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STOICHIOMETRIC MODELS FOR PREDICTION OF ENTERIC METHANE PRODUCTION

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Abstract
This study aimed to empirically evaluate the adjustment quality of two stoichiometric models of methane production from diets that used different rumen fermentation modulators. We used the models proposed by Moss et al. (2000) and Blümmel et al. (1997). The data set consisted of 169 observations of *in vitro* methane production and volatile fatty acids (acetate, propionate, and butyrate) from dissertations, theses, and scientific articles. The model's adequacy evaluation was only possible through combination of several statistical analyzes. The models were unable to predict the enteric methane precisely and accurately. Despite this, the model proposed by Moss et al. (2000) showed a better fit based on the mean bias (% of observed, MB), MEF, RMSEP, Cb and CCC measures compared to the model proposed by Blümmel et al. (1997). However, both models presented strong evidence against the general hypothesis H0: a = 0 & b = 1. Thus, the model proposed by MOSS et al. (2000) was more efficient, whereas the model by Blümmel et al. (1997) showed a better fit for diets with high availability of H2.

Keywords: Empirical evaluation. Predictive power. Short-chain fatty acids.

1. Introduction

Methane (CH4), produced as a result of digestion, represents energy loss for animals. The energy lost through CH4 production can vary from 2 to 12% of the gross energy intake (Johnson and Johnson 1995). This range is due to differences in the nutritional composition of the feed, type of processing, addition of lipids, additives (ionophores, non-ionophores, essential oils, etc.), the amount of concentrate in the diet, and other factors that can lead to different fermentation patterns (Sejian et al. 2011). Thus, the result is the different proportions of the final fermentation products such as volatile fatty acids (VFAs), acetate, propionate, and butyrate, which represent about 95% of the total short-chain fatty acid production (Bannink et al. 2006). The VFAs are an important metabolizable energy source for ruminants. Along with these fermentative processes, CO2 and CH4 are produced, in a higher or lower scale, depending on the individual proportion of the acids, which are influenced by the organic matter composition of diets, mainly the nature and rate of carbohydrates fermentation (Schönfeld and Wojtczak 2016).

The proportions of acetate, propionate, and butyrate determine the amounts of hydrogen gas (H2) available in the rumen to be used in methanogenesis. The formation of acetate and butyrate is largely the result from the fermentation of fibrous carbohydrates (although reasonable amounts of butyrate are produced from soluble carbohydrates), resulting in H2 production that is used by methanogenic *Archeas* for CH4 production (Hegarty and Nolan 2007). On the other hand, propionate is produced mostly by the fermentation of non-fibrous carbohydrates and it a competitive pathway for the utilization of H2 in the rumen. The consequence of this route is the reduction in the production of CH4 (4). For this reason, CH4 emission can be estimated stoichiometrically from the production of VFAs. So, the accuracy of the predictions of CH4 production strongly depends on the accuracy in quantifying the VFAs produced in the rumen (Wolin 1960).

For St-Pierre (2001), most of the prediction equations for methane production in the rumen reported in literature are based on specific types of diets. In addition, traditional approaches using simple regression analysis, without considering the effect of the randomized study, can result in estimated parameter values with considerable bias.

Therefore, this study aimed to empirically evaluate the adjustment quality of two stoichiometric models of methane production from diets with different ruminal fermentation modulators.

2. Material and Methods

Data set description

We collected data from dissertations, theses, and scientific articles published by Lima (2016), Becker (2015), Cieślak et al. (2006), Rodrigues (2017), Benetel (2018), Araujo (2010), Souza (2014), Kara et al. (2016), Kara et al. (2018), Ramirez-Bribiesca et al. (2018), Ugwuowo et al. (2017), Mallmann (2013), Medjekal et al. (2018), Nguluve (2014), Rivera (2006), Molina-Alcaide et al. (2017) and Garcia (2013).

The data set consisted of elements that can change ruminal fermentation when they are in the animals' diet, such as: fat, essential oils, protein feedstuff, different roughages, and additives (ionophore antibiotics and non-ionophores, yeasts, and algae).

The values of volatile fatty acids that were used as input in the models and the methane values that were used for comparison between stoichiometric models are described in Table 1.

The chemical composition, quantity, and rate of carbohydrates fermentation of the diet directly influence the concentration and proportion of total VFAs from ruminal fermentation and, consequently, affect the CH4 amount produced by the animal. Thereby, we used the studies by Lima (2016), Becker (2015), Cieślak et al. (2006) and Rodrigues (2017) on the inclusion of fat in the animals' diet, as well as the studies by Benetel (2018), Araujo (2010) and Souza (2014) who analyzed the impact of essential oils on rumen fermentation. Rivera (2006), Molina-Alcaide et al. (2017) and Garcia (2013) evaluated the additives in diets. The data set composed by Mallmann (2013), Kara et al. (2018), Ramirez-Bribiesca et al. (2018) and Ugwuowo et al. (2017) were feedstuffrich in protein. CH4 production tends to increase when the diet contains forages with different phenological ages. The inclusion of legumes may result in less enteric CH4 production compared to grass fermentation, for this we used the studies by Rodrigues (2017), Medjekal et al. (2018), Nguluve (2014) and Kara et al. (2016).

Methane Prediction

We used the models proposed by Moss et al. (2000) and Blümmel et al. (1997). Moss et al. (2000) developed this model based on the model proposed by Demeyer and Van Nevel (1975): $2C2+C3+4C4=4CH_4+2C3+2C4$. Where, C2 is acetate, C3 is propionate, C4 is butyrate, and CH_4 is methane. The metabolic hydrogen (H2) recovery rate considered was 90%, allowing the development of the equation:

$$CH_4=0.45\times C2-0.275\times C3+0.40\times C4$$

Another model evaluated was the one developed by Wolin (1960), which considers the quantity and molar proportion of C2,C3, andC4 from molar quantities of CO_2 and CH_4. The stoichiometry described by Wolin (1960), and exemplified by Van Soest (1994), assumes that the net oxidation balance of the products is zero ($C2+C3+C4+CO_2+CH_4=0$). Thus, the model by Blümmel et al. (1997) was developed using derivations of the equations described by Van Soest (1994).

$$CO_2 = C2/2 + C3/4 + 1.5 \times C4$$
$$CH_4 = C2 + 2 \times C4 - CO_2$$

Table 1. Mean, median, standard deviation (SD), maximum (Max), minimum (Min), and number of observations (n) of the amounts of acetate (C2), propionate (C3), butyrate (C4), and volatile fatty acids (VFAs), expressed as mmol /l and methane (CH4, ml/g of dry matter).

	Mean	Median	SD	Max	Min	n
Fat addition in the diet						*23*
C2	38.56	24.9	17.639	79.59	19.4	
C3	14.33	13.3	2.320	22.84	10.3	
C4	8.41	7.6	2.114	12.74	5.2	
VFAs	61.29	45.9	21.871	100	35	
CH_4	14.22	12.4	4.538	28.2	0.4	
Essencial oils						*83*
C2	25.29	24.60	14.208	56.3	2.27	
C3	6.26	5.11	3.732	13.4	0.43	
C4	4.15	3.43	2.681	9.1	0.93	
VFAs	35.71	33.11	20.596	74.4	3.93	
CH_4	9.90	9.50	3.362	22.19	0.17	
Additives						*26*
C2	47.12	55.40	14.311	64.6	25.27	
C3	14.37	14.10	3.896	26	8.69	
C4	10.39	11.50	5.996	21.4	2.52	
VFAs	71.88	89.60	23.420	94.52	36.61	
CH_4	8.66	5.96	4.878	22.29	0.91	
Protein feedstuff						*16*
C2	42.83	49.33	17.430	67.75	16.59	
C3	16.39	19.53	6.493	26.68	5.36	
C4	13.81	11.17	7.109	32.97	4.25	
VFA's	73.03	68.19	19.712	105.6	29.34	
CH_4	20.81	21.9	3.019	26.37	12.67	
Types of roughage						*21*
C2	28.37	10.18	23.974	63.6	6.86	
C3	9.13	4.0	7.905	28.38	1.37	
C4	5.50	1.27	5.575	18.97	0.39	
VFA's	42.98	15.25	37.454	93.98	9.62	
CH_4	19.66	20.15	5.306	33.9	1.69	

Assessment of the adequacy of the predictions

The assessment of the adequacy of the models was only possible through the combination of several statistical and empirical analyses and proper investigation on the model initially conceptualized (Tedeschi 2006). We used several techniques in this study. The coefficient of determination (r^2) (Neter et al. 1996), and the simultaneous test for the intercept and slope (Dent and Blackie 1979).

We also used additional techniques as discussed by Tedeschi (2006).evaluation of accuracy with concordance correlation coefficient (CCC; Lin 1989), mean bias (Cochran and Cox 1957), mean square error of prediction (MSEP; Bibby and Toutenburg 1977), and the model efficiency factor (Loague and Green 1991; Zacharias et al. 1996).

Pearson's correlation coefficient was used to measure the intensity of the linear relationship between volatile fatty acids and methane production using the PROC CORR procedure from SAS program (University Edition version, SAS Institute Inc., Cary, NC, USA).

3. Results

The data set had a large range (e.g., C2, C3, C4, total VFAs, and methane), this is considered adequate for the evaluation of different models (Table 1). Pearson's correlation test (Table 2) showed that only total VFAs did not (p > 0.05) correlate with C2, C3, C2/C3, C2 + C4/C3, and methane. However, the other variables were positively correlated (p <0.05), mainly with methane.

Table 2. Pearson's correlation between volatile fatty acids (VFAs), their proportions (mmol/l), and methane production (ml/g of dry matter).

	C2	C3	C4	C2/C3	C2/C4	C2+C4/C3	Total VFAs	CH_4
C2	1.000							
C3	0.805*	1.000						
C4	0.709*	0.849*	1.000					
C2/C3	0.757*	0.498	0.776*	1.000				
C2/C4	0.673*	0.881*	0.992**	0.694*	1.000			
C2+C4/C3	0.937**	0.904**	0.908**	0.810*	0.887*	1.000		
Total VFAs	0.128	0.427	0.681*	0.379	0.699*	0.412	1.000	
CH_4	0.803*	0.801*	0.807*	0.697*	0.793*	0.873*	0.404	1.000

*p < 0.05; ** p < 0.0001.

The models were unable to predict enteric methane (Figure 1) precisely and accurately. The lines from the predictions of the models by Blümmel et al. (1997) and Moss et al. (2000) presented significant distances from the unit line (Y = X) and may present biased and inaccurate predictions (Figure 1).

The model proposed by Moss et al. (2000) presented a better fit than Blümmel et al. (1997) based on the mean bias (% of observed, MB), MEF, RMSEP, Cb, and CCC. model. However, both models presented strong evidence against the general hypothesis H0: a = 0 & b = 1 (Table 3).

Table 3. Evaluation of the models' performance for methane prediction (ml/g of dry matter) using data from literature (n = 169).

	Models									
Methane	Blümmel et al. (1997)					Moss et al. (2000)				
	Fat[a]	EO[b]	Adittives	Protein[c]	Roughage[d]	Fat	EO	Adittives	Protein	Roughage
Observed (O)	14.85	9.89	9.24	20.22	19.42	14.85	9.89	9.24	20.22	19.42
Predicted (P)	32.74	13.15	25.32	24.52	14.26	16.77	11.32	21.56	20.61	12.11
P - O	17.89	3.26	16.08	4.3	-5.16	1.92	1.43	12.32	0.39	-7.31
Mean bias (% of O)	-123.48	-29.40	-174.07	-21.18	26.58	-18.09	-19.62	-133.21	-1.97	37.62
MEF[e]	-10.24	-2.19	-10.22	-2.73	-4.77	-0.9	-0.94	-6.66	-2.49	-3.16
RMSEP[f]	20.81	7.20	21.07	10.58	13.40	8.55	5.98	17.41	8.97	12.97
R^{2g}	0.10	0.42	0.22	0.02	0.12	0.20	0.40	0.21	0.03	0.13
P[h]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C_b[i]	0.31	0.71	0.31	0.70	0.74	0.89	0.83	0.40	0.89	0.69
Pc[j]	0.09	0.46	-0.15	-0.10	0.26	0.40	0.52	-2.04	-0.16	0.25

[a]Fat addition in the diet. [b]Essencial oils. [c]Protein feedstuff. [d]Types of roughage. [e]MEF = model efficiency factor. [f]RMSEP = root of mean squared error prediction. [g]R2 = coefficient of determination of the best fit regression line not forced through the origin. [h]P = probability associated to F-test to reject the simultaneous hypothesis with the slope = 1 and intercept = 0; when NS (P > 0.1) in the hypothesis is not rejected (Dent and Blackie 1979). iAccuracy of the model (Lin 1989). [j]Concordance correlation coefficient (CCC) (Lin 1989).

4. Discussion

The formation of the main VFAs is directly associated with the production of CO_2 and CH_4. The formation of acetate releases 2 moles of CO_2 and 4 moles of H_2 per mole of fermented glucose. Four moles of H_2 are used in the methanogenesis to reduce one mole of CO_2 to form CH_4. Consequently, the acetate formation results in the production of one mole of CO_2 and one of CH_4 per mole of fermented glucose. Similarly, the butyrate production results in the production of 1.5 mol of CO_2 and 0.5 mol of CH_4 per mol of fermented glucose. Differently, the formation of propionate does not result in the CO_2 and, consequently, reduces the CH_4 production. The formation of propionate conserves more energy in the glucose fermentation, and it is used by the animal (Ellis et al. 2008). Thus, the stoichiometric relationships developed *in vitro* can explain more than 90% of the H_2 observed in VFAs and CH_4 (Demeyer and Van Nevel 1975).

The enteric CH_4 produced by methanogenic *Archaeas* depends on the ruminal balance of H_2, and it is influenced by the production rates of acetate and propionate (Johnson and Johnson 1995).

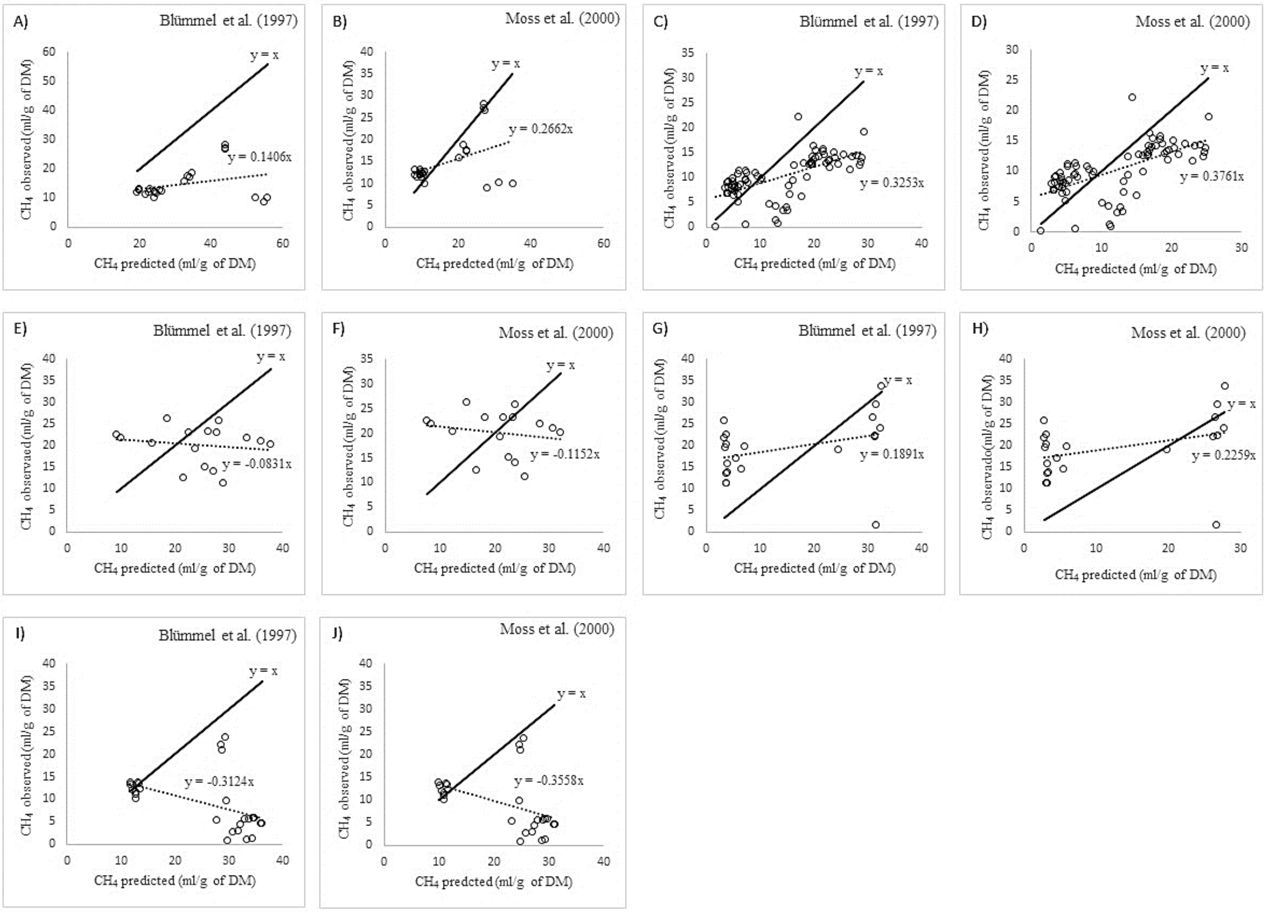

Figure 1. Observed versus predicted methane production (ml/g of DM) regarding diets with addition of fat (A and B), essencial oils (C and D), adittives (E and F), protein feedstuff (G and H), and types of roughage (I and J) as proposed by Blümmel et al. (1997) and Moss et al. (2000) and observed in these studies. The solid line indicates unitary equivalence (X =Y). DM = Dry matter.

The fermentation products are partially determined by the diet's nature. For example, when we add fat to the animal's diet, biohydrogenation occurs in the rumen (transfer of H_2 to saturation of unsaturated fatty acids), decreasing the availability of H_2 for CH_4 synthesis (Van der Honing et al. 1981). Johnson and Johnson (1995) attributed the reduction in CH_4 production to lipid supplementation, mainly associated with a decrease in fermentable substrate in the rumen than to a direct effect on methanogenesis. However, Cone and Van Gelder (1999) emphasize that the amount of CH_4 produced by carbohydrate fermentation is different from that produced by protein fermentation. Thus, the stoichiometric model of Wolin (1960) was developed considering feeds rich in carbohydrates, as well as the model by Moss et al. (2000) that was based on the model proposed by Demeyer and Van Nevel (1975), this fact may justify the reduction of accuracy in the models when we use feeds rich in fat. This corroborates the findings of this study (Table 3, Figure 1 A and B). The same assumption holds for the data set of protein-rich feeds (i.e., canola flour and wet brewery residue). Still, the model by Moss et al. (2000) had better adjustment (P - O = 0.39 units; MEF of -2.49, RMSEP of 8.97, and Cb of 0.89) for the estimate of methane production (Table 3, Figures 1G and H).

The antibacterial activity of some essential oils (e.g., anise oil) and some additives such as ionophores, algae, and yeasts are very similar, promoting changes in the fermentative profile (Calsamiglia et al. 2007; Gunal et al., 2014). The change in the fermentative pattern is due to the reduction of C2:C3 ratio, it makes the rumen more energy efficient, consequently the reduction of CH4, as the propionate serves as an H2 drain (Van Soest 1994). Regarding the data set of essential oils, we observed that the model by Moss et al. (2000) presented the best fit (MEF of -0.94, RMSEP of 5.98, and Cb of 0.83) for the estimate of methane production (Table 3, Figures 1C and D). However, when we evaluated the data set of additives, the models showed low precision to estimate methane production (CCC = -0.15 and -2.04 for the Blümmel et al. (1997) and Moss et al. (2000) models, respectively) (Table 3, Figures 1E and F). It is worth mentioning that ionophores, such as monensin, can reduce degradation and total concentration of VFAs depending on the dose (Russel and Strobe 1989). The less accurate model of Moss et al. (2000) when we used the data set of additives may be due to the decrease in the number of protozoa, because they supply H2 to methanogenic *Archeas* maintaining a symbiosis relationship (Russel and Strobe, 1989).

Diets rich in fibrous carbohydrates allow the proliferation of fibrolytic bacteria, triggering an increase in C2 and C4 production. With this, there is some release of a large amount of H2 in the rumen thatis used for the production of CH4. We evaluated the data set of different types of roughage and observed that the two models overestimated the production of CH4, but the model proposed by Blümmel et al. (1997) had a better fit (Table 3, Figures 1I and J). Probably the model by Blümmel et al. (1997) considers 100% H2 recovery. For the fermentative modulators, we observed that the model by Moss et al. (2000) fits better for the enteric CH4 prediction, possibly due to the lower amount of H2 available for CH4 production.

So, the low precision of stoichiometric models for predicting enteric CH4 may be due to the model's inability to detect changes in the ruminal fermentation profile. In addition, Alemu et al. (2011) point out that enteric CH4 production would be better predicted through the VFAs production rates and not by their concentration in the rumen. However, there is a shortage of data on VFAs production rate in literature, most studies focus on VFAs concentrations.

5. Conclusions

The change in VFAs proportions caused by ruminal fermentation modulators reduced the accuracy of methane prediction by the models.

The model proposed by Moss et al. (2000) was more efficient, whereas the model by Blümmel et al. (1997) showed a better fit for diets that allow high availability of H2.

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