17 Analysis of Covariance

The use of additional information in the experimental units as a local control practice to reduce the estimate of experimental error is the primary subject of this chapter. The values for treatment means in a research study may depend on covariates that vary among the experimental units and have a significant relationship with the primary response variable. The analysis of covariance is used in this chapter to remove the influence of the covariates on treatment comparisons in completely randomized and complete block designs.

17.1 Local Control with a Measured Covariate

A number of local control techniques are used in experiments to control experimental error variance. Local control practices reduce experimental error variance and increase the precision for estimates of treatment means and tests of hypotheses. Concomitant variables are often used to select and group units to control experimental error variation.

Many concomitant variables, or *covariates,* can be measured at any time during the course of the experiment, and their influence on the response variable can be assessed when analyzing the results. The analysis of covariance, combining regression methodology with the analysis of variance, evaluates the influence of the covariate on the response variable and enables the comparison of treatments on a common basis relative to the values of the covariates.

Often, many factors external to the treatment factors influence the response variable. Blocking on the basis of these influential factors is one of the primary means used by researchers to control experimental error. When blocks of units are constructed with similar values for the factors, the experimental treatments can be compared with one another in a more homogeneous environment.

Frequently, the experimental setting may prohibit blocking of like units for a variety of reasons. There may be incomplete knowledge about the experimental material or the effects of external factors may not appear until after the experiment has begun. Too few units of like value may exist for adequate blocking. Even though the investigator may have knowledge of the influential factors, the number of additional factors may prohibit the use of all of them as blocking criteria.

Typical studies with additional variables that influence treatment comparisons include:

- Clinical trials where age, weight, sex, previous medical history, or occupation of patients can influence their response to the treatments. Although the investigators can block on two or three of these factors, it is not possible to ignore the influence of the remaining variables.
- Trials with fruit trees, which are blocked on the basis of soil or irrigation gradients, but the influence of previous treatments and historical yield records cannot be ignored.
- Feeding trials where blocks of animals can be constructed on the basis of litter and initial weights, but the amount of feed consumed during the course of the study will also have influence on the measured weight gains.

The study described in Example 17.1 illustrates an experiment in which the response variable is affected not only by the treatment applied to the subjects of the study but also by a covariate that was measured on each subject prior to the study.

Example 17.1 Effects of Exercise on Oxygen Ventilation

A common clinical method to evaluate an individual's cardiovascular capacity is through treadmill exercise testing. One of the measures obtained during treadmill testing, maximal oxygen uptake, is considered the best index of work capacity and maximal cardiovascular function.

The measured maximal oxygen uptake by an individual depends on a number of factors, including the mode of testing, test protocol, and the subjects' physical condition and age. A common test protocol on the treadmill is the inclined protocol where grade and speed incrementally increase until exhaustion occurs.

Research Hypothesis: Researchers in exercise physiology were of the opinion that the conditions for treadmill testing should simulate as closely as possible the mode of subject cardiovascular training to attain their maximal oxygen uptake during the test. It was hypothesized that step aerobic training was better simulated by the inclined protocol than a flat terrain running regimen.

Treatment Design: Two treatments selected for the study were a 12-week step aerobic training program and a 12-week outdoor running regimen on flat terrain. The subjects were to be tested on a treadmill before and after the 12-week training period with the inclined protocol. If the hypothesis were true, the subjects from the step aerobic training would show greater increases in maximal oxygen uptake than the subjects trained only on flat terrain. Those trained on flat terrain might be limited by localized muscle fatigue when tested on inclined protocols and thus not attain maximal oxygen uptake before exhaustion.

Experiment Design: The subjects were 12 healthy males who did not participate in a regular exercise program. Six men were randomly assigned to each group in a completely randomized design. Various respiratory measurements were made on the subjects while on the treadmill before the 12-week period. There were no differences in the respiratory measurements of the two groups of subjects prior to treatment.

The measurement of interest for this example is the change in maximal ventilation (liters/minute) of oxygen for the 12-week period. The observations on the 12 subjects and their ages are shown in Table 17.1, along with the group means and standard errors.

Table 17.1 Maximal ventilation change (liters/minute) following a 12-week exercise program

Group	*Age*	*Change*	*Group*	*Age*	*Change*
Aerobic	31	17.05	Running	23	– 0.87
	23	4.96		22	– 10.74
	27	10.40		22	– 3.27
	28	11.05		25	– 1.97
	22	0.26		27	7.50
	24	2.51		20	– 7.25
Mean	25.83	7.71		23.17	– 2.77
Std. Err.	1.40	2.55		1.01	2.54

Source: D. Allen, Exercise Physiology, University of Arizona.

Is Treadmill Performance Related to Age?

A graph of the change in maximal ventilation (y) for each of the subjects with their age (x) on the horizontal axis is shown in Figure 17.1. The plot suggests a strong positive relationship between the age of the subjects and their change in maximal ventilation on the treadmill regardless of the treatment group to which they belong. Thus; there appears to be considerable experimental error variation within each group associated with age differences.

The study protocol required the eligible subjects to be healthy males between the ages of 20 and 35 with a sedentary lifestyle. Although the two groups of subjects did not differ in their average pretest maximal ventilation measures, a one-way analysis of variance reveals a significant difference between the two groups in the maximal ventilation change after training. The aerobic exercise group had a larger change in ventilation rate than the running group, but the aerobic group consists of

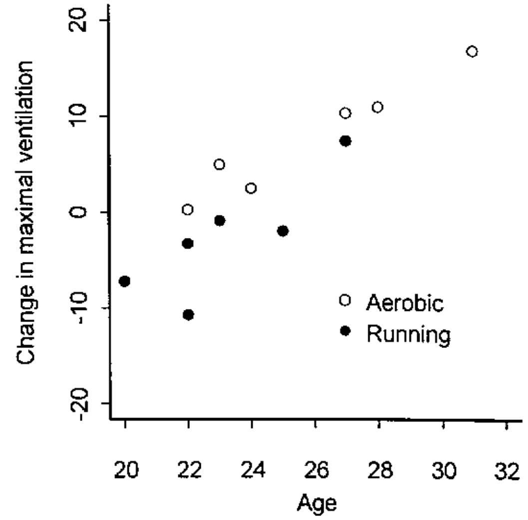

Figure 17.1 The relationship between maximal ventilation and age in the exercise physiology study

an older group of males. It must be determined whether the difference is a result of the exercise or the age differences in the groups. That is, suppose the mean changes in maximal ventilation were compared at the same age for both groups. Would the aerobic exercise group mean still be significantly greater than the running group mean? The analysis of covariance can be used to help answer that question and to determine whether the relationship between maximal ventilation change and age contributes significantly to experimental error variation.

17.2 Analysis of Covariance for Completely Randomized Designs

The Linear Model and Analysis of Covariance

The experiment on exercise physiology was conducted in a completely randomized design with two treatment groups. Assuming a linear relationship between the response variable y and a covariate x the linear model for the completely randomized design is

$$y_{ij} = \mu_i + \beta(x_{ij} - \overline{x}_{..}) + e_{ij} \qquad (17.1)$$

$$i = 1, 2, \ldots, t \quad j = 1, 2, \ldots, r$$

where μ_i is the treatment mean, β is the coefficient for the linear regression of y_{ij} on x_{ij}, and the e_{ij} are independent, normally distributed random experimental errors with mean 0 and variance σ^2. Two additional key assumptions for this model are that the regression coefficient β is the same for all treatment groups, and the treatments do not influence the covariate x.

The first objective of the covariance analysis is to determine whether the addition of the covariate has reduced the estimate of experimental error variance. If the reduction is significant, then we obtain estimates of the treatment group means $\overline{y}_{ia}$ adjusted to the same value of the covariate x for each of the treatment groups and determine the significance of treatment differences on the basis of the adjusted treatment means.

Alternative Models to Evaluate the Covariate Contribution

The analysis will require least squares estimates of the parameters for the alternative full and reduced models, which are

- the full model $y_{ij} = \mu_i + \beta(x_{ij} - \overline{x}_{..}) + e_{ij}$
- a reduced model without the covariate $y_{ij} = \mu_i + e_{ij}$
- a reduced model without treatment effects $y_{ij} = \mu + \beta(x_{ij} - \overline{x}_{..}) + e_{ij}$

The reduced model without the covariate is required to assess the influence of the covariate, and the reduced model without the treatment effects is required to assess the significance of treatment effects in the presence of the covariate.

Least squares estimates of the parameters are derived for the full model to obtain

$$SSE_f = \Sigma[y_{ij} - \widehat{\mu}_i - \widehat{\beta}(x_{ij} - \overline{x}_{..})]^2$$

with $N - t - 1$ degrees of freedom; the reduced model without the covariate for

$$SSE_r = \Sigma\,(y_{ij} - \widehat{\mu}_i)^2$$

with $N - t$ degrees of freedom; and the reduced model without treatment effects for

$$SSE_r^* = \Sigma\,[y_{ij} - \widehat{\mu} - \widehat{\beta}(x_{ij} - \overline{x}_{..})]^2$$

with $N - 2$ degrees of freedom.

The sum of squares reduction due to the addition of the covariate x to the model is obtained as the difference

$$SS(\text{Covariate}) = SSE_r - SSE_f$$

with 1 degree of freedom. The adjusted treatment sum of squares after fitting the covariate is

$$SST(\text{adjusted}) = SSE_r^* - SSE_f$$

with $t - 1$ degrees of freedom.

The SSE for each of the three models fit to the exercise physiology data (Table 17.1) are $SSE_f = 70.39$, $SSE_r = 389.30$, and $SSE_r^* = 142.18$.

Sum of Squares Partitions for the Analysis of Covariance

These sum of squares reductions can be computed by most computer programs for the analysis of covariance, and they will produce the required information found in the following discussion. The sum of squares partitions for the change in maximal ventilation rate with the age covariate in the exercise physiology study is shown in the analysis of variance in Table 17.2. Notice without the age covariate the estimated experimental error variance is $MSE_r = SSE_r/(N - t) = 389.3/10 = 38.93$. The addition of the covariate has reduced the estimate to $MSE = 7.82$ in Table 17.2. Thus, use of the age covariate as local control to reduce σ^2 through covariates appears to be effective. The gain in efficiency due to the covariate is illustrated later in this section.

Table 17.2 Analysis of covariance for maximal ventilation change with age covariate in an exercise physiology study

Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*F*	*Pr > F*
Regression	1	318.91	318.91*	40.78	.000
Group	1	71.79	71.79†	9.18	.014
Error	9	70.39	7.82‡		

*MS(Covariate) †MST(adjusted) ‡MSE_f

Tests of Hypotheses About Covariates and Treatments

Determine the significance of the reduction due to the covariate with a test of the null hypothesis H_0: $\beta = 0$. The test statistic is

$$F_0 = \frac{MS(\text{Covariate})}{MSE} = \frac{318.91}{7.82} = 40.78 \qquad (17.2)$$

with critical value $F_{.05,1,9} = 5.12$. The null hypothesis is rejected with $Pr > F = .000$ in Table 17.2; the addition of the covariate has significantly reduced experimental error variance.

The significant relationship between change in maximal ventilation rate and age of the subjects indicates the necessity to assess the significance of the treatment effects after the covariance adjustment. The null hypothesis for the adjusted treatment means is H_0: $\mu_1 = \mu_2$, and the test statistic is

$$F_0 = \frac{MST(\text{adjusted})}{MSE} = \frac{71.79}{7.82} = 9.18 \qquad (17.3)$$

with critical value $F_{.05,1,9} = 5.12$. The null hypothesis is rejected with $Pr > F = 0.014$ in Table 17.2, and we conclude that treatment means adjusted for age are different.

Treatment Means Adjusted to a Common Covariate Value

The estimates of the treatment means are adjusted to a common value for the covariate if inclusion of the covariate in the model significantly reduced experimental error variance. The treatment means can be adjusted to any value of the covariate, but ordinarily they are adjusted to the overall mean $\overline{x}_{..}$ as

$$\overline{y}_{ia} = \overline{y}_{i.} - \widehat{\beta}(\overline{x}_{i.} - \overline{x}_{..}) \qquad (17.4)$$

The estimate of the regression coefficient, which most programs compute automatically, is

$$\widehat{\beta} = \frac{\sum_{i=1}^{t}\sum_{j=1}^{r}(x_{ij} - \overline{x}_{i.})(y_{ij} - \overline{y}_{i.})}{\sum_{i=1}^{t}\sum_{j=1}^{r}(x_{ij} - \overline{x}_{i.})^2} = \frac{169.10}{89.67} = 1.886 \qquad (17.5)$$

and the estimated regression equation for the ith treatment group will be

$$\widehat{y}_i = \overline{y}_{i.} + \widehat{\beta}(x_i - \overline{x}_{i.}) \qquad (17.6)$$

and are

$$\widehat{y}_{1j} = 7.70 + 1.886(x_{1j} - 25.83)$$

for the aerobic group and

$$\widehat{y}_{2j} = -2.77 + 1.886(x_{2j} - 23.17)$$

for the running group. The regression line for each of the treatment groups is shown in Figure 17.2.

The treatment means adjusted to the mean age $\overline{x}_{..} = 24.5$ are

$$\overline{y}_{1a} = 7.70 - 1.886(25.83 - 24.5) = 5.19$$

$$\overline{y}_{2a} = -2.77 - 1.886(23.17 - 24.5) = -0.26$$

The unadjusted and adjusted treatment means are shown with the computed regression line for each of the treatment groups in Figure 17.2.

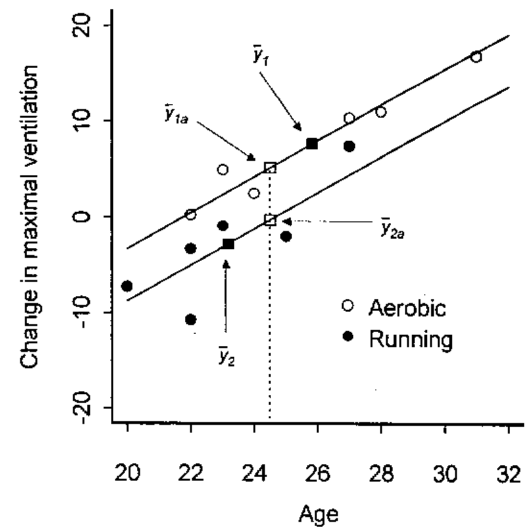

Figure 17.2 Regression between maximal ventilation and age in the exercise physiology study with adjusted treatment means

The difference between the unadjusted treatment means $\overline{y}_{1.} - \overline{y}_{2.} = 7.71 - (-2.77) = 10.48$ was much greater than the difference between the treatment means adjusted to a common age, $\overline{y}_{1A} - \overline{y}_{2A} = 5.19 - (-0.26) = 5.45$. Part of the difference between the unadjusted means was due to the difference of over two years in the average ages of the subjects in the two treatment groups, $\overline{x}_{1.} = 25.83$ and $\overline{x}_{2.} = 23.17$. The adjusted means are estimates of the mean maximal ventilation change at a common age. Thus, the difference between the adjusted means reflects only the effect of exercise on the change in maximal ventilation rate.

Standard Errors for the Adjusted Treatment Means

Two quantities useful for calculation of standard errors and relative efficiency in the analysis of covariance are the sums of squares for treatments, T_{xx}, and experimental error, E_{xx}, from an analysis of variance for the age covariate, x. These sums of squares and their values for this study are

$$T_{xx} = r\sum_{i=1}^{t}(\overline{x}_{i.} - \overline{x}_{..})^2 = 21.31$$

and

$$E_{xx} = \sum_{i=1}^{t}\sum_{j=1}^{r}(x_{ij} - \overline{x}_{i.})^2 = 89.67$$

The standard error estimator for an adjusted treatment mean is

$$s_{\overline{y}_{ia}} = \sqrt{MSE\left[\frac{1}{r_i} + \frac{(\overline{x}_{i.} - \overline{x}_{..})^2}{E_{xx}}\right]} \tag{17.7}$$

The estimated standard error for the adjusted aerobic exercise group mean is

$$s_{\overline{y}_{1a}} = \sqrt{7.82\left[\frac{1}{6} + \frac{(25.83 - 24.50)^2}{89.67}\right]} = 1.21$$

The standard error estimate for the adjusted running group mean will be the same as that for the exercise group mean since the quantity $(\overline{x}_{i.} - \overline{x}_{..})^2$ in Equation (17.7) is the same for both treatment groups.

The standard error estimator for the difference between two adjusted treatment means is not always available from computer programs without some clever programming on the part of the user. It is calculated as

$$s_{(\overline{y}_{ia} - \overline{y}_{ja})} = \sqrt{MSE\left[\frac{1}{r_i} + \frac{1}{r_j} + \frac{(\overline{x}_{i.} - \overline{x}_{j.})^2}{E_{xx}}\right]} \tag{17.8}$$

The difference between the adjusted treatment means for the aerobic exercise and running groups is $\overline{y}_{1a} - \overline{y}_{2a} = 5.19 - (-0.26) = 5.45$ with standard error estimate

$$s_{(\overline{y}_{1a} - \overline{y}_{2a})} = \sqrt{7.82\left[\frac{1}{6} + \frac{1}{6} + \frac{(25.83 - 23.17)^2}{89.67}\right]} = 1.80$$

Even when all r_i are equal, the standard error of the difference will vary among the pairs of treatments with more than two treatment groups because of the term $(\overline{x}_{i.} - \overline{x}_{j.})$ in Equation (17.8). In practice the variation in the estimate is slight. A single average standard error of the difference suggested by Finney (1946) to simplify the analysis with equal replication numbers is

$$s_{(\overline{y}_{ia} - \overline{y}_{ja})} = \sqrt{\frac{2MSE}{r}\left[1 + \frac{T_{xx}}{(t-1)E_{xx}}\right]} \tag{17.9}$$

The substitution of T_{xx} for $(\overline{x}_{i.} - \overline{x}_{..})^2$ in Equation (17.7) provides the average standard error for the adjusted treatment means.

Was There Increased Efficiency with a Covariate?

Whether the covariance adjustment has been worth the required effort depends on the gain in efficiency of the estimated means. The efficiency of the covariance adjustment relative to the analysis without the adjustment is based on the ratio of respective variances for the estimates of treatment means. The estimate of experimental error variance from the reduced model without the covariate is $MSE_r = SSE_r/(N-t) = 389.3/10 = 38.93$. The average variance suggested by Finney (1946) can be used for the estimate with the covariance adjustment. The efficiency is calculated as

$$E = \frac{MSE_r}{MSE\left[1 + \frac{T_{xx}}{(t-1)E_{xx}}\right]} \tag{17.10}$$

Given $MSE_r = 38.93, T_{xx} = 21.33, E_{xx} = 89.67, MSE = 7.82$, and $t = 2$ the efficiency of the covariance adjustment is $E = 38.93/9.68 = 4.0$. Thus, without covariance adjustment for age, four times as many subjects would be required for the exercise study to achieve the same precision on the estimated treatment means.

Critical Assumptions for a Valid Covariance Analysis

The validity of inferences from the analysis of variance requires an assumption of independent and homogeneous normally distributed errors. An evaluation of the assumptions regarding homogeneous and normally distributed experimental errors can be achieved with an estimate of the residuals for each observation as $\widehat{e}_{ij} = y_{ij} - \overline{y}_{i.} - \widehat{\beta}(x_{ij} - \overline{x}_{i.})$. Many computer programs will supply estimates of the residuals that can be used for the normal plots and plots of residuals versus estimated values to evaluate the assumptions as outlined in Chapter 4.

Additional assumptions critical to the validity of inferences from the analysis of covariance are (1) the covariate x is unaffected by the treatments, (2) a linear relationship exists between the response variable y and the covariate x, and (3) the regression coefficient β is the same for all treatment groups.

Do the Treatments Affect the Covariate?

If the covariate x as well as the primary response variable y is affected by the treatments the resultant response is multivariate, and the covariance adjustment for treatment means is inappropriate. In these cases, an analysis of the bivariate response (x, y) utilizing multivariate analysis methods is in order. Adjustment for the covariate is appropriate if it is measured prior to treatment administration since the treatments have not yet had the opportunity to affect its value. If the covariate is measured concurrently with the response variable, then it must be decided whether it could be affected by the treatments before the covariance adjustments are considered.

Is the Regression Coefficient the Same for All Treatments?

Comparisons among adjusted treatment means are independent of the covariate value if the regression lines for the treatment groups are parallel. If the relationship between y and x differs among the treatment groups as shown in Figure 17.3, then differences between adjusted treatment means depend crucially on the level of x chosen for the adjustment.

This heterogeneity of regression coefficients among treatment groups resembles interaction between factors in the standard factorial treatment design. In

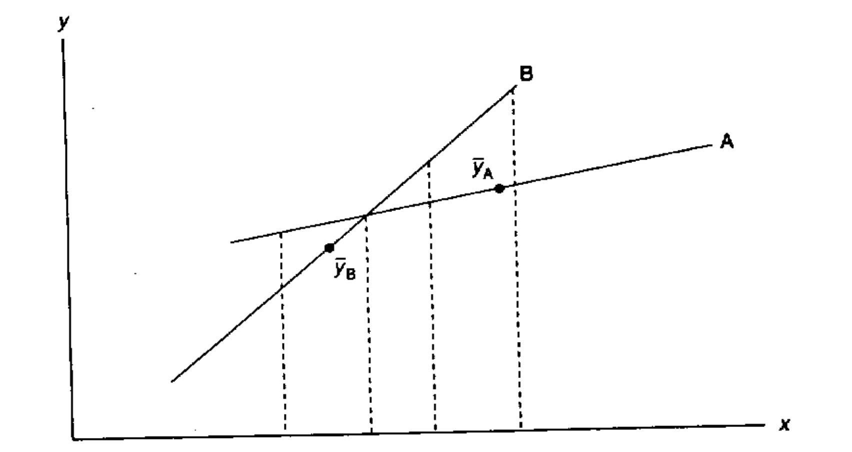

Figure 17.3 Differing regression relationships between the response variable y and the covariate x

that case comparisons are made with simple effects of one factor at each level of other factors. A similar strategy must be used in the case of continuous covariates with different regression coefficients for the treatment groups.

Similar difficulties occur with nonlinear relationships. Under these circumstances the inferences regarding the responses must include a complete description involving the effects of the treatments and the covariate.

Evaluation of the Separate Regressions Model

The Linear Model with Separate Regressions for Each Treatment

The linear model with different regression coefficients for each of the treatment groups is

$$y_{ij} = \mu_i + \beta_i(x_{ij} - \overline{x}_{i.}) + e_{ij} \tag{17.11}$$

$$i = 1, 2, \ldots, t \quad j = 1, 2, \ldots, r$$

where β_i is the regression coefficient for the ith treatment group. A test for the equality of the regression coefficients, H_0: $\beta_1 = \beta_2 = \cdots = \beta_t$, can be derived from an analysis of the two alternative models, the model with a common regression for all treatments and the model with separate regressions for the treatments. Different regressions imply the presence of an interaction between the treatments and the covariate; thus, the model alternatively can be written to include a term for interaction between the covariate and treatments.

Example 17.2 Auditory Discrimination and Cultural Environment

Hendrix, Carter, and Scott (1982) described a study conducted to determine the difference in the ability to discriminate aurally between environmental sounds with respect to several factors. The study was designed to test the effects of a treatment on auditory discrimination. Subjects belonging to two different cultural groups were given pre-treatment and post-treatment tests on auditory discrimination.

Only a portion of the data is used for this example to illustrate the effects of heterogeneous regression coefficients for the covariate. The analysis will be conducted to determine whether the gain in auditory score between the pre- and post-treatment administration differed between the subjects with pre-test scores as a covariate. The data for gain in scores and pre-test scores from female subjects in the auditory treatment group are shown in Table 17.3.

Table 17.3 Pre-test and gain scores for auditory discrimination in two cultural groups

Culture	*Pre-Test*	*Gain*	*Culture*	*Pre-Test*	*Gain*
1	64	7	2	52	2
	39	32		50	12
	69	2		59	6
	56	20		42	10
	67	4		62	1
	39	26		35	9
	32	34		41	6
	62	8		36	8
	64	4		37	8
	66	2		64	6
Mean	55.8	13.6		47.8	6.8

A Sum of Squares Partition for Homogeneity of Regressions

Let the model $y_{ij} = \mu_i + \beta(x_{ij} - \overline{x}_{..}) + e_{ij}$ in Equation (17.1), assuming a common slope, be model 1 with the usual $t + 1$ parameters, μ_i and β. Let the model $y_{ij} = \mu_i + \beta_i(x_{ij} - \overline{x}_{i.}) + e_{ij}$ in Equation (17.11), assuming different slopes for each treatment, be model 2 with $2t$ parameters, μ_i and β_i. The sum of squares for experimental error from model 1, say SSE_1, will have $(N - t - 1)$ degrees of freedom, and the sum of squares for experimental error from model 2, say SSE_2, will have $(N - 2t)$ degrees of freedom.

The analysis of variance of the auditory discrimination data for model 1 in Table 17.4 contains $SSE_1 = 519.38$ with 17 degrees of freedom. The analysis of variance for model 2 is shown in Table 17.5 with 2 degrees of freedom for the sum of squares reduction for separate regressions calculated within each culture group.

The error sum of squares has been reduced to $SSE_2 = 147.22$ with 16 degrees of freedom.

Table 17.4 Analysis of covariance for score gain with pre-test score covariate in the auditory discrimination study

Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*F*	*Pr > F*
Regression	1	1061.12	1061.12	34.73	.000
Group	1	647.47	647.47	21.19	.000
Error	17	519.38*	30.55		

*SSE_1

Table 17.5 Analysis of covariance score gain assuming separate regressions of gain on pre-test scores for each cultural group

Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*F*	*Pr > F*
Group	1	610.24	610.24	66.33	.000
Regression within groups	2	1433.28	716.64	77.89	.000
Error	16	147.22*	9.20		

*SSE_2

The sum of squares to test homogeneity of regression coefficients for the treatment groups is the difference in the experimental error sum of squares for the two models or

$$SS(\text{Homogeneity}) = SSE_1 - SSE_2 \tag{17.12}$$
$$= 519.38 - 147.22$$
$$= 372.16$$

with $(N - t - 1) - (N - 2t) = (t - 1)$ or $17 - 16 = 1$ degrees of freedom. The F_0 statistic to test the null hypothesis of equal regression coefficients, H_0: $\beta_1 = \beta_2 = \cdots = \beta_t$ is

$$F_0 = \frac{MS(\text{Homogeneity})}{MSE_2} \tag{17.13}$$

with critical value $F_{\alpha,(t-1),(N-2t)}$. The test for the exercise study is $F_0 = (372.16/1)/9.20 = 40.45$ exceeds $F_{.05,1,16} = 4.49$, and the null hypothesis of equal regression coefficients for the two cultural groups is rejected. The regression of gain in score on pre-test scores is different for the two cultural groups, and adjustment of the cultural group means to the same pre-test score with a common regression is not appropriate.

Many statistical programs are capable of directly computing the required sums of squares for homogeneity of regression coefficients in Equation (17.12) when specified as a *treatment by covariate* interaction effect in the program. The analysis of variance for the covariance model with a treatment by covariate interaction term is shown in Table 17.6. Note the sum of squares for the Groups × Regression interaction is equivalent to the sum of squares for homogeneity, Equation (17.12), derived from the alternative models with common and different regressions for the treatment groups.

Table 17.6 Analysis of covariance for score gain, including the interaction between the pre-test score covariate and cultural groups

Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*F*	*Pr > F*
Group	1	610.24	610.24	66.32	.000
Regression	1	770.63	770.63	83.75	.000
Groups × Regression	1	372.16	372.16	40.45	.000
Error	16	147.22	9.20		

A Regression Coefficient Estimate for Each Treatment

If the regressions are significantly different among the treatment groups the least squares estimate of the regression coefficient for the ith treatment group is computed from the x_{ij} and y_{ij} values within the treatment group as

$$\widehat{\beta}_i = \frac{\sum_{j=1}^{r}(x_{ij} - \overline{x}_{i.})(y_{ij} - \overline{y}_{i.})}{\sum_{j=1}^{r}(x_{ij} - \overline{x}_{i.})^2} \tag{17.14}$$

The estimated regression equation for the ith treatment group is $\widehat{y}_i = \overline{y}_{i.} + \widehat{\beta}_i(x_{ij} - \overline{x}_{i.})$. The estimates of the regression coefficients for the two cultural groups and their standard errors (in parentheses) are $\widehat{\beta}_1 = -0.91(0.07)$ and $\widehat{\beta}_2 = -0.16(0.09)$. Both of these estimates are available from computer programs for linear models. The standard error estimate for the estimated regression coefficient, $\widehat{\beta}_i$, is the square root of $MSE/\sum_{j=1}^{r}(x_{ij} - \overline{x}_{i.})^2$.

The estimated regression equations for the two cultural groups are

$$\text{Group 1}: \quad \widehat{y}_1 = 55.8 - 0.91(x - 13.6)$$

$$\text{Group 2}: \quad \widehat{y}_2 = 47.8 - 0.16(x - 6.8)$$

The regression equations are plotted in Figure 17.4 along with the observations for each cultural group. Clearly, the regression lines are different from one another and a comparison of cultural group means adjusted to the overall pre-test mean of $\overline{x} = 51.8$ would be relatively meaningless.

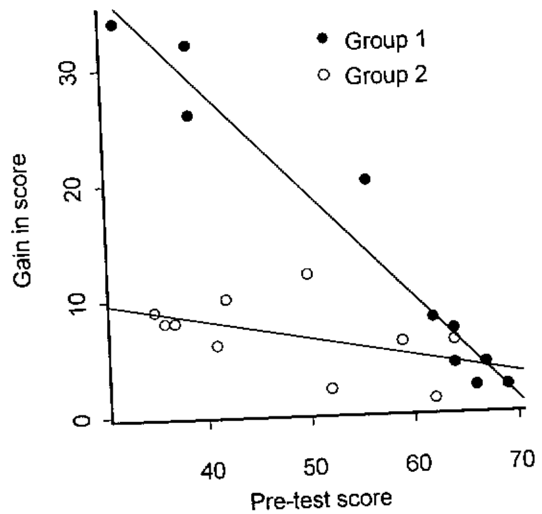

Figure 17.4 Regression between gain in score and pre-test scores with separate regressions for cultural groups

Rather, consider the regression lines as estimates of the simple effect of pre-test scores at each level of cultural group and make inferences on that basis. In both cultural groups the gain in score decreased with higher pre-test scores, but the rate of decrease was much greater with Group 1 than with Group 2. In fact a simple Student t test for $H_0 : \beta_2 = 0$ is $t_0 = -0.16/0.09 = -1.78$, which would lead to nonrejection of the null hypothesis; so no convincing evidence exists to indicate gain changes with pre-test scores in Group 2.

It is evident that the gain in scores from treatment in Group 1 are much greater when the subjects had low initial auditory discrimination and appear to benefit much more from training than do those in Group 2 with similar low initial auditory discrimination. When subjects from either group had high initial auditory discrimination on the pre-test the gain from training was relatively low or negligible.

Confidence intervals or tests of hypotheses between the estimated group means for any given value of pretest score $x = x_0$, say , $\widehat{y}_{1|x_0} - \widehat{y}_{2|x_0}$, can be constructed with the variance of the contrast as

$$s^2_{\widehat{y}_{1|x_0} - \widehat{y}_{2|x_0}} = MSE\left[\frac{1}{r_1} + \frac{1}{r_2} + \frac{(\overline{x}_0 - \overline{x}_{1.})^2}{\Sigma(x_{1j} - \overline{x}_{1.})^2} + \frac{(\overline{x}_0 - \overline{x}_{2.})^2}{\Sigma(x_{2j} - \overline{x}_{2.})^2}\right] \tag{17.15}$$

17.3 The Analysis of Covariance for Blocked Experiment Designs

The analysis of covariance can be applied to any experimental design with a straightforward extension of the principles applied to the completely randomized design in the previous section. However, a test for equality of regression coefficients among treatment groups is not possible with blocked designs unless there is more than one experimental unit for each treatment within each block. The analysis is illustrated with a randomized complete block design.

Example 17.3 Nutrient Availability Tests with Barley in the Greenhouse

Management methods on forest and range watersheds affect the nutrient status and availability of nutrients in any vegetation and soil-type complex. Knowledge of the soil, plant, and nutrient relationships is essential to properly manage watershed vegetation and soils.

The availability of certain soil nutrients in these watershed soils is evaluated by a pot culture technique in a greenhouse with barley plants. In principle, the method is based on the law of limiting factors. The test plants are grown in the soil fertilized to an optimum level and in the soil fertilized in identical fashion but without the nutrient in question. If the nutrient is deficient in the soil, the plants cultured in the complete nutrient soil will exhibit more plant growth than those cultured in the soil with the nutrient omitted from the fertilizer.

Research Objective: In one such study an investigator wanted to determine the availability of nitrogen and phosphorus in a watershed dominated by chaparral vegetation. He had collected soil samples from under the canopies of the dominant vegetation in the watershed, mountain mahogany, and composited the samples for a pot culture evaluation of nitrogen and phosphorus availability.

Treatment Design: Four nutrient treatments used for the study were (1) check, no fertilizer added; (2) full, a complete fertilizer; (3) N_0, nitrogen omitted from full, and (4) P_0, phosphorus omitted from full. The nutrient treatments were added as solutions to the soil, mixed, and placed in plastic pots in the greenhouse.

Experimental Design: The treatment pots were arranged on a greenhouse bench in a randomized complete block design to control experimental error variation caused by gradients in light and temperature in the greenhouse.

The barley plants were grown in the pots for seven weeks when plants were harvested, dried, and weighed. A leaf blight infected the plants part way through the experiment. It was assumed the blight would affect the growth of the plants and at the end of the experiment the percentage of the leaf area affected by the blight was measured in each container before the barley plants were harvested. The total dry weight of the barley plants and the percent leaf area affected with the blight is shown in Table 17.7 for each container in the experiment.

Table 17.7 Total dry matter y in grams and percent blighted leaf area x of barley plants

	Treatment							
	Check		*Full*		N_0		P_0	
Block	y	x	y	x	y	x	y	x
1	23.1	13	30.1	7	26.4	10	26.2	8
2	20.9	12	31.8	5	27.2	9	25.3	9
3	28.3	7	32.4	6	28.6	6	29.7	7
4	25.0	9	30.6	7	28.5	6	26.0	7
5	25.1	8	27.5	9	30.8	5	24.9	9
Mean	24.48	9.8	30.48	6.8	28.3	7.2	26.42	8.0

Source: Dr. J. Klemmedson, Renewable Natural Resources, University of Arizona.

The Linear Model for a Randomized Complete Block Design

The effects model for the randomized complete block design can be expressed as

$$y_{ij} = \mu + \tau_i + \rho_j + \beta(x_{ij} - \bar{x}_{..}) + e_{ij} \qquad \textbf{(17.15)}$$

$$i = 1, 2, \ldots, t \qquad j = 1, 2, \ldots, r$$

where μ is the general mean, τ_i is the treatment effect, ρ_j is the block effect, β is the regression of y on x, and the e_{ij} are independent and normally distributed random errors with mean 0 and variance σ^2. It is further assumed that the covariate is unaffected by the treatments or blocks and the regression is the same for all treatments.

Alternative Models to Evaluate the Covariate Influence

Alternative full and reduced models are used to evaluate the influence of the covariate and also the significance of the treatment effects after adjustment for the covariate if necessary. The required models and their experimental error sums of squares are

- the full model, $y_{ij} = \mu + \tau_i + \rho_j + \beta(x_{ij} - \bar{x}_{..}) + e_{ij}$, and SSE_f with $(r-1)(t-1) - 1$ degrees of freedom
- the usual randomized complete block model with no covariate, $y_{ij} = \mu + \tau_i + \rho_j + e_{ij}$, and SSE_r with $(r-1)(t-1)$ degrees of freedom

- no treatment effects, $y_{ij} = \mu + \rho_j + \beta(x_{ij} - \bar{x}_{..}) + e_{ij}$, and SSE_r^* with $(N - r - 1)$ degrees of freedom

Finally, although not entirely necessary for the analysis, the adjusted sum of squares for blocks can be computed using the model with

- no block effects, $y_{ij} = \mu + \tau_i + \beta(x_{ij} - \bar{x}_{..}) + e_{ij}$, and SSE_r^{**} with $(N - t - 1)$ degrees of freedom

The SSE for each of the models fit to the barley data, Table 17.7, are $SSE_f = 12.577$, $SSE_r = 39.167$, $SSE_r^* = 37.437$, and $SSE_r^{**} = 20.363$.

Sum of Squares Partitions for the Analysis of Covariance

The sum of squares reduction upon adding the covariate x to the usual randomized complete block model is obtained as the difference

$$SS(\text{Covariate}) = SSE_r - SSE_f$$

$$= 39.167 - 12.577 = 26.590$$

with 1 degree of freedom. The adjusted treatment sum of squares after fitting the covariate and block effects is

$$SST(\text{adjusted}) = SSE_r^* - SSE_f$$

$$= 37.437 - 12.577 = 24.860$$

with $(t - 1) = 3$ degrees of freedom. The adjusted block sum of squares after fitting the covariate and treatment effects is

$$SSB(\text{adjusted}) = SSE_r^{**} - SSE_f$$

$$= 20.363 - 12.577 = 7.786$$

The analysis of covariance for the nutrient availability test with barley is shown in Table 17.8.

The significance of the covariate requires a test of the null hypothesis H_0: $\beta = 0$ with the statistic

Table 17.8 Analysis of covariance for dry matter production of barley plants with percent blight damaged leaf area as a covariate

Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*F*	*Pr > F*
Regression	1	26.590	26.590	23.263	0.001
Block	4	7.786	1.947	1.703	0.219
Treatment	3	24.860	8.287	7.250	0.006
Error	11	12.577	1.143		

$$F_0 = \frac{MS(\text{Covariate})}{MSE} = \frac{26.590}{1.143} = 23.263$$

which is significant with $Pr > F = .001$ in Table 17.8. Thus, the relationship between the percent blight damaged leaf area and dry matter production of the barley plants is significant.

The estimate of the regression coefficient, which will be provided by most computer programs, is $\widehat{\beta} = -0.863$. The negative coefficient indicates the dry matter production decreases with an increase in the incidence of the disease on the plant.

The null hypothesis of no differences among the treatment means is tested with the statistic

$$F_0 = \frac{MST(\text{adjusted})}{MSE} = \frac{8.287}{1.143} = 7.25$$

and the null hypothesis is rejected with $Pr > F = .006$ in Table 17.8.

Adjusted Treatment Means and Their Standard Errors

The adjusted treatment means are calculated the same as for the completely randomized design with Equation (17.4) and are shown in Table 17.9 along with their standard errors. The standard errors shown in Equations (17.7) and (17.9) can be used for the adjusted treatment means with the sums of squares for treatments, $T_{xx} = 26.55$, and error, $E_{xx} = 35.70$, from the analysis of variance for the covariate, blight infection, using the data in Table 17.7. The estimated average standard error of the difference between two adjusted treatment means is

$$s_{(\bar{y}_{ia}-\bar{y}_{ja})} = \sqrt{\frac{2(1.143)}{5}\left[1 + \frac{26.55}{(3)35.7}\right]} = 0.76 \qquad \textbf{(17.16)}$$

Interpretations with Multiple Contrasts

The one-sided Dunnett 95% simultaneous confidence with the "Full" treatment as a control can be used to determine whether the soil was deficient in nutrients. The soil is deficient if the plant grown in the control or Full treatment exceeds that for any treatments deficient in one or more of the nutrients; therefore, if the upper bound of the interval for treatment *minus* control is negative the treatment is deficient in nutrients.

Table 17.9 Adjusted means and their standard errors from the analysis of covariance for dry matter production of barley plants with percent blight damaged leaf area as a covariate

Treatment	*Adjusted Mean*	*Standard Error*
Check	26.08	0.58
Full	29.49	0.52
N_0	27.65	0.50
P_0	26.46	0.48

From Appendix Table VI the critical value of the Dunnett statistic for a one-sided interval is $d_{.05,3,11} = 2.31$. The Dunnett criterion is $D(3, .05) = 2.31(0.76) = 1.76$. The 95% SCI upper bounds for differences between the treatments and the control, Full, treatment are shown in Table 17.10.

Table 17.10 Upper bounds 95% simultaneous confidence intervals using the Dunnett method to compare the control treatment, Full, to other treatments

Treatment	*Adjusted Mean* $(\bar{y}_i)$	$(\bar{y}_i - \bar{y}_c)$	*95% SCI Upper Bounds*
Check	26.08	− 3.41	− 1.65
Full	$\bar{y}_c = 29.49$	–	–
N_0	27.65	− 1.84	− 0.08
P_0	26.46	− 3.03	− 1.27

The difference between the Check treatment with no added nutrients and the control treatment is $26.08 - 29.29 = -3.41$ with an upper bound, -1.65, on the one-sided confidence interval. Thus, the soil is deficient in some unspecified nutrients. Specific tests for deficiencies in nitrogen and phosphorus require the comparisons between the full treatment and the N_0 and P_0 treatments. The difference between the nitrogen deficient treatment N_0 and the control treatment is $27.65 - 29.49 = -1.84$ with an upper bound of -0.08. The difference between the phosphorus deficient treatment P_0 and the control is $26.46 - 29.49 = -3.03$ with an upper bound of -1.27. Although the soil was deficient in both nitrogen and phosphorous, the upper bound for the phosphorous comparison was much farther removed from 0 than that for the nitrogen comparison, indicating a greater deficiency in phosphorous than in nitrogen.

17.4 Practical Consequences of Covariance Analysis

Practical application of the analysis of covariance has been demonstrated only with completely randomized and randomized complete block designs. However, the use of covariates can be extended to any treatment and experiment design as well as to comparative observational studies of complex structure and studies requiring the use of multiple covariates for adjustment. The objective in this chapter was to introduce the basic ideas underlying the use of additional information on basic units of the study. The specific manual formulae for the analysis will depend on the specific treatment and experiment design employed for the study. However, in all cases the use of full and reduced models, as illustrated with the two designs in this chapter, will enable an assessment of the covariates influence on the reduction of experimental error and the significance of adjusted treatment means.

Extensive discussions on the uses and misuses of covariates in research studies were provided in two special issues of *Biometrics* (1957), Volume 13, No. 3 and (1982), Volume 38, No. 3. Of particular interest are articles by Cochran (1957), Smith (1957), and Cox and McCullagh (1982). A number of issues arise relevant to the use of covariates. Among those concerns are the applicability in certain situations and the relationship between blocking and covariates.

Analysis of Covariance Combines the Features of Two Models

The analysis of covariance combines the features of models for the analysis of variance and regression to partition the total variation into components ascribable to (1) the treatment effects, (2) the effects attributable to any covariates, and (3) random experimental error as well as the variation associated with any design blocking factors. The basic intention is to compare treatments at a common value for the covariate.

When Covariates Are Superior to Blocks for Error Control

On the surface, covariance analysis appears to offer an alternative to blocking for reducing experimental error. Blocking designs restrict the number of criteria that reasonably can be used for local control. On the other hand, covariance permits the use of any number of factors thought necessary. Covariance also makes better use of exact values for quantitative factors, whereas blocking groups the same factors into classes of values. The advantage of covariates seems great when there are more than a few factors available as potential candidates for blocking variables.

When Blocks Are Superior to Covariates for Error Control

Covariance may be at a distinct disadvantage without blocking because random allocation of treatments to experimental units can result in an uneven distribution of treatments among the covariate classes. Any association between the covariate and the treatments confounds the effects of both on the response variable. Blocking on the values of the covariates distributes the treatments evenly among the covariate classes and avoids confounding the effects of the treatments and covariates.

Blocking is most effective with qualitative factors that produce recognizable variation among the experimental units. These factors include study management practices when tasks have to be performed by several technicians or on different days. Batches of raw materials provide natural effective qualitative blocking criteria.

Whenever the systematic differences are highly recognizable blocking is a most effective means of reducing experimental error variation that maintains the orthogonality required to avoid confounding the effects of the covariates with the treatments. In summary, blocking is recommended as a first course to reduce experimental error variance with adjustments on additional information if necessary with the analysis of covariance to further improve precision.

Comparative Observational Studies at a Disadvantage

The analysis of comparative observational studies can benefit equally from an analysis that includes covariates to reduce error variance and adjust group means for differences in their covariate values. Observational studies suffer from the disadvantage that units cannot be randomized to the defined treatment groups. The possibility exists for an influence on the response by additional unobserved covariates that are associated with the treatment groups, thus introducing an unknown bias into the group comparisons. Experimental studies have the advantage that the effects of these variables are distributed among the units by randomization and their influence is much less likely to be confounded with the effects of the treatments.

The Dangers of Extrapolation Beyond the Data

Finally, caution must be used when treatment means are adjusted to a common value for the covariate. Even though the regressions are parallel and there is no possibility that the treatments affect the covariate, the values of the covariate could be quite different for the treatment groups. If the covariate values are widely separated for the treatment groups, then adjustments would have to be extrapolated to a value of the covariate that is not common to either of the groups. An extreme example for illustration is a situation where income is used as a covariate for adjustment in comparing a group of corporate executives to entry-level clerical workers. Clearly, there would be no overlap of the income levels for the two groups. The adjustment would apply to the extrapolated region of an average income not included in either group and a comparison would be made between two groups in a nonsense setting. Even if the extrapolated adjustments were valid the standard errors of extrapolated values would be quite large.

EXERCISES FOR CHAPTER 17

1. An experiment was conducted on the shear strength of spot welds for three types of steel alloy. Six welds were made on each of the alloys and the force required to shear the weld was measured. The diameter of the weld was measured because it was believed that the strength of the weld was affected by its diameter. The data are shown in the table where y = weld strength and x = weld diameter.

Alloy	y	x	*Alloy*	y	x	*Alloy*	y	x
1	37.5	12.5	2	57.5	16.5	3	38.0	15.5
1	40.5	14.0	2	69.5	17.5	3	44.5	16.0
1	49.0	16.0	2	87.0	19.0	3	53.0	19.0
1	51.0	15.0	2	92.0	19.5	3	55.0	18.0
1	61.5	18.0	2	107.0	24.0	3	58.5	19.0
1	63.0	19.5	2	119.5	22.5	3	60.0	20.5

a. Use weld diameter as a covariate for weld strength, and write a linear model for the experiment, identify each of the terms in the model, and state the assumptions for the model.
b. Conduct the analysis of covariance, and test the significance of the covariate and adjusted treatment means.
c. Compute the adjusted treatment means, their standard errors, and an average standard error of the difference between two adjusted means.
d. Plot the regression line for each alloy showing the observed means and adjusted means for each alloy.
e. Compute the efficiency of the covariance adjustment.
f. Test the hypothesis of homogeneous regressions for each of the alloys.
g. Discuss the results of the experiment and the effectiveness of the covariance adjustment.
h. The significance level of the test for homogeneous regressions was $Pr > F = .068$. When the Group × Regression source of variation is not significant we end up with the sum of squares for error in the regular analysis of covariance assuming homogeneous regression coefficient. Effectively we are pooling the Group × Regression sum of squares partition with the sum of squares for error from the analysis of covariance for the model with separate regression coefficients for each treatment group. Hendrix et al. (1982) suggested in that case we should use a significance level of $\alpha = .20$ or .25 since it was similar to the problem of testing incompletely specified models as discussed by Bozivich, Bancroft, and Hartley (1956). What do you think about this strategy?
i. Suppose you subscribe to the philosophy of a significance level of $\alpha = .20$ or .25 when testing the null hypothesis of equal regression coefficients for all treatment groups. The null hypothesis in part (f) is then rejected. Conduct the analysis of covariance with separate regression estimates for each treatment group, and compute the estimated regression coefficients and their standard errors for each alloy. What is your inference from the study at this point?

2. A nutrition scientist conducted an experiment to evaluate the effects of four vitamin supplements on the weight gain of laboratory animals. The experiment was conducted in a completely

randomized design with five separately caged animals for each treatment. The caloric intake will differ among animals and influence weight gain so the investigator measured the caloric intake of each animal. The data on weight gain (y = grams) and calorie intake (x = calories/10) are shown in the table.

Diet	y	x	*Diet*	y	x	*Diet*	y	x	*Diet*	y	x
1	48	35	2	65	40	3	79	51	4	59	53
1	67	44	2	49	45	3	52	41	4	50	52
1	78	44	2	37	37	3	63	47	4	59	52
1	69	51	2	73	53	3	65	47	4	42	51
1	53	47	2	63	42	3	67	48	4	34	43

a. Determine whether diet influenced calorie intake to the extent that the latter would be invalidated as a covariate.
b. Use calorie intake as a covariate for weight gain, and write a linear model for the experiment. Identify each of the terms in the model, and state the assumptions for the model.
c. Conduct the analysis of covariance, and test the significance of the covariate and adjusted treatment means.
d. Compute the adjusted treatment means, their standard errors, and an average standard error of the difference between two adjusted means.
e. Plot the regression line for each diet showing the observed means and adjusted means for each diet.
f. Compute the efficiency of the covariance adjustment.
g. Test the hypothesis of homogeneous regressions for each of the diets.
h. Discuss the results of the experiment and the effectiveness of the covariance adjustment.

3. A plant scientist conducted an experiment to study the effects of drip irrigation water level on sweet corn growth, yield, and quality. Three levels of irrigation were used in the experiment (15.8, 24.0, and 28.5 inches of water applied), and the experiment was arranged in a randomized complete block design to control for soil variability in the field. One of the response variables measured was the weight of culls per plot or the amount of sweet corn in the plot that was unsuitable for market. The number of plants per plot varied and this would affect the crop yield on the plot. Since the soil moisture was optimized to establish the crop stand the variation in plants per plot was not affected by the irrigation levels imposed after the crop was established. The observed yield of culls (y = metric tons/hectare) and x = plants in a 40-foot section of row are shown in the table.

	Irrigation Level					
	1		*2*		*3*	
Block	y	x	y	x	y	x
1	1.5	45	1.9	54	1.1	43
2	3.1	58	1.8	57	1.8	60
3	3.8	61	2.9	55	3.7	71
4	3.3	59	2.3	56	1.8	48

a. Use plants-per-40-feet of row as a covariate for yield of culls, and write a linear model for the experiment, identify each of the terms in the model, and state the assumptions for the model.
b. Conduct the analysis of covariance, and test the significance of the covariate and adjusted treatment means.
c. Show the value of the regression coefficient. Compute the adjusted treatment means, their standard errors, and an average standard error of the difference between two adjusted means.
d. Compute the efficiency of the covariance adjustment.
e. Discuss the results of the experiment and the effectiveness of the covariance adjustment.

4. An experiment was conducted in a randomized complete block design to study the effect of natural control, *Bacillus*, and a standard chemical insecticide for control of hornworm infestations on a crop plant. The treatments included four sources of *Bacillus* (Treatments 1–4), a standard chemical treatment (Treatment 5), and a control of no treatment (Treatment 6). The treatments were applied to plants grown in field plots in the field. The number of hornworms (*count*) on each plant were counted prior to treatment. The number of live hornworms (*live*) were counted 20 hours after application of the treatment. The data for each plot are shown in the table.

	Block							
	1		*2*		*3*		*4*	
Treatment	*Count*	*Live*	*Count*	*Live*	*Count*	*Live*	*Count*	*Live*
Bacillus 1	15	17	25	26	18	21	23	26
Bacillus 2	19	18	21	22	20	19	19	20
Bacillus 3	19	19	19	21	21	23	25	22
Bacillus 4	22	14	31	26	17	17	19	19
Chemical	17	5	22	6	26	13	18	10
Control	22	25	14	19	22	26	23	27

a. Use the count of hornworms before treatment as a covariate for the number of live hornworms 20 hours after treatment, and write a linear model for the experiment, identify each of the terms in the model, and state the assumptions for the model.
b. Conduct the analysis of covariance, and test the significance of the covariate and adjusted treatment means.
c. Show the value of the regression coefficient. Compute the adjusted treatment means, their standard errors, and an average standard error of the difference between two adjusted means.
d. Compute the efficiency of the covariance adjustment.
e. Discuss the results of the experiment and the effectiveness of the covariance adjustment.
f. The response variable is a count measure and likely does not have a normal distribution. Did you check the assumptions of homogeneous variance and normal distributions of experimental errors for the model? If not, do so now, and if necessary take corrective actions (according to discussions in Chapter 4) and repeat parts (b) through (e).

5. The analysis of covariance can be used to estimate a missing value in a blocked design and provide an unbiased estimate of the treatment sum of squares to test the hypothesis of no differences among the treatment means with a missing value (Coons, 1957).

A covariate x is introduced for the missing value. The covariate is assigned the value $x = -1$ for the missing y value and $x = 0$ for all other values of y that are not missing. A value of $y = 0$ is assigned to the missing value. Compute a regular analysis of covariance with the values assigned to y and the covariate x. The estimate of the missing value is the estimate of the regression coefficient $\widehat{\beta} = E_{xy}/E_{xx}$, and the adjusted treatment sum of squares in the analysis of covariance is the correct sum of squares to test the null hypothesis of no differences among the treatment means.

As a demonstration of the technique use the data for the randomized complete block design in Exercise 17.3 and ignore the covariate, $x =$ number of plants, for this exercise. Assume the observation $y = 2.9$ on irrigation level 2 in block 3 is missing and assign it the value $y = 0$. Construct the new covariate with $x = -1$ for the missing value and $x = 0$ for all other values of y, and conduct the analysis of covariance. Estimate the missing value, and use the adjusted mean square for treatments to test the hypothesis of no difference among the treatment means.

6. Describe a study in your own field in which a covariate is used in addition to or in place of a blocking criterion to reduce the error variance and adjust treatment means. Provide justification for the use of the covariate values directly in the statistical model in place of blocking on the covariate, and justify its use on the basis that it is not affected by the treatments. Use an example from a journal article or your own research experiences if possible.