

REPEATED MEASURES ANALYSIS AND SOME EXPERIMENTAL DESIGN CONSIDERATIONS IN ANIMAL SCIENCE

Dr. Özkan GÖRGÜLÜ

Mustafa Kemal University, Agriculture Faculty, Antakya/Hatay

Assoc. Prof. Dr. Suat ŞAHİNLER

Mustafa Kemal University, Agriculture Faculty, Antakya/Hatay

1. Introduction

Repeated measurements, as the name suggests, are observations of the same characteristic, which are made several times. What distinguishes such observations from those in more traditional statistical data modeling is that the same variable is measured on the same observational unit more than once. The responses are not independent as in the usual regression analysis and more than one observational unit is involved. The responses do not form a simple time series. To many animal scientists, a mention of the term, repeated measurements evokes the idea either of the fisheries study of growth curves or split plot designs. However, once one begins to delve into the subject, one realizes that these two subjects, in no way, completely cover the field of repeated observations. In fact, repeated measurements are very frequent not only in animal science experiments but also in almost all scientific fields where statistical models are used [1].

Few animals may be available (or few used, because of complex technique) in experiments with non-random repeated measurement (e.g. p animals in each of r treatment groups, each measured in p periods). In such cases, the use of summary statistics for each animal to eliminate the time factor, or ordinary univariate split-plot tests of the treatment means or multivariate analysis is inadvisable, because comparison of the treatments are not sufficiently sensitive for any of those procedures. The problem is that main effects of treatments must be tested by the mean square for the animals within treatments, which is inflated by positive correlations among repeated observations. Even conditional tests (e.g. comparisons of treatments within periods), as well as tests of means of summary statistics, can not be very sensitive; because, with low replication, the standard errors of mean differences are not much smaller than the ordinary (error) standard deviation among animals treated alike, without the influence of correlations induced by repeated measurement. In severe restriction of numbers of animals leaves few degrees of freedom for error, either reducing statistical power drastically or preventing multivariate analysis entirely [2]. In such cases, the primary benefit of a Repeated Measures Design (RMD) is statistical power relative to sample size, which is important in many real researches. RMD use the same subjects throughout different treatments and thus, require fewer subjects overall. Because the

subjects are constant, the variance due to subjects can be partitioned into the error variance term, thereby making any statistical test more powerful [3, 4].

RMDs are quite versatile, and researchers use many different designs and call the designs by many different names. For example, a one way repeated measures ANOVA may be considered as a one-factor within subjects ANOVA. A two way repeated measures ANOVA may be referred to as a two-way within subjects ANOVA. These designs are called related samples models, matched samples models, longitudinal studies and within-subject designs [4,5]. In RMDs, total variation consist of two parts as Between-Subject Factor(s) (BSF) or non-repeated factor(s) and Within-Subject Factor(s) (WSF) or repeated factor(s), and an error terms are computed for each source of variation. Having effect of animal within treatment removed from experimental error reduces experimental error, this provides having the researchers make a more reliable decision [6]. A BSF is a non-repeated or grouping factor, such as race or experimental group, for which subjects will appear in only one level. A WSF is repeated factors for which subjects will participate in each level e.g. subjects participate in both experimental conditions, albeit at different times.

In repeated measures experimental design the following assumptions should be validated;

The measurement errors are independent, and identically normally distributed with mean 0 and the same variance.

The subjects are considered to be a random sample from the subject population of interest, so that the subject effect is random.

Measurements from the same subject will be positively correlated. It is assumed that the variance of the difference between the estimated means for any two different factor levels will be the same. This property is called sphericity. A slightly more restrictive assumption is that the covariance between observations within any be the same for any two different factor levels. This property is called compound symmetry. Compound symmetry is a special case of more general property sphericity. If compound symmetry exists, then sphericity also exists, but it is possible for sphericity to exist when compound symmetry does not [7,8].

Alternative analytic techniques are available when assumptions validity is dubious. These include an ϵ adjustment procedure based on Geisser and Greenhouse (1958) and a multivariate analysis using Hotelling's T^2 statistic [4, 8, 9, 10].

In this paper, the analysis methods of RMD with one, two and three factors containing repeated measures with one and two factors are given for comparing treatments in a way that permit sensitive tests, often when the number of animals per treatment is inadequate. Four real data sets obtained from real animal science experiments in different disciplines were used to illustrate the procedures.

2. Material and Methods

In this study, four real data sets obtained from four real animal science experiments in different disciplines are used to illustrate the different repeated measures design procedure.

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I) Data taken from an experiment conducted determine differences with regard to fat tissue content (g) among four sections (long leg, flank, the first five ribs and remaining ribs) of ten fat tail Awassi lamb carcasses were used. Here, WSF is a different part of lamb carcasses. These data were used to illustrate analysis of one-way RMD.

II) Data from a fattening experiment on Awassi lambs. The objective of this experiment was to determine the effects of sex and different feeding periods on daily gain weight of lambs. In the experiment, twelve lambs were used, six of which are male and six of which are female. The daily gain weight of lambs (kg) was measured in each 15 days period from birth to 60 days old. Here, WSF is periods and BSF is gender as well. This data were used to illustrate analysis of two-way RMD.

III) Data were used relating to the experiment on broiler. The objective of this experiment was to determine the effects of dehydroepiandrosterone (0mg, 30mg, 60mg) and corticosteron (0mg, 10mg, 20mg) hormones on feed consumption of broilers. In the experiment, 54 broilers were used, which were 24 days old. All broilers were divided in to 9 groups and in each group there are 6 broilers, and nine (3x3=9) different rations each of which contain combination of three levels of dehydroepiandrosterone (0mg, 30mg, 60mg) and corticosteron (0mg, 10mg, 20mg) hormone was given to animal groups randomly. The daily feed consumptions of broilers (g/day) were measured during 28 days and the measurements were put in order as periods weekly. In this study, WSF is periods and BSF are also dehydroepiandrosteron and corticosteron hormone. These data were used to illustrate analysis of repeated measures designs with three factors, one of which is WSF (C) with p levels (p treatments or periods).

IV) Data were used relating to the experiment on broiler. The objective of this experiment was to determine the effects of sex, feeding and heat on feed consumption of broilers. In the experiment, 14 broilers were used, seven of which are male and seven of them are female. Each of group was fed with two feeds, which contain different protein (concentrated, corn) at three heat levels (28 °C, 30 °C and 32 °C) and the daily feed consumptions of broilers (g/day) were measured. Here, BSF is sex, and WSF is also feed and heat. These data were used to illustrate analysis of repeated measures designs with three factors of which two are WSFs (B and C) with q and r levels.

The experiments with repeated measures are designed in different ways as to number of BSF and WSF.

One-Way Repeated Measures Design

It is the simplest design among the RMDs. There is one factor in this design and all of the experimental units are taken into experiment within the factor levels. A repeated factor might be different time points (periods), different treatments or the different levels of the same treatment [11].

The model of the design is,

$$y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij} \quad i=1,2,3,\dots,n ; j=1,2,3,\dots,p, \quad (1)$$

where μ is overall mean, α_i is the effect of i^{th} animal, β_j is the effect of j^{th} period or treatment ε_{ij} is error term [3].

Table 1 presents a general illustration of one-way repeated measures designs with n subject and p treatments or periods (repeated measures).

In one-way RMD, the sources of total variation are the between subject and within subject variations, and the aim is to test the differences among the periods or treatments. The sources of within subject variation are also variation among the treatments and error [11,12,13]. Thus,

$$SS_{TOTAL} = SS_{BETWEEN-SUBJECT} + SS_{WITHIN-SUBJECT}$$

$$= SS_{BETWEEN-SUBJECT} + SS_{TREATMENT} + SS_E$$

All sources of variations are computes as follows;

$$CT = \left[\sum_{i=1}^n \sum_{j=1}^p y_{ij} \right]^2 / np ,$$

$$SS_{TOTAL} = \sum_{i=1}^n \sum_{j=1}^p (y_{ij} - \bar{y}_{..})^2 = \sum_{i=1}^n \sum_{j=1}^p y_{ij}^2 - CT ,$$

$$SS_{TREATMENT} = p \sum_{i=1}^n (\bar{y}_{i.} - \bar{y}_{..})^2 = \sum_{i=1}^n y_{i.}^2 / p - CT ,$$

$$SS_{BETWEEN-SUBJECT} = \sum_{j=1}^p y_{.j}^2 / n - CT ,$$

$$SS_{WITHIN-SUBJECT} = SS_{TOTAL} - SS_{BETWEEN-SUBJECT} ,$$

$$SS_E = SS_{TOTAL} - SS_{BETWEEN-SUBJECT} - SS_{TREATMENT} = SS_{WITHIN-SUBJECT} - SS_{TREATMENT}$$

Table 2 presents analysis of variance (ANOVA) summary table contains the results of all computations for general.

The general ANOVA table with one way repeated measures differs from one way independent samples ANOVA table such that, the row for subjects acts as another factor and the residual or error term is the interaction between subjects and treatments, not real error (The real error is the differences among the experimental units, which subject to same treatment). This difference arises because subjects are constant through the treatments and; thus, subject effects may be partitioned out of the error variance. There is still only one effect of interest, treatments, with only one test statistic.

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Two- Way Repeated Measures Design

There are two factors, one of them BSF (A) and the other is WSF (B) in this designs. Because the experimental units were classified as one inside the other with factor levels of A but as factorial with factor levels of B, interaction between experimental units and factor A could not be considered.

In two-way RMD, the sources of total variation are separated two parts as the between subject ($SS_{\text{BETWEEN-SUBJECT}}$) and within subject variations ($SS_{\text{WITHIN-SUBJECT}}$). Therefore,

$$SS_{\text{BETWEEN-SUBJECT}} = SS_A + SS_{E1} \text{ and } SS_{\text{WITHIN-SUBJECT}} = SS_B + SS_{A \times B} + SS_{E2}$$

Thus, the model of the design is [2,14,15,16],

$$y_{ijk} = \mu + \alpha_i + \gamma_{(i)k} + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{(i)jk}, \quad i=1,2,\dots,n; j=1,2,\dots,p; k=1,2,\dots,q,$$

where μ is overall mean, α_i is the effect of i^{th} level of factor A, β_j is the effect of j^{th} period or treatment, $(\alpha\beta)_{ij}$ is interaction effect of A and B, $\gamma_{(i)k}$ is the effect of k^{th} experimental unit in i^{th} level of the A factor (Error 1) and $\varepsilon_{(i)jk}$ is error term (Error 2) [3].

Table 3 presents a general illustration for two-way repeated measures designs with n levels of BSF, q subjects and p treatments or periods (repeated measures). As seen in Table 3, while the measurements are taken from different experimental units in each levels of BSF (A), they are taken from the same experimental unit in all levels of WSF (B).

All sources of variations are calculated as follows [10,13];

$$CT = \left[\sum_{i=1}^n \sum_{j=1}^p \sum_{k=1}^q y_{ijk} \right]^2 / npr,$$

$$SS_{\text{TOTAL}} = \sum_{i=1}^n \sum_{j=1}^p \sum_{k=1}^q y_{ijk}^2 - CT,$$

$$SS_{\text{BETWEEN -SUBJECT}} = \sum_{i=1}^n \sum_{k=1}^q y_{i.k}^2 / p - CT,$$

$$SS_A = \sum_{i=1}^n y_{i..}^2 / pq - CT,$$

$$SS_{E1} = SS_{\text{BETWEEN -SUBJECT}} - SS_A,$$

$$SS_{\text{WITHIN -SUBJECT}} = SS_{\text{TOTAL}} - SS_{\text{BETWEEN -SUBJECT}},$$

$$SS_B = \sum_{j=1}^p y_{.j}^2 / nq - CT ,$$

$$SS_{AxB} = \left[\sum_{i=1}^n \sum_{j=1}^p y_{ij}^2 / q - CT \right] - SS_A - SS_B ,$$

$$SS_{E2} = SS_{WITHIN-SUBJECT} - SS_B - SS_{AxB} .$$

Table 4 presents analysis of variance (ANOVA) summary table contains the results of all computations for general.

Error 1 is used to test factor A, and Error 2 is used to test B and AxB [14]. Testing AxB interaction effect is more as than comparing the main effects (A and B).

ρ in Table 4 is coefficient of correlation which denotes the total correlation between two levels of factor that come to one after another and contain repeated measurement [2,14], and computed as;

$$\rho = [MS_{E1} - MS_{E2}] / p \sigma^2$$

where p is the number of periods or treatments, σ^2 is the variation among the experimental units which are in the same treatment and $\sigma^2 = [MS_{E1} + (p-1)MS_{E2}] / p$. If the measurements are taken from different experimental units, $\rho = 0$.

Three- Way Repeated Measures Design

There are differences among these designs based on the number of factors included in the repeated measures.

Table 5 presents a general three-way repeated measures design; one of which is WSF (C is a factor contains repeated measures) with p levels (p treatments or periods) and two of which are BSFs (A and B) with n and q levels. As seen in Table 5, while the measurements are taken from different experimental units in each combination of levels of BSFs A and B, they are taken from the same experimental unit in all levels of WSF (C).

The model of the design is

$$y_{ijkm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \pi_{m(ij)} + \gamma_k + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\pi\gamma)_{km(ij)} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkm},$$

$$i = 1, 2, \dots, n; \quad j = 1, 2, \dots, q; \quad k = 1, 2, \dots, p; \quad m = 1, 2, \dots, r ,$$

where μ is overall mean, α_i is the effect of i^{th} level of factor A, β_j is the effect of j^{th} level of factor B, $(\alpha\beta)_{ij}$ is interaction effect of A and B, $\pi_{m(ij)}$ is the effect of m^{th} experimental unit in i^{th} level of the A factor and j^{th} level of B factor (Error 1), γ_k is the

effect of k^{th} level of factor C, $(\alpha\gamma)_{ik}$ is interaction effect of A and C, $(\beta\gamma)_{jk}$ is interaction effect of B and C, $(\pi\gamma)_{km(ij)}$ is interaction effect of experimental unit and factor C, $(\alpha\beta\gamma)_{ijk}$ is interaction effect of A, B and C, and ε_{ijkm} is error term which is the interaction between factor C (repeated factor) and Error 1 (Error 2)[17].

All sources of variations are computes as follows;

$$CT = \left[\sum_{i=1}^n \sum_{j=1}^q \sum_{k=1}^p \sum_{m=1}^r y_{ijkm} \right]^2 / nqpr$$

$$SS_{TOTAL} = \sum_{i=1}^n \sum_{j=1}^q \sum_{k=1}^p \sum_{m=1}^r y_{ijkm}^2 - CT ,$$

$$SS_{BETWEEN-SUBJECT} = \sum_{i=1}^n \sum_{j=1}^q \sum_{m=1}^r y_{ij..m}^2 / p - CT ,$$

$$SS_A = \sum_{i=1}^n y_{i...}^2 / qpr - CT ,$$

$$SS_B = \sum_{i=1}^n \sum_{j=1}^q y_{.j..}^2 / npr - CT ,$$

$$SS_{AxB} = \left[\sum_{i=1}^n \sum_{j=1}^q y_{ij..}^2 / pr - CT \right] - (SS_A + SS_B) ,$$

$$SS_{E1} = SS_{BETWEEN-SUBJECT} - (SS_A + SS_B + SS_{AxB}) ,$$

$$SS_{WITHIN-SUBJECT} = SS_{TOTAL} - SS_{BETWEEN-SUBJECT} ,$$

$$SS_C = \sum_{k=1}^p y_{..k.}^2 / nqr - CT ,$$

$$SS_{AxC} = \left[\sum_{i=1}^n \sum_{k=1}^p y_{i.k.}^2 / qr - CT \right] - (SS_A + SS_C) ,$$

$$SS_{BxC} = \left[\sum_{j=1}^q \sum_{k=1}^p y_{.jk.}^2 / nr - CT \right] - (SS_B + SS_C) ,$$

$$SS_{AxBxC} = \left[\sum_{i=1}^n \sum_{j=1}^q \sum_{k=1}^p y_{ijk}^2 / r - CT \right] - [(SS_{AxB} + SS_{AxC} + SS_{BxC}) + (SS_A + SS_B + SS_C)],$$

$$SS_{E2} = SS_{WITHIN-SUBJECT} - (SS_C + SS_{AxC} + SS_{BxC} + SS_{AxBxC}).$$

Table 6 presents analysis of variance (ANOVA) summary table three-way repeated measures design one of which is WSF with p levels (p treatments or periods) in general. As seen in Table 6, Error 1 is used to test the effects of factor A, B and AxB, and Error 2 is used to test the effects of factor C and AxC, BxC and AxBxC..

Table 7 presents a general three-way repeated measures design, two of which are WSFs (B and C contain repeated measures) with q and r levels and one of them is BSF (A) with n levels [6]. As seen in Table 7, while the measurements are taken from different experimental units in each level of BSF A, they are taken from the same experimental unit in each combination of levels of WSFs B and C.

The model of the design is [15, 17]

$$y_{ijkl} = \mu + \alpha_i + \beta_k + \pi_l + (\alpha\beta)_{ik} + (\alpha\pi)_{il} + (\beta\pi)_{kl} + (\alpha\beta\pi)_{ikl} + \gamma_{(i)j} + (\pi\gamma)_{(i)jl} + (\beta\gamma)_{(i)jk} + \varepsilon_{(i)jkl}, \quad i = 1, 2, \dots, n; \quad j = 1, 2, \dots, t; \quad k = 1, 2, \dots, r; \quad l = 1, 2, \dots, q,$$

where μ is overall mean, α_i is the effect of i^{th} level of factor A, β_k is the effect of k^{th} level of factor C, π_l is l^{th} level of factor B, $(\alpha\beta)_{ik}$ is interaction effect of A and C, $(\alpha\pi)_{il}$ is interaction effect of A and B, $(\beta\pi)_{kl}$ is interaction effect of B and C, $(\alpha\beta\pi)_{ikl}$ is interaction effect of A, B and C, $\gamma_{(i)j}$ is the effect of j^{th} experimental unit in i^{th} level of the A factor (Error 1), $(\pi\gamma)_{(i)jl}$ is interaction effect of A and subject (Error 2), $(\beta\gamma)_{(i)jk}$ is the interaction effect of C and subject (Error 3), $\varepsilon_{(i)jkl}$ is the interaction effect of B, C and subject (Error 4).

All sources of variations are computes as follows;

$$CT = \left[\sum_{i=1}^n \sum_{k=1}^r \sum_{j=1}^t \sum_{l=1}^q y_{ijkl} \right]^2 / ntrq,$$

$$SS_{TOTAL} = \sum_{i=1}^n \sum_{k=1}^r \sum_{j=1}^t \sum_{l=1}^q y_{ijkl}^2 - CT,$$

$$SS_{BETWEEN-SUBJECT} = \sum_{i=1}^n \sum_{j=1}^t y_{ij..}^2 / rq - CT,$$

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$$SS_A = \sum_{i=1}^n y_{i...}^2 / tqr - CT ,$$

$$SS_{E1} = SS_{BETWEEN-SUBJECT} - SS_A ,$$

$$SS_{WITHIN-SUBJECT} = SS_{TOTAL} - SS_{BETWEEN-SUBJECT} ,$$

$$SS_B = \sum_{l=1}^q y_{...l}^2 / ntr - CT ,$$

$$SS_{AxB} = \left[\sum_{i=1}^n \sum_{l=1}^q y_{i..l}^2 / tr - CT \right] - SS_B - SS_A ,$$

$$SS_{E2} = \left[\sum_{i=1}^n \sum_{j=1}^t \sum_{l=1}^q y_{ij.l}^2 / r \right] - CT - SS_{BETWEEN-SUBJECT} - SS_B - SS_{AxB} ,$$

$$SS_C = \sum_{l=1}^q \sum_{k=1}^r y_{..kl}^2 / ntq - CT ,$$

$$SS_{AxC} = \left[\sum_{i=1}^n \sum_{k=1}^r y_{i.k.}^2 / qt - CT \right] - SS_C - SS_A ,$$

$$SS_{E3} = \left[\sum_{i=1}^n \sum_{j=1}^t \sum_{k=1}^r y_{ijk.} / q - CT \right] - SS_{BETWEEN-SUBJECT} - SS_C - SS_{AxC}$$

$$SS_{BxC} = \left[\sum_{k=1}^r \sum_{l=1}^q y_{..kl}^2 / nt - CT \right] - SS_B - SS_C ,$$

$$SS_{AxBxC} = \left[\sum_{i=1}^n \sum_{k=1}^r \sum_{j=1}^q y_{i.kl}^2 / t - CT \right] - SS_A - SS_C - SS_{AxC} - SS_B - SS_{AxB} - SS_{BxC} ,$$

$$SS_E = SS_{W.SUBJECT} - SS_B - SS_{AxB} - SS_{E2} - SS_C - SS_{AxC} - SS_{E3} - SS_{BxC} - SS_{AxBxC}$$

Table 8 presents analysis of variance (ANOVA) summary table for three-way repeated measures design, two of them are WSFs with q and r levels in general. As seen in Table 8, Error 1 is used to test the effects of factor A, Error 2 is used to test the effects of factor B and AxB, Error 3 is used to test the effects of factor C and AxC and Error 4 is used to test the effects of BxC and AxBxC.

The statistical packages, EXCEL and SPSS FOR WINDOWS, were used for the statistical analysis of these data.

In SPSS, input the repeated measures data and analyzed are easier than other experimental design. Within subject factor levels input different columns side by side, on the contrary between subject levels input the same column categorically (Figure 1a). When the researchers follow the Analyze > General Linear Model > Repeated Measures paths (Figure 1b), Repeated Measures define factor(s) dialog box opens (Figure 1c). In this dialog box are defined within subject factor and this factor levels. Later, click on the define button and then Repeated measures dialog box opens (Figure 1d). In this dialog box within subject factor is moved into the within subject factor box. Between subject factor is moved into the between subject factor box. In addition, using the buttons on this dialog box might do some definitions shown in Figure 1d. SPSS gives effects of WSF and BSF in the individual ANOVA table at the end of the analysis. As a result of the later analyzed researcher can easily interpret their outputs [6,18].

3. Results

The data from the studies I, II, III, and IV were analyzed by using equations presented in material and method. The results were given in Table 9, 10, 11 and 12 respectively.

The results in Table 9 were found by analyzing with one way repeated measures design for the experiment I. In the analysis the source of within subject variation consists of sections and Error. Error originated variation among lambs in the same carcass sections (e.g. long leg, shoulder, neck, flank, the first five ribs or remaining ribs). Sections effects were analyzed according to error 1 and were found significant ($p < 0.01$). For this reason we concluded that there were significance differences among the different carcass sections with regard to fat contents in Awassi lambs.

Analyzing with two-way repeated measures design for the experiment II in which period and sex effect on daily gain weight of lambs were examined generated the results in Table 10. The source of between subject variation and within subject variation consists of sex and Error 1, and period, period x sex and Error 2, respectively. Error 1 was originated from variation lambs in the same sex and period. Error 2 was originated from error1 x period interaction effect. In the experiment II, sex effect was analyzed according to Error 1, while period effect was analyzed according to Error 2. Results showed that sex, period and period x sex interaction effects were not significant for daily gain weight of lambs ($p > 0.05$) (Table 10).

In the experiment III, the source of between subject variation and within subject variation consists of dehydroepiandrosterone, corticosteron, dehydroepiandrosterone x corticosteron and Error 1 effects; and week, dehydroepiandrosterone x week, corticosteron x week, dehydroepiandrosterone x corticosteron x week and Error 2, respectively. In the experiment III, dehydroepiandrosterone, corticosteron and dehydroepiandrosterone x corticosteron effects were analyzed according to Error 1. Week, dehydroepiandrosterone x week, corticosteron x week and dehydroepiandrosterone x corticosteron x week effects were analyzed according to Error 2. Results showed (Table 11) that week and week x

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corticosteron interaction effects were found significant on feed consumption of broilers ($p < 0.01$). Dehydroepiandrosterone and corticosteron effects were not significant ($p > 0.05$). Similarly dehydroepiandrosterone x week, dehydroepiandrosterone x corticosteron, dehydroepiandrosterone x corticosteron x week effects were not significant on feed consumption of broilers ($p > 0.05$).

In the experiment IV, there were four error terms. Error 1, 2, 3 and 4 originated from variation of subjects in the same sex, heat x subject interaction effect, feed x subject interaction effect and feed x heat x subject interaction effect, respectively. Between subject variation and within subject variation consists of sex and Error 1, and heat, heat x sex, Error 2, feed, feed x sex, Error 3, feed x heat, feed x heat x sex and Error 4, respectively. Sex effect was analyzed according to Error 1. Heat and heat x sex effects were analyzed according to Error 2. Feed and feed x sex effects were analyzed according to Error 3. Feed x heat and feed x heat x sex effects were analyzed according to Error 4. Results (Table 12) showed that heat, feed and feed x heat effects on feed consumption of broilers were found significant ($p < 0.01$). While sex, heat x sex, feed x sex, and feed x heat x sex effects were not found significant ($p > 0.05$).

4. Discussion

Finding uniform subjects for animal science research is quite difficult. Repeated measures design deals with this problem because researchers observe on the same subject under the different periods and treatments in repeated measured design. It may be more economical in terms of time and effort to test the same subjects under each treatment or each period. Because of the reason explained above repeated measures design needs fewer subjects than the other traditional experimental design. Another advantage is that the number of treatment or periods does not affect amount of subject in experiments. Thus researchers can act in a more flexible.

Besides mentioned advantages of RMD, there are two disadvantages: carry-over effect and latent effect. Carry-over effect is the effect of earlier treatment on the following one. For example, each subject may receive each of three different drugs for two weeks. In a situation like this, the order of the different drugs should be randomized for each subject, to reduce carry-over or trend effects. It may also be necessary to allow one drug time to wear off before applying the next, to avoid carry-over effects. It is possible to prevent from the effect of disadvantage by regulating the treatment intervals. Therefore the researcher should not apply the second treatment before the effect of earlier treatment terminated. On the other hand, if one or more of the treatments will have a long-lasting or permanent effect, making it impossible to avoid carry over effects, then a repeated measures design cannot be used. Latent effect is the interaction of between successive treatments. This negative effect may be discarded by randomized design of the treatments [6, 10, 13, 19].

Since the treatment in RMD due to time the usage of the treatments with long-term effect reduce usefulness of this experimental design. Thus, these designs are mostly used in human and animal when a treatment with short-term effect applied.

There are some similarities between RMD and traditional experimental designs. A one-way blocked ANOVA with random blocks is analyzed the same way as a repeated measures design with one repeated measures factor. The subjects are the blocks, and each subject either receives each treatment over time, or the same treatment evaluated at different times. If the main goal of the analysis is simply to test the significance of the treatment effect, the assumption of no interaction between subjects and treatments can be relaxed for a one repeated measures factor ANOVA with random subjects. The overall F test is the same as for the no interaction case. Under compound symmetry, the correlation between two observations from the same subject will still be constant, but will not be the same as in the no-interaction case.

If there are only two levels of the repeated measures, the overall F test is equivalent to a paired t test, and sphericity is guaranteed.

The structure of three-ways RMD with two Within-Subject factors is similar to split-plot in time experimental design. In time experimental design with split-plot measures are taken the same subject and the different period by observation. Each main plots in this design equals to a subject in RMD. Main plots are homogenous between each other. Analysis of variance tables are similar for both experimental designs. The only difference between them is that being block factor and block x year interaction in time experimental design with split-plot. In time experimental design with split-plot it is not possible to analyze the data when there is no block. Since, the blocks are taken as repeats. Although repeats are the subjects in RMD, there is no need for blocks. Therefore, there is no mixed effect originated from block. Similarly, RMD with two WSF is similar to split plot experimental designs. Main plots in split-plots experimental design equivalent of BSF, subplots equivalent of WSF or different times in period. The difference between two-way RMD and split plot experimental design is that being not possible to assign random levels of WSF to the subject in RMD due to impossibility of time intervene. Each subject has different covariance matrix because of unrandomized assign. These matrixes are not suitable for split-plot experimental design.

It is assumed that main plots are homogenous between each other in the split plots experimental design. There are uncontrolled effects between subplots. These effects are attributed and increasing the errors. The error is fewer in RMD, because taken measures on the same subject. For this reason, the outcomes are more confident.

The analysis of data in a RMANOVA is quite complex. The complexity of RMANOVA stems largely from the fact that RMANOVA is quite sensitive to violations of one of major assumptions of the test. If data for one or more of samples to be analyzed by repeated measures factor ANOVA come from a population whose distributions violates the assumption of normality, or outliers are present, the ANOVA on the original data may provide misleading results, or may not be the most powerful test available. In such cases, transforming the data or using nonparametric tests may provide a better analysis. Transformations are applied to correct problems of nonnormality or unequal variances. Nonparametric tests are tests that do not make the usual distributional assumptions of the normal-theory-based tests. For the one-way

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RMANOVA and two-way RMANOVA, the most common nonparametric alternative tests are Friedman's test.

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Table 1. A general illustration of one-way repeated measures designs

Subject (BSF)	Within Subject Factor (WSF)					Σ
	1	2	3	. . .	p	
1	Y_{11}	Y_{12}	Y_{13}	. . .	Y_{1p}	$Y_{1\cdot}$
2	Y_{21}	Y_{22}	Y_{23}	. . .	Y_{2p}	$Y_{2\cdot}$
.
.
.
n	Y_{n1}	Y_{n2}	Y_{n3}	. . .	Y_{np}	$Y_{n\cdot}$
Σ	$Y_{\cdot 1}$	$Y_{\cdot 2}$	$Y_{\cdot 3}$. . .	$Y_{\cdot p}$	$Y_{\cdot \cdot}$

Table 2. Summary of analysis of variance for a general one way repeated measures design

Source of Variation	DF	MS (SS/DF)	F	E(MS)
Between Subject	n-1			
Within Subject	n(p-1)			
Treatment	p-1	$SS_{TREATMENT} / p - 1$	$MS_{TREATMENT} / MS_E$	$\sigma_\varepsilon^2 + \sigma_{\alpha\beta}^2 + r\sigma_\alpha^2$
Error	(n-1)(p-1)	$SS_E / (n - 1)(p - 1)$		$\sigma_\varepsilon^2 + \sigma_{\alpha\beta}^2$
Total	np-1			

Table 3. A general illustration for two-way repeated measures designs

Between Subject Factor	Subject	Within Subject Factor				Σ	Σ
		B ₁	B ₂	...	B _p		
A ₁	1	Y ₁₁₁	Y ₁₂₁	...	Y _{1p1}	Y _{1·1}	Y _{1··}
	2	Y ₁₁₂	Y ₁₂₂	...	Y _{1p2}	Y _{1·2}	
	·	·	·	...	·	·	
	·	·	·	...	·	·	
	q	Y _{11q}	Y _{12q}	...	Y _{1pq}	Y _{1·q}	
A ₂	1	Y ₂₁₁	Y ₂₂₁	...	Y _{2p1}	Y _{2·1}	Y _{2··}
	2	Y ₂₁₂	Y ₂₂₂	...	Y _{2p2}	Y _{2·2}	
	·	·	·	...	·	·	
	·	·	·	...	·	·	
	q	Y _{21q}	Y _{22q}	...	Y _{2pq}	Y _{2·q}	
·	·	·	...	·	·	·	
·	·	·	...	·	·	·	
A _n	1	Y _{n11}	Y _{n21}	...	Y _{np1}	Y _{n·1}	Y _{n··}
	2	Y _{n12}	Y _{n22}	...	Y _{np2}	Y _{n·2}	
	·	·	·	...	·	·	
	·	·	·	...	·	·	
	q	Y _{n1q}	Y _{n2q}	...	Y _{npq}	Y _{n·q}	
Σ		Y _{·1·}	Y _{·2·}	...	Y _{·p·}	Y _{···}	

Table 4. Summary of analysis of variance for a general two-way

repeated measures design

Source of variation	DF	MS (SS/DF)	F	E(MS)
Between Subject	n (q-1)			
A	n-1	$SS_A / (n - 1)$	MS_A / MS_{E1}	$\sigma_\epsilon^2 [1 + (q-1)\rho] + pq\sigma_\alpha^2$
Error 1	n (q-1)	$SS_{E1} / n(q - 1)$		$\sigma_\epsilon^2 [1 + (q - 1)\rho]$
Within Subject	nq (p-1)			
B	p-1	$SS_B / (p - 1)$	MS_B / MS_{E2}	$\sigma_\epsilon^2 (1 - \rho) + nq\sigma_\beta^2$
AxB	$(n-1)(p-1)$	$SS_{AxB} / (n-1)(p-1)$	MS_{AxB} / MS_{E2}	$\sigma_\epsilon^2 (1 - \rho) + q\sigma_{\alpha\beta}^2$
Error 2	$n(q-1)(p-1)$	$SS_{E2} / n(q-1)(p-1)$		$\sigma_\epsilon^2 (1 - \rho)$
TOTAL	npq-1			

Table 5. A general illustration of three-way repeated measures design, which of them is WSF (C) with p levels (p treatments or periods).

Between Subject Factor		Subject	Within Subject Factor				Σ	Σ	Σ
A	B		C ₁	C ₂	...	C _p			
A ₁	B ₁	1	Y ₁₁₁₁	Y ₁₁₂₁	...	Y _{11p1}	Y _{11·1}	Y _{11··}	Y _{1···}
		2	Y ₁₁₁₂	Y ₁₁₂₂	...	Y _{11p2}	Y _{11·2}		
		·	·	·	...	·	·		
		·	·	·	...	·	·		
		r	Y _{111r}	Y _{112r}	...	Y _{11pr}	Y _{11·r}		
	·	·	·	·	...	·	·	·	
	·	·	·	·	...	·	·		
	·	·	·	·	...	·	·		
	B _q	1	Y _{1q11}	Y _{1q21}	...	Y _{1qp1}	Y _{1q·1}	Y _{1q··}	
		2	Y _{1q12}	Y _{1q22}	...	Y _{1qp2}	Y _{1q·2}		
		·	·	·	...	·	·		
		·	·	·	...	·	·		
r		Y _{1q1r}	Y _{1q2r}	...	Y _{1qpr}	Y _{1q·r}			
·	·	·	·	...	·	·	·		
·	·	·	·	...	·	·	·		
·	·	·	·	...	·	·	·		
A _n	B ₁	1	Y _{n111}	Y _{n121}	...	Y _{n1p1}	Y _{n1·1}	Y _{n1··}	Y _{n···}

		2	Y _{n112}	Y _{n122}	...	Y _{n1p2}	Y _{n1·2}	
		
		
		
		r	Y _{n11r}	Y _{n12r}	...	Y _{n1pr}	Y _{n1·r}	
	
	
	
	B _q	1	Y _{nq11}	Y _{nq21}	...	Y _{nqp1}	Y _{nq·1}	Y _{nq··}
		2	Y _{nq12}	Y _{nq22}	...	Y _{nqp2}	Y _{nq·2}	
.			
.			
r		Y _{nq1r}	Y _{nq2r}	...	Y _{nqpr}	Y _{nq·r}		
Σ		Y _{·1·}	Y _{·2·}	...	Y _{·p·}	Y _{·...}		

Table 6. Summary of analysis of variance for repeated measures desing with three factors one of which is WSF with p levels (p treatments or periods)

Source of variation	DF	MS (SS/DF)	F	E(MS)
Between Subject	nqr-1			
A	n-1	$SS_A / (n - 1)$	MS_A / MS_{E1}	$\sigma_\epsilon^2 + q\sigma_\pi^2 + prq\sigma_\alpha^2$
B	q-1	$SS_B / q - 1$	MS_B / MS_{E1}	$\sigma_\epsilon^2 + q\sigma_\pi^2 + nqr\sigma_\beta^2$
AB	(n-1)(q-1)	$SS_{AB} / (n - 1)(q - 1)$	MS_{AB} / MS_{E1}	$\sigma_\epsilon^2 + q\sigma_\pi^2 + qr\sigma_{\alpha\beta}^2$
Error 1	nq(r-1)	$SS_{E1} / nq(r - 1)$		$\sigma_\epsilon^2 + q\sigma_\pi^2$
Within Subject	rnq (p-1)			
C	p-1	$SS_C / (p - 1)$	MS_C / MS_{E2}	$\sigma_\epsilon^2 + \sigma_{\gamma\pi}^2 + nqr\sigma_\gamma^2$
AxC	(n-1)(p-1)	$SS_{AC} / (n - 1)(p - 1)$	MS_{AC} / MS_{E2}	$\sigma_\epsilon^2 + \sigma_{\gamma\pi}^2 + qr\sigma_{\alpha\gamma}^2$
BxC	(q-1)(p-1)	$SS_{BC} / (q - 1)(p - 1)$	MS_{BC} / MS_{E2}	$\sigma_\epsilon^2 + \sigma_{\gamma\pi}^2 + nr\sigma_{\beta\gamma}^2$
AxBxC	(n-1)(q-1)(p-1)	$SS_{ABC} / (n - 1)(q - 1)(p - 1)$	MS_{ABC} / MS_{E2}	$\sigma_\epsilon^2 + \sigma_{\gamma\pi}^2 + r\sigma_{\alpha\beta\gamma}^2$
Error 2	nq(r-1)(p-1)	$SS_{E2} / nq(r - 1)(p - 1)$		$\sigma_\epsilon^2 + \sigma_{\gamma\pi}^2$
Total	nqpr-1			

Table 7. A general illustration of three-way repeated measures design, two of them are WSFs (B and C) with q and r levels

Between Subject Factor	Subject	Within Subject Factor								Σ	Σ	
		B ₁				...	B _q					
		C ₁	C ₂	...	C _r	...	C ₁	C ₂	...			C _r
A ₁	1	Y ₁₁₁₁	Y ₁₁₂₁	...	Y _{11r1}	...	Y _{111q}	Y _{112q}	...	Y _{11rq}	Y _{11..}	Y _{1...1}
	2	Y ₁₂₁₁	Y ₁₂₂₁	...	Y _{12r1}	...	Y _{121q}	Y _{122q}	...	Y _{12rq}	Y _{12..}	
	
	
	t	Y _{1t11}	Y _{1t21}	...	Y _{1tr1}	...	Y _{1t1q}	Y _{1t2q}	...	Y _{1trq}	Y _{1p..}	
A ₂	1	Y ₂₁₁₁	Y ₂₁₂₁	...	Y _{21r1}	...	Y _{211q}	Y _{212q}	...	Y _{21rq}	Y _{21..}	Y _{2...1}
	2	Y ₂₂₁₁	Y ₂₂₂₁	...	Y _{22r1}	...	Y _{221q}	Y _{222q}	...	Y _{22rq}	Y _{22..}	
		
		
	t	Y _{2t11}	Y _{2t21}	...	Y _{2tr1}	...	Y _{2t1q}	Y _{2t2q}	...	Y _{2trq}	Y _{2p..}	
.	
.	
.	
A _n	1	Y _{n111}	Y _{n121}	...	Y _{n1r1}	...	Y _{n11q}	Y _{n12q}	...	Y _{n1rq}	Y _{n1..}	Y _{n...1}
	2	Y _{n211}	Y _{n221}	...	Y _{n2r1}	...	Y _{n21q}	Y _{n22q}	...	Y _{n2rq}	Y _{n2..}	
		
		
	t	Y _{nt11}	Y _{nt21}	...	Y _{ntr1}	...	Y _{nt1q}	Y _{nt2q}	...	Y _{ntrq}	Y _{np..}	
Σ		Y _{..11}	Y _{..21}	Y _{..1q}	Y _{..2q}	Y _{....}	
Σ		Y _{...1}				...	Y _{...q}					

Table 8. Summary of analysis of variance for three-way repeated measures design, two of them are WSFs with q and r levels

Source of variation	DF	MS(SS/DF)	F	E(MS)
Between Subject	nt-1			
A	n-1	$SS_A / (n - 1)$	MS_A / MS_{E1}	$\sigma_\epsilon^2 + qr\sigma_\gamma^2 + tqr\sigma_\alpha^2$
Error 1	n(t-1)	$SS_{E1} / n(t - 1)$		$\sigma_\epsilon^2 + qr\sigma_\gamma^2$
Within Subject	nt(rq-1)			

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B	q-1	$SS_B / (q - 1)$	MS_B / MS_{E2}	$\sigma_\epsilon^2 + r\sigma_{\pi\gamma}^2 + ntr\sigma_\pi^2$
AxB	(n-1)(q-1)	$SS_{AxB} / (n-1)(q-1)$	MS_{AxB} / MS_{E2}	$\sigma_\epsilon^2 + r\sigma_{\pi\gamma}^2 + tr\sigma_{\pi\alpha}^2$
Error 2	n(t-1)(q-1)	$SS_{E2} / n(t-1)(q-1)$		$\sigma_\epsilon^2 + r\sigma_{\pi\gamma}^2$
C	r-1	$SS_C / (r - 1)$	MS_C / MS_{E3}	$\sigma_\epsilon^2 + q\sigma_{\beta\gamma}^2 + ntq\sigma_\beta^2$
AxC	(r-1)(n-1)	$SS_{AxC} / (r-1)(n-1)$	MS_{AxC} / MS_{E3}	$\sigma_\epsilon^2 + q\sigma_{\beta\gamma}^2 + tq\sigma_{\beta\alpha}^2$
Error 3	n(t-1)(r-1)	$SS_{E3} / n(t-1)(r-1)$		$\sigma_\epsilon^2 + q\sigma_{\beta\gamma}^2$
BxC	(r-1)(q-1)	$SS_{BxC} / (r-1)(q-1)$	MS_{BxC} / MS_{E4}	$\sigma_\epsilon^2 + \sigma_{\beta\pi\gamma}^2 + nt\sigma_{\beta\pi}^2$
AxBxC	(r-1)(q-1)(n-1)	$\frac{SS_{AxBxC}}{(r-1)(q-1)(n-1)}$	$\frac{MS_{AxBxC}}{MS_{E4}}$	$\sigma_\epsilon^2 + \sigma_{\beta\pi\gamma}^2 + t\sigma_{\beta\pi\alpha}^2$
Error 4	n(t-1)(q-1)(r-1)	$\frac{SS_{E4}}{n(t-1)(q-1)(r-1)}$		$\sigma_\epsilon^2 + \sigma_{\beta\pi\gamma}^2$
Total	nrq-1			

Table 9. Variance analysis table belonging to experiment I in material

Source of variation	DF	SS	MS	F	Sig.
B.Subject	(10-1)=9	93627.75			
W.Subject	10(4-1)=30	759099.25			
Sections	(4-1)=3	634278.9	211426.3	45.73	0.000
Error	(4-1)(10-1)=27	124815.35	4622.79		
Total	(4*10-1)=39	852724			

Table 10. Variance analysis table belonging to experiment II in material

Source of variation	DF	SS	MS	Sig.
B.Subject	2*6-1=11	29.98		
Sex	(2-1)=1	1.184	1.184	0.536
Error 1	2(6-1)=10	28.79	2.879	
W.Subject	2*6(4-1)=36	53.06		
Period	(4-1)=3	2.49	0.830	0.671
Period x Sex	(4-1)(2-1)=3	2.84	0.940	0.623
Error 2	2(6-1)(4-1)=30	47.73	1.591	
Total	47	83.04		

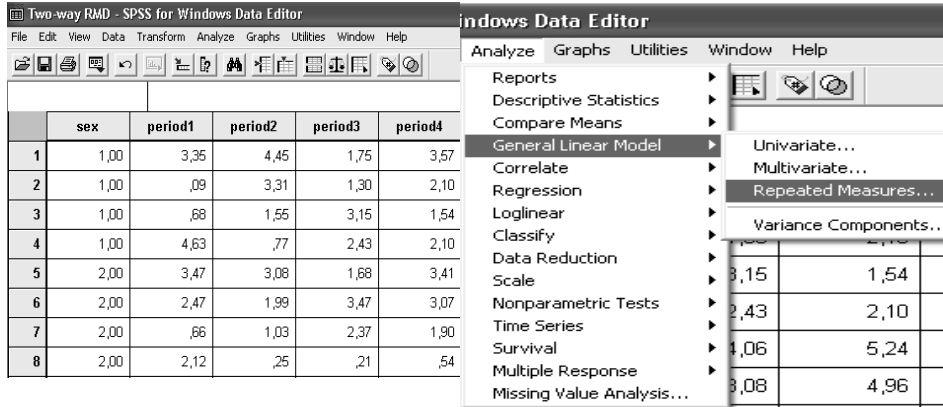
Table 11. Variance analysis table belonging to experiment III in material

Source of variation	DF	SS	MS	F	Sig.
B.Subject	3*3*6-1=53	5.20			
Dehydroepiandrosterone	(3-1)=2	0.32	0.160	1.77	0.181
Corticosterone	(3-1)=2	0.44	0.220	2.44	0.098
Dhyd x Cort	(3-1)(3-1)=4	0.34	0.080	0.88	0.467
Error 1	3*3(6-1)=45	4.10			
W.Subject	6*3*3*(4-1)=162	4.00			
Week	(4-1)=3	1.15	0.380	25.33	0.000
Dhyd x Week	(3-1)(4-1)=6	0.13	0.020	1.33	0.212
Cort x Week	(3-1)(4-1)=6	0.46	0.070	4.66	0.001
Dhyd x Cort x Week	(3-1)(3-1)(4-1)=12	0.11	0.009	0.60	0.567
Error 2	3*3(6-1)(4-1)=135	2.15	0.015		
Total	3*3*4*6-1=215	9.20			

Table 12. Variance analysis table belonging to experiment IV in material

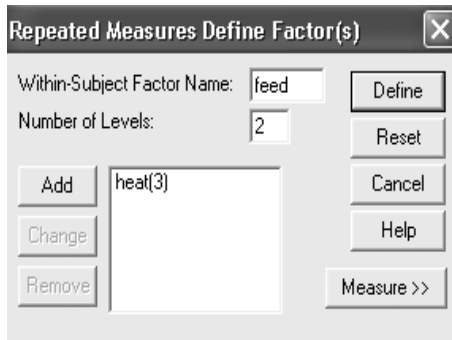
Source of variation	DF	SS	MS	F	Sig.
B.Subject	2*7-1=13	7664.23			
Sex	2-1=1	1008.1	1008.1	1.817	0.203
Error 1	2(7-1)=12	6656.143	554.679		
W.Subject	2*7(2*3-1)=70	155382.07			
Heat	3-1=2	5347.57	2673.75	18.91	0.000
HeatxSex	(2-1)(3-1)=2	97.87	48.935	0.346	0.711
Error 2	2(7-1)(3-1)=24	3392.23	141.345		
Feed	2-1=1	122591.52	122591.52	101.59	0.000
Feed x sex	(2-1)(2-1)=1	112	112	0.093	0.766
Error 3	2(7-1)(2-1)=12	14480.05	1206.671		
Feed x Heat	(2-1)(3-1)=2	5925.1	2962.583	20.896	0.000
Feed x Heat x Sex	(2-1)(3-1)(2-1)=2	33.167	16.583	0.117	0.890
Error 4	2(7-1)(3-1)(2-1)=24	3402.6	141.778		
Total	2*7*3*2-1=83	163046.3			

FEN BİLİMLERİ DERGİSİ
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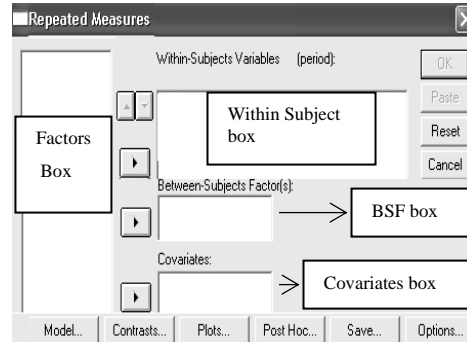


(a)

(b)



(c)



(d)

Figure 1. Repeated measures analysis diagram in SPSS package. (a) data input box, (b) repeated measures analyze paths, (c) repeated measures define factor(s) dialog box, (d) repeated measures dialog box