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4 **Comparison of Two Mathematical Models to**
5 **Describe the Rumen Fermentation Parameters**
6 **of Some Sources of Plant and Animal Protein**
7 **Using *In Vitro* Gas Method**

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12 **ABSTRACT**
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Aims: To compare, on an experimental basis, the respective relevance of two mathematical models estimating the rumen fermentation parameters of some plant and animal protein sources: the “exponential” model by Ørskov & McDonald (EXP) and the “sigmoid” model by France *et al.* (FRC).

Study design:

The study was conducted at the University of Ardebil (Iran) between 2014 and 2016. In order to conduct the experimental part of the study, 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 was measured for 6 feeding contents in 3 repeats at 3 separate periods. The volume of gas produced after 2, 4, 6, 8, 10, 12, 16, 24, 36, 48 and 72 hours incubation was measured and checked against two models estimating gas production parameters and ruminal fermentation kinetics.

Results: The amounts of gas production potential and the rate constant gas production according to both models, EXP and FRC, was not significantly different. However, the two models differ significantly regarding the length of the lag phase (T lag) which is significantly longer in the model EXP, than in the model FRC; due to model EXP substantially overestimating the actual time-lags.

Conclusion: The sigmoid model FRC, proposed by France *et al.*, appears providing more relevant estimates than does the exponential model EXP by Ørskov & McDonald, at least regarding the duration of the lag phase before starting of the fermentation process. Accordingly, it seems that the sigmoid FRC model should be preferred over the exponential EXP model.

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15 **Keywords:** *In vitro* fermentation, Mathematical models, Protein sources.
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18 1. INTRODUCTION

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20 Gas production *in vitro*, is related to fermentation parameters, and rumen digestion kinetics
21 are valuable descriptions in the evaluation feeds [1]. In this *in vitro* gas production
22 fermentation, a certain amount of feed in the rumen fluid was incubated and the volume of
23 gas produced at regular intervals and row that showed the speed of feed digestion is
24 measured. The results of the tests is described mainly by fitting them into two models of EXP
25 and FRC is done [2]. Therefore, comparing the performance and capability of two models
26 can highly be influential model for choice. Some of the differences between the two models
27 may be related to the test conditions and the type of feed. Some of models, like the model
28 France sigmoid structure have established that due to the use of this structure; the presence
29 of microbial activity in the rumen has been reported [3]. But some other of models like the
30 model of Ørskov and McDonald have non-Sigmoid structure. So today, for greater reliability
31 of gas production test results by the researchers, a variety of models non- Sigmoid and
32 Sigmoid structure is used and in this regard, various formulas have been proposed [4,5]. In
33 most studies related to rumen fermentation parameters by *in vitro* gas production of the
34 exponential equation Ørskov and McDonald [9] as (EXP) $y=A(1-e^{-ct})$ is used. Ørskov and
35 McDonald model is one of the most well-known models used-in predicting rumen
36 fermentation parameters. This model assumes that the rate of gas production in the rumen
37 depends only on the availability of feed has been reported [6]. Another model that is used to
38 predict gas production, is the model of France (FRC). As mentioned, France model had
39 sigmoid structure and great flexibility in fitting the data of gas production. France model
40 assumes that the rate of gas production is directly linked to the rate of feed degradation and
41 this condition is dependent on fermentation time and time identification or adherence of
42 bacteria to feed components (lag phase) [2]. In addition, there are models that have been
43 proposed by other researchers but have received little attention. However, you need to
44 expantiate on the statement for better understanding of the concept [7]. According to the
45 comprehensive comparison between the two models of France and Ørskov and McDonald
46 for described ruminal fermentation parameters plant and animal some protein sources using
47 gas test method and since the evaluation tests of feed has been done more than alfalfa hay
48 as a standard feed and with important in ruminant nutrition. Therefore, in this study the
49 accuracy of the proposed methods in terms of goodness of fit and to describe the ruminal
50 fermentation parameters in some plant and animal protein sources evaluated using the gas
51 production method.

52 2. MATERIALS AND METHODS

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54 In order to conduct the experiment, sources of plant protein (soybean meal, Rapeseed meal
55 and cottonseed meal) and sources of animal protein (poultry offal meal, fish meal and blood
56 meal) were obtained from the agricultural sector and the local slaughterhouse. The chemical
57 composition of the feed by conventional methods [8] was carried out. The *in vitro* method [1]
58 was used to measure the amount of produced gas in laboratory conditions and the amount
59 of gas production measured and recorded at 2, 4, 6, 8, 10, 12, 16, 24, 36, 48 and 72 hours
60 of incubation, respectively. In this study, the different mathematical models have been
61 developed to analyze gas production data by two models of digestion by France et al [2] and
62 Ørskov and McDonald [9] with regard to the lag phase was used to evaluate the digestive
63 process. For this purpose of 54 series data obtained from the tests (three separate periods
64 with 3 repeat and 3 levels of feed and 2 feed per period) for fitted data's and T-test was used
65 to compare their means for each parameter of the two models.

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67 Models include:

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69 Ørskov and McDonald model [9] with regard to the lag phase

70 $G=A(1-e^{-ct+L})$

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72 Model France et al. [2]

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74 $G = A(1-e-c(t-L)-d(\sqrt{t}-\sqrt{L}))$

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76 Where G is equal to the accumulation of gas produced per unit time, A is equal to the total
 77 amount of gas produced (ml), c is equal to a fixed rate of gas production (ml per hour), d is
 78 equal to a fixed rate of gas production (ml at ½ h), L equal to the lag phase, t time and t ½
 79 equal to half of the total gas production time is cumulative.

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81 3. RESULTS AND DISCUSSION

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83 3.1 CHEMICAL COMPOSITION

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

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

Protein sources	DM	CP	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						
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 (%)

98 However, when the individual protein sources were fitted in terms of the two models of
 99 France and Ørskov and McDonald, it was observed that rapeseed meal had a significant
 100 difference in gas production rate. Only the two models had a significant difference in terms of
 101 the lag time (T lag) except for cotton seed meal ($P < 0.05$). According to the results of the
 102 tables, T lag was higher in the Ørskov and McDonald's model than the France model. T lag
 103 or the time colony production is an important parameter that is associated with feed fibre
 104 degradability [10]. There was less time to start the colony by the France model for all plant
 105 and animal protein sources. The lag phase for France was 0.44 hours as against 1.96 hours
 106 for the Ørskov and McDonald Model observed as shown in Table 2. The longer lag phase for
 107 all protein sources in the Ørskov and McDonald model indicated that in this model,
 108 microorganisms were observed to have started to recognize and colonize on the digestible
 109 substrate in a delayed and time-consuming behaviour compared to the France model.
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Table 2. Comparison of two models (France and Ørskov and McDonald) based on the estimated parameters between the plant and animal protein sources

		Model		
	Parameters	France	Ørskov and McDonald	P value for T-test
Plant and animal protein sources	A	133.41	131.79	0.93
	c	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)

112 It is desirable to reduce the production time of the colony for a fermentable substrate and
 113 easily fermented, and especially for samples containing fiber and cell wall and certain
 114 physicochemical characteristics in the cell wall. Among the studied protein sources, cotton
 115 seed meal had the lowest T lag (Table 7) in both models. However, other sources of plant
 116 and animal protein in this study, despite their high fibre and cell wall structure (NDF) had
 117 less T lag than that of cottonseed meal but the two models in the T lag have shown
 118 significantly different values for the protein sources. In this comparison, the France model
 119 has the lowest lag phase for either plant or animal protein sources ($P < 0.05$).
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Table 3. Comparison of two models (France and Ørskov and McDonald) based on the estimated parameters between the plant and animal protein sources

		Model		
	Parameters	France	Ørskov and McDonald	P value for T-test
	A	204.74	202.09	0.90
	c	0.06	0.05	0.27

Plant protein	T lag	0.37	1.48	0.002
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*A = potential gas production (ml) c = constant rate gas production (ml per hour) T lag = lag phase (hours)

121 This shows that the Ørskov and McDonald model could have an overestimate for lag phase.
 122 Therefore, it can be concluded that the French model estimates less lag phase for sources
 123 of protein with less fibre. Reis, Sidnei Tavares Dos, et al., [11] stated that the correlation
 124 between the cumulative production phase and the total carbohydrate degradation is strong
 125 and high, but some differences in this relation could be due to the model used for the
 126 analysis.

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Table 4. Comparison of two models (France and Ørskov and McDonald) based on the estimated parameters between the plant and animal protein sources

		Model		
	Parameters	France	Ørskov and McDonald	P value for T-test
Animal protein	A	62.08	61.49	0.96
	c	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)

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

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Table 5. Comparison of France and Ørskov and McDonald models based on the estimated potential gas production parameters of the individual protein sources

		Model		
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Source protein	France	Ørskov and McDonald	P value For T-test
	A	A	
Soybean meal	287.04	287.48	0.96
Rapeseed meal	215.99	219.68	0.79
Cottonseed meal	111.16	99.12	0.28
poultry offal meal	118.33	117.75	0.95
Fish meal	38.12	37.67	0.94
Blood meal	29.78	29.03	0.81

*A = potential gas production (ml)

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Table 6. Comparison of France and Ørskov and McDonald models based on the estimated constant rate gas production parameters of the individual protein sources

Source protein	Model		P value For T-test
	France	Ørskov and McDonald	
	c	c	
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

*c = constant rate gas production (ml per hour)

Table 7. Comparison of France and Ørskov and McDonald models based on the estimated lag phase parameters of the individual protein sources

Source protein	Model		P value For T-test
	France	Ørskov and McDonald	
Soybean meal	T lag 0.34	T lag 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

*T lag = lag phase (hours)

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4. CONCLUSION

According to the goodness-of-fit tests, the two compared models differ substantially from each other, in particular regarding the estimation of the time-lag preceding the fermentation process. Namely, the sigmoid model FRC, proposed by France et al., appears providing more relevant estimates, in this respect, than does the exponential model EXP by Ørskov & McDonald. For this reason, the sigmoid model FRC should arguably be preferred over the exponential model.

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