

REPEATED MEASUREMENT ANALYSES OF FORAGES IN CROPPING SYSTEMS ¹

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Abstract

Repeated measurements (RM) are common in forage experiments. The data used in this study were accumulated ammonia losses by volatilization (N) and dry matter production (DM) of *Cynodon dactylon* cv. Coastcross pasture from an experiment in blocks with five levels of urea: 0, 25, 50, 100 and 200 kg of N ha⁻¹, applied in five periods (cuttings). For N, RM were the averages of cuttings and nine days of observation. The F test for the hypothesis of no effect for Period and Level x Period interaction (DM) and for Days and Level x Days interaction was not affected by univariate and multivariate tests. However, Greenhouse-Geisser epsilon estimate was biased downwards. Polynomial contrasts in univariate ANOVA and Logistic function agreed in explaining accumulated N. For DM, unequal population variances on different periods was detected and the assumption that the pairs of observations on the same subject are equally correlated was rejected.

Keywords: Analysis of variance, Statistical analysis, GLM, MIXED and REG procedures, non-linear model

Introduction

In forage experiments, repeated measurements (RM) are taken from the same experimental unit or subject. In univariate analysis, such as a split-plot analysis, subjects are the whole-plot units and the subjects at a particular time are the sub-plot units. It is assumed that the pairs of observations on the same subject are equally correlated. Tests for within-subject effects and interactions involving these effects require that the within-subject variance-covariance matrix has a Huynh-Feldt condition (H-F). In most RM data, this assumption is not valid. Adjustments such as “Greenhouse-Geisser epsilon” (G-G) and H-F tests provided by GLM are necessary. The multivariate tests used are Wilks’ Lambda, Pillai’s Trace, Hotelling-Lawley Trace and Roy’s Maximum Root tests. These tests do not require H-F condition. The MIXED procedure can be used to fit within-subject variance-covariance matrices, to select the most appropriate of them. The REG procedure is used for fitting linear regression models by least squares. Sometimes the behavior of RM over time in forages is best described by a non-linear model of the parameters of interest. The purpose of this paper is to provide a unified presentation of modeling strategies for analyzing RM data of forages.

Material and Methods

The data used were accumulated ammonia losses by volatilization (N) and dry matter production (DM) of a *Cynodon dactylon* cv. Coastcross pasture from an experiment carried out from November 1998 to April 1999 in São Carlos, São Paulo State, in randomized blocks with five levels of urea: 0, 25, 50, 100 and 200 kg of N ha⁻¹, applied in five periods (cuttings). For N, RM were the average of cuttings and nine days of observation (Period). For DM, RM were the cuttings. The data were analyzed using procedures of SAS (SAS, 1993a, b), as

follows: a) GLM: adjusted univariate test, using Greenhouse-Geisser (G-G) and H-F epsilon-adjusted tests; multivariate analysis tests, using the options: POLYNOMIAL, which specifies orthogonal polynomials contrasts that may be appropriate for a continuous within-subject effect; HELMER, which specifies contrasts between each level of the factor and the mean of subsequent levels; and PROFILE, which generates contrasts between adjacent levels on the factor; b) MIXED procedure, to fit compound symmetry and unstructured within-subject variance-covariance matrices and to select the most appropriate of them; c) REG procedure, to estimate responses to N levels by polynomial regressions; d) nonlinear model named Logistic function $y_i = A / (1 + e^{-kx}) + e_i$ (Draper and Smith, 1980), in order to estimate accumulated ammonia losses by volatilization.

Results and Discussion

The P values for the F test produced by univariate and multivariate tests (Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace and Roy's Maximum Root tests) for the hypothesis of no effect for Period and Level x Period interaction (DM) and for Days and Level x Days interaction (N) were significant ($P = .0001$), except Pillai's Trace for Level x Period interaction ($P = .0593$). This result indicates that the F test significance was not affected by univariate tests (adjusted and unadjusted) and multivariate tests. The G-G epsilon values were .6001 and .2077 and the Huynh-Feldt epsilon, .9129 and .2879 for DM and N losses, respectively, showing that G-G estimates tend to be biased downwards. Little et al. (1998) found discrepancies between Roy's Maximum Root tests and Pillai's Trace in the interaction tests. Based in their experiences they recommended G-G adjusted P value instead of the multivariate tests. Table 1 shows the ANOVA of contrast variables. These results from the REPEATED statement indicate interaction between Levels and Days and interaction between Levels and Periods. The label Day.1 refers to a difference between the losses response on

Day 1 (D1) and the mean of responses on Day 2 (D2) through Day 8 (D8), *i.e.*, $\text{Day.1} = \text{D1} - (\text{D2} + \dots + \text{D8})/7$; likewise, $\text{Day.2} = \text{D2} - (\text{D3} + \dots + \text{D8})/6$, and so forth. For ammonia losses, P values were significantly different ($P=.0001$), indicating that profiles for all levels, within each day, are not parallel. The only exception was the contrast variables in Day.2 ($P=.2374$), indicating lack of interaction between Levels and Day 2, *i.e.*, that profiles for all levels are parallel. For DM, there was no interaction between levels in Period 4 ($P=.2260$), *i.e.*, profiles for all levels are parallel for this Period. For Periods 1, 2 and 3, profiles for all levels are not parallel. In the Mean Squares for polynomial contrasts in univariate ANOVA, Day.n and Period.n represent the n^{th} degree polynomial contrast for Day and Period. For N losses, the P value was significant ($P = .0001$) from Day 1 to Day 5, showing that this variable reached a plateau at Day 5. This behavior was shown by the adjustment of the Logistic function $y_i = A(1 - Be^{-Kt}) + e_i$ in Figure 1, which shows the upper-and-lower 95% confidence limits (CL) for the mean expected values of ammonia losses considering four levels of urea: 25, 50, 100 and 200 kg of N ha⁻¹. For DM, the effect of Levels was significant ($P = .0001$) for all days. Figure 1 illustrates the CL for the mean expected values of DM and N losses considering five levels of urea in Period 4, showing that the effect of level in this period was estimated by a quadratic polynomial function. Using the unstructured R matrix in dry matter production, the variance associated to period 1 through period 5 were, respectively, 58166.79; 97660.78; 97660.78; 171050.74; and 103182.40. The largest variance (171050.74) was approximately three times as large as the smallest (58166.79), showing evidence of unequal population variances on different periods with increasing trends in the variances. The correlation between periods ranged from -.29 to .59, *i.e.*, the assumption that the pairs of observations on the same subject are equally correlated was rejected. The trend observed in the correlation indicates no evidence of use of auto-regressive structure. The F test significance was not affected by univariate and multivariate tests for the hypothesis of no effect for Period and

Level x Period interaction and for Days and Level x Days interaction. However, G-G epsilon estimate tended to be biased downwards. Both, polynomial contrast in univariate ANOVA (PC) and the Logistic function did agree in explaining accumulated ammonia losses; PC and quadratic polynomial function did agree in explaining the effect of five levels of urea in Period 4. For DM, evidence of unequal population variances on different periods was detected and the assumption that the pairs of observations on the same subject are equally correlated was rejected.

References

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Table 1 - Analysis of Variance of Contrast Variables. Day.n and Period.n represent, respectively, the contrast between the nth level of Day and Period and the mean of subsequent levels; b) Mean Squares for univariate ANOVA. Day.n and period.n represent the nth degree polynomial contrast for Day and Period.

a) Analysis of Variance of Contrast Variables

		Ammonia loss by volatilization						
Mean Squares	df	Day.1	Day.2	Day.3	Day.4	Day.5	Day.6	Day.7
level	4	26.780	0.302	0.368	0.494	0.841	2.520	8.993
Error	15	0.813	0.187	0.011	0.019	0.037	0.047	0.224

		Dry matter production			
		Period.1	Period.2	Period.3	Period.4
level	4	2500260.464	3519671.394	1107934.632	480803.155
Error	15	33412.052	106150.598	205615.671	301287.802

a) Mean Squares for univariate ANOVA

		Ammonia loss by volatilization						
Mean squares	df	Day.1	Day.2	Day.3	Day.4	Day.5	Day.6	Day.7
level	3	16.980	0.978	11.478	.260	1.438	.050	.016
Error	12	.585	.126	0.230	.047	.451	.023	.005

		Dry matter production			
		Period.1	Period.2	Period.3	Period.4
level	4	483711.314	731200.942	1168394.736	3235679.590
Error	15	38247.259	34880.776	1842.878	209092.692

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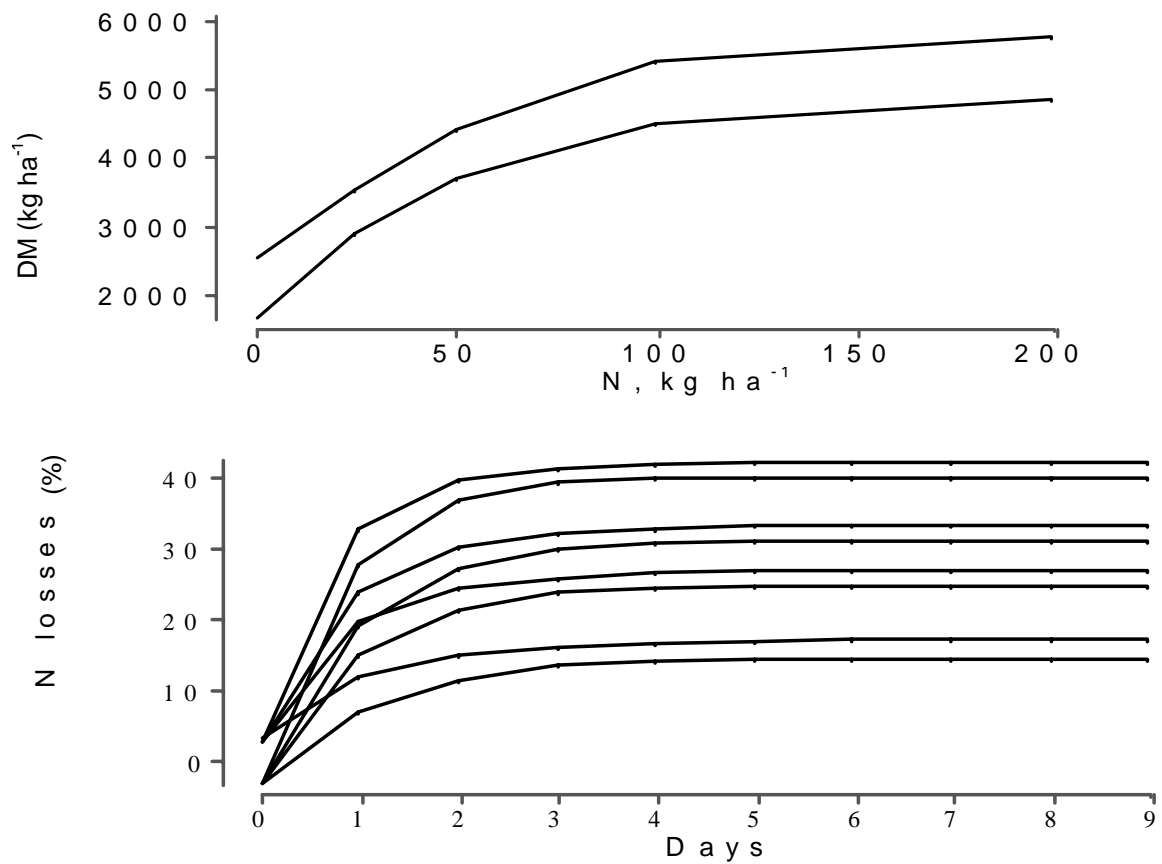


Figure 1 - Upper-and-lower 95% confidence limits (CL) for the mean expected values: Left) Dry matter production, kg ha⁻¹, estimated by quadratic polynomial regression in Period 4. Right) Ammonia losses, %, estimated by Logistic function. The CL, in decreasing order of losses, are associated to N doses of 200, 100, 50 and 25 kg ha⁻¹ per cutting, respectively.