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Ruminal pH and N-NH_3 behavior: a Bayesian approach

Comportamento do pH e N-NH_3 ruminal: uma alternativa por meio da abordagem Bayesiana

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Abstract

In this work, we present the Bayesian approach as an alternative to frequentist analysis regarding correlated data of pH and N-NH_3 in the Holstein cow rumen. It was observed that for pH and N-NH3 data, a posteriori estimates of coefficients of the regression models were significant, which was not observed for least-squares estimates. Thus, the Bayesian approach allowed inferences that were directly linked to the sampling of parameters of interest and statistical comparisons of non-linear functions of the estimated parameters.
Key words: Gibbs sampling, statistical inference, least squares estimators

Resumo

Neste trabalho objetivou-se estudar a abordagem Bayesiana como alternativa à análise frequentista, para tratar dados correlacionados de pH e N-NH_3 coletados no rúmen de vacas Holandesas. Observou-se que tanto para os dados de pH quanto N-NH_3, as estimativas *a posteriori* dos coeficientes dos modelos de regressão foram significativas, o que não foi observado nas estimativas de mínimos quadrados. Desta forma, a abordagem Bayesiana permitiu inferências ligadas diretamente ao conceito de amostragem dos parâmetros de interesse, assim como comparações estatísticas sobre funções não-lineares dos parâmetros estimados.
Palavras-chave: Amostrador de Gibbs, dados correlacionados, inferência estatística, estimadores mínimos quadrados

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Introduction

Typically, statisticians and technicians from related fields use linear models associated with least-squares estimation methods without defining the data distribution or consider the likelihood of the analyzed variables under an arbitrary data distribution.

In such frequentist methods, assumptions regarding parameters are made with respect to probabilistic models, wherein the component (error) is assumed normal, with null mean (zero), constant variance, and null covariance, for every observation (FERREIRA, 2005).

These assumptions often do not match the reality of data and parameters, leading to unreliable estimates in exceptionally small samples. In other situations, the complexity of the model makes inferences difficult.

Bayesian methods are alternative methods based on Bayes' theorem, which states that the joint probability of two or more events can be described by the product of their single probabilities. In this context, the probability of the evaluated parameters based on experimental data, or a posteriori probability, is a function of the product of a priori probability and the likelihood function; thus, every inference is based on the posteriori distribution of the parameters (ROSSI, 2011).

In this paper, we aimed to apply the Bayesian methodology to experimental analysis in animal science, as an alternative to frequentist analysis, to assess the behavior of ruminal pH and $N\text{-}NH_3$ in cattle.

Material and Methods

The data analyzed here was obtained from Aguiar et al. (2014), who studied the effect of diets with 59.19% corn silage and 40.81% concentrate on ruminal pH and $N\text{-}NH_3$ levels. Four treatments with different inclusion doses of LLOS[5] products were used: T1, control (no additive); T2, LLOS B1 (3.81 g phenolic compounds/kg ingested dry matter); T3, LLOS C1 (3.27 g phenolic compounds/kg ingested dry matter); and T4, LLOS C3 (1.93 g phenolic compounds/kg ingested dry matter).

Two methods, the least-squares (frequentist) and Bayesian methods, were applied to analyze pH and N-NH3 data via Markov Chain Monte Carlo (MCMC) techniques.

The frequentist analysis was performed on the basis of the model described in equation 1.

$$y_{ijkl} = u + p_i + a_j + t_k + \boldsymbol{h}_l + t\boldsymbol{h}_{kl} + e_{ijkl} \qquad (1)$$

where y_{ijkl} is the observation of the ith period in animal j receiving treatment k at the lth time after feeding; u is the overall mean; p_i is the effect of the ith period, i = 1,...4; a_j is the effect of animal j, j = 1,...4; t_k is the effect of treatment k, k = 1,...4; h_l is the effect of the lth time after feeding l, l = 0, 2, 4, 6, and 8 h; th_{kl} is the effect of the interaction between treatment and time; and e_{ijkl} is the random error associated with each observation of the ith period.

To evaluate the behavior of pH and N-NH3 over time after feeding, data from each treatment were fitted using second order polynomial regression, as described in equation 2

$$y_{ijkl} = u + p_i + a_j + t_k + [b_1 (\boldsymbol{h}_l - \bar{\boldsymbol{h}}) + b_2 (\boldsymbol{h}_l - \bar{h})^2] | t_k + e_{ijkl} \qquad (2)$$

5 Propolis-based preparation described in Aguiar et al. (2014).

where b_1 and b_2 are linear and quadratic regression coefficients, respectively, of the dependent variable over time after feeding and $\bar{h}$ is the average time after feeding. The other parameters are as described for equation 1.

After fitting, the minimum value for pH and maximum value for $N\text{-}NH_3$ were computed by minimizing the times at which they occur in each treatment. Data were analyzed using the R software (R CORE TEAM, 2015). The Bayesian analysis considered the model (2) as described in equation 3:

$$y = f(\beta, X) + \epsilon_i \; \epsilon_i \sim N(0, \sigma_e^2) \qquad (3)$$
$$[y|X, \beta, \sigma_e^2] \sim N(f(\beta, x), \sigma_e^2),$$

where X is the incidence matrix of non-informative priori distributions for all model parameters such as and *Gama*.

It was assumed that c = 1.000 and, according to the OpenBugs parametrization (SPIEGELHALTER et al., 1994) (*Model.bug* attached).

Initial values for regression coefficients were set at frequentist estimates. To obtain marginal posterior distributions for all parameters, the *Brugs* R package was used (THOMAS et al., 2006). This program generated 5,100,000 values in an MCMC process with a burn-in of 100,000 initial values. The final sample, taken in jumps of size 50, generated 100,000 values. The convergence of chains was verified by the criteria of Heidelberger and Welch (1983) by using the *coda* R package (PLUMMER et al., 2006). Parameters whose 95% credibility intervals did not include the zero value were considered significant at a 5% level of significance (ROSSI, 2011).

Additionally, for each of 100,000 equations of generated samples, minimum values for pH and maximum values for $N\text{-}NH_3$ were computed to obtain the posteriori distribution of the data from each treatment.

Results and Discussion

The frequentist analysis did not detect interaction between treatment and time for pH and $N\text{-}NH_3$. The average ruminal pH was not affected ($p > 0.05$) by treatments applied, unlike the average concentration of $N\text{-}NH_3$ ($p < 0.05$, Table 1) (AGUIAR et al., 2014).

Table 1. Average pH and $N\text{-}NH_3$ in the ruminal liquid of dairy cattle fed diets with and without LLOS. Frequentist approach.

Variables	Treatments				SE
	T_1 (Control)	T_2 (LLOS B1)	T_3 (LLOS C1)	T_4 (LLOS C3)	
pH	6.24^a	6.17^a	6.22^a	6.23^a	0.0261
$N\text{-}NH_3$ (mg/dL)	27.27^a	27.37^a	25.94^b	27.63^a	2.2705

Means followed by the same letter, into the row, do not differ at 5% of significance, according to the test of Tukey.
SE = Standard error.
Source: Aguiar et al. (2014).

Aguiar et al. (2014), on evaluating rumen pH as a function of time after feeding, found quadratic behavior (pH = 6.86944 – 0.42107h + 0.042898h^2; R^2 = 73.6%) and estimated a minimum pH value of 5.83 at 4 h 54 min after feeding. Furthermore, $N\text{-}NH_3$ behavior as function of time after feeding was found to be quadratic ($N\text{-}NH_3$ = 16.4810 + 7.96253h – 0.871208h^2; R^2 = 95.1%) and maximum value of $N\text{-}NH_3$ was estimated as 34.67 mg dL^{-1} of ruminal fluid at 4 h 36 min after feeding.

These results allow comparison of only the mean values of pH and N-NH3 for each treatment over time after feeding and not the evaluation of the behavior of pH and N-NH3 values over time. The latter may show that despite no statistical difference in the means of pH and N-NH3 values, ruminal acidification dynamics and ammonia production are different for each treatment.

One way of analyzing ruminal acidification dynamics in each treatment is to assume the polynomial regression model of pH and N-NH3 over time after feeding (equation 2).

Using the frequentist approach, it can be observed that the fitted equations for each treatment were significant, since the regression coefficients are non-zero; however, this does not imply that these equations differ from each other (Table 2). For this comparison, one possible approach is a model identity analysis (REGAZZI, 1999), which tests the difference between residual mean squares of complete model and considers different models for each treatment and a reduced model, assuming that all treatments follow the same model. Further analysis can be performed by comparing coefficients of different curves (GRAYBILL, 1976) by comparing fitting patterns, wherein same-order coefficients are considered equal or not, for different models.

Table 2. Estimates of regression parameters for modeling pH and $N\text{-}NH_3$ over time after feeding (hours), using the least-squares method.

Treatment	Parameter	pH			$N\text{-}NH_3$		
		Estimate	SE[1]	p-value[2]	Estimate	SE[1]	p-value[2]
	μ	5.8713	0.0287	2×10^{-16}	33.6451	0.2484	2×10^{-16}
T_1	b_1	-0.0383	0.0391	0.3317	0.7787	0.3387	0.0249
	b_2	0.0456	0.0041	2.92×10^{-16}	-0.8327	0.0356	2×10^{-16}
T_2	b_3	-0.1043	0.0391	0.0098	0.8386	0.3387	0.0161
	b_4	0.0394	0.0041	9.32×10^{-14}	-0.8039	0.0356	2×10^{-16}
T_3	b_5	0.0016	0.0391	0.9662	-0.7882	0.3387	0.0233
	b_6	0.0435	0.0041	2.09×10^{-15}	-0.8122	0.0356	2×10^{-16}
T_4	b_7	-0.0467	0.0391	0.2371	1.4577	0.3387	6.18×10^{-5}
	b_8	0.0431	0.0041	3.02×10^{-15}	-0.8478	0.0356	2×10^{-16}

[1] Standard error for the estimate; [2] Probability for the Student *t*-test.

Important information in the analysis of ruminal acidification dynamics is the minimum value assumed by the pH, maximum value assumed by $N\text{-}NH_3$, and time at which these critical values occurred in each treatment. Estimates for these values are presented in tables 3 and 4.

A numerical analysis of the results indicates that **LLOS C1** reached its minimum pH fastest and **LLOS B1** took longest, while **control** and **LLOS C3** had intermediate times. It is known that ruminal pH directly affects the microbial growth rate, because ruminal microorganisms grow better in specific pH ranges. A diet with high grain content favors the growth of amylolytic bacteria and production of lactic acid, which leads to a drop in pH and consequently inhibits the growth of certain bacteria that grow better at higher pH (AGUIAR et al., 2014).

Table 3. Fitted regression models for ruminal pH over time after feeding (hours), minimum value for pH, and corresponding time (hours), per treatment. Frequentist approach.

Treatment	Fitted model	Minimum pH	Time (h)
T_1	$pH = 6.75 - 0.4038h + 0.0456h^2$	5.85	4 h 25 min
T_2	$pH = 6.91 - 0.4197h + 0.0394h^2$	5.79	5 h 19 min
T_3	$pH = 6.56 - 0.3465h + 0.0435h^2$	5.87	3 h 59 min
T_4	$pH = 6.74 - 0.3916h + 0.0431h^2$	5.85	4 h 32 min

Table 4. Fitted regression models for ruminal $N\text{-}NH_3$ over time after feeding (hours), maximum value for $N\text{-}NH_3$, and corresponding time (hours), per treatment. Frequentist approach.

Treatment	Fitted model	Maximum $N\text{-}NH_3$	Time (h)
T_1	$N\text{-}NH_3 = 17.20 + 7.441h - 0.8327h^2$	33.82	4 h 28 min
T_2	$N\text{-}NH_3 = 17.42 + 7.2702h - 0.8039h^2$	46.65	4 h 31 min
T_3	$N\text{-}NH_3 = 23.80 + 5.7096h - 0.8122h^2$	41.01	3 h 31 min
T_4	$N\text{-}NH_3 = 14.24 + 8.2402h - 0.8478h^2$	50.16	4 h 51 min

The limitation of this approach using regression coefficients (Tables 3 and 4) is that the maximum or minimum of a quadratic equation is set as the product of the ratios between its coefficients and such quantities probably do not follow a normal distribution.

This limitation could be overcome by using frequentist approach for fitting a model for each cow and for each treatment, producing 16 equations with their respective minima for pH, maxima for $N\text{-}NH_3$, and corresponding estimates to reach these values. Thus, these estimates could be considered dependent variables and subjected to further analysis. However, one-per-cow models are fitted very poorly owing to the small number of observations for each animal in each treatment. Besides, such minima and maxima do not adhere to normality assumptions. Thus, the use of the Bayesian approach is presented as a viable alternative, as follows.

Notably, for each estimate, the respective standard deviation and credibility interval are computed by sampling the posteriori distribution for each parameter (Table 5), unlike the frequentist approach that provides, for each estimate, the standard error and the Student *t*-test p-value based on the mean square of the error.

The standard deviation indicates the accuracy of the estimate based on parameter sampling, while the standard error indicates the precision based on an estimate of the residual variation. The credibility interval indicates the significance of the estimate, which is also based on parameter sampling. If the credibility interval does not include the value 0, it is inferred that the obtained estimate is statistically different from null. On the other hand, the *t*-test directly indicated probability of the estimate being equal to zero, based on an estimate of the residual variance.

Table 5. Estimates of regression parameters for modeling pH and $N\text{-}NH_3$ over time after feeding (hours), using the Bayesian approach.

Treatment	Parameter	pH			$N\text{-}NH_3$		
		Mean	SD	CrI	Mean	SD	CrI
	μ	5.871	0.029	(5.814; 5.928)*	33.640	0.2911	(33.07; 34.21)*
T_1	b_1	- 0.0717	0.0133	(-0.098; -0.045)*	1.1242	0.1311	(0.86; 1.38)*
	b_2	0.0457	0.0042	(0.037; 0.054)*	-0.8325	0.0417	(-0.91; -0.74)*
T_2	b_3	- 0.0808	0.0134	(-0.107; -0.054)*	1.0786	0.1319	(0.81; 1.33)*
	b_4	0.0394	0.0043	(0.030; 0.047)*	-0.8036	0.0424	(-0.88; -0.72)*
T_3	b_5	- 0.0612	0.0134	(-0.087; -0.034)*	0.7771	0.1319	(0.51; 1.03)*
	b_6	0.0435	0.0042	(0.035; 0.052)*	-0.8121	0.0418	(-0.89; -0.72)*
T_4	b_7	- 0.0981	0.0136	(-0.124; -0.070)*	1.5245	0.1344	(1.26; 1.79)*
	b_8	0.0430	0.0042	(0.034; 0.051)*	-0.8481	0.0418	(-0.93; -0.76)*

SD: standard deviation; CrI: 95% credibility interval; * significant at 5% probability.

A comparison of the significance of regression coefficient estimates obtained from both methods shows that in the frequentist approach (Table 2), linear coefficients for pH in treatments **control**, **LLOS C1,** and **LLOS C3** were not significant, while those in the Bayesian approach were (Table 5). For other factors, no significant differences were detected between both methods.

It was observed using the Bayesian approach that **LLOS C1** reached its minimum pH quickest, while **LLOS C3** took longest (Table 6). This result differs from that one found using the frequentist approach, which suggests that treatment **LLOS B1** is the slowest. **Control** and **LLOS B1** had values between those of **LLOS C1** and **LLOS C3** (Figure 1).

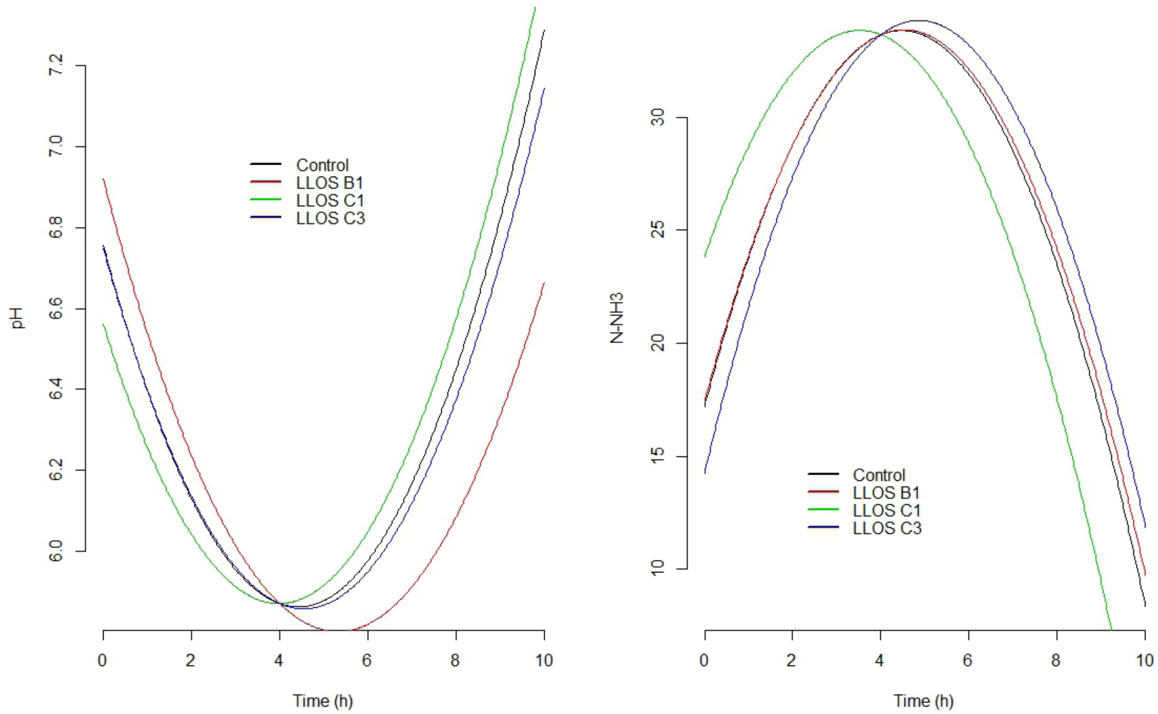
Table 6. Fitted regression models for ruminal pH over time after feeding (hours), minimum value for pH, and corresponding time (hours), per treatment. Bayesian approach.

Treatment	Fitted model	Minimum pH	Time (h)
T_1	$pH = 6.88 – 0.4374h + 0.0457h^2$	5.84	4 h 46 min
T_2	$pH = 6.82 – 0.3964h + 0.0394h^2$	5.82	5 h 02 min
T_3	$pH = 6.81 – 0.4094h + 0.0435h^2$	5.84	4 h 42 min
T_4	$pH = 6.95 – 0.4427h + 0.0430h^2$	5.81	5 h 08 min

Regarding $N\text{-}NH_3$, **LLOS C1** reached the maximum quickest, while **LLOS C3** was the slowest. **Control** and **LLOS B1** showed intermediate behavior (Table 7).

So far, both methods have the same application potential for inference. However, because the Bayesian approach sampled regression coefficients, this method can be used to obtain samples of the minima and maxima and infer about differences among treatments for these estimates.

Figure 1. Fitted models of Bayesian estimates for pH (a) and $N\text{-}NH_3$ (b) in the ruminal liquid of dairy cattle, fed diets with and without addition of LLOS.



(a) (b)

Table 7. Fitted regression models for ruminal $N\text{-}NH_3$ along time after feeding (hours), maximum value for $N\text{-}NH_3$, and corresponding time (hours), per treatment. Bayesian approach.

Treatment	Fitted model	Maximum $N\text{-}NH_3$	Time (h)
T_1	$N\text{-}NH_3 = 15.82 + 7.7843h - 0.8325h^2$	34.02	4 h 40 min
T_2	$N\text{-}NH_3 = 16.47 + 7.5078h - 0.8036h^2$	34.00	4 h 40 min
T_3	$N\text{-}NH_3 = 23.75 + 5.7198h - 0.8121h^2$	33.83	3 h 31 min
T_4	$N\text{-}NH_3 = 13.97 + 8.3099h - 0.8481h^2$	34.3	4 h 53 min

It was found that all minima for pH and corresponding times differ between treatments; the same was observed for the maxima and corresponding times for N-NH3, except for time until maximum in **control** and **LLOS B1**, which were equal (Tables 8 and 9).

Notably, in the results presented in Tables 8 and 9, there is a discrepancy in estimates in relation to those presented in Tables 6 and 7. This is because estimates of the minima or maxima and corresponding times, when obtained from the sample as shown in Tables 8 and 9, are equivalent to the a posteriori average of ratios between linear and quadratic regression coefficients, while those presented in Tables 6 and 7 are ratios between a posteriori averages of the linear and quadratic regression coefficients.

In other words, the minima or maxima of random variables are non-linear functions of linear and quadratic regression coefficients; therefore, the estimate of the ratio between coefficients is different from the ratio between estimates of coefficients. This finding indicates that the estimates presented in Tables 8 and 9 should be considered, which have no equivalence to frequentist method.

Table 8. Bayesian estimates of time for minimum pH and contrasts between treatments.

Treatment	Mean	SD	CrI
T_1	4.7913	0.1662	(4.4830; 5.1396)
T_2	5.0374	0.2109	(4.6689; 5.4916)
T_3	4.7106	0.1716	(4.3876; 5.0644)
T_4	5.1507	0.1985	(4.7877; 5.5696)
Contrast			
1-2	-0.2461	0.0456	(-0.3522; -0.1829)*
1-3	0.0806	0.0063	(0.0713; 0.0961)*
1-4	-0.3594	0.0327	(-0.4270; -0.3044)*
2-3	0.3268	0.0406	(0.2795; 0.4271)*
2-4	-0.1133	0.0143	(-0.1265; -0.0768)*
3-4	-0.4401	0.0276	(-0.5012; -0.3983)*
$\widehat{pH}_{minimum}$	Mean	SD	CrI
T_1	5.8419	0.0303	(5.7826; 5.9016)
T_2	5.8282	0.0308	(5.7674; 5.8884)
T_3	5.8485	0.0301	(5.7894; 5.9085)
T_4	5.8137	0.0313	(5.7525; 5.8752)
Contrast			
1–2	0.0137	0.0007	(0.0127; 0.0153)*
1–3	-0.0065	0.0005	(-0.0077; -0.0057)*
1–4	0.0282	0.0012	(0.0259; 0.0302)*
2–3	-0.0202	0.0009	(-0.0225; -0.0187)*
2–4	0.0144	0.0007	(0.0129; 0.0153)*
3–4	0.0347	0.0014	(0.0325; 0.0373)*

SD: standard deviation; CrI: 95% credibility interval; * significant at 5% probability.

Table 9. Bayesian estimates of time for the maximum$_3$ and contrasts between treatments.

Continue ...

Treatment	Mean	SD	CrI
T_1	4.6769	0.0861	(4.5129; 4.8519)
T_2	4.6729	0.0899	(4.5006; 4.8510)
T_3	4.4797	0.0855	(4.3166; 4.6543)
T_4	4.9009	0.0912	(4.7302; 5.0918)
Contrast			
1–2	0.0039	0.0041	(-0.0026; 0.0122) ns
1–3	0.1971	0.0020	(0.1948; 0.1998)*
1–4	-0.2240	0.0054	(-0.2382; -0.2170)*
2–3	0.1931	0.0050	(0.1845; 0.1996)*
2–4	-0.2279	0.0032	(-0.2377; -0.2251)*
3–4	-0.4211	0.0062	(-0.4373; -0.4146)*
$\hat{N} - \widehat{NH}_{3\ máx}$	Mean	SD	CrI
T_1	34.0303	0.2965	(33.4503; 34.6181)
T_2	34.0130	0.2954	(33.4309; 34.5969)

... Continuation

T_3	33.8364	0.2925	(33.2649; 34.4147)
T_4	34.3367	0.3006	(33.7480; 34.9274)
Contrast			
1-2	0.0173	0.0039	(0.0122; 0.0224)*
1-3	0.1938	0.0055	(0.1821; 0.2014)*
1-4	-0.3064	0.0072	(-0.3180; -0.2982)*
2-3	0.1765	0.0054	(0.1659; 0.1869)*
2-4	-0.3237	0.0077	(-0.3389; -0.3146)*
3-4	-0.5002	0.0101	(-0.5175; -0.4858)*

SD: standard deviation; CrI: 95% credibility interval; * significant at 5% probability.

Conclusions

The Bayesian approach to data analysis allows inferences directly linked to the sampling of parameters of interest and allows statistical comparisons on non-linear functions of estimated parameters of the concerned models.

Additionally, the results suggest that fitting one regression model per treatment is advisable.

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