UNIVERSIDAD AUTONÓMA DEL ESTADO DE MÉXICO
DOCTORADO EN CIENCIAS AGROPECUARIAS Y RECURSOS NATURALES

"EL EFECTO DE LOS ACEITES DE OLEAGINOSAS EN RUMEN EN EL SISTEMA DE FERMENTACIÓN IN VITRO"

TESIS

QUE PARA OBTENER EL GRADO DE DOCTOR EN CIENCIAS AGROPECUARIAS Y RECURSOS NATURALES

PRESENTA

YONG LIU

El Cerrillo Piedras Blancas, Toluca, Estado de México
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RESUMEN

Los subproductos de plantas oleaginosas han sido utilizadas para mejorar el desempeño productivo en ruminates ya sea por la alta energía que proporciona o por su composición proteica, sin embargo, pocos artículos reportan la cantidad que debe proporcionarse en forma adecuada en la dieta de ruminates como suplemento. Es por lo anterior, que el trabajo fue elaborado para evaluar el efecto de cártamo (*Carthamus tinctorius L.,* SFM)/canola (*Brassica napus,* CAS) con semilla de sorgo en una proporción (0%, 25%, 50%, 75%, 100%, respectivamente) en una proporción concentrado-forraje (1:1) en una prueba *in vitro* de fermentación ruminal de ovino, como es la cinética de producción de gas, nitrógeno amoniacal (NH_3-N), pH, producción de metano (CH_4) y la desaparición de materia seca *in vitro* (*IVDMD*) de rastrojo de maíz. Los resultados mostraron que la producción de gas, y de metano significativamente disminuyeron, pero *IVDMD* y pH incrementaron con el incremento de ambas proporciones de SFM y CAS en alimentación a base de concentrado. Para tomar en consideración el desempeño de fermentación y ambiente, nuestros resultados sugieren que la suplementación más adecuada de canola y cartamo en alimentación a base de concentrado son de 25% a 50%, y de 25% a 75%, respectivamente.

Modificar el metabolismo microbiano en rumen a través de la adición de aceites derivados de plantas es una manera efectiva de aumentar los ácidos grasos funcionales de los productos derivados de ruminates. Poco es conocido de la influencia de ácidos grasos exogenos en los procesos del metabolismo de lípidos en las membranas de bacterias y protozoarios. Es por lo anterior, que el presente trabajo se enfocó en investigar los ácidos grasos de cadena larga (LCFA), ácidos grasos volétiles (VFA) y metano (CH_4) a las 48 horas de suplemtento exógeno de aceites principalmente conteniendo ácidos grasos poli/mono insaturados, PUFA/MUFA, (C18:3, C18:2 y C18:1 de aceites de linaza, cártamo y canola respectivamente) y ácidos

grasos de cadena mediana MCFA, (C12:0 de aceite de coco). Los resultados mostraron que la composición de ácidos grasos entre bacteria y protozoarios fueron diferentes. La suplementación de aceite de linaza, principalmente ácido linoleico (C18:3), al incrementar las proporciones de C18:2 n6*c*, C18:2 n6*t*, C18:1 n9*c*, C18:1 n9*t* y *cis*-ácidos grasos en la membrane de bacterias y protozoarios en diferente medida, mejora ligeramente las concentraciones de ácido acético y propiónico pero no tiene impacto en CH_4. La suplementación con aceite de coco, aumenta la composición de MCFA, y por lo tanto aumenta la biosíntesis de MCFA en fracciones tanto de bacterias como de protozoarios (del C12:0 al C14:0), para inhibir la actividad de metanogénesis en cierta medida. Los mayores ácidos grasos saturados, saturados/insaturados, *trans*-, *even*-carbon insaturados fueron obtenidos de membranas de bacterias cuando se suplementaba con aceites de cártamo y canola, principalmente C18:2 y C18:1, respectivamente, pero más bajo que aceite de coco. Sin embargo, en ambos con cártamo y canola aumentaron las concentraciones de ácido acético y propiónico, disminuye la Ac/Pro ratio pero no el impacto de la actividad de metanogénesis. En resumen, los resultados implican que diferentes grados de ácidos grasos insaturados de cadena corta o larga puede impactar en la fermentación ruminal.

Palabras clave: cártamo, canola, cinética de producción de gas, metano, fermentación in vitro, estudio teórico experimental, producción de metano, redes de distribución de ácidos grasos,composición de membrana, bacteria, protozoarios.

ABSTRACT

Oleaginous plant by-products are widely used to improve ruminant growth performance for high energy or protein composition, however, few article reports the suitable fit amount for oleaginous by-products to the ruminant diets in a large supplementation range. Therefore, this work was conducted to evaluate the effect of Safflower meal (*Carthamus tinctorius L.,* SFM)/ Canola seed (*Brassica napus,* CAS) with sorghum seed in theoretic proportion (0%, 25%, 50%, 75%, and 100%, respectively) in a specific concentrate-roughage ratio of (1:1) on *in vitro* ovine rumen fermentation performance, such as the kinetics of gas production, ammonia nitrogen (NH_3-N), pH, methane production (CH_4) and *in vitro* dry matter disappearance (*IVDMD*) of maize stover. The results showed that gas production performance, and methane production were significantly decreased, but *IVDMD* and pH were obviously increased with the increasing proportions of both SFM and CAS in concentrated feed. To take a consideration of fermentation performance and environment/ cost factors, our results suggest that most suitable supplementation dosage of canola seed and safflower meal in concentrated feed are from 25% to 50%, and 25% to 75%, respectively. However, in practical production of completed feed, the supplementation amount of safflower meal/ canola seed also depend on concentrate-roughage ratios.

Unbalanced uptake of Omega 6 / Omega 3 (ω-6/ω-3) ratios could increase chronic disease occurrence, such as inflammation, atherosclerosis, or tumor proliferation *etc.*, and the methylation methods for measuring the ruminal microbiome fatty acids (FA) composition/distribution play a vital role in discovering the contribution of food components to ruminant products (*e.g.*, meat and milk) when pursuing a healthy diet. Hansch's models based on Linear Free Energy Relationships (LFER) using physicochemical parameters, such as partition coefficients, molar refractivity, polarizability, *etc.*, as input variables (V_k), are advocated. In this work, a new

combined experimental-theoretical strategy was proposed to study the effect of ω-6/ω-3 ratios, FA chemical structure, and other factors over FA distribution networks in the ruminal microbiome. In step 1, experiments were carried out to measure long chain fatty acids (LCFA) profiles in rumen microbiome (bacteria and protozoan), and volatile fatty acids (VFA) in fermentation media. In step 2, the proportions and physicochemical parameter values of LCFA and VFA were calculated in different boundary conditions (c_j) like c_1 = acid and/or base methylation treatments, c_2 = with/without fermentation, c_3 = FA distribution phase (media, bacterial, or protozoan microbiome), *etc*. In step 3, Perturbation Theory (PT) and LFER ideas were combined to develop a PT-LFER model of a FA distribution network using physicochemical parameters (V_k), the corresponding Box-Jenkins (ΔV_{kj}) and PT Operators ($\Delta\Delta V_{kj}$) in statistical analysis. The best PT-LFER model found predicted the effects of perturbations over the FA distribution network with Sensitivity, Specificity, and Accuracy > 80% for 407,655 cases in training + external validation series. In step 4, alternative PT-LFER and PT-NLFER models were tested for training Linear and Non-Linear Artificial Neural Networks (ANN). PT-NLFER models based on ANNs presented better performance but are more complicated than the PT-LFER model. Last, in step 5, the PT-LFER model based on LDA was used to reconstruct the complex networks of perturbations in the FA distribution and compared the giant components of the observed and predicted networks with random Erdős–Rényi network models. In short, our new PT-LFER model is a useful tool for predicting a distribution network in terms of specific fatty acids distribution.

Keywords: safflower meal, canola seed, gas production kinetics, methane, *in vitro* fermentation, experimental-theoretical study, methane production, fatty acid distribution networks, membrane composition, long chain fatty acids, bacteria, protozoan.

INTRODUCCIÓN

La técnica de fermentación in vitro ha sido considerada ser adecuado para evaluar la contribución de la fermentación microbiana ruminal en la digestión general de rumiantes (Gosselink et al, 2004b;.. Getachew et al, 2005). La cinética de la producción de gas, y la desaparición de materia seca (Menke y Steingass, 1988) se ha aplicado con éxito a digestivo de los rumiantes investiga para evaluar los valores nutricionales de la alimentación y las correspondientes rumen rendimiento fermentación microbiana, proporcionando alguna información virtual sobre in vivo o métodos in situ (Gosselink et al, 2004a;. Wulf y Südekum, 2005).

Las extensiones cambiantes de in vitro cinética de producción de gas, la producción de CH4 y digestibilidad de la fibra están relacionadas con la naturaleza o la forma de la semilla oleaginosa (extruido, presionado comida o todo el estado), y sus interacciones con la composición de la dieta basal (Lerch et al. , 2012). Sin embargo, están disponibles para la elaboración de las cantidades adecuadas de semillas de canola sin procesar y harina de cártamo extraída en las raciones de las especies ovina pocos datos. Por lo tanto, este trabajo fue diseñado para estudiar el efecto integral de una variedad de proporción de SFM / CAS con semilla de sorgo en el rendimiento de la fermentación in vitro por la cinética de producción de gas, la concentración de NH3-N, pH, producción de CH4 e in vitro de materia seca digestibilidad (DIVMS) del rastrojo de maíz.

La relación ω-6 / ω-3 juega un papel importante no sólo en la patogénesis de enfermedades cardiovasculares, sino también en el cáncer, enfermedades inflamatorias y autoinmunes (Simopoulos, 2002, 2006; Simopoulos, 2008). Se considera un alto ω-6 / ω-3 relación perjudicial para la salud humana, un valor cercano a 1 es considerada protectora contra las patologías degenerativas (Simopoulos, 1999). Enriquecimiento de la carne de rumiantes o de leche con ω-3 PUFAs, para disminuir aún más ω-6 / ω-3 ratios de captación, es una manera eficaz para introducir estos PUFAs beneficiosos en la dieta, pero proceso complejo biohidrogenación ruminal limita su biodisponibilidad (Kronberg et al. , 2007). Petit et

al. informó añadiendo riquezas linaza enteros en ALA a las raciones de vacas lecheras, lo que resultó en el ω-6 bajo cociente / ω-3 en la leche en comparación con la soja micronizadas o semillas de girasol (Petit, 2002). Hess et al. (Hess et al., 2012) demostró que la incorporación de ω-3 PUFAs en sangre de los animales y el músculo depende directamente de la dieta de alimentación de ácido graso específico. Además, los ácidos grasos de cadena larga (LCFA) tiene que ser metilado por ácido y / o la metilación del valor inicial antes de determinar con cromatógrafo de gases (GC). Existen diferentes métodos para medir la metilación LCFA de leche, músculo o membrana microbiana ruminal (Kramer et al., 1997; O-Rashid et al, 2007;. Ichihara y Fukubayashi, 2010), acompañan con la generación de resultados diferentes. Las propiedades estructurales de LCFA (sobre todo el número, ubicación o estructura topológica de dobles enlaces) son altamente relacionado con la enfermedad crónica. Para abordar este problema, se postuló que los LCFAs en las membranas microbianas ruminales cambian con el suministro de ω-6 / ω-3 ratios.

La Quimioinformática está relacionado con Aprendizaje Automático, Quimiometría y Bioinformática (Varnek y Baskin, 2011), y combina los campos científicos de trabajo de Química, Ciencias de la Información y las áreas de la topología, teoría de grafos química y la minería de datos en el espacio químico. Es bien sabido que, electrostático, y factores de hidrofobicidad estéricos pueden ser biológicamente relevante (Chou y Cai, 2005; Estrada et al., 2006). En esta ecuación, los diferentes parámetros se pueden utilizar como entradas para dar cuenta de los factores tales como: coeficientes de partición agua / n-octanol (PI), refractividad molecular (MR), constantes de acidez logarítmica (PKA), y otros parámetros fisicoquímicos para cuantificar diferentes propiedades moleculares globales. (Ehresmann et al., 2005) las salidas del modelo son los valores de una propiedad molecular (εi) o una función de esta propiedad f (εi) para un compuesto químico dado o entidad molecular (mi).

Las innovaciones de estos modelos se describen como sigue. 1) El uso de la regresión lineal para buscar ecuaciones lineales multivariantes es capaz de predecir los valores de f (εi), empleando varias variables de entrada. 2) Hánsch también

generaliza el uso de parámetros lipofilia por la formulación de modelos parabólicos para las relaciones no lineales. 3) Los términos logarítmicos (logPi) de Pi se utilizan comúnmente como las medidas de lipofilia molecular y juegan un papel importante en el modelo. A su vez, los valores logPi se pueden predecir ya sea por métodos atómicos (como XlogP o ALogP) o por métodos químicos de fragmentos (como CLogPi o métodos similares) (Tetko et al, 2001;. Tetko y Poda, 2004). A partir de una química física punto de vista, el modelo de Hánsch es un enfoque extra-termodinámico estrechamente relacionado con relaciones lineales Gratis Energía (LFER) (Roy y Leonard, 2005; Anslyn y Dougherty, 2006). La designación de los modelos de Hansch como ecuaciones LFER provienen del uso de parámetros en función de energía libre de Gibbs (Gi) del proceso i. (Gibbs, 1871)

Sin embargo, otros parámetros fisicoquímicos o descriptores moleculares también se pueden usar para cuantificar el efecto de los cambios en la estructura química más de una característica de interés. Esto significa que los descriptores moleculares para una molécula dada se pueden utilizar, que no sólo son constantes termodinámicas, sino también otras medidas teóricas de lipofilia molecular, electronegatividad, polarizabilidad, o propiedades de topología molecular (Ehresmann et al., 2005), etc. Los valores de estas variables de entrada (IVK) puede calcularse como parámetros fisicoquímicos o descriptores moleculares de diferentes tipos (k) para una molécula dada (mi).

En general, la suposición básica para el análisis de Hánsch es que las moléculas similares tienen actividades similares (de Hansch et al, 2001;.. De Hansch et al, 2002; de Hansch et al., 2003). Este principio también se denomina Relación Estructura-Actividad (SAR). La paradoja SAR se refiere al hecho de que no todos los casos que las moléculas similares a lo largo con actividades similares. Por tanto, el problema de fondo es cómo definir una pequeña diferencia en un nivel molecular. El problema es relevante ya que cada tipo de propiedad, por ejemplo, solubilidad, reactividad, o el metabolismo, se espera que depender de otra diferencia. Esto significa que las variaciones o perturbaciones "pequeños" tienen que ser cuantificados a nivel

estructural molecular, que a su vez implica un "pequeño" cambio lineal en la energía libre de la interacción del fármaco con el receptor.

En nuestra opinión, las ideas de la teoría de perturbaciones (PT) (González-Díaz et al., 2013b) pueden utilizarse para dar cuenta de este problema en el contexto de Quimioinformática. Es por eso que; PT y LFER las ideas se utilizaron para formular un nuevo enfoque PT-LFER. Este enfoque PT-LFER es una generalización del método clásico Hánsch extra-Termodinámica para Quimioinformática. La prueba de concepto-También se demostró con un estudio experimental, teórica sobre redes complejas de distribución de FA en lipidómica. La metodología de aprendizaje de máquina, como Redes Neuronales Artificiales (RNAs) se utilizaron para probar modelos PT-NLFER (análogos no lineal de PT-LFER). El mejor modelo PT-LFER encontró fue utilizado para predecir el efecto de las perturbaciones en las condiciones de contorno iniciales sobre un gran compleja red de distribución / absorción de FA en el microbioma ruminal. La compleja red observada para los datos reportados se construyó y se compara por primera vez con la red y modelo predijo redes aleatorias de gran similar. Por último, la sección teórica se completa con un estudio comparativo del modelo de clasificación PT-LFER encontrado con otros modelos no lineales. Este estudio fue de gran relevancia debido a los resultados anteriores que apuntan a una fuerte relación entre las relaciones de ω-6 / ω-3 de la ingesta de FA y la salud humana. (Calder et al, 2011; Calviello et al, 2013) En consecuencia, esta trabajo allana el camino para evaluar el efecto de las perturbaciones en los sistemas moleculares complejas implicadas en las estructuras químicas y las condiciones experimentales de contorno.

REVISIÓN BIBLIOGRÁFIA

1. Plantas oleaginosas utilizadas por el rumen

1.1. Cártamo y Canola

El Cártamo (Carthamus tinctorius L.), a menudo se utiliza para extraer aceite y se siembra en suelos con bajos requerimientos de insumos y de agua. México es una de las principales regiones de producción de cártamo (FAO, 2011), su rendimiento es de alrededor de 2.5 mt/ha (Gilbert, 2008). Es ampliamente utilizado para las dietas de aves de compañía, vacas lecheras y carneros (Smith, 1996 (Bäumler et al., 2006); Sudhamayee et al., 2004). El alto nivel de suplementación de cártamo disminuye el rendimiento animal, (Goss y Otagaki, 1954a; Dixon et al., 2003). La proteína de la harina de cártamo contiene proteína altamente degradable (Walli, 2005b) que varía entre el 60% (Chandrasekharaiah et al., 2001a), y 70% (Dixon et al., 2003) y se puede mezclar con semillas de soya o harina de linaza en diferentes proporciones.

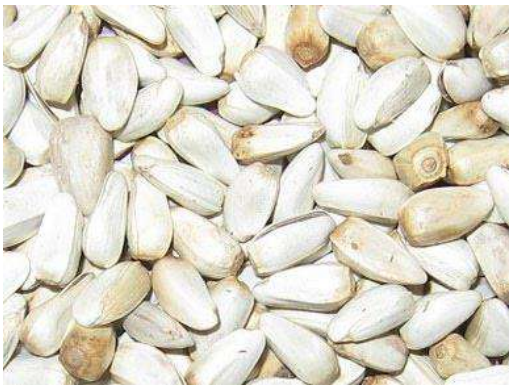
Brassica napus (Canola) ha sido utilizada ampliamente como fuente energética y proteica en rumiantes con alto valor biológico (CP, 20-43%; aceite, >40%) (Ebrahimi et al., 2009), (Maxin et al., 2013). Sin embargo, la proteina de canola es fácilmente degradable por los microorganismos del rumen (Khorasani et al., 1993), aunque también contiene sustancias anti-nutricionales (Tripathi and Mishra, 2007), (Al-Kaiesy et al., 2003).

1.2 Usos de diferentes fracciones de semilla de Cártamo

El cártamo (*Carthamus tinctorius L.*), se utiliza normalmente para obtener aceites, al igual que algunos usos medicinales como para el tratamiento para reumatismo, analgésico y desinflamatorio. El cártamo se produce principalmente en Estados Unidos, México e India (FAO, 2011), mientras que Japón, Canada e inclusive México

son el mayor mercado de acuerdo a los reportes de Los Servicios Nacionales de Estadística en Agricultura (NASS, 2012).

a. Flor de Cártamo



b. Semilla de Cártamo

c. Flor de Canola

d. Semilla de Canola

Figura 1. Flores y semillas de Cártamo y Canola.

2. Características de ácidos grasos de plantas.

Los ácidos grasos se clasifican por su longitud. Se dividen por el número de átomos de carbono de sus estructuras químicas, ya sea de cadena corta, de cadena media y de cadena larga. Los aceites naturales vegetales son ricos en ácidos grasos de cadena media (AGCM) con las colas alifáticos de menos de 18 átomos de carbono, y ácidos grasos de cadena larga (LCFA), con colas alifáticos de 18 a 21 carbonos.

2.1. Ácidos grasos de cadena corta.

Los ácidos grasos de cadena corta (AGCC) son ácidos grasos alifáticos con colas de menos de seis carbonos (por ejemplo, acetato, propionato, y butirato). En los rumiantes, algunos ácidos grasos volátiles (AGV) ruminales, son el ácido acético, ácido propiónico, ácido butírico y al concluir isobutirato, valerato, y isovalerato. Estos AGV se digieren de alimentación o forraje, y son absorbidos y utilizados por los rumiantes.

2.2. Ácidos grasos de cadena mediana.

Los ácidos grasos de cadena media (AGCM) contienen menos de 18 átomos de carbono y, en general, más de 6 carbonos. Estos AGCM son fuentes ideales naturales de biodiesel y también para algunas materias primas oleoquímicos, es decir, recubrimientos y detergentes. En las plantas, la biosíntesis de ácidos grasos de novo se produce en el estroma del plástido, con el alargamiento plastidial de cadenas de acilo está terminada por la actividad de proteína portadora de acilo-acilo (acil-ACP) tioesterasas (Tjellström et al., 2013). Por tanto, estas enzimas se consideran determinantes principales de longitudes de cadena de carbono de ácidos grasos, y además a los perfiles generales de ácidos grasos de la planta. Sin embargo, se ha demostrado que sintetasas-acil-ACP puede reactivar la liberación de ácidos grasos, permitiendo por lo tanto un alargamiento adicional (Koo et al., 2005). Tioesterasas-acil ACP se clasifican típicamente como Fat-A o Fat-B, los cuales son responsables de la liberación de ácido oleico (C18: 1Δ9), ácido esteárico (C18: 0), ácido láurico (C12: 0), mirístico ácido (C14: 0), y el ácido palmítico (C16: 0). Después de exportación desde el plástido, estos ácidos grasos pueden ser esterificados a una cadena principal

glicerofosfato a través de la ruta de Kennedy en el retículo endoplásmico para formar triacilglicerol (Umbellularia californica) (Eccleston et al, 1996).

Los MCFAs tienen ventajas sobre los ácidos grasos de cadena larga ya que son fáciles de digerir y son quemados como energía. Es un error pensar que todas las grasas son malas para el corazón. De hecho, el corazón necesita ácidos grasos para proporcionar con energía (Eccleston et al, 1996).

2.3. Ácidos grasos de cadena larga.

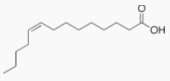
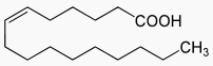
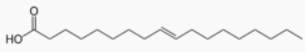
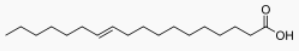
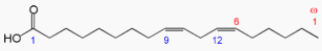
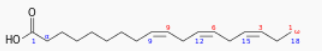
Los ácidos grasos de cadena larga (AGCL) juegan un papel importante en la función fisiológica. Proporcionan una fuente de energía importante para la mayoría de los tejidos. Por ejemplo, el tejido del corazón puede derivar hasta el 70% de sus necesidades de energía de ácidos grasos (FA) de oxidación (van der Vusse et al., 1992). Los LCFAs son componentes de los triglicéridos y ésteres de colesterol producidos por los adipocitos, las glándulas suprarrenales, hepatocitos, y macrófagos, etc., como parte de las vías centrales para el metabolismo general. Los LCFAs son precursores de las prostaglandinas, que tienen una variedad de efectos reguladores (Galli y Marangoni, 1997), y bloques de construcción para fosfolípidos, que son cruciales para la integridad y la función de la membrana (Yamashita et al., 1997). La acilación de LCFAs en proteínas de membrana puede influir en su localización y función biológica (Konrad et al, 2014;. Vartak et al, 2014). Los LCFAs también pueden modular directamente la activación de canales de iones (Boland et al., 2009), la función de la enzima, la transmisión sináptica (Liu et al., 2011), y la memoria (Yamada et al., 2014). Además, los LCFAs se muestran para regular la expresión de genes que implican en el metabolismo de lípidos, la diferenciación celular, el envejecimiento, el desarrollo neural y neurodegeneración (Janssen y Kiliaan, 2014). Gracias a múltiples funciones, los LCFAs han sido implicados en condiciones tales como la inflamación, la aterosclerosis, las respuestas inmunes, el cáncer, la diferenciación celular, y la tumorogénesis (Sun et al., 2014).

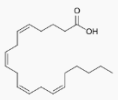
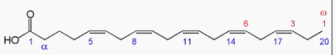
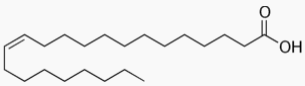
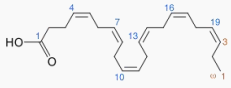
2.4. Ácidos grasos insaturados.

Los ácidos grasos insaturados tienen al menos un doble enlace, mientras que los saturados no tienen. Los ácidos grasos saturados son más comunes en la fabricación de alimentos procesados ya que son menos vulnerables a la rancidez y en general más estables a temperatura ambiente. Para los humano y animales, los ácidos grasos monoinsaturados o poliinsaturados ayuda a mantener niveles más bajos de colesterol total y de lipoproteínas de baja densidad (LDL) en la sangre (Catapano et al., 2011).

Se observó que en un consumo relativamente bajo de grasa poliinsaturada se asoció positivamente con la progresión de la aterosclerosis coronaria (Mozaffarian et al., 2004). Mientrás que en otro estudio, los ácidos grasos poliinsaturados disminuyeron el riesgo de desarrollar esclerosis lateral amiotrófica (ALS, también conocida como enfermedad de Lou Gehrig) (Veldink et al., 2007). Un Alto nivel de ácido docosahexaenoico (DHA), se asoció con un menor riesgo de cáncer de mama (Pala et al., 2001).

Tabla 1. Los ejemplos de ácidos grasos insaturados que existían o puedan existir en rumen

Examples of Unsaturated Fatty Acids in Rumen bacteria or protozoan				
Name	**Chemical structure**	***cis* or *trans*-Δ^x**	***Unsaturated degree***	***ω−x***
Myristoleic acid		*cis*-Δ^9	14:1	*ω*−5
Palmitoleic acid		*cis*-Δ^9	16:1	ω−7
Sapienic acid		*cis*-Δ^6	16:1	ω−10
Oleic acid		*cis*-Δ^9	18:1	ω−9
Elaidic acid		*trans*-Δ^9	18:1	ω−9
Vaccenic acid		*trans*-Δ^{11}	18:1	ω−7
Linoleic acid		*cis*,*cis*-Δ^9,Δ^{12}	18:2	ω−6
Linoelaidic acid		*trans*,*trans*-Δ^9,Δ^{12}	18:2	ω−6
α-Linolenic acid		*cis*,*cis*,*cis*-Δ^9,Δ^{12},Δ^{15}	18:3	ω−3

Arachidonic acid		*cis*,*cis*,*cis*,*cis*-$\Delta^5\Delta^8,\Delta^{11},\Delta^{14}$	20:4	ω−6
Eicosapentaenoic acid		*cis*,*cis*,*cis*,*cis*,*cis*-$\Delta^5,\Delta^8,\Delta^{11},\Delta^{14},\Delta^{17}$	20:5	ω−3
Erucic acid		*cis*-Δ^{13}	22:1	ω−9
Docosahexaenoic acid		*cis*,*cis*,*cis*,*cis*,*cis*,*cis*-$\Delta^4,\Delta^7,\Delta^{10},\Delta^{13},\Delta^{16},\Delta^{19}$	22:6	ω−3

2.5. Ácidos grasos Omega 3 y Omega 6.

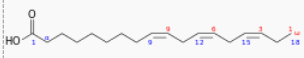
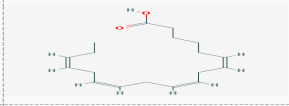
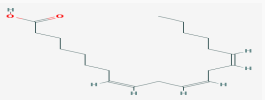
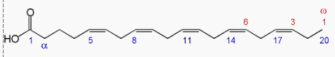
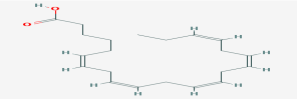
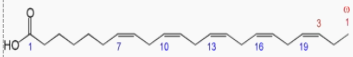
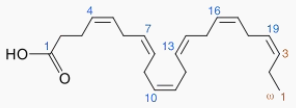
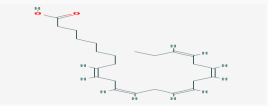
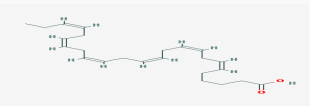
Los ácidos grasos también se pueden clasificar en ácidos grasos esenciales y no esenciales dependiendo de las funciones para animales o humanos. Los ácidos grasos esenciales, son los que los humanos y otros animales deben ingerir porque el cuerpo los necesita para un estado saludable, pero no puede sintetizarlos o no puede proporcionar la cantidad suficiente de otros sustratos. Hay dos series de ácidos grasos esenciales: uno tiene un doble enlace de tres átomos de carbono eliminado del extremo metilo (omega-3); el otro tiene un doble enlace seis átomos de carbono eliminado del extremo metilo (Omega-6) (Blasbalg et al, 2011).

2.5.1. Ácidos grasos omega 3.

Los ácidos grasos omega-3 (ω-3) son ácidos grasos esenciales poliinsaturados (PUFAs) con el primer doble enlace (C=C) situado en el tercer átomo de carbono desde el final de la cadena de carbono. Existen dos clases de ácidos grasos ω-3 (Hu y Manson, 2012). La primer clase de ellos es el ácido α-linolénico (ALA), que es un ácido graso esencial derivado de fuentes vegetales, aceites, por ejemplo, la linaza, arándano, perilla y nogales, etc. La segunda clase son los ácidos grasos de cadena larga, ácido eicosapentaenoico (EPA) y ácido docosahexaenoico (DHA), que se deriva principalmente de peces abisales y aceite de huevo. Con los métodos de metanálisis (Bucher et al, 2002; Burdge y Calder, 2006; Kwak et al, 2012; Rizos et al, 2012; Blasbalg et al, 2011), se han observado las contribuciones que tienen los ácidos grasos ω-3, tanto el ácido linolénico-α como EPA o DHA, en las enfermedades

cardiovasculares y cerebrovasculares inflamación, aterosclerosis, o la proliferación tumoral (Aldai et al., 2005).

Tabla 2. Las listas de los ácidos grasos Omega-3 en la naturaleza

Common name	Lipid name	Chemical name	Chemical Structure
Hexadecatrienoic acid (HTA)	16:3 (ω-3)	*all-cis*-7,10,13-hexadecatrienoic acid	
α-Linolenic acid (ALA)	18:3 (ω-3)	*all-cis*-9,12,15-octadecatrienoic acid	
Stearidonic acid (SDA)	18:4 (ω-3)	*all-cis*-6,9,12,15-octadecatetraenoic acid	
Eicosatrienoic acid (ETE)	20:3 (ω-3)	*all-cis*-11,14,17-eicosatrienoic acid	
Eicosapentaenoic acid (EPA)	20:5 (ω-3)	*all-cis*-5,8,11,14,17-eicosapentaenoic acid	
Heneicosapentaenoic acid (HPA)	21:5 (ω-3)	*all-cis*-6,9,12,15,18-heneicosapentaenoic acid	
Docosapentaenoic acid (DPA)	22:5 (ω-3)	*all-cis*-7,10,13,16,19-docosapentaenoic acid	
Docosahexaenoic acid (DHA)	22:6 (ω-3)	*all-cis*-4,7,10,13,16,19-docosahexaenoic acid	
Tetracosapentaenoic acid	24:5 (ω-3)	*all-cis*-9,12,15,18,21-tetracosapentaenoic acid	
Tetracosahexaenoic acid (Nisinic acid)	24:6 (ω-3)	*all-cis*-6,9,12,15,18,21-tetracosahexaenoic acid	

2.5.2. Ácidos grasos omega 6.

Se han realizado investigaciones comparativas entre ω-3 (FA) y ω-6. La participación de los peroxisomas en el metabolismo de ácido graso, descrito inicialmente para los ácidos ω-3, ahora se ha demostrado también para el ω-6 FA

(formación de 22 carbono insaturado Δ4 FA, formación de productos recién identificados de β-oxidación del ácido araquidónico, AA). Vías adicionales de conversión de AA, más allá de los eicosanoides clásicos, dan lugar a una serie de productos biológicamente activos, tales como los epóxidos, que participan en la modulación de las funciones vasculares, a través del sistema del citocromo P450, y para el AA-etanolamida, la anandamida, y ligando endógeno de los receptores de cannabinoides, a través de un proceso de fosfolipasa mediada.

Como efectos funcionales de ω-6 FA, se ha observado que son reductores de lípidos, están presentes en recién nacidos, y en cooperación con ω-3 contribuyen directa o indirectamente a modular parámetros funcionales en el nivel celular, tales como la función del receptor, canales de iones, y la expresión génica. Desde un punto de vista nutricional, PUFA representa el componente biológicamente más activo de grasa de la dieta, y los ω-6 son cuantitativamente la fracción más relevante en nuestra dieta. Dado las diversas actividades de ω-6 y ω-3 PUFA, se recomienda un correcto equilibrio entre los diversos ácidos grasos (Galli y Marangoni, 1997).

2.5.3. Ácidos grasos omega 3/omega6.

La relación ω-6 / ω-3 juega un papel importante no sólo en la patogénesis de enfermedades cardiovasculares sino también en el cancer, en enfermedades inflamatorias y autoinmunes (Simopoulos, 2002, 2006; Simopoulos, 2008). Se considera que una alta relación ω-6 / ω-3 puede ser perjudicial para la salud humana, mientrás que un valor cercano a 1 es considerada protectora contra las patologías degenerativas (Simopoulos, 1999). Hess et al. (Hess et al., 2012) demostraron que la incorporación de ω-3 PUFAs en sangre de los animales y el músculo depende directamente de la dieta de alimentación de ácido graso específico.

3. Influencia de ácidos grasos en la fermentación ruminal.

3.1. Técnicas de fermentación *in vitro*.

La técnica de fermentación *in vitro* ha sido considerado para simular la fermentación microbiana ruminal y así reducir de los costos experimentales y mejorar la eficiencia (Getachew et al., 2005). La producción de gas en una etapa temprana es causada principalmente por la fermentación de los componentes solubles en agua en piensos, tales como hidratos de carbono solubles, pectina y componentes de la proteína (Cone et al., 1997). La tasa de degradación se define como la tasa fraccional de degradación (FRD) (Francia et al., 1993; France et al., 2000).

Una FRD alta puede indicar una fermentación rápida, lo que significa una mayor producción de gas en un cierto tiempo de fermentación (Wang et al., 2013). Por otra parte, la cinética dinámica de la producción de gas, así como diferentes tipos de digestibilidad de la fibra (Menke y Steingass, 1988) se han aplicado con éxito a la investigación de la digestión animal para evaluar los valores nutricionales de los forrajes bajo condiciones de fermentación microbiana ruminal (Gosselink et al, 2004a; Wulf y Südekum, 2005).

Los cambios en la cinética de producción de gas, producción de CH_4 y la digestibilidad de la fibra están relacionados con la naturaleza o forma de la semilla oleaginosa, y de sus interacciones con el resto de los componentes de la dieta basal (Lerch et al., 2012).

3.2. Producción de Gas.

La fermentación ruminal *in vitro*, es afectada por la suplementación de lípidos exógenos, y está en función de la estructura de los ácidos grasos. Sin embargo, hay trabajos de investigación que difieren en esto, por ejemplo, el aceite de carotino no afectó a la producción de gas, mientrás que el aceite de girasol la disminuyó (Adeyemi et al., 2015).

3.3. Producción de Metano.

En los últimos años, las emisiones de metano han atraído cada vez más la atención de los nutriólogos y científicos del medio ambiente. Existen métodos o sustancias químicas desarrolladas para mejorar el desempeño del crecimiento de rumiantes y al mismo tiempo reducir la emisión de gases de efecto invernadero (metano) (Kumar et al, 2014; Kim et al, 2015). Por ejemplo, los aceites esenciales o ácidos grasos funcionales son el tipo de métodos eficientes para reducir las emisiones de metano del rumen (Patra y Saxena, 2010; Sallam et al, 2011; Patra y Yu, 2012, 2015). Entre los ácidos grasos ω-3 también se ha observado que pueden reducir la emisión de metano (Czerkawski et al, 1966; Rasmussen y Harrison, 2011;. Li et al, 2015). Las bacterias y los protozoarios participan en los procesos de metabolismo de fibra exógena, lípidos, proteínas. Existe una correlación entre la actividad microbiana (polaridad, la fluidez, permeabilidad, etc.) y las composiciones estructura de la membrana celular, compuesta principalmente de ácidos grasos (por ejemplo, fosfolípidos, glicolípidos y esteroles) (Czerkawski et al, 1966; Rasmussen y Harrison, 2011; Li et al, 2015.).

3.4. Ácidos grasos de cadena larga en membranas de bacterias y protozoarios ruminales.

En los rumiantes, la microflora del rumen participa en el metabolismo de la grasa, la carne y los productos lácteos ricos en algunos ácidos grasos funcionales (FA) componentes incluyen 9cis, ácido linoleico conjugado (11trans- Aldai et al., 2005), el ácido vaccénico (Blewett et al., 2009), ácido ruménico (Chisaguano et al., 2014), el ácido eicosapentaenoico (Gillies et al., 2012) y ácido docosahexaenoico. Una forma eficaz de mejorar los ácidos grasos funcionales se logra mediante la modificación de metabolismo microbiano ruminal por la suplementación de exógena FA con diferentes dobles enlaces o números de carbono (Mouradian et al., 2014).

Las principales vías del metabolismo de FA en rumen incluyen dos tipos, una es por la degradación o la hidrólisis de los ácidos grasos poliinsaturados (PUFA) exógenos, la otra es la síntesis de novo a partir de ácido graso de cadena corta o ácidos grasos

volátiles. Sin embargo, los ácidos grasos de cadena larga se almacenan en la membrana bacteriana o protozoarios.

Ajuste de la fermentación en el rumen o vía de metabolismo de lípidos exógenos con recursos abundantes en derivado de plantas ω-3 PUFAs es un método eficaz para el enriquecimiento ω-3 PUFAs contenidos en la carne de rumiantes o de leche. Sin embargo, los complejos procesos del metabolismo o de biohidrogenación reducen significativamente los PUFAs exógenos derivación rumen. Para abordar este problema, se postula que las composiciones de ácidos grasos en las membranas bacterianas y protozoarias cambiaron con el suministro de diferentes ω-6 / ω-3 proporciones basadas en el ámbito de aplicación favorable y perjudicial en la dieta. Por ejemplo, Petit et al. Enrich de linaza informado de ácido linolénico proporcionado a las vacas raciones, resultó en la relación más baja ω-6 / ω-3 en la leche en comparación con la soja micronizadas o semillas de girasol (Petit, 2002). La incorporación de ácidos grasos ω-3 de sangre animal y el músculo depende directamente del tipo de ácido graso de la dieta (Hess et al., 2012). Por otro lado, LCFAs deben ser metilado por un medio de ácido / metilación basal antes determinado por cromatografía de gases (Kramer et al., 1997; O-Rashid et al., 2007; Ichihara y Fukubayashi, 2010).

4. Bases teóricas de quimioinformática y QSPR.

4.1. Teoría del modelo de Hansch.

Quimioinformática, relacionado con el aprendizaje de máquina, quimiometría y la bioinformática (Varnek y Baskin, 2011), combina los campos de trabajo científicos de la química, ciencias de la información, y las áreas de la topología, teoría de grafos química y la minería de datos en el espacio químico. Corwin Hánsch fue uno de los fundadores de Quimioinformática modernos, que se basa en la relación lipofilia-actividad. Un tipo de modelo de Hansch es la siguiente (de Hansch et al., 1965).

$$f(\varepsilon_i) = a_0 + a_1 \cdot \log P_i + a_2 \cdot pK_a + a_3 \cdot MR - a_4 \cdot (\log P_i)^2 \quad (1)$$

Es bien sabido que, electrostático, y factores de hidrofobicidad estéricos pueden ser biológicamente relevante (Chou y Cai, 2005; Estrada et al., 2006). En esta ecuación, los diferentes parámetros pueden ser utilizados como insumos para dar cuenta de los factores: como coeficientes de reparto de agua / ω-octanol (Pi), refractividad molecular (MR), constantes de acidez logarítmica (PKA) y otros parámetros físico-químicos para cuantificar diferentes propiedades moleculares globales (Ehresmann et al., 2005).

Las salidas del modelo son los valores de una propiedad molecular (εi) o una función de esta propiedad f (εi) para un compuesto químico dado o entidad molecular (mi). Las innovaciones de estos modelos se describen como sigue. 1) El uso de la regresión lineal para buscar ecuaciones lineales multivariantes es capaz de predecir los valores de f (εi), empleando varias variables de entrada. 2) Hánsch también generaliza el uso de parámetros lipofilia por la formulación de modelos parabólicos para las relaciones noω lineal. 3) Los términos logarítmicos (logPi) de Pi se utilizan comúnmente como las medidas de lipofilia molecular y juegan un papel importante en el modelo. A su vez, los valores logPi se pueden predecir ya sea por métodos atómicos (como XlogP o ALogP) o por métodos químicos de fragmentos (como CLogPi o métodos similares) (Tetko et al, 2001;. Tetko y Poda, 2004)..

4.2. Relación de la energía linear libre.

Desde un punto de vista físico-química, el modelo de Hánsch es un enfoque extra-termodinámico estrechamente relacionado con relaciones lineales Gratis Energía (LFER) (Roy y Leonard, 2005; Anslyn y Dougherty, 2006). _ENREF_10 La designación de los modelos de Hansch como ecuaciones LFER provienen de la utilización de parámetros en función de energía libre de Gibbs (Gi) del proceso de ITH (Gibbs, 1871). Los cambios en los valores de este potencial durante un proceso obedecen a

una relación logarítmica con termodinámico estadístico constante de equilibrio Ki (Greiner et al., 2000).

$$\Delta G_i = -RT\log(K_i) \qquad (2)$$

Sin embargo, en estos tipos de ecuaciones, otros parámetros fisicoquímicos o descriptores moleculares también se pueden usar para cuantificar el efecto de los cambios en la estructura química más de una característica de interés. Esto significa que los descriptores moleculares para una molécula dada se pueden utilizar, que no sólo son constantes termodinámicas, sino también otras medidas teóricas de lipofilia molecular, electronegatividad, polarizabilidad, o propiedades de topología molecular (Ehresmann et al., 2005), etc. Los valores de estas variables de entrada (IVK) puede calcularse como parámetros fisicoquímicos o descriptores moleculares de diferentes tipos (k) para una molécula dada (mi). De hecho, la notación se puede ampliar incluyendo funciones o parámetros termodinámicos extra de la siguiente.

$$f(\varepsilon_i) = \sum_{j=1}^{jmax} a_k \cdot {}^{i}V_k + \sum_{j=1}^{jmax} b_j \cdot \left({}^{i}V_k\right)^2 + e_0 \qquad (3)$$

4.3. Relación estructura-actividad (SAR).

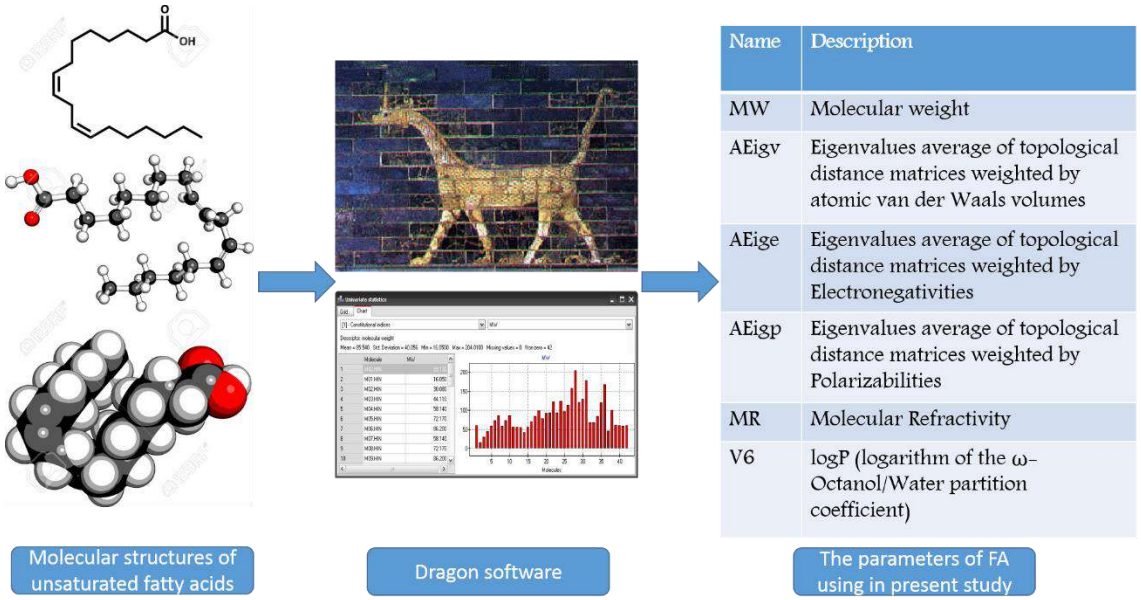
En general, la suposición básica para el análisis de Hánsch es que las moléculas similares tienen actividades similares (de Hansch et al, 2001;.. De Hansch et al, 2002; de Hansch et al., 2003). Este principio también se denomina Relación Estructura-Actividad (SAR). La paradoja SAR se refiere al hecho de que no todos los casos que las moléculas similares a lo largo con actividades similares. Por tanto, el problema de fondo es cómo definir una pequeña diferencia en un nivel molecular. El problema es relevante ya que cada tipo de propiedad, por ejemplo, solubilidad, reactividad, o el metabolismo, se espera que depender de otra diferencia. Esto significa que las variaciones o perturbaciones "pequeños" tienen que ser cuantificados a nivel estructural molecular, que a su vez implica un "pequeño" cambio lineal en la energía libre de la interacción del fármaco con el receptor.

En nuestra opinión, las ideas de la teoría de perturbaciones (PT) (González-Díaz et al., 2013A) pueden utilizarse para dar cuenta de este problema en el contexto de Quimioinformática. Es por eso que; en este trabajo se utilizaron PT y LFER ideas para formular un nuevo enfoque PT-LFER. Este enfoque PT-LFER es una generalización del método clásico Hánsch extra-Termodinámica para Quimioinformática. La prueba de concepto-También se demostró con un estudio experimental, teórica sobre redes complejas de distribución de FA en lipidómica. Con este fin, primero los experimentos se llevaron a cabo para determinar la composición LCFA en el microbioma rumen. A continuación, se incluyó el estudio Quimioinformática, empezando por la definición, la formación, y la validación de nuevos modelos de clasificación PT-LFER. Metodología de aprendizaje de máquina, como Redes Neuronales Artificiales (RNAs) se utilizaron para probar modelos PT-NLFER (análogos Noω-lineales de PT-LFER). A continuación, el mejor modelo PT-LFER encontró fue utilizado para predecir el efecto de las perturbaciones en las condiciones de contorno iniciales sobre un gran compleja red de distribución / absorción de FA en el microbioma ruminal. La compleja red observada para los datos reportados se construyó y se compara por primera vez con la red y modelo predijo redes aleatorias de gran similar. Por último, la sección teórica se completa con un estudio comparativo del modelo de clasificación PT-LFER encontrado con otros modelos noω lineal. Este estudio fue de gran relevancia debido a los resultados anteriores que apuntan a una relación fuerte entre la ω-6 / ω-3 ratios de consumo de FA y la salud humana (Calder et al, 2011; Calviello et al., 2013).. Por consiguiente, este trabajo allana el camino para evaluar el efecto de las perturbaciones en los sistemas moleculares complejas implicadas en las estructuras químicas y las condiciones experimentales de contorno.

4.4. Cálculo de descriptores moleculares.

En la primera obra de esta serie, se utilizó el valor medio de los valores de electronegatividad atómicos de los descriptores de la estructura química (IVK) de un medicamento (Hong et al., 2008). En este trabajo, los modelos PT anteriores se extienden en dirección contraria. En este documento, se combinan los modelos PT y

ecuaciones LFER de Hansch para llevar a cabo un análisis de PT-LFER por primera vez. El sistema de entrada de línea (sonríe) códigos simplificado Molecular-entrada de cada ácido graso fueron descargados de los datos del sitio web de entidades químicas de interés biológico (Chebi: http://www.ebi.ac.uk/chebi/). Y luego, esta forma SMILES códigos de cada ácido graso se han subido a DRAGON43 software, 44 para obtener algunos descriptores moleculares (IVK). Códigos sonrisas son muy útiles para administrar estructuras moleculares (Siani y col., 1994; Karwath y De Raedt, 2006; Toropov y Benfenati, 2007). Y para su posterior cálculo de descriptores moleculares (Tetko et al., 2001; Vidal et al, 2005). En primer lugar, se utilizaron variables estructurales (IVK) de cada ácido graso como un nuevo conjunto de descriptores moleculares. Estas variables son las mismas clases de descriptores moleculares utilizados en el análisis de Hansch para cuantificar las estructuras moleculares. La primera clase de descriptor molecular calculado era V1 = Mw (peso molecular). La segunda clase de descriptores moleculares V2 = AEigv, V3 = AEige, y V4 = AEigp también fueron incluidos, que son la media de valores propios de matrices de distancia topológicos ponderado con atómica van der Waals volúmenes (v), polarizabilidad (p), o Electronegatividad (e). Por último, también se propusieron V5 = MR (Molecular Refractividad), V6 = LogP (logaritmo del coeficiente de ω-reparto octanol / agua.



Name	Description
MW	Molecular weight
AEigv	Eigenvalues average of topological distance matrices weighted by atomic van der Waals volumes
AEige	Eigenvalues average of topological distance matrices weighted by Electronegativities
AEigp	Eigenvalues average of topological distance matrices weighted by Polarizabilities
MR	Molecular Refractivity
V6	logP (logarithm of the ω-Octanol/Water partition coefficient)

Fig. 2 El ejemplo de la transformación de las estructuras moleculares en descriptores moleculares con el software de dragón

4.5. Cálculo de operadores de Teoría de la Perturbación.

Cuando se amplió la ecuación anterior del modelo PT-LFER, se pueden observar dos tipos de términos de entrada. El primer tipo de término es la función f (εij) nueva. Esta función toma los valores, f (εij) nueva = IPA (%) nuevos para cada muestra. IPA (%) i es la proporción Área Pico Interna, que se utiliza para cuantificar la proporción experimental de un ácido graso determinado por GC (O-Rashid et al, 2007;. Na, 2011). Esto significa que f (εij) nuevo es la valor proporción esperada de un ácido graso en las mismas condiciones cj. La segunda clase se refiere a los términos de perturbación ΔΔiVk (cj). Los parámetros ΔΔVk (cj) son útiles para cuantificar el efecto de las perturbaciones de diferentes condiciones de contorno (cj) sobre el 'f salida (μnr) nuevo, que se define aquí como una función de valor discreto (ocurrencia o no de enlaces en la red) para los efectos de clasificación. La diferencia ΔΔVk (cj) entre el estado nuevo o final (ΔVk (cj) nueva) y el (ref ΔVk (cj)) de referencia o estado inicial es la perturbación aditiva para un componente en el ΔVk (cj).

$$\Delta\Delta V_k(c_j) = p(c_j)_{new} \cdot \left({}^{i}V_k - \langle V_k(c_j)\rangle\right)_{new} - p(c_j)_{ref} \cdot \left({}^{i}V_k - \langle V_k(c_j)\rangle\right)_{ref} \quad (4)$$

4.6. Cálculo de operadores de la Caja de Jenkins

Una inspección detallada de los términos de perturbación muestra que son diferencias de probabilidad ponderada (Δ) de Box-Jenkins Operadores ΔiVk (cj). Se calcularon los valores de Box-Jenkins Operadores ΔiVk (cj) de los descriptores moleculares (IVK) para cuantificar el efecto de las desviaciones de una molécula (millas) de los valores propios medios de todas las moléculas medidos con el mismo conjunto de condiciones (cj) de el sistema complejo. Las desviaciones debido a los cambios en diferentes condiciones de contorno cj se tuvieron en cuenta, por ejemplo, c1 = el tratamiento experimental de la muestra, c2 = con / sin fermentación, c3 = la fase de distribución de los ácidos grasos (medios ruminales, organismos bacterianos o protozoos), etc . Todos los datos medidos con GC fueron procesados en un archivo de Excel. En Excel, se calcularon los valores propios de ΔiVk (cj) considerando diversas condiciones de contorno experimentales (cj). Las probabilidades son p (cj) = nj / ntotal; nj número de entradas experimentales para la misma condición cj y ntotal = 744 el número total de entradas experimentales. El valor medio <ΔVk (cj)> es la diferencia del valor de la función IVK con los descriptores moleculares medios <Vk> para una condición de contorno cj específico, consulte las ecuaciones:

$$\Delta V_k(c_j) = \left({}^{i}V_k - \langle V_k(c_j)\rangle\right) \quad (5)$$

$$\langle V_k(c_j)\rangle = \frac{1}{n_j}\left(\sum_{m_i \subset c_j}^{n_j} {}^{i}V_k\right) \quad (6)$$

5. Método quimioinformático para establecer las redes de distribución de ácidos grasos en micoorganismos ruminales.

En resumen, la estructura molecular de los ácidos grasos exógenos juega un papel importante en el mecanismo de regulación en rumen vía y los procesos de metabolismo. Esto implica que es razonable combinar las propiedades de la

estructura molecular y la cantidad de ácidos grasos en la membrana bacteriana o protozoo para predecir las propiedades de metabolismo de lípidos rumen. En este sentido, los modelos Quimioinformática pueden convertirse en una herramienta útil (Varnek y Baskin, 2011). En nuestra opinión, podemos combinar la idea de Perturbación Teoría (PT) (González-Díaz et al, 2013A.) Y lineales Free-Energy Relaciones (LFER) (Roy y Leonard, 2005; Anslyn y Dougherty, 2006) para manejar este asunto. Como resultado de esta combinación podemos obtener modelos PT-LFER que pueden manejar los datos complejos generados en estos estudios. Además, los métodos de aprendizaje de máquina noω lineal tales como Redes Neuronales Artificiales (RNA) (González-Díaz et al, 2013b;. Duardo-Sánchez et al, 2014). Se puede utilizar para mejorar la capacidad de predicción de técnicas Quimioinformática en el estudio de los sistemas sociales bio-molecular compleja, ecológico, y. Como resultado podemos obtener modelos Noω lineal PT-LFER modelos (PT-NLFER).

En el presente trabajo queremos introducir una nueva metodología teórico basado métodos PT-LFER que es capaz de procesar los datos experimentales complejas a partir de los estudios de metabolómica de la producción de metano. Es por eso que; el presente trabajo consta de dos etapas principales. La primera etapa de la obra tiene como objetivo reportar conjunto de datos experimentales original de distribución de ácidos grasos en la membrana biológica. En la segunda etapa de este trabajo vamos a desarrollar unos nuevos modelos Quimioinformática PT-LFER / PT-NLFER para los datos generados. A continuación, el mejor modelo PT-LFER encontró fue utilizado para predecir el efecto de las perturbaciones en las condiciones de contorno iniciales sobre un gran compleja red de distribución / absorción de FA en el microbioma ruminal. Por consiguiente, este trabajo allana el camino para estudiar el efecto de la teoría de la perturbación complejo molecular en la estructura química de ácido graso, los parámetros de la fermentación y las condiciones experimentales correspondientes de contorno.

5.1. Detalles del modelo PT-LFER.

En nuestra opinión, podemos combinar la idea de Perturbación Teoría (PT) (González-Díaz et al, 2013A.) Y lineales Free-Energy Relaciones (LFER) (Roy y Leonard, 2005; Anslyn y Dougherty, 2006) para manejar este asunto. Corwin Hánsch fue uno de los fundadores en Quimioinformática modernos, que se basa en la relación lipofilia-actividad. El supuesto para el análisis de Hánsch se basa en las moléculas similares con actividades similares (Hánsch et al, 2001;.. Hánsch et al, 2002; Hánsch et al., 2003) o la llamada Relación Estructura-Actividad (SAR). Un tipo de modelo de Hansch es la siguiente (de Hansch et al., 1965).

$$f(\mu_i) = a_0 + \varepsilon_1 \cdot \log P_i + \varepsilon_2 \cdot pK_a + \varepsilon_3 \cdot MR + \varepsilon_4 \cdot (\log P_i)^2 \quad (7)$$

Es bien sabido que estéricos, electrostático, y factores de hidrofobicidad de sustancias pueden ser biológicamente relevante (Chou y Cai, 2005;. Estrada et al, 2006). En esta ecuación diferentes parámetros de descriptores moleculares se utilizan como entradas para reflejar propiedades biológicas, por ejemplo, los coeficientes de agua / ω-octanol partición (Pi), refractividad molecular (MR), constantes de acidez logarítmicas (PKA) etc., para cuantificar diferentes propiedades moleculares. (Ehresmann et al., 2005) la salida del modelo es los valores de una función de esta propiedad f (μi) o una propiedad molecular (μi) para una sustancia química dada o entidad molecular (mi). Las innovaciones de este modelo se presentan a continuación. La regresión lineal se utilizó para buscar ecuaciones lineales multivariables empleando varias variables de entrada para predecir el μi de salida o f (μi); parámetros lipofilia también fueron generalizadas por Corwin Hánsch a una formulación de un modelo parabólico para las relaciones noω lineal; logarítmica plazo (logPi) del Pi se utiliza normalmente para descripta más la lipofilia molecular y desempeñar un papel muy importante en el modelo. Mientras tanto, logPi se puede predecir ya sea por métodos atómicos (XlogP o ALogP) o por métodos químicos de fragmentos (CLogPi o métodos similares). (Tetko et al, 2001;. Tetko y Poda, 2004) Desde un punto de vista físico-química, el modelo de Hánsch es un enfoque extra-

termodinámico estrechamente relacionado con los modelos LFER (Roy y Leonard, 2005; Anslyn y Dougherty, 2006). (Anslyn et al., 2006). Además, otros parámetros fisicoquímicos o descriptores moleculares también se pueden utilizar para cuantificar la influencia de los cambios (GI) en una estructura química más de una característica de interés. Esto significa que algunos descriptores moleculares, que no sólo son constantes termodinámicas, sino también valores propios teóricos de lipofilia molecular, electronegatividad, polarizabilidad, o propiedades de topología molecular, (Ehresmann et al., 2005) para una molécula dada pueden ser aplicadas como entradas a utilizar en el desarrollo de un modelo. Los valores de estas variables de entrada (IVk) se pueden calcular como parámetros fisicoquímicos o descriptores moleculares de diferentes tipos (k) para una molécula dada (mi). De hecho, la notación se puede ampliar incluyendo funciones termodinámicas extra o descriptores moleculares como sigue.

Recientemente, González-Díaz et al. (González-Díaz et al., 2013A) formuló un modelo PT-propósito general para múltiples-frontera problemas Quimioinformática. En trabajos anteriores, esta teoría se extiende para el estudio de modelo PT-LFER de la teoría de perturbaciones (PT) en combinación con relaciones lineales de energía libre (LFER) en redes complejas. Ajuste de una función general f (μnr) útil para cuantificar la ocurrencia (μnr = 1) o no (μnr = 0) de un proceso que implica un conjunto de moléculas de mi en un sistema complejo. Se considera que todos los posibles estados forman una red de estados. Los nodos de la red son los estados iniciales o de referencia (r) vinculados a sus respectivos estados finales o nuevos (n) alcanzado por el sistema después de una perturbación de las condiciones iniciales. En el modelo PT-LFER, un conjunto de múltiples condiciones de contorno experimentales iniciales refcj ≡ (c0, c1, c2, c3 ... cn) y un conjunto de condiciones de contorno diferente o nuevo newcj ≡ (c0, c1, c2, c3 ... cn) son considerados al principio o al final del proceso después de que uno o múltiples perturbaciones. El modelo PT-LFER propuesto en este documento es una ecuación lineal con el siguiente formulario.

$$'f\left(\mu_{nr}\right)_{new} = a_0 + a_1 \cdot f_1\left(\varepsilon_{ij}\right)_{new} + a_2 \cdot \left\langle f_2\left(\varepsilon_{ij}\right)_{ref} \right\rangle + \sum_{q=1}^{q=qmax} a_q \cdot f_q\left(v_q\right)_{ref} \qquad (8)$$

$$+ \sum_{q=1}^{q=qmax} a_q \cdot f_q\left(\Delta v_q\right) + \sum_{q=1}^{q=qmax} a_q \cdot f_q\left(\log\left|\Delta v_q\right|\right) + \sum_{(i,k,j)=1}^{(i,k,j)=(i,k,j)max} a_{ikj} \cdot \Delta\Delta^i V_k\left(c_j\right)$$

F La función de salida '(μnr) nueva es una puntuación utilizado en lineal discriminante Análisis (LDA) para calcular la probabilidad de clasificación binaria de un insumo como μnr = 1 o μnr = 0 (salidas) (Hill y Lewicki, 2006).

JUSTIFICACIÓN

Los productos de origen animal desempeñan un papel fundamental en la dieta del hombre. A las grasas de la carne y de la leche, provenientes de rumiantes, se les ha puesto especial atención para ser consideradas en una dieta saludable.

Los metabolitos de ácidos grasos en el rumen están relacionados con la cantidad de depósitos de ácidos grasos en la leche o la grasa intermuscular, es decir, están en función del alimento que ingiere el rumiante. A los ácidos grasos omega 3 y omega 6 se les relaciona como benéficos para el hombre ya que se ha observado que pueden prevenir o curar las enfermedades de la vida moderna como lo son las cardiovasculares, las autoinmunes, etc.

Actualmente existen herramientas quimioinformáticas (QSAR, minería de datos, modelado de datos y aprendizaje automático), que nos permiten predecir algunas tendencias potenciales del alimento de los rumiantes en relación con los depósitos de ácidos grasos que tendrán. Estos métodos permiten disminuir el costo y el tiempo de investigación al igual que la reducción del uso de animales

HIPÓTESIS

Los métodos de reemplazo de alto rendimiento *In silico* (QSAR, minería de datos (DATA MINING), modelado de datos y aprendizaje automático) permitirán predecir las redes complejas de distribución de ácidos grasos en los sistemas de fermentación ruminal *in vitro.*

OBJETIVOS

Objetivo General

Predecir y evaluar los efectos de cártamo y canola en los sistemas de fermentación ruminal *in vitro* de ovino por métodos métodos de reemplazo (QSAR, minería de datos (DATA MINING), modelado de datos y aprendizaje automático).

Objetivos Específicos

1) Determinar las proporciones de harina de cártamo o de semillas de canola para los ovinos por sistemas de fermentación ruminal *in vitro*.
2) Analizar las diferentes partes de las semillas de cártamo en sistemas de fermentación ruminal *in vitro*.
3) Determinar el efecto de aceites vegetales en las composiciones de ácidos grasos de membrana microbiana ruminal.
4) Diseñar modelo de aprendizaje automático de las redes complejas de distribución de los ácidos grasos basado en los estudios computacionales de la minería de datos, datos con la teoría de la estructura cuantitativa relación de actividad (QSAR) para predecir y evaluar los efectos de cártamo y canola en los sistemas de fermentación ruminal *in vitro* de ovino .

MATERIALES Y MÉTODOS

1. Parte Experimental.

1.1 Métodos para determiner proporciones de harina de cártamo o semilla de canola con semillas de sorgo

Este experimento se llevó a cabo para estudiar el efecto de mezcla (semilla de canola o harina de cártamo: semillas de sorgo en las diferentes proporciones de 0: 100; 25:75; 50:50; 75:25; 100: 0, respectivamente) y el rastrojo de maíz (que fueron puestos en una pequeña bolsa) con la cantidad de 0.5 g: 0.5 g, sobre la cinética de la producción de gas, la producción de CH4, además se determinó el rendimiento de fermentación de *in vitro* (NH3-N, el pH y la DIVMS de rastrojo de maíz).

1.1.1. Animales donantes y sustratos materiales

Se utilizaron cuatro adultos machos ovinos de la raza Pelibuey con fistula ruminal (peso corporal, 45 ± 5.0 kg) los cuales fueron utilizados como donantes de inóculo según la norma oficial mexicana (NOM-220-SSA1-2002). Cada ovino se mantuvo de forma individual y con libre acceso al agua, y una alimentación a base de 1.0 kg de forraje con 0.5 kg de rastrojo de maíz achicalado. Los ingredientes del forraje que se proporcionó fueron 64.2% de sorgo, 19.3% heno de alfalfa, 4.6% de harina de canola, 2.9% de aceite de canola, 5.0% rastrojo de maíz, 2.0% de melaza, 1.0% de sebo purificado, y 1.0% de urea, con una composición declarada de 87% de materia seca, proteína bruta 13.9%, 12.1 MJ / kg energía total, 5.3% de grasa cruda, 0.54% de calcio, 9.5% de fibra detergente ácido, y el 21.4% de fibra detergente neutro. Los sustratos (harina de cártamo, sorgo, semillas de canola y el rastrojo de maíz (500 g en cada uno)) se obtuvieron de la planta del Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (CENID-INIFAP), México.

2.Fermentación ruminal in vitro

En la fermentación in vitro se llevó a cabo según la descripción de Tang et al., (2008). En 0700 am antes de la alimentación, el total de 600 ml de líquido rumen de ovejas Pelibuey cuatro adulto macho se obtuvieron, se mezcla y filtrar a través de cuatro capas de tela de queso en un matraz Erlenmeyer lleno de CO2. Las soluciones de fermentación in vitro se prepararon mezclando mediante la mezcla de las ovejas fluido ruminal libre de partículas con una solución de tampón de saliva artificial (Menke y Steingass, 1988) en una proporción de 1: 4 (v / v) a 39 ºC bajo lavado continuo con CO2 durante 30 min.

Sustratos (0,5 g paja de maíz con 0,5 g mezcla de cártamo comida (SFM) o semilla de canola (CAS) con semilla de sorgo en diferentes proporciones de 0: 100, 25:75, 50:50, 75:25, 100: 0) fueron incubadas con 100 solución tampón rumen ml a 39 ºC en 4 repeticiones de cada 72 h, para determinar la cinética de producción de gas con el equipo de determinación de gas ANKOM (ANKOM Technology Corp., Fairport, Nueva York, EE.UU.).

Otros lotes de fermentación in vitro se llevó a cabo para determinar el pH, la concentración de NH3-N y DIVMS de rastrojo de maíz. Soluciones de fermentación (50 ml) se añadieron en las botellas pre-calentado con la mezcla de 0,5 g y 0,5 g rastrojo de maíz que fueron ponderados previamente en una bolsa de nylon (tamaño de poro, 52 m; área de superficie específica, 44 cm2 / g) fue poco más alto que informe anterior (Valentin et al., 1999) con 33 cm2 / g. Las botellas fueron sellados con tapones de goma y tornillos de tapones y se incubaron a 39 ºC en un oscilador de baño de agua a temperatura constante durante 24 h y 48 h en 3 repeticiones de cada tratamiento. Los volúmenes de gas se midieron y muestras de gas se obtuvieron de cada botella después de 24 hy 48 h de incubación a 25 ºC. La fermentación se termina haciendo girar las botellas en hielo, sin tope salarial, y luego para determinar el valor de pH de inmediato, sacó la bolsa de nylon se lava con agua desionizada durante 4 veces hasta que el agua estaba limpia. Mientras tanto, 1 ml de líquido de fermentación

se mezcla con 0,25 ml de ácido Meta-fosfórico (25%; w / v) se almacenaron en -20 ºC para la determinación de la concentración de NH3-N.

2.3 Análisis químico

Muestras de alimento fueron analizados mediante los métodos estándar de la AOAC (Cunniff, 1995) para DM (No. 967.03), proteína cruda (N ° 984.13) y el extracto de éter (No. 954.02), contenido de cenizas (No. 942.05), la energía total y el se expresan inclusive de la ceniza residual. Concentración de NH3-N de líquido de incubación a 24 hy 48 h se determinó con fenol-hipoclorito y ninhidrina procedimientos colorimétricos descritos por Broderick y Kang (1980). La concentración de metano de las muestras de gas se determina mediante cromatografía de gases con columna de HP-SOLAR / Q (longitud 30 m; Identificación 0.530 mm; PELÍCULA: 40 micrómetros; gato, No. 19095P-QO4).

2.4 Cálculos y Análisis de datos

En las curvas de gas in vitro fueron equipados con el modelo logístico-Exponencial (LE0) utilizando NLREG Versión 5.0 (Sherrod PH, 1995), El modelo LE0 descrito por Wang et al, (2011; 2013) fue descrito de la siguiente ecuación:

$$V = V_F \cdot \frac{1 - \exp(-\kappa t)}{1 + \exp(b - \kappa t)} \qquad (1)$$

Donde V es la producción de gas asintótico final (ml / g) en el tiempo t punto, VF es el volumen de gas asintótico final con dimensión de "ml ', k es la tasa fraccional de la producción de gas con dimensión de' / h '; yb es parámetro de forma y sin dimensión.

Tasa fraccional de degradación inicial (FRD0), tasa de producción de gas (RGT), medio tiempo (t0,5) y la tasa fraccional de la producción de gas en la vida media (μ0.5) propuesto por Wang et al, (2013) como. siguiente:

$$FRD_0 = \frac{\kappa}{1+\exp(b)} \qquad (2)$$

$$RG_t = V_F \cdot \kappa \frac{(1+\exp(b)) \cdot \exp(-\kappa t)}{(1+\exp(b - \kappa t))^2} \qquad (3)$$

$$t_{0.5} = \frac{\ln(2+\exp(b))}{\kappa} \qquad (4)$$

$$\mu_{0.5} = \frac{k(d+0.5)}{1+d} \qquad (5)$$

Los análisis estadísticos se realizaron utilizando el procedimiento GLM de SAS 9.0 (2001) y los medios dentro se compararon los errores estándar con medios mínimos cuadrados. Se reportaron menos medios plazas en todo el texto, y significaciones estadísticas fueron declarados si P <0,05.

1.2. Métodos para influir en las diferentes partes de la semilla de cártamo en fermentación in vitro

Diseño experimental

Este experimento se llevó a cabo en dos partes: En primer lugar, para separar toda semilla en casco y partes del núcleo, y preparar la comida de cártamo (extraído con hexano, SMH; extrajo con éter de petróleo, SMP), después de que para determinar la composición nutricional de cada parte de semilla de cártamo obtenerse. En segundo lugar, para hacer en el experimento de fermentación in vitro, cada parte de semilla de cártamo se fermentó por separado para obtener los datos de la producción de gas, aparente fibra detergente neutro / ácido (/ ADF NDF) digestibilidad y digestibilidad de la materia seca en tres ejemplares cada uno, después de que el uso de la ecuación de calcular la energía metabolizable (EM) y digestibilidad de la materia orgánica (DMO).

Animales donantes y preparación de sustratos

Cuatro adultos varones ovinos Pelibuey (45 ± 5,0 kg / cada uno) con permanente rumen-fístula, ubicado de forma individual y el libre acceso al agua potable, fueron utilizados como donantes de inóculo de acuerdo a la Norma Oficial Mexicana (NOM-220-SSA1-2002). Cada oveja fue proporcionado diariamente 1,0 kg de forraje, que compuesto con el 64,2% del sorgo, el 19,3% de heno de alfalfa, 4,6% de harina de canola, 2,9% de aceite de canola, 5,0% rastrojo de maíz, 2,0% de melaza, 1,0% de sebo purificado, y 1,0% de urea, con una composición declarada de materia seca del 87%, proteína cruda 13,9%, 12,1 MJ / kg de energía total, el 5,3% de grasa cruda, 0.54% de calcio, 9,5% de fibra detergente ácido, y el 21,4% de fibra detergente neutro.

Semilla de cártamo (0,5 kg) fueron comprados de Querétaro en México, separa artificialmente en casco y kernel, y luego molida esas muestras en pequeñas partículas para la solución como casco de cártamo, kernel y particiones de semillas enteras. Otros 0,5 kg de semilla de cártamo se trituraron y se extrajo con hexano y éter de petróleo para preparar dos tipos de harina de cártamo extraído (SMH y SMP).

Fermentación in vitro

El líquido ruminal obtenido, se mezcló y se tensa a través de cuatro capas de queso de tela en un matraz Erlenmeyer con un espacio de cabeza-O2 libre, de animales donantes a las 07:00 am antes de alimentar. Fluido ruminal libre de partículas se mezcló con la solución tampón de saliva artificial (Menke y Steingass, 1988) en una proporción de 1:. 4 (v / v) a 39 ºC bajo lavado continuo con CO2 (Mauricio et al, 1999; Tang et al., 2008). Experimento 1: La producción de gas. Cada parte de la semilla de cártamo (casco, de almendra, harina de cártamo, cártamo suelo, 1,0 g, respectivamente, como sustrato de fermentación, respectivamente) se incubó con 100 ml del rumen solución tampón a 39ºC en cuádruples de cada uno, para determinar la cinética de la producción de gas con la determinación de gas ANKOM equipo (ANKOM Technology Corp., Fairport, Nueva York, EE.UU.) durante 48 h descritos por Cone et al.

(1996). **Experimento 2:** Botellas eran precalentado (39ºC) antes de añadir 50 ml de fluido ruminal tamponada (casco, de almendra, harina de cártamo, cártamo suelo, 0,5 g como sustrato de fermentación, respectivamente) en virtud de lavado de CO2, sellados con tapones de goma y nillador en los casquillos y se incubaron a 39ºC en la temperatura del baño de agua oscilador constante durante 48 h. Cada se llevó a cabo en 3 repeticiones dentro de 3 espacios en blanco (sin sustrato) para la corrección. Las botellas fueron retirados del oscilador baño de agua después de 48 h de incubación, para filtrar el sustrato de cada uno, se secó en 105ºC durante la noche y se pesaron el residuo de cada sustrato, y luego para calcular ADF / NDF digestibilidad y en la desaparición de materia seca in vitro (DIVMS).

Análisis de composición química

Dieta y muestras (sustratos) se midieron por los métodos estándar de la AOAC (Cunniff, 1995) para la materia seca (Nº 967.03), proteína cruda (N ° 984.13) y el extracto de éter (Nº 954.02), contenido de cenizas (Nº 942.05), la energía total, y se expresaron como el integrador de la ceniza residual.

Cálculo y análisis de datos

En las curvas de producción de gas in vitro fueron equipados con NLREG Versión 5.0 (Sherrod, 1995), utilizando la combinación de la Gauss-Newton y Métodos Levenberg-Marquardt (John et al., 1981). Para calcular los parámetros de la cinética de producción de gas, se utilizó el modelo logístico de Exponencial (LE0) como se describe por Wang et al. (2013), y los parámetros se obtuvieron basa en la inspección visual de las parcelas con el fin de llegar a la convergencia.

$$V = V_F \cdot \frac{1 - \exp(-\kappa t)}{1 + \exp(b - \kappa t)} \qquad (6)$$

donde V es la producción acumulada de gas (ml) en t, VF es el volumen de gas asintótica final con dimensión de 'ml', k es la tasa fraccional de la producción de gas

con la dimensión de '/ h'; yb es parámetro de forma y sin dimensión. Tasa fraccional de degradación inicial, FRD0 = k / (1 + exp (b)) (Wang et al, 2013.); la tasa de producción de gas, RG = VF k (1 + exp (b)) exp (kt) / (1 + exp (b-kt) 2 (Jiao et al, 2013);. la media hora en la que un medio de la producción de gas final, t0,5 = ln (2 + exp (b)) / k; tasa fraccional de la producción de gas en un medio de vida, μ0.5 = k (d + 0,5) / (1 + d) (Wang et al., 2013).

La energía metabolizable (ME, MJ / kg MS) y la digestibilidad de la materia orgánica (DMO,%) de diferentes partes de semillas de cártamo se calcularon utilizando el modelo de Menke et al. (1979) como sigue:

$$\text{ME (MJ/kg DM)} = 2.20 + 0.136 \cdot \text{GP} + 0.057 \cdot \text{CP} + 0.0029 \cdot \text{CF}^2 \qquad (7)$$

$$\text{OMD (\%)} = 14.88 + 0.889 \cdot \text{GP} + 0.45 \cdot \text{CP} + 0.0651 \cdot \text{Ash} \qquad (8)$$

where GP is 24 h net gas production (ml/200 mg); CP is crude protein (%); CF is crude fat (%).

NDF/ ADF, fiber and Non-fiber disappearance were calculated with formula as follows:

$$\{\text{IVDMD}\}\,(\text{g/kg}) = \frac{\text{W.}_{(\text{original})} - \text{W.}_{(\text{residue})}}{\text{W.}_{(\text{original})}} \qquad (9)$$

$$\{\text{NDF/ ADF}\}_{\text{d}}\,(\%) = \frac{[\{\text{NDF/ ADF}\}_{(\text{original})} - \{\text{NDF/ ADF}\}_{(\text{residue})}]}{[\{\text{NDF/ ADF}\}_{(\text{original})}]} * 100 \qquad (10)$$

$$\text{Fiber}_{\text{d}}\,(\text{g/kg}) = [NDF + ADF]_{(\text{difference between original and residue})} \qquad (11)$$

$$\text{Non - fiber}_{\text{d}}\,(\text{g/kg}) = [IVDMD - (NDF + ADF)]_{(\text{difference between original and residue})} \qquad (12)$$

Los análisis estadísticos se realizaron con el de un solo sentido procedimiento ANOVA de SPSS y los medios se compararon con los medios mínimos cuadrados. La diferencia significativa entre las diferentes partes de la semilla de cártamo en los

parámetros de producción de gas y NDF / ADF y digestibilidad de la fibra se marca como letras minúsculas en las Mesas correspondientes si había diferencias de significación. Se informó de mínimos cuadrados en todo el texto, y los efectos del tratamiento fueron declarados importancia si P <0,05.

1.3. Métodos para diferentes de recursos de plantas oleaginosas in vitro

Diseño experimental

En este trabajo, los aceites derivados de plantas tales como semillas de lino, cártamo, canola y aceite de coco, que son abundantes en poli- o mono insaturados (C18: 3, C18: 2 y C18: 1) y ácidos grasos de cadena media (C12 : 0) (refiérase a la γ-linolénico, ácido linoleico, ácido oleico, y el ácido láurico, respectivamente) se utilizaron para investigar el efecto de estos exógenos diferentes dobles enlaces y números de carbonos de los ácidos grasos en los perfiles de LCFA de la biología bacteriana y protozoo membrana, AGV en medios de fermentación, y el metano (CH4) la producción in vitro de fermentación micro-nicho ecológico.

Animales donantes e ingresientes de la alimentación

El macho adulto ovejas Pelibuey (3 personas) con fístula ruminal-permanente (peso corporal, 45 ± 5,0 kg) fueron utilizados como donantes de inóculo de acuerdo con la norma oficial mexicana (NOM-220-SSA1-2002). Composiciones nutricionales del forraje fueron de acuerdo a las necesidades nutricionales del NRC (2007). Cada oveja, alojados individualmente y acceso libre al agua, se alimentó con 1,0 kg de alimento y 0,5 kg rompieron rastrojo de maíz. La alimentación se composited de 38% de grano de maíz, 30% de heno de alfalfa, 2% de harina de canola, 2,5% de aceite de canola, 2% de harina de cártamo, 2,5% de aceite de cártamo, 18% rastrojo de maíz, 2,0% de melaza, y 1,0% de urea.

Sustrato, heno de alfalfa, se obtiene de una planta del Instituto de Investigación Natural de Forestales, Agrícolas y Ganadería en Querétaro, México. Y todos los ácidos

grasos se adquirieron de Sigma-Aldrich con los números de serie de cada uno, el aceite de cártamo (S8281), aceite de linaza (430021), el aceite de canola (46961), y aceite de coco (46949). FA composiciones de estos cuatro aceites vegetales se presentan en la Tabla 1. El aceite de cártamo es abundante en ácido linoleico con una proporción de 70%, aceite de linaza con aproximadamente 50% de ácido γ-linolénico, aceite de canola contiene aproximadamente de ácido oleico 60%, y el aceite de coco incluye ácido láurico en torno al 60%. Estos cuatro aceites elegidos en base a la diferencia de composición principal, representan ácidos grasos de cadena medios (ácido láurico, C12: 0), ácido mono-poliinsaturados (ácido oleico, C18: 1), ácidos poliinsaturados (ácido linoleico, C18: 2; y ácido linolénico, C18: 3), respectivamente.

Table 1. The ingredients of exogenous plant-derived oils using in this study

Name of fatty acids	Chemical Structure	The name of plant-derived oils (%)			
		Safflower	Linseed	Canola	Coconut
1. Lauric Acid	C 12:0	0.85	1.31	1.17	59.78
2. Myristic Acid	C14:0	0.49	0.61	0.65	19.97
3. Pentadecanoic Acid	C15:0	0.26	0.44	0.51	0.59
4. Palmitic Acid	C 16:0	6.79	5.90	5.59	8.63
5. Palmitoleic Acid	C16:1	0.16	0.10	0.27	0.00
6. *cis*-10-heptadecanoic acid	C17:1	0.06	0.00	0.00	0.00
7. Stearic acid	C18:0	2.76	4.22	2.19	3.33
8. Elaidic acid	C18:1 n9t	0.07	0.09	0.11	0.04
9. Oleic acid	C18:1 n9c	16.23	20.92	59.60	5.29
10. Linolelaidic acid	C18:2 n6t	0.09	0.00	0.00	0.00

11. Linoleic acid	C18:2 n6c	69.79	14.90	20.48	1.36
12. Arachidic acid	C20:0	0.39	0.00	0.49	0.11
13. γ-Linolenic acid	C18:3 n6	0.00	50.15	0.00	0.00
14. cis-11-Eicosadienoic acid	C20:1	0.24	0.26	1.12	0.00
15. Linolenic acid	C18:3 n3	0.18	0.00	4.98	0.00
16. Heneicosanoic acid	C21:0	0.94	0.19	0.38	0.00
17. Behenic acid	C22:0	0.21	0.14	0.36	0.00
18. *cis*-11,14,17-Eicosatrienoic acid	C20:3 n3	0.04	0.12	0.01	0.08
19. Arachidonic acid	C20:4 n6	0.06	0.11	0.14	0.33
20. Tricosanoic acid	C23:0	0.17	0.22	0.16	0.48
21. *cis*-13,16-Docosadienoic acid	C22:2	0.09	0.18	0.13	0.00
22. Lignoceric acid	C24:0	0.12	0.14	0.13	0.00
23. *cis*-5,8,11,14,17-Eicosapentaenoic acid	C20:5 n3	0.00	0.00	1.21	0.00
24. Nervonic acid	C24:1	0.00	0.00	0.33	0.00

***Fermentación in vitro**.*

La manipulación in vitro fue de acuerdo a la descripción de Tang el al (2008), el fluido de rumen libre de partículas se mezcló con solución tampón de saliva artificial (Menke y Steingass, 1988) en una proporción de 1:. 2 (v / v) a los 39 ºC bajo lavado continuo de CO2 durante 30 minutos. Cada aceite, se mezcla con 0,5 g de sustrato en una proporción de 5%, se incubó con 50 ml de solución tampón rumen a 39 ºC en 3 repeticiones de cada uno por 48 h. Después de 48 h, bacterias y protozoos fracciones

se obtuvieron de cada botella de fermentación para determinar las distribuciones de ácidos grasos en la membrana bacteriana y biología protozoo, mientras tanto el volumen de gas y la concentración de metano se determinaron, además para calcular la cantidad de producción de metano. Fracciones bacterianas y protozoarias se prepararon de acuerdo a la descripción de O-Rashid (2007). Además, 1 ml de sobrenadante, se recogió para la determinación de perfiles de AGV en los medios de comunicación, se mezcla con solución de ácido meta-fosfórico 25% (HPO3) en una proporción de 10: 1 y se almacena en 4 hasta que el análisis.

Análisis químico

La concentración de CH4 de las muestras de gas se determina por cromatografía de gases (GC, Agilent Technologies, sistema 6890N Red GC) con la columna de HP-SOLAR / Q. VFA y perfiles LCFA metilados también se determinaron mediante GC con la columna de DB-FFAP y HP-88 (112-88A7), respectivamente. Perfiles LCFA en la membrana bacteriana y la biología protozoos, se expresaron como valor proporción interna bajo la misma condición, sobre la base de que, se calcularon algunos ingredientes ácidos grasos interesante, los detalles calculative se presentan en la Tabla 2.

Table 2. The calculation details of some biological significance FA items

Items	**Calculation details or functions**
a) Saturated fatty acids	= *sum* (C12:0, C14:0, C15:0, C16:0, C17:0, C18:0 and C20:0)
b) Long chain fatty acids ≥ 18 C, %	All fatty acids composited with equal or larger than 18 carbons
c) 18 carbons unsaturated fatty acids, %	= *sum* (C18:1 n9t, C18:1 n9c, C18:2 n6t, C18:2 n6c, and C18:3 n6)
d) cis-fatty acids, %	= *sum* (C18:1 n9c, C18:2 n6c)

e) trans-fatty acids, %	= *sum* (C18:1 n9t, C18:2 n6t)
f) Ratios (*cis*-/*trans*-)	= *sum* (C18:1 n9c, C18:2 n6c)/*sum* (C18:1 n9t, C18:2 n6t)
g) Ratios (Stearic acid: Palmitic acid)	= C18:0/C 16:0
h) *odd*-carbon fatty acids, %	= *sum* (C15:0, C15:1, C17:0, C17:1)
i) *even*-carbon saturated fatty acids, %	= *sum* (C12:0, C14:0, C16:0, C18:0, C20:0)
j) *even*-carbon unsaturated fatty acids, %	= *sum* (C14:1, C16:1, C18:1 n9t, C18:1 n9c, C18:2 n6t, C18:2 n6c, C18:3 n6)
k) Saturated/unsaturated fatty acids	= *sum* (C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0)/*sum* (C14:1, C15:1, C16:1, C17:1, C18:1 n9t, C18:1 n9c, C18:2 n6t, C18:2 n6c, C18:3 n6)

Statistical analyses were performed using the GLM procedure of SPSS (2011) and means within standard errors were compared with least squares means. Least squares means were reported throughout the text, and statistical significances were declared if $P< 0.05$.

2. Partes teóricas

2.1. Sección experimental

En el flujo de trabajo de esta parte experimental (Fig. 1). Los datos generales de los procedimientos experimentales utilizados en el experimento 1 y el experimento 2 se explican de la siguiente manera.

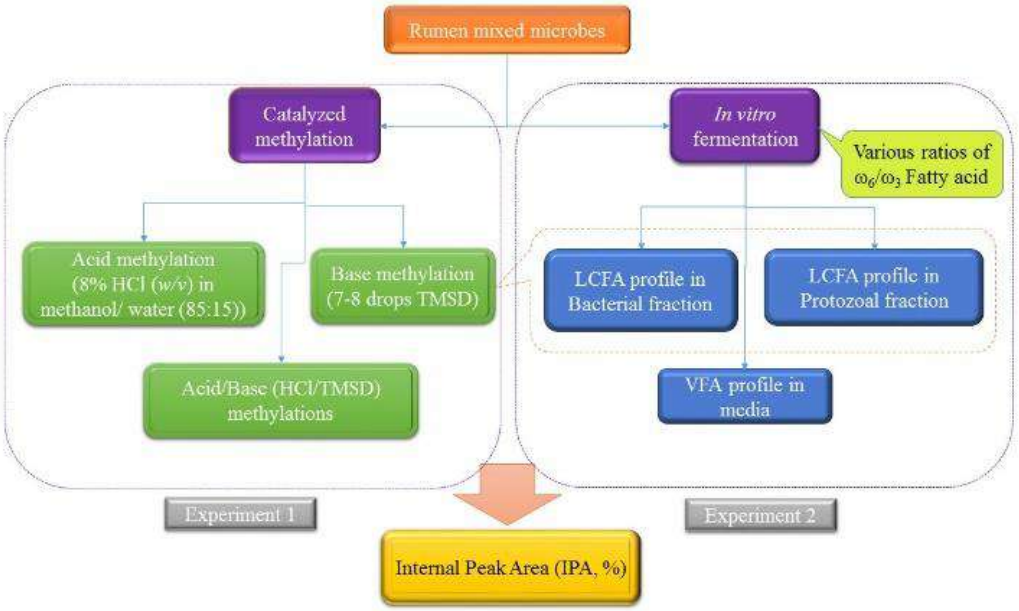


Fig. 1. Workflow of the experimental section (dataset): IPA values of each FA based on bacterial membrane FAs catalyzed with methylation methods (**experiment 1**) and IPA values obtained from bacteria, protozoan, and media fractions with the fermentation of various exogenous ω-6/ω-3 ratios supplementation with base methylation (**experiment 2**)

2.1.1. Bienestar animal.

Tres hombre adulto Pelibuey oveja con rumen-fístula permanente (peso corporal, 45,0 ± 5,0 kg) fueron utilizados como donantes de inóculo de acuerdo a la Norma Oficial Mexicana (NOM-220-SSA1-2002). Composición nutricional del forraje para animales donantes fue de acuerdo con el Consejo Nacional de Investigación (NRC). (2007)

2.1.2. Detalles de fermentación in vitro.

Los detalles in vitro están de acuerdo con la descripción de Tang, el al., (Tang et al., 2008) con el fluido de rumen libre de partículas mezclado con la solución tampón de saliva artificial (Menke y Steingass, 1988) en una proporción de 1 : 2 (v / v) a 39 °C bajo lavado continuo de CO2. Ácidos grasos microbianas se prepararon de acuerdo

con el método desarrollado por O-Rashid. (O-Rashid et al., 2007) Más específicamente, las muestras microbianas y protozoarios se separaron por centrifugación diferencial de acuerdo con el método descrito por Legay-Carmier y Bauchart. (1989)

2.1.3. Procedimientos específicos de parte 1.

Microbios ruminales mezclados sin fermentación fueron catalizadas con la metilación del ácido (8% de HCl (w / v) disuelto en metanol / agua (85/15)) (Ichihara y Fukubayashi, 2010), la metilación de base (trimetilsilildiazometano, TMSD) (O-Rashid et al., 2007) (prensatelas et al., 2004) y ácido combinado de bases metilaciones (primera catalizadas con 8% de HCl, y posteriormente catalizadas con TMSD), respectivamente. Los valores de la zona Peak, PA (i), para cada LCFA bajo diferentes conjuntos de condiciones experimentales cj (diferentes muestras) se determinaron con GC (O-Rashid et al, 2007;. Na, 2011) (Modelo 6890N, Agilent Technologies Inc ., EE.UU.) con HP-88 columna en el laboratorio de CENID FYMA, INIFAP, y AGV se determinaron con la columna DB-FFAP. Se utilizaron los valores de área de pico obtenido para calcular el área del pico interna, IPA (%), como sigue.

$$\mathrm{IPA}(\%)_{ij} = 100 \cdot \left(\frac{\mathrm{PA}_{(i)}}{\sum_{m_i \subset cj}^{nj} \mathrm{PA}_{(i)}} \right) \quad (13)$$

2.1.4. Especificación de procedimientos experimentales 2.

Este estudio se realizó para evaluar el efecto de diversos ω-6 exógenos / ω-3 proporciones sobre el metabolismo biohidrogenación del microbioma microbiana. Los ω-6 y ω-3 PUFAs, ácido linoleico (LA, L1376-5g, Sigma-Aldrich) y el ácido α-linolénico (ALA, L2376-500mg, Sigma-Aldrich) con un importe total de 100 mg / g en sustratos , se establecieron como las proporciones de 100: 0, 90:10, 80:20, 66:33, 50:50 y 20:80, respectivamente.

Componentes de los alimentos utilizados para alimentar a los animales fueron los mismos que los utilizados en el experimento 1. Todas las muestras de lípidos de fermentación se extrae con una mezcla de cloroformo-metanol (2: 1, v / v) a partir de fracciones bacterianas y protozoarias (Folch et al, 1957). fueron catalizadas con la metilación de base (TMSD, en este documento) (O-Rashid et al., 2007). LCFA perfiles extraídos de fracciones bacterianas y protozoarias y los perfiles de AGV se determinaron para calcular IPA (%), y también se calculó la concentración (mM) de los perfiles de VFA.

2.2. Sección teórica

2.2.1. Workflow used for PT-LFER Estudio quimioinformática.

En la segunda sección, un estudio Quimioinformática de los resultados obtenidos en la sección experimental se llevó a cabo. Fig. 2 muestra el diagrama de flujo de trabajo que establece la integración de ambas secciones (experimentales y teóricos). Para el análisis, los valores de los datos cromatográficos sobre IPA (%) de ácidos grasos se recogieron bajo diferentes ω-6 / ω-3 ratios y condiciones experimentales cj. A continuación, definimos el modelo PT-LFER. Después de eso, se calcularon los valores de las variables de entrada, incluyendo descriptores moleculares (IVK) de la clase de orden k para cada ITH molécula de ácido graso, y los operadores de perturbación ΔΔVk (cj). Después de que se realizó el análisis estadístico y obtuvimos el modelo PT-LFER. En la siguiente manera, más detalles se explican en algunos pasos.

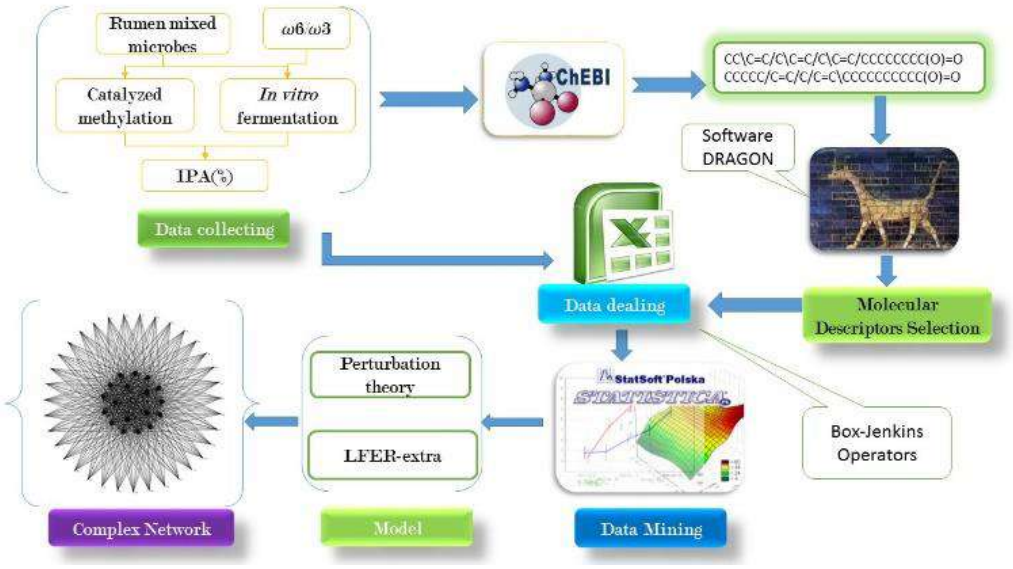


Fig. 2 Workflow used herein to seek PT-LFER models

2.2.2. Detalles teóricos de modelos PT-LFER.

En un estudio reciente, González-Díaz et al. (González-Díaz et al., 2013) ha formulado un modelo PT-propósito general para múltiples-frontera problemas Quimioinformática. En este trabajo, esta teoría se extiende al estudio de los modelos PT-LFER de perturbaciones en redes complejas. Dejado ser una función general f (Lnr) útil para cuantificar la ocurrencia (Lnr = 1) o no (Lnr = 0) de un proceso que implica un conjunto de moléculas de (mi) en un sistema complejo. Se considera que todos los posibles estados forman una red de estados. Los nodos de la red son los estados iniciales o de referencia (r) vinculados a sus respectivos estados finales o nuevos (n) alcanzado por el sistema después de una perturbación de las condiciones iniciales. Se separa en un conjunto de múltiples condiciones de contorno experimentales iniciales refcj ≡ (c0, c1, c2, c3 ... cn) (condiciones de referencia) y un conjunto de condiciones de contorno newcj ≡ (c0, c1, c2, c3 ... cn diferente o nuevo) (condiciones de nuevo) después de uno o múltiples perturbaciones (cambios en estas condiciones). El modelo PT-LFER propuesto en este documento es una ecuación lineal con el siguiente formulario:

$$'f\left(L_{nr}\right)_{new} = a_0 + a_1 \cdot f\left(\varepsilon_{ij}\right)_{ref} + a_2 \cdot \left\langle f\left(\varepsilon_{ij}\right)\right\rangle_{ref} + \sum_{j=1,k=1}^{j=jmax,\ k=kmax} a_{kj} \cdot \Delta\Delta V_k\left(c_j\right) \quad (14)$$

F La función de salida '(Lnr) nueva es una puntuación utilizado en el análisis discriminante lineal (LDA) para calcular las salidas o probabilidad a posteriori de la clasificación binaria de las entradas Lnr = 1 o Lnr = 0 (Hill y Lewicki, 2006). Los vectores Vi = [f (εij) REF, <f (εij)> ref, ω-3, ω-6, IV1, ... iVkmax, ΔV1 (c1), ... ΔVk (cj), ... ΔVkmax (cjmax), ΔΔV1 (c1), ... ΔΔVk (cj), ... ΔΔVkmax (cjmax)] son las entradas de este modelo. Cada vi vector, representa un caso estadístico (caso ITH), de un total de n = 407.655 casos (perturbaciones). Estos casos estadísticos codificadas por vi vectores son perturbaciones de una entrada o estado de referencia (cambios en los parámetros de entrada) que producen una salida o nuevo estado. Los vectores de entrada vi incluyen el valor de f (εij) ref para el estado de referencia (valor conocido). Los vectores vi también tienen en cuenta las cantidades de ω-6 y ω-3 para el nuevo estado (después de la perturbación). También se incluyeron los valores de descriptores moleculares (IVK) utilizados en el análisis de un clásico de Hánsch. Por último, las entradas también se consideran los valores de la operadores ΔΔVk PT-LFER (cj).

2.2.3. Cálculo de descriptores moleculares.

En la primera obra de esta serie, hemos utilizado los valores medios de electronegatividad atómica de los descriptores de la estructura química (IVK) de un medicamento (Hong et al., 2008). En otro trabajo reciente, el método para la predicción de epítopos de péptidos fue adaptada utilizando la teoría de perturbaciones (González-Díaz et al., 2014). En el presente trabajo, los modelos PT anteriores se extienden a otra dirección. En este documento, se combinan los modelos PT y ecuaciones LFER de Hansch para llevar a cabo un análisis de PT-LFER por primera vez. Para ello, se tomaron los siguientes pasos. En primer lugar, se utilizaron variables estructurales (IVK) como un nuevo conjunto de descriptores moleculares. Los valores de estas variables se calcularon con el software DRAGON (Papa et al, 2005;.. Mauri et al, 2006;. Helguera et al, 2008) (. Mauri et al, 2006). La primera

descriptor molecular calculado fue V1 = Mw (peso molecular). Los descriptores moleculares V2 = AEigv, V3 = AEige, y V4 = AEigp fueron incluidos, que son los valores propios medios de las matrices de distancia topológicos ponderados con volúmenes atómicos de van der Waals (v), polarizabilidades (p), o Electronegatividad (e). Por último, también se propusieron V5 = MR (Molecular Refractividad), V6 = LogP (logaritmo de la n-octanol / Coeficiente de reparto de agua). Las estructuras de los ácidos grasos se han subido al dragón en una forma de códigos simplificado Molecular Input-Portero Line (sonríe). Códigos sonrisas son muy útiles para administrar estructuras moleculares (Siani y col., 1994; Karwath y De Raedt, 2006; Toropov y Benfenati, 2007). Y para su posterior cálculo de descriptores moleculares (Tetko et al., 2001; Vidal et al, 2005) (Tabla 1). En nuestro trabajo, los códigos de sonrisas de los ácidos grasos correspondientes fueron descargados de los datos del sitio web de entidades químicas de interés biológico (ChEBI: **http://www.ebi.ac.uk/chebi/**).

Table 1. Molecular descriptors ($^{i}V_{k}$) of fatty acids obtained from the ChEBI database

Name of fatty acids in ChEBI [a]	*cis*/*trans* pattern [b]	Molecular descriptors of FA- Inputs [c]					
		V_1	V_2	V_3	V_4	V_5	V_6
Lauric Acid	*l*	200.4	137.4	127.6	139.5	58.7	4.5
Myristic Acid	*l*	228.4	179.1	169.0	181.2	67.9	5.5
Myristoleic Acid	*c*	226.4	172.1	162.0	174.2	69.0	5.0
Pentadecanoic Acid	*l*	242.5	202.0	191.8	204.1	72.5	5.9
cis-10-Pentadecenoic acid	*c*	240.4	194.8	184.5	196.9	73.6	5.5
Palmitic Acid	*l*	256.5	226.3	216.0	228.4	77.1	6.4
Palmitoleic Acid	*c*	254.5	217.9	207.6	220.1	78.2	5.9
Heptadecanoic acid	*l*	270.5	252.0	241.6	254.1	81.7	6.8
Stearic acid *cis*-10-Heptadecenoic acid	*c*	268.5	243.2	232.8	245.4	82.8	6.4
Stearic acid	*l*	284.5	279.1	268.6	281.2	86.3	7.3
Elaidic acid	*t*	282.5	269.5	259.0	271.7	87.4	6.8
Oleic acid	*c*	282.5	269.5	259.0	271.7	87.4	6.8
Linolelaidic acid	*tt*	280.5	260.9	250.4	263.1	88.5	6.4
Linoleic acid	*cc*	280.5	260.9	250.4	263.1	88.5	6.4
Arachidic acid	*l*	312.6	337.4	326.8	339.6	95.5	8.2
γ-Linolenic acid	*ccc*	278.5	251.5	240.9	253.7	89.6	5.9
Linolenic acid	*ccc*	278.5	255.1	244.5	257.3	89.6	5.9
cis-11.14-Eicosadienoic acid	*ct*	308.6	318.0	307.3	320.2	97.7	7.3

Behenic acid	*l*	340.7	401.3	390.6	403.5	104.7	9.1
cis-8.11.14-Eicosatrienoic acid	*ctt*	306.5	307.4	296.7	309.7	98.8	6.9
Erucic acid	*t*	338.6	390.2	379.4	392.4	105.8	8.7
Acetic acid	*l*	60.1	13.1	6.4	14.4	12.6	-0.2
Propionic acid	*l*	74.1	19.2	11.8	20.7	17.3	0.4
Isobutyric acid	*l*	88.1	24.6	16.7	26.2	21.8	0.9
Butyric acid	*l*	88.1	26.7	18.8	28.3	21.9	0.9
Isovaleric acid	*l*	102.2	33.2	24.9	34.9	26.4	1.1
Valeric acid	*l*	102.2	35.6	27.4	37.4	26.5	1.3

[a] Fatty acids measured for our linear discriminant analysis PT-LFER model.

[b] *cis*/*trans* pattern, *l* represents *linear*, *c* as *cis*-, *t* as *trans*- PUFAs. The order of *c* or *t* represents the order of initial isomerization characteristics with the tails of PUFAs.

[c] Molecular descriptors (${}^{i}V_k$) calculated with DRAGON software: V_1 = Mw, V_2 = Aeigv, V_3 = Aeige, V_4 = Aeigp, V_5 = AMR, and V_6 = LogP.

2.2.4. Calculo de operadores.

Cuando se amplió la ecuación anterior del modelo PT-LFER, se pueden observar dos tipos de términos de entrada. El primer tipo de término es la función f (εij) ref. Esta función toma los valores, f (εij) ref = <εij> ref = IPA (%) i para cada muestra. IPA (%) i = 100 · (PAI / PAjmax) es la proporción Área Pico interna, que se utiliza para cuantificar la proporción experimental de un ácido graso determinado por GC. Esto significa que f (εij) ref es el valor medido de la proporción de un ácido graso en las mismas condiciones cj. La segunda clase se refiere a los términos de perturbación ΔΔVk (cj). Los parámetros ΔΔVk (cj) son útiles para cuantificar el efecto de las perturbaciones de diferentes condiciones de contorno (cj) sobre el 'f salida (Lnr) nuevo, que se define aquí como una función de valor discreto (ocurrencia o no de enlaces en la red) para los efectos de clasificación. La diferencia ΔΔVk (cj) entre el estado final o una nueva (ΔVk (cj) nuevo) y el estado inicial o de referencia (ΔVk (cj) ref) es la perturbación aditiva para un componente en el ΔVk (cj). Cuando la salida de esta ecuación es 'f (Lnr) nueva> 'f (Lnr) REF => Lnr = 1 => IPA (%) nueva> IPA (%) ref; y por lo tanto (=>) la distribución o proporción de la FA en el nuevo estado es mayor que en el estado de referencia, de lo contrario Lnr = 0.

$$'f\left(L_{nr}\right)_{new} = a_0 + a_1 \cdot 'f\left(L_{nr}\right)_{ref} + a_1 \cdot \left\langle \varepsilon_{ij} \right\rangle_{ref} + \sum_{j=1,\,k=1}^{j=jmax,\ k=kmax} a_{ijk} \cdot \Delta\left({}^{i}V_k - \left\langle V_k\left(c_j\right)\right\rangle\right) \quad (15)$$

$$\Delta\Delta V_k\left(c_j\right) = p\left(c_j\right)_{new} \cdot \left({}^{i}V_k - \left\langle V_k\left(c_j\right)\right\rangle\right)_{new} - p\left(c_j\right)_{ref} \cdot \left({}^{i}V_k - \left\langle V_k\left(c_j\right)\right\rangle\right)_{ref} \quad (16)$$

2.2.5. Caja de jenkins.

Una inspección detallada de los términos de perturbación muestra que son diferencias de probabilidad ponderada (Δ) de Box-Jenkins Operadores ΔVk (cj). Se calcularon los valores de Box-Jenkins Operadores ΔVk (cj) de los descriptores moleculares (IVK) para cuantificar el efecto de las desviaciones de una molécula (millas) desde el comportamiento promedio de todas las moléculas medidos con el mismo conjunto de condiciones (cj) de el sistema complejo. Las desviaciones debidas a los cambios en las diferentes condiciones de contorno (cj) se tuvieron en cuenta. Las condiciones de contorno se refieren a las condiciones operativas preliminares, c1 = se refiere a la utilización de diferentes tratamientos experimentales, c2 = con / sin fermentación, c4 = es el protocolo de cromatografía de gases utilizada, y c5 = se refiere al uso de experimentos replicados. Otros están más directamente relacionados con la distribución posterior y la naturaleza de la FA, c3 = la fase biológica de distribución de LCFAs y c6 = cuantificar información sobre cis / patrón geométrico trans presentes en los LCFAs. Todos los datos se procesan en un archivo de Excel. En Excel, se calcularon los valores de ΔVk (cj) considerando diversas condiciones de contorno experimentales (cj). Las probabilidades son p (cj) = nj / ntotal; nj número de entradas experimentales para la condición cj y ntotal = 744 el número total de entradas experimentales (el número total de IPA (%) medido en este trabajo). El valor medio <ΔVk (cj)> (**Table 2**) es la diferencia de la IVK valor de la función con los descriptores moleculares medios <Vk> para una condición de contorno cj específica, ver las ecuaciones:

$$\Delta V_k(c_j) = \left({}^{i}V_k - \langle V_k(c_j)\rangle\right) \quad (17)$$

$$\langle V_k(c_j)\rangle = \frac{1}{n_j}\left(\sum_{m_i \subset c_j}^{n_j} {}^{i}V_k\right) \quad (18)$$

Table 2. Average values of input variables (<V_k>) for experimental boundary conditions (c_j)

Experimental boundary condition		**Average eigenvalues of input variables <$V_k(c_j)$> *aa***						***p*(c_j) *ab***
Conditions (c_j)	level	V_1	V_2	V_3	V_4	V_5	V_6	
	BM	274.4	258.3	247.9	260.5	84.8	6.6	0.113
	AM	274.4	258.3	247.9	260.5	84.8	6.6	0.113
c_1 =>	CM	274.4	258.3	247.9	260.5	84.8	6.6	0.113
Treatments *a*	BA	261.9	234.6	224.3	236.8	80.3	6.2	0.258
	PA	261.9	234.6	224.3	236.8	80.3	6.2	0.258
	MA	85.8	25.4	17.7	27.0	21.1	0.7	0.145
c_2 =>	***0***	274.4	258.3	247.9	260.5	84.8	6.6	0.339
fermentation *b*	***1***	223.2	188.7	178.9	190.7	67.3	5.0	0.661
	Bacteria fraction	269.0	248.1	237.7	250.3	82.9	6.4	0.597
c_3 => Phase *c*	Protozoa fraction	261.9	234.6	224.3	236.8	80.3	6.2	0.258
	Media fraction	85.8	25.4	17.7	27.0	21.1	0.7	0.145
c_4 => Column of GC *d*	HP-88 (112-88A7)	266.8	244.0	233.6	246.2	82.1	6.4	0.855
	DB-FFAP	85.8	25.4	17.7	27.0	21.1	0.7	0.145
	0	242.8	213.9	203.8	216.0	73.9	5.6	0.391
c_5 => Replicate	***0.1***	242.8	213.9	203.8	216.0	73.9	5.6	0.391
(*r*-error) *e*	***0.2***	205.8	173.6	164.2	175.6	61.6	4.4	0.133
	0.3	274.4	258.3	247.9	260.5	84.8	6.6	0.085
	linear	203.2	180.8	171.5	182.8	59.6	4.6	0.238
	cis	278.5	269.1	258.6	271.3	86.1	6.7	0.111
	trans	310.6	329.8	319.2	332.1	96.6	7.7	0.032
c_6 => *cis*/*trans*	*trans, trans*	280.5	260.9	250.4	263.1	88.5	6.4	0.016
pattern *f*	*cis, cis*	308.6	320.8	310.1	323.0	97.7	7.3	0.032
	cis, trans	289.9	279.6	269.0	281.8	91.6	6.7	0.048
	trans, cis	280.5	261.1	250.5	263.3	88.5	6.4	0.016

cis, cis, cis	286.5	269.9	259.3	272.1	92.3	6.2	0.111
cis, trans, trans	292.5	280.0	269.3	282.2	94.2	6.4	0.032
trans, cis, trans	278.5	254.6	244.0	256.8	89.6	5.9	0.016
trans, trans, trans	278.5	252.0	241.4	254.2	89.6	5.9	0.032
cis, cis, trans	278.5	252.5	241.9	254.8	89.6	5.9	0.016
cis, trans, cis	278.5	252.0	241.4	254.2	89.6	5.9	0.032

[a] "BM" means base methylation without fermentation; "AM" means acid methylation without fermentation; "CM" means acid- and base-combined methylation; "BA" means fatty acids from bacterial fraction after 48h fermentation; "PA" means fatty acids from protozoan fraction after 48h fermentation; "MA" means volatile fatty acids from media fraction after 48h fermentation.

[b] "***0***" means the dataset from experiment 1 without fermentation; "***1***" means the dataset from experiment 2 with fermentation of omega 6 and omega 3.

[c] "phase", means the dataset: long chain fatty acids including from the bacterial membrane (bacterial fraction), protozoan membrane (protozoan fraction), volatile fatty acids from fermentation media (media fraction).

[d] Column of GC, "HP-88 (112-88A7)" means the column of GC for determining long chain fatty acids; "DB-FFAP" means the column for determining volatile fatty acids.

[e] "***0***" means the original data, "***0.1***, ***0.2***, or ***0.3***" means the 1, 2, or 3 replicates, respectively.

[f] *cis*/*trans* pattern: "*linear*" means LCFA without double bonds; "*cis*" means LCFA with *cis* isomerization; "*trans*" means LCFA with *trans* isomerization; and the number of cis or trans means LCFA with the same number of *cis* or *trans* double bonds.

[aa] $\langle V_k(c_j) \rangle$ means the average of Molecular descriptors (V_k) for different conditions (c_j); the descriptors are V_1 = Mw, V_2 = Aeigv, V_3 = Aeige, V_4 = Aeigp, V_5 = AMR, and V_6 = LogP.

[ab] $p(c_j) = n_j / n_{total}$; n_j number of experimental entries for condition c_j and n_{total} = 744 total number of experimental entries.

2.2.6. Clasificación de modelos.

El análisis discriminante lineal (LDA) (Hill y Lewicki, 2006) se utilizó el algoritmo implementado en el software STATISTICA para encontrar el mejor modelo PT-LFER. A veces la relación entre las variables de entrada y la salida es más compleja y la linealidad no puede resolver el problema. Por lo tanto, los modelos no lineales podrían proporcionar una mejor solución, pero con el inconveniente de no ser capaz de interpretar el modelo y las relaciones entre las variables. De este modo, las redes neuronales artificiales (RNAs) (Haykin, 1998) se pusieron a prueba: Redes Neuronales lineales (LNNs), que son similares a los modelos de LDA, y no lineales Multi-Layer Perceptron (MLP) (Hill y Lewicki, 2006) . Los datos completos se dividieron aleatoriamente en serie de entrenamiento ("t", el 75%) que se utiliza para la

construcción de modelo y serie de validación ("v", el 25%) que se utiliza para la validación del modelo. Además, se añadió una variable validación cruzada para el conjunto de datos con los valores de la prueba de "t" y "v". Todas las variables independientes fueron unificados y estandarizados con el software STATISTICA, antes de la construcción del modelo.

2.2.7. Estudio de complejo de redes.

Tanto las redes observadas y predichas se construyeron en Excel y se guardan en el .net (listas de pares de nodos) formato de archivo. Los enlaces de la red observada coinciden con las clases para ser predichos por el modelo anterior LDA. La existencia de un enlace correspondiente a la condición Lnr = 1, si IPA (%) obs> IPA (%) ref para cada ácido graso en ambos los estados inicial y final, Lnr = 0 en caso contrario. Se generó una serie de pares de estados lo más alto posible, el cálculo de la existencia de vínculos observados con la regla anterior, y que también estaba previsto con el modelo. Estas archivos se procesaron con el software CentiBiN descrito por Junker et al. (Junker et al., 2006), para calcular los índices promedio de la topología de la red. Los índices calculados eran los valores medios de la distancia topológica-vértice vértice (Newman et al., 2001), el grado de nodo, y la cercanía del componente gigante de lo observado, predijeron y los dos más aleatorias similares. También se construyeron dos modelos de redes aleatorias (red aleatoria 1 y 2). El modelo de la red aleatoria seleccionada fue la gráfica Erdös-Rényi (ER), que a menudo se utiliza como un modelo de red aleatoria.

2.2. Métodos quimioinformáticos 2

2.1. Sección experimental

El experimento se presenta en la (Fig. 1). (a) el pH, la presión del gas producción de metano y la temperatura, así como (b) las áreas cromatográficas internos pico (IPA%)

de (b1) ácidos grasos de cadena larga (LCFA) en la membrana bacteriana y protozoos y (b2) grasos volátiles ácidos (VFA) se determinará en las diferentes experimentos. Los datos generales de los procedimientos experimentales utilizados se explican de la siguiente manera.

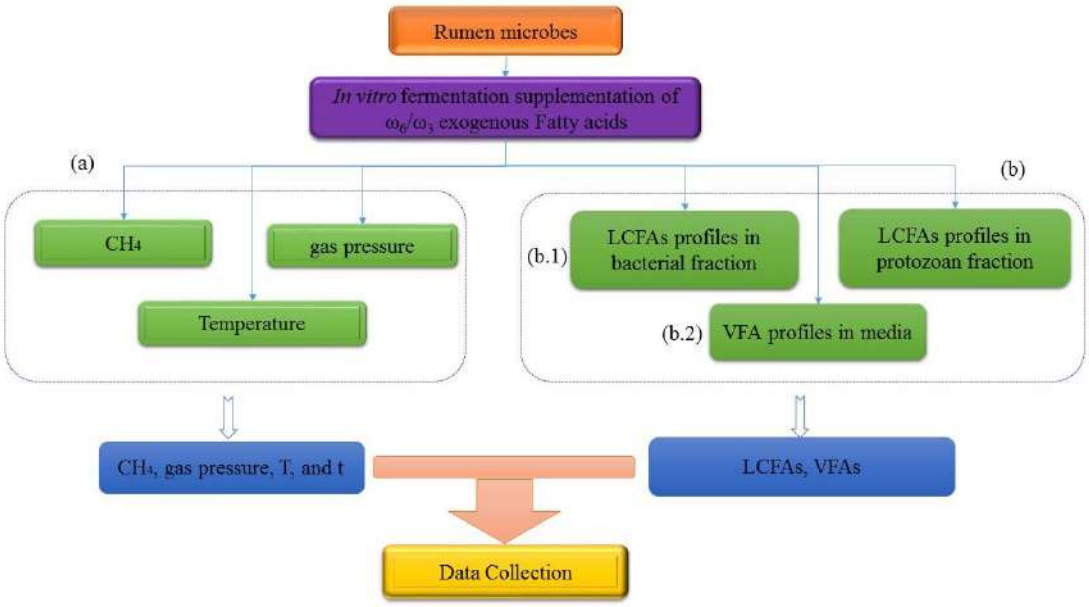


Fig. 1. Workflow of the experimental parts

2.1.1. Inóculo.

Los microbios de inóculo fueron proporcionados por tres adultos varones Pelibuey oveja con rumen-fístula permanente (peso corporal, 45,0 ± 5,0 kg). Los métodos de instalación del rumen-fístula estaban de acuerdo con la Norma Oficial Mexicana (NOM-220-SSA1-2002). Composiciones nutricionales del forraje para animales donantes fueron de acuerdo con la descripción de la NRC (2007).

2.1.2. Fermentación in vitro experimental.

Los detalles de fermentación in vitro fueron de acuerdo a la descripción de Tang el al. (. Tang et al, 2008) fluido ruminal libre de partículas se mezcló con solución

tampón de saliva artificial (Menke y Steingass, 1988) en una proporción de 1: 2 (v / v) a 39 ºC con lavado continuo de CO2. Las fracciones microbianas y por protozoos se separaron por centrifugación diferencial de acuerdo con los métodos de Legay-Carmier y Bauchart (1989). Ácidos grasos microbianas se prepararon de acuerdo con el método desarrollado por O-Rashid (O-Rashid et al., 2007), y catalizadas por metilación catalizada por base.

La parte conjunto de datos (a) se constaba de CH4, la presión del gas (GP) y la temperatura (T). Estos parámetros se determinaron después de fermentado dentro exógenas diferentes proporciones ω-6 / ω-3 PUFAs. El PUFAs ω-6 / ω-3 fueron compuestas de ácido linoleico (LA, L1376-5 g, Sigma-Aldrich) / α-linolénico (ALA, L2376-500 mg, Sigma-Aldrich) en las proporciones de 100: 0 ; 90:10; 80:20, 66:33, 50:50 y 20:80, respectivamente, con una cantidad total de 100 mg / g en sustrato. La parte de datos (b) se composited del Área Interna Peak, IPA (%), de (b.1) LCFA de la membrana bacteriana y protozoo de dos tratamientos diferentes (metilaciones y fermentación con exógenos ω-6 / ω-3 PUFAs catalizada) y (b.2) VFA en el medio de fermentación. Entre las metilaciones catalizadas incluyen la metilación del ácido (solución de HCl de 8% en metanol / agua (v / v, 85/15)) (Ichihara y Fukubayashi, 2010), la metilación de base (trimetilsilildiazometano, TMSD) (O-Rashid et al ., 2007) (Presser et al., 2004) combinación de ácido / base-metilación (catalizado con HCl, a su vez con TMSD). Experimento de fermentación fue de los mismos tratamientos con la parte (a) usando exógenos ω-6 / ω-3 PUFAs. Los valores de área de pico, PA (i), para cada ácido graso bajo diferentes conjuntos de condiciones experimentales cj (diferentes muestras) se determinaron con cromatografía de gases (modelo 6890N, EE.UU.) con HP-88 Columna, mientras que VFAs se determinaron con la columna de DB-FFAP. Se utilizaron los valores de área del pico obtenido por GC para calcular el área interna Peak, IPA (%), como sigue.

$$\mathrm{IPA}(\%)_{ij} = 100 \cdot \left(\frac{\mathrm{PA(i)}}{\sum_{m_i \subset c_j}^{m_1 \to m_i} \mathrm{PA(i)}} \right) \quad (19)$$

Donde, mi se refiere a un ácido graso de una muestra, mi cj se refiere a un ácido graso en un conjunto de especificar condición experimental.

2.2. Sección teórica

Se calcularon los conjuntos de datos de producción LCFAs, AGV, CH4, además de la temperatura y presión del gas en un conjunto diferente de condiciones experimentales y el tratamiento de acuerdo con la teoría de perturbaciones combinado con relaciones lineales de energía libre. Se hicieron las modelos de clasificación basado en estas variables de entrada con el software STATISTICA.

2.2.1. PT-LFER Estudio quimioinformático.

Un estudio Quimioinformática de los resultados obtenidos en la sección experimental se llevó a cabo. La Figura 2 muestra un diagrama de flujo de trabajo que establece la integración de ambas secciones (experimentales y teóricos). El diagrama explica brevemente la sección experimental, centrándose principalmente en el desarrollo posterior de modelo PT-LFER. Para el análisis, se calcularon los parámetros (Ω) de la sección experimento (a) y (b) basado en el conjunto de condiciones experimentales cj. Después de eso, la diferencia (Δ) y los valores absolutos de logística de la diferencia (log (| Δ |)) desde el estado inicial o de referencia (ref) vinculado a su respectivo nuevo o final (nuevo) se calcularon. A continuación, algunos valores de las variables de entrada, incluyendo descriptores moleculares (IVK) de orden k de clase para cada i-ésima y molécula de ácido graso, y los operadores de perturbación ΔΔVk (cj) se calcularon. A continuación, se definió el modelo PT-LFER basado en estas variables de entrada obtenidos a partir de piezas experimentales y los descriptores moleculares de cada ácido graso. En el análisis estadístico final y obtuvo se realizaron

el modelo PT-LFER. A continuación, explicamos más detalles en algunos de estos pasos.

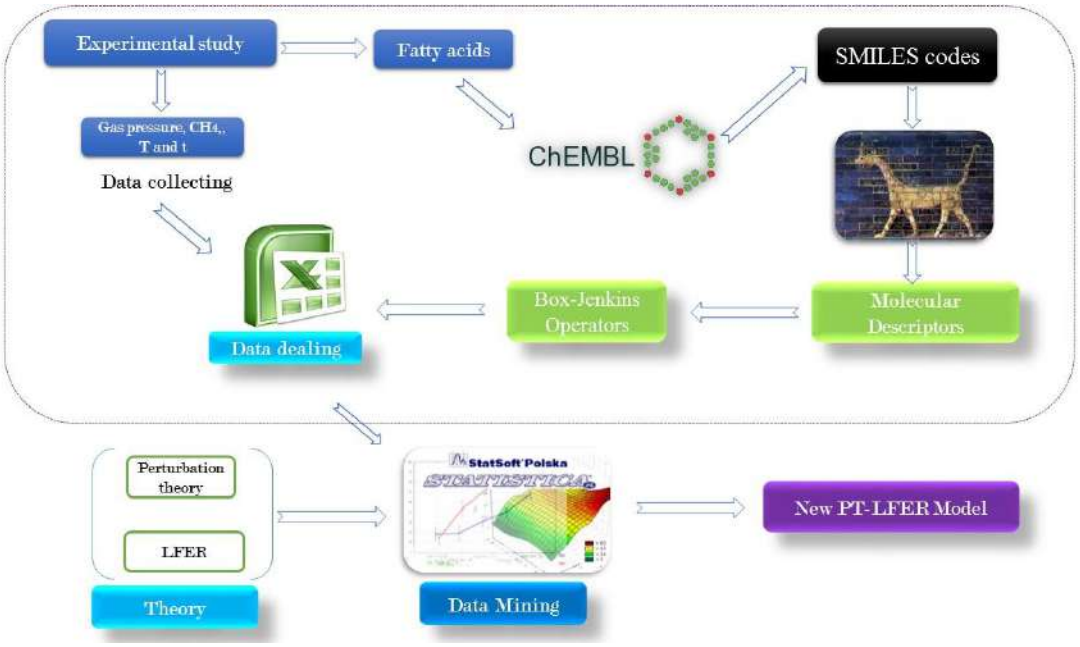


Fig. 2. Workflow diagram that states the integration of both (experimental and theoretical) sections.

2.2.2. PT-LFER modelo.

En nuestra opinión, podemos combinar la idea de Perturbación Teoría (PT) y relaciones lineales Libre-energía (LFER) (Roy y Leonard, 2005; Anslyn y Dougherty, 2006) (González-Díaz et al, 2013). Para manejar esto asunto. Recientemente, González-Díaz et al. (González-Díaz et al., 2013) formuló un modelo PT-propósito general para múltiples-frontera problemas Quimioinformática. El modelo calcula una función f general (μnr) para cuantificar la ocurrencia (μnr = 1) o no (μnr = 0) de un proceso que implica un conjunto de moléculas de mi en un sistema complejo. Se considera que todos los posibles estados forman una red de estados. Los nodos de la red son los estados iniciales o de referencia (r) vinculados a sus respectivos estados finales o nuevos (n) alcanzado por el sistema después de una perturbación de las condiciones iniciales. En el modelo PT-LFER, un conjunto de múltiples condiciones de

contorno experimentales iniciales refcj ≡ (c0, c1, c2, c3 ... cn) y un conjunto de condiciones de contorno diferente o nuevo newcj ≡ (c0, c1, c2, c3 ... cn) son considerados al principio o al final del proceso después de que uno o múltiples perturbaciones. El modelo PT-LFER propuesto en este documento es una ecuación lineal con el siguiente formulario:

$$'f\left(\mu_{nr}\right)_{new} = a_0 + a_1 \cdot f_1\left(\varepsilon_{ij}\right)_{new} + a_2 \cdot \left\langle f_2\left(\varepsilon_{ij}\right)_{ref} \right\rangle + \sum_{q=1}^{q=qmax} a_q \cdot f_q\left(v_q\right)_{ref} \qquad (20)$$

$$+ \sum_{q=1}^{q=qmax} a_q \cdot f_q\left(\Delta v_q\right) + \sum_{q=1}^{q=qmax} a_q \cdot f_q\left(\log\left|\Delta v_q\right|\right) + \sum_{(i,k,j)=1}^{(i,k,j)=(i,k,j)max} a_{ikj} \cdot \Delta\Delta^i V_k\left(c_j\right)$$

F La función de salida '(μnr) nueva es una puntuación utilizado para calcular la probabilidad de clasificación binaria de un insumo como μnr = 1 o μnr = 0 (salidas). (Hill y Lewicki, 2006) Los vectores vi = [f (εij) nueva, <f (εij)> ref, ω-6, ω-3, CH4, IV1, ... iVkmax, ΔV1 (c1), ... ΔVk (cj), ... ΔVkmax (cjmax), ΔΔV1 (c1), ... ΔΔVk (cj), ... ΔΔVkmax (cjmax)] son las entradas de este modelo. Cada vi vector representa un caso estadístico (caso ITH), de un total de n = 545.695 casos (perturbaciones). Estos casos estadísticos codificadas por vi vectores son perturbaciones de una entrada o estado de referencia (cambios en los parámetros de entrada) que producen una salida o nuevo estado. Los vectores de entrada vi incluyen el valor de f (εij) ref para el estado de referencia (valor conocido). Los vectores vi también toman las cantidades de ω-6 y ω-3 en el nuevo estado (después de la perturbación) en cuenta. Los valores de los descriptores moleculares (IVK) y operadores PT-LFER ΔΔVk (cj) también se incluyeron en los vectores vi (Ver detalles en Complementario SM01.pdf archivo de materiales, disponibles en la revista web o a petición del autor).

2.3. Calculation of molecular descriptors.

F La función de salida '(μnr) nueva es una puntuación utilizado para calcular la probabilidad de clasificación binaria de un insumo como μnr = 1 o μnr = 0 (salidas). (Hill y Lewicki, 2006) Los vectores vi = [f (εij) nueva, <f (εij)> ref, ω-6, ω-3, CH4, IV1,

... iVkmax, ΔV1 (c1), ... ΔVk (cj), ... ΔVkmax (cjmax), ΔΔV1 (c1), ... ΔΔVk (cj), ... ΔΔVkmax (cjmax)] son las entradas de este modelo. Cada vi vector representa un caso estadístico (caso ITH), de un total de n = 545.695 casos (perturbaciones). Estos casos estadísticos codificadas por vi vectores son perturbaciones de una entrada o estado de referencia (cambios en los parámetros de entrada) que producen una salida o nuevo estado. Los vectores de entrada vi incluyen el valor de f (εij) ref para el estado de referencia (valor conocido). Los vectores vi también toman las cantidades de ω-6 y ω-3 en el nuevo estado (después de la perturbación) en cuenta. Los valores de los descriptores moleculares (IVK) y operadores PT-LFER ΔΔVk (cj) también se incluyeron en los vectores vi (Ver detalles en Complementario SM01.pdf archivo de materiales, disponibles en la revista web o a petición del autor)*2.4. Chemometric classification models.*

El análisis discriminante lineal (LDA) (Hill y Lewicki, 2006) se utilizó el algoritmo implementado en el software STATISTICA para encontrar el mejor modelo PT-LFER. A veces la relación entre las variables de entrada y la salida es más compleja y la linealidad no puede resolver el problema. Por lo tanto, los modelos no lineales podrían proporcionar una mejor solución. De este modo, la red neuronal artificial (RNA) (Haykin, 1998) se pusieron a prueba:. Multi-Layer no lineal Perceptron (MLP) (Hill y Lewicki, 2006) Los datos completos se dividieron en formación y validación subconjuntos con una proporción de 75 : 25 utilizado para la construcción del modelo y validación, respectivamente. Todas las variables independientes están unificados y estandarizados con el software STATISTICA, antes de la construcción del modelo.

RESULTADOS

CAPÍTULO 1

Yong Liu, Claudia Giovanna Peñuelas-Rivas, German Buendía-Rodríguez, Zhiliang Tan, Ricardo Basurto-Gutiérrez, Ming Wang and María Rivas-Guevara, 2015. In vitro the Oretic Evaluation on Ruminal Fermentation Performance by Varying Proportion Supplementation of *Carthamus Tinctorius* Meal/*Brassica napus* Seed with Sorghum Seed in Ovine Rations. *Journal of Animal and Veterinary Advances*, 14: 43-53.

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In vitro the Oretic Evaluation on Ruminal Fermentation Performance by Varying Proportion Supplementation of Carthamus Tinctorius Meal/*Brassica napus* Seed with Sorghum Seed in Ovine Rations

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Abstract: Oleaginous plant by products are widely used to improve ruminant growth performance for high energy or protein composition, however, few article reports the suitable fit amount for oleaginous by products to the ruminant diets in a large supplementation range. Therefore, this research was conducted to evaluate the effect of Safflower meal (*Carthamus tinctorius* L., SFM)/Canola seed (*Brassica napus*, CAS) with sorghum seed in theoretic proportion (0, 25, 50, 75 and 100%, respectively) in a specific concentrate-roughage ratio of (1:1) on *in vitro* ovine rumen fermentation performance such as the kinetics of gas production, ammonia nitrogen (NH_3-N), pH, methane production (CH_4) and *In Vitro* Dry Matter Disappearance (IVDMD) of maize stover. The results showed that gas production performance and methane production were significantly decreased but IVDMD and pH were obviously increased with the increasing proportions of both SFM and CAS in concentrated feed. To take a consideration offermentation performance and environment/cost factors, our results suggest that most suitable supplementation dosageof canola seed and safflower meal in concentrated feed are from 25-50 and 25-75%, respectively. However, in practical production of completed feed, the supplementation amount of safflower meal/canola seed also depend on concentrate-roughage ratios.

Key words: Safflower meal, canola seed, gas production kinetics, methane, *in vitro* dry matter disappearance

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) an annual herb of Compositae is often used to extract oil and reclaim new soils in low input and water requirements. Mexico is one of the main production region of safflower (FAO, 2011), its yield was about 2.5 mt ha^{-1} (Gilbert, 2008). Safflower seeds contain 33-60% hull and 40-67% kernel with less palatability (Baumler *et al*., 2006) and are widely employed to the diets of pet bird, dairy cows and rams (Smith, 1996; Sudhamayee *et al*., 2004). High supplementation level of safflower decreases animal performance and undecorticated/decorticated safflower meal have only 45 and 68% *in vivo* Organic Matter (OM) digestibility in ruminants (Goss and Otagaki, 1954; Dixon *et al*., 2003). Protein degradability of safflower meal with highly degradable protein (Walli, 2005) varies between 60% (Chandrasekharaiah *et al*., 2002) and 70% (Dixon *et al*., 2003) to mix with rapeseed, soybean or linseed meal in different ratios. In fact, high content of hull in safflower mealhampers the utilization efficiency of nutrients in ruminant (Chandrasekharaiah *et al*., 2002). However, safflower meal with low price and high protein has special superiority in the practical ruminant production.

Brassica napus (Canola) has been widely utilized as an energy and protein source in ruminant with high biological value (CP: 20-43%; oil: >40%) (Ebrahimi *et al*., 2009) as a protein resource widely used to replace other

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protein resource, e.g., soybean, cottonseed or corn gluten meals (Maxin *et al*., 2013). However, canola proteins are extensively and easily degraded by rumen microbes (Khorasani *et al*., 1993) and also contains many anti-nutritional substances which hamper the widespread utilization in ruminant. For instance, glucosinolates reduce feed intake, induce iodine deficiencies and depress fertility (Tripathi and Mishra, 2007), phytic acid and the hexaphosphate of myoinositol chelating minerals and amino acids to form insoluble precipitates to reduce digestibility of proteins (Al-Kaisey *et al*., 2003).

In vitro fermentation technique has widely been considered to be suitable for evaluating the contribution of rumen microbial fermentation in the overall digestion of ruminant (Gosselink *et al*., 2004; Getachew *et al*., 2005). The kinetics of gas production and dry matter disappearance (Menke and Steingass, 1988) has been successfully applied to ruminant digestive researches to evaluate nutritional values of feed and corresponding rumen microbial fermentation performance, providing some virtualinformation over *in vivo* or *in situ* Methods (Gosselink *et al*., 2004; Wulf and Sudekum, 2005).

The changeable extents of *in vitro* gas production kinetics, CH_4 production and fibre digestibility are related to the nature or form of oleaginous seed (extruded, pressed meal or whole unprocessed) and their interactions with the composition of the basal diet (Lerch *et al*., 2012). However, little data are available to elaborate the appropriate amounts of unprocessed canola seed and extracted safflower meal in ovine rations. Therefore, this research was designed to study the integral effect of a variety of proportion of SFM/CAS with sorghum seed on *in vitro* fermentation performance by gas production kinetics, NH_3-N concentration, pH, CH_4 production and *In Vitro* Dry Matter Digestibility (IVDMD) of maize stover.

MATERIALS AND METHODS

Experiment design: This experiment was conducted to study the effect of 0.5 g mixtures (canola seed, CAS or safflower meal, SFM with sorghum seed in a series of ratios (λ, %), e.g., 0, 25, 50, 75 and 100, respectively) and 0.5 g maize stover (which were put into a little bag) on kinetics of gas production, CH_4 production, NH_3-N concentration, pH and IVDMD of maize stover. The amount of CAS or SFM in total substrate as follow, we set the ratios (λ, %) of SFM/CAS in the concentrated feed composited with SFM/CAS with sorghum seed in a concentrate-roughage ratio (1:1):

$$f_{(SFM/CAS)}(\%,\ substrate) = \frac{\lambda_{(SFM/CAS)} \times 0.5\ g^{(sorghum)}}{0.5\ g^{(MStover)} + \left[\lambda_{(SFM/CAS)} + (1-\lambda)_{(roughage)}\right] \times 0.5\ g^{(sorghum)}} \times 100 \quad (1)$$

$(\lambda = 0\%,\ 25\%,\ 50\%,\ 75\%,\ 100\%)$

where, $f_{(SFM/CAS)}$ (%, substrate) represents the proportion of SFM/CAS in the whole fermentation substrate (represented the supplementation dosage in concentrated feed of ovine diets). The 0.5 g mixture was composition of SFM/CAS with sorghum seed in various ratios. MStover represents maize stover.

Animal donor and substrate material: Four adult male Pelibuey sheep with permanent rumen-fistula (body weight, 45±5.0 kg) were used as inoculum donor according to Mexican official standard (NOM-220-SSA1, 2002). Each sheep was housed individually and free access to the water and fed 1.0 kg feed and 0.5 kg smashed sorghum seed. The ingredients of feed was provided with 64.2% sorghum, 19.3% alfalfa hay, 4.6% canola meal, 2.9% canola oil, 5.0% sorghum seed, 2.0% molasses, 1.0% purified tallow and 1.0% urea with a declared composition of 87% dry matter, 13.9% crude protein, 12.1 MJ kg^{-1} total energy, 5.3% crude fat, 0.54% calcium, 9.5% acid detergent fiber and 21.4% neutral detergent fiber. The substrates (safflower meal, canola seed and sorghum seed and maize stover, 500 g in each) were obtained from the plant of Natural Research Institute of Forestry, Agricultural and Livestock in Queretaro, Mexico. Substrate constituent in each treatment was shown in Table 1. The dry matter, CP, ashes and energy of substrates in SFM and CAS treatments increased and ether extract in CAS increased but which in SFM had minor changed with high proportion of SFM/CAS, respectively. The data of digestible starch was from 64.34-69.7% (Souilah *et al*., 2014) in this study we selected 64% as the digestible starch content in sorghum and to calculate the digestible starch content according the different sorghum proportion in different substrate treatments.

***In vitro* rumen fermentation:** *In vitro* fermentation was carried out according to the description by Tang *et al*. (2008). At 0700 a.m. before feeding, total 600 mL of rumen liquid from four adult Pelibuey male sheep were obtained, mixed and strained through four layers of cheese-cloth into an Erlenmeyer flask filled with CO_2. The *in vitro* fermentation solution was prepared by mixing the particle-free sheep ruminal fluid with artificial saliva buffer solution (Menke and Steingass, 1988) in a proportion of 1:4 (v/v) at 39°C under continuous flushing with CO_2 for 30 min. This experiment were divided into two parts for *in vitro* fermentation, one part was conducted to determine the gas production kinetics while other

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Table 1: Chemical constituents of substrates for *in vitro* fermentation

Substrates	Ratio	Dry matter (%)	Crude protein (%)	Ether extract (%)	Ashes (%)	Total energy (MJ kg^{-1})	Digestible starch (%)[1]
Sorghum	/	88.97±0.06	8.96±0.08	1.42±0.01	1.19±0.06	16.20±0.02	64.0
Safflower meal	/	94.18±0.03	21.72±0.13	0.33±0.02	3.85±0.13	17.84±0.04	-
Canola seed	/	91.43±0.03	14.84±0.12	31.86±0.57	3.96±0.02	26.49±0.07	-
Maize stover	/	95.88±0.12	3.42±0.02	/	6.48±0.01	16.30±0.01	-
Nutritional ingredients of concentrated feed composited of safflower meal with sorghum seed							
SFM1	100:0	89.85±0.81	8.99±0.05	1.42±0.00	1.17±0.04	16.19±0.01	64.0
SFM2	75:25	90.29±0.03	12.17±0.06	1.15±0.01	1.87±0.06	16.61±0.02	48.0
SFM3	50:50	91.59±0.01	15.35±0.08	0.87±0.01	2.53±0.08	17.02±0.05	32.0
SFM4	25:75	92.87±0.03	18.52±0.11	0.61±0.01	3.19±0.10	17.43±0.04	16.0
SFM5	0:100	94.18±0.03	21.72±0.13	0.34±0.01	3.85±0.12	17.84±0.04	0.0
Nutritional ingredients of concentrated feed composited of canola seed with sorghum seed							
CAS1	100:0	88.95±0.04	8.93±0.05	1.42±0.00	1.21±0.04	16.20±0.01	64.0
CAS2	75:25	89.60±0.04	10.45±0.07	8.98±0.09	1.90±0.02	18.79±0.01	48.0
CAS3	50:50	90.21±0.03	11.91±0.08	16.74±0.19	2.59±0.01	20.50±0.88	32.0
CAS4	25:75	90.82±0.03	13.37±0.10	24.40±0.29	3.27±0.02	23.07±0.90	16.0
CAS5	0:100	91.43±0.03	14.84±0.12	31.67±0.38	3.96±0.02	25.64±0.92	0.0

[1]The data of digestible starch was from 64.34-69.7% (Souilah *et al*., 2014) in this study we selected 64% as the digestible starch content in sorghum and to calculate the digestible starch content according the different sorghum proportion in different substrate treatments; SFM-n/CAS-n (among, n = 1, 2, 3, 4, 5) represent the proportion of Safflower Meal (SFM)/Canola Seed (CAS) in concentrated feed (composited of safflower meal/canola seed with sorghum seed in this study) as 0, 25, 50, 75 and 100%, respectively

part was incubated in a separated bottle to obtain samples for CH_4, NH_3-N and IVDMD, however, all the determined indexes obtained/determined at the similar condition.

Part 1: Substrates (1.0 g in total each) were incubated with 100 mL rumen buffer solution at 39°C in 4 replicates of each treatment for 72 h to determine the gas production kinetics with ANKOM gas determination equipment (ANKOM Technology Corp., Fairport, NY, USA).

Part 2: Other substrates (0.5 g in total each) were carried out to determine the pH, NH_3-N concentration and IVDMD of maize stover for 24 and 48 h. Fermentation solution (50 mL) were added into the pre-warmed bottles with 0.25 g mixture and 0.25 g maize stover which were previously weighted into a nylon bag (pore size, 52 μm; specific surface area, 44 cm^2/g) was little higher than previous report (Valentin *et al*., 1999) with 33 cm^2/g. Bottles were sealed with rubber stoppers and screw-on caps and incubated at 39°C in a constant temperature water bath oscillator for 24 and 48 h in 3 replicates of each treatment. The gas volume measured at 25°C and gas sample obtained in each at 24 and 48 h incubation. The fermentation terminated by swirling the bottles in ice, uncapped and then to determine pH value immediately, took out the nylon bag washed with deionized water for 4 times until the water was clear. Meanwhile, 1 mL fermentation liquid free of substrate and microbes mixed with 0.25 mL meta-phosphoric acid (25%; w/v) were stored in -20°C for the determination of NH_3-N concentration.

Chemical analysis: Feed samples were analyzed using the standard methods of AOAC (Cunniff, 1995) for DM (No. 967.03), crude protein (No. 984.13) and ether extract (No. 954.02), ash content (No. 942.05), total energy and are expressed inclusive of residual ash. NH_3-N concentration of incubation liquid collected at 24 and 48 h were measured by phenol-hypochlorite and ninhydrin colorimetric procedures described by Broderick and Kang (1980). The CH_4 concentration was determined by gas chromatography with column of HP-PLOT/Q (Length 30 m; I.D 0.530 mm; FILM: 40 μm; Cat, No. 19095P-QO4).

Calculations and data analysis: *In vitro* gas curves were fitted with Logistic-Exponential (LE_b) Model descripted by Wang *et al*. (2011, 2013) using NLREG Version 5.0 (Sherrod, 1995):

$$V = V_f \cdot \frac{1-\exp(-\kappa \cdot t)}{1+\exp(b-\kappa \cdot t)} \times 100 \quad (2)$$

Where:

- V = The final asymptotic gas production (mL/g) at time point t
- V_f = The final asymptotic gas volume with dimension of 'mL'
- κ = The fractional rate of gas production with dimension of '/h'
- b = Shape parameter without dimension

Initial Fractional Rate of Degradation (FRD_i), Rate of Gas production (RG_i), half time ($t_{0.5}$) and fractional rate of gas production at half-life ($\mu_{0.5}$) proposed by Wang *et al*. (2013) as follows:

$$FRD_i = \frac{\kappa}{1+\exp(b)} \quad (3)$$

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$$RG_t = V_F \cdot \frac{\kappa \cdot (1+\exp(b)) \cdot \exp(-\kappa \cdot t)}{(1+\exp(b-\kappa \cdot t))^2} \quad (4)$$

$$t_{0.5} = \frac{\ln(2+\exp(b))}{\kappa} \quad (5)$$

$$\mu_{0.5} = \frac{\kappa \cdot (d+0.5)}{1+d} \quad (6)$$

Statistical analyses and Pearson correlations were performed using the GLM and CORR procedures of SAS (2001) 9.0, respectively and means within standard errors were compared with least squares means. Least squares means were reported throughout the text and statistical significances were declared if $p<0.05$.

RESULTS

Kinetics of *in vitro* gas production: The shape and final asymptotic gas production of different proportion of SFM or CAS treatments were obvious different in macroscopic as shown in Fig 1, the changeable of gas cumulative production was minor from 48-72 h. V_F ($p<0.05$, Quadratic), k, FRD_0, RG_t and $\mu_{0.5}$ ($p<0.001$, Linear) of SFM treatments were decreased with increasing proportions of safflower meal in whole *in vitro* fermentation system yet $t_{0.5}$ ($p<0.001$, Linear) was increased with that of SFM (Table 2).

For canola seed, V_F and RG_t ($p<0.001$, Quadratic), $t_{0.5}$ ($p<0.05$, Quadratic) were decreased with increasing proportions of CAS (Table 3). Meanwhile, k ($p<0.001$, Quadratic), FRD_0 ($p<0.01$, Cubic) with that value of CAS 5 significant lower than others, $\mu_{0.5}$ ($p<0.001$, Quadratic) were increased with increasing proportions of CAS. However, the shape of gas production curve (b) ($p<0.05$, Cubic) was increased with increasing proportions of CAS.

NH_3-N, pH, CH_4 and IVDMD of maize stover: In this study, the methane production of different proportions of CAS ($p<0.001$, Cubic) and SFM ($p<0.001$, Cubic) were decreased with the increasing proportions of CAS and SFM at 24 h of *in vitro* fermentation time and that of CAS ($p<0.05$, Quadratic) and SFM ($p<0.001$, Linear) were also decreased with increasing ratios of canola seed or safflower meal at 48 h of *in vitro* fermentation (Fig. 2). In addition, methane production at 48 h was higher than that at 24 h while there was statistically insignificant difference between SFM1 and SFM2 at 48 h in addition of CAS1 and CAS2 at 48 h.

IVDMD of maize stover pushed into a nylon bag of CAS treatment ($p<0.01$, Linear) and SFM treatment ($p<0.001$, Linear) were increased with the increasing

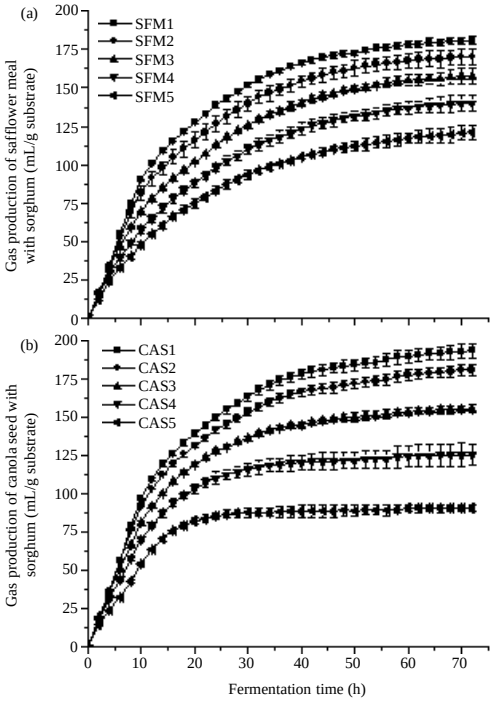

Fig. 1: a, b) Gas production dynamic curve of SFM-n or CAS-n in concentrated feed (composited of SFM/CAS with sorghum seed) with maize stover (ratio, 1:1) as substrate; SFM-n/CAS-n (among, n = 1, 2, 3, 4, 5) representthe proportion of Safflower Meal (SFM)/Canola Seed (CAS) in concentrated feed (composited of safflower meal/canola seed with sorghum seed in this study) as 0, 25, 50, 75 and 100%, respectively

proportion of canola seed and safflower meal at 24 h (Fig. 2, Table 4), however, IVDMD of maize stover in CAS ($p<0.05$, Quadratic) and SFM treatment ($p<0.05$, Cubic) were increased with both proportion of CAS and SFM with Sorghum at 48 h (Table 5). In addition, this study showed IVDMD in CAS 5 was higher than others.

NH_3-N concentrates were range from 33-66 mg dL^{-1}, NH_3-N concentration in CAS treatments at 24 h ($p<0.01$, Linear) (Table 4) and 48 h ($p<0.01$, Cubic) (Table 5) were changeable with increasing proportions of canola seed. For safflower meal treatment, NH_3-N concentration at 24 h ($p<0.001$, Cubic) and 48 h ($p<0.05$, Cubic) were varied with increasing proportions of safflower meal in substrate. Meanwhile that of high contents of SFM or CAS were higher than that of low contents in rumen *in vitro* fermentation liquid.

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Table 2: Gas parameters of safflower meal with sorghum and maize stover on *in vitro* fermentation system (1 g substrate)

Treatments	$V_{f(mL/g)}$	k (%/h)	b	FRD_0 (%/h)	RG	$t_{0.5}$	$\mu_{0.5}$ (%/h)
SFM1 (0%)	182.2±2.32^a	6.1±0.06^a	-22.0±1.23	6.1±0.06^a	10.5±0.08^a	11.3±0.15^a	6.3±0.12^a
SFM2 (25%)	172.1±4.20^b	5.8±0.23^b	-24.3±1.70	5.8±0.23^b	9.4±0.49^b	12.0±0.44^b	5.9±0.17^b
SFM3 (50%)	161.2±4.18^c	5.2±0.21^c	-23.3±3.68	5.2±0.21^c	7.9±0.12^c	13.4±0.55^a	5.3±0.26^c
SFM4 (75%)	144.2±4.05^d	4.9±0.12^{cd}	-26.3±4.34	4.9±0.12^{cd}	6.7±0.26^d	14.3±0.29^a	5.0±0.12^c
SFM5 (100%)	124.5±4.56^e	4.7±0.26^{cd}	-23.6±3.42	4.7±0.26^{cd}	5.5±0.20^e	14.9±0.79^a	4.8±0.26^{cd}
SEM	2.277	0.111	1.798	0.111	0.157	0.287	0.115
p-values							
Linear	***	***	NS	***	***	***	***
Quadratic	*	NS	NS	NS	NS	NS	NS
Cubic	NS	NS	NS	NS	NS	NS	NS

SFM-n (among, n = 1-5) represent the proportion of safflower meal in concentrated feed (composited of safflower meal with sorghum seed in this study) as 0, 25, 50, 75 and 100%, respectively; V_f: Final asymptotic gas volume with dimension of 'mL'; k: fractional rate of gas production with dimension of '%/h'; 'Shape parameter without dimension; FRD_0: Initial Fractional Rate of Degradation; RG: Rate of Gas production, $t_{0.5}$: The half time at which half of the final gas production; $\mu_{0.5}$: Fractional rate of gas production at half-life. The significant difference was labeled within different proportions of CAS or SFM with sorghum and treatment effects were declared significance if $p<0.05$; *$p<0.05$; **$p<0.01$; ***$p<0.001$; NS: $p>0.10$; Ω: $p<0.10$; values are expressed as mean±SE

Table 3: Gas parameters of various proportions of canola seed in concentrated feed on *in vitro* fermentation system (1 g substrate)

Treatments	$V_{f(mL/g)}$	k (%/h)	b	FRD_0 (%/h)	RG	$t_{0.5}$	$\mu_{0.5}$ (%/h)
CAS1 (0%)	193.5±4.53^a	6.5±0.20^a	-15.8±10.92^{ab}	6.4±0.12^a	11.6±0.97^a	10.8±0.26^a	6.5±0.15^a
CAS2 (25%)	178.0±3.78^b	6.6±0.21^a	-20.5±2.12^b	6.6±0.21^{ab}	11.2±0.12^a	10.4±0.30^a	6.6±0.21^a
CAS3 (50%)	155.1±2.74^c	7.6±0.76^a	-8.5±9.96^{ab}	7.0±0.26^{ab}	10.1±0.36^b	9.7±0.10^b	7.3±0.26^a
CAS4 (75%)	124.3±6.72^d	10.7±1.61^b	-0.8±0.54^a	7.1±0.23^b	8.6±0.54^c	8.6±0.30^c	8.9±0.65^b
CAS5 (100%)	90.0±3.13^e	17.4±1.99^a	0.7±0.25^a	5.9±0.31^b	5.6±0.20^d	7.9±0.29^d	11.6±0.90^a
SEM	2.546	0.694	3.858	0.135	0.18	0.152	0.301
p-values							
Linear	***	***	**	NS	***	***	***
Quadratic	***	***	NS	***	***	Ω	***
Cubic	NS	NS	Ω	**	NS	NS	NS

CAS-n (among, n = 1, 2, 3, 4, 5) represent the proportion of Canola Seed (CAS) in concentrated feed (composited of seed with sorghum seed in this study) as 0, 25, 50, 75 and 100%, respectively; V_f: Final asymptotic gas volume with dimension of 'mL'; k: fractional rate of gas production with dimension of '%/h'; 'Shape parameter without dimension; FRD_0: Initial Fractional Rate of Degradation; RG: Rate of Gas production; $t_{0.5}$: The half time at which half of the final gas production; $\mu_{0.5}$: Fractional rate of gas production at half-life. The significant difference was labeled within different proportions of CAS or SFM with sorghum and treatment effects were declared significance if $p<0.05$; *$p<0.05$; **$p<0.01$; ***$p<0.001$; NS: $p>0.10$; Ω: $p<0.10$; values are expressed as mean±SE

Table 4: The fermentation characteristics of different ratios of safflower meal, canola seed with sorghum at 24 h

	Different ratios of SFM and CAS[1] (%)								
Treatments	0	25	50	75	100	SEM	Linear	Quadratic	Cubic
CH_4 production (μmol/g substrate)									
Canola seed	45.80±0.57^a	34.30±1.640^b	33.50±1.29^{bc}	31.6±0.490^c	29.3±0.300^d	0.579	***	***	***
Safflower meal	45.80±0.57^a	43.60±1.460^a	38.10±1.19^b	36.6±0.430^b	19.8±0.430^c	0.532	***	***	***
DMD of maize stover (g kg^{-1})									
Canola seed	230.00±1.78	227.00±10.19	234.70±9.47	245.3±11.22	251.4±9.820	5.287	**	NS	NS
Safflower meal	230.00±1.78^b	248.20±8.280^b	269.60±7.39^a	269.7±16.06^a	297.8±25.89^a	8.385	***	NS	NS
NH_3-N of fermentation liquid (mg/100 mL)									
Canola seed	33.10±1.36^d	38.90±0.290^c	44.00±0.91^b	48.2±3.410^b	57.3±0.240^a	0.987	***	NS	NS
Safflower meal	33.10±1.36^b	32.70±0.620^b	41.50±1.53^a	43.3±0.280^a	40.6±0.870^a	0.598	***	***	***
pH value of fermentation liquid									
Canola seed	5.81±0.05^a	5.97±0.020^b	6.11±0.01^c	6.23±0.01^b	6.30±0.02^a	0.015	***	**	NS
Safflower meal	5.81±0.05^a	6.01±0.050^b	6.14±0.03^c	6.30±0.01^b	6.38±0.03^a	0.021	***	Ω	NS

Table 5: The fermentation characteristics of different ratios of safflower meal, canola seed with sorghum at 48 h

	Different ratios of SFM and CAS[1] (%)								
Treatments	0	25	50	75	100	SEM	Linear	Quadratic	Cubic
CH_4 production (μmol/g substrate)									
Canola seed	48.0±0.28^a	47.5±0.89^a	40.5±1.93^b	39.0±2.00^{bc}	31.2±1.040^d	0.800	***	Ω	NS
Safflower meal	48.0±0.28^a	46.1±2.57^a	43.4±0.98^b	40.0±2.46^c	35.7±1.700^d	1.055	***	NS	NS
DMD of maize stover (g kg^{-1})									
Canola seed	301.4±15.80^b	304.3±1.79^b	314.2±4.88^b	324.3±11.79^b	349.1±11.19^a	5.997	***	Ω	NS
Safflower meal	301.4±15.80^d	323.5±18.51^c	366.1±18.10^b	414.0±3.15^a	419.7±21.25^a	9.597	***	NS	Ω
NH_3-N of fermentation liquid (mg/100 mL)									
Canola seed	50.1±2.35^b	40.7±1.68^c	50.0±6.66^b	62.4±0.72^a	66.3±0.300^a	1.892	***	**	**
Safflower meal	50.1±2.35^b	51.9±5.24^b	42.3±4.82^c	47.5±5.77^c	57.8±4.190^a	2.672	NS	**	Ω
H value of fermentation liquid									
Canola seed	5.74±0.05^a	5.91±0.01^b	6.05±0.03^c	6.15±0.01^b	6.24±0.02^a	0.015	***	**	NS
Safflower meal	5.74±0.05^a	5.93±0.03^b	6.07±0.01^c	6.16±0.00^b	6.29±0.03^a	0.016	***	*	NS

[1]The values were expressed as an average with standard error within 3 replicates; the significant difference was labeled within different proportions of CAS or SFM with sorghum and the treatment effects were declared significance if $p<0.05$; *$p<0.05$; **$p<0.01$; ***$p<0.001$; NS: $p>0.10$; Ω: $p<0.10$

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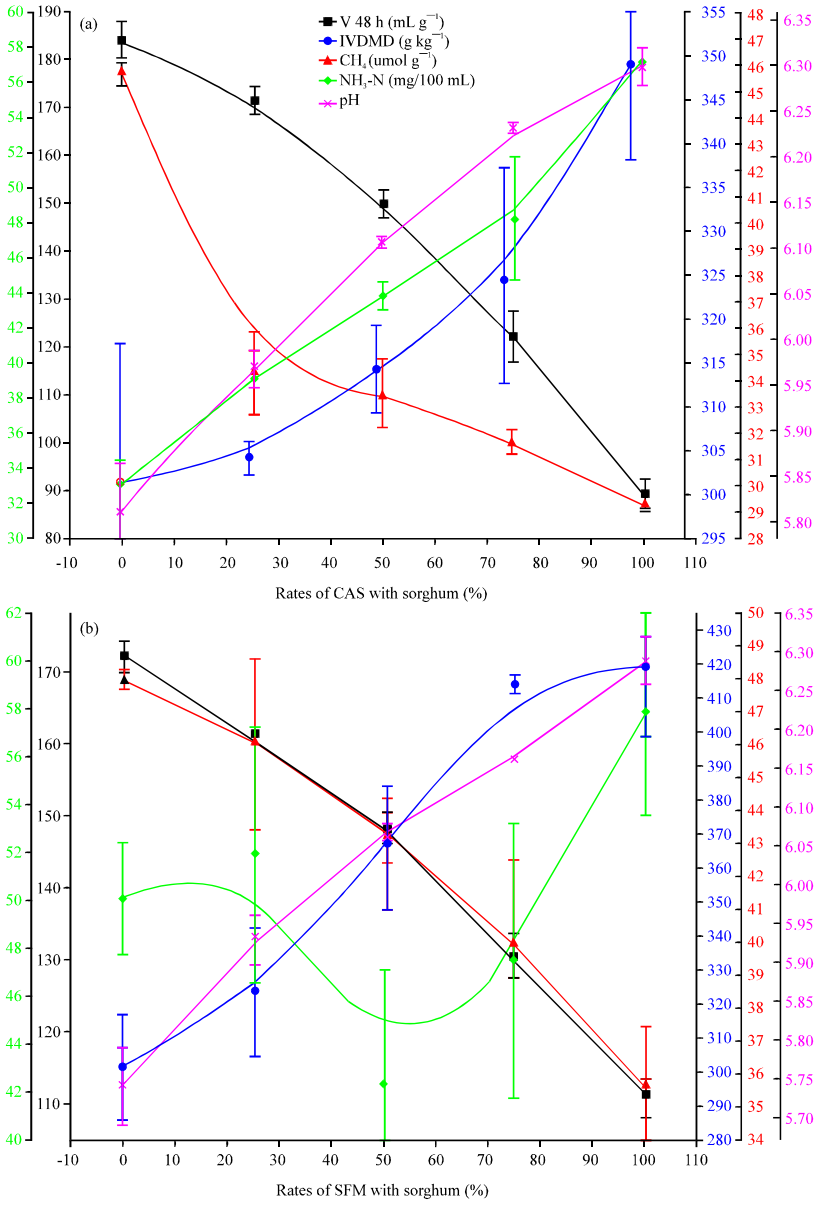

Fig. 2: a, b) Values tendency of fermentation parametersunder different ratios of SFM/CAS in concentrated feed fixed ratio with maize stover (1:1) *in vitro* fermentation at 48

The pH of all treatment were higher than 5.5 (Table 4 and 5), pH value of CAS treatment ($p<0.01$, Quadratic) and SFM treatment ($p<0.05$, Quadratic) at 24 h fermentation were increased with the increasing content of CAS and SFM. The changeable of pH value of CAS ($p<0.01$, Quadratic) and SFM ($p<0.01$, Quadratic) at 48 h were similar with that of 24 h however that of 48 h was lower than that of 24 h for each single treatment.

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Table 6: The Pearson correlation coefficients of different chemical ingredients with fermentation performance for SFM and CAS at 24 and 48 h

Groups	CP	EE	Starch[1]	V_f[2]	CH_4	NH_3-N	IVDMD	pH
CP		0.879	-0.835	-0.818	-0.638	0.256	0.537	0.856
EE	0.879		-0.473	-0.461	-0.329	-0.071	0.510	0.543
Starch	-0.835	-0.473		0.977	0.801	-0.550	-0.404	-0.958
V_f	-0.818	-0.461	0.977		0.802	-0.558	-0.396	-0.919
CH_4	-0.638	-0.329	0.801	0.802		-0.225	0.048	-0.831
NH_3-N	0.256	-0.071	-0.550	-0.558	-0.225		0.515	0.356
IVDMD	0.537	0.510	-0.404	-0.396	0.048	0.515		0.240
pH	0.856	0.543	-0.958	-0.919	-0.831	0.356	0.240	

CP: Crude Protein; EE: Ether Extract; V_f: Final asymptotic gas volume; NH_3-N: Ammonia nitrogen; IVDMD: *In Vitro* Dry Matter Disappearance; [1]The data of digestible starch was from 64.34-69.7% (Souilah *et al*., 2014). [2]The number of V_f was 30; n: Analysis number within all of the treatments and repeats

Pearson correlation coefficients: V_f had significant correlation with CP (r = -0.818, negative) and starch (r = 0.977, positive), methane production had high correlation with CP (r = -0.638), starch (r = 0.801) and V_f (r = 0.802) (Table 6); IVDMD of maize stover had positive relation with CP (r = 0.539), energy (r = 0.535) and negative relation with starch contents (r = -0.404). Value of pH had high positive correlation with DM, CP (r = 0.856), energy and EE but had negative correlation with starch (r = -0.958) and V_f (r = -0.919).

DISCUSSION

The statistical differences in gas kinetic among SFM and CAS treatments could be due to the proportion and nature of their composition (Rubanza *et al*., 2003). High content of soluble carbohydrates could be due to one factor resulting to higher gas production in high sorghum proportion treatments (Zerbini *et al*., 2002; Amer *et al*., 2012). Meanwhile, some hull or anti-nutritional compositions in safflower meal or canola seed could also give rise to lower *in vitro* gas production parameters (V_f, RG, and $t_{0.5}$ in this study) such as matairesinol-β-glucoside, 2-hydroxyarctiin-β-glucoside (Jin *et al*., 2010) and cyanogenic glucoside (Satish and Shrivastava, 2011) in safflower meal and sinapine, tannins, phytic acid (Brand *et al*., 2008) and glucosinolates (Bell, 1993) in canola seed.

V_f of SFM treatments was decreased with its ratio increasing in substrates that could be due to its the combination effects of non-digestible structural carbohydrate-hull improvement (Baumler *et al*., 2006), absolute high soluble carbohydrate reducing (Lechartier and Peyraud, 2011) as well as the anti-nutritional factors (such as cyanogenic glucoside (Satish and Shrivastava, 2011)). Some parameters of gas production (k, $FRDF_0$, RGt and $\mu_{0.5}$) were decreased while half time ($t_{0.5}$) was increased with high proportion content of safflower meal in the mixed substrate had further improved the most important factor influencing kinetics of gas production was the high soluble carbohydrate contents (Souilah *et al*., 2014). However, the changeable of FRD_0, k, $t_{0.5}$, Rg, and $\mu_{0.5}$ implied SFM have some anti-nutritional or undigested ingredientsdecreased the efficiency of digestibility because of the hydration, removal of digestion inhibitors and/or attachment of microbes with substrate (Merters, 1993) increased adsorption time, obviously those process should be more difficulty at occurring in structural carbohydrate (Tan *et al*., 2002). $t_{0.5}$ increased but $\mu_{0.5}$ decreased could be due to SFM's hull impede the attachment, adhesion, colonization and degradation processes of ruminal microorganisms (Varga and Kolver, 1997). Furthermore, the value of b (negative, -22.0 to -26.3 for all of SFM) reflected all of the synthetic gas production were the typical parabolic curve without lag time, resulted to no difference between fractional rate of gas production (k) and initial Fractional Rate of Degradation (FRD_0).

For CAS, V_f was the same reason as mentioned before. The gas parameters of k and FRD_0 in CAS were increased while V_f decreased, it seemed meaningless but high oil content in canola seed made it more reasonable as to some fatty acids have the potential to change the microbial ecosystem and the activity of some bacterial adhesion ability (Benchaar *et al*., 2008) or due to the easily degraded carbohydrate, some unknown nutritional factors from CAS to increase the initial fermentation performance. Those factors could activate some rumen bacterial bioactivity in the beginning period of fermentation but V_f have strong relationship with non-structural digestible carbohydrate (Tan *et al*., 2002). The curve shape b was increased with the increasing proportion of canola seed which means the asymptotic gas production was like as s curve with fermentation time accompany with a lag time. We speculate the ether extract, protein and energy of feed are incorrect way to judge the nutritional functions of feed for ruminant under the especial function of rumen microorganisms.

Rumen microorganism digests macromolecular of protein, carbohydrates and long chain polyunsaturated fatty acids to generate gas production, Volatile Fatty Acids (VFAs), secondary metabolites, microbial protein (Baba *et al*., 2002; Camacho *et al*., 2010). Beneficial diets added to the rumen results in an inhibition of deamination

and methanogenesis, resulting in lower NH_3-N, CH_4 and acetate and in higher propionate and butyrate concentrations (Calsamiglia *et al*., 2007). Microbial protein and feed protein are insufficient to supply adequate amounts of amino acids for optimal growth performance of ruminant (Kung Jr. and Rode, 1996), so NH_3-N as non-protein nitrogen or degradation metabolites of protein also plays an important role in keeping nutritive equilibrium of ruminant. In this study, NH_3-N were range from 33.1-66.3 mg dL^{-1} and were close to previously reports (Khorasani *et al*., 1989; Cherdthong and Wanapat, 2013), changeable of NH_3-N could be due to the different ratios of feed protein and the balance of microbial synthesis and degradation; NH_3-N at 48 h were a bit higher than 24 h implied the rate of microbial protein digested were faster than that of synthesis accumulation in 48 h incubation.

Rumen pH always reverses with concentrate ratios (Pina *et al*., 2009). The pH value which could be deemed as an important determinant of *in vitro* fermentation was range from 5.5-7.5 (Yuan *et al*., 2010), highly correlated with volatile fatty acid amounts, the population and activities of microbes, e.g., most acids like lactic acid which inhibits microbial activity at high concentration when pH is low, presumably due to greater penetration of cell membranes by lactic acid in non-ionized than in ionized form, protozoal population which are responsible for about 25% of rumen microbial cellulolytic and help maintain a higher pH by engulfing starch granules (Mould *et al*., 2005). In this study showed that pH was higher than 5.5 as to 50% fiber content (maize stover) added to fermentation system, similar with anterior research work (Poulsen *et al*., 2012). When ruminal pH is below to 5.5, rumen and gastrointestinal function are usually abnormal due to acidosis and many ruminal microbes cease growing despite an ability to survive even higher concentrations of H^+. In addition, the changeable of pH value was similar with reported by Russell (1998) when a cow, fed high concentrates, lower ruminal pH as to the starch-rich diets compared to forage based diets.

IVDMD of maize stover increased with increasing proportion of CAS and SFM could be due to the high starch content in sorghum reduced pH value of fermentation liquid and increase the production of short volatile fatty acid (Lechartier and Peyraud, 2011), further to effect the fibrolytic activity. This was consistency with high concentrate portion decreases the apparent crude fibre digestibility (Flachowsky and Schneider, 1992). It also implied that digestible rumen protein should be the major restricted factor for fibre digestibility when addition of sufficient soluble carbohydrate. During adaptation to a high-concentrate diet, pH exerts selective pressure against microbes intolerant of a low pH value. As pH drops, amylolytic and acid-tolerant bacteria increase while cellulolytic microbes decrease, excessive Non Structural Carbohydrate (NSC) may depress the energy available from propionic and lactic acid production reduce microbial protein synthesis (Tan *et al*., 2002) and decrease fiber digestibility as well as cause abnormalities in rumen tissue which may lead to ulcers and liver abscesses in animal (Ishler, 1996).

High starch diets as well as addition of lipid are the alternative methods to lower enteric CH_4 production (Beauchemin *et al*., 2009) which is consistency with high ether extract content of CAS significantly decrease the methane production in our study. In addition, as starch degradation in the rumen, pH drops to <5.5 due to overgrowth of starch-fermenting lactate-producing *Streptococcus bovis* and *Lactobacillus* sp. which is another factor to inhibit methanogenic activity for reducing methane production (Poulsen *et al*., 2012). However, pH value in our study was range of 5.6-6.4 corresponding with optimum for methanogenic activity in the pH range of 6.0-6.5 (Jarvis *et al*., 2000), so this could not be the major reason to reduce CH_4 for SFM and CAS, we speculated the high content of hull from SFM, oils from CAS or lower degradability of substrate could be due to reduce methane production in this study.

CONCLUSION

This research is a rare but valuable result to supplementary the system information (firstly, large range, from 0-100% usage amount in concentrated feed; secondly, different type: extracted meal and whole seed) of an oleaginous plant by-product used in practical ruminant production by mimicking the rumen digestive characteristics *in vitro*. Our results show that high proportion of safflower meal in concentrated feed have potential to lower *in vitro* gas production performance and methane production but increase the IVDMD of maize stover and pH while high proportion of canola seed obvious reduce *in vitro* gas production, methane production but increase IVDMD of maize stover. In addition, considering of fermentation performance and environment factor, the most suitable usage dosageof canola seed and safflower meal in concentrated feed are from 25-50 and 25-75%, respectively. However, in practical production of completed feed, the supplementation amount of safflower meal and canola seed are not only depend on those proportions in concentrated feed mentioned before but also on ratio of these concentrated feed with forage for ruminants. Therefore, our group suggest further research work might continue to discover

the suitable fit proportions of whole corresponding concentrated feed (containing a certain of canola seed/safflower meal) with other forage (corn stover, rice straw, alfalfa, etc.) by *in vitro* fermentation technique on rumen micro-niche balance performance.

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CAPÍTULO 2

Y. Liu, Z.L. Tan, S.X. Tang, T. Ran, G. Buendía-Rodríguez, C.G. Peñuelas-Rivas, M. Rivas-Guevara. Ruminal in vitro Gas Production Kinetics Combined with Metabolizable Energy, and Fiber Digestibility to Evaluate the Nutritional Contribution of Different Fractions of Safflower Seed on Ovine System. *Journal of Applied Animal Research* (Submitted)

Submitted letter:

11-May-2015

Dear Mr LIU:

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Ruminal *in vitro* Gas Production Kinetics Combined with Metabolizable Energy, and Fiber Digestibility to Evaluate the Nutritional Contribution of Different Fractions of Safflower Seed on Ovine System

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Safflower seeds are widely utilized for extracting editable oil, its by-products are always using as a fodder. This study was conducted to evaluate the nutritional contribution of different safflower seed fractions for ovine production by *in vitro* fermentation. First, safflower seeds were artificially separated into kernel and hull, meanwhile, two different extracting solvents (hexane and petroleum ether) were used to extract safflower whole seed to prepare safflower meal. Then the nutritional compositions of safflower seed fractions (safflower whole seed, hull, kernel, and two types of safflower meal, extracted with hexane/ petroleum ether, SMH and SMP) were determined. After that, we conducted to evaluate these safflower seed fractions on *in vitro* fermentation characteristics, including gas production kinetics, pH, and *in vitro* dry matter disappearance (*IVDMD*), NDF/ ADF digestibility, metabolizable energy (ME) and organic matter digestibility (OMD). The results showed that safflower meal (V_F, 70 ml/g) had high gas production performance compared with 48 ml/g in kernel, 46 ml/g in safflower whole seed, and 36 ml/g in hull part, respectively. Safflower meal also had high OMD (around 347 g/kg in two types of safflower meals), in comparison with 332 g/kg in kernel, 291 g/kg in whole seed and 218 g/kg in hull parts. However, safflower kernel had higher ME (14.5 MJ/kg) than any other parts of safflower seed *in vitro* fermentation, *e.g.*, 7.8 MJ/kg in whole seed, 5.0 MJ/kg in safflower meal, and 3.2 MJ/kg in hull parts. In conclusion, this work have proved that safflower meal has higher utilization efficiency than other fractions of safflower seed, and there is no difference in nutritional values between two extracting methods based on extracting solvents of hexane and petroleum ether.

Keywords: safflower seed fraction, *in vitro*, metabolizable Energy, NDF, ADF, *in vitro* dry matter disappearance

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1. Introduction

Safflower (*Carthamus tinctorius L.*), an annual herb of Compositae, is usually utilized to provide editable oil because of high oil contents in its seed, and also is an important medicine resource for curing rheumatism, emergency analgesic, and anti-inflammatory bleeding because of essential oils from its flower. Safflower seed are mainly produced in USA, Mexico and India (FAO 2011), while Japan, Canada and even Mexico are the largest market requirements for safflower oil, and this tendency is growing year by year, according to the reports of National Agriculture Statistical Service (NASS, 2012). Safflower seeds contain around 33-60% hull and 40-67% kernel with undesirable palatability (Baümler et al. 2006), and are widely employed to diets of pet bird, dairy cows and rams (Smith 1996, Sudhamayee et al. 2004). However, for some unknown factors, high level supplementation of safflower seeds may decrease animal performance. Its protein degradability with highly degradable protein (Walli 2005) varies between 60% (Chandrasekharaiah et al. 2001), and 70% (Dixon et al. 2003) when they are mixed with rapeseed, soybean or linseed meal according to different ratios. Other researches have reported that high hull content hampers the utilization efficiency of safflower meal or whole seed in ruminants (Chandrasekharaiah et al. 2002).

Whole safflower seed can be fed to beef cattle without processing, however, high level of safflower seeds in the diet could result in low animal performance due to lignification of fiber and high level lipid content. In another way, safflower kernel cannot be fed to animals as a protein or high energy fodder as to the high cost for separating small kernel from hard hull. The incorporation of safflower hulls in cattle rations lead to a reduction of feed efficiency because of their low digestibility, but growth performance is not impaired when adequate energy and protein are supplied by other sources. Safflower hulls are used to provide bulk in high grain rations for beef cattle (Kohler et al. 1966). Hulls contain about 60 % crude fiber and 21 % lignin, and about 15% of the non-fiber, non-ash, non-protein part of the defatted safflower kernel is of unknown composition (Guggolz et al. 1968).

Safflower meal is a little bitter and less palatable being relative to other common protein supplementary for beef cattle but no impact on dairy cows and rams (Smith 1996, Sudhamayee et al. 2004). Live weight and wool growth performance were increased by supplementation of safflower meal to ram rations based on low quality grass hay or cereal

straw compared with barley/urea supplements (Dixon et al. 2003). Intake, digestibility, feed efficiency and body gain performances were improved by adding 350 g/day of concentrate mix with 25% safflower meal to the lambs (18 kg body weight) diet based on hay-fed (Dessie et al. 2010). Other reports shown that safflower meal has a low organic matter digestibility (OMD) in ruminants by *in vivo* (around 45%) in a ratio of 0.60 or 0.70 with barley or linseed meal, respectively (Dixon et al. 2003), compared with 68% OMD of decorticated safflower meal (Goss & Otagaki 1954).

In vitro fermentation technique has widely been considered to be suitable for mimicking rumen microbial fermentation by reducing experimental costs and improving experimental efficiency (Getachew et al. 2005). Gas production at early stage is mainly caused by fermentation of water-soluble components in fodders, such as soluble carbohydrates, pectin and protein components (Cone et al. 1997). Among, the fractional rate of degradation (FRD), defined as the rate of degradation, divided by the weight of degradable substrate, and changed with the alteration of hydration of particle, microbial attachment and numbers (France et al. 1993, France et al. 2000). A higher FRD may indicate faster *in vitro* fermentation, which means a greater gas production in a certain fermentation time (Wang et al. 2013). On the other hand, the dynamic kinetics of gas production, as well as different types of fiber digestibility (Menke & Steingass 1988) have been successfully applied to animal digestion research to assess the nutritional values of fodder underneath corresponding rumen microbial fermentation conditions, providing additional virtual information over *in vivo* or *in situ* methods (Gosselink et al. 2004, Wulf & Südekum 2005). The variance of *in vitro* gas production kinetics, or fiber digestibility are related to the nature or processing of oleaginous seed (extruded, pressed meal or whole unprocessed), and complicated interaction with ingredients of the basal diet (Lerch et al. 2012).

In a word, it's a pity there is not yet a systematic work to study safflower seeds as a potential supplements for ovine rations combined with *in vitro* fermentation technique. To make full use of safflower seeds, and to discover nutritional potential as ruminants' feedstuffs, this work was thereby to be conducted to evaluate the characteristics of *in vitro* ruminal fermentation of different parts of safflower seeds.

2. Materials and methods

2.1. Experimental design

This experiment was conducted in two parts: First, to separate whole seed into hull and kernel parts, and prepare safflower meal (extracted with hexane, SMH; extracted with petroleum ether, SMP), after that to determine the nutritional composition of each part of safflower seed obtained. Second, to do *in vitro* fermentation experiment, each part of safflower seed was individually fermented to obtain the data of gas production, apparent neutral/ acid detergent fiber (NDF/ ADF) digestibility and dry matter digestibility in triplicate each, after that using equation to calculate metabolizable energy (ME), and organic matter digestibility (OMD).

2.2. Animal donors and substrate sample preparation

Four adult male Pelibuey sheep (45 ± 5.0 kg/ each) with permanent rumen-fistula, housed individually and free access to the clean water, were used as inoculum donor according to Mexican Official Standard (NOM-220-SSA1-2002). Each sheep was daily provided 1.0 kg fodder, which composited with 64.2% sorghum, 19.3% alfalfa hay, 4.6% canola meal, 2.9% canola oil, 5.0% maize stover, 2.0% molasses, 1.0% purified tallow, and 1.0% urea, with a declared composition of 87% dry matter, 13.9% crude protein, 12.1 MJ/kg total energy, 5.3% crude fat, 0.54% calcium, 9.5% acid detergent fiber, and 21.4% neutral detergent fiber.

Safflower seed (0.5 kg) were bought from Queretaro in Mexico, artificially separated into hull and kernel, and then ground those samples into small particles for settling as safflower hull, kernel and whole seed partitions. Other 0.5 kg safflower seed were ground and extracted with hexane and petroleum ether for preparing two types of extracted safflower meal (SMH and SMP).

2.3. In vitro fermentation

The rumen liquid obtained, was mixed and strained through four layers of cheese-cloth into an Erlenmeyer flask with an O_2-free headspace, from donor animals at 07:00 am before feeding. Particle-free rumen fluid was mixed with the artificial saliva buffer solution (Menke & Steingass 1988) in a proportion of 1:4 (*v/v*) at 39 ºC under continuous flushing with CO_2 (Mauricio et al. 1999, Tang et al. 2008). **Experiment 1**: Gas production. Each part of safflower seed (hull, kernel, safflower meal, ground safflower, 1.0 g respectively as fermentation substrate, respectively) was incubated with 100 ml rumen buffer solution at

39ºC in quadruple of each, to determine kinetics of gas production with ANKOM gas determination equipment (ANKOM Technology Corp., Fairport, NY, USA) for 48 h described by Cone et al. (1996). **Experiment 2**: Bottles were pre-warmed (39ºC) prior to add 50 ml of buffered ruminal fluid (hull, kernel, safflower meal, ground safflower, 0.5 g as fermentation substrate, respectively) under CO_2 flushing, sealed with rubber stoppers and screw-on caps and incubated at 39ºC in constant temperature water bath oscillator for 48 h. Each was conducted in 3 replicates within 3 blanks (no substrate) for correction. Bottles were withdrawn from the water bath oscillator after 48 h incubation, to filter the substrate of each, dried in 105ºC overnight and weighed the residue of each substrate, and then to calculate ADF/ NDF digestibility and *in vitro* dry matter disappearance (*IVDMD*).

2.4. Chemical composition analysis

Diet and samples (substrates) were measured by the standard methods of AOAC (Cunniff 1995) for dry matter (No. 967.03), crude protein (No. 984.13) and ether extract (No. 954.02), ash content (No. 942.05), total energy, and were expressed as the inclusive of residual ash.

2.5. Calculations and data analysis

In vitro gas production curves were fitted with NLREG Version 5.0 (Sherrod 1995), using the combination of the Gauss-Newton and Levenberg-Marquardt Methods (John et al. 1981). To calculate the parameters of gas production kinetics, the Logistic-Exponential (LE_0) model was used as described by Wang *et al.* (2013), and the parameters were obtained based on visual inspection of the plots in order to reach convergence.

$$V = V_F \cdot \frac{1 - \exp(-\kappa t)}{1 + \exp(b - \kappa t)} \qquad (1)$$

where, V is the cumulative gas production (ml) at t, V_F is the final asymptotic gas volume with dimension of 'ml', k is the fractional rate of gas production with dimension of '/h'; and b is shape parameter without dimension. Initial fractional rate of degradation, $FRD_0 = k/(1+\exp(b))$ (Wang et al. 2013); the rate of gas production, $RG = V_F k(1+\exp(b))\exp(-kt)/(1+\exp(b-kt)^2$ *(Jiao et al. 2013)*; the half time at which half of the final gas production, $t_{0.5} = \ln(2+\exp(b))/k$; fractional rate of gas production at half-life, $\mu_{0.5} = k(d+0.5)/(1+d)$ (Wang et al. 2013).

Metabolizable energy (ME, MJ/kg DM) and organic matter digestibility (OMD, %) of different parts of safflower seeds were calculated using model of Menke et al. (1979) as follows:

$$\text{ME (MJ/kg DM)} = 2.20 + 0.136 \cdot \text{GP} + 0.057 \cdot \text{CP} + 0.0029 \cdot \text{CF}^2 \quad (2)$$

$$\text{OMD (\%)} = 14.88 + 0.889 \cdot \text{GP} + 0.45 \cdot \text{CP} + 0.0651 \cdot \text{Ash} \quad (3)$$

where GP is 24 h net gas production (ml/200 mg); CP is crude protein (%); CF is crude fat (%).

NDF/ ADF, fiber and Non-fiber disappearance were calculated with formula as follows:

$$\{\text{IVDMD}\}\text{(g/kg)} = \frac{\text{W.}_{\text{(original)}} - \text{W.}_{\text{(residue)}}}{\text{W.}_{\text{(original)}}} \quad (4)$$

$$\{\text{NDF/ ADF}\}_{\text{d}}\ (\%) = \frac{[\{\text{NDF/ ADF}\}_{\text{(original)}} - \{\text{NDF/ ADF}\}_{\text{(residue)}}]}{[\{\text{NDF/ ADF}\}_{\text{(original)}}]} * 100 \quad (5)$$

$$\text{Fiber}_{\text{d}}\ (\text{g/kg}) = [NDF + ADF]_{\text{(difference between original and residue)}} \quad (6)$$

$$\text{Non-fiber}_{\text{d}}\ (\text{g/kg}) = [IVDMD - (NDF + ADF)]_{\text{(difference between original and residue)}} \quad (7)$$

Statistical analyses were performed using the one-way ANOVA procedure of SPSS and means were compared with least squares means. The significant difference between different parts of safflower seed on gas production parameters, and NDF/ ADF and fiber digestibility were marked as lowercase letters in the corresponding Tables if there were significance differences. Least squares means were reported throughout the text, and treatment effects were declared significance if $P < 0.05$.

3. Results

3.1. Chemical composition

The chemical composition of different parts of safflower seeds were presented in **Table 1**. Safflower seed consisted of 40% hull, and 60% kernel in weight. The whole safflower seed contained 14.25% crude protein, 35.19% crude fat, and 53.91% neutral detergent fiber (NDF) and 36.25% acid detergent fiber (ADF). Hull has high level of fiber than kernel, whole seed and meal of safflower seed. Meanwhile, high level of protein and low fiber were shown in kernel parts of safflower seed.

Table 1 comes about here

3.2. Kinetics of in vitro gas production

In this study, the gas production were determined for 48 h fermentation, some of parameters were calculated using LE0 model (Shown in **Table 2**). We found that the theoretical maximal gas production of safflower meal (around 70 ml/g, safflower meal both extracted with hexane and petroleum ether, represented as SMH and SMP, respectively) was greater (P<0.05) than those of kernel (48 ml/g) and whole seed (46.4 ml/g), and the least theoretical maximal gas production occurred at the hull part (35.9 ml/g). Fractional rate of gas production of kernel was higher (P<0.05) than those of whole seed and hull, and numerically higher than that of safflower meal (SMH, SMP). Initial fractional rate of degradation (FRD_0) and the rate of gas production (RG_t) had the similar changeable tendency between different parts of safflower seeds, both of these parameters in kernel and whole seed were lower than those of safflower seed meal (including SMH and SMP), but higher than that of hull. About half time at half of the final gas production ($t_{0.5}$), and fractional rate of gas production at half-life ($\mu_{0.5}$), both of these specific time parameters in safflower meal were lower than that in hull treatment, but higher than that in kernel and whole seed.

Table 2 comes about here

3.3. Gas production, metabolizable energy and organic matter digestibility

The gas production (ml/g substrate) at relevant fermentation points (6, 12, 24, 36, and 48 h), pH, metabolizable energy (ME, MJ/kg), and organic matter digestibility (OMD, g/kg) were given in **Table 3**. At all fermentation time points, the gas production values of kernel and whole seed were similar with each other, the same phenomenon occurred (viewing in **Figure 1**) in safflower meal (SMH and SMP). However, the values of gas production of kernel and whole seed were lower than that of safflower meal, and higher than that of hull. After 48 h fermentation, pH values of each treatment were similar with each other, however, which in whole seed was higher than that in safflower meal, kernel and hull. The ME in kernel (14.5 MJ/kg) were double of that in whole seed, and near to triple of that in safflower seed (5.0 MJ/kg) and quintuple of that in hull (3.2 MJ/kg). We also calculated organic matter digestibility (OMD, g/kg) for different parts of safflower seed in this study, the values of that in safflower meal (around 347 g/kg) were similar with each other but

slightly higher than that in kernel (332 g/kg), and much higher than whole seed (290 g/kg) and hull (218 g/kg).

Figure 1, Table 3 come about here

3.4. In vitro fibre digestibility

The *in vitro* ruminal NDF, ADF and DM digestibility of different parts of safflower seeds are listed in **Table 4**. Higher ruminal *in vitro* dry matter disappearance (*IVDMD*) was noted in kernel (676.62 g/kg), following by SMH (397.92 g/kg), SMP (374.28 g/kg), and whole seed (382.22 g/kg). In this study, the sum of non-fiber and fiber digestibility were equal to *IVDMD*, the fiber digestibility was equal to sum of NDF and ADF digestibility. NDF digestibility (in proportion, %) of kernel (98.53%) was greater than that of whole seed (36.84%), and much higher than those of safflower meal (16.60% of SMH, and 15.82% of SMP) and safflower hull (12.50%). Digestibility of ADF (%) had a similar tendency with that of NDF for different fractions of safflower seeds.

Table 4 comes about here

4. Discussion

4.1. Kinetics of in vitro ruminal gas production

In 1968, Guggolz *et al.* systematically analyzed the chemical composition of different parts of safflower seed (Guggolz et al. 1968), subsequently a few valuable researches reported the nutritional contribution of safflower seeds on animal feed and production (*in vivo* or *in situ*) (Alizadeh et al. 2010, Bozan & Temelli 2008, Dschaak et al. 2010, Lee et al. 2004, Moon et al. 2001). Some valuable utilization of safflower seed have been reported for bird feed (http://birding.about.com/od/Foods/a/Safflower-Seeds.htm), and medicinal function of safflower seed composition (http://www.webmd.com/vitamins-supplements/ingredientmono-96-safflower.aspx?activeingredientid=96&activeingredientname=safflower). From our results, *in vitro* gas production parameters (V_F, FRD_0, RG_t) in safflower hull were lower than that in other safflower seed fractions. The differences in *in vitro* gas production among different fractions of safflower seed, could be due to the proportion and nature of their composition (Rubanza et al. 2003).

We used the pure different parts of safflower seed as substrate for *in vitro* fermentation, which resulted in higher fermentation performance *in vitro* of safflower meal than that in whole seed and hull, even in kernel. As previously reported, we combined safflower meal with sorghum seed for *in vitro* fermentation, the high content of starch in sorghum seed leaded to the high gas production *in vitro*. Souilah and Djabali (2014) reported that high soluble carbohydrate contents had further improved the most important gas production parameters. Anti-nutritional ingredients, such as matairesinol-β-glucoside, 2-hydroxyarctiin-β-glucoside (Jin et al. 2010) and cyanogenic glucoside (Satish & Shrivastava 2011) in safflower meal, in safflower meal could give rise to the reduction of *in vitro* gas production parameters (V_F, RG_t, and $t_{0.5}$). In this work, we also obtained some valuable parameters about shape parameter (*b*) in hull treatment is positive, whole safflower seed and kernel were around to 0, however, the value of shape parameter (*b*) in safflower seed were negative (around -20), that data did not show in the Tables. About the value of shape parameter, positive represents the gas production curve is similar with *s* curve with a lag time for one sealed fermentation system, otherwise, the gas production curve is like a parabolic curve, represents that this sealed system has no fermentation initial-limited, because of the balance or high energy composition was supplemented (Wang et al. 2013).

V_F was a corresponding parameter related to the non-digestible structural carbohydrate-hull (Baümler et al. 2006), high soluble carbohydrate (Lechartier & Peyraud 2011) as well as the anti-nutritional factors (such as cyanogenic glucoside) (Satish & Shrivastava 2011). In this study, we had the corresponding gas production per **g** of different parts of safflower seed, from the results we concluded safflower meal had the highest theoretical maximal gas production than those of whole safflower seed, hull even kernel partition. As there was high oil content in safflower seed, most of them deposited in the kernel part of safflower seed, resulted in low gas production *in vitro* fermentation. Fatty acids have the potential to change the microbial ecosystem, influence activity of some bacterial adhesion ability (Benchaar et al. 2008). Anti-nutritional or undigested ingredients deposited in safflower whole seed/ kernel could also decrease the digestion efficiency reflected by the gas production parameters (V_F, FRD_0, k, $t_{0.5}$, RG_t, and $\mu_{0.5}$), for the hydration, removal of digestion inhibitors and/or the attachment of microbes with substrates (Mertens 1993)

increase the adsorption time, so as to be more difficulty against the structural carbohydrate (Tan et al. 2002). $t_{0.5}$ of hull was much higher than that in safflower meal and kernel or whole seed, it might be ascribed to that SFM's hull impeded the attachment, adhesion, colonization and degradation processes of ruminal microorganisms (Varga & Kolver 1997).

4.2. Rumen pH

Rumen pH is inconsistent with metabolite concentrates, high pH value always adjunct with lower secondary metabolites, otherwise (Pina et al. 2009). The pH value, ranging from 5.5 to 7.5, is deemed as an important determinant in the rumen *in vitro* or *in vivo* fermentation (Yuan et al. 2010), highly correlated with VFAs, the population and enzyme activities of microbes. Fatty acids like lactic acid, which inhibits membranes by lactic acid in non-ionized than in ionized form, protozoal population which are responsible for about 25% of rumen microbial cellulolytic and help maintain a higher pH by engulfing starch granules (Mould et al. 2005). In our study, pH value was around 6.7 to 6.9 during the processes of *in vitro* fermentation of different parts of safflower seed for 48 h. The stability of pH showed that it was beneficial for the entire fermentation and to kept microbes to survive in a comfortable micro-environment.

4.3. Digestibility of fibre

Other very important index to evaluate the nutritional values of feed is the digestibility (Naga & el-Shazly 1963). In this study, hull had high fibre content, its *in vitro* dry matter disappearance (*IVDMD*) was the lowest compared with other partitions of safflower seed. It would be due to the high fibre lignification, as we know the lignified fibre is indigestible composition for rumen microbes. Apparent *IVDMD* of kernel was the highest among different parts of safflower seed, because it had high soluble ingredients, *e.g.*, soluble carbohydrates, protein, and lipids. Some compositions were washed out by the neutral or acid fiber detergents, which had been calculated into *IVDMD* result. That's why we also calculated non-fiber digestibility, NDF and ADF digestibility (in percentage). NDF/ ADF digestibility of Kernel were around to 100% or a little higher than 100%, as high soluble capacities of ingredients in safflower kernel or might be the microbes adjunct to the nylon bag fermented *in vitro*. Whole seed have higher NDF and ADF digestibility than safflower meal, because calculating the original NDF or ADF contents (in percentage) in whole seed was lower than that of safflower meal as high lipid content deposited in whole seed, other

while, safflower meal have high NDF/ ADF in percentage for getting rid out of lipid in it. However, *IVDMD* of whole seed was similar with that of safflower meal, as to co-contributions of lipid content which increase and some anti-nutritional factors which decrease *IVDMD* (apparent) in whole seed, in total, this two important parts resulted to *IVDMD* of safflower seed was similar with that of safflower meal, just according to the truth *IVDMD* of safflower meal should be higher than that of safflower seed, what a pity, this need a further work to prove it.

5. Conclusion

This work give us the information about utilization of safflower seed in ovine rations by *in vitro* ruminal fermentation, the results implied that there was no significant difference in nutritional contribution to *in vitro* fermentation characteristics between safflower meals extracted with hexane or petroleum ether, meanwhile safflower meal had higher ruminal fermentation characteristics and digestibility *in vitro* than those of whole safflower whole seed, as well as kernel and hull partitions, in spite of digestibility of kernel was very high because of high soluble capability of kernel compositions. Our research work had proved that safflower meal has higher utilization efficiency than other fractions of safflower seed, and there is no difference in nutritional values between two extracting methods based on extracting solvents of hexane and petroleum ether. We expected this work could provide a very clear idea for producers or farmers in ruminant industry to choose a good resource of safflower by-products as feedstuff, in addition of consideration of the cost for production.

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FIGURE LEGENDS

Figure 1. The *in vitro* ruminal gas production curves of safflower seed fractions

(SMH is safflower meal extracted with hexane, SMP is safflower meal extracted with petroleum ether)

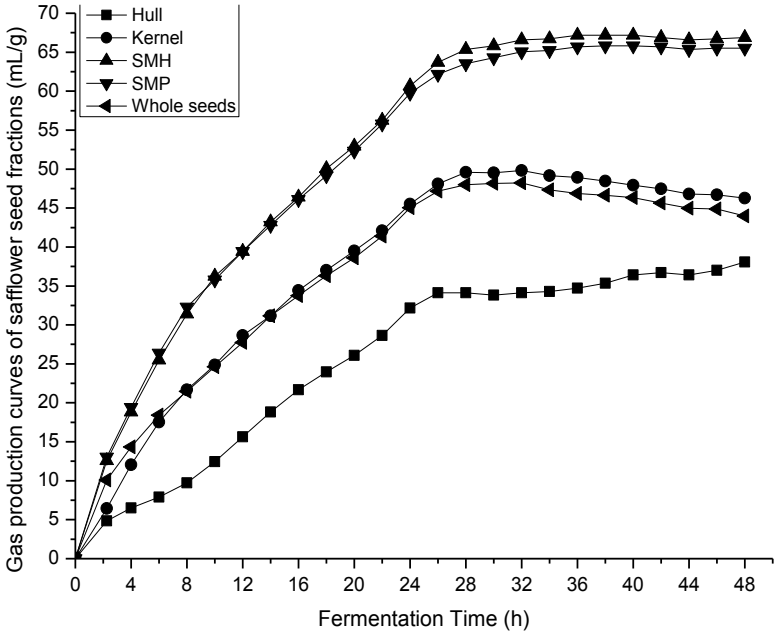

Figure 1. The *in vitro* ruminal gas production curves of safflower seed fractions

SMH is safflower meal extracted with hexane, SMP is safflower meal extracted with petroleum ether.

Table lists:

TABLES LISTS

Table 1. Chemical composition of safflower seed fractions

Items [i]	**Chemical compositions of safflower seed fractions (%)** [ii]				
	Protein	**Crude fat**	**NDF**	**ADF**	**Ashes**
Hull	3.72 ± 0.40	0.85 ± 0.30	83.29 ± 1.90	56.57 ± 1.17	1.57 ± 0.03
Kernel	22.92 ± 2.15	58.26 ± 0.85	24.05 ± 4.70	13.04 ± 2.10	3.00 ± 0.12

Whole seed	14.25 ± 1.32	35.19 ± 0.28	53.91 ± 5.12	36.25 ± 2.67	2.32 ± 0.09
SMH	20.15 ± 0.93	0.36 ± 0.08	63.67 ± 5.00	42.05 ± 1.71	3.38 ± 0.27
SMP	19.44 ± 1.17	3.23 ± 0.48	64.63 ± 0.20	44.57 ± 0.05	3.60 ± 0.02

Note: [i] SMH, safflower meal extracted with hexane; SMP, safflower meal extracted with petroleum ether; [ii] NDF is neutral detergent fiber; ADF is acid detergent fiber.

Table 2. Gas production characteristics of *in vitro* gas production of safflower seed fractions

Items [i]	Gas production characteristics [ii]					
	V_F *(mL/g)*	k (%)	FRD_0 (%)	RG_t	$t_{0.5}$	$u_{0.5}$ (%)
Hull	35.9 ± 2.60 [c]	11.7 ± 2.35 [a]	3.3 ± 0.93 [c]	1.3 ± 0.39 [c]	13.0 ± 0.62 [a]	8.5 ± 0.03
Kernel	48.0 ± 4.37 [b]	13.3 ± 1.72 [a]	6.1 ± 0.26 [b]	2.9 ± 0.33 [b]	8.7 ± 0.24 [b]	7.3 ± 0.03
Whole seed	46.4 ± 3.32 [b]	11.7 ± 0.62 [a]	7.7 ± 1.45 [a]	3.5 ± 0.77 [b]	7.9 ± 0.83 [b]	6.7 ± 0.13
SMH	69.5 ± 1.59 [a]	7.8 ± 0.81 [b]	7.8 ± 0.75 [a]	5.0 ± 0.34 [a]	8.9 ± 0.86 [b]	8.4 ± 0.01
SMP	70.1 ± 1.10 [a]	8.0 ± 0.07 [b]	8.0 ± 0.07 [a]	5.2 ± 0.12 [a]	8.6 ± 0.08 [b]	8.2 ± 0.00

Note: [i] SMH, safflower meal extracted with hexane; SMP, safflower meal extracted with petroleum ether; [ii] V_F is the final asymptotic gas volume with dimension of 'mL', k is the fractional rate of gas production with dimension of '/h'; FRD_0 is the initial fractional rate of degradation; RG_t is the rate of gas production; $t_{0.5}$ is the half time at which half of the final gas production; $\mu_{0.5}$ is fractional rate of gas production at half-life.

Table 3. Gas production at specified times, metabolizable energy and organic matter digestibility of safflower seed fractions *in vitro*

Items [i]	Gas production **(mL/g substrate)**					pH **(48 h)**	ME [ii] **(MJ/kg)**	OMD **(g/kg)**
	6 h	12 h	24 h	36 h	48 h			
Hull	8.2 ± 1.82	15.5 ± 2.05	29.9 ± 1.67	33.0 ± 1.59	36.4 ± 1.93	6.74 ± 0.02	3.2 ± 0.05 [d]	218.2 ± 3.9 [d]
Kernel	18.4 ± 2.05	30.3 ± 1.82	44.4 ± 2.73	47.3 ± 3.87	47.5 ± 3.64	6.75 ± 0.03	14.5 ± 0.43 [a]	331.6 ± 11.32 [b]
Whole	19.3 ±	29.3 ±	43.9 ±	45.3 ±	45.3 ±	6.95 ±	7.8 ± 0.02 [b]	290.9 ±

seed	3.41	3.18	3.64	3.18	3.18	0.01		3.73 [c]
SMH	26.4 ± 0.91	41.0 ± 2.10	59.5 ± 1.46	65.5 ± 0.95	68.2 ± 1.14	6.83 ± 0.12	5.0 ± 0.04 [c]	347.0 ± 3.25 [a]
SMP	28.4 ± 0.68	42.5 ± 0.91	60.3 ± 0.91	66.9 ± 1.14	68.7 ± 1.14	6.87 ± 0.04	5.0 ± 0.07 [c]	346.6 ± 6.40 [a]

Note: [i] SMH, safflower meal extracted with hexane; SMP, safflower meal extracted with petroleum ether; [ii] ME is metabolizable energy (MJ/kg); OMD is organic matter digestibility (g/kg).

Table 4. *In vitro* ruminal digestibility characteristics of safflower seed fractions (g/kg) at 48 h

Items [i]	***In vitro* ruminal digestibility characteristics** [ii]				
	IVDMD	**Non-fiber_*d***	**Fiber_*d***	**NDF*d*** (%)	**ADF*d*** (%)
Hull	177.47 ± 17.74 [c]	11.70 ± 3.05 [d]	165.78 ± 22.37 [b]	12.50 ± 1.55 [c]	10.50 ± 1.77 [c]
Kernel	676.62 ± 56.92 [a]	297.05 ± 42.66 [a]	379.57 ± 22.33 [a]	98.53 ± 4.50 [a]	107.73 ± 8.77 [a]
Whole seed	382.22 ± 26.18 [b]	36.04 ± 14.40 [c]	346.18 ± 31.85 [a]	36.84 ± 2.79 [b]	39.66 ± 4.56 [b]
SMH	397.92 ± 20.48 [b]	239.12 ± 14.01 [b]	158.80 ± 18.09 [b]	16.60 ± 1.35 [c]	11.58 ± 2.20 [c]
SMP	374.28 ± 14.66 [b]	204.73 ± 19.08 [b]	169.54 ± 11.42 [b]	15.82 ± 1.33 [c]	14.58 ± 0.68 [c]

Note: [i] SMH, safflower meal extracted with hexane; SMP, safflower meal extracted with petroleum ether; [ii] *IVDMD*, *in vitro* dry matter disappearance; Non-fiber_d is non fiber digestibility; Fiber_d is fiber digestibility; NDF*d*, *in vitro* neutral detergent fiber disappearance including ashes; ADF*d*, *in vitro* acid detergent fiber disappearance including ashes.

CAPÍTULO 3

Yong Liu, Zhiliang Tan, Claudia Giovanna Peñuelas-Rivas, Germàn Buendía-Rodríguez, María Rivas-Guevara, Alejandro Pazos. Long chain fatty acid, volatile fatty acid profiles in ruminal bacteria and protozoan fractional membrane, and methane production varied with supplementation of plant oils rich in lauric, oleic, linoleic and linolenic acid. *Letters in Applied Microbiology*. (Submitted)

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Variations of long chain fatty acids in ruminal bacteria-protozoan membrane and volatile fatty acids as fermented with poly-, mono-unsaturated and medium chain fatty acids

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Key Words:	Fermentation, Membrane, Metabolism, Protozoa, Mechanism of action

Variations of long chain fatty acids in ruminal bacteria-protozoan membrane and volatile fatty acids as fermented with poly-, mono-unsaturated and medium chain fatty acids

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Running head: ruminal microbiome lipid metabolism

Significance and Impact of the Study: Ruminal microbiomes play an important role in nutrimental components of ruminant products (meat or milk). A lot of fatty acids, macronutrients or micronutrients of feed are digested or resynthesized by ruminal microbiomes. Fatty acid compositions of ruminal microbial membrane could directly reflect the fatty acids metabolism processes as both of bacteria and protozoan play a key role in the ruminal lipid metabolism processes. Therefore, this study was conducted to determine the long chain fatty acids composition in ruminal microbial membrane by supply of mono-, poly-unsaturated fatty acids and medium chain fatty acids. The results showed that exogenous different unsaturated degree, long/short chains fatty acids can impact on ruminal microbe fermentation performance by changing the fatty acid composition, further to influence the microbial activity of bacteria and protozoan.

Abstract: Modifying ruminal microbial metabolism in addition of plant-derived oils is an effective way to enhance the functional fatty acids in ruminant-derived products. Little is known about the influence of exogenous fatty acids on lipid metabolism processes in both bacteria and protozoan membrane. Thus, this work focused on investigating the long chain fatty acid (LCFA) and volatile fatty acids (VFA) at 48 h supply of exogenous oils mainly containing poly-/mono-unsaturated fatty acid, PUFA/MUFA, (C18:3, C18:2 and C18:1 from linseed, safflower and canola oils, respectively) and medium chain fatty acids MCFA, (C12:0 from coconut oil). The results showed that fatty acid compositions between bacteria and protozoan were different. Linseed oil supplementation, mainly linolenic acid (C18:3), increased the proportions of C18:2 n6*c*, C18:2 n6*t*, C18:1 n9*c*, C18:1 n9*t* and *cis*- fatty acid in bacteria and protozoan membrane to a different extent, and also slightly improves acetic and propionic acid concentrations. Coconut oil supplementation enhanced the MCFA compositions, and further to increase MCFA biosynthesis in both bacteria and protozoan fractions (from C12:0 to C14:0). Higher saturated, saturated/unsaturated, *trans*-, *even*-carbon unsaturated fatty acids obtained in bacterial membrane when supply of C18:2 and C18:1, but lower than that in C12:0. In addition, both of safflower/canola oils increased the acetic and propionic acid concentrations, decreased the Ac/Pro ratio. In brief, the results implies that exogenous different unsaturated degree, long/short chains fatty acids can impact on ruminal microbe fermentation performance by changing the fatty acid compositions, further to influence the microbial activity of bacteria and protozoan.

Keywords: poly-/mono-unsaturated fatty acids, *in vitro*, long chain fatty acids, volatile fatty acids, bacteria/protozoan, lipid metabolism.

Introduction

Consumers are gradually noticing that foods containing micronutrients to the benefits of health maintenance and disease prevention. In ruminant, as the particular biology function of rumen microbes in fat metabolism, the meat and milk products rich in some functional fatty acid (FA) components include 9*cis*, 11*trans*- conjugated linoleic acid (Aldai et al. 2005), vaccenic acid (Blewett et al. 2009), rumenic acid (Chisaguano et al. 2014), eicosapentaenoic acid (Gillies et al. 2012), and docosahexaenoic acid (Mouradian et al. 2014). It is an effective way to enhance these functional fatty acids in ruminant-derived food by modifying ruminal FA microbial metabolism by supplementation of exogenous FA with different structure and molecular properties. FAs in ruminant rations go through extensive hydrolysis or biohydrogenation by the ruminal microbes.

In general, the metabolism pathways in rumen are very complicated, for instance, poly-unsaturated FA are comprehensively hydrogenated into monounsaturated, saturated fatty acid, or further to degradation into less-carbon FA. These pathways result in lower exogenous polyunsaturated fatty acids (PUFA) but with the accumulation of various biohydrogenation (BH) products in ruminal biology membrane or further to influence the FA composition in ruminant

products, *e.g.*, milk and meat (Hoffmann et al. 2015). In particularly, C18:3 is converted to stearic acid (C18:0) via *t*-C18:1, C18:2, 9*c*, 11*t*- CLA as intermediates (van de Vossenberg and Joblin 2003). Oleic acid and *cis*-vaccenic acid are converted to a series of *trans*- monounsaturated isomers as well as C18:0 (Murakami et al. 2004). Meanwhile, different microbes play the different role in lipid metabolism processes. For instance, *B. hungatei Su6* is able to convert alpha-linolenic acid to C18:0 (van de Vossenberg and Joblin 2003), *B. fibrisolvens* has the ability to convert C18:2 into C18:1 but not to C18:0 (Wilde and Dawson 1966), whereas *B. proteoclasticus* can convert C18:1 to C18:0 (Vasta et al. 2010).

The representative of PUFA is C18:3, which has been proved to increase *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA in milk fat (Chilliard et al. 2009). Meanwhile, linoleic acid (C18:2) can enhance both *trans*-10 C18:1 and *trans*-10, cis-12 C18:2 yields, and increase propionic acid and CLA (Li et al. 2011) but lower milk fat yield, fat concentration, and C12:0, C16:0 yield (Stoffel et al. 2015). In addition, supply of 400g lauric acid in cow rations decreased the proportions of rumen acetate and butyrate but with an increase of propionate (Rindsig and Schultz 1974). Canola oil reduced fat-corrected milk yield and milk fat concentration but not reduce the dry matter intake (Jenkins 1998). Coconut oil is recommended as a healthy oil for replacing the high content unhealthy plant/animal-derived oils. In ruminant, coconut oil improves the beneficial fatty acids in meat and milk (Lourenco et al. 2010), increases the percentage of *trans*- C18:1, C12:0 and C14:0, and biohydrogenation (Reveneau et al. 2012). In short, it is an efficiency way to modify the lipid biohydrogenation processes by supply of exogenous oils in ruminant rations, further to increase the functional micronutrients accumulation go through the rumen metabolism.

The compositional and functional of FA in bacterial and protozoan membranes are different in ruminal micro-ecological niche. Different species had different compositions, such as *Epidinium ecaudatum* (Devillard et al. 2006) contains more than ten times of CLA and VA, compared to other species. The main FA metabolism pathways in rumen include two types, one is by degradation or hydrolysis of exogenous polyunsaturated fatty acids (PUFA), the other one is the *de novo* synthesis from short chain fatty acid or volatile fatty acids. However, the main existed form of long chain fatty acids are stored in bacterial or protozoan biology membrane. Manipulation of ruminant rations might increase functional FA, *e.g.*, either conjugated linoleic acid or *trans*-11 C18:1/ *trans*-10 C18:1, and in associated with decreasing the saturated fatty acid contents in the ruminant products.

Keep that in mind, this work is focused on investigating the effect of different plant-derived oils rich in poly-unsaturated FA (PUFA; C18:3, C18:2 in linseed and safflower oil), mono-unsaturated FA (MUFA, C18:1 in canola oil) and medium chain saturated FA (MCFA, C12:0 in coconut oil) on bacterial and protozoan biology membrane and VFAs. The objectives of this work were to describe and compare the fatty acid compositions of bacteria and protozoa membrane in supplementation of PUFA, MUFA and MCFA, and to attempt to disclosure the role or function of these exogenous different types of FA in ruminal fatty acid metabolism processes.

Results and Discussion

LCFA profiles in bacterial fractions

C18:0 and C16:0 of bacteria membrane in supply of PUFA (C18:3, C18:2 and C18:1), were higher than that supply of MCFA (C12:0) (**Table 1**). It implies that exogenous plant-derived FA can deposit or assimilate into bacteria membrane to some extent; in addition, mixed bacteria has the ability to hydrogenate either mono- or poly- unsaturated FA to saturated FA. Both of C12:0 and C14:0 in bacterial membrane in supply of C12:0 were higher than that in other oil treatments. It speculates that bacteria might assimilate C12:0 into its own biological membrane or further to prolong carbon chains from C12 to C14 in a complex unknown metabolism process. A very high percent (12.31%) of C18:1 in bacteria membrane in linseed oil treatment compared to safflower oil (5.82%), canola oil (7.61%) and coconut oil (3.69%), that reflects that some of exogenous original C18:3 transforms into C18:1 by some bacterial FA desaturase, and then C18:1 as an intermediate was assimilated or deposited into bacterial biological membrane. Other reports presented supplementation of linolenic acid increased *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA in milk fat (Chilliard et al. 2009), and Linoleic acid (C18:2) can enhance both *trans*-10 C18:1 and *trans*-10, *cis*-12 C18:2 yields in milk but lower C12:0 and C16:0 yield (Stoffel et al. 2015).

Table 1. Come about here

The proportions of LCFA ≥ 18 carbons and unsaturated FA of bacteria fraction in supply of PUFA or MUFA were higher than that in MCFA. The significant difference between LCFA and unsaturated FA showed that exogenous plant-derived FA could influence the composition distribution of microbial membrane, and further to influence the function of biological membrane. The proportion of *cis*-FA in coconut oil (4.79%) was obvious ($p<0.05$) lower than that in other treatments with an average value of 10.90%. The proportion of *trans*- FA of bacterial fraction supplied with linseed oil (5.91%) was a little lower than that in other treatments. This is associated with *cis*-/*trans*- ratio which in linseed oil was threefold than that in coconut oil. In addition, the proportion of *even*-carbon unsaturated fatty acids in the treatment of linseed oil (67.72%) was lower than that in safflower oil (76.91%), canola oil (72.66%) and coconut oil (81.25%).

LCFA profiles in protozoan fractions

The proportion values of MCFA in protozoal fraction, including C12:0, C14:0, C14:1, C15:0 and C16:0, in coconut oil treatment was very higher than that supply with poly- or mono-unsaturated FA. In particular, the proportion of C16:0 in protozoan membrane in coconut oil (riches in C12:0) was 21.49%, which was significantly higher ($P<0.05$) than supply of other plant oils, 13.29% in safflower, 14.36% in canola or 15.73% in linseed oil, respectively. In addition, LCFA (≥ 18 carbons) compositions in protozoal membrane in supply of coconut oil (51.51%) was significantly lower than that in other treatments with the average of 80%, while that in linseed oil was higher than other treatments. The *cis*- and *trans*- PUFA

compositions in protozoal membrane implies that more content of *cis*- PUFAs are coming from the exogenous plant canola oil which is mainly composited of C18:1. Meanwhile, more *trans*- PUFAs are synthesized by addition of exogenous linseed oil which is mainly composited of C18:3.

It is interesting to notice that proportion of *odd*- carbon FA in protozoan fraction with supplementation of coconut oil (4.48%) was approximately double times compared to exogenous poly- or mono-unsaturated FA. And proportions of C15:0, C17:0, and 10 *cis*- C17:1 in coconut oil were around double times compared to that in others plant oils. It implies that exogenous C12:0 might be prolonged to high carbons *odd*- FA and synthesized into the protozoan membrane. Lower proportion of *even*-carbon saturated FA but higher proportion of *even*-carbon unsaturated FA were obtained in linseed oil, it was consistent with the saturated/unsaturated FA ratios obtained in here with the value of 3.83 in linseed oil compared to other oils with the average value of 5.27. The saturated/unsaturated FA of microbial biological membrane seems related to the hydrophobicity (Kundu et al. 2013), fluidity (Calder et al. 1994), osmotic stress and permeability (Machado et al. 2004).

Comparing the mean value of FA compositions in bacterial and protozoan fractions, there was obviously difference between medium (39.05% vs. 26.48%) and long chain FA (60.95% vs. 73.52%). A higher proportion value of medium FA composition in bacteria, it implies that bacteria has more capacity to assimilate MCFA than protozoa. Meanwhile, the higher C16:0 proportion in bacterial membrane compared to higher C18:0 proportion in protozoal membrane. The cell membrane hydrophobicity of microbes, except strong related to hydroxyl fatty acids, is also extremely dependent on the long/medium chain, saturated/unsaturated FA composition in membrane (Kaczorek et al. 2013). It can also be concluded, protozoan can extend exogenous medium chain FA from C12:0 to C16:0 to some extent. Furthermore, the proportion of C18:0 in protozoan and bacterial biological membranes was increasing with supplementation of exogenous mono- and poly- unsaturated FA compared to just supply of MCFA. The FA composition of bacterial and protozoan membrane is not just refers to the difference between these typical fatty acids, but also mention to some functional micronutrient fatty acids such as the conjugated linolenic acid (Lee 2013), and docosahexaenoic acid (DHA) (Torok et al. 2014). As mentioned by previous reports, protozoan and bacteria are involving in some different biological metabolism processes (Ranilla et al. 2007), which might be strong related to the difference composition in bacterial and protozoan membrane, both bacteria and protozoan have different function in the rumen micro-environment because that the function of microorganisms are high related to its structure and composition (Belanche et al. 2011).

VFA in fermentation media

All the values of VFA were the eigenvalues do not remove the value in blank treatment, in addition, the control was a treatment with the same condition but no exogenous oil (**Table 2**). Acetic acid and propionic acid in presence of linseed oil was higher than that in canola oil, safflower oil, and in turn coconut oil. The eigenvalues of total VFA in the existence

of exogenous fatty acids from linseed, canola, safflower, and coconut oils were increased but without statistical difference between in the presence or absence of plant oils, that is consistent with other reports presented a little enhance (Lin et al. 2013). The Ac/Pro values of all treatments were ranged from 3.4 to 3.8 obtained in present study, the results showed that a poor nutritional condition, provided in blank treatment of presence study, had decreased Ac/Pro values. Meanwhile, Ac/Pro values in presence of exogenous plant oils were also decreased, which was even lower than that in the poor nutritional condition. That implies that the eigenvalue of propionic acid in presence of those exogenous oils was increased greater than acetic acid.

Table 2. Come about here

However, isobutyric acid in the presence of coconut oil was a little low, compared to the presence of other oils or control. In addition, eigenvalues of isovaleric acid and valeric acid in the presence of canola oil were higher than that in the order of linseed, safflower and in turn coconut oil. In spite of that, eigenvalues of total VFA were not changed with the presence of exogenous plant oils. Both eigenvalues of acetate and total VFA in presence of coconut oil were lower than that in safflower, linseed and canola oils. That might mean exogenous fatty acids are mainly absorbed and assimilated into the biological membrane by microorganisms, at least not degraded into the short chain fatty acids in some extent, or without more metabolite intermediates such as VFAs to participate in further metabolite processes.

Synergism of VFAs and LCFAs of bacteria and protozoan

It is interesting to mention that combination of LCFAs profiles in bacterial and protozoal membrane and the short chain fatty acids (VFAs) to further explanation effect of exogenous fatty acids on the fatty acids metabolism or biological assimilation of ruminal microbial ecosystem. The high proportion of MCFA obtained in the presence of coconut oil, which is mainly composited of lauric acid, combined with the lowest production of VFAs, that rumen microbes prefer to prolong exogenous lauric acid (C12:0) to synthesis MCFAs such as C14:0, C14:1, C16:0 and C16:1, rather than to degrade lauric acid into short chain fatty acids (VFAs). It implies that fatty acid elongase related to lauric acid has higher activity in some extent. Linseed oil decreases FA from C4:0 to C16:0 and increases in C18:0, *trans*-11 C16:1, all *cis*- and *trans*- C18:1 (except *trans*-11 C18:1), and non-conjugated *trans* C18:2 isomers (Chilliard et al. 2009).

In the presence of γ-linolenic acid (linseed oil) has increased the oleic acid, *even*-carbon, *cis*-, and *trans*-, C18 unsaturated fatty acids proportion in both bacterial and protozoal fractions, combined with VFA profiles, acetic acid and propionic acid were also increased. It means that γ-linolenic acid has been converted into short or less double-bond FA by desaturases or degrading enzymes in a complex environment.

Materials and Methods

Experimental design

In present work, plant-derived oils such as linseed, safflower, canola, and coconut oil, which are abundant in poly- or mono- unsaturated (C18:3, C18:2 and C18:1) and medium chain fatty acids (C12:0) (refer to γ-linolenic acid, linoleic acid, oleic acid, and lauric acid, respectively) were used to investigate the effect of these exogenous different double-bonds location and numbers of fatty acids on fatty acids distribution in bacteria-protozoan-media phases (LCFA in bacteria and protozoan membrane and VFAs in fermentation media) *in vitro* fermentation micro-ecological niche.

Animal donors and feed ingredients

Adult male Pelibuey sheep (3 individuals) with permanent rumen-fistula (body weight, 45 ± 5.0 kg) were used as inoculum donors according to Mexican official standard (NOM-220-SSA1-2002). Nutritional compositions of fodder were according to the nutritional requirement of NRC (2007). All the animal procedures and protocols were approved by the Animal Care Committee, CENID FyMA, INIFAP, Queretaro, Mexico. Each sheep, housed individually and free access to water, was fed with 1.0 kg feed and 0.5 kg smashed maize stover. The feed was composited of 38% corn grain, 30% alfalfa hay, 2% canola meal, 2.5% canola oil, 2% safflower meal, 2.5% safflower oil, 18% corn stover, 2.0% molasses, and 1.0% urea.

Substrate, alfalfa hay, was obtained from a plant of CENID FyMA, INIFAP in Querétaro, Mexico. And all plant oils were purchased from Sigma-Aldrich with the series numbers of each, safflower oil (S8281), linseed oil (430021), canola oil (46961), and coconut oil (46949). FA compositions of these four plant oils were presented in **Table 3**. Safflower oil is abundant in linoleic acid with a proportion of 70%, linseed oil with about 50% of γ-Linolenic acid, canola oil approximately contains of 60% oleic acid, and coconut oil includes around 60% lauric acid. These four oils chosen based on the difference of main composition, represent media chain fatty acids (lauric acid, C12:0), mono-polyunsaturated acid (oleic acid, C18:1), polyunsaturated acids (linoleic acid, C18:2, and linolenic acid, C18:3), respectively.

Table 3 comes about here

In vitro fermentation.

The *in vitro* manipulation was according to the description of Tang *el at.*(2008), the particle-free rumen fluid was mixed with artificial saliva buffer solution (Menke and Steingass 1988) in a ratio of 1:2 (*v/v*) at 39 °C under continuous flushing of CO_2 for 30 min. Each oil, mixed with 0.5 g substrate in a proportion of 5%, was incubated with 50 mL rumen buffer solution at 39 °C in 3 replicates of each for 48 h. After 48 h, bacterial and protozoan fractions were obtained and prepared according to the description of Or-Rashid (2007). In addition, 1 mL supernatant, collected for determining VFA profiles in media, mixed with 25% meta-phosphoric acid solution (HPO_3) in a ratio of 10:1 and stored in 4℃ until analysis.

Chemical and data analysis

VFA and methylated LCFA profiles were also determined by GC with the column of DB-FFAP and HP-88 (112-88A7), respectively. LCFA profiles in bacterial and protozoal biology membrane, were expressed as internal proportion value under the same condition, on the basis of that, some interesting fatty acid ingredients were calculated, the calculative details are presented in **Table 4**.

Table 4 comes about here

Statistical analyses were performed using the GLM procedure of SPSS (2011) and means within standard errors were compared with least squares means. Least squares means were reported throughout the text, and statistical significances were declared if $P < 0.05$.

Conclusion

Exogenous C18:3 increased the proportions of PUFA, *e.g.*, C18:2 n6c, C18:2 n6t, C18:1 n9c, C18:1 n9t and *cis*- fatty acids in both of bacteria and protozoan membrane as well as slightly improves acetic and propionic acid concentrations, compared to other supplementations. Coconut oil supplementation enhanced MCFA in bacterial and protozoa membrane, proved that they can synthesis MCFA from exogenous C12:0 compared to others oils. However, both of safflower oil and canola oil increased the saturated FA, saturated/unsaturated FA, *trans*- FA, *even*-carbon unsaturated FA in bacterial and protozoan membrane, acetic and propionic acid concentrations, decreased the Ac/Pro ration. In brief, the results implies that different exogenous FA can impact on ruminal microbe fermentation performance by changing FA compositions in bacterial and protozoan membrane, further to influence their microbial activity.

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Conflict of interest

All authors declare no conflict of interest.

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315 **Lists of Tables**

316 **Table 1.** LCFA profiles of bacterial and protozoal fraction supply of plant-derived oils (%)

Name of fatty acids	Chemical structure	Bacteria fraction				Protozoan fraction				Averages	
		Safflower	Canola	Linseed	Coconut	Safflower	Canola	Linseed	Coconut	bacteria	Protozoa
1. Lauric Acid	C12:0	1.31	0.46	2.82	18.56	0.46	0.61	0.45	11.07	5.79	3.15
2. Myristic Acid	C14:0	2.64	1.89	1.87	7.30	0.74	0.24	0.70	6.99	3.42	2.17
3. Myritoleic Acid	C14:1	1.42	2.24	1.89	1.38	0.33	0.23	0.56	1.50	1.73	0.66
4. Pentadecanoic Acid	C15:0	1.81	2.02	1.87	1.24	0.56	0.64	0.50	1.06	1.73	0.69
5. cis-10-Pentadecenoic acid	C15:1	0.60	1.11	0.90	0.98	0.38	0.11	0.28	0.34	0.90	0.28
6. Palmitic Acid	C16:0	26.13	20.76	20.26	18.80	13.29	14.36	15.73	22.91	21.49	16.57
7. Palmitoleic Acid	C16:1	0.96	0.85	0.94	1.39	0.56	1.02	1.08	1.55	1.04	1.05
8. Heptadecanoic acid	C17:0	2.13	2.28	3.75	2.24	0.98	1.62	1.24	2.56	2.60	1.60
9. cis-10-Heptadecenoic acid	C17:1	0.30	0.47	0.47	0.19	0.37	0.11	0.28	0.51	0.36	0.32
10. Stearic acid	C18:0	45.85	45.34	40.50	35.55	68.20	65.38	59.96	38.04	41.81	57.90
11. Elaidic acid	C18:1 n9t	5.86	5.93	4.82	5.20	6.66	4.82	6.04	5.80	5.45	5.83
12. Oleic acid	C18:1 n9c	5.82	7.61	12.31	3.69	4.36	7.96	7.65	4.59	7.36	6.14
13. Linolelaidic acid	C18:2 n6t	0.67	0.55	1.10	0.83	0.61	1.20	3.02	0.64	0.78	1.37
14. Linoleic acid	C18:2 n6c	2.37	2.09	2.50	1.09	1.41	0.75	0.95	1.07	2.01	1.04
15. Arachidic acid	C20:0	0.99	4.21	2.27	1.04	0.80	0.61	0.73	0.91	2.13	0.76
16. γ-Linolenic acid	C18:3 n6	1.16	2.18	1.73	0.53	0.29	0.35	0.83	0.47	1.40	0.48
a) saturated fatty acids, %		80.85	76.96	73.34	84.72	85.03	83.46	79.31	83.55	78.97	82.84
b) Long chain fatty acids ≥ 18 C, %		62.71	67.91	65.23	47.93	82.33	81.06	79.18	51.51	60.95	73.52
c) 18 carbons unsaturated fatty acids, %		14.72	16.18	20.72	10.81	13.04	14.72	17.66	12.09	15.61	14.38
d) *cis*-fatty acids, %		8.20	9.70	14.81	4.79	5.76	8.71	8.60	5.65	9.37	7.18
e) *trans*-fatty acids, %		6.52	6.48	5.91	6.03	7.27	6.01	9.06	6.43	6.24	7.20
f) Ratios (*cis*-/*trans*-)		1.26	1.50	2.50	0.79	0.79	1.45	0.95	0.88	1.50	1.00
g) Ratios (Stearic acid: Palmitic acid)		1.75	2.18	2.00	1.89	5.13	4.55	3.81	1.66	1.95	3.49
h) *odd*-carbon fatty acids, %		4.85	5.88	6.99	4.64	2.29	2.48	2.30	4.48	5.59	2.89
i) *even*-carbon saturated fatty acids, %		76.91	72.66	67.72	81.25	83.49	81.20	77.56	79.92	74.64	80.54
j) *even*-carbon unsaturated fatty acids, %		18.25	21.45	25.29	14.11	14.22	16.32	20.13	15.61	19.77	16.57
k) Saturated/unsaturated fatty acids		4.22	3.34	2.75	5.55	5.68	5.05	3.83	5.08	3.75	4.83

317 **Table 2.** VFA profiles *in vitro* fermented with four exogenous plant oils at 48h ((μmol·ml^{-1}))

Volatile fatty acids (VFA)	VFA values as supplementation of four exogenous plant-derived oils					
	Blank	Control	Safflower	Linseed	Canola	Coconut
Acetic acid	17.7	47.0 ± 0.47	50.9 ± 0.63	52.4 ± 0.58	51.5 ± 0.42	47.2 ± 0.10
Propionic acid	4.8	12.4 ± 0.15	13.9 ± 0.21	14.5 ± 0.20	14.3 ± 0.12	13.6 ±0.15
Isobutyric acid	0.9	1.2 ± 0.03	1.4 ± 0.04	1.3 ± 0.09	1.4 ± 0.08	1.0 ± 0.02
Butyric acid	4.8	7.3 ± 0.09	8.3 ± 0.16	8.7 ± 0.14	8.7 ± 0.09	8.2 ± 0.15
Isovaleric acid	2.3	2.1 ± 0.07	2.6 ± 0.15	2.7 ± 0.05	3.3 ± 0.01	2.6 ± 0.21
Valeric acid	1.4	1.8 ± 0.03	2.9 ± 0.10	3.0 ± 0.04	3.1 ± 0.02	2.8 ± 0.15
Ac/Pro	3.69	3.78	3.66	3.62	3.59	3.47

318 **Table 3.** The fatty acid compositions of four exogenous plant-derived oils

Name of fatty acids	Chemical Structure	The name of plant-derived oils (%)			
		Safflower	Linseed	Canola	Coconut
1. Lauric Acid	C 12:0	0.85	1.31	1.17	59.78
2. Myristic Acid	C14:0	0.49	0.61	0.65	19.97
3. Pentadecanoic Acid	C15:0	0.26	0.44	0.51	0.59
4. Palmitic Acid	C 16:0	6.79	5.90	5.59	8.63
5. Palmitoleic Acid	C16:1	0.16	0.10	0.27	0.00
6. *cis*-10-heptadecanoic acid	C17:1	0.06	0.00	0.00	0.00
7. Stearic acid	C18:0	2.76	4.22	2.19	3.33
8. Elaidic acid	C18:1 n9t	0.07	0.09	0.11	0.04
9. Oleic acid	C18:1 n9c	16.23	20.92	59.60	5.29
10. Linolelaidic acid	C18:2 n6t	0.09	0.00	0.00	0.00
11. Linoleic acid	C18:2 n6c	69.79	14.90	20.48	1.36
12. Arachidic acid	C20:0	0.39	0.00	0.49	0.11
13. γ-Linolenic acid	C18:3 n6	0.00	50.15	0.00	0.00
14. cis-11-Eicosadienoic acid	C20:1	0.24	0.26	1.12	0.00
15. Linolenic acid	C18:3 n3	0.18	0.00	4.98	0.00
16. Heneicosanoic acid	C21:0	0.94	0.19	0.38	0.00
17. Behenic acid	C22:0	0.21	0.14	0.36	0.00
18. *cis*-11,14,17-Eicosatrienoic acid	C20:3 n3	0.04	0.12	0.01	0.08
19. Arachidonic acid	C20:4 n6	0.06	0.11	0.14	0.33
20. Tricosanoic acid	C23:0	0.17	0.22	0.16	0.48
21. *cis*-13,16-Docosadienoic acid	C22:2	0.09	0.18	0.13	0.00
22. Lignoceric acid	C24:0	0.12	0.14	0.13	0.00
23. *cis*-5,8,11,14,17-Eicosapentaenoic acid	C20:5 n3	0.00	0.00	1.21	0.00
24. Nervonic acid	C24:1	0.00	0.00	0.33	0.00

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320 **Table 4.** The calculation details of some types of fatty acids with biological functions

Fatty acid items	Calculation details or functions
a) Saturated fatty acids	= *sum* (C12:0, C14:0, C15:0, C16:0, C17:0, C18:0 and C20:0)
b) Long chain fatty acids ≥ 18 C, %	All fatty acids composited with equal or larger than 18 carbons
c) 18 carbons unsaturated fatty acids, %	= *sum* (C18:1 n9t, C18:1 n9c, C18:2 n6t, C18:2 n6c, and C18:3 n6)
d) *cis*-fatty acids, %	= *sum* (C18:1 n9c, C18:2 n6c)
e) *trans*-fatty acids, %	= *sum* (C18:1 n9t, C18:2 n6t)
f) Ratios (*cis*-/*trans*-)	= *sum* (C18:1 n9c, C18:2 n6c)/*sum* (C18:1 n9t, C18:2 n6t)
g) Ratios (Stearic acid: Palmitic acid)	= C18:0/C 16:0
h) *odd*-carbon fatty acids, %	= *sum* (C15:0, C15:1, C17:0, C17:1)
i) *even*-carbon saturated fatty acids, %	= *sum* (C12:0, C14:0, C16:0, C18:0, C20:0)
j) *even*-carbon unsaturated fatty acids, %	= *sum* (C14:1, C16:1, C18:1 n9t, C18:1 n9c, C18:2 n6t, C18:2 n6c, C18:3 n6)
k) Saturated/unsaturated fatty acids	= *sum* (C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0)/*sum* (C14:1, C15:1, C16:1, C17:1, C18:1 n9t, C18:1 n9c, C18:2 n6t, C18:2 n6c, C18:3 n6)

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CAPÍTULO 4

Yong Liu, Germán Buendía-Rodríguez, Claudia Giovanna Peñuelas-Rívas, Zhiliang Tan, María Rívas-Guevara, Esvieta Tenorio-Borroto, Cristian R. Munteanu, Alejandro Pazos, Humberto González-Díaz. Experimental-Computational Studies of Fatty Acids Distribution Networks. *Molecular BioSystems*. 2015 (In press)

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Experimental and computational studies of fatty acid distribution networks

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Unbalanced uptake of Omega 6/Omega 3 (ω-6/ω-3) ratios could increase chronic disease occurrences, such as inflammation, atherosclerosis, or tumor proliferation, and methylation methods for measuring the ruminal microbiome fatty acid (FA) composition/distribution play a vital role in discovering the contribution of food components to ruminant products (*e.g.*, meat and milk) when pursuing a healthy diet. Hansch's models based on Linear Free Energy Relationships (LFERs) using physicochemical parameters, such as partition coefficients, molar refractivity, and polarizability, as input variables (V_k) are advocated. In this work, a new combined experimental and theoretical strategy was proposed to study the effect of ω-6/ω-3 ratios, FA chemical structure, and other factors over FA distribution networks in the ruminal microbiome. In step 1, experiments were carried out to measure long chain fatty acid (LCFA) profiles in the rumen microbiome (bacterial and protozoan), and volatile fatty acids (VFAs) in fermentation media. In step 2, the proportions and physicochemical parameter values of LCFAs and VFAs were calculated under different boundary conditions (c_j) like c_1 = acid and/or base methylation treatments, c_2 = with/without fermentation, c_3 = FA distribution phase (media, bacterial, or protozoan microbiome), *etc.* In step 3, Perturbation Theory (PT) and LFER ideas were combined to develop a PT-LFER model of a FA distribution network using physicochemical parameters (V_k), the corresponding Box Jenkins (ΔV_{kj}) and PT operators ($\Delta\Delta V_{kj}$) in statistical analysis. The best PT-LFER model found predicted the effects of perturbations over the FA distribution network with sensitivity, specificity, and accuracy > 80% for 407 655 cases in training + external validation series. In step 4, alternative PT-LFER and PT-NLFER models were tested for training Linear and Non-Linear Artificial Neural Networks (ANNs). PT-NLFER models based on ANNs presented better performance but are more complicated than the PT-LFER model. Last, in step 5, the PT-LFER model based on LDA was used to reconstruct the complex networks of perturbations in the FA distribution and compared the giant components of the observed and predicted networks with random Erdős–Rényi network models. In short, our new PT-LFER model is a useful tool for predicting a distribution network in terms of specific fatty acid distribution.

1. Introduction

The ω-6/ω-3 ratio plays an important role not only in the pathogenesis of cardiovascular diseases, but also in cancer, inflammatory and autoimmune diseases.[1–3] A high ω-6/ω-3 ratio is considered detrimental for human health, a value close to 1 is considered protective against the degenerative pathologies.[4] The inconsistent results[5–8] combined with meta-analysis methods reported the contributions of ω-3 fatty acids to cardio- and cerebrovascular diseases, inflammation, or tumor proliferation. Some researchers tend to explain the metabolism mechanism not only in terms of absolute amounts of ω-6 and ω-3, but also their balance.

Enrichment of ruminant meat or milk with ω-3 PUFAs, further to decrease ω-6/ω-3 uptake ratios, is an efficient method

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to introduce these beneficial PUFAs into diet, but the biohydrogenation process of ruminal complexes limits their bioavailability.[9] Petit *et al.* reported adding whole linseeds rich in ALA to the ratios of dairy cows, which resulted in the lowest ω-6/ω-3 ratio in milk compared to micronized soybeans or sunflower seeds.[10] Hess *et al.*[11] proved that the incorporation of ω-3 PUFAs into animal blood and muscle depends directly on the dietary supply of specific fatty acid. In addition, the long chain fatty acids (LCFAs) have to be methylated by acid- and/or base-methylation before determining it using a gas chromatograph (GC). There are different methylation methods for measuring LCFAs of milk, muscle or ruminal microbial membranes,[12–14] accompanied by generating different results. The structure properties of LCFAs (especially the number, location or topology structure of double bonds) are highly related to the chronic disease. To address this problem, it was postulated that the LCFAs in ruminal microbial membranes change with the supply of ω-6/ω-3 ratios. This work is aimed to look for a new classification model by means of Chemoinformatics, combined with an original experimental fatty acid distribution in ruminal microbial membranes.

On the other hand, Chemoinformatics is related to Machine Learning, Chemometrics and Bioinformatics,[15] and it combines the scientific working fields of Chemistry, Information Science, and the areas of topology, chemical graph theory, and data mining in the chemical space. Corwin Hansch was one of the founders of modern Chemoinformatics, which is based on the lipophilicity-activity relationship. A type of Hansch model is as follows:[16]

$$f(\varepsilon_i) = a_0 + a_1 \cdot \log P_i + a_2 \cdot \mathrm{p}K_a + a_3 \cdot \mathrm{MR} - a_4 \cdot (\log P_i)^2 \qquad (1)$$

It is well known that steric, electrostatic, and hydrophobicity factors may be biologically relevant.[17,18] In this equation, the different parameters can be used as inputs to account for the factors: such as water/*n*-octanol partition coefficients (P_i), molecular refractivity (MR), logarithmic acidity constants ($\mathrm{p}K_a$), and other physicochemical parameters to quantify different global molecular properties.[19] The outputs of the model are the values of a molecular property (ε_i) or a function of this property $f(\varepsilon_i)$ for a given chemical compound or molecular entity (m_i). The innovations of these models are described as follows. (1) The use of the linear regression to seek multivariate linear equations is able to predict the values of $f(\varepsilon_i)$, employing several input variables. (2) Hansch also generalized the use of lipophilicity parameters by the formulation of parabolic models for non-linear relationships. (3) The logarithmic terms ($\log P_i$) of P_i are commonly used as the measures of molecular lipophilicity and play an important role in the model. In turn, $\log P_i$ values can be predicted either by atomic methods (like $X \log P$ or $A \log P$) or by chemical fragment methods (like $C \log P_i$ or similar methods).[20,21] From a physical-chemistry point of view, Hansch's model is an extra-thermodynamic approach closely related to Linear Free Energy Relationships (LFERs).[22,23] The designation of Hansch's models as LFER equations comes from the use of parameters depending on Gibbs free energy (G_i) of the *i*th process.[24] The changes in the values of this potential during a process obey a logarithmic statistical thermodynamic relationship with equilibrium constant K_i.[25]

$$\Delta G_i = -RT \log(K_i) \qquad (2)$$

However, in these types of equations, other physicochemical parameters or molecular descriptors can also be used to quantify the effect of changes on the chemical structure over a characteristic of interest. It means that molecular descriptors for a given molecule can be used, which are not only thermodynamic constants, but also other theoretical measures of molecular lipophilicity, electronegativity, polarizability, or molecular topology properties,[19] *etc.* The values of these input variables (^iV_k) may be calculated as physicochemical parameters or molecular descriptors of different types (k) for a given molecule (m_i). In fact, the notation can be extended including extra-thermodynamic functions or parameters as follows.

$$f(\varepsilon_i) = \sum_{j=1}^{j_{max}} a_k \cdot {}^iV_k + \sum_{j=1}^{j_{max}} b_j \cdot \left({}^iV_k\right)^2 + e_0 \qquad (3)$$

Overall, the basic assumption of Hansch's analysis is that similar molecules have similar activities.[26–28] This principle is also called the Structure–Activity Relationship (SAR). The SAR paradox refers to the fact that not in all cases similar molecules have similar activities. The underlying problem is therefore how to define a *small* difference at a molecular level. The problem is relevant since each kind of property, *e.g.*, solubility, reactivity, or metabolism, is expected to depend on another difference. It means that "*small*" variations or perturbations need to be quantified at the molecular structural level, which in turn implies a "*small*" linear change in the free energy of interaction of a drug with a receptor.

In our opinion, the ideas of the Perturbation Theory (PT)[29] can be used to account for this problem in the context of Chemoinformatics. That is why in this work PT and LFER ideas were used to formulate a new PT-LFER approach. This PT-LFER approach is a generalization of the classic Hansch Extra-Thermodynamics method for Chemoinformatics. The proof-of-concept was also demonstrated by an experimental–theoretical study on complex networks of FA distribution in Lipidomics. To this end, first the experiments were carried out to determine LCFA composition in the ruminal microbiome. Next, the Chemoinformatics study was included, starting with the definition, training, and validation of new PT-LFER classification models. Machine Learning methods such as Artificial Neural Networks (ANNs) were used to test PT-NLFER models (non-linear analogues of PT-LFER). Next, the best PT-LFER model found was used to predict the effect of perturbations on initial boundary conditions over a large complex network of FA distribution/uptake in the ruminal microbiome. The observed complex network for the data reported was constructed and compared for the first time with the predicted network and model random networks of similar size. Last, the theoretical section was completed with a comparative study of the PT-LFER classification model found using other non-linear models. This study was of major relevance due to previous results that point to a strong relationship between ω-6/ω-3 ratios of

FA intake and human health.[30,31] Accordingly, this work paves the way for evaluating the effect of perturbations on complex molecular systems involved in chemical structures and boundary experimental conditions.

2. Materials and methods

2.1. Experimental section

In the workflow of this experimental part (Fig. 1), the general details of the experimental procedures used in experiment 1 and experiment 2 are explained as follows.

2.1.1. Animal welfare. Three adult male Pelibuey sheep with permanent rumen-fistula (body weight, 45.0 ± 5.0 kg) were used as an inoculum donor according to the Mexican Official Standard (NOM-220-SSA1-2002). Nutritional composition of fodder for animal donors was according to the National Research Council (NRC).[32] All the animal procedures and protocols were approved by the Animal Care Committee, National Center for Disciplinary Research in Animal Breeding and Physiology (CENID FyMA), and National Institute of Forestry, Agriculture and Livestock (INIFAP), Queretaro, Mexico.

2.1.2. Details of *in vitro* fermentation. The *in vitro* details are according to the description of Tang *et al.*,[33] with the particle-free rumen fluid mixed with the artificial saliva buffer solution[34] in a proportion of 1 : 2 (v/v) at 39 °C under continuous flushing of CO_2. Microbial fatty acids were prepared according to the method developed by Or-Rashid.[14] More specifically, the microbial and protozoan samples were separated by differential centrifugation according to the method described by Legay-Carmier and Bauchart.[35]

2.1.3. Specific procedures of experiment 1. Ruminal mixed microbes without fermentation were catalyzed by acid methylation (8% HCl (w/v) dissolved in methanol/water (85/15)),[12] base methylation (trimethylsilyldiazomethane, TMSD),[14] combined acid- and base-methylations (first catalyzed with 8% HCl, and subsequently catalyzed with TMSD), respectively. The values of Peak Area, $PA_{(i)}$, for each LCFA under different sets of experimental conditions c_j (different samples) were determined by GC[14,36] (Model 6890N, Agilent Technologies Inc., USA) using a HP-88 Column at laboratory of CENID FyMA, INIFAP, and VFAs were determined using a DB-FFAP column. The values of peak area obtained were used to calculate the internal peak area, IPA(%), as follows.

$$\mathrm{IPA}(\%)_{ij} = 100 \cdot \left(\frac{\mathrm{PA}_{(i)}}{\sum_{m_i \subset c_j}^{n_j} \mathrm{PA}_{(i)}} \right) \quad (4)$$

2.1.4. Specific procedures of experiment 2. This study was conducted to evaluate the effect of various exogenous ω-6/ω-3 ratios on the biohydrogenation metabolism of the microbial microbiome. The ω-6 and ω-3 PUFAs, linoleic acid (LA, L1376-5g, Sigma-Aldrich) and α-linolenic acid (ALA, L2376-500 mg, Sigma-Aldrich) with a total amount of 100 mg g^{-1} in substrates were set at the ratios of 100 : 0, 90 : 10, 80 : 20, 66 : 33, 50 : 50 and 20 : 80, respectively.

Food components used to feed the animals were the same as those used in experiment 1. All fermentation lipid samples extracted with a chloroform–methanol mixture (2 : 1, v/v)[37] from bacterial and protozoan fractions were catalyzed by base methylation (TMSD, herein).[14] LCFA profiles extracted from bacterial and protozoan fractions and the VFA profiles were determined to calculate IPA(%), and the concentration (mM) of VFA profiles was also calculated.

2.2. Theoretical section

2.2.1. Workflow used for the PT-LFER Chemoinformatics study. In the second section, a Chemoinformatics study of the results obtained in the Experimental section was carried out. Fig. 2 shows the workflow diagram that states the integration of both (experimental and theoretical) sections. For the analysis, the chromatographic data about IPA(%) values of fatty acids were collected under different ω-6/ω-3 ratios and experimental conditions c_j. Next, we defined the PT-LFER model. After that, we calculated the values of input variables, including molecular descriptors (^iV_k) of class k for every ith fatty acid molecule, and perturbation operators $\Delta\Delta V_k(c_j)$. After that we performed the statistical analysis and obtained the PT-LFER model. More details are explained in some steps as follows.

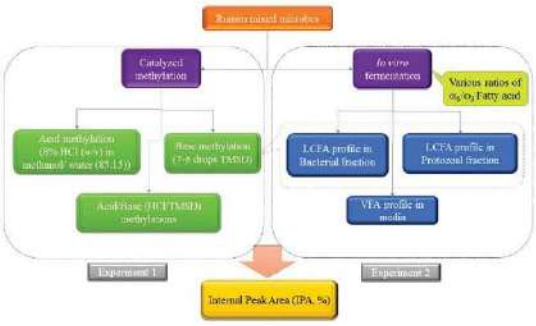

Fig. 1 Workflow of the experimental section (dataset): IPA values of each FA based on bacterial membrane FAs catalyzed by methylation methods (experiment 1) and IPA values obtained from bacterial, protozoan, and media fractions by the fermentation of various exogenous ω-6/ω-3 ratio supplementation by base methylation (experiment 2).

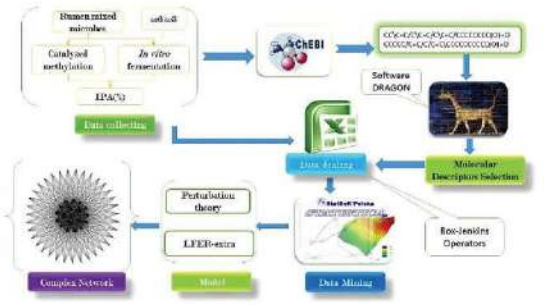

Fig. 2 Workflow used herein to seek PT-LFER models.

2.2.2. Theoretical details of the PT-LFER models. In a recent study, Gonzalez-Díaz *et al.*[29] has formulated a general-purpose PT model for multiple-boundary Chemoinformatics problems. In this work, this theory is extended to the study of PT-LFER models of perturbations in complex networks. Let a general function $f(L_{nr})$ be useful to quantify the occurrence ($L_{nr} = 1$) or not ($L_{nr} = 0$) of a process involving a set of molecules (m_i) in a complex system. It is considered that all the possible states form a network of states. The network nodes are the initial or reference states (r) linked to their respective final or new states (n) reached by the system after a perturbation of the initial conditions. It separates into a set of multiple initial experimental boundary conditions ${}^{ref}c_j \equiv (c_0, c_1, c_2, c_3, \ldots, c_n)$ (conditions of reference) and a different or new set of boundary conditions ${}^{new}c_j \equiv (c_0, c_1, c_2, c_3, \ldots, c_n)$ (conditions of new) after one or multiple perturbations (changes in these conditions). The PT-LFER model proposed herein is a linear equation with the following form:

$$'f(L_{nr})_{new} = a_0 + a_1 \cdot f(\varepsilon_{ij})_{ref} + a_2 \cdot \langle f(\varepsilon_{ij}) \rangle_{ref} + \sum_{j=1,k=1}^{j=j_{max},k=k_{max}} a_{kj} \cdot \Delta\Delta V_k(c_j) \quad (5)$$

The output function $'f(L_{nr})_{new}$ is a score used in Linear Discriminant Analysis (LDA) to calculate the outputs or *posteriori* probability of binary classification of inputs $L_{nr} = 1$ or $L_{nr} = 0$.[38] The vectors $v_i = [f(\varepsilon_{ij})_{ref}, \langle f(\varepsilon_{ij}) \rangle_{ref}, \omega\text{-}3, \omega\text{-}6, {}^iV_1, \ldots, {}^iV_{k_{max}}, \Delta V_1(c_1), \ldots, \Delta V_k(c_j), \ldots, \Delta V_{k_{max}}(c_{j_{max}}), \Delta\Delta V_1(c_1), \ldots, \Delta\Delta V_k(c_j), \ldots, \Delta\Delta V_{k_{max}}(c_{j_{max}})]$ are the inputs of this model. Each vector, v_i, represents a statistical case (ith case) out of a total of $n = 407\,655$ cases (perturbations). These statistical cases encoded by v_i vectors are perturbations of one entry or state of reference (changes in input parameters) that yield an output or new state. The input vectors v_i include the value of $f(\varepsilon_{ij})_{ref}$ for the state of reference (known value). The vectors v_i also take into account the amounts of ω-6 and ω-3 for the new state (after perturbation). The values of molecular descriptors (^iV_k) used in a classic Hansch analysis were also included. Last, the inputs also consider the values of the PT-LFER operators $\Delta\Delta V_k(c_j)$.

2.2.3. Calculation of molecular descriptors. In the first work of this series, we used the mean values of atomic electronegativity of the chemical structure descriptors (^iV_k) of a drug.[39] In another recent work, the method for the prediction of peptide epitopes was adapted using the perturbation theory.[40] In the present work, the previous PT models are extended to other directions. Herein, PT models and Hansch's LFER equations are combined to carry out a PT-LFER analysis for the first time. To this end, the following steps were taken. First, structural variables (^iV_k) were used as a new set of molecular descriptors. The values of these variables were calculated using the DRAGON software.[41–43] The first molecular descriptor calculated was V_1 = Mw (Molecular weight). The molecular descriptors V_2 = AEigv, V_3 = AEige, and V_4 = AEigp were included, which are the average eigenvalues of the topological distance matrices weighted with atomic van der Waals volumes (v), polarizabilities (p), or electronegativities (e). Last, V_5 = MR (Molecular Refractivity) and $V_6 = \log P$ (logarithm of the *n*-octanol/water partition coefficient) were also proposed. The structures of fatty acids were uploaded to DRAGON in a form of Simplified Molecular-Input Line Entry System (SMILES) codes. SMILES codes are very useful to manage molecular structures[44–46] and for further calculations of molecular descriptors[21,47] (Table 1). In our work, the SMILES codes of corresponding fatty acids were downloaded from the website data of Chemical Entities of Biological Interest (ChEBI: http://www.ebi.ac.uk/chebi/).

2.2.4. Calculation of PT operators. When the previous equation of the PT-LFER model was expanded, two types of input terms can be observed. The first type of term is the function $f(\varepsilon_{ij})_{ref}$. This function takes the values, $f(\varepsilon_{ij})_{ref} = \langle \varepsilon_{ij} \rangle_{ref} = IPA(\%)_i$ for each sample. $IPA(\%)_i = 100\text{-}(PA_i/PA_{j_{max}})$ is the internal peak area proportion, used to quantify the experimental proportion of a fatty acid determined by GC. It means that $f(\varepsilon_{ij})_{ref}$ is the measured value of the proportion of a fatty acid under the same conditions c_j. The second class refers to the perturbation terms $\Delta\Delta V_k(c_j)$. The parameters $\Delta\Delta V_k(c_j)$ are useful to quantify the effect of perturbations of different boundary conditions (c_j) over the output $'f(L_{nr})_{new}$, which was defined herein as a discrete value function (occurrence or not of links in the network) for the classification purposes. The difference $\Delta\Delta V_k(c_j)$ between the final or new state ($\Delta V_k(c_j)_{new}$) and the initial or reference state ($\Delta V_k(c_j)_{ref}$) is the additive perturbation for a component in $\Delta V_k(c_j)$. When the output of this equation is $'f(L_{nr})_{new} > 'f(L_{nr})_{ref} \Rightarrow L_{nr} = 1 \Rightarrow IPA(\%)_{new} > IPA(\%)_{ref}$ consequently ($\Rightarrow$) the distribution or proportion of the FA in the new state is higher than that in the reference state, otherwise $L_{nr} = 0$.

$$'f(L_{nr})_{new} = a_0 + a_1 \cdot 'f(L_{nr})_{ref} + a_1 \cdot \langle \varepsilon_{ij} \rangle_{ref} + \sum_{j=1,k=1}^{j=j_{max},k=k_{max}} a_{ijk} \cdot \Delta({}^iV_k - \langle V_k(c_j) \rangle) \quad (6)$$

$$\Delta\Delta V_k(c_j) = p(c_j)_{new} \cdot ({}^iV_k - \langle V_k(c_j) \rangle)_{new} - p(c_j)_{ref} \cdot ({}^iV_k - \langle V_k(c_j) \rangle)_{ref} \quad (7)$$

2.2.5. Calculation of Box–Jenkins operators. A close inspection of the perturbation terms shows that they are probability-weighted differences (Δ) of Box–Jenkins operators $\Delta V_k(c_j)$. The values of Box–Jenkins operators $\Delta V_k(c_j)$ of the molecular descriptors (^iV_k) were calculated to quantify the effect of deviations of a molecule (m_i) from the average behavior of all molecules measured under the same set of conditions (c_j) of the complex system. Deviations due to the changes in different boundary conditions (c_j) were taken into account. The boundary conditions refer to preliminary operational conditions, c_1 refers to the use of different experimental treatments, c_2 is with/without fermentation, c_4 is the gas chromatography protocol used, and c_5 refers to the use of replicate experiments. Others are more directly related to the posterior distribution and nature of the FA, c_3 is the biological phase of distribution of LCFAs, and c_6 quantifies information about the *cis*/*trans* geometric pattern present in the LCFAs. All data were processed in an Excel file.

Table 1 Molecular descriptors (^iV_k) of fatty acids obtained from the ChEBI database

Name of fatty acids in ChEBI[a]	*cis/trans* pattern[b]	Molecular descriptors of FA-inputs[c]					
		V_1	V_2	V_3	V_4	V_5	V_6
Lauric acid	*t*	200.4	137.4	127.6	139.5	58.7	4.5
Myristic acid	*t*	228.4	179.1	169.0	181.2	67.9	5.5
Myristoleic acid	*c*	226.4	172.1	162.0	174.2	69.0	5.0
Pentadecanoic acid	*t*	242.5	202.0	191.8	204.1	72.5	5.9
cis-10-Pentadecenoic acid	*c*	240.4	194.8	184.5	196.9	73.6	5.5
Palmitic acid	*t*	256.5	226.3	216.0	228.4	77.1	6.4
Palmitoleic acid	*c*	254.5	217.9	207.6	220.1	78.2	5.9
Heptadecanoic acid	*t*	270.5	252.0	241.6	254.1	81.7	6.8
Stearic acid *cis*-10-heptadecenoic acid	*c*	268.5	243.2	232.8	245.4	82.8	6.4
Stearic acid	*t*	284.5	279.1	268.6	281.2	86.3	7.3
Elaidic acid	*t*	282.5	269.5	259.0	271.7	87.4	6.8
Oleic acid	*c*	282.5	269.5	259.0	271.7	87.4	6.8
Linolelaidic acid	*tt*	280.5	260.9	250.4	263.1	88.5	6.4
Linoleic acid	*cc*	280.5	260.9	250.4	263.1	88.5	6.4
Arachidic acid	*t*	312.6	337.4	326.8	339.6	95.5	8.2
γ-Linolenic acid	*ccc*	278.5	251.5	240.9	253.7	89.6	5.9
Linolenic acid	*ccc*	278.5	255.1	244.5	257.3	89.6	5.9
cis-11.14-Eicosadienoic acid	*ct*	308.6	318.0	307.3	320.2	97.7	7.3
Behenic acid	*t*	340.7	401.3	390.6	403.5	104.7	9.1
cis-8.11.14-Eicosatrienoic acid	*ctt*	306.5	307.4	296.7	309.7	98.8	6.9
Erucic acid	*t*	338.6	390.2	379.4	392.4	105.8	8.7
Acetic acid	*t*	60.1	13.1	6.4	14.4	12.6	−0.2
Propionic acid	*t*	74.1	19.2	11.8	20.7	17.3	0.4
Isobutyric acid	*t*	88.1	24.6	16.7	26.2	21.8	0.9
Butyric acid	*t*	88.1	26.7	18.8	28.3	21.9	0.9
Isovaleric acid	*t*	102.2	33.2	24.9	34.9	26.4	1.1
Valeric acid	*t*	102.2	35.6	27.4	37.4	26.5	1.3

[a] Fatty acids measured for our linear discriminant analysis PT-LFER model. [b] *cis/trans* pattern, *t* represents *linear*, and *c* represents *cis*-, and *t* represents *trans*-PUFAs. The order of *c* or *t* represents the order of initial isomerization characteristics with the tails of PUFAs. [c] Molecular descriptors (^iV_k) calculated using DRAGON software: V_1 = Mw, V_2 = Aeigv, V_3 = Aeige, V_4 = Aeigp, V_5 = AMR, and V_6 = log *P*.

In the Excel, the values of $\Delta V_k(c_j)$ were calculated considering various experimental boundary conditions $\{c_j\}$. The probabilities are $p\{c_j\} = n_j/n_{\text{total}}$; n_j is the number of experimental entries for condition c_j and $n_{\text{total}} = 744$ total number of experimental entries (the total number of IPA(%) measured in this work). The average value $\langle \Delta V_k(c_j) \rangle$ (Table 2) is the difference in the function value ^iV_k with the average molecular descriptors $\langle V_k \rangle$ for a specific boundary condition c_j, see the equations:

$$\Delta V_k(c_j) = \left({}^iV_k - \langle V_k(c_j) \rangle \right) \quad (8)$$

$$\langle V_k(c_j) \rangle = \frac{1}{n_j} \left(\sum_{m_i \subset c_j}^{n_j} {}^iV_k \right) \quad (9)$$

2.2.6. Dataset. Predicting the effect of perturbations in input conditions over the output properties is the aim of this model. For this, we need to infer the value of the property in a new set of conditions using a known experimental value as reference. It means that we need to predict the variation of the experimental properties for pairs of data cases (reference and new). Consequently, if we have an original dataset with n cases we need to explore a total of n^2 cases for an exhaustive investigation of the data space (all pairs of data). If n is large, the number of pairs increases notably. Consequently, we carry out a random sampling procedure. We generated as many as possible pairs of data that we can process with a MS Excel sheet selecting at random both the reference and the new state. If a random MS Excel function has been used to generate pairs of random numbers between one and n, the very high number of 407 655 perturbations was the higher number of pairs of cases we were able to handle in the Excel with our processing power.

2.2.7. Classification models. The Linear Discriminant Analysis (LDA)[38] algorithm implemented in the STATISTICA software was used to find the best PT-LFER model. Sometimes the relationship between the input variables and the output is more complex and the linearity cannot solve the problem. Therefore, the non-linear models could provide a better solution, but with the drawback of not being able to interpret the model and the relations between the variables. Thus, the Artificial Neural Networks (ANNs)[48] were tested: Linear Neural Networks (LNNs), which are similar to the LDA models, and non-linear Multi-Layer Perceptrons (MLPs).[38] The full datasets were randomly split into training series ("*t*", 75%) used for model construction and validation series ("*v*", 25%) used for model validation. In addition, a cross-validation variable was added to the dataset with the test values of "*t*" and "*v*". All independent variables were unified and standardized using the STATISTICA software, prior to model construction.

2.2.8. Complex network study. Both the observed and predicted networks were constructed in the Excel and saved in the .net (lists of pairs of nodes) file format. The links of the observed network coincide with the classes to be predicted by

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Table 2 Average values of input variables ($\langle V_k \rangle$) for experimental boundary conditions (c_j)

Experimental boundary condition		Average eigenvalues of input variables $\langle V_k(c_j) \rangle^g$						
Conditions (c_j)	Level	V_1	V_2	V_3	V_4	V_5	V_6	$p(c_j)^h$
$c_1 \Rightarrow$ treatments[a]	BM	274.4	258.3	247.9	260.5	84.8	6.6	0.113
	AM	274.4	258.3	247.9	260.5	84.8	6.6	0.113
	CM	274.4	258.3	247.9	260.5	84.8	6.6	0.113
	BA	261.9	234.6	224.3	236.8	80.3	6.2	0.258
	PA	261.9	234.6	224.3	236.8	80.3	6.2	0.258
	MA	85.8	25.4	17.7	27.0	21.1	0.7	0.145
$c_2 \Rightarrow$ fermentation[b]	0	274.4	258.3	247.9	260.5	84.8	6.6	0.339
	1	223.2	188.7	178.9	190.7	67.3	5.0	0.661
$c_3 \Rightarrow$ phase[c]	Bacterial fraction	269.0	248.1	237.7	250.3	82.9	6.4	0.597
	Protozoan fraction	261.9	234.6	224.3	236.8	80.3	6.2	0.258
	Media fraction	85.8	25.4	17.7	27.0	21.1	0.7	0.145
$c_4 \Rightarrow$ column of GC[d]	HP-88 (112-88A7)	266.8	244.0	233.6	246.2	82.1	6.4	0.855
	DB-FFAP	85.8	25.4	17.7	27.0	21.1	0.7	0.145
$c_5 \Rightarrow$ replicate (r-error)[e]	0	242.8	213.9	203.8	216.0	73.9	5.6	0.391
	0.1	242.8	213.9	203.8	216.0	73.9	5.6	0.391
	0.2	205.8	173.6	164.2	175.6	61.6	4.4	0.133
	0.3	274.4	258.3	247.9	260.5	84.8	6.6	0.085
$c_6 \Rightarrow$ *cis*/*trans* pattern[f]	*linear*	203.2	180.8	171.5	182.8	59.6	4.6	0.238
	cis	278.5	269.1	258.6	271.3	86.1	6.7	0.111
	trans	310.6	329.8	319.2	332.1	96.6	7.7	0.032
	trans, trans	280.5	260.9	250.4	263.1	88.5	6.4	0.016
	cis, cis	308.6	320.8	310.1	323.0	97.7	7.3	0.032
	cis, trans	289.9	279.6	269.0	281.8	91.6	6.7	0.048
	trans, cis	280.5	261.1	250.5	263.3	88.5	6.4	0.016
	cis, cis, cis	286.5	269.9	259.3	272.1	92.3	6.2	0.111
	cis, trans, trans	292.5	280.0	269.3	282.2	94.2	6.4	0.032
	trans, cis, trans	278.5	254.6	244.0	256.8	89.6	5.9	0.016
	trans, trans, trans	278.5	252.0	241.4	254.2	89.6	5.9	0.032
	cis, cis, trans	278.5	252.5	241.9	254.8	89.6	5.9	0.016
	cis, trans, cis	278.5	252.0	241.4	254.2	89.6	5.9	0.032

[a] "BM" means base methylation without fermentation; "AM" means acid methylation without fermentation; "CM" means acid- and base-combined methylation; "BA" means fatty acids from bacterial fraction after 48 h of fermentation; "PA" means fatty acids from protozoan fraction after 48 h of fermentation; "MA" means volatile fatty acids from media fraction after 48 h of fermentation. [b] "0" means the dataset from experiment 1 without fermentation; "1" means the dataset from experiment 2 with fermentation of omega 6 and omega 3. [c] "Phase", means the dataset: long chain fatty acids including from the bacterial membrane (bacterial fraction), protozoan membrane (protozoan fraction), volatile fatty acids from fermentation media (media fraction). [d] Column of GC, "HP-88 (112-88A7)" means the column of GC for determining long chain fatty acids; "DB-FFAP" means the column for determining volatile fatty acids. [e] "0" means the original data, "0.1, 0.2, or 0.3" means the 1, 2, or 3 replicates, respectively. [f] *cis*/*trans* pattern: "*linear*" means LCFAs without double bonds; "*cis*" means LCFAs with *cis* isomerization; "*trans*" means LCFAs with *trans* isomerization; and the number of *cis* or *trans* means LCFAs with the same number of *cis* or *trans* double bonds. [g] $\langle V_k(c_j) \rangle$ means the average of molecular descriptors (V_k) for different conditions (c_j); the descriptors are V_1 = Mw, V_2 = Aeigv, V_3 = Aeige, V_4 = Aeigp, V_5 = AMR, and V_6 = log P. [h] $p(c_j) = n_j/n_{\text{total}}$; n_j number of experimental entries for conditions c_j and n_{total} = 744 total number of experimental entries.

the previous LDA model. If the existence of a link corresponding to the condition $L_{nr} = 1$, means $\text{IPA}(\%)_{obs} > \text{IPA}(\%)_{ref}$ for each fatty acid at both the initial and final states; or $L_{nr} = 0$ otherwise. A number of pairs of states as high as possible was generated, calculating the existence of observed links with the previous rule, and they were also predicted using the model. These files were processed using the CentiBiN software described by Junker *et al.*,[49] to calculate the average indices of the topology of the network. The indices calculated were the average values of the vertex–vertex topological distance,[50] node degree, and closeness of the giant component of the observed, predicted and the two similar random ones. Two models of random networks (random networks 1 and 2) were also built. The model of the random network selected was the Erdös–Rényi graph (ER), which is often used as a random network model.

3. Results and discussion

3.1. Catalyzed methylation in ruminal microbes

Saponification followed by methylation is a classic method for the preparation of FA methyl esters. Table 3 shows the results obtained in the experimental determination of the values of IPA(%) after different acid-/base-methylations. This table reports the average and standard deviation (SD) values of IPA(%) for those fatty acids for the first time. In general, base-catalyzed methylation proceeds more rapidly under mild temperature conditions than acid-catalyzed reactions.[51]

3.2. LCFA profiles in bacteria and protozoan

This work is focused on the lipid metabolism of exogenous FAs by direct determination of the IPA(%) values of LCFA from

Table 3 Internal peak area values, IPA(%)[a], of LCFA profiles of ruminal mixed microbes by acid- and/or base-catalyzed methylation

Name of fatty acids		Average[b]			SD		
		B	A	A&B	B	A	A&B
Lauric acid	C12:0	0.55	0.52	1.87	0.07	0.16	0.76
Myristic acid	C14:0	1.06	1.24	2.12	0.05	0.26	0.69
Myritoleic acid	C14:1	1.95	2.31	2.38	0.11	0.49	0.69
Pentadecanoic acid	C15:0	1.20	1.47	1.60	0.02	0.35	0.43
cis-10-Pentadecenoic acid	C15:1	0.40	0.53	0.68	0.08	0.10	0.25
Palmitic acid	C16:0	17.91	18.86	19.70	0.54	1.73	0.98
Palmitoleic acid	C16:1	1.85	2.30	2.14	0.07	0.32	0.18
Heptadecanoic acid	C17:0	0.99	1.39	1.49	0.25	0.20	0.28
cis-10-Heptadecanoic acid	C17:1	0.00	0.17	0.53	0.00	0.20	0.29
Stearic acid	C18:0	49.76	49.17	40.21	1.02	1.62	0.80
Elaidic acid	C18:1 n9t	8.68	8.23	8.76	0.65	0.69	1.30
Oleic acid	C18:1 n9c	9.00	8.69	8.17	0.09	0.88	2.12
Linolelaidic acid	C18:2 n6t	0.65	0.74	0.85	0.04	0.05	0.64
Linoleic acid	C18:2 n6*c*	2.37	2.63	2.81	0.06	0.11	0.36
Arachidic acid	C20:0	0.84	0.68	1.23	0.17	0.07	1.50
γ-Linolenic acid	C18:3 n6	0.45	0.58	0.80	0.06	0.14	0.34
Linolenic acid	C18:3 n3	0.00	0.00	1.52	0.00	0.00	1.25
cis-11,14-Eicosadienoic acid	C20:2	0.34	0.14	0.75	0.67	0.16	0.63
Behenic acid	C22:0	0.59	0.13	1.33	0.15	0.15	0.78
cis-8,11,14-Eicosatrienoic acid	C20:3 n6	0.58	0.05	0.82	0.22	0.10	1.11
Erucic acid	C22:1 n9	0.82	0.17	0.24	0.42	0.19	0.47
Unsaturated fatty acids		27.10	26.55	30.44	1.46	1.08	3.29
Long chain fatty acids ≥ 18 carbons		74.07	71.20	67.50	0.95	3.51	3.76
18 carbons unsaturated fatty acids		21.15	20.88	22.91	0.68	1.46	4.03
cis-fatty acids		11.36	11.32	10.98	0.06	0.79	1.88
trans-fatty acids		9.34	8.97	9.61	0.61	0.64	1.89
Ratios (*cis*/*trans*)		1.22	1.26	1.14	0.07	0.05	0.11
Ratios (stearic acid : palmitic acid)		2.78	2.61	2.04	0.03	0.32	0.12
Odd-carbon fatty acids		2.60	3.56	4.30	0.31	0.59	0.85
Even-carbon saturated fatty acids		70.71	70.59	66.47	1.25	0.69	2.96
Even-carbon unsaturated fatty acids		26.70	25.85	29.23	1.52	1.13	3.76
Saturated/unsaturated fatty acids		2.69	2.77	2.28	0.19	0.15	0.40

[a] Internal peak area values, IPA(%), mean the relative proportion of different fatty acids in the corresponding individual sample. [b] "A" = acid methylation, "B" = base methylation, or "A&B" = acid methylation with subsequent base methylation. Average and standard deviation (SD) of IPA(%) values for long chain fatty acids.

ruminal microbe/protozoan biological membranes, including FAs from bacterial (Table 4) and protozoan (Table 5) biological membranes under different experimental conditions (c_j).

It is well known that the imbalance of ω-6/ω-3 ratios in the diet has the potential to induce inflammation, asthma, arthritis, and vascular diseases,[52] but high levels of ω-3 exert a suppressive effect.[53–56] As expected, the FA composition of ruminal bacterial and protozoan biological membranes, and VFAs in media was indeed changeable with the exogenous ω-6/ω-3 PUFA ratios. This study had no significant statistical difference in the main IPA(%) of fatty acids (*e.g.*, C16:0, C18:0), but some valuable information has still been extracted from the results. First of all, *cis*-FA content increased with exogenous ω-3 PUFAs, and *trans*-FAs decreased in the biological phase of bacteria. For example, the *cis*-FA profiles in ω-6/ω-3 = 20 : 80 were 1.76 times (bacterial phase) and 1.60 times (protozoan phase) than that in ω-6/ω-3 = 100 : 0, and *trans*-FA profiles in ω-6/ω-3 = 100 : 0 were 1.24 times (bacterial phase) and 0.98 time (protozoan phase) than that in ω-6/ω-3 = 20 : 80. This directly results in the increasing ratio of *cis*/*trans*-fatty acid compositions with the increase of the exogenous ω-3 PUFAs, such as 2.18 times in the bacterial phase and 1.58 times in the protozoan phase, when ω-6/ω-3 = 20 : 80 compared with ω-6/ω-3 = 100 : 0, respectively. It means that exogenous PUFAs are degraded by rumen microorganisms, or have more complex metabolism processes leading to intermediary metabolism with both *cis*- and *trans*-unsaturated FA formulations. The biohydrogenation of linoleic acid (LA, *cis* 9, *cis* 12-C18:2) in rumen is isomerized to *cis* 9, *trans* 11-C18:2 isomer (conjugated linoleic acid, CLA), conversion of this isomer to *trans* 11-C18:1 (vaccenic acid), and reduction to stearic acid (C18:0).[57] Whereas the bio-hydrogenation of α-linolenic acid (ALA) is characterized by isomerization to 9, 11, 15-*cis*, *trans*, *cis*-C18:3 isomer and subsequent reduction *via cis*, *trans* isomers C18:2, C18:1 and then to stearic acid.[58] This research showed that ω-3 PUFAs (α-linolenic acid) could increase the *cis*-FA content compared to ω-6 PUFAs (linoleic acid) on both of bacterial and protozoan phases.

Secondly, IPA values of C16:0 and C18:0 in the bacterial phase were 18.7% and 57.8%, whereas those in the protozoan phase were 13.3% and 67.5%, respectively. The exogenous ω-6/ω-3 ratios have no significant effect on these two major fatty acids in both bacterial and protozoan phases. However, the minor difference in lipid composition like ratios of palmitic/stearic acid, or unsaturated/saturated fatty acids on bacterial and protozoan biological membranes may trigger a great difference in the function of membranes of bacteria and protozoa (*e.g.*, membrane fluidity, permeability, hydrophobicity and stability),[59,60] or further

Table 4 Internal peak area values, IPA(%), of LCFA profiles in bacterial fraction[a]

	Various exogenous ω-6/ω-3 PUFA ratios (x:[100 − x]; in total of 100 mg g^{-1} substrate)						
Name of fatty acids	100-0	90-10	80-20	66-33	50-50	20-80	Average
Lauric acid	0.62	0.67	0.56	0.55	1.89	0.52	0.80
Myristic acid	1.53	1.45	1.31	1.27	1.71	1.23	1.42
Myritoleic acid	2.66	2.61	2.32	2.20	1.90	2.06	2.29
Pentadecanoic acid	2.13	1.86	1.82	1.68	1.53	1.61	1.77
cis-10-Pentadecenoic acid	0.83	0.73	0.64	0.58	0.45	0.44	0.61
Palmitic acid	18.38	18.73	18.82	18.82	18.79	18.39	18.65
Palmitoleic acid	1.45	1.91	1.97	1.29	1.72	1.94	1.71
Heptadecanoic acid	1.77	2.16	2.01	1.73	1.65	1.92	1.87
cis-10-Heptadecenoic acid	0.62	0.41	0.16	0.00	0.25	0.25	0.28
Stearic acid	56.93	56.17	58.64	58.97	58.22	57.58	57.75
Elaidic acid	5.95	6.61	5.71	5.59	4.89	5.01	5.63
Oleic acid	2.93	3.86	3.07	3.59	3.90	5.33	3.78
Linolelaidic acid	2.09	0.97	1.18	1.65	1.05	1.47	1.40
Linoleic acid	1.03	1.07	1.04	1.02	1.24	1.62	1.17
Arachidic acid	0.56	0.59	0.46	0.68	0.55	0.33	0.53
γ-Linolenic acid	0.53	0.22	0.28	0.37	0.25	0.31	0.33
Unsaturated fatty acids, %	18.07	18.38	16.38	16.29	15.66	18.43	17.20
Long chain fatty acids ≥18 carbons, %	70.02	69.49	70.39	71.87	70.09	71.65	70.58
18 carbon unsaturated fatty acids, %	12.52	12.73	11.28	12.22	11.33	13.74	12.30
cis-Fatty acids, %	3.95	4.93	4.12	4.61	5.13	6.95	4.95
trans-Fatty acids, %	8.04	7.58	6.89	7.24	5.94	6.48	7.03
Ratios (*cis*-/*trans*-)	0.492	0.650	0.598	0.637	0.864	1.072	0.72
Ratios (stearic acid : palmitic acid)	3.098	2.999	3.116	3.134	3.098	3.131	3.10
Odd-carbon fatty acids, %	5.34	5.15	4.63	4.00	3.89	4.22	4.54
Even-carbon saturated fatty acids, %	78.03	77.60	79.80	80.29	81.16	78.04	79.15
Even-carbon unsaturated fatty acids, %	16.63	17.24	15.58	15.71	14.95	17.74	16.31
Saturated/unsaturated fatty acids	4.53	4.44	5.11	5.14	5.39	4.43	4.81

[a] Internal peak area values, IPA(%), mean the relative proportion (%) of different fatty acids in the corresponding individual sample.

Table 5 Internal peak area values, IPA(%), of LCFAs in protozoan fraction[a]

	Various exogenous ω-6/ω-3 PUFA ratios (x:[100 − x]; in total of 100 mg g^{-1} substrate)						
Name of fatty acids	100-0	90-10	80-20	66-33	50-50	20-80	Average
Lauric acid	0.61	0.50	1.09	0.25	0.28	0.21	0.49
Myristic acid	0.76	1.07	1.16	0.73	0.93	0.68	0.89
Myritoleic acid	0.63	0.96	0.88	0.75	0.87	0.59	0.78
Pentadecanoic acid	0.79	1.24	1.04	0.93	1.00	0.73	0.96
cis-10-Pentadecenoic acid	0.48	0.67	0.66	0.32	0.59	0.48	0.53
Palmitic acid	11.39	13.46	15.78	13.16	14.07	11.93	13.30
Palmitoleic acid	0.39	0.89	0.92	0.99	1.10	0.89	0.86
Heptadecanoic acid	0.44	1.72	1.70	1.26	1.59	1.19	1.31
cis-10-Heptadecenoic acid	0.20	0.15	0.17	0.17	0.19	0.10	0.16
Stearic acid	71.29	66.40	63.53	68.44	66.89	68.55	67.52
Elaidic acid	7.29	6.67	6.63	6.50	6.20	5.83	6.52
Oleic acid	3.04	3.50	3.90	4.04	3.98	5.08	3.93
Linolelaidic acid	0.69	0.59	0.60	0.88	0.95	2.25	0.99
Linoleic acid	0.78	1.44	1.05	0.83	0.94	1.05	1.02
Arachidic acid	1.01	0.42	0.53	0.47	0.24	0.31	0.50
γ-Linolenic acid	0.21	0.33	0.35	0.28	0.16	0.14	0.24
Unsaturated fatty acids, %	13.71	15.20	15.17	14.77	14.99	16.41	15.04
Long chain fatty acids ≥18 carbons, %	84.31	79.34	76.60	81.45	79.37	83.21	80.71
18 carbon unsaturated fatty acids, %	12.01	12.52	12.54	12.54	12.24	14.36	12.70
cis-Fatty acids, %	3.82	4.94	4.96	4.87	4.92	6.13	4.94
trans-fatty acids, %	7.98	7.25	7.23	7.38	7.16	8.09	7.51
Ratios (*cis*-/*trans*-)	0.480	0.681	0.685	0.661	0.688	0.758	0.66
Ratios (stearic acid : palmitic acid)	6.258	4.933	4.027	5.202	4.753	5.746	5.15
Odd-carbon fatty acids, %	1.90	3.78	3.56	2.68	3.37	2.50	2.96
Even-carbon saturated fatty acids, %	85.07	81.85	82.10	83.05	82.42	81.67	82.69
Even-carbon unsaturated fatty acids, %	13.04	14.38	14.34	14.28	14.21	15.83	14.34
Saturated/unsaturated fatty acids	6.29	5.58	5.60	5.78	5.67	5.09	5.65

[a] Internal peak area values, IPA(%), mean the relative proportion (%) of different fatty acids in the corresponding individual sample.

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in the functional groups[61,62] such as specific peptide, enzymes, or channels, *etc.* Stearic acid in a protozoan membrane is higher (about 14.0%) than that in a bacterial membrane, unlike other fatty acids.

Thirdly, even-carbon saturated FAs in the treatment of ω-6/ω-3 = 100 : 0 and ω-6/ω-3 = 20 : 80 were 85.1% and 81.7% in the protozoan phase, whereas even-carbon unsaturated FAs in those treatments were 13.0% and 15.8%, respectively (Table 5). Herein, the highest value of even-carbon unsaturated FA appeared in the treatment of ω-6/ω-3 = 20 : 80, this phenomenon also occurred in the bacterial phase, which means that, according to this study, a high amount of ω-3 PUFAs has the tendency to increase the even-carbon unsaturated FAs compared to ω-6 PUFAs.

An interesting fact is that linolelaidic acid (C18:2, 6*t*) proportion in ω-6/ω-3 = 100 : 0 was a little higher than other treatments in the bacterial phase, whereas a higher proportion appeared in the protozoan phase when ω-6/ω-3 = 20 : 80. This might be due to the biohydrogenation of PUFAs (such as linoleic and linolenic acid) in rumen resulting in the production of primarily *trans*-fatty acids and stearic acid.[63] All the differences in fatty acid distributions between bacterial and protozoan phases can reflect protozoa and bacteria having a different and complex metabolism in the processes of assimilation, absorption, degradation or *de novo* synthesis. Thanks to these differences, that make the different phases (bacterial and protozoan phases), as important input variables, more reasonable in our new Hansch Perturbation Theory – LFER model. The LCFA and VFA distribution was stated in the entire fermentation system of a ruminal micro-niche environment, as shown in Fig. 3.

3.3. VFA profiles in media provided with exogenous ω-6/ω-3 PUFAs

In this study, the peak area (PA) of VFAs was determined in each sample to calculate the internal peak area, IPA(%), at 48 h fermentation. On the other hand, the absolute concentration of VFAs was also calculated, using the PA combined with the corresponding standard curve in the same situation (Table 6). Volatile fatty acids (VFAs) are widely regarded as secondary metabolites to reflect the hydrogenation metabolism of lipids, microbial degradation enzyme activity, and the lifecycle of microbial organisms in the rumen micro-ecological niche.[64,65]

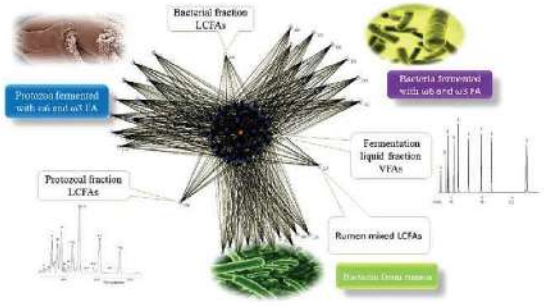

Fig. 3 Illustration of the sub-network of LCFAs and VFAs in rumen micro-niches.

The acetic, propionic and butyric acids are the major VFAs, with proportions of 43.6%, 23.1% and 19.2%, respectively. Meanwhile, the residue VFAs, including isobutyric, isovaleric and valeric acid had a total proportion of 14.1%. It is noteworthy that there was no significant difference in VFAs with the supply of different exogenous ω-6/ω-3 PUFA ratios. The values of all VFAs in the treatment of ω-6/ω-3 = 80 : 20 were lower than others, which might be the result of the bottle cap of storage containers which was broken or was not sealed properly. However, this study is focused on the proportion peak areas of VFAs on the same sample or treatment. It can be concluded that acetic acid had a minor decrease, but propionic acid had a slight increase, with an increasing proportion of ω-3 PUFAs in the total supplementation exogenous PUFAs. Even if both acetic acid and propionic acid changed a little in terms of different proportions of ω-6 and ω-3 PUFAs in a total of 100 mg g^{-1} of substrate, the ratio of acetic and propionic acid was regularly decreased, with increasing proportion of ω-3 PUFAs with an average of 1.89.

3.4. PT-LFER model for a FA distribution network

A new model was developed which is useful to predict the proportion of FAs (LCFAs and VFAs) in different phases of ruminal microbiome with/without exogenous PUFAs after perturbations in chemical molecular descriptors (V_k) and under initial experimental boundary conditions (c_j). Each value represents a corresponding coefficient in the new model for predicting the IPA(%)$_{new}$ of each FA (Table 7). As explained, this model can classify as high (L_{nr} = 1)/low (L_{nr} = 0) the expected proportion of FAs (LCFA/VFA) between the new and reference states after changing the boundary conditions c_j. The parameter $n(L_{nr} = 1)$ represents the number of cases in the sub-set with L_{nr} = 1 (links in the network), or the same with IPA(%)$_{new}$ of a new sub-set higher than that of reference IPA(%)$_{ref}$. On the other hand, $n(L_{nr} = 0)$ represents the number of cases observed and predicted in the sub-set with L_{nr} = 0 (not connected nodes) or explained that the IPA(%)$_{new}$ value is lower than the IPA(%)$_{ref}$ value. The best PT-LFER model found using the LDA algorithm has only 12 variables and it is described by the following algorithm.

$$\begin{aligned} {}'f(L_{nr})_{new} = {} & -0.021 \cdot f(\varepsilon_{ij})_{ref} + 0.0026 \cdot \langle f(\varepsilon_{ij}) \rangle_{ref} \\ & + 0.3713 \cdot {}^{new}V_6 + 1.0709 \cdot {}^{new}\omega 6 - 1.1264 \cdot {}^{new}\omega 3 \\ & + 0.0237 \cdot \Delta\Delta V_5(c_1) - 0.0063 \cdot \Delta\Delta V_6(c_2) \\ & + 0.0044 \cdot \Delta\Delta V_1(c_4) - 0.0037 \cdot \Delta\Delta V_1(c_5) \\ & - 0.0036 \cdot \Delta\Delta V_4(c_6) - 0.1682 \cdot ({}^{new}V_6)^2 \\ & + 0.0182 \cdot (\Delta\Delta V_6(c_3))^2 - 13.7236 \end{aligned}$$

$$N = 407,\ 655; \quad \chi^2 = 244,\ 532.9; \quad p < 0.005 \qquad (10)$$

Where, the output function ${}'f(L_{nr})_{new}$ is a function of the connectivity pattern (L_{nr}) in the complex network for the co-distribution of FAs in the reference and new state (predicted values). The output function ${}'f(L_{nr})_{new}$ is useful to classify the pairs of states (pairs of nodes).The statistical parameters used were specificity (Sp), sensitivity (Sn), and accuracy (Ac).

Table 6 Internal peak area values, IPA(%), and absolute concentration (mM) of volatile fatty acids (VFAs) in media fraction[a]

	IPA(%) values of VFAs supplemented with various exogenous ω-6/ω-3 PUFA ratios[b]						
VFA name	100-0	90-10	80-20	66-33	50-50	20-80	Average
Acetic acid	44.72	43.86	43.61	43.62	42.83	42.74	43.56
Propionic acid	22.80	22.62	22.90	23.15	23.36	23.66	23.08
Isobutyric acid	2.48	2.57	2.78	2.37	2.39	2.65	2.54
Butyric acid	18.31	19.06	19.55	19.21	19.66	19.62	19.24
Isovaleric acid	5.93	5.99	5.84	5.89	5.77	5.81	5.87
Valeric acid	5.77	5.89	5.32	5.77	5.99	5.51	5.71
Ac/Pro	1.96	1.94	1.90	1.88	1.83	1.81	1.89
	Absolute concentration (mM) of VFAs supplemented with various exogenous ω-6/ω-3 PUFA ratios[c]						
VFA name	100-0	90-10	80-20	66-33	50-50	20-80	$(r^2)^d$
Acetic acid	124.4 ± 1.66	104.2 ± 2.89	75.6 ± 2.26	114.1 ± 1.85	109.4 ± 1.01	110.5 ± 0.71	0.9964
Propionic acid	31.6 ± 0.54	26.8 ± 0.71	19.8 ± 0.56	30.2 ± 0.59	29.7 ± 0.37	30.5 ± 0.22	0.9974
Isobutyric acid	2.3 ± 0.04	2.1 ± 0.02	1.6 ± 0.07	2.1 ± 0.02	2.1 ± 0.02	2.3 ± 0.27	0.9985
Butyric acid	19.1 ± 0.41	16.9 ± 0.87	12.6 ± 0.22	18.8 ± 1.15	18.8 ± 0.35	19.0 ± 0.10	0.9983
Isovaleric acid	5.0 ± 0.11	4.3 ± 0.13	3.1 ± 0.10	4.6 ± 0.21	4.4 ± 0.04	4.5 ± 0.04	0.9988
Valeric acid	4.8 ± 0.10	4.2 ± 0.10	2.8 ± 0.22	4.5 ± 0.03	4.6 ± 0.04	4.3 ± 0.25	0.9986
Ac/Pro[e]	3.93 ± 0.02	3.89 ± 0.01	3.83 ± 0.01	3.78 ± 0.02	3.68 ± 0.04	3.63 ± 0.01	—

[a] Internal peak area values, IPA(%), mean the relative proportion (%) of different fatty acids in the same individual sample. [b] The entire supplementation amount of ω-6/ω-3 PUFAs was standard: 100 mg g^{-1} alfalfa substrate, means ± standard errors. [c] Standard curve was used to calculate the values of each VFA; the standard curve equation is $f(x) = a \cdot x + b$, with $f(x)$ = concentration of the VFAs, x = Peak Area (PA), a = coefficient of peak area, and b = intercept. The values of (a, b) found for different VFAs are as follows: for acetic acid (3 000 000; 3 000 000), propionic acid (6 000 000; 1 000 000), isobutyric acid (9 000 000; 465 874), butyric acid (8 000 000; 767 690), isovaleric acid (10 000 000; 501 483), and valeric acid (10 000 000; 644 543). [d] r^2 the correlation coefficient square of the standard curve for calculating each corresponding volatile fatty acid. [e] Ac/Pro = the ratio of acetic acid with propionic acid.

Table 7 Details of the PT-LFER model for the distribution network of fatty acids

Coeff.	Variable[a]	Value	Classic symbols[b]	PT operators ($\Delta\Delta V_k(c_j)$)[c]
a_0	Intercept	−13.7236	—	—
a_1	$f(\varepsilon_{ij})_{ref}$	−0.0210	—	—
a_2	$\langle f(\varepsilon_{ij})\rangle_{ref}$	0.0026	—	—
a_3	${}^{new}V_6$	0.3713	$V_6 = \log P$	—
a_4	${}^{new}V_7$	1.0709	$V_7 = (\log P)^2$	—
b_5	${}^{new}\omega$-6	−1.1264	—	—
b_6	${}^{new}\omega$-3	0.0237	—	—
b_7	$\Delta\Delta V_5(c_1)$	−0.0063	V_5 = MR	$= \Delta V_5(c_1)_{new} - \Delta V_5(c_1)_{ref} = p(c_1)_{new}\cdot({}^{new}V_5 - \langle V_5(c_1)\rangle_{new}) - p(c_1)_{ref}\,({}^{ref}V_5 - \langle V_5(c_1)\rangle_{ref})$; $\langle V_5(c_1)\rangle$ = average of MR for c_1
b_8	$\Delta\Delta V_6(c_2)$	0.0044	$V_6 = \log P$	$= \Delta V_6(c_2)_{new} - \Delta V_6(c_2)_{ref} = p(c_2)_{new}\cdot({}^{new}V_6 - \langle V_6(c_2)\rangle_{new}) - p(c_2)_{ref}\,({}^{ref}V_6 - \langle V_6(c_2)\rangle_{ref})$; $\langle V_6(c_2)\rangle$ = average of $\log P$ for c_2
b_9	$\Delta\Delta V_1(c_4)$	−0.0037	V_1 = Mw	$= \Delta V_1(c_4)_{new} - \Delta V_1(c_4)_{ref} = p(c_2)_{new}\cdot({}^{new}V_1 - \langle V_1(c_4)\rangle_{new}) - p(c_2)_{ref}\,({}^{ref}V_1 - \langle V_1(c_4)\rangle_{ref})$; $\langle V_1(c_4)\rangle$ = average of Mw for c_2
b_{10}	$\Delta\Delta V_1(c_5)$	−0.0036	V_1 = Mw	$= \Delta V_1(c_5)_{new} - \Delta V_1(c_5)_{ref} = p(c_5)_{new}\cdot({}^{new}V_1 - \langle V_1(c_5)\rangle_{new}) - p(c_5)_{ref}\,({}^{ref}V_1 - \langle V_1(c_5)\rangle_{ref})$; $\langle V_1(c_5)\rangle$ = average of Mw for c_5
b_{11}	$\Delta\Delta V_4(c_6)$	−0.1682	V_4 = Aeigp	$= \Delta V_4(c_6)_{new} - \Delta V_4(c_6)_{ref} = p(c_6)_{new}\cdot({}^{new}V_4 - \langle V_4(c_6)\rangle_{new}) - p(c_6)_{ref}\,({}^{ref}V_4 - \langle V_4(c_6)\rangle_{ref})$; $\langle V_4(c_6)\rangle$ = average of Aeigp for c_6
b_{12}	$(\Delta\Delta V_6(c_3))^2$	0.0182	$V_6 = \log P$	$= \Delta V_6(c_3)_{new} - \Delta V_6(c_3)_{ref} = p(c_3)_{new}[({}^{new}V_6 - \langle V_6(c_3)\rangle_{new}) - p(c_3)_{ref}\,({}^{ref}V_6 - \langle V_6(c_3)\rangle_{ref})]^2$; $\langle V_6(c_3)\rangle$ = average of $\log P$ for c_3

[a] $f(\varepsilon_{ij})_{ref} = \langle\varepsilon_{ij}\rangle_{ref} = \langle IPA(\%)\rangle_{ref}$ average of reference entries for conditions of c_1 = treatments, c_2 = with/without fermentation, c_3 = phase, c_4 = gas chromatography protocol, c_5 = replicates, and c_6 = *cis*/*trans* pattern. [b] Symbols of molecular descriptors calculated using the DRAGON software: V_1 = Mw, V_2 = Aeigv, V_3 = Aeige, V_4 = Aeigp, V_5 = AMR, V_6 = log P, and $V_7 = (\log P)^2$. The parameters $\Delta V_k(c_j)$ are moving averages, and $\Delta\Delta V_k(c_j) = p(c_j)^{new}\cdot({}^{new}V_k - \langle V_k(c_j)\rangle^{new}) - p(c_j)ref\cdot({}^{ref}V_k - \langle V_k(c_j)\rangle^{ref})$ are PT operators.

Consequently, the other input terms were expanded as follows. For instance, $\Delta\Delta V_k(c_j) = p(c_j)_{new}\cdot\Delta V_k(c_j)_{new} - p(c_j)_{ref}\,\{\Delta V_k(c_j)_{ref}\}$. This can be further expanded in turn as $\Delta\Delta V_k(c_j) = p(c_j)_{new}\,\{{}^{new}V_k - \langle V_k(c_j)\rangle_{new}\} - p(c_j)_{ref}\,\{{}^{ref}V_k - \langle V_k(c_j)\rangle_{ref}\}$, where $\langle V_k(c_j)\rangle$ = average of V_k for c_j. This new model found predicted the effects of perturbations under the initial conditions (c_j) over FA distribution with sensitivity, specificity, and accuracy > 80% for a total of = 303 712 cases in training and = 103 943 cases in external validation series (Table 8). These results are considered good for any LDA model.

3.5. PT-NLFER model for a FA distribution network

Additional tests have been conducted using STATISTICA, using linear and non-linear ANN (LNNs and MLPs) methods in order

Table 8 Results of the LDA PT-LFER model for the perturbation network of fatty acid distribution in ruminal microbiome

Data sub-set[a]	Statistical parameter	Prediction rates (%)	Prediction cases No. (L_{nr} = 0)	Prediction cases No. (L_{nr} = 1)
Training dataset				
No. (L_{nr} = 0)	Specificity	82.9	127 292	26 275
No. (L_{nr} = 1)	Sensitivity	91.1	13 403	136 742
Train total	Accuracy	86.9	90.5%[b]	83.9%[c]
Validation dataset				
No. (L_{nr} = 0)	Specificity	81.7	40 118	8986
No. (L_{nr} = 1)	Sensitivity	84.9	8282	46 557
Validation total	Accuracy	83.4	82.9%[b]	83.8%[c]

[a] Number in total = 407 655; No. (L_{nr} = 0) represents the number of cases in the sub-set with L_{nr} = 0 (not connected nodes) or the same with new and reference states when $IPA(\%)_{new} \leq IPA(\%)_{ref}$. No. ($L_{nr}$ = 1) represents the number of cases in the sub-set with L_{nr} = 1 (links in the network), or the same with new and reference states when $IPA(\%)_{new} > IPA(\%)_{ref}$. [b] NPV: negative predictive value. [c] PPV: positive predictive value.

to compare them with the above LDA model. LNNs have one input layer and one output layer, but no hidden layer. Therefore, the predicted output is a linear combination of the input neuron values, similar to the LDA model. MLPs have at least one hidden layer of neurons. The ANNs have been used in the literature to find diverse classification models.[66–68] Accordingly, and strictly speaking, the LNN models are also PT-LFER models because they are linear relationships. However, they are included in this section because they are a particular case of ANNs. In contrast, the MLP models can be classified as PT-NLFERs (PT-Non-Linear Free Energy Relationships), because they consider non-linear relationships between the input PT operators and the output.

Table 9 presents the best 11 ANN models with the corresponding statistics for the best LDA classification. The MLP models have different input variables, from 5 to 12 and the LNN models are based on 8 to 12 variables. The results demonstrate the prediction power of the non-linear ANNs (MLPs) against the linear models (LDA and LNNs). The best MLP model (no. 6: MLP 12:12-11-1:1) has 12 input variables and only one hidden layer with 11 neurons. It can predict 93.73% of the test cases and it classifies 92.54% of the training cases. This model has around 10% more prediction power compared to the LDA PT-LFER model but only 5.6% more classification power in training. The PT-NLFER model obtained with MLP number 6 classified our dataset better than the LDA PT-LFER model. However, PT-LFER is notably simpler and shows a direct relationship between the input variables and the output. If the results are sorted by the test classification (validation preference), the order of the models is the following: MLPs-6, 5, 4, 3, 2, 1; 12 (LDA model); LNNs-9, 8, 10, 11, 7. Thus, the LDA model has a better prediction capacity than all LNNs but less than MLPs. Between MPLs, the models with only 5 input variables can be observed (compared with the 12 ones for LDA), but the LDA model classifies 4.70% more of the training set, even if the MLP one can predict 4.92% more of the test set. Another advantage of the LDA model is the low training and validation errors compared to all ANNs (around 25% of the ANN errors). In conclusion, the MLP models were better problem solvers, but notably more complicated.

Table 9 Comparative study of PT-LFER *vs.* PT-NLFER models[a]

Model no. description	Typical topology	Statistical parameters[b] TP (%)	VP (%)	TE (%)	VE (%)
PT-NLFER models					
1-MLP 12:12-13-1:1		87.61	87.91	59.97	85.07
2-MLP 5:5-10-1:1		82.25	88.31	56.22	47.65
3-MLP 6:6-8-1:1		87.60	90.07	49.93	51.87
4-MLP 7:7-10-1:1		88.22	90.40	46.13	40.90
5-MLP 12:12-10-1:1		92.54	92.10	43.61	53.88
6-MLP 12:12-11-1:1		92.54	93.73	41.81	41.18
PT-LFER models					
7-LNN 8:8-1:1		85.68	81.73	33.94	35.85
8-LNN 9:9-1:1		86.60	82.12	33.50	36.09
9-LNN 10:10-1:1		86.73	82.14	33.46	36.11
10-LNN 11:11-1:1		86.63	82.00	33.45	36.16
11-LNN 12:12-1:1		86.62	81.98	33.43	36.23
12-LDA 12:12-1:1		86.94	83.39	15.03	19.92

[a] PT-LFERs: Perturbation Theory-Linear Free Energy Relationships; PT-NLFERs: Perturbation Theory-Non-Linear Free Energy Relationships. [b] TP (%) = Training Performance, VP (%) = Validation Performance, TE (%) = Training Error, VE (%) = Validation Error.

The number of nodes = fatty acids (sum of input results i) of the complex networks was 744 (the full details are presented in http://dx.doi.org/10.6084/m9.figshare.1408852). It can be concluded that the classification results, obtained using this new PT-LFER equation, are promising and confirm the potential of the present methodology. The present model is the result of combining Hansch analysis with LDA models, Box–Jenkins operators, and Perturbation Theory ideas. Our group and other authors[69–74] have used LDA models alone or combined with Box–Jenkins operators to predict the properties of complex systems,[75–78] and these models may include or not perturbation theory considerations.[79] However, in this paper these ideas are extended to the Hansch analysis for the first time.

3.6. Construction of a FA distribution network using the PT-LFER model

Network biology[80] is accepted as a very useful approach to shed light on the functional organization of the cell. With this idea in mind, the observed complex networks were built for perturbations in FA metabolism/distribution between ruminal media and bacterial or protozoan individuals. In so doing it was considered that two states are connected (L_{nr} = 1) if both $f(\varepsilon_{ij})_{new} = IPA(\%)_{obs}$ and $f(\varepsilon_{ij})_{ref} = IPA(\%)_{obs} - IPA(\%)_{ref} > 0$, and L_{nr} = 0 otherwise. This condition indicates that the level of both fatty acids in the new state is higher than that of fatty acids in the state of

Table 10 Giant components of the observed, predicted, and random networks

Observed networks	Value	Average indices[a]	Value	Predicted networks
	1.8 72.7 0.0008	Distance Degree Closeness	1.8 80.3 0.0008	
ER random network[b] 1	**Value**	**Average indices**	**Value**	**ER random network 2**
	1.9 68.6 0.0007	Distance Degree Closeness	1.9 80.0 0.0008	

[a] Distance means the average values of the vertex–vertex topological distance, degree and closeness mean the node degree, and closeness of the giant component, respectively. [b] ER random network means the Erdős–Rényi random network.

reference (initial state). Consequently, our network is a network of co-distribution of fatty acids. In addition, the model was used to predict the same complex network. To this end, it was considered that $L_{nr} = 1$ (nodes linked) when both values of ${}^{'}f(e_{ij})_{new}$ and ${}^{'}f(e_{ij})_{ref}$ predicted by the model have the probability $p(c_{ij}) > 0.5$ of having $f(e_{ij})_{ref} = IPA(\%)_{obs} - IPA(\%)_{ref} > 0$.

Last, two models of random networks (random networks **1** and **2**) were also built (Table 10). Each model was defined with a number of nodes and links as similar as possible to the observed and predicted networks, respectively. The objective was to understand the overall nature of the FA metabolism/distribution data (similar to a random process or not). The average values of some topological indices were calculated to compare quantitatively the structure of these networks. The indices calculated were the average values of the vertex–vertex topological distance,[50] node degree, and closeness of the giant component of the observed, predicted network models and the two similar random ones. Erdős–Rényi (ER) random networks were, apparently, similar to the observed and predicted networks. In fact, the average values of the topological distance, node degree, and closeness are similar, halfway between the observed and predicted network (1.83 *vs.* 1.77, 72.75 *vs.* 80.29, and 0.000755 *vs.* 0.000836, respectively).

4. Conclusions

A mixed experimental–theoretical methodology can be used to study the effect of multiple factors over fatty acid distribution networks on ruminal microbiome. PT and LFER ideas can be combined to develop a PT-LFER model of fatty acid distribution networks. Box–Jenkins and PT operators of physicochemical parameters are useful inputs in this sense. ANN algorithms are also useful to test the performance of alternative PT-NLFER; non-linear models. Last, ER random network models can be employed to carry out comparative studies with the observed and predicted networks in order to study the overall effect of perturbations on the fatty acid distribution processes.

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CAPÍTULO 5

Yong Liu, Zhiliang Tan, Claudia Giovanna Peñuelas-Rívas, Alejandro Pazos, Germán Buendía-Rodríguez, María Rívas-Guevara, Esvieta Tenorio-Borroto, Rafael García-Vázquez, and Humberto González-Díaz. Perturbation Theory-Linear Free Energy Relationships (PT-LFER) to study Fatty Acid Distribution Networks in Ruminal Microbiome combined with fermentation factors by Experimental-Computational Method. *Chemometrics and Intelligent Laboratory Systems*. (Submitted)

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Chemometric Approach to Bacteria – Rumen – Protozoa Distribution Networks in Ruminal Microbiome: Parallel Experiments and PT-LFER Model of Methane Production and Fatty Acid Metabolism

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ABSTRACT: Methane emission has attracted more and more attention by nutrition and environmental scientists. Unfortunately, the number of factors influencing the phenomena is very high (chemical structure of metabolites, time, methane pressure, microbiome composition, diet, *etc*.). This fact determines the accumulation of a large amount of experimental data from different experiments; which in turn difficult the study. In this work, we propose a new Chemometric methodology to integrate different laboratory experiments in this field. Firstly, we report (1) new laboratory experiments to measure by separate (1a) methane production (gas phase), (1b) volatile fatty acid distribution (liquid phase) and (1c) fatty acid distribution in rumen microbiome. Next, we report the new (2) Chemometric methodology that integrates all the data in a single theoretical model. The laboratory work includes two experimental sections (**a**) to measure the methane production, pH, gas pressure and temperature and (**b**) fatty acid distribution. The section (**b**) include two different experimental parts (**b.1**) chromatographic determination of internal peak areas (IPA%) of long chain fatty acids and (**b.2**) volatile fatty acids. In all studies, we can use different treatments, distribution phase (media, bacteria, or protozoan microbiome), *cis*/*trans* patterns, experimental protocols, *etc*. Next, we combined Perturbation Theory (PT), Linear Free-Energy Relationships (LFER), Linear Discriminant Analysis (LDA), and Artificial Neural Networks (ANNs) to develop linear and non-linear models of perturbations in Methane production – fatty acid distribution network. The best PT-LFER model found presented values of Sensitivity, Specificity, and Accuracy >94% for 545,695 cases of perturbations in experimental data. This methodology may be useful to quantify the effect of perturbations due to changes in experimental conditions in the study of fatty acid distribution when we need to carry out parallel experiments in different phases.

Keywords: Complex Networks; Artificial Neural Networks; Perturbation Theory; Linear Free-Energy Relationships; Methane Production; Fatty Acids in Ruminal Microbiome.

1. INTRODUCTION

In recent years, methane emission has attracted more and more attention by nutrition and environmental scientists. There are lots of methods or chemical substances developed to improve the ruminant growth performance and meanwhile to reduce the emission of greenhouse gases (methane) [1, 2]. For instance, essential oils or functional fatty acids are the one type of efficient methods to reduce methane emission from rumen [3-6]. Among, ω-3 fatty acids also proved to efficient to reduce the methane emission [7-9]. Bacteria and protozoan, as the protagonists, participate in the metabolism processes of exogenous fibre, lipid, or protein resources. There is a strong correlation between the microbe activity (polarity, fluidity, permeability *etc*.) and the cell membrane structure compositions, mainly composited of various fatty acids (*e.g*., phospholipids, glycolipids, and sterols). Herein, combined with inside fatty acids composition changeable in bacterial and protozoan membrane and other parameters (such as pH, gas pressure and temperature) with some outputs (methane mission, or other nutritional elements) of this complex system should be an interesting thing to reflex the reality of rumen complex metabolism processes [7-9]. Adjusting the rumen fermentation or metabolism pathway with exogenous lipid resources abundant in plant-derived ω-3 PUFAs is an efficient method to enrichment ω-3 PUFAs contents in ruminant meat or milk. However, the complex metabolism or biohydrogenation processes significantly reduce the rumen bypass exogenous PUFAs. To address this problem, it is postulated that fatty acid compositions in bacterial and protozoan membranes changed with supply of different ω-6/ω-3 ratios based on the favorable and detrimental scope in diet. For instance, Petit *et al*. reported linseed enrich of linolenic acid provided to cows rations, resulted in the lowest ω-6/ω-3 ratio in milk compared to micronized soybeans or sunflower seeds [10]. Hess *et al*.[11] proved that incorporation of ω-3 fatty acids of animal blood and muscle depends directly on the dietary fatty acid type. On the other hand, LCFAs must be methylated by a means of acid-/base- methylation before determined by gas chromatograph [12-14].

In short, the molecular structure of exogenous fatty acids plays a very important role in regulatory mechanism on rumen metabolism pathway and processes. It implies that it is reasonable to combine the molecular structure properties and the amount of fatty acids in bacterial or protozoan membrane to predict the rumen lipid metabolism properties. In this sense, Chemoinformatics models may become a useful tool [15]. In our opinion, we can combine the idea of Perturbation Theory (PT) [16] and Linear Free-Energy Relationships (LFER) [17, 18] to handle this issue. As the result of this combination we can obtain PT-LFER models that can handle the complex data generated in these studies. In addition, non-linear Machine Learning methods such as Artificial Neural Networks (ANN) [19, 20] can be used to improve the predictive power of Chemoinformatics techniques in the study of complex bio-molecular, ecological, and social systems. As result we can obtain Non-Linear PT-LFER models (PT-NLFER) models.

In the present work we want to introduce a new theoretical methodology based PT-LFER methods that is able to process complex experimental data from metabolomics studies of methane production. That is why; the present work consists of two main stages. The first stage of the work is aimed to report original experimental dataset of fatty acid distribution in biological membrane. In the second stage of this work we are going to develop a new Chemoinformatics PT-LFER / PT-NLFER models for the data generated. Next, the best PT-LFER model found was used to predict the effect of perturbations on initial boundary conditions over a large complex network of FA distribution/uptake in the ruminal microbiome. Accordingly, this work paves the way to study the effect of complex molecular perturbation theory in fatty acid chemical structure, the corresponding fermentation parameters and boundary experimental conditions.

2. MATERIALS AND METHODS

2.1. Experimental section.

The experiment was presented in (**Figure 1**). (**a**) the methane production, pH, gas pressure and temperature as well as (**b**) the chromatographic internal peak areas (IPA%) of (**b1**) long chain fatty acids (LCFA) in bacterial and protozoan membrane and (**b2**) volatile fatty acids (VFA) were determined under the different experiments. The general details of the experimental procedures used are explained as follows.

Figure 1 comes about here

2.1.1. Inoculum microbes.

The inoculum microbes were provided by three adult male Pelibuey sheep with permanent rumen-fistula (body weight, 45.0 ± 5.0 kg). The installation methods of the rumen-fistula were according to the Mexican Official Standard (NOM-220-SSA1-2002). Nutritional compositions of fodder for animal donors were according to the description of NRC [21].

2.1.2. Experimental in vitro fermentation.

The *in vitro* fermentation details were according to the description of Tang *el at.* [22] Particle-free rumen fluid was mixed with artificial saliva buffer solution [23] in a ratio of 1:2 (*v/v*) at 39 ºC with continuous flushing of CO_2. The microbial and protozoal fractions were separated by differential centrifugation according to the methods of Legay-Carmier and Bauchart [24]. Microbial fatty acids were prepared according to the method developed by Or-Rashid [14], and catalyzed by base-catalyzed methylation.

The dataset part (**a**) was consisted of CH_4, gas pressure (GP) and temperature (T). These parameters were determined after fermented within exogenous different ratios ω-6/ω-3 PUFAs. The ω-6/ω-3 PUFAs were composited of linoleic acid (LA, L1376-5 g, Sigma-Aldrich)/α-linolenic acid (ALA, L2376-500 mg, Sigma-Aldrich) in the ratios of 100:0; 90:10; 80:20, 66:33, 50:50, and 20:80, respectively, with a total amount of 100 mg/g in substrate. The dataset part (**b**) was composited of Internal Peak Area, IPA (%), of (**b.1**) LCFA of bacterial and protozoan membrane from two different treatments (catalyzed methylations and fermentation with exogenous ω-6/ω-3 PUFAs), and (**b.2**) VFA in the fermentation media. Among, the catalyzed methylations included acid methylation (HCl solution of 8% dissolved in methanol/water (*v*/*v*, 85/15)) [12], base methylation (Trimethylsilyldiazomethane, TMSD)[14] ENREF 30, combination of acid-/base-methylation (catalyzed with HCl, in turn with TMSD). Fermentation experiment was the same treatments with part (**a**) using exogenous ω-6/ω-3 PUFAs. The values of Peak Area, PA(i), for each fatty acid under different sets of experimental conditions c_j (different samples) were determined with Gas Chromatography (Model 6890N, USA) with HP-88 Column, whereas VFAs were determined with column of DB-FFAP. The values of peak area obtained by GC were used to calculate the Internal Peak Area, IPA (%), as follows.

$$\mathrm{IPA}(\%)_{ij} = 100 \cdot \left(\frac{\mathrm{PA(i)}}{\sum_{m_i \subset c_j}^{m_1 \to m_i} \mathrm{PA(i)}} \right) \quad (1)$$

Where, m_i refers to a fatty acid of a sample, $m_i \subset c_j$ refers to a fatty acid in a specify set of experimental condition.

2.2. Theoretical section.

The datasets of LCFAs, VFAs, CH_4 production in addition of temperature and gas pressure in a different set of experimental condition were calculated and dealing with according to the perturbation theory combined with linear free energy relationships. The classification models were made based on these input variables with STATISTICA software.

2.2.1. Datasets and Workflow for PT-LFER Chemoinformatics study.

A Chemoinformatics study of the results obtained in the experimental section was carried out. **Figure 2** shows a workflow diagram that states the integration of both (experimental and theoretical) sections. The diagram briefly explains the experimental section, mainly focusing on the subsequent development of PT-LFER model. For the analysis, the parameters (Ω) from experiment section (**a**) and (**b**) were calculated based on the set of experimental conditions c_j. After that, the difference (Δ) and logistical absolute values of difference (log(|Δ|)) from the initial or reference state (ref) linked to their respective new or final (new) were calculated. Then, some input variable values, including molecular descriptors (^iV_k) of class k^{th} for every i^{th} fatty acid molecule, and perturbation operators $\Delta\Delta V_k(c_j)$ were calculated. Next, we defined the PT-LFER model based on these input variables obtained from experimental parts and molecular descriptors of each fatty acid. In final, the statistical analysis and obtained the PT-LFER model were performed. At follow, we explained more details in some of these steps.

Figure 2 comes from here

2.2.2. PT-LFER model details.

In our opinion, we can combine the idea of Perturbation Theory (PT) [16] and Linear Free-Energy Relationships (LFER) [17, 18] to handle this issue. Recently, Gonzalez-Díaz *et al.* [16] formulated a general-purpose PT model for multiple-boundary Chemoinformatics problems. The model calculates a general function $f(\mu_{nr})$ to quantify the occurrence ($\mu_{nr} = 1$) or not ($\mu_{nr} = 0$) of a process involving a set of molecules m_i in a complex system. It is considered that all the possible states form a network of states. The network nodes are the initial or reference states (r) linked to their respective final or new states (n) reached by the system after a perturbation of the initial conditions. In the PT-LFER model, a set of multiple initial experimental boundary conditions $^{ref}c_j \equiv (c_0, c_1, c_2, c_3 \ldots c_n)$ and a different or new set of boundary conditions $^{new}c_j \equiv (c_0, c_1, c_2, c_3 \ldots c_n)$ are considered at the beginning or end of the process after one or multiple perturbations. The PT-LFER model proposed herein is a linear equation with the following form:

$$'f(\mu_{nr})_{new} = a_0 + a_1 \cdot f_1(\varepsilon_{ij})_{new} + a_2 \cdot \langle f_2(\varepsilon_{ij})_{ref} \rangle + \sum_{q=1}^{q=qmax} a_q \cdot f_q(v_q)_{ref} \quad (2)$$

$$+ \sum_{q=1}^{q=qmax} a_q \cdot f_q(\Delta v_q) + \sum_{q=1}^{q=qmax} a_q \cdot f_q(\log|\Delta v_q|) + \sum_{(i,k,j)=1}^{(i,k,j)=(i,k,j)max} a_{ikj} \cdot \Delta\Delta^i V_k(c_j)$$

The output function $'f(\mu_{nr})_{new}$ is a score used to calculate the probability of binary classification of an input as $\mu_{nr} = 1$ or $\mu_{nr} = 0$ (outputs).[25] The vectors $\mathbf{v}_i = [f(\varepsilon_{ij})_{new}, \langle f(\varepsilon_{ij})\rangle_{ref}$, ω-6, ω-3, CH_4, $^iV_1, \ldots {}^iV_{kmax}, \Delta V_1(c_1), \ldots \Delta V_k(c_j), \ldots \Delta V_{kmax}(c_{jmax}), \Delta\Delta V_1(c_1), \ldots \Delta\Delta V_k(c_j), \ldots \Delta\Delta V_{kmax}(c_{jmax})]$ are the inputs of this model. Each vector $\mathbf{v}_i$ represents a statistical case (i^{th} case) out of a total of n = 545,695 cases (perturbations). These statistical cases encoded by $\mathbf{v}_i$ vectors are perturbations of one entry or state of reference (changes in input parameters) that yield an output or new state. The input vectors $\mathbf{v}_i$ include the value of $f(\varepsilon_{ij})_{ref}$ for the state of reference (known value). The vectors $\mathbf{v}_i$ also take the amounts of ω-6 and ω-3 in the new state (after perturbation) into account. The values of molecular descriptors (^iV_k), and PT-LFER operators $\Delta\Delta V_k(c_j)$ were also included in vectors $\mathbf{v}_i$ (See details in Supplementary Material file SM01.pdf, available in journal web or upon author request).

2.3. Calculation of molecular descriptors.

The Simplified Molecular-Input Line Entry System (SMILES) codes of each fatty acid were downloaded from the website data of Chemical Entities of Biological Interest (ChEBI: http://www.ebi.ac.uk/chebi/). Then, SMILES codes of each fatty acid were uploaded to software DRAGON[43, 44] to obtain some molecular descriptors (^iV_k). SMILES codes are very useful to manage molecular structures [26-28] and for further calculation of molecular descriptors [29, 30]. First, structural variables (^iV_k) of each fatty acid were used as a new set of molecular descriptors. These variables are the same classes of molecular descriptors used in Hansch analysis to quantify the molecular structures. The first class of molecular descriptors calculated was V_1 = Mw (Molecular weight). The second class of molecular descriptors V_2 = AEigv, V_3 = AEige, and V_4 =

AEigp were also included, which are the Eigenvalues average of topological distance matrices weighted with atomic van der Waals volumes (v), Polarizabilities (p), or Electronegativities (e). Last, V_5 = MR (Molecular Refractivity), V_6 = LogP (logarithm of the n-Octanol/Water partition coefficient) were also proposed.

2.4. Chemometric classification models.

The Linear Discriminant Analysis (LDA) [25] algorithm implemented in the STATISTICA software was used to find the best PT-LFER model. Sometimes the relationship between the input variables and the output is more complex and the linearity cannot solve the problem. Therefore, the non-linear models could provide a better solution. Thus, the Artificial Neural Network (ANNs) [31] were tested: non-linear Multi-Layer Perceptron (MLPs) [25]. The full datasets were split into training and validation subsets with a ratio of 75: 25 used for model construction and validation, respectively. All independent variables are unified and standardized with the STATISTICA software, prior to model construction.

3. RESULTS AND DISCUSSION

3.1. Experimental study, catalyzed methylation.

Table 1 shows the results obtained in the experimental determination of the values of IPA (%) after different acid/base methylations. In general, the base-catalyzed methylation proceeds more rapidly under a mild temperature condition than acid-catalyzed methylation [32]. In this work, the results showed that combined base-/acid- methylation seems to yield higher proportions of C18:3 n3, C18:3 n6, C20:2, C22:0 and C20:3 n6, unsaturated FAs, *odd*-carbon FAs, and *even*-carbon unsaturated FAs, but with lower proportions of C18:0 and *cis*-FAs compared to base- or acid-catalyzed methylation methods, respectively. It implied the base-/acid- methylation was the best methylation method to catalyze the free fatty acids for determining the fatty acids proportion with GC, compared to acid- and base- methylation.

Table 1 comes about here

3.2. Experimental study, LCFAs in bacteria and protozoa.

This work was focused on the lipid metabolism and fermentation performance of ruminal microbes in addition of exogenous ω-6/ω-3 PUFAs by direct determination of the IPA (%) values of LCFA from ruminal microbe/protozoan biological membrane, including FAs from bacteria (**Table 2**) and protozoa (**Table 3**) biological membrane under different experimental conditions (c_j).

Table 2 and Table 3 come from here

As expected, the FA composition of ruminal bacteria and protozoa biological membrane in present work were indeed changeable with the exogenous ω-6/ω-3 ratios. First of all, *cis*-FA increased with exogenous ω-3 PUFA, whereas *trans*-FAs decreased in bacteria biological phase. For example, *cis*- fatty acids in ω-6/ω-3 = 20:80 was 1.76 times (bacterial phase)/1.60 times (protozoan phase) than that in ω-6/ω-3 = 20:80; and *trans*- fatty acids in ω-6/ω-3 = 100:0 was 1.24 times (bacterial phase)/0.98 time (protozoan phase) than that in ω-6/ω-3 = 20:80. Thus, *cis*-/*trans*- FA ratios were decreasing with exogenous ω-3 PUFA. It means exogenous PUFAs are degraded by ruminal microorganisms, or have more complex metabolism processes to produce the intermediate metabolism with both of *cis*- and *trans*- unsaturated FAs formulation. The bio-hydrogenation of linoleic acid (LA, *cis* 9, *cis* 12- C18:2) in rumen is isomerized to *cis* 9, *trans* 11- C18:2 isomer (conjugated linoleic acid, CLA), conversion of this isomer to *trans* 11- C18:1 (vaccenic acid), and reduction to stearic acid (C18:0) [33]. Whereas the bio-hydrogenation of α-linolenic acid (ALA) is characterized by isomerization to 9, 11, 15- *cis*, *trans*, *cis*- C18:3 isomer and subsequent reduction via *cis*-, *trans*- isomers C18:2, C18:1 and then to stearic acid [34]. However, ω-3 PUFA could produce more *cis*- FAs compared to ω-6 on both of bacteria and protozoa phases.

Secondly, the main FA composition in both bacterial and protozoan membrane. The IPA values of palmitic acid (C16:0) and stearic acid (C18:0) in bacterial phase were 18.7% and 57.8%, whereas

these in protozoan phase were 13.3% and 67.5%, respectively. The exogenous ω-6/ω-3 ratios have no significant influence on these two fatty acids in both bacterial and protozoan phases. However, the minor deviation of lipid composition such as the ratios of palmitic/stearic acid or unsaturated/saturated FA on bacterial and protozoan biological membrane, might trigger a great difference in the function of membranes of bacteria and protozoan (*e.g.*, membrane fluidity, hydrophobicity, stability and permeability)[35, 36], or further in the associated functional groups such as specific peptide, enzymes, or channels *etc*. [37, 38]. In addition, C18:0 in protozoa membrane is higher than that in bacteria membrane.

Thirdly, *even*-carbon saturated FA in ω-6/ω-3 = 100:0 and ω-6/ω-3 = 20:80 were 85.1% and 81.7% in the protozoan phase, whereas *even*-carbon unsaturated FA in these treatments were 13.0% and 15.8%, respectively (**Table 3**). Herein, the highest value of *even*-carbon unsaturated FA existed in the treatment of ω-6/ω-3 = 20:80, this phenomenon also appeared in the bacterial phase, which means that, a high amount of ω-3 PUFAs has a tendency to increase *even*-carbon unsaturated FAs compared to ω-6 PUFAs. An interesting thing is that linolelaidic acid (C18:2 n6*t*) proportion in ω-6/ω-3 = 100:0 was a little higher than other treatments in bacterial phase, whereas a higher proportion existed in protozoan phase when ω-6/ω-3 = 20:80. This might be due to the biohydrogenation of PUFAs (linoleic and linolenic acid in present work) resulting in the accumulation of *trans* FA and stearic acid in biological membrane [39].

3.3. Experimental study, VFA profiles.

Volatile fatty acids (VFAs) are widely regarded as secondary metabolites to reflect the hydrogenation metabolism of lipids, microbial degradation enzyme activity, and the lifecycle of microbial organism in the rumen micro-ecological niche [40, 41]. For the new model dataset, the IPA(%) was calculated as a part of $f(\varepsilon_{ij})$ inserted into the model construction. In this study, the peak area (PA) of VFA was determined in each sample to calculate the internal peak area at 48 h fermentation (**Table 4**). The acetic, propionic and butyric acids are among the major VFAs, with proportions of 43.6%, 23.1% and 19.2%, respectively. Meanwhile, the residue VFAs, including isobutyric, isovaleric and valeric acid had a total proportion of 14.1%. It is noteworthy that there was no significant difference among the different exogenous ω-6/ω-3 PUFAs ratios supplementation. However, this study is focused on the proportion peak areas of VFA on the same sample or treatment. It can be concluded that acetic acid had a minor decrease, whereas propionic acid had a slight increase, with an increasing proportion of ω-3 PUFAs in the total supplementation exogenous PUFAs. Even if both acetic acid and propionic acid changed a little in terms of different proportion of ω-6 and ω-3 PUFAs in a total of 100 mg/g substrate, the ratio of acetic and propionic acid was regularly decreased, increasing proportion of ω-3 PUFAs with an average of 1.89.

Table 4 comes about here

3.4. Data generated for the PT-LFER study.

The PT-LFER model developed combined the information of two *in vitro* fermentation studies (methane production and FA distribution). It includes methane production levels, pH values, gas pressure, and fermentation temperature after supply of different ratios of ω-6/ω-3 PUFAs. **Table 10** showed the changeable of CH_4, pH values and a part dataset of gas pressure and temperature during the fermentation process, after treated with different ratios of ω-6/ω-3 PUFAs. All the eigenvalues, the difference ($\Delta\Omega$) and logistic eigenvalue of difference ($\log(|\Delta\Omega|)$) of CH_4, pH value, gas pressure and temperature in initial/ reference or final/ new state were calculated. Methane production at 48 h was significantly decreased with supplementation of exogenous ω-6/ω-3 PUFAs, control (108.81 mM/g in substrate) *vs.* average eigenvalues of different ratios of ω-6/ω-3 PUFAs (79.19 mM/g). It is interesting to notice that methane production was lower in treatment of high level of ω-3 PUFAs (ω-6/ω-3 = 20:80) than other treatments supply of different ratios of ω-6/ω-3 PUFAs and control. It implied that omega 3 fatty acids might have more strong ability to inhibit methanogenesis process compared to omega 6 fatty acids. This is consistent with the previous works [42]. In present study, the gas pressure and fermentation temperature were measured with detectors of ANKOM in every

15 min during 72 h. All dataset of gas pressure and temperature was published online by Liu and Buendía-Rodríguez [43], however, only some fermentation time points were shown in **Table 5**.

Table 5 comes about here

3.5. Chemometric PT-LFER model of methane production and fatty acid distribution.

A new model was developed based both on the dataset from ruminal fermentation performance, including fatty acid composition in different phase of fermentation (LCFAs of bacterial and protozoan phase, VFAs of fermentation media), gas pressure, pH values, methane production, fermentation temperature and ω-6/ω-3 ratios, and the chemical molecular descriptors ($^{i}V_k$) of each fatty acid molecular structure. The details of input variables (more than 80 variables in all) were shown in **Table 6**. The difference ($\Delta\Omega$) and logistic absolute values of difference ($\log(|\Delta\Omega|)$) of first class of dataset, including gas pressure P(psi), pH(72h), CH_4, T, ω-6 and ω-3, were calculated as a set of input variables. On the other hand, molecular descriptors ($^{i}V_k$), moving averages ($\Delta^{i}V_k$), and PT operators ($\Delta\Delta^{i}V_k(c_j)$) in addition of the square of molecular descriptors $(^{i}V_{k\ new})^2$ and PT operators $(\Delta\Delta^{i}V_6(c_j))^2$ of each fatty acid on the initial and final states were also taken into account.

Table 6 comes about here

The new PT-LFER model was developed useful to predict the proportion of fatty acids, including LCFAs and VFAs, in different phases of ruminal microbiome within/without exogenous PUFAs. This model was performed with perturbation calculation of P(psi), pH(72h), CH_4, T, ω-6, ω-3 and chemical molecular descriptors ($^{i}V_k$) combined with the initial or final experimental boundary conditions (c_j). The best PT-LFER model obtained using linear discriminant analysis (LDA) algorithm has only 21 variables from more than 80 variables and it is described by the following algorithm:

$$
\begin{aligned}
{}'f(\mu_{nr}) = {} & 1.1352 - 4.1554\cdot(IPA)_{new} - 4.8007\cdot{}^{new}\omega_6 + 4.0087\cdot{}^{new}\omega_3 + 0.0669\cdot\langle IPA\rangle_{ref}^{avg.} - 0.0173\cdot pH(72h) - 8.6897\cdot\Delta\omega_6 \\
& - 4.7979\cdot\Delta\omega_3 + 0.0091\cdot\Delta P(psi) + 0.0848\cdot(CH_4)_{ref} + 0.0011\cdot\log(|\Delta T|) + 4.0914\cdot(V_5)_{new} - 7.7553\cdot(V_2)_{ref} \\
& - 134.5729\cdot\Delta V_1 + 89.3481\cdot\Delta V_5 - 10.4400\cdot\Delta\Delta V_1(c_1) + 34.5450\cdot\Delta\Delta V_6(c_3) + 10.8253\cdot\Delta\Delta V_1(c_4) \\
& - 0.4531\cdot\Delta\Delta V_5(c_5) + 7.4454\cdot(V_6)_{new}^2 + 0.2977\cdot(\Delta\Delta V_6(c_2))^2 - 0.3115\cdot(\Delta\Delta V_6(c_6))^2 \\
& No. = 545{,}695; \qquad \chi^2 = 619{,}988.9; \qquad p < 0.001
\end{aligned}
\tag{3}
$$

In this model, description or calculation details of each input variable was represented in **Table 7**, with a corresponding coefficient for predicting $IPA(\%)_{pred.}$ of each fatty acids under the boundary condition c_j. Meanwhile, all the dataset for this model were published online by Liu et al. [44, 45]. The values of molecular descriptors ($^{i}V_k$) of each fatty acid, including LCFAs and VFAs, were shown in **Table 8**. That includes V_1 = Mw (molecular weight), V_2 = Aeigv, V_3 = Aeige, V_4 = Aeigp, V_5 = MR (molecular refractivity), and V_6 = LogP; V_2, V_3, and V_4 are average eigenvalues of the topological distance matrix weighted with atomic van der Waals volumes (*v*), Electronegativities (*e*), or Polarizabilities (*p*).

Table 7 and 8 come about here

As explained, this model can classify the expected proportion of FAs between the *new* and *ref* states as high (μ_{nr} = 1)/low (μ_{nr}= 0) after changing the boundary conditions c_j. The parameter *No.*(μ_{nr} = 1) represents the number of cases in the sub-set with μ_{nr} = 1 (links in the network), or the same with $IPA(\%)_{new}$ of new sub-set is higher than that of reference $IPA(\%)_{ref}$. On the other hand, *No.*(μ_{nr} = 0) represents the number of cases observed and predicted in the sub-set with μ_{nr} = 0 (not connected nodes) or explained as that the $IPA(\%)_{new}$ value is lower than $IPA(\%)_{ref}$ value. The output function $'f(\mu_{nr})_{new}$ is a function of connectivity pattern (μ_{nr}) in the complex network for the co-distribution of FAs in reference (*ref*) and new state (predicted values). The output function $'f(\mu_{nr})_{new}$ is useful to classify the pairs of states (pairs of nodes). This new model found predicted the effects of perturbations under the initial conditions (c_j) over fatty acid distribution with Sensitivity,

Specificity, and Accuracy >94% for a total of 409,434 cases in training and 136,261 cases in external validation series (**Table 9**). These results are considered very well for any LDA model.

Table 9 comes about here

The perturbation calculation of each molecular descriptors ($^{i}V_k$) were associated with the boundary conditions c_j. Two classes of the boundary conditions were provided. Preliminary operational conditions were explained as follows, c_1 refers to the use of different experimental treatments, c_2 refers to with/without fermentation, c_4 is the gas chromatographic column used for GC, and c_5 is the use of replicate experiments. Others are more directly related to the posterior distribution and nature of the FA c_3 = the biological phase of distribution of fatty acids, and c_6 refers to the quantify information about *cis*/*trans* geometric pattern present in the LCFAs. Consequently, the other input terms were expanded as follows. For instance, $\Delta\Delta V_k(c_j) = p(c_j)_{new} \cdot \Delta Vk(c_j)_{new} - p(c_j)_{ref} \cdot \Delta V_k(c_j)_{ref}$. This can be further expanded in turn as $\Delta\Delta V_k(c_j) = p(c_j)_{new} (^{new}V_k - \langle V_k(c_j)\rangle_{new}) - p(c_j)_{ref} (^{ref}V_k - \langle V_k(c_j)\rangle_{ref})$, among $\langle V_k(c_j)\rangle$ = Average of V_k for c_j. The details of each chemical descriptor of input variable based on the boundary conditions, and the frequency $p(c_j) = n_j/n_{total}$ of the number of each level c_j of experimental boundary condition were shown in **Table 10**.

Table 10 comes about here

It can be concluded that the classification results, obtained with this new PT-LFER equation, are promising and confirm the potential of the present methodology. The present model is the result of combining Hansch analysis with LDA models, Box-Jenkins Operators, and Perturbation Theory ideas. Our group and other authors [20, 46-50] have used LDA models alone or combined with Box-Jenkins Operators to predict properties of complex systems[51-54]; however, these models may not take the perturbation theory into account ENREF_21 ENREF_46. In present work, these ideas are extended to the Hansch analysis. To this end, it was considered that $\mu_{nr} = 1$ (nodes linked) when both the values of $'f(\varepsilon_{ij})_{new}$ and $'f(\varepsilon_{ij})_{ref}$ predicted by the model have the probability $p(c_{ij}) > 0.5$ of having $f(\varepsilon_{ij})_{ref} = IPA(\%)_{obs} - IPA(\%)_{ref} > 0$.

3.6. Chemometric PT-NLFER model of methane production and fatty acid distribution.

Additional tests have been conducted with ANN algorithms in order to seek non-linear models. **Table 11** presents the best 17 MLP models with the corresponding statistics for the best LDA classification. The MLP models have different input variables from 10 to 19. The best MLP model (No.15: MLP 18:18-15-1:1) has 18 input variables and only one hidden layer with 15 neurons. It can predict 99.68% of the test cases and it classifies 99.70% of the training cases. This model has around 10% more prediction power compared to the LDA PT-LFER model but only 5.6% more classification power in training. The PT-NLFER models obtained with MPL classified our dataset better than the LDA PT-LFER model. However, PT-LFER is considered better than PT-NLFER, because of more simple and direct relationship between the input and output variables.

Table 11 comes about here

▪ ASSOCIATED CONTENT

Supporting information

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Notes

The authors declare no competing financial interest.

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FIGURES CAPTIONS

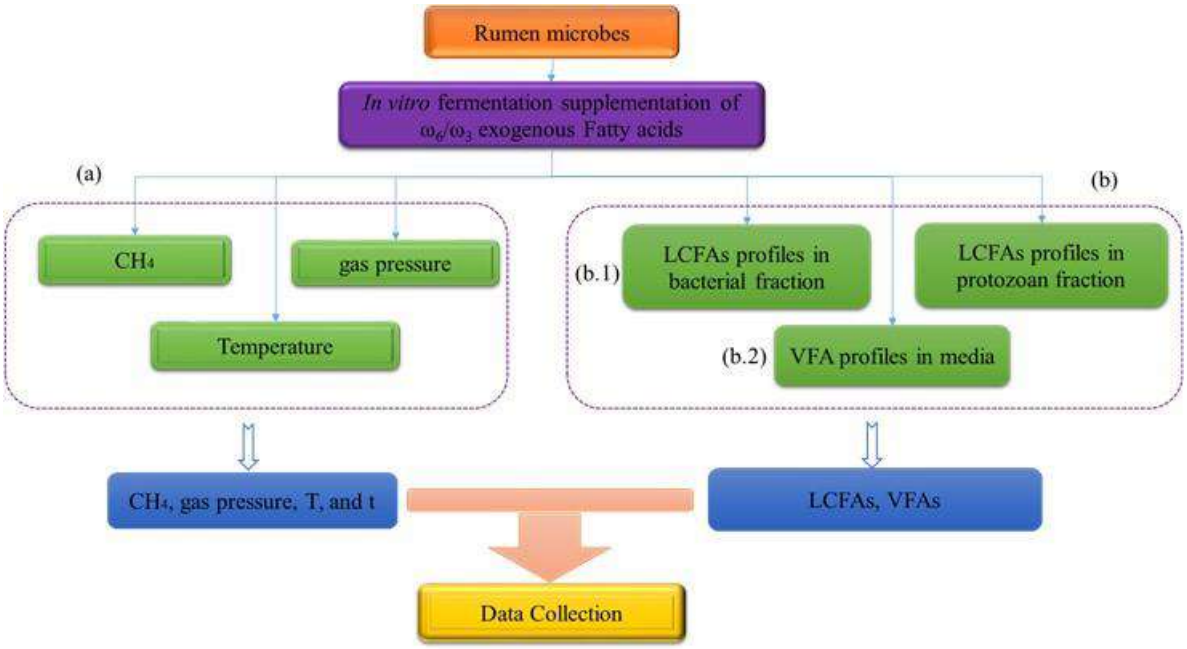


Figure 1. Illustration of the general workflow for experimental part

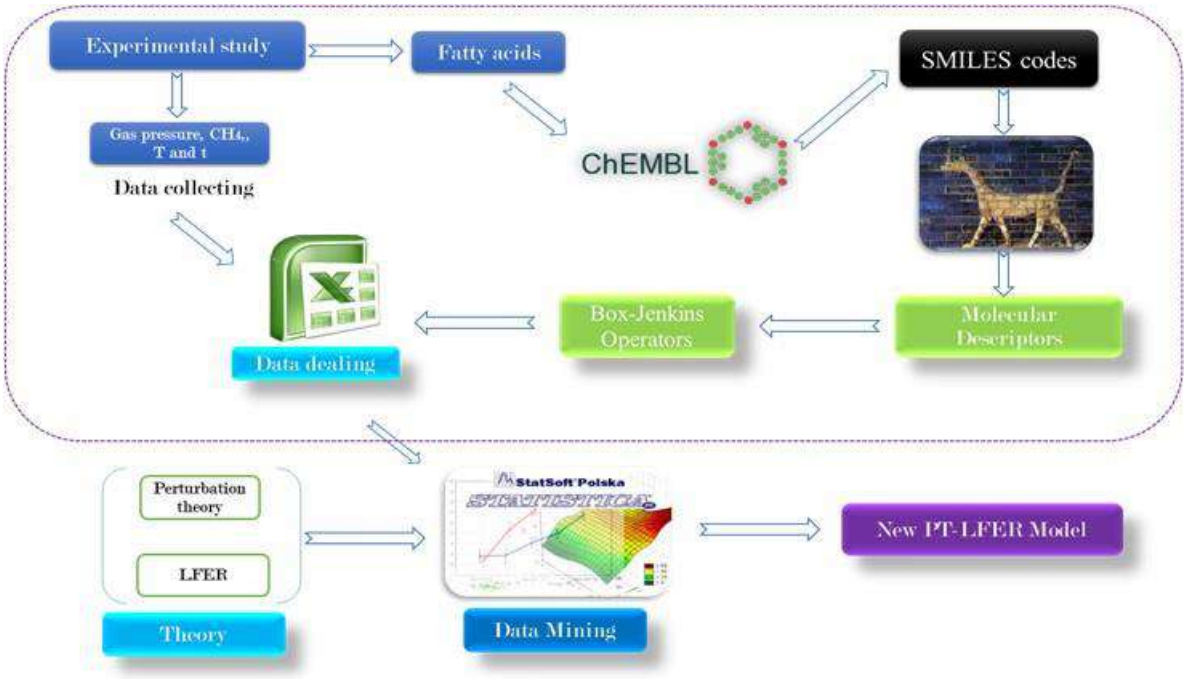
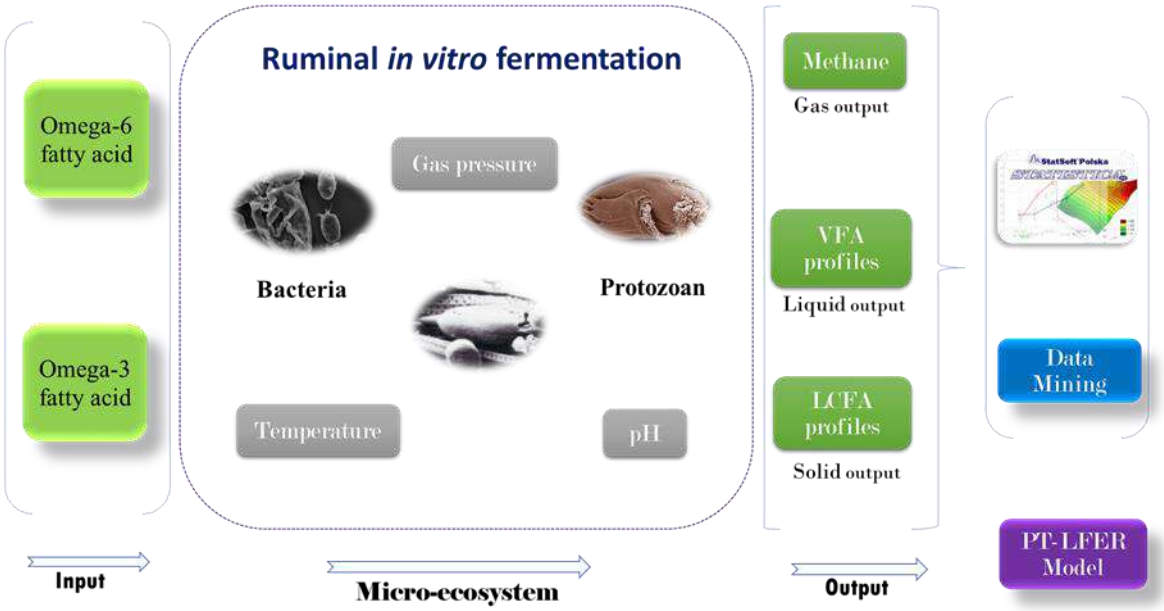


Figure 2. Illustration of the general workflow for experimental + theoretical study



TOC. Graphical abstract

Table 1. Eigenvalues of Internal Peak Area, IPA (%) [a], of LCFA profiles from ruminal mixed microbes by acid- and/or base- catalyzed methylation

Items	Different methylation methods		
	Base methylation	Acid methylation	Acid- and base-methylation
C12:0	0.55	0.52	1.87
C14:0	1.06	1.24	2.12
C14:1	1.95	2.31	2.38
C15:0	1.20	1.47	1.60
C15:1	0.40	0.53	0.68
C16:0	17.91	18.86	19.70
C16:1	1.85	2.30	2.14
C17:0	0.99	1.39	1.49
C17:1	0.00	0.17	0.53
C18:0	49.76	49.17	40.21
C18:1 n9*t*	8.68	8.23	8.76
C18:1 n9*c*	9.00	8.69	8.17
C18:2 n6*t*	0.65	0.74	0.85
C18:2 n6*c*	2.37	2.63	2.81
C20:0	0.84	0.68	1.23
C18:3 n6	0.45	0.58	0.80
C18:3 n3	0.00	0.00	1.52
C20:2	0.34	0.14	0.75
C22:0	0.59	0.13	1.33
C20:3 n6	0.58	0.05	0.82
C22:1 n9	0.82	0.17	0.24
Unsaturated fatty acids	27.10	26.55	30.44
Long chain fatty acids >= 18 carbons	74.07	71.20	67.50

18 carbons unsaturated fatty acids	21.15	20.88	22.91
cis-fatty acids	11.36	11.32	10.98
trans-fatty acids	9.34	8.97	9.61
Ratios (*cis*/*trans*)	1.22	1.26	1.14
Ratios (Stearic acid: Palmitic acid)	2.78	2.61	2.04
Odd-carbon fatty acids	2.60	3.56	4.30
Even-carbon saturated fatty acids	70.71	70.59	66.47
Even-carbon unsaturated fatty acids	26.70	25.85	29.23
Saturated/unsaturated fatty acids	2.69	2.77	2.28

[a] Internal peak area values, IPA(%), means the relative proportion of different fatty acids in the corresponding individual sample.

Table 2. Eigenvalues of Internal Peak Area of LCFA profiles in bacterial membrane [a]

Items	A various of exogenous ω-6/ω-3 LCFA Ratios (x: [100-x]; in total of 100 mg/g substrate)						Average
	100-0	*90-10*	*80-20*	*66-33*	*50-50*	*20-80*	
Lauric acid	0.62	0.67	0.56	0.55	1.89	0.52	0.80
Myristic acid	1.53	1.45	1.31	1.27	1.71	1.23	1.42
Myritoleic acid	2.66	2.61	2.32	2.20	1.90	2.06	2.29
Pentadecanoic acid	2.13	1.86	1.82	1.68	1.53	1.61	1.77
cis-10-Pentadecenoic acid	0.83	0.73	0.64	0.58	0.45	0.44	0.61
Palmitic acid	18.38	18.73	18.82	18.82	18.79	18.39	18.65
Palmitoleic acid	1.45	1.91	1.97	1.29	1.72	1.94	1.71
Heptadecanoic acid	1.77	2.16	2.01	1.73	1.65	1.92	1.87
cis-10-heptadecanoic acid	0.62	0.41	0.16	0.00	0.25	0.25	0.28
Stearic acid	56.93	56.17	58.64	58.97	58.22	57.58	57.75
Elaidic acid	5.95	6.61	5.71	5.59	4.89	5.01	5.63
Oleic acid	2.93	3.86	3.07	3.59	3.90	5.33	3.78
Linolelaidic acid	2.09	0.97	1.18	1.65	1.05	1.47	1.40
Linoleic acid	1.03	1.07	1.04	1.02	1.24	1.62	1.17
Arachidic acid	0.56	0.59	0.46	0.68	0.55	0.33	0.53
γ-Linolenic acid	0.53	0.22	0.28	0.37	0.25	0.31	0.33
Unsaturated fatty acids, %	18.07	18.38	16.38	16.29	15.66	18.43	17.20
Long chain fatty acids ≥ 18 carbons, %	70.02	69.49	70.39	71.87	70.09	71.65	70.58
18 carbons unsaturated fatty acids, %	12.52	12.73	11.28	12.22	11.33	13.74	12.30
cis- fatty acids, %	3.95	4.93	4.12	4.61	5.13	6.95	4.95
trans- fatty acids, %	8.04	7.58	6.89	7.24	5.94	6.48	7.03
Ratios (*cis*-/*trans*-)	0.492	0.650	0.598	0.637	0.864	1.072	0.72
Ratios (Stearic acid: Palmitic acid)	3.098	2.999	3.116	3.134	3.098	3.131	3.10
Odd-carbon fatty acids, %	5.34	5.15	4.63	4.00	3.89	4.22	4.54
Even-carbon saturated fatty acids, %	78.03	77.60	79.80	80.29	81.16	78.04	79.15
Even-carbon unsaturated fatty acids, %	16.63	17.24	15.58	15.71	14.95	17.74	16.31
Saturated/unsaturated fatty acids	4.53	4.44	5.11	5.14	5.39	4.43	4.81

[a] Internal peak area values, IPA(%), means the relative proportion (%) of different fatty acids in the corresponding individual sample.

Table 3. Internal peak area values, IPA(%), of LCFAs in protozoal fraction [a]

Items	A various of exogenous ω-6/ω-3 LCFAs Ratios (x: [100-x]; in total of 100 mg/g substrate)						Average
	100-0	*90-10*	*80-20*	*66-33*	*50-50*	*20-80*	
Lauric Acid	0.61	0.50	1.09	0.25	0.28	0.21	0.49
Myristic Acid	0.76	1.07	1.16	0.73	0.93	0.68	0.89
Myritoleic Acid	0.63	0.96	0.88	0.75	0.87	0.59	0.78
Pentadecanoic Acid	0.79	1.24	1.04	0.93	1.00	0.73	0.96
cis-10-Pentadecenoic acid	0.48	0.67	0.66	0.32	0.59	0.48	0.53
Palmitic Acid	11.39	13.46	15.78	13.16	14.07	11.93	13.30
Palmitoleic Acid	0.39	0.89	0.92	0.99	1.10	0.89	0.86
Heptadecanoic acid	0.44	1.72	1.70	1.26	1.59	1.19	1.31
cis-10-Heptadecenoic acid	0.20	0.15	0.17	0.17	0.19	0.10	0.16
Stearic acid	71.29	66.40	63.53	68.44	66.89	68.55	67.52
Elaidic acid	7.29	6.67	6.63	6.50	6.20	5.83	6.52
Oleic acid	3.04	3.50	3.90	4.04	3.98	5.08	3.93
Linolelaidic acid	0.69	0.59	0.60	0.88	0.95	2.25	0.99
Linoleic acid	0.78	1.44	1.05	0.83	0.94	1.05	1.02
Arachidic acid	1.01	0.42	0.53	0.47	0.24	0.31	0.50
γ-Linolenic acid	0.21	0.33	0.35	0.28	0.16	0.14	0.24
Unsaturated fatty acids, %	13.71	15.20	15.17	14.77	14.99	16.41	15.04
Long chain fatty acids ≥ 18 carbons, %	84.31	79.34	76.60	81.45	79.37	83.21	80.71
18 carbons unsaturated fatty acids, %	12.01	12.52	12.54	12.54	12.24	14.36	12.70
cis- fatty acids, %	3.82	4.94	4.96	4.87	4.92	6.13	4.94
trans- fatty acids, %	7.98	7.25	7.23	7.38	7.16	8.09	7.51
Ratios (*cis*-/*trans*-)	0.480	0.681	0.685	0.661	0.688	0.758	0.66
Ratios (Stearic acid: Palmitic acid)	6.258	4.933	4.027	5.202	4.753	5.746	5.15
Odd-carbon fatty acids, %	1.90	3.78	3.56	2.68	3.37	2.50	2.96
Even-carbon saturated fatty acids, %	85.07	81.85	82.10	83.05	82.42	81.67	82.69
Even-carbon unsaturated fatty acids, %	13.04	14.38	14.34	14.28	14.21	15.83	14.34
Saturated/unsaturated fatty acids	6.29	5.58	5.60	5.78	5.67	5.09	5.65

[a] Internal peak area values, IPA(%), means the relative proportion (%) of different fatty acids in the corresponding individual sample.

Table 4. Internal peak area values, IPA(%), of volatile fatty acids (VFAs) in media fraction [a]

Items	IPA (%) values of VFAs within exogenous ω-6/ω-3 LCFAs Ratios supplementation [b]						Average
	100-0	90-10	80-20	66-33	50-50	20-80	
Acetic acid	44.72	43.86	43.61	43.62	42.83	42.74	43.56
Propionic acid	22.80	22.62	22.90	23.15	23.36	23.66	23.08
isobutyric acid	2.48	2.57	2.78	2.37	2.39	2.65	2.54
Butyric acid	18.31	19.06	19.55	19.21	19.66	19.62	19.24
isovaleric acid	5.93	5.99	5.84	5.89	5.77	5.81	5.87
Valeric acid	5.77	5.89	5.32	5.77	5.99	5.51	5.71
Ac/Pro	1.96	1.94	1.90	1.88	1.83	1.81	1.89

[a] Internal peak area values, IPA(%), means the relative proportion (%) of different fatty acids in the same individual sample. Experiments were in ruminal fermented media on supplementation of exogenous ω-6/ ω-3 long chain fatty acids (or mM in fermentation media). We used 100 mg of total ω-6 and ω-3 per each gram (g) of alfalfa substrate;
[b] The whole amount of ω-6/ ω-3 LCFAs were standard as 100 mg/g alfalfa substrate, means ± standard errors.

Table 5. *in vitro* fermentation performance supplementation of ω-6/ω-3 PUFAs

Items	In vitro fermentation characteristics within exogenous ω-6/ω-3 LCFAs Ratios supplementation						
	Control	100-0	90-10	80-20	66-33	50-50	20-80
CH_4 (mMol/g) 48	108.81	87.44	83.12	74.28	87.84	86.96	58.50
pH	6.14	6.20	6.30	6.15	6.21	6.14	6.15
Time (h)	Gas pressure (psi) determined with the detectors of ANKOM during 72 h						
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	1.219	1.322	1.286	1.334	1.237	1.298	1.383
4	2.165	2.001	1.989	2.099	1.977	2.050	2.147
6	3.039	2.657	2.632	2.778	2.657	2.681	2.747
8	3.821	3.299	3.263	3.506	3.287	3.312	3.293
12	4.749	4.124	4.051	4.355	4.136	4.100	4.149
16	5.258	4.561	4.452	4.791	4.549	4.549	4.549
20	5.604	4.804	4.719	5.131	4.840	4.816	4.840
24	5.877	5.022	4.937	5.422	5.058	4.998	5.040
30	6.059	5.192	5.167	5.689	5.192	5.180	5.186
36	6.205	5.289	5.325	5.895	5.289	5.252	5.240
42	6.296	5.374	5.434	5.980	5.313	5.265	5.258
48	6.387	5.507	5.580	6.101	5.374	5.410	5.331
54	6.387	5.519	5.628	6.089	5.325	5.398	5.313
60	6.350	5.519	5.641	6.114	5.289	5.337	5.277
66	6.277	5.495	5.641	6.138	5.180	5.252	5.204
72	6.277	5.519	5.677	6.199	5.155	5.313	5.222

Time (h)	Fermentation temperature (℃) determined with the detectors of ANKOM during 72 h						
0	39.000	39.000	39.052	39.000	39.000	39.000	39.000
2	41.429	42.344	42.709	41.926	41.351	41.874	41.194
4	41.116	40.933	41.769	41.142	41.247	41.978	41.273
6	41.194	41.351	41.874	41.194	41.090	42.030	41.038
8	41.429	41.717	41.978	41.299	40.933	42.083	41.038
12	41.586	41.926	42.187	41.612	41.456	42.396	41.273
16	41.743	41.978	42.135	41.665	41.665	42.448	41.351
20	41.429	41.717	41.821	41.247	41.299	42.135	41.038
24	40.489	40.776	41.247	40.776	40.829	41.456	40.646
30	40.567	40.567	40.933	40.202	40.567	40.985	40.019
36	40.411	40.411	40.463	39.731	40.202	40.672	39.549
42	39.862	40.515	40.567	39.888	39.888	40.254	39.313
48	40.019	40.202	40.149	39.470	39.784	40.515	39.549
54	39.862	40.202	40.149	39.261	39.575	40.463	39.392
60	39.549	39.940	39.993	39.261	39.104	39.888	39.000
66	39.313	39.470	39.679	38.582	38.425	39.313	38.373
72	38.765	39.000	38.791	38.112	38.739	39.366	38.530

The gas pressure (psi) and fermentation temperature (℃) was presented a part of all the dataset obtained in this table, the full details of these fermentation parameters were shown in http://dx.doi.org/10.6084/m9.figshare.1428561.

Table 6. All input variables for developing a new RT-LFER model

Symbols	**The details of input variables for new RT-LFER model** [b]
ε_{ij}	IPA $(\%)_{new}$, IPA $(\%)_{ref}$
$<\varepsilon_{ij}>$ [a]	$<IPA(\%)_{new}>$, $<IPA(\%)_{ref}>$
Ω	P(psi), pH(72h), CH_4, T, t, ω-6, ω-3
$\Delta\Omega$	ΔP(psi), ΔpH(72h), ΔCH_4, ΔT, Δt, Δω-6, Δω-3
$\log(\lvert\Delta\Omega\rvert)$	$\log(\lvert\Delta P(psi)\rvert)$, $\log(\lvert\Delta pH(72h)\rvert)$, $\log(\lvert\Delta CH_4\rvert)$, $\log(\lvert\Delta T\rvert)$, $\log(\lvert\Delta t\rvert)$
${}^iV_k$	$({}^iV_1, {}^iV_2, {}^iV_3, {}^iV_4, {}^iV_5, {}^iV_6)_{new}$, $({}^iV_1, {}^iV_2, {}^iV_3, {}^iV_4, {}^iV_5, {}^iV_6)_{ref}$
Δ^iV_k	$\Delta^iV_1, \Delta^iV_2, \Delta^iV_3, \Delta^iV_4, \Delta^iV_5, \Delta^iV_6$
$\Delta\Delta^iV_k(c_j)$	$\Delta\Delta^iV_1(c_1), \Delta\Delta^iV_1(c_2), \Delta\Delta^iV_1(c_3), \Delta\Delta^iV_1(c_4), \Delta\Delta^iV_1(c_5), \Delta\Delta^iV_1(c_6)$; $\Delta\Delta^iV_2(c_1), \Delta\Delta^iV_2(c_2), \Delta\Delta^iV_2(c_3), \Delta\Delta^iV_2(c_4), \Delta\Delta^iV_2(c_5), \Delta\Delta^iV_2(c_6)$; $\Delta\Delta^iV_3(c_1), \Delta\Delta^iV_3(c_2), \Delta\Delta^iV_3(c_3), \Delta\Delta^iV_3(c_4), \Delta\Delta^iV_3(c_5), \Delta\Delta^iV_3(c_6)$; $\Delta\Delta^iV_4(c_1), \Delta\Delta^iV_4(c_2), \Delta\Delta^iV_4(c_3), \Delta\Delta^iV_4(c_4), \Delta\Delta^iV_4(c_5), \Delta\Delta^iV_4(c_6)$; $\Delta\Delta^iV_5(c_1), \Delta\Delta^iV_5(c_2), \Delta\Delta^iV_5(c_3), \Delta\Delta^iV_5(c_4), \Delta\Delta^iV_5(c_5), \Delta\Delta^iV_5(c_6)$; $\Delta\Delta^iV_6(c_1), \Delta\Delta^iV_6(c_2), \Delta\Delta^iV_6(c_3), \Delta\Delta^iV_6(c_4), \Delta\Delta^iV_6(c_5), \Delta\Delta^iV_6(c_6)$;
$(V_{k\,new})^2$	$(V_{1\,new})^2, (V_{2\,new})^2, (V_{3\,new})^2, (V_{4\,new})^2, (V_{5\,new})^2, (V_{6\,new})^2$
$(\Delta\Delta^iV_6(c_j))^2$	$(\Delta\Delta^iV_6(c_1))^2, (\Delta\Delta^iV_6(c_2))^2, (\Delta\Delta^iV_6(c_3))^2, (\Delta\Delta^iV_6(c_4))^2, (\Delta\Delta^iV_6(c_5))^2, (\Delta\Delta^iV_6(c_6))^2$

[a] $f(\varepsilon_{ij})_{ref} = \langle\varepsilon_{ij}\rangle_{ref} = \langle IPA(\%)\rangle_{ref}$ average of reference entries for conditions of c_1, c_3, c_4, and c_5; among, c_1 = treatments, c_2 = with/without fermentation, c_3 = phase, c_4 = gas chromatography column, c_5 = replicates, and c_6 = *cis*/*trans* pattern.
[b] Symbols of molecular descriptors were calculated with DRAGON software:V_1 = Mw, V_2 = Aeigv, V_3 = Aeige, V_4 = Aeigp, V_5 = AMR, and V_6 = LogP; V_2, V_3, and V_4 are function of eigenvalues of the topological distance matrix weighted with atomic van der Waals volumes (v), Electronegativities (e), or Polarizabilities (p). The parameters $\Delta V_k(c_j)$ are Moving Averages, and $\Delta\Delta^i V_k(c_j) = p(c_j)_{new}\cdot({}^{new\ i}V_k - \langle {}^iV_k(c_j)\rangle_{new}) - p(c_j)_{ref}\cdot({}^{ref\ i}V_k - \langle {}^iV_k(c_j)\rangle_{ref})$ are PT operators.

Table 7. Details of PT-LFER model for fatty acids distribution network

Coeff.	Variable [a]	Eigenvalues	Classic Symbols [b]	Description of input variables [c]
a_0	Intercept	1.1352		
a_1	ω-6$_{_new}$	4.8007	ω-6	Exogenous ω-6 PUFAs in the new state
a_2	ω-3$_{_new}$	4.0087	ω-3	Exogenous ω-3 PUFAs in the new state
a_3	pH_72h$_{_new}$	-0.0173	pH	pH value at 72h fermentation in the new state
a_4	IPA$_{_new}$	-4.1554	FA proportion	Internal peak area value of each fatty acid in the new state
b_5	$\Delta\omega$-6	-8.6897	ω-6	Difference (Δ) of ω-6 in the new and reference states
b_6	ΔP(psi)	0.0091	Gas pressure	Difference of gas pressure (ΔP) in the new and reference states
b_7	$\Delta\omega$-3	-4.7979	ω-3	Difference (Δ) of ω-3 in the new and reference states
b_8	CH_4_48h$_{_ref}$	0.0848	Methane	Methane production at 48h fermentation in the reference state
b_9	Log($\lvert\Delta T\rvert$)	0.0011	Temperature	Logistic absolute value of Difference (Δ) of temperature in the new state and reference states
b_{10}	$\langle IPA(\%)_{ref}\rangle$	0.0669	avg. (IPA $_{ref}$)	The mean value of the same fatty acid in different boundary condition of reference state
b_{11}	V_{5_new}	4.0914	V_5 = MR	Magnetoresistence value in the new state
b_{12}	V_{2_ref}	-7.7553	V_2 = Aeigv	Aeigv values in the new state
b_{13}	ΔV_1	-134.5729	V_1 = Mw	$= V_{1\_new} - V_{1\_ref}$
b_{14}	ΔV_5	89.3481	V_5 = MR	$= V_{5\_new} - V_{5\_ref}$
b_{15}	$\Delta\Delta V_1(c_1)$	-10.4400	V_1 = Mw	$= \Delta V_1(c_1)_{new} - \Delta V_1(c_1)_{ref} = p(c_1)_{new}\cdot({}^{new}V_1 - \langle V_1(c_1)\rangle_{new}) - p(c_1)_{ref}\cdot({}^{ref}V_1 - \langle V_1(c_1)\rangle_{ref})$; $\langle V_1(c_1)\rangle$ = Average of MR for c_1
b_{16}	$\Delta\Delta V_6(c_3)$	34.5450	V_6 = LogP	$= \Delta V_6(c_3)_{new} - \Delta V_6(c_3)_{ref} = p(c_3)_{new}\cdot({}^{new}V_6 - \langle V_6(c_3)\rangle_{new}) - p(c_3)_{ref}\cdot({}^{ref}V_6 - \langle V_6(c_3)\rangle_{ref})$; $\langle V_6(c_3)\rangle$ = Average of Mw for c_3
b_{17}	$\Delta\Delta V_1(c_4)$	10.8253	V_1 = Mw	$= \Delta V_1(c_4)_{new} - \Delta V_1(c_4)_{ref} = p(c_4)_{new}\cdot({}^{new}V_1 - \langle V_1(c_4)\rangle_{new}) - p(c_4)_{ref}\cdot({}^{ref}V_1 - \langle V_1(c_4)\rangle_{ref})$; $\langle V_1(c_4)\rangle$ = Average of Aeigp for c_4
b_{18}	$\Delta\Delta V_5(c_5)$	-0.4531	V_5 = MR	$= \Delta V_5(c_5)_{new} - \Delta V_5(c_5)_{ref} = p(c_5)_{new}\cdot[({}^{new}V_5 - \langle V_5(c_5)\rangle_{new}) - p(c_5)_{ref}\cdot({}^{ref}V_5 - \langle V_5(c_5)\rangle_{ref})]^2$; $\langle V_5(c_5)\rangle$ = Average LogP for c_5
b_{19}	$(V_{6new})^2$	7.4454	V_6 = LogP	$= (\text{LogP}_{new})^2$
b_{20}	$(\Delta\Delta V_6(c_2))^2$	0.2977	V_6 = LogP	$= \Delta V_6(c_2)_{new} - \Delta V_6(c_2)_{ref} = p(c_2)_{new}\cdot({}^{new}V_6 - \langle V_6(c_2)\rangle_{new}) - p(c_2)_{ref}\cdot({}^{ref}V_6 - \langle V_6(c_2)\rangle_{ref})$; $\langle V_6(c_2)\rangle$ = Average of Mw for c_2
b_{21}	$(\Delta\Delta V_6(c_6))^2$	-0.3115	V_6 = LogP	$= \Delta V_6(c_6)_{new} - \Delta V_6(c_6)_{ref} = p(c_6)_{new}\cdot({}^{new}V_6 - \langle V_6(c_6)\rangle_{new}) - p(c_6)_{ref}\cdot({}^{ref}V_6 - \langle V_6(c_6)\rangle_{ref})$; $\langle V_6(c_6)\rangle$ = Average of Mw for c_6

[a] $f(\varepsilon_{ij})_{ref} = \langle\varepsilon_{ij}\rangle_{ref} = \langle IPA(\%)\rangle_{ref}$ average of reference entries for conditions of c_1, c_3, c_4, and c_5; among, c_1 = treatments, c_2 = with/without fermentation, c_3 = phase, c_4 = gas chromatography column, c_5 = replicates, and c_6 = *cis*/*trans* pattern.

[b] Symbols of molecular descriptors were calculated with DRAGON software:V_1 = Mw, V_2 = Aeigv, V_3 = Aeige, V_4 = Aeigp, V_5 = AMR, and V_6 = LogP; V_2, V_3, and V_4 are function of eigenvalues of the topological distance matrix weighted with atomic van der Waals volumes (v), Electronegativities (e), or Polarizabilities (p).
[c] The parameters $\Delta^{i}V_k(c_j)$ are Moving Averages, and $\Delta\Delta^{i}V_k(c_j) = p(c_j)_{new}\cdot({}^{new}\,{}^{i}V_k - \langle {}^{i}V_k(c_j)\rangle_{new}) - p(c_j)_{ref}\cdot({}^{ref}\,{}^{i}V_k - \langle {}^{i}V_k(c_j)\rangle_{ref})$ are PT operators.

Table 8. Molecular descriptors ($^{i}V_k$) of fatty acids obtained from ChEBI database

Name of fatty acids in ChEBI [a]	*cis*/*trans* pattern [b]	DRAGON software variables- outputs [c]					
		V_1	V_2	V_3	V_4	V_5	V_6
Lauric Acid	*l*	200.4	137.4	127.6	139.5	58.7	4.5
Myristic Acid	*l*	228.4	179.1	169.0	181.2	67.9	5.5
Myristoleic Acid	*c*	226.4	172.1	162.0	174.2	69.0	5.0
Pentadecanoic Acid	*l*	242.5	202.0	191.8	204.1	72.5	5.9
cis-10-Pentadecenoic acid	*c*	240.4	194.8	184.5	196.9	73.6	5.5
Palmitic Acid	*l*	256.5	226.3	216.0	228.4	77.1	6.4
Palmitoleic Acid	*c*	254.5	217.9	207.6	220.1	78.2	5.9
Heptadecanoic acid	*l*	270.5	252.0	241.6	254.1	81.7	6.8
Stearic acid *cis*-10-Heptadecenoic acid	*c*	268.5	243.2	232.8	245.4	82.8	6.4
Stearic acid	*l*	284.5	279.1	268.6	281.2	86.3	7.3
Elaidic acid	*t*	282.5	269.5	259.0	271.7	87.4	6.8
Oleic acid	*c*	282.5	269.5	259.0	271.7	87.4	6.8
Linolelaidic acid	*tt*	280.5	260.9	250.4	263.1	88.5	6.4
Linoleic acid	*cc*	280.5	260.9	250.4	263.1	88.5	6.4
Arachidic acid	*l*	312.6	337.4	326.8	339.6	95.5	8.2
γ-Linolenic acid	*ccc*	278.5	251.5	240.9	253.7	89.6	5.9
Linolenic acid	*ccc*	278.5	255.1	244.5	257.3	89.6	5.9
cis-11.14-Eicosadienoic acid	*ct*	308.6	318.0	307.3	320.2	97.7	7.3
Behenic acid	*l*	340.7	401.3	390.6	403.5	104.7	9.1
cis-8.11.14-Eicosatrienoic acid	*ctt*	306.5	307.4	296.7	309.7	98.8	6.9
Erucic acid	*t*	338.6	390.2	379.4	392.4	105.8	8.7
Acetic acid	*l*	60.1	13.1	6.4	14.4	12.6	-0.2
Propionic acid	*l*	74.1	19.2	11.8	20.7	17.3	0.4
Isobutyric acid	*l*	88.1	24.6	16.7	26.2	21.8	0.9
Butyric acid	*l*	88.1	26.7	18.8	28.3	21.9	0.9
Isovaleric acid	*l*	102.2	33.2	24.9	34.9	26.4	1.1
Valeric acid	*l*	102.2	35.6	27.4	37.4	26.5	1.3

[a] Measured fatty acids with our linear discriminant analysis PT-LFER model.
[b] *cis*/*trans* pattern, *l* represents *linear*, *c* as *cis*-, *t* as *trans*- LCFAs. The orders of *c* or *t* represents the order of isomerization characteristics initial with the tails of LCFAs.
[c] Molecular descriptors ($^{i}V_k$) calculated with software DRAGON: V_1 = Mw (molecular weight), V_2 = Aeigv, V_3 = Aeige, V_4 = Aeigp, V_5 = MR (magnetoresistence), and V_6 = LogP; V_2, V_3, and V_4 are average eigenvalues of the topological distance matrix weighted with atomic van der Waals volumes (v), Electronegativities (e), or Polarizabilities (p).

Table 9. Results of LDA PT-LFER model for perturbation network of fatty acid distribution in ruminal microbiome

Data sub-set [a]	Statistical parameter	Prediction rates (%)	Prediction cases	
			$N(L_{nr} = 0)$	$N(L_{nr} = 1)$
Training dataset				
$N(L_{nr} = 0)$	Specificity (Sp)	93.5	215,010	14,983
$N(L_{nr} = 1)$	Sensitivity (Sn)	96.3	6,600	172,841
	Accuracy (Ac)	94.7		
Validation dataset				
$N(L_{nr} = 0)$	Specificity (Sp)	93.3	71,496	5,127
$N(L_{nr} = 1)$	Sensitivity (Sn)	96.6	2,056	57,582
	Accuracy (Ac)	94.7		

[a] Number in total (N) = 545,695; $N(L_{nr} = 0)$ represents the number of cases in sub-set with $L_{nr} = 0$ (no connected nodes) or the same with new and reference states when $IPA(\%)_{new} \leq IPA(\%)_{ref}$. $N(L_{nr} = 1)$ represents the number of cases in sub-set with $L_{nr} = 1$ (links in the network), or the same with new and reference states when $IPA(\%)_{new} > IPA(\%)_{ref}$.

Table 10. Average values of input parameters ($<^iV_k>$) for experimental boundary conditions (c_j)

Experimental boundary condition	Average values of input variables $<^iV_k(c_j)>$ [aa]							$p(c_j)$ [ab]
Conditions (c_j)	level	V_1	V_2	V_3	V_4	V_5	V_6	
c_1 => Treatments [a]	BM	274.4	258.3	247.9	260.5	84.8	6.6	0.113
	AM	274.4	258.3	247.9	260.5	84.8	6.6	0.113
	CM	274.4	258.3	247.9	260.5	84.8	6.6	0.113
	BA	261.9	234.6	224.3	236.8	80.3	6.2	0.258
	PA	261.9	234.6	224.3	236.8	80.3	6.2	0.258
	MA	85.8	25.4	17.7	27.0	21.1	0.7	0.145
c_2 => fermentation [b]	***0***	274.4	258.3	247.9	260.5	84.8	6.6	0.339
	1	223.2	188.7	178.9	190.7	67.3	5.0	0.661
c_3 => Phase [c]	Bacteria fraction	269.0	248.1	237.7	250.3	82.9	6.4	0.597
	Protozoa fraction	261.9	234.6	224.3	236.8	80.3	6.2	0.258
	Media fraction	85.8	25.4	17.7	27.0	21.1	0.7	0.145
c_4 => column of GC [d]	HP-88 (112-88A7)	266.8	244.0	233.6	246.2	82.1	6.4	0.855
	DB-FFAP	85.8	25.4	17.7	27.0	21.1	0.7	0.145
c_5 => replicates (*r*-error) [e]	***0***	242.8	213.9	203.8	216.0	73.9	5.6	0.391
	0.1	242.8	213.9	203.8	216.0	73.9	5.6	0.391
	0.2	205.8	173.6	164.2	175.6	61.6	4.4	0.133
	0.3	274.4	258.3	247.9	260.5	84.8	6.6	0.085
c_6 => *cis*/*trans*	*linear*	203.2	180.8	171.5	182.8	59.6	4.6	0.238
	cis	278.5	269.1	258.6	271.3	86.1	6.7	0.111

pattern [f]	*trans*	310.6	329.8	319.2	332.1	96.6	7.7	0.032
	trans, trans	280.5	260.9	250.4	263.1	88.5	6.4	0.016
	cis, cis	308.6	320.8	310.1	323.0	97.7	7.3	0.032
	cis, trans	289.9	279.6	269.0	281.8	91.6	6.7	0.048
	trans, cis	280.5	261.1	250.5	263.3	88.5	6.4	0.016
	cis, cis, cis	286.5	269.9	259.3	272.1	92.3	6.2	0.111
	cis, trans, trans	292.5	280.0	269.3	282.2	94.2	6.4	0.032
	trans, cis, trans	278.5	254.6	244.0	256.8	89.6	5.9	0.016
	trans, trans, trans	278.5	252.0	241.4	254.2	89.6	5.9	0.032
	cis, cis, trans	278.5	252.5	241.9	254.8	89.6	5.9	0.016
	cis, trans, cis	278.5	252.0	241.4	254.2	89.6	5.9	0.032

[a] "BM" means base methylation without fermentation; "AM" means acid methylation without fermentation; "CM" means acid- and base- combined methylation; "BA" means fatty acids from bacterial fraction after 48h fermentation; "PA" means fatty acids from protozoal fraction after 48h fermentation; "MA" means the volatile fatty acids from media fraction after 48h fermentation; [b] "***0***" means the dataset from experimental 1 without fermentation; "***1***" means the dataset from experimental 2 with fermentation of omega 6 and omega 3; [c] "phase", means the dataset: long chain fatty acids from including bacterial membrane (bacterial fraction), protozoal membrane (protozoa fraction), volatile fatty acids from fermentation media (media fraction); [d] Column of GC, "HP-88 (112-88A7)" means the column of GC for determining long chain fatty acids; "DB-FFAP" means the column for determining volatile fatty acids; [e] "***0***" means the original data, "***0.1***, ***0.2***, or ***0.3***" means the 1, 2, or 3 replicates, respectively; [f] *cis*/*trans* pattern: "*linear*" means LCFA without double bonds; "*cis*" means LCFA with *cis* isomerization; "*trans*" means LCFA with *trans* isomerization; and the number of cis or trans means LCFA with the same number of *cis* or *trans* double bonds; [aa] $<V_k(c_j)>$ means the average of Molecular descriptors (V_k) for different conditions (c_j); the descriptors are V_1 = Mw, V_2 = Aeigv, V_3 = Aeige, V_4 = Aeigp, V_5 = AMR, and V_6 = LogP; V_2, V_3, and V_4 are function of eigenvalues of the topological distance matrix weighted with atomic van der Waals volumes (*v*), Electronegativities (*e*), or Polarizabilities (*p*); [ab] $p(c_j) = n_j / n_{total}$; n_j number of experimental entries for condition c_j and n_{total} = 744 total number of experimental entries.

Table 11. Comparative study of PT-LFER *vs.* PT-NLFER models [a]

No.	Model-Description	Statistical Parameters [a]			
		TP **(%)**	***VP*** **(%)**	***TE*** **(%)**	***VE*** **(%)**
1	MLP 10:10-9-1:1	88.56	88.44	77.12	77.45
2	MLP 11:11-8-1:1	97.57	97.57	42.19	41.92
3	MLP 14:14-10-1:1	98.44	98.43	35.54	35.48
4	MLP 14:14-14-1:1	98.97	98.95	32.06	31.58
5	MLP 15:15-10-1:1	98.86	98.80	30.24	30.44
6	MLP 15:15-12-1:1	99.27	99.25	24.16	24.31
7	MLP 16:16-11-1:1	99.03	99.03	27.11	27.16
8	MLP 16:16-13-1:1	99.37	99.34	22.33	21.99
9	MLP 17:17-12-1:1	99.30	99.26	19.07	19.99
10	MLP 17:17-13-1:1	99.55	99.53	16.73	17.20
11	MLP 17:17-14-1:1	99.69	99.68	16.97	17.31
12	MLP 18:18-12-1:1	99.51	99.47	15.02	15.72
13	MLP 18:18-13-1:1	99.53	99.56	13.49	12.98
14	MLP 18:18-14-1:1	99.42	99.45	16.37	15.93

15	**MLP 18:18-15-1:1**	99.70	99.68	10.85	11.13
16	MLP 19:19-13-1:1	99.35	99.37	16.81	16.47
17	MLP 19:19-14-1:1	99.67	99.69	15.61	15.59
18	LDA	94.7	94.7	**5.56**	**5.56**

[a] PT-LFER: Perturbation Theory- Linear Free Energy Relationships; PT-NLFER: Perturbation Theory- Non Linear Free Energy Relationships; MLPs: Multilayer perceptron.
[b] *TP* (%) = Training Performance, *VP* (%) = Validation Performance, *TE* (%) = Training Error, *VE* (%) = Validation Error.

DISCUSIÓN

Parte 1. Proporciones de cártamo y canola con sorgo

Las diferencias estadísticas en la cinética de gases entre la comida de cártamo (SFM) y semilla de canola (CAS) los tratamientos podrían deberse a la proporción, y la naturaleza, de su composición. Alto contenido de hidratos de carbono solubles podría ser debido a un factor importante resultante a una mayor producción de gas en tratamientos proporción alta de sorgo (Zerbini et al., 2002; Amer et al, 2012.). Mientras tanto, algunas composiciones de casco o anti-nutricionales en la harina de cártamo o de semilla de canola también podrían dar lugar a disminuir los parámetros de producción de gas in vitro, como matairesinol-β-glucósido, 2-hydroxyarctiin-β-glucósido (Jin et al., 2010) y glucósido cianogénico (Satish y Shrivastava, 2011) en SFM y sinapina, taninos, ácido fítico (Brand et al., 2007) y de glucosinolatos (Bell, 1993) en el CAS.

Rumen microorganismo digiere macromolecular de proteínas, carbohidratos y ácidos grasos poliinsaturados de cadena larga para generar la producción de gas, ácidos grasos volátiles (AGV), metabolitos secundarios, proteína microbiana (Baba et al., 2002; Camacho et al., 2010). Dietas beneficiosos añadidos a los resultados del rumen en una inhibición de la desaminación y la metanogénesis, lo que resulta en una menor NH3-N, CH4, y acetato, y en las concentraciones de propionato y butirato más altas (Calsamiglia et al., 2007). Proteína microbiana y proteínas de los alimentos son insuficientes para suministrar cantidades adecuadas de aminoácidos para un rendimiento óptimo crecimiento de los rumiantes (Kung Jr y Rode, 1996), por lo NH3-N como no proteicos de nitrógeno o de degradación de los metabolitos de la proteína también juega un papel importante en mantener equilibrio nutritivo de los rumiantes. En este estudio, los concentrados de amoniaco N eran rango 33,1-66,3 mg / dl y estaban cerca de los informes anteriormente (Cherdthong y Wanapat, 2013), cambiante de NH3-N podría ser debido a las diferentes proporciones de proteínas de

los alimentos, y el saldo de la síntesis y la degradación microbiana (Khorasani et al., 1989); NH3-N a las 48 h eran un poco más alto que 24 h implicaban la tasa de proteína microbiana digeridos fueron más rápido que el de la acumulación en la síntesis de 48 h de incubación.

DIVMS de rastrojo de maíz aumentó con el aumento de la proporción de CAS y SFM podría ser debido al alto contenido de almidón en el sorgo reducido valor del pH del líquido de fermentación y aumentar la producción de ácidos grasos volátiles corto (Lechartier y Peyraud, 2011), además de efectuar la fibrolítica actividad. Implicaba que la proteína digestible rumen debe ser el factor restringido importante para digestibilidad de la fibra cuando adición de suficientes carbohidratos solubles. Durante la adaptación a una dieta alta en concentrado, pH ejerce una presión selectiva contra los microbios intolerantes de un bajo valor de pH. Como el pH cae, amilolítica y bacterias ácido-tolerantes aumentan mientras que los microbios celulolíticas disminuyen, hidratos de carbono no estructural excesiva (NSC) puede deprimir la energía disponible de la producción de ácido propiónico y láctico reducir la síntesis de proteína microbiana (Tan et al., 2002b), y disminuyen la digestibilidad de la fibra así como causar anormalidades en el tejido rumen, lo que puede conducir a úlceras y abscesos hepáticos en animales (Ishler, 1996).

Parte 2. Fracciones

2.1. Cinética de producción de gas in vitro

En 1968, Guggolz et al. analizó sistemáticamente la composición química de las diferentes partes de semilla de cártamo (Guggolz et al., 1968), posteriormente una pocas investigaciones valiosas informaron el aporte nutricional de semillas de cártamo en la alimentación animal y de producción (in vivo o in situ) (Moon et al., 2001; Lee et al., 2004; Bozan y Temelli, 2008; Alizadeh et al, 2010;.. Dschaak et al, 2010). Algunos valiosa utilización de semilla de cártamo se han reportado para la alimentación de aves, y la función medicinal de la composición de la semilla de cártamo. En este estudio, en los parámetros de producción de gas in vitro (VF, FRD0,

RGT) en el casco cártamo fueron menores que en otras fracciones de semillas de cártamo. Las diferencias en la producción de gas in vitro en entre diferentes fracciones de semilla de cártamo, podría ser debido a la proporción y naturaleza de su composición (Rubanza et al., 2003).

Utilizamos las partes puras diferentes de semilla de cártamo como sustrato para la fermentación in vitro, lo que resultó en un mayor rendimiento de fermentación in vitro de la harina de cártamo de que en toda la semilla y el casco, incluso en kernel. Como se informó anteriormente, combinamos comida cártamo con semilla de sorgo para la fermentación in vitro, el alto contenido de almidón en plomo semilla de sorgo a la alta producción de gas in vitro. Souilah y Djabali (2014) reportaron que un alto contenido de carbohidratos solubles habían mejorado aún más los parámetros de producción de gas más importantes. Ingredientes anti-nutricionales, tales como matairesinol-β-glucósido, 2-hydroxyarctiin-β-glucósido (Jin et al., 2010) y glucósido cianogénico (Satish y Shrivastava, 2011) en la harina de cártamo, en la harina de cártamo podrían dar lugar a la reducción de la in vitro parámetros de producción de gas (VF, RGT y t0,5). En este trabajo, también obtuvimos algunos parámetros valiosas sobre parámetro de forma (b) en el casco tratamiento es semilla de cártamo positivo, entero y kernel estaban alrededor de 0, sin embargo, el valor del parámetro de forma (b) en las semillas de cártamo fueron negativas (alrededor de - 20), que los datos no se presentó en las Tablas. Sobre el valor del parámetro de forma, representa positiva de la curva de la producción de gas es similar con s curva con un tiempo de retardo para un sistema de fermentación sellada, de lo contrario, la curva de producción de gas es como una curva parabólica, representa que este sistema de sellado no tiene ninguna fermentación inicialización limitado, debido a la composición de equilibrio o de alta energía se complementó (Wang et al., 2013).

VF era un parámetro correspondiente relacionado con el carbohidrato no digerible-casco estructural (Bäumler et al., 2006), alta en carbohidratos solubles (Lechartier y Peyraud, 2011), así como los factores anti-nutricionales (tales como glucósido cianogénico) (Satish y Shrivastava, 2011). En este estudio, hemos tenido la producción de gas correspondiente por g de diferentes partes de la semilla de cártamo, de los

resultados concluimos comida cártamo tuvo la mayor producción de gas máxima teórica que los de semilla de cártamo conjunto, casco, incluso partición del núcleo. Como no había alto contenido de aceite en semilla de cártamo, la mayoría de ellos depositado en la parte del núcleo de semilla de cártamo, resultó en la producción de gas en baja fermentación in vitro. Los ácidos grasos tienen el potencial de cambiar el ecosistema microbiano, la actividad influencia de alguna capacidad de adhesión bacteriana (Benchaar et al., 2008). Ingredientes anti-nutricionales no digeridas o depositados en cártamo semilla entera / kernel también podrían disminuir la eficacia de la digestión reflejada por los parámetros de producción de gas (VF, FRD0, k, t0,5, RGT, y μ0.5), para la hidratación, la eliminación de inhibidores de la digestión y / o la unión de microbios con sustratos (Mertens, 1993) incrementan el tiempo de adsorción, de modo que sea más dificultad contra el carbohidrato estructural (Tan et al., 2002a). t0,5 de casco era mucho más alta que en la harina de cártamo y el núcleo o semilla entera, podría ser atribuida a que el casco del SFM impedido los procesos de fijación, adhesión, colonización y de degradación de los microorganismos ruminales (Varga y KOLVER, 1997).

2.2. Rumen pH

Rumen pH es incompatible con concentrados de metabolitos, alto valor de pH siempre adjunto con metabolitos secundarios menores, de lo contrario (Pina et al., 2009). El valor de pH, que varía entre 5,5 y 7,5, se considera como un determinante importante en el rumen in vitro o in vivo de fermentación (Yuan et al., 2010), altamente correlacionado con VFAs, las actividades de población y de la enzima de microbios. Los ácidos grasos como el ácido láctico, que inhibe las membranas de ácido láctico en no ionizado que en forma ionizada, la población de protozoos que son responsables de aproximadamente el 25% de celulolítica microbiana ruminal y ayudar a mantener un pH más alto por que envuelve los gránulos de almidón (Mould et al., 2005). En nuestro estudio, el valor de pH fue de alrededor de 6.7 a 6.9 durante los procesos de fermentación in vitro de diferentes partes de la semilla de cártamo para 48 h. La estabilidad de pH mostró que era beneficioso para toda la fermentación y a los microbios guardados para sobrevivir en un ambiente cómodo micro-.

2.3. Digestibilidad de fibra

Otro índice muy importante para evaluar los valores nutricionales de la alimentación es la digestibilidad (Naga y el-Shazly, 1963). En este estudio, el casco tenía un alto contenido de fibra, su in vitro importa desaparición seca (DIVMS) fue la más baja en comparación con otras particiones de semilla de cártamo. Sería debido a la lignificación alto contenido de fibra, como sabemos la fibra lignificada es composición digerible para los microbios del rumen. DIVMS aparente del kernel fue la más alta entre las diferentes partes de la semilla de cártamo, porque tenía ingredientes de alta solubles, por ejemplo, los carbohidratos solubles, proteínas y lípidos. Algunas composiciones se lavaron a cabo por los detergentes de fibra neutros o ácidos, que habían sido calculados en DIVMS resultado. Es por eso que también se calculó no digestibilidad de la fibra, FDN y FDA digestibilidad (en porcentaje). NDF / ADF digestibilidad de Kernel eran alrededor de 100% o un poco mayor que 100%, como altas capacidades solubles de ingredientes en kernel de cártamo o podría ser los microbios adjuntos a la bolsa de nylon fermentado in vitro. Toda semilla tienen una mayor digestibilidad de FDN y FDA que la harina de cártamo, porque el cálculo de los contenidos originales NDF o ADF (en porcentaje) en toda la semilla fue inferior a la de la harina de cártamo como alto contenido de lípidos depositados en toda la semilla, mientras que otros, harina de cártamo tiene alta NDF / ADF en porcentaje para deshacerse de los lípidos en el mismo. Sin embargo, DIVMS de toda semilla fue similar a la de harina de cártamo, como a los compañeros de las contribuciones de contenido en lípidos que aumentan y algunos factores anti-nutricionales que disminuyen DIVMS (aparente) en toda la semilla, en total, este dos piezas importantes resultaron DIVMS de semillas de cártamo fue similar a la de harina de cártamo, sólo de acuerdo a la verdad DIVMS de harina de cártamo debe ser mayor que el de semilla de cártamo, qué pena, esto necesita un trabajo más para probarlo.

Parte 3. Suplementación de acietes ricos en ácidos laúrico, oleico, linoleico y linolénico

3.1. LCFA fracciones de bacterias y protozoarios.

Para fracción bacteriana (BAC), ácido esteárico (C18: 0) y ácido palmítico (C16: 0) de la membrana de bacteria biología como la administración de suplementos de ácidos

grasos poli- o mono-insaturados (C18: 3, C18: 2 y C18: 1) de semillas de lino, cártamo y canola, fueron mayores que el suministro de aceite de coco, que las riquezas de AGCM (ácido láurico, C12: 0). Implica que los ácidos grasos vegetales exógenas pueden depositar o asimilar en la membrana de bacterias en cierta medida; Además, bacteria mixto tiene la capacidad de hidrogenar los ácidos grasos insaturados o bien mono- o poli- a ácidos grasos saturados. 12: 0 y C14: 0 fue mayor que éstos en otros tratamientos de aceite cuando se suministra con aceite de coco. Se especula que la bacteria podría asimilar a 12: 0 en su propia membrana biológica y, además de prolongar la cadena de carbono de C12 a C14 en un proceso de metabolismo desconocido compleja. Un muy alto porcentaje de ácido oleico en el aceite de linaza en comparación con el aceite de cártamo, aceite de canola y aceite de coco, que refleja que algunos de exógena original de PUFA (ácido γ-linolénico, C18: 3) se transforma en el ácido oleico (C18: 1) por algunas enzimas de degradación bacteriana, y luego el ácido oleico se asimilan o depositados en la membrana biológica bacteriana. Esto es consistente con informes anteriores (Chilliard et al, 2009;. Li et al., 2011). Chilliard et al. (. Chilliard et al, 2009) informó la administración de suplementos de ácido linolénico aumentó trans-11 C18: 1 y cis-9, trans-11 CLA en la grasa láctea. Mientras tanto, el ácido linoleico (C18: 2) puede mejorar tanto la trans-10 C18: 1 y trans-10, cis-12 C18: 2 rendimientos en leche (. Li et al, 2011), pero inferior C12: 0 y C16: 0 rendimiento (Stoffel et al., 2015).

La proporción de ácidos grasos de cadena larga (LCFA) ≥ 18 átomos de carbono y ácidos grasos insaturados en la administración de suplementos de PUFA o MUFA eran más alto que en AGCM del aceite de coco. La diferencia significativa mostró que los ácidos grasos de recursos planta exógenos podrían influir en la distribución de la composición de la membrana microbiana, y aún más para influir en la función de la membrana biológica. Es una interesante mencionar que cambiable de ácidos grasos cis y trans y cis / trans en relación membranas microbianas como la administración de suplementos de diversos tipos de FA. La proporción de trans- FA de la fracción bacteriana suministrado con aceite de linaza (5,91%) era un poco más baja que en

otros tratamientos. Esto se asocia con una relación cis / trans que en el aceite de linaza era triple que la de aceite de coco.

En fracción protozoo, composiciones FA también se cambiaron con suplementos de aceites de plantas exógenas. En primer lugar, los valores de proporción de AGCM en la fracción de protozoos, incluyendo el ácido láurico (C12: 0), ácido mirístico (C14: 0), ácido myritoleic (C14: 1), ácido pentadecanoico (C15: 0) y ácido palmítico (C16: 0), en el tratamiento de aceite de coco fue muy alta en comparación con la suplementación de poli- o mono-insaturados FA. En particular, la proporción de ácido palmítico en la adición de aceite de coco era 21,49%, que fue significativamente mayor ($P < 0,05$) que complementado con otros aceites vegetales, 13,29% en aceite de cártamo, 14,36% en el aceite de canola y 15,73% en aceite de linaza , respectivamente. En segundo lugar, sobre composiciones LCFA en la membrana protozoo. LCFA (≥ 18 carbonos) composiciones en la administración de suplementos de membrana por protozoos del aceite de coco (51.51%) fue significativamente menor que en otros aceites vegetales, 82,33% en aceite de cártamo, 81,06% en el aceite de canola, y 79,18% en aceite de linaza, respectivamente, sin diferencia estadística entre estos tres aceites vegetales. En tercer lugar, el cis- y trans- composiciones de PUFA en la membrana protozoos cambiaron con la administración de suplementos de aceites vegetales. Mientras tanto, la proporción de PUFA trans- membrana protozoo en aceite de linaza era 9,06%, que tenía 37,90% veces superior a la media (6,57%) de que en otros aceites vegetales. Aquí, es necesario mencionar que el cis / trans PUFA relación de la administración de suplementos de aceite de canola estaba cerca de 1,5 o 2 veces que la de otros aceites vegetales. Esto implica que más contenido de AGPI cis provienen de la planta de aceite de canola exógeno que se composited principalmente con ácido oleico. Es interesante notar que proporción de carbono impar FA en la administración de suplementos de aceite de coco (4,48%) fue veces aproximadamente el doble en comparación a los aceites de cártamo, linaza y canola con la composición principal de poli- o mono-insaturados FA. Eso es debido a que la proporción de ácido pentadecanoico (C15: 0), ácido heptadecanoico (C17: 0), ácido y cis-10-heptadecenoic (C17: 1) en aceite de coco eran alrededor de veces el doble que en otros aceites vegetales. También implica

que el ácido láurico exógena (C12: 0) podría prolongarse hasta más carbonos FA y sintetiza en la membrana protozoo. Baja proporción de ácidos grasos incluso en carbono saturado pero mayor proporción de ácidos grasos incluso-carbono insaturado se obtuvieron en aceite de linaza que en otros aceites vegetales, que era consistente con las proporciones de ácido / grasos insaturados saturados obtenidos aquí con los valores de 3,83 en aceite de linaza en comparación con otros aceites con el valor promedio de 5.27. El / insaturado FA saturado parece estar relacionado con la hidrofobicidad (Kundu et al., 2013), la fluidez (Calder et al., 1994), el estrés osmótico y la permeabilidad (Machado et al., 2004).

Obviamente, hubo diferencia entre media (39,05% frente a 26,48%) y la larga cadena FA (60,95% frente a 73,52%) en fracciones bacterianas y por protozoos. Un valor mayor proporción de la composición de FA medio en el que las bacterias que en la fracción de protozoos, implica que las bacterias tiene más capacidad de asimilar ácidos grasos de cadena media de los protozoos. Mientras tanto, la mayor proporción de ácido palmítico en la membrana bacteriana como la correspondiente que con mayor proporción de ácido esteárico en la membrana protozoos. La hidrofobicidad de la membrana celular de microbios, excepto fuerte relacionada con ácidos grasos hidroxilo, también es extremadamente dependiente de la larga cadena / medio, saturado / insaturado composición FA en la membrana (Kaczorek et al., 2013). También se puede concluir, protozoo puede extender de cadena media exógeno FA a partir de ácido láurico (C12: 0) a ácido palmítico (C16: 0) en cierta medida. Además, la proporción de ácido esteárico en protozoo y membranas biológicas bacterianas fue aumentando con la administración de suplementos de mono- y poli- exógeno FA insaturado en comparación con la administración de suplementos de MCFA. Sin embargo, la proporción de ácido palmítico (C16: 0) composición de tratamientos de aceite en protozoo fue menor que en la membrana bacteriana. La composición FA de membrana bacteriana y protozoo no es sólo se refiere a la diferencia entre estos ácidos grasos típicos, pero también menciona a algunos ácidos grasos de micronutrientes funcionales tales como el ácido linolénico conjugado (Lee, 2013), y ácido docosahexaenoico (DHA) (Boeckaert et al., 2008; Torok et al, 2014).. Como se ha

mencionado en informes anteriores, protozoos y bacterias implican de alguna diferente proceso de metabolismo biológico (Ushida y Jouany, 1996;. Ranilla et al, 2007), lo que podría ser fuerte en relación con la composición de diferencia en la membrana bacteriana y protozoos, bacterias y protozoos que tienen diferente función en el rumen micro-entorno porque que la función de los microorganismos son altos en relación con su estructura y composición (Huws et al, 2009;.. Belanche et al, 2011).

3.2. VFA en fermentación

Ácidos grasos volátiles (AGV), como productos intermedios segundo metabolizar en todo el sistema de fermentación ruminal, se han demostrado de manera eficiente la reflexión el rendimiento de la fermentación de microorganismos del rumen en las correspondientes condiciones de fermentación complejos. Todos los valores propios de ácido acético, ácido propiónico, ácido butírico, ácido isovalérico y ácido valérico en presencia de aceites de cártamo, linaza, colza y coco fueron mayores en comparación a la de control. Entre, ácido acético y ácido propiónico en presencia de aceite de linaza fue mayor que en el aceite de canola, aceite de cártamo, aceite de coco y en turno. El valor propio de AGV totales en la existencia de ácidos grasos exógenos de linaza, canola, cártamo, aceites de coco y se aumentó pero sin diferencia estadística entre en la presencia o ausencia de aceites de plantas, que es consistente con otros informes presentados mejorar un poco (Lin et al., 2013). Los Ac / valores Pro de todos los tratamientos se variaron desde 3,4 hasta 3,8 obtenido en este estudio, los resultados mostraron que un estado nutricional deficiente, siempre en el tratamiento en blanco del estudio presencia, había disminuido Ac / valores Pro. Mientras tanto, Ac / valores Pro en presencia de aceites vegetales exógenas también se redujeron, además, que los valores en presencia de aceites de cártamo, linaza, canola y coco eran incluso más baja que en el mal estado nutricional. Esto implica que el valor propio de ácido propiónico en presencia de aceites de cártamo, linaza, colza y coco se incrementó rápidamente que el ácido acético.

Sin embargo, el ácido isobutírico en presencia de aceite de coco era un poco más baja, en comparación con la presencia de cártamo, linaza o aceite de canola, incluso con el tratamiento control. Además, los valores propios de ácido isovalérico y ácido valérico

en presencia de aceite de canola fueron más altos que en el orden de aceite de linaza, aceite de cártamo y aceite en vez de coco. A pesar de que, los valores propios de AGV totales no cambiaron con la presencia de aceites vegetales exógenas, en cierta medida, similar con otros informes (Ueda et al., 2003). Como Hristov et al. (2011) informaron de que el ácido láurico redujo los recuentos de protozoos en el rumen, así como de etilo, AGV totales, en comparación con ácido esteárico y ácido mirístico. En este trabajo, los dos valores propios de etilo y AGV totales en presencia de aceite de coco fueron más bajos que en los aceites de cártamo, linaza y canola. Eso podría significar ácidos grasos exógenos fueron absorbidos principalmente y asimilados en la membrana biológica por microorganismos, al menos no degrada en los ácidos grasos de cadena corta, en cierta medida, o sin más intermediarios de metabolitos tales como VFAs para participar en otros procesos de metabolitos.

3.3. Producción de metano in presencia de de plantas oleaginosas

El metano (CH4) de producción a las 48 h de fermentación se midieron por triplicado, y todos los valores en presencia de aceites exógenos y tratamiento de control se presentaron con el valor incluyendo valor propio de blanco. La producción de metano del tratamiento en blanco era 7,4 mmol / ml. En este trabajo, excepto el valor propio de CH4 en presencia de aceite de linaza fue 46,2 mmol / g sustrato, que un poco más alto que el de cártamo o canola (42,6 vs. 42,5 mmol g sustrato /), y los valores propios de estos eran mayor que en el aceite de coco (39,4 mmol / g). Como otros informes, presentó el valor propio de metano fue significativa reducción en la presencia de aceite de coco, aceite de linaza (Panyakaew et al, 2013). (Fiorentini et al, 2014;.. Li et al, 2015), el aceite de cártamo (Li et al., 2011), pero no cambió in situ según lo informado por Hristov et al. (2011). Como el complejo entorno de fermentación, incluso con el mismo suministro de aceite vegetal en las dietas de rumiantes o como un suplementario añadido a la fermentación in vitro, no hay excepción de que la producción de metano es diferente distinción en el experimento y el medio ambiente.

3.4. Sinergismo de VFAs, CH4 and LCFAs of bacteria y protozoarios

Es interesante mencionar que la combinación de perfiles LCFAs en la membrana bacteriana y por protozoos y los ácidos grasos de cadena corta (AGV) a una

explicación más detallada del afecto de los ácidos grasos exógenos en la biosíntesis y la asimilación biológica de membrana de los microorganismos en el rumen. La alta proporción de AGCM obtuvo en presencia de aceite de coco, que es importante compuesta de ácido láurico, combinado con la menor producción de AGV, incluyendo ácido acético, ácido propiónico, ácido isobutírico, ácido butírico, ácido isovalérico y ácido valérico, implica que microorganismos del rumen (incluyendo bacterias y protozoos) tienen más potente para prolongar ácido láurico exógeno (C12: 0) a la síntesis de ácidos grasos de cadena larga medianas tales como C14: 0, C14: 1, C16: 0 y C16: 1, en lugar de degradar ácido láurico en los ácidos grasos de cadena corta, tales como ácido acético, ácido propiónico, ácido isobutírico, ácido butírico, ácido isovalérico y ácido valérico. Esto implica que el ácido láurico relacionada con sintetasa prolongada tiene una mayor actividad, en comparación con las enzimas de degradación. Chilliard et al. (2009) utilizaron el aceite de linaza como un regulador para modular las dietas de vacas Holstein lactantes, para obtener la composición y producción de metano FA, entonces a análisis de la correlación entre la FA con metano. Los resultados sugieren que el aceite de linaza disminuye FA desde C4: 0 a C16: 0 y el aumento de C18: 0, trans-11 C16: 1, todo cis- y trans C18: 1 (excepto trans-11 C18: 1), y no conjugado trans C18: 2 isómeros. Las correlaciones más positiva (r = 0,87 a 0,91) entre la leche FA concentraciones de metano y de salida se observaron para saturada de FA C6: 0 a C16: 0 y C10 para: 1, y las correlaciones más negativos (r = -0,86 a -0,90) se observaron para trans-16, cis-14 C18: 1; cis-9, trans-13 C18: 2; trans-11 C16: 1; y trans-12 C18: 1. Se sugirió que la leche perfil FA puede ser considerado un indicador potencial de la producción de metano in vivo en los rumiantes.

Por otro lado, en presencia de aceite de linaza ha aumentado el ácido oleico, incluso en carbono ácidos grasos insaturados, ácidos grasos cis, y los ácidos grasos trans, 18 carbonos ácidos grasos insaturados proporción en ambas fracciones bacterianas y protozoarias, combinado con perfiles de AGV, ácido acético y ácido propiónico también se incrementaron en presencia de ácido γ-linolénico (aceite de linaza). Esto significa que el ácido γ-linolénico se ha convertido en dobles enlaces cortos o menos FA por desaturasas o enzimas degradantes en un entorno complejo. Es una pena

mencionar que la falta de algunos ácidos grasos rastro de valor, tales como los ácidos conjugados grasos, o isómeros de C18: 1 y C18: 2 en este estudio debido a la limitada variedad de ácidos grasos en la muestra patrón para identificar algunos picos en el cromatograma.

Parte 4. Estudios computacionales experimentales de complejos de redes de distribución basado en ω-3/ ω-6

4.1. LCFA within ω-6/ω-3 PUFAs

Es bien conocido que el desequilibrio de ω-6 / ω-3 proporciones en la dieta tiene el potencial de inducir la inflamación, asma, artritis, enfermedad vascular (Calder, 2006a), pero alto nivel de ω-3 ejercen un efecto supresor (Calder, 2006b, 2008, 2013;. Cittadini et al, 2013). Todo el contenido cis-AF aumentó con exógeno ω-3 PUFA, trans-AF disminuyó en bacterias fase biológica. Esto se traduce directamente en la proporción cada vez mayor de composiciones de ácidos grasos cis / trans con el aumento de la exógeno ω-3 PUFA. Esto significa PUFAs exógenos son degradados por los microorganismos del rumen, o tienen procesos de metabolismo más complejos para que conduce a metabolismo intermediario con ambos de formulación AF insaturado cis y trans. La biohidrogenación de ácido linoleico (LA, 9 cis, cis 12- C18: 2) en el rumen se isomeriza a cis 9, trans 11- C18: 2 isómero (ácido linoleico conjugado, CLA), la conversión de este isómero trans a 11- C18 : 1 (ácido vaccénico), y la reducción de ácido esteárico (C18: 0) (Nam y Garnsworthy, 2007). Considerando que el bio-hidrogenación de α-linolénico (ALA) se caracteriza por isomerización a 9, 11, 15- cis, trans, cis C18: 3 de isómeros y posterior reducción a través de cis, isómeros trans C18: 2 C18: 1 y luego en ácido esteárico (Wilde y Dawson, 1966). Esta investigación mostró que ω-3 PUFA (ácido α-linolénico) podría aumentar el contenido de cis-AF en comparación con ω-6 PUFA (ácido linoleico) en ambos de las bacterias y los protozoos fases.

En segundo lugar, los valores de IPA de C16: 0 y C18: 0 en la fase bacteriana fueron 18,7% y 57,8%, mientras que aquellos en fase protozoo eran 13,3% y 67,5%, respectivamente. El ω-6 exógena / ω-3 proporciones no tienen ningún efecto significativo sobre estos dos ácidos grasos principales en ambas fases bacterianas y

protozoarias. Sin embargo, la menor diferencia de composición de lípidos como relaciones de ácido esteárico / palmítico, o ácidos insaturados / saturados grasos en la membrana biológica bacteriana y protozoo, puede desencadenar una gran diferencia en la función de las membranas de bacterias y protozoos (por ejemplo, fluidez de la membrana, la permeabilidad , hidrofobicidad y estabilidad) (Goldfine, 1984;. Kankaanpää et al, 2004), o más de los grupos funcionales (Janmey y Kinnunen, 2006; Bay y Turner, 2013) como específicos de péptidos, enzimas, o canales, etc. esteárico ácido en la membrana protozoos es mayor (aproximadamente 14,0%) que en la membrana de las bacterias, a diferencia de otros ácidos grasos.

En tercer lugar, incluso en carbono saturado FA en el tratamiento de ω-6 / ω-3 = 100: 0 y ω-6 / ω-3 = 20:80 fueron 85,1% y 81,7% en la fase protozoo, mientras que incluso insaturados carbono- FA en aquellos tratamientos eran 13,0% y 15,8%, respectivamente. Aquí, el valor más alto de incluso-carbono insaturado FA apareció en el tratamiento de ω-6 / ω-3 = 20:80, este fenómeno también ocurrió en la fase bacteriana, lo que significa que una alta cantidad de ω-3 PUFAs tiene la tendencia a aumentar las AF incluso en carbono insaturados en comparación con ω-6 PUFAs.

Un hecho interesante es que el ácido linolelaídico (C18: 2, 6t) en proporción ω-6 / ω-3 = 100: 0 era un poco más alto que otros tratamientos en fase bacteriana, mientras que una proporción superior apareció en fase protozoo cuando ω-6 / ω-3 = 20:80. Esto podría ser debido a la biohidrogenación de PUFAs (tales como ácido linoleico y ácido linolénico) en el rumen que resulta en la producción de ácidos grasos trans y principalmente ácido esteárico. (Lock y Bauman, 2004) Toda la diferencia en la distribución de ácidos grasos entre bacteriana y fases protozoarios pueden reflejar el protozoo y bacterias que tienen un metabolismo diferente y compleja en los procesos de asimilación, la absorción, la degradación o la síntesis de novo.

4.2. VFA ω-6/ω-3 PUFAs

Ácidos grasos volátiles (AGV) son ampliamente consideradas como metabolitos secundarios para reflejar el metabolismo de los lípidos de la hidrogenación, microbiano actividad de la enzima de degradación, y el ciclo de vida del organismo microbiano en el rumen-micro nicho ecológico (Allison et al, 1962;.. Fontanille et al .,

2012) La acético, propiónico y butírico son la principal VFAs, con proporciones de 43,6%, 23,1% y 19,2%, respectivamente. Mientras tanto, los AGVs de residuos, incluyendo el ácido isobutírico, isovalérico y valérico tenían una proporción total de 14,1%. Es de destacar que no hubo diferencia significativa en VFAs cuando el suministro de diferentes proporciones exógenos ω-6 / ω-3 PUFAs. Los valores de todos los AGV en el tratamiento de ω-6 / ω-3 = 80:20 fueron inferiores a los demás, que pueden ser el resultado de la tapa de la botella de los contenedores de almacenamiento que se rompen o no se sellan correctamente. Sin embargo, este estudio se centra en las áreas de los picos proporción de VFA en la misma muestra o tratamiento. Se puede concluir que el ácido acético tuvo una disminución menor, pero el ácido propiónico tenido un ligero aumento, con una proporción creciente de ω-3 PUFAs en los suplementación total de PUFAs exógenos. Incluso si tanto el ácido acético y ácido propiónico cambiado un poco diferente en términos de proporción de ω-6 y ω-3 PUFAs en un total de 100 mg / g de sustrato, la relación de ácido acético y propiónico se redujo con regularidad, como el aumento de la proporción de ω-3 PUFAs con un promedio de 1,89.

4.3. PT-LFER model for FA

Fases Un nuevo modelo fue desarrollado útil para predecir la proporción de AF (LCFAs y AGV) en el rumen bacterias protozoo líquidos dentro / sin exógenos ω-6 / ω-3 PUFA después de las perturbaciones en los descriptores moleculares químicas (VK) y límite experimental inicial condiciones (cj). Cada valor representa un coeficiente correspondiente en el nuevo modelo para predecir la nueva API (%) de cada FA. Como se ha explicado, este modelo puede clasificar como alto (Lnr = 1) / baja (Lnr = 0) la proporción esperada de AF (LCFA / VFA) entre los nuevos y los estados de referencia después de cambiar las condiciones de contorno cj. El parámetro n (Lnr = 1) representa el número de casos en el sub-conjunto con LNR = 1 (enlaces en la red), o el mismo con IPA (%) nuevo de la nueva sub-conjunto es superior a la de referencia de la API (%) ref, lo contrario. El mejor modelo PT-LFER encontrado utilizando el algoritmo LDA tiene sólo 12 variables.

Cuando, f la función de salida '(Lnr) nueva es una función del patrón de conectividad (Lnr) en el complejo de la red para la co-distribución de AF en referencia y el nuevo estado (valores previstos). F La función de salida '(Lnr) nuevo es útil clasificar los pares de estados (pares de nodos) .Los parámetros estadísticos utilizados fueron especificidad (Sp), sensibilidad (Sn) y exactitud (Ac). En consecuencia, los otros términos de entrada se ampliaron los siguientes. Por ejemplo,$\Delta\Delta V_k(c_j) = p(c_j)_{new} \cdot \Delta Vk(c_j)_{new} - p(c_j)_{ref} \cdot \Delta V_k(c_j)_{ref}$. This can be further expanded in turn as $\Delta\Delta V_k(c_j) = p(c_j)_{new} ({}^{new}V_k - \langle V_k(c_j)\rangle_{new}) - p(c_j)_{ref} ({}^{ref}V_k - \langle V_k(c_j)\rangle_{ref})$, among $\langle V_k(c_j)\rangle$ = Average of V_k for c_j. This new model found predicted los efectos de las perturbaciones en las condiciones iniciales (cj) sobre la distribución de la FA con la sensibilidad, especificidad y precisión> 80% para un total de 303.712 casos = en la formación y = 103.943 casos en serie validación externa. Estos resultados se consideran buenas para cualquier modelo LDA.

4.4. PT-NLFER model for a fatty acid distribution network

Pruebas adicionales se han realizado con STATISTICA, utilizando lineales y no lineales RNAs métodos (LNNs y MLP) con el fin de compararlos con el modelo LDA arriba. LNNs tienen una capa de entrada y una capa de salida, pero sin capa oculta. Por lo tanto, la salida predicha es una combinación lineal de los valores de neuronas de entrada, de forma similar con el modelo de LDA. MLP tienen al menos una capa oculta de las neuronas. Los RNAs han sido utilizados en la literatura para encontrar diversos modelos de clasificación (González-Díaz et al, 2011;.. Duardo-Sánchez et al, 2013; González-Díaz et al, 2014).. En consecuencia, y en rigor, los modelos LNN también son modelos PT-LFER porque son relaciones lineales. Sin embargo, se incluyen en esta sección, ya que son un caso particular de las RNA. Por el contrario, los modelos MLP se pueden clasificar como PT-NLFER (Linear Energy Relaciones PT para no gratis), porque consideran que las relaciones no lineales entre los operadores de TP de entrada y el de salida.

Los resultados demuestran la potencia de predicción de los RNAs no lineales (MLP) en contra de los modelos lineales (LDA y LNNs). El mejor modelo MLP (sin 6:. MLP 12: 12-11-1: 1) tiene 12 variables de entrada y una sola capa oculta con 11 neuronas. Se

puede predecir 93,73% de los casos de prueba y se clasifica 92,54% de los casos de formación. Este modelo tiene alrededor de 10% más de potencia de predicción en comparación con el modelo LDA PT-LFER pero sólo 5,6% más de potencia de clasificación en la formación. El modelo PT-NLFER obtenido con MLP número 6 clasificado nuestra base de datos mejor que el modelo LDA PT-LFER. Sin embargo, PT-LFER es notablemente más simple y muestra una relación directa entre las variables de entrada y el de salida. Si los resultados son ordenados por la clasificación de la prueba (preferencia validación), el orden de los modelos son los siguientes: MLP - 6, 5, 4, 3, 2, 1; 12 (modelo LDA); LNNs - 9, 8, 10, 11, 7. Así, el modelo LDA tiene una mejor capacidad de predicción de todos los LNNs pero menos de MLP. Entre LMP, se pueden observar los modelos con sólo 5 variables de entrada (en comparación con los 12 de los LDA), pero el modelo LDA clasifica 4,70% más que el conjunto de entrenamiento, incluso si la MLP se puede predecir 4,92% más que el equipo de prueba . Otra de las ventajas del modelo LDA son los de formación y validación errores bajos en comparación con todas las RNA (alrededor del 25% de los errores ANN). En conclusión, los modelos MLP eran mejores solucionadores de problemas, pero sobre todo más complicado.

El número de nodos = ácidos grasos (suma de los resultados de entrada ITH) de las redes complejas era 744. Se puede concluir que los resultados de la clasificación, obtenidos con esta nueva ecuación PT-LFER, son prometedores y confirman el potencial de la presente metodología. El modelo actual es el resultado de combinar el análisis Hánsch con modelos LDA, Box-Jenkins Operadores e ideas Perturbación Teoría. Nuestro grupo y otros autores (Agüero-Chapin et al, 2008a;. Agüero-Chapin et al, 2008b;. González-Díaz y Riera-Fernández, 2012; Alonso et al, 2013;. Duardo-Sánchez et al, 2014.; . González-Díaz et al, 2014A) han utilizado modelos LDA solo o combinado con Box-Jenkins Operadores para predecir propiedades de los sistemas complejos (mota-Planche et al, 2012;. Speck-Planche et al, 2013A;. Speck-Planche et al., 2013c, b), estos modelos puede incluir o no la perturbación consideraciones teóricas (González-Díaz et al., 2013A). Sin embargo, en este trabajo estas ideas se extienden al análisis Hánsch por primera vez.

4.5. Construction of FA distribution network using the PT-LFER model

La biología de red (Barabási y Oltvai, 2004) es aceptado como un enfoque muy útil para arrojar luz sobre la organización funcional de la célula. Mantenga esta idea en mente, las redes complejas observadas fueron construidos para las perturbaciones en el metabolismo FA / distribución en fases bacterias protozoarios-líquido. Al hacerlo se consideró que dos estados están conectados (Lnr = 1) si ambos f (εij) nueva = IPA (%) obs yf (εij) ref = IPA (%) obs - IPA (%) ref> 0, y Lnr = 0 en caso contrario. Esta condición indica que el nivel de ambos ácidos grasos en el nuevo estado es mayor que la de los ácidos grasos en el estado de referencia (estado inicial). Por consiguiente, nuestra red es una red de co-distribución de los ácidos grasos. Además, se usó el modelo para predecir la misma red compleja. Para ello, se consideró que Lnr = 1 (nodos enlazados) cuando ambos valores de 'f (εij) nuevo y 'f (εij) REF predicha por el modelo tienen la probabilidad p (CIJ)> 0,5 de tener f (εij) ref = IPA (%) obs - IPA (%) ref> 0.

Por último, también se construyeron dos modelos de redes aleatorias (red aleatoria 1 y 2). Cada modelo se definió con un número de nodos y enlaces como similares como sea posible a las redes observadas y predichas, respectivamente. El objetivo era entender la naturaleza global del metabolismo FA / datos de distribución (similitud con un proceso aleatorio o no). Se calcularon los valores medios de algunos índices topológicos para comparar cuantitativamente la estructura de estas redes. Los índices calculados eran los valores medios de la distancia topológica-vértice vértice (Newman et al., 2001), el grado de nodo, y la cercanía del componente gigante, de los modelos de red predichos observados y los dos más aleatorias similares. Erdős-Rényi (ER) redes aleatorias donde, al parecer, similar a las redes observados y pronosticados. De hecho, los valores medios de la distancia topológica, el grado de nodo, y la cercanía son similares, a medio camino entre la red observada y predicha (1,83 vs. 1,77, 72,75 vs. 80,29, y 0.000755 vs. 0.000836, respectivamente).

Parte 5. Acercamiento quimiométrico de redes de distribución de Bacteria – Rumen – Protozoa

5.1. Metilación.

En general, la metilación catalizada por base procede más rápidamente bajo una condición de temperatura leve que la metilación catalizada por ácido (Salimon et al., 2014). En este trabajo, los resultados mostraron que combinado basal / ácido metilación parece producir mayores proporciones de C18: 3 n3, C18: 3 N6, C20: 2, C22: 0 y C20: 3 N6, insaturado Fas, odd-carbono AF, e incluso en carbono insaturado AF, pero con menor proporción de C18: 0 y cis-AF en comparación con el valor inicial o por ácidos métodos de metilación catalizada, respectivamente. Implicaba el valor inicial / metilación ácido era el mejor método para catalizar la metilación de los ácidos grasos libres para la determinación de la proporción de ácidos grasos con GC, en comparación con ácido y la metilación basal.

5.2. PT-LFER

El modelo PT-LFER desarrollado combina la información de dos estudios in vitro de fermentación (producción de metano y distribución FA). Incluye los niveles de metano de producción, los valores de pH, la presión del gas, y la temperatura de fermentación después de la oferta de diferentes proporciones de ω-6 / ω-3 PUFAs. Todos los valores propios, la diferencia (ΔΩ) y valor propio logístico de diferencia (log (| ΔΩ |)) de CH4, valor de pH, la presión del gas y la temperatura en referencia inicial / o estado final / nuevo se calcularon. La producción de metano a las 48 h se redujo significativamente con la suplementación de exógenas ω-6 / ω-3 PUFAs, control (108,81 mM / g en el sustrato) vs. valores propios medios de diferentes proporciones de ω-6 / ω-3 PUFAs (79,19 mM / g). Es interesante notar que la producción de metano fue menor en el tratamiento de alto nivel de PUFAs ω-3 (ω-6 / ω-3 = 20:80) que otros tratamientos suministro de diferentes proporciones de ω-6 / ω-3 PUFAs y control. Es implícito que los ácidos grasos omega 3 podrían tener más fuerte capacidad de inhibir la metanogénesis proceso en comparación con los ácidos grasos omega 6. Esto es consistente con las obras anteriores (nges et al., 2012). En este estudio, la temperatura de la presión del gas y la fermentación se midió con detectores de ANKOM en cada 15 min durante 72 h. Todo el conjunto de datos de la presión del gas y la temperatura fue publicado en línea por Liu y Buendía-Rodríguez (Liu y

Buendía-Rodríguez, 2015).5.3. Chemometric PT-LFER model of methane production and fatty acid distribution.

Un nuevo modelo fue desarrollado en base tanto en el conjunto de datos de rendimiento de la fermentación ruminal, incluida la composición de ácidos grasos en diferentes fases de la fermentación (LCFAs de la fase de bacterias y protozoos, AGV de medios de fermentación), la presión del gas, los valores de pH, la producción de metano, temperatura de fermentación y ω-6 / ω-3 las proporciones, y los descriptores moleculares químicos (IVK) de cada estructura molecular de ácidos grasos. La diferencia (ΔΩ) y valores absolutos logísticos de diferencia (log (| ΔΩ |)) de la primera clase del conjunto de datos, incluyendo la presión del gas P (psi), pH (72h), CH4, T, ω-6 y ω- 3, se calcularon como un conjunto de variables de entrada. Por otro lado, descriptores moleculares (IVK), las medias móviles (ΔiVk) y operadores (PT ΔΔiVk (cj)), además de la plaza de descriptores moleculares (IVK nuevo) 2 y operadores de TP (ΔΔiV6 (cj)) 2 de cada ácido graso en los estados inicial y final también se tuvieron en cuenta.

El nuevo modelo PT-LFER fue desarrollado útil para predecir la proporción de ácidos grasos, incluyendo LCFAs y VFAs, en diferentes fases de microbioma ruminal dentro / sin PUFAs exógenos. Este modelo se realizó con el cálculo de perturbación de P (psi), pH (72h), CH4, T, ω-6, ω-3 y descriptores moleculares químicos (IVK) combinado con las condiciones de contorno experimentales iniciales o finales (cj). El mejor modelo PT-LFER obtuvo utilizando el análisis discriminante lineal (LDA) algoritmo tiene sólo 21 las variables de más de 80 variables.

En este nuevo modelo, todo el conjunto de datos para este modelo fueron publicados en línea por Liu et al. (Liu et al., 2015A, b). Los valores de los descriptores moleculares (IVK) de cada ácido graso, incluyendo LCFAs y AGV, eran representar algunas propiedades físicas de los ácidos grasos. Eso incluye V1 = Mw (peso molecular), V2 = Aeigv, V3 = Aeige, V4 = Aeigp, V5 = MR (refractividad molecular), y V6 = LogP; V2, V3, V4 y son valores propios medios de la matriz de distancia topológica ponderado con volúmenes atómica de van der Waals (v), Electronegatividad (e), o polarizabilidad (P).

Como se ha explicado, este modelo puede clasificar la proporción esperada de AF entre las nuevas y ref estados tan alto (μnr 1 =) / baja (μnr = 0) después de cambiar las condiciones de contorno cj. El parámetro No. (μnr = 1) representa el número de casos en el sub-conjunto con μnr = 1 (enlaces en la red), o el mismo con IPA (%) nuevo de la nueva sub-conjunto es superior a la de referencia IPA (%) ref. F La función de salida '(μnr) nueva es una función del patrón de conectividad (μnr) en el complejo de la red para la co-distribución de AF en la referencia (ref) y el nuevo estado (valores previstos). F La función de salida '(μnr) nuevo es útil clasificar los pares de estados (pares de nodos). Este nuevo modelo encontrado predijo los efectos de las perturbaciones en las condiciones iniciales (cj) sobre distribución de ácidos grasos con la sensibilidad, especificidad y precisión> 94% para un total de 409.434 casos en la formación y 136.261 casos en series de validación externa.El cálculo perturbación de cada descriptores moleculares (IVK) se asociaron con las condiciones de contorno cj. Se proporcionan dos clases de las condiciones de contorno. Condiciones operacionales preliminares se explican a continuación, c1 se refiere a la utilización de diferentes tratamientos experimentales, c2 se refiere a con / sin fermentación, c4 es la columna de cromatografía de gases utilizada para GC, y c5 es el uso de replicar los experimentos. Otros están más directamente relacionados con la distribución posterior y la naturaleza de la c3 FA = la fase biológica de distribución de los ácidos grasos, y c6 se refiere a la información sobre cuantificar cis / patrón geométrico trans presentes en los LCFAs. En consecuencia, los otros términos de entrada se ampliaron los siguientes. Por ejemplo, ΔΔVk (cj) = p (cj) nueva · ΔVk (cj) nueva - p (cj) ref · ΔVk (cj) ref. Esto se puede ampliar a su vez como ΔΔVk (cj) = p (cj) nueva (newVk - <Vk (cj)> nuevo) - p (cj) ref (refVk - <Vk (cj)> ref), entre <Vk (cj)> = Promedio de Vk para cj.Se puede concluir que los resultados de la clasificación, obtenidos con esta nueva ecuación PT-LFER, son prometedores y confirman el potencial de la presente metodología. El modelo actual es el resultado de combinar el análisis Hánsch con modelos LDA, Box-Jenkins Operadores e ideas Perturbación Teoría. Nuestro grupo y otros autores (Agüero-Chapin et al, 2008a;. Agüero-Chapin et al, 2008b;. González-Díaz y Riera-Fernández, 2012; Alonso et al, 2013;. Duardo-Sánchez et al, 2014.; .

González-Díaz et al, 2014A) han utilizado modelos LDA solo o combinado con Box-Jenkins Operadores para predecir propiedades de los sistemas complejos (mota-Planche et al, 2012;. Speck-Planche et al, 2013A;. Speck-Planche et al, 2013c, b).; Sin embargo, estos modelos no pueden tomar la teoría de perturbaciones en cuenta. En este trabajo, estas ideas se extienden al análisis Hánsch. Para ello, se consideró que μnr = 1 (nodos enlazados) cuando tanto los valores de 'f (εij) nuevo y 'f (εij) REF predichas por el modelo tienen la probabilidad p (CIJ)> 0,5 de tener f (εij) ref = IPA (%) obs - IPA (%) ref> 0.5.4.

5.3 Modelo quimiométrico de producción de metano y distribución de ácidos grasos

Pruebas adicionales han llevado a cabo con algoritmos ANN para buscar modelos no lineales. Los mejores 17 modelos MLP con las estadísticas correspondientes a la mejor clasificación LDA. Los modelos MLP tienen diferentes variables de entrada de 10 a 19. El mejor modelo MLP (No.15: MLP 18: 18-15-1: 1) tiene 18 variables de entrada y una sola capa oculta con 15 neuronas. Se puede predecir 99,68% de los casos de prueba y se clasifica 99,70% de los casos de formación. Este modelo tiene alrededor de 10% más de potencia de predicción en comparación con el modelo LDA PT-LFER pero sólo 5,6% más de potencia de clasificación en la formación. Los modelos PT-NLFER obtenidos con MPL clasifican nuestro conjunto de datos mejor que el modelo LDA PT-LFER. Sin embargo, PT-LFER se considera mejor que PT-NLFER, debido a la relación más simple y directa entre las variables de entrada y de salida..

CONCLUSIONES

1. Las mejores proporciones de harina de cártamo con semilla de sorgo, a las que disminuye significativamente la producción de gas en sistemas de fermentación *in vitro* de ovinos, fueron de 25% a 50%, al igual que las proporciones de semilla de canola con semilla de sorgo.

2. En la investigación de las fracciones de semilla de cártamo con fermentación *in vitro*, se observó que las características nutricionales son diversas entre sí, siendo que la harina de cártamo tiene mayor eficiencia en la utilización que las otras fracciones y no se observó diferencia entre los dos métodos de extracción basados en disolventes de hexano y éter de petróleo en relación a los valores nutricionales.

3. El suministro de ácidos grasos mono y poli-insaturados, así como los de cadena media en los sistemas de fermentación *in vitro*, mostró que pueden cambiar de manera efectiva la distribución de ácidos grasos o de su composición en la membrana microbiana (LCFA) y líquido (VFA) ruminal. Esto implica que pueden influir aún más en la actividad de bacterias y protozoarios del rumen.

4. Las redes de distribución de ácidos grasos se establecieron con éxito a partir de los ácidos grasos de bacterias y protozoarios ruminales bajo la condición de las diferentes relaciones de ácidos grasos omega-3 y omega-6. El mejor modelo PT-LFER encontrado predijo los efectos de las perturbaciones sobre la red de distribución FA con sensibilidad, especificidad y exactitud > 80% para 407,655 casos en formación + serie de validación externa.

5. Un nuevo modelo PT-LFER de red de distribución de metano-ácido graso basado en la producción de metano (fase gaseosa), la distribución de ácidos grasos volátiles (fase líquida) y la distribución de ácidos grasos en microbioma ruminal (fase sólida), también se estableció con la sensibilidad, especificidad, exactitud > 94% para los

545,695 casos. Esta metodología puede llevar a cabo experimentos paralelos en diferentes fases en el estudio de la distribución de ácidos grasos.

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