Original Research Article

Comparison of the Fitting of Two Mathematical Models to Describe the Ruminal Fermentation Parameters of Some Sources of Plant and Animal Protein Using In Vitro Gas Method

ABSTRACT

Aims: In this study of two mathematical models was used for described rumen fermentation parameters of plant and animal some protein sources using test gas method. **Study design:** The two models include the exponential model Ørskov and McDonald (EXP) and sigmoid model the France (FRC).

Place and Duration of Study: The study was conducted at the University of Ardebil, between 2014 and 2016. In order to conduct the experiment, sources of plant protein (soybean meal, Rapeseed meal and cottonseed meal) and sources of animal protein (poultry offal meal, fish meal and blood meal) were obtained from the agricultural sector and the local slaughterhouse.

Methodology: Gas production tested for 6 feed in 3 repeat in 3 separate periods was conducted. The volume of gas produced at 2, 4, 6, 8, 10, 12, 16, 24, 36, 48 and 72 hours incubation were measured by two model gas production parameters and ruminal fermentation were fitted.

Results: The results showed that the amount of gas production potential (A) and the rate constant gas production (c) in both model of EXP and FRC was the same and had not significant difference together. However, two model at lag phase (T lag) had the significant difference that the amount lag phase in the model EXP than model FRC was higher. **Conclusion:** Therefore, the FRC model instead EXP model can often be a useful technique

for describe the gas production profiles.

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1. INTRODUCTION

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Gas production in vitro, in related with fermentation parameters and ruminal digestion kinetics <u>are</u> valuable descriptions in the evaluation feed provides [4]. In this in vitro gas production, a certain amount of feed in the rumen fluid incubated and the volume of gas

23 produced at regular intervals and row that showed the speed of feed digestion feed is measured. Described the results of the tests is described mainly by fitting the

Keywords: Gas test, Mathematical models, Protein sources.

24 is measured. Described the results of the tests is described mainly by fitting them by two 25 models of EXP and FRC is done [1]. Therefore, comparing the performance and capability of

two models can highly be influential model for <u>choice choosing</u>. Some of the differences between the two models may be related to the test conditions and the type of feed. But some

of these differences in the ability to model and flexible models at predict and describe the

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29 results related to fermentation [7]. Since the gas production curve is non-linear structure, the 30 models that for describe it used, it should have such a structure [11]. Some of models, like 31 the model France sigmoid structure have established that due to the use of this structure; 32 the presence of microbial activity in the rumen has been reported [11]. But some other of models likes mode of Ørskov and McDonald have non-Sigmoid structure. So today, for 33 34 greater reliability of gas production test results by the researchers, a variety of models non-35 Sigmoid and Sigmoid structure is used and in this regard, various formulas have been 36 proposed [3,8]. In most studies related to rumen fermentation parameters by in vitro gas 37 production of the exponential equation Ørskov and McDonald (1979) as (EXP) y=A (1- e-ct) 38 is used. McDonald and Ørskov model is one of the most well-known models used-in predict 39 rumen fermentation parameters. This model assumes that the rate of gas production in the 40 rumen depends only on the availability of feed [13]. One another of the models that is used 41 to predict gas production, is the model of France (FRC). As mentioned, France model had 42 sigmoid structure and great flexibility in fitting the data of gas production. France model 43 assumes that the rate of gas production is directly linked to the rate of feed degradation feed 44 and this condition is dependent on fermentation time and time identification or adherence of 45 bacteria to feed components (lag phase) [1]. In addition, there are models that by other researchers for this purpose have been proposed that have received little attention [9]. 46 47 according to the comprehensive comparison between the two models of France and Ørskov 48 and McDonald for described ruminal fermentation parameters plant and animal some protein sources using gas test method and since the evaluation tests of feed has been done more 49 50 than alfalfa hay as a standard feed and with important in ruminant nutrition. Therefore, in this 51 study the accuracy of the proposed methods of terms of goodness of fit and this is used to 52 describe the ruminal fermentation parameters some plants and animal some-protein sources 53 evaluated using gas method. 54 2. MATERIALS AND METHODS

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56 In order to conduct the experiment, sources of plant protein (soybean meal, Rapeseed meal 57 and cottonseed meal) and sources of animal protein (poultry offal meal, fish meal and blood 58 meal) were obtained from the agricultural sector and the local slaughterhouse. The chemical 59 composition of the feed by conventional methods [12] was carried out. The in vitro method 60 [4] was used to measure the amount of produced gas in laboratory conditions and the amount of gas production measured and recorded at 2, 4, 6, 8, 10, 12, 16, 24, 36, 48 and 72 61 62 hours of incubation, respectively. In this study, among of the different mathematical models 63 have been developed to analyze gas production data by two models digestion France et al 64 (1993) and Ørskov and McDonald (1979) with regard to the lag phase was used to evaluate 65 the digestive process. For this purpose of 54 series data obtained from the tests (three 66 separate periods with 3 repeat and 3 levels of feed and 2 feed per period) for fitted data's and T-test was used to compare their mean for each parameter of the model. 67

68 69 Models include:

7071 Ørskov and McDonald model (1979) with regard to the lag phase

72 G=A (1-e^{-ct+L})

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74 Model France et al., (1993).

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76 G = A (1-e-c (t-L)-d ($\sqrt{t} - \sqrt{L}$)) 77

78 Where G is equal to the accumulation of gas produced per unit time, A is equal to the total 79 amount of gas produced (ml), c is equal to a fixed rate of gas production (ml per hour), d is **Comment [#4]:** This is ambiguous, please recast or delete

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equal to a fixed rate of gas production (ml at h1/2), L equal to the lag phase, t time and t $\frac{1}{2}$ equal to half of the total gas production time is cumulative.

83 3. RESULTS AND DISCUSSION

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3.1 CHEMICAL COMPOSITION

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87 The chemical compositions of test feed are presented in Table 1. Blood meal contents has higher percentage of protein than any of the other plant and animal protein. The maximum 88 89 amount of crude fat 31.3% for POM and highest ash content of 20% was observed for FM. Highest of NDF and ADF (70.6% and 58.4%) for CM and the lowest NDF and ADF were 90 91 obtained 45.7 and 33.3% for SM, respectively. The results related to predicted parameters 92 by the model France (FRC) and the Ørskov and McDonald (EXP) are presented in Table 2. As observed the gas production potential (A) for all feed samples testing in the model FRC 93 94 and EXP respectively, 133.407 and 131.790 ml per gram dry matter was predicted and 95 significant difference was observed between the two models in terms of gas production 96 potential. The gas production rate constant (c) for all feed tested in the FRC and EXP 97 respectively 0.089 and 0.082 ml per hour, which was not significantly different between the 98 two models.

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Table 1. Chemical composition of some plant and animal protein sources

Protein sources	DM	СР	EE	Ash	NDF	ADF
Plant						
Soybean meal	92.4	50	1.6	6.1	45.7	33.3
Rapeseed meal	91.4	37	1.2	8	51.5	46.1
Cottonseed meal	93	24	1.4	4.7	70.6	58.4
Animal	\mathcal{A}					
Poultry offal meal	94.4	55	31.3	7.3	48.9	34.8
Fish meal	93.6	50	18.1	20	61.2	40.6
Blood meal	70.6	59	1.6	5	55.3	33.4

*DM = dry matter (percent), CP = crude protein (%DM), EE= crude fat (%DM), Ash = ash (%DM) NDF = Neutral detergent fiber (%), ADF= Acid detergent fiber (%)

However, when the individual feed was fitted in terms of the two models of France and Ørskov and McDonald, it was observed that rapeseed meal had a significant difference in gas production rate. Only the two models had a significant difference in terms of the lag time (T lag) except for cotton seed meal (P <0.05). According to the results of the tables, T lag was higher in the Ørskov and McDonald's model than the France model. T lag or the time colony production is an important parameter that is associated with feed fiber degradability [5]. Less time to start the colony by France the model for all plant and animal protein sources were received. The lag phase for the France 0.435 hours and against 1.964 hours for the
Ørskov and McDonald were observed. The longer lag phase for all protein sources in the
Ørskov and McDonald model indicates that in this model, microorganisms were observed to
have started to recognize and colonize on the digestible substrate in a delayed and timeconsuming behavior compared to the France model.

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 Table 2. Comparison of two models France and Ørskov and McDonald based the

 estimated parameters these to between the plant and animal protein sources

	Parameters	France	Ørskov and McDonald	P value for T-test
	А	133.41	131.79	0.93
Total feeds	С	0.09	0.08	0.59
	T lag	0.44	1.96	<0.001

*A = potential gas production (ml) c = constant rate gas production (ml per hour) T lag = lag phase (hours)

114 It is desirable to reduce the production time of the colony for a fermentable substrate and 115 easily fermented, and especially for samples containing fiber and cell wall and certain 116 physicochemical characteristics in the cell wall. In the case of studied protein sources, cotton 117 seed meal had a lower T lag in both models. However, other sources of plant and animal 118 protein in this study, despite the fact that fiber and cell wall structure (NDF) were less than

that of cottonseed meal but, two models in the T lag have shown significant different values

120 for our protein sources. In this comparison, the France model has the lowest lag phase for

121 these sources (P <0.05). 122

Table 3. Comparison of two models France and Ørskov and McDonald based the estimated parameters these to between the plant protein sources
Model

	Parameters	France	Ørskov and McDonald	P value for T-test
	A	204.74	202.09	0.90
Plant protein	С	0.06	0.05	0.27
	T lag	0.37	1.48	0.002

*A = potential gas production (ml) c = constant rate gas production (ml per hour) T lag = lag phase (hours)

123	This shows	that th	ie Ørskov	and	McDonald	model	has	an	over	estimate	for	lag	phase.
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124 Therefore, it can be concluded that the French model estimates less lag phase for sources 125 of protein with less fiber. Reis, Sidnei Tavares Dos, et al., (2016) stated that the correlation Comment [#10]: Indicated

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126 between the cumulative production phase and the total carbohydrate degradation is strong 127 and high, but some differences in this relation are concerned to the model used model for 128 the analysis.

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Table 4. Comparison of two models France and Ørskov and McDonald based the					
estimated parameters these to between the animal protein sources					
Model					

	Parameters	France	Ørskov and McDonald	P value for T-test
	А	62.08	61.49	0.96
Animal protein	С	0.12	0.11	0.74
	T lag	0.50	2.45	<0.001

*A = potential gas production (ml) c = constant rate gas production (ml per hour) T lag = lag phase (hours)

132 T Lag represents the amount of time that microbes spent for attachment to raw material or substrate fermentable and adhesion to the insoluble substrate is as a predigesting condition 133 and beginning the process of digestion. Shorter lag phase may be faster fermentation rate. 134 135 So among those protein sources, those with a lower lag phase have been shown more fermentation or degradation rates, as well as more gas production. The structure of the 136 137 solution fraction of each feed is as an energy substrate for rapid fermentation by attachment 138 microbes, and the suitable colonization of microorganisms onto substrate materials, followed 139 by increased fermentation and ultimately reduced lat phase.

140 However, the importance of the solution fraction to start the degradation and gas production 141 is significant when larger amounts of cell wall components can be provided to 142 143 microorganisms by better colony and more microbes [10].

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> Table 5. comparative models France and Ørskov and McDonald based the estimated parameters of these to between each sources of study

		Model	
	France	Ørskov and McDonald	
_			-
Source protein	А	Α	P value For T-test
Soybean meal	287.04	287.48	0.96
Rapeseed meal	215.99	219.68	0.79

*A = potential gas production (ml) c = constant rate gas production (ml per hour) T lag = lag phase (hours)

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Table 6. comparative models France and Ørskov and McDonald based the estimated parameters of these to between each sources of study Model

	France Ø	ðrskov and McDonald	
Source protein	C	C	P value For T-test
Soybean meal	0.08	0.07	0.23
Rapeseed meal	0.06	0.04	0.01
Cottonseed meal	0.04	0.04	0.89
poultry offal meal	0.12	0.10	0.29
Fish meal	0.10	0.09	0.60
Blood meal	0.13	0.14	0.89

*A = potential gas production (ml) c = constant rate gas production (ml per hour) T lag = lag phase (hours)

Table 7. comparative models France and Ørskov and McDonald based the estimated parameters of these to between each sources of study Model

	Model					
-	France	Ørskov and McDonald				
Source protein	T lag	T lag	P value For T-test			
Soybean meal	0.34	1.35	0.02			
Rapeseed meal	0.62	2.47	0.002			

Cottonseed meal	0.16	0.63	0.31
poultry offal meal	0.52	2.21	0.002
Fish meal	0.51	2.39	0.008
Blood meal	0.46	2.74	0.001

*A = potential gas production (ml) c = constant rate gas production (ml per hour) T lag = lag phase (hours)

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4. CONCLUSION

150 According to the goodness of fitness is done between the two models, the French model seems to be a better model for describing the ruminal fermentation parameters than the 152 model_Ørskov and McDonald model because of the shorter lag phase or less colony production time. Also this fact does not lead to an underestimation of fermentation level or degradability and the potential of gas production for ruminant feeds.

157 REFERENCES

- 1. France J, Dhanoa M, Theodorou M, Lister S, Davies D, Isac D. A model to interpret gas accumulation profiles associated with in vitro degradation of ruminant feeds. J Theor Biol. (1993):163(1):99-111.
- 2. France J, Dijkstra J, Dhanoa MS, Lopez S, Bannink A. Estimating the extent of degradation of ruminant feeds from a description of their gas production profile observed in vitro: derivation of models and other mathematical considerations. Br J Nutr. (2000): 83(2): 143-150.
- 3. France J, Lopez S, Kebreab E, Bannink A, Dhanoa MS, Dijkstra J. A general compartmental model for interpreting gas production profiles. Anim Feed Sci Tech. (2005):123-124(1): 473-485.
- 4. Menke K, Steinggass H. The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor in vitro. J Agri Sci. (1979): 93(1): 217-222.
- 5. Mertens DR, Loften JR. The effect of starch on forage fiber digestion kinetics in vitro. J Dairy Sci. (1980):63(9): 1437-1446.
- 6. Ørskov ER, McDonald I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rates of passage. J Agri Sci. (1979):92(2): 499-503.
- 7. Peripolli V, Prates ER, Barcellos JOJ, Mcmanus CM, Wilbert CA, Braccini Neto J, Camargo CM, Lopes B. Models for gas production adjustment in ruminant diets containing crude glycerol. Livestock Res Rural Dev. (2014): 26:2.
- 8. Sahin M, Ückardes F, Canbolat Ö, Kamalak A, Atalay Ai. Estimation of partial gas production times of some feedstuffs used in ruminant nutrition. Kafkas Univ Vet Fak Derg J. (2011): 17(5):731-734.

- Tedeschi LO, Schofield P, Pell AN. Determining feed quality for ruminants using in vitro gas production technique. Building an anaerobic fermentation chamber, In: The 4th Workshop on Modeling in Ruminant Nutrition: Application of the Gas production Technique, Juiz de fora, MG. (2008). Brazil.
 - Tosto MSL, Araujo GGL, Ribeiro LGP, Heriques LT, Menezes DR, Barbosa AM, Romão CO. In vitro rumen fermentation kinetics of diets containing old man saltbush hay and forage cactus, using a cattle inoculum. Arq Bras Med Vet Zootec. (2015):67(1)149-158.
 - Uckardes F, Korkmaz M, Ocal P. Comparison of models and estimation of missing parameters of some mathematical models related to *in situ* dry matter degradation. J Anim Plant Sci. (2013):23(4):999-1007.
 - 12. Association of Official Analytical Chemists (AOAC). Official Methods of Analysis, 16th ed. USDA, Washington, DC. (2000).
 - Wang M, Tang S, Tan Z. Modeling in vitro gas production kinetics: derivation of logistic-exponential (le) equations and comparison of models. Anim Feed Sci Technol.(2011):165(3-4):137–150.
 - Reis, Sidnei Tavares dos, Lima, Marcus Vinícius Gonçalves, Sales, Eleuza Clarete Junqueira de, Monção, Flávio Pinto, Rigueira, João Paulo Sampaio, and Santos, Leonardo David Tuffi. Fermentation kinetics and in vitro degradation rates of grasses of the genus Cynodon. Acta Scientiarum Anim Sci. (2016):38(3):249-254.