Objective Bayesian vs. least squares estimation for by-products degradability with different rumen fluids

Antonio Martínez-Teruel[1], Maria Dolores Megías[1], Fuensanta Hernández[1], Josefa Madrid[1], Diego Salmerón[2], and Juan Antonio Cano[3]

[1]Departamento de Producción Animal, Facultad de Veterinaria, Universidad de Murcia, Campus Espinardo, 30071, Murcia, Spain; [2]Murcia Health Council, Epidemiology, CIBER Epidemiología y Salud Pública, (CIBERESP), 30008, Murcia, Spain; and [3]Departamento de Estadística e I.O., Facultad de Matemáticas, Universidad de Murcia, Campus Espinardo, 30071, Murcia, Spain. Received 10 September 2008, accepted 4 March 2009.

Martínez-Teruel, A., Megías, M. D., Hernández, F., Madrid, J., Salmerón, D. and Cano, J. A. 2009. **Objective Bayesian vs. least squares estimation for by-products degradability with different rumen fluids**. Can. J. Anim. Sci. **89**: 273–277. The degradation kinetic curves of different by-products have been obtained. The considered by-products were lemon and several types of treated and untreated barley straw, and they were degraded by in vitro incubation with rumen fluid extracted from two herds of Murciano-Granadina goats, one of them fed alfalfa hay and the other one fed barley straw. The feeds were incubated at 39°C for 12, 24, 36, 48 and 72 hours with each rumen fluid. The resulting fitted exponential-type degradation curves obtained with a frequentist statistical analysis were compared with those resulting from an objective Bayesian statistical analysis. The use of the objective Bayesian analysis smoothed the estimates of the frequentist fit using least squares, which did not suitably process the involved restrictions and avoided biologically unacceptable results. On the other hand, the rumen fluid from goats fed alfalfa hay fomented the greatest effective degradability and the degradabilities of the different by-products were also compared, with the result that the lemon by-product was the best degraded one under both statistical analyses.

Key words: In vitro fermentation, by-product, degradation curve

Martínez-Teruel, A., Megías, M. D., Hernández, F., Madrid, J., Salmerón, D. et Cano, J. A. 2009. **Estimation Bayesienne objectif vs moindres carrés pour la dégradation de sous-produits avec différents fluides ruminales.** Can. J. Anim. Sci. **89**: 273–277. Les courbes cinétiques de dégradation de différents sous-produits ont été obtenues. Les sous-produits considérés sont du citron et plusieurs types de la paille d'orge traitée et non traitée, et ils ont été degradés par incubation in vitro avec du fluide de panse extrait de deux troupeaux de chèvres Murcia-Grenadines. Un de ces troupeaux fut nourri avec du foin de luzerne, alors que le deuxième fut nourri avec de la paille d'orge. Les fourrages furent incubés à 39°C pendant 12, 24, 36, 48 and 72 heures avec chaque type de fluide de panse. Les courbes de dégradation associées, type exponentiel, qui furent obtenues par une analyse statistique classique furent comparées à celles obtenues par une analyse statistique Bayésienne objectif. L'analyse Bayésienne objectif nous permis d'une part de surmonter les difficultés associées à une optimisation par moindres carrés qui ne tient pas en compte avec precision les restrictions naturelles des paramètres, et d'autre part d'éviter les résultats biologiquement non admissibles. De même, le fluide de panse de chèvres nourries avec du foin de luzerne fomenté le plus efficace dégradabilité et la degradabilité des différents sous-produits ont également été comparés en resulte que le citron sous-produit est le meilleur degradées dans les deux analyses statistiques.

Mots clés: Fermentation in vitro, sous-produits, courbe de dégradation

Ruminant feed contains ingredients that are either indigestible or potentially digestible. The latter is degraded in the rumen by microorganisms at a given rate that depends on the speed with which it passes through the rumen (Mertens 1973; Orskov and McDonald 1979). The degradation kinetics is widely used to estimate the digestibility of feed in the rumen because it provides insight into their quality and nutritional characteristics.

The use of mathematical models to describe degradation curves provides information on the ruminal digestion of feedstuff. Several types of feed degradation curves have been described as a function of time (Mertens 1973; Orskov and McDonald 1979). In this work the percentage of feed degraded up to time t, $y(t)$ is modelled with a non-linearizable curve of the type $y = a + b\ (1 - e^{-ct})$ (Orskov and McDonald 1979), where a is the percentage of soluble feed, b is the percentage of insoluble, potentially degradable feed and c controls the degradation velocity of the fraction b. Of course, there are more recent references on degradation curves, such as Dhanoa et al. (2004) and Fathi Nasri et al. (2006). The latter considers extensions of the Orskov and McDonald model allowing for lagged versions. Here, the model without the lag term is good from both perspectives in terms of their corresponding errors and

Abbreviations: **AHRF**, alfalfa hay rumen fluid; **BSRF**, barley straw rumen fluid; **DM**, dry matter; **LB**, lemon by-product

deviations, and because of this we decided to use it; nevertheless, the presence or absence of a lag term cannot be determined from this experiment, in which the earliest sampling time point is at 12 h. since a model without a lag term can fit the data of a degradation profile possessing a lag term perfectly if the sampling time points are not chosen around the lag time. Note that to carry out Bayesian inference in the model with the lag term, a similar theoretical study to that in Cano and Salmerón (2007) would be necessary to determine the prior objective and the Bayesian inferences for this model. For these reasons we have limited ourselves to analyse this model. We analyse this model from the two current statistical methodologies, the frequentist and the Bayesian ones. The frequentist approach considers the parameters as unknown constants, and their uncertainty is stated in terms of the typical confidence intervals and least squares estimators and their standard errors. In the Bayesian approach, parameters are treated as random variables so that a prior distribution is assigned to them and their uncertainty is stated in terms of their posterior distribution, which is obtained according to the rules of computation of probabilities and from which all inferences are to be made, including credible intervals, point estimators and their Bayesian standard errors. The point estimates we use here are the posterior means as is usual in Bayesian analysis, since they minimize the posterior variance and their associated error is the standard deviation of the posterior distribution.

One of the main difficulties of using this model is that, due to the biological meaning of the parameters, they have to necessarily satisfy the following natural restrictions $a \geq 0$, $b \geq 0$, $(a+b) \leq 100$ and $c \geq 0$ and fitting this type of curve can comes up against the limitations of the commercial frequentist statistics packages that use the least squares method, some of which do not allow restrictions to be established in the parameters of the curves, thus hindering the estimation of admissible regression curves. These packages sometimes produce negative values of a, which, according to Naranjo et al. (2005), are unacceptable from a biological point of view because they suppose the existence of negative pre-fermentation times. McDonald (1981) argued that it makes no biological sense that the values of b, the slowly degradable fraction, or $(a+b)$, the potential degradation, be larger than 100. However, as demonstrated by Cano and Salmerón (2007), Bayesian analysis avoids these disadvantages automatically since the non admissible values for the parameters are excluded in the prior distribution, and consequently they do not appear in the posterior distribution. To learn about the advantages of the Bayesian analysis in a general biological setting see Blasco (2001). Note that theoretically with optimally designed sampling time points, the boundary constraints will not be needed and biologically reasonable estimates will be made because the input data will represent the biological process under investigation, although a large number of sampling time points could be needed.

The objective of this work was to study the degradability of untreated and treated barley straw and of a by-product of the lemon processing industry when they were incubated in vitro with rumen fluid extracted from two herds of goats fed with alfalfa hay and barley straw using the two current statistical methods, the frequentist and the Bayesian ones. The statistical Bayesian analysis we have carried out is an objective analysis that starts with an objective model dependent prior avoiding the arbitrariness of prior selection.

MATERIALS AND METHODS

Five by-products were used in the experiment: a by-product from the lemon processing industry (LB), and four by-products derived from the barley straw: untreated barley straw, urea-supplemented barley straw, urea-treated barley straw, and barley straw treated with both urea and NaOH, all dried at 60°C and ground through a 1-mm screen. Two types of rumen fluid, alfalfa hay rumen fluid (AHRF) and barley straw rumen fluid (BSRF), were used, which were obtained from animals of two herds of Murciano-Granadina goats fed alfalfa hay and barley straw ad libitum, respectively. The guidelines of the Animal Wellbeing Committee of the University of Murcia were followed. Fourteen days were allowed to pass for the animals to adapt to the diet before the rumen fluid samples were taken. Three goats per diet were sampled and the rumen liquor was collected from the dorsal sac of the rumen. The rumen fluids were combined per diet. Each one of the resulting rumen fluids mixtures was maintained in anaerobic conditions at 39°C and quickly used as fermentation inocula. The collection of rumen fluids and the full detailed in vitro procedure is described in Madrid et al. (2002), where it is stated that duplicate bottles were incubated in a 39°C shaking water bath for 12, 24, 36, 48 and 72 h. After incubation, the non-degraded residue was obtained by filtering, drying and weighing. The dry matter (DM) that had been digested was calculated as the difference between the starting material and the resulting residue. Table 1 shows the values obtained for each by-product, type of rumen fluid and incubation time, which were used to obtain the degradation curves; there are two data points per time point, and numbers in Table 1 are the mean of these two data points. In summary, we have an experimental design with two factors, type of rumen fluid and type of by-product, and a covariate, time. However, the dependence of the response variable, percentage of degraded feed, is not linear with respect to the covariate, and standard techniques of linear models like a factorial design analysis cannot be applied here. Therefore, we have to study the evolution of the response variable for each combination of type of rumen fluid and type of by-product by fitting a non linear curve of the type $y = a + b(1 - e^{-ct})$. These curves were fitted using both statistical methodologies, the frequentist and the Bayesian ones. However, as we wanted to study the degradability of

Table 1. Average degraded % DM for different by-products at different incubation times when rumen fluid is used from goats fed with alfalfa hay or barley straw

	12 h Mean ± SEM[z]	24 h Mean ± SEM	36 h Mean ± SEM	48 h Mean ± SEM	72 h Mean ± SEM
Rumen fluid (alfalfa hay)					
Lemon by-product	45.3 ± 1.41	69.3 ± 0.21	79.2 ± 3.19	75.5 ± 0.69	78.0 ± 4.24
Untreated barley straw	11.8 ± 0.15	29.1 ± 0.84	39.9 ± 1.21	41.0 ± 2.03	50.9 ± 1.31
Urea-supplemented barley straw	11.9 ± 0.10	33.8 ± 2.47	40.6 ± 0.13	41.4 ± 1.76	47.4 ± 2.05
Urea-treated barley straw	15.7 ± 2.45	32.3 ± 2.83	43.6 ± 2.00	45.9 ± 0.84	54.1 ± 2.80
Urea + NaOH treated barley straw	15.6 ± 1.50	36.6 ± 1.38	49.3 ± 2.17	54.0 ± 1.88	55.9 ± 1.61
Rumen fluid (barley straw)					
Lemon by-product	29.6 ± 0.02	31.2 ± 1.00	49.2 ± 3.31	62.9 ± 0.51	71.7 ± 0.48
Untreated barley straw	13.9 ± 0.03	15.9 ± 0.66	20.2 ± 0.22	25.1 ± 0.50	31.4 ± 0.23
Urea − supplemented barley straw	18.7 ± 0.04	23.3 ± 0.40	32.8 ± 1.19	33.6 ± 2.22	38.6 ± 2.06
Urea − treated barley straw	21.7 ± 0.58	24.4 ± 0.21	38.7 ± 0.34	39.1 ± 0.10	45.1 ± 0.20
Urea + NaOH treated barley straw	16.7 ± 0.09	27.9 ± 0.98	45.2 ± 0.53	54.7 ± 0.05	58.0 ± 0.86

[z]SEM, standard error of the mean.

feed and not individual time responses, once the degradation curves were obtained we used the parameter estimates and the potential and effective degradability as response variables about which to make inferences according to a factorial design, because now the assumptions to carry out a factorial analysis were satisfied.

The data were first analysed by least squares non linear regression. To prevent the results from being biologically illogical, the following restrictions were established for the parameters: $a \geq 0$, $b \geq 0$, $(a+b) \leq 100$ and $c \geq 0$. The objective Bayesian statistical analysis designed for this type of curve in Cano and Salmerón (2007) was also used, wherein a non informative prior distribution was given to the parameters to compute the posterior distribution. Both statistical analyses were done with the aid of the program Mathematica, version 4.0 for Windows (2000) copyright© Wolfram Research, Inc. The computation of the constrained least squares estimates was carried out by simulating 50 million values for the parameters in the admissible region and minimizing the sum of square errors and their standard errors were computed using a parametric bootstrap [see Efron and Tibshirani (1993)], while to obtain the Bayesian estimates the simulation techniques stated in Cano and Salmerón (2007) were used. A few lines program that are available from the authors were needed in each case.

RESULTS AND DISCUSSION

Frequentist versus Bayesian Estimations

Note that computing the constrained least squares estimates in the way mentioned above improved the results previously obtained using commercial statistics packages yielding a bigger R^2 and smaller standard errors (SE) and root mean square errors (RMSE). Table 2 shows the degradation kinetic values obtained using constrained least squares estimation along with their standard errors, R^2 and root mean square errors and the potential and the effective degradability. In all cases the soluble fraction a was non negative, while the potentially degradable fraction $(a+b)$ was less than or equal to 100, leading to models with an R^2 greater than 0.91. However, several values of a were 0 and two values of $(a+b)$ were 100 meaning they are extreme and therefore not smoothed estimations.

Table 3 shows the values of the parameters and their standard deviations obtained with the Bayesian statistical analysis developed in Cano and Salmerón (2007) with the same restrictions that were used in the least squares analysis. In this case, the values of the soluble fraction a are bigger than their corresponding values in Table 2 when they are 0 and similar in the other cases, the greater value corresponding to the incubation of LB with AHRF (10.97%); that said, and taking into consideration that errors are similar for both statistical procedures, we conclude that Bayesian estimates are preferable, since it is sensible to assume that a small percentage of soluble feed is always going to be present. For the sake of brevity, confidence and credible intervals have not been included. Credible intervals can be computed using the percentiles of the posterior distribution and they are entirely contained in the parametric space providing similar conclusions to that obtained from the estimates. No potential degradability value was close to 100. In other words, as stated by Cano and Salmerón (2007), the use of Bayesian analysis avoid the inconveniences described above smoothing the values of the fraction a and the potential degradability $(a+b)$ when they are extreme. Likewise, the Bayesian statistical analysis provides a value that cannot be obtained with the least squares method; the probability, $P(a+b)$, that one by-product will be degraded more than any other for each inocolum. Table 3 shows that the probability that the lemon by-product is more degradable is 0.705 and 0.603 (AHRF and BSRF, respectively), which is by

Table 2. a, b and c values fitted to the equation $y(t) = a + b(1 - e^{-ct})$ to predict degradability when rumen fluid from goats fed different diets is used. Constrained least squares analysis

	a (%)		b (%)		c (h^{-1})					
	Estimate	SE	Estimate	SE	Estimate	SE[w]	Deg P[z] (%)	Deg E[y] (%)	R^2	RMSE[x]
Rumen fluid (alfalfa hay)										
Lemon by-product	0.00	8.79	79.87	7.98	0.077	0.012	79.87	44.91	0.91	3.73
Untreated barley straw	0.00	2.73	61.12	8.39	0.025	0.006	61.12	18.08	0.95	2.90
Urea − supplemented barley straw	0.00	5.29	50.40	6.07	0.040	0.010	50.40	20.26	0.89	3.49
Urea − treated barley straw	0.00	2.80	62.54	7.37	0.029	0.006	62.54	20.27	0.96	2.68
Urea + NaOH treated barley straw	0.00	5.15	62.52	6.04	0.037	0.008	62.52	24.02	0.92	3.54
Rumen fluid (barley straw)										
Lemon by-product	9.30	5.02	90.70	7.66	0.016	0.005	100.00	28.69	0.93	4.58
Untreated barley straw	8.76	0.89	91.24	18.83	0.004	0.003	100.00	14.38	0.98	0.88
Urea-supplemented barley straw	8.10	4.01	35.32	7.41	0.028	0.011	43.42	19.35	0.92	2.04
Urea-treated barley straw	9.26	4.71	44.11	11.12	0.024	0.010	53.36	21.91	0.92	2.65
Urea + NaOH treated barley straw	0.00	2.94	74.60	8.96	0.023	0.005	74.60	20.95	0.96	3.35

[z]Deg P = potential degradability: $(a+b)$.
[y]Deg E = effective degradability: $a+(bc/(c+k))$, with $k = 0.06\ h^{-1}$.
[x]RMSE = root mean square error.
[w]SE = standard error.

far higher than that of the other by-products. This value, then, permits the level of potential degradability of the by-products studied to be quantitatively compared, since for each inocolum the corresponding probabilities sum up to 1. The probabilities $P(a+b)$ were computed by simulation using the techniques in Cano and Salmerón (2007).

Table 4 shows the results of the ANOVA that has been carried out for the factorial design considering the parameter estimates and the potential and effective degradability as response variables and inoculum, by-products and statistical procedures as factors from where the main conclusions are to be drawn. Regarding the comparison of the two statistical procedures a significance difference was found for the values of a ($P = 0.04$) reinforcing the conclusions stated above.

Effect of the Rumen Fluid Type

From Table 4 we see that no significance difference was found for the potential degradability ($P = 0.23$). However, when we studied the effective degradability, which takes into account all the kinetic parameters of degradation (a, b and c) obtained in the analysis and the rate at which the particles pass through the rumen, the values found for the samples incubated with AHRF were mostly greater than those incubated with BSRF ($P = 0.01$). Thus, effective degradability depends on the type of

Table 3. a, b and c values fitted to the equation $y(t) = a + b(1 - e^{-ct})$ to predict degradability when rumen fluid from goats fed with different diets is used. Bayesian analysis

	a (%)		b (%)		c (h^{-1})				
	Estimate	SD[w]	Estimate	SD	Estimate	SD	Deg P[z] (%)	Deg E[y] (%)	P (a+b)[x]
Rumen fluid (alfalfa hay)									
Lemon by-product	10.97	9.19	70.47	8.76	0.066	0.013	81.44	47.88	0.705
Untreated barley straw	2.84	2.63	63.62	9.73	0.022	0.006	66.46	19.91	0.093
Urea − supplemented barley straw	5.80	5.15	49.36	8.85	0.033	0.011	55.16	23.31	0.023
Urea − treated barley straw	3.82	3.27	65.00	10.11	0.024	0.007	68.82	22.39	0.133
Urea + NaOH treated barley straw	5.20	4.95	61.07	8.16	0.032	0.009	66.27	26.44	0.043
Rumen fluid (barley straw)									
Lemon by-product	7.87	5.06	80.54	9.02	0.022	0.005	88.41	29.48	0.603
Untreated barley straw	7.92	1.26	62.94	16.41	0.007	0.004	70.86	14.50	0.228
Urea − supplemented barley straw	8.14	3.98	38.37	7.69	0.028	0.011	46.51	20.35	0.004
Urea − treated barley straw	9.28	4.37	47.67	9.31	0.024	0.010	56.95	22.90	0.029
Urea + NaOH treated barley straw	3.03	2.81	75.41	9.19	0.021	0.005	78.44	22.58	0.133

[z]Deg P = potential degradability: $(a+b)$.
[y]Deg E = effective degradability: $a+(bc/(c+k))$, with $k = 0.06\ h^{-1}$.
[x]$P\ (a+b)$ = probability that one by-product will be degraded more than any other.
[w]SD = standard deviation of the posterior distribution.

Table 4. *P* values of the factorial analysis that considers the parameter estimates and the potential and effective degradability as response variables

	a (%)	*b* (%)	*c* (h^{-1})	Deg P[z] (%)	Deg E[y] (%)
Inoculum	0.01	0.75	0.00	0.23	0.01
By-product	0.24	0.00	0.03	0.00	0.00
Statistical procedure	0.04	0.43	0.64	0.86	0.36

[z]Deg P = potential degradability: $(a+b)$.
[y]Deg E = effective degradability: $a+(bc/(c+k))$, with $k = 0.06\ h^{-1}$.

inoculum used for the incubation, which would be related with the capacity of adaptation of the microorganisms to the fermentable materials. These results coincide with those of Van Soest (1994), who reported that forages rich in nitrogen and highly fermentable, like alfalfa hay, promote microorganism growth in the rumen, while poor forages, like the straw, with very lignified cell walls, yield a deficiency of energy and nitrogen in the rumen promoting lower microorganism growth.

Effect of the By-product

From Table 4 we see that highly significant differences were found for the potential ($P = 0.00$) and effective ($P = 0.00$) degradability. Tables 2 and 3 show that the largest degradabilities, both effective and potential, correspond to the lemon by-product, adding support to the above-mentioned fact that this by-product is more degradable than any other. As regards the barley straw, despite the fact the largest potential degradability corresponded mostly to the untreated material, the lowest effective degradability also corresponded to the untreated material, since the degradation rate, *c*, was substantially improved in the treated materials, mainly when the BSRF was used and there were small differences between the treated materials. These results, obtained in vitro, coincide with those found in vivo by Madrid et al. (1999) in studies in which they observed that treatment with urea treated and NaOH improved the digestibility of the barley straw DM. As the same authors noted (Madrid et al. 2002), the by-product of lemon is efficiently degraded regardless of the inoculum used, since it contains the cell material that is largely unlignified.

In summary, the type of feed given to the donor animal affects the effective degradability of the incubated by-product, in our case the inoculum from goats fed with alfalfa hay foments the greater effective degradability. Of the studied feeds, the lemon by-product showed the highest potential degradability, regardless of the inoculum used. With both inocula, the feed showing the greatest effective degradability was the lemon by-product, while the untreated straw showed the lowest effective degradability. The effect on degradability of the type of donor and the type of by-product was revealed by both statistical analyses. However, Bayesian estimations are smoother than the corresponding frequentist ones, and when these are extreme Bayesian analysis can result in biologically allowable values.

Blasco, A. 2001. The Bayesian controversy in animal breeding. J. Anim. Sci. **79**: 2023–2046.

Cano, J. A. and Salmerón, D. 2007. Objective Bayesian analysis of an exponential regression model with constrained parameters applied to animal digestibility. Commun. Stat. Theory Meth. **36**: 2463–2473.

Dhanoa, M. S., France, J., Crompton, L. A., Mauricio, R. N., Kebreab, E., Mills, J. A. N., Sanderson, R., Dijkstra, J. and López, S. 2004. Technical note: A proposed method to determine the extent of degradation of a feed in the rumen from the degradation profile obtained with the in vitro gas production technique using feces as the inoculum. J. Anim. Sci. **82**: 733–746.

Efron, B. and Tibshirani, R. J. 1993. An introduction to the bootstrap. Chapman & Hall. London, UK.

Fathi Nasri, M. H., Danes Mesgaran, M., France, J., Cant, J. P. and Kebreab, E. 2006. Evaluation of models to describe ruminal degradation kinetics from *in situ* ruminal incubation of whole soybeans. J. Dairy Sci. **89**: 3087–3095.

Madrid, J., Hernández, F., Megías, M. D. and Cid, J. M. 1999. Suplementación y tratamiento en el valor nutritivo de la paja de cebada. Digestibilidad. Cuban J. Agric. Sci. **33**: 273–281.

Madrid, J., Megías, M. D. and Hernández, F. 2002. *In vitro* determination of ruminal dry matter and cell wall degradation, and production of fermentation end – products of various by-products. Anim. Resch. **51**: 189–199.

McDonald, I. 1981. A revised model for estimation of protein degradability in the rumen. J. Agric. Sci. (Camb.) **96**: 251–252.

Mertens, D. R. 1973. Application of theorical mathematical models to cell wall digestion and forage intake in ruminants. Ph.D. thesis. Cornell University, Ithaca, NY.

Naranjo, J. F., Cuartas, C. A. and Correa, H. J. 2005. Comparación de cuatro modelos matemáticos para la caracterización de la cinética de degradación ruminal de algunos rercursos forrajeros. Livest. Res. Rural Develop. **17**: 1–13.

Orskov, E. R. and McDonald, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. (Camb.) **92**: 499–503.

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. 2nd ed. Cornell University Press, Ithaca, NY.