased slightly without significant differences. In the period when the cow would be pregnant, the fatty acids synthesized in the mammary gland (14:0, 16:0) increase while those that originate from dietary lipids absorbed by the small intestine and from lipolysis of adipose tissue decrease. This means that at this stage, the LC-FAs do not pass to the udder so it could be accumulating in the adipose tissue in form of reserves for the time of parturition. If milk is tested immediately after birth (colostrum), it may be possible to see the variations on lipid profile. Zou *et al.* (Zou et al., 2015) analyzed in their study the lipid profile of colostrum and mature milk, obtained significant differences in the three groups of FAs: SFAs were lower in colostrum while MUFAs and PUFAs were higher. So, high levels of LC-FAs are present in colostrum because, at parturition, cows are in a negative energy balance, resulting in the mobilization of adipose tissue fatty acids which are incorporated into

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milk fat and the high levels of LC-FAs inhibit *de novo* synthesis of SC-FAs (McGrath, Fox, McSweeney, & Kelly, 2016).

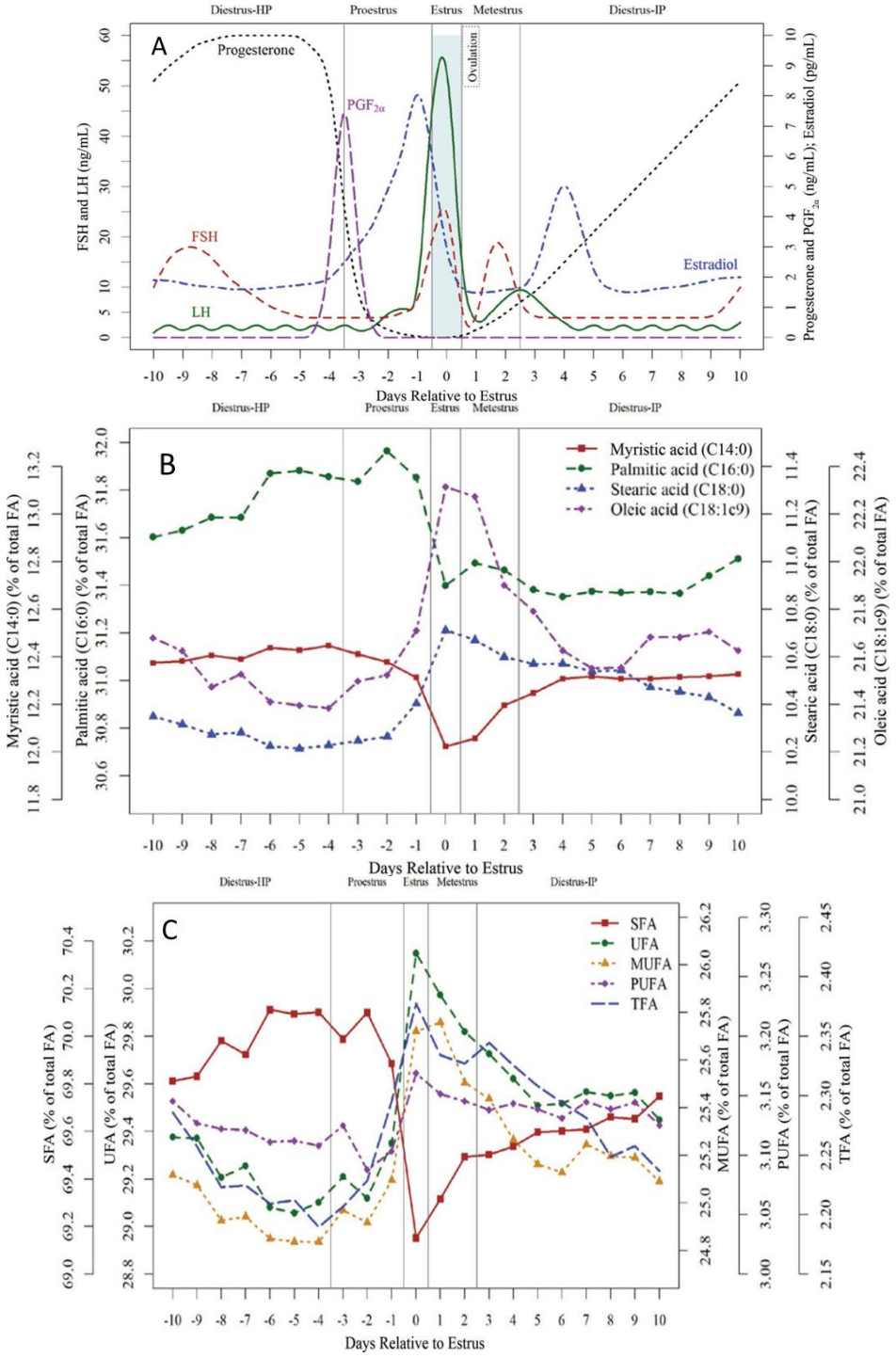


Figure 15. (A) Schematic depiction of the patter of secretion of various hormones during the estrous cycle in cattle. (B) Least squares means of the most abundant FAs in milk for each day nested in the estrous cycle phases. (C) Least squares means of FAs groups for each day nested in the estrous cycle phases. Reprinted from Toledo-Alvarado *et al.* (2018) with permission from Elsevier.

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This fact also occurs in human milk, in comparison with mature milk, human colostrum was characterized by a lower percentage of SFAs (including MC-FAs) and higher percentage of MUFAs and AA, EPA and DHA (Barreiro et al., 2018a; Gibson & Kneebone, 1981). The perinatal period in humans, is a period of intensive growth of the brain, which contains high amounts of LC-PUFAs (AA and DHA). Furthermore, they are involved in inflammatory and immunological processes (Demmelmair & Koletzko, 2018). Multivariate analysis was performed to visualize the global FAs profiles of two groups of animals. Figure 16 shows OPLS-DA plot where a separation between the groups of pregnant and non-pregnant cows in the two investigated farms (Farm A and B). The FAs relative contributing to this separation are those for which significant differences are found in the univariate analysis (Table 9): C12:0, C14:0, SFAs, OA, MUFAs, and DHA.

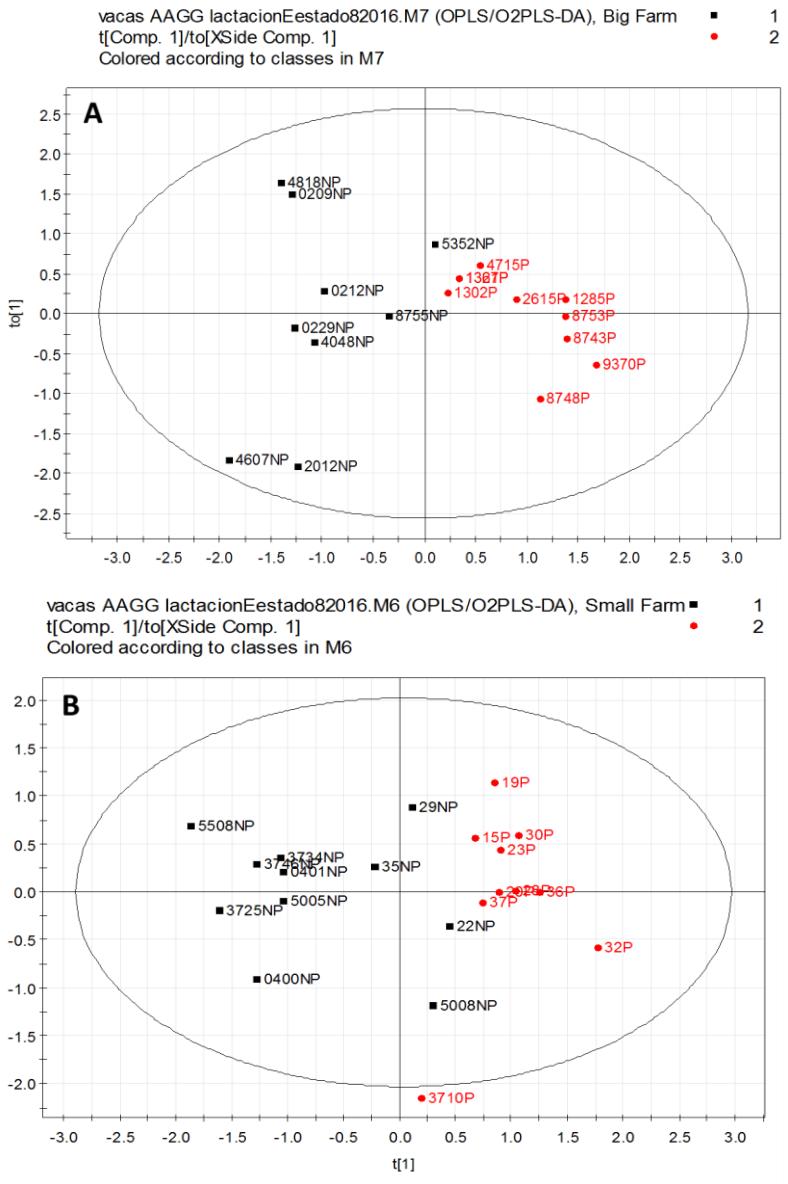


Figure 16. OPLS-DA plot (A and B) showing the discrimination of milk from pregnant and non- pregnant cows in the two investigated farms. The upper plot corresponding to Farm A and bottom plot corresponding to Farm B. P (red): pregnant cows. NP (black): non-pregnant cows.

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5.3.2 Variations of bovine milk fatty acid profile by an exogenous factor: study of bovine recombinant somatotropin

The recombinant bovine somatotropin (rbST) is a genetically engineered copy of the natural bovine pituitary product, somatotropin. This product is marketed to increase milk production and efficiency of milk synthesis in cows. The natural and recombinant forms of the somatotropin hormone, stimulate the activity and longevity of mammary secretory cells by increasing levels of insulin-like growth factor (IGF-1) hormone produced by either liver or mammary gland (Rath, Panda, Pandey, Routray, & Sethy, 2018). rbST stimulates liver that initiates the release of growth factor IGF-1 and, this hormone, enhances the milk production in cows. rbST-treated cows must consume sufficient quantities of nutrients to meet the energy demands for more milk production. But, as a result, body lipid mobilization doesn't get increased; rather decreases the capability of the body to synthesize fat and making free fatty acids available for milk production (Kumar, Rajan, Divya, & Sasikumar, 2018; Rath et al., 2018).

Treatment of cows with rbST is approved in more than 20 countries while in the European Union (EU), Canada, Australia, Argentina, New Zealand, Israel and Japan have banned its use. However, due to globalization, trade agreements have been launched in recent years as the TTIP (Transatlantic Trade and Investment Partnership, 2013) that aims to remove barriers to trade and investment between the EU and the United States of America (USA) (Pietrzyck, Driouech, & Petersen, 2018). USA is one of the most important countries where the use of rbST is not prohibited since 1993 when the Food and Drug Administration approved its use. A fraudulent labelling of rbST-free dairy products can exist since in those countries where its use is permitted is not monitored presumably due to the absence of feasible analytical options (Regal, P., Lamas, A., Barreiro, R., Franco, C.M., Cepeda, A., 2017). For these reasons, is important to know the measurable effects or rbST on the nutritional properties of bovine milk. *Profiling* and *omics* technologies offer a good opportunity to assess various components simultaneously in food as fatty acid profiles.

The last experimental study of this Doctoral Thesis (Barreiro et al., 2022) profiled the measurable effects of biweekly doses of rbST on the bovine milk fatty acid profile over the course of eight months. This research project was financial for the Spanish Innovation Program "Programa Estatal de Investigación, Desarrollo e Innovación Orientada a los Retos de la Sociedad" (Project AGL2014-58881-R). All procedures were preformed after evaluation and with the permission of the corresponding regional authorities (Service of Livestock Farming of the Provincial Government of Lugo, Regional Ministry of Rural Affairs, Galicia) in accordance with EU guidelines and national laws on animal experiments in particular Directive 2010/63/EU. All procedures were performed respecting animal welfare and causing no more pain, suffering, distress or lasting harm accordance with good veterinary practice. For this purpose, nine Holstein cows were selected for the study, six cows were selected to be treated with rbST and the other three were the control group. The nine animals were never separated and the diet was the same. The rbST group was treated subcutaneously with 500 mg of rbST (Lactotropina, Elanco, Eli Lilly, Mexico) every 14 days, a total of 12 doses were administered and the time elapse between the first and the last dose was 168 days (24 weeks), the last collection data of the study was in 240 day (32 weeks) to observe how long the effect of the hormone lasted. A wide profile of fatty acids (*fatty acidome*) was determined in bovine milk collected at different time points after treatment. The galactopoietic effects of rbST on lactating dairy cattle was demonstrated, reaching the highest point in cycle 12 of the doses administered with 43% of more production of treated animals as reflected in the bibliography (Lamas et al., 2019). As for the gross composition of milks, protein increased in treated animals giving significant differences, while lactose remained constant. Differences were also

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observed in some minerals analysed (calcium and potassium) (Regal, P., Lamas, A., Barreiro, R., Franco, C.M., Cepeda, A., 2017).

Tables 10 and 11 present the evolution of main fatty acid groups in bovine milk, for animals treated with rbST and control cows. Week -1 reflects the two groups of animals 7 days before starting the study (first dose or rbST) and it can be observed how the values are similar for all FAs groups (SFAs, MUFAs and PUFAs) in the two groups of animals. The modifications were especially evident in the first weeks of treatment and until week 10 and the differences became smaller the following weeks. It is worth mentioning that in week 10 the group of treated animals should have received a new dose of rbST following the biweekly pattern, but at this point the injection was not applied to observe if the production would drop (Lamas et al., 2019). The values for SFAs were always lower in the group of animals treated up to week 20. After the end of the study at week 24, SFAs content begins to increase in the treated animals to equal control animals. MUFAs and OA values were higher in treated animals than in the control group. Some previous studies have found differences in the FA profile cows treated with rbST (Bitman et al., 1984; Dahl, 1991; Ravi, Saikia, Porteen, Sarath, & Kumar, 2007; Soderholm et al., 1988) noticing a decrease in SFAs and an increase in OA for treated animals compared with control group coinciding with the results of the present study were the percentage of C14:0 and SFAs were significantly lower in rbSt group in comparison to the control group and the percentage of MUFAs and OA were significantly higher in rbST group in comparison to non-treated animals.

Table 10. Means of SFAs, MUFAs and OA (% wt/wt of total fatty acids) for milk from rbST-treated and control animals throughout all weeks of the study (Project AGL2014-58881-R).

Days	Week	SFAs				MUFAs				OA			
		rbST (N=6)		Control (N=3)		rbST (N=6)		Control (N=3)		rbST (N=6)		Control (N=3)	
		Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD	
Predose	-1	63.60 ± 2.62		64.92 ± 3.20		29.74 ± 2.74		29.19 ± 3.16		22.91 ± 2.84		24.20 ± 3.54	
1to7	1	67.51 ± 3.44		71.01 ± 3.72		26.04 ± 3.28		22.71 ± 3.78		19.08 ± 3.09		16.81 ± 4.14	
8to14	2	67.74 ± 5.11		72.63 ± 2.58		25.77 ± 4.88		20.86 ± 2.24		18.43 ± 3.98		14.63 ± 1.93	
15to21	3	69.63 ± 3.77		73.31 ± 0.44		23.84 ± 3.44		20.11 ± 0.65		16.67 ± 2.79		14.01 ± 0.43	
22to28	4	72.89 ± 2.28		74.05 ± 0.66		20.87 ± 2.11		19.42 ± 0.46		14.51 ± 1.59		13.82 ± 0.63	
29to35	5	69.11 ± 4.08		72.15 ± 0.72		23.78 ± 4.15		20.37 ± 0.66		17.61 ± 3.99		14.68 ± 0.59	
36to42	6	68.83 ± 4.03		72.72 ± 0.99		24.19 ± 3.86		20.20 ± 0.63		17.43 ± 3.16		14.72 ± 0.51	
43to49	7	68.06 ± 6.32		74.02 ± 0.65		25.02 ± 5.96		19.33 ± 0.65		18.23 ± 5.25		14.08 ± 0.72	
50to56	8	69.01 ± 3.06		72.91 ± 2.26		24.35 ± 2.77		20.47 ± 1.63		17.89 ± 2.39		14.95 ± 1.13	
57to63	9	69.07 ± 3.91		72.44 ± 1.62		23.95 ± 3.75		20.49 ± 1.32		17.24 ± 3.30		14.88 ± 1.06	
64to70	10	70.29 ± 2.21		71.71 ± 1.25		22.98 ± 2.11		21.24 ± 1.21		16.29 ± 1.77		14.68 ± 1.05	
71to77	11	69.93 ± 3.86		71.52 ± 0.73		23.29 ± 4.06		21.34 ± 0.36		16.62 ± 3.76		15.22 ± 0.46	
78to84	12	71.24 ± 2.71		72.34 ± 1.61		21.91 ± 2.60		20.60 ± 1.27		15.89 ± 2.39		14.93 ± 0.89	
85to91	13	69.30 ± 5.07		70.49 ± 2.92		23.67 ± 4.58		22.39 ± 2.64		17.15 ± 3.93		16.32 ± 2.14	
129to126	18	70.23 ± 2.92		72.14 ± 0.11		23.30 ± 2.70		21.08 ± 0.14		16.19 ± 1.63		14.75 ± 0.32	
134to140	20	69.02 ± 2.66		68.83 ± 1.04		24.05 ± 2.57		24.27 ± 0.50		16.33 ± 1.35		16.81 ± 0.73	
162to168	24	69.90 ± 1.95		68.43 ± 0.94		22.58 ± 1.70		24.03 ± 0.38		15.74 ± 1.36		17.47 ± 0.83	
190to196	28	72.46 ± 1.60		70.69 ± 0.02		20.74 ± 1.58		21.77 ± 0.04		14.80 ± 1.37		15.93 ± 0.13	
218to224	32	72.83 ± 2.20		72.54 ± 0.51		21.05 ± 2.25		21.32 ± 0.55		13.89 ± 1.46		14.68 ± 0.53	
Mean		69.51 ± 3.36		71.52 ± 1.37		23.74 ± 3.22		21.64 ± 1.17		16.99 ± 2.71		15.66 ± 1.15	

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Table 11 shows the data for total PUFAs, n-3 and n-6 where no differences were found between the two groups of animals, showing that rbST has no effect on this group of FAs throughout the treatment. It is necessary to emphasize that no such a long-term study has been performed so far and neither publish.

Table 11. Means of PUFAs, n-3 and n-6 (% wt/wt of total fatty acids) for milk from rbST-treated and control animals throughout all weeks of the study (Project AGL2014-58881-R).

Days	Week	PUFAs				n-3				n-6			
		rbST (N=6)		Control (N=3)		rbST (N=6)		Control (N=3)		rbST (N=6)		Control (N=3)	
		Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD	
Predose	-1	6.60 ± 0.46		5.85 ± 0.31		0.85 ± 0.06		0.78 ± 0.02		4.13 ± 0.35		3.55 ± 0.13	
1to7	1	6.38 ± 0.57		6.22 ± 0.56		0.86 ± 0.10		0.85 ± 0.11		3.99 ± 0.35		3.78 ± 0.24	
8to14	2	6.40 ± 0.44		6.44 ± 0.44		0.87 ± 0.10		0.90 ± 0.09		4.12 ± 0.31		4.08 ± 0.31	
15to21	3	6.41 ± 0.59		6.48 ± 0.56		0.84 ± 0.19		0.90 ± 0.11		4.20 ± 0.34		4.10 ± 0.24	
22to28	4	6.12 ± 0.53		6.43 ± 0.38		0.82 ± 0.13		0.88 ± 0.10		4.15 ± 0.34		4.29 ± 0.23	
29to35	5	7.01 ± 0.54		7.40 ± 0.37		0.94 ± 0.11		1.00 ± 0.07		4.63 ± 0.31		4.81 ± 0.15	
36to42	6	6.91 ± 0.38		7.00 ± 0.45		0.94 ± 0.07		0.98 ± 0.05		4.58 ± 0.22		4.56 ± 0.27	
43to49	7	6.86 ± 0.53		6.58 ± 0.49		0.89 ± 0.13		0.87 ± 0.09		4.62 ± 0.30		4.33 ± 0.25	
50to56	8	6.57 ± 0.47		6.54 ± 0.73		0.86 ± 0.07		0.88 ± 0.11		4.36 ± 0.28		4.24 ± 0.45	
57to63	9	6.92 ± 0.52		7.00 ± 0.55		0.97 ± 0.11		0.99 ± 0.09		4.48 ± 0.32		4.46 ± 0.36	
64to70	10	6.67 ± 0.28		6.99 ± 0.05		1.00 ± 0.11		1.00 ± 0.03		4.24 ± 0.26		4.40 ± 0.08	
71to77	11	6.73 ± 0.42		7.08 ± 0.43		0.93 ± 0.12		1.01 ± 0.04		4.28 ± 0.21		4.47 ± 0.21	
78to84	12	6.77 ± 0.47		6.98 ± 0.36		0.98 ± 0.04		1.11 ± 0.08		4.40 ± 0.24		4.37 ± 0.21	
85to91	13	6.96 ± 0.66		7.04 ± 0.44		1.10 ± 0.10		1.16 ± 0.12		4.42 ± 0.45		4.52 ± 0.37	
129to126	18	6.41 ± 0.38		6.71 ± 0.03		1.01 ± 0.12		1.01 ± 0.04		4.04 ± 0.26		4.21 ± 0.10	
134to140	20	6.84 ± 0.37		6.82 ± 0.55		1.10 ± 0.07		1.20 ± 0.11		4.38 ± 0.18		4.18 ± 0.39	
162to168	24	7.45 ± 0.45		7.49 ± 0.55		1.30 ± 0.12		1.29 ± 0.06		4.45 ± 0.24		4.46 ± 0.52	
190to196	28	6.75 ± 0.47		7.49 ± 0.01		1.19 ± 0.07		1.20 ± 0.01		4.23 ± 0.36		4.58 ± 0.13	
218to224	32	6.07 ± 0.51		6.09 ± 0.04		1.09 ± 0.17		1.02 ± 0.02		3.80 ± 0.25		3.74 ± 0.14	
Mean		6.68 ± 0.48		6.77 ± 0.38		0.98 ± 0.10		1.00 ± 0.07		4.29 ± 0.29		4.27 ± 0.25	

Baer R. (Baer, 1991) discussed in their article that the use of rbST may be considered an advantage in milk production because this hormone results in a better lipid profile of the final product for human standards. Calcedo V. (Calcedo Ordóñez, 2000) declared that rbST is a healthy, safe and efficient product to increase milk production in cattle, and free of any risks for both consumers and cows. If this is so, why is its administration prohibited in some countries? Will its use have consequences for the animal or humans that consume these milk and meat products? Ravi P. *et al.* (Ravi et al., 2007) discussed in their review the rbST effects on the health and well-being of animals and on the health and safety of humans that consume milk, milk products and other livestock products as meat. They emphasized that the use of rbST can increases the risk of clinical mastitis and infertility in cows and can increases the risk of clinical lameness. For humans, highlighted as the main problem an increased level of the Insulin-like Growth Factor-1 (IGF-1) in milk derived from rbST-treated cow because is

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associated with a risk of various cancers Lamas *et al.* (Lamas et al., 2019) studied the gene expression profile in milk somatic cells in the same cows of the present study (Barreiro et al., 2022). The transcription of some genes (include IGF-1R) resulted strongly influenced by rbST treatment. Finally, if the risk of mastitis increases, the use of antibiotic treatment will increase too, subsequently the risk of antibiotic residues in milk and meat. So, the profiling of FAs in milk is an interesting alternative to assess the effects of rbST in animals but, analysts should bear in mind that FAs can be influenced by other factors as pregnancy, feeding or health status.

5.4 TYPES OF MILK THAT HUMANS CAN CONSUME AT DIFFERENT STAGES OF LIFE

The fatty acid profile of the human diet has changed in the last 100-150 years. The diets rich in SFAs and the increased intake of omega-6 over omega-3 counterparts, might have shifted the physiological state of humans to one more pro-inflammatory, pro-thrombotic and pro-aggregatory, because the ratio of n-6 / n-3 has increased in westernized diets to 20-50/1 when the recommendation is 4-5/1 (Mariamenatu & Abdu, 2021). Historically, milk from domestic cows has been an important nutrient source and it's believed that this relation has led to a gene-culture coevolution between human culture and domestic cattle. Thus, bovine milk has been part of the human diet, from birth to old age, for millennia (Marangoni et al., 2018). In recent years, the consumption of this food has been decreasing in some countries although preliminary evidence suggest potentially protective effects of milk against overweight, obesity, diabetes and cardiovascular disease, amongst others (Marangoni et al., 2018), and all international guidelines recommend daily consumption of milk and yogurt despite the cross-country variability of recommendations (Haug et al., 2007; Muehlhoff, Bennett, & McMahon, 2013). The replacement of bovine milk with other types of milk of plant-based drinks to gain health effects is becoming a widespread practice. However, this fact is not supported by adequate scientific evidence for the general healthy population, including children. Some FAs that are present in milk have very specific health effects as it is the case of short chain-SFAs, OA, PUFAs, and CLAs. CLA has been deeply studied because it offers an important protection against cancer, especially in the breast (Masters, McGuire, Beerman, Dasgupta, & McGuire, 2002). During human pregnancy, bovine milk may be a good source of proteins and calcium. In fact, the Spanish Nutrition Foundation recommends consuming 3-4 dairy products a day during pregnancy: 1 glass of milk, 2 yogurts, fresh or semi-cured cheese (Llano et al., 2017). In summary, milk and dairy are considered nutritious foods in all stages of human life.

The only stage of life in which it is not recommended to ingest cow's milk is during the first year of life. During the first 6 months life, the only food the infant should ingest is breast milk or formula milk. Between 6 and 12 months, breast milk continues to be the main food and different institutions, such as the Spanish Paediatric Association, recommend keeping breast milk on demand and introducing whole cow's milk after 12 months of age. It should be considered that breast milk continues to be the dairy of first choice and is recommended over any other milk, as long as mother and child wish it, up to 2 years of age or more. In the case of non-breastfed infants, the indicated formula after 6 months of age is the follow-on formula.

Table 12 shows the values of the main FAs in the dairy lipids representing all types of milk potentially consumed by humans through life. The great differences in the fatty acid profiles of bovine milk, breast milk and formula milks can be appreciated. The adequate intakes (IA) of EFAs recommended by WHO for infants (0-24 months) and children (2-18 years) and the acceptable macronutrient distribution range for adults were included in the table. In addition to the raw bovine milk samples, a sample of UHT (Ultra-High Temperature) whole milk was analysed and added to the table since cow's milk are not normally consumed raw. The UHT

milk changes slightly having a lower content in SFAs and higher in MUFAs. These data agree with the findings of Pestana *et al.* (Pestana, Gennari, Wissman Monteiro, Neutzling Lehn, & Volken de Souza, 2015), who concluded that UHT process slightly alter the composition of milk but it does not substantially change FAs profile regarding its potential nutritive properties and consequent benefits for human health.

With the data compiled in Table 12 from the various studies in this Thesis project, it can be clearly understood why the milk of each species is indicated for the survival of its progeny and why bovine milk is not the best choice for the infant as it differs significantly from human milk. Bovine milk has a much higher saturated content than human or formula milk. The saturated fatty acid 12:0 is higher in breast milk than in bovine milk, while 14:0 and 16:0 are higher in bovine milk than in human milk. Usually, MUFAs become the major fraction of human milk (Barreiro et al., 2018a), and OA is the main fatty acid in % of total fatty acids, while in bovine milk the fatty acid with the highest percentage is a saturated fatty acid, *i.e.* palmitic acid or 16:0. The major difference between bovine and human milk, is the greater contribution of PUFAs and EFAs offered by breast milk, with all the advantages that these FAs provide to the infant for the neural development both in the first months of life and in prolonged lactation.

The PUFAs AA, ALA and DHA, of different types of milk together with AI for each fatty acid as recommended by WHO, have been represented in Figure 17. Bovine milk contains an acceptable value of ALA but is deficient in AA and DHA compared to human milk and WHO recommendations. Bovine milk-based infant formulas are fortified with LC-PUFAs to make up for this deficit in EFAs but still fall short of WHO recommendations and human milk content. The ALA content of FSF for infants 0-6 months is much higher than in breast milk and AI, indicating that infant formulas are over-enriched with LC-PUFAs. One reason may be that the different brands of baby milk usually enrich their formulas with diverse oils (eggs and fish) or from unicellular microorganism (fungus and algae).

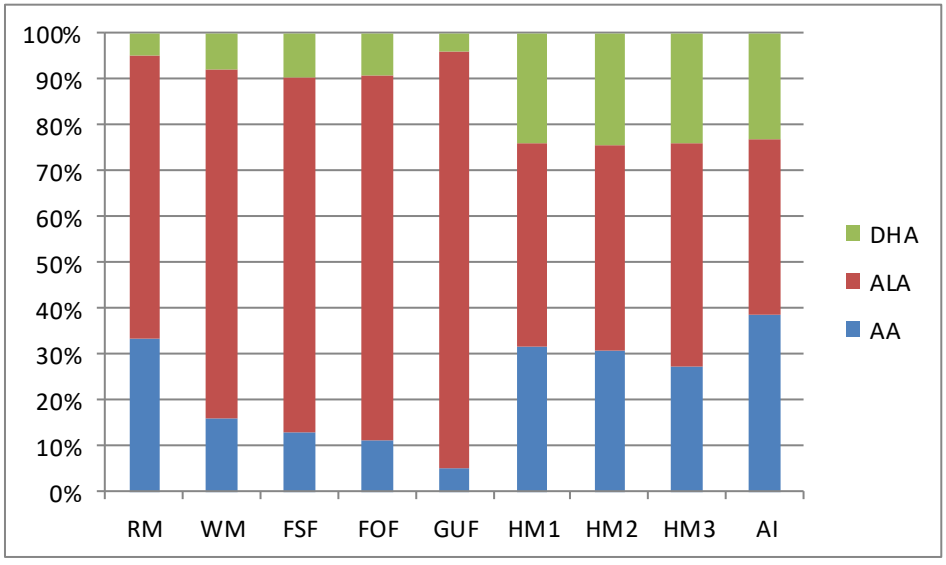


Figure 17. DHA, ALA and AA values in different types of milk and AI for each fatty acid. RM: raw milk, WM: whole milk, FSF: first- stage formula, FOF: follow-on formulas, GUF: growing-up formulas, HM1: human milk 0-6 months, HM2: human milk 6 months to 12 months, HM3: human milk from prolonged lactation, AI: adequate intake indicated by WHO.

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Table 12. Main fatty acids in different types of milk potentially consumed by humans through life, expressed as %w/w of total FAs, and recommended dietary intakes for infants and adults. Data collected from the studies of Barreiro and co-workers (2017, 2018, 2020); data for whole UHT milk is unpublished.

	Bovine milk		Infant formulas			Human milk			AI (INFANTS 0 - 6 m)	AI (ADULTS)
	n=20	n=5	n=5	n=13	n=5	N=24	N=12	N=20	% of FAs	Energy (%)
Fatty Acids (%)	Raw milk	Whole milk	FSF	FOF	GUF	0-6m	6m-1y	PL		
ΣSFAs	67.67	66.48	39.67	40.73	38.70	47.85	40.12	47.06	-	< 10
12:0	5.58	5.32	7.15	7.78	6.25	9.99	8.77	10.68	-	-
14:0	12.76	12.50	4.54	4.64	4.54	6.69	7.06	9.46	-	-
16:0	31.77	30.90	18.24	18.62	18.10	20.95	16.22	18.84	-	-
ΣMUFAs	26.15	27.72	40.51	40.37	41.30	31.83	41.37	31.08	-	10 - 15%
OA	19.94	21.50	38.48	38.04	39.60	26.48	36.12	25.39	-	-
ΣPUFAs	6.13	5.75	19.61	18.71	20.60	20.24	18.46	21.86	-	6 – 11%
Σn-6	3.91	2.82	17.02	16.08	17.30	17.54	16.13	18.68	-	2.5 - 9%
LA	3.11	2.31	16.26	15.63	16.5	15.62	14.77	16.74	-	2.5 - 9%
AA	0.27	0.12	0.28	0.26	0.11	0.61	0.45	0.69	0.4 - 0.6%	-
Σn-3	0.82	1.19	2.02	2.13	2.10	1.77	1.59	2.27	-	0.5 - 2%
ALA	0.5	0.57	1.69	1.88	1.96	0.70	0.69	1.12	0.4 - 0.6%	1 - 2%
EPA	0.07	0.09	0.07	0.07	0.07	0,14	0.12	0.13	-	-
DHA	0.04	0.06	0.21	0.22	0.09	0.49	0.46	0.60	0.2 - 0.36%	-

FSF: first-stage infant formulas, FOF: follow-on infant formulas, GUF: growing-up infant formulas, m: months, y: years, n=number of samples, N: number of women, PL: prolonged lactation, AI: adequate intake indicated by WHO; AMDR: acceptable macronutrient distribution range by WHO.

Figure 18 shows the main fatty acid groups in different types of milk consumed by humans over life. Bovine milk has the highest percentage of SFA of all milks, and it was observed that heat treatment did not noticeably change the values of total fatty acids. Breast milk from 0-6 months and during extended lactation (1-8 years of lactation) were very similar. At the beginning of lactation, breast milk has to supply all the baby's needs as it is the only nutrient. In prolonged lactation, the intake of breast milk is reduced in many cases to only one a day and the amount that the child can take is less as it ingests a complete diet. Even so, it is still a great source of nutrients and EFAs. If breast milk cannot be produced and the infant is fed with formula, the graph shows that SFA and MUFA values are balanced and PUFA values are very similar to those of human milk from 0 to 6 months. With breastfeeding time, these PUFAs increase their content in breast milk.

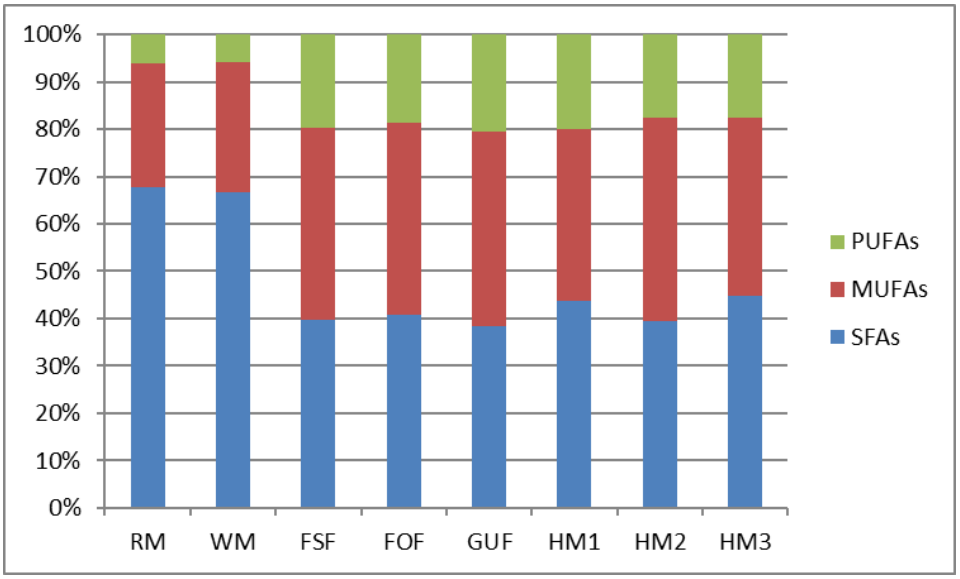


Figure 18. Total values of the fatty acid groups in the different types of milk. RM: raw milk, WM: whole milk, FSF: first- stage formula, FOF: follow-on formulas, GUF: growing-up formulas, HM1: human milk 0- 6 months, HM2: human milk 6 months to 12 months, HM3: human milk from prolonged lactation.

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6 CONCLUSIONS

Based on the results achieved in the five original studies that constitute this Doctoral Thesis, the following conclusions have been drawn:

1. The fatty acid profiles of human milk are rather similar worldwide, with the clear exception of Asiatic breast milk. The latter showing high levels of n-3 PUFAs, some particular MUFAs (derived from 18:0, 20:0 and 22:0), while low in 12:0 and 14:0 and high in long chain saturated fatty acids 20:0 and 22:0. Oils, meat and fish intake are clearly reflected in the fatty acid composition of breastmilk, and diet confers a clear and distinguishable *fingerprint* in the fat of this outstanding biological fluid.

2. Breast milk from Galician women shows a profile similar to the milk from mothers living in other European countries adhered to the Atlantic Diet (Portugal, Sweden, Netherlands…), and different from those countries considered purely Mediterranean and bordering the Mediterranean Sea (Greece and Italy). Typical food products of the northwestern part of Spain such as milk and dairy, pork, beef and sunflower and olive oils, are reflected in Galician breast milk as relatively high levels of linoleic acid (LA) and lower α-linolenic acid (ALA), 15:0 and 17:0.

3. The breast milk fatty acid profiles (*fatty acidome*) differ widely between individual women showing woman-specific features and demonstrating that the composition of each woman's milk is unique and adapted to the child.

4. As lactation goes on, the fatty acid profile of milk evolves, increasing the levels of α-linolenic acid (ALA), arachidonic acid (AA), and docosahexaenoic acid (DHA), while its saturated fraction increases, particularly for 14:0. In terms of AA, EPA and DHA, human milk never ceases to be a complete nutritional food that does not lose its properties over time and meets adequate intake recommendations for infants.

5. Infant formulas are suitable to replace breast milk, in terms of fatty acids, with the formulas indicated for 0-6 months (*first stage*) being most similar to breastmilk. The fatty acids included in the formulation of infant formulas are very similar between them, not even close to the woman-specific features observed in human milk. The values for AA and DHA are lower in formula than human milk, and ALA is much higher, altogether suggesting that formulas are over-enriched with ALA. The balance between AA and DHA in formulas is similar while in breast milk, the value of AA is always higher than that of DHA.

6. Pregnancy is a natural state of the animal that can vary the lipid composition of the bovine milk, giving a profile richer in SFAs, especially 12:0, 14:0 and 16:0 (pro-atherogenic fatty acids), lower levels of PUFAs such as oleic acid, and similar levels of PUFAs. The fatty acid profiles of milk from non-pregnant dairy cows appears more adequate for human consumption.

7. Recombinant bovine somatotropin (rbST) administered to dairy cattle, not only increases milk yield but it can also modify the lipid profile of the milk, lowering SFAs and increasing MUFAs, particularly oleic acid, while PUFAs remain stable in comparison to

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control animals. The fatty acid profiles of milk from rbST-treated dairy cows appears more adequate for human consumption.

8. Human milk is the ultimate personalized food for infant humans, providing a very particular fatty acid profile that does not match that of composition of bovine milk and/or formulas, evolves over time, and is influenced by diet and individual characteristics of the mother and child.

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Fatty acid composition of breast milk in Galicia (NW Spain): A cross-country comparison

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Publication: Prostaglandins, Leukotrienes and Essential Fatty Acids

Publisher: Elsevier

Date: August 2018

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Evolution of breast milk fatty acids in Spanish mothers after one year of uninterrupted lactation

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Publication: Prostaglandins, Leukotrienes and Essential Fatty Acids

Publisher: Elsevier

Date: August 2020

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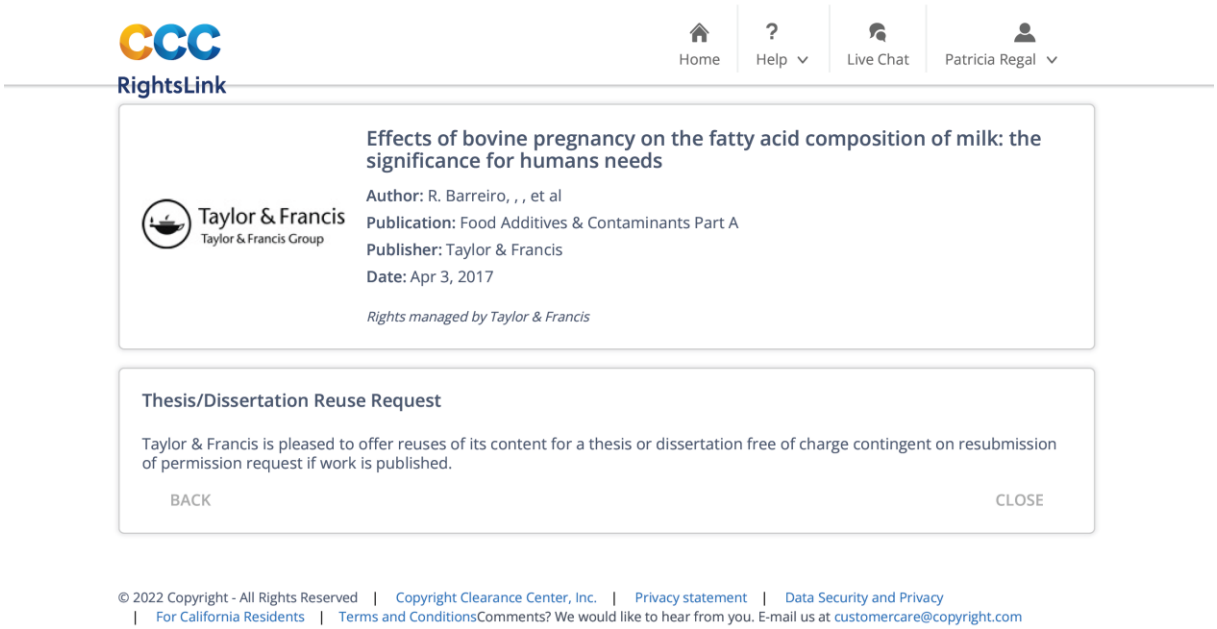
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DITAME DO COMITÉ DE ÉTICA DA INVESTIGACIÓN DE SANTIAGO-LUGO

Juan Manuel Vázquez Lago, Secretario do Comité de Ética da Investigación de Santiago-Lugo

CERTIFICA:

Que este Comité avaliou na súa reunión do día 21/06/2016 o estudo:

Título: Composición de la leche materna: variaciones interindividuales y condicionantes dietéticos
Promotor: Olga López Racamonde
Tipo de estudo: Outros
Versión: Versión: 1, fecha 02/05/2016
Código do Promotor: XXXX/2016
Código de Rexistro: 2016/280

E, tomando en consideración as seguintes cuestións:

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Centros	Investigadores Principais
Centro de Salud de San Roque	Olga López Racamonde

En Santiago de Compostela, a 21 de xuño de 2016
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Juan M. Vázquez Lago

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11. O Comité Territorial de Ética da Investigación de Santiago-Lugo cumpre tanto na súa composición coma nos seus PNTs os requisitos legais vixentes (R.D 1090/2015 de ensaios clínicos, e a Lei 14/2007 de Investigación Biomédica).

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Para que conste onde proceda, e a petición do promotor / investigador, en Santiago de Compostela, a 21 de xuño de 2016.

O Secretario do Comité Territorial de Ética da Investigación de Santiago Lugo,



Juan M. Vázquez Lago

ANNEX 3. AUTHOR'S DECLARATION ON ETHICS APPROVAL AND HUMAN STUDY REGISTRATION.

Declaración sobre a aprobación polo comité de ética

Dona Rocío Barreiro Tomé, autora da tese titulada *Fatty acids contribution of bovine and maternal milk to humans: assessment and influential factors,* presento a miña tese para defendida en base á modalidade de COMPENDIO DE PUBLICACIÓNS e declaro que:

1) A tese abarca os resultados do meu traballo de investigación, parte dos cales foron obtidos de seres humanos, as súas mostras, e/ou os seus datos de carácter persoal.
2) O estudio realizado sobre humanos é de carácter observacional, e foi levado a cabo seguindo as directrices da declaración de Helsinki, e co consentimento informado de tódolos voluntarios.
3) O traballo que implica a seres humanos recibiu a aprobación do Comité de Ética da Investigación de Santiago-Lugo, con código de rexistro 2016/280 (Anexos da Tese).
4) O estudio realizado en humanos está rexistrado no rexistro de ensaios clínicos ClinicalTrials.gov da Biblioteca Nacional de Medicina dos Estados Unidos, baixo o código identificador NCT03245697 (https://clinicaltrials.gov/show/NCT03245697).

E para que conste ós efectos oportunos, e co visto e prace dos meus directores de tese, asino a presente en Lugo, a 3 de outubro de 2022.

Asdo.: Rocío Barreiro Tomé (a autora)

Visto e Prace:

Asdo.: Patricia Regal López (a Directora)

Asdo.: Alberto Cepeda Sáez (o Director)

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ANNEX 4. INFORMED CONSENT FOR HUMAN PARTICIPANTS

ANEXO II. CONSENTIMENTO INFORMADO.

FOLLA DE INFORMACIÓN Ó/Á PARTICIPANTE ADULTO/A

TÍTULO DO ESTUDO: Composición do leite materno: variacións interindividuais e condicionantes dietéticos

INVESTIGADORES: Olga López Racamonde, Patricia Regal López y Cristina Fente Sampayo.

CENTRO/S: Laboratorio de Hixiene, Inspección e Control de Alimentos (LHICA), Facultade de Veterinaria de Lugo, Universidade de Santiago de Compostela. Centro de Saúde de San Roque de Lugo.

Este documento ten por obxecto ofrecerlle información sobre un **estudo de investigación** no que se lle invita a participar. Este estudo foi aprobado polo Comité de Ética da Investigación de Galicia.

Se decide participar no mesmo, debe recibir información personalizada do investigador, **ler antes este documento** e facer todas as preguntas que precise para comprender os detalles sobre o mesmo. Se así o desexa, pode levar o documento, consultalo con outras persoas, e tomar o tempo necesario para decidir se participar ou non.

A participación neste estudio é completamente **voluntaria**. Vostede pode decidir non participar ou, se acepta facelo, cambiar de parecer retirando o consentimento en calquera momento sen obriga de dar explicacións. Asegurámoslle que esta decisión non afectará á relación co seu médico nin á asistencia sanitaria á que vostede ten dereito.

Cal é o propósito do estudo?

En base aos antecedentes científicos existentes, como obxectivo principal deste estudo preténdese determinar a composición do leite materno, as variacións existentes entre individuos (nais lactantes), e a posible existencia dunha relación entre a composición do leite e os hábitos dietéticos das nais.

Por que me ofrecen participar a min?

Vostede é convidado a participar porque está aleitando o/s seu/s fillo/s.

En que consiste a miña participación?

A súa participación consiste en aportar unha mostra de leite materno, recollida por vostede mesma de xeito individual, en envases/recipientes estériles que lle proporcionarán os

investigadores responsables do estudo. Para a obtención dunha mostra de leite nas mellores condicións para o estudo, a nai deberá tomar como referencia os seguintes pasos:

- Lavar ben con auga e xabón as mans, e secar con toalliña de papel ou similar.
- As mostras deben ser recollidas inmediatamente antes de aleitar o seu fillo/a, descartando uns mililitros. Non debería de aplicarse nada na mama antes da toma de mostra, como pomadas ou cremas. En caso contrario, deberá indicarlle ao investigador responsable da recollida da mostra qué tipo de crema/pomada (nome) empregou.
- Despois da recollida da mostra, cerrar ben o recipiente estéril e entregar inmediatamente a algún dos investigadores responsables do estudo. Se a entrega non é posible no momento da toma de mostra, conxelar e entregar cando sexa posible.

Aceptando participar neste estudo, vostede acepta que a investigadora Olga López Racamonde consulte datos da súa historia clínica directamente relacionados co obxectivo deste estudio. O remanente das mostras biolóxicas (leite), de habelo, será destruído cando finaliza o estudo ou anonimizado.

Existe a posibilidade de que contactemos con vostede con posterioridade para conseguir datos adicionais ou novas mostras.

A análise do seu leite materno englobará composición nutricional e funcional (graxas, vitaminas, hormonas, bacterias, ...), centrándose en compostos que benefician ou prexudican ó/á seu/súa fillo/a e se poden ver modificados polos alimentos que consume ou os seus hábitos diarios. Coa fin de buscar unha posible relación entre os seus hábitos dietéticos ou alimenticios e a composición do seu leite, pediráselle que cubra un cuestionario de frecuencia de consumo de alimentos, e un cuestionario de adherencia a Dieta Mediterránea (DM) e a Dieta Atlántica. Tamén lle solicitaremos información sobre parámetros socio-demográficos básicos: nacionalidade, profesión, idade, peso, estatura, duración da última xestación, peso e sexo do bebé nacido, problemas en xestación/parto, hábitos nocivos –tabaquismo–, estado de saúde/presenza de enfermidades (prevalencia de factores de risco cardiovascular), e actividade física diaria.

A súa participación terá unha duración total estimada de media hora, e de vinte minutos a media hora para a realización dos cuestionarios.

O investigador pode decidir finalizar o estudo antes do previsto ou interromper a súa participación. En todo caso informaráselle dos motivos da súa retirada.

Que molestias ou inconvenientes ten?

O procedemento está exento de riscos, tanto para vostede como para o seu fillo.

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Obterei algún beneficio por participar?

Non se espera que vostede obteña beneficio directo por participar no estudo. A investigación pretende descubrir aspectos descoñecidos ou pouco claros sobre a composición do leite materno, e tamén da súa relación coa alimentación das nais. Esta información poderá ser de utilidade nun futuro para outras nais e lactantes, así como para vostede mesma. Igualmente, se durante a análise do seu leite os investigadores observasen algunha anomalía ou alteración que poida ser prexudicial para vostede ou o seu fillo/a, comunicaráselle de xeito inmediato.

Recibirei a información que se obteña do estudo?

Se vostede o desexa, si. Facilitaráselle un resumo dos resultados do estudo. Tamén poderá recibir os resultados das probas que se realicen coas súas mostras se así o solicita, dirixíndose ós investigadores. Estes resultados poden non ter aplicación clínica nin unha interpretación clara, polo que, se quere dispor deles, deberían ser comentados cos responsables do estudo. Algúns dos resultados poderían descubrir certas condicións que afecten no futuro á súa saúde. Estas condicións deberían ser compartidas por familiares seus. Chegado o caso, seralle comunicado e sería conveniente que vostede lles transmita esta información a familiares.

Publicaranse os resultados deste estudo?

Os resultados deste estudo serán remitidos a publicacións científicas para a súa difusión, pero non se transmitirá ningún dato que poida levar á identificación dos participantes.

Como se protexerá a confidencialidade dos meus datos e mostras?

O tratamento, comunicación e cesión dos seus datos farase conforme ao disposto pola Lei Orgánica 15/1999, de 13 de decembro, de protección de datos de carácter persoal. En todo momento, vostede poderá acceder aos seus datos, opoñerse, corrixilos ou cancelalos, solicitando ante o equipo investigador.

Unicamente o equipo investigador, e as autoridades sanitarias, que teñen deber de gardar a confidencialidade, terán acceso a todos os datos recollidos polo estudo. Poderase transmitir a terceiros información que non poida ser identificada. No caso de que algunha información sexa transmitida a outros países, realizarase cun nivel de protección dos datos equivalente, como mínimo, ao esixido pola normativa do noso país.

Os seus datos e mostras biolóxicas serán recollidos e conservados até rematar o estudo, **codificados,** que quere dicir que posúen un código có que o equipo investigador poderá coñecer a quen pertencen. O responsable da custodia dos datos e mostras *é Patricia Regal López*, e o lugar de realización dos análises previstos neste estudo *é o Laboratorio de Hixiene, Inspección e Control de Alimentos (LHICA)*, na facultade de Veterinaria de Lugo.

Versión: 2, data: 03/06/2016.
Deberán firmarse tres modelos, un será entregado ao participante, outro será conservado no centro no que se obtivo a mostra, e o terceiro será conservado polo responsable do estudo de investigación

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Ao rematar o estudo, conforme ao RD 1716/2011, as súas mostras biolóxicas serán ***anonimizadas***, e dicir, que se rompeu todo vínculo que poida identificar a persoa doante da mostra, non podendo ser identificado nin sequera polo equipo investigador. Igualmente, vostede poderá solicitar a destrución ou anonimización da súa mostra en calquera momento, dirixíndose ós investigadores. Ten que saber que isto non será de aplicación aos datos resultantes dos análises que xa foran feitos.

Existen intereses económicos neste estudo?

Esta investigación non esta promovida por ningún ente financeiro. Os investigadores non recibirán retribución específica pola dedicación ao estudo.

Vostede non será retribuído por participar. Se dos resultados do estudo se derivasen produtos comerciais ou patentes, vostede non participará dos beneficios económicos orixinados.

Como contactar có equipo investigador deste estudo?

Vostede pode contactar con:

Olga López Racamonde, no teléfono 627537647, ou enderezo electrónico: olga.lopez.racamonde@sergas.es

Patricia Regal López no teléfono 645930000, ou enderezo electrónico: patricia.regal@usc.es

Moitas grazas pola súa colaboración.

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DOCUMENTO DE CONSENTIMENTO PARA A PARTICIPACIÓN NUN ESTUDO DE INVESTIGACIÓN

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Eu, _____

- *Lin a folla de información ao participante do estudo arriba mencionado que se me entregou, puiden conversar con _____ e facer todas as preguntas sobre o estudo necesarias.*
- *Comprendo que a miña participación é voluntaria, e que podo retirarme do estudo cando queira, sen ter que dar explicacións e sen que isto repercuta nos meus coidados médicos.*
- *Accedo a que se utilicen os meus datos e mostras nas condicións detalladas na folla de información ao participante.*
- *Presto libremente a miña conformidade para participar neste estudo.*

Respecto á conservación DAS MOSTRAS recollidas neste estudo:

☐ Non accedo a que sexan conservadas unha vez terminado o estudo

☐ Accedo a que sexan conservadas anonimizadas.

Asdo.: O/a participante,	Asdo.:O/a investigador/a que solicita o consentimento
Nome e apelidos:_____	Nome e apelidos:_____
Data:	Data:

Versión: 2, data: 03/06/2016.
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ANNEX 5. CONFLICT OF INTEREST STATEMENT

The doctoral candidate declares no conflicts of interest related to her thesis.

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AGRADECEMENTOS

Chegado ó final deste manuscrito, non podo esquecer dar as grazas a todos os que contribuíron, dunha maneira ou doutra, a que agora mesmo estea a escribir ó final.

Grazas ós meus directores de tese, Patricia Regal e Alberto Cepeda, por facer posible levar a cabo os proxectos de toda a parte experimental. Aínda non me creo que todos eses centos de mostras e horas deran o seu froito. Grazas a profesora Cristina Fente por deixarnos entrar no extenso mundo do leite materno. Grazas especialmente a Patri, que confía máis en min do que ás veces o fago eu mesma. Nunca me cansarei de agradecerlle a Mo e Chelo tanta axuda e ánimos. Moitas veces panos de bágoas pero moitas máis risas infinitas, facendo que as horas en residuos sexan máis levadías.

Grazas a todos os profesores e compañeiros do laboratorio, dos que sempre hai algo novo por aprender cada día. Grazas por novos proxectos e retos nos que poder mellorar e superarse todos xuntos. Grazas tamén a todos eses compañeiros cos que tiven a sorte de coincidir, unhas veces por máis tempo outras pouco, pero dos que me quedei con moitas leccións.

As grazas máis grandes quedan para o final e van para a familia e amig@s porque sen eles nada é o mesmo, porque aínda que estean lonxe están sempre aquí, porque sodes o primeiro sempre. Grazas polos ánimos, confianza e forza que me dades , sobre todo nos momentos duros, facendo que o camiño sexa sempre cara adiante. Grazas infinitas porque sodes vida.

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The fatty acids occurring in mammalian milk are one of the most studied components for their effects in human health. Several endogenous and exogenous factors can vary their levels in milk, making this food more beneficial or harmful to the human organism. In this Doctoral Thesis project, the fatty acid profiles of the three types of milk most consumed by humans, i.e. bovine milk, breast milk and/or infant formula, were analysed in detail. Diverse influential factors have been investigated, and their composition has been compared and discussed to assess the adaptation of each type of milk to the specific dietary requirements of humans.