

COMPARISON OF SOME TESTS FOR CORRELATED ERROR IN ANIMAL STUDIES

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ABSTRACT: Some assumptions should be checked because of autocorrelation of residuals, when data collected on the same subject at each time or space. There is no problem for testing the treatments, whereas some assumptions should be valid for testing the period and period treatment interaction in case of repeated measures. In this study, 53 male lambs were assigned to the three treatments and each one was measured with 15 days interval through 70 days. Four different analysis methods, standard analysis, transformed data analysis, auto-regressive error model, and multivariate analysis were performed to compare for repeated measures. There were no differences in terms of improvement of F test among standard analysis, transformed data analysis and multivariate analysis methods. The autoregressive error model improved the F test by %20 whereas assumptions were not valid for this analysis.

Keywords: Repeated measures, univariate, multivariate, auto-regressive error model

HAYVANCILIK UYGULAMALARINDA HATALARIN BİRBİRİ İLE İLİŞKİLİ OLDUĞU DURUMLARDA BAZI TESTLERİN KARŞILAŞTIRILMASI

ÖZET: Aynı birey üzerinde yer veya zamana bağlı olarak birden fazla gözlem yapıldığı zaman hataların birbirleriyle otokorelasyonu söz konusu olacağından analizlerden önce bazı varsayımların kontrol edilmesi gerekir. Zira, hataların oto-korelasyonu durumunda faktörlerin ana etkisinin test edilmesinde herhangi bir sorun olmamasına karşın, zaman ve zaman*faktör interaksiyonunu test

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edebilmesi için bazı varsayımların geçerli olması gerekir. Bu çalışmada 53 erkek kuzu üç ayrı yemleme sistemine tabi tutulmuş ve her biri üzerinde 15 gün aralıklarla 5 kez ölçüm yapılmıştır. Elde edilen gözlemlere 4 ayrı analiz yöntemi uygulanmıştır. Standart analiz, transforme edilmiş verilerin analizi ve çokdeğişkenli (multivariate) analiz yöntemleri arasında fark olmamıştır. Otoresgressive hata modeli ile F testinde %20 düzeyinde bir iyileşme olmuş ancak bu testin uygulanabilmesi için varsayımların geçerli olmadığı belirlenmiştir.

Anahtar kelimeler: tekrarlanan ölçümler, tekdeğişkenli, çokdeğişkenli, otoresgressive hata modeli.

INTRODUCTION

Repeated measures designs have structure that involve more than one size of the experimental unit (1). It is important to make distinction between repeated measurement data and data collected on different subject at each time or space. Because, the validity of analysis of variance is main concern in such data. Certain assumptions require about of independence pattern of correlation for repeated or unrepeated measures.

In standard analysis context, split-plot type methods frequently are used to analyze data of repeated measures. An assumption required for validity of the standard analysis is that the $p \times p$ variance-covariance matrix pooled over treatments is homogeneous with respect to period or time (2). When assumption is violated, standard statistical techniques should not be valid. As it will be always the case, when measurements are taken over seasons, in other words repeated factors cannot be randomized this assumption is likely to be violated (3). Because the pattern of correlations among the measures that are taken sequentially introduce a structure in the residuals that violates the assumptions of standard analysis of variance model.

Properties of repeated measures in terms of homogeneity of variance-covariance structure were studied by many researchers (1-6). In recent years different, both parametric and nonparametric methods have advocated to adopt the repeat measurement problems. Some categorical data analysis techniques based on the nominal or ordinal scale like McNamare test, test of symmetry, Freedmen, Aligned ranks, Marginal homogeneity tests and Quasi symmetry tests are frequently used in repeated measures (3). In addition, methods of time-series analysis for repeated measures had considerable interest on that topic. In particular using of a first-order auto-regressive error model to reduce error by filtering residual error that suggested by Milikan and Johnsen (2) is a powerful technique in

improving the power of test. On the other hand, properties of the repeated measures in binary response and the efficiency of some tests were studied by Lipsitz et al. (4, 5)

The purpose of this study is to show the effect of repeated measures on validity of assumptions for classical analysis and to compare some model, that are suggested in literatures. An example was given for that reason.

MATERIAL AND METHOD

An example was given to illustrate the concept of repeated measures analysis of variance with using different methods. Hence, three different feeding types were carried out on 54 males lambs. All lambs were assigned randomly to the feeding types. But only 53 lambs were used in the final analysis. Only body weight was considered in this study. Data were collected with 15 days interval through 70 days.

Properties of auto-correlation in repeated measures.

When repeated measures are the main concern of study, then the relation of error terms over time in the model is more important for checking assumption. Consider a linear model for repeated measures as:

$$Y_t = XB + e_t \quad t = 1, 2, \dots, p \quad (1)$$

Where, Y_t is an $n \times 1$ of observed responses, X is an $n \times r$ incidence matrix, β is $p \times 1$ of parameters and e_t is residual error in the t^{th} period. For repeated data, e_t has a first auto-regressive error. $e_t = r e_{t-1} + u_t$

Here $|r| < 1$ and u_t provide some assumption as (7):

$$\begin{aligned} E(u_t) &= 0 \\ E(u_t, u_{t-m}) &= \begin{cases} \sigma^2 & m = 0 \\ 0 & m \neq 0 \end{cases} \quad \text{for all } t \end{aligned}$$

Hence, $e_t = r e_{t-1} + u_t$

$$e_{t-1} = r e_{t-2} + r^2 u_{t-1}$$

replacing e_{t-1} in the e_t equation the one obtain,

$$e_t = r^2 e_{t-2} + r u_{t-1} + u_t$$

and continuing in this custom then equation for e_t is to be

$$e_t = \sum_{m=0}^{\infty} r^m u_{t-m} \quad 2.$$

Consequently $E(e_t) = 0$ (because $E(u_t) = 0$ for all t) and

$$\sigma_{e_t}^2 = rE(u_t^2) + r^2E(u_{t-1}^2) + r^4E(u_{t-2}^2) + r^6E(u_{t-3}^2) + \dots + r^{2m}E(u_{t-m}^2)$$

$$\sigma_e^2 = (r + r^2 + r^4 + \dots + r^{2m}) \sigma_u^2 = \sigma_u^2 \sum_{m=1}^{\infty} r^{2m} \quad 3.$$

Therefore,

$$\sigma_e^2 = \sigma_u^2 / (1-r) \quad \text{for } |r| < 1 \quad 4.$$

In addition, the covariance between e_t and e_{t-1} is $\text{Cov}(e_t, e_{t-1}) = \sigma(e_t, e_{t-1}) = r^m(\sigma^2/(1-r^2))$, $m \neq 0$

This means that all errors are auto-correlated since $r \neq 0$. Durbin-Watson test in general using for test at auto-correlation (See Neter and Wasserman (9) for details to test the auto-correlation).

Checking assumptions for repeated measures.

Usually, the classical split-plot model is applied to animal experiment for repeated data. But, validity of assumption is required for that analysis, that is, $p \times p$

$$S = \begin{bmatrix} S_{11} & S_{12} & \dots & S_{1t} \\ S_{21} & S_{22} & \dots & S_{2t} \\ \dots & \dots & \dots & \dots \\ S_{t1} & S_{t2} & \dots & S_{tt} \end{bmatrix}$$

$$S_0 = \begin{bmatrix} S_{tt}^2 & S_{tt} & S_{tt} & \dots & S_{tt} \\ S_{tt} & S_{tt}^2 & S_{tt} & \dots & S_{tt} \\ \dots & \dots & \dots & \dots & \dots \\ S_{tt} & S_{tt} & S_{tt} & \dots & S_{tt}^2 \end{bmatrix} \quad 5a$$

pooled variance-covariance matrix is homogenous with respect to periods. Consider the observations obtained from several treatments. When the variance-covariance has structure this matrix is called to have compound symmetry. Where S , S_0 and S^2 are the estimators of Σ , Σ_0 and σ^2 , respectively for hypothesis of $H_0 : S = S_0$. The S is sample variance-covariance matrix and S_0 is the perfect uniform matrix (10). S^2_{it} is mean of S^2 's in S matrix. Similarly, the S_{it} is mean of all covariance terms in S . That is,

$$S^2 = \sum_{i=1}^t S^2_i / p \quad 5b$$

$$S_{it} = \sum_{l < l'} \Sigma_{ll'} / [(p-1)/2] \quad 5c$$

The natural log of the ratio of the two determinants for $H_0 : S = S_0$ are used for hypothesis (See appendix). A chi-square test with v_1 degree of freedom (where $v_1 = p^2 + p - 4)/2$) was used for test. The compound symmetry of errors provides sufficient essential for usual F test in classical split-plot or any other linear model. In equation 4 the r is least square estimate of the first order auto-correlation coefficient.

$$r = [\sum \sum \sum \sum e_{ij1t} \cdot e_{ij1(t-1)}] / (\sum \sum \sum \sum e_{ij1t}^2) \quad 6.$$

Gill (2) suggested that using filtrated data for analysis with

$$Y^* = Y - r e_{ij1(t-1)}$$

equation will be more powerful than those performed an unfiltered data (2).

Models

1. Four different methods were used for that analysis. The first one was a classical linear model. Description of this model was as follow:

$$Y_{ijkt} = \mu + a_i + b_j + p_k + (b^*p)_{jt} + b_1(X_{1ijkt} - X_{1j}) + b_2(X_{2ijkt} - X_2) + b_3(X_{3ijkt} - X_3) + e_{ijkt}$$

Where Y is a measured response variable, μ is a general mean, a_i random effect of animals ($i = 1, 2, \dots, r$, $r = 53$), b_j the fixed effect of feeds ($j = 1, \dots, n$, $n = 3$), p_k is the fixed effect of a period of measurement ($k = 1, 2, \dots, p$). b_1, b_2, b_3 are the

partial regression coefficient of age of lambs, birth weight and initial weight of experiment to involve trait. $x_{(i)}$'s are known constant, namely value of the any independent variable in the involved trait. $e_{(i)}$ is a random error term with $E(e_{ij,t}) = 0$ and variance σ_e^2 ; $e_{ij,t}$, $e_{ij,t+1}$ are uncorrelated, so that the covariance, $\sigma(e_{ij,t}, e_{ij,t+1}) = 0$. The PROC GLM (12) used to analysis for linear model described above.

2. The second technique used in analysis was auto-regressive error model (filtered data), suggested by Gill (2). The model suggested by Gill was without covariates. But three covariate sources introduced above were used in our analysis. The filtered data for dependent and independents (covariates) continuous variables obtained as follows;

$$\begin{aligned} Y'_{ij,t} &= Y_{ij,t} - re_{ij,t-1} \\ X'_{1ij,t} &= (X_{1ij,t} - re_{ij,t-1}) \\ X'_{2ij,t} &= (X_{2ij,t} - re_{ij,t-1}) \\ X'_{3ij,t} &= (X_{3ij,t} - re_{ij,t-1}) \end{aligned}$$

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where k is period and r is first-order auto-correlation coefficient. These coefficients were calculated as follow;

$$r = [\sum \sum \sum e_{ij,t} \cdot e_{ij,t-1}] / (\sum \sum \sum e_{ij,t}^2)$$

After data filtered with using 7. The Durbin-Watson test was employed to test whether the error uncorrelated for filtered data analysis. In addition, auto-regressive residual error variance-covariance was constructed for checking compound symmetry. Calculating auto-regressive residual error variance-covariance matrix was based on auto-correlation coefficient and mean square error of filtered data as follow (2);

$$e_{ij,t} = [\sigma_e^2 / (1-r^2)] \begin{vmatrix} 1 & r & r^2 & r^3 & \dots & r^{p-1} \\ r & 1 & & & & r^{p-2} \\ & & & & & \\ & & & & & \\ r^{p-1} & r^{p-2} & & & & 1 \end{vmatrix}$$

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where σ_e^2 are the variance of filtered residual error r is first-order auto-correlation and p is periods involved. An estimate of σ_e^2 (S_e^2) was obtained from analysis of variance by GLM. A second matrix named sample matrix of pooled error variance-covariance matrix, was built to compare with auto-regressive residual error variance-covariance matrix for symmetry compound of filtered data. The diagonal elements of second matrix were calculated as,

$$\text{Var}(P_t) = \left(\sum_{i=1}^r \sum_{j=1}^n c_{ij}^2 / n(r-1) \right) \quad (n = 3, \quad r = 53) \quad 9$$

and off-diagonal elements,

$$\text{Cov}(P_t, P_t') = \left(\sum_{i=1}^r \sum_{j=1}^n c_{ij} c_{ij}' \right) / n(r-1) \quad 10$$

These two matrices were compared to each other to justify the assumption of uniformity of variance-covariance among periods. Therefore, hypothesis of $H_0: \Sigma = \Sigma_0$ was tested for this purpose (see appendix for detail).

3. The third model used other than two univariate analysis described above was multivariate analysis. For validity of multivariate analysis (also univariate analysis), firstly variance-covariance matrix of treatment over periods hypothesized as $H_0: S_1 = S_2 = S_3$ (10, 11). For this purpose estimates of Σ_1, Σ_2 and Σ_3 were constructed (S_1, S_2 and S_3). Compound symmetry condition was tested. $H_0: \Sigma = \Sigma_0$ in case of accepting $H_0: \Sigma_1 = \Sigma_2 = \Sigma_3$. Hence S and S_0 were estimated for this hypothesis. Where S is sample variance-covariance matrix and S_0 is the perfect uniform matrix (See appendix).

4. The last method used in this analysis based on transformed data. This analysis procedure was used by Bliss (2). Thus, pooled standard deviation over treatments (S_t) was considered as dependent and pooled means (Y_t) were independent to provide a regression equation,

$$S_t = b_0 + b_1(\bar{Y}_t) \quad 11$$

For that analysis, original data was transformed into $\log(Y + \text{intercept/slope})$. The GLM SAS (12, 13) procedure was used for analysis of transformed data.

RESULTS AND DISCUSSION

Some results obtained from the univariate test for analysis of variance for filtered, unfiltered and transformed data are shown in Table 1. Filtered data analysis yielded a decreasing residual error mean square by 20%, transformed data decreased by 3%. In particular, filtering consistently increased the power of F-test statistic for treatment, period and treatment*period interaction. Increasing in power of test obtained by transformed data seems to be trivial. But, trend between mean and standard deviations, indicates that a transformation may be more appropriate (Figure 1)

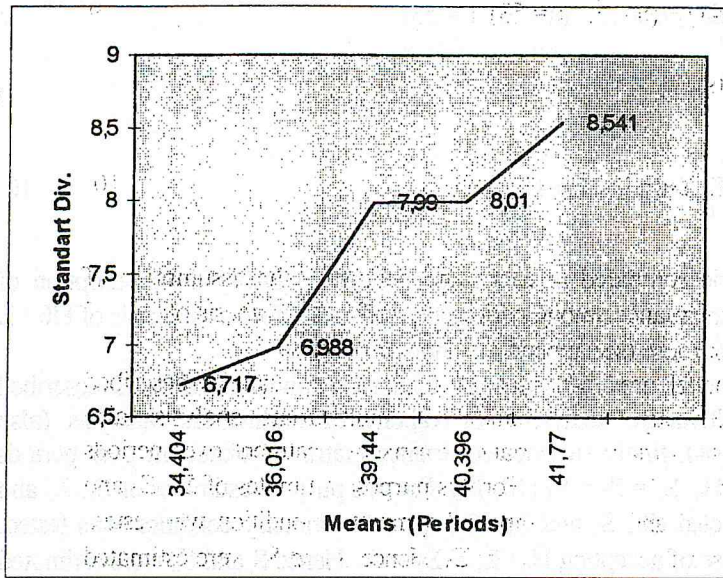


Figure 1. Relation between means standart division of periods

Table 1. Some statistics obtained from three different analysis

Type of analysis	MSE	R ²	C.V.	F (Treatments)
Filtered data	17.967	0.72	11.06	79.72**
Unfiltered data	24.120	0.65	12.79	40.41**
Transformed data	0.068	0.66	4.54	48.74**

Apparently, a considerable improvement is present for filtered data and trivial from transformed data analysis. But this improvements obtained from two different analysis are not answer whether the error model is valid or not. The Huynh-Feldt (H-F) or sphericity condition, a condition which covariance structure for repeated measurement must be homogenous, was performed for validity of analysis. Test for sphericity Mauchly's Criterion was 0.1497463 and chi-square approximation was 76.744 ($P < 0.01$). This indicates that conditions for tests for period, period*treatments and period covariate interactions are significantly questionable for filtered data analysis. Because validity of test for treatments do not demand the H-F condition in repeated measures since it is a element among animals fragment of the analysis. The invalidity of period period*treatments and period covariate interactions can be concluded from partial correlation among

weights measured along five weeks. The magnitude of correlations were decreased as the length of period between weights increased (Table 3). The trend of correlations

Table 2. Partial correlation among weights observed with 15 days interval through 70 days

	Weigth1	Weigth2	Weigth3	Weigth4	Weigth5
Weigth1	1.00				
Weigth2	0.93**	1.00			
Weigth3	0.92**	0.95**	1.00		
Weigth4	0.89**	0.94**	0.96**	1.00	
Weigth5	0.83**	0.92**	0.94**	0.94**	1.00

** (p<0.01)

imply doubt on validity of H-F condition. In addition, Table 3 and Table 4 are constructed to determine that whether filtrated data analysis is correct or not in terms of assumption. The test involves the natural log of ratio of the two determinants from Table 3 and Table 4 matrixes, in an approximate chi-square statistic with $(p^2 + p - 4)/2$ degree of freedom.

Table 3. Auto-regressive residual error variance-covariance matrix (calculated from equation 8)

	Period 1	2	3	4	5
1	621.70	105.69	17.96	3.05	0.52
2	105.69	621.70	105.69	17.96	3.05
3	17.96	105.69	621.70	105.69	17.96
4	3.05	17.96	105.69	621.70	105.69
5	0.52	3.05	17.96	105.69	621.70

Clearly, uniformity is not valid ($P<0.01$) for analysis of data acquired from auto-regressive error model. Therefore assumptions are not valid for that analysis. So, improvement in power of F test by auto-regressive error model has no practical result for inferences. Information obtained from both analysis of variance and partial correlation among weights indicate that time-series method(auto-regressive error model) doesn't support the assumption for repeated measures in animal for

some cases. This finding agree with Gill (2) results. But this conclusion may change depending on the treatments structure (applying drugs at the different growth stages and so on).

Table 4. Sample matrix of pooled residual error variance and covariance

	Period 1	2	3	4	5
1	19.3090	18.0300	16.2170	15.1710	16.4938
2	18.0300	19.4220	17.9906	14.5960	15.5100
3	16.2170	17.9906	29.5022	14.1868	14.4950
4	15.1710	14.5960	14.1868	21.9880	21.6442
5	16.4938	15.5100	14.4950	21.6442	24.1188

The power of F tests of univariate unfiltered for period and first order interactions with period were outperformed by multivariate analysis technique (Table 5). For instance, the F value of multivariate for period was 2.303, whereas this value was 0.27 for unfiltered univariate analysis. Although assumption of uniformity of variance-covariance among periods is justified for univariate analysis (See appendix for test of uniformity). In other words, there is no question for testing period test and the first order interaction with period. However, existing a auto-correlation ($r = 0.158$) over time (period) suggests that the analytical results of multivariate seems to be more powerful then univariate analysis for repeated data (3). The significance of interaction implies that compare of treatment within period and of periods within treatments are in order (2).

Table 5. The F value for repeated measures analysis of variance univariate tests of hypotheses and multivariate for within subject effects

	Period	Period*Treatment	Period*X1	Period*X2	Period*X3
Unfiltered(Uni.)	0.270	2.58**	0.11	0.070	0.04
Multivariate(T ²)	2.303	16.60**	1.404	1.203	0.43

Some of statistical analysis methods interested in analyzing repeated measurement data among a varies of statistical procedure were discussed in this paper. Test of treatments with univariate analysis can be accomplished whether the $H_0: \Sigma_1 = \dots = \Sigma_r$ is rejected. The most important part of analysis is the test and inferences the period and period-treatments or period covariates interaction, accurately. For this purpose some assumption should be checked before using the

univariate analysis. In case of assumption is violated a multivariate test, time-series analysis procedure or transformed data may be used for continuous variables.

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Appendix

Let $\Sigma_1, \Sigma_2, \dots, \Sigma_t$ be the variance-covariance of t treatments. Because the natural tendency of variance to related to means of treatment, one should check the variance-covariance uniformity structure associated t treatments for comparison effects of treatment in a univariate analysis (10). In case of rejecting the $H_0: \Sigma_1 = \Sigma_2 = \dots, \Sigma_t$ test, neither the univariate nor multivariate tests can be used. In the most cases Σ 's are not known and estimators of Σ 's can be estimated from samples. Table 1a, is presented the estimates of three treatments variance-covariance matrices (S_1, S_2, S_3). For test the $H_0: \Sigma_1 = \Sigma_2 = \Sigma_3$ one need q_1, q_3 and q for test the H_0 . These estimated as follows (10, 11),

$$q_1 = [(t+1)/(n-t)] [(2p^2+3p-1)/6(p+1)]$$

$$q_3 = [(n-6) \log_e |S| - \sum_{i=1}^{t-1} (n-1) \log_e |S_i|]$$

$$q = q_3 (1 - q_1) \quad (t=3 \quad p=5 \text{ and } n=53)$$

q was compared with $X^2_{(p^2+p-4)/2, \alpha}$.

The next step after accepting the $H_0: \Sigma_1 = \Sigma_2 = \dots, \Sigma_t$ is to pool the variance-covariance from t different matrices ($t = 1, 2, 3$) and S (Table 1a), estimates of common structure variance-covariance of all treatments (Σ). To test $H_0: \Sigma = \Sigma_0$, chi-square test with $(p^2+p-4)/2$ degree of freedom was used. Where Σ is the mean of population variance-covariance and Σ_0 is uniform variance-covariance matrix of population. Estimates of Σ and Σ_0 are obtained from sample (S and S_0). Both are shown in Table 1a. To test $H_0: \Sigma = \Sigma_0$ one needs h_1, h_3 and q . These were estimated as follows,

$$h_1 = [p(p + 1)^2(2p - 3)]/[6(r - 1)(p - 1)(p^2 + p - 4)].$$

$$h_3 = [-(r - 1)\log_e (|S| / |S_0|)].$$

$$q = (1 - h_1)h_3$$

Table 1a. Variance-covariance matrices for three treatments (S_1 , S_2 , S_3), common variance-covariance matrix (S) and uniformity matrix averaged across periods

	P_1	P_2	P_3	P_4	P_5	
P_1	31.58	16206.70	18479.2	19344.60	20565.60	= S_1
P_2		30.03	19694.9	20622.70	21906.00	
P_3			39.8	23529.50	25005.70	
P_4				35.88	26207.60	
P_5		Symmetry			36.84	
P_1	36.36	16699.20	17917.50	17828.10	18170.30	= S_2
P_2		38.19	18423.40	18426.80	18789.01	
P_3			46.79	19793.70	20173.04	
P_4				37.94	20078.70	
P_5		Symmetry			37.58	
P_1	39.69	13186.90	14129.40	14331.80	14541.90	= S_3
P_2		45.56	14725.40	14951.20	15136.30	
P_3			51.55	16023.40	16227.70	
P_4				46.37	16477.30	
P_5		Symmetry			48.44	
P_1	35.88	15364.27	16842.03	17168.17	17759.27	= S
P_2		37.93	17647.90	18000.23	18610.47	
P_3			45.99	19782.20	20468.93	
P_4				40.06	20822.37	
P_5		Symmetry			40.95	
P_1	40.16	18246.58	18246.58	18246.58	18246.58	= S_0
P_2		40.16	18246.58	18246.58	18246.58	
P_3			40.16	18246.58	18246.58	
P_4				40.16	18246.58	
P_5		Symmetry			40.16	