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Model Comparison to Interpret the Kinetics of *in vitro* Gas Production with Bovine Excreta Used as Inoculum

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ABSTRACT

New models to help interpret the kinetics of *in vitro* gas production with bovine excreta used as inoculum were suggested. Samples of *L. leucocephala*, *G. sepium* and *P. maximum*, with different gas production profiles, were used. The samples were submitted to the procedure for gas production and the volumes were determined every 3 h, in the first 30 h; then at 36; 48; 72 and 96 h. Five models were compared (two monophase and three biphase), and the parameters for better adjustment were determined. Mean squared error and the Durbin-Watson test were used as comparison criteria. It was concluded that the monophase models fall short to describe the kinetics of *in vitro* gas production with bovine excreta; whereas the biphase models with simple exponential components are useful. The biphase equation, where V = 0 for t < L and V = B * (1 – EXP (- C * (t - L))) for t ≥ L classified as the most useful work is done with syringes.

Key Words: in vitro *gas, excreta, models, better adjustment*

INTRODUCTION

In order to have gas production profiles suitable to predict animal behavior toward a particular food, a great deal of work must be done to standardize the method and improve interpretation of the results, instead of emulating *in vivo* environments (Rymer *et al.*, 2005).

It occurs that the profile slope tends to zero at some point during the first fermentation stages —more often with excreta than with ruminal fluid— when there is very little or no gas production (*lag* phase), to then sharply increase and tend back to zero (Martínez *et al*, 2008). Ideally, a function would be required that can model this kind of sigmoidal movement. Model designers are challenged with finding an equation to describe the family of curves (France *et al.*, 2005). Several equations have been suggested (Ørskov and McDonald, 1979; Correa, 2004; France *et al.*, 2005). However, these models not always have parameters with a direct biological meaning, and the data from gas production must be useful —along with chemical composition of the substrate, or in vitro degradability, or both— as input for more complex models to predict rumen behavior. The purpose of this work was to suggest models that can help interpret the best possible way the kinetics of *in vitro* gas production with bovine excreta used as inoculum.

MATERIALS AND METHODS

Samples of *L. leucocephala*, *G. sepium* and *P. maximum* with different gas profiles are different, were used (Martínez, 2005).

Sample processing and gas determinations were performed according to Martínez (2005). The output volume was measured every 3 h, during the first 30 h; then, measurements were done 36; 48; 72 and 96 h following inoculation.

The models shown in table 1 were selected for evaluation, where:

L: time of the first phase or *lag* phase.

A: for models 1 and 2 there is no accurate biological meaning; it is the intersection with the ordinates. In bio exponential models, it is the gas produced for t = T.

A + B: potential of gas produced by the food (model No. 3, A = 0).

B' and C': relation with the curve shape, inflection points, etc.

As a value to minimize, and for model comparison, the residual sum of the square between the experimental and estimated values was used.

Other parameters used to contrast adjustment mildness were,

Mean squared error (or residue) (CME).

$$CME = \frac{\sum \left(e_i - \bar{e}\right)^2}{n - K}$$

Where $\bar{e}$, is the mean of *n* residuals (number of observations) and *K* is the number of parameters estimated in the model.

Durbin-Watson (DW) coefficient

$$DW = \frac{\sum_{t=2}^{n} (e_t - e_{t-1})^2}{\sum_{t=1}^{n} e_t^2}$$

Where e_t = time t residual, and e_{t-1} = time t-1 residual

Modeling in the dynamics of *in vitro* gas production, both with excreta and ruminal fluid was performed to the 3 mean values of the gas volume measured at each time.

In order to determine the best adjustment for dynamic models Microsoft Excel Solver was used. The r^2 values and estimation of standard error were calculated with the applications of the same software, also used to determine CME and DW.

RESULTS AND DISCUSSION

The results of adjustments to different models and comparisons between them are shown in tables 1 and 2. The Ørskov and McDonald (1979) model has been the one most commonly used so far by researchers at the Center for Animal Production Studies (CEDEPA), University of Camagüey, Cuba, and by several other researchers in other countries (Martínez, 2005; Hernández, 2006). The model by McDonald (1981) includes the time subtracted *lag* phase, but it is monophase like the previous one.

The model by de France *et al.* (2005) has been reported as one of the most widely used and studied for *in vitro* gas kinetics follow up, using ruminal fluid supported by pressure transductors (Aparicio *et al*., 2007). The disadvantage the model has is that it is empirical and as a result, not all its parameters make biological sense.

The model suggested by Correa (2004) is a modification to Ørskov and McDonald´s (1979), also suggested by Posada and Noguera (2007), and all its parameters make biological sense in describing the *in vitro* gas production using excreta. The bio exponential model was included from criteria presented in the literature (Rymer *et al.*, 2005; Posada and Nogueras, 2007), on the kinetics of in vitro gas production using ruminal fluid, apart from the fact that the *lag* phase is greater when excreta are used. This model assumes that during digestion of the soluble and the insoluble fractions, first order kinetics occurs.

The models of Correa (2004), France *et al.* (2005) and the bio exponential model, have a lower mean squared error (CME), which groups variability of factors that exclude the researcher, besides including the influence of the number of parameters (Posada and Nogueras, 2007). These models also have a better behavior in relation to the Durbin-Watson coefficient, and are, therefore, better in the first-order serial correlation among the residuals (Posada and Nogueras, 2007).

Both for the sum of the quadratic error (whose minimum value was to find the best adjusting parameters), and all the other indicators used to contrast adjustment mildness, the models that include more than one phase are observed to achieve a better description than those that try to explain the process with monotone curves; residual behavior observation (Fig. 1) confirms the previous assertion.

Table 3 contains a compendium of the parameters best adjusting to each of the models suggested for forage included in the experiment.

The model by de France *et al.* (2005) fails when it tries to find a value in the *lag* phase, indicating that *P. maximum* takes less time than *L. leucocephala*, which contradicts the experimental data (Fig. 2). The reason for it may be that such model has been conceived for use when there is a higher data concentration in the first phase (Van Laar *et al.*, 2006) or, simply, because it fails to describe the dynamics of excreta and their more extensive *lag* phase.

The values of the parameters achieved using models with a simple exponential phase are perfectly comparable. The adjustment to the bio exponential model demands a great deal of point concentration in the first hours, which is difficult to achieve if no automation is applied. The limitation of Correa´s (2004) model is that in the *lag* phase no gas is produced, which disables it for wider food kinetics with increased soluble fraction, quickly digestible, as in the case of highly digestible concentrates and forage. However, it could be very useful for forage with a small soluble fraction and does not need so many mea-

surements in the first hours, as does the bio exponential model. For *in vitro* gas from excreta or ruminal fluid, only the A values from the exponential biphase model make biological sense. It would also be the volume at the end of the *lag* phase, basically produced by the soluble fraction of the food, which was demonstrated in four digestive contrasting forages (Pedraza, 1998).

Values A + B obtained with the monophase models (with or without the lag phase inclusion) do not seem to offer the real value of the food. For instance, according to these models, *P. maximum* has a much greater potential than *L. leucocephala* and *G. sepium*, which contrasts with previous results (Keir *et al.*, 1997; Pedraza, 1998 and La O, 2001), and the predictions made with the other models.

The disadvantage lies in that the models by Ørskov and McDonald (1979) and McDonald (1981) do not include inflection points, and they could hardly describe the dynamics in in vitro gas production using excreta as inoculum, which tend to show a solid inflection point at the end of the first phase (Martínez *et al*, 2008), which is longer when ruminal fluid is used.

When comparing the values of these parameters for the different models studied, the Ørskov and McDonald (1979), and McDonald (1981) were observed to be equal and different from the rest, confirming the assertions made above.

The graph (Fig. 2) shows the cause of the result achieved previously, especially in its first phase (before quick gas production). The models without a component to recognize the sigmoidal shape of the curve are unable to describe this phase, which is steeper when excreta are used, even when compared with substrates that are hard to colonize, as in the case of *P. maximum*.

The models should correspond with the objective set for analysis, and the laboratory equipment availability. There is no single method to choose among models (Jay and Torres, 2007; Posada and Nogueras, 2007), as no model can be authentically universal.

CONCLUSIONS

Monophase models are unable to correctly describe the kinetics of *in vitro* gas production using excreta.

Biphase models with a simple exponential component are useful to represent the behavior of *in vitro* gas production kinetics using excreta as inoculum.

The biphase equation where V = 0 for t < L and V = B * (1 - EXP (- C * (t - L))) for t ≥ L is the most functional of all, if syringes are used.

REFERENCES

APARICIO, R.; GONZÁLEZ-RONQUILLO, M.; TORRES, R.; ASTUDILLO, L.; CORDOBA, L. y CARRASQUEL, J. (2007). Degradabilidad de los pastos lambedora (*Leersia hexandra*) y paja de agua (*Hymenachne amplexicaulis*) en cuatro épocas del año de una sabana inundable del estado Apure, Venezuela. *Zootecnia Trop*., *25* (3), 225-228. Extraído en mayo de 2008, desde www.scielo.org.ve/scielo.php?script=sci_arttext&pid=S0798-72692007000300014&lng=en&nrm=iso>. ISSN 0798-7269.

CORREA, H. J. (2004). RUMENAL: Procedimiento para estimar los parámetros de cinética ruminal mediante la función Solver de Microsoft Excel. *Rev. Col. Cienc. Pec.*, *17* (3), 2004.

FRANCE, J.; LOPEZ, S.; KEBREA, E.; BANNINK, A.; DHANOA, M. S. y DIJKSTRA, J. (2005). A General Compartmental Model for Interpreting Gas Production Profiles. *Animal Feed Science and Technology*, *123* (2), 473-485.

HERNÁNDEZ, J. E. (2006). *Valoración de la caprinocultura en la Mixteca Poblana: socioeconomía y recursos arbóreo-arbustivos*. Tesis de doctorado en Ciencias Veterinarias, Universidad de Camagüey, Cuba.

JAY, O. y TORRES, V. (2007). *Bondad de ajuste y supuestos básicos en la modelación de la degradación ruminal de forraje de CT-115*. II Congreso Internacional de Producción Animal Tropical, ICA, La Habana.

KEIR, B.; LAI, N. V.; PRESTON, T. R. y ØRSKOV, E. R. (1997). Nutritive Value of Leaves from Tropical Trees and Shrubs: 1. *In vitro* Gas Production and *in sacco* Rumen Degradability. *Livestock Research for Rural Development*, *9* (4).

LA O, O. (2001). *Contribución al estudio del valor nutritivo de diferentes ecotipos del género* Leucaena *para la alimentación de rumiantes*. Tesis de doctorado en Ciencias Veterinarias, Instituto de Ciencia Animal, Universidad Agraria de La Habana, Cuba.

MARTÍNEZ, S. J.; PEDRAZA, R. M.; RESÍLLEZ, A.; GUEVARA, G.; GONZÁLEZ, CECILIA y LEÓN, MARLENE (2008). Correlación entre la degradabilidad ruminal *in situ* y la producción de gas *in vitro* con el uso de heces vacunas depuestas como inóculo. *Rev. prod. anim., 20* (2), 110-114.

MARTÍNEZ, S. J. (2005). *Implementación de la técnica de producción de gas* in vitro *con heces vacunas*

como inóculo y su empleo para evaluar el follaje de algunas leguminosas arbustivas. Tesis de maestría en Producción Bovina Sostenible, Facultad de Ciencias Agropecuarias, Universidad de Camagüey, Cuba.

MCDONALD, I. (1981). A Revised Model for the Estimation of Protein Degradability in the Rumen. *J. Sci. Agric. Camb*., *96*, 251-252.

ØRSKOV, E. R. y MCDONALD, I. (1979). The Estimation of Protein Degradability in the Rumen from Incubation Measurements Weighted According to Rate of Passage. *Journal of Agricultural Science*, 92, 499-503.

PEDRAZA, R. M. (1998). Use of *in vitro* Gas Production Technique to Assess the Contribution of Both Soluble and Insoluble Fractions on the Nutritive Value of Forages. Tesis de maestría, Universidad de Aberdeen, Escocia.

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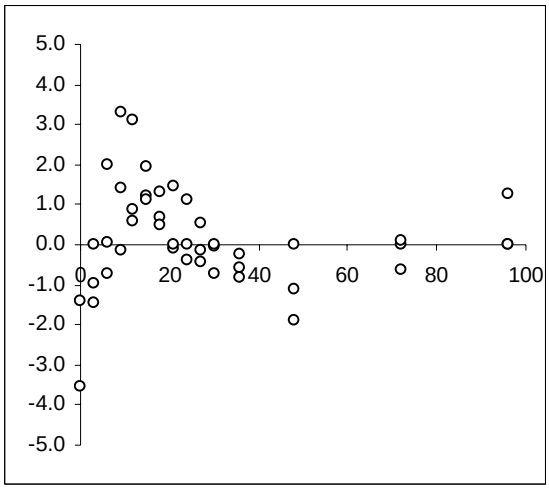
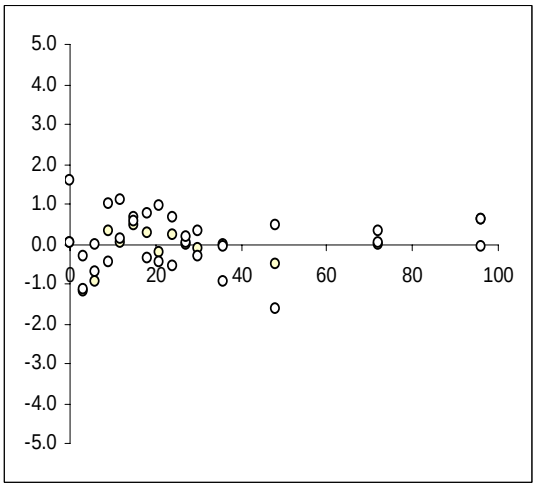
POSADA, S. L. y NOGUERA, R. R. (2007). Comparación de modelos matemáticos: una aplicación en la evaluación de alimentos para animales. *Revista Colombiana de Ciencias Pecuarias*. Extraído en julio de 2007, desde http://rccp.udea.edu.co/v_actual/.

RYMER, C.; HUNTINGTON, J. A.; WILLIAMS, B. A. y GIVENS, D. I. (2005). *In vitro* Cumulative Gas Production Techniques: History, Methodological Considerations and Challenges. *Animal Feed Science and Technology*, *25* (2), 9-30.

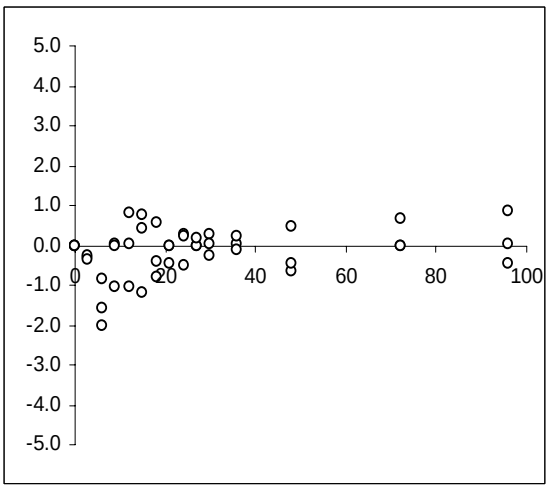
VAN LAAR, H.; VAN STRAALEN, W. M.; VAN GELDER, A. H.; DE BOEVER, J. L.; D'HEER, B.; VEDDER, H.; KROES, R.; DE BOT, P.; VAN HEES, J. y CONEB, J. W. (2006). Repeatability and Reproducibility of an Automated Gas Production Technique. *Animal Feed Science and Technology*, *27* (2), 133-150.



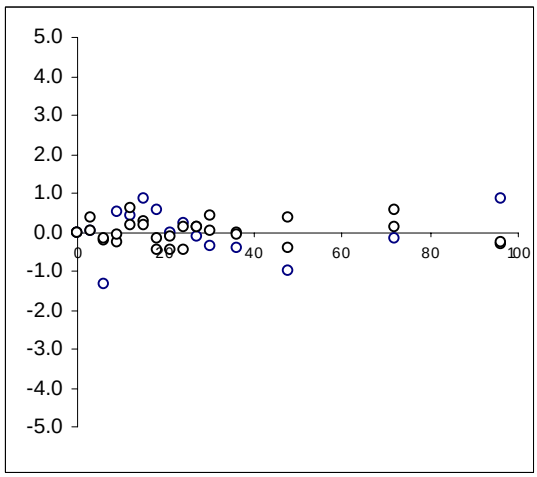
Ørskov and McDonald (1979)



France *et al.* (2005)



Correa (2004)



Bio exponential

The model suggested by McDonald (1981) behaves identically to Ørskov and McDonald´s (1979) concerning residuals

Fig. 1. Residuals of the four models studied

Table 1. Models studied for better adjustment of data collected from *in vitro* gas production with bovine excreta

	Name	Model	Reference
1	Ørskov and McDonald (1979)	$V = A + B(1-EXP(-Ct))$	Ørskov and McDonald, (1979)
2	McDonald (1979)	$V = A + B(1-EXP(-C(t-L)))$	McDonald (1981)
3	Correa (2004)	$V = 0$ si $t < L$ $V = B(1-EXP(-Ct))$ si $t \geq L$	Correa (2004)
4	France (2005)	$V = B\ (1 - EXP(-B'(t-L)-C'(\sqrt{t} - \sqrt{L}\ ))$	France *et al.* (2005)
5	Bio exponential	$V = B_1\ (1-EXP(-C_1t))$ si $t \leq L$ $V = A + B\ (1-EXP(-C(t-L)))$ si $t > L$ In that case, $A = B_1(1-EXP(-C_1L))$	Suggested by Posada and Nogueras (2007)

Table 2. Model comparison to adjust dynamics data from in vitro gas production using excreta

	Model	Sum of squared error	r^2	Mean squared error	Durbin-Watson
1	Ørskov-McDonald (1979)	26.55[a]	0.9754	2.21	0.554
2	McDonald (1981)	26.55[a]	0.9754	2.21	0.554
3	Correa (2004)	6.65[b]	0.9938	0.55	1.174
4	France (2005)	7.80[b]	0.9898	0.71	1.092
5	Bio exponential	2.99[b]	0.9977	0.30	1.677
	ES	1.90			
	Significance	<0.01			

Different exponents indicate a significant difference for the sum of the squared error

Table 3. Some gas production parameters for the different models, and forage studied

Model	Forage	A (ml)	B (ml)	A+B (ml)	C (h^{-1})	L (h)
Ørskov-McDonald (1979)	*L. leucocephala*	-3.1	32.9	29.8	0.034	
	G. sepium	-3.2	40.9	37.6	0.033	
	P. maximum	-1.2	61.0	59.9	0.004	
McDonald (1981)	*L. leucocephala*	0.1	29.9	30.0	0.034	2.80
	G. sepium	3.5	34.5	38.0	0.033	5.08
	P. maximum	0.4	59.5	59.9	0.004	6.83
Correa (2004)	*L. leucocephala*		27.9	27.9	0.051	6.68
	G. sepium		36.2	36.2	0.041	4.68
	P. maximum		20.3	20.3	0.020	13.82
France (2005)	*L. leucocephala*		28.0	28.0	0.062	4.20
	G. sepium		35.6	35.6	0.055	3.35
	P. maximum		24.8	24.8	0.017	3.73
Biexponential	*L. leucocephala*	2.5	25.5	28.0	0.045	8.39
	G. sepium	4.1	31.8	35.9	0.051	8.28
	P. maximum	1.2	16.7	18.3	0.023	19.75

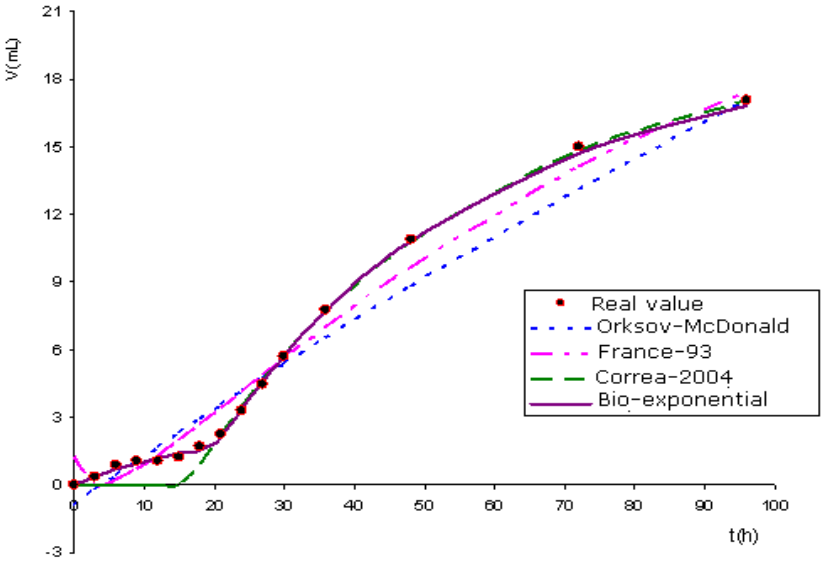


Fig. 2. Model comparison using *P. maximum*