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Chemometric approach to fatty acid metabolism-distribution networks and methane production in ruminal microbiome☆

Yong Liu [a,b,c], Claudia Giovanna Peñuelas-Rivas [a], Esvieta Tenorio-Borroto [a], María Rivas-Guevara [d], Germán Buendía-Rodríguez [e], Zhiliang Tan [b], Humberto González-Díaz [f,g,*]

[a] Faculty of Veterinary Medicine and Animal Science, Autonomous University of the State of Mexico (UAEM), Toluca 50090, Mexico
[b] Key Laboratory of Subtropical Agro-ecological Engineering, Institute of Subtropical Agriculture, Chinese Academy of Sciences (CAS), Changsha, Hunan 410125, P.R. China
[c] Department of Information and Communications Technologies - INIBIC, University of A Coruna (UDC), Campus Elviña, 15071 A Coruña, Spain
[d] Ethnobiology and Biodiversity Research Center, Chapingo Autonomous University, Texcoco 56230, Mexico
[e] National Center for Disciplinary Research on Animal Physiology and Breeding, National Institute of Forestry, Agriculture and Livestock Research, Queretaro 76280, Mexico
[f] Department of Organic Chemistry II, University of the Basque Country UPV/EHU, 48940 Leioa, Spain
[g] IKERBASQUE, Basque Foundation for Science, 48011 Bilbao, Spain

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ABSTRACT

Methane emission has been attracting more and more attention. Unfortunately, a lot of factors influence methane emission (chemical structure of metabolites, time, methane, gas pressure, microbiome composition, diet, *etc.*). We propose a new chemometric methodology to integrate different laboratory experiments in this field. Firstly, we report (1) new laboratory experiments to measure by separating (1a) methane production (gas phase), (1b) volatile fatty acid (VFA) distribution (liquid phase) and (1c) fatty acid (FA) distribution in rumen microbiome. Next, we also report a 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, temperature and (b) FA distribution. Section (b) includes two different experimental parts: chromatographic determination of internal peak areas (IPA%) of (b.1) long-chain fatty acids (LCFA) and (b.2) VFA. In all studies, we can use different treatments, distribution phases (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 > 0.94, and Matthews correlation coefficient (MCC) > 0.894 for 545,695 cases of perturbations in experimental data. This methodology may be useful to quantify the effect of perturbations due to the changes in experimental conditions in the study of fatty acid distribution when we need to carry out parallel experiments in different phases.

1. Introduction

In recent years, both nutritional and environmental scientists have paid more and more attention to the pollution from greenhouse gas (methane) emissions. Some methods and chemical substances have been developed to improve ruminant growth performance and reduce methane emission [1,2]. For instance, fatty acids (FAs) were proved to restrain methane emission [3–5]. In addition, bacteria and protozoans 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. Herein, combined with intrinsic fatty acid compositions and exogenous parameters (*e.g.*, pH, gas pressure, and temperature) with some outputs (methane emission or other nutritional elements) of this complex system should be an interesting topic to reflect the intrinsic characteristics of complex ruminal metabolic pathways. To address this problem, it is postulated that FA compositions in bacterial and protozoan membranes change with the supply of different ω-6/ω-3 ratios based on the favorable and detrimental scope in the diet. On the other hand, LCFAs must be methylated by acid-/base-methylation before measurements [6].

The molecular structure of exogenous FA plays a very important role in the accommodation of rumen metabolism pathways and processes. It implies that it is reasonable to combine the molecular structure properties and amount of FA in bacterial and protozoan membranes to predict the rumen's lipid metabolism properties. In

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* Corresponding author at: Department of Organic Chemistry II, University of the Basque Country UPV/EHU, 48940 Leioa, Spain. Tel.: +34 94 601 3547; fax: +34 94 6012748.
E-mail address: humberto.gonzalezdiaz@ehu.eus (H. González-Díaz).

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this sense, chemoinformatics models may become a useful tool [7]. In our opinion, we can combine the ideas of perturbation theory (PT) [8] and linear free-energy relationships (LFER) [9,10] to handle this issue. As the result of this combination, we 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) [11,12] can be used to improve the predictive power of chemoinformatical techniques in the study of complex bio-molecular, ecological, or social systems. As a result, we can obtain non-linear PT-LFER (PT-NLFER) models.

In a previous work, we developed a PT-LFER method for the first time able to predict the FAs distribution taking multiple factors into consideration [13]. However, the model fails to account for some important factors like fermentation pressure, time, and temperature. In the present work, we want to introduce a new theoretical methodology based PT-LFER method that is able to process complex experimental data from metabolomics studies of methane production incorporating many new factors. That is why the present work consists of two main stages. The first stage of the work is aimed to report on the original experimental dataset of fatty acid distribution in biological membranes. In the second stage of this work, we are going to develop new chemoinformatics PT-LFER/PT-NLFER models for the data generated. Next, the best PT-LFER/PT-NLFER 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 studying the effect of complex molecular perturbation theory in FA chemical structure, the corresponding fermentation parameters and boundary experimental conditions.

2. Materials and methods

2.1. Experimental section

The experiment is presented in Fig. 1. Panel (a) shows the methane production, pH, gas pressure (V_g), and temperature as well as (b) the chromatographic internal peak areas (IPA%) of (b1) LCFAs in bacterial and protozoan membrane and (b2) VFAs that were determined under the same experimental conditions but in different experiments. The general details of the experimental procedures used are explained as follows.

2.1.1. Donor animal

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 donor animals refer to the description of the NRC [14]. All animal protocols and procedures were approved by the Animal Care Committee of the National Institute of Forestry, Agriculture and Livestock (INIFAP), Queretaro, Mexico.

2.1.2. Experimental in vitro fermentation

The *in vitro* fermentation details were according to the description of Tang et al. [15]. Particle-free rumen fluid was mixed with artificial saliva buffer solution [16] at a ratio of 1:2 (v/v) at 39 °C with continuous flushing of CO_2. The microbial and protozoan fractions were separated by differential centrifugation according to the description of Legay-Carmier and Bauchart [17]. Microbial FAs were obtained according to the method developed by Or-Rashid [6], and catalyzed by base-catalyzed methylation. The PA(i) value of each fatty acid obtained by gas chromatography (GC) was 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 FA of a sample, $m_i \subset c_j$ refers to a FA in a specific set of experimental conditions. The denominator of Eq. (1) refers to the sum of all PA(i) values under the same specific set of experimental conditions. One section of experimental materials and methods are similar to our previous paper [13]. However, in the present work, we included new results related to the variable values of gas pressure, CH_4 production, temperature (T), time (t), and pH (see Supplementary material SM01)

2.2. Theoretical section

The datasets of LCFAs, VFAs, CH_4 production in addition to T, t, and gas pressure in a different set of experimental conditions were calculated and dealt with according to the perturbation theory combined with

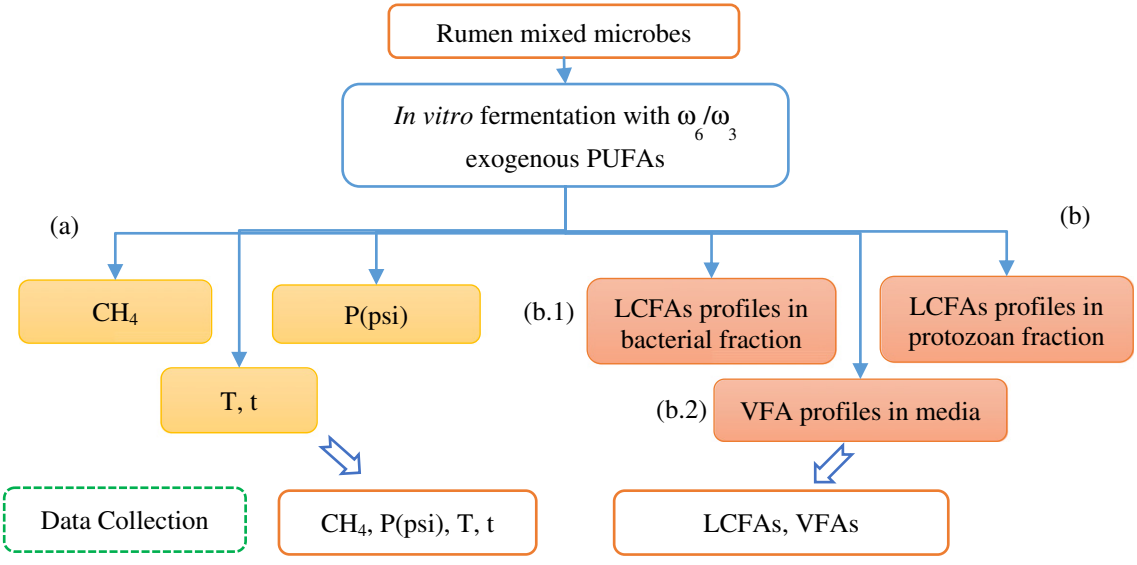

Fig. 1. Illustration of the general workflow for *in vitro* fermentation experiments.