UNIVERSIDAD COMPLUTENSE DE MADRID
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TESIS DOCTORAL

Ácidos grasos y aminoácidos como marcadores de salud intestinal en perros con enteropatías inflamatorias crónicas

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Cristina Higueras López

DIRIGIDA POR

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Memoria presentada para optar al grado de doctora por

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A mi familia

"A veces sentimos que lo que hacemos es tan solo una gota en el mar, pero el mar sería menos si le faltara una gota"

Madre Teresa de Calcuta

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ÍNDICES

AA	Ácido araquidónico (C20:4 n-6)
AAAs	Aminoácidos aromáticos
AACR	Aminoácidos de cadena ramificada
ACTH	Hormona adrenocorticótropa
AGCC	Ácidos grasos de cadena corta
AGCI	Ácidos grasos de cadena impar
AGRs	Ácidos grasos ramificados
AhR	Receptor aril-hidrocarburos
ALA	Ácido alfa-linolénico (C18:3n-3)
ARE	*Antibiotic-responsive enteropathy*
ATP	Adenosín trifosfato
BCAT	Enzima transaminasa de aminoácidos de cadena ramificada
BCS	*Body condition score*
BKD	Enzima deshidrogenasa de α-cetoácidos de cadena ramificada
C12:0	Ácido láurico
C15:0	Ácido pentadecanoico
C16:0	Ácido palmítico
C16:1n-7	Ácido palmitoleico
C17:0	Ácido heptadecanoico
C18:0	Ácido esteárico
C18:1n-9	Ácido oleico
C2:0	Ácido acético
C3:0	Ácido propiónico
C4:0	Ácido butírico
CC	Condición corporal
CCECAI	*Canine chronic enteropathy clinical activity index*
CIBDAI	*Canine Inflammatory bowel disease activity index*

CM	Condición muscular
COX	Ciclooxigenasa
CU	Colitis ulcerosa
DHA	Ácido docosahexaenoico (C22:6 n-3)
EC	Enteropatía crónica
ECR	Enfermedad de Crohn
EFA	*Essential fatty acids*
ELOVLs	Elongasas
EPA	Ácido eicosapentaenoico (C20:5 n-3)
F/B	Firmicutes/Bacteroidetes
FRE	*Food-responsive enteropathy*
GIA	*Giardia* spp.
GLA	γ-linolénico
GLP-1	Péptido similar al glucagón tipo 1
GPRs	Receptores acoplados a proteínas G
HC	Hidratos de carbono
HDAC	Histona desacetilasa
HIF-1a	Factor de transcripción que regula la respuesta celular a la hipoxia
IBD	*Inflammatory bowel disease*
IC4:0	Ácido isobutírico
IC5:0	Ácido isovalérico
IgA	Inmunoglobulina A
IL-18	Interleuquina 18
IRE	*Immunosuppressant-responsive enteropathy*
LA	Ácido linoleico (C18:2 n-6)
LOX	Lipoxigenasa
LTB_4	Leucotrieno B4

MCS	*Muscle condition score*
MUFA	Ácidos grasos monoinsaturados
NF-KB	Factor nuclear κB
NLR	Neutrófilos/linfocitos
NO	Óxido nítrico
NRE	*Non-responsive enteropathy*
pANCA	*Perinuclear anti-neutrophilic cytoplasmic antibodies* (anticuerpos perinucleares anticitoplasma de los neutrófilos)
PGE_2	Prostaglandina E2
PLE	*Protein-losing enteropathy*
PLI	*Pancreatic lipase immunoreactivity*
PLR	Plaquetas/linfocitos
PPARγ	Receptor activado por proliferadores peroxisómicos gamma
PUFA	Ácidos grasos poliinsaturados
PYY	Péptido YY
S100A12	Calgranulina C
S100A8/A9	Calprotectina fecal
SAT	Ácidos grasos saturados
SII	Plaquetas*neutrófilos/linfocitos
SNPs	Polimorfismos de nucleótido único
sRAGE	Receptor soluble para productos finales de glicación avanzada
T4	Tiroxina
TAG	Triglicéridos
TGF-β	Factor de crecimiento transformante
Th	*T helper Lymphocytes* (linfocitos T colaboradores)
TLI	*Trypsin-like immunoreactivity*
TLR	Receptor tipo Toll
Tregs	Linfocitos T reguladores

TSH	Hormona estimulante de la tiroides
α1- PI	Inhibidor de la proteinasa α1

RESUMEN

RESUMEN

Las enteropatías crónicas (ECs) en perros son un grupo de enfermedades de etiología desconocida en cuya patogenia participan factores genéticos y una respuesta inmunitaria exacerbada frente a antígenos dietéticos o microbianos. Actualmente, su diagnóstico se basa en la respuesta al tratamiento, clasificándose en enteropatía que responde a dieta (FRE), enteropatía que responde a antibióticos (ARE), enteropatía que responde a inmunosupresores (IRE) y enteropatía que no responde (NRE).

El diagnóstico de estas enfermedades requiere un protocolo diferencial y un ensayo dietético con una dieta altamente digestible basada en proteína novel o hidrolizada. En ausencia de respuesta, se precisan pruebas más invasivas, como la endoscopia y la toma de biopsias, para confirmar IRE. Debido al prolongado tiempo necesario para alcanzar un diagnóstico, se están investigando biomarcadores que permitan predecir la respuesta al tratamiento y evitar procedimientos invasivos. Aunque existen biomarcadores clínicos utilizados para la monitorización y el pronóstico, aún no se dispone de un biomarcador que diferencie de manera fiable entre FRE e IRE. En medicina humana, el estudio de la metabolómica ha permitido identificar alteraciones en metabolitos como ácidos grasos y aminoácidos en pacientes con enfermedad inflamatoria intestinal (IBD), análoga a la IRE en perros. Sin embargo, en veterinaria, estos estudios son escasos.

Esta tesis doctoral investiga las alteraciones en el perfil de ácidos grasos y aminoácidos en perros con FRE e IRE principalmente, así como en otras patologías que cursan con sintomatología similar, como la parasitosis por *Giardia* spp., con el objetivo de identificar posibles biomarcadores y profundizar en el conocimiento de la patogenia de estas enfermedades. Hasta la fecha, los estudios sobre ECs en perros han analizado exclusivamente la IRE o han agrupado los distintos grupos en la misma categoría de ECs. Por ello, esta investigación aporta un análisis diferencial entre FRE e IRE.

Para la consecución de los objetivos, se llevaron a cabo 2 experimentos. En un primer experimento piloto, participaron nueve perros con FRE y seis perros sanos, de los cuales se recogió información mediante anamnesis y exploración física relacionada con los signos clínicos y la gravedad de la enfermedad en base a determinados índices. Además, se recogió información de las dietas consumidas por los perros control y los perros

diagnosticados con FRE antes del inicio del ensayo dietético. Se compararon los perfiles de ácidos grasos y aminoácidos sistémicos (plasma) y fecales, mediante cromatografía de gases y cromatografía de líquidos.

Los perros con FRE mostraron antes del tratamiento dietético niveles plasmáticos reducidos de C20:5n-3 (EPA), C22:5n-3, ácidos grasos poliinsaturados (PUFA) y omega-6, mientras que la proporción de C20:3n-6 aumentó respecto a los perros control. A nivel fecal, los perros con FRE exhibieron concentraciones inferiores de ácidos grasos de cadena corta (AGCC) totales, ácido acético, propiónico, isobutírico e isovalérico, así como de ácidos grasos de cadena larga, incluidos C15:0, C16:1n-7, C16:1n-9, EPA y ácidos grasos monoinsaturados (MUFA). Además, los indicadores de desaturación (C16:1n-7/C16:0 y C18:1n-9/C18:0) estuvieron reducidos, mientras que los niveles de ácido araquidónico y los indicadores de elongación fueron superiores, sugiriendo una alteración del metabolismo lipídico (artículo 1).

En cuanto al perfil aminoacídico, los perros con FRE mostraron antes del tratamiento dietético niveles plasmáticos reducidos de asparagina, histidina, glicina, cistina, leucina y de la relación aminoácidos de cadena ramificada/aminoácidos aromáticos (AACR/AAAs), mientras que la fenilalanina se encontró aumentada. En muestras fecales, se observaron niveles elevados de cistina y reducidos de fenilalanina. Se identificó una asociación entre AACR (leucina, isoleucina) y fenilalanina con indicadores de salud intestinal como los AGCC. Además, se encontró una correlación positiva entre el índice de gravedad CIBDAI (*canine inflammatory bowel disease activity index*) y la fenilalanina plasmática, mientras que la leucina, lisina, los AACR y la proporción AACR/AAAs sistémicos mostraron correlaciones negativas, lo que sugiere que una mayor gravedad de la enfermedad se asocia con niveles más elevados de AAAs y reducidos de AACR (artículo 2).

En términos generales, se evidenció que las muestras fecales reflejaban mejor el estado de la enfermedad que las plasmáticas al correlacionarse con indicadores de gravedad, y al ser un procedimiento no invasivo de más fácil recolección, en el experimento 2 se procedió a la recogida exclusivamente de este tipo de muestras.

En el segundo experimento, se investigaron distintos tipos de enfermedades inflamatorias crónicas incluyendo perros con IRE y perros con infección por *Giardia* spp.

(uno de los principales diagnósticos diferenciales de las ECs), junto con perros con FRE y perros sanos, y se aumentó el tamaño muestral respecto al primer experimento piloto (control = 22; FRE = 35; IRE= 18; *Giardia* spp. = 9). De manera similar al experimento 1, se recogió información sobre los animales y las dietas consumidas previo al ensayo dietético con el objeto de tener grupos lo más homogéneos posibles en cuanto a alimentación, y se procedió a la determinación del perfil de ácidos grasos por cromatografía de gases y al perfil de aminoácidos por cromatografía de líquidos.

Los ácidos grasos C16:1n-7, la ratio C16:1n-7/C16:0, el ácido acético y el total de AGCC en heces permitieron diferenciar entre perros con FRE e IRE. Además, se encontró que un mayor índice de gravedad CIBDAI se asoció con menores concentraciones de PUFA, omega-6 y de su precursor (el ácido linoleico, LA); pero con mayores niveles de ácidos grasos saturados (SAT), Δ6-desaturasa y elongasa C22:5n-3/C20:5n-3, lo que sugiere una hiperactivación del metabolismo de los omega-6 en estados más graves. El deterioro en las características de las heces, medido mediante la puntuación fecal de Purina®, se correlacionó con niveles elevados de SAT y elongasa, y con valores reducidos de PUFA y omega-6. Niveles altos de CIBDAI y de grasa fecal también se asociaron con menores concentraciones de C16:1n-7, C16:1n-7/C16:0 y Δ9-desaturasa en heces, lo que confirma una mayor alteración del metabolismo lipídico en IRE (artículo 3).

En el análisis discriminante llevado a cabo, el perfil de ácidos grasos fecales permitió diferenciar un 75% de las muestras correctamente, siendo las variables seleccionadas para las ecuaciones lineales discriminantes C16:1n-7/C16:0, C18:1n-9/C18:0, ácido acético y omega-3. Los perros con IRE se diferenciaron correctamente en un 92% de los casos con respecto al resto de grupos, mostrando mayores cambios en dichos ácidos grasos en comparación con los perros con FRE y los controles, entre los cuales se observó cierto solapamiento (artículo 3).

Por otra parte, se estudió el hemograma, la bioquímica y diversos índices inflamatorios sistémicos en relación con el perfil lipídico fecal (artículo 4) con el objeto de profundizar en el conocimiento de los grupos FRE e IRE antes del tratamiento dietético. Se identificaron diferencias significativas entre los perros con FRE y aquellos con IRE en los niveles de plaquetas, NLR (neutrófilos/linfocitos), PLR (plaquetas/linfocitos) y SII

(plaquetas*neutrófilos/linfocitos). Además, se destacó el papel de C16:1n-7, la ratio C16:1n-7/C16:0, el ácido acético, el butírico y los AGCC totales en la regulación del metabolismo sistémico, influyendo en linfocitos, plaquetas, índice SII y glucosa, observándose que los perros con IRE presentaron una mayor disrupción metabólica que los perros con FRE.

En cuanto al perfil aminoacídico fecal, no se observaron diferencias entre perros con FRE y perros con IRE antes del tratamiento dietético. Sin embargo, ambos grupos de ECs se diferenciaron de los perros sanos, presentando niveles superiores de treonina y AAAs, especialmente tirosina, así como una relación AACR/AAAs más baja. Además, la relación AACR/AAAs se asoció con niveles elevados de MUFA, ácido propiónico y AGCC, mientras que con niveles inferiores de SAT en heces, lo que sugiere que los niveles de AACR podrían estar relacionados con un mejor estado de salud. Por otra parte, el perfil de aminoácidos de los perros infectados por *Giardia* spp. mostró diferencias marcadas con respecto al resto de los grupos experimentales, con niveles significativamente superiores (artículo 5).

El análisis discriminante del perfil de aminoácidos permitió clasificar correctamente el 56% de las muestras, seleccionando como variables discriminantes la tirosina, el ácido glutámico, la arginina y la fenilalanina. Sin embargo, el mayor porcentaje de clasificación correcta (78%) se obtuvo al combinar los perfiles de ácidos grasos y aminoácidos en un mismo análisis discriminante. En este caso, las variables seleccionadas fueron: tirosina, ácido glutámico, MUFA, C16:1n-7/C16:0, ácido acético, AACR/AAAs, C22:5n-3, C18:1n-9/C18:0, elongasa C22:5n-3/C20:5n-3, cistina y EPA. En este sentido se mostró una mayor capacidad del perfil de ácidos grasos sobre el de aminoácidos en la diferenciación entre los distintos grupos, aunque es la combinación de ambos la que obtiene mejores resultados.

La caracterización de la composición y características de las heces como procedimiento no invasivo previo al tratamiento dietético en perros con distintos tipos de ECs, ha permitido observar principalmente diferencias en el metabolismo de ácidos grasos de distinta intensidad y abre nuevas expectativas en el diseño más personalizado de dietas dirigidas a estos animales.

SUMMARY

Chronic inflammatory enteropathies (CIEs) in dogs are a group of diseases with an unknown etiology, in which genetic factors and an exaggerated immune response to dietary or microbial antigens play a role in their pathogenesis. Currently, diagnosis is based on treatment response, classifying them as food-responsive enteropathy (FRE), antibiotic-responsive enteropathy (ARE), immunosuppressant-responsive enteropathy (IRE), and non-responsive enteropathy (NRE).

The diagnosis of these diseases requires a differential protocol and a dietary trial with a highly digestible diet based on novel or hydrolyzed protein. In the absence of response, more invasive tests, such as endoscopy and biopsy collection, are needed to confirm IRE. Due to the prolonged time required to reach a diagnosis, biomarkers are being investigated to predict treatment response and avoid invasive procedures. Although there are clinical biomarkers used for monitoring and prognosis, there is still no reliable biomarker that differentiates between FRE and IRE. In human medicine, metabolomics studies have identified alterations in metabolites such as fatty acids and amino acids in patients with inflammatory bowel disease (IBD), analogous to IRE in dogs. However, in veterinary medicine, such studies are scarce.

This doctoral thesis primarily investigates alterations in the fatty acid and amino acid profiles in dogs with FRE and IRE, as well as in other diseases that present similar clinical signs, such as *Giardia* spp. parasitosis to identify potential biomarkers and gain a deeper understanding of the pathogenesis of these diseases. To date, studies on CIEs in dogs have exclusively analyzed IRE or grouped the different types under the same category of CIEs. Therefore, this research provides a differential analysis between FRE and IRE.

To achieve the objectives, two experiments were carried out. In the first pilot experiment, nine dogs with FRE and six healthy dogs participated. Information was collected through anamnesis and physical examination related to clinical signs and the severity of the disease based on certain indices. Additionally, information on the diets consumed by the control dogs and the dogs diagnosed with FRE before the start of the dietary trial was gathered. The systemic (plasma) and fecal fatty acid and amino acid profiles were compared using gas chromatography and liquid chromatography,

respectively, with significant differences observed between groups in both types of samples.

Dogs with FRE showed reduced plasma levels of C20:5n-3 (EPA), C22:5n-3, polyunsaturated fatty acids (PUFAs), and omega-6, while C20:3n-6 showed elevated values. In feces, dogs with FRE exhibited lower concentrations of total short-chain fatty acids (SCFAs), acetic acid, propionic acid, isobutyric acid, and isovaleric acid, as well as long-chain fatty acids, including C15:0, C16:1n-7, C16:1n-9, EPA, and monounsaturated fatty acids (MUFAs). Furthermore, desaturation indicators (C16:1n-7/C16:0 and C18:1n-9/C18:0) were reduced, while arachidonic acid levels and elongation indicators were higher, suggesting a lipid metabolism disturbance (Article 1).

Regarding the amino acid profile, dogs with FRE showed reduced plasma levels of asparagine, histidine, glycine, cystine, leucine, and the branched-chain amino acid/aromatic amino acid ratio (BCAA/AAAs), while phenylalanine was increased. In fecal samples, elevated levels of cystine and reduced levels of phenylalanine were observed. An association between BCAA (leucine, isoleucine) and phenylalanine with intestinal health indicators such as SCFAs was identified. Additionally, a positive correlation between the Canine Inflammatory Bowel Disease Activity Index (CIBDAI) and plasma phenylalanine was found, while leucine, lysine, BCAA, and the systemic BCAA/AAA ratio showed negative correlations, suggesting that higher disease severity is associated with higher levels of AAAs and lower levels of BCAAs (Article 2).

In general terms, it was found that fecal samples better reflected the disease state than plasma samples, as they correlated with severity indicators. Being a non-invasive and easier collection procedure, in Experiment 2, only fecal samples were collected.

In the second experiment, different types of chronic inflammatory diseases were studied, including dogs with IRE and dogs with *Giardia* spp. infection (one of the main differential diagnoses for CIEs), along with dogs with FRE and healthy dogs. The sample size was increased compared to the first pilot experiment (control = 22; FRE = 35; IRE = 18; *Giardia* spp. = 9). Similar to Experiment 1, information was collected on the animals and the diets consumed before the dietary trial to ensure as homogeneous groups as possible

regarding their diet, and the fatty acid profile was determined using gas chromatography, and the amino acid profile was determined using liquid chromatography.

Fatty acids C16:1n-7, the C16:1n-7/C16:0 ratio, acetic acid, and total SCFAs in feces allowed differentiation between dogs with FRE and IRE. Additionally, it was found that a higher CIBDAI was associated with lower concentrations of PUFAs, omega-6, and its precursor (linoleic acid, LA) but higher levels of saturated fatty acids (SAT), Δ6-desaturase, and elongase C22:5n-3/C20:5n-3, suggesting a hyperactivation of omega-6 metabolism in more severe states. Deterioration in fecal characteristics, measured by the Purina® fecal score, was correlated with elevated SAT and elongase levels and reduced PUFAs and omega-6 levels. High CIBDAI and fecal fat levels were also associated with lower concentrations of C16:1n-7, C16:1n-7/C16:0, and Δ9-desaturase in feces, confirming a greater disturbance in lipid metabolism in IRE (Article 3).

In the discriminant analysis, the fecal fatty acid profile correctly differentiated 75% of the samples, with the selected variables for the discriminant linear equations being C16:1n-7/C16:0, C18:1n-9/C18:0, acetic acid, and omega-3. Dogs with IRE were correctly differentiated in 92% of cases from the other groups, showing more significant changes in these fatty acids compared to dogs with FRE and the controls, among which some overlap was observed (Article 3).

Additionally, a blood count, biochemical profile, and various systemic inflammatory indices were studied in relation to the fecal lipid profile (Article 4) to deepen the understanding of FRE and IRE groups before dietary treatment. Significant differences were identified between dogs with FRE and those with IRE in platelet levels, NLR (neutrophil/lymphocyte ratio), PLR (platelet/lymphocyte ratio), and SII (platelet*neutrophil/lymphocyte ratio). Moreover, the role of C16:1n-7, the C16:1n-7/C16:0 ratio, acetic acid, butyric acid, and total SCFAs in regulating systemic metabolism was highlighted, influencing lymphocytes, platelets, SII index, and glucose. Dogs with IRE showed greater metabolic disruption than dogs with FRE.

Regarding the fecal amino acid profile, no differences were observed between dogs with FRE and dogs with IRE before dietary treatment. However, both CIE groups differed from healthy dogs, showing higher levels of threonine and AAAs, particularly tyrosine, as

well as a lower BCAA/AAA ratio. Furthermore, the BCAA/AAA ratio was associated with elevated MUFAs, propionic acid, and SCFAs, while it was inversely related to SAT levels in feces, suggesting that BCAA/AAA levels may be linked to better health status. On the other hand, the amino acid profile of dogs infected with *Giardia* spp. showed marked differences compared to the other experimental groups, with significantly higher levels (Article 5).

The discriminant analysis of the amino acid profile correctly classified 56% of the samples, selecting tyrosine, phenylalanine, arginine, and glutamic acid as discriminant variables. However, the highest percentage of correct classification (78%) was obtained by combining the fatty acid and amino acid profiles in a single discriminant analysis. In this case, the selected variables were tyrosine, glutamic acid, MUFA, C16:1n-7/C16:0, acetic acid, BCAA/AAA, C22:5n-3, C18:1n-9/C18:0, elongase C22:5n-3/C20:5n-3, cystine, and EPA (Article 5). In this regard, the fatty acid profile showed a higher capacity than the amino acid profile in differentiating between the different groups, although combining both provided the best results.

The characterization of the composition and characteristics of feces as a non-invasive procedure before dietary treatment in dogs with different types of CIEs has mainly revealed differences in the intensity of fatty acid metabolism disturbances and opens new expectations for more personalized diet designs for these animals.

INTRODUCCIÓN

1. INTRODUCCIÓN

1.1 ENTEROPATÍAS CRÓNICAS

1.1.1 Definición

La **enteropatía crónica (EC) canina** (del inglés *Canine chronic inflammatory enteropathy*) es un término utilizado para referirse a enfermedades gastrointestinales que presentan tres o más semanas de duración, habiéndose descartado enfermedades extra-gastrointestinales o enfermedades intestinales como las parasitarias, infecciosas o neoplásicas (Dandrieux, 2016).

1.1.2 Clasificación

A día de hoy existen distintos tipos de ECs y se clasifican de forma retrospectiva en función de su respuesta al tratamiento en **enteropatía que responde a dieta (del inglés, *food-responsive enteropathy*, FRE)**, **enteropatía que responde a antibióticos (*antibiotic-responsive enteropathy*, ARE)**, **enteropatía que responde a inmunosupresores (*immunosuppressant-responsive enteropathy*, IRE)** y **enteropatía que no responde (*non-responsive enteropathy,* NRE)** (Dandrieux, 2016; Jergens & Heilmann, 2022). Un grupo adicional de las ECs denominado **enteropatía perdedora de proteínas (*protein-losing enteropathy*, PLE)** hace referencia a todas aquellas enteropatías que presentan hipoalbuminemia, cursando con un pronóstico mucho más desfavorable (Dandrieux, 2016; Jergens & Heilmann, 2022).

A pesar de que esta es la clasificación utilizada en la actualidad, recientemente en el estudio de Dupouy-Manescau *et al.,* en 2024 se ha propuesto una nueva clasificación en la que se sustituye la ARE por la enteropatía que responde a la modulación de la microbiota (*microbiota-related modulation-responsive enteropathy).* Esto es debido a que, como se verá más adelante, uno de los objetivos del tratamiento de estas enfermedades consiste en la restauración del microbioma intestinal (Marchesi *et al.,* 2017). Para ello es posible emplear diversos mecanismos que están siendo investigados en la actualidad y parecen presentar resultados prometedores, por lo que estos autores sugieren una redefinición de

la categoría en función de la respuesta favorable a este tipo de tratamiento (Dupouy-Manescau *et al.,* 2024).

Por otra parte, es importante destacar que el término IRE también se conoce como **enfermedad inflamatoria crónica intestinal o *inflammatory bowel disease* (IBD).** Esta enfermedad se caracteriza por la presencia de un infiltrado inflamatorio en la mucosa gastrointestinal de etiología desconocida (Cerquetella *et al.,* 2010; Jergens & Simpson, 2012; Hall & Day, 2017). La IRE se considera semejante a la enfermedad de Crohn, una variante de la enfermedad inflamatoria crónica intestinal en medicina humana (Dandrieux, 2016). Esta enfermedad, junto con la colitis ulcerosa, son las dos variantes de la IBD humana. Las diferencias entre ellas radican en la localización y la afección de capas de la pared intestinal. Así, la enfermedad de Crohn afecta a toda la pared intestinal principalmente del íleon y colon (aunque puede encontrarse en otras áreas del aparato digestivo), mientras que la colitis ulcerosa se limita a las capas más superficiales (mucosa y superficie de submucosa) del colon y del recto (Stange *et al.,* 2006; Stange *et al.,* 2008).

1.1.3 Prevalencia

La prevalencia real de las ECs en perros es actualmente desconocida, aunque se estima que representa entre el 1 y el 2% de los casos en centros de referencia (Dandrieux, 2016). Sin embargo, es posible que estos porcentajes estén subestimados ya que hasta el 20-30% de las visitas veterinarias en animales de compañía están relacionadas con vómitos o diarrea (O'Neill *et al.,* 2014).

La mayoría de los casos de enteropatía se engloban en la **FRE** (50-65%) (Allenspach *et al.,* 2016; Dandrieux, 2016; Kawano *et al.,* 2016; Dandrieux & Mansfield, 2019). En esta categoría, los perros afectados suelen ser más jóvenes, presentan signos clínicos menos graves y, predominantemente, diarrea de intestino grueso (Münster *et al.,* 2006; Allenspach *et al.,* 2007; Allenspach *et al.,* 2016). Además, suelen presentar una menor puntuación en uno de los índices que evalúa la gravedad de la enfermedad, el CCECAI (*Canine chronic enteropathy clinical activity index*), así como niveles normales de albúmina en comparación con el resto de grupos (Allenspach *et al.,* 2007; Allenspach *et al.,* 2016).

Sin embargo, recientemente se han descrito animales con hipoalbuminemia que responden al tratamiento dietético (Okanishi *et al.,* 2014; Rudinsky *et al.,* 2017; Wennogle *et al.,* 2021). En el estudio de Wennogle *et al.,* en 2021 se demostró que 8/10 perros con PLE sin una respuesta previa a una combinación de dieta más glucocorticoides o inmunosupresores, pudieron conseguir una remisión completa de los signos clínicos con una dieta baja en grasa. Es más, en 3/8 perros se retiró el tratamiento inmunosupresor y mantuvieron remisión clínica durante 12-36 meses simplemente con la dieta (Wennogle *et al.,* 2021). En 4/8 perros la dieta ayudó a reducir la dosis de los inmunosupresores administrados (Wennogle *et al.,* 2021). La restricción de la grasa en la dieta ha demostrado ser efectiva en la reducción de las dosis de prednisona en 19/24 perros con PLE con linfangiectasia (Okanishi *et al.,* 2014), una presentación común en esta enteropatía que se caracteriza por la dilatación de los vasos linfáticos (Jablonski, 2022). Además, en el estudio de Hodel *et al.,* en 2024 se observó que perros que habían sido clasificados con IRE pasaron a la categoría de FRE al año de evaluación, sugiriendo que si se hubieran llevado a cabo varios ensayos dietéticos, quizás algunos perros clasificados con IRE hubieran sido clasificados con FRE. Por ello, es posible que la prevalencia del grupo FRE sea incluso mayor de lo que se estima, ya que no se suelen realizar varios ensayos dietéticos y distintos estudios han demostrado que perros que no habían respondido inicialmente a la dieta, sí respondieron a un segundo o tercer ensayo dietético (Mandigers *et al.,* 2010; Dandrieux *et al.,* 2019; Schramm & Kook, 2022), demostrando la importancia de la dieta en el manejo de esta enfermedad.

Por el contrario, la **IRE** engloba de un 10 a un 25% de los casos (Dandrieux & Mansfield, 2019). En esta categoría los perros suelen ser de mediana edad o mayores, presentan signos clínicos más graves y, predominantemente, diarrea de intestino delgado (Allenspach *et al.,* 2007).

En cuanto a la **ARE**, la necesidad de tratar a los perros con EC con estos fármacos (principalmente metronidazol y tilosina) ha sido cuestionada en los últimos años (Cerquetella *et al.,* 2020). El tratamiento antimicrobiano puede tener un efecto prolongado en la alteración del microbioma intestinal (Suchodolski *et al.,* 2009; Manchester *et al.,*

2019; Werner *et al.,* 2020; Pilla *et al.,* 2021), además de que la mayoría de los perros que inicialmente respondieron a los antibióticos recaen tras suspender el tratamiento (Allenspach *et al.,* 2016; Volkmann *et al.,* 2017). Todo ello sumado al aumento de la resistencia a los antimicrobianos a nivel global, ha resultado en la propuesta de que el uso de los mismos se lleve a cabo al final del estudio diagnóstico, una vez hayan fallado el resto de las opciones terapéuticas (la dieta y los inmunosupresores) (Cerquetella *et al.,* 2020; Hodel *et al.,* 2024). Sin embargo, es importante puntualizar que en la colitis ulcerosa histiocítica o granulomatosa del Bóxer y del Bulldog francés sí se ha identificado a *E.coli* como agente causal de la enfermedad y, por tanto, está justificado el uso de antibióticos como el enrofloxacino (Hostutler *et al.,* 2004; Simpson *et al.,* 2006; Mansfield *et al.,* 2009; Manchester *et al.,* 2012).

Por último, un 5-45% de perros entrarían dentro de la **NRE** (Dandrieux & Mansfield, 2019), teniendo un pronóstico más desfavorable y una alta tasa de eutanasia (Craven *et al.,* 2004; Allenspach *et al.,* 2007). La **figura 1.1** muestra la clasificación y frecuencia de las enteropatías crónicas en el perro.

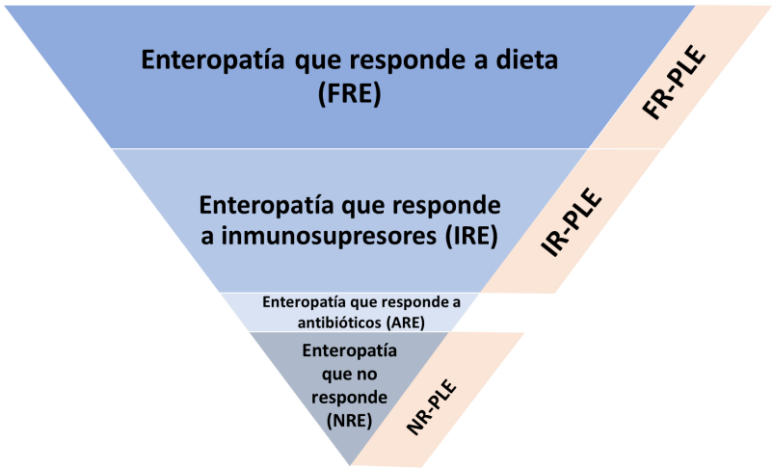

Figura 1.1. Clasificación y frecuencia de las ECs en los perros. Adaptado de Jergens & Heilmann, (2022).

1.1.4 Etiopatogenia

La **etiología** de las ECs es actualmente **desconocida**. Se trata de una **enfermedad multifactorial** que tiene lugar en individuos **genéticamente predispuestos** en los que el **sistema inmunitario** responde de manera exacerbada a desencadenantes ambientales (antígenos **dietéticos** y **microbianos**). Esto provoca la alteración de la inmunotolerancia y se desencadena la activación excesiva de la respuesta inmunitaria innata y adquirida del huésped, dando lugar a una inflamación de origen desconocido (**Figura 1.2**) (Dandrieux, 2016; Jergens & Heilmann, 2022).

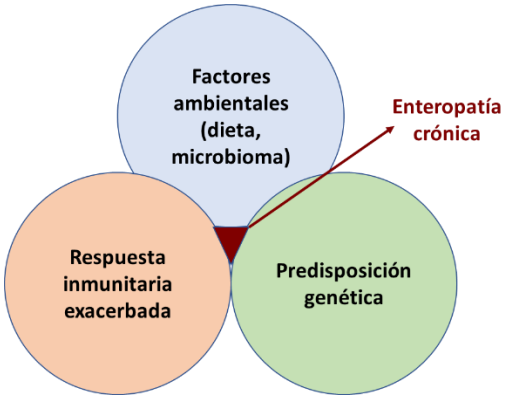

Figura 1.2. Factores implicados en la etiopatogenia de la EC canina. Adaptado de Jergens & Heilmann, (2022).

1.1.4.1 Genética

La **predisposición genética** juega un papel clave en la aparición de la enfermedad de Crohn y la colitis ulcerosa en humanos (McGovern *et al.,* 2015). De manera similar, existen ciertas razas predispuestas a la IBD en medicina veterinaria. Diversos estudios han descrito que razas como el Pastor Alemán, Border Collie, Yorkshire Terrier, Bóxer, Rottweiler o Braco de Weimar se encuentran predispuestas a padecer IBD (Kathrani *et al.,* 2011). La mayoría de los estudios genéticos se han realizado en el Pastor Alemán ya que es una de las razas más predispuestas a la enfermedad (Kathrani *et al.,* 2010; Peiravan *et al.,* 2018). Estos estudios han encontrado la presencia de polimorfismos de nucleótido único (SNPs) en los genes que codifican los receptores tipo Toll (TLR4, TLR5) (Kathrani *et al.,* 2010; Kathrani *et al.,* 2011), en el gen que codifica el receptor NOD2 (Kathrani *et al.,* 2014) y en

el gen que codifica el complejo mayor de histocompatibilidad II (Peiravan *et al.,* 2016). Los receptores Toll y NOD son "receptores de reconocimiento de patrones", encargados de reconocer productos microbianos, permitiendo distinguir entre lo infeccioso y lo no infeccioso (Kigerl *et al.,* 2014). Ambos identifican estructuras moleculares asociadas a los patógenos (Kigerl *et al.,* 2014). En cuanto a los polimorfismos de nucleótido único, son la forma más común de variación genética en la población (Gambini *et al.,* 2016). Cada SNP representa el cambio de un solo nucleótido en una secuencia genética (Gambini *et al.,* 2016). Por ello, el hecho de encontrar estos SNPs en genes que codifican para estos receptores de reconocimiento de patrones, puede suponer una alteración en la función de los mismos, aumentando así la predisposición a determinadas enfermedades como la IBD.

1.1.4.2 Microbioma

El **microbioma** intestinal está compuesto por bacterias, hongos, protozoos, arqueas y virus, siendo el componente bacteriano el más abundante y estudiado (Pilla & Suchodolski, 2021).

El microbioma fecal de los perros sanos se compone mayoritariamente de 3 filos: Fusobacterium, Bacteroidetes y Firmicutes (Middelbos *et al.,* 2010; Hand *et al.,* 2013). En animales enfermos, y particularmente en aquellos con patologías gastrointestinales como las ECs, la diversidad del microbioma se reduce (Minamoto *et al.,* 2019; Pilla & Suchodolski, 2021; Díaz-Regañón *et al.,* 2023).

En perros con IBD se ha descrito una reducción en la abundancia del filo Fusobacteria, así como del filo Bacteroidetes, especialmente en las familias Bacteroidaceae y Prevotellaceae (por ejemplo, el género *Prevotella*) (Suchodolski *et al.,* 2012; Minamoto *et al.,* 2019; Díaz-Regañón *et al.,* 2023). Dentro del filo Firmicutes, se han descrito disminuciones en las familias Ruminococcaceae (género *Ruminococcus*), Veillonellaceae (género *Megamonas*) y Lachnospiraceae (Suchodolski *et al.,* 2012; Minamoto *et al.,* 2015; Minamoto *et al.,* 2019).

Por otra parte, las Gamma-Proteobacteria (por ejemplo, Enterobacteriaceae) están sobrerrepresentadas en muestras fecales de perros con IBD (Xenoulis *et al.,* 2008; Suchodolski *et al.,* 2010; Minamoto *et al.,* 2019).

Se desconoce si esta alteración del microbioma intestinal (disbiosis) es la causa o la consecuencia de la inflamación de la mucosa intestinal en los perros con EC (Jergens & Heilmann, 2022). La disbiosis genera una alteración de los metabolitos microbianos producidos por la microbiota intestinal, cuyos efectos ejercen una función beneficiosa para el individuo, como es el caso de los ácidos grasos de cadena corta (Minamoto *et al.,* 2019). Actualmente, no se ha identificado un patrón universal de disbiosis que pueda emplearse para distinguir de manera definitiva entre las diferentes ECs (Suchodolski, 2016). No obstante, se ha desarrollado un **índice de disbiosis fecal** que facilita la diferenciación entre un estado de normobiosis y disbiosis en perros que padecen ECs (Suchodolski, 2016; AlShawaqfeh *et al.,* 2017). Este índice puede utilizarse para monitorizar la alteración del microbioma en el tiempo y en relación a la recuperación de condiciones fisiológicas en respuesta a un tratamiento (Pilla & Suchodolski, 2020).

1.1.4.3 Dieta

Los factores dietéticos han sido ampliamente estudiados como posibles desencadenantes de la IBD en humanos (Yan *et al.,* 2022). Sin embargo, existe poca literatura científica sobre la **dieta** como factor de riesgo previo a la enfermedad en perros con ECs.

Recientemente se ha descrito que el consumo de una dieta alta en hidratos de carbono (HC), moderada en proteína y baja en grasa en la etapa neonatal y postnatal se asoció a un mayor riesgo de IBD en perros adultos y, por el contrario, una dieta baja en HC, proteína moderada y alta en grasa, con un menor riesgo de desarrollo de la enfermedad (Hemida *et al.,* 2021). De manera similar, en el estudio de Vuori *et al.,* en 2023 el consumo de una dieta alta en HC durante la etapa de cachorro (2-6 meses) y adolescencia (6-18 meses) supuso un factor de riesgo en el desarrollo de EC en el futuro. Por el contrario, el consumo de una dieta en base a proteína cruda (huesos y cartílago), y restos de comida humana se asoció a un menor riesgo de EC (Vuori *et al.,* 2023). Los resultados en relación

al consumo de dieta cruda se asocian a la "teoría de la higiene", en la cual, se sugiere que una mayor exposición en edades tempranas a los microorganismos genera un sistema inmunitario más robusto (Klement *et al.,* 2008). Sin embargo, es fundamental resaltar el riesgo de las dietas crudas sobre la transmisión de agentes bacterianos y parasitarios, especialmente a animales y personas inmunocomprometidas, por lo que la evidencia actual recomienda evitar el uso de este tipo de dietas (Freeman *et al.,* 2013; van Bree *et al.,* 2018).

Por último, en el estudio retrospectivo de Trewin & Kathrani, en 2023 se evaluó la dieta como posible factor de riesgo en 48 perros con EC (25 presuntiva y 23 confirmada) y 47 perros control. Los resultados demostraron que, al evaluar todos los casos de EC (tanto los presuntivos como los confirmados), una dieta principal que contenía HC era un posible factor de riesgo en el desarrollo de la enfermedad. Sin embargo, dejó de ser un factor de riesgo al analizar únicamente los casos confirmados de EC. Los controles consumían más carne roja como fuente principal de proteína en comparación con los enfermos. Además, un contenido de humedad ≤14% en materia fresca al inicio de los signos clínicos iniciales se asoció con el desarrollo de la enfermedad únicamente en casos confirmados de EC (Trewin & Kathrani, 2023).

En medicina humana, el consumo de una dieta occidental ("Western") rica en azúcares refinados, grasas animales, carne roja y carbohidratos complejos se ha asociado con aumento de la incidencia de IBD, mientras que las dietas ricas en ácidos grasos omega-3, vegetales, frutas y fibra parecen ofrecer protección contra la misma (Yan *et al.,* 2022). La dieta Western y las dietas ultra procesadas reducen la diversidad del microbioma provocando disbiosis intestinal (Broussard & Devkota, 2016; Cuevas-Sierra *et al.,* 2021).

Aunque los hallazgos son aún inconsistentes, en medicina humana la ingesta elevada de HC refinados y azúcares está asociada con el riesgo de desarrollar IBD como consecuencia de la alteración del microbioma y de sus metabolitos microbianos (Yan *et al.,* 2022). Este resultado podría ser similar en el caso de los perros. Trewin & Kathrani en 2023 sugerían que los niveles inferiores al 14% de humedad, junto con niveles altos de HC, son típicos de dietas sometidas a un proceso de extrusión, en el cual la generación de productos finales de glicación avanzada ha demostrado presentar un efecto perjudicial sobre la

microbiota intestinal en vivo, y estar implicados en el desarrollo de condiciones inflamatorias en las personas (Qu *et al.,* 2017).

Por otra parte, en relación a los niveles de proteína en la dieta, los resultados en perros serían contradictorios en comparación con la literatura científica en la IBD humana, en donde el consumo de carne roja se trata de un factor de riesgo en el desarrollo de la misma (Yan *et al.,* 2022). Sin embargo, en los perros la pérdida de tolerancia frente a los antígenos dietéticos es uno de los mecanismos por los que se produce una respuesta inflamatoria exacerbada a nivel de la mucosa intestinal en las ECs (Eissa *et al.,* 2019). De hecho, los antígenos dietéticos más comunes son de origen proteico, siendo para la especie canina y en orden decreciente la carne de vacuno, los lácteos, el trigo, el cordero, el huevo y el pollo (Verlinden *et al.,* 2006). De ahí que la primera línea de tratamiento consista en el cambio de dieta hacia una fuente nueva de proteína o bien hacia proteínas hidrolizadas, para evitar que se desencadene la respuesta inmunitaria (Tolbert *et al.,* 2022).

En el caso de la grasa, la ratio de los ácidos grasos polinsaturados (PUFA) omega-6/omega-3 se encuentra muy elevado en las dietas occidentales para personas, habiéndose demostrado la asociación de una dieta alta en grasa con la incidencia de la IBD humana y disbiosis intestinal (Yan *et al.,* 2022). En la especie canina, como se ha comentado con anterioridad, los datos al respecto son escasos, aunque se han demostrado los beneficios de la reducción de los niveles de grasa en el tratamiento de las ECs, concretamente en la PLE con linfangiectasia (Okanishi *et al.,* 2014; Rudinsky *et al.,* 2017; Wennogle *et al.,* 2021).

Las dietas altas en grasa han demostrado aumentar la expresión de ARNm de los receptores NOD2 y TLR5 en un modelo experimental de la IBD (Martinez-Medina *et al.,* 2013). Por otro lado, la suplementación con aceite de pescado durante 21 días redujo los niveles de ARNm de ambos receptores en cerdos tratados con lipopolisacáridos (Liu *et al.,* 2012). En enfermedades gastrointestinales, la falta de digestión y absorción de la grasa en el intestino delgado puede provocar su paso hacia el intestino grueso, generando disbiosis, daño epitelial y aumentos en la secreción en el colon (Ramakrishna *et al.,* 1994).

1.1.4.4 Respuesta inmunitaria

En condiciones fisiológicas, la homeostasis intestinal se mantiene mediante un equilibrio entre la inmunidad contra los patógenos y las respuestas tolerantes hacia los microorganismos beneficiosos (simbiontes) u otros antígenos luminales inofensivos, con el fin de evitar respuestas inmunitarias patológicas exacerbadas (Eissa *et al.,* 2019).

En las ECs caninas, se produce una alteración en ese equilibrio, inclinándose hacia una inflamación patológica, en la cual existe una ruptura de la integridad de la barrera epitelial, y una pérdida de tolerancia hacia los simbiontes o los antígenos dietéticos (Eissa *et al.,* 2019) como se muestra en la **figura 1.3**. Esto culmina en el reclutamiento y activación de respuestas inflamatorias caracterizadas por la infiltración de la mucosa con neutrófilos, eosinófilos, células dendríticas maduras y macrófagos proinflamatorios, así como linfocitos T (Eissa *et al.,* 2019).

Los linfocitos T parecen jugar un papel importante en la patogénesis de la IBD, tanto en medicina humana como en veterinaria (Eissa *et al.,* 2019). Los linfocitos T reguladores (Tregs) mantienen una respuesta tolerante mediante la secreción de citoquinas como IL-10 y el factor de crecimiento transformante (TGF-β) (Larosa & Orange, 2008; Eissa *et al.,* 2019). Por otra parte, los linfocitos T citotóxicos se encargan de eliminar células infectadas, mientras que los linfocitos T colaboradores (*T helper lymphocytes*, Th) organizan y dirigen la respuesta inmunitaria (Larosa & Orange, 2008). Estos se clasifican principalmente según las citoquinas que producen en Th1 (IFN-γ), Th2 (IL-4, IL-5 e IL-13), y Th17 (IL-17 e IL-22) (Boden & Lord, 2017).

En personas con enfermedad de Crohn se conoce que existe predominantemente una respuesta Th1, mientras que en la colitis ulcerosa una respuesta Th2 (Neurath *et al.,* 2002). En el caso de las ECs caninas se ha descrito un perfil de citocinas equilibrado entre ambos tipos de respuesta (Jergens *et al.,* 2009; Heilmann & Suchodolski, 2015). No obstante, en el estudio de Agulla *et al.,* en 2024 se observó un aumento del porcentaje de linfocitos productores de IFN-γ en el epitelio intestinal en perros con IBD en comparación con perros control, sugiriendo un posible predominio de la respuesta Th1 al igual que en la

enfermedad de Crohn. En este estudio se evidenciaron alteraciones en distintos tipos de linfocitos T en 3 compartimentos diferentes: a nivel periférico (sangre), a nivel intraepitelial y a nivel de la lámina propia (Agulla *et al.,* 2024). Así, a nivel periférico se observó un mayor porcentaje de linfocitos Th y Tregs, y de linfocitos T citotóxicos (estos últimos no significativos) (Agulla *et al.,* 2024), coincidiendo estos resultados con los descritos en sangre de pacientes humanos con IBD (Funderburg et al., 2013; Kosoy et al., 2021). Por otra parte, se observó un aumento del porcentaje de linfocitos T citotóxicos a nivel del epitelio intestinal y una disminución de las células Th (Agulla *et al.,* 2024). El aumento de los niveles de linfocitos T citotóxicos a nivel intraepitelial es un hallazgo común tanto en la enfermedad de Crohn como en la colitis ulcerosa y podría ser la causa del daño al epitelio con la consiguiente invasión bacteriana de la mucosa, aumentando así la activación celular (Kontoyiannis et al., 2002; Geng et al., 2023).

Por otra parte, no existe evidencia concluyente que respalde la participación de la respuesta Th17 en la EC canina (Schmitz *et al.,* 2012; Ohta *et al.,* 2014), a diferencia de lo que sucede en la IBD humana (Boden & Lord, 2017). Sin embargo, diversos estudios han descrito aumentos en la expresión de citoquinas derivadas de esta respuesta (IL-17A, IL-23p19 e IL-12p35) en la mucosa de perros enfermos en comparación con perros control (Ohta *et al.,* 2013; Tamura *et al.,* 2013; Kinjo *et al.,* 2017).

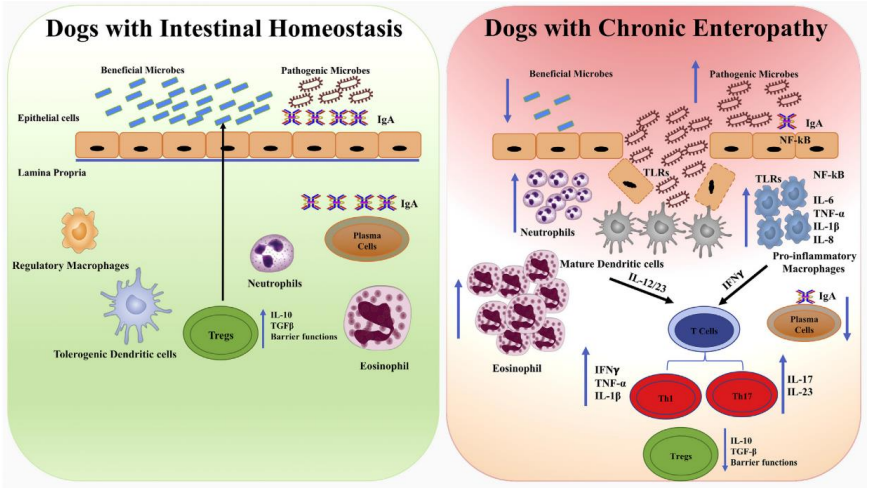

Figura 1.3. Patogenia de las ECs caninas (Eissa *et al.,* 2019).

1.1.5 Sintomatología

La manifestación clínica de la EC canina se caracteriza por la presencia de **signos** clínicos **gastrointestinales** persistentes o recurrentes durante un **mínimo de tres semanas**. Estos signos clínicos están condicionados por la localización anatómica de la inflamación (intestino delgado o intestino grueso), el estado de la enfermedad (fase activa o fase de remisión) y la presencia de complicaciones asociadas (hipoproteinemia e hipoalbuminemia, entre otras) (Jergens & Simpson, 2012). Los signos clínicos más frecuentes incluyen diarrea y vómitos, acompañados de alteraciones en el apetito, pérdida de peso, dolor abdominal, borborigmos y flatulencias (German *et al.,* 2003a; Jergens *et al.,* 2003; Rodríguez-Franco *et al.,* 2004; Hall, 2013a).

La **diarrea** y los **vómitos** son los signos clínicos más comunes en la IBD canina que afecta al intestino delgado. Las heces suelen ser voluminosas, de color claro, con esteatorrea y restos de alimentos sin digerir, acompañadas de borborigmos y flatulencias. La frecuencia de defecación puede ser normal o ligeramente aumentada (Marks, 2013). Generalmente se observa en estos animales pérdida de peso y alteraciones en el apetito (Hall, 2013a; Hall & Day, 2017). En los casos en los que exista afectación del intestino grueso, la diarrea se caracteriza por aumento en el número de defecaciones, pudiendo presentar moco o sangre fresca (Lecoindre & Gaschen, 2011). Suele ser una defecación de urgencia con dificultad (tenesmo) y/o dolor (disquecia) (Lecoindre & Gaschen, 2011). Cuando la diarrea es mixta aparecen signos de afectación del intestino delgado y del intestino grueso (Hall, 2013a; Hall & Day, 2017).

El **vómito** puede presentarse como único signo clínico, especialmente con vómitos frecuentes en ayuno que contienen bilis, líquido o espuma (Hall & Day, 2017). Menos frecuentemente, se expulsa contenido alimenticio digerido o parcialmente digerido. El vómito se origina por estimulación neural del centro del vómito (Washabau, 2013) y suele asociarse a inflamación gástrica crónica debida a reflujo duodenogástrico, incompetencia pilórica e hipersecreción ácida gástrica (Rodríguez-Franco *et al.,* 1997; García-Sancho *et al.,* 2005).

La inflamación intestinal genera un síndrome de malabsorción que provoca **pérdida de peso** progresiva y deficiencias nutricionales (Hall, 2013a; Hall & Day, 2017). Tras el tratamiento, suele observarse una recuperación de peso y mejoría en la absorción de nutrientes (García-Sancho *et al.,* 2007; Allenspach *et al.,* 2016; Rudinsky *et al.,* 2018). Las **alteraciones del apetito** incluyen polifagia, asociada a malabsorción, e hiporexia o anorexia en casos graves (Craven *et al.,* 2004; García-Sancho *et al.,* 2006).

El **dolor abdominal** es un síntoma común, percibido durante la palpación abdominal o mediante posturas antiálgicas como el encorvamiento o la posición de rezo (Hall, 2013b; Hall & Day, 2017). Este dolor se relaciona con la liberación de mediadores inflamatorios, distensión intestinal por acumulación de gas y las lesiones en la mucosa intestinal (Hall, 2013b; Rychlik *et al.,* 2020).

Aunque en humanos son comunes las manifestaciones extraintestinales (Ott & Schölmerich, 2013), estas complicaciones son raras en perros. No obstante, se han observado signos como cambios en la actividad y actitud, letargia y apatía (Jergens *et al.,* 2003; García-Sancho *et al.,* 2007; Hall & Day, 2017). En perros con PLE es posible observar ascitis y edema periférico como consecuencia de la hipoalbuminemia (Craven & Washabau, 2019; Allenspach & Iennarella-Servantez, 2021). Además, una de las complicaciones más problemáticas en esta enteropatía es el tromboembolismo pulmonar, vinculado a un estado de hipercoagulabilidad (Goodwin *et al.,* 2011; Jacinto *et al.,* 2017).

Existen 2 índices clínicos que se utilizan para valorar la gravedad de la enfermedad y su evolución en el tiempo: el **CIBDAI** (*canine IBD activity index*) (Jergens *et al.,* 2003) y el **CCECAI** (Allenspach *et al.,* 2007). En el CIBDAI se establece una puntuación de 0 a 3 para cada uno de los siguientes signos clínicos: actividad/actitud, apetito, frecuencia de vómitos, consistencia de las heces, frecuencia de defecación y pérdida de peso. En el CCECAI se añaden los niveles de albúmina, la presencia de ascitis o edema periférico y la presencia de prurito. Una vez sumada la puntuación, un resultado total de 0-3 en el CIBDAI indica enfermedad insignificante clínicamente; 4-5 enfermedad leve; 6-8 enfermedad moderada; y ≥ 9 enfermedad grave (Jergens *et al.,* 2003). Los perros con IRE suelen presentar niveles más elevados en estos índices en comparación con los perros con FRE, indicando una mayor

gravedad de la presentación clínica de la enfermedad (Allenspach *et al.,* 2007; Allenspach *et al.,* 2016).

1.1.6 Diagnóstico

El diagnóstico de las ECs suele alargarse en el tiempo ya que, como hemos comentado previamente, consiste en la respuesta favorable a un tratamiento, tras un protocolo previo de exclusión de otras causas asociadas a la sintomatología clínica. Esta situación se debe a la incapacidad de la historia clínica, los hallazgos de laboratorio, los biomarcadores de inflamación o las anomalías histológicas de diferenciar entre una forma de enteropatía u otra (Jergens & Heilmann, 2022).

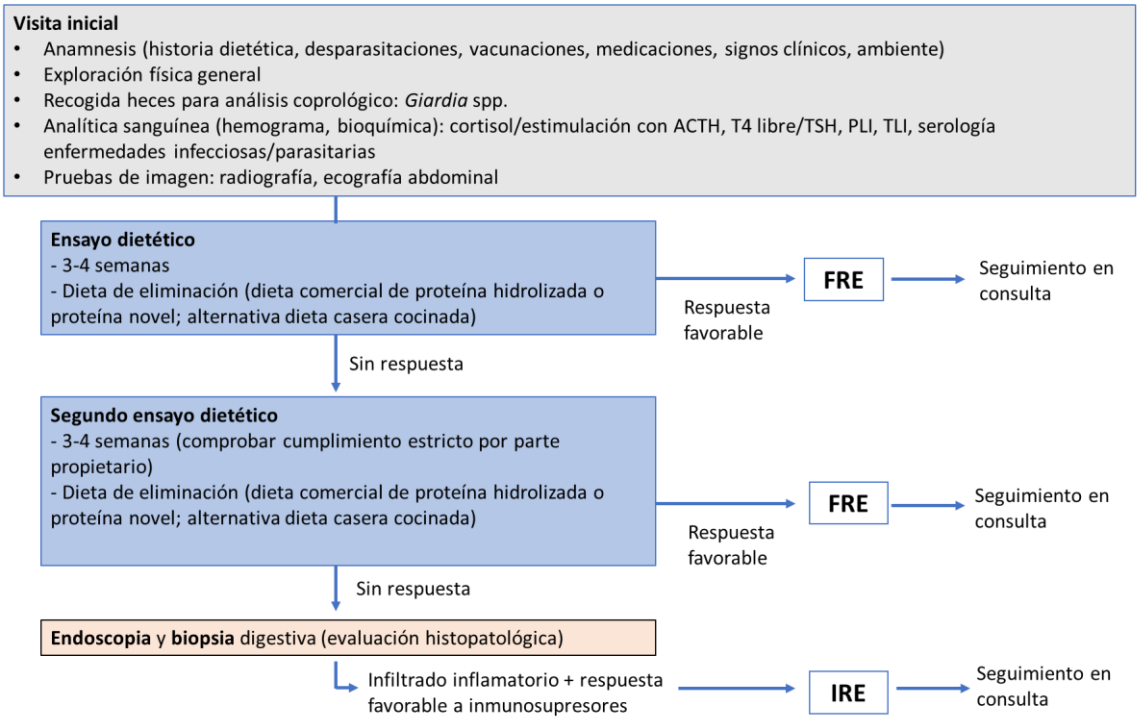

Figura 1.4. Protocolo diagnóstico de las ECs en perros. Adaptado de Jergens & Heilmann, (2022). Abreviaturas: ACTH: hormona adrenocorticotropa, TSH: hormona estimulante de la tiroides, T4: tiroxina, TLI: *trypsin-like immunoreactivity*, PLI: *pancreatic lipase immunoreactivity.*

Es importante llevar a cabo un protocolo diagnóstico que permita excluir otras enfermedades sistémicas que cursen con la misma sintomatología que las ECs, o enfermedades que causan inflamación intestinal crónica, pero cuya etiología es conocida

(Jergens & Simpson, 2012; Hall, 2013a; Hall & Day, 2017). La **figura 1.4** muestra un esquema representativo del protocolo a seguir.

Este protocolo incluye:

- **Anamnesis**: es importante recoger información sobre la historia dietética del animal, vacunas, desparasitaciones, medicamentos, hábitat, etc. La historia clínica del paciente y la descripción de las heces son útiles para distinguir entre diarrea aguda y crónica, estimar la gravedad de la enfermedad y caracterizar la localización más evidente (intestino delgado frente a intestino grueso) (Luckschander-Zeller, 2020).
- **Exploración física general**: permite determinar la gravedad y el posible origen (intestinal o extraintestinal) de la enfermedad (Luckschander-Zeller, 2020). Será importante evaluar la condición corporal (CC) del paciente (del inglés *body condition score,* BCS) así como la condición muscular (CM) (*muscle condition score*, MCS) (Freeman *et al.,* 2011). Además, la palpación abdominal permitirá detectar malestar o dolor abdominal, alteraciones en las asas intestinales o en su contenido, organomegalias, masas o ascitis en los casos más graves (Hall, 2013a). Por otra parte, la palpación rectal proporciona una muestra de heces que permite evaluar su consistencia, color y olor, así como la presencia de moco, sangre fresca, melena o material extraño (Luckschander-Zeller, 2020). Además, se puede realizar una extensión en fresco de heces para visualizar formas parasitarias. Asimismo, mediante la palpación rectal es posible detectar masas, estenosis o un engrosamiento irregular de la pared (Luckschander-Zeller, 2020).
- **Análisis coprológico**: Entre otros parásitos, *Giardia duodenalis* es uno de los diagnósticos diferenciales más comunes de las ECs caninas, siendo necesaria la recogida de muestras de heces de 3 días consecutivos para aumentar las probabilidades de detección del parásito (ESCCAP; Ridyard, 2020). Esta recogida permitirá evaluar las características de las heces según una escala de puntuación fecal, valorando la gravedad de la diarrea (Cavett *et al.,* 2021).

- **Hemograma y bioquímica**: permiten descartar enfermedades metabólicas (hipoadrenocorticismo, hipertiroidismo, hipotiroidismo), pancreáticas (pancreatitis, con la determinación de PLI, insuficiencia pancreática exocrina, con TLI), hepáticas, renales o enfermedades infecciosas/parasitarias sistémicas (*Leishmania* spp.) que cursen con la misma sintomatología (Luckschander-Zeller, 2020).
    - En el **hemograma**, las anomalías hematológicas asociadas con la inflamación suelen ser inespecíficas (Ridyard, 2020). En algunos casos puede presentarse anemia microcítica, hipocrómica y no regenerativa, secundaria a inflamación crónica o a un sangrado gastrointestinal prolongado (Jergens & Simpson, 2012). Esta condición se ha relacionado con un pronóstico desfavorable y con una falta de respuesta al tratamiento (Volkmann *et al.,* 2017). En la serie leucocitaria se pueden observar alteraciones como neutrofilia con o sin desviación a la izquierda y eosinofilia (Jergens & Simpson, 2012; Hall, 2013a). Asimismo, es posible detectar linfocitos reactivos en perros con enteritis linfoplasmocitaria (un subtipo de la IBD), mientras que la linfopenia puede estar presente en perros con linfangiectasia intestinal asociada a PLE (Hall & Day, 2017). Al igual que en la IBD humana, se han descrito niveles elevados de plaquetas (trombocitosis), relacionados con la gravedad clínica de la enfermedad (Marchetti *et al.,* 2010; Cristóbal *et al.,* 2022; Agulla *et al.,* 2024). Además, recientemente se han descrito alteraciones en índices inflamatorios sistémicos como la ratio neutrófilos/linfocitos, plaquetas/linfocitos, monocitos/linfocitos y (plaquetas x neutrófilos)/linfocitos en perros con EC (Benvenuti *et al.,* 2020; Becher *et al.,* 2021; Pierini *et al.,* 2021; Cristóbal *et al.,* 2022; Agulla *et al.,* 2024; Marchesi *et al.,* 2024).
    - Las alteraciones en el perfil **bioquímico** son poco frecuentes, excepto en perros con PLE en los que se describe hipoproteinemia e hipoalbuminemia (Ridyard, 2020). De forma general, se han descrito aumentos en las enzimas hepáticas como consecuencia del proceso inflamatorio (Ridyard, 2020), así

como alteraciones en los niveles de cobalamina (vitamina B12) y folatos (vitamina B9) (Hall, 2013a).

- **Pruebas de diagnóstico por imagen**: las pruebas de imagen, y especialmente la ecografía abdominal, son útiles para la detección de causas extra-gastrointestinales, así como para la definición de la afectación de la enfermedad mediante la identificación de lesiones focales o difusas en la mucosa, determinación del espesor de la pared intestinal y determinación de linfadenopatías mesentéricas, observadas no solo en la IBD sino en otras patologías infiltrativas como neoplasias (linfoma) (Jergens & Simpson, 2012; Ridyard, 2020).
- **Ensayo dietético**: una vez descartadas otras enfermedades, el ensayo dietético consiste en administrar al animal, de modo exclusivo y estricto, una dieta comercial hipoalergénica basada en proteínas hidrolizadas o bien, una dieta con una fuente de proteína *novel* que el animal nunca haya consumido. Esta dieta de eliminación se administra durante 3-4 semanas para observar si desaparece la sintomatología digestiva. Si este es el caso, se sugiere volver a administrar su dieta habitual (dieta de provocación) para confirmar que ésta era la causante del cuadro digestivo gracias a la aparición, nuevamente, de sintomatología (Ridyard, 2020). Sin embargo, en un gran número de casos los propietarios prefieren mantener la dieta de eliminación en base a la buena respuesta clínica del animal (Ridyard, 2020). Los animales que respondan de forma favorable a la dieta de eliminación se diagnostican con la FRE.
- **Endoscopia**: en caso de falta de respuesta a la dieta de eliminación, se debe llevar a cabo una endoscopia digestiva para la toma de biopsias intestinales (Ridyard, 2020). La evaluación histológica de la mucosa intestinal es necesaria para un diagnóstico definitivo de la IBD (Ridyard, 2020). En función del infiltrado celular, se puede clasificar a la IBD en varios tipos, siendo la enteritis linfoplasmocitaria la forma más común (Steiner & Allenspach, 2008). Además de la infiltración de células inflamatorias en la mucosa, la inflamación crónica se asocia con anomalías arquitectónicas, como atrofia de vellosidades, distorsión glandular y/o necrosis (Ridyard, 2020), así como con lesiones ultraestructurales (lesión mitocondrial,

vacuolización citoplasmática, aumento del espacio entre microvellosidades y entre uniones estrechas proteicas) (Walker *et al.,* 2013; Fietz *et al.,* 2024). Aquellos animales en los que se confirme un infiltrado inflamatorio intestinal de etiología desconocida, habiendo descartado el resto de causas, y sin respuesta favorable a la dieta de eliminación, recibirán un tratamiento con inmunosupresores.

1.1.7 Tratamiento

La terapia de las ECs tiene como objetivo reducir la inflamación intestinal y modular la microbiota del animal, consiguiendo la desaparición o la reducción parcial de los signos clínicos (Marchesi *et al.,* 2017). Este objetivo puede alcanzarse mediante diferentes estrategias como la dieta, los inmunosupresores y, en última instancia, los antibióticos. Actualmente, otros tratamientos moduladores de la microbiota como el uso de prebióticos, probióticos, simbióticos o el trasplante de microbiota fecal están siendo evaluados (Jergens & Heilmann, 2022).

1.1.7.1 Dieta

Las dietas que a menudo se consideran para perros con EC incluyen aquellas altamente digestibles, enriquecidas con fibra, bajas en grasa, con una fuente de proteína *novel* (dieta comercial o dieta casera) o bien de proteínas hidrolizadas (Tolbert *et al.,* 2022). Es importante que la dieta casera esté formulada por un veterinario nutricionista certificado (Kathrani, 2020).

Muchas de estas dietas tienen como objetivo evitar la respuesta antigénica por parte del sistema inmunitario (Tolbert *et al.,* 2022). En este sentido, la efectividad de cualquier proteína *novel* para reducir los signos clínicos asociados con la sensibilidad alimentaria se debe principalmente a su novedad o a la falta de exposición previa (Mars *et al.,* 2002). Así, dietas comerciales a base de pato, pescado o venado, entre otras, sirven para este propósito (Villaverde & Hervera, 2015). En el caso de dietas caseras, además de las fuentes proteicas anteriores, se utiliza con frecuencia carne de caballo o conejo (Villaverde & Hervera, 2015). Por otra parte, la respuesta favorable a una fuente de proteínas hidrolizadas se debe a la incapacidad de reconocimiento del sistema inmunitario

de estos fragmentos de polipéptidos, que presentan un tamaño de partícula inferior (Marks *et al.,* 2002; Mandigers *et al.,* 2010).

Tabla 1.1. Ventajas e inconvenientes de las diferentes categorías de dietas utilizadas para el tratamiento de las ECs (Kathrani, 2020).

TIPO DE DIETA	VENTAJAS	INCONVENIENTES
Dieta gastrointestinal	• Generalmente de elevada digestibilidad • Posiblemente menos cara que la dieta hidrolizada • Mayor palatabilidad que la dieta hidrolizada • Algunas son bajas en grasa	• Menos exitosas para mantener la remisión de las enteropatías crónicas (ECs) en perros en comparación con la dieta hidrolizada
Dieta comercial con una fuente de proteína *novel*	• Mayor palatabilidad • Algunas presentan niveles totales de fibra superiores para el manejo exitoso de la diarrea de intestino grueso • Se ha demostrado su eficacia en el tratamiento de las ECs	• Necesidad de historial dietético • Generalmente son más elevadas en grasa • Posibles recaídas por la reacción a nuevos antígenos a lo largo del tiempo • Contaminación cruzada con otros productos
Dieta comercial de proteína hidrolizada	• Historial dietético no necesario • Menos recaídas en comparación con dietas de proteína *novel*, de forma anecdótica • Algunas contienen menos grasa que las dietas de proteína *novel* • Se ha demostrado su eficacia en el tratamiento de las ECs	• Posiblemente menor palatabilidad en comparación con otras dietas • Posiblemente más caras en comparación con otras dietas comerciales
Dieta casera	• Generalmente elevada digestibilidad • Mayor palatabilidad • Capacidad de definir de forma precisa el perfil de macronutrientes, como el contenido de grasa • Evita antígenos de contaminación cruzada o emulsificantes • Se ha demostrado su eficacia en el tratamiento de la linfangiectasia	• Elevado precio en comparación con otras dietas • Exige mayor implicación por parte del propietario • Necesidad de un veterinario nutricionista certificado • Posibles recaídas por la reacción a nuevos antígenos a lo largo del tiempo • Posible dificultad de encontrar una fuente de proteína *novel* si ya ha habido una exposición a antígenos extensa • Necesidad de controles hematológicos si la biodisponibilidad de la dieta es desconocida

La ventaja del uso de las dietas hidrolizadas frente a una dieta de proteína novel radica en la dificultad de elaborar una buena historia dietética del animal para identificar aquellas proteínas a las que ya ha estado expuesto (Mandigers et al., 2010). De hecho, la mayoría de las dietas comerciales de mantenimiento suelen incluir una combinación de proteínas, lo que expone a los perros a múltiples alérgenos potenciales desde temprano (Mandigers et al., 2010). Asimismo, la práctica común de ofrecer restos de comida y chuches para perros puede intensificar este problema, complicando la identificación de una dieta de exclusión adecuada en algunos casos (Mandigers et al., 2010). No obstante, estas dietas hidrolizadas presentan una serie de inconvenientes como su baja palatabilidad en comparación con otras dietas, y su precio (Kathrani, 2020).

Las dietas de eliminación regulan el microbioma y son capaces así de reducir los signos clínicos de la IBD (Marchesi *et al.,* 2017; Kalenyak *et al.,* 2018). Recientemente se ha evaluado el uso de "dietas elementales" para el tratamiento de la NRE (Manchester *et al.,* 2023). Estas dietas proporcionan proteínas en forma de aminoácidos individuales y están diseñadas para ser no inmunogénicas y de fácil asimilación (Damas *et al.,* 2019). Así en el estudio de Manchester *et al.*, en 2023 se demostró la remisión clínica en 16/23 perros que no habían respondido de forma favorable a ninguno de los tratamientos de la EC. Además, se observaron diferencias en el microbioma de los perros que respondieron a la dieta elemental frente a aquellos que no respondieron (Manchester *et al.,* 2023). Por ello, es importante considerar varios ensayos dietéticos antes de descartar la FRE ya que no todos los pacientes con EC responden a la misma estrategia nutricional (Kathrani, 2020; Tolbert *et al.,* 2022). Los cambios en la dieta deben realizarse de forma individualizada, valorando los signos clínicos del animal y su respuesta o falta de respuesta a dietas previas (Kathrani, 2020; Tolbert *et al.,* 2022).

1.1.7.2 Inmunosupresores

El tratamiento con inmunosupresores siempre debe realizarse tras la confirmación histológica de la IBD, habiendo descartado el resto de causas.

Los glucocorticoides como la prednisona y la prednisolona son la primera línea de tratamiento (García-Sancho *et al.,* 2007; Jergens *et al.,* 2010; Allenspach *et al.,* 2016; Atherly *et al.,* 2019). La dosis inicial consiste en 1-2mg/kg por vía oral cada 12 horas, durante 1 a 4 semanas para luego ir reduciendo cada 2-4 semanas hasta llegar a dosis de 0,5mg/kg cada 48 horas, intentando la retirada del fármaco (Hall & Day, 2017). En algunos casos, es necesario mantener una dosis mínima efectiva, no siendo posible retirar el medicamento (Jergens & Simpson, 2012).

En animales que no toleran dosis elevadas, no muestran respuesta al tratamiento convencional con estos inmunosupresores, o desarrollan efectos secundarios, se recomienda combinar o reemplazar estos medicamentos con otros agentes inmunosupresores como la ciclosporina (Allenspach *et al.,* 2006). Las dosis recomendadas son de 5mg/kg/24 horas por vía oral durante 10 semanas (Allenspach *et al.,* 2006). Se deben tener en cuenta los posibles efectos adversos de la mayoría de estos fármacos (Viviano, 2022).

1.1.7.3 Moduladores de la microbiota (prebióticos, probióticos, simbióticos, trasplante de microbiota fecal)

En los últimos años, se han explorado diversas estrategias para restaurar la funcionalidad de la microbiota intestinal alterada, incluyendo el uso de prebióticos, probióticos, simbióticos y el trasplante de microbiota fecal (Jergens & Heilmann, 2022).

Las publicaciones revisadas por pares que investigan el uso de probióticos o simbióticos en perros con EC son escasas (Jergens & Heilmann, 2022). Los posibles beneficios de estos suplementos se han investigado mediante ensayos clínicos y experimentos ex vivo. En perros con FRE el uso de un simbiótico (Schmitz *et al.,* 2015) o una mezcla de probióticos (Sauter *et al.,* 2006) no logró mejorar los resultados clínicos ni los patrones de citoquinas intestinales proinflamatorias en comparación con el placebo (dieta de eliminación). Este mismo simbiótico tampoco alteró la riqueza o diversidad de la microbiota fecal en perros con FRE (Pilla *et al.,* 2019). Sin embargo, en perros con IBD el uso de probióticos ha demostrado mejorar la integridad de la barrera epitelial intestinal favoreciendo la remisión clínica en comparación con una terapia combinada de prednisona

y metronidazol (Rossi *et al.,* 2014) así como aumentar la presencia de bacterias beneficiosas en la mucosa intestinal (White *et al.,* 2017), ofreciéndose como una herramienta potencial en el tratamiento de la enfermedad.

Por otra parte, el trasplante de microbiota fecal es una técnica que se utiliza en el tratamiento de las infecciones por *Clostridium difficile* en medicina humana (Chapman *et al.,* 2016). Diversos estudios sugieren el beneficio de esta técnica en pacientes con colitis ulcerosa moderada, aunque no existe suficiente evidencia científica para su aplicación práctica (Lopetuso *et al.,* 2023). En perros con EC y más concretamente con IBD, se han llevado a cabo escasos estudios clínicos evaluando el uso de esta técnica (Bottero *et al.,* 2017; Niina *et al.,* 2019; Niina *et al.,* 2021; Sugita *et al.,* 2021; Cerquetella *et al.,* 2022; Collier *et al.,* 2022; Innocente *et al.,* 2022; Pérez-Accino et al., 2025). El estudio de Toresson *et al.*, en 2023 evalúa el mayor número de casos hasta la fecha. En el mismo, 41 perros con EC sin respuesta a dieta, probióticos o inmunosupresores recibieron de 1 a 5 trasplantes con heces congeladas en forma de enemas por vía rectal, observándose una mejoría en los signos clínicos en un 76% de los casos (Toresson *et al.,* 2023). Además, en 20/41 perros la dosis de glucocorticoides se redujo y el tratamiento antibiótico se retiró (Toresson *et al.,* 2023). Los niveles del CIBDAI se redujeron significativamente. Sin embargo, el estudio no evaluó un grupo control (Toresson *et al.,* 2023). Los resultados por el momento podrían apoyar el uso de esta técnica en perros con EC, aunque la evidencia es todavía escasa y requiere de futuras investigaciones.

1.1.8 Pronóstico

La mayoría de los estudios sugieren que hay una buena respuesta a largo plazo (> 6 meses) para los perros que responden inicialmente a la dieta (Craven *et al.,* 2004; Dandrieux *et al.,* 2019; Dandrieux & Mansfield, 2019) mientras que, por el contrario, el pronóstico no es tan favorable en el caso de los perros con ARE e IRE, en los que es común que se produzcan recaídas (Allenspach *et al.,* 2007; Allenspach *et al.,* 2016; Dandrieux & Mansfield, 2019).

1.1.9 Biomarcadores

En las últimas décadas, ha habido un gran interés en la búsqueda de biomarcadores que puedan ser útiles para identificar la gravedad de la enfermedad, determinar el pronóstico, predecir la respuesta al tratamiento y las posibles recaídas (Chantawong, 2023).

1.1.9.1 Biomarcadores funcionales

La **cobalamina** puede encontrarse disminuida (hipocobalaminemia) en perros con EC, considerándose un factor pronóstico negativo (Berghoff *et al.,* 2013; Volkmann *et al.,* 2017). Sin embargo, se debe tener en cuenta que la hipocobalaminemia también puede estar presente en múltiples patologías como la insuficiencia pancreática exocrina o el linfoma intestinal y, por tanto, no es específica de las ECs (Kather *et al.,* 2020a; Sacoor *et al.,* 2021; Volkmann *et al.,* 2017). Además, también se han encontrado aumentos en los niveles de esta vitamina en perros con signos gastrointestinales crónicos (Kather *et al.,* 2020b). En cualquier caso, una baja concentración sérica de cobalamina indica la necesidad de suplementación (Toresson *et al.,* 2016).

Por otra parte, el **folato** se ha encontrado aumentado, disminuido o normal en las ECs (Heilmann *et al.,* 2018). El folato se absorbe principalmente en el intestino delgado proximal (duodeno y yeyuno proximal) (Berghoff & Steiner, 2011). El daño causado a la mucosa a este nivel puede producir su malabsorción intestinal dando lugar a hipofolatemia (German *et al.,* 2003b; Allenspach *et al.,* 2007; Allenspach *et al.,* 2016). Por el contrario, la disbiosis puede producir un aumento en la producción de folato por determinados microorganismos (Sacoor *et al.,* 2021). Recientemente se ha descrito la falta de asociación entre los niveles de folato con parámetros indicadores de la gravedad de la enfermedad (índices clínicos y biomarcadores laboratoriales) sugiriendo que la hipofolatemia es un biomarcador inferior en las ECs en comparación con la hipocobalaminemia (Ullal *et al.,* 2023).

El **inhibidor de la proteinasa α1** (**α1- PI)** es una proteína con un tamaño similar a la albúmina, por lo que es probable que ambas proteínas se excreten desde la luz intestinal

hacia las heces en casos de enfermedades gastrointestinales que causen pérdida de proteínas, como la PLE (Melgarejo *et al.,* 1996; Cerquetella *et al.,* 2010). Debido a su resistencia a la degradación proteolítica, su determinación a nivel fecal sirve para detectar la pérdida de proteínas gastrointestinales en etapas tempranas, incluso antes de la presencia de hipoalbuminemia (Allenspach, 2013). Por ello puede ser utilizada para monitorizar la progresión de la enfermedad y evaluar la eficacia del tratamiento (Collins, 2013).

1.1.9.2 Biomarcadores inflamatorios

La **proteína C-reactiva** es una proteína de fase aguda, sintetizada en el hígado como respuesta a lesión tisular, infección, inflamación o cáncer (Nakamura *et al.,* 2008). Niveles séricos superiores a 9,1mg/L junto con un aumento en los niveles de **calprotectina fecal** fueron capaces de diferenciar a los perros con IRE de los perros con FRE o ARE, con una sensibilidad del 72% y una especificidad del 100% (Heilmann *et al.,* 2018). No obstante, la utilidad clínica de este biomarcador para las ECs en perros es limitada debido a su falta de especificidad para el tracto gastrointestinal (Jergens & Heilmann, 2022). Puede utilizarse como indicador del estado de la enfermedad y la respuesta al tratamiento ya que diversos estudios han demostrado que se encuentra elevada en el suero de perros con IBD y que se reduce cuando la respuesta terapéutica es favorable (Jergens *et al.,* 2003; Allenspach *et al.,* 2007; Jergens *et al.,* 2010; Otoni *et al.,* 2018).

La concentración de la **calprotectina fecal (S100A8/A9)** parece ser un biomarcador útil de inflamación intestinal en perros (Grellet *et al.,* 2013; Heilmann *et al.,* 2018; Otoni *et al.,* 2018). Esta proteína se acumula en sitios de inflamación y actúa como ligando del receptor TLR4 (Vogl *et al.,* 2007). Puede utilizarse para predecir la respuesta al tratamiento entre la IRE y la NRE con una sensibilidad del 80% y una especificidad del 75%, además de correlacionarse con la gravedad de las ECs (Heilmann *et al.,* 2018). Aunque este biomarcador también se eleva en condiciones inflamatorias gastrointestinales agudas (Heilmann *et al.,* 2017), es muy valorado por su utilidad en monitorizar la progresión de la enfermedad y por diferenciar entre periodos de actividad y de remisión (Otoni *et al.,* 2018; Sacoor *et al.,* 2021).

La **calgranulina C (S100A12)** presenta una distribución celular similar a la calprotectina (Heilmann *et al.,* 2019). Se libera como consecuencia de la activación de neutrófilos, macrófagos y monocitos (Wdowiak *et al.,* 2013; Hanifeh *et al.,* 2018) y su determinación en heces es más específica de inflamación intestinal que en suero (Sacoor *et al.,* 2021). Se han descrito niveles superiores de esta proteína en perros con EC, asociándose a un peor pronóstico (Heilmann *et al.,* 2014a; Heilmann *et al.,* 2018). Además, niveles superiores a 490 ng/g han demostrado la capacidad de distinguir perros con IRE o con NRE de perros con FRE o ARE, con una sensibilidad del 64% y una especificidad del 77% (Heilmann *et al.,* 2016; Heilmann & Steiner, 2018). Aunque la determinación de calgranulina fecal puede ser más específica para la inflamación que las concentraciones de calprotectina fecal, su medición no está disponible en perros de forma rutinaria en la actualidad (Jergens & Heilmann, 2022).

Los **anticuerpos perinucleares anticitoplasma de los neutrófilos o pANCA** (*perinuclear anti-neutrophilic cytoplasmic antibodies*) están asociados a inflamación y enfermedades autoinmunes (Wdowiak *et al.,* 2013; Heilmann & Steiner, 2018). Se ha demostrado mayor prevalencia de seropositividad en perros con FRE en comparación con IRE (Luckschander *et al.,* 2006; Mancho *et al.,* 2010). Además, este biomarcador parece ser útil para la detección temprana de la PLE, habiéndose encontrado seropositividad hasta 2 años antes de la manifestación de los signos clínicos acompañados de una disminución en los niveles de albúmina (Allenspach *et al.,* 2008). No obstante, un resultado positivo en los pANCA no es exclusivo de las ECs, sino que también puede encontrarse en enfermedades infecciosas u otras enfermedades autoinmunes (Allenspach & Mochel, 2022), por lo que se ha sugerido su uso como una herramienta diagnóstica suplementaria en la diferenciación de perros con EC de otras enfermedades gastrointestinales (Mancho *et al.,* 2010).

La **N-metilhistamina**, un metabolito derivado de la histamina, es un indicador de la activación de los mastocitos. Se han descrito niveles superiores de este biomarcador en heces (Vaden *et al.,* 2000; Berghoff *et al.,* 2008) y en orina (Berghoff *et al.,* 2014) de perros con EC correlacionándose con la gravedad de las lesiones histológicas (Berghoff *et al.,* 2014). Sin embargo, no se encontró ninguna asociación con los niveles de mastocitos en la

mucosa intestinal o la gravedad de la enfermedad (Anfinsen *et al.,* 2014; Berghoff *et al.,* 2014) por lo que futuros estudios son necesarios en relación a este biomarcador.

La **3-bromotirosina** es un biomarcador de inflamación eosinofílica que se ha encontrado elevado en suero de perros con IBD (Sattasathuchana *et al.,* 2015). De hecho, sus niveles han sido más altos en estos perros en comparación con aquellos que solo requirieron de dieta de eliminación para la resolución de los signos clínicos (Sattasathuchana *et al.,* 2017). Sin embargo, aún se debe determinar la precisión diagnóstica del mismo.

El **receptor soluble para productos finales de glicación avanzada (sRAGE)** es un receptor que mitiga respuestas proinflamatorias secuestrando ligandos (Cabrera-García *et al.,* 2021). Se han descrito niveles séricos disminuidos de este receptor en perros con IBD, y aumentos con una buena respuesta al tratamiento (Heilmann *et al.,* 2014b), por lo que podría utilizarse como biomarcador para monitorizar a los perros durante el tratamiento.

1.1.9.3 Biomarcadores microbiómicos

En esta categoría destacaría el **índice de disbiosis fecal** que, como se ha comentado con anterioridad, sirve para diferenciar entre un estado de normobiosis y de disbiosis (Suchodolski, 2016; AlShawaqfeh *et al.,* 2017). Está basado en la determinación de 7 grupos bacterianos, así como las bacterias totales que típicamente se ven afectados en las ECs (*E. coli, Fecalibacterium* spp., *Turicibacter* spp., *Streptococcus* spp., *Blautia* spp., *Fusobacterium* spp., *C. hiranonis*) (AlShawaqfeh *et al.,* 2017). Por ello, puede ser de utilidad para evaluar los efectos del tratamiento y la eficacia del mismo (Pilla & Suchodolski, 2020).

1.1.9.4 Biomarcadores metabolómicos

La metabolómica es una herramienta ómica innovadora que ofrece información valiosa sobre la interacción compleja entre el organismo, la microbiota intestinal y factores ambientales, como la dieta (Nicholson & Lindon, 2008). Mediante el análisis metabolómico, es posible cuantificar pequeñas moléculas como ácidos orgánicos, lípidos o aminoácidos presentes en muestras biológicas, tales como suero/plasma, orina, o heces (Nicholson & Lindon, 2008). El plasma o suero es indicativo del metabolismo sistémico mientras que los

perfiles fecales son más representativos del metabolismo digestivo, incluyendo los metabolitos microbianos o huésped-microbianos y la transformación microbiana de los componentes dietéticos (Bjerrum *et al.,* 2015).

Estudios previos han identificado diferencias significativas en diversas clases de metabolitos en la IBD humana en comparación con individuos sanos e incluso entre la enfermedad de Crohn y la colitis ulcerosa (Gallagher *et al.,* 2020; Aldars-García *et al.,* 2021; Bauset *et al.,* 2021). Entre las principales clases de metabolitos descritas se encuentran los ácidos grasos de cadena corta, los aminoácidos y los ácidos biliares. Estos descubrimientos están todavía en fases iniciales y están contribuyendo al conocimiento de estas enfermedades, así como a las interacciones entre el huésped y la microbiota, sugiriendo que el análisis metabolómico podría desempeñar un papel potencial como biomarcador diagnóstico complementario (Jagt *et al.,* 2022).

A pesar de que se han identificado **ácidos grasos** y **aminoácidos** como compuestos implicados en la salud digestiva y metabólica del individuo, existe poca información sobre su papel como potenciales biomarcadores entre los distintos tipos de enteropatías. En el punto 4 se describirán los estudios científicos hasta la fecha que evalúan las alteraciones en estos metabolitos en enfermedades como la IBD humana y en las ECs en medicina veterinaria.

1.2 ÁCIDOS GRASOS Y SALUD INTESTINAL Y SISTÉMICA

Los ácidos grasos son ácidos carboxílicos que se caracterizan por tener una estructura compuesta por un extremo metilo (CH_3), una cadena de hidrocarburos (R) y un grupo carboxilo (COOH) en el extremo opuesto (Stryer *et al.,* 2019). Estos ácidos grasos son componentes esenciales de los lípidos en el organismo y desempeñan funciones clave en la nutrición y el metabolismo.

Las dos funciones esenciales de los ácidos grasos son actuar como reserva energética y desempeñar un papel estructural (Stryer *et al.,* 2019), además de participar en

funciones más específicas. Normalmente, no se encuentran en su forma libre, sino integrados en biomoléculas formando ésteres de glicerol. Solo durante procesos metabólicos y por periodos breves se presentan como ácidos grasos libres (Hidalgo, 2013).

1.2.1 Tipos de ácidos grasos

Los ácidos grasos se pueden clasificar de acuerdo a diferentes criterios, principalmente por la longitud de su cadena de carbono, el grado de insaturación o la ramificación de la cadena. Así, se distinguen ácidos grasos de cadena corta (< 6 carbonos), de cadena media (6-12 carbonos), y de cadena larga (más de 12 carbonos) (Burdge & Calder, 2014). Además, dependiendo de la presencia de dobles enlaces, se pueden dividir en ácidos grasos saturados (sin dobles enlaces), monoinsaturados (un doble enlace), y poliinsaturados (dos o más dobles enlaces) (Burdge & Calder, 2014). Si presentan o no un número par de átomos de carbono serán ácidos grasos de cadena par o de cadena impar (Zhang *et al.,* 2020). Por último, los ácidos grasos de cadena ramificada se diferencian del resto de ácidos grasos de cadena lineal por la presencia de ramificaciones en la cadena en forma de grupos metilo (He *et al.,* 2023).

Para su denominación, presentan tanto un nombre común como sistemático (por ejemplo, el ácido C18:0 u esteárico, también es conocido como octadecanoico). A menudo también se expresan mediante una fórmula esquemática (notación abreviada), como en *CN:p n-x*, donde *CN* (número de carbono) representa el total de átomos de carbono, *p* es el número de enlaces dobles, y *x* indica la posición del primer enlace doble contando desde el extremo metilo (*n*). Por ejemplo, la notación abreviada para el ácido linoleico (ácido 9,12-octadecadienoico) es 18:2 n-6, ya que contiene 18 átomos de carbono y 2 enlaces dobles, de los cuales el primero se encuentra en el sexto átomo de carbono contando desde el extremo metilo (IUPAC-IUB). Algunos ejemplos de las fórmulas estructurales, así como los tipos de notaciones abreviadas, se muestran en la **figura 1.5.**

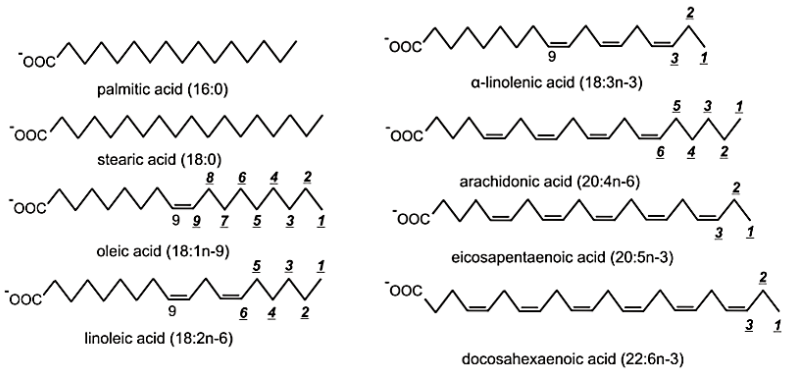

Figura 1.5. Fórmulas estructurales y tipos de abreviaciones de los ácidos grasos (Tvrzicka *et al.,* 2011).

Siguiendo la clasificación en función del grado de insaturación, los **ácidos grasos saturados (SAT)** se caracterizan por la ausencia de dobles enlaces o insaturaciones en su estructura. Todos los enlaces carbono-hidrógeno están saturados, lo que les confiere una estructura lineal y rígida. Son sólidos a temperatura ambiente (Gillingham *et al.,* 2011) siendo los más representativos el ácido láurico (C12:0), el ácido palmítico (C16:0) y el esteárico (C18:0) (Calder, 2015). El consumo elevado de estos ácidos se ha asociado con enfermedad cardiaca, infarto o diabetes tipo II en medicina humana (Judge & Dodd, 2020).

Los **ácidos grasos monoinsaturados (MUFA)** contienen un solo doble enlace en su cadena de carbono, lo que les confiere una estructura más flexible que los saturados. Son líquidos a temperatura ambiente y se consideran beneficiosos para la salud cardiovascular (Gillingham *et al.,* 2011). Los más representativos son el ácido oleico (C18:1n-9) y el palmitoleico (C16:1n-7).

Los **ácidos grasos poliinsaturados (PUFA)** contienen dos o más dobles enlaces en su cadena de carbono, lo que les otorga una estructura más flexible y menor estabilidad frente a la oxidación. Son esenciales para el organismo, lo que significa que deben ser obtenidos a través de la dieta. Los más destacados de los omega-6 son el ácido linoleico (LA, C18:2 n-6) y el ácido araquidónico (AA, C20:4 n-6); y dentro de los omega-3 el ácido alfa-linolénico (ALA, C18:3n-3), ácido eicosapentaenoico (EPA, C20:5 n-3) y el ácido docosahexaenoico (DHA, C22:6 n-3).

En relación con la clasificación en función de la longitud de la cadena, los **ácidos grasos de cadena corta (AGCC)** son unos de los metabolitos microbianos más estudiados hasta la fecha. Se obtienen principalmente de la fermentación de los hidratos de carbono no digeribles por parte de la microbiota intestinal del animal (Morrison & Preston, 2016), favoreciendo una respuesta inmunitaria intestinal tolerante frente a antígenos dietéticos y microbianos inocuos para el hospedador (Gonçalves *et al.,* 2018). Incluyen mayoritariamente el ácido acético (C2:0), propiónico (C3:0), y butírico (C4:0) (Tvrzicka *et al.,* 2011). A pesar de que el isobutírico (IC4:0) e isovalérico (IC5:0) son considerados AGCC, también se encuentran en la categoría de los ácidos grasos ramificados (Gozdzik *et al.,* 2023).

Los **ácidos grasos de cadena media** tienen cadenas de carbono entre 6 y 12 átomos. Se absorben y metabolizan más rápidamente que los ácidos grasos de cadena larga, lo que los hace una fuente rápida de energía (Tvrzicka *et al.,* 2011).

Los **ácidos grasos de cadena larga** tienen cadenas de carbono de 13 o más átomos. Son los más comunes en la dieta y en el cuerpo humano. Los ácidos grasos esenciales como los omega-3 y omega-6 son parte de este grupo (Tvrzicka *et al.,* 2011).

Los ácidos grasos también se pueden diferenciar en función de si presentan un número par o impar de átomos de carbono en la cadena. Así, a pesar de que su contenido es bajo, los **ácidos grasos de cadena impar (AGCI)** están ampliamente presentes en animales superiores, plantas y microorganismos heterótrofos (Yang *et al.,* 2023). Los más comunes son el ácido pentadecanoico (C15:0) y el ácido heptadecanoico (C17:0) ya que son biomarcadores de la ingesta de grasa láctea (Pfeuffer & Jaudszus, 2016). Estos ácidos se han relacionado con enfermedades cardiovasculares, obesidad, síndrome metabólico, diabetes tipo II o inflamación crónica (Venn-Watson *et al.,* 2020), y el consumo elevado de los mismos se ha relacionado con niveles inferiores de colesterol, menor riesgo de enfermedad cardiovascular y de síndrome metabólico (Mika *et al.,* 2016). Además, presentan interés a nivel intestinal, ya que los microorganismos son una de las vías principales para la producción de estos ácidos (Zhang *et al.,* 2020).

Por último, encontramos los **ácidos grasos ramificados (AGRs)**. Estos ácidos saturados se caracterizan por presentar uno (monometilados) o varios (polimetilados) grupos metilo en la cadena de carbono. Por lo general, los AGRs tienen una estructura "iso", cuando el grupo metilo se encuentra en el penúltimo átomo de carbono, o una estructura "anteiso", cuando se sitúa en el antepenúltimo átomo de carbono (Taormina *et al.,* 2020). Uno de los aspectos más interesantes de estos ácidos es que parecen presentar efectos beneficiosos sobre la salud, ya que se han visto relacionados con enfermedades como la diabetes, la obesidad o enfermedades inflamatorias. Además, numerosos estudios han evidenciado su papel a nivel intestinal, ya que se encuentran en el vérnix caseosa, un biofilm que recubre la piel de los recién nacidos (Taormina *et al.,* 2020), así como en el meconio, por lo que se cree que podrían jugar un papel fundamental en la generación del microbioma (Gozdzik *et al.,* 2023). Por otra parte, en rumiantes se ha observado que son marcadores de la microbiota intestinal, ya que son los componentes principales de las membranas bacterianas (Lu *et al.,* 2023). A pesar de estas características, su estudio en perros es muy escaso o prácticamente nulo.

Tabla 1.2. Clasificación de los distintos tipos de ácidos grasos, nomenclatura y fuentes. Adaptado de Calder, (1998).

Clasificación	Nombre sistemático	Nombre común	Notación abreviada	Fuentes
SAT	Ácido etanoico	Ácido acético	C2:0	*De novo* síntesis
	Ácido propanoico	Ácido propiónico	C3:0	*De novo* síntesis
	Ácido butanoico	Ácido butírico	C4:0	*De novo* síntesis/mantequilla
	Ácido pentanoico	Ácido valérico	C5:0	*De novo* síntesis
	Ácido dodecanoico	Ácido láurico	C12:0	*De novo* síntesis/ leche
	Ácido tetradecanoico	Ácido mirístico	C14:0	*De novo* síntesis/ aceite de coco
	Ácido pentadecanoico	Ácido pentadecílico	C15:0	Productos lácteos/ *de novo* síntesis sobre todo rumiantes
	Ácido hexadecanoico	Ácido palmítico	C16:0	*De novo* síntesis/ leche, huevos, grasa animal, carne, manteca de cacao, aceite de palma, aceite de pescado
	Ácido heptadecanoico	Ácido margárico	C17:0	Productos lácteos/*de novo* síntesis rumiantes
	Ácido octadecanoico	Ácido esteárico	C18:0	*De novo* síntesis/ leche, huevos, grasa animal, carne, manteca de cacao
		Ácidos grasos de cadena ramificada (AGRs)	AGRs-iso/anteiso	Leche, queso, carne, pescado, semillas de chía, o alimentos fermentados/ de novo síntesis a partir de aminoácidos ramificados
MUFA	Ácido delta-9-cis-hexadecénico	Ácido palmitoleico	C16:1n-7	Desaturación del ácido palmítico/aceite de pescado
	Ácido 9-octadecanoico	Ácido oleico	C18:1n-9	Desaturación del ácido esteárico/aceite de oliva, leche, huevos, grasa animal, carne, manteca de cacao
PUFA	Ácido 9,12-octadecadienoico	Ácido linoleico	C18:2n-6	No puede ser sintetizado en mamíferos/ algunas leches, huevos, grasa animal, carne, verduras, vegetales, aceite de girasol, de cártamo y de soja
	Ácido 9,12,15-octadecatrienoico	Ácido α-Linolénico	C18:3n-3	No puede ser sintetizado en mamíferos/ Hojas verdes, algunos aceites vegetales especialmente de colza, soja y aceites de linaza
	Ácido 6,9,12-octadecatrienoico	Ácido γ-Linolénico	C18:3n-6	Sintetizado a partir del ácido linoleico/ aceite de borraja o de onagra
	Ácido 8,11,14-eicosatrienoico	Ácido dihomo-γ-linolénico	C20:3n-6	Sintetizado a partir del γ-Linolénico
	Ácido 8,11,14,17-eicosatetraenoico	Ácido araquidónico	C20:4n-6	Sintetizado a partir del ácido linoleico via γ-Linolénico y dihomo-γ-linolénico
	Ácido 5,8,11,14,17-eicosapentaenoico	Ácido eicosapentaenoico	C20:5n-3	Sintetizado a partir del α-Linolénico/ aceite de pescado
	Ácido 4,7,10,13,16,19-docosahexaenoico	Ácido docosahexaenoico	C22:6n-3	Sintetizado a partir del α-Linolénico vía ácido eicosapentaenoico/ aceite de pescado

1.2.2 Origen y metabolismo de los ácidos grasos

Los ácidos grasos se obtienen a partir de la dieta o a partir de síntesis endógena en el organismo.

La mayor parte de la digestión de las grasas ocurre en el intestino delgado, específicamente en el duodeno, donde las sales biliares emulsionan los lípidos, dividiéndolos en pequeñas gotitas (micelas), aumentando la superficie sobre la cual las lipasas pueden actuar (Maldonado-Valderrama *et al.,* 2011). Estas enzimas hidrolizan los triglicéridos (TAG) en monoglicéridos y ácidos grasos libres (van Tilbeurgh *et al.,* 1999), que serán absorbidos por los enterocitos. A continuación, se reensamblan en TAG y se empaquetan en quilomicrones. Los quilomicrones se liberan en el sistema linfático y, finalmente, ingresan al torrente sanguíneo (Omer & Chiodi, 2024). Los ácidos grasos de cadena corta y media son más fácilmente digeridos y absorbidos ya que pueden ser transportados directamente al torrente sanguíneo sin necesidad de quilomicrones y llegar al hígado para ser metabolizados (Schönfeld & Wojtczak, 2016).

En cuanto al proceso de síntesis endógena de los ácidos grasos, en el caso de los **AGCC**, su obtención es llevada a cabo gracias a la acción de la microbiota intestinal. Bajo condiciones anaeróbicas, las bacterias del colon fermentan los HC para la síntesis de estos ácidos (Blaak *et al.,* 2020). Se ha descrito que las fuentes proteicas también son fermentadas para su obtención, aunque en menor medida (Macfarlane *et al.,* 1992). Tras la hidrólisis microbiana de los HC se obtienen monosacáridos, posteriormente fermentados para la obtención de fosfoenolpiruvato. Este intermediario será utilizado para la producción de los AGCC a través de diferentes mecanismos (Blaak *et al.,* 2020).

El filo Bacteroidetes produce principalmente ácido acético y propiónico, mientras que Firmicutes se especializa en la producción de butírico (Louis *et al.,* 2017). A partir del acetil-CoA en el proceso de glucólisis se obtiene el ácido acético. La mayor concentración de este producto de fermentación se encuentra en el colon proximal, donde es absorbido por las células epiteliales intestinales, o transportado a la sangre hacia el hígado a través de la vena porta hepática. Este ácido participa mayoritariamente en la síntesis lipídica y es

metabolizado en el hígado, cerebro, corazón y el músculo (Zhang *et al.,* 2023). Generalmente es el que se encuentra en mayor proporción en relación al propiónico y el butírico (relación 3:1:1) (Blaak *et al.,* 2020).

El ácido propiónico deriva del metabolismo de los HC durante la glucólisis, principalmente a través de la vía del succinato (Zhang *et al.,* 2023). Es reabsorbido en menor medida que el ácido acético por la circulación portal, transportándose al hígado y transformándose en glucosa (Tvrzicka *et al.,* 2011).

Por último, el butírico se produce a través de la glucólisis, mediante la combinación de dos moléculas de acetil-CoA para formar acetoacetil-CoA, seguido de una reducción escalonada a butiril-CoA. El paso final en la formación de butírico a partir de butiril-CoA puede proceder de la ruta de butiril-CoA:acetil-CoA transferasa (a partir del acético), o de las vías de fosfo-butirato y butirato quinasa (Zhang *et al.,* 2023). Su principal destino son los colonocitos, ya que es la principal fuente de energía de los mismos (den Besten *et al.,* 2013). Los niveles que no hayan sido utilizados son transportados por la circulación portal hasta el hígado, donde serán metabolizados (Schönfeld & Wojtczak, 2016). La **figura 1.6** muestra las diferentes vías de síntesis de estos ácidos.

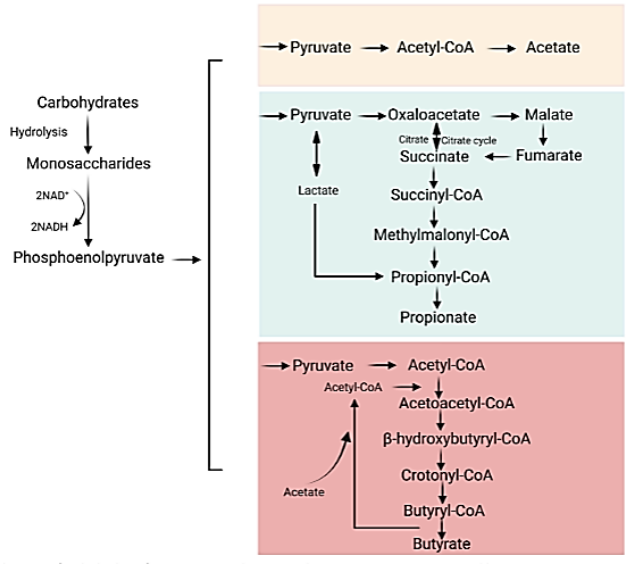

Figura 1.6. Diagrama esquemático de las vías de fermentación de los hidratos de carbono para la obtención de los AGCC (Zhang *et al.,* 2023).

Los productos lácteos son la mayor fuente de los **AGCI** para los humanos y los no rumiantes (Abdoul-Aziz *et al.,* 2021). A pesar de que se están investigando posibles rutas de síntesis endógena de los mismos, estas cobran una mínima importancia en comparación con la síntesis interna que se produce en los rumiantes. En general, estos ácidos se biosintetizan a partir de precursores de cadena impar como el propionil-CoA o mediante el acortamiento de la cadena de ácidos grasos de cadena par por α-oxidación. Una de las principales fuentes del propionil-CoA es el ácido propiónico, siendo esta la ruta más destacada en el caso de los rumiantes para la producción de los AGCI a nivel ruminal. Además, se ha descrito que el metabolismo de los aminoácidos ramificados valina e isoleucina genera también este precursor; por tanto, pueden actuar en la síntesis de estos ácidos grasos. De manera similar, la fitosfingosina puede metabolizarse a C15:0 e incorporarse en los glicerofosfolípidos, tanto en levaduras como en células de mamíferos (Zhang *et al.,* 2020). En estudios humanos, se han observado resultados contradictorios con relación al aumento de los niveles plasmáticos de AGCI tras el consumo de dietas ricas en fibra, acompañadas de una mayor producción de ácido propiónico (Ciesielski *et al.,* 2024).

Al igual que con los AGCI, las fuentes mayoritarias de los **AGRs** son los alimentos (Mao *et al.,* 2023). Sin embargo, una pequeña cantidad de estos es producida endógenamente a nivel intestinal gracias a la acción bacteriana sobre los aminoácidos de cadena ramificada (Gozdzik *et al.,* 2023; He *et al.,* 2023). La valina, leucina e isoleucina se transforman primero en α-cetoácidos ramificados mediante la eliminación del grupo amino por la enzima transaminasa de aminoácidos de cadena ramificada (BCAT) (Vlaeminck *et al.,* 2006). Estos productos α-cetoácidos, como el α-cetoisovalerato, el α-ceto-β-metilvalerato y el α-cetoisocaproato, son posteriormente descarboxilados por la enzima deshidrogenasa de α-cetoácidos de cadena ramificada (BKD), produciendo los respectivos ácidos carboxílicos de cadena corta ramificada: isobutiryl-CoA, isovaleryl-CoA y 2-metilbutiryl-CoA (Harper *et al.,* 1984). Finalmente, los ácidos carboxílicos de cadena corta ramificada son alargados por la sintetasa de AGRs, con malonil-CoA como extensor de la cadena, para formar AGRs iso- y anteiso- (Kaneda, 1991). Los productos obtenidos son iso-14:0 e iso-16:0, derivados de la valina; iso-15:0 e iso-17:0 de la leucina; y anteiso-15:0 y anteiso-17:0 de la isoleucina (**figura 1.7**).

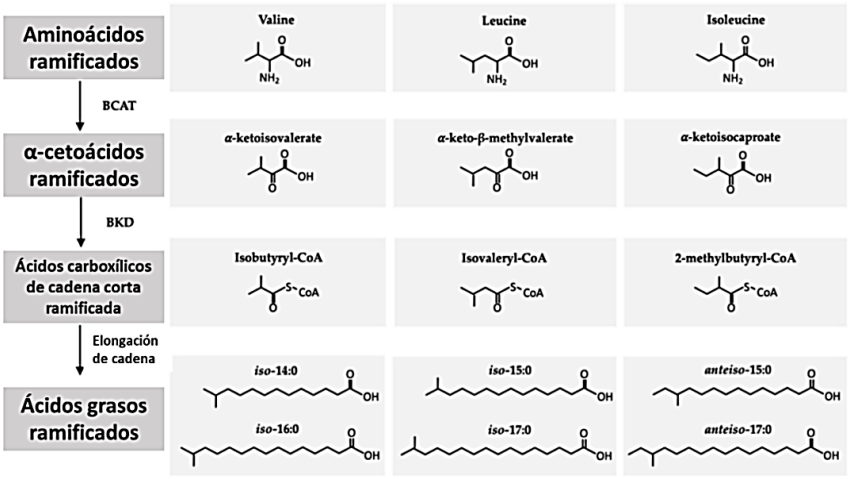

Figura 1.7. Biosíntesis de los AGRs a partir de los aminoácidos de cadena ramificada. BCAT: branched-chain amino acid transferase enzyme; BKD: branched-chain α-ketoacid dehydrogenase. Adaptado de Taormina *et al.* (2020).

En cuanto a los **ácidos grasos de cadena larga**, algunos pueden ser sintetizados por el organismo en lo que se conoce como lipogénesis a partir de precursores como el acetil-CoA, obtenidos del metabolismo de los carbohidratos, aminoácidos y ácidos grasos (Judge & Dodd, 2020; Xu *et al.,* 2021). La lipogénesis tiene lugar en el hígado y en el tejido muscular. Este proceso de síntesis involucra una serie de enzimas clave, como las desaturasas y elongasas, que permiten la formación de ácidos grasos más complejos a partir de precursores simples.

El acetil-CoA, primer compuesto implicado en la síntesis de ácidos grasos, puede originarse a partir de tres rutas principales. Primero, puede derivar de la descarboxilación oxidativa del piruvato, el cual es un producto final de la oxidación de la glucosa (Xu *et al.,* 2021). En segundo lugar, puede provenir de la degradación de ácidos grasos, ya sean de origen endógeno o exógeno (Judge & Dodd, 2020). Finalmente, el acetil-CoA también puede obtenerse a través del catabolismo de aminoácidos, principalmente aquellos glucogénicos, como la treonina, alanina y arginina (Judge & Dodd, 2020). Este acetil-CoA dentro de la mitocondria necesita ser transportado al citosol, donde ocurre la síntesis de los ácidos grasos (Beld *et al.,* 2015). Para ello, el citrato, resultado de la condensación de acetil-CoA con el ácido oxaloacético, es transportado fuera del ciclo de Krebs hacia el

citosol, donde se descompone nuevamente en oxaloacetato y acetil-CoA mediante la acción de la enzima ATP citrato liasa. El oxaloacetato regresa a la mitocondria en forma de ácido málico (Ferre & Foufelle, 2007). La carboxilación del acetil-CoA citosólico a malonil-CoA, catalizada por la acetil-CoA carboxilasa, es el primer paso clave en la síntesis de ácidos grasos (**Figura 1.8**). El primer compuesto liberado en el citoplasma es un ácido carboxílico de 4 carbonos (ácido butírico). A través de ciclos repetidos de elongación de este ácido butírico, se forman ácidos grasos de cadena par más largos, como el ácido palmítico (C16:0) y el ácido esteárico (C18:0) (Burgy *et al.,* 2022). En algún caso puede ocurrir que participe un propionilo, que consta de tres átomos de carbono, en la elongación. En este caso el resultado sería una cadena de número impar de átomos de carbono. Al finalizar este proceso, los ácidos grasos suelen unirse a glicerol para formar TAG, la principal forma en la que se almacenan los ácidos grasos en el cuerpo, actuando como la principal fuente de energía.

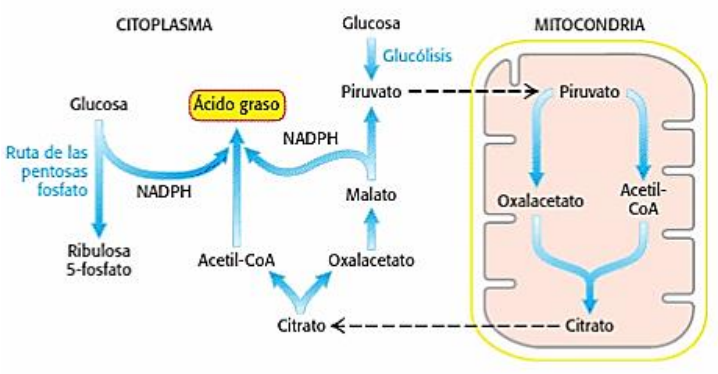

Figura 1.8. Resumen esquemático de síntesis de ácidos grasos (Stryer *et al.,* 2019).

A continuación, por la acción de las desaturasas y elongasas se obtienen los MUFA y los PUFA. Los **MUFA** se sintetizan a través de la elongación y desaturación de los ácidos grasos palmítico (C16:0) y esteárico (C18:0) en animales y humanos (He *et al.,* 2023). Una de las enzimas más destacadas en este proceso es la **Δ9-desaturasa**. Esta enzima, localizada en la membrana del retículo endoplásmico, introduce un doble enlace cis en la posición C9 de los ácidos como el palmítico (C16:0) o el esteárico (C18:0), formando así ácidos grasos monoinsaturados, como el ácido palmitoleico (C16:1n-7) o el ácido oleico (C18:1n-9),

respectivamente (**Figura 1.9**) (Kubota & Espenshade, 2022). La actividad de la Δ9-desaturasa influye en la composición de TAG y fosfolípidos dentro de la célula, ayudando a equilibrar la relación entre ácidos grasos saturados e insaturados. Esta relación, a su vez, es un determinante importante de la fluidez de la membrana, la función de los orgánulos y las interacciones entre células (Kubota & Espenshade, 2022). Además, alteraciones en la relación SAT/MUFA, debido a niveles disminuidos de ácidos grasos monoinsaturados, pueden ser un factor determinante en la aparición de enfermedades como la diabetes, enfermedades cardiovasculares o cáncer, ya que el acúmulo de grasa saturada induce la apoptosis celular desencadenada por estrés en el retículo endoplásmico (Kubota & Espenshade, 2022). Por lo tanto, los niveles de MUFA están siendo cada vez más considerados como marcadores de salud en alteraciones de procesos metabólicos (Bermúdez *et al.,* 2022).

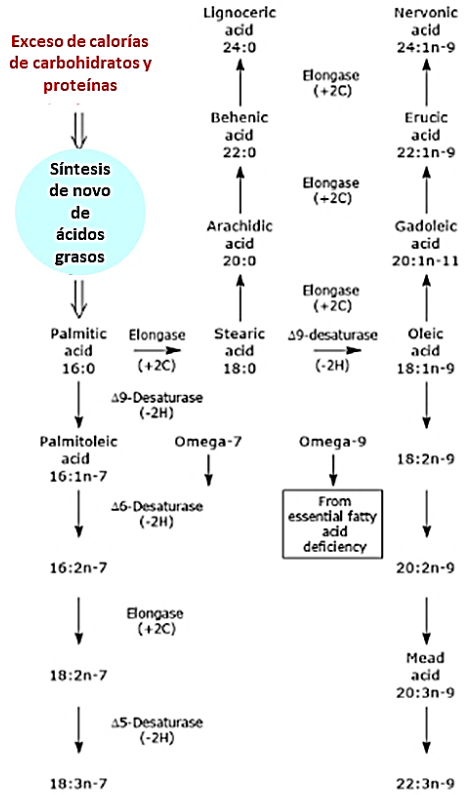

Figura 1.9. Biosíntesis de MUFA. Adaptado de Tvrzicka *et al.* (2011).

Por otra parte, las elongasas (ELOVLs) son las encargadas del alargamiento de las cadenas de ácidos grasos y desempeñan un papel crucial en la regulación de la biosíntesis de lípidos, el metabolismo de ácidos grasos y en la aparición de algunas enfermedades metabólicas. Es importante señalar que se han identificado siete miembros de la familia de proteínas ELOVLs (ELOVL1-7) en mamíferos, cada uno con especificidades de sustrato y distribuciones tisulares diferentes (Wang *et al.,* 2024).

Además de la síntesis de MUFA, los **PUFA** también necesitan de los procesos de desaturación y elongación para su producción. Sin embargo, tanto el LA de la familia omega-6, como el ALA de la familia omega-3, son ácidos grasos esenciales (del inglés, *essential fatty acids,* EFA) que no pueden ser sintetizados endógenamente, por lo que deben ser incorporados a través de la dieta (Tvrzicka *et al.,* 2011). Normalmente, solo una pequeña proporción del LA y ALA de la dieta se convierten en PUFAs de cadena más larga, ya que la mayor parte de estos ácidos grasos se somete a β-oxidación para proporcionar energía (Cunnane & Anderson, 1997).

Los genes FADS1 y FADS2 parecen ser responsables de toda la desaturación de los PUFAs omega-3 y omega-6 en los mamíferos. FADS1 exhibe actividad de Δ5-desaturasa y, aunque FADS2 fue originalmente identificado como una Δ6-desaturasa, se ha demostrado que también posee actividades de Δ4- y Δ8-desaturasa (Dyall *et al.,* 2022). Dado que ambos EFA comparten rutas de desaturación y elongación, se va a producir una competición entre ambos para la síntesis de sus respectivos productos de la familia omega-6 (AA) y omega-3 (EPA y DHA) (Nelson *et al.,* 2013). La **figura 1.10** muestra el metabolismo de ambas familias de ácidos grasos.

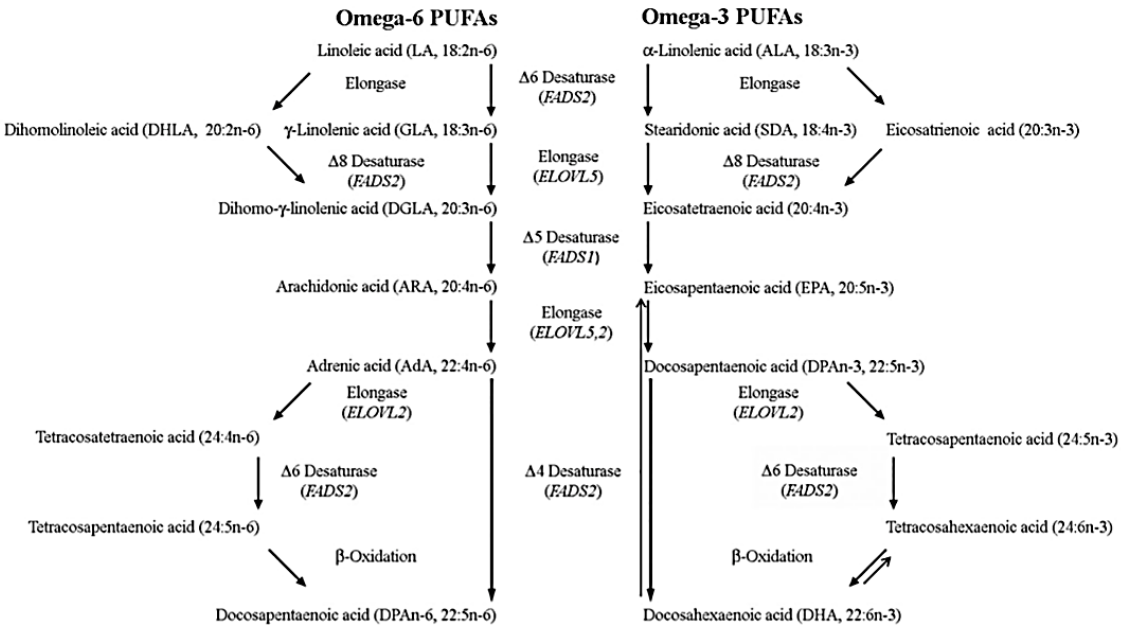

Figura 1.10. Biosíntesis de ácidos grasos omega-6 y omega-3 (Dyall *et al.,* 2022).

1.2.3 Implicación de los ácidos grasos en la salud sistémica e intestinal

1.2.3.1 Ácidos grasos como fuente de energía

Los ácidos grasos juegan un papel crucial como fuente de energía en los animales y los humanos, siendo componentes fundamentales de las reservas energéticas almacenadas en el cuerpo. El proceso de utilización de los ácidos grasos comienza con su movilización desde los tejidos de almacenamiento, principalmente el tejido adiposo, donde se encuentran en forma de TAG (Stryer *et al.,* 2019).

Debido a su naturaleza hidrofóbica, los ácidos grasos no pueden circular libremente en el torrente sanguíneo. Para ser transportados a los tejidos que los utilizarán, como el músculo y el hígado, se unen a la albúmina plasmática, una proteína que facilita su transporte en la sangre. En estos tejidos, los ácidos grasos se internalizan y se dirigen a las mitocondrias. Una vez en las mitocondrias, se produce lo que se conoce como β-oxidación (**figura 1.11**), el proceso por el cual los ácidos grasos se descomponen para obtener energía en forma de ATP (adenosín trifosfato) (Xu *et al.,* 2021). En este proceso, la cadena de carbono de los ácidos grasos se acorta secuencialmente mediante la eliminación de

fragmentos de dos carbonos en forma de acetil-CoA. Esta es una molécula intermediaria del metabolismo de proteínas, lípidos e hidratos de carbono. Cuando toda la cadena ha sido completamente degradada en unidades de acetil-CoA, cada molécula generada entra en el ciclo de Krebs donde es procesada para producir ATP (Stryer *et al.,* 2019). La oxidación completa de un ácido graso, dependiendo de su longitud, genera grandes cantidades de ATP. Comparado con los HC o las proteínas, los ácidos grasos otorgan el doble de energía, y su almacenamiento en el tejido adiposo requiere menor cantidad de agua (Kremmyda *et al.,* 2011).

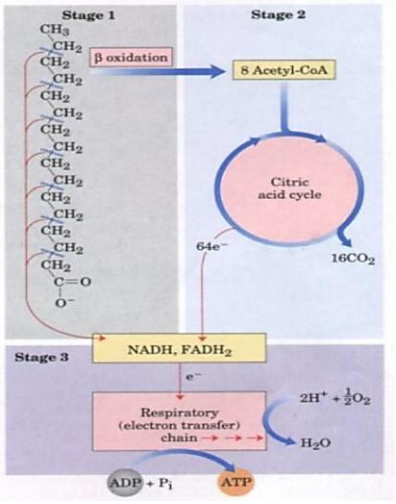

Figura 1.11. Resumen esquemático de la β-oxidación mitocondrial (Prakash, 2018).

En términos de producción energética, el grado de utilización de lípidos depende del grado de insaturación, así como de la longitud de la cadena. Así, se ha demostrado que los AGCC y media se utilizan de forma más rápida que los de cadena larga (Leyton *et al.,* 1987; DeLany *et al.,* 2000; Rey *et al.,* 2020). Una posible explicación consiste en que estos pueden difundirse de forma pasiva en la mitocondria sin la presencia de la L-carnitina, necesaria para el paso de los de cadena larga (Noguchi *et al.,* 2002). En cuanto al grado de insaturación, estudios en animales y humanos parecen demostrar que los ácidos grasos insaturados como el oleico (C18:1n-9) y el ALA (C18:3n-3) se utilizan de forma más rápida que los saturados como el palmítico (C16:0) y el esteárico (C18:0) (DeLany *et al.,* 2000).

A nivel intestinal, los ácidos grasos juegan un papel determinante como fuente de energía. Los AGCC son el principal sustrato para los colonocitos. Hasta un 60-70% de la energía de los mismos proviene de la oxidación de los AGCC, sobre todo, del ácido butírico (den Besten *et al.,* 2013). Una vez absorbidos en las células, se difunden hasta la membrana mitocondrial, entrando en el ciclo de Krebs para generar energía en forma de ATP (Blaak *et al.,* 2020).

1.2.3.2 Ácidos grasos y salud sistémica

Los ácidos grasos son capaces de regular el metabolismo lipídico, el metabolismo de la glucosa, además de procesos inflamatorios e inmunitarios.

En este sentido los AGCC cobran un especial protagonismo respecto a otros ácidos grasos en la salud general del animal. Una vez producidos en el intestino se transportan al hígado a través de la vena porta y de ahí a la sangre periférica. Una vez en los diversos tejidos actúan como sustratos para la síntesis de otros ácidos grasos (acético) o glucosa (propiónico), o bien como moléculas de señalización para la regulación del metabolismo (den Besten *et al.,* 2013). El ácido acético es el que mayor absorción presenta de entre los AGCC (hasta un 70%), y es utilizado en diversos tejidos como el tejido adiposo, hígado, cerebro, corazón, riñón, y el músculo. El propiónico y el butírico se encuentran en menor proporción en la circulación periférica. Estos ácidos actúan a través de dos mecanismos de transducción de señales: la inhibición de la histona desacetilasa (HDAC) y la activación de los receptores acoplados a proteínas G (GPRs) (He *et al.,* 2020).

A través de los GPRs los AGCC regulan el metabolismo lipídico y de la glucosa. La oxidación de ácidos grasos es activada por los AGCC, mientras que la síntesis *de novo* y la lipólisis son inhibidas. El resultado es una reducción de las concentraciones de ácidos grasos libres en el plasma (Ge *et al.,* 2008) y una disminución del peso corporal (Gao *et al.,* 2009; Kondo *et al.,* 2009).

Los AGCC además parecen afectar de manera beneficiosa al metabolismo de la glucosa al normalizar sus niveles en plasma y mejorar la captación de la misma por parte de los tejidos (**figura 1.12**). Existe evidencia indirecta de la reducción de la gluconeogénesis

en el hígado gracias a la acción de estos ácidos. Además, también pueden afectar los niveles de glucosa en plasma al incrementar las hormonas intestinales PYY (péptido YY) y GLP-1 (péptido similar al glucagón tipo 1) mediante la activación de los receptores GPR43 y GPR41 (He *et al.,* 2020). El PYY es conocido como una hormona de saciedad, pero también refuerza la acción de la insulina en la captación de glucosa en el músculo y el tejido adiposo. El GLP-1 regula indirectamente los niveles de glucosa en sangre al aumentar la secreción de insulina y disminuir la secreción de glucagón por parte del páncreas (Barrera *et al.,* 2011). Además, otros autores han encontrado que estos ácidos pueden aumentar la secreción de leptina (encargada de regular la ingesta de alimentos, el peso corporal y el metabolismo energético a través del sistema nervioso central) (Sakakibara *et al.,* 2006), así como la expresión de GLUT4, una proteína clave que facilita la entrada de glucosa en las células, principalmente en las células del músculo esquelético (Deng, 2018).

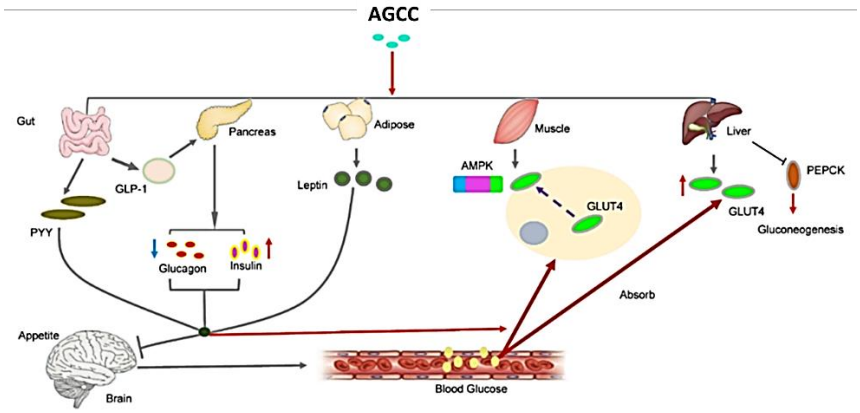

Figura 1.12. Regulación de los niveles de glucosa sanguínea por los AGCC. Adaptado de He *et al.* (2020).

La inhibición de la HDAC provoca una hiperacetilación de las histonas, facilitando el acceso al ADN para iniciar la transcripción génica (Gonçalves *et al.,* 2018). Esta capacidad otorga a estos ácidos un papel crucial en la modulación de la respuesta inmunitaria, ya que les permite controlar procesos celulares clave. Así, se ha demostrado que estos ácidos actúan sobre las células inmunitarias regulando su actividad y activación. Los receptores GPR41 y GPR43 se expresan en monocitos, células dendríticas, eosinófilos y neutrófilos, lo que permite a los AGCC influir directamente en su actividad (Gonçalves *et al.,* 2018).

Actualmente, estudios en humanos y modelos animales han descrito que estos ácidos disminuyen la diferenciación de monocitos a macrófagos (Millard *et al.,* 2002) o células dendríticas, alteran la maduración y diferenciación de las células dendríticas (Singh *et al.,* 2010; Nascimento *et al.,* 2011; Zimmerman *et al.,* 2012), y promueven la diferenciación y proliferación de linfocitos B (Newman *et al.,* 1989; Yamamoto *et al.,* 1997; Yamaguchi *et al.,* 2010). Además, afectan al reclutamiento de células del sistema inmunitario hacia la periferia, disminuyen la secreción de citoquinas proinflamatorias y quimiotácticas, y regulan la diferenciación de los linfocitos T hacia linfocitos Tregs (Wang *et al.,* 2009), implicados en la tolerancia inmunitaria intestinal. Por otra parte, en condiciones patológicas, aumentan la generación de células Th1 y Th17 acompañadas de citoquinas adecuadas, además de atraer a los neutrófilos hacia el intestino durante la respuesta inflamatoria (Kim *et al.,* 2014). En su participación en el inmunometabolismo, el ácido acético ha demostrado ser sustrato energético para el funcionamiento adecuado de los linfocitos B reguladores (Daïen *et al.,* 2021) y las células T CD8 de memoria (Balmer *et al.,* 2016). La estimulación de linfocitos B reguladores favorece la liberación de la citoquina antiinflamatoria IL-10 así como la síntesis de Inmunoglobulina A (IgA), manteniendo la tolerancia a antígenos ambientales y previniendo la inflamación intestinal (Gonçalves *et al.,* 2018).

En cuanto a los **ácidos grasos de cadena larga**, estos también participan en importantes procesos metabólicos en el organismo. Dentro de los MUFA, el **ácido palmitoleico (C16:1n-7)** se ha considerado como una "lipoquina" u hormona lipídica debido a su participación en numerosos procesos metabólicos (Cao *et al.,* 2008). A pesar de que los estudios en humanos no son consistentes por el momento, estudios in vitro y con animales demuestran que el palmitoleico regula el metabolismo de la glucosa aumentando la secreción de insulina por parte del páncreas, y mejorando la sensibilidad a la insulina en el hígado y en el tejido muscular (Yang *et al.,* 2013; Bolsoni-Lopes *et al.,* 2014). El **C16:1n-7** ha demostrado favorecer la lipólisis (Bolsoni-Lopes *et al.,* 2013) y la oxidación lipídica aumentando los niveles de ATP en el tejido adiposo blanco (Cruz *et al.,* 2018). También se le atribuye un carácter antinflamatorio ejerciendo numerosos efectos positivos en el sistema cardiovascular y el hígado (**figura 1.13**).

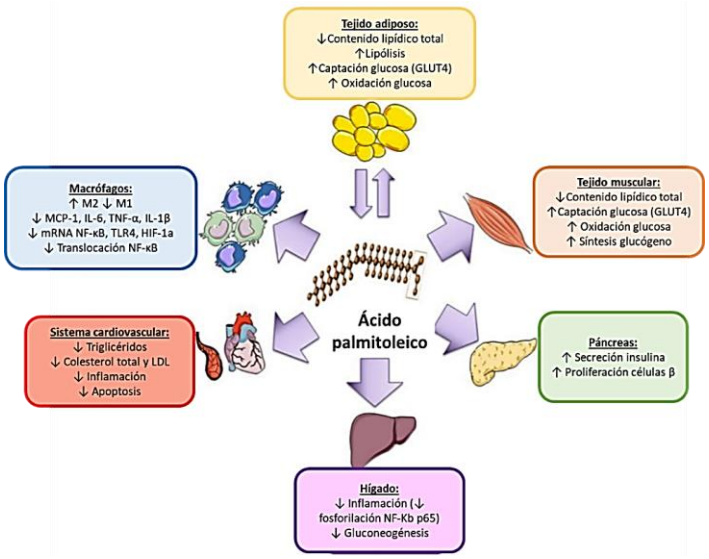

Figura 1.13. Efectos reguladores del ácido palmitoleico sobre el metabolismo. Adaptado de de Souza *et al.* (2017).

Además, parece presentar un efecto antiinflamatorio sobre los macrófagos (Neto *et al.,* 2021). De hecho, los MUFA y la actividad de la Δ9-desaturasa son necesarios para la regulación de la respuesta inmunitaria. En concreto, los linfocitos B necesitan de estos ácidos para su diferenciación (Zhou *et al.,* 2021). El consumo de aceite de oliva rico en ácido oleico (C18:1n-9) se ha asociado a una disminución en la proliferación linfocitaria en estudios con animales (Yaqoob *et al.,* 1994). Tanto el oleico como el **palmitoleico** han demostrado en estudios *in vitro* regular la expresión de linfocitos Tregs, controlando la respuesta inflamatoria. En el estudio de Passos *et al.,* en 2016 se demostró la capacidad del **palmitoleico** de inhibir la proliferación de linfocitos B, así como de citoquinas proinflamatorias (IL6, IFN-γ, TNF-α, e IL17A) *in vitro*. Estas citoquinas favorecen una respuesta Th1 y Th17. Sin embargo, estos autores encontraron que el **palmitoleico** reducía los niveles de Tregs, influyendo en la activación periférica de los linfocitos (Passos *et al.,* 2016).

En el caso de los **PUFA**, actúan como precursores de eicosanoides, reguladores del crecimiento y hormonas, además de formar parte de los fosfolípidos de las membranas involucrados en la transducción de señales (Jump, 2001). Por ello, tienen efectos importantes en procesos fisiológicos, como la función cognitiva, y desempeñan acciones

inmunosupresoras y antiinflamatorias (Simopoulos, 2002). Los eicosanoides son reguladores de la inflamación y la inmunidad. Incluyen las prostaglandinas, tromboxanos, leucotrienos, resolvinas, maresinas, lipoxinas, etc (Calder, 2010). Del AA se obtienen, sobre todo, mediadores lipídicos con carácter inflamatorio como la prostaglandina E2 (PGE_2) y el leucotrieno B4 (LTB_4). Por el contrario, el EPA y el DHA liberan mediadores menos inflamatorios o antiinflamatorios (Calder, 2010). La **figura 1.14** muestra la competición de estos ácidos como sustratos por las enzimas ciclooxigenasa (COX) o la lipoxigenasa (LOX) para la síntesis de estos mediadores.

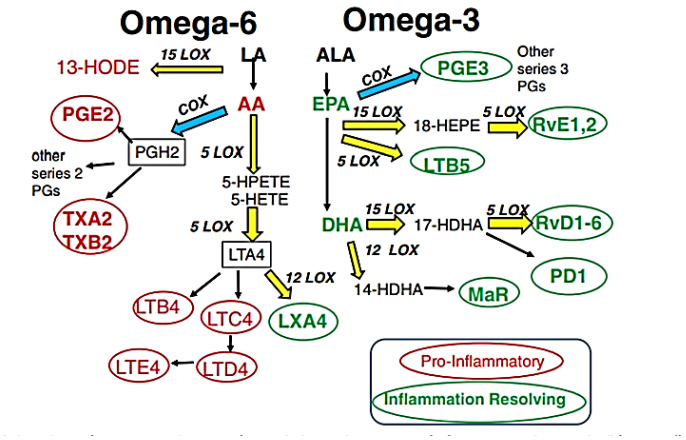

Figura 1.14. Rutas metabólicas de la síntesis de mediadores lipídicos a partir de los ácidos grasos omega-6 y omega-3 (Fabian *et al.,* 2015).

Los **ácidos grasos omega-3** presentan fundamentalmente propiedades antiinflamatorias, ejercidas mediante diferentes mecanismos. Uno de ellos consiste en la prevención de la conversión del AA en eicosanoides proinflamatorios, reduciendo así la activación de células inmunitarias (**Figura 1.15**). Para ello, se ha visto que son capaces de reducir la liberación del AA de los fosfolípidos de membrana al disminuir el contenido intracelular del mismo, o de inhibir a la fosfolipasa A_2, encargada de la liberación de este ácido (Fu *et al.,* 2021).

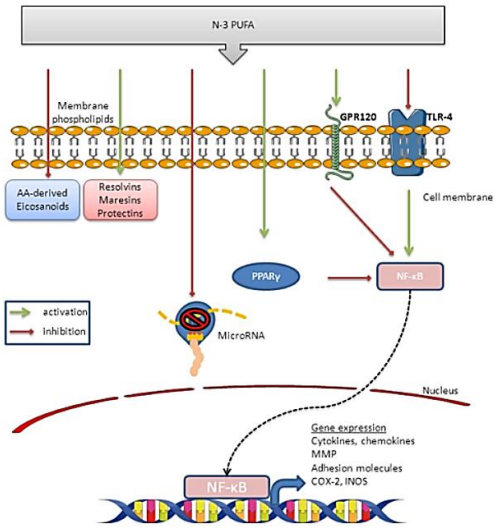

Figura 1.15. Mecanismos de acción de los omega-3 sobre la regulación inflamatoria (Marion-Letellier *et al.,* 2015).

Sin embargo, aunque algunos de los efectos de los **omega-3** pueden ser provocados por la modulación de la cantidad y el tipo de eicosanoides producidos, también ejercen sus efectos actuando sobre las vías de señalización intracelular y la actividad de los factores de transcripción. Así, los **omega-3** regulan la inmunidad mediante la inhibición del factor nuclear κB (NF-κB), gracias a la activación del receptor activado por proliferadores peroxisómicos gamma (PPARγ), reduciendo así la transcripción de moléculas inflamatorias como la interleuquina 17 (IL-17) (Fu *et al.,* 2021). Además, los **omega-3** promueven la liberación de citoquinas como IL-10 por parte de los macrófagos, y la inducción de Tregs (Fu *et al.,* 2021). Otro posible mecanismo en la supresión de la inflamación consiste en la activación de GPR120, expresado en células dendríticas y macrófagos derivados de la médula ósea (Fu *et al.,* 2021). Por último, el estudio de Liu *et al.,* en 2012 demostró que la suplementación con aceite de pescado rico en **omega-3** inhibió la expresión del receptor TLR4, un potente estimulador de respuestas inflamatorias. Sin embargo, a pesar de los efectos antinflamatorios observados en modelos experimentales, la suplementación de estos ácidos en humanos con IBD ha demostrado efectos contradictorios (Belluzzi *et al.,* 1996; Barbosa *et al.,* 2003; Nielsen *et al.,* 2005; John *et al.,* 2010; Turner *et al.,* 2011; Chan *et al.,* 2014).

Por otra parte, se considera que los **omega-6** presentan mayoritariamente un efecto proinflamatorio como consecuencia de los eicosanoides derivados del AA. De hecho, el AA es el mayor PUFA en las membranas de las células involucradas en procesos inflamatorios (Innes & Calder, 2018). Sin embargo, recientemente se ha demostrado que determinadas lipoxinas y resolvinas con carácter antiinflamatorio, también son sintetizadas a partir de los **omega-6** (Marion-Letellier *et al.,* 2013). De hecho, la PGE_2 es capaz de ejercer una actividad antiinflamatoria al inhibir la liberación de TNF-α (Innes & Calder, 2018). Además, en el estudio de Mobraten *et al.,* en 2013 se demostró que el AA actuaba al igual que el EPA y DHA como agonistas del receptor GPR120. Actualmente la interacción entre los **omega-3** y **omega-6**, y sus derivados, en el contexto de la inflamación es compleja y no está completamente elucidada. Es posible que la alta susceptibilidad de los PUFA al proceso de oxidación con la liberación de radicales libres como los superóxidos sea una de las causas del daño en las enfermedades inflamatorias (Fritsche, 2015) como es el caso de la IBD, en la que existe evidencia científica del daño causado por estrés oxidativo (Bourgonje *et al.,* 2020).

1.2.3.3 Ácidos grasos y homeostasis intestinal

El tracto intestinal representa la principal barrera entre el organismo y su entorno. Para cumplir esta función, posee un sistema protector robusto que lo defiende de toxinas externas, antígenos alimentarios, bacterias y otras sustancias peligrosas. Este sistema protector es complejo y está compuesto por varios mecanismos, incluyendo una capa de moco, un epitelio en constante renovación, uniones estrechas proteicas, el microbioma intestinal, y las células inmunitarias que controlan la respuesta inflamatoria. Todos ellos trabajan en conjunto para mantener la salud del intestino y evitar daños al huésped (Rohr *et al.,* 2019).

1.2.3.3.1 Implicación de los ácidos grasos en la regulación del mucus intestinal

El mucus consiste en una barrera protectora que separa el epitelio intestinal de las bacterias luminales. Está formado por las mucinas, las cuales le otorgan características de gel. Las células caliciformes son las encargadas de sintetizar este mucus. Se sugiere que el

traspaso de un gran número de bacterias a través de esta capa protectora sea uno de los mecanismos que expliquen la inflamación intestinal (Pelaseyed *et al.,* 2014). Se ha demostrado en estudios con líneas celulares, modelos experimentales en animales y muestras frescas de tejido intestinal humano que los **AGCC**, principalmente el butírico, inducen de manera dosis-dependiente la formación de mucinas al aumentar la expresión de genes encargados de la síntesis de las mismas (Gaudier *et al.,* 2004; Burger-van Paassen *et al.,* 2009; Nielsen *et al.,* 2018; Giromini *et al.,* 2022). Por ejemplo, MUC2 y MUC5AC, dos de las mucinas principales sintetizadas por las células caliciformes, han visto aumentada su producción bajo determinadas dosis de estos ácidos, confirmando la importancia de los mismos en el mantenimiento de la salud intestinal.

1.2.3.3.2 Implicación de los ácidos grasos en el mantenimiento del epitelio gastrointestinal

La membrana del epitelio intestinal es uno de los tejidos con mayor capacidad de autorrenovación en mamíferos adultos. Cada ciclo de renovación se completa en un lapso de 3 a 5 días (Funk *et al.,* 2020). Por ello, la biodisponibilidad de los ácidos grasos para esta función es de carácter fundamental teniendo en cuenta que son los componentes principales de las membranas celulares del organismo. Como se muestra en la **figura 1.16**, los ácidos grasos se encuentran en forma de fosfolípidos formando una bicapa lipídica (Ali & Szabó, 2023). Su composición influye en el grosor y la fluidez de la membrana, afectando a la actividad de proteínas como enzimas, canales iónicos y receptores (Kremmyda *et al.,* 2011). La longitud de la cadena, el grado de insaturación, la posición de los dobles enlaces y la hidroxilación de los ácidos grasos que se integran en fosfolípidos y esfingolípidos determinan su impacto en las propiedades biofísicas de la membrana. Los **PUFA** son componentes estructurales clave que proporcionan fluidez y contribuyen a la permeabilidad selectiva (Leonard et al., 2004), siendo el **AA** uno de los componentes mayoritarios de la membrana celular (Ali & Szabó, 2023).

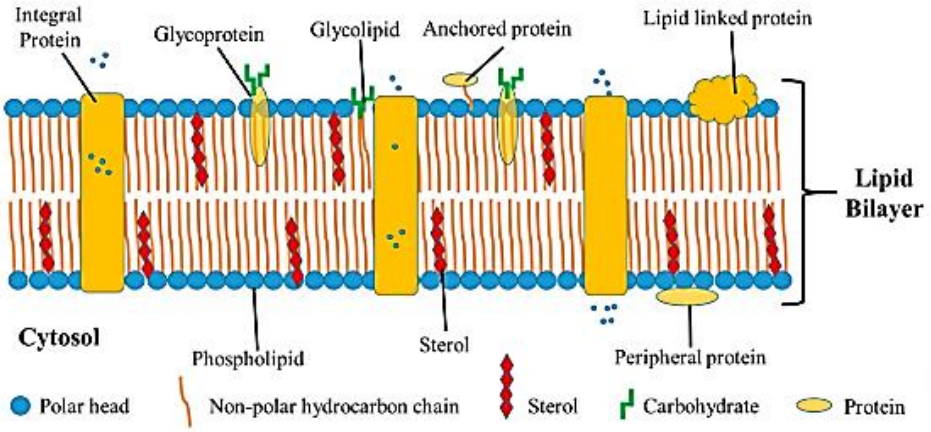

Figura 1.16. Representación esquemática de la estructura de la membrana plasmática (Ali & Szabó, 2023).

Los **AGCC**, los de **cadena media**, y los de **cadena larga** participan en el cuidado de la membrana epitelial mediante la estimulación de la secreción de péptidos antimicrobianos por parte de las células epiteliales (Zhou *et al.,* 2019). Además, los **AGCC** modulan la producción de la interleuquina 18 (IL-18) encargada de la reparación y mantenimiento de la integridad epitelial (Corrêa-Oliveira *et al.,* 2016). Asimismo, el metabolismo del ácido butírico promueve un microambiente hipóxico al aumentar el consumo de oxígeno, produciendo la estabilización de un factor de transcripción que regula la respuesta celular a la hipoxia (HIF-1a). Esta condición anaeróbica favorece la regulación positiva de genes involucrados en la función de la barrera intestinal (Tan *et al.,* 2023).

1.2.3.3.3 Implicación de los ácidos grasos en la regulación de la permeabilidad intestinal y mantenimiento de las uniones estrechas proteicas

Otro mecanismo en el cual participan los ácidos grasos es en la regulación de la permeabilidad de las uniones estrechas proteicas entre las células intestinales (**Figura 1.17**). Las uniones estrechas son componentes de sellado intercelular que controlan la difusión de iones, solutos y agua a través del espacio entre cada célula epitelial intestinal. La pérdida de la integridad de estas uniones se conoce como “leaky gut” y parece estar relacionada con el desarrollo de enfermedades intestinales como la IBD (Hashimoto *et al.,* 2019). Su permeabilidad puede ser regulada por diversos **ácidos grasos de cadena larga**

como el AA, LA, EPA, DHA y γ-linolénico (GLA). Sin embargo, algunas investigaciones han presentado resultados contradictorios. En estudios con células intestinales Caco-2, algunos de estos ácidos (como LA, EPA, DHA y GLA) han demostrado incrementar la permeabilidad de las uniones estrechas (Usami *et al.,* 2001; Usami *et al.,* 2003). Sin embargo, en otras células intestinales como las T84, algunos de los mismos (GLA, AA, EPA, y DHA) disminuyeron la permeabilidad, protegiendo la barrera intestinal frente a estímulos dañinos (Willemsen *et al.,* 2008).

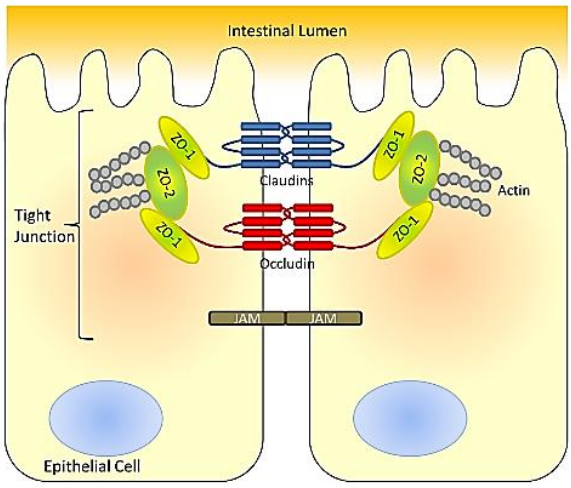

Figura 1.17. Proteínas de unión estrecha intestinales (Collins *et al.,* 2017).

En el caso de los **ácidos grasos de cadena media**, estos han demostrado aumentar la permeabilidad de las uniones estrechas mediante la activación de vías como la proteína quinasa C y la quinasa de cadena ligera de miosina (Tomita *et al.,* 1996; Lindmark *et al.,* 1998a; Lindmark *et al.,* 1998b). Estos efectos se han observado tanto en células como en estudios con ratas y humanos.

En el caso de los **AGCC**, estos juegan un papel determinante en la regulación de las uniones estrechas. En concreto, el ácido butírico promueve la función de la barrera intestinal aumentando la inducción de genes que codifican componentes de las uniones estrechas (como la claudina-1, o zonula occludens-1) (Wang *et al.,* 2012) y la reorganización de proteínas mediante la activación de otros factores de transcripción, incluyendo STAT3 y SP1. Como resultado, el butírico mantiene y/o aumenta la resistencia eléctrica

transepitelial en células Caco-2 y T84 del colon humano (Peng *et al.,* 2009), en células cdx2-IEC del intestino delgado de ratas (Wang *et al.,* 2012) y en células IPEC-J2 del intestino delgado porcino, incluso bajo condiciones inflamatorias (Yan & Ajuwon, 2017); de ahí su elevada importancia en enfermedades como la IBD, donde la reparación de la mucosa intestinal es uno de los objetivos terapéuticos.

1.2.3.3.4 Ácidos grasos y microbioma intestinal

Existe una estrecha relación entre el microbioma y los ácidos grasos. Tanto la cantidad como el tipo de grasa en la dieta son capaces de regular al propio microbioma. Un aumento en la ratio Firmicutes/Bacteroidetes (F/B) se ha asociado con fenotipos obesos/delgados y puede modular el equilibrio energético (Yang *et al.,* 2020). Los estudios clínicos sugieren que las dietas altas en grasa o en **SAT** presentan efectos adversos en el microbioma intestinal, mediante el aumento de la ratio F/B y de grupos bacterianos perjudiciales y, por el contrario, la disminución de los beneficiosos (Yang *et al.,* 2020). Sin embargo, las dietas ricas en **MUFA** han demostrado aumentar los niveles de bacterias beneficiosas como *Akkermansia* y *Bifidobacterium* y disminuir perjudiciales (*Streptococcus* y *Escherichia* spp.) (Yang *et al.,* 2020). La suplementación enteral con **PUFA** se ha asociado con una disminución en la abundancia de bacterias perjudiciales, así como con una mayor diversidad bacteriana en bebés prematuros con una enterostomía (Younge *et al.,* 2017). No obstante, en otro estudio no tuvieron efecto en la riqueza y diversidad de la microbiota intestinal (Wolters *et al.,* 2019). Sin embargo, parece que los **omega-3** presentan efectos beneficiosos en la regulación de esta microbiota, incluso en enfermedades como la IBD, en la cual su estudio se encuentra actualmente en desarrollo (Fu *et al.,* 2021).

Por otra parte, gracias a la acción de la microbiota se obtienen ácidos grasos beneficiosos como los AGCC o los AGRs. Los **AGRs** son un componente importante de las membranas celulares bacterianas, donde, al igual que los PUFA, aumentan la fluidez de la membrana y, por tanto, ayudan en la adaptación al medio externo (Gozdzik *et al.,* 2023). La variación en estos ácidos grasos de membrana microbiana entre especies de rumiantes refleja diferencias en el tipo y la abundancia de microorganismos en el rumen (He *et al.,* 2023).

1.2.3.3.5 Implicación de los ácidos grasos en la regulación de las células inmunitarias y la respuesta inflamatoria intestinal

Como se ha comentado previamente, los **AGCC** presentan una actividad antiinflamatoria a nivel intestinal (**figura 1.18**). Son capaces de interactuar con la mayoría de las células del sistema inmunitario a través de los GPRs, que se encuentran en la superficie celular (Kim *et al.,* 2014). Además, la inhibición de las HDAC ha demostrado reducir la síntesis de citoquinas proinflamatorias del colon como TNF-α, IFN-γ e IL-6 en la colitis murina experimental (Glauben *et al.,* 2006).

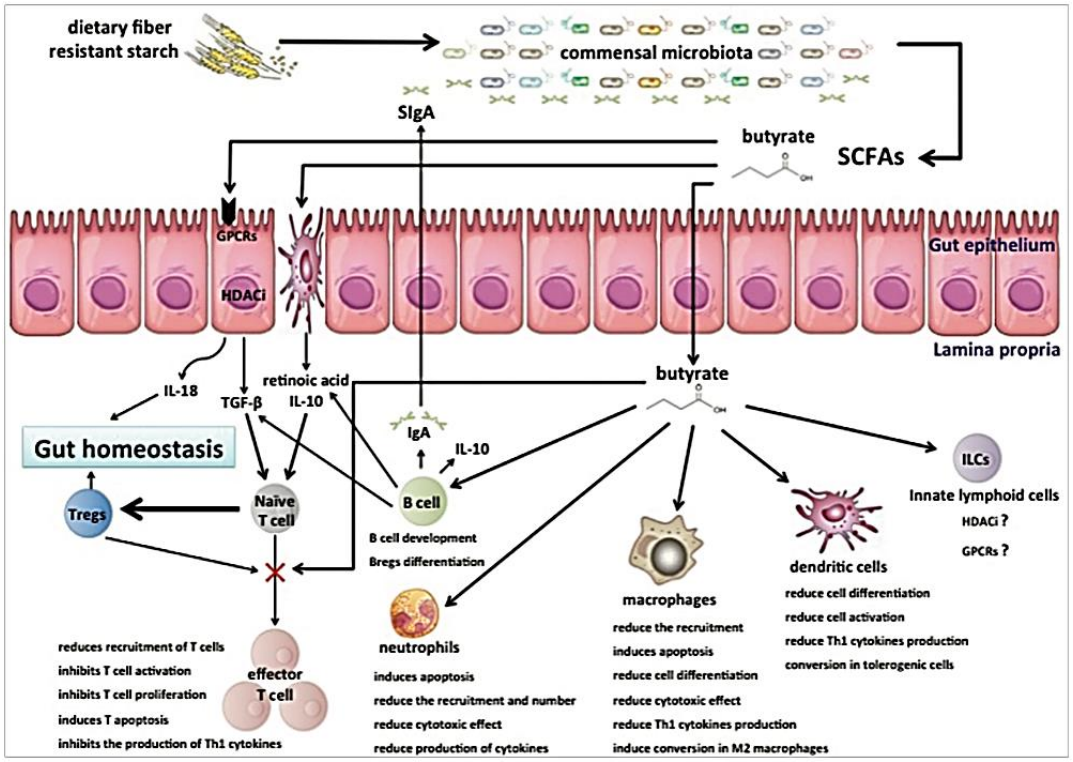

Figura 1.18. Efecto de los AGCC sobre las células inmunitarias intestinales y el mantenimiento de una correcta homeostasis intestinal (Gonçalves *et al.,* 2018).

Los **ácidos grasos de cadena media y larga** también pueden ser reconocidos por receptores como el GPR40, GPR84 y GPR120 (Tan *et al.,* 2017). Existe cierta controversia sobre el efecto proinflamatorio de los ácidos grasos de cadena media, ya que su receptor GPR84 expresado en macrófagos y neutrófilos, una vez inducido, parece aumentar la quimiotaxis y la liberación de citoquinas proinflamatorias como IL-8 y TNF-α (Tan *et al.,*

2017). Sin embargo, en el estudio de Papada *et al.,* en 2013, una dieta rica en estos ácidos demostró reducir por encima de los ácidos grasos de cadena larga algunas de estas citoquinas proinflamatorias en ratones con colitis experimental.

En relación a los **ácidos grasos de cadena larga**, la literatura científica es más extensa sobre los efectos de los ácidos grasos insaturados en la inflamación intestinal. Diversos estudios han demostrado que los **MUFA** modulan factores de transcripción involucrados en los procesos inflamatorios, como el factor NF-κB, (Passos *et al.,* 2016). Recientemente se ha descubierto el posible potencial del **ácido palmitoleico** como regulador del proceso inflamatorio intestinal. En ratones con colitis, demostró ejercer un papel antiinflamatorio suprimiendo la producción de TNF-α e IL-6 (Chen *et al.,* 2023). Los autores sugirieron la posibilidad de la regulación de las vías IL-6/STAT3 y TNF-α/NF-κB por parte de este ácido.

Los efectos de los **omega-6 y omega-3** en la inflamación intestinal no están claros. Numerosos estudios en animales demuestran los efectos beneficiosos y antiinflamatorios de los **omega-3** en la IBD (Whiting *et al.,* 2005; Bassaganya-Riera & Hontecillas, 2006; Cho *et al.,* 2010), aunque otras investigaciones han descrito un empeoramiento de los signos clínicos con dietas ricas en estos ácidos (Matsunaga *et al.,* 2008; Woodworth *et al.,* 2010). Lo mismo sucede con los **omega-6**. Su consumo elevado se ha asociado con riesgo de IBD (Marion-Letellier *et al.,* 2015). No obstante, en modelos de colitis experimental en cerdos, el LA conjugado ha demostrado reducir los signos de inflamación intestinal, acompañado de una reducción de los niveles séricos de TNF-α y NF-κB (Hontecillas *et al.,* 2002). Estos resultados fueron también descritos en estudios experimentales con ratones (Bassaganya-Riera *et al.,* 2004). Sin embargo, el aumento del ALA en la dieta con el consiguiente desplazamiento del LA y la reducción de sus niveles demostró reducir la inflamación intestinal y la gravedad de la colitis murina en el estudio de Tyagi *et al.,* en 2012. En los ensayos en medicina humana existe la misma controversia. Algunos estudios demuestran que el consumo de **omega-3** se correlaciona de manera negativa con el riesgo de la enfermedad de Crohn (Chan *et al.,* 2014) y la colitis ulcerosa (John *et al.,* 2010). De hecho, las dietas enriquecidas con **omega-3** han presentado efectos beneficiosos en parámetros

clínicos e histopatológicos (Belluzzi *et al.,* 1996; Barbosa *et al.,* 2003; Nielsen *et al.,* 2005). En cambio, en otros estudios no han demostrado capacidad para prevenir las recaídas y mantener la remisión de los signos clínicos (Turner *et al.,* 2011). En general, los estudios realizados hasta ahora son ambiguos y actualmente se sigue investigando el efecto de estos ácidos en la inflamación intestinal, así como de sus derivados lipídicos.

Por último, algunos estudios demuestran el efecto antiinflamatorio de los **AGRs** sobre la salud intestinal de los recién nacidos. Los niños nacidos a término presentan concentraciones mayores de estos ácidos en el vérnix caseosa y el meconio en comparación con los niños prematuros (Li *et al.,* 2021). De hecho, la enterocolitis necrotizante es una enfermedad común en los últimos. En el estudio de Ran-Ressler *et al.,* en 2011 se demostró que los **AGRs** administrados en la dieta reducían la incidencia de esta enfermedad en ratones al aumentar la expresión de citoquinas como IL-10. Estudios que han utilizado líneas celulares gastrointestinales han demostrado la subexpresión de mediadores proinflamatorios como IL-8, TLR4 y el factor NF-κB gracias a estos ácidos (Yan *et al.,* 2017).

1.3 AMINOÁCIDOS Y SALUD INTESTINAL Y SISTÉMICA

Los aminoácidos se definen como sustancias orgánicas que contienen tanto un grupo amino (-NH2) como uno ácido unidos a un átomo de carbono (Wu, 2013). Cada aminoácido presenta una cadena lateral única, la cual le otorga diferentes propiedades y funciones. Existen más de 700 aminoácidos en la naturaleza, pero solo 20 de ellos sirven para la formación de estructuras proteicas. Aquellos que sirven para la formación de polipéptidos se conocen como aminoácidos proteicos, mientras que el resto se conocen como aminoácidos no proteicos (Wu, 2013).

1.3.1 Tipos de aminoácidos

Se consideran diez aminoácidos esenciales en el perro debido a que la capacidad de síntesis es insuficiente para cubrir las demandas fisiológicas y, por ello, deben ser incorporados a partir de la dieta (NRC, 2006) (**figura 1.19**). Los aminoácidos condicionalmente esenciales son aquellos que, de forma general, pueden ser sintetizados

en cantidades adecuadas por el organismo pero que deben ser administrados a partir de la dieta en condiciones en las que el balance de utilización sea superior al de la capacidad de síntesis (crecimiento, estrés, trauma, sepsis, etc) (Wu, 2009). Por último, los aminoácidos no esenciales son aquellos que pueden ser sintetizados por el organismo en cantidades suficientes para cubrir sus requerimientos (Wu, 2009).

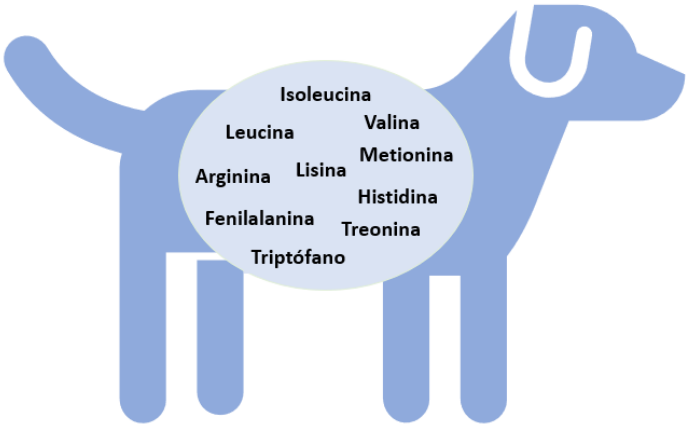

Figura 1.19. Aminoácidos esenciales en el perro. Elaborado a partir de NRC, (2006).

Por otra parte, los aminoácidos pueden clasificarse en función de su estructura general, la estructura de su cadena lateral y en función de sus características químicas generales. Así, la clase alifática incluye glicina, alanina, valina, leucina e isoleucina (Wu, 2013). La clase de aminoácidos que contienen azufre incluye cisteína y metionina. La prolina es el único aminoácido clasificado como un iminoácido (Wu, 2013). La clasificación de aminoácidos básicos incluye histidina, lisina y arginina, y la clasificación de aminoácidos ácidos incluye aspartato y glutamato (Wu, 2013). La clase aromática (aminoácidos aromáticos, AAAs) incluye fenilalanina, tirosina y triptófano. Por último, aquellos que presentan una cadena lateral alifática que se ramifica se conocen como aminoácidos de cadena ramificada (AACR) donde se incluyen la leucina, la isoleucina y la valina (Wu, 2013).

1.3.2 Funciones fisiológicas de los aminoácidos sobre la salud intestinal

Los aminoácidos circulantes en la sangre derivan de la digestión del alimento, la degradación proteica o de la síntesis *de novo*. Estos aminoácidos tendrán destinos metabólicos distintos, como la incorporación en estructuras proteicas, síntesis de

aminoácidos indispensables, conversión en moléculas de señalización (hormonas, neurotransmisores) o uso como fuente de energía (Wu, 2013).

A nivel intestinal, los aminoácidos presentan un papel crucial sirviendo como componentes principales en el recambio proteico, así como moléculas reguladoras de las funciones intestinales. Los requerimientos para el mantenimiento del metabolismo proteico intestinal son incluso más elevados que en otros tejidos como el hígado o el músculo (Bertrand *et al.,* 2012). Un correcto equilibrio en la cantidad total de aminoácidos es indispensable para llevar a cabo funciones sobre la barrera intestinal, el desarrollo inmunitario y antioxidante, y la regulación de la microbiota para la obtención de metabolitos beneficiosos derivados de la fermentación microbiana.

1.3.2.1 Función de los aminoácidos sobre la barrera intestinal

Los aminoácidos son esenciales para mantener la integridad de la barrera intestinal. La **glutamina** es particularmente importante para esta función. Sirve como fuente principal de energía para los enterocitos y apoya la producción de proteínas de unión estrecha (Beaumont & Blachier, 2020; Ji *et al.,* 2023). Se ha demostrado que la suplementación con **glutamina** mejora la integridad de la barrera y reduce la permeabilidad de la misma (Beaumont & Blachier, 2020). Junto con la glutamina, los aminoácidos **glutamato** y **aspartato** son los siguientes más destacados en el aporte de energía para el epitelio intestinal (Beaumont & Blachier, 2020; Ji *et al.,* 2023).

La **arginina** también contribuye a la función de la barrera al promover por una parte la síntesis de óxido nítrico (NO), que ayuda a regular el flujo sanguíneo y la permeabilidad (Liu *et al.,* 2017; Ji *et al.,* 2023), y por otra parte la síntesis de poliaminas, esenciales para la división celular y el crecimiento de la mucosa (Liu *et al.,* 2017; Ji *et al.,* 2023). Además, se ha demostrado que mejora la integridad y alivia el daño en varios modelos de lesión intestinal (Liu *et al.,* 2017).

Junto con estos aminoácidos, la **treonina** también presenta una elevada importancia en esta función al estar estrechamente implicada en la síntesis de mucinas,

fortaleciendo así la barrera física contra los patógenos (Wang *et al.,* 2008; Beaumont & Blachier, 2020).

En el caso de los **AACR**, la **valina**, **leucina** e **isoleucina** se han considerado como importantes moduladores de la salud y desarrollo intestinal en modelos porcinos, debido a su capacidad de promover la síntesis proteica (anabolismo) (Murgas *et al.,* 2010; Nie *et al.,* 2018; Zhou *et al.,* 2018).

1.3.2.2 Función de los aminoácidos sobre la defensa inmunitaria y antioxidante

Los aminoácidos desempeñan roles vitales en el apoyo de la función inmunológica intestinal. En primer lugar, conforman las proteínas, componentes estructurales de las células inmunitarias, así como de los anticuerpos y citoquinas producidas (Kelly & Pearce, 2020). Además, contribuyen a la replicación rápida de las mismas gracias a su aporte de energía en forma de ATP, así como a la síntesis de nucleótidos para la generación de ARN y ADN (Kelly & Pearce, 2020).

La **glutamina** es esencial para la proliferación y función de células inmunitarias, incluyendo linfocitos y macrófagos (Beaumont & Blachier, 2020; Ji *et al.,* 2023). Ayuda a mantener el equilibrio entre las respuestas proinflamatorias y antiinflamatorias en el intestino. Los **AACR** como la **leucina** aportan un grupo α-amino para la síntesis endógena de glutamina en el músculo esquelético, participando de manera indirecta en la respuesta inmunitaria (Newsholme & Calder, 1997). Además, se ha descrito que las células inmunitarias pueden oxidar los **AACR** como fuente de energía e incorporarlos como precursores para la síntesis de nuevas células inmunitarias, moléculas efectoras y moléculas protectoras (Negro *et al.,* 2008), colaborando en el aporte de energía para las células que necesitan de una rápida diferenciación.

La **arginina** participa en la función de los linfocitos T y en la producción de NO, que posee propiedades antimicrobianas (Liu *et al.,* 2017; Ji *et al.,* 2023). Se ha demostrado que mejora las respuestas inmunitarias y reduce la inflamación en el intestino. Junto con la

arginina, la **fenilalanina** también ha demostrado regular la producción de NO por parte de los leucocitos (Wu & Meininger, 2002).

El **triptófano** es metabolizado en el intestino en compuestos que pueden reducir la inflamación al interactuar con receptores aril-hidrocarburos (AhR), los cuales están involucrados en la regulación de la inmunidad en la mucosa intestinal (Beaumont & Blachier, 2020).

Además, los aminoácidos regulan el proceso inflamatorio actuando sobre diferentes vías de señalización en las células epiteliales intestinales. Así, como muestra la **figura 1.20**, los aminoácidos activan vías de señalización antiinflamatorias (por ejemplo, los **AACR** actúan sobre la vía GCN2) o inhiben vías de señalización pro-inflamatorias como NF-κB (He *et al.,* 2018).

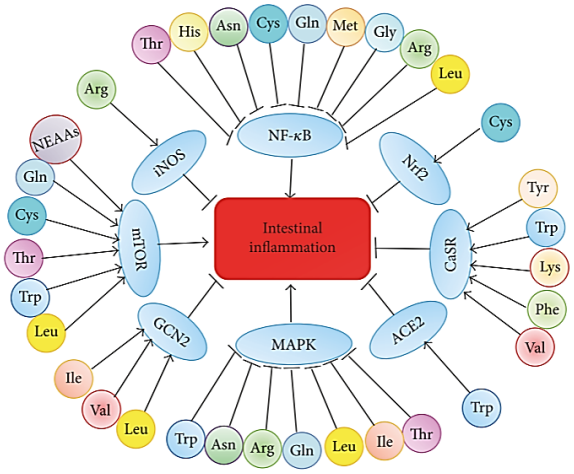

Figura 1.20. Regulación de la inflamación intestinal mediante diferentes vías de señalización por parte de los aminoácidos (He *et al.,* 2018).

Aparte de la respuesta inmunitaria, el control del estrés oxidativo es una función determinante, sobre todo en enfermedades como la IBD, en la cual se trata de un factor agravante de la enfermedad (Bourgonje *et al.,* 2020). De esta forma, los aminoácidos que más actividad antioxidante presentan son la **glutamina**, **cisteína** y **glicina**, ya que forman uno de los mayores antioxidantes celulares del organismo: el glutatión (Roth *et al.,* 2002; Wu *et al.,* 2004). Niveles adecuados son esenciales para proteger a las células intestinales

de la liberación de radicales libres de oxígeno generados como subproductos normales de los procesos metabólicos o como resultado de ciertas respuestas ante situaciones patológicas de estrés e inflamación (Wu *et al.,* 2004).

1.3.2.3 Función de los aminoácidos sobre la regulación de la microbiota intestinal

Las proteínas que escapan a la digestión en el intestino delgado y llegan al colon, sirven como sustratos fermentables para la microbiota intestinal y experimentan una intensa proteólisis para convertirse en aminoácidos. De forma seguida, estos aminoácidos pueden ser metabolizados por la microbiota, dando lugar a metabolitos microbianos que participan en la homeostasis intestinal (Diether & Willing, 2019).

Este es el caso de los **AAAs,** como el triptófano, la fenilalanina y la tirosina. El **triptófano** se metaboliza a nivel intestinal por tres rutas diferentes (**figura 1.21**): (A) la vía de la quinurenina, que ocurre principalmente en células inmunitarias y epiteliales a través de la enzima IDO1, dando lugar a varios metabolitos de quinurenina, incluidos ligandos para los AhR; (B) la vía de producción de serotonina, que tiene lugar en las células enteroendocrinas (un subtipo de células epiteliales intestinales) a través de TpH1; y finalmente, (C) la conversión directa del **triptófano** por la microbiota intestinal en varios indoles y derivados, incluidos ligandos de los AhR (Ghiboub *et al.,* 2020).

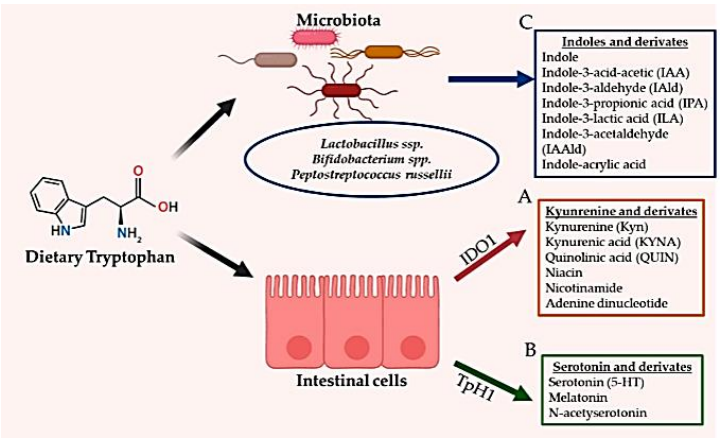

Figura 1.21. Metabolismo intestinal del triptófano (Ghiboub *et al.,* 2020).

Una gran proporción de estudios ha descrito los efectos de los metabolitos del **triptófano** como moduladores de la función gastrointestinal. Varios derivados del indol

funcionan como reguladores de la integridad epitelial y la respuesta inmunitaria de la mucosa a través del eje AhR/interleuquina 22 (Liu *et al.,* 2020). Además, se ha descrito que algunos de estos metabolitos pueden inducir apoptosis en las células Th1, inhibiendo así la inflamación mediada por estas células, y estimular a los linfocitos T vírgenes para desarrollarse en linfocitos Tregs, aumentando la producción de citoquinas inmunosupresoras (Zhang *et al.,* 2014). Es por lo que el **triptófano** es uno de los aminoácidos más estudiados en enfermedades como la IBD.

En el caso de la **tirosina** (derivada de la degradación de la **fenilalanina**), sirve como precursora para la síntesis de catecolaminas (epinefrina y norepinefrina), hormonas tiroideas, así como dopamina y melanina (Li *et al.,* 2007). Tanto la **tirosina** como su derivado obtenido por acción microbiana, el *p*-cresol, actúan como reguladores de hormonas a nivel intestinal y del sistema nervioso central, lo que se conoce con el término de "eje intestino-cerebro" (Chen *et al.,* 2021). De modo que algunos de estos neurotransmisores regulan la respuesta inmunitaria como es el caso de la epinefrina y norepinefrina, actuando sobre los linfocitos Th1 y linfocitos B (Kin, 2006); o la dopamina y melanina sobre linfocitos, monocitos, macrófagos, neutrófilos y plaquetas (Basu & Dasgupta, 2000; Mohagheghpour *et al.,* 2000). Por otra parte, el *p*-cresol es genotóxico para los colonocitos y se ha asociado a una menor diversidad de la microbiota del intestino (Passmore *et al.,* 2018).

Por último, como se ha mencionado con anterioridad, las proteínas y aminoácidos sirven como sustratos de la fermentación microbiana para la obtención de otras moléculas beneficiosas. Este es el caso de los AGCC, obtenidos a partir de aminoácidos como la **glicina**, **lisina**, **glutamato** y **treonina** (Neis *et al.,* 2015); o bien el caso de los AGRs, obtenidos a partir de los **AACR** (Gozdzik *et al.,* 2023; He *et al.,* 2023). Sin embargo, no solo se obtienen metabolitos a partir de la acción de estos microorganismos, sino que ellos mismos se sirven de los aminoácidos para su propio desarrollo. Así, se ha descrito que tanto la cantidad como el tipo de proteína influyen en la composición de la microbiota intestinal (Yang *et al.,* 2020). Todo ello demuestra la estrecha relación entre el metabolismo de ácidos grasos, aminoácidos y la funcionalidad de la microbiota intestinal.

1.4 ALTERACIONES EN EL PERFIL DE ÁCIDOS GRASOS O AMINOÁCIDOS EN LA IBD HUMANA Y EN LAS ENTEROPATÍAS CRÓNICAS CANINAS

En medicina humana se ha descrito una alteración en el metabolismo lipídico y aminoacídico en pacientes con IBD, tanto en la enfermedad de Crohn como en la colitis ulcerosa, en comparación con individuos sanos. Estas diferencias también se han documentado entre las dos variantes de la enfermedad. Las **tablas 1.3 y 1.4** presentan un resumen de los estudios realizados hasta la fecha sobre los perfiles de ácidos grasos y aminoácidos, respectivamente.

Aunque algunos resultados en la literatura son contradictorios, en términos generales se observan diferencias en los **ácidos grasos** dependiendo del tipo de muestra analizada. En estudios con muestras de **plasma o suero,** los hallazgos más consistentes indican niveles reducidos de SAT (Esteve-Comas *et al.,* 1992; Wiese *et al.,* 2016) mientras que los resultados relacionados con los MUFA y los PUFA son menos concluyentes. No obstante, suele observarse una disminución en los niveles de PUFA omega-6 con el aumento de la gravedad de la enfermedad, lo que podría explicarse por procesos de malabsorción o un mayor consumo energético relacionado con la inflamación crónica (Esteve-Comas *et al.,* 1992; Scoville *et al.,* 2018; Jiang *et al.,* 2023).

En las muestras **fecales**, se ha documentado una reducción en los niveles de AGCC (Marchesi *et al.,* 2007; Bjerrum *et al.,* 2015; Franzosa *et al.,* 2018; Lloyd-Price *et al.,* 2019; Wang *et al.,* 2021a; Kaczmarczyk *et al.,* 2022; Vich *et al.,* 2023) especialmente en pacientes con enfermedad de Crohn, lo que sugiere un cuadro de disbiosis intestinal e inflamación más grave. Además, se han encontrado niveles elevados de ácidos grasos de cadena larga, probablemente debido a procesos de malabsorción (Jansson *et al.,* 2009; Alghamdi *et al.,* 2018; Franzosa *et al.,* 2018) o a la necesidad de la síntesis de compuestos precursores involucrados en los procesos inflamatorios (Scoville *et al.,* 2018).

En relación a los **aminoácidos**, los resultados más destacados en muestras de **plasma o suero** describen niveles reducidos de aminoácidos debido a las alteraciones en el proceso de absorción o al incremento del catabolismo proteico asociado a la inflamación (Williams *et al.,* 2012). Entre estos, destacan niveles bajos de histidina, considerada un

posible predictor de recaídas (Hisamatsu *et al.,* 2012; Hisamatsu *et al.,* 2015), así como de glutamina, triptófano y alteraciones en sus metabolitos, como el aumento de quinurenina (Nikolaus *et al.,* 2017; Murgia *et al.,* 2018; Scoville *et al.,* 2018; Lai *et al.,* 2019; Cioffi *et al.,* 2023; Tews *et al.,* 2023). También se han descrito alteraciones en los AACR, con aumentos en isoleucina (Schicho *et al.,* 2012), y disminuciones o aumentos variables en valina y leucina, según el estudio consultado (Schicho *et al.,* 2012; Williams *et al.,* 2012; Dawiskiba *et al.,* 2014; Murgia *et al.,* 2018; Scoville *et al.,* 2018; Cioffi *et al.,* 2023; Tews *et al.,* 2023)

En las muestras **fecales,** se han descrito incrementos en los niveles de aminoácidos, posiblemente como resultado de malabsorción por daño en la mucosa intestinal o del metabolismo de las bacterias intestinales, que utilizan estos compuestos como sustratos para la generación de metabolitos (Marchesi *et al.,* 2007; Jansson *et al.,* 2009; Bjerrum *et al.,* 2015; Bosch *et al.,* 2018a; Bosch *et al.,* 2020; Diederen *et al.,* 2020; Jagt *et al.,* 2022).

En **medicina veterinaria**, el estudio del metaboloma en las ECs es relativamente reciente. De hecho, el primer estudio que evaluó el perfil de **ácidos grasos** en **heces** fue realizado por Xu *et al.,* en 2016, mientras que el perfil de **aminoácidos** fue estudiado por Minamoto *et al.,* en 2015. Este campo aún cuenta con una literatura científica limitada.

Las **Tablas 1.5 y 1.6** resumen los resultados obtenidos hasta la fecha sobre las alteraciones en los perfiles de ácidos grasos y aminoácidos en las ECs. Al igual que en medicina humana, la mayoría de los estudios describen alteraciones significativas en el metabolismo de estos compuestos. Sin embargo, la mayoría de estas investigaciones han evaluado estas diferencias entre animales con EC en general, o en individuos con IRE (IBD), siendo necesaria la investigación diferenciando entre cada una de las ECs, con el objetivo de poder caracterizarlas en mejor medida y determinar la posible existencia de biomarcadores que permitan su diferenciación de forma más temprana, o bien que ayuden en el conocimiento de la patogenia, así como en el avance de su tratamiento dietético.

Tabla 1.3. Estudios que evalúan el perfil de ácidos grasos en pacientes con IBD (enfermedad de Crohn y Colitis ulcerosa).

Grupo de estudio	Especie	Muestra	Hallazgos representativos	Referencia
Enfermedad de Crohn (ECR) (n=32) Colitis ulcerosa (CU) (n=41) Control (n=107)	Humana	Plasma	-ECR y CU: PUFA: ↑ ALA, DHA (n-3); ↓ C20:3n-6 (n-6) SAT: ↓ C18:0 ↓ n-6 con mayor severidad de enfermedad	(Esteve-Comas *et al.*, 1992)
Enfermedad de Crohn (n=64) Colitis ulcerosa (n=40) Control (n=101)	Humana	Suero	-ECR: PUFA: ↓ LA, C18:3n-6, AA, C22:5n-6, ALA, EPA, DHA, C22:5n-3 -CU: PUFA: ↓ AA, EPA, DHA, C22:5n-6, C22:5n-3 ↓ PUFA con mayor severidad de enfermedad	(Jiang *et al.*, 2023)
Enfermedad de Crohn (n=20) Colitis ulcerosa (n=20) Control (n=20)	Humana	Suero	- IBD: ↓PUFA	(Scoville *et al.*, 2018)
Colitis ulcerosa (n=101) Control (n=23)	Humana	Suero	-CU: ↓ SAT ↑ MUFA, C18:1n-9 ↑ PUFA n-3, LA, EPA	(Wiese *et al.*, 2016)
Enfermedad de Crohn (n=20) Control (n=10)	Humana	Suero	-ECR: ↓ ALA, DHA, AA	(Lai *et al.*, 2019)
Enfermedad de Crohn (n=9) Colitis ulcerosa (n=10) Control (n=10)	Humana	Suero	-CU: ↓ DHA, AA	(Daniluk *et al.*, 2019)
Enfermedad de Crohn (n=24) Colitis ulcerosa (n=20) Control (n=23)	Humana	Suero	-ECR y CU: ↓ PUFA inespecíficos	(Williams *et al.*, 2012)
Enfermedad de Crohn (n=52) Control	Humana	Fosfolípidos del plasma	-ECR: ↓PUFA n-3, DHA, AA; ↑ C22:5n-3	(Geerling *et al.*, 1999)
Enfermedad de Crohn (n=26) Control (n= 16)	Humana	Fosfolípidos del plasma	-ECR: ↓ SAT (C16:0, C18:0) MUFA: ↑ C18:1n-7; ↓ C18:1n-9 PUFA: ↑ n-3 (DHA, EPA, C22:5n-3), AA; ↓ ratio n6/n3, LA	(Schwarz *et al.*, 2021)
Enfermedad de Crohn (n=21) Colitis ulcerosa (n=30) Control (n=24)	Humana	Fosfolípidos del plasma	-ECR y CU: ↓ SAT (C16:0), ↑ PUFA (C18:3n-6, C22:4n-6), C20:3n-3, DHA, n-3 -CU: PUFA: ↑ n-6 y AA, C22:6n-3, ↓ n-6/n-3	(Figler *et al.*, 2007)
Enfermedad de Crohn (n=21) Colitis ulcerosa (n=30) Control (n=13)	Humana	Colon	-ECR y CU: ↑ SAT (C16:0, C18:0) ↓ MUFA (C18:1n-9) ↑ PUFA n-6 y n-3 pero ↓ de precursores (LA, ALA)	(Fernández-Bañares *et al.*, 1997)
Colitis inducida (n=30)	Rata	Colon	↓ MUFA PUFA: ↑ n-6 y n-3 pero ↓ de precursores (LA, ALA)	(Fernández-Bañares *et al.*, 1997)
Enfermedad de Crohn (n=15) Control (n=16)	Humana	- Íleon - Colon	- Íleon: ↓ precursores PUFA (LA, ALA) y ↑ PUFA (AA, C22:4n-6, C22:6n-3), ↑ elongación y desaturación - Colon: igual que íleon más ↑ de SAT(C18:0), ratio SAT/MUFA, ↓ de MUFA (C16:1n-7, C18:1n-9)	(Buhner *et al.*, 1994)
Colitis ulcerosa (n=9) Control (n=8)	Humana	Fosfolípidos mucosa colon	-CU: PUFA: ↑ AA y DHA MUFA: ↓ oleico y palmitoleico	(Nishida *et al.* , 1987)
Colitis ulcerosa (n=63) Control (n=30)	Humana	- Plasma - Mucosa (CU n=26; HC n=13)	- Plasma: ↑ SAT (C18:0), ↓ MUFA (C18:1n-9) y ALA - Mucosa: ↑ SAT (C18:0), PUFA n-6 (AA); ↓ MUFA (C18:1n-9), precursores PUFA (LA, ALA)	(Esteve *et al.*, 1998)
Colitis ulcerosa inducida(n=20) Control (n=20)	Rata	- Plasma - Mucosa	- Plasma: ↑ SAT, MUFA; ↓ de AA y C20:2n-6 - Mucosa: ↑ n-3 PUFA; ↓ ratio C18:2n-6/C20:4n-6	(Nieto *et al.*, 1998)
Colitis ulcerosa activa (n=18) Colitis ulcerosa remisión (n=10) Control (n=14)	Humana	Mucosa colon	-CU activa:↓ LA, ↑PUFA (C20:3, C20:4n-6 AA) Diferencias metabolismo colitis activa y en remisión	(Diab *et al.*, 2019)

Tabla 1.3. (Continuación)

Grupo de estudio	Especie	Muestra	Hallazgos representativos	Referencia
Enfermedad de Crohn (n= 50) Colitis ulcerosa (n= 78) Control (n= 60)	Humana	Plasma	-ECR y CU: ↑ C16:0 ↑ C16:1n-7 (MUFA) ↑ LA, ALA (PUFA)	(Murgia *et al.*, 2018)
Enfermedad de Crohn (n=21) Control (n=14)	Humana	Heces	-ECR íleon vs EC colon/controles: ↑ C16:0, C18:0, C18:1n-9, LA, ALA, AA	(Jansson *et al.*, 2009)
Enfermedad de Crohn (n=173) Colitis ulcerosa (n=107) Control (n=42)	Humana	Heces	-ECR y CU: SAT: ↓ ac. araquídico (C20:0), tridecanoico ↓ C18:1n-9 (MUFA)	(Weng *et al.*, 2019)
Enfermedad de Crohn (n=43) Control (n=11)	Humana	Heces	-ECR: ↑ C20:3, C22:3, AA	(Alghamdi *et al.*, 2018)
Enfermedad de Crohn (n=83) Colitis ulcerosa (n=68) Control (n=40)	Humana	Heces	-ECR y CU: ↓ ácidos grasos de cadena media	(De Preter *et al.*, 2014)
Enfermedad de Crohn Colitis ulcerosa Control	Humana	Heces	-ECR y CU: PUFA: ↑ C22:5, C20:3 AGCC: ↓ ácido butírico y propiónico	(Franzosa *et al.*, 2018)
Enfermedad de Crohn (n=238) Colitis ulcerosa (n=174) Control (n=255)	Humana	Heces	-ECR y CU: ↑ AA; ↓ ratio n-6/n-3 -CU: ↓ AGCC (acético, propiónico, butírico)	(Vich *et al.*, 2023)
Enfermedad de Crohn (n=10) Colitis ulcerosa (n=10) Control (n=13)	Humana	Heces	-ECR: ↓ ácido acético y butírico (AGCC) -EC vs CU: ↓ acético	(Marchesi *et al.*, 2007)
Enfermedad de Crohn (n=44) Colitis ulcerosa (n=48) Control (n=21)	Humana	Heces	-ECR y CU:↓ ácido propiónico y butírico (AGCC)	(Bjerrum *et al.*, 2015)
Enfermedad inflamatoria intestinal (IBD) (n= 132)	Humana	Heces	-IBD: ↑ AA; ↓ ácido butírico (AGCC)	(Lloyd-Price *et al.*, 2019)
Enfermedad de Crohn (n=18) Colitis ulcerosa (n=43) Control (n=16)	Humana	Heces	-ECR y CU: ↓ ácido acético, butírico, valérico	(Kaczmarczyk *et al.*, 2022)
Enfermedad de Crohn (n=29) Control (n=20)	Humana	Heces	-ECR: ↓ AGCC: acético, propiónico, butírico	(Wang *et al.*, 2021)
Enfermedad de Crohn (n=43) Control (n=31)	Humana	Heces	-ECR: ↑ propiónico	(Diederen *et al.*, 2020)
Enfermedad de Crohn (n=132) Colitis ulcerosa (n=119) Control (n=205)	Humana	Plasma	-ECR y CU:Sin diferencias significativas en niveles de AGCC plasmáticos	(Kiasat *et al.*, 2023)
Enfermedad de Crohn (n=30) Colitis ulcerosa (n= 30) Control (n=60)	Humana	Orina	-ECR y CU:↓ ácido acético (AGCC)	(Stephens *et al.*, 2013)

Tabla 1.4. Estudios que evalúan el perfil de aminoácidos en pacientes con IBD (enfermedad de Crohn y Colitis ulcerosa).

Grupo de estudio	Especie	Muestra	Hallazgos representativos	Referencia
Enfermedad de Crohn (ECR) (n=55) Colitis ulcerosa (CU) (n=34) Control (n=40)	Humana	Suero	-ECR y CU: ↓ alanina, glutamina, histidina, valina, leucina, fenilalanina, tirosina	(Tews *et al.*, 2023)
Enfermedad de Crohn (n=20) Colitis ulcerosa (n=20) Control (n=20)	Humana	Suero	-ECR:↓ lisina, valina, leucina, arginina, glutamina, serina -CU: no diferencia con control EC vs CU: ↓ valina, leucina, serina, glutamina	(Scoville *et al.*, 2018)
Enfermedad de Crohn (n= 50) Colitis ulcerosa (n= 78) Control (n= 60)	Humana	Plasma	-ECR: ↓ alanina, treonina, prolina, valina, metionina, tirosina ↑ histidina -CU: ↓ arginina, alanina, treonina, prolina, metionina, tirosina, triptófano ↑cisteína	(Murgia *et al.*, 2018)
Enfermedad de Crohn (n= 102) Colitis ulcerosa (n= 102) Control (n= 102)	Humana	Plasma	-ECR y CU: ↓ histidina, triptófano	(Hisamatsu *et al.*, 2012)
Colitis ulcerosa (n=369)	Humana	Plasma	-CU: ↓ histidina predictor riesgo de recaída	(Hisamatsu *et al.*, 2015)

Tabla 1.4. (Continuación)

Grupo de estudio	Especie	Muestra	Hallazgos representativos	Referencia
Enfermedad de Crohn (n=103)	Humana	Suero	-ECR activa vs inactiva:↓ triptófano, valina, leucina, lisina, glutamina ↑ ác. aspártico, glutamato, glicina	(Cioffi *et al.,* 2023)
Enfermedad de Crohn (n=20) Colitis ulcerosa (n=20) Control (n=40)	Humana	Plasma	-ECR y CU: ↓ valina, tirosina, serina ↑ isoleucina, metionina, lisina, glicina, arginina, prolina	(Schicho *et al.,* 2012)
Enfermedad de Crohn (n=29) Ileon-colon (n=17) Ileon (n=7) Colon (n= 5)	Humana	Plasma	No diferencias significativas entre las clasificaciones Los aminoácidos valina, metionina, leucina, histidina y triptófano se correlacionaron con el índice de gravedad	(Chiba *et al.,* 2018)
Enfermedad de Crohn (n=19) Colitis ulcerosa (n=24) Control (n=17)	Humana	Suero	-ECR y CU: ↑ leucina, isoleucina, glicina, fenilalanina ↓ histidina	(Dawiskiba *et al.,* 2014)
Enfermedad de Crohn (n=20) Control (n=10)	Humana	Suero	-ECR: ↓ fenilalanina y metabolitos derivados del triptófano	(Lai *et al.,* 2019)
Enfermedad de Crohn (n= 234) Colitis ulcerosa (n= 211) Control (n= 291)	Humana	Suero	-ECR y CU: ↓ triptófano	(Nikolaus *et al.,* 2017)
Colitis ulcerosa (n22) Control (n=14)	Humana	Suero	-CU: ↑ arginina Sus niveles se correlacionaron con el nivel de daño histológico	(Hong *et al.,* 2010)
Enfermedad de Crohn (n=24) Colitis ulcerosa (n=20) Control (n=23)	Humana	Suero	-ECR vs control/CU: ↓ alanina, isoleucina -CU vs control: ↓ alanina, isoleucina	(Williams *et al.,* 2012)
Enfermedad de Crohn (n=26) Colitis ulcerosa (n=31) Control (n=26)	Humana	Mucosa	-ECR y CU: ↓ glutamina, ácido glutámico, AACR (valina, leucina, isoleucina), alanina En la fase inactiva las concentraciones fueron parecidas a los controles	(Balasubramanian *et al.,* 2008)
Colitis ulcerosa activa (n=18) Colitis ulcerosa remisión (n=10) Control (n=14)	Humana	Mucosa colon	-CU activa vs control:↓ triptófano ↑ácido glutámico, asparagina Diferencias metabolismo colitis activa y en remisión	(Diab *et al.,* 2019)
Enfermedad de Crohn (n= 15) Colitis ulcerosa (n= 15) Control (n= 15)	Humana	Heces	-ECR y CU: ↑ Histidina, triptófano, fenilalanina, valina, leucina, tirosina -ECR: ↑ histidina, fenilalanina, triptófano -CU: ↑ serina	(Bosch *et al.,* 2018)
Enfermedad de Crohn (n= 5) Colitis ulcerosa (n= 6) Control (n= 8)	Humana	- Suero - Heces	-ECR y CU: ↑ alanina, glutamina, fenilalanina, valina, leucina, lisina, serina, tirosina Sin diferencias en niveles de aa séricos	(Bosch *et al.,* 2020)
Enfermedad de Crohn (n=21) Control (n=14)	Humana	Heces	-ECR: ↑ AAA (triptófano, tirosina, fenilalanina)	(Jansson *et al.,* 2009)
Enfermedad de Crohn (n=10) Colitis ulcerosa (n=10) Control (n=13)	Humana	Heces	-ECR y CU: ↑ lisina -ECR: ↑ alanina, AACR (valina, leucina, isoleucina), lisina -CU: ↑ glutamato, lisina -ECR vs CU: ↑ alanina, AACR, lisina	(Marchesi *et al.,* 2007)
Enfermedad de Crohn (n=44) Colitis ulcerosa (n=48) Control (n=21)	Humana	Heces	- ECR activa vs control: ↑ AACR (valina, leucina, isoleucina), lisina, alanina, fenilalanina, tirosina - ECR inactiva vs Control: sin diferencias - CU activa vs control/CU inactiva:↑ AACR (valina, leucina, isoleucina), lisina, alanina, taurina, tirosina	(Bjerrum *et al.,* 2015)
Enfermedad de Crohn (n=40) Colitis ulcerosa (n=38) Control (n=105)	Humana	Heces	-ECR y CU:↑ triptófano, taurina, alanina, ornitina, valina, leucina, histidina	(Jagt *et al.,* 2022)
Enfermedad de Crohn (n=43) Control (n=31)	Humana	Heces	-ECR: ↑ alanina, triptófano, tirosina, valina, leucina, isoleucina, fenilalanina	(Diederen *et al.,* 2020)
Enfermedad de Crohn (n=29) Control (n=20)	Humana	Heces	-ECR: ↑ leucina	(Wang *et al.,* 2021)
Enfermedad de Crohn (n=43) Control (n=11)	Humana	Heces	-ECR: ↓ tirosina	(Alghamdi *et al.,* 2018)
Enfermedad de Crohn (n=50) Colitis ulcerosa (n= 82) Control (n=51)	Humana	heces	-ECR: ↑ alanina, fenilalanina	(Santoru *et al.,* 2017)
Enfermedad de Crohn (n=10) Colitis ulcerosa (n=11) Control (n=15)	Humana	Heces	-ECR y CU: ↑ alanina, tirosina, leucina	(Lins *et al.,* 2020)
Enfermedad de Crohn (n=30) Colitis ulcerosa (n= 30) Control (n=60)	Humana	Orina	-ECR y CU: ↓ histidina, lisina, asparagina	(Stephens *et al.,* 2013)

Tabla 1.5. Estudios que evalúan el perfil de ácidos grasos en animales de compañía con ECs.

Grupo de estudio	Especie	Muestra	Hallazgos representativos	Referencia
Enteropatía crónica (EC) (n=13) Control (n=20)	Canina (Yorkshire terrier)	Plasma	-EC: ↓ ácido oleico (MUFA), PUFA (C20:2n-6, C20:3n-6)	(Galler *et al.*, 2022a)
Enteropatía crónica (n=55) Control (n=204)	Canina	Suero	-EC: ↓ ácidos grasos totales, SAT, C16:0, ácido oleico, PUFA, (LA, n-6)	(Walker *et al.*, 2022)
Enteropatía crónica (n=48): Enteropatía que responde a dieta (FRE) (n= 28) Enteropatía que responde a antibióticos (ARE) (n= 5) Enteropatía que responde a inmunosupresores (IRE) (n= 15) Control (n=68)	Canina	Fosfolípidos de membrana eritrocitos	-EC vs control: SAT:↓ SAT (C16:0), ↑ C18:0 PUFA: ↓ LA, ↑ C20:3n-6, EPA, DHA, n-3 ↓ SAT/MUFA ↓ n-6/n-3 ↑ elongasa (C18/C16) ↑ Δ6- desaturasa, ↓ Δ5-desaturasa, ↓ Δ9-desaturasa Sin diferencias significativas entre grupos de EC	(Crisi *et al.*, 2021)
Enteropatía que responde a dieta (FRE) (n= 16) Enteropatía que responde a inmunosupresores (IRE) (n= 16)	Canina	Fosfolípidos de sangre y plasma	Diferencias de fosfolípidos entre ambos grupos	(Kalenyak *et al.*, 2019)
Enfermedad inflamatoria intestinal (IBD) (n=12) Control (n=10)	Canina	Suero	-IBD: no diferencias en metabolismo de lípidos con los controles	(Minamoto *et al.*, 2015)
Enteropatía crónica activa (n=14) Enteropatía crónica en remisión (n=11) Control (n=26)	Canina (Yorkshire terrier)	Heces	-EC activa: ↑ ácidos largos (↑ MUFA n-9, LA, ALA). Disminución de los mismos tras tratamiento	(Galler *et al.*, 2022b)
Enteropatía crónica (n=15) Control (n=15)	Canina	Heces	-EC: SAT: ↑C16:0, C18:0 ↑ MUFA n-9 PUFA: ↑ C22:3n-3, AA, C22:2n-6	(Honneffer *et al.*, 2017)
Enteropatía perdedora de proteínas (PLE) (n= 38) Control (n= 47)	Canina	Heces	-PLE: ↑ SAT (C16:0, C18:0) ↑ MUFA n-9 (oleico) PUFA: ↑ LA, AA ↑ácidos grasos totales Tras tratamiento: ↓ MUFA n-9 y C18:0	(Cagnasso *et al.*, 2024)
Enteropatía perdedora de proteínas (PLE) (n= 8) Control (n= 22)	Canina	Heces	-PLE: ↑ C18:0, AA, ácido nervónico ↓ ALA	(Tolbert *et al.*, 2025)
Enteropatía crónica (n=73) Control (n=49)	Canina	Heces	-EC: ↓ ácido acético (C2), propiónico (C3), total AGCC en materia seca ↓ C2, C3 y ácido butírico (C4) en materia húmeda	(Minamoto *et al.*, 2019)
Enfermedad inflamatoria intestinal (n= 6) Control (n= 16)	Canina	Heces	-IBD: ↓ acético (C2) y propiónico (C3)	(Kaga *et al.*, 2023)
Enfermedad inflamatoria intestinal (n= 15) Control (n= 10)	Canina	Heces	No diferencias en niveles de AGCC entre grupos	(Xu *et al.*, 2016)
Enfermedad inflamatoria intestinal (n= 11) Linfoma de célula pequeña fenotipo T (n= 11) Control (n= 14)	Felina	Heces	-IBD y linfoma:↑ ácidos grasos de cadena larga: PUFA, AA, DHA, MUFA n-9 No diferencias entre sanos e IBD	(Marsilio *et al.*, 2021)
Enteropatía crónica (n=34): Enteropatía que responde a dieta (n= 13) Enfermedad inflamatoria intestinal (n= 15) Linfoma de célula pequeña fenotipo T (n=6) Control (n=27)	Felina	Heces	-EC: SAT: ↑ C14:0, C18:0 ↑ erúcico y nervónico (MUFA n-9) ↑ AA (PUFA) Diferencias significativas entre controles y IBD+linfoma para los ácidos C14:0, C18:0, nervónico, AA El metaboloma de los FRE se pareció más a los controles que a IBD o linfoma	(Giordano *et al.*, 2024)
Enteropatía crónica (n=41): Enteropatía que responde a dieta (n= 17) Enfermedad inflamatoria intestinal (n= 15) Linfoma de célula pequeña fenotipo T (n=9) Control (n=43)	Felina	Fosfolípidos de membrana eritrocitos	-EC vs control: PUFA: ↑ C22:5n-3, DHA, n-3 PUFA, balance PUFA (n-3/(n-3 + n-6)); ↓ LA, ratio n-6/n-3 ↑ Δ6- desaturasa No se observaron diferencias entre grupos de EC	(Crisi *et al.*, 2024)
Enteropatía crónica (n=15): Enfermedad inflamatoria intestinal (n= 6) Linfoma de célula pequeña fenotipo T (n=6) Enteropatía no identificada (n=3) Control (n=13)	Felina	Heces	-EC: ↓ ácido propiónico (C3) e isobutirico (IC4); ↑ ácido butírico (C4) y de AGCC totales	(Miller *et al.*, 2023)
Enteropatía crónica (n=56): Enfermedad inflamatoria intestinal (n= 22) Linfoma de célula pequeña fenotipo T (n=34) Control (n=77)	Felina	Heces	-EC: SAT: ↑ C14:0, C18:0 MUFA: ↑ C18:1n-9, erúcico, gondoico, nervónico PUFA: ↑ ALA, AA	(Sung *et al.*, 2023)

Tabla 1.6. Estudios que evalúan el perfil de aminoácidos en animales de compañía con ECs.

Grupo de estudio	Especie	Muestra	Hallazgos representativos	Referencia
Enfermedad inflamatoria intestinal (IBD) (n=51) Control (n=26)	Canina	Suero	-IBD: ↓ tirosina, fenilalanina, triptófano (AAA) ↑ serina, ac. glutámico, arginina, treonina, prolina, cistina, lisina, valina, isoleucina	(Benvenuti *et al.,* 2020)
Enfermedad inflamatoria intestinal (n=10) Control (n=12)	Canina	Plasma	-IBD: ↓ triptófano, serina, metionina, prolina. Correlación negativa entre Serina- CCECAI	(Tamura *et al.,* 2019)
Enfermedad inflamatoria intestinal (n=12) Control (n=10)	Canina	Suero	-IBD: Alteración metabolismo aminoacídico (↓ histidina, glutamina, tirosina, triptófano, cisteína, prolina, hidroxiprolina)	(Minamoto *et al.,* 2015)
Enteropatía crónica (EC) (n=55) Control (n=204)	Canina	Suero	-EC: ↑ fenilalanina ↓ glicina	(Walker *et al.,* 2022)
Enfermedad inflamatoria intestinal (n= 15) Control (n= 10)	Canina	Plasma	-IBD: ↑ valina Valina y alanina se correlacionaron con CCECAI	(Xu *et al.,* 2016)
Enteropatía perdedora de proteínas (PLE) (n=30) Control (n=12)	Canina	Suero	-EPP: ↓ triptófano Correlación triptófano-niveles albúmina	(Kathrani *et al.,* 2018)
Enteropatía crónica (n=10) Control (n=11)	Canina	-Suero -Heces	- Suero: ↑ valina - Heces: ↑ triptófano	(Blake, 2022)
Enfermedad inflamatoria intestinal (n=9) Control (n=13)	Canina	Heces	-IBD: ↑ prolina, valina, leucina, isoleucina, alanina, triptófano, asparagina, ac.aspártico, cisteína, cistina, ac. glutámico, glicina, metionina, fenilalanina, serina, treonina	(Pilla *et al.,* 2021)
Enteropatía crónica (n=15) Control (n=15)	Canina	Heces	-EC: ↑cisteína, glicina, fenilalanina, valina, leucina, lisina ↓ metabolitos derivados del triptófano	(Honneffer *et al.,* 2017)
Enteropatía crónica (n=8) Control (n=16)	Felina	-Plasma -Heces -Orina	-EC: Plasma: ↑ alanina, glutamina, valina, isoleucina, fenilalanina Metabolitos plasmáticos (alanina, glutamina, betaína, glicerol) y urinarios sirvieron como predictores de respuesta a dieta	(Kathrani *et al.,* 2023)
Enfermedad inflamatoria intestinal (n= 13) Linfoma de célula pequeña fenotipo T (n= 13) Control (n= 14)	Felina	Suero	-IBD y linfoma: ↑ alanina, histidina, metionina, lisina, valina ↓ Metabolitos derivados del triptófano -IBD vs Linfoma: diferencias en tirosina y otros compuestos	(Questa *et al.,* 2024)
Enfermedad inflamatoria intestinal (n= 11) Linfoma de célula pequeña fenotipo T (n= 11) Control (n= 14)	Felina	Heces	-IBD y linfoma: ↑ aspartato, cisteína, fenilalanina, leucina, valina ↓ Metabolitos derivados del triptófano Alteración metabolismo aminoácidos	(Marsilio *et al.,* 2021)

JUSTIFICACIÓN Y OBJETIVOS

2. JUSTIFICACIÓN Y OBJETIVOS

Las ECs son un grupo de enfermedades de etiología desconocida (Dandrieux, 2016). Entre los factores implicados en su desarrollo se consideran la genética, la dieta y el propio ambiente intestinal que puede determinar cambios en el microbioma o en la respuesta inmunitaria a este nivel (Dandrieux, 2016; Jergens & Heilmann, 2022). De hecho, como se ha indicado en la revisión bibliográfica, el tratamiento de elección consiste en un cambio de dieta que incorpore, en muchos casos, nutrientes más fácilmente digestibles, proteína hidrolizada o proteína novel con el objetivo de paliar la sintomatología (Tolbert *et al.,* 2022). Sin embargo, no todos los perros presentan la misma respuesta al tratamiento dietético, lo que determina a su vez la existencia de distintos tipos de enteropatías, entre perros que responden y los que no responden (Dandrieux, 2016; Jergens & Heilmann, 2022) y, por tanto, se hace necesario el uso de métodos invasivos que confirmen la existencia de inflamación a nivel intestinal y de un tratamiento antiinflamatorio específico (Jergens & Simpson, 2012; Hall & Day, 2017). El motivo por el que unos perros responden al tratamiento dietético y otros no, es desconocido (Dandrieux, 2016). Todo ello hace que el diagnóstico se prolongue mucho en el tiempo, lo que supone un inconveniente tanto para el paciente como para el propietario. En este sentido, en los últimos años se han investigado distintos biomarcadores con el objeto de conocer si un animal responderá o no a la dieta y diferenciar, de forma más temprana, entre perros con FRE y con IRE. Así, biomarcadores como la proteína C-reactiva, la calprotectina fecal, la calgranulina, los pANCA, y la 3-bromotirosina parecen presentar cierto potencial en la diferenciación de estas enteropatías. Sin embargo, no presentan una elevada especificidad ni sensibilidad, por lo que su uso en la práctica se limita a la determinación de la proteína C-reactiva y la calprotectina fecal para valorar el pronóstico y monitorizar la progresión de la enfermedad (Chantawong, 2023).

El estudio del metaboloma en este tipo de enfermedades inflamatorias crónicas ha permitido recientemente conocer alteraciones en algunos compuestos orgánicos específicos, como los ácidos grasos y los aminoácidos. Ambos compuestos pueden ser proporcionados con el alimento y algunos son incluso de síntesis endógena.

Los ácidos grasos ejercen su papel principal como fuente de energía y como componentes estructurales de las membranas celulares (Stryer *et al.,* 2019). Además, participan en la regulación del metabolismo y de los procesos inflamatorios a través de la síntesis de mediadores lipídicos (Calder, 2010; Calder, 2015) y algunos de ellos son de síntesis microbiana y, por tanto, podrían ser indicadores de salud intestinal (Zhang *et al.,* 2023).

Por su parte, los aminoácidos forman estructuras proteicas indispensables para el mantenimiento de la arquitectura celular, participando en el elevado metabolismo proteico a nivel intestinal (Wang *et al.,* 2008). Así mismo, ejercen funciones energéticas, antioxidantes y reguladoras de procesos inflamatorios e inmunitarios mediante su participación en vías de señalización, actuando como precursores de hormonas o neurotransmisores (Wu, 2009). Además, también pueden dar lugar a distintos metabolitos microbianos con efectos variables a nivel intestinal (Liu *et al.,* 2020).

De este modo, se han descrito alteraciones en el perfil de ambos compuestos orgánicos, tanto en muestras de suero/plasma como en heces en humanos con IBD (Gallagher *et al.,* 2020; Aldars-García *et al.,* 2021; Bauset *et al.,* 2021). En el campo de la veterinaria los estudios son escasos y han avanzado más lentamente. Además, la mayoría de los estudios llevados a cabo han evaluado estas diferencias entre animales sanos y animales con EC en general, o por separado en algún tipo concreto de enteropatía, fundamentalmente en perros con IRE (Minamoto *et al.,* 2015; Xu *et al.,* 2016; Tamura *et al.,* 2019; Benvenuti *et al.,* 2020; Pilla *et al.,* 2021; Galler *et al.,* 2022b; Kaga *et al.,* 2023). Por otra parte, los estudios en los que se estudia el perfil de ácidos grasos o aminoácidos previo a la administración de la dieta comparando distintos tipos de enteropatías se centran en la evaluación de muestras sanguíneas (Kalenyak *et al.*, 2019; Crisi *et al.*, 2021), sin que existan estudios que caractericen la composición de muestras no invasivas como las heces. Ello podría aportar nuevos hallazgos que permitan su diferenciación de forma más temprana antes de establecer un tratamiento concreto o más personalizado en función del perfil lipídico o aminoacídico encontrado.

En consecuencia, la **hipótesis** de partida de la presente tesis consiste en que **la determinación del perfil de ácidos grasos y de aminoácidos podría permitir establecer diferencias entre los distintos tipos de ECs y esclarecer la falta de respuesta a la dieta, pudiendo así obtener un biomarcador no invasivo que permita diferenciarlas y ayudar al establecimiento de un tratamiento más personalizado.**

Por lo tanto, los **objetivos específicos** que se plantean son:

1. Evaluar diferencias en el perfil de ácidos grasos en sangre y heces entre perros sanos y perros con FRE.
2. Evaluar diferencias en el perfil de aminoácidos en sangre y heces entre perros sanos y perros con FRE.
3. Evaluar diferencias en el perfil de ácidos grasos y aminoácidos en heces entre perros sanos y perros con distintos tipos de enteropatía (FRE e IRE) con el objeto de buscar un biomarcador que permita diferenciarlas, así como con otras enfermedades digestivas que cursan con sintomatología similar (*Giardia* spp.).
4. Establecer relaciones entre el perfil de ácidos grasos y el de aminoácidos con las características de las heces, así como con indicadores de gravedad de la enfermedad.
5. Profundizar en los cambios entre los distintos tipos de ECs mediante el estudio del perfil hematológico y su posible relación con el perfil de ácidos grasos o aminoácidos con el objeto de tratar de establecer diferencias entre los perros que responden a dieta y los que no responden, pero sí lo hacen a inmunosupresores.

DISEÑO EXPERIMENTAL

3. DISEÑO EXPERIMENTAL

Los experimentos de esta tesis doctoral se llevaron a cabo en la consulta de Gastroenterología del Hospital Clínico Veterinario Complutense (HCVC) y en el laboratorio de Nutrición Animal del Departamento de Producción Animal de la Facultad de Veterinaria de la Universidad Complutense de Madrid (UCM). Todos los procedimientos fueron aprobados por el Comité de Ética de Experimentación Animal del HCVC (número de referencia 11/2021) y se realizaron conforme al Real Decreto 53/2013 sobre la protección de los animales utilizados en experimentación y otros fines científicos. La participación de los animales requirió el consentimiento informado de sus propietarios.

Para evaluar las hipótesis planteadas, se diseñaron dos experimentos. En el Experimento 1, se realizó un ensayo piloto para analizar los perfiles lipídico y aminoacídico en sangre y heces de perros sanos y perros con FRE. En el Experimento 2, se amplió el tamaño muestral e incluyeron perros con otras patologías gastrointestinales, como la IRE y la parasitosis por *Giardia* spp. Con el fin de identificar biomarcadores mediante un método menos invasivo, en este segundo experimento solo se utilizaron muestras fecales para la determinación de ambos perfiles.

3.1 Diseño del experimento 1

Este experimento incluyó 6 perros sanos (grupo control) y 9 perros con FRE (**figura 3.1**).

Los perros sanos que participaron en este experimento fueron aquellos sin signos clínicos que acudieron al HCVC para consultas rutinarias (por ejemplo, controles de salud, ovariohisterectomías u orquiectomías) o perros de los propios estudiantes de la facultad. Los criterios de inclusión para el grupo control fueron la ausencia de signos gastrointestinales en los cuatro meses previos al muestreo y valores dentro de los parámetros normales en la anamnesis, exploración física y análisis sanguíneo. Se incluyeron perros de diferentes edades, sexos, razas y condiciones corporales para garantizar la aplicabilidad de los resultados a la población canina general.

Los perros con FRE debían presentar signos de enfermedad digestiva crónica (vómitos, diarrea, pérdida de peso o anorexia/hiporexia) durante al menos tres semanas. Se descartaron otras enfermedades mediante el protocolo diagnóstico descrito en la sección 1.1.6 de la Introducción. El diagnóstico de FRE se confirmó tras una respuesta satisfactoria a una dieta de eliminación (Purina HA *Hypoallergenic*® o Royal Canin *Hypoallergenic*®) durante un mes.

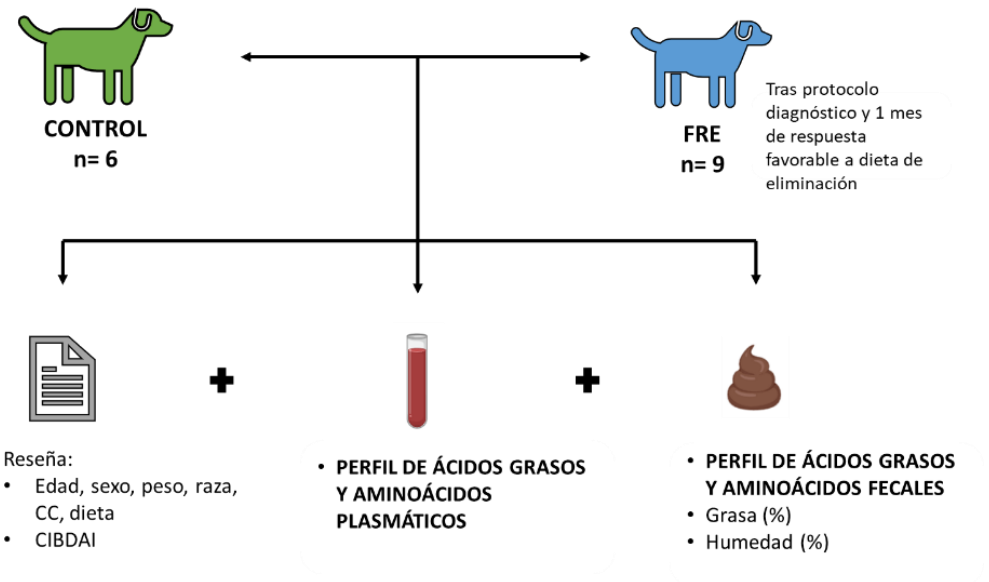

Figura 3.1. Diseño experimental 1. FRE (enteropatía que responde a dieta); CC (condición corporal); CIBDAI (*canine inflammatory bowel disease activity index*).

En la primera consulta, se recopiló información de los animales mediante anamnesis, exploración física y un cuestionario, donde se registraron edad, sexo, peso, raza, CC (1-9) (**figura 3.2**), dieta y gravedad de la enfermedad según el índice CIBDAI (**Tabla 3.1**). Una vez sumada la puntuación según los distintos signos clínicos evaluados por este índice, la gravedad de la enfermedad se clasificó como insignificante clínicamente (0-3); leve (4-5); moderada (6-8); y grave (≥9) (Jergens *et al.,* 2003).

Tabla 3.1. Índice de actividad clínica CIBDAI (*canine inflammatory bowel disease activity index*).

Puntuación	Actividad/actitud	Apetito	Vómitos	Consistencia heces	Frecuencia defecación	Pérdida de peso
0	Normal	Normal	Ninguno	Normal	Normal	No
1	Ligeramente disminuida	Ligeramente disminuido	Aproximada-mente 1/semana	Blandas o pastosas o presencia de sangre o moco	Ligeramente aumentada (2-3 veces/día)	Ligera <5%
2	Moderadamente disminuida	Moderadamente disminuido	2-3/semana	Muy blandas	Moderadamente aumentada (4-5 veces/día)	Moderada 5-10%
3	Gravemente disminuida	Gravemente disminuido	>3/semana	Diarrea líquida	Muy aumentada (>5 veces/día)	Grave >10%

Se recogió información de las dietas consumidas por todos los animales que participaron en el experimento, tanto de los perros sanos como de los perros con FRE previo al ensayo dietético con la dieta de eliminación, presentando una composición similar de los principales nutrientes (proteína bruta: 26,8 % ± 3,4; grasa bruta: 11,7 % ± 4,4; cenizas: 5,9 % ± 1,7; fibra bruta; 1,8 % ± 0,5; fibra soluble: 6,0 % ± 0,7; extractivos libres de nitrógeno: 38,3 % ± 12,5; humedad: 9,5 % ± 0,0; energía metabolizable/1000g: 3332,3 kcal ± 645,2).

Figura 3.2. A: valoración de la condición corporal del animal según la escala de puntuación de la WSAVA (*World Small Animal Veterinary Association)*. **B:** muestra de heces.

Se recolectaron muestras de sangre y heces en la primera consulta clínica y antes del inicio de la dieta de eliminación (**figura 3.2**). Se extrajeron 2 mL de sangre por venopunción yugular o cefálica en tubos con heparina; el plasma obtenido tras centrifugación se almacenó a -80 °C. Las muestras fecales recolectadas por los propietarios

para el análisis parasitológico incluido en el protocolo diagnóstico diferencial sirvieron para emplear parte de la muestra fresca para la determinación del perfil de ácidos grasos y aminoácidos fecales. De este modo, los propietarios recogieron heces durante tres días consecutivos: dos muestras se mantuvieron refrigeradas hasta la consulta y la tercera se recolectó la misma mañana de la visita, dividiéndose ésta última en dos recipientes estériles en la consulta clínica. Uno de estos dos recipientes fue el que se destinó a la determinación del perfil de ácidos grasos y aminoácidos, almacenándose a -20 °C hasta su análisis.

El perfil de ácidos grasos en plasma y heces se determinó por cromatografía de gases, y el perfil de aminoácidos, por cromatografía de líquidos, siguiendo los procedimientos descritos en los artículos 1 y 2.

3.2 Diseño del experimento 2

En este experimento, se amplió el tamaño de muestra y se incluyeron 22 perros sanos, 35 perros con FRE, 18 perros con IRE y 9 perros con *Giardia* spp., (**figura 3.3**).

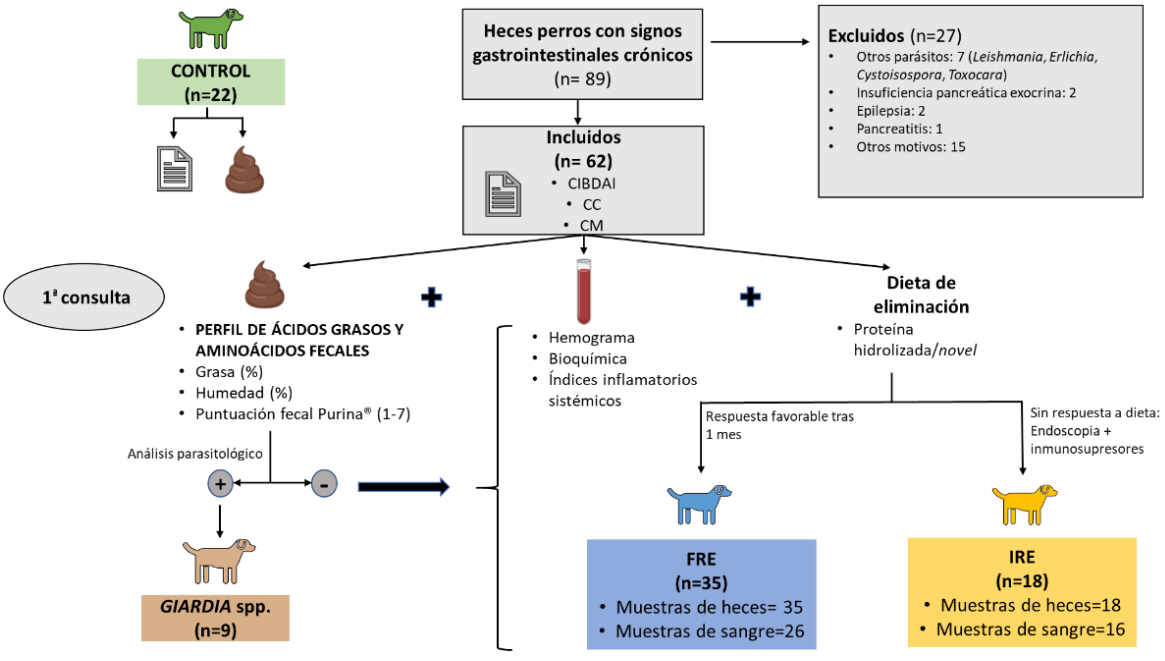

Figura 3.3. Diseño experimental 2. CIBDAI (*canine inflammatory bowel disease activity index*); CC (condición corporal); CM (condición muscular); FRE (enteropatía que responde a dieta); IRE (enteropatía que responde a inmunosupresores).

Los criterios de inclusión para los perros control y con FRE fueron los mismos que en el Experimento 1.

La información de los animales se obtuvo mediante anamnesis, exploración física y el mismo cuestionario utilizado en el Experimento 1. En comparación con este experimento, en la exploración física se añadió la determinación de la condición muscular según la WSAVA, clasificándose como normal (3), atrofia muscular leve (2), moderada (1) o grave (0) (**figura 3.4**).

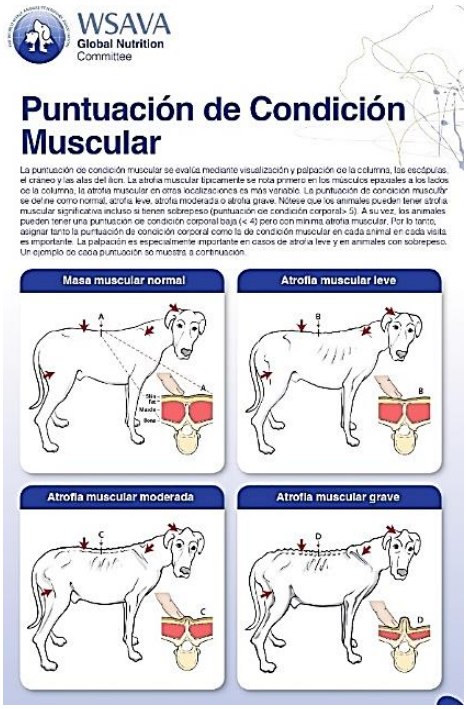

Figura 3.4. Puntuación de la condición muscular según la WSAVA (*World Small Animal Veterinary Association).*

Se recogió información sobre las dietas previas al ensayo dietético recibidas por cada uno de los grupos (**Tabla 3.2**), de forma que se eliminaron del estudio aquellos animales que recibieron dietas caseras o que recibían dietas específicas con alto contenido en fibra como dietas *light* o similares.

Tabla 3.2. Composición general de las dietas (g/100 g ± desviación estándar) antes del cambio dietético en perros control (HC), perros con enteropatía que responde a dieta (FRE), perros con enteropatía que responde a inmunosupresores (IRE) y perros parasitados por *Giardia* spp. (GIA).

Variable	HC (n=22)	FRE (n=35)	IRE (n=18)	GIA (n=9)
Humedad	9.50 ± 0.71	8.38 ± 0.48	8.50 ± 0.49	8.50 ± 0.50
Proteína bruta	23.05 ± 4.68	23.71 ± 4.50	23.73 ± 5.07	22.70 ± 4.90
Grasa bruta	13.78 ± 4.10	14.87 ± 4.30	13.46 ± 4.21	13.54 ± 4.41
Fibra bruta	2.83 ± 0.78	2.83 ± 1.89	2.12 ± 1.63	2.57 ± 1.01
Cenizas	7.64 ± 1.20	6.70 ± 0.87	5.83 ± 1.00	6.23 ± 2.07
Extractivos libres de nitrógeno	43.18 ± 7.91	43.49 ± 7.42	46.34 ± 8.36	46.44 ± 8.20
Energía metabolizable (kcal/kg)	3583 ± 193	3711 ± 201	3742 ± 163	3684 ± 221

Se recolectaron muestras fecales antes de la dieta de eliminación. De manera similar al Experimento 1, las muestras fecales recogidas por parte de los propietarios se utilizaron para el análisis parasitológico (dos muestras refrigeradas y una muestra fresca) y para la determinación del perfil de ácidos grasos y aminoácidos fecales (muestra fresca). La infección por *Giardia* spp. se diagnosticó mediante el método Telemann modificado y la técnica MIF (Mertiolato-Iodo-Formaldehído), realizadas en el Servicio de Microbiología y Parasitología del HCVC. Las muestras destinadas a la determinación de ambos perfiles se almacenaron a −20 °C. Además, como novedad en comparación con el Experimento 1, se procedió a la clasificación de la muestra de heces fresca según la escala de puntuación fecal de Purina® (**figura 3.5**).

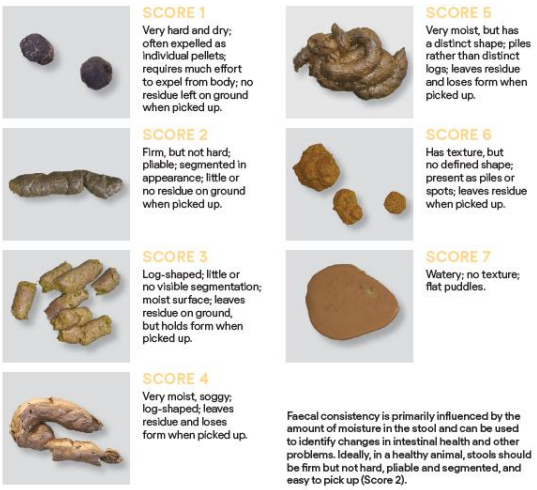

Figura 3.5. Puntuación escala fecal de Purina®.

Asimismo, se tomaron muestras de sangre en ayuno en un subgrupo de perros (FRE n=26; IRE n=16) por venopunción yugular o cefálica para la determinación del hemograma, el perfil bioquímico y los índices inflamatorios sistémicos. Se recolectaron en tubos con EDTA para el hemograma y con heparina para el perfil bioquímico.

Aquellos perros cuyo análisis parasitológico fue negativo, no respondieron de forma favorable a la dieta de eliminación y en los cuales se descartaron otras enfermedades gracias al protocolo diagnóstico, fueron sometidos, tras un protocolo de diagnósticos diferenciales completo, a una endoscopia digestiva superior permitiendo la toma de biopsias para el estudio histopatológico del estómago (cuerpo gástrico y antro pilórico) y duodeno. Estos animales debieron acudir en ayuno de 24 horas de sólidos y de 12 horas de líquidos. Todas las biopsias fueron introducidas en formol tamponado comercial al 10% estabilizado con metanol a pH 7 (Panreac®, Barcelona, España) hasta su procesado. Las muestras fueron procesadas siguiendo el protocolo del Servicio de Anatomía Patológica del HCVC. Se diagnosticaron con IRE aquellos animales que presentaron un infiltrado inflamatorio intestinal, con respuesta favorable al tratamiento inmunosupresor.

Los perfiles de ácidos grasos y aminoácidos en las muestras fecales se determinaron por cromatografía de gases y cromatografía líquida, respectivamente, según lo descrito en los artículos 3 y 5.

El análisis estadístico de ambos experimentos se realizó con un modelo lineal general en el programa SAS. Las correlaciones y análisis discriminantes se efectuaron con Statgraphics-19 (Statgraphics Centurion XIX, X64). Se consideraron resultados estadísticamente significativos cuando la $P < 0,05$.

RESULTADOS

4. RESULTADOS

4.1 Experimento 1

4.1.1 Artículo 1: Higueras *et al.*, 2022,

Short-Chain and Total Fatty Acid Profile of Faeces or Plasma as Predictors of Food-Responsive Enteropathy in Dogs: A Preliminary Study

Cristina Higueras, Ana I. Rey, Rosa Escudero, David Díaz-Regañón, Fernando Rodríguez-Franco, Mercedes García-Sancho, Beatriz Agulla and Ángel Sainz.

Animals. Índice de impacto = 3. **Q1.**

Este artículo investiga los objetivos 1 y 4 de la presente tesis doctoral, en relación con la evaluación de las diferencias en el perfil de ácidos grasos en sangre y heces entre perros con FRE y perros sanos, así como su posible relación con indicadores de la gravedad de la enfermedad, entre ellos las características fecales.

animals

MDPI

Article

Short-Chain and Total Fatty Acid Profile of Faeces or Plasma as Predictors of Food-Responsive Enteropathy in Dogs: A Preliminary Study

Cristina Higueras [1], Ana I. Rey [1,*], Rosa Escudero [1], David Díaz-Regañón [2], Fernando Rodríguez-Franco [2], Mercedes García-Sancho [2], Beatriz Agulla [3] and Angel Sainz [2]

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Simple Summary: Food-responsive enteropathy is the most common diagnosis given for dogs with chronic enteropathy, and there are no tests that can replace treatment trials. Furthermore, there is a lack of information on the specific nutritional status of these patients regarding the lipid profile that could relate them to the state of health/disease. This study evaluated differences in short-chain fatty acids and the total fatty acid profile of faeces and plasma as possible indicators of food-responsive enteropathy (FRE), as well as its relationship with body condition and the chronic enteropathy activity index. Changes in the long-chain fatty acid of plasma, and short-chain, branched and odd-chain fatty acids of faeces were detected in sick dogs, and high correlations were observed between some of these compounds and the existing calculated indices.

Abstract: The aim of this study was to evaluate differences in short-chain fatty acids (SCFAs) and the total fatty acid profile of faeces or plasma as possible indicators of FRE in comparison with healthy dogs. FRE dogs had a lower concentration (p = 0.026) of plasma α-tocopherol as an indicator of the oxidative status of the animal, and lower C20:5n-3 (p = 0.033), C22:5n-3 (p = 0.005), polyunsaturated fatty acids (PUFA) (p = 0.021) and n-6 (p = 0.041) when compared with the control dogs; furthermore, sick dogs had higher proportions of plasma C20:3n-6 (p = 0.0056). The dogs with FRE showed a decrease in the production of faecal levels of SCFAs, mainly propionic acid (C3) (p = 0.0001) and isovaleric acid (iC5) (p = 0.014). FRE dogs also had a lower proportion of C15:0 (p = 0.0003), C16:1n-9 (p = 0.0095), C16:1n-7 (p = 0.0001), C20:5n-3 (p = 0.0034) and monounsaturated fatty acids (p = 0.0315), and tended to have lower n-3 (p = 0.058) and a reduced desaturase activity index in the stool when compared with the control group. However, the dogs with chronic enteropathy tended to have greater C20:4n-6 (p = 0.065) in their faeces as signs of damage at the intestinal level. The faecal parameters were better predictors than plasma. The highest correlations between faecal odd-chain, medium- or long-chain fatty acids and SCFAs were observed for C15:0 that correlated positively with faecal acetic acid (C2) (r = 0.72, p = 0.004), propionic acid (r = 0.95, p = 0.0001), isobutyric acid (iC4) (r = 0.59, p = 0.027) and isovaleric acid (r = 0.64, p = 0.0136), as well as with total SCFAs (r = 0.61, p = 0.02). Conversely, faecal C20:4n-6 showed a high inverse correlation (r = −0.83, p = 0.0002) with C2 and C3 (r = −0.59, p = 0.027). Canine inflammatory bowel disease (IBD) activity (CIBDAI) index correlated negatively mainly with faecal measurements, such as C3 (r = −0.869, p = 0.0005) and C15:0 (r = −0.825, p = 0.0018), followed by C16:1/C16:0 (r = −0.66, p= 0.0374) and iC5 (r = −0.648, p = 0.0310), which would indicate that these fatty acids could be good non-invasive indicators of the chronic inflammatory status, specifically FRE.

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1. Introduction

Chronic enteropathy (CE) is a very common diagnosis given for dogs with chronic digestive signs, with an estimated prevalence of 70% in cases with chronic diarrhoea [1]. CE can be further subdivided retrospectively by the response to treatment into food-responsive enteropathy (FRE), antibiotic-responsive enteropathy (ARE), immunosuppressant-responsive enteropathy (IRE) and non-responsive enteropathy (NRE) [2]. Several retrospective studies suggested that FRE is probably the most common CE in dogs, with a prevalence greater than 60–70% of cases [1,3]. The significance of the effect of diet in different subtypes of CE is increasing. In fact, it was recently described that dogs with protein-losing enteropathy with a previous non-response to a combination of dietary therapies, glucocorticoids and immunosuppressive medications can achieve remission following a dietary change [4–6].

During recent years, the scientific focus has been directed to identifying biomarkers of interest in prognosis and treatment. However, to date, there are no tests that can replace treatment trials [7–10].

Short-chain fatty acids (SCFAs) are major end products of dietary fibre formed by bacteria [11]. Their role in health and disease was previously evaluated, taking into account their relationship with digestive microbiota and their effects on the immune system and gastrointestinal motility [12–14]. Previous studies in human medicine have shown a decrease in faecal concentrations of SCFAs in different chronic digestive diseases, such as inflammatory bowel disease [15]. Similar results were found in human populations with a high risk of colon cancer [16]. In veterinary sciences, the effect of dietary change or intervention on SCFAs was widely evaluated in healthy dogs [17–19], but information about faecal SCFAs in different canine digestive diseases is limited. To the best of our knowledge, systematic evaluation of faecal SCFAs has only been performed in dogs with acute diarrhoea [20] and CE [21].

In addition, some studies revealed the potential interest of other fatty acids present in faeces as diagnostic indicators of certain chronic intestinal diseases. This is due to the important functions that lipids have in the body, from being part of cellular structures to being a source of energy, participating in metabolic regulation or as precursors of certain substances [22]. Thus, DePreter et al. [23] found that faecal medium-chain fatty acids were decreased in human patients with inflammatory intestinal diseases and considered hexanoate levels as a good tool for gut disease prediction. Song et al. [24] observed a certain relationship between humans with colorectal cancer and the amount of monounsaturated or polyunsaturated fatty acids in the stool, although there were contradictory results when associating certain long-chain fatty acids with the appearance of disease. Furthermore, some of these fatty acids, such as odd-chain fatty acids (OCFA), were described as coming mainly from gut-derived propionic acid synthesised endogenously in the body [25]. Hence, disorders of propionate were detected through plasma total odd-chain fatty acids determination [26]. OCFA was also used as a potential indicator of rumen function, as well as bacterial matter [27]; however, in non-ruminant species, OCFA are present in small proportions [28]. Recent studies carried out in humans show that plasma and tissue OCFA may be associated not only with lipid status in the organism [28] but also with the gut microbiota [29]. However, there is a lack of information on the diagnostic utility of the fatty acid profile (SCFAs, OCFAs or long-chain fatty acids) in the stool or plasma in chronic intestinal diseases in dogs. In addition, some studies reported that long-chain fatty acids could be involved in the development and treatment of some chronic inflammatory diseases [30]; therefore, the complete fatty acid profile in faeces and plasma as non-invasive procedures deserve more attention.

Thus, the aim of this study was to evaluate differences in SCFAs and the total fatty acid profile of faeces and plasma as possible indicators of inflammatory chronic disease in the dog, specifically food-responsive enteropathy, and to study the possible correlation between SCFAs as indicators of gut homeostasis and the fatty acid profile in order to determine the most adequate fatty acid for intestinal disease prediction.

2. Materials and Methods

2.1. Animals and Sample Collection

Healthy dogs included in the study did not have any clinical signs, including digestive signs, within the past 4 months before the sample collection. Physical examinations and routine bloodwork of these dogs had to be normal to be enrolled in the study. Asymptomatic dogs with chronic diseases were excluded from the study.

The criteria for the inclusion of sick dogs in the study included the persistence of clinical signs of chronic digestive disease (vomiting, diarrhoea, weight loss or anorexia/hyporexia) for at least 3 weeks. All dogs included in the study had a favourable response to an elimination diet (hydrolised protein or novel protein diet) after one month. Based on the response to dietary therapy, the disease of these dogs was classified as FRE. No dog included in the study had protein-losing enteropathy.

Signalment, including age, sex, breed, sexual status, body weight and body condition score, was collected for every dog. Information about specific clinical signs related to chronic digestive disease was obtained in order to calculate the chronic enteropathy activity index (CIBDAI), as previously described [31].

Faecal samples were collected by the owners after spontaneous defecation and received at the clinic in less than 3 h, where they were immediately frozen at −20 °C until analysis. Blood (2 mL) extracted using jugular or cephalic venipuncture were collected in heparine tubes. Plasma obtained after subsequent centrifugation was stored at −80 °C. Faecal and blood samples of dogs with FRE were collected before starting the dietary treatment. The pre-experimental diet for all dogs was mainly based on cereals, animal proteins and vegetable/animal fats (averaged percentages according to the manufacturer's composition: humidity, 9.5 ± 0.0; crude protein, 26.8 ± 3.4; crude fat, 11.7 ± 4.4; ash, 5.9 ± 1.7; crude fibre, 1.8 ± 0.5; soluble fibre, 6.0 ± 0.7; nitrogen-free extractives, 38.3 ± 12.5; Ca, 0.9 ± 0.1; *p*, 0.7 ± 0.1; C18:2, 2.9 ± 1.1; ∑n-6, 2.7 ± 1.2; ∑n-3, 0.7 ± 0.1; mg/kg vitamin E: 619.2 ± 245.5; metabolic energy/100 g: 3332.3 ± 645.2. No vitamins, minerals, energy or any other supplements were administered at least 4 days before sample collection.

Their participation in the study was always carried out through the informed consent of the owners. All procedures and protocols were approved by the Animal Research Committee of the Veterinary Medicine Teaching Hospital, Complutense University of Madrid (reference number 11/2021).

2.2. Laboratory Analysis

2.2.1. Concentration of Vitamin E in Plasma Samples

The concentration of vitamin E in the plasma samples was quantified as described elsewhere [32]. Vitamin E (α-tocopherol) was extracted directly without saponification. Duplicate plasma aliquots were mixed with a dibasic sodium phosphate buffer (0.054 M) adjusted to pH 7.0. Tocopherol was extracted via centrifugation (600× *g* for 10 min at 4 °C) after the addition of hexane to the mixture. After the evaporation of the upper layer, the remaining residue was dissolved in ethanol. Tocopherol was analysed using reverse-phase HPLC (HP 1100, equipped with a diode array detector) (Agilent Technologies, Waldbronn, Germany) [32]. Identification was carried out using the pure compound (Sigma-Aldrich, Alcobendas, Madrid) and quantification (μg of α-tocopherol per mL of plasma) was carried out by means of a standard curve built with the pure compound.

2.2.2. Analysis of Short-Chain Fatty Acids in Faecal Samples

Determination of short-chain fatty acids in the faecal samples was carried out as previously described [33]. Frozen dried stool samples were accurately weighed in a 2 mL safe-lock micro test tube. Two glass balls (2 mm Ø) and 1.0 mL distilled water were added. After being tightly capped, the tubes were placed on the adapters and homogenised for 5 min at 30 Hz in a Mixer Mill MM400 (Retsch technology, Haan, Germany). The final system was allowed to separate via centrifugation (10 min, 10,000 rpm). The extraction was repeated three times. Then, the faecal suspension was transferred into a vial and the internal standard (20 mM 4-methylvaleric acid solution) was spiked and the pH was adjusted to 2–3 by adding 25% phosphoric acid. Finally, this solution was placed in vials for gas chromatography injection. Chromatographic analysis was carried out using an Agilent 6850N GC system equipped with a flame ionisation detector (FID) (Agilent Technologies, Waldbronn, Germany). A fused-silica capillary column with a free fatty acid phase (DB-FFAP 125-3237, J&W Scientific, Agilent Technologies Inc., Santa Clara, CA, USA) of 30 m × 0.53 mm i.d. coated with a 0.50 µm thickness film was used. Nitrogen was used as the carrier gas at a constant pressure of 15 psi. The initial oven temperature was 100 °C maintained for 0.5 min, raised to 180 °C at 8 °C/min and held for 1.0 min, then increased to 200 °C at 20 °C/min and finally held at 200 °C for 5 min. The temperatures of the FID and the injection port were 240 °C and 200 °C, respectively. The flow rates of hydrogen, air and nitrogen as makeup gases were 40, 300 and 30 mL/min, respectively. Data handling was carried out with HP ChemStation Plus software (Agilent Technologies, Waldbronn, Germany). Identification and quantification were carried out using pure standards (Sigma-Aldrich, Alcobendas, Spain). An aqueous stock standard solution was prepared for each acid with a concentration of 400 mM for acetic acid, propionic acid and n-butyric acid; 200 mM for n-valeric acid and i-valeric acid; 100 mM for i-butyric acid; 50 mM for n-caproic acid; and 15 mM for n-heptanoic acid.

2.2.3. Extraction of Total Fat and Fatty Acid Profile of Plasma and Faecal Samples

Plasmatic and faecal total lipids were extracted and then analysed for fatty acid profile determination. A solvent mixture of dichloromethane-methanol 8:2 was added to lyophilised weighted samples (Lyoquest, Telstar, Tarrasa, Spain) and after homogenisation in a mixer mill (MM400, Retsch technology, Haan, Germany) and centrifugation (8 min at 10,000 rpm), the upper layer containing lipids were collected. The lipid content was quantified gravimetrically after evaporation of the solvent in a nitrogen stream [34]. Fatty acid methyl esters (FAMEs) were obtained by heating the lipids (80 °C for 1 h) in the presence of methanol:toluene:H_2SO_4 (88:10:2 by volume), as described elsewhere [35]. After esterification, FAMEs were extracted with hexane and separated in a gas chromatograph (HP 6890 Series GC System; Hewlett Packard, Avondale, PA, USA) after direct injection of the sample. The gas chromatograph was provided with an automatic injector (hold at 170 °C), a flame ionisation detector (hold at 250 °C) and a capillary column (HP-Innowax polyethylene glycol, 30 m × 0.316 mm × 0.25 µm). After injection, the oven temperature was increased to 210 °C at a rate of 3.5 °C/min, then to 250 °C at a rate of 7 °C/min [35]. Identification and quantification of the FAMEs were made by comparing the retention times with those of authentic standards (Sigma–Aldrich, Alcobendas, Spain). Results were expressed as grams per 100 grams of quantified fatty acids.

Different indices were measured to estimate the desaturase or elongase activities.

The Δ9 desaturase index was calculated as the ratio of C18:0 to C18:1n-9 and as the ratio of C16:0 to C16:1.

The elongase index was calculated as the ratio of C18:0 to C16:0 and as the ratio of C20:5 to C22:5.

2.3. Statistical Analysis

Data were analysed following a completely randomised design using the general linear model (GLM) procedure contained in SAS (version 9; SAS Inst. Inc., Cary, NC, USA).

Data were presented as the mean of each group and the standard error of the mean (SEM), together with significance levels (p-values). Tukey's test was used to separate the treatment means. The differences between means were considered statistically significant at $p < 0.05$. Pearson correlations (among SCFAs and plasma or faecal fatty acids, or CIBDAI index and the other variables) were calculated using the Statgraphics-18 program. A linear adjustment between variables was carried out by means of the Statgraphics-18 program (Statgraphics Centurion XVIII, version 18.1.12).

3. Results and Discussion

3.1. Signalment of Dogs

Data regarding the age, sex, sexual status, body weight, body condition score (BCS) and canine inflammatory bowel disease activity index (CIBDAI) are shown in Table 1. The breeds of dogs with FRE ($n = 9$) were three mongrel dogs and one each of labrador retriever, cocker spaniel, miniature schnauzer, Maltese, short-haired dachshund and chihuahua. The breeds of the healthy dogs ($n = 6$) were 5 mongrel dogs and one Gordon setter. Differences concerning age, body weight or body condition score between the FRE and control groups were not statistically affected. The breed or reproductive status was not evaluated because of the insufficient numbers of individuals to study these effects. Previous studies indicated a higher prevalence of chronic enteropathy in purebreds, such as German shepherd, rottweiler, Weimaraner, border collie, or boxer when compared with mixed-breed dogs [36]. However, based on the study of these factors on the microbiome in dogs with IBD, other authors did not observe changes due to breed or reproductive status, although they did observe changes due to the disease [37].

Table 1. Data of signalment and CIBDA index (chronic enteropathy activity index) in the dogs with FRE (food-responsive enteropathy) and control dogs included in the study.

	FRE Average ± SD (Range)		**Control Average ± SD (Range)**		p [1]
Age (years)	6.2 ± 3.6	(3–13)	4.5 ± 2.1	(3–6)	NS [2]
Male	4 (2 entire, 2 castrated)		3 (3 entire)		
Female	5 (1 entire, 4 spayed)		3 (3 spayed)		
Body weight	11.4 ± 9.4	(2.3–31)	15.5 ± 0.7	(15–16)	NS
Body condition score (scale 9)	4.2 ± 1.4	(2–6)	4.5 ± 0.40	(4–5)	NS
CIBDAI index	7 ± 1.8	(5–10)	0	(0–0)	0.0001

[1] p: differences were statistically significant when $p < 0.05$; [2] NS: not significant.

3.2. Oxidative Status and Lipid Plasma Profile of the Dogs

The oxidative status, total fat and fatty acid profile of the plasma are presented in Table 2. The FRE dogs had a lower concentration ($p = 0.026$) of α-tocopherol as an indicator of the oxidative status of the animal, whereas no changes were observed in the proportion of plasma fat. Other authors reported reduced plasma antioxidant concentrations (mainly vitamin E and A) in human patients with inflammatory chronic disease [38]. The pathogenesis studies of inflammatory bowel disease (IBD) revealed that human patients with IBD had an excessive amount of oxidised molecules compared with healthy controls [39,40]. According to Rezaie et al. [39], in order to counteract this state, the organism responds with higher antioxidant production, which reduces the deposits and the capacity of the organism's response to oxidative stress. Yuksel et al. [41] also reported that total antioxidant status in humans could be a good predictor of IBD. Hence, the use of substances based on the assessment of the redox status was recently proposed as a therapeutic alternative [42,43]. In addition, in rats, a relationship was found between different regions in the gastrointestinal

tract and the oxidative status according to the microbiota since some microorganisms are able to produce endogenous antioxidants [40]. It is interesting to highlight that in the present study, the oxidative status, measured as the plasma vitamin E concentration in the alpha-tocopherol form, was also an indicator of greater C3 (r = 0.56, $p = 0.044$) in faeces, and a positive trend was observed for IC4 (r = 0.51, $p = 0.072$) and total SCFAs (r = 0.53, $p = 0.064$). Therefore, the higher the concentration of vitamin E in the plasma, the higher the content of C3 in faeces. There is no previous information on this result in dogs with chronic diseases, but some studies carried out in mice point to a direct connection between SCFAs and the inhibition of oxidative stress and inflammation [44].

Table 2. Concentration of α-tocopherol, fat and fatty acid profile of plasma samples from the FRE (food-responsive enteropathy) and control dogs.

	FRE		Control		SEM [6]	p [7]
Plasma α-tocopherol (μg/g)	24.61	b	44.62	a	3.431	0.0266
Plasma total fat (%)	24.59		29.99		1.630	0.1746
Plasma fatty acids (g per 100 g)						
C14:0	0.35		0.34		0.041	0.9139
C14:1	0.05		0.03		0.010	0.3887
C15:0	0.14		0.16		0.016	0.5581
C16:0	15.00		15.78		0.399	0.4115
C16:1n-9	0.57		0.41		0.060	0.2655
C16:1n-7	1.36		1.13		0.119	0.4007
C17:0	0.40		0.48		0.034	0.3097
C17:1	0.19		0.21		0.011	0.3325
C18:0	18.22		17.67		0.569	0.6785
C18:1n-9	16.77		11.29		1.208	0.0721
C18:1n-7	2.51		2.50		0.078	0.9412
C18:2n-6	22.81		27.72		0.983	0.0511
C18:3n-6	0.27		0.23		0.022	0.4109
C18:3n-3	0.49		0.43		0.083	0.7858
C18:4n-3	0.17		0.15		0.024	0.6721
C20:0	0.09	b	0.14	a	0.006	0.0032
C20:1n-9	0.18		0.15		0.018	0.5024
C20:2	0.37		0.26		0.023	0.0627
C20:3n-6	0.94	a	0.49	b	0.058	0.0056
C20:4n-6	14.59		14.33		0.794	0.8886
C20:5n-3	1.39	b	2.80	a	0.254	0.0335
C22:4n-6	1.84		1.78		0.247	0.9142
C22:5n-3	0.42	b	0.73	a	0.039	0.0053
C22:6n-3	0.88		0.81		0.053	0.5579
∑SAT [1]	34.20		34.57		0.689	0.8196
∑MUFA [2]	21.63		15.71		1.336	0.0783
∑PUFA [3]	44.17	b	49.72	a	0.910	0.0217
∑n-6 [4]	40.45	b	44.54	a	0.775	0.0410
∑n-3 [5]	3.35		4.92		0.321	0.0560
∑n-6/∑n-3	13.63		9.12		1.180	0.1218

[1] ∑SAT: sum of total saturated fatty acids; [2] ∑MUFA: sum of total monounsaturated fatty acids; [3] ∑PUFA: sum of total polyunsaturated fatty acids; [4] ∑n-6: sum of total n-6 fatty acids; [5] ∑n-3: sum of total n-3 fatty acids; [6] SEM: standard error of the mean; [7] p: differences were statistically significant when $p < 0.05$. Values with different superscripts (a,b) were statistically significant.

Concerning the lipid plasma profile, previous investigations did not find any changes in plasma triglyceride content in human patients with IBD [45]. In the present study, the specific fatty acid profile of the plasma (Table 2) revealed that the FRE dogs had higher C20:3n-6 ($p = 0.0056$) and tended to have higher C18:1n-9 ($p = 0.072$) and C20:2n-6

(p = 0.062), although the differences were not statistically significant; meanwhile, these FRE dogs had lower C20:0 (p = 0.003), C20:5n-3 (p = 0.033), C22:5n-3 (p = 0.005), polyunsaturated fatty acids (PUFA) (p = 0.021) and n-6 (p = 0.041), and tended to have lower proportions of C18:2n-6 (p = 0.051) and n-3 (p = 0.056), although these last two were not statistically different when compared with the control group. Other authors [46–48] reported decreased polyunsaturated fatty acids in different intestinal chronic diseases. In a detailed study carried out in humans, Esteve-Comas et al. [48] found a relationship between the severity of the disease (ulcerative colitis and Crohn's disease) and the degree of decrease in n-3 and n-6 fatty acids. These authors also observed an increase in monounsaturated fatty acids in both diseases corresponding to the status severity. Similarly, Kuroki et al. [47] found negative correlations between the Crohn's disease activity index and serum polyunsaturated fatty acids. The main essential polyunsaturated fatty acids come mainly from the diet, while monounsaturated fatty acids can be synthesised endogenously in the body. A lower proportion of polyunsaturated fatty acids could be related to malabsorption processes, or to a higher lipolytic activity of these fatty acids that are used preferentially for energy supply [49] as structural parts of cell membranes or as precursors of inflammation-regulating substances [50] in a high-requirement status, such as chronic digestive disease [47], consequently resulting in a higher proportion of other plasma fatty acids. In addition, a predominance of monounsaturated fatty acids due to desaturation phenomena to obtain energy in metabolic states in which there was a significant decrease in polyunsaturated fatty acids was described [22,49]. Conversely, in humans with ulcerative colitis, Bazarganipour et al. [51] reported increases of EPA and DHA (20:5n-3 and C22:6n-3) in the blood of patients with a more severe stage of the disease since these fatty acids are precursors of resolvins and maresins that are synthesised in order to repair the barrier disruption and were attributed to anti-inflammatory properties; however, dietary supply of these essential fatty acids should have been considered. As indicated by Hengstermann et al. [52], discrepancies in results between studies could be attributed to the malnutrition status and dietary therapeutic alternatives used to counteract the disease state. Furthermore, in the present research, it is interesting to highlight the greater proportions of some n-6 fatty acids (C20:3n-6 and C20:2n-6) observed in plasma from FRE dogs when compared with the control dogs, contrary to the decrease in n-6 fatty acids observed by other authors [47]. It was described that C20:3n-6 is the immediate precursor of PGE1 and C20:4n-6 (the main component of the phospholipids membranes) [22], and C20:3n-6 can be obtained directly from C20:2n-6 via Δ8-desaturase in a direct alternative route that is mainly activated with high eicosanoid requirements, such as in inflammation [53]. This is the first study in which the plasma fatty acid profile of dogs with inflammatory digestive chronic diseases was studied, specifically in dogs with food-responsive CE. The specific increase in these long-chain n-6 fatty acids (C20:2 and C20:3) in the blood could indicate their diagnostic potential as indicators of inflammation.

3.3. Short-Chain Fatty Acid (SCFA) Profile in Faecal Samples

In the present research, the short-chain fatty acid profile of faecal samples was also quantified (Table 3). Dogs with FRE showed a decrease in faecal levels of SCFAs. Similar results were found in dogs with different acute and chronic digestive diseases [20,21]. It was suggested that decreased SCFAs might contribute to the inflammatory status of these disorders [21]. Intestinal microbiota of dogs with CE showed a decrease in the phylum Firmicutes, especially in Clostridium XIVa and IV, which are significant producers of SCFAs [54,55].

Table 3. Faecal parameters and concentration of short-chain fatty acids (SCFAs) in the faeces from the FRE (food-responsive enteropathy) and control dogs.

	FRE		Control		SEM [1]	p [2]
Faecal parameters						
Moisture (%)	68.912	a	62.138	b	1.271	0.0437
Fat (%)	3.619		2.403		0.288	0.0975
Fat (% DM)	11.965		6.332		1.118	0.0541
Short-Chain Fatty Acids (mmol/g DM)						
Acetic acid	1.901	b	3.852	a	0.312	0.0219
Propionic acid	0.400	b	3.049	a	0.128	0.0001
Isobutyric acid	0.236	b	0.939	a	0.128	0.0386
Butyric acid	1.454		1.634		0.419	0.8562
Isovaleric acid	0.074	b	0.387	a	0.042	0.0089
Valeric acid	0.723		0.000		0.469	0.5196
Total SCFAs	4.787	b	9.862	a	0.922	0.0384
∑C2,C3 [3]	2.300	b	6.901	a	0.379	0.0004
∑C2,C3,C4 [3]	3.754	b	8.535	a	0.560	0.0041
∑IC4,IC5 [3]	0.310	b	1.326	a	0.141	0.0108

[1] SEM: standard error of the mean; [2] p: differences were statistically significant when $p < 0.05$. Values with different superscripts (a,b) were statistically significant; [3] C2: acetic acid; C3: propionic acid; C4: butyric acid; IC4: isobutyric acid; IC5: isovaleric acid.

When analysing the specific content of SCFAs in the present study, the propionic acid concentration decreased in the dogs with FRE. A similar decrease in the concentrations of faecal propionic acid was also found in dogs with acute diarrhoea [20] and in dogs with CE [21]. These similar results were not unexpected, taking into account the fact that the previous study performed in dogs with CE [21] included some dogs with FRE, but also dogs with other types of CE. The significance of the difference in faecal propionate concentrations found in our study when comparing healthy dogs and dogs with FRE was the most prominent among all SCFAs, similarly to what was previously found in dogs with CE [21]. Propionate can play a role in the pathogenesis of chronic intestinal inflammation in dogs [21]. Its role in the immune system and intestinal inflammation has been widely evaluated, especially in vivo and in rodents. Among these functions, propionate is able to regulate the size and function of the colonic Treg cells that express the transcription factor Foxp3 and protect against colitis in mice [56]. The decreased levels of propionate in dogs with different CE could be potentially significant, taking into account the fact that dogs with IBD have decreased numbers of Foxp3-positive Treg cells in the duodenal mucosa [57,58].

Concerning other SCFAs, information in the literature about faecal branched-chain fatty acids (BCFAs) in dogs is very limited. The relative concentration of isovalerate was associated with increased colitis and the IL-1β concentration of the intestinal mucosa in experimental models of IBD [59]. Our study showed that dogs with FRE also had lower concentrations of faecal isovaleric acid ($p = 0.014$), similar to what was found in rodent models of colitis [60]. Conversely, Guard et al. [20] reported that dogs with acute diarrhoea have similar levels of BCFA to healthy dogs. In contrast, in the present research, faecal butyric acid in dogs with FRE was not altered in comparison with healthy dogs. Similar results were found in dogs with chronic intestinal disease [21], while dogs with acute diarrhoea had higher levels of faecal butyric acid [20]. It was hypothesised that these results could be due to a reduction in the utilisation of butyrate by epithelial cells or a loss into the intestinal lumen in dogs with chronic intestinal inflammation [21].

3.4. Total Fatty Acid Profile in Faecal Samples

The total fatty acid profile of faeces from dogs affected with intestinal chronic disease, specifically FRE, or the control group is presented in Table 4. The FRE dogs had a lower proportion of C15:0 ($p = 0.0003$), C16:1n-9 ($p = 0.0095$), C16:1n-7 ($p = 0.0001$), C20:5n-3 ($p = 0.0034$) and monounsaturated fatty acids (MUFA) ($p = 0.0315$), and tended to have

lower ∑n-3 ($p = 0.058$) when compared with the control group. However, the FRE dogs had a greater proportion of C18:0 ($p = 0.017$) and tended to have greater C20:4n-6 ($p = 0.065$) in faeces. This fatty acid profile was different from that found in plasma samples; however, some results were connected with those observed in blood, such as the proportion of ∑n-3 fatty acids. It is also interesting to highlight the high proportion of arachidonic acid (C20:4n-6), which could be the result of excessive membrane destruction in sick dogs or to greater production of this fatty acid to repair cellular damage at the intestinal level. This fact coincided with the higher proportions of C20:3n-6 and C20:2n-6 in blood as a faster alternative route for the synthesis of C20:4 [53], which could point to these fatty acids as possible indicators of inflammatory processes.

Table 4. Fatty acid profile (g/100 g of total fatty acids) in the faeces from the FRE (food-responsive enteropathy) and control dogs.

	FRE		Control		SEM [6]	*p* [7]
C14:0	1.499		2.131		0.244	0.2212
C14:1	0.052		0.055		0.010	0.6906
C15:0	0.341	b	1.169	a	0.064	0.0003
C16:0	24.685		25.545		1.055	0.5925
C16:1n-9	0.253	b	0.313	a	0.008	0.0095
C16:1n-7	1.526	b	2.955	a	0.094	0.0001
C17:0	0.653		0.564		0.105	0.8873
C17:1	1.129		1.058		0.111	0.8607
C18:0	17.264	a	11.677	b	0.744	0.0172
C18:1n-9	20.931		22.557		1.050	0.7389
C18:1n-7	5.506		5.385		0.716	0.8425
C18:2n-6	14.784		16.953		1.794	0.9021
C18:3n-6	0.096		0.083		0.010	0.6815
C18:3n-3	1.178		1.383		0.143	0.7735
C18:4n-3	0.373		0.480		0.041	0.1855
C20:0	0.491		0.420		0.065	0.9366
C20:1n-9	0.659		0.444		0.051	0.2151
C20:2	0.353		0.465		0.029	0.1096
C20:3n-6	1.010		1.017		0.148	0.7916
C20:4n-6	3.707		1.436		0.421	0.0657
C20:5n-3	0.448	b	0.816	a	0.043	0.0034
C22:4n-6	0.482		0.353		0.067	0.5617
C22:5n-3	0.510		0.490		0.058	0.9505
C22:6n-3	2.068		2.253		0.164	0.354
∑SAT [1]	44.934		41.506		2.073	0.6699
∑MUFA [2]	30.058	b	32.767	a	0.428	0.0315
∑PUFA [3]	25.009		25.727		1.925	0.9294
∑n-6 [4]	20.079		19.840		1.913	0.7381
∑n-3 [5]	4.577		5.422		0.200	0.0584
C18:1/C18:0	1.212		1.932		0.112	0.0584
C16:1/C16:0	0.062	b	0.116	a	0.004	0.0005
C20:4n-6/C20:2n-6	11.219		3.130		1.554	0.0536
Elongase C16/C18	0.698	a	0.458	b	0.024	0.0023
Elongase C225/C205	1.206		0.612		0.123	0.0693

[1] ∑SAT: sum of total saturated fatty acids; [2] ∑MUFA: sum of total monounsaturated fatty acids; [3] ∑PUFA: sum of total polyunsaturated fatty acids; [4] ∑n-6: sum of total n-6 fatty acids; [5] ∑n-3: sum of total n-3 fatty acids; [6] SEM: standard error of the mean; [7] *p*: differences were statistically significant when $p < 0.05$. Values with different superscripts (a,b) were statistically significant.

The lower presence of some fatty acids, such as C15:0, in faecal samples could be in part associated with the different microbiome activity in the digestive system in these sick dogs. It was observed that odd-chain fatty acids (OCFA), such as C15:0 and C17:0, can be endogenously synthesised in the body from short-chain fatty acids, such as propionic acid (C3:0), which mainly come from processes of microbial fermentation in the gut [25]. Sick dogs also had lower levels of MUFA, such as C16:1n-9 and C16:1n-7, in the faeces, which could be explained by the higher metabolic use of these fatty acids, although no significant differences were observed in the blood levels. Other authors reported the high predisposition of C16:1n-7 to undergo β-oxidation [47,61], as well as the preferential use of monounsaturated fatty acids to obtain energy after polyunsaturated fatty acids [35,47]. The poorer utilisation of nutrients and malnutrition associated with animals with inflammatory digestive disease could induce this greater metabolic utilisation rate of certain fatty acids in the present study. Furthermore, the lower presence of these monounsaturated fatty acids and high level of C18:0 in dogs with inflammatory disease could also be explained by a possible lower desaturase activity at the level of the enterocyte membrane. This was confirmed by the lower C16:1/C16:0 and C18:1/C18:0 indices as indicators of the desaturase activity, which were observed in the faecal samples from the sick dogs. Garg et al. [62] reported that although the activity of desaturases in the intestine was lower than in the liver, it had an important effect on the properties of the enterocyte membrane. Therefore, according to the results of the present study, a greater alteration of the intestinal cell membrane could affect such a desaturation capacity. However, it is very interesting to observe in the present study how the elongase capacity found in the intestinal sample was higher in sick dogs. This was more marked in the case of the elongase activity of monounsaturated fatty acids C16:0/C18:0 than in the elongase activity of polyunsaturated fatty acids C22:5/C20:5. Oreshko et al. [63] reported a tendency towards increased serum elongase activity in human patients with celiac disease when compared with healthy controls. The diseased animals probably adapted their metabolic pattern to achieve a greater synthesis of saturated and long-chain polyunsaturated fatty acids as precursors of other energy-supplier fatty acids and main constituents of the membrane structure [63], as well as the synthesis of compounds with anti-inflammatory characteristics.

3.5. Correlations between Fatty Acids and SCFAs

There is no previous information on the global diagnostic possibilities of SCFAs, plasma or faeces fatty acid profile for FRE in dogs; therefore, this is the first study in which these compounds were evaluated in healthy dogs and dogs with food-responsive chronic enteropathy. Since some of these measurements are considered of interest in humans, we looked for possible relationships between the different compounds. In the faecal SCFAs, the greater correlations were detected for propionic acid (C3), isobutyric acid (IC4) and isovaleric acid (IC5), followed by acetic acid (C2) and valeric acid (C5) (Table 5). The faecal fatty acids that presented the greatest correlations were C15:0, C16:1n-7 and C20:5n-3, followed by C18:0, MUFA and C20:4n-6. The highest correlations between faecal OCFA, medium- or long-chain fatty acids and SCFAs were observed for C15:0, which correlated positively with C2 (r = 0.72, p = 0.004), C3 (r = 0.95, p = 0.0001), IC4 (r = 0.59, p = 0.027) and IC5 (r = 0.64, p = 0.0136), as well as with total SCFAs (r = 0.61, p = 0.02). Conversely, C20:4n-6 showed a high inverse correlations with C2 (r = −0.83, p = 0.0002) and C3 (r = −0.59, p = 0.027). C16:1n-7 correlated positively with C3 (r = 0.85, p = 0.0001), IC4 (r = 0.66, p = 0.0102) and IC5 (r = 0.72, p = 0.0037); meanwhile, C20:5 correlated to a lesser extent with C3 (r = 0.62, p = 0.017), IC4 (r = 0.55; p= 0.042) and IC5 (r = 0.68, p = 0.0072). These fatty acids could therefore be considered as indicators of the intestinal health status and mucosa integrity.

Table 5. Correlation coefficients between short-chain fatty acids (SCFAs) and the fatty acid profile in faeces.

Faeces	Acetic Acid	Propionic Acid	Isobutyric Acid	Butyric Acid	Isovaleric Acid	Valeric Acid	Total SCFAs
Fat	−0.39	−0.61	−0.47	−0.24	−0.23	0.02	−0.55
Moisture	−0.21	−0.68 [b]	−0.55 [a]	−0.50	−0.56 [a]	−0.38	−0.80 [b]
C14:0	0.46	0.10	−0.09	−0.41	−0.11	−0.35	−0.10
C14:1	0.26	−0.24	−0.35	−0.24	−0.36	−0.15	−0.21
C15:0	0.72 [b]	0.95 [b]	0.59 [a]	−0.05	0.64 [a]	−0.34	0.61 [a]
C16:0	0.49	0.34	0.08	0.04	0.08	−0.32	0.22
C16:1n-7	0.46	0.85 [b]	0.66 [a]	0.07	0.72 [b]	−0.29	0.55 [a]
C16:1n-9	0.36	0.25	0.10	−0.22	0.12	−0.05	0.16
C17:1	−0.43	0.21	0.41	0.38	0.30	0.33	0.26
C18:0	−0.33	−0.70 [b]	−0.32	−0.21	−0.73 [b]	−0.08	−0.60 [a]
C18:1n-7	0.19	0.02	0.19	0.01	−0.13	−0.11	0.06
C18:1n-9	−0.03	0.10	−0.14	−0.15	0.23	−0.12	−0.09
C18:2n-6	−0.10	0.02	−0.04	0.23	0.21	0.38	0.21
C18:3n-3	0.02	0.00	−0.25	−0.32	−0.02	−0.12	−0.20
C18:3n-6	−0.55 [a]	−0.11	−0.20	0.42	0.04	0.31	−0.01
C18:4n-3	−0.05	0.50	0.35	0.36	0.16	−0.03	0.35
C20:0	−0.23	0.07	0.21	−0.16	−0.10	−0.24	−0.20
C20:1n-9	−0.30	−0.42	−0.30	−0.15	−0.54	−0.07	−0.44
C20:2n-6	0.42	0.55 [a]	0.35	−0.31	0.25	−0.50	0.12
C20:3n-6	−0.08	0.16	−0.22	0.49	0.05	0.60 [a]	0.43
C20:4n-6	−0.83 [b]	−0.59 [a]	0.00	0.08	−0.28	0.36	−0.39
C20:5n-3	0.21	0.62 [a]	0.55 [a]	−0.18	0.68 [b]	−0.28	0.26
C22:4n-6	0.09	−0.03	0.00	0.34	−0.03	0.25	0.25
C22:5n-3	−0.12	−0.17	0.02	−0.05	0.14	0.28	−0.01
C22:6n-3	−0.10	0.37	0.58 [a]	−0.07	0.40	−0.24	0.09
∑SAT [1]	0.23	−0.11	−0.11	−0.18	−0.34	−0.32	−0.19
∑MUFA [2]	0.26	0.59 [a]	0.34	−0.18	0.56 [a]	−0.42	0.17
∑PUFA [3]	−0.33	−0.06	0.01	0.26	0.20	0.48	0.15
∑n-6 [4]	−0.09	−0.33	−0.13	−0.05	0.29	0.12	0.51
∑n-3 [5]	−0.05	0.44	0.45	−0.22	0.51	−0.21	0.08

[1] ∑SAT: sum of total saturated fatty acids; [2] ∑MUFA: sum of total monounsaturated fatty acids; [3] ∑PUFA: sum of total polyunsaturated fatty acids; [4] ∑n-6: sum of total n-6 fatty acids; [5] ∑n-3: sum of total n-3 fatty acids. [a] Significant at <0.05 probability level (blue color); [b] significant at <0.01 probability level (red color).

On the other hand, faeces moisture and fat were negatively correlated with SCFA (Table 5). A higher number of correlations were observed for faeces moisture than for fat. Hence, faeces moisture correlated negatively with C3 (r = −0.68, p = 0.0077), IC4 (r = −0.55, p = 0.040) and IC5 (r = −0.56, p = 0.035), and showed a greater correlation for total SCFAs (r = −0.80, p = 0.0007) than the other variables. Other authors found a greater SCFA proportion and faecal moisture in the colons of healthy mice [64]; however, in the present research sick dogs were affected with diarrhoea and higher moisture in the stool could be associated with a clinical sign of disease. Therefore, according to the present results and as stated before, C3 could be a good indicator of chronic diarrhoea, but more research is needed in order to know if this compound could be affected between different CE.

The correlations between SCFA and plasma fatty acids, plasma fat and tocopherol concentration were also determined (Table 6). A lower number of significant correlations were observed in the plasma than in the faeces. IC4 and IC5, followed by C3, were the SCFAs that had the greatest number of correlations with the other plasma variables. IC4 correlated negatively with C18:0 (r = −0.61, p = 0.025) and positively with C18:3n-3 (r = 0.64, p = 0.018), C20:1n-9 (r = 0.57, p = 0.042), C20:5n-3 (r = 0.60, p = 0.031), C22:4n-6 (r = 0.68, p = 0.010) and total n-3 (r = 0.61, p = 0.025). In the same way, IC5 correlated positively with C20:0 (r = 0.56, p = 0.046), C20:5n-3 (r = 0.68, p = 0.010), C22:5n-6 (r = 0.68, p = 0.011), total PUFA (r = 0.56, p = 0.048) and n-3 (r = 0.59, p = 0.013). According to these results,

plasma n-3 fatty acids levels could be interesting predictors of intestinal health, probably in connection with the nutritional status of the animal.

Table 6. Correlation coefficients between short-chain fatty acids (SCFAs) and fatty acid profile in the plasma.

	Acetic Acid	Propionic Acid	Isobutyric Acid	Butyric Acid	Isovaleric Acid	Valeric Acid	Total SCFA
Plasma fat	0.39	0.45	0.39	−0.26	0.68 [a]	−0.11	0.26
Plasma α-toc	−0.08	0.56 [a]	0.36	0.51	0.47	0.21	0.53
C14:0	0.23	0.07	−0.16	0.60 [a]	0.08	0.38	0.48
C15:0	−0.07	0.25	−0.04	−0.09	0.48	−0.17	−0.02
C16:0	0.43	0.09	0.06	−0.31	0.25	−0.38	−0.05
C16:1n-7	−0.03	−0.10	−0.08	0.67 [a]	−0.24	0.52	0.40
C16:1n-9	−0.41	−0.29	0.33	−0.12	−0.39	−0.11	−0.32
C17:0	0.71 [b]	0.39	0.05	0.04	0.24	−0.13	0.39
C17:1	0.25	0.30	0.36	0.39	0.23	0.07	0.41
C18:0	−0.01	−0.07	−0.61 [a]	−0.09	0.13	0.19	−0.07
C18:1n-7	−0.49	0.10	0.27	0.25	0.02	0.20	0.06
C18:1n-9	−0.38	−0.40	−0.10	−0.08	−0.57 [a]	−0.05	−0.38
C18:2n-6	0.38	0.41	0.35	−0.03	0.42	−0.18	0.28
C18:3n-3	−0.19	−0.22	0.64 [a]	−0.15	−0.15	−0.23	−0.21
C20:0	0.29	0.74 [b]	0.36	−0.02	0.56 [a]	−0.28	0.33
C20:1n-9	−0.38	−0.28	0.57 [a]	−0.19	−0.20	−0.28	−0.35
C20:3n-6	−0.54	−0.66 [a]	−0.34	−0.08	−0.53	0.22	−0.46
C20:4n-6	0.12	0.04	−0.45	0.29	0.02	0.44	0.29
C20:5n-3	0.03	0.54	0.60 [a]	0.00	0.68 [a]	−0.29	0.21
C22:4n-6	−0.39	−0.17	0.68 [a]	−0.08	−0.12	−0.06	−0.17
C22:5n-3	0.26	0.62 [a]	0.21	−0.01	0.68 [a]	−0.11	0.33
C22:6n-3	−0.28	−0.35	−0.29	0.30	−0.18	0.37	−0.01
∑SAT [1]	0.30	0.04	−0.48	−0.22	0.30	−0.06	−0.04
∑MUFA [2]	−0.39	−0.38	−0.06	−0.01	−0.55	0.00	−0.33
∑PUFA [3]	0.34	0.48	0.37	0.15	0.56 [a]	0.04	0.46
∑n-6 [4]	0.43	0.43	0.21	0.19	0.45	0.15	0.51
∑n-3 [5]	0.00	0.43	0.61 [a]	−0.01	0.59 [a]	−0.26	0.17

[1] ∑SAT: sum of total saturated fatty acids; [2] ∑MUFA: sum of total monounsaturated fatty acids; [3] ∑PUFA: sum of total polyunsaturated fatty acids; [4] ∑n-6: sum of total n-6 fatty acids; [5] ∑n-3: sum of total n-3 fatty acids. [a] Significant at <0.05 probability level (blue color); [b] significant at <0.01 probability level (red color).

Finally, the correlation between BCS and CIBDAI scores and the other parameters of plasma and faeces were also evaluated (Table 7). The significant correlations were mainly detected in faecal parameters. Moreover, the CIBDAI score presented a higher number of significant correlations than BCS. Hence, the CIBDAI score correlated negatively mainly with faecal measurements, such as C3 (r = −0.869, p = 0.0005) and C15:0 (r = −0.825, p = 0.0018), followed by C16:1/C16:0 (r = −0.66, p = 0.0374) and iC5 (r = −0.648, p = 0.0310), as well as the sum of C2 + C3 (r = −0.659, p= 0.0273) and the sum of C2 + C3 + C4 (r = −0.632, p = 0.0369). In addition, the CIBDAI score was also positively correlated with faeces moisture (r = 0.790, p= 0.0039). This index is considered a reliable measure of inflammatory activity in canine IBD [31]; therefore, according to the results of the present study, the CIBDAI score would be a good indicator of chronic inflammatory status. Its quantification, together with C3, other or total SCFAs, C15:0 or desaturase capacity in faecal samples would reinforce this non-invasive diagnosis technique in dogs with chronic inflammatory diseases.

Table 7. Correlation coefficients between BCS (body condition score) and CIBDAI (chronic enteropathy activity index) and the other parameters of the faeces from the FRE (food-responsive enteropathy) and control dogs.

	BCS		**CIBDAI Index**	
	r	***p*-Value**	**r**	***p*-Value**
Faeces fat	−0.15	0.6689	0.50	0.1177
Faeces moisture	−0.55	0.0794	0.79	0.0039
Acetic acid (C2)	0.13	0.7091	−0.35	0.2962
Propionic acid (C3)	0.35	0.287	−0.87	0.0005
Isobutyric acid (iC4)	−0.27	0.4192	−0.45	0.1610
Butiryc acid (C4)	0.55	0.0806	−0.13	0.7141
Isovaleric acid (iC5)	0.16	0.637	−0.65	0.0310
Valeric acid (C5)	0.45	0.1688	−0.05	0.8733
∑SCFAs [1]	0.54	0.0831	−0.59	0.0552
C2 + C3	0.26	0.4413	−0.66	0.0273
C2 + C3 + C4	0.52	0.1011	−0.63	0.0369
IC4 + IC5	−0.18	0.6062	−0.57	0.0668
C14:0	0.23	0.4919	−0.10	0.7644
C14:1	−0.61	0.0451	0.42	0.2040
C15:0	0.35	0.2851	−0.82	0.0018
C16:0	0.43	0.1874	−0.05	0.8833
C16:1n-7	−0.16	0.631	−0.54	0.0865
C16:1n-9	0.18	0.5939	−0.39	0.2402
C17:0	0.53	0.0927	0.02	0.9454
C17:1	0.45	0.1636	−0.46	0.1569
C18:0	0.20	0.549	0.48	0.1306
C18:1n-7	0.29	0.3835	−0.05	0.8912
C18:1n-9	−0.48	0.1371	0.08	0.8243
C18:2n-6	−0.44	0.1737	0.00	0.9924
C18:3n-3	−0.06	0.8692	0.21	0.5412
C18:3n-6	−0.32	0.3362	−0.02	0.9473
C18:4n-3	0.21	0.5391	−0.26	0.4327
C20:0	0.38	0.2493	−0.26	0.4428
C20:1n-9	0.39	0.2302	0.16	0.6400
C20:2	0.20	0.5541	−0.52	0.0997
C20:3n-6	0.64	0.0335	−0.34	0.3052
C20:4n-6	−0.03	0.9385	0.14	0.6802
C20:5n-3	−0.13	0.6926	−0.53	0.0923
C22:4n-6	0.52	0.1031	−0.04	0.8956
C22:5n-3	0.06	0.8668	−0.07	0.8469
C22:6n-3	0.35	0.2869	−0.46	0.1571
∑SAT [2]	0.43	0.1913	0.13	0.7040
∑MUFA [3]	−0.44	0.1779	−0.24	0.4781
∑PUFA [4]	−0.34	0.3049	−0.07	0.8277
∑n-6 [5]	−0.35	0.2939	0.00	0.9894
∑n-3 [6]	0.05	0.8747	−0.52	0.0994
C16:1/C16:0	−0.33	0.3536	−0.66	0.0374

[1] ∑SCFAs: sum of total short-chain fatty acids; [2] ∑SAT: sum of total saturated fatty acids; [3] ∑MUFA: sum of total monounsaturated fatty acids; [4] ∑PUFA: sum of total polyunsaturated fatty acids; [5] ∑n-6: sum of total n-6 fatty acids; [6] ∑n-3: sum of total n-3 fatty acids.

4. Conclusions

In conclusion, the dogs with FRE had a lower oxidative status and higher plasma proportions of C20:2 and C20:3 as indicators of chronic inflammation, as well as lower propionic acid and branched-chain fatty acids, such as isovaleric acid, in their stools. The short-chain fatty acids correlated better with the total fatty acid profile of the faeces. The high correlations observed between most of the SCFAs and OCFA, such as C15:0, in the faeces indicates the diagnostic potential of this compound. Sick dogs also showed signs of damage at the intestinal level with a greater presence of arachidonic acid (C20:4), as well as

a reduced desaturase activity in the stool. Further studies would be warranted in order to elucidate whether specific profiles of faecal SCFAs, OCFA or long-chain fatty acids could be found in dogs with different CEs, such as FRE, ARE and IRE.

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4.1.2 Artículo 2: Higueras *et al.*, 2023,

Changes in Faecal and Plasma Amino Acid Profile in Dogs with Food-Responsive Enteropathy as Indicators of Gut Homeostasis Disruption: A Pilot Study

Cristina Higueras, Rosa Escudero, Almudena Rebolé, Mercedes García-Sancho, Fernando Rodríguez-Franco, Ángel Sainz and Ana I. Rey

Veterinary sciences. Índice de impacto = 2. **Q2.**

Este artículo investiga los objetivos 2 y 4 de la presente tesis doctoral en relación con las diferencias en el perfil de aminoácidos en sangre y heces entre perros con FRE y perros sanos, así como su relación con indicadores de gravedad de la enfermedad, entre ellos las características fecales.

veterinary sciences

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Article

Changes in Faecal and Plasma Amino Acid Profile in Dogs with Food-Responsive Enteropathy as Indicators of Gut Homeostasis Disruption: A Pilot Study

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Simple Summary: Food-responsive enteropathy (FRE) has the greatest prevalence among the different groups of chronic enteropathies. However, information is lacking on the specific amino acid profile for FRE in dogs and its diagnostic utility. This study evaluated differences in the plasma and faecal amino acid profile between control and FRE in dogs as possible indicators of disease. We also searched for correlations between amino acids and parameter indicators of gut health, including body condition score (BCS), and indices, such as canine inflammatory bowel disease activity index (CIBDAI), to evaluate whether the amino acid profile could serve as an indicator of disease severity. Several alterations were observed in plasma and faecal amino acid profiles in sick dogs, and high correlations were found between amino acids and disease activity index or faecal characteristics. More information on the amino acid profile in dogs with FRE could help with diagnoses and lead to more precise and specific amino acid formulation, dietary interventions, better response to diet, and recovery of animals.

Abstract: Dogs suffering from food-responsive enteropathy (FRE) respond to an elimination diet based on hydrolysed protein or novel protein; however, studies regarding the amino acid profile in FRE dogs are lacking. The aim of this pilot study was to evaluate whether the plasma and faecal amino acid profiles differed between control and FRE dogs and whether these could serve as indicators of severity of illness. Blood, faecal samples, body condition score, and severity of clinical signs based on the canine inflammatory bowel disease activity index were collected before starting the elimination diet. FRE dogs had lower proportions of plasma Asparagine, Histidine, Glycine, Cystine, Leucine, and branched-chain/aromatic amino acids; however, Phenylalanine increased. In faecal samples, Cystine was greater whereas Phenylalanine was lesser in sick dogs compared to control. Leucine correlated negatively with faecal humidity (r = −0.66), and Leucine and Phenylalanine with faecal fat (r = −0.57 and r = −0.62, respectively). Faecal Phenylalanine (r = 0.80), Isoleucine (r = 0.75), and Leucine (r = 0.92) also correlated positively with total short-chain fatty acids, whereas a negative correlation was found with Glycine (r = −0.85) and Cystine (r = −0.61). This study demonstrates the importance of Leucine and Phenylalanine amino acids as indicators of the disease severity in FRE dogs.

Keywords: canine; chronic enteropathy; food-responsive enteropathy; faecal amino acids; plasma amino acids

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1. Introduction

Amino acids are important compounds in the organism as they constitute the main components of proteins and various bioactive molecules [1,2]. Recent studies have proved

that amino acids play an important role in the gut and regulation of inflammation [3]. They participate in the proliferation and apoptosis of intestinal epithelial cells (IECs), expression of tight junction proteins (TJPs), inflammatory processes, and oxidative stress through the regulation of signalling pathways [4]. Consequently, amino acids can regulate this common process taking place in inflammatory diseases such as chronic enteropathies (CEs). Some studies carried out in human medicine have shown alterations of the amino acid profile in patients suffering from gastrointestinal diseases, such as inflammatory bowel disease (IBD), with decreased levels of several amino acids in serum samples and increased levels in faecal samples of these patients [5]. This finding also means that some amino acids could be used to monitor clinical disease activity [6] or even serve as part of the treatment by improving clinical symptoms [7].

In veterinary medicine, studies regarding metabolomics in CEs are focused on the search for potential biomarkers. It has been observed that cobalamin, folate, C-reactive protein or dysbiosis index (DI) could help in diagnostic evaluation, prognosis, and monitoring clinical activity [8]. However, studies regarding the nutritional profile of food-responsive enteropathy (FRE) in dogs are lacking. In previous research, we evaluated the short-chain and total fatty acid profiles in FRE dogs, finding alterations and correlations between some of these compounds and the intestinal disease activity index [9]. Currently, scientific reports concerning the amino acid profile in dogs with CEs are very limited, although a few studies have been conducted in dogs with immunosuppressant-responsive enteropathy (IRE) and protein-losing enteropathy (PLE), finding alterations in the amino acid profile when compared to healthy individuals [10–13]. Since FRE seems to have the greatest prevalence (60–70% of cases of CE) [14] and dogs suffering from this disease respond to an elimination diet based on hydrolysed protein or novel protein, the amino acid profile evaluation deserves more attention.

Thus, the aim of this study was, first, to compare the plasma and faecal amino acid profiles between control and FRE dogs in order to expand our current knowledge and characterize the disease. Our second aim was to search for correlations between amino acids and metabolites such as short-chain fatty acids (SCFAs) or other parameter indicators of gut health, including body condition score (BCS) or indices such as canine inflammatory bowel disease activity index (CIBDAI), to evaluate whether the amino acid profile could serve as an indicator of disease severity.

2. Materials and Methods

2.1. Animals and Sample Collection

All procedures and protocols were approved by the Animal Research Committee of the Veterinary Medicine Teaching Hospital, Complutense University of Madrid (reference number 11/2021). The owners of all the patients accepted their participation in the study through informed consent.

The criteria inclusion for healthy dogs (n = 6) were a normal physical examination, blood test, and the absence of any clinical signs, including digestive signs, for at least four months. Asymptomatic dogs with chronic diseases were excluded from the study. Only sick dogs (n = 9) that had been suffering from digestive clinical signs (weight loss, anorexia, hiporexia, vomiting, or diarrhoea) for at least three weeks were to be enrolled in the study. Moreover, they had to respond to an elimination diet based on novel protein or hydrolysed protein after one month of administration. Based on the successful response to the diet, they were diagnosed as dogs with FRE. No dog included in the study suffered from hypoproteinemia.

Data including sex, age, breed, sexual status, body weight, and BCS were collected from every patient (Table 1). Breeds of dogs with FRE were three mongrel dogs, one of each breed of Labrador Retriever, Cocker Spaniel, Miniature Schnauzer, Maltese, short-haired Dachshund, and Chihuahua. Breeds of healthy dogs included five mongrel dogs and one Gordon Setter. Information about specific digestive clinical signs was measured based on the CIBDAI, as previously described [15].

Table 1. Data of signalment and CIBDAI (canine inflammatory bowel disease activity index) in dogs with FRE (food-responsive enteropathy) and healthy dogs included in the study.

	FRE Average ± SD	Range	Control Average ± SD	Range
Age (years)	6.2 ± 3.6	(3–13)	4.5 ± 2.1	(3–6)
Male	4 (2 entire, 2 castrated)		3 (3 entire)	
Female	5 (1 entire, 4 spayed)		3 (3 spayed)	
Body weight	11.4 ± 9.4	(2.3–31)	15.5 ± 0.7	(15–16)
Body condition score (scale 9)	4.2 ± 1.4	(2–6)	4.5 ± 0.40	(4–5)
CIBDAI index	7 ± 1.8	(5–10)	0	(0–0)

SD: standard deviation of the mean.

Blood samples (2 mL) were obtained following the regular procedure by jugular or cephalic venipuncture and collected in heparine tubes in fasted dogs for a period of at least 8 h. Plasma was obtained after centrifugation and stored at −80 °C. Faecal samples were collected by the owners after defecation (the same morning under fasting conditions) and brought to the clinic in less than three hours where they were stored at −20 °C. Blood and faecal samples of dogs with FRE were collected before starting the elimination diet. The commercial diets consumed by the dogs before the dietary treatment consisted of cereals, animal proteins, and vegetable/animal fats (averaged % according to manufacturer's composition: humidity, 9.5 ± 0.0; crude protein, 26.8 ± 3.4; crude fat, 11.7 ± 4.4; ash, 5.9 ± 1.7; crude fibre, 1.8 ± 0.5; soluble fibre, 6.0 ± 0.7; nitrogen-free extractives, 38.3 ± 12.5; Ca, 0.9 ± 0.1; P, 0.7 ± 0.1; C18:2, 2.9 ± 1.1; ∑n-6, 2.7 ± 1.2; ∑n-3, 0.7 ± 0.1; mg/kg vitamin E: 619.2 ± 245.5; metabolic energy/1000 g: 3332.3 ± 645.2).

2.2. Concentration of Free Amino Acids in Plasma Samples

Plasma-free amino acids were extracted as described elsewhere [16]. Essentially, plasma samples (100 µL) were mixed with 500 µL of a mixture of acetronile:methanol:acetone. After centrifugation, the supernatant was removed, leaving the dry residue. The supernatant was evaporated in N_2 stream, redissolved in 500 µL water (MilliQ) and stored at −20 °C until analysis. The plasma amino acids and their standards were then derivatised with OPA (*o*-phtalaldehyde) as described by Jones et al. [17]. Samples were derivatised in an HPLC (Hewlet-Packard 1100 Agilent Technologies GmbH, Walbronn, Germany) equipped with a fluorescent detector, a phase reverse column Porshell HPH-C18 (4.6 × 100 mm, 2.7 µm, Agilent Technologies, Walbronn, Germany), and a pre-column HPH-C18 (Infinitylab Porshell 120, 3.0 mm, UHPLC, Agilent Technologies, Germany). Two mobile phases were used: phase A, a dilution of 10 mM Na_2HPO_4, 10 mM $Na_2B_4O_7$ pH 8.2, and 0.5 mM NaN_3; and phase B, a mixture of acetonitrile:methanol:water. The detector was adjusted at 340 nm for excitation and 450 nm for emission. The determination of amino acids was made by comparing their retention times with those of a standard sample of nineteen amino acids: aspartic acid (Asp), glutamic acid (Glu), serine (Ser), alanine (Ala), arginine (Arg), cystine (Cys-Cys), histidine (His), glycine (Gly), leucine (Leu), isoleucine (Ile), lysine (Lys), methionine (Met), threonine (Thr), phenylalanine (Phe), tyrosine (Tyr), and valine (Val) (1 nm/µL in 0.1 M HCl, Agilent Technologies), along with a dilution of asparagine (Asn), glutamine (Gln) (0.01 M HCl), and tryptophan (Trp) (0.1 M HCl, Agilent Technologies).

2.3. Concentration of Amino Acids in Faecal Samples by Acid Hydrolysis

Lyophilized samples (50–80 mg) (Lyoquest, Telstar, Tarrasa, Spain) were placed in screw-capped glass tubes and hydrolysed with 15 mL of 6 M HCl. These tubes were then flushed with N_2 and heated to 110 °C for 22 h. After cooling at room temperature, samples were filtered through filter paper to a beaker, and the pH was adjusted to 5.6 by the addition of NaOH solution (phmeter Crison Basic 20+). The solution was placed in a 100 mL volumetric flask and levelled up to that volume. Then, 20 mL were collected

with a syringe and filtered by Sep-pak silica cartridge. Subsequently, 2 mL of the sample extract was isolated in a vial and stored at −20 °C. Protein hydrolysates and an amino acid calibration mixture were derivatised by o-phtaldialdehyde. Finally, an analysis of these samples was properly carried out by HPLC under the same conditions previously described by plasma samples analysis.

2.4. Statistical Analysis

For the analysis of variance, data were analysed following a completely randomised design using the general linear model (GLM) procedure contained in SAS (version 9; SAS Inst. Inc., Cary, NC, USA) following the model: $Y_{ij} = \mu + T_i + \xi_{ij}$ (where Y is the data observed of the dog j of the status i, μ is the average, T is the dog status (i = 1, 2), and ξ is the residual error). Data were presented as the mean of each group and the standard deviation of the mean (SD) together with significance levels (p values). Differences were considered significant at $p < 0.05$. Pearson correlation among different amino acids and condition indices or other components of plasma/faeces, such as humidity, fat, and α-tocopherol (determined in a previous paper [9]), were carried out using the Statgraphics-19 program. The linear adjustments between amino acids and faecal characteristics or SCFAs (analysed in a previous study) [9] were quantified by Statgraphics-19.

3. Results

The proportion of plasma amino acids is shown in Figure 1. FRE dogs had lower proportions of Asn ($p = 0.034$), His ($p = 0.009$), Gly ($p = 0.005$), Cys-Cys ($p = 0.028$), Leu ($p = 0.017$), and ratio branched-chain amino acids/aromatic amino acids (BCAA/AAA) ($p = 0.018$) when compared to control dogs. However, FRE dogs had a greater proportion of Phe ($p = 0.013$).

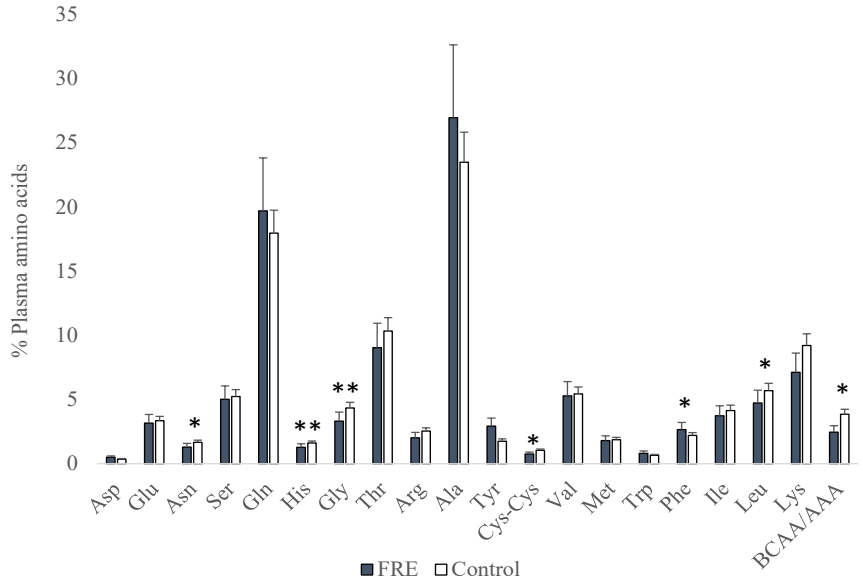

Figure 1. Amino acid proportions (%) in plasma from FRE (food-responsive enteropathy) and control dogs (* $p < 0.05$; ** $p < 0.01$).

Correlations between plasma-free amino acids and plasma fat content, α-tocopherol concentrations, BCS, and CIBDAI indices are presented in Table 2. Total plasma fat content correlated negatively with Trp (r = −0.58, $p = 0.014$) and Phe (r = −0.50, $p = 0.040$). Next, α-Tocopherol (as an indicator of the oxidative status) correlated positively with Asn (r = 0.56, $p = 0.020$), Gly (r = 0.51, $p = 0.035$), Arg (r = 0.65, $p = 0.004$), and Cys-Cys (r = 0.60, $p = 0.024$). On the contrary, Ala correlated negatively with α-tocopherol (r = −0.49, $p = 0.045$). In addition, BCS correlated positively with Leu (r = 0.48; $p < 0.05$). Finally, CIBDAI correlated

positively with Phe (r = 0.53, $p = 0.027$), whereas it correlated negatively with Leu (r = −0.69, $p = 0.002$), Lys (r = −0.59, $p = 0.012$), BCAA (r = −0.49, $p = 0.043$), and BCAA/AAA ratio (r = −0.67, $p = 0.003$).

Table 2. Correlation coefficients between plasma amino acids proportions and plasma fat, plasma α-tocopherol, body condition score (BCS), and CIBDAI (canine inflammatory bowel disease activity index).

%	Fat	α-Tocopherol	BCS	CIBDAI
Aspartic acid	−0.39	0.27	0.35	0.19
Glutamic acid	−0.20	0.48	0.28	−0.35
Asparagine	0.04	0.56 [b]	−0.39	−0.32
Serine	0.41	−0.07	−0.14	−0.07
Glutamine	−0.30	0.26	0.36	0.15
Histidine	0.23	0.15	−0.30	−0.38
Glycine	0.20	0.51 [b]	0.20	−0.47
Threonine	0.35	−0.02	−0.22	−0.12
Arginine	−0.08	0.65 [a]	−0.02	−0.42
Alanine	0.15	−0.49 [b]	−0.22	0.20
Tyrosine	−0.25	0.08	−0.29	0.41
Cystine	−0.13	0.60 [b]	0.28	−0.40
Valine	−0.13	−0.03	0.28	−0.25
Methionine	0.10	0.18	−0.39	0.08
Tryptophan	−0.58 [b]	0.06	0.36	0.32
Phenylalanine	−0.50 [b]	0.03	0.14	0.53 [b]
Isoleucine	−0.08	0.32	0.12	−0.37
Leucine	0.15	0.38	0.48 [b]	−0.69 [a]
Lysine	0.04	0.36	0.43	−0.59 [b]
BCAA [1]	0.00	0.26	0.33	−0.49 [b]
AAA [2]	−0.32	0.08	−0.24	0.47
BCAA/AAA	0.40	0.03	0.21	−0.67 [a]

[1] BCAA, Branched-chain amino acids: sum of amino acids valine, leucine, and isoleucine. [2] AAA, Aromatic amino acids: sum of tyrosine, phenylalanine, and tryptophan. [a] Significant at <0.01 probability level (red colour); [b] significant at <0.05 probability level (blue colour).

Proportions of faecal amino acids are shown in Figure 2. Faecal Cys-Cys proportion was greater ($p = 0.005$), whereas Phe was lesser ($p = 0.032$) in sick dogs compared to the control. The other faecal amino acids were not statistically affected.

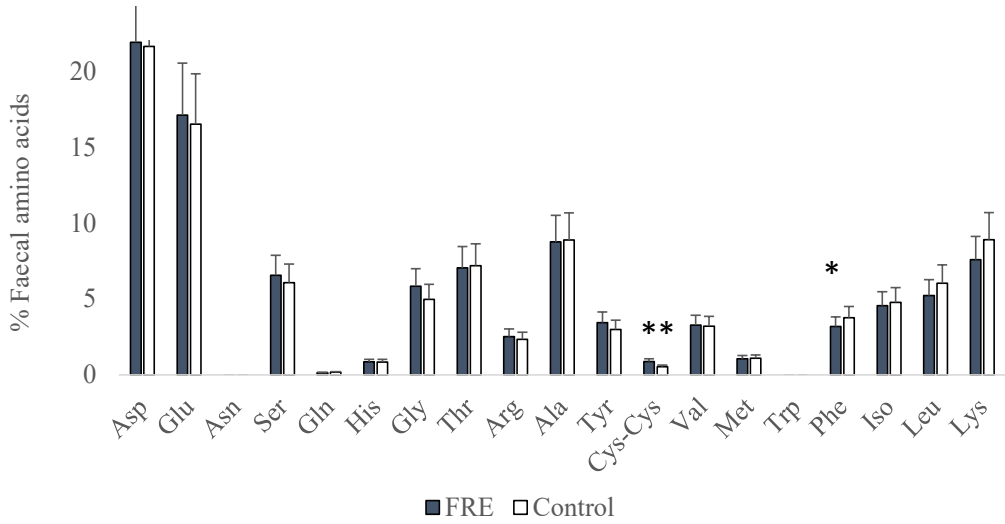

Figure 2. Amino acid proportions (%) in faeces from FRE (food-responsive enteropathy) and control dogs (* $p < 0.05$; ** $p < 0.01$).

Correlations between faecal amino acids and faecal parameters (fat and humidity), BCS, and CIBDAI indices are shown in Table 3. Faecal fat percentage was negatively correlated with Phe (r = −0.62, p = 0.030), Lys (r = −0.65, p = 0.022), and Leu (r = −0.57, p = 0.050). Faecal humidity percentage correlated positively with Gly (r = 0.59, p = 0.045) and negatively with Leu (r = −0.66, p = 0.018). No correlation was found with BCS or CIBDAI.

Table 3. Correlation coefficients between faecal amino acids proportions and faeces composition (% humidity, % fat), body condition score (BCS), and CIBDAI (canine inflammatory bowel disease activity index).

% Faeces	% Faecal Fat	% Faecal Humidity	BCS	CIBDAI
Aspartic acid	0.37	0.07	−0.18	−0.12
Glutamic acid	0.25	−0.05	−0.02	0.22
Serine	0.45	0.48	−0.21	0.52
Histidine	−0.13	−0.38	0.33	−0.09
Glycine	0.26	0.59 [b]	−0.39	0.40
Threonine	0.41	0.29	−0.03	0.15
Arginine	0.29	0.16	0.40	0.23
Alanine	−0.24	0.27	0.13	0.15
Tyrosine	−0.39	0.32	−0.04	0.41
Cystine	0.35	0.35	0.22	0.48
Valine	−0.43	−0.21	0.47	0.13
Methionine	−0.10	−0.34	0.31	−0.1
Phenylalanine	−0.62 [b]	−0.53	0.28	−0.45
Isoleucine	−0.57	−0.47	0.44	−0.19
Leucine	−0.57 [b]	−0.66 [b]	0.44	−0.46
Lysine	−0.65 [b]	−0.43	−0.02	−0.39

[b] Significant at <0.05 probability level (blue colour).

The correlation between faecal amino acids and SCFAs was also investigated (Table 4). The concentration of SCFAs between FRE and healthy dogs was quantified in a previous study [9]. Total SCFAs correlated positively with Phe (r = 0.80, p = 0.002), Ile (r = 0.75, p = 0.005), and Leu (r = 0.92, p = 0.0001); whereas a negative correlation was observed with Gly (r = −0.85, p = 0.0005) and Cys-Cys (r = −0.61, p = 0.035). The SCFA that presented the highest number of correlations was butyric acid (C4), which correlated positively with amino acids Val (r = 0.67, p = 0.017), Met (r = 0.68, p = 0.015), Ile (r = 0.77, p = 0.003), and Leu (r = 0.80, p = 0.001), while negatively with Gly (r = −0.78, p = 0.002). Valeric acid (C5) and isovaleric acid (IC5) also presented a high number of correlations. Isovaleric acid correlated negatively with Gly (r = −0.59, p = 0.042), Tyr (r = −0.59, p = 0.042), and Cys-Cys (r = −0.60, p = 0.039), and C5 correlated positively with Leu (r = 0.59, p = 0.043) and negatively with Gly (r = −0.61, p = 0.034). However, the C2, C3, and IC4 presented a lower number of correlations with faecal amino acids. Thus, C2 correlated negatively with Ser (r = −0.58, p = 0.049), and IC4 correlated positively with His (r = 0.64, p = 0.026). However, C3 correlated positively with Phe (r = 0.62, p = 0.030), and a tendency was observed with Leu (r = 0.56, p = 0.060).

Table 4. Correlation coefficients between faecal amino acids proportions and faecal short-chain fatty acids (SCFAs, mmol/g dry matter).

% Faeces	∑SCFAs	C2	C3	IC4	C4	IC5	C5
Aspartic acid	−0.42	−0.12	−0.11	−0.14	−0.54	0.02	−0.24
Glutamic acid	0.04	−0.55	−0.24	0.04	0.37	0.29	0.39
Serine	−0.49	−0.58	−0.53	0.10	−0.03	−0.47	−0.10
Histidine	0.25	−0.33	0.05	0.64 [b]	0.39	0.13	0.24
Glycine	−0.85 [a]	−0.10	−0.48	−0.22	−0.78 [a]	−0.59 [b]	−0.61 [b]
Threonine	0.01	−0.24	0.07	−0.19	0.25	0.14	0.01
Arginine	0.27	0.12	−0.04	−0.36	0.42	−0.24	0.32
Alanine	0.14	0.53	0.19	−0.16	0.05	−0.23	−0.26
Tyrosine	−0.15	0.24	−0.29	−0.29	−0.01	−0.59 [b]	−0.13
Cystine	−0.61 [b]	−0.42	−0.53	−0.40	−0.23	−0.60 [b]	−0.16
Valine	0.40	−0.05	−0.01	0.07	0.67 [b]	−0.24	0.38
Methionine	0.53	−0.24	0.25	0.23	0.68 [b]	0.34	0.50
Phenylalanine	0.80 [a]	0.41	0.62 [b]	0.39	0.56	0.50	0.26
Isoleucine	0.75 [a]	0.22	0.31	0.06	0.77 [a]	0.19	0.54
Leucine	0.92 [a]	0.24	0.56	0.22	0.80 [a]	0.47	0.59 [b]
Lysine	0.51	0.48	0.42	0.54	0.12	0.30	0.04

∑SCFAs sum of short-chain fatty acids; DM, dry matter; C2, Acetic acid; C3, propionic acid; IC4, Isobutyric acid; C4, butyric acid; IC5, Isovaleric acid; C5, valeric acid; [a] significant at <0.01 probability level (red colour); [b] significant at <0.05 probability level (blue colour).

Finally, linear adjustments were observed between faecal characteristics (faecal fat or humidity), faecal Leu (R^2 = 0.32), Phe (R^2= 0.41), and Gly (R^2= 0.42) (Figure 3a–c, respectively). These faecal amino acids also presented high linear responses with SCFAs. Thus, more than 70% of the variation of these amino acids in faeces was linearly explained by the concentration of SCFAs: R^2 = 0.84; R^2 = 0.70; and R^2 = 0.70 for Leu, Phe, and Gly, respectively (Figure 3d–f).

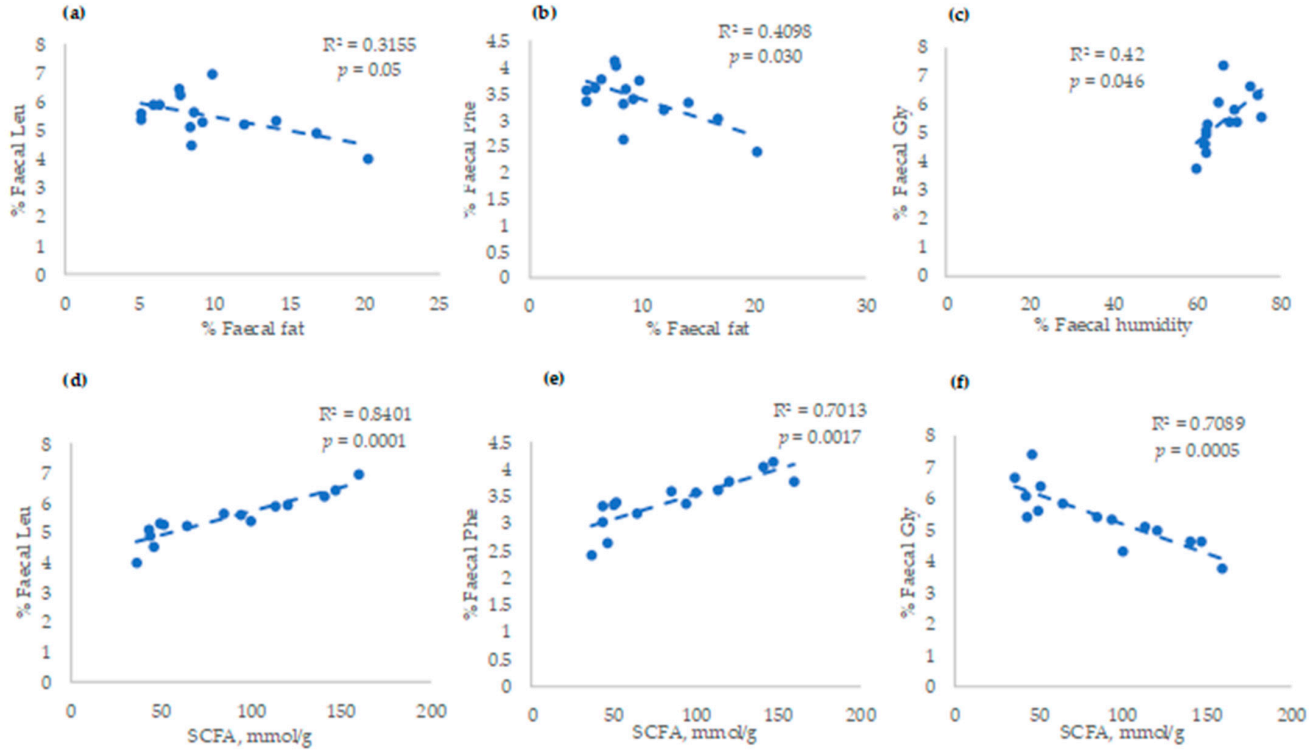

Figure 3. Linear adjustments between (**a**) % fat and Leu; (**b**) % fat and Phe; (**c**) humidity and Gly; (**d**) ∑SCFAs and Leu; (**e**) ∑SCFAs and Phe; and (**f**) ∑SCFAs and Gly in faecal samples.

4. Discussion

Academic literature concerning the amino acid profile in dogs with gastrointestinal disorders is currently quite limited. Some research has been actually carried out in dogs with IRE or PLE [10–13]. However, current information on the amino acid profile in FRE dogs is not yet available. In the present study, FRE dogs had lower proportions of Asn, His, Gly, Cys-Cys, Leu, and ratio BCAA/AAA in plasma. Studies carried out in human medicine have also shown alterations in the amino acid profile of patients suffering from IBD [5,7], and the benefits of supplementing some amino acids on the reduction of symptomatology have been described [7,18,19]. Also in human studies, some authors have suggested the utility of amino acids like His as monitoring tools for predicting the risk of relapse in patients with ulcerative colitis (UC) [20]. In addition, prior research points to Gly and Cys as important amino acids for the maintenance of oxidative status linked to the inflammatory process, as they are part of antioxidant enzymes such as glutathione [21]. In this study, a positive correlation was found between these amino acids and vitamin E, which is one of the most important antioxidants that participate to a great extent in cell oxidative control in connection with other antioxidant systems to ensure the homeostasis of the individual [16]. Finding lower proportions of Gly and Cys-Cys in FRE dogs could be due in part to the higher use of these amino acids to synthesize glutathione and control the augmented reactive oxygen species (ROS) production that takes place in the inflammatory process. Moreover, these amino acids, together with His, Asn, and Leu, regulate intestinal inflammation, downregulating the production of proinflammatory cytokines [4]. In contrast, Cys, Gly, Asn, and Leu are also responsible for maintaining the normal functioning of the intestinal epithelial barrier by enhancing tight junction proteins [4]. Therefore, the lack of adequate long-term levels would exacerbate the inflammatory process and aggravate the integrity of the mucosal barrier. This lack would thus lead to bacterial adhesion and alteration of transporters responsible for the absorption of nutrients which, in turn, would result in nutritional deficits [22].

It is worth emphasizing that, in the present study, plasma Phe proportion was the only one increased in dogs with FRE. A recent study by Walker et al. [13] also found greater Phe serum concentration in dogs with CEs. It has also been reported that both inflammation and infection often lead to increased levels of Phe in human patients [23] since cytokines induce a strong metabolic disruption, muscle tissue breakdown, and a catabolic state. This state is associated with a higher release and increased Phe plasma levels in demand of the high metabolic rate [24], with Phe being a good indicator of body protein breakdown [25,26]. It is interesting, therefore, to observe that Phe correlated positively with CIBDAI in the present study, indicating a severe state of the disease based on weight and muscle loss. Moreover, the BCAA/AAA ratio decreased in FRE in comparison to that of healthy dogs. Phe is considered an AAA that is converted into Tyr. Branched-chain amino acids (BCAA), including Leu, Ile, and Val, are responsible for regulating the metabolism of glucose, lipid and protein synthesis, intestinal health, and immunity. Thus, BCAAs represent the major nitrogen source for the synthesis of Ala, Gln, and Glu [27] which are essential components for rapidly dividing cells such as enterocytes and immune cells [28]. Other authors reported lower BCAA/AAA in gastrointestinal or hepatic diseases [26] in association with the malnutrition process or with increased protein catabolism [29]. This result was confirmed in the present study by the negative correlation between AAA (Phe) and plasma fat. Some studies carried out in humans found that plasma AAA were higher in obese patients and were positively correlated to adiposity [30–32]. In addition, in the present study, Leu, Lys, BCAA, and the BCAA/AAA ratio correlated negatively with CIBDAI. Leu and Lys are essential amino acids, having a significant role in protein anabolism. The amino acid Leu [33] has especially been considered the major regulator of muscle protein synthesis in neonates [34]. Moreover, it has been confirmed that, among the BCAA, the response of muscle protein synthesis is unique to Leu, whereas Val and Ile failed to stimulate protein synthesis activation [35]. This finding is in line with the present results since one of the clinical signs evaluated by the CIBDAI index is weight loss that

is associated with muscle loss, a typical sign of CE. Therefore, dogs with greater weight loss would have lower levels of these essential amino acids in plasma and, consequently, a greater CIBDAI classification. According to the results of the present study, Leu was the amino acid that showed the highest correlation, together with BCAA/AAA and the illness state, followed by Lys and Phe. These might then represent potential novel biomarkers for FRE. Commercially available diets containing hydrolysed protein formulated for dogs with CEs do not specify, in most cases, any amino acid profile. More information on the amino acid profile of dogs with FRE could lead to more precise and specific amino acid formulation in dietary interventions, better response to diet, and the recovery of the animal.

Contrary to what was observed in plasma samples, FRE dogs had a lower proportion of Phe in the stool, which might indicate a greater metabolic use of this compound. However, faecal Cys-Cys was high in sick dogs which could be a consequence of an increase of Cys metabolism at this level as reported previously in IBD patients, possibly due to perturbed gut microbiota [36]. The proportions of amino acids were lower in faeces than in plasma as expected, except for the amino acids Asp and Glu. It has been reported that, during acid hydrolysis, the amino acids Asn and Gln are completely converted to Asp and Glu, respectively, while Trp is destroyed [37]. The correlations observed between faecal amino acids and faeces characteristics confirm again the importance of Phe and Leu as possible indicators of intestinal disease severity. Faecal Phe reached the highest negative correlation with the fat proportion in the stool, whereas faecal Leu was negatively correlated with proportions of fat and humidity. Balasubramanian et al. [38] also found lesser levels of Leu, as part of BCAA (Ile, Leu, Val), in the colonic mucosa of IBD patients compared with healthy subjects and considered these as potential biomarkers. Other amino acids involved in the endogenous antioxidant capacity that could be associated with illness, such as Gly, showed a positive correlation with stool humidity in the present study. Bjerrum et al. [39] also found increased levels of Gly in the faecal samples of IBD individuals compared to healthy controls, since this amino acid plays a key role in oxidative homeostasis and the regulation of inflammation [40]. It seems that gut alterations could induce a higher proportion of amino acids involved in oxidative functions such as Cys or Gly, since Cys-Cys was also greater in the stool of FRE dogs. However, no correlations were observed between Cys-Cys and faecal characteristics, although it should be pointed out that both presented a negative correlation with SCFAs.

It has been reported the importance of SCFAs for keeping intestinal health and their levels are reduced in the faeces of adults suffering from IBD [41] or other CEs [9] with numerous studies suggesting that they play an important role in the treatment of inflammation-related diseases [9,41]. Although SCFA production comes mainly from the fermentation of carbohydrates, bacterial fermentation of protein sources serves as well for their obtention [42,43]. It has been reported that protein fermentation by intestinal bacteria in humans could account for 17 % of SCFAs found in the caecum and 38% of SCFAs produced in the rectum [42]. The ratio of available carbohydrates to protein determines substrate utilization by the gut microbiota. Therefore, when energy is scarce, proteins are catabolized by bacteria to produce amino acid-derived end products [43]. In the present research, the amino acid that presented the highest positive correlation with total SCFA proportion was Leu, which was mainly positively related to faecal C4 proportion, followed by Phe, and Ile. Leu and Ile, as BCAA, play an important role in gut health by promoting intestinal development, nutrient transporters, and immune-related function [27]. To the best of our knowledge, there is no previous information on the correlation between faecal SCFAs and faecal amino acids in dogs with CE. According to our results, the higher proportion of faecal Leu and Phe, the higher the faecal SCFAs, which would be associated with better gut health. These results confirm again their importance as indicators of disease severity and faecal characteristics.

5. Conclusions

Our results show that dogs with FRE had different plasma and faecal amino acid profiles than control dogs. The high correlation observed between plasma Leu and Phe with CIBDAI suggests that they could be used as disease biomarkers. Furthermore, statistically significant correlations observed for Leu, Phe, Gly, and Cys-Cys with SCFAs might indicate gut microbiota functionality, as well as homeostasis disruption. Consequently, these amino acids might have a role to play in food-responsive enteropathy.

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Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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4.2 Experimento 2

4.2.1 Artículo 3: Higueras *et al.*, 2024,

Faecal Short-Chain, Long-Chain, and Branched-Chain Fatty Acids as Markers of Different Chronic Inflammatory Enteropathies in Dogs

Cristina Higueras, Ángel Sainz, Mercedes García-Sancho, Fernando Rodríguez-Franco and Ana I. Rey

Animals. Índice de impacto (2023) = 2,7. **Q1.**

Este artículo investiga los objetivos 1, 3 y 4 de la presente tesis doctoral en relación con la búsqueda de diferencias en el perfil de ácidos grasos en heces entre perros con distintas ECs (FRE e IRE), así como en perros con sintomatología similar diagnosticados con *Giardia* spp. Además, explora las correlaciones entre los ácidos grasos y diversos indicadores de la gravedad de la enfermedad, como las características fecales.

animals

MDPI

Article

Faecal Short-Chain, Long-Chain, and Branched-Chain Fatty Acids as Markers of Different Chronic Inflammatory Enteropathies in Dogs

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Simple Summary: Canine chronic inflammatory enteropathies and *Giardia* infection are gastrointestinal diseases characterised by inflammation of the digestive mucosa. This inflammatory process can induce alterations in components of the intestinal structure, such as lipids, as well as in the homeostasis and intestinal environment. Consequently, we have theorised that variations in the composition of faecal fatty acids could exist between different digestive disorders and could help in their differentiation as well as a more personalised dietary treatment.

Abstract: Chronic inflammatory enteropathies (CIEs) are classified based on treatment trials, and new methods are being sought for earlier differentiation and characterization. *Giardia* infection (GIA) is one of the first differential diagnoses and may be present in CIE-affected dogs. The aim of this study was to evaluate the faecal characteristics and faecal fatty acid profile (short, medium, long, and branched-chain fatty acids) in dogs with food-responsive enteropathy (FRE), immunosuppressant-responsive enteropathy (IRE), and dogs infected with *Giardia* compared to healthy control (HC) animals as a potential non-invasive indicator of intestinal health that helps in the differentiation of CIEs. The C16:1n-7 percentage ($p = 0.0001$) and C16:1n-7/C16:0 ratio ($p = 0.0001$) served to differentiate between HC, FRE, and IRE. IRE dogs presented lower levels of short-chain fatty acids (∑SCFAs) ($p = 0.0008$) and acetic acid (C2) ($p = 0.0007$) compared to the other three groups and lower propionic acid (C3) ($p = 0.0022$) compared to HCs. IRE and GIA presented higher faecal fat content ($p = 0.0080$) and ratio of iso/anteiso branched-chain fatty acids (BCFAs) compared to HC and FRE. Correlations between some fatty acids and desaturation indices with the canine inflammatory bowel disease activity index and faecal characteristics were observed, suggesting that these compounds could play an important role in the pathogenesis of these diseases.

Keywords: gut health; desaturase index; elongase index; faecal fatty acid profile; food-responsive enteropathy; immunosuppressant-responsive enteropathy; *Giardia* infection; dogs

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1. Introduction

Lipids are one of the major constituents of biological membranes and are organised into a bilayer configuration in which proteins with crucial functions are partially or completely embedded [1]. These phospholipid-based bilayers are important for the preservation of structural integrity, but also allow the regulation of membrane fluidity for signal molecules, metabolites, and ions [2]. Among the fatty acids that are mainly involved in membranes, polyunsaturated fatty acids (PUFAs) have demonstrated a crucial role in gut homeostasis by reducing membrane rigidity and adequately facilitating nutrient absorption [3]. Thus,

some changes in the fatty acid profile of the phospholipid membrane or mucus have been associated with inflammatory bowel diseases (IBDs) [2,4].

A gut homeostasis state also contributes to the good functioning of the microbiome, which in turn collaborates in the synthesis of some compounds (short-chain and branched-chain fatty acids) [5]. Short-chain fatty acids (SCFAs) are produced in the colon and derive mostly from anaerobic fermentation of dietary fibres by intestinal bacteria [5]. They are important energy sources for colonocytes and have anti-inflammatory and immunomodulatory effects, increasing the expression of mucins and antimicrobial peptides, promoting crypt differentiation and modulation of tight-junction proteins, and regulating innate and adaptive immune cell generation [6,7]. Other fatty acids, such as branched-chain fatty acids (BCFAs), can also be synthesised by bacteria from branched-chain amino acids (valine, leucine, and isoleucine) [8,9]. Recent investigations have pointed to their potential beneficial effects in developing and maintaining the microbiota and enterocyte health since they are the main component of membrane lipids in numerous bacteria [10,11]. Other fatty acids associated with gut microbiota are odd-chain fatty acids (OCFAs), which can be synthesised from SCFAs, mostly from propionic acid (C3) [12,13].

Previous research on dogs with chronic inflammatory enteropathies (CIEs) reported changes in some of these fatty acids. Thus, some authors found reduced C18:1n-9 [14,15], C18:2n-6 or linoleic acid [15] and n-6 [15,16] in blood samples from sick dogs compared to healthy individuals. Moreover, Crisi et al. [17] observed altered elongase and desaturase indices in the erythrocyte membrane phospholipids as a perturbation of the lipid metabolism. On the contrary, in faecal samples, there are controversial results. Galler et al. [18] found higher levels of linoleic acid and monounsaturated fatty acids (MUFAs) in sick animals, whereas other authors observed lower MUFA proportions as a consequence of a lower desaturase activity [16]. Furthermore, reductions in faecal acetic acid (C2), propionic acid (C3), and ∑SCFAs have also been reported [16,19] in dogs with CIEs. However, most of these studies were carried out to characterize CIEs in general or in specific cases, such as food-responsive enteropathy (FRE) [16] or immunosuppressant-responsive enteropathy (IRE) [18], without there being a comparative study that allows us to evaluate different types of enteropathies from the point of view of the lipid profile of the faeces. Moreover, there are no studies that evaluate to what extent long BCFAs can change in different canine CIEs and if it can help to establish differences between them.

On the other hand, there is no current information on the potential modifications of these compounds in dogs with parasitic disorders such as *Giardia* (GIA), which is one of the first differential diagnoses of CIEs. This parasite is a worldwide intestinal protozoan that infects humans and domestic and wild animals causing gastrointestinal signs [20]. *Giardia* diagnosis is well established using coprological examination, but the risk of false negatives depends on the sensitivity of the diagnostic method [21]. It is well known that *Giardia* depends on exogenous lipids for its growth and differentiation, since it has a limited ability to synthesize its own lipid molecules [22]. As a result, lipids contained in the gut are incorporated into *Giardia* membranes, probably reducing their availability to the host [22].

Since the current classification of CIEs as a group of idiopathic disorders with chronic, persistent, or recurrent gastrointestinal signs [23] relies on treatment response [24], new biomarkers are being sought for earlier differentiation. In consequence, we hypothesize that the general composition and lipid profile of faecal samples in dogs with gastrointestinal chronic disorders could be a non-invasive method for the characterization of these diseases and that the lipid profile may change depending on the severity of the damage to the intestinal structure or homeostasis, and consequently its determination could help in the diagnosis and a more accurate dietary treatment.

Thus, the aims of this study were firstly to evaluate the faecal characteristics and faecal fatty acid profile (short, medium, long, and branched-chain fatty acids) in dogs with chronic digestive diseases (food-responsive enteropathy, immunosuppressant-responsive enteropathy, and *Giardia* infection) compared to healthy individuals, and secondly to look

for possible correlations between these fatty acids and the illness severity, as well as its discriminating potential.

2. Materials and Methods

All procedures and protocols developed in the present research were approved by the Animal Research Committee of the Complutense Veterinary Medicine Teaching Hospital (CVMTH), (reference 11/2021, approval date: 26 May 2021). Owners of all the dogs accepted their participation through informed consent.

2.1. Experimental Design and Animal Signalment

A total of eighty-four dogs were part of this experimental study. Of these, sixty-two were sick dogs that attended the CVMTH Gastroenterology and Endoscopy Service between January 2022 and March 2023. The criteria for inclusion of these dogs were the presence of gastrointestinal signs for at least three weeks consisting of vomiting, diarrhoea, weight loss, and/or anorexia/hyporexia. In addition, twenty-two healthy control (HC) dogs for elective or routine consultations were included in the study. The criteria for inclusion of the latter were a normal physical examination, negative serology for the most common vector-borne diseases in our geographical area (*Ehrlichia* spp., *Anaplasma* spp. and *Leishmania infantum*), negative faecal parasite detection, and the absence of any clinical signs, including digestive signs, or treatments for at least four months. Asymptomatic dogs with chronic diseases were also excluded.

Data on age, sex, fertile status, body weight, body condition score (BCS), muscle condition score (MCS), and canine inflammatory bowel disease activity index (CIBDAI) were collected on the day of the visit (Table 1). The CIBDAI [25] was used for clinical severity evaluation. BCS and MCS were evaluated based on the World Small Animal Veterinary Association (WSAVA) nutritional guidelines [26]. MCS was classified as normal (3), mild (2), moderate (1), or severe (0) muscle loss.

The diagnostic procedure in sick dogs consisted of a complete physical examination, followed by blood sample collection (by jugular or cephalic venipuncture) to rule out other systemic diseases presenting gastrointestinal symptoms (hypoadrenocorticism, pancreatitis, systemic parasites, or infectious diseases), trypsin-like immunoreactivity (TLI) to dismiss exocrine pancreatitis insufficiency, abdominal ultrasound, and a faecal parasitological analysis based on the determination of a modified Telemann method and MIF (merthiolate iodine formaldehyde concentration) technique [27] for the diagnosis of *Giardia* infection. Nine dogs were diagnosed with *Giardia* infection in the present research. Dogs that tested negative were subjected to a dietary trial based on at least two different elimination diets based on novel protein or hydrolysed protein.

Thirty-five dogs were diagnosed with FRE based on a good response to the diet after one month of administration. Dogs that did not respond to the diet change were subjected to endoscopy for biopsy and histological samples [24,28].

Eighteen dogs were diagnosed with IRE based on a poor response to the diet, good response to immunosuppressants, and evidence of an inflammatory process on histological samples. Other causes of gastrointestinal inflammation or systemic diseases were excluded.

Information about the consumed diet was collected for every dog included in the study before starting the dietary trial for diagnosis. The commercial diet's average composition (percentage) according to the manufacturer was (mean $\pm$ standard deviation): humidity, 9.5 $\pm$ 0.0; crude protein, 23.8 $\pm$ 4.7; crude fat, 14.1 $\pm$ 4.2; ash, 6.8 $\pm$ 1.3; crude fibre, 2.6 $\pm$ 1.4; nitrogen-free extractives, 53.4 $\pm$ 8.5; and metabolic energy/1000 g: 4327.2 $\pm$ 229.2). A detailed composition of each diet is presented in Supplementary Materials (Table S1).

Table 1. Signalment, epidemiological data, and clinical scores of healthy control (HC) dogs, food-responsive enteropathy (FRE) dogs, immunosuppressant-responsive enteropathy (IRE) dogs, and dogs parasitised with *Giardia* (GIA).

Variables	HC (n = 22)		FRE (n = 35)		IRE (n = 18)		GIA (n = 9)		RMSE [4]	p-Value
Age (months; mean[range])	60.23	[7–144]	57.31	[8–144]	76.17	[9–136]	64.22	[4–144]	42.066	0.4786
Sex (male/female)	9/13		19/16		6/12		7/2		0.494	0.1286
Fertile status (neutered/entire)	12/10		20/15		12/6		4/5		0.503	0.7351
Breed (pure/mixed)	15/7		30/5		13/5		8/1		0.411	0.3344
Weight (kg); (median [range])	19.40 ^{a}	[7.5–55]	10.60 ^{ba}	[2.6–45.5]	7.32 ^{ba}	[1.19–36.2]	12.60 ^{b}	[2.7–26.3]	12.358	0.0059
CIBDAI [1] (median [range])	0.00 ^{b}	[0]	5.00 ^{a}	[1–8]	6.00 ^{a}	[1–9]	6.00 ^{a}	[1–10]	2.004	0.0001
BCS [2] (1–9); (median [range])	5.00	[3–6]	5.00	[2–8]	4.00	[2–8]	3.00	[3–6]	1.467	0.2625
MCS [3] (0–3); (median [range])	3.00 ^{a}	[3]	3.00 ^{b}	[1–3]	2.50 ^{b}	[1–3]	2.00 ^{b}	[1–3]	0.538	0.0006
Faecal characteristics										
Purina® faecal score (1–7); (mean [range])	2.50 ^{b}	[1–5]	3.14 ^{ba}	[1–7]	3.56 ^{ba}	[1–7]	4.11 ^{a}	[2–6]	1.586	0.0497
Moisture (%); (mean [range])	67.53	[57.05–75.22]	68.40	[55.63–90.73]	71.15	[53.46–91.14]	71.51	[58.24–84.90]	7.651	0.3391
Fat (%); (mean [range])	6.53 ^{b}	[4.25–11.93]	6.76 ^{b}	[2.97–15.05]	10.02 ^{a}	[3.40–23.39]	10.40 ^{a}	[3.85–19.48]	3.839	0.0080

[1] CIBDAI: canine inflammatory bowel disease activity index; [2] BCS: body condition score; [3] MCS: muscle condition score; [4] RMSE: root mean squared error; p-value significant when <0.05. Values with different superscript letters (^{a,b}) statistically significant.

2.2. Faecal Sample Collection

Samples were collected before the start of the dietary trial. Three faecal samples were collected by the owners after defecation on sterile plastic containers for three consecutive days: two were kept under refrigeration until the day of the clinical practice, and the other one was collected the same morning of the visit (these faeces were divided into two different sterile containers). Then, faecal samples were distributed as follows: two refrigerated faecal samples and one fresh were used for parasite detection and the other fresh sample was stored at −20 °C for fatty acid determination. Faecal samples were graded using the Purina® faecal scoring chart [29] on the same day of the visit: from one (very hard and dry sample) to seven (watery sample).

2.3. Laboratory Analysis

2.3.1. Total Moisture, Fat Percentage, Total Fatty Acid, and Branched-Chain Fatty Acid Profile in Faecal Samples

Total moisture percentage was calculated by the difference between weighed fresh samples and lyophilised samples [16].

For fatty acid analysis, fat was initially extracted by the addition of a solvent mixture of dichloromethane–methanol (8:2) to lyophilised weighted (0.2 g) samples (Lyoquest, Telstar, Tarrasa, Spain). After homogenisation in a mixer mill (MM400, Retsch technology, Haan, Germany), samples were centrifuged at 10,000 rpm for 10 min. The upper layer containing lipids was collected and placed in 4 mL vials. This process was repeated a second time. After that, the solvents were evaporated in a nitrogen stream and vials were left for 24 h for drying. Total fat percentage (% on a dry matter basis) was calculated by the difference between the 4 mL vial containing extracted fat after evaporation of the solvent and the empty weighed vial with respect to the amount of lyophilised sample weight and multiplied by 100. After that, fatty acid methyl esters (FAMEs) were obtained by heating the lipids at 80 °C for 1 h in the presence of methanol:toluene:H_2SO_4 (88:10:2 by volume), as described elsewhere [30]. After esterification, FAMEs were extracted with hexane and separated in a gas chromatograph (HP 6890 Series GC System; Hewlett Packard, Avondale, PA, USA) after direct injection of the sample.

The gas chromatograph was provided with an automatic injector (hold at 170 °C), a flame ionisation detector (hold at 250 °C), and a capillary column (HP-Innowax polyethylene glycol, 30 m × 0.316 mm × 0.25 µm). After injection, the oven temperature was increased to 210 °C at a rate of 3.5 °C/min, then to 250 °C at a rate of 7 °C/min [16]. FAMEs were identified and quantified by comparing their retention times with those of authentic standards (Sigma–Aldrich, Alcobendas, Spain). Results are expressed as grams per 100 g of quantified fatty acids. For branched-chain fatty acid identification, bacterial acid methyl ester (BAME) mix was used (47080-U, Sigma Aldrich, Alcobendas, Spain).

The desaturase indices were calculated as the ratio of C14:1n-5 to C14:0, C16:1n-7 to C16:0, C18:1n-9 to C18:0, C20:1n-9 to C20:0 for Δ-9-desaturase, and C18:3n-6 to C18:2n-6 C18:4n-3 to C18:3n-3 for Δ-6-desaturase. Also, the total Δ-9 and Δ-6-desaturase indices were calculated with the following equations:

$$\Delta\text{-9-desaturase} = (\text{C14:1n-5/C14:0}) + (\text{C16:1n-7/C16:0}) + (\text{C18:1n-9/C18:0}) + (\text{C20:1n-9/C20:0})$$

$$\Delta\text{-6-desaturase} = (\text{C18:3n-6/C18:2n-6}) + (\text{C18:4n-3/C18:3n-3})$$

The elongase index was calculated as the ratio of C18:0 to C16:0 and C22:5 to C20:5.

2.3.2. Short-Chain Fatty Acid Analysis in Faecal Samples

Short-chain fatty acid determination was carried out as described elsewhere [16]. One millilitre of distilled water and two glass balls (2 mm Ø) were added to 0.5 g of frozen stool samples. The tubes were homogenised for 5 min at 30 Hz in an MM400 Mixer Mill (Retsch technology, Haan, Germany). Then, separation was conducted via centrifugation (10 min, 10,000 rpm) and the extraction was repeated three times. The superior phase was transferred into a vial, where 20 mM 4-methylvaleric acid solution and 25% phosphoric

acid were added to adjust pH at 2–3. Finally, the solution was placed in vials for gas chromatography injection.

Chromatographic analysis was performed using an Agilent 6850N GC system equipped with a flame ionisation detector (FID) (Agilent Technologies, Waldbronn, Germany). A fused-silica capillary column with a free fatty acid phase (DB-FFAP 125-3237, J&W Scientific, Agilent Technologies Inc., Santa Clara, CA, USA) of 30 m × 0.53 mm i.d. coated with a 0.50 µm-thickness film was used. Nitrogen was the carrier gas and maintained at a constant pressure of 15 psi. Initially, the oven temperature was 100 °C for 0.5 min, then raised to 180 °C at 8 °C/min and held for 1 min. After that, it increased to 200 °C at 20 °C/min and finally held at 200 °C for 5 min. The temperatures of the FID and the injection port were 240 °C and 200 °C, respectively. The flow rates of hydrogen, air, and nitrogen as makeup gases were 40, 300, and 30 mL/min, respectively. Data handling was carried out with HP ChemStation Plus software (Rev. B.04.02.98) (Agilent Technologies, Waldbronn, Germany). Pure standards were used for identification and quantification (Sigma-Aldrich, Alcobendas, Spain). An aqueous stock standard solution was prepared for each acid with a concentration of 400 mM for acetic acid, propionic acid, and n-butyric acid; 200 mM for n-valeric acid and i-valeric acid; and 100 mM for i-butyric acid.

2.4. Statistical Analysis

Data were analysed following a completely randomised design using the general linear model (GLM) procedure contained in SAS (version 9; SAS Inst. Inc., Cary, NC, USA).

Data are presented as the mean of each group and the standard deviation of the mean (RMSE), together with significance levels (p-values). Duncan's test was used to separate means. The differences between means were considered statistically significant at $p < 0.05$. Pearson correlations (among SCFAs and faecal fatty acids, or CIBDAI and the other variables) were calculated using the Statgraphics-19 program. A linear adjustment between variables was carried out by means of the Statgraphics-19 program (Statgraphics Centurion XIX, X64). Linear discriminant analysis was carried out to discriminate between groups (Statgraphics-19).

3. Results

Dog signalment (age, sex, fertile status, breed), body state (weight, BCS, or MCS) and faecal characteristics (Purina® faecal score, faecal moisture and fat content) are presented in Table 1.

Differences concerning age, BCS, and faecal moisture percentage were not statistically affected between groups. On the contrary, HC dogs differed from the other three groups presenting the lowest CIBDAI ($p = 0.0001$) and the highest MCS ($p = 0.0006$). These variables (CIBDAI and MCS) were similar in FRE, IRE, and GIA dogs. Furthermore, body weight ($p = 0.0059$) and Purina® faecal score ($p = 0.0497$) were different in HC dogs when compared to GIA dogs, whereas the FRE and IRE groups presented intermediate values.

The faecal fat content was the stool characteristic that was most affected among the different enteropathies: IRE and GIA dogs had faeces with higher fat percentages than HC and FRE dogs ($p = 0.008$).

Data concerning the total fatty acid profile are presented in Table 2.

HC dogs were differentiated from the other three groups presenting the lowest percentage of C18:0 ($p = 0.0002$), ∑SAT ($p = 0.0002$), and ECFAs ($p = 0.0001$); and the highest percentage of C18:1n-9 ($p = 0.0007$), ∑MUFA ($p = 0.0008$), ratios C16:1n-7/C16:0 ($p = 0.0001$), C18:1n-9/C18:0 ($p = 0.0003$), and Δ-9-desaturase index ($p = 0.0051$). Moreover, this group had the highest percentage of ∑PUFA ($p = 0.0032$) and ∑n-6 ($p = 0.0055$) compared to IRE and GIA dogs, although without statistical differences for FRE dogs.

FRE dogs also presented intermediate percentages of C18:2n-6 ($p = 0.0128$), C20:1n-9 ($p = 0.0336$), C14:1/C14:0 ($p = 0.0412$), C20:1n-9/C20:0 ($p = 0.0314$), and C18:3n-6/C18:2n-6 ($p = 0.0019$) between the HC and IRE groups.

Table 2. Faecal fatty acid profile (g per 100 g) of healthy control (HC) dogs, food-responsive enteropathy (FRE) dogs, immunosuppressant-responsive enteropathy (IRE) dogs and dogs parasitised with *Giardia* (GIA).

%	HC (*n* = 22)		FRE (*n* = 35)		IRE (*n* = 18)		GIA (*n* = 9)		RMSE [10]	*p*-Value
C14:0	1.231	b	1.393	b	1.773	a	1.503	ba	0.513	0.0122
C14:1	0.319		0.261		0.300		0.250		0.107	0.1591
C15:0	0.716		0.641		0.647		0.617		0.233	0.6041
C16:0	25.158	b	26.852	b	28.088	ba	30.564	a	4.078	0.0079
C16:1n-9	0.238		0.229		0.264		0.259		0.090	0.5417
C16:1n-7	1.968	a	1.571	b	1.054	c	1.834	ba	0.475	0.0001
C17:0	0.526		0.533		0.581		0.547		0.156	0.6829
C18:0	15.613	b	21.654	a	25.164	a	20.821	a	6.599	0.0002
C18:1n-9	25.155	a	19.165	b	17.640	b	19.338	b	6.111	0.0007
C18:1n-7	4.726		5.221		5.299		6.145		1.845	0.2838
C18:2n-6	16.343	a	14.099	ba	11.448	b	11.490	b	5.053	0.0128
C18:3n-6	0.098		0.114		0.126		0.109		0.040	0.1718
C18:3n-3	1.248	a	1.072	a	1.087	a	0.754	b	0.399	0.0240
C18:4n-3	0.061		0.057		0.063		0.048		0.021	0.3244
C20:0	0.686		0.764		0.700		0.890		0.255	0.1938
C20:1n-9	0.550	a	0.513	ba	0.412	b	0.503	ba	0.149	0.0336
C20:2	0.250		0.248		0.265		0.277		0.087	0.7631
C20:3n-6	1.540		1.666		1.467		1.136		0.582	0.1070
C20:4n-6	1.658		1.903		1.684		1.400		0.638	0.1562
C20:5n-3	0.577		0.437		0.440		0.489		0.167	0.1119
C22:4n-6	0.361		0.325		0.319		0.272		0.121	0.3011
C22:5n-3	0.978	ba	1.282	a	1.180	a	0.755	b	0.491	0.0160
∑SAT [1]	43.930	b	51.837	a	56.953	a	54.940	a	9.143	0.0002
∑MUFA [2]	32.956	a	26.961	b	24.969	b	28.329	b	6.401	0.0008
∑PUFA [3]	22.865	a	20.954	ba	17.813	bc	16.453	c	5.177	0.0032
∑n-6 [4]	20.000	a	18.107	ba	15.043	bc	14.408	c	5.046	0.0055
∑n-3 [5]	2.864	a	2.847	a	2.770	a	2.046	b	0.607	0.0020
C14:1n-5/C14:0	0.259	a	0.188	ba	0.170	b	0.166	b	0.102	0.0412
C16:1n-7/C16:0	0.078	a	0.059	b	0.038	c	0.060	b	0.018	0.0001
C18:1n-9/C18:0	1.611	a	0.885	b	0.701	b	0.929	b	0.780	0.0003
C20:1n-9/C20:0	0.802	a	0.672	ba	0.589	b	0.566	b	0.287	0.0314
C18:3n-6/C18:2n-6	0.006	b	0.008	ba	0.011	a	0.010	ba	0.005	0.0019
C18:4n-3/C18:3n-3	0.049		0.053		0.058		0.063		0.033	0.6247
Δ-9-desaturase [6]	2.750	a	1.803	b	1.497	b	1.721	b	0.825	0.0001
Δ-6-desaturase [7]	0.055		0.061		0.069		0.073		0.036	0.3470
Elongase C18/C16	0.621	c	0.806	ba	0.896	a	0.681	bc	0.236	0.0028
Elongase C22:5/C20:5	1.695		2.936		2.679		1.543		2.949	0.0943
OCFAs [8]	1.241		1.174		1.229		1.163		0.305	0.8098
ECFAs [9]	42.689	b	50.663	a	55.724	a	53.777	a	9.122	0.0001

[1] ∑SAT: sum of total saturated fatty acids; [2] ∑MUFA: sum of total monounsaturated fatty acids; [3] ∑PUFA: sum of total polyunsaturated fatty acids; [4] ∑n-6: sum of total n-6 fatty acids; [5] ∑n-3: sum of total n-3 fatty acids; [6] Δ-9-desaturase = (C14:1n-5/C14:0) + (C16:1n-7/C16:0) + (C18:1n-9/C18:0) + (C20:1n-9/C20:0); [7] Δ-6-desaturase = (C18:3n-6/C18:2n-6) + (C18:4n-3/C18:3n-3); [8] OCFAs: odd-chain fatty acids; [9] ECFAs: even-chain fatty acids; [10] RMSE: root mean squared error; *p*-value was significant when <0.05. Values with different superscripts ([a,b,c]) are statistically significant.

IRE dogs presented the highest faecal percentage of C14:0 ($p = 0.0122$), but the lowest of C16:1n-7 ($p = 0.0001$). This C16:1n-7 was the fatty acid that differed among the HC, FRE, and IRE groups, whereas GIA presented intermediate percentages of C16:1n-7 between HC and FRE dogs ($p = 0.0001$). A similar trend was observed for the ratio C16:1n-7/C16:0, which was different between HC, FRE, and IRE dogs, whereas the GIA group presented similar percentages to FRE ($p = 0.0001$). On the contrary, IRE dogs had higher elongase C18/C16 ($p = 0.0028$) than HC and GIA and presented similar values to FRE dogs. Moreover, IRE

dogs presented similar percentages of C14:0, C16:0, C18:0, C18:1n-9, C18:2n-6 and C20:1n-9, ∑SAT, ∑MUFA, ∑PUFA, ∑n-6, Δ-9, C18:3n-6/C18:2n-6 ratio to GIA dogs.

However, GIA dogs differed in the percentage of C18:3n-3 (α-linolenic acid) ($p = 0.0240$) and ∑n-3 ($p = 0.0020$), compared to IRE, HC, and FRE dogs and in C22:5n-3 ($p = 0.0160$) compared to FRE and IRE dogs. Also, the GIA group had a higher percentage of C16:0 ($p = 0.0079$) than the HC and FRE groups.

No statistical differences were observed for the percentages of C14:1, C15:0, C16:1n-9, C17:0, C18:1n-7, C18:3n-6, C18:4n-3, C20:0, C20:2, C20:3n-6, C20:4n-6 (arachidonic acid; AA), C20:5n-3 (eicosapentaenoic acid; EPA), C22:4n-6, total Δ-6-desaturase, elongase C22:5/C20:5, or OCFAs between the experimental groups.

Table 3 presents the long branched-chain fatty acid profile of the experimental groups.

Table 3. Faecal long branched-chain fatty acid profile (g per 100 g) of healthy control (HC) dogs, food-responsive enteropathy (FRE) dogs, immunosuppressant-responsive enteropathy (IRE) dogs and dogs parasitised with *Giardia* (GIA).

Variable	HC ($n = 22$)		FRE ($n = 35$)		IRE ($n = 18$)		GIA ($n = 9$)		RMSE [3]	*p*-Value
Iso C15:0	11.57	b	11.21	b	13.41	ba	14.38	a	3.473	0.0313
Anteiso C15:0	57.87	a	54.76	ba	47.80	bc	43.09	c	12.121	0.0054
Iso C16:0	4.92		4.30		4.80		6.05		1.755	0.0651
Iso C17:0	20.40	b	24.97	ba	28.51	a	30.26	a	10.119	0.0333
Anteiso C17:0	5.24		4.76		5.48		6.21		1.983	0.2195
Total iso	36.89	c	40.49	bc	46.72	ba	50.69	a	11.291	0.0054
Total anteiso	63.11	a	59.51	ba	53.28	bc	49.31	c	11.291	0.0054
Iso/anteiso	0.61	b	0.74	b	1.05	a	1.15	a	0.433	0.0017
∑OCBFAs [1]/ ∑ECBFAs [2]	20.55	ba	27.08	a	22.95	ba	17.19	b	9.360	0.0122

[1] ∑OCBFAs: odd-chain branched fatty acids (iso C15:0 + anteiso C15:0 + iso C17:0 + anteiso C17:0); [2] ∑ECBFAs: even-chain branched fatty acids (iso C16:0); [3] RMSE: root mean squared error; *p*-value was significant when <0.05. Values with different superscripts (^{a,b,c}) are statistically significant.

IRE and GIA dogs presented higher ratio iso/anteiso compared to HC and FRE dogs ($p = 0.0017$) and higher iso C17:0 compared to HC dogs, whereas FRE dogs presented intermediate values ($p = 0.0333$). Moreover, the GIA group presented lower levels of anteiso C15:0 ($p = 0.0054$) and total anteiso ($p = 0.0054$) and higher levels of total iso ($p = 0.0054$) compared to HC and FRE dogs. Also, the FRE group showed a higher ∑OCBFA/∑ECBFA ratio when compared to the GIA group ($p = 0.0122$); however, this ratio did not differ between FRE, HC, or IRE dogs. The percentages of iso C16:0 and anteiso C17:0 were not statistically different among the groups of dogs.

Faecal short-chain fatty acid profiles are shown in Table 4. IRE dogs presented lower acetic acid (C2) ($p = 0.0007$) and total ∑SCFAs ($p = 0.0008$) when compared to the other three groups. Propionic acid (C3) of faeces was also different between groups and IRE dogs had lower levels of this SCFA compared to HC dogs, whereas FRE and GIA dogs showed intermediate values ($p = 0.0022$). No differences were observed in the faecal content of isobutyric acid (iC4), butyric acid (C4), isovaleric acid (iC5), or valeric acid (C5) among the experimental groups.

Significant correlations of fatty acid profile, BCFAs, and SCFAs with clinical scores and faecal characteristics are shown in Table 5. CIBDAI and Purina® faecal scores were the variables that correlated to a greater extent with fatty acids.

Table 4. Faecal short-chain fatty acid (SCFAs) profile (mM) of healthy control (HC) dogs, food-responsive enteropathy (FRE) dogs, immunosuppressant-responsive enteropathy (IRE) dogs and dogs parasitised with *Giardia* (GIA).

Variable	HC (*n* = 22)		FRE (*n* = 35)		IRE (*n* = 18)		GIA (*n* = 9)		RMSE [7]	*p*-Value
C2 [1]	3.196	a	3.113	a	1.630	b	2.742	a	1.134	0.0007
C3 [2]	3.019	a	2.046	ba	1.326	b	2.111	ba	1.222	0.0022
iC4 [3]	0.140		0.154		0.090		0.122		0.108	0.3451
C4 [4]	1.319		1.131		0.834		1.163		0.763	0.3577
iC5 [5]	0.259		0.312		0.184		0.244		0.205	0.2910
C5 [6]	0.141		0.119		0.058		0.070		0.197	0.6709
Total SCFAs	8.075	a	6.850	a	4.111	b	6.451	a	2.632	0.0008
∑C2 + C3 + C4	7.535	a	6.291	a	3.791	b	6.016	a	2.452	0.0008
∑iC4 + iC5	0.399		0.465		0.274		0.365		0.309	0.3045

[1] C2: acetic acid; [2] C3: propionic acid; [3] iC4: isobutyric acid; [4] C4: butyric acid; [5] iC5: isovaleric acid; [6] C5: valeric acid; [7] RMSE: root mean squared error; *p*-value was significant when <0.05. Values with different superscripts ([a,b]) are statistically significant.

Table 5. Significant correlation coefficients between faecal fatty acids (%), branched-chain (%), and short-chain fatty acids (mM) and canine inflammatory bowel disease activity index (CIBDAI), muscle condition score (MCS) or faecal characteristics (Purina® faecal score, fat, moisture contents).

Variable	CIBDAI		MCS		Purina® Faecal Score		Faecal Moisture (%)		Faecal Fat (%)	
Faecal fatty acids										
C14:1	–0.18		0.05		–0.33	b	–0.14		–0.27	a
C15:0	–0.11		0.27	a	–0.25	a	–0.22		–0.23	a
C16:1n-7	–0.39	b	0.25	a	–0.08		0.08		–0.34	b
C18:0	0.40	b	–0.14		0.24	a	0.18		0.16	
C18:2n-6	–0.32	b	0.11		–0.18		–0.14		–0.24	a
C20:3n-6	0.08		0.08		–0.28	b	–0.22	a	–0.21	
C20:4n-6	0.13		0.00		–0.27	a	–0.12		–0.21	
C20:5n-3	–0.22	a	0.14		–0.33	b	–0.05		–0.02	
C22:5n-3	0.33	b	–0.26	a	–0.01		–0.07		0.22	
∑SAT [1]	0.36	b	–0.13		0.22	a	0.16		0.15	
∑PUFA [2]	–0.28	a	0.10		–0.26	a	–0.18		–0.30	b
∑n-6 [3]	–0.30	b	0.12		–0.25	a	–0.18		–0.31	b
C16:1n-7/C16:0	–0.46	b	0.27	a	–0.20		–0.04		–0.33	b
Δ-9-desaturase [4]	–0.47	b	0.21		–0.12		–0.08		–0.09	
Δ-6-desaturase [5]	0.32	b	–0.10		0.11		0.07		–0.14	
Elongase C18/C16	0.38	b	–0.16		0.25	a	0.18		0.13	
Elongase C22:5/C20:5	0.29	b	–0.24		0.22		0.07		0.15	
Branched-chain fatty acids										
Anteiso C15:0	–0.04		0.12		–0.23	a	–0.20		–0.12	
Short-chain fatty acids										
C2 [6]	–0.20		0.10		–0.11		–0.01		–0.27	a
iC4 [7]	0.06		–0.04		–0.16		–0.25	a	–0.04	

[1] ∑SAT: sum of total saturated fatty acids; [2] ∑PUFA: sum of total polyunsaturated fatty acids; [3] ∑n-6: sum of total n-6 fatty acids; [4] Δ-9-desaturase = (C14:1n-5/C14:0) + (C16:1n-7/C16:0) + (C18:1n-9/C18:0) + (C20:1n-9/C20:0); [5] Δ-6-desaturase = (C18:3n-6/C18:2n-6) + (C18:4n-3/C18:3n-3); [6] C2: acetic acid; [7] iC4: isobutyric acid. [a] Significant at <0.05 probability level; [b] significant at <0.01 probability level.

The greatest number of correlations were observed for C15:0, C16:1n-7, ∑PUFA, ∑n-6 and C16:1n-7/C16:0. CIBDAI correlated positively with faecal ∑SAT (r = 0.36, p = 0.0012), C18:0 (r = 0.40, p = 0.0002), C22:5n-3 (r = 0.33, p = 0.0024), Δ-6-desaturase (r = 0.32, p = 0.0040), elongase C18/C16 (r = 0.37, p = 0.0006) and elongase C22:5/C20:5 (r = 0.29, p = 0.0090). On the contrary, a negative correlation was observed between CIBDAI with C16:1n-7 (r = −0.39, p = 0.0003), C18:2n-6 (r = −0.32, p = 0.0030), C20:5n-3 (r = −0.22, p = 0.0475), ∑PUFA (r = −0.27, p = 0.0123), ∑n-6 (r = −0.30, p = 0.0062), C16:1n-7/C16:0 (r = −0.45, p = 0.0001) and Δ-9-desaturase (r = −0.46, p = 0.0001).

C14:1 and C15:0 correlated negatively with Purina® faecal score (r = −0.33, p = 0.0025; r = −0.25, p = 0.0249) and faecal fat (r = −0.27, p = 0.0205; r = −0.23, p = 0.0415), respectively. On the contrary, a positive correlation between C15:0 and MCS was found (r = 0.27, p = 0.0270). The C16:1n-7 percentage and C16:1n-7/C16:0 ratio correlated positively with MCS (r = 0.25, p = 0.0431; r = 0.27, p = 0.0329) and negatively with faecal fat (r = −0.34, p = 0.0029; r = −0.33, p = 0.0041), respectively. A positive correlation was found between C18:0 and Purina® faecal score (r = 0.24, p = 0.0298). C18:2n-6 correlated negatively with fat percentage (r = −0.24, p = 0.0341). Also, a negative correlation was found between C20:3n-6 and Purina® faecal score (r = −0.28, p = 0.0100) and faecal humidity (r = −0.22, p = 0.0439). Moreover, C20:5n-3 correlated negatively with Purina® faecal score (r = −0.33, p = 0.0030) and C22:5n-3 with MCS (r = −0.26, p = 0.0374). Both, ∑PUFA and ∑n-6 correlated negatively with Purina® faecal score (r = −0.26, p = 0.0203; r = −0.25, p = 0.0230), and fat percentage (r = −0.30, p = 0.0095; r = −0.31, p = 0.0072), respectively. On the contrary, ∑SAT and elongase C18/C16 correlated positively with Purina® faecal score (r = 0.22, p = 0.0486; r = 0.25, p = 0.0256).

The BCFA profile also correlated with some faecal characteristics. Thus, anteiso C15:0 correlated negatively with Purina® faecal score (r = −0.23, p = 0.0325).

Concerning significant correlations between the SCFAs and faecal characteristics, only negative relationships were found between C2 and faecal fat content (r = −0.27, p = 0.0273), as well as between iC4 and moisture content of the stool (r = −0.25, p = 0.0352).

The correlations between BCFAs and SCFAs are presented in Figure 1. C3 was the SCFA that correlated the most with BCFAs. A positive correlation was found between C3 and anteiso C15:0 (r = 0.44, p = 0.0002) and total anteiso (r = 0.43, p = 0.0003), whereas a negative one was found between C3 with iso C17:0 (r = −0.35, p = 0.0030) and total iso (r = −0.43, p = 0.0003).

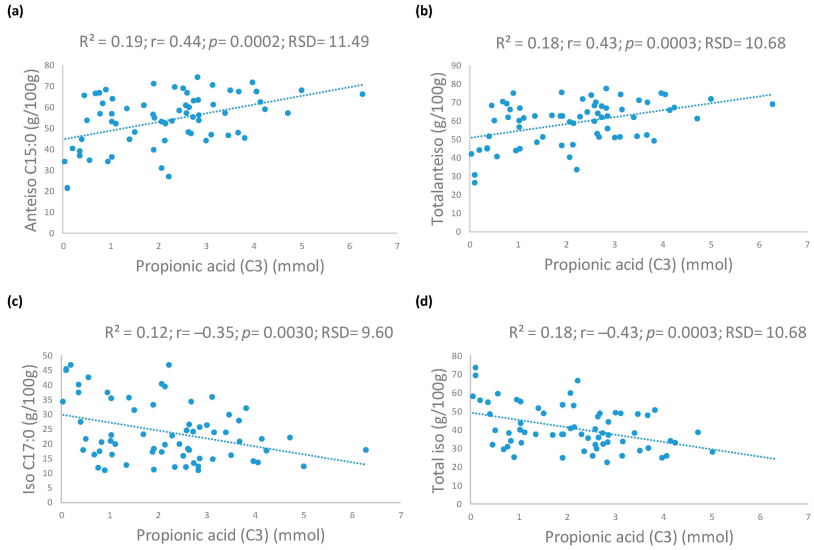

Figure 1. Linear adjustments between (**a**) propionic acid (C3) and anteiso C15:0; (**b**) propionic acid (C3) and total anteiso; (**c**) propionic acid (C3) and iso C17:0; (**d**) propionic acid (C3) and total iso in faecal samples.

Additionally, a discriminant analysis was carried out in order to evaluate the discriminant potential of the studied variables (Figure 2). The most determinant variables that contributed to functions 1 and 2 in the canonical discriminant functions were C16:1n-7/C16:0, C18:1n-9/C18:0, ∑n-3, and C2. Taking into consideration this multivariable analysis, the IRE and GIA groups were the ones that differed the most from the HC and FRE groups, while the FRE and HC groups showed some overlap between them.

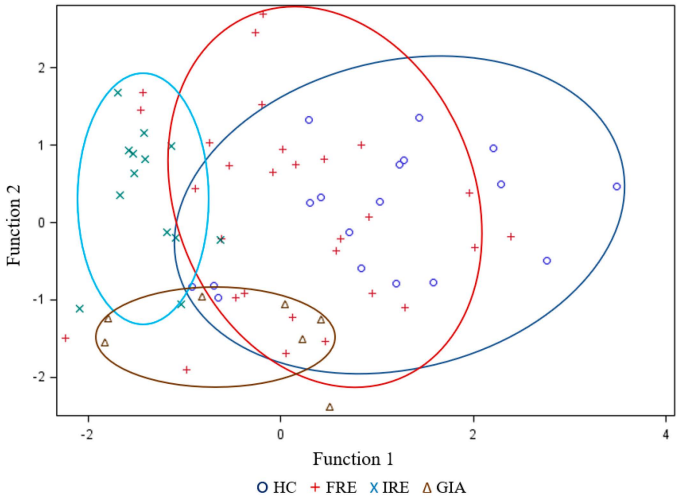

Figure 2. Linear discriminant analysis for faecal samples of healthy control (HC; dark blue) dogs, food-responsive enteropathy (FRE; red) dogs, immunosuppressant-responsive enteropathy (IRE; light blue) dogs and dogs parasitised with *Giardia* (GIA; brown).

The validation results of the discriminant functions (Table 6) showed that 25% of stool samples were not correctly classified, giving a 75% success rate. For the FRE group, 41% were assigned to the correct group; however, 24% were considered GIA, 14% IRE, and 21% HC. The group with the lowest percentage of wrong classifications was GIA (100% correct assignments). However, they had a high percentage of false positives (24% were assigned to FRE, 17% to HC and 8% to IRE). Overall, 92% of faecal samples from IRE dogs were correctly classified, but 8% were wrongly assigned to the GIA group. Finally, 17% of HC samples were considered FRE and 17% GIA.

Table 6. Classification of healthy control (HC) dogs, food-responsive enteropathy (FRE) dogs, immunosuppressant-responsive enteropathy (IRE) dogs, and dogs parasitised with *Giardia* (GIA) (% assigned correctly) according to discriminant functions (cross-validation).

	FRE	GIA	IRE	HC	TOTAL (%)
FRE	41.38	24.14	13.79	20.69	100
GIA	0	100	0	0	100
IRE	0	7.69	92.31	0	100
HC	16.67	16.67	0	66.67	100

4. Discussion

This is the first study to evaluate changes in faecal characteristics and fatty acid profile among FRE, IRE, GIA and HC dogs. The study was focused on stool samples, since the objective was to search for markers that would establish differences between these chronic gastrointestinal diseases and whose collection would be as little invasive as possible for this purpose.

In the present research, IRE and GIA dogs presented higher fat content in the stools. Taking into account that the fat percentage in the diet was similar in all groups, this result could be attributed to a possible indicator of worse nutrient absorption, probably caused

by greater intestinal damage. *Giardia* is a primary alternative diagnosis for CIEs, since it can cause acute or chronic diarrhoea. Despite this, many hosts are still asymptomatic, leading to a debate if the parasite alone is responsible for the gastrointestinal signs or other factors such as dysbiosis, drugs, antimicrobial treatments, infections, and nutritional or environmental changes are implied [31]. However, mechanical mucosal damage caused by *Giardia* infection can lead to fat malabsorption, which in turn can cause intestinal steatosis and increased lipid transit into the distal small intestine and colon [31]. This result was accompanied by worse stool consistency based on Purina® faecal score. On the other hand, the increase in faecal fat in dogs with IRE could be due to a greater disruption of bile acid metabolism resulting from alterations in their transporters or the dysbiosis process [32]. One of the primary functions of bile acids is acting as micelle-forming surfactants that solubilise lipids, facilitating their absorption in the intestine [33]. Alterations in the metabolism of these bile acids have been described in human patients with IBD, especially Crohn's disease [32], as well as in CIE dogs [18,34], as a consequence of dysbiosis and alterations in the transporters responsible for their reabsorption. Only a very small portion of these acids is not reabsorbed and passes into the colon, where bacteria convert primary bile acids into secondary ones [18]. However, when losses exceed a certain threshold and the compensatory synthetic capacity of the liver is limited, there is a reduction in the intraduodenal pool of bile acids, limiting micelle formation and thereby resulting in deficient lipid digestion with increased faecal fat content [32]. Fat malabsorption is accompanied by an increase in colonic fluid secretion, with fatty acids significantly involved in this phenomenon [32].

Interestingly, although no significant differences in BCS were observed between groups, sick dogs did present lower MCS compared to HC, with the GIA group being the most affected one, followed by IRE. It has been reported that the profile of circulating fatty acids available for metabolic use could influence MCS values, since fat is the major source of energy for the muscle. Thus, a relationship between muscle mass indices and fatty acid profile has been observed in humans [35], with the polyunsaturated/saturated ratio being the most positively associated with higher muscle mass indices. Furthermore, a direct relationship has been reported between the loss of muscle mass and chronic inflammation, related to a greater synthesis of products involved in the inflammatory process [36]. Polyunsaturated fatty acids play a fundamental role in the development of inflammatory diseases through the synthesis of mediators such as prostanoids, leukotrienes, lipoxins, etc. [37]. The lower proportion of ∑PUFA, mainly ∑n-6, found in faecal samples of IRE and GIA dogs could indicate the higher use of these fatty acids for the synthesis of compounds involved in the inflammation process and therefore a lowered source of energy for the muscle that could lead to greater loss of mass. These results agree with a higher CIBDAI score in sick dogs, mainly IRE and GIA dogs, indicative of a worse clinical status in these animals.

Moreover, sick dogs also displayed alterations in fatty acid metabolism, characterised by higher ∑SAT, lower ∑MUFA and lower Δ-9-desaturase index. These results coincide with previous studies in FRE dogs [16] and CIE dogs [17]. In cases of active human ulcerative colitis, studies have indicated a decrease in the gene expression of Δ-9-desaturase [38], while in mice with colitis, there was a significant inhibition observed in the liver expression of this enzyme [39,40]. However, in the present study, it is interesting to highlight that although IRE dogs did not present significant changes in the desaturase indices (Δ-9) compared to the other groups of sick animals, this group appeared to be the one with the greatest alteration in fatty acid profile. Thus, IRE dogs presented a clear difference in the percentage of C16:1n-7 and ratio of C16:1n-7/C16:0, which were the lowest compared to the other groups. In consequence, this C16:1n-7 could be an interesting fatty acid to consider in the differentiation of IRE dogs. C16:1n-7 has received much attention owing to its anti-inflammatory and metabolic properties, being described as a lipid hormone. The anti-inflammatory character of this fatty acid suggests that its release is a crucial regulatory step for the initiation of pathways aimed at reducing inflammatory damage [41]. Akawaza et al. [42] reported higher serum levels of this fatty acid in humans with Crohn's disease,

and they found a positive correlation with the severity clinical index. However, in the present research, in which faeces samples were evaluated, a negative relationship between C16:1n-7 and CIBDAI score was found. C16:1n-7 may have anti-inflammatory effects on colon inflammation by modulating the IL-6/STAT3 and TNF-α/NF-κB pathways in mouse models [43]. The lower levels of C16:1n-7 in faecal samples in dogs with IRE could in part be explained by a possible lower Δ-9-desaturase activity at the intestinal membrane due to the gut damage [44] or down-regulation by other fatty acids [45,46]. More research is needed to understand changes in this fatty acid in IRE dogs.

On the other hand, IRE dogs presented the highest levels of C18:3n-6/C18:2n-6 (as an indicator of Δ-6-desaturase) in the stool, and this parameter differed from the HC group, but not from the others. This fact, added to a diminution in linoleic acid (C18:2n-6), a main precursor of the n-6 route, suggests that C18:2n-6 is being used to synthesize longer-chain n-6-PUFAs, which are major components of gut cell membranes [47,48], or the synthesis of compounds involved in the inflammatory process [37]. This result agrees with previous studies in the erythrocyte membrane of CIE dogs [17] and humans with Crohn's disease [49] where the metabolism of linoleic acid by means of the Δ-6-desaturase enzyme is accelerated to a greater extent compared to that of healthy individuals. Thus, lower levels of ∑PUFA and ∑n-6 in faeces (mainly observed in IRE and GIA dogs) were correlated with higher levels of Purina® faecal score and fat percentage, indicators of a worse clinical state. This result aligns with a study where n-6 was negatively correlated with canine chronic enteropathy clinical activity index (CCECAI) scores, although in blood membranes [17]. In addition, the elongase C22:5n-3/C20:5n-3 ratio positively correlated with CIBDAI scores, suggesting that the demand for these fatty acids was higher in animals with more marked intestinal damage. Polyunsaturated fatty acid oxidation products generated have a potent influence on inflammation processes. Thus, oxylipins formed from n-3 PUFAs have more potent anti-inflammatory properties than those formed from n-6 [50]. Therefore, these fatty acids may be consumed as the clinical activity of the disease increases for the repair of intestinal membranes or the synthesis of derivatives involved in the inflammation [37].

Although the GIA group showed similar trends and values for desaturase indices, they differed from the other three groups, presenting the lowest α-linolenic acid (C18:3n-3) and ∑n-3 in the stool. C16:0, C18:0, and C18:1 are the major fatty acids in *Giardia*; however, small amounts of C18:3n-3 have been observed in their composition [51]. It might be possible that GIA dogs presented lower levels of these acids in their faeces to incorporate them into their structure and continue their cycle. Moreover, as suggested in the IRE group, these fatty acids could be used to synthesise pro-resolving mediators during chronic inflammation [37].

Concerning the long BCFAs, these saturated fatty acids are classified as iso (iBCFAs) or anteiso (aiBCFAs) depending on the position of their methyl branch [11]. They have been used as markers of ruminal colonization as one of the main components of ruminal bacteria membranes [52]. In the present research, IRE and GIA dogs had lower anteiso BCFAs than HC and FRE dogs, but higher iso forms. Similar results were observed in the study of Xin et al. [10], where total iso and ratio of iso/anteiso BCFAs were more elevated in the faeces of diarrheic calves compared to healthy controls. However, to the authors' knowledge, there is no further information, and this study provides for the first time the long BCFA profile in faecal samples of dogs diagnosed with CIEs and *Giardia* infection. Yan et al. [53] showed that anteiso BCFAs presented a more potent inhibitory effect on proinflammatory interleukin 8 production than iso forms in a group of intestinal cells cultivated with BCFAs under lipopolysaccharide stimulation. Both anteiso and iso forms presented anti-inflammatory properties through inhibition of nuclear factor kappaB expression, but anteiso forms reported comparable inhibitory proinflammatory effects to docosahexaenoic acid (DHA) and EPA [53]. Hence, lower anteiso BCFAs in faecal samples of IRE and GIA dogs could be caused by a different bacterial population or a greater utilization of anteiso forms to counteract the inflammatory process that seems to be more severe in these pathologies. This hypothesis could be valid, considering that clinical

severity increases from FRE to IRE [54]. Nevertheless, more studies regarding the BCFA profile are needed.

Unlike BCFAs, SCFAs have been more studied to date since they are key prebiotics for maintaining intestinal health, exerting anti-inflammatory, anticancer and immunomodulatory functions in the gut [55]. Recent investigations have reported alterations of SCFAs in dogs with CIEs [16,19] and gastrointestinal diseases such as IBD and colorectal cancer in humans and rodent models [55]. Previous research found lower content of C2, C3, and ∑SCFAs in FRE dogs compared to healthy animals [16]. However, the present research provides for the first time an evaluation of SCFAs between FRE, IRE, and GIA dogs. It is interesting to highlight that IRE differed from the other three groups, presenting the lowest levels of C2, ∑SCFAs, and ∑C2 + C3 + C4 and the lowest levels of C3 compared to HC dogs, but not to FRE or GIA dogs, which presented intermediate values. This result could be attributed to a higher degree of dysbiosis in this IRE group compared to the others, in which reductions of the Bacteroidetes and Firmicutes phyla (SCFAs producers) have been observed [56]. However, only a differential abundance of bacteria belonging to the phylum Proteobacteria was found between samples of intestinal mucosal microbiota in FRE and IRE dogs, whereas no differences in the overall species richness were observed [57]. Although it seems that both groups have similar dysbiotic microbiomes, studies evaluating both lipid profiles and microbiome differences in faecal samples between groups are needed to prove or disprove this hypothesis. In the present research, however, lower levels of these microbial metabolites in IRE dogs could suggest a greater disruption in their bacterial producers. This could lead to a new reclassification of this category in the future [58]. On the other hand, some research points out that dogs with giardiasis do not experience significant dysbiosis [59]. *Giardia* has an impact on gut microbiota that is enriched in protective taxa against gut inflammation and depleted in lipid-producing taxa, which limits *Giardia* infection and reduces host inflammation [31,59]. This supports the idea that there may be a delicate relationship between this parasite and the gut microbiota.

Another result to comment on from the present research is related to OCFAs. C15:0 and C17:0 are OCFAs that can be endogenously synthesised from propionic acid (C3) [12]. In the present research, these OCFAs were not significantly differently affected between the different groups, despite the fact that C15:0 was related to better faecal characteristics (lower Purina® score and faecal fat content). This result does not coincide with previous research in which healthy dogs had higher faecal C15:0 when compared to sick animals [16]. The authors suggested that higher levels of this fatty acid could be attributed to greater microbiome activity [60,61]. However, discrepancies found in the current investigation could be due to the fact that this fatty acid can also be obtained from the diet, which was not exactly the same in both experiments. Jenkins et al. [13] found that circulating C15:0 was linearly related to dietary C15:0 intake, and hardly any contribution was observed on C15:0/C17:0 levels by the gut microbiota.

Concerning the BCFAs, positive correlations were found between C3 and anteiso C15:0 and total anteiso, whereas the relationship was negative with iso C17:0, total iso, and ratio iso/anteiso. These BCFAs are predominant in certain bacteria [62] and are mainly synthesised from malonyl-CoA esters that carry methyl branches, which can be obtained from the degradation of branched amino acids [63]. Thus, although C3 and iso and anteiso BCFAs come from different substrates, both are related to microbial action. The IRE group, which had the significantly lowest C3 content, also presented significantly lower anteiso BCFAs but higher iso forms compared to healthy individuals. Moreover, similar relationships have been found between these BCFAs and clinical parameters such as Purina® faecal score, suggesting that anteiso forms in faecal samples are associated with a better clinical state compared to iso. Since the function of these fatty acids is largely unknown in mammals, more studies are necessary to understand these changes.

In addition, CIBDAI correlations demonstrated that sick dogs presented an altered metabolic state with lower desaturase activity (Δ-9-desaturase, responsible for the conversion of C16:1n-7/C16:0 and C18:1n-9/C18:0) and thus lower C16:1n-7, and higher elongase

activity and Δ-6-desaturase, responsible for a faster metabolisation of linoleic acid and α-linolenic acid for long-PUFA production.

Finally, the discriminant analysis of the lipid profile to differentiate different diseases would indicate that the IRE and GIA groups were the ones best differentiated from the rest of the groups considering the variables C16:1n-7/C16:0, C18:1n-9/C18:0, ∑n-3, and C2. FRE dogs differentiated the worst from the rest of the groups. However, only 13.79% of false negatives from FRE dogs were confused with IRE dogs. Therefore, the lipid profile and specifically the desaturase index seem to be interesting indicators of the state of severity and could be a variable to consider to establish more precise diagnosis and dietary treatments between FRE and IRE dogs.

5. Conclusions

In conclusion, the faecal fatty acid profile showed alterations between dogs affected by different chronic inflammatory enteropathies and *Giardia* infection compared to healthy dogs. Among sick animals, IRE dogs showed clear differences in the faecal percentage of C16:1n-7 and ratio of C16:1n-7/C16:0 and also differed in the content of C2 from the other groups and in C3 from HC dogs. However, FRE dogs did not present such marked differences in SCFAs or BCFAs. Future studies combined with microbiota analysis or systemic changes will allow for a better understanding of the use of lipid compounds and how to utilize them to improve earlier diagnosis and dietary treatment response.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/ani14121825/s1. Table S1. General composition of diets of healthy control dogs (HC), food-responsive enteropathy dogs (FRE), immunosuppressant-responsive enteropathy dogs (IRE) and dogs parasitized with *Giardia* (GIA).

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4.2.2 Artículo 4: Higueras *et al.*, en revisión

Associations between hematological variables and fecal fatty acid profile: new insights into the metabolic changes in dogs with chronic inflammatory enteropathies

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Research in Veterinary Science. Índice de impacto (2023) = 2,2. **Q1.**

Este artículo investiga el objetivo 5 de la presente tesis doctoral sobre las diferencias entre los perros con FRE y aquellos con IRE en el perfil hematológico y en los índices inflamatorios sistémios, así como su relación con el perfil de ácidos grasos fecales.

Associations between hematological variables and fecal fatty acid profile: new insights into the metabolic changes in dogs with chronic inflammatory enteropathies

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ABSTRACT

The diagnosis of food-responsive enteropathy (FRE) and immunosuppressant-responsive enteropathy (IRE) relies on dietary response followed by other invasive methods, since the pathogenesis is unknown. This study aimed firstly, to evaluate differences in main blood cells, biochemical profile, and hematological inflammatory indices between (FRE) and (IRE) dogs; and secondly, to study and quantify possible associations between these blood variables and the fecal lipid profile to better understand both diseases. Dogs with IRE showed higher platelet counts, plateletcrit, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio, and systemic immune-inflammation index (SII) compared to dogs with FRE. Lymphocytes correlated with C16:1n-7 and the C16:1n-7/C16:0 ratio, with significant linear responses differing between FRE and IRE dogs. Positive correlations between fecal fat levels and immune cells or systemic indices of inflammation were observed. Acetic acid (C2) correlated and followed a significant linear response with platelet counts, and the SII index. Fecal C2 levels higher than 2 mM were associated with platelet counts within the reference range in FRE dogs; whereas the opposite was observed in IRE. Blood glucose and fecal short-chain fatty acids also presented significant correlations, quantified by regression equations in FRE dogs. Significant changes were found in NLR as fecal iso-C15:0 levels increased, differing between FRE and IRE dogs. This research confirms that systemic changes in FRE and IRE dogs are directly related to the metabolism of fatty acids. Combining the hemogram or inflammatory indices with fecal fatty acids would allow these two enteropathies to be distinguished more accurately and establish a more appropriate dietary treatment.

Key words: blood-cell inflammatory ratios, food-responsive enteropathy, immunosuppressant-responsive enteropathy, palmitoleic acid, acetic acid, butyric acid.

1. INTRODUCTION

Chronic inflammatory enteropathies (CIEs) are processes characterized by an exacerbated pro-inflammatory reaction due to inappropriate recognition of metabolites in the intestinal lumen (Chantawong, 2023). The etiology is unknown but it is believed that idiopathic inflammation occurs in genetically predisposed individuals, with diet, environmental factors, and microbiota playing determining roles (Dandrieux, 2016).

Studies in humans suggest that alterations in lipid metabolism could contribute to the development of CIEs, as lipids are crucial for membrane fluidity, structure, integrity and eicosanoid production (Shores et al., 2011) which are involved in immunoregulation (Calder et al., 2002). Thus, specific research on the fatty acid profile of the intestinal mucosa in people with CIEs has revealed a higher proportion of saturated fatty acids (SFA) and arachidonic acid (Nishida et al., 1987), which have a pro-inflammatory nature (Harvey et al., 2010; Nishida et al., 1987; Shores et al., 2011) to the detriment of a decrease in monounsaturated fatty acids (MUFAs) (Fernández-Bañares et al., 1997; Nishida et al., 1987). MUFAs have been associated with greater membrane stability and fluidity (Maulucci et al., 2016), as well as anti-inflammatory properties (Howe et al., 2022). Among them, C16:1n-7 (palmitoleic acid) has gained attention in recent years, for its role in modulating lymphocyte proliferation (Zhou et al., 2021), production of pro-inflammatory cytokines (Passos et al., 2016; Schirmer et al., 2016), and regulation of insulin sensitivity and lipogenic activity (Bermúdez et al., 2022).

In veterinary medicine, the pathogenesis of these chronic inflammatory diseases is also unknown. Diagnosis is based on the response to a specific diet and the administration of immunosuppressants, requiring an histological study to confirm the existence of an inflammatory infiltrate (Jergens and Heilmann, 2022). Cases that respond favorably to an elimination diet are classified as food-responsive enteropathy (FRE) (Dandrieux, 2016); while those that do not respond and require endoscopy to confirm inflammation and immunosuppressant treatment are classified as immunosuppressant-responsive enteropathy (IRE) (Cerquetella et al., 2020; Dandrieux, 2016). Among the studies based on the lipid profile in dogs with CIEs, recent investigations (Crisi et al., 2021) have found reduced Δ-9-desaturase indices and increased elongases in red blood cell membrane fatty acids including populations of FRE and IRE dogs. Recently changes in the fecal C16:1n-7/C16:0 ratio and

palmitoleic acid levels have been reported, with the latter showing the most significant differences between different types of enteropathies (Higueras et al. 2021; Higueras et al., 2024). Alterations in short-chain fatty acid (SCFA) production as indicators of microbial activity have also been reported in IRE and FRE dogs (Higueras et al., 2021; Higueras et al. 2024; Minamoto et al., 2019). However, the reasons for these differences in fatty acids and their relationship with systemic changes in the animal were not evaluated in any case. This is an aspect of interest since these fatty acids play crucial roles not only in promoting a tolerant immune response at the intestinal level (Chen et al., 2023; Zhang et al., 2023), but also in systemic regulation by controlling metabolism (Kim et al., 2014; Passos et al., 2016; Rizzetto et al., 2018; Schirmer et al., 2016). Hence, it has been observed in humans that patients with CIEs presented glucose homeostasis alterations (Maconi et al., 2014), and an increase in platelet count in inflammatory bowel disease (IBD) that was directly related to the disease activity (Matowicka-karna, 2016), with these patients presenting a high risk of thrombotic events (Voudoukis et al., 2014). Furthermore, indices such as the neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), and platelet-to-lymphocyte ratio (PLR) (Al-Rshaidat et al., 2023; Feng et al., 2017; Fu et al., 2021) appear to hold potential for diagnostic and prognostic use.

In veterinary medicine, few studies have evaluated the hematological profile of these CIEs. Some have compared this profile between IRE and healthy dogs (Agulla et al., 2024; Cristobal et al., 2022; Marchesi et al., 2024; Mehain et al., 2019) and alterations in platelets have been reported as the clinical condition worsens (Marchetti et al., 2010; Mehain et al., 2019; Pierini et al., 2021). The systemic immune-inflammation index (SII), which was related with disease severity, has also been reported to present higher values in IRE dogs when compared to healthy individuals (Agulla et al., 2024; Cristobal et al., 2022). Moreover, although there is a lack of information, some authors (Becher et al., 2021) have observed differences between FRE and IRE dogs in NLR levels. However, no studies have related the inflammatory state or systemic changes determined by the blood profile with the lipid profile, which could provide a better understanding of the pathogenesis of both enteropathies, faster diagnosis, and more adjusted dietary treatments.

Thus, the aims of this study were to: (1) evaluate the differences in main blood cells, biochemical profile, and hematological inflammatory indices between FRE and IRE dogs; and (2) assess and quantify

the possible associations between the main blood cells or biochemical changes with the fecal lipid profile for a better understanding of both diseases.

2. MATERIALS AND METHODS

The Animal Research Committee of the Complutense Veterinary Medicine Teaching Hospital (CVMTH), under reference number 11/2021, approved all procedures and protocols developed in the current research.

2.1. Animal signalment

Fecal samples from eighty-nine dogs attending the CVMTH Gastroenterology and Endoscopy Service were collected in this prospective study (Fig. 1). All dog owners provided informed consent for their participation in the study. The inclusion criteria for dogs required the presence of digestive clinical signs lasting at least three weeks, including anorexia, reduced appetite, vomiting, diarrhea and weight loss. The diagnostic procedure included a complete physical examination, detection of fecal parasites, complete blood count and basic serum biochemistry (to exclude hypoadrenocorticism, pancreatitis, infectious diseases or systemic parasites), abdominal ultrasound and/or radiographs, trypsin-like immunoreactivity (TLI) and a switch to, at least, two elimination diets based on novel protein or hydrolyzed protein (Allenspach et al., 2007). Furthermore, demographic information (age, sex, reproductive status, body weight, body condition score (BCS), muscle condition score (MCS) and the canine inflammatory bowel disease activity index (CIBDAI) was evaluated and reported in a previous study (Higueras et al., 2024).

Prior to initiating the dietary trial, information regarding the dog´s diets was obtained for each participant in the study. The average percentage composition of commercial diets, as provided by the manufacturer, was as follows: moisture content, 8.2 ± 0.5; crude protein, 23.8 ± 4.7; crude fat, 14.1 ± 4.2; ash, 6.3 ± 0.9; crude fiber, 2.6 ± 1.8; nitrogen-free extractives, 44.1 ± 7.9; metabolic energy per 1000 g: 3726 ± 182.

Thirty-five dogs were diagnosed with FRE (n = 35) after one month of good response to diet change. FRE group consisted of 19 males (11 spayed and 8 entire) and 16 females (9 spayed and 7 entire) with a median age of 3.25 ± 3.50 years (range: 1-12 years) and a median body weight of 10.6 ±

12.73 kg (range: 2.60-45.50 kg) (Higueras et al., 2024). The breeds included 5 crossbred, and 30 purebred dogs (3 Spanish Greyhound, 3 Maltese, 2 German Shepherd, 2 Golden Retriever, 2 American Staffordshire, 2 Caniche, and one of each of the following: Yorkshire Terrier, Miniature Schnauzer, Miniature Pinscher, Italian Greyhound, Andalusian wine-cellar rat-hunting, Hungarian Puli, Dachshund, West Highland white terrier, Czechoslovakian Wolfdog, Chihuahua, Labrador Retriever, Cavalier King Charles spaniel, Border collie, Ibizan Hound, Boxer, and Shiba Inu).

Dogs that did not respond to the diet change, underwent endoscopy for biopsy and histological sampling and were diagnosed with IRE (*n* =18) based on the poor response to diet, the good response to immunosuppressants, and the evidence of an inflammatory process on histologic duodenal samples. IRE group consisted of 6 males (1 spayed and 5 entire) and 12 females (11 spayed and 1 entire) with a median age of 7 ± 2.95 years (range: 1.70-11 years) and a median body weight of 7.32 ± 9.45 kg (range: 1.19-36.20 kg) (Higueras et al., 2024). In IRE dogs, 5 were crossbred and 13 were purebred (4 Yorkshire Terrier, 2 German Shepherd, 2 Maltese, Shiba Inu, Miniature Pinscher, Cocker Spaniel, Shih Tzu, Spanish Water dog).

Thirty-six dogs were excluded from the study for other reasons (parasites, exocrine pancreatitis insufficiency, etc).

2.2. Laboratory analysis

2.2.1. Blood samples collection, hemogram and biochemistry determination

Blood samples were collected (FRE *n*= 26; IRE *n*= 16) from fasted dogs using standard jugular or cephalic venipuncture techniques and transferred into heparin and ethylenediaminetetraacetic acid (EDTA) tubes. Fasting period lasted for a minimum of 8 hours prior to sample collection. Hematological parameters of whole blood were assessed using the ADVIA 120 flow cytometer (Siemens Healthcare Diagnosis) in accordance with standardized laboratory procedures. The analysis included a full blood count, measuring red blood cell count (RBC), hemoglobin (HGB), hematocrit (HTC), platelet count, and white blood cell count (WBC).

Biochemical analyses were conducted using colorimetric enzymatic techniques to measure alanine aminotransferase (ALT), urea, creatinine, and glucose levels (Bradford Diagnostics, Sigma-

Aldrich, and Thermo Scientific). Serum protein electrophoresis was performed using the Hydrasys system (Sebia Hispania S.A.) (Villaescusa et al., 2012).

Inflammatory indices were calculated as follows: neutrophil/lymphocyte count (NLR), monocyte/lymphocyte count (MLR), platelet/lymphocyte count (PLR) and [platelet x neutrophil]/lymphocyte counts as systemic immune-inflammation index (SII).

2.2.2. Fecal samples collection and composition.

Fecal samples were collected over three consecutive days preceding the clinical practice to detect parasites. Fresh samples taken on the day of the visit were stored at -20ºC until analysis.

Fecal composition (% moisture, % fat and fatty acid profile) were quantified in a previous paper (Higueras et al., 2024). Briefly, fat extraction was conducted by adding a solvent mixture of dichloromethane-methanol (8:2) to lyophilized samples (0.2 grams). The samples were then homogenized using a mixer mill (MM400, Retsch technology, Haan, Germany), centrifuged (10,000 rpm for 10 minutes) to separate the upper lipid-containing layer that was evaporated (N_2 stream) and analyzed via gas chromatography (HP 6890 Series GC System; Hewlett Packard, Avondale, PA, USA) after the fatty methyl esters obtention (Higueras et al., 2021).

2.3. Statistical Analysis

Normality of data was evaluated according to Shapiro-Wilkinson test (SAS 9.4, SAS Inst. Inc., Cary, NC, USA). Then, the dataset was subjected to analysis employing a completely randomized design using the general linear model (GLM) procedure within SAS (version 9.4.; SAS Inst. Inc., Cary, NC, USA). Results were expressed as group means alongside their respective standard error of the mean (SEM) values, with associated significance levels (*p*-values). Statistical significance was established at $p < 0.05$ and a tendency was considered between 0.05-0.1. Pearson correlations were evaluated using the Statgraphics-19 software to explore associations among hematological variables and fecal characteristics or fecal fatty acids. Additionally, the relationship between blood parameters and fecal fatty acids was quantified by regression equations using the Statgraphics-19 program (Statgraphics Centurion XIX, X64). Differences between slopes in regression equations were analysed by Student's t-test.

3. RESULTS

Concerning the hemogram (Table 1), significant differences were found in platelets ($p = 0.0033$) and plateletcrit ($p = 0.0204$) among the different groups. Thus, IRE dogs had higher platelet counts and plateletcrit percentage than FRE. A trend was observed in segmented neutrophils ($p = 0.0892$), and IRE dogs tended to have higher concentration than dogs from FRE group. Furthermore, regarding hematological inflammatory indices, significant differences were observed in NLR ($p = 0.0001$), PLR ($p = 0.0007$) and SII ($p = 0.0004$) (Fig. 2). For these indices IRE dogs also had higher levels than FRE dogs. No differences in RBC, HGB, HTC, WBC, lymphocytes, monocytes, eosinophils, basophils and MLR were found between groups.

The blood biochemistry results are presented in Table 2. No differences were observed in total protein, albumin, urea, creatinine or ALT concentrations among the different groups. Concerning glucose concentration, IRE dogs tended to have higher levels than FRE dogs ($p = 0.0676$).

Although hardly any significant changes were observed in the hematological and biochemical profile of the blood, significant correlations of these variables or hematological inflammatory indices with the fecal fatty acid profile (analysed in Higueras et al., 2024) were found. Thus, a significant positive correlation and linear responses between lymphocytes and C16:1n-7 were observed for FRE and IRE groups (FRE: $r = 0.46$, $R^2 = 0.21$, $p = 0.0177$; IRE: $r = 0.62$, $R^2 = 0.38$, $p = 0.0136$) (Figure 3a). Also, lymphocytes showed a significant positive correlation that fit to linear equations with C16:1n-7/C16:0 ratio (FRE: $r = 0.56$, $R^2 = 0.31$, $p = 0.0026$; IRE: $r = 0.50$, $R^2 = 0.25$, $p = 0.050$) (Figure 3b). Significant differences in the slope of the regression equations that relate lymphocyte counts with C16:1n-7 (0.54 vs 3.47) or ratio C16:1n-7/C16:0 (17.97 vs 52.87) between FRE and IRE dogs respectively, confirm differences in the response for these variables between these groups (Table 3).

Moreover, a significant negative correlation between platelets and C2 was observed for FRE and IRE dogs, and the relationship was quantified by means of regression equations that showed significant linear adjustments in both groups (FRE: $r = -0.49$, $R^2 = 0.24$, $p = 0.0096$; IRE: $r = -0.53$, $R^2 = 0.29$, $p = 0.0382$) (Figure 4a). Also SII was inversely and linearly related with C2 levels (FRE: $r = -0.39$, $R^2 = 0.15$, $p = 0.0464$; IRE: $r = -0.55$, $R^2 = 0.30$, $p = 0.0325$) (Figure 4b); and, on the contrary, there was a positive correlation and linear response between NLR and iso C15:0 (FRE: $r = 0.37$, $R^2 =$

0.14, $p = 0.050$; IRE: r = 0.63, $R^2 = 0.39$, $p = 0.0116$) (Figure 5). Changes in the slopes of the regression equations in FRE and IRE dogs confirm again clear differences between these groups of dogs (Table 3). Thus, the highest platelet counts were observed in IRE dogs and this was directly associated with lower C2 levels; whereas FRE dogs presented lower platelet counts and higher C2. Then, in IRE dogs the decrease in the slope of the regression equation according to the increase in C2 was higher than in FRE dogs (− 115.04 vs − 27.50) (Fig. 4a, Table 3). A similar response was observed for the SII index and C2, and higher changes in the SII index were observed in IRE dogs that presented lower levels of C2 when compared to FRE dogs (− 1088.5 vs − 113.5) (Fig. 4b, Table 3). In addition, significant changes were observed in the slopes of the regression equations relating the NLR to iso C15:0 (0.20 vs 0.08). Thus, in IRE dogs, a greater increase in fecal iso C15:0 produced higher values of the NLR blood index than in the FRE dogs (Table 3).

Finally, Figures 6a and 6b show correlations of glucose concentration with total SCFAs and butyric acid (C4), respectively. A significant negative correlation was found between glucose and the sum of SCFAs and between glucose and C4 for FRE dogs, and these relationships that significantly fit to linear responses were quantified by means of regression equations (SCFAs: r = − 0.47, $R^2 = 0.22$, $p = 0.0362$; C4: r = − 0.58, $R^2 = 0.34$, $p = 0.0102$). Thus, higher levels of faecal SCFAs and C4 found in the FRE dogs were linearly associated with lower blood glucose concentrations. However, in IRE dogs that had lower levels of fecal SCFAs (< 5mM) and C4 (< 1mM), no such significant relationships were observed.

Lastly, there were significant correlations between hemogram and hematological inflammatory indices with fecal fat and moisture contents. Fecal fat correlated positively with WBC (r = 0.58, $p = 0.0001$), segmented neutrophils (r = 0.66, $p = 0.0001$), monocytes (r = 0.57, $p = 0.0004$), platelets (r = 0.65, $p = 0.0001$), plateletcrit (r = 0.47, $p = 0.0069$), NLR (r = 0.50, $p = 0.0021$), SII (r = 0.62, $p = 0.0001$) and MLR (r = 0.50, $p = 0.0024$); whereas negatively with RBC (r = − 0.38, $p = 0.0159$), HGB (r = − 0.40, $p = 0.0112$) and HTC (r = − 0.39, $p = 0.0144$). On the other hand, only platelets (r = 0.34, $p = 0.0315$) and plateletcrit (r = 0.39, $p = 0.0195$) correlated positively with fecal moisture respectively.

4. DISCUSSION

The diagnosis of FRE and IRE in dogs relies initially on dietary response, followed by other invasive methods. This diagnostic process typically extends over a prolonged period. Consequently, there has been a continuous effort in recent years to identify blood or fecal compounds that can help characterize these diseases. Many of these investigations focus on compounds that indirectly indicate inflammation (Chantawong, 2023). More recent studies suggest that the lipid profile could be a valuable tool (Crisi et al., 2021; Higueras et al., 2024). However, no studies have yet established a link between the lipid profile and the systemic response in individuals. Additionally, there is a lack of works that quantify this relationship, which would facilitate distinguishing between the two diseases, enhance understanding of their pathogenesis, and lead to more effective dietary treatments.

In the present research there were some significant differences between FRE and IRE dogs at the systemic level. IRE dogs presented higher platelet counts and plateletcrit compared to FRE. Higher platelet counts have been reported in previous investigations comparing CIE dogs to healthy control (Agulla et al., 2024; Cristobal et al., 2022; Marchetti et al., 2010) suggesting that it could be a marker of clinical activity (Mehain et al., 2019). In human medicine, people diagnosed with ulcerative colitis and Crohn's disease are commonly identified as being at high risk for systemic thrombotic events (Voudokis et al., 2014). Thus, it has been observed that higher platelet counts may reflect a hypercoagulable state due to the increased release of inflammatory mediators or loss of antithrombin associated with albumin loss (Craven and Washabau, 2019; Goodwin et al., 2011). Moreover, elevated platelets may be influenced by stress-induced hypercortisolemia, leading to platelet release into the peripheral blood, and transient lymphopenia (Pierini et al., 2021). Consequently, PLR index in the present study was significantly increased in IRE when compared to FRE dogs, even though no significant differences were found in lymphocyte counts between groups. Elevated PLR has also been found in human IBD studies (Akpinar et al., 2018; Feng et al., 2017; Jaoude et al., 2018; Xie et al., 2021). In veterinary medicine, several studies have shown higher PLR levels in IBD dogs compared to control (Cristobal et al., 2022; Marchesi et al., 2024). The study of Pierini et al. (2021) found positive correlations of PLR with serum total protein, albumin and Canine Chronic Enteropathy Clinical Activity

Index (CCECAI), whereas no correlation with endoscopic and histopathological scores. However, other authors (Agulla et al., 2024) did not find significant differences in this inflammatory index.

Concerning other inflammatory indices, IRE dogs also presented higher NLR and SII than FRE dogs. Various studies have shown higher NLR values in IBD dogs compared to healthy control (Becher et al., 2021; Benvenuti et al., 2020; Cristobal et al., 2022; Marchesi et al., 2024). Also, Becher et al. (2021) reported higher NLR in IRE dogs compared to FRE. Benvenuti et al. (2020) connected NLR to clinical severity indicators such as CCECAI, protein loss, and histological lymphangiectasia. During inflammatory conditions, the neutrophil count can rise, while the lymphocyte count may drop as a result of inflammation or a "stress" leukogram (Blackwood, 2016). Neutrophils are crucial in the active inflammatory response to stress and are intimately involved in destructive tissue cascades through cytokine secretion (Torun et al., 2012). Conversely, previous studies on patients (Torun et al., 2012) and dogs with IBD (Agulla et al., 2024) have shown abnormal lymphocyte function in the bloodstream and at the mucosal level.

The systemic immune-inflammation index (SII), which involves platelet, neutrophil and lymphocyte counts, has been reported as a novel biomarker of the inflammatory state (Cristobal et al., 2022). In the present research, IRE dogs presented higher values compared to FRE. Other studies have reported higher SII index in IBD dogs compared to control (Agulla et al., 2024; Cristobal et al., 2022) and its correlation with disease severity (Cristobal et al., 2022) and histopathologic lesions (Agulla et al., 2024), indicating that it might serve as a biomarker for prognosis and response to treatment. Thus, the systemic neutrophilic response could be linked with mucosal damage, bacterial translocation, local necrosis and significant systemic complications (Craven and Washabau, 2019; Rosero et al., 2014), even though clinical severity appears to align more closely with mucosal architectural alterations than with the quantities of infiltrating lymphocytes and plasma cells in the lamina propia (Allenspach and Mochel, 2021).

Although in the present research no significant differences were observed in lymphocyte counts between FRE and IRE, these cells significantly and linearly correlated with C16:1n-7 and C16:1n-7/C16:0 ratio (as an indicator of desaturation index). Mono-unsaturated fatty acids like C16:1n-7 or palmitoleic acid, and the enzyme responsible for their endogenous production (Δ-9-desaturase), are

notably important in the development and activation of B lymphocytes (Zhou et al., 2021). C16:1n-7 is obtained not only endogenously by Δ-9-desaturase but also from the diet (Passos et al., 2016). This fatty acid is considered a lipokine (Bermúdez et al., 2022) capable of regulating inflammatory processes at the intestinal level in mice with colitis (Chen et al., 2023). Moreover, several studies have demonstrated an anti-inflammatory effect of C16:1n-7 concerning lymphocytes and pro-inflammatory cytokines (IL-1β, TNFα, IFN-γ, IL-6) (Passos et al., 2016; Schirmer et al., 2016); whereas an increase in saturated (by desaturase inactivation) can lead to inflammation (Harvey et al., 2009).

In addition, in the present study, it is interesting to highlight that the linear response between lymphocytes and C16:1n-7 was different in FRE and IRE dogs as observed by significant changes in the slope of the regression equations. A higher quantity of blood lymphocytes was observed as levels of C16:1n-7 and the C16:1n-7/C16:0 ratio increased in feces. However, these changes were more pronounced in IRE dogs, which exhibited the lowest blood lymphocyte counts and a lower range of fecal C16:1n-7 and the C16:1n-7/C16:0 ratio compared to FRE dogs. A previous study showed lower fecal levels of this fatty acid and C16:1n-7/C16:0 desaturase index in dogs with IRE compared to FRE and healthy dogs, and these could discriminate between both diseases (Higueras et al., 2024). It has been found an under-expression of Δ-9-desaturase in the liver of mice with colitis, possibly as a consequence of damage caused by the migration of endotoxins and pro-inflammatory cytokines through the portal vein (Chen et al., 2008). Thus, alteration of this enzyme at the enterocyte level could be partially responsible for systemic inflammation, and an increase in their activity has been correlated with a lower inflammatory state (Mukherjee et al., 2018). Moreover, the pronounced changes in lymphocytes in IRE dogs could be due to "stress" leukogram (Blackwood, 2016), and possible migration towards the intestinal mucosa. Thus, according to Galler et al. (2017), immune cells circulating in the blood can migrate to the intestine epithelium or lamina propria, reducing their circulating levels.

Using the regression equations obtained for the first time in the present study, we could determine that dogs with C16:1n-7 percentage lower than 1.1 at the same time that lymphocyte levels below 1.8 $x10^3/\mu L$ are unlikely to respond to any of the elimination diets. These dogs are more likely to have a complex inflammatory condition, such as IRE.

This inflammatory state of the IRE animals was also related to the greater presence of fat in feces (Higueras et al., 2024), as observed by the positive correlations between fecal fat levels and immune cells or systemic indices of inflammation observed in the present study. Also, this would be related to the lower levels of C16:1n-7 and consequently C16:1n-7/C16:0 index compared to the FRE group. The production of palmitoleyl-CoA by the enzyme desaturase is needed to synthesize enough cholesterol esters (Miyazaki et al., 2000), which are precursors of the bile acids necessary for micelle formation and lipid absorption (Di Ciaula et al., 2017). It must be taken into account that fat induces alterations in the microbiome, as well as a state of intestinal hyperpermeability (Rohr et al., 2020), and consequently, an inflammatory state. An excessive presence of fat in fecal material could play a determining role in the pathogenesis of CIEs, and it might be closely related to changes in the intestinal microbiome in IRE dogs.

Additionally, in the present study, significant and linear relationships were found between certain blood parameters and fecal SCFAs (microbial metabolites). SCFAs are saturated organic acids containing between one and six carbon atoms (den Besten et al., 2013), produced through anaerobic fiber fermentation by intestinal microorganisms (He et al., 2020). Acetic (C2), propionic (C3), and butyric acid (C4) are the most prevalent SCFAs in the intestine. These SCFAs influence various cell types through G protein-coupled receptors (GPCRs), playing a crucial role in regulating key biological functions such as host metabolism, intestinal health, and immune responses (Zhang et al., 2023).

In this study, C2 was correlated with blood platelet counts and the SII index and these relationships adjusted to significant linear responses in a different way in FRE and IRE dogs. Thus, fecal C2 levels higher than 2 mM were associated with platelet counts within the reference range (150-337 $x10^3/\mu L$) in FRE dogs. Conversely, values lower than 2 mM of C2 observed in IRE dogs were associated with a more pronounced ascending slope in platelet counts, exceeding the reference range. Lower levels of C2 have been reported in the feces of dogs with IBD compared to FRE dogs (Higueras et al., 2024). Of the SCFAs, acetic acid is the most likely to enter peripheral circulation because it is primarily absorbed by the liver (up to 70%) (den Besten et al., 2013). According to Jing et al. (2015), acetic acid inhibits platelet aggregation by inhibiting platelet-aggregating agonists (such as collagen, arachidonic acid, adenosine diphosphate, and thrombin). Additionally, it inhibits the enzyme COX-1 and exhibits

fibrinolytic activity by activating plasminogen, preventing thrombus formation. Consequently, it could be directly involved in platelet regulation, as observed in the present study. Notably, acetic acid also showed the same behavior with the SII index. This different relationship in FRE and IRE dogs between platelets or SII index and acetic acid is of special relevance, since it has not been described before, and would allow us to estimate according to these variables what type of enteropathy we are dealing with and establish the most adjusted dietary treatment. Thus, levels higher than 2 mM of C2 were directly related with less SII index and consequently a lower inflammatory state; whereas levels lower than 2 mM of C2 were associated with a more inflammatory state presented in IRE dogs. Immune cells such as lymphocytes and neutrophils have cell surface GPCRs, which allows SCFAs to regulate their activity (Zhang et al., 2023). Some studies have demonstrated that acetic acid can inhibit tumor necrosis factor-alpha (TNF-α)-induced inflammation by suppressing the activation of nuclear factor-kappa B (NF-κB) p65 (Xie et al., 2020), spleen tyrosine kinase, and mitogen-activated protein kinase (MAPK) (Hung and Suzuki, 2017), thereby reducing colon inflammation and improving intestinal epithelial barrier dysfunction (Chen et al., 2018). Additionally, SCFAs promote the polarization of intestinal T cells toward Tregs in mice with colitis to inhibit the inflammatory response (Smith et al., 2013).

Moreover, the observed correlations and linear relationships between blood glucose and fecal SCFAs, specifically C4, demonstrated the importance of these fatty acids in maintaining blood glucose concentrations. Thus, the higher the fecal SCFA values the lower the blood glucose concentrations. According to the regression equation of the present research, fecal SCFA values higher than 5 mM were associated with regulated blood glucose concentrations observed in FRE dogs (within the reference range 77-120 mg/dL). Conversely, IRE dogs exhibited fecal SCFA values lower than 5 mM and glucose concentrations seem not to decrease as in FRE dogs. This result agrees with the slight tendency in IRE dogs to exhibit higher levels of blood glucose concentrations. Other studies observed that some of the extraintestinal manifestations of IBD include insulin resistance (Tigas and Tsatsoulis, 2012). Thus, IBD could elevate the risk of diabetes by triggering inflammatory and metabolic signaling pathways (Shanahan, 2012). Moreover, some reports suggest that gut microbiota dysbiosis in IBD patients could contribute to the development of type 2 diabetes (Kang et al., 2019). The results of the present study would indicate that SCFAs, which are microbial metabolites, were involved in glucose regulation but at

concentrations higher than >5 mM. It is known that SCFAs stimulate the release of gut hormones peptide YY and glucagon-like peptide-1 (GLP-1) through the activation of GPCRs (den Besten et al., 2013). Peptide YY enhances glucose absorption in muscle and adipose tissue, by reinforcing insulin action (He et al., 2020). Furthermore, GLP-1 regulates blood glucose levels by increasing insulin secretion and reducing pancreatic glucagon secretion (He et al., 2020). Additionally, Sakakibara et al. (2006) found that SCFAs can boost leptin secretion, which regulates food intake, body weight, and energy metabolism through the central nervous system. Some researchers have also discovered that SCFAs can increase the expression of GLUT4, a key protein that facilitates glucose entry into cells, primarily found in skeletal muscle cells (Deng, 2018). Thus, SCFAs help regulate glucose metabolism mainly by maintaining blood glucose stability. Moreover, Sato et al. (2020) found that obese mice treated with butyrate showed improved glucose metabolism and reduced adipose tissue inflammation via GPCR activation. Consequently, it is possible that dogs with IRE, whose SCFA levels were lowered in feces, do not benefit from the mentioned effects, presenting an altered metabolic state.

In addition, in the present research, NLR index showed a greater increase in IRE dogs than in FRE dogs as iso C15:0 levels increased. This fatty acid belongs to the branched-chain fatty acid (BCFA) group. BCFAs can be obtained through the metabolism of branched-chain amino acids (valine, leucine, and isoleucine) by the gut microbiome (Gozdzik et al., 2023). Previous studies found that IRE dogs had higher iso/anteiso ratio compared to FRE dogs, and this was related to a worse clinical state (Higueras et al., 2024). A greater increase in the NLR index in IRE dogs as the iso forms increase could be associated with changes in the microbial population and, consequently, with different production of iso or anteiso forms. Intestinal dysbiosis has been reported by other authors in this IRE group (Díaz-Regañón et al., 2023). Also, Higueras et al. (2024) found that as iso forms increased, there was a linear decrease in SCFAs (mainly propionic acid), which have been related to antiinflammatory properties (Smith et al., 2013). Unlike SCFAs, the specific physiological functions of BCFAs are still not well understood. It has been reported that BCFAs also exhibit anti-inflammatory effects in the gut (He et al., 2023; Yan et al., 2017) by the regulation of NF-κB and its synthesis of pro-inflammatory cytokines, although the iso forms seem to be less effective (Yan et al., 2017). The results of the present research

would confirm that alterations in microbial metabolites in IRE dogs, probably by dysbiosis, could affect their antiinflammatory function and consequently present a more markedly NLR index than FRE dogs.

5. CONCLUSIONS

This study suggests that platelet counts and blood cell ratios such as NLR, PLR, and SII could be clinically useful in differentiating between FRE and IRE dogs. Additionally, different regression equations quantified for the first time in the present research by the combination of blood parameters or fecal fatty acids indicate their potential in distinguishing these two conditions. This research confirms that systemic changes in FRE or IRE dogs are directly related to the metabolism of fatty acids such as palmitoleic acid, acetic acid or butyric acid; and consequently, these might determine different levels of supplementation to achieve a response to the dietary treatment. Further research, including microbiome analysis, is needed for a better understanding of the role of these fatty acids in the pathogenesis of CIEs and to develop more accurate dietary treatments.

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AUTHORS CONTRIBUTIONS

Conceptualisation, Á.S. and A.I.R.; methodology, C.H., A.I.R., F.R.-F., M.G.-S., and Á.S.; software, C.H. and A.I.R.; formal analysis, C.H. and A.I.R.; data curation, C.H and A.I.R.; writing—original draft preparation, C.H. and A.I.R.; writing—review and editing, C.H., F.R.-F., M.G.-S., Á.S. and A.I.R. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors do not declare any conflict of interest

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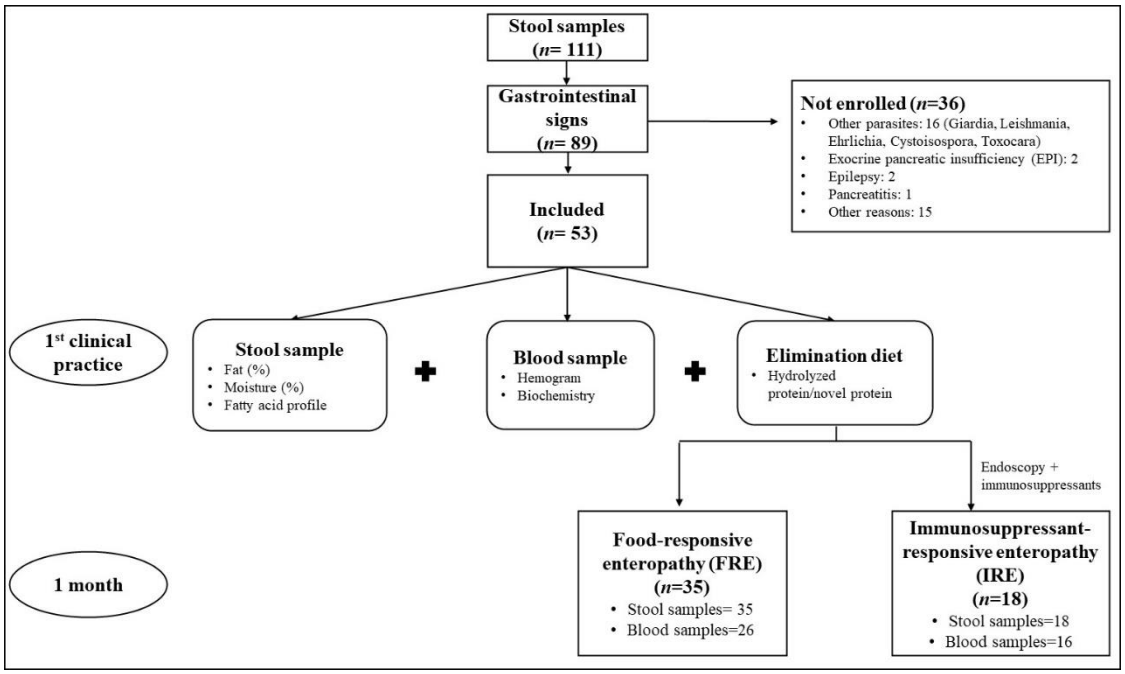

Fig. 1. Study protocol, including data collection of stool and blood samples of food-responsive enteropathy dogs (FRE) and immunosuppressant-responsive enteropathy dogs (IRE).

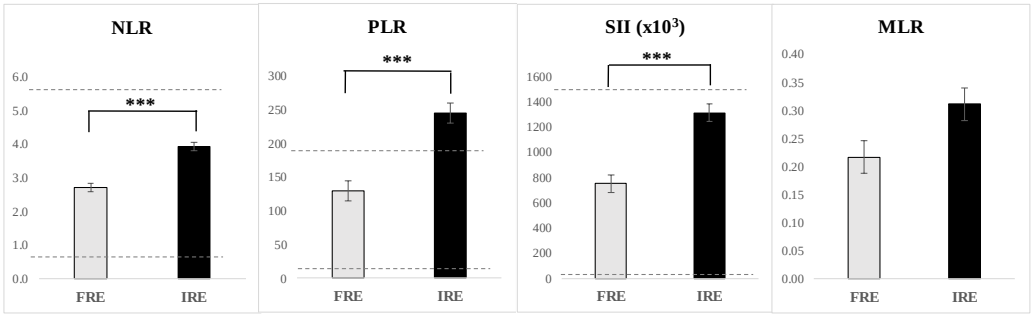

Fig. 2. Hematological inflammatory indices of food-responsive enteropathy dogs (FRE) and immunosuppressant-responsive enteropathy dogs (IRE). Data are presented as mean ± standard error of the mean (SEM). NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; SII: systemic immune-inflammation index (platelet * neutrophil/lymphocyte counts); MLR: monocyte-to-lymphocyte ratio. A dashed gray line is indicative of the reference range. * $p<0.05$; ** $p<0.01$; *** $p<0.001$

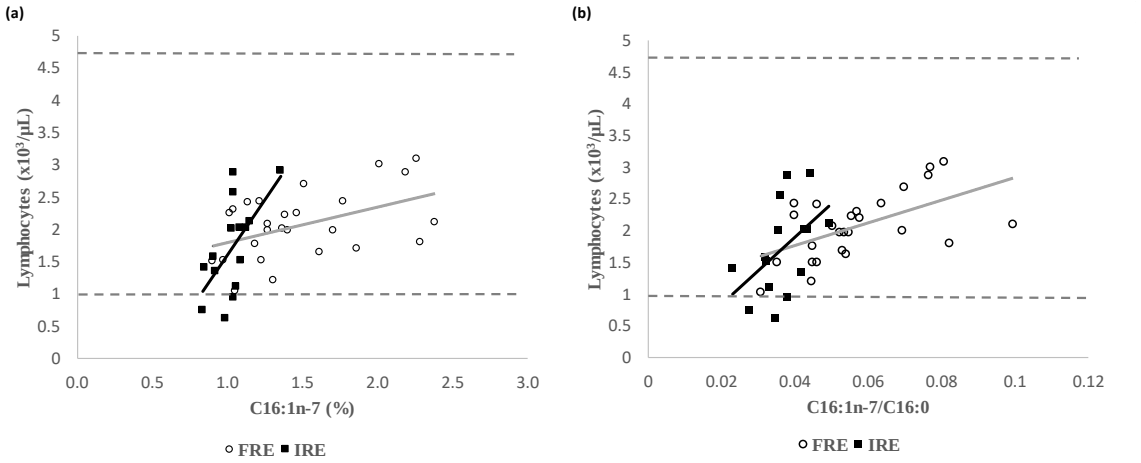

Fig. 3. Relationship between (a) lymphocytes and C16:1n-7; (b) lymphocytes and C16:1n-7/C16:0 ratio in food-responsive enteropathy (FRE) and immunosuppressant-responsive enteropathy (IRE) dogs. A dashed gray line is indicative of the reference range.

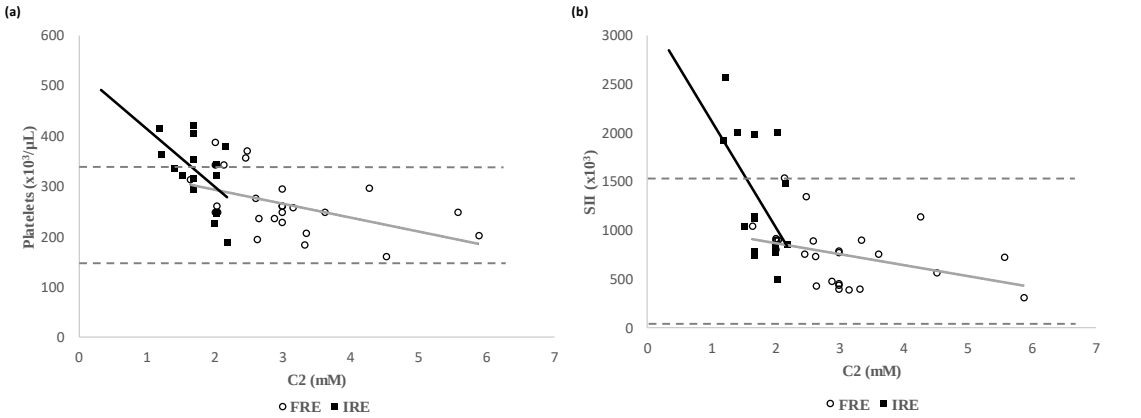

Fig. 4. Relationship between (a) platelets and acetic acid (C2); (b) systemic immune-inflammation index (SII= platelet * neutrophil/lymphocyte counts) and C2 in food-responsive enteropathy (FRE) and immunosuppressant-responsive enteropathy (IRE) dogs. A dashed gray line is indicative of the reference range.

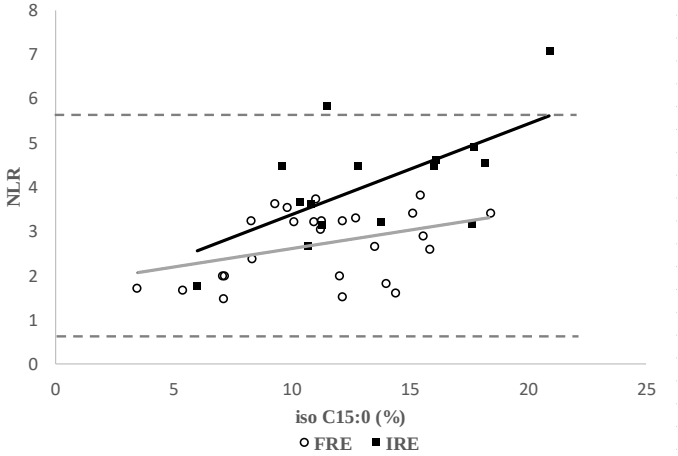

Fig. 5. Relationship between neutrophil-to-lymphocyte ratio (NLR) and iso C15:0 in food-responsive enteropathy (FRE) and immunosuppressant-responsive enteropathy (IRE) dogs. A dashed gray line is indicative of the reference range.

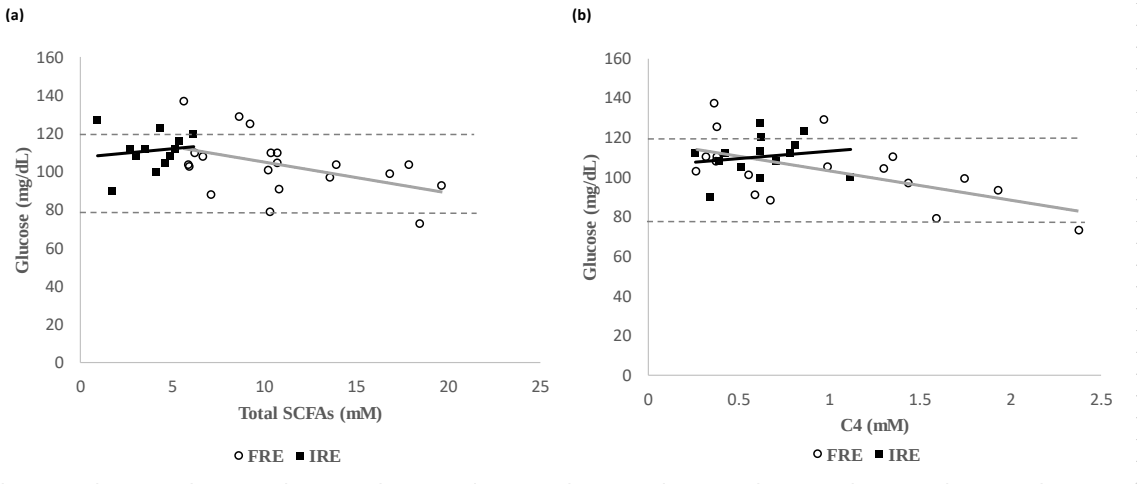

Fig. 6. Relationship between (a) glucose and total short-chain fatty acids (SCFAs); (b) glucose and butyric acid (C4) in food-responsive enteropathy (FRE) and immunosuppressant-responsive enteropathy (IRE) dogs. A dashed gray line is indicative of the reference range.

Table 1.

Hemogram of food-responsive enteropathy dogs (FRE) and immunosuppressant-responsive enteropathy dogs (IRE).

VARIABLES	FRE (*n*=26)	IRE (*n*=16)	REFERENCE VALUE	SEM [5]	*p*-Value
RBC ($x10^6/\mu L$) [1]	7.35	7.33	5.70-8.90	0.20	0.9638
HGB (g/dL) [2]	17.15	17.34	13-21	0.34	0.7854
HTC (%) [3]	50.33	51.23	40-63	1.11	0.6978
WBC ($x10^3/\mu L$) [4]	8.67	10.97	6-17	0.78	0.1585
Segmented neutrophils ($x10^3/\mu L$)	5.73	7.98	3-11.50	0.62	0.0892
Lymphocytes ($x10^3/\mu L$)	2.08	1.90	1-4.80	0.11	0.4795
Monocytes ($x10^3/\mu L$)	0.36	0.35	0.15-1.35	0.03	0.9132
Eosinophils ($x10^3/\mu L$)	0.32	0.48	0.1-1.25	0.05	0.1642
Basophils ($x10^3/\mu L$)	0.00	0.00	0-0.1	0.00	0.7800
Platelets ($x10^3/\mu L$)	265.65	328.73	150-337	9.59	0.0033
Plateletcrit (%)	0.37	0.47	NA	0.02	0.0204

[1] RBC: red blood cells; [2] HGB: hemoglobin; [3] HTC: hematocrit; [4] WBC: white blood cells; [5] SEM: standard error of the mean. *p*-value was significant when <0.05. NA: not applicable.

Table 2.

Biochemistry of food-responsive enteropathy dogs (FRE) and immunosuppressant-responsive enteropathy dogs (IRE).

VARIABLES	FRE (*n*=26)	IRE (*n*=16)	REFERENCE VALUE	SEM [2]	*p*-Value
Total protein (g/dL)	6.47	6.27	5.50-7.10	0.10	0.3734
Albumin (g/dL)	3.26	3.18	2.30-4.00	0.06	0.5307
Glucose (mg/dL)	104.39	113.00	77.00-120.00	2.23	0.0676
Urea (mg/dL)	32.38	32.05	15.00-53.00	2.26	0.9462
Creatinine (mg/dL)	0.99	0.91	0.50-1.60	0.04	0.4004
ALT(GPT) (U/L) [1]	28.78	32.07	15.00-58.00	2.32	0.5051

[1] ALT: alanine aminotransferase; [2] SEM: standard error of the mean. *p*-value was significant when <0.05.

Table 3.

Regression equations between blood cells or systemic inflammatory indices and fecal fatty acids in food-responsive enteropathy dogs (FRE) or immunosuppressant-responsive enteropathy dogs (IRE).

y	Group	*n*	Intercept	x	R^2	r	RSD [6]	*p*-Value
Lymphocytes				**C16:1n-7**				
	FRE	26	1.24a ± 0.33	0.54a ± 0.21	0.21	0.46	0.47	0.0177
	IRE	15	− 1.88b ± 1.27	3.47b ± 1.22	0.38	0.62	0.58	0.0136
Lymphocytes				**C16:1n-7/C16:0**				
	FRE	26	1.04a ± 0.31	17.97a ± 5.35	0.31	0.56	0.43	0.0026
	IRE	15	− 0.22b ± 0.94	52.87b ± 25.19	0.25	0.5	0.64	0.050
Platelets				**C2** [3]				
	FRE	24	348.27a ± 31.07	−27.50a ± 9.77	0.24	−0.49	51.69	0.0096
	IRE	15	529.05b ± 88.19	−115.04b ± 49.87	0.29	−0.53	59.72	0.0382
SII [1]				**C2** [3]				
	FRE	22	1093.89a ± 171.79	−113.55a ± 54.05	0.15	−0.39	285.79	0.0464
	IRE	15	3209.98b ± 804.38	−1088.5b ± 454.85	0.3	−0.55	544.65	0.0325
NLR [2]				**iso C15:0**				
	FRE	26	1.78a ± 0.48	0.08 ± 0.04	0.14	0.37	0.73	0.050
	IRE	15	1.35b ± 0.97	0.20 ± 0.06	0.39	0.63	1.05	0.0116
Glucose				**Total SCFAs** [4]				
	FRE	20	121.33 ± 8.47	− 1.63 ± 0.72	0.22	−0.47	13.92	0.0362
	IRE	12				0.13		> 0.05
Glucose				**C4** [5]				
	FRE	18	117.90 ± 5.90	− 14.77 ± 5.07	0.34	− 0.58	13.53	0.0102
	IRE	14				0.16		> 0.05

[1] SII: Systemic immune-inflammation index (platelet * neutrophil/lymphocyte counts); [2] NLR: neutrophil-to-lymphocyte ratio; [3] C2: acetic acid; [4] Total SCFAs: total short-chain fatty acids; [5] C4: butyric acid; [6] RSD: relative standard deviation. *p*-value was significant when <0.05.

CONFLICT OF INTEREST

The authors do not declare any conflict of interest.

4.2.3 Artículo 5: Higueras *et al.*, 2025,

Differentiating canine chronic inflammatory enteropathies using faecal amino acid profiles: potential and limitations

Cristina Higueras, Claudia Ruiz-Capillas, Ana Herrero, Ángel Sainz, Mercedes García-Sancho, Fernando Rodríguez-Franco, Mar Larrosa and Ana I. Rey

Animals. Índice de impacto (2023) = 2,7. **Q1.**

Este artículo investiga los objetivos 2, 3 y 4 de la presente tesis doctoral en relación con la búsqueda de diferencias en el perfil de aminoácidos en heces entre perros con distintas ECs (FRE e IRE), así como en perros con sintomatología similar diagnosticados con *Giardia* spp. Además, explora las correlaciones entre los aminoácidos y diversos indicadores de la gravedad de la enfermedad, como las características fecales.

animals

MDPI

Article

Differentiating Canine Chronic Inflammatory Enteropathies Using Faecal Amino Acid Profiles: Potential and Limitations

Cristina Higueras [1], Claudia Ruiz-Capillas [2], Ana Herrero [2], Angel Sainz [3], Mercedes García-Sancho [3], Fernando Rodríguez-Franco [3], Mar Larrosa [4] and Ana I. Rey [1,*]

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Simple Summary: This study explores the potential of faecal amino acids as non-invasive biomarkers for distinguishing different forms of canine chronic inflammatory enteropathies. The findings suggest that faecal amino acid profiles, particularly threonine and aromatic amino acids such as tyrosine, could serve as indicators of chronic digestive disorders. However, the faecal amino acid profile alone cannot fully differentiate dogs that respond to diet from those that do not. Dogs with clinical signs but infected with *Giardia* spp. show significantly greater excretion of faecal amino acids compared to the others.

Abstract: The aims of this study were to characterise the faecal amino acid profile of dogs with different chronic digestive diseases (food-responsive enteropathy (FRE), immunosuppressant-responsive enteropathy (IRE)) prior to dietary change, and *Giardia* infection (GIA), compared to healthy control (HC), and to evaluate their discriminating potential. The HC group presented lower faecal tyrosine (Tyr) and aromatic amino acids (AAAs) compared to FRE or IRE dogs ($p = 0.0001$). Additionally, the HC group had lower levels of threonine (Thr) ($p = 0.0005$) than the IRE group, while FRE dogs showed intermediate values. No statistically significant differences in faecal amino acids were observed between FRE and IRE dogs. In contrast, the GIA group had higher faecal amino acid values (except glutamic acid (Glu)) compared to the other dogs. The most determinant variables contributing to the discriminant functions were Tyr, Glu, arginine, and phenylalanine. Validation results of the discriminant functions showed that 44% of stool samples were misclassified, resulting in a 56% success rate. The faecal amino acid profile did not accurately distinguish FRE from IRE dogs; however, faecal excretion of AAs was generally higher in dogs with GIA.

Keywords: aromatic amino acids; branched-chain amino acids; tyrosine; threonine; gut health; chronic inflammatory enteropathies; dogs

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1. Introduction

Chronic inflammatory enteropathies (CIEs) in dogs are characterised by a multifactorial origin, in which an exacerbated immune response, environmental factors (such as diet and microbiota), and a genetic predisposition are thought to play a role [1,2]. In this context,

it has been observed that certain food components can affect the integrity of the intestinal epithelium, which is essential for maintaining homeostasis, gut microbiota balance, and the mucosal immune system [3]. Regarding protein, it remains unclear whether it exacerbates inflammation or, conversely, has positive effects through bioactive peptides or amino acids. These components may influence intestinal homeostasis, modulate immune responses, or impact intestinal tight junction proteins [4,5].

Thus, diets based on easily digestible protein or novel protein are commonly used in veterinary medicine for CIEs, although a wide variability in response to dietary treatment has been observed [1]. Dogs responding to diet (FRE) typically experience clinical remission after adopting these specialised diets, whereas non-responders require further invasive diagnostic tests that reveal an inflammatory process in the intestinal mucosa and treatment with immunosuppressants (IRE). The reason for these differing dietary responses remains unknown [1]. Recent studies suggest that dietary protein content may influence gut bacterial diversity, and microbiome changes may significantly affect the host's amino acid metabolism [6].

Considering that these are idiopathic diseases whose diagnosis is based on the response to diet, in recent years, there has been an intensified search for compounds that allow for faster differentiation. Thus, in human medicine, significant changes in circulating amino acids (AAs) have been observed in individuals with inflammatory bowel disease (IBD) compared to healthy controls [7,8]. These changes have been considered useful for diagnosis, monitoring and understanding the pathogenesis of these diseases. In veterinary medicine, studies have also shown differences in the AA profiles of dogs with gastrointestinal diseases compared to healthy dogs. Thus, Tamura et al. [9] observed decreased serum AA levels and correlated serine (Ser) with the canine chronic enteropathy clinical activity index (CCECAI). Similarly, Benvenuti et al. [10] reported significant changes in serum phenylalanine (Phe), tryptophan (Trp), and histidine (His) levels based on the severity of the enteropathy. Other studies have evaluated serum AA concentrations in protein-losing enteropathy [11]. In cats with chronic gastrointestinal diseases, plasma AA levels have been considered more sensitive nutritional markers than other parameters, such as total proteins or albumin levels [12].

Most research on CIEs in animals has focused on blood AA levels, with little information on the potential use of non-invasive methods, such as the faecal amino acid profile to differentiate these diseases. In humans, Bosch et al. [13] highlighted the high potential of faecal AAs as novel non-invasive, low-cost biomarkers for diagnosing pediatric IBD. Recent studies on dogs with CIEs versus controls have also reported increased faecal Trp levels [14]. Moreover, Higueras et al. [15] observed some variations in the faecal AA profile, although the most notable changes occurred in serum AAs; this study, however, only compared FRE dogs to healthy controls.

To date, no study has evaluated the changes in faecal AA profile in dogs with different CIEs or the potential of these compounds as discriminating factors for correct diagnosis and tailored dietary strategies. Given that serum and faecal AA composition reflects the host's metabolic state and possible alterations in gut microbiota [6], their study deserves further attention.

On the other hand, among the diseases that can potentially cause chronic diarrhea, and other associated clinical signs are parasitic infections. These processes must be diagnosed first in order to determine whether we are dealing with a parasitic problem or, on the contrary, whether it is an inflammatory process with idiopathic characteristics such as CIEs. *Giardia* (GIA) is one of the most frequent protozoan infections affecting both humans and animals [16]. However, there is a lack of information on the changes that this parasite can produce in the faecal AA profile or how they differ from those in CIEs.

Thus, the aim of this study was, firstly, to characterise the faecal amino acid profile of dogs with different chronic digestive diseases (FRE, IRE), prior to dietary change and GIA in comparison to healthy individuals; and secondly, to evaluate their discriminating potential and clinical utility.

2. Materials and Methods

The procedures and protocols outlined in this study received approval from the Animal Research Committee of the Complutense Veterinary Medicine Teaching Hospital (CVMTH) under reference number 11/2021 (approval date: 26 May 2021). All dog owners provided informed consent for their pets' participation.

2.1. Experimental Design, Animal Signalment and Diets

This study involved 62 sick dogs presenting with gastrointestinal signs, referred to the CVMTH between January 2022 and March 2023. To be included, dogs had to exhibit digestive signs such as vomiting, diarrhoea, weight loss, and/or anorexia/hyporexia for at least three weeks. A comprehensive diagnostic process was carried out, which included, at least, physical exams, faecal parasite screening, blood analysis, serum biochemistry including trypsin-like immunoreactivity (TLI) and cortisol, and ultrasound. In addition, each dog underwent trials with at least two elimination diets featuring novel or hydrolysed proteins [17]. For those unresponsive to dietary changes, endoscopic biopsies and histological samples were taken for further evaluation.

The dogs were grouped into three diagnostic categories: thirty-five were identified with FRE due to their positive response to dietary changes within one month. Eighteen were diagnosed with IRE, characterised by a lack of response to diet alone, improvement with immunosuppressants, and histological signs of inflammation. Nine dogs were confirmed to have GIA, verified through modified Telemann and Merthiolate iodine formaldehyde tests (ESCCAP), as well as a favourable response to specific treatment against this parasite. Other potential causes of gastrointestinal inflammation or systemic diseases were ruled out to refine the diagnoses.

Dogs with FRE consisted of 16 females (7 intact, 9 spayed) and 19 males (8 intact, 11 neutered). The median age in this group was 3.25 $\pm$ 3.50 years (range: 1–12 years), and the median body weight was 10.60 $\pm$ 12.73 kg (range: 2.60–45.50 kg). The breeds included 5 crossbreds and 30 purebreds. In the IRE group, there were 12 females (1 intact, 11 spayed) and 6 males (5 intact, 1 neutered), with a median age of 7 $\pm$ 2.95 years (range: 1.70–11 years) and a median body weight of 7.32 $\pm$ 9.45 kg (range: 1.19–36.20 kg). There were 5 crossbred and 13 purebred dogs. The GIA group consisted of 2 females (1 intact, 1 spayed) and 7 males (4 intact, 3 neutered). The median age of this group was 8 $\pm$ 4.38 years (range: 1–12 years), and the median body weight was 12.60 $\pm$ 7.43 kg (range: 2.70–26.30 kg). There were 1 crossbred and 8 purebred dogs.

Alongside the affected dogs, a control group of 22 healthy control dogs (HC) was included as a baseline. These control dogs were selected based on normal physical exams, blood tests, and the absence of clinical signs for at least four months. Dogs with asymptomatic chronic diseases were excluded from the control group. The control group consisted of 13 females (4 intact, 9 spayed) and 9 males (6 intact, 3 neutered), with a median age of 5 $\pm$ 2.96 years (range: 1–12 years) and a median body weight of 19.40 $\pm$ 15.02 kg (range: 7.50–55 kg). Regarding breed, 15 dogs were purebred and 7 were crossbred.

Faecal samples were collected by owners over three consecutive days using sterile containers. Two of the samples were refrigerated until the clinical examination day, while the third one was collected on the morning of the visit. Once delivered by the owner, the fresh sample was immediately split into two containers and frozen at -20 °C for amino acid

determinations. For testing, two refrigerated samples and the other fresh sample were used for parasite detection. Faecal consistency was assessed using the Purina® Faecal Scoring Chart (Société des Produits Nestlé S.A., Vevey, Switzerland), which ranges from one (very hard and dry) to seven (watery). Additional data collected during the visit included age, sex, fertility status, breed, body weight, body condition score (BCS), muscle condition score (MCS), and the canine inflammatory bowel disease activity index to assess clinical symptom severity (CIBDAI) [18].

Prior to the dietary trial, information regarding the dogs' commercial diets was gathered for every dog included in the study (Table 1). In all diets, the main source of protein was of animal origin. Those animals receiving a homemade diet were excluded from the study.

Table 1. Composition of diets (averaged g/100 g according to manufacturer's composition) prior to the dietary change of healthy control dogs (HC), food-responsive enteropathy dogs (FRE), immunosuppressant-responsive enteropathy dogs (IRE), and dogs parasitised with *Giardia* (GIA).

Variable	HC (*n* = 22)	FRE (*n* = 35)	IRE (*n* = 18)	GIA (*n* = 9)
Humidity	9.50 ± 0.71	8.38 ± 0.48	8.50 ± 0.49	8.50 ± 0.50
Crude protein	23.05 ± 4.68	23.71 ± 4.50	23.73 ± 5.07	22.70 ± 4.90
Crude fat	13.78 ± 4.10	14.87 ± 4.30	13.46 ± 4.21	13.54 ± 4.41
Crude fibre	2.83 ± 0.78	2.83 ± 1.89	2.12 ± 1.63	2.57 ± 1.01
Crude ash	7.64 ± 1.20	6.70 ± 0.87	5.83 ± 1.00	6.23 ± 2.07
Nitrogen-free extractives	43.18 ± 7.91	43.49 ± 7.42	46.34 ± 8.36	46.44 ± 8.20
Calcium	1.47 ± 0.16	1.09 ± 0.30	0.91 ± 0.13	1.43 ± 0.06
Phosphorus	1.03 ± 0.17	0.77 ± 0.21	0.64 ± 0.09	0.92 ± 0.10
Sodium	0.30 ± 0.08	0.37 ± 0.08	0.35 ± 0.07	0.40 ± 0.08
∑n-3	0.67 ± 0.47	1.03 ± 0.89	2.00 ± 1.83	0.55 ± 0.35
Metabolic energy (kcal/kg) [1]	3583 ± 193	3711 ± 201	3742 ± 163	3684 ± 221

[1] Calculated according to NRC [19].

2.2. Measurement of Amino Acids in Faecal Samples

Lyophilised samples (0.1 g) (Lyoquest, Telstar, Tarrasa, Spain) were placed in screw-capped glass tubes and hydrolysed with 25 mL of 6 M HCl. These tubes were then flushed with N_2 and heated to 110 °C for 22 h. Then, samples (after cooling at room temperature) were filtered through filter paper to a beaker, and the pH was adjusted to 5.6 by the addition of NaOH solution (pH meter Euthech, Thermo Scientific, Waltham, MA, USA). The solution was placed in a 100 mL volumetric flask and levelled up to that volume. Then, 20 mL were collected with a syringe and filtered using a Sep-pak silica cartridge. Subsequently, 2 mL of the sample extract was isolated in a vial and stored at −20 °C.

The amino acid content was determined using a Biochrom 20 amino acid analyser (Amersham Pharmacia Biotech., Biocom, Uppsala, Sweden), using the methodology of amino acid analysis based on ion exchange chromatography and post-column derivatisation with ninhydrin. After chromatographic separation, the ninhydrin–amino acid derivative eluted from the column was detected by absorbances at 570 and 440 nm (proline).

The determination of amino acids was made by comparing their retention times with those of a standard sample of seventeen amino acids (Supelco-Sigma-Aldrich, Alcobendas, Madrid, Spain): aspartic acid (Asp), glutamic acid (Glu), serine (Ser), alanine (Ala), arginine (Arg), cysteine (Cys), histidine (His), glycine (Gly), leucine (Leu), isoleucine (Ile), lysine (Lys), methionine (Met), threonine (Thr), phenylalanine (Phe), tyrosine (Tyr), valine (Val), and proline (Pro) (1 µm/mL). The determinations were performed in duplicate, and the results were expressed as nmol per sample.

Given the positive relationship observed in previous studies [15] between branched amino acids and certain parameters related to intestinal health, a separate calculation of BCAAs, AAAs, and their corresponding ratio was performed. Total branched-chain amino acids (BCAAs) were calculated as the sum of Leu, Ile, and Val; whereas total aromatic amino acids (AAAs) were the sum of Tyr and Phe.

2.3. Statistical Analysis

The dataset was analysed using a completely randomised design applying the General Linear Model (GLM) procedure in SAS (version 9; SAS Institute Inc., Cary, NC, USA). Comparative analysis of means was conducted using Duncan's test (version 9; SAS Institute Inc., Cary, NC, USA). The diagnosed group was considered as a fixed effect according to the following model: $Y_d = \mu + \alpha_d$, where Y_d represents the diagnosed group dependent variable, μ the overall mean, and α_d the effect of the diagnosed group. Given the wide range of ages and weights of the dogs, the possible relationship between these variables and the faecal amino acid profile was tested using Pearson's correlation analysis (Statgraphics Centurion XIX, X64, version 19.2.01). Since no relationships were observed between animal weight or age and faecal amino acids, these variables were not included in the statistical model. Group means and their corresponding root mean square error (RMSE) were reported, with statistical significance set at $p < 0.05$. Pearson's correlation analysis was performed to investigate potential relationships between faecal amino acid levels and CIBDAI (measured in Higueras et al. [16]) using Statgraphics (Statgraphics Centurion XIX, X64, version 19.2.01). For the linear discriminant analysis, the variables included in the model were using a stepwise algorithm (SAS STEPDISC), and the resulting model was executed and validated using the SAS DISCRIM procedure (version 9.4; SAS Institute Inc., Cary, NC, USA).

3. Results

Concerning the faecal amino acid profile, no statistically significant differences were observed between the HC group and FRE or IRE dogs in levels of faecal Asp, Ser, Glu, Pro, Gly, Ala, Cys, Lys, Met, Arg, Val, Ile, Leu, Phe, or His (Figures 1 and 2). However, HC presented lower faecal Tyr levels than FRE or IRE dogs ($p = 0.0001$) (Figure 1). The amino acid profile was not influenced by age or body weight, although body weight differed significantly between treatments ($p = 0.0059$). This was confirmed by the absence of a relationship between body weight and each of the amino acids ($p > 0.05$). Moreover, its inclusion as a covariate in the statistical model did not alter the results; therefore, it was ultimately excluded from the final analysis.

Additionally, the HC group had lower levels of Thr ($p = 0.0005$) compared to IRE dogs; however, no significant differences were found in faecal Thr amount between HC and FRE, whereas FRE dogs presented intermediate levels of Thr between HC and IRE (Figure 2).

In no case did the faecal amino acid levels between FRE and IRE dogs vary statistically. However, significant differences were observed between the GIA group and the other experimental groups for all amino acids except Glu. Specifically, the GIA group had higher faecal levels of Asp ($p = 0.0035$), Ser ($p = 0.0005$), Pro ($p = 0.0027$), Gly ($p = 0.0030$), Ala ($p = 0.0037$), Cys ($p = 0.0015$), Tyr ($p = 0.0001$), Lys ($p = 0.0085$), Met ($p = 0.0084$), Arg ($p = 0.0002$), Thr ($p = 0.0005$), Val ($p = 0.0011$), Ile ($p = 0.0045$), Leu ($p = 0.0044$), Phe ($p = 0.0023$), and His ($p = 0.0044$) (Figures 1 and 2).

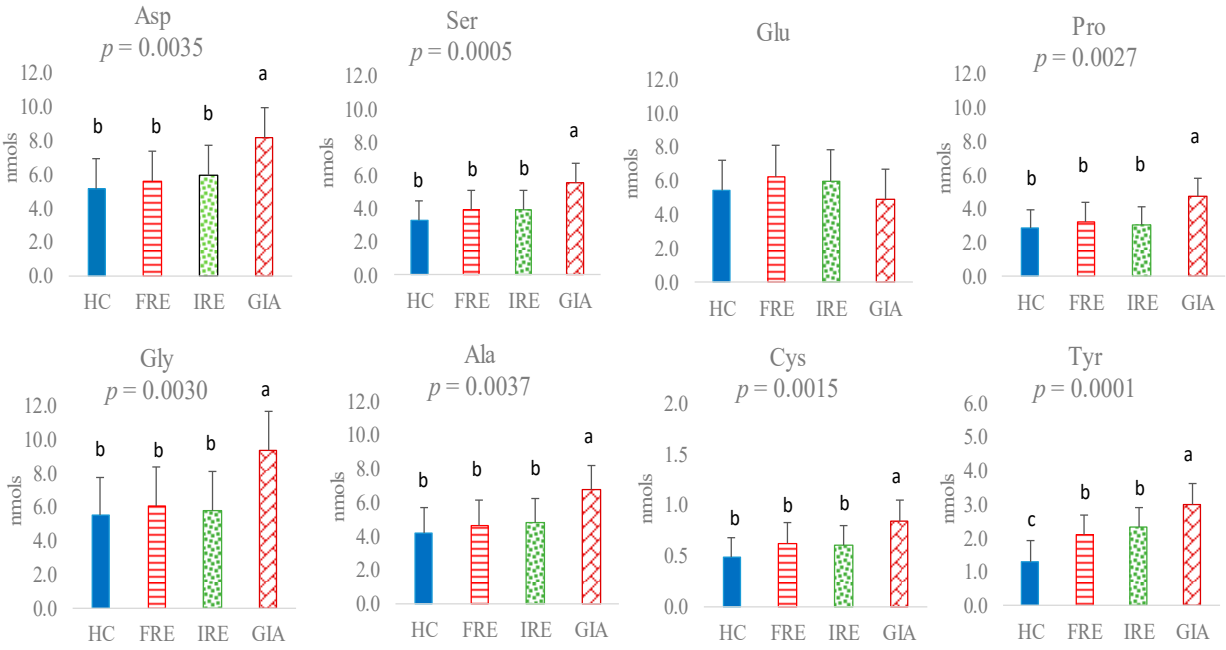

Figure 1. Non-essential amino acids (nmol) in faeces of healthy control (HC) dogs, food-responsive enteropathy (FRE) dogs, immunosuppressant-responsive enteropathy (IRE) dogs, and dogs parasitised with *Giardia* (GIA). Aspartic acid (Asp), serine (Ser), glutamic acid (Glu), proline (Pro), glycine (Gly), alanine (Ala), cysteine (Cys), tyrosine (Tyr). Values with different superscripts (a,b,c) are statistically significant.

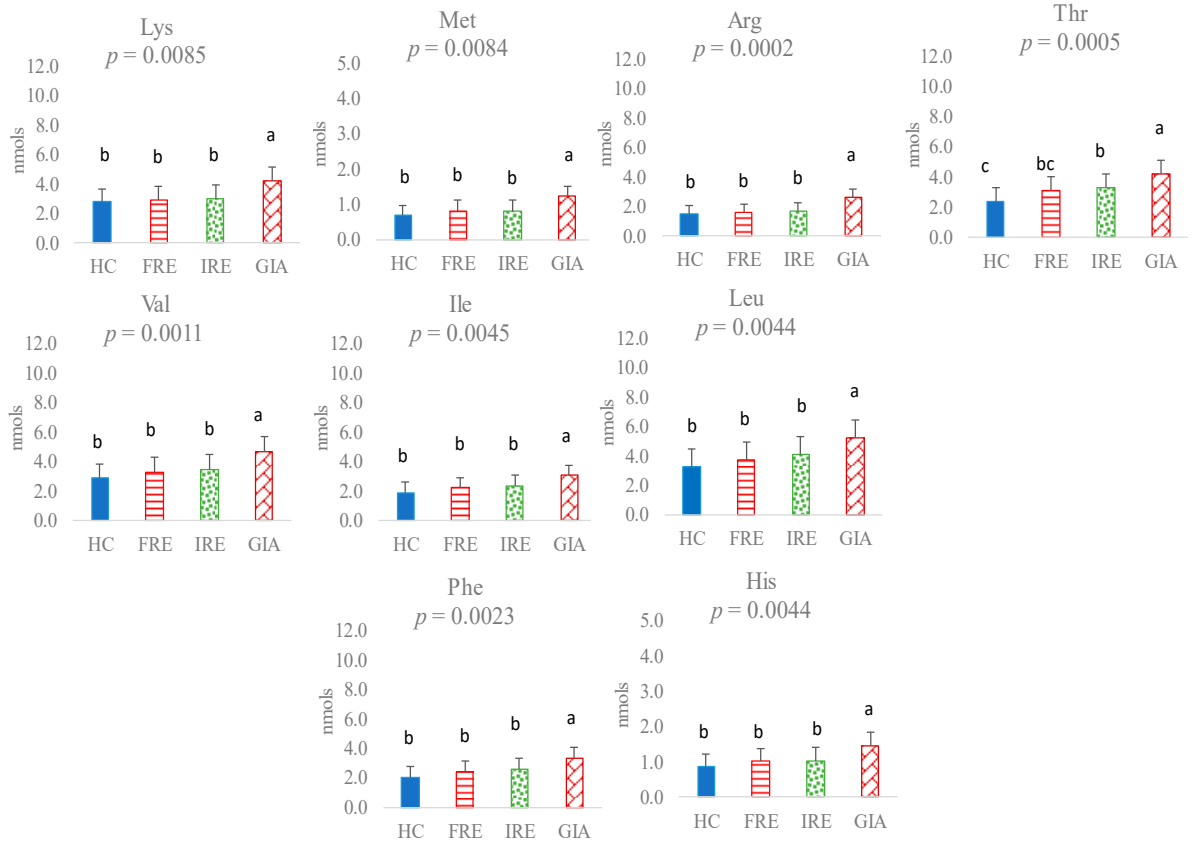

Figure 2. Essential amino acids (nmol) in faeces of healthy control (HC) dogs, food-responsive enteropathy (FRE) dogs, immunosuppressant-responsive enteropathy (IRE) dogs, and dogs parasitised with *Giardia* (GIA). Lysine (Lys), methionine (Met), arginine (Arg), threonine (Thr), valine (Val), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), and histidine (His). Values with different superscripts (a,b,c) are statistically significant.

Concerning BCAAs, no significant differences were observed between the HC, FRE, or IRE groups (Figure 3). However, the GIA group presented higher BCAA values than the other dog groups ($p = 0.0019$). On the other hand, AAAs showed more pronounced differences. Thus, the HC group was the one that presented the lowest AAA values compared to the other groups, while GIA had the highest ($p = 0.0001$). The FRE and IRE groups did not present significant differences between them in the AAA levels, generally presenting intermediate values between the HC and GIA groups. Consequently, the BCAAs/AAAs ratio was the highest in HC dogs compared to the rest of the other groups, which showed no significant differences among themselves ($p = 0.0001$).

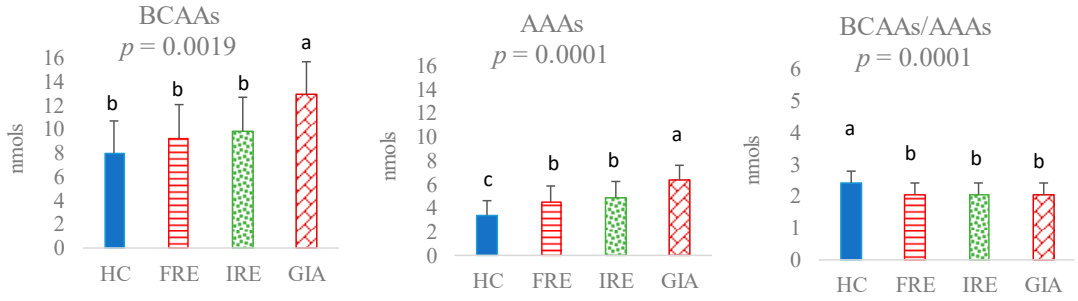

Figure 3. Branched-chain amino acids (BCAAs = sum of Leu, Ile, and Val), aromatic amino acids (AAAs = sum of Tyr and Phe), and the BCAAs/AAAs ratio in faeces of healthy control (HC) dogs, food-responsive enteropathy (FRE) dogs, immunosuppressant-responsive enteropathy (IRE) dogs, and dogs parasitised with *Giardia* (GIA). Values with different superscripts (a,b,c) are statistically significant.

Regarding CIBDAI, this was positively related to Tyr (r = 0.63; $p = 0.0001$), Thr (r = 0.48; $p = 0.0001$), Phe (r = 0.42; $p = 0.0001$), and AAAs (r = 0.55; $p = 0.0001$) and negatively related to the BCAAs/AAAs ratio (r = −0.27; $p = 0.0134$).

The discriminant potential of the amino acid profile to differentiate between different enteropathies was evaluated through linear discriminant analysis (Figure 4). Multivariable analysis revealed that HC and GIA groups differed the most from the FRE and IRE groups, while the FRE and IRE groups overlapped to some extent.

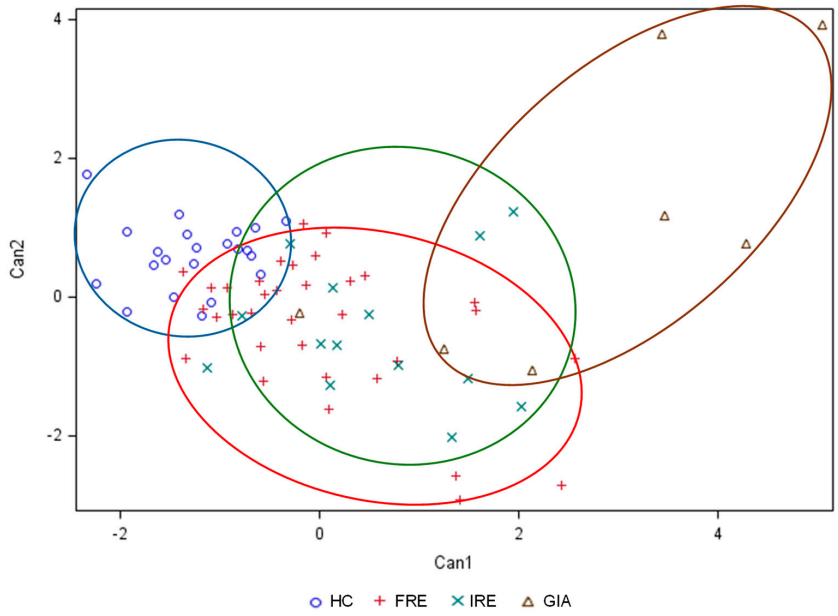

Figure 4. Linear discriminant analysis for faecal amino acids of healthy control (HC; dark blue) dogs, food-responsive enteropathy (FRE; red) dogs, immunosuppressant-responsive enteropathy (IRE; light green) dogs, and dogs parasitised with *Giardia* (GIA; brown).

The most influential variables contributing to canonical discriminant functions 1 and 2 were Tyr ($p = 0.0001$), Glu ($p = 0.0003$), Arg ($p = 0.0001$), and Phe ($p = 0.1435$).

Cross-validation results of the discriminant functions (Table 2) indicated that 44% of stool samples were not correctly assigned to their group, giving a 56% success rate. The HC group had the highest correct classification rate (100% correct assignments), followed by the GIA group (57%). However, 29% of GIA were misclassified as FRE and 14% as IRE. For the FRE group, 38% were assigned to the correct group, whereas 3% were misclassified as GIA, 27% as IRE, and 32% as HC. The IRE group had the lowest correct classification rate (29%), with 50% of stool samples classified as FRE, 14% as GIA, and 7% as HC.

Table 2. Classification accuracy (%) based on faecal amino acid profile of healthy control (HC) dogs, food-responsive enteropathy (FRE) dogs, immunosuppressant-responsive enteropathy (IRE) dogs, and dogs parasitised with *Giardia* (GIA) (% assigned correctly) according to discriminant functions (cross-validation).

	FRE	GIA	IRE	HC	TOTAL (%)
FRE	38.24	2.94	26.47	32.35	100
GIA	28.7	57.14	14.29	0.00	100
IRE	50.00	14.29	28.57	7.14	100
HC	0.00	0.00	0.00	100.00	100

4. Discussion

Few studies in veterinary medicine have evaluated the amino acid profile in the faeces of dogs with CIEs, and in some cases, the results have not followed similar trends. However, these comparisons have been limited to healthy dogs and a single group of sick animals, without including a broader evaluation of dogs with different types of enteropathies.

In the present study, no significant changes were observed in the essential or non-essential amino acid profiles of FRE and IRE dogs, nor in the total BCAAs or AAAs or the BCAAs/AAAs ratio. However, FRE and IRE dogs had higher levels of Tyr and total AAAs in faeces than healthy dogs. As previously mentioned, this is the first study to evaluate the amino acid profile of dogs with different enteropathies together, thereby providing novel information into the potential differences between these two groups of dogs based on their faecal amino acid profiles. Comparisons between sick animals with the HC group align with findings from previous studies in humans, which reported elevated Tyr levels in the faeces of individuals with IBD compared to healthy controls [20–22]. Tyr is an AAA obtained from Phe via phenylalanine hydroxylase in the liver, which is further metabolised into neurotransmitters, such as dopamine, norepinephrine, and adrenaline, as well as melanin. Impaired conversion of Phe to Tyr could result in reduced cognitive performance [23], and alterations in the Phe/Tyr ratio have also been observed in inflammatory conditions [24]. Furthermore, it is interesting to note that Tyr can give rise to the compound *p*-cresol [25] by the action of certain anaerobic microorganisms, a compound that is genotoxic to colonocytes and reduces mitochondrial oxygen consumption in these cells [26]. In addition, other metabolites such as *p*-cresyl sulfate can be synthesised from *p*-cresol in the host mucosa [26], subsequently entering circulation or being excreted in urine [27]. These metabolites have been associated with increased oxidative stress and, in some cases, cell death [27]. Excessive levels of such compounds have also been linked to reduced gut microbiota diversity [28]. Other investigations have reported significant changes in other faecal AAAs such as Phe or Trp. Thus, Marchesi et al. [20] observed a greater abundance of Phe in faecal samples from patients with various CIEs. Conversely, Higueras et al. [15] reported a reduced proportion of Phe in faecal samples from sick animals compared to the control group, whereas this amino acid was found to be increased in blood. More numerous are the authors who

have identified alterations, primarily increases in the aromatic amino acid Trp, in both humans and animals [14,21,22], with evidence linking microbial Trp metabolites to gut disorders [29]. The discrepancies between studies regarding the presence of amino acids in faeces may be due to factors related to the sample heterogeneity across investigations, as well as methodological differences, including whether prior hydrolysis was employed in the analytical process [15].

It is interesting to note that although the levels of Tyr, Phe, and AAAs were not different between FRE and IRE dogs, a direct relationship was observed with the severity index. In other studies, IRE dogs have also shown more severe signs of disease [18], although based on the levels of these AAs in faeces and with the methodology used for their measurement, it would not be possible to predict what treatment an individual CIE dog would respond to based on faecal AAs analysis. On the other hand, neither the total BCAA levels nor the BCAAs/AAAs ratio were significantly affected between FRE, IRE, and control groups in the present study. Higueras et al. [15] did not observe any significant changes in these AAs in a previous study involving FRE dogs compared to a control. However, some of the BCAAs, such as Leu, showed a direct relationship with indicators of intestinal health, such as the presence of short-chain fatty acids (SCFAs) in faeces. This relationship was also observed in the present study (data not presented), confirming the previous results. It has been reported that BCAA catabolism contributes to the synthesis of all fatty acids [28]. Specifically, Val and Ile contribute to the lipogenic propionyl-CoA pool, which acts as a primer for fatty acid synthase in the production of odd-chain fatty acids [30]. Moreover, the catabolism of AAAs by the gut microbiome has revealed that the derived metabolites are bioactive compounds that may exert varying effects on the gut and other organs [29]. Thus, under certain environmental conditions, such as changes in the gut pH or other factors associated with gut diseases, a dysregulation of the microbiome may occur, altering metabolite production [29].

Another remarkable result of the present study was the higher Thr content in the faeces of IRE dogs compared to control dogs, with FRE dogs showing intermediate values. In children with ulcerative colitis, Kolho et al. [31] observed elevated levels of Thr in faecal metabolic fingerprinting compared to controls. Moreover, an in-depth metabolomics study by Filmoniuk et al. [32] on children with IBD found significant changes in the metabolism of 16 compounds. Among these, the metabolism of Thr was notably affected, followed by changes in the metabolism of Phe and its derived products, which aligns with the results of the present study. In fact, in the present study, Thr had a significant direct relationship with the severity index, as did Tyr and Phe. Thr is an essential amino acid that serves as a substrate for mucin synthesis [33] and plays a role in lipid metabolism regulation [34]. The importance of Thr in mucus formation is such that, even when Thr supply is deficient, other tissue growth functions may be limited while mucin production is maintained [35]. Thus, Thr is critical for barrier function and gut homeostasis and may influence the intestinal immune system [36]. The higher levels of this amino acid found in the faeces of sick animals in the present study could be explained by its preferential use by mucosal cells for mucin synthesis when present in the intestinal lumen [37]. In addition, it has been reported that under intestinal inflammation, Thr uptake is enhanced by intestinal cells for mucus production, contributing to gut protection [38].

In relation to the group of dogs infected with *Giardia* that also experienced gastrointestinal problems, they presented the highest levels of faecal amino acids, including Thr and Tyr, compared to the other groups. Additionally, these animals presented the highest faecal levels of most amino acids (except Glu), as well as AAAs, and BCAAs in comparison to the HC, FRE, and IRE groups. *Giardia* spp. is a protozoan parasite that infects humans and other vertebrates, residing in the upper small intestine and causing severe diarrhoea,

malabsorption, and other gastrointestinal diseases worldwide [39]. This parasite has been described to utilise amino acids differently [40] and to produce secretory/excretory proteins linked to infection [41]. It also induces structural damage that compromises epithelial integrity and function [42]. Additionally, some studies indicate that *Giardia* proteins secreted upon contact with epithelial cells may facilitate colonisation of the host's small intestine and deactivate host innate immune factors, such as nitric oxide production [43]. These changes might explain the increased presence of AAs in the faeces of GIA dogs. It is important to note that many GIA infections are asymptomatic and have even been considered to protect against other gastrointestinal processes or resolve spontaneously [42]. However, this infection can also lead to irritable bowel syndrome and other intestinal disorders [42], making its differentiation from other digestive conditions essential. According to the results of the present study, the faecal amino acid profile could serve as a non-invasive tool complementary to existing diagnostic techniques for this infection.

It is interesting to note that in general the AAs that presented the greatest discriminating power between the different groups were Tyr, Phe, Glu, and Arg. The differences in Tyr and Phe between the groups of dogs have been previously discussed, with Tyr, derived from the metabolism of Phe, showing the most significant variation. However, Glu and Arg levels did not significantly differ among the groups, except in GIA dogs, which presented higher Arg levels compared to the others. Arg is a semi-essential amino acid that serves as a precursor for the synthesis of proteins, nitric oxide (NO), creatinine, and urea [44]. Einarsson et al. [42] reported that Giardia trophozoites use the arginine dihydrolase pathway for energy production and that the arginine deiminase is one of the metabolic enzymes secreted by the parasite upon interaction with the epithelium. Giardia-induced Arg depletion is a mechanism to prevent the production of NO by the host since this compound is toxic to the parasite [42,43]. Interestingly, Arg can be synthesised from glutamine [45]. The fact that Glu was the only faecal AA that did not show higher levels in the GIA group compared to the other experimental groups could indicate its increased consumption for Arg synthesis. Glutamate is a non-essential amino acid that can be synthesised in the body and serves as an important energy source for the proliferation of intestinal lymphocytes and epithelial cells [46]. Consequently, it plays significant roles in the maintenance of mucosal structure, tight junctions, and mucosal permeability [47]. In a metabolomic study of individuals with IBD, Filimoniuc et al. [32] also highlighted the metabolism of Glu and Arg being most influential in relation to the presence of disease, along with the metabolism of Thr or Phe [32]. Additionally, patients with IBD often exhibit Arg deficiency and alterations in the Arg metabolic pathway [48]. Both Glu and Arg, have beneficial effects on the modulation of inflammatory cytokines, which may influence gut integrity [49].

Despite the discriminating potential of some of the amino acids mentioned, the faecal AA analysis could not accurately distinguish between the FRE and IRE dogs, as both groups showed considerable overlap. Therefore, based on the results, we could not say that the two groups have distinct amino acid metabolism. Furthermore, although the GIA dogs had a markedly different AA profile compared to the other groups, the statistical model built to distinguish between groups did not accurately differentiate this group from FRE or IRE.

5. Conclusions

In conclusion, dogs infected with GIA showed significantly greater faecal amino acid excretion compared to the other groups. Additionally, healthy dogs presented some significant differences in their faecal amino acid profiles compared to dogs with FRE or IRE, particularly in Thr and AAAs (especially Tyr). However, the faecal amino acid profile did not differ between dogs with FRE and IRE. Therefore, it would not be possible to predict an individual CIE-affected dog's response to treatment based on faecal AA analysis. Further

studies are necessary to better understand the different responses to dietary treatment in these two groups of dogs.

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Data Availability Statement: Data are contained within the article.

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Abbreviations

The following abbreviations are used in this manuscript:

AA	amino acid
AAA	aromatic amino acid
Ala	alanine
Arg	arginine
Asp	aspartic acid
BCAA	branched-chain amino acid
BCS	body condition score
CCECAI	canine chronic enteropathy clinical activity index
CIE	chronic inflammatory enteropathy
CIBDAI	canine inflammatory bowel disease activity index
CSIC	Spanish National Research Council
CVMTH	Complutense Veterinary Medicine Teaching Hospital
Cys	cysteine
DNA	deoxyribonucleic acid
FRE	food-responsive enteropathy
GIA	*Giardia* infection
Glu	glutamic acid
Gly	glycine
HC	healthy control
His	histidine
HCl	hydrochloric acid
Ile	isoleucine
IBD	inflammatory bowel disease
IRE	immunosuppressant-responsive enteropathy
Leu	leucine
Lys	lysine

Met	methionine
MCS	muscle condition score
NaOH	sodium hydroxide
Phe	phenylalanine
Pro	proline
RMSE	root mean square error
Ser	serine
Thr	threonine
TLI	trypsin-like immunoreactivity
Trp	tryptophan
Tyr	tyrosine
Val	valine

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DISCUSIÓN

5. DISCUSIÓN

El estudio integral de pequeñas moléculas presentes en muestras biológicas (metabolómica) es un método emergente que podría permitir profundizar en la fisiopatología de las ECs y las interacciones entre el huésped y los microorganismos, con el potencial de desarrollar enfoques novedosos de diagnóstico y/o tratamiento.

En medicina veterinaria, solo unos pocos estudios han abordado este enfoque, evidenciando el papel de determinadas moléculas orgánicas, como los ácidos grasos y los aminoácidos en estas enfermedades. Estas moléculas pueden ser administradas a través del alimento o ser sintetizadas de forma endógena. Por este motivo en la presente tesis doctoral, se trató de evaluar el perfil de ácidos grasos y aminoácidos en muestras biológicas de distinta naturaleza con el objetivo de caracterizar los distintos tipos de enteropatías, evaluar su potencial como moléculas biomarcadoras de enfermedad, así como abrir paso a un manejo dietético más personalizado.

5.1 Evaluación del perfil de ácidos grasos en perros con distintos tipos de ECs

En un primer experimento piloto (artículo 1) llevado a cabo en el desarrollo de la presente tesis doctoral, se evaluaron las posibles diferencias en el perfil de ácidos grasos (ácidos grasos totales y AGCC como indicadores de actividad microbiana) entre perros sanos y perros con FRE, tanto a nivel sistémico (plasma) como en heces. Los resultados mostraron una mayor correlación entre el perfil lipídico de las muestras fecales y los índices de gravedad de la enfermedad (el CIBDAI y la CC) en comparación con las muestras plasmáticas. Por ello, y teniendo en cuenta uno de los objetivos de la presente tesis de buscar biomarcadores en muestras de la manera menos invasiva posible, en el artículo 3 se procedió a la evaluación del perfil lipídico exclusivamente en muestras fecales.

La determinación del perfil de ácidos grasos a nivel sistémico (artículo 1) mostró que los perros con FRE presentaron niveles inferiores de PUFA y de omega-6 en comparación con los perros sanos. Aunque los estudios en humanos con IBD muestran resultados contradictorios respecto a los niveles sistémicos de PUFA, las investigaciones sugieren una disminución de estos, especialmente de los omega-6, a medida que aumenta la gravedad de la enfermedad (Esteve-Comas *et al.,* 1992; Scoville *et al.,* 2018; Jiang *et al.,* 2023).

En medicina veterinaria, los estudios sobre el perfil lipídico sistémico son escasos y emplean diversos tipos de muestras para su evaluación (Kalenyak *et al.,* 2019; Crisi *et al.,* 2021; Galler *et al.,* 2022a; Walker *et al.,* 2022). No obstante, los hallazgos de Walker *et al.,* en 2022 describieron niveles reducidos de PUFA y omega-6 en muestras séricas de perros con EC en comparación con perros sanos, en concordancia con los resultados de la presente tesis. Estos hallazgos sugieren una posible malabsorción intestinal, un mayor consumo de PUFA para la síntesis de otros compuestos derivados, o para obtener energía como consecuencia de la elevada demanda energética que tiene lugar en la patogénesis de estas enfermedades (Scoville et al., 2018), donde se requiere de energía para reclutar células inmunitarias, así como de estos precursores para sintetizar mediadores lipídicos o reparar las membranas celulares afectadas por el proceso inflamatorio.

Es más, Crisi *et al.,* en 2021 también describieron una hiperactivación del metabolismo del precursor de los omega-6, el LA, en perros con EC, con un consumo incrementado de PUFA a medida que la enfermedad se agravaba. Además, describieron un aumento de la actividad elongasa con el objetivo de sintetizar ácidos grasos de cadena larga (Crisi *et al.,* 2021).

Estos resultados son consistentes con los hallazgos en las muestras fecales de los perros con EC en el experimento 2 de esta investigación (artículo 3), donde los perros con IRE mostraron mayores alteraciones en el metabolismo lipídico. Así, en estos animales se observaron niveles fecales reducidos del total de PUFA y omega-6 en comparación con perros sanos, junto con una disminución del precursor LA. Además, el aumento de la ratio C18:3n-6/C18:2n-6 (indicador de la actividad de la Δ6-desaturasa) sugiere una hiperactivación de la ruta metabólica de los omega-6 para la síntesis de PUFA, similar a lo observado por Uchiyama *et al.,* en 2013 en humanos con enfermedad de Crohn.

Se detectaron además niveles elevados en la ratio elongasa C18:0/C16:0 y una tendencia ascendente en la ratio elongasa C22:5n-3/C20:5n-3 (**Figura 5.1**), apoyando la síntesis de PUFA omega-3 y ácidos grasos de cadena larga durante el proceso inflamatorio. Los niveles superiores del índice de gravedad CIBDAI y del porcentaje de grasa fecal se correlacionaron significativamente con niveles más bajos de PUFA, omega-6 y LA, y con

valores mayores de los índices Δ6-desaturasa y elongasa C22:5n-3/C20:5n-3 (esta última correlación solo se observó con el CIBDAI).

Estos hallazgos indican que los perros con IRE presentan una síntesis de PUFA más activa en comparación con los perros sanos, mientras que los perros con FRE muestran valores intermedios.

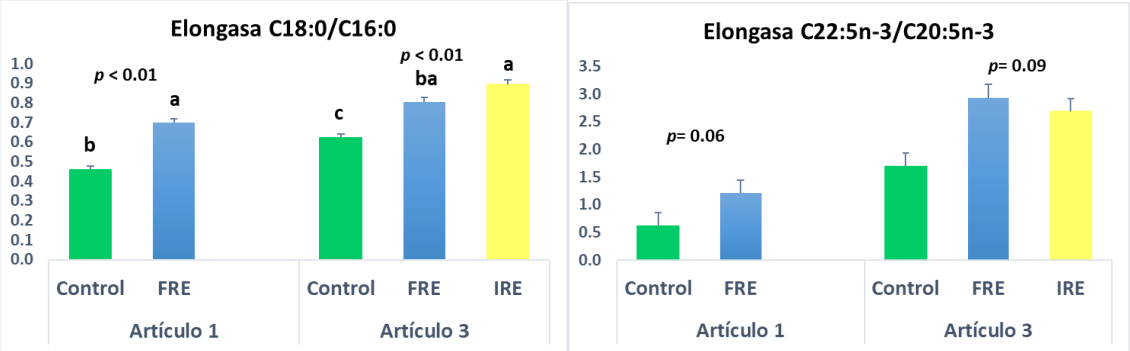

Figura 5.1. Comparación del perfil de ácidos grasos fecales entre los artículos 1 y 3.

Es importante señalar que en el estudio de Galler *et al.* (2022a) no se detectaron niveles inferiores de PUFA sistémicos en perros con EC, aunque sí se observó una reducción en los niveles de algunos PUFA, como el C20:2n-6 y el C20:3n-6. Curiosamente, estos dos ácidos mostraron niveles superiores a nivel sistémico en los perros con FRE en la presente tesis, lo que se asoció a una posible activación de la ruta alternativa que cataliza la síntesis de C20:3n-6 a partir del C20:2n-6 en condiciones inflamatorias, para la obtención final de AA (Park *et al.,* 2009). Estos resultados coincidieron con la tendencia observada en el aumento del AA en las muestras fecales de los perros con FRE del artículo 1, sugiriendo que estos ácidos podrían servir como indicadores sistémicos del proceso inflamatorio en este tipo de enteropatía. A pesar de que en el estudio de Crisi *et al.,* en 2021 también se demostraron niveles sistémicos elevados de C20:3n-6 en perros con EC en comparación con perros sanos, es importante considerar la diferencia en la muestra utilizada: la membrana de los eritrocitos plasmáticos.

En cuanto a otros ácidos grasos de cadena larga de interés como el AA, los perros con FRE en el primer experimento piloto (artículo 1) mostraron una tendencia a presentar niveles superiores de AA en las muestras fecales en comparación con los perros sanos. Sin

embargo, en el segundo experimento (artículo 3) en el que se utilizó un mayor número de individuos, apenas se observaron cambios en este ácido graso en muestras de heces procedentes de perros con distintos tipos de enteropatías (FRE e IRE). La mayoría de los estudios realizados en humanos con IBD han observado un aumento de los niveles de AA en las heces como consecuencia del proceso inflamatorio (Jansson *et al.,* 2009; Alghamdi *et al.,* 2018; Lloyd-Price *et al.,* 2019; Vich *et al.,* 2023). Además, estos resultados también se han observado en perros (Honneffer *et al.,* 2017; Cagnasso *et al.,* 2024) y gatos con ECs (Marsilio *et al.,* 2021; Sung *et al.,* 2023; Giordano *et al.,* 2024). En esta misma línea, se ha demostrado un aumento del AA en los fosfolípidos del plasma (Figler *et al.,* 2007; Schwarz *et al.,* 2021), los fosfolípidos de la mucosa del colon (Nishida *et al.,* 1987) y en la propia mucosa intestinal (Buhner *et al.,* 1994; Esteve *et al.,* 1998; Nieto *et al.,* 1998; Diab *et al.,* 2019) en humanos con IBD. Este ácido graso es el componente mayoritario de las membranas celulares y participa en la síntesis de mediadores lipídicos con carácter proinflamatorio (Calder, 2010; Innes & Calder, 2018). Los niveles elevados de AA en las heces observados en el artículo 1 se correlacionaron negativamente con el ácido acético y el propiónico, dos AGCC indicadores de salud intestinal. En consecuencia, los niveles elevados de AA podrían reflejar una mayor destrucción celular o un aumento de la síntesis para reparar las membranas dañadas o sintetizar mediadores lipídicos involucrados en el proceso inflamatorio.

Uno de los resultados más interesantes de la presente tesis doctoral ha sido el cambio en los niveles de ácidos grasos MUFA y SAT, así como en las ratios indicadores del índice de desaturación en muestras fecales de perros con EC en comparación con los perros sanos. Así, en el artículo 1 los perros enfermos mostraron niveles inferiores de MUFA y del índice Δ9-desaturasa en heces. Los resultados del artículo 3 corroboraron esta afirmación, en relación a las proporciones en el C18:0 (SAT), ∑MUFA, y los índices indicadores de la Δ9 (C16:1n-7/C16:0, C18:1n-9/C18:0) (**Figura 5.2**).

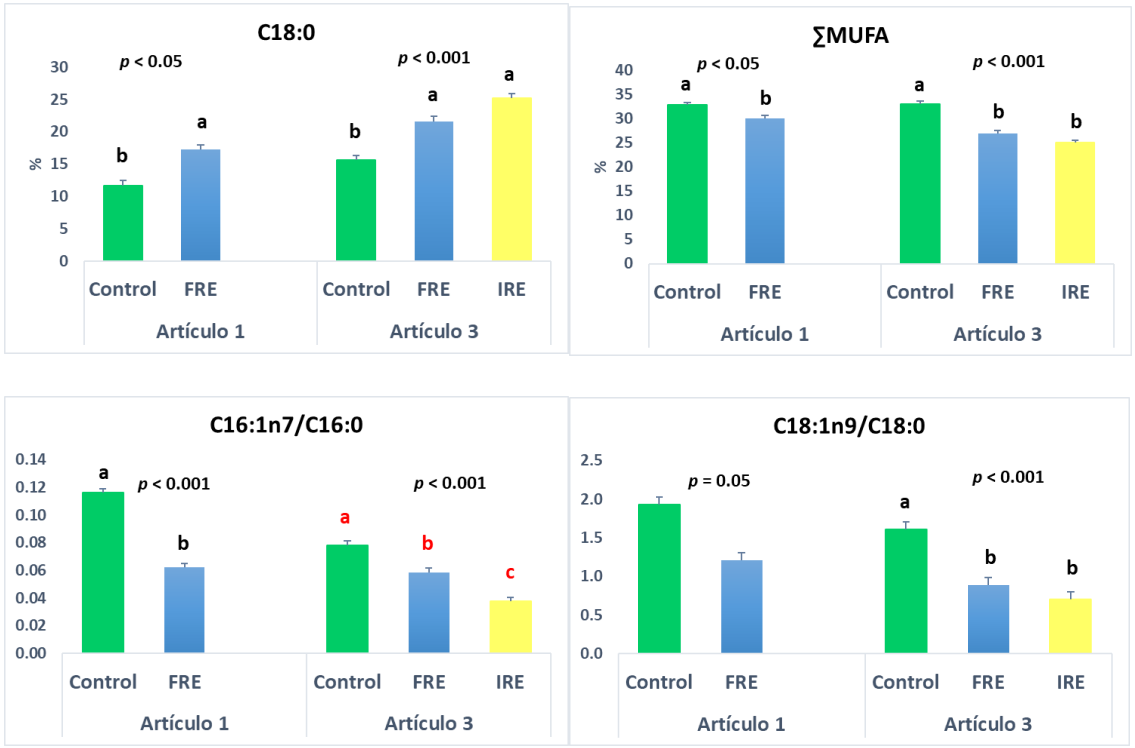

Figura 5.2. Comparación del perfil de ácidos grasos fecales entre los artículos 1 y 3.

La Δ9-desaturasa es la enzima encargada de catalizar el paso de SAT a MUFA; por tanto, su falta de actividad podría explicar, en parte, la alteración observada. Numerosos estudios en muestras de íleon o colon de humanos con IBD han descrito estos mismos resultados (Nishida *et al.,* 1987; Buhner *et al.,* 1994; Fernández-Bañares *et al.,* 1997; Esteve *et al.,* 1998). Además, se ha documentado la falta de expresión de los genes de esta enzima en humanos con colitis ulcerosa (Bueno-Hernández *et al.,* 2011), y una inhibición de su actividad a nivel hepático en modelos murinos con colitis (Chen *et al.,* 2008; Wang *et al.,* 2016), lo cual podría deberse al daño causado por la migración de endotoxinas y citoquinas proinflamatorias a través de la vena porta (Chen *et al.,* 2008). En el estudio de Ducheix *et al.,* en 2018 se demostró que la falta de expresión de esta enzima a nivel intestinal aumentaba la susceptibilidad a la inflamación y al desarrollo de tumores en un modelo murino y, que el ácido monoinsaturado C18:1n-9 en la dieta prevenía tanto la inflamación como la tumorogénesis.

Este menor valor del índice indicador de la actividad de la Δ9-desaturasa podría ser responsable, en parte, de los niveles inferiores de dos de los MUFA más importantes obtenidos de la actividad de esta enzima, el C16:1n-7 y el C18:1n-9, en los perros enfermos,

tanto con FRE como con IRE. De hecho, uno de los hallazgos más destacados de la presente tesis ha sido demostrar que los niveles inferiores de C16:1n-7 (ácido palmitoleico), así como la ratio C16:1n-7/C16:0 fueron capaces de diferenciar a los perros con IRE de los perros con FRE y de los sanos (**Figura 5.3**), siendo este el primer estudio científico en describir estas diferencias (artículo 3).

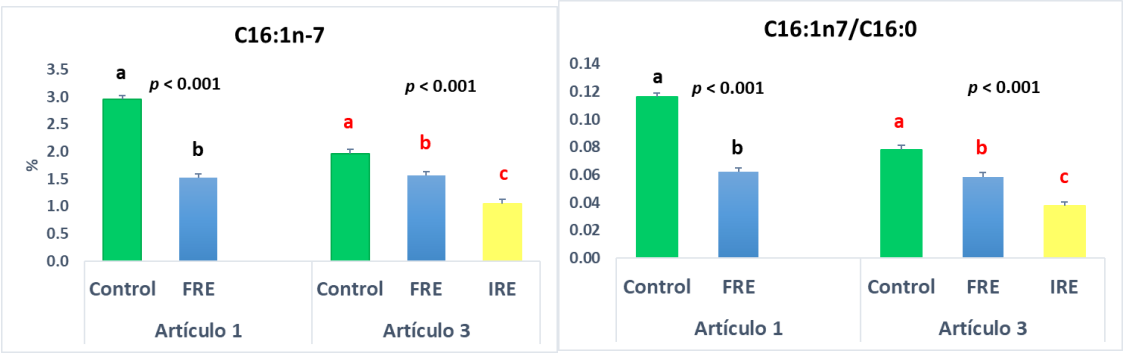

Figura 5.3. Comparación del perfil de ácidos grasos fecales entre los artículos 1 y 3.

Como se mencionó en la revisión bibliográfica, el ácido palmitoleico es considerado una hormona lipídica que participa en numerosos procesos metabólicos (Cao *et al.,* 2008), y se le atribuye un carácter antiinflamatorio (Bermúdez *et al.,* 2022). Aunque puede ser incorporado a partir de la dieta, parte de su síntesis es endógena (Guillou *et al.,* 2010; Hodson & Karpe, 2013). Se han descrito niveles elevados de este ácido en suero de humanos con enfermedad de Crohn y una correlación positiva con el índice de gravedad (Akazawa *et al.,* 2021). En modelos murinos con colitis, se ha sugerido que el ácido palmitoleico inhibe la inflamación y repara la mucosa intestinal, incluso por encima del ácido butírico (Chen *et al.,* 2023). Además, también ha demostrado facilitar el crecimiento *in vivo* e *in vitro* de la bacteria beneficiosa *Akkermansia muciniphila (*Chen *et al.,* 2023).

Los perros con IRE fueron los que presentaron los valores significativamente inferiores en comparación con otros perros afectados con otro tipo de enteropatía, aunque los niveles de Δ9-desaturasa no se vieron afectados entre los animales enfermos. En cualquier caso, existe muy poca literatura científica relacionada con este ácido graso en estas enfermedades, por lo que futuros estudios son necesarios para entender los cambios más marcados observados en los perros con IRE. Aun así, tanto el ácido palmitoleico como la ratio C16:1n-7/C16:0 y la Δ9-desaturasa se correlacionaron negativamente con el CIBDAI

y el porcentaje de grasa fecal, lo que sugiere que podrían jugar un papel importante en la patogénesis de las ECs.

Otro de los resultados más interesantes de la presente tesis ha sido la identificación de diferencias en los niveles de AGCC entre los perros con IRE y los perros con FRE en comparación con animales sanos (artículos 1 y 3), hallazgo que no había sido descrito previamente. Los perros con IRE se diferenciaron tanto de los controles como de los perros con FRE presentando los niveles más bajos del total de AGCC y del ácido acético en las muestras fecales (**Figura 5.4**). Además, mostraron los niveles más bajos de ácido propiónico en comparación con los controles, aunque no en comparación con los perros con FRE, que presentaron valores intermedios (**Figura 5.4**).

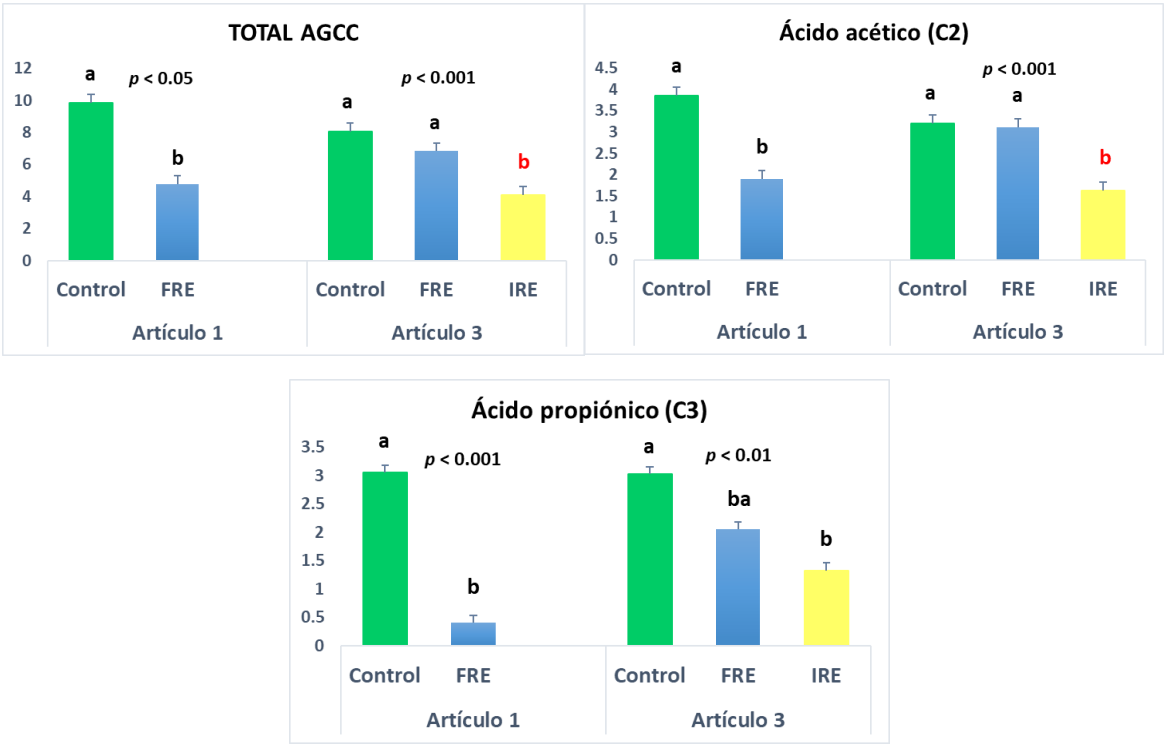

Figura 5.4. Comparación del perfil de AGCC en heces entre los artículos 1 y 3.

En términos generales, se han descrito niveles reducidos de AGCC en enfermedades gastrointestinales como la IBD y el cáncer colorrectal en humanos y modelos murinos (Zhang *et al.,* 2022). En medicina humana los resultados más comunes han sido niveles reducidos de los tres AGCC más prevalentes: ácido acético, propiónico y butírico (Marchesi *et al.,* 2007; Bjerrum *et al.,* 2015; Franzosa *et al.,* 2018; Lloyd-Price *et al.,* 2019; Wang *et al.,*

2021a; Kaczmarczyk *et al.,* 2022; Vich *et al.,* 2023). En veterinaria, los escasos estudios realizados hasta la fecha han descrito niveles reducidos de ácido acético y propiónico en perros con ECs en general (Minamoto *et al.,* 2019) o con IBD en específico (Kaga *et al.,* 2023). Por tanto, los resultados de la presente investigación coinciden con los estudios mencionados; sin embargo, nunca antes se habían observado diferencias entre los perros con FRE y los perros con IRE en relación a estos ácidos grasos.

La disbiosis intestinal y la reducción de bacterias responsables de la síntesis de AGCC son los principales factores que explican la disminución de estos compuestos en las muestras fecales asociadas a estas enfermedades (Minamoto *et al.,* 2019). Los niveles reducidos observados en los perros con IRE, en comparación con los perros con FRE, podrían reflejar un mayor grado de disbiosis vinculado a su peor estado clínico. Sin embargo, aunque no se han encontrado diferencias en la riqueza global de especies bacterianas entre ambos grupos en muestras intestinales, sí se han identificado variaciones en bacterias del filo Proteobacteria (Kalenyak *et al.,* 2018). En cualquier caso, son necesarios más estudios que analicen tanto el perfil lipídico como las diferencias en la microbiota fecal entre los grupos para confirmar o desmentir esta hipótesis.

Por otra parte, no se han detectado diferencias en los niveles de ácido butírico en ninguno de los dos experimentos. Una posible explicación podría ser su distinto patrón de uso y absorción en comparación con el ácido acético y el propiónico (den Besten *et al.,* 2013). Como se destacó en la revisión bibliográfica, el ácido butírico se emplea principalmente como fuente de energía para los colonocitos, con una mínima transferencia hacia la circulación portal (den Besten *et al.,* 2013). No obstante, en perros con diarrea aguda sí se han descrito niveles superiores en heces (Guard *et al.,* 2015).

El total de AGCC, junto con el ácido acético, propiónico, isobutírico e isovalérico se correlacionó positivamente en el artículo 1 con el AGCI C15:0. Este resultado era previsible dado que los AGCI como el C15:0 y el C17:0 pueden sintetizarse a partir del ácido propiónico (Zhang *et al.,* 2020). Los perros con FRE presentaron niveles reducidos de C15:0 en las muestras fecales, los cuales se asociaron con niveles superiores en el CIBDAI. Por ello, se sugirió que este ácido podría ser un biomarcador prometedor para diagnosticar la gravedad de esta enteropatía y estaría vinculado a alteraciones en la actividad microbiana intestinal.

Sin embargo, aunque en el segundo experimento (artículo 3) los niveles inferiores de C15:0 se relacionaron con características fecales desfavorables (mayor porcentaje de grasa y puntuación más alta en la escala fecal), no se observaron diferencias significativas entre los grupos. Esta discrepancia podría explicarse por las diferencias dietéticas entre ambos experimentos, ya que, como se mencionó en la revisión bibliográfica, a diferencia de los rumiantes, la mayor parte de los AGCI en perros se obtiene a partir de la dieta (Abdoul-Aziz *et al.,* 2021).

En cuanto a los AGCC ramificados isobutírico e isovalérico, se observaron diferencias entre los controles y los perros con FRE en el artículo 1. Sin embargo, estas diferencias no fueron significativas en el artículo 3 (**Figura 5.5**), si bien sí que se observó una correlación negativa con el porcentaje de humedad fecal. Existe muy poca literatura científica sobre estos ácidos en las ECs. En el estudio de Kaga *et al.,* en 2023 tampoco se encontraron diferencias significativas en estos ácidos entre perros con IBD y perros sanos, a pesar de que en modelos experimentales de IBD sí se han descrito alteraciones (Koleva *et al.,* 2014; Liu *et al.,* 2020).

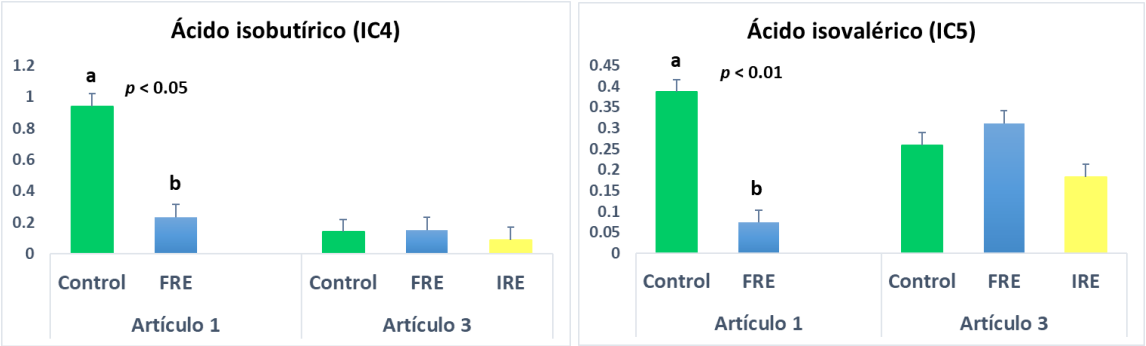

Figura 5.5. Comparación del perfil de AGCC en heces entre los artículos 1 y 3.

Sin embargo, es interesante destacar las diferencias en los niveles de los AGRs de cadena larga. Los perros con IRE presentaron niveles superiores de AGRs iso- e inferiores de anteiso en comparación con los controles y con los perros con FRE (artículo 3). Actualmente no existe literatura científica sobre estos ácidos grasos en las ECs, siendo este el primer estudio en documentar dichas diferencias. En otras especies como los rumiantes, se han observado aumentos de AGRs iso- y disminuciones de anteiso en terneros con diarrea (Xin *et al.,* 2021).

En un estudio *in vitro* realizado por Yan *et al.,* en 2017 en células intestinales, los AGRs anteiso- mostraron un efecto antiinflamatorio superior al de los iso, mediante la inhibición del factor NF-κB. Dado que estos ácidos forman parte de las membranas celulares bacterianas a nivel intestinal (Gozdzik *et al.,* 2023; Lu *et al.,* 2023), es posible que las diferencias observadas entre grupos reflejen cambios en el microbioma intestinal o un mayor consumo de anteiso por parte de los perros con IRE, como un mecanismo para contrarrestar un proceso inflamatorio más exacerbado.

De hecho, estos ácidos grasos se correlacionaron con los niveles de ácido propiónico. En este sentido, niveles superiores de propiónico se asociaron con concentraciones más altas de anteiso y más bajas de iso, lo que sugiere un posible carácter beneficioso de los AGRs anteiso-. No obstante, debido a su estrecha relación con el microbioma intestinal, futuros estudios que combinen su cuantificación junto con el análisis del microbioma proporcionarán información valiosa sobre su papel en la función digestiva.

Con el objetivo de profundizar en la caracterización de los dos tipos principales de enteropatías (experimento 2, artículo 4), se midieron parámetros relativos al hemograma, la bioquímica y los índices inflamatorios sistémicos, evaluando además su posible relación con los cambios en el perfil lipídico de las heces.

Así, en el artículo 4, los perros con IRE se diferenciaron significativamente de los perros con FRE al presentar niveles más elevados de plaquetas, plaquetocrito, NLR (neutrófilos/linfocitos), PLR (plaquetas/linfocitos) y SII (plaquetas*neutrófilos/linfocitos).

El aumento en el número de plaquetas se ha descrito tanto en humanos (Voudokis *et al.,* 2014) como en perros con IBD (Marchetti *et al.,* 2010; Cristóbal *et al.,* 2022; Agulla *et al.,* 2024), y se asocia con diversos factores, como la liberación de mediadores inflamatorios, la pérdida de antitrombina debido a hipoalbuminemia, o la presencia de hipercortisolemia (Goodwin *et al.,* 2011; Craven & Washabau, 2019; Pierini *et al.,* 2021). Este incremento en el recuento plaquetario también explicaría los valores superiores de PLR observados en los perros con IRE. Aunque estudios previos han descrito un aumento de PLR tanto en medicina humana (Feng *et al.,* 2017; Akpinar *et al.,* 2018; Jaoude *et al.,*

2018; Xie *et al.,* 2021) como en veterinaria en pacientes con IBD (Cristóbal *et al.,* 2022; Marchesi *et al.,* 2024), los resultados no siempre han sido consistentes (Agulla *et al.,* 2024).

A diferencia del PLR, el NLR cuenta con un mayor respaldo en la literatura veterinaria (Benvenuti *et al.,* 2020; Becher *et al.,* 2021; Cristóbal *et al.,* 2022; Marchesi *et al.,* 2024). En el estudio de Becher *et al.,* en 2021 el NLR permitió diferenciar a los perros con IRE de aquellos con FRE, hallazgos que son consistentes con los resultados obtenidos en esta tesis. Además, se ha demostrado que el NLR se correlaciona con parámetros clínicos importantes, como el CCECAI, la pérdida de proteínas y la linfangiectasia intestinal (Benvenuti *et al.,* 2020). Estos hallazgos refuerzan el valor del NLR como biomarcador relevante en la evaluación y caracterización de las ECs.

De manera similar, el índice SII mostró valores significativamente más elevados en los perros con IRE, lo que sugiere un estado de mayor gravedad en esta condición. Estos resultados son consistentes con los estudios de Cristóbal *et al.* (2022) y Agulla *et al.* (2024), que documentaron hallazgos similares en perros con IBD.

La elevación de los neutrófilos observada en este contexto podría estar relacionada con su papel en condiciones inflamatorias, como el daño a la mucosa intestinal, el incremento en la permeabilidad intestinal y la traslocación bacteriana, factores que pueden contribuir a complicaciones sistémicas (Rosero *et al.,* 2014; Craven & Washabau, 2019). Asimismo, las alteraciones en los linfocitos, comúnmente descritas en la patogénesis de las ECs (Agulla *et al.,* 2024), refuerzan la idea de que tanto el aumento en los neutrófilos como los cambios en los linfocitos contribuyen significativamente a la elevación descrita de los índices inflamatorios sistémicos.

De manera interesante, algunas de las variables sistémicas evaluadas se correlacionaron con los ácidos grasos que mostraron diferencias significativas entre los perros con IRE y los perros con FRE en el artículo 3, como el C16:1n-7, la ratio C16:1n-7/C16:0, el ácido acético y los AGCC. Específicamente, el C16:1n-7 y la ratio C16:1n-7/C16:0 se correlacionaron de forma lineal con los niveles de linfocitos, mostrando diferencias en la pendiente de la ecuación de regresión entre los grupos de perros con FRE e IRE (artículo 4).

Diversos estudios han señalado la importancia de los MUFA, como el C16:1n-7, y de la Δ9-desaturasa en la activación de los linfocitos B (Zhou *et al.,* 2021). En los perros con IRE, se observaron niveles marcadamente inferiores de linfocitos, junto con niveles más bajos de estas variables lipídicas. Esto es consistente con las alteraciones linfocitarias que suelen encontrarse en la patogénesis de las ECs (Agulla *et al.,* 2024). Es posible que la migración de linfocitos hacia la mucosa intestinal reduzca sus niveles circulantes (Galler *et al.,* 2017), un proceso que podría asociarse al daño intestinal y a una disminución de la actividad de la Δ9-desaturasa, lo que explicaría la reducción del C16:1n-7. De hecho, las ecuaciones de regresión entre los niveles de linfocitos y este ácido graso permitieron determinar que los perros con niveles de C16:1n-7 inferiores al 1,1 % y linfocitos por debajo de 1,8 x 10^3/µL correspondían mayoritariamente al grupo con IRE.

De manera similar, se identificaron diferencias significativas entre los grupos de enteropatías en las correlaciones del ácido acético con las plaquetas y con el SII (artículo 4). En los perros con FRE, niveles de ácido acético superiores a 2 mM se asociaron a recuentos plaquetarios dentro del rango de referencia. Por el contrario, en los perros con IRE, niveles de ácido acético inferiores a 2 mM se asociaron con recuentos plaquetarios por encima de los valores de referencia. Estos hallazgos, que no habían sido descritos previamente en perros con ECs, coinciden con estudios recientes que sugieren que el ácido acético puede inhibir la agregación plaquetaria al actuar sobre agonistas plaquetarios y la enzima COX-1 (Jing *et al.,* 2015). Como se observa en esta tesis, esta capacidad podría estar directamente implicada en la regulación de la actividad plaquetaria en las ECs.

Los AGCC, a través de su interacción con los receptores GPRs, regulan la actividad de las células inmunitarias (Kim *et al.,* 2014). En este sentido, niveles de ácido acético inferiores a 2 mM se asociaron con un estado inflamatorio más marcado, evidenciado por niveles elevados del SII (artículo 4). Esto destaca la importancia de mantener niveles adecuados de ácido acético para reducir la inflamación intestinal y preservar la integridad de la barrera epitelial intestinal (Chen *et al.,* 2018).

Por último, la relación observada entre los AGCC y el ácido butírico con los niveles de glucosa sanguíneos confirmaron el papel esencial de estos compuestos en la regulación del metabolismo de la glucosa a nivel sistémico (Sakakibara *et al.,* 2006; Deng, 2018; He *et*

al., 2020). En los perros con FRE, niveles de AGCC superiores a 5 mM se asociaron significativamente con la regulación de la glucosa dentro del rango de referencia. Por el contrario, en los perros con IRE, que presentaron niveles de AGCC inferiores a 5 mM, no se observó esta capacidad reguladora, y estos perros tendieron a mostrar niveles de glucosa más altos en comparación con los perros con FRE (artículo 4). Aunque las alteraciones en los niveles de glucosa no son comunes en la bioquímica de perros con ECs (Ridyard, 2020), en humanos con IBD se ha descrito resistencia a la insulina (Tigas & Tsatsoulis, 2012) y un mayor riesgo de desarrollar diabetes (Shanahan, 2012). Esto sugiere que la incapacidad de los AGCC para regular eficazmente la glucosa en los perros con IRE podría contribuir a las alteraciones de su estado metabólico.

5.2 Evaluación del perfil de aminoácidos en perros con distintos tipos de ECs

Al igual que en la evaluación del perfil lipídico, para la determinación del perfil de aminoácidos se llevó a cabo un experimento piloto inicial en el que se analizaron los perfiles de aminoácidos en sangre y heces de perros con FRE y perros sanos (artículo 2). Con el objetivo de identificar un biomarcador de la manera menos invasiva posible, en el siguiente experimento (artículo 5) se realizó exclusivamente la evaluación del perfil aminoacídico en heces, comparando distintos tipos de enteropatías.

A nivel sistémico, los perros con FRE mostraron niveles reducidos de asparagina, histidina, glicina, cistina, leucina, y de la ratio AACR/AAAs (artículo 2). Estos resultados coinciden con la mayoría de los estudios realizados en humanos con IBD, en los que se observa una reducción de los niveles sistémicos de los aminoácidos, posiblemente como consecuencia de la malabsorción debido al daño intestinal o del aumento del catabolismo proteico asociado a estas enfermedades, caracterizadas por una mayor demanda energética (Williams *et al.,* 2012; Gallagher *et al.,* 2020; Aldars-García *et al.,* 2021; Bauset *et al.,* 2021).

Sin embargo, dentro de la categoría de los AAAs, en el experimento 1 (artículo 2), la fenilalanina mostró niveles plasmáticos superiores en los perros con FRE en comparación con los perros sanos. El papel de este aminoácido en las ECs no está tan claro como el de otros aminoácidos, y los resultados en distintos estudios son controvertidos (Bauset *et al.,*

2021). Varios trabajos en humanos (Dawiskiba, 2014), perros (Walker *et al.,* 2022) y gatos (Kathrani *et al.,* 2023) con ECs también han descrito niveles elevados de fenilalanina en muestras sanguíneas. Este incremento podría atribuirse a procesos inflamatorios e infecciosos, como resultado del elevado catabolismo proteico (Huang *et al.,* 2019) o, como sugirieron Dawiskiba *et al.,* en 2014, a una inhibición inmunitaria de la enzima responsable de la conversión de fenilalanina a tirosina.

En cualquier caso, los niveles elevados de fenilalanina sanguínea observados en la presente tesis se asociaron con valores más altos de CIBDAI, mientras que la relación AACR/AAAs mostró una correlación inversa con este índice de gravedad. Estos hallazgos sugieren que los AAAs (incluyendo la fenilalanina), podrían estar relacionados con la presencia de enfermedad en los perros afectados. Además, los resultados del experimento 2 (artículo 5) respaldaron esta hipótesis al analizar las muestras fecales, donde los perros enfermos, tanto con FRE como con IRE, presentaron niveles superiores de AAAs (suma de fenilalanina y tirosina), y sobre todo del aminoácido tirosina, así como una relación AACR/AAAs más baja respecto a los perros control, coincidiendo con hallazgos previos en medicina humana (Jansson *et al.,* 2009; Bjerrum *et al.,* 2015; Diederen *et al.,* 2020).

Otros autores han descrito alteraciones en los niveles del AAA triptófano y de sus metabolitos en humanos (Gallagher *et al.,* 2020; Bauset *et al.,* 2021) y perros con IBD (Minamoto *et al.,* 2015; Tamura *et al.,* 2019; Benvenuti *et al.,* 2020), describiendo también aumentos de este aminoácido a nivel fecal (Jansson *et al.,* 2009; Bosch *et al.,* 2018; Diederen *et al.,* 2020; Pilla *et al.,* 2021; Blake, 2022; Jagt *et al.,* 2022). Sin embargo, en los estudios de la presente tesis doctoral se empleó un procedimiento de extracción de aminoácidos basado en hidrólisis ácida que destruye en gran medida este compuesto, por lo que no fue posible analizarlo (Ovalles, 2015).

El catabolismo de los AAAs por parte del microbioma intestinal ha revelado que algunos de los metabolitos derivados son compuestos bioactivos capaces de ejercer diversos efectos en el intestino y otros órganos (Liu et al., 2020). Sin embargo, bajo ciertas condiciones ambientales, como la disbiosis intestinal, o en enfermedades como la IBD (Jansson *et al.,* 2009; Lai *et al.,* 2019) se han descrito alteraciones en los metabolitos derivados de los AAAs. Algunos de ellos favorecen la producción de sustancias genotóxicas

para los enterocitos, como el *p*-cresol (Passmore *et al.,* 2018). Los niveles elevados de los AAAs en las heces de los perros con enteropatías podrían deberse a la disbiosis, la malabsorción o la pérdida de proteínas hacia la luz intestinal (Bosch *et al.,* 2018b).

Por otra parte, otro de los resultados más interesantes de la presente investigación fue la asociación de los AACR con un mejor estado de salud intestinal. En el experimento 1 (artículo 2), niveles plasmáticos superiores de los AACR, entre ellos la leucina, se asociaron con valores más bajos de CIBDAI. Los AACR desempeñan un papel clave en la regulación del metabolismo de la glucosa, los lípidos y la síntesis proteica, además de contribuir a la salud intestinal y la inmunidad (Nie *et al.,* 2018). Proveen energía en forma de nitrógeno para la síntesis de otros aminoácidos esenciales para la división celular rápida, como la de los enterocitos y las células inmunitarias (Holeček, 2002).

En la presente tesis, los perros enfermos mostraron niveles reducidos de la ratio AACR/AAAs en comparación con los perros sanos, tanto a nivel sistémico (artículo 2) como en las heces (artículo 5). Otros autores han descrito reducciones en este índice en enfermedades gastrointestinales y hepáticas (Dam *et al.,* 2015), asociadas a procesos de malnutrición o al aumento del catabolismo proteico (Eghtesad *et al.,* 2013). De hecho, los perros con EC de la presente tesis presentaron puntuaciones inferiores en el índice de CM en comparación con los perros sanos, lo cual podría deberse al aumento del catabolismo proteico como consecuencia del proceso inflamatorio, o a una falta de absorción de los nutrientes. Además, se han descrito niveles reducidos de los AACR en la mucosa de pacientes con IBD en comparación con pacientes sanos (Balasubramanian *et al.,* 2008; Diab *et al.,* 2019) posiblemente asociados a su mayor uso para la reparación de la mucosa intestinal afectada por el proceso inflamatorio crónico (Gallagher *et al.,* 2020).

Asimismo, los AACR se asociaron con niveles más altos de AGCC en las muestras fecales, mostrando una correlación positiva con el ácido butírico, el ácido propiónico y el total de AGCC (artículo 1). Hecho que fue confirmado en el segundo experimento al observarse una relación entre una mayor ratio AACR/AAAs y los AGCC (**Figura 5.6**).

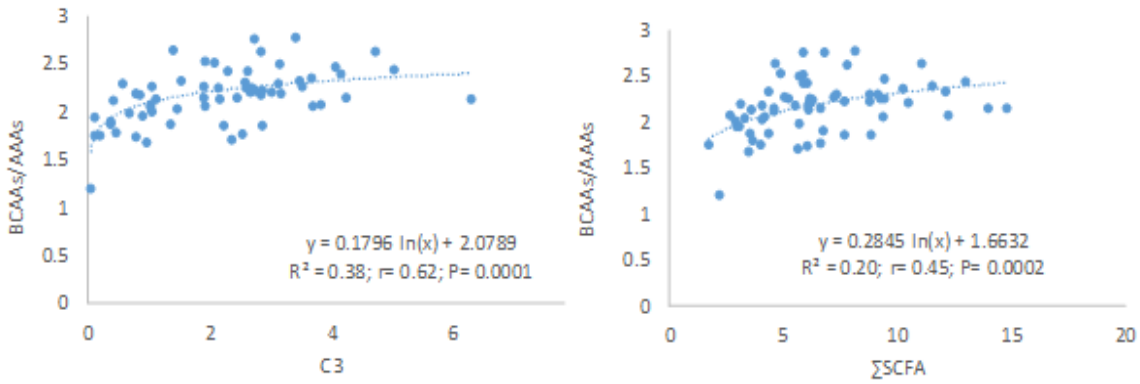

Figura 5.6. Relación entre la ratio AACR/AAAs y los ácidos grasos fecales de cadena corta (AGCC o SCFAs).

Esta relación observada se debe en buena medida a que el catabolismo de algunos aminoácidos, sobre todo los AACR, contribuyen a la síntesis de ácidos grasos (Crown et al., 2015).

Otro resultado destacable de la presente tesis fue la relación positiva observada entre alguno de los AAAs, especialmente tirosina, y la presencia de SAT en las heces, mientras que la relación entre la ratio AACR/AAAs fue inversa con los SAT, pero directa con los MUFA (**Figura 5.7**). Varios autores han descrito que el ambiente intestinal y la existencia de mayor o menor grado de disbiosis puede verse alterado por la presencia de grasa o un determinado tipo de ácido graso (Tsutsumi el al. 2021). La mayor presencia de SAT como el C16:0 y menos MUFA como el C16:1n-7 en las heces de los perros IRE por tanto podría haber contribuído a agravar la enfermedad respecto a los perros FRE que, como se ha indicado, presentaron valores intermedios en los niveles de estos ácidos grasos. Esto subraya la importancia de cómo determinados ácidos grasos o aminoácidos pueden contribuir en el mantenimiento de una adecuada salud intestinal.

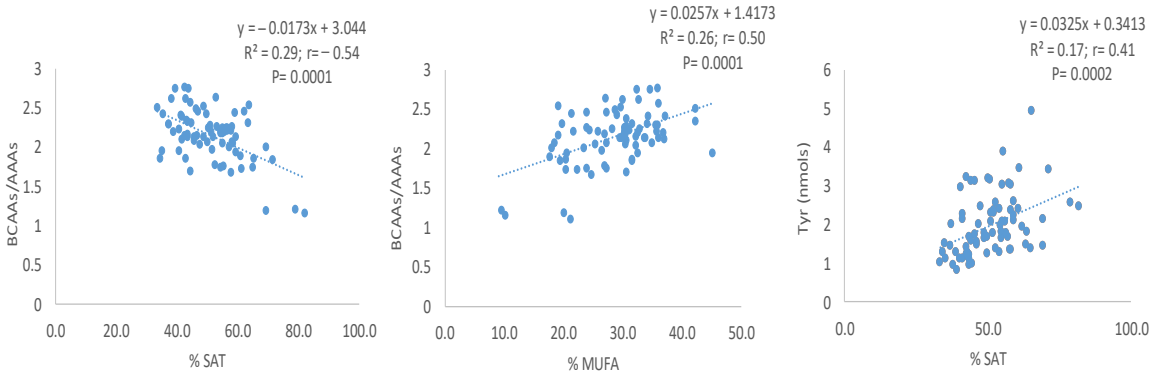

Figura 5.7. Relación entre la ratio fecal AACR/AAAs o la tirosina y los ácidos grasos saturados (SAT), o monoinsaturados (MUFA) en las heces.

Por otro lado, otro de los aminoácidos que presentó ciertas diferencias entre los distintos grupos de animales con ECs y, sobre todo, entre los perros enfermos y los sanos fue la treonina. Los niveles elevados de treonina en el artículo 5 estuvieron en concordancia con el estudio de Pilla *et al.* (2021) en perros con IBD. Este aminoácido desempeña un papel fundamental en el fortalecimiento de la mucosa intestinal mediante la síntesis de mucinas (Wang *et al.,* 2008; Beaumont & Blachier, 2020). Bajo condiciones inflamatorias, se ha descrito un aumento en el consumo de este aminoácido por parte de las células intestinales para asegurar la producción de mucus (Rémond *et al.,* 2009). Los niveles más altos de treonina en las heces de los animales enfermos podrían explicarse por su uso preferencial por parte de las células de la mucosa para proteger la barrera intestinal (Law *et al.,* 2007).

Contrario a la hipótesis planteada, no se encontraron diferencias en el perfil de aminoácidos fecales entre los perros con FRE y aquellos con IRE con las técnicas analíticas empleadas. Cabe destacar que los estudios en veterinaria son limitados y que serían necesarios más estudios utilizando similares técnicas de extracción o modificaciones que confirmen los resultados observados.

5.3 Capacidad de diferenciación entre los grupos experimentales mediante el análisis de ácidos grasos y aminoácidos fecales

Como se ha indicado en la revisión bibliográfica, actualmente se están investigando nuevos biomarcadores que permitan diferenciar de manera más temprana y menos invasiva a los perros con ECs que responden a dieta de aquellos que no lo hacen y requieren, para su diagnóstico, de métodos más invasivos y costosos, como la endoscopia digestiva. La recogida de muestras de heces es un método menos invasivo y podría proporcionar información valiosa sobre las diferencias en metabolitos entre los perros con FRE y aquellos con IRE.

Los análisis discriminantes mediante el uso de variables basadas en el estudio del perfil lipídico o aminoacídico fecales mostraron una capacidad de diferenciación diferente entre los distintos grupos de animales. La combinación conjunta del perfil de ácidos grasos y aminoácidos fecales mostró la mayor efectividad en la diferenciación de los casos (78%), seguidos del perfil de ácidos grasos (75%) y del perfil de aminoácidos fecales (56%) de forma independiente (**Tabla 5.1**).

Tabla 5.1. Clasificación (%) según los ácidos grasos fecales en solitario, los aminoácidos fecales en solitario o la combinación de ambos, de perros sanos (HC), perros con enteropatía que responde a dieta (FRE), perros con enteropatía que responde a inmunosupresores (IRE) y perros parasitados por *Giardia* spp. (GIA), de acuerdo con las funciones discriminantes.

	FRE	GIA	IRE	HC	TOTAL (%)
Ácidos grasos fecales					
FRE	41.38	24.14	13.79	20.69	100
GIA	0	100	0	0	100
IRE	0	7.69	**92.31**	0	100
HC	16.67	16.67	0	66.67	100
Aminoácidos fecales					
FRE	38.24	2.94	26.47	32.35	100
GIA	28.7	57.14	14.29	0	100
IRE	50	14.29	28.57	7.14	100
HC	0	0	0	**100**	100
Aminoácidos más ácidos grasos fecales					
FRE	**60.71**	3.57	17.86	17.86	100
GIA	16.67	83.33	0	0	100
IRE	9.09	0	**90.91**	0	100
HC	16.67	0	5.56	77.78	100

Tanto la combinación de ácidos grasos y aminoácidos fecales como el perfil de ácidos grasos en solitario mostraron un mayor éxito en la diferenciación de los perros con IRE respecto al resto de los grupos, con una precisión similar del 91% utilizando ambos perfiles combinados y del 92% utilizando únicamente el perfil de ácidos grasos (**Tabla 5.1**).

Las variables que fueron seleccionadas a partir del análisis del perfil lipídico en solitario y que formaron parte de las ecuaciones lineales discriminantes fueron: C16:1n-7/C16:0, C18:1n-9/C18:0, ácido acético y omega-3 (artículo 3). Los perros con IRE mostraron mayores cambios en dichos ácidos grasos en comparación con los perros con FRE y los controles, entre los cuales se observó cierto solapamiento. Estos resultados refuerzan la evidencia de que las alteraciones en el perfil lipídico son más pronunciadas en los perros con IRE, mientras que los perros con FRE presentaron valores intermedios.

Por otra parte, el perfil de aminoácidos fecales fue el menos efectivo para clasificar los distintos grupos experimentales; sin embargo, fue el que mejor permitió diferenciar a los controles de los perros enfermos (**Tabla 5.1**). Los aminoácidos fecales seleccionados que formaron parte de las ecuaciones lineales discriminantes fueron la tirosina, el ácido glutámico, la arginina y la fenilalanina (artículo 5).

Los perros con FRE presentaron un mayor porcentaje de muestras correctamente clasificadas utilizando la combinación del perfil lipídico y aminoacídico en heces, aunque sin alcanzar valores tan elevados como los perros con IRE, evidenciando su solapamiento con los mismos, así como con los controles. Estos hallazgos sugieren una menor alteración del metabolismo en la FRE. Asimismo, estos resultados son similares a los descritos por Giordano *et al.* (2024) en gatos con ECs, donde se observó una mayor similitud entre los gatos con FRE y los controles, en comparación con los gatos con IBD y linfoma intestinal, a nivel del perfil lipídico fecal.

Como se ha comentado anteriormente, la combinación del perfil de ácidos grasos y aminoácidos fecales fue la que presentó mayor efectividad para diferenciar los distintos grupos **(Figura 5.8)**. Las variables seleccionadas en este análisis discriminante fueron: tirosina, ácido glutámico, MUFA, C16:1n-7/C16:0, ácido acético, AACR/AAAs, C22:5n-3, C18:1n-9/C18:0, elongasa C22:5n-3/C20:5n-3, cistina y EPA. La **tabla 5.2** muestra las ecuaciones lineales discriminantes construidas a partir de las variables seleccionadas.

Algunas de estas variables también habían sido seleccionadas en los análisis discriminantes de cada perfil por separado.

Tabla 5.2. Ecuaciones lineales discriminantes combinando el perfil de ácidos grasos y aminoácidos fecales. Perros sanos (HC), perros con enteropatía que responde a dieta (FRE), perros con enteropatía que responde a inmunosupresores (IRE) y perros parasitados por *Giardia* spp. (GIA).

Variable	Etiqueta	FRE	GIA	IRE	HC
Constante		-74,298	-120,130	-73,438	-85,131
Tirosina	Tirosina	23,365	41,391	27,982	18,208
Ac. Glutámico	Ac. Glutámico	-3,336	-6,055	-2,922	-2,939
MUFA	MUFA	1,888	3,037	1,668	1,621
C16:1n-7/C16:0	C16:1n-7/C16:0	-1,163	-108,686	-77,440	57,738
Ácido acético	Ácido acético	2,308	2,435	0,748	2,082
AACR/AAA	AACR/AAA	36,227	40,640	40,825	41,676
C22:5n-3	C22:5n-3	-5,744	-12,707	-5,455	-6,511
C18:1n-9/C18:0	C18:1n-9/C18:0	-5,975	-10,249	-5,314	-3,852
Elongasa C22:5n-3/C20:5n-3	Elongasa C22:5n-3/C20:5n-3	0,236	0,221	0,049	0,31731
Cistina	Cistina	-15,607	-28,676	-26,267	-9,854
EPA (C20:5n-3)	EPA (C20:5n-3)	53,948	44,004	39,513	79,256

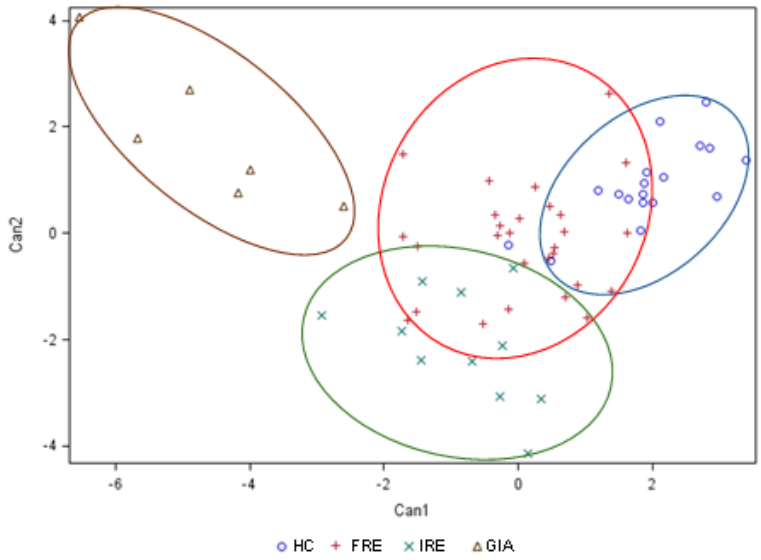

Figura 5.8. Diferenciación por las ecuaciones discriminantes obtenidas por combinación del perfil lipídico y aminoacídico de las heces de los distintos grupos experimentales (HC: control; FRE: perros que responden a dieta; IRE: perros que responden a inmunosupresores; GIA: perros parasitados por *Giardia* spp.).

En el apartado 5.1. de la discusión se describió la importancia de las ratios C16:1n-7/C16:0 y C18:1n-9/C18:0 como indicadores de la actividad Δ9-desaturasa, enzima que convierte los SAT en MUFA (Kubota & Espenshade, 2022). Estas alteraciones pueden sugerir diferencias en el metabolismo lipídico entre ambas enteropatías, posiblemente influenciadas por el grado de inflamación intestinal y la composición de la microbiota. En particular, una menor actividad de la desaturasa podría estar relacionada con un estado inflamatorio más grave en los perros con IRE.

Esta alteración y los cambios en los niveles de MUFA, podrían conllevar posibles consecuencias negativas, ya que estos ácidos grasos desempeñan un papel importante en la regulación de la inflamación y el metabolismo celular, favoreciendo la integridad de las membranas y participando en la modulación de la respuesta inmunitaria (Passos *et al.,* 2016; Zhou *et al.,* 2021).

El ácido acético, un AGCC, es un producto clave de la fermentación bacteriana y desempeña un papel crucial en la homeostasis intestinal (Gonçalves *et al.,* 2018). Diferencias en los niveles entre los grupos experimentales podrían reflejar alteraciones en la microbiota, con una menor producción en perros con IRE debido a una disbiosis más pronunciada en comparación con la FRE.

En cuanto a los PUFA, el EPA (C20:5n-3) destaca por sus propiedades antiinflamatorias (Fu *et al.,* 2021). Diferencias en sus concentraciones pueden estar relacionadas con un mayor consumo en respuesta a la inflamación crónica (Wang *et al.,* 2021b). Además, la actividad de la elongasa C22:5n-3/C20:5n-3, encargada de la conversión de EPA en DPA (C22:5n-3), podría estar afectada en función del estado inflamatorio intestinal, sugiriendo una hiperactivación metabólica para la síntesis de estos ácidos grasos, posiblemente para la reparación de las membranas intestinales o la síntesis de mediadores con carácter antiinflamatorio (Wang *et al.,* 2021b).

Otros autores han descrito alteraciones en los niveles de omega-3 en la IBD; sin embargo, como se mencionó en la revisión bibliográfica, los resultados sobre los PUFA en esta enfermedad siguen siendo controvertidos (Bauset *et al.,* 2021; Upadhyay *et al.,* 2022). No obstante, en muestras de mucosa es común encontrar un aumento de los omega-3, una disminución de sus precursores y alteraciones en los procesos de elongación y desaturación

(Buhner *et al.,* 1994; Fernández-Bañares *et al.,* 1997; Nieto *et al.,* 1998; Diab *et al.,* 2019), mientras que en muestras de plasma y heces los resultados son contradictorios. Esto sugiere que los PUFA desempeñan un papel importante en la patogénesis de la IBD, ya que cumplen múltiples funciones en la respuesta inflamatoria intestinal, incluyendo la síntesis de mediadores inflamatorios, como los eicosanoides, y la regulación de las membranas celulares de las células inmunitarias (Esteve-Comas *et al.,* 1992; Scoville *et al.,* 2018).

Por otra parte, en cuanto a los aminoácidos, la tirosina, el ácido glutámico y la cistina ejercen un papel fundamental en la función inmunitaria y la protección de la mucosa intestinal. La tirosina está involucrada en la síntesis de neurotransmisores y en la regulación del sistema inmunológico (Li *et al.,* 2007). El ácido glutámico, al ser precursor de la síntesis de glutamina, es esencial para la salud intestinal, ya que la glutamina es la principal fuente de energía para los enterocitos (Beaumont & Blachier, 2020; Ji *et al.,* 2023). Asimismo, el glutamato, en combinación con la cisteína y la glicina, participa en la síntesis de glutatión, un importante antioxidante a nivel celular (Roth *et al.,* 2002; Wu *et al.,* 2004). Es posible que estos aminoácidos se vean involucrados en el desequilibrio oxidativo descrito en la patogénesis de estas enfermedades (Bourgonje *et al.,* 2020).

La relación entre AACR y AAAs es un indicador del equilibrio entre el catabolismo proteico y la síntesis de proteínas (Eghtesad *et al.,* 2013). Los AACR, como la leucina, juegan un papel crucial en la regulación de la síntesis de proteínas y en la salud intestinal (Nie *et al.,* 2018). En perros con ECs, la disminución de la relación AACR/AAAs podría reflejar una alteración en este equilibrio, sugiriendo un mayor catabolismo proteico asociado con la inflamación crónica y el daño intestinal.

En conjunto, estos resultados indican que el perfil de ácidos grasos fecales tiene un mayor poder discriminante que el perfil de aminoácidos fecales en la diferenciación entre perros con FRE e IRE, pero que la combinación de ambos es el modelo más efectivo. Estos hallazgos concuerdan con el mayor número de diferencias observadas en el perfil de ácidos grasos entre ambos grupos, resaltando el papel clave de estos compuestos en la patogénesis de estas enfermedades, más específicamente en los perros con IRE.

Es importante mencionar que el grupo *Giardia* spp. se incluyó en los estudios debido a la disponibilidad de muestras, ya que esta parasitosis es uno de los principales

diagnósticos diferenciales de las ECs. Aunque esta patología es diagnosticada mediante otras pruebas específicas, en la presente tesis se ha podido observar cómo su diferenciación mediante el estudio del perfil lipídico y aminoacídico es posible y puede llevarse a cabo con bastante precisión, si bien estas analíticas pueden resultar más costosas y más complejas.

CONCLUSIONES

6. CONCLUSIONES

1. Los perros con enteropatía que responde a dieta presentan, antes del tratamiento dietético, cambios significativos en los ácidos grasos poliinsaturados de cadena larga omega-6 y omega-3 a nivel plasmático. A nivel fecal, los ácidos grasos de cadena corta, los ácidos grasos monoinsaturados, el ácido impar C15:0 y los índices de desaturación son los más afectados, con niveles inferiores en los perros enfermos y en correlación inversa con el índice de gravedad CIBDAI.

2. Los perros con enteropatía que responde a dieta presentan, antes del tratamiento dietético, cambios significativos en los niveles de aminoácidos libres en sangre y, en menor medida, en heces, en comparación con los perros sanos. Los niveles plasmáticos de aminoácidos ramificados o la ratio entre aminoácidos ramificados y aromáticos son los que guardan mayor correlación inversa con el índice de gravedad, mientras que los niveles en heces de aminoácidos ramificados presentan relación directa con indicadores de salud intestinal.

3. Los perros que no responden a dieta, pero sí a inmunosupresores, presentan antes del tratamiento, la proporción más baja en heces de ácido graso C16:1n-7, ratio C16:1n-7/C16:0 y cantidad de ácido acético y ácidos grasos de cadena corta totales, en comparación con los perros que sí responden a dieta o los sanos. La relación entre el índice de gravedad CIBDAI con el C16:1n-7 fecal y la ratio C16:1n-7/C16:0 como indicador de actividad Δ9-desaturasa, nos indica que los perros que no responden a dieta presentan una mayor alteración metabólica.

4. El análisis hematológico revela diferencias entre los perros con enteropatía que responde a dieta y aquellos que responden a inmunosupresores, en concreto, en los niveles de plaquetas, ratio neutrófilos/linfocitos, ratio plaquetas/linfocitos y el índice de inflamación sistémico, que son inferiores en los perros que responden a dieta. El grado de inflamación, además, guarda relación directa con la mayor presencia de grasa en heces.

5. Los modelos de regresión entre los valores de plaquetas y el índice de inflamación sistémico con los niveles de ácido acético en heces muestran una respuesta distinta entre los perros que no responden a dieta y los que responden. Niveles de ácido acético en heces por debajo de 2mM en los perros que no responden se asocian con niveles de plaquetas más elevados que incluso superan el rango de referencia.

6. En los perros que responden a dieta, los niveles de ácidos grasos de cadena corta y ácido butírico se asocian con los niveles de glucosa sanguínea, mientras que en los perros que no responden no se observa esa asociación. Esto demuestra que los cambios sistémicos observados entre estos dos grupos de perros están relacionados con el metabolismo de los ácidos grasos en distinta intensidad.

7. Los perros que no responden a dieta, pero sí a inmunosupresores, no presentan apenas cambios en el perfil de aminoácidos fecales, en comparación con perros que sí responden, aunque sí presentan cambios con los animales sanos, sobre todo, en los niveles de treonina y aminoácidos aromáticos como la tirosina. Sin embargo, los perros infectados con *Giardia* spp. presentan un perfil de aminoácidos fecales muy diferente al resto.

8. La ratio entre aminoácidos ramificados y aminoácidos aromáticos fecales presenta una relación directa con la mayor presencia de ácidos grasos monoinsaturados y ácido propiónico en heces, mientras que esta ratio está inversamente relacionada con la proporción de ácidos grasos saturados.

9. El perfil de ácidos grasos fecales permite diferenciar mediante funciones discriminantes lineales con mayor precisión que el perfil de aminoácidos fecales a los perros con distintas enteropatías (los que responden a dieta y los que responden a inmunosupresores), antes del tratamiento dietético. Los ácidos grasos que presentan mayor poder discriminante son las ratios C16:1n-7/C16:0 y C18:1/C18:0, los omega-3 y el ácido acético.

10. La combinación del perfil de ácidos grasos y aminoácidos fecales mejora la eficiencia en la diferenciación mediante funciones discriminantes, con hasta un 78 % de éxito entre los perros con distintos tipos de enteropatías antes del tratamiento dietético.

BIBLIOGRAFÍA

7. BIBLIOGRAFÍA

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