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Using multivariate factor analysis to characterize the unbranched fatty acid profile in bovine rumen fluid

Uso del análisis multivariado factorial para caracterizar el perfil de ácidos grasos no ramificados en fluido ruminal de bovinos

Uso da análise multivariada fatorial para caracterizar o perfil de ácidos graxos não ramificados em fluido ruminal de bovinos

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Abstract

Background: Multivariate factor analysis (MFA) could be used for analyzing the complex pattern of correlations among fatty acids (FAs) in the rumen.

Objective: To investigate the potential use of MFA to extract information on unbranched FAs metabolism in bovine rumen fluid.

Methods: A dataset containing 107 individual records of 26 unbranched FAs from two *in vitro* ruminal incubation studies was constructed. The MFA was performed using the SPSS (Statistical Package for the Social Sciences) software.

Results: The MFA extracted four latent factors, accounting for 86.7% of the total variance. The first factor was positively associated with short (6:0), medium (8:0, 10:0, 12:0, 14:0, and 16:0), and long (17:0, 20:0, and 23:0) saturated FAs (SFAs), as well as with *cis* and *trans* monounsaturated FAs (MUFAs) from 14 to 16 carbon atoms (14:1- *t*5, 14:1-*c*9, 15:1-*t*10, 16:1-*c*9, and 16:1-*t*9). The second factor was positively correlated with 18:0 and the majority of *cis* and *trans* MUFAs of 18 carbon atoms (18:1-*t*9, 18:1-*t*11, 18:1-*c*6, 18:1-*c*9, and 18:1-*c*11). The third factor was positively related to 18:3-*c*9,*c*12,*c*15 and 18:2-*t*11,*c*15, and negatively to 18:2-*c*9,*t*11. The fourth factor was positively correlated with 18:1-*t*6 and 19:0; however, 19:0 was also negatively associated with the second factor. The 18:2-*c*9,*c*12 was negatively correlated with the second and third factors.

Conclusion: Multivariate factor analysis (MFA) allowed the reduction of a large number of fatty acids in bovine ruminal fluid to a few latent factors with biological meaning.

Keywords: *cattle; lipid profile; lipid metabolism; multivariate statistics; multivariate factor analysis; ruminant; ruminal fluid; unbranched fatty acids.*

Resumen

Antecedentes: El análisis multivariado factorial (MFA) podría usarse para evaluar el complejo patrón de correlaciones entre ácidos grasos (FAs) en el rumen.

Objetivo: Investigar el uso potencial del AMF para extraer información sobre el metabolismo de FAs no ramificados en fluido ruminal de bovinos.

Métodos: Se construyó una base de datos conformada por 107 registros individuales de 26 FAs no ramificados, provenientes de dos estudios de incubación *in vitro*. El MFA se desarrolló usando el programa SPSS (Statistical Package for the Social Sciences).

Resultados: El MFA extrajo cuatro factores latentes, cuantificando el 86.7% de la varianza total. El primer factor se correlacionó positivamente con FAs saturados (SFAs) de cadena corta (6:0), media (8:0, 10:0, 12:0, 14:0 y 16:0) y larga (17:0, 20:0 y 23:0) como también con FAs monoinsaturados (MUFAs) de 14 a 16 átomos de carbono (14:1-*t*5, 14:1-*c*9, 15:1-*t*10, 16:1-*c*9 y 16:1-*t*9). El segundo factor se correlacionó positivamente con 18:0 y con la mayoría de MUFAs *cis* y *trans* de 18 átomos de carbono (18:1- *t*9, 18:1-*t*11, 18:1-*c*6, 18:1-*c*9 y 18:1-*c*11). El tercer factor se correlacionó positivamente con 18:3-*c*9,*c*12,*c*15 y 18:2-*t*11,*c*15 y negativamente con 18:2-*c*9,*t*11. El cuarto factor se correlacionó positivamente con 18:1-*t*6 y 19:0; sin embargo, 19:0 también se correlacionó negativamente con el segundo factor. El 18:2-*c*9,*c*12 se correlacionó negativamente con los factores segundo y tercero.

Conclusión: El análisis multivariado factorial (MFA) permitió reducir el gran número de ácidos grasos del fluido ruminal a unos pocos factores latentes con significado biológico.

Palabras clave: *ácidos grasos no ramificados; análisis multivariado factorial; estadística multivariada; liquido ruminal; ganado; perfil lípidico; metabolismo de lípidos; rumiante.*

Resumo

Antecedentes: A análise multivariada fatorial (MFA) poderia ser aplicada na análise do padrão complexo de correlações entre ácidos graxos (FAs) no rúmen.

Objetivo: Investigar o potencial de uso do MFA para extrair informação sobre o metabolismo de FAs não ramificados no fluido ruminal de bovinos.

Métodos: Construiu-se um banco de dados formado por 107 registros individuais de 26 FAs não ramificados, oriundos de dois estudos de incubação *in vitro*. A MFA foi desenvolvida usando o programa SPSS (Statistical Package for the Social Sciences).

Resultados: A MFA extraiu quatro fatores latentes, quantificando 86.7% da variância total. O primeiro fator correlacionou-se positivamente com FAs saturados (SFAs) de cadeia curta (6:0), meia (8:0, 10:0, 12:0, 14:0 e 16:0) e longa (17:0, 20:0 e 23:0) bem como com os FAs monoinsaturados (MUFAs) de 14 a 16 átomos de carbono (14:1-*t*5, 14:1-*c*9, 15:1-*t*10, 16:1-*c*9 y 16:1-*t*9). O segundo fator correlacionou-se positivamente com 18:0 e com a maioria de MUFAs *cis* y *trans* de 18 átomos de carbono (18:1-*t*9, 18:1-*t*11, 18:1- *c*6, 18:1-*c*9 e 18:1-*c*11). O terceiro fator correlacionou-se positivamente com 18:3-*c*9,*c*12,*c*15 e 18:2-*t*11,*c*15 e negativamente com 18:2-*c*9,*t*11. O quarto fator correlacionou-se positivamente com 18:1-*t*6 e 19:0; porém, 19:0 também se correlacionou negativamente com o segundo fator. O 18:2-*c*9,*c*12 se correlacionou negativamente com os fatores segundo e terceiro.

Conclusão: A análise multivariada fatorial (MFA) permitiu reduzir um número grande de ácidos graxos no fluido ruminal a poucos fatores latentes com significado biológico.

Palavras-chave: *ácidos graxos não ramificados; análise multivariada fatorial; estatística multivariada; fluido ruminal; gado; perfil lipídico; metabolismo de lipídeos; ruminante.*

Introduction

The diversity of fatty acids (FAs) in ruminant meat and milk is mainly derived from ruminal dietary FA metabolism (Jenkins *et al*., 2008). Some FA classes, such as unbranched *cis* and *trans* isomers of 18:1, 18:2, and 18:3 FAs, are associated with biological activity in humans (Hur *et al*., 2017). Therefore, a better understanding of unbranched FA metabolism in the rumen may aid in designing nutritional strategies to enhance the quality of beef and milk.

A combination of gas chromatography and multivariate statistics may enable us to infer metabolic relations in the complex ruminal FA profile. Gas chromatography has greatly improved over the last 20 years, and it is now possible to get a detailed ruminal FA profile, including the detection and quantification of several positional and geometrical isomers of the 16:1, 18:1, 18:2, and 18:3 groups of unbranched FAs (Delmonte *et al*., 2012; Mele *et al*., 2016). Additionally, multivariate statistics offers different techniques that can capture the covariance structures of complex patterns of variables. One of these techniques is multivariate factor analysis (MFA), which allows to explain the maximum amount of (co) variance among the original variables (Snedecor and Cochran, 1989). This technique divides the total variance of a multivariate system into two components: the variance that all the variables share (i.e., communality) and the particular variance of each variable (i.e., uniqueness). The MFA has been used extensively to analyze the complex milk FA profile (Macciotta *et al*., 2004; Macciotta *et al*., 2015; Mele *et al*., 2016), to model milk composition (Todaro *et al*., 2005), and to study milk coagulation properties (Macciotta *et al*., 2012; Dadousis *et al*., 2018). However, to date, MFA has not been used to analyze the complex ruminal FA profile. Therefore, in this study we investigated the potential of using MFA to obtain information on unbranched FA metabolism in bovine rumen fluid.

Materials and methods

Ethical considerations

All procedures used across studies were approved by the Bioethics Committee of the Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional de Colombia (Act 001 of 2010).

Data collection

A dataset containing 107 individual records from two ruminal *in vitro* incubation studies (Vargas *et al*., 2012; Vargas *et al*., 2018) was constructed (Table 1). The studies were conducted at the facilities of Universidad Nacional de Colombia (Bogotá Campus, Colombia). The dataset used was composed of 26 short, medium, and long unbranched saturated and unsaturated FAs (6:0; 8:0; 10:0; 12:0; 14:0; 14:1-*t*5; 14:1-c9; 15:1-*t*10; 16:0; 16:1-*c*9; 16:1-*t*9; 17:0; 18:0; 18:1-*t*6; 18:1-*t*9; 18:1-*t*11; 18:1-*c*6; 18:1-*c*9; 18:1-*c*11; 19:0; 18:2-*c*9,*c*12; 18:2-*t*11,*c*15; 18:2-*c*9,*t*11; 18:3-*c*9,*c*12,*c*15; 20:0; 23:0),expressed as grams per 100 grams of total FAs.

The concentrations of FAs in the incubation systems were determined using gas chromatography coupled to a flame ionization detector (GC-FID). Briefly, FAs in the incubation systems were extracted and methylated according to Garcés and Mancha (1993). Methylated FAs were quantified using a Shimadzu GC-2014 gas chromatograph (Shimadzu Manufacturing, Inc., Canby, OR, USA). The column was a fused silica capillary (Rt- 2560, 100 m x 0.25 mm i.d. x 0.2 μm film thickness; Restec®, Inc, Belefonte, PA, USA). Helium was used as the carrier gas. Detector and injector temperatures were 260 and 270 ℃, respectively, and the split ratio was 30:1. Oven temperature was 140 ℃ for 5 min, increased by 4℃/min to 220 ℃, held for 5 min, increased by 2.0 ℃/ min to 240 ℃, and held for 10 min. The FAs in samples were identified by comparison of their retention times with those observed in commercial standards as Nu- Chek® Prep (Elysian, MN, USA), and quantified by direct comparison of the peak areas.

Table 1. Descriptive statistics of bovine rumen fluid fatty acid profile

Fatty acid (g/100 g of total FAs)	Common name	Min	Max	Mean	SD
6:0	capronic acid	0.44	4.16	1.22	0.69
8:0	caprilic acid	0.19	1.15	0.43	0.19
10:0	capric acid	0.01	0.28	0.07	0.05
12:0	lauric acid	0.02	0.28	0.10	0.05
14:0	miristic acid	0.20	0.72	0.38	0.12
14:1-*t*5	transmiristelaidic acid	0.10	0.36	0.18	0.06
14:1-*c*9	miristoleic acid	0.07	0.34	0.15	0.06
15:1-*t*10	trans-pentadecenoic acid	0.62	3.38	1.54	0.61
16:0	palmitic acid	7.27	22.2	11.3	3.62
16:1-*t*9	palmitelaidic acid	0.00	0.10	0.04	0.02
16:1-*c*9	palmitoleic acid	0.13	0.38	0.21	0.06
17:0	heptadecanoic acid	0.31	0.84	0.47	0.14
18:0	stearic acid	12.6	44.9	24.2	7.25

18:1-*t*6	Petroselaidic	0.05	0.26	0.14	0.05
18:1-*t*9	elaidic acid	0.08	0.90	0.36	0.21
18:1-*t*11	transvaccenic acid	2.39	20.8	10.6	5.34
18:1-*c*6	petroselinic acid	0.26	3.23	1.10	0.63
18:1-*c*9	oleic acid	0.52	1.68	0.97	0.26
18:1-*c*11	vaccenic acid	0.12	0.50	0.25	0.09
19:0	nonadecanoic acid	0.06	5.54	0.85	1.16
18:2-*c*9,*c*12	linoleic acid	0.77	58.2	17.6	14.7
18:2-*t*11,*c*15	ND	0.09	6.67	2.59	1.79
18:2-*c*9,*t*11	conjugated linoleic acid	0.00	12.2	2.42	2.39
18:3-*c*9,*c*12,*c*15	alpha-linolenic acid	3.42	48.6	17.9	11.8
20:0	metil araquidic	0.16	0.66	0.30	0.12
23:0	tricosanoic acid	0.08	0.30	0.15	0.06

Min = minimum; Max = maximum; SD = standard deviation; ND = common name is not defined in the literature; c = cis; t = trans.

Statistical analysis

An MFA was performed using the IBM SPSS (Statistical Package for the Social Sciences; IBM Corp, 2013) Statistics software, version 22 (IBM® Corporation, Armonk, NY, USA), to explore the metabolic relationships between unbranched FAs in bovine rumen fluid (Johnson and Wichern, 2014). The main aim of MFA is to explain the (co) variance of a system defined by n measured variables (Y_1, … ,Y_n) by deriving a smaller number p (p<n) of latent unobservable variables (X_1, … ,X_p), named common latent factors. The MFA accepts that the variance of each original variable can be decomposed into its common (i.e., communality) and unique (i.e., uniqueness) components. The factor model decomposes the covariance matrix of the measured variables (Q) as follows:

$$Q = AA' + \varphi,$$

where AA' and φ are the communality and the uniqueness (co) variance matrices, respectively (Snedecor and Cochran, 1989; Hair et al., 2014).

As stated in the (co) variance model, the measured variables can be represented as a combination of p unobservable common factors (X) plus a unique variable (e):

$$y_1 = a_{11}X_1 + \ldots + a_{1p}X_p + e_1$$
$$y_n = a_{n1}X_1 + \ldots + a_{np}X_p + e_n,$$

Where a are the loadings that quantify the correlation between the ith latent factor and the measured variable. Loadings are the elements of the A matrix of the theoretical factor variance model.

The MFA was performed on the correlation matrix of 26 individual unbranched FAs. The number of factors to be extracted was based on their eigenvalue (> 1), their readability in terms of the relationship with the original variables, and the amount of variance explained. Factor readability was improved through a VARIMAX rotation. To extract the latent factors, a variable was considered to be associated with a specific latent factor if the absolute value of its correlation with the specific factor was greater than or equal to 0.5 (Mele *et al.*, 2016). The suitability of the dataset used in MFA was further checked by calculating the Kaiser-Meyer-Olkin Measure of Sampling Adequacy (KMO), which determines the difference between Pearson and partial correlations (Hair *et al.*, 2014).

Results

In this study, the KMO was 0.812 ($p < 0.01$), close to the empirical threshold of 0.80 (Macciotta *et al.,* 2012), which allowed to conclude that dataset used was suitable for MFA.

The overall patterns of the composition of ruminal FAs (Table 1) consisted of 11 saturated FAs (SFAs), 11 monounsaturated FAs (MUFAs), and 4 polyunsaturated FAs (PUFAs). The SFAs accounted for almost 42% of total FAs, MUFAs accounted for almost 42%, and PUFAs were a minor part, only accounting for almost 16%. Palmitic acid (16:0) and stearic acid (18:0) were the main SFAs, t*rans*-vaccenic acid (18:1-*t*11) was the main MUFAs, and linoleic acid (18:2-*c*9,*c*12) and alpha-linolenic acid (18:3-*c*9,*c*12,*c*15) were the main PUFAs.

The MFA was able to extract four latent factors from the 26 FAs quantified in the ruminal fluid (Figure 1), accounting for about 86.7% of the total variance. The first latent factor accounted for the 46.3% of the total variance, and it was positively associated with short (6:0), medium (8:0, 10:0, 12:0, 14:0 and 16:0), and long (17:0, 20:0, and 23:0) SFAs, as well as with *cis* and *trans* MUFAs from 14 to 16 carbon atoms (14:1-*t*5, 14:1-*c*9, 15:1-*t*10, 16:1-*c*9, and 16:1-*t*9). Therefore, this factor was named "*de novo* FAs and Δ desaturase activity" (Table 2). The second latent factor explained 26.6% of the variance behind the first factor. It was named the "biohydrogenation (18:0 and MUFAs)", and was positively correlated with 18:0 and the majority of *cis* and *trans* MUFAs of 18 carbon atoms (18:1-*t*9, 18:1-*t*11, 18:1-*c*6, 18:1-*c*9, and 18:1-*c*11) (Table 2).

The third latent factor was named as "biohydrogenation (PUFAs)"; it accounted for 8.6% of the total variance and was positively related to 18:3-*c*9,*c*12,*c*15 and 18:2-*t*11,*c*15, and negatively to 18:2-*c*9,*t*11 (Table 2). Finally, the fourth factor was named "novel pathway"; it explained 5.2% of the total variance and was positively correlated with 18:1-t6 and 19:0 (Table 2). However, 19:0 was also negatively associated with the second factor. The 18:2-*c*9,*c*12 was negatively correlated with the second and third factors.

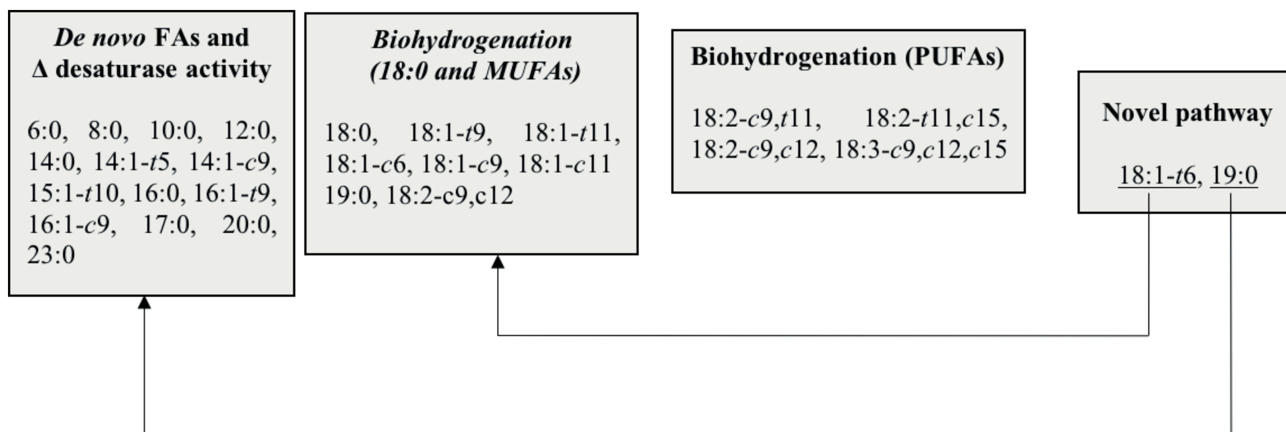

Figure 1. Metabolic groups of FAs in ruminal fluid and their interrelations derived from the multivariate factor analysis. FAs = fatty acids, *c = cis, t = trans.* Despite 19:0 and 18:1-t6 were mainly associated with the fourth factor, 19:0 and 18:1-t6 had a second significant interrelation with the first and second factors, respectively, which were represented using arrows.

Table 2. Rotated factor (F) pattern and proposed factor name, for F_1 through F_4.

Fatty acid	Common name	F_1 *De novo* FAs and Δ desaturase activity	F_2 Biohydrogenation (18:0 and MUFAs)	F_3 Biohydrogenation (PUFAs)	F_4 (Novel pathway)
6:0	capronic acid	0.854	-0.244	0.144	-0.038
8:0	caprilic acid	0.947	-0.141	0.031	-0.078
10:0	capric acid	0.786	-0.378	-0.010	-0.160
12:0	lauric acid	0.883	-0.355	0.059	0.166
14:0	miristic acid	0.939	0.135	-0.023	0.138
14:1-*t*5	transmiristelaidic acid	0.891	0.331	0.072	0.084
14:1-*c*9	miristoleic acid	0.945	0.038	0.014	0.091
15:1-*t*10	trans-pentadecenoic acid	0.689	0.396	0.096	-0.509
16:0	palmitic acid	0.973	0.139	0.071	0.020
16:1-*t*9	palmitelaidic acid	0.836	-0.232	-0.006	0.107
16:1-*c*9	palmitoleic acid	0.837	0.464	0.038	0.029
17:0	heptadecanoic acid	0.906	0.375	0.054	0.038
18:0	stearic acid	0.351	0.897	-0.064	0.164
18:1-*t*6	Petroselaidic	0.550	0.276	-0.211	0.619
18:1-*t*9	elaidic acid	-0.069	0.919	-0.105	0.018
18:1-*c*11	vaccenic acid	0.263	0.848	-0.001	-0.397
19:0	nonadecanoic acid	0.292	-0.558	0.149	0.658
18:2-*c*9,*c*12	linoleic acid	-0.324	-0.590	-0.588	-0.138
18:2-*t*11,*c*15	ND	-0.331	0.481	0.607	-0.321
18:2-*c*9,*t*11	conjugated linoleic acid	-0.128	0.141	-0.808	0.066
18:3-*c*9,*c*12,*c*15	alpha-linolenic acid	0.056	-0.491	0.766	0.116
20:0	metil araquidic	0.932	-0.048	-0.033	0.226
23:0	tricosanoic acid	0.818	0.134	0.215	0.171

FAs = fatty acids; MUFAs = monounsaturated FAs; PUFAs = polyunsaturated FAs; ND = common name is not defined by the literature; *c* = *cis; t* = *trans;* numbers in bold correspond to main correlations between a variable and a specific latent factor.

Discussion

The aim of this study was to investigate the potential of using MFA to extract information on unbranched FA metabolism in bovine rumen fluid. The results suggested that, unbranched ruminal FAs could be categorized under four latent factors with metabolic significance. This implies that multivariate factor analysis could be applied to studying FA metabolism in the rumen.

The results revealed that 16:0 and 18:0 were the most abundant SFAs in ruminal fluid. These SFAs are the most predominant FAs in ruminal bacteria and protozoa (Jenkins *et al*., 2008). This is in accordance with Or-Rashid *et al*. (2007), who revealed that 16:0 content was 74% greater in the protozoal FAs than in the bacterial FAs, whereas bacteria had 2.25-times greater 18:0 content than protozoa. Among MUFAs, 18:1-*t*11 was the main FA. This was expected because 18:2-*c*9,*c*12 and 18:3-*c*9,*c*12,*c*15 tend to be mainly converted into 18:1-t11 during their ruminal biohydrogenation (Jenkins *et al*., 2008; Ferlay *et al*., 2017; Vargas *et al.,* 2018). With respect to PUFAs, 18:2-*c*9,*c*12 and 18:3-*c*9,*c*12,*c*15 were the main FAs. High concentrations of 18:2-*c*9,*c*12 and 18:3-*c*9,*c*12,*c*15 were expected in ruminal fluid because the *in vitro* studies used to build dataset incubated pure 18:2-*c*9,*c*12 and 18:3-*c*9,*c*12,*c*15.

The MFA extracted four latent factors with biological meaning. The factor pattern (i.e., correlations

between each factor and original variables) was easy to read. In particular, after examining the patterns across factors, we observed that many variables correlated highly with only one factor and poorly with others, showing correlation greater than or equal to 0.5 with one particular factor. This type of structure was an indicator of the suitability of multivariate factor analysis for examining the dataset used in this study (Snedecor and Cochran, 1989; Hair *et al.*, 2014).

We observed that the first latent factor was positively associated with short, medium, and long-SFAs, as well as with *cis* and *trans* MUFAs from 14 to 16 carbon atoms, naming this latent factor as "*de novo* FAs and Δ desaturase activity". Short, medium, and long-SFAs are endogenously synthesized by ruminal microorganisms from acetate and butyrate by the acetyl-CoA carboxylase and FA synthase enzyme (Emmanuel, 1974; Or-Rashid *et al.*, 2007). Conversely, short and medium *cis* and *trans* MUFAs derive from Δ desaturase activities on the respective SFAs. Also, Ntambi *et al.* (1999) and Or-Rashid *et al.* (2007) demonstrated that bacteria and protozoa produce Δ9 and Δ11 desaturase enzymes, which produce MUFAs in the rumen. Therefore, MFA confirmed that these two groups of FAs (i.e., SFAs and short and medium-chain MUFAs) are metabolically linked under lipid ruminal metabolism, demonstrating that this statistical technique may be a valuable tool for analyzing the complex FA metabolism in the rumen.

A better understanding of the metabolic link between SFAs and MUFAs could help to enhance the physicochemical properties of milk. Considering that FA metabolism in the rumen significantly influences the milk FA profile (Jenkins *et al.*, 2008, Hur *et al.*, 2017), and milk fat fluidity is strongly affected by the relative abundance of SFAs (Mele *et al.*, 2016), such understanding could be useful to design nutritional strategies for optimizing milk composition.

The 18:0 is considered as the main final product of 18:2-*c*9,*c*12 and 18:3-*c*9,*c*12,*c*15 ruminal biohydrogenation (Jenkins *et al.*, 2008). However, Jouany *et al.* (2007), Shingfield *et al.* (2013), and Ferlay *et al.* (2017) demonstrated that these PUFAs are not completely biohydrogenated to 18:0, being interconverted in a great diversity of *cis* and *trans* MUFAs of 18 carbon atoms. Moreover, Or-Rashid *et al.* (2007) suggested that rumen protozoa have Δ11-desaturase activity, allowing conversion from 18:0 to 18:1-*t*11, and Kemp *et al.* (1984) and Ferlay *et al.* (2017) demonstrated that the ruminal fungus *P. communis* can dehydrogenate 18:0 to 18:1-*c*9. Therefore, a metabolic link between 18:0, 18:1 *cis* and *trans* FAs, and PUFAs may be expected, as demonstrated by the second latent factor named as "Biohydrogenation (18:0 and MUFAs)", which was mainly composed by 18:0, a majority of *cis* and *trans* MUFAs of 18 carbon atoms, and of 18:2- *c*9,*c*12.

The third latent factor was mainly composed by 18:2-*c*9,*c*12 (which was also a representative FA of the second latent factor), 18:3-*c*9,*c*12,*c*15, 18:2-*t*11,*c*15, and 18:2-*c*9,*t*11. This suggests that the 18:2-*c*9,*c*12 could be a metabolic link between the second and third factors. This is as expected, considering that 18:2-*c*9,*c*12 and 18:3-*c*9,*c*12,*c*15 have common metabolic intermediates (i.e., 18:1-*t*11 and 18:0) (Jenkins *et al.*,2008).

The 18:2-*t*11,*c*15 and 18:3-*c*9,*c*12,*c*15 presented a positive correlation with the third latent factor. This is because 18:2-*t*11,*c*15 is an intermediary key of 18:3-*c*9,*c*12,*c*15 biohydrogenation (Hur *et al.*, 2017). Similarly, 18:2-*c*9,*t*11 and 18:2-*c*9,*c*12 presented a negative correlation with the third latent factor, due to 18:2-*c*9,*t*11 is an intermediary key of 18:2-*c*9,*c*12 biohydrogenation (Ferlay *et al.*, 2017).

Unlike 18:0, 18:1, 18:2, and 18:3 FAs are interrelated during lipid metabolism in the rumen (Jenkins *et al.*, 2008), MFA revealed that 18:0 and 18:1 *cis* and *trans* FAs need to be described by a factor (i.e., second factor), and 18:2 and 18:3 FAs need to be described by another factor (i.e., third factor). This suggests that these two groups of FAs may also be metabolized by independent biochemical pathways. This is in accordance with Harfoot and Hazlewood (1997), who suggested that despite ruminal FA biohydrogenation occurs by interaction between several microorganism species, there are microorganisms (i.e., *Fusocillus*) capable to completely biohydrogenate 18:1-*c*9 and 18:2-

*c*9,*c*12 to 18:0, as well as 18:3-*c*9,*c*12,*c*15 to 18:1-*c*15 without interaction with another microbial species. In consequence, with the use of MFA, we could highlight the metabolic differences between these groups of FAs (18:0, MUFAs, and PUFAs) by extracting two different latent factors.

Finally, the fourth latent factor was mainly composed by 18:1-*t*6 and 19:0. Also, 19:0 was also highly correlated with the second factor. This suggests that the 18:1-*t*6 may be metabolically linked to 18:1 *cis* and *trans* FAs (second factor) using 19:0 as a potential intermediate. A metabolic relation between 18:1-*t*6 and 19:0 in PUFAs biohydrogenation has not been reported in the literature. However, Kim *et al.* (2005) demonstrated that 18:1-*t*11 was strongly associated with odd and branched-chain FAs during lipid ruminal metabolism. Therefore, a potential metabolic relation between 18:1-*t*6 and 19:0 FAs could be possible as well. Thus, MFA could be used in future studies to find potential novel ruminal biohydrogenation pathways.

We would expect a high correlation of 19:0 and 18:1-*t*6 to the first and second factors, respectively, considering that the first factor was mainly described by SFAs and the second factor was mainly composed by 18:1 *cis* and *trans* FAs. However, they constituted an independent factor. This may suggest that 19:0 and 18:1-*t*6 have different metabolic origins of their chemical families. This is in accordance with Harfoot and Hazlewood *et al.* (1997), who suggested that the metabolic origins of FAs during ruminal biohydrogenation may be affected by microbial populations and ruminal conditions. Moreover, the findings of Kemp *et al.* (1975) and Shingfield *et al.* (2013) demonstrated that several bacterial species are capable of independently producing specific SFAs, and 18:1 *cis* and *trans* FAs, not necessarily involving the general biohydrogenation process.

The data demonstrated that multivariate factor analysis has many positive outcomes when applied to a dataset derived from the analysis of bovine rumen fluid samples. A first positive outcome concerned the reduction of a great number of variables to a few latent factors with biological meaning. Second, the statistical approach classified groups of fatty acids with common metabolic origins or linked to defined metabolic pathways. Considering the recent emerging analytical techniques for determining novel fatty acids involved in ruminal metabolism, we suggest that multivariate factor analysis could be useful to design studies aiming to determine novel fatty acid metabolic pathways. Moreover, this information may aid in designing better nutritional strategies to optimize the nutritional quality of ruminant milk and fat.

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Conflicts of interest

The author declares he has no conflicts of interest with regard to the work presented in this report.

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