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Invited Review: Integrating Quantitative Findings from Multiple Studies Using Mixed Model Methodology[1]

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ABSTRACT

In animal agriculture, the need to understand complex biological, environmental, and management relationships is increasing. In addition, as knowledge increases and profit margins shrink, our ability and desire to predict responses to various management decisions also increases. Therefore, the purpose of this review is to help show how improved mathematical and statistical tools and computer technology can help us gain more accurate information from published studies and improve future research. Researchers, in several recent reviews, have gathered data from multiple published studies and attempted to formulate a quantitative model that best explains the observations. In statistics, this process has been labeled meta-analysis. Generally, there are large differences between studies: e. g., different physiological status of the experimental units, different experimental design, different measurement methods, and laboratory technicians. From a statistical standpoint, studies are blocks and their effects must be considered random because the inference being sought is to future, unknown studies. Meta-analyses in the animal sciences have generally ignored the Study effect. Because data gathered across studies are unbalanced with respect to predictor variables, ignoring the Study effect has as a consequence that the estimation of parameters (slopes and intercept) of regression models can be severely biased. Additionally, variance estimates are biased upward, resulting in large type II errors when testing the effect of independent variables. Historically, the Study effect has been considered a fixed effect not because of a strong argument that such effect is indeed fixed but because of our prior inability to efficiently solve even modest-sized mixed models (those containing both fixed and random effects). Modern statistical software has, however, overcome this limitation. Consequently, meta-analyses should now incorporate the Study effect and its interaction effects as random components of a mixed model. This would result in better prediction equations of biological systems and a more accurate description of their prediction errors.
(**Key words:** meta-analysis, mixed-model, regression)

Abbreviation key: MSE = mean square error.

INTRODUCTION

Frequently, scientists want to summarize prior knowledge in the form of a review. In such instances, the approach may be narrative, and the reviewer uses mental integration to combine the findings from a collection of studies. Results are then described qualitatively. A more modern approach is to use statistical methods to quantify research evidence. When such methods are applied to a set of different experiments (or studies) they are labeled as meta-analyses (Glass, 1976). Meta-analytic methods have progressed markedly in disciplines such as psychology, in which multitudes of studies are conducted without the ability to fully randomize and control experiments to the same extent as is expected in the animal sciences (Bangert-Drowns, 1986; Bushman and Cooper, 1990; Hedges et al., 1992; Wang and Bushman, 1999).

Several reviews of animal science research typically rely on regression methods in an attempt to extract quantitative relationships between measurements of interest (Broderick and Clayton, 1997; Nocek and Russell, 1988). Generally, the intent is to derive a regression for the prediction of future observations. In such reviews, however, it is customary for the authors to ignore the fact that observations within a given study have more in common than observations across studies. Additionally, differences in accuracy of measurements within and across studies are generally ignored. Unfortunately, these two common oversights (ignoring the blocking effect of studies and heterogeneity of variances) have as consequences that the parameters in the regression equation under consideration are estimated with considerable bias. In many instances, the wrong

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conclusions likely have been reached by the investigators.

Developments in statistical theory and recent advances in computer technology have produced new methods to solve models that are a better representation of the true structure underlying experimental observations. Mixed models incorporating both fixed and random effect variables can now be solved easily using powerful software applications such as PROC MIXED (SAS, 1999). The objectives in this paper are 1) to illustrate the errors and biases induced by traditional regression methods when the observations are gathered across many studies, and 2) to demonstrate the proper analysis of such data using mixed model procedures.

MATERIALS AND METHODS

Data Generation

Data used in my example are from a synthetic dataset with known parameters. Monte Carlo methods have been used extensively in statistics to investigate properties of statistical procedures (Bechhofer, 1954). For the example dataset, we refrained from using real data to avoid the inevitable confusion between the biology underlying the observations and the quantitative methodologies used to extract the information. Synthetic data, often referred to as simulated data, provide a unique opportunity because the analyst knows the true value of the parameters to be estimated before the analysis is performed so that the appropriateness of statistical methods can be gauged accurately. The goal in using a synthetic data set was not to prove that mixed model methodologies are better suited than other traditional methods for the analysis of summary data. The scientific evidence on this is very clear (Hedges and Holkin, 1985; Rosenthal, 1995). The synthetic dataset refers to generic X and Y variables without defining their biological meanings.

Data were generated using Monte Carlo methods (Fishman, 1978) according to the following model:

$$Y_{ij} = B_o + s_i + B_1X_{ij} + b_i\,X_{ij} + e_{ij} \qquad [1]$$

where:

Y_{ij} = the expected outcome for the dependent variable Y observed at level j of the continuous variable X in the study i,
B_o = the overall intercept across all studies (fixed effect),
s_i = the random effect of study i (i = 1, . . . , 20),
B_1 = the overall regressing coefficient of Y on X across all studies (fixed effect),
X_{ij} = the synthetic datum value j of the continuous variable X in study i,
b_i = the random effect of study i on the regression coefficient of Y on X in study i, and
e_{ij} = the unexplained residual error.

The e_{ij} was modeled as N (0, 0.25) (i.e., normally distributed with a mean of 0 and a variance of 0.25). The s_i were generated from N (0, 4); and b_i, from N (0, 0.04) and a correlation r = 0.5 with the random s_i effects. In short, this model assumes that there is an overall relationship (regression) between Y and X across all studies. The Study effect induces a random shift on the intercept and a random change in the slope of the regression. Furthermore, this random change in the slope of the regression attributable to studies is positively correlated with the random shift of the intercept. B_o was set at a mean value of 0.0, and the overall slope B_1 was set at a mean value of 1.0. Levels of the regression variable X were generated within each study using a uniform distribution between 1 and 10 ($X \sim U(1, 10)$). Levels of X were randomly truncated according to the mean to reflect the inevitable imbalance in regression levels across studies (i.e., levels and range of regressor X are not the same across all studies).

The complete dataset is reported in the Appendix so that interested readers can duplicate the analyses.

Simple Regression Analysis

As is generally done in published reviews, we analyzed the data using a simple regression model of the form:

$$Y_i = B_o + B_1X_i + e_i \quad i = 1, \ldots, 108 \qquad [2]$$

PROC GLM of SAS (1999) was used for convenience, but other SAS procedures or other commercial software could be used with identical results.

Fixed Effects Model Analysis

The potential effect of studies and their interaction with the regression slope of Y on X were analyzed in the context of a fixed effect model using PROC GLM with the following model:

$$Y_{ij} = B_o + S_i + B_1X_{ij} + B_iX_{ij} + e_{ij} \qquad [3]$$

where S_i is the fixed effect of study i (i = 1, . . . , 20).

B_i is the fixed effect of study i on the regression coefficient of Y on X in study i and all other symbols are as defined in equation [1]. Under this model, all effects are assumed to be fixed, except for the residual error.

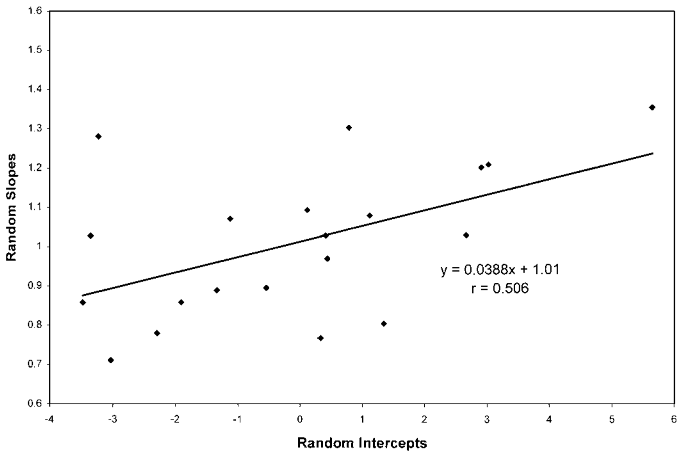

Figure 1. Relationship between true random slopes and true random intercepts for 20 studies with 108 simulated observations.

Mixed Model Analysis

Data were analyzed according to the following model:

$$Y_{ij} = B_o + B_1X_{ij} + s_i{*} + b_i{*}\ X_{ij} + e_{ij} \qquad [4]$$

where

$i = 1, \ldots, 20$ studies
$j = 1, \ldots, n_i$ values
$B_o + B_1X_{ij}$ is the fixed effect part of the model
$s_i{*} + b_i{*}\ X_{ij} + e_{ij}$ is the random effect part of the model

$$\begin{bmatrix} s_i^* \\ b_i^* \end{bmatrix} \sim \text{iid N}\left[\begin{pmatrix} o \\ o \end{pmatrix}, \Sigma\right]$$

$e_{ij} \sim$ iid N (o, σ_e^2)

$$\Sigma = \begin{bmatrix} \sigma_s^2 & \sigma_{sb} \\ \sigma_{sb} & \sigma_b^2 \end{bmatrix},$$

that is $s_i{*}$ and $b_i{*}$ have means of 0 and Σ is their variance – covariance matrix.

PROC MIXED as implemented in version 8.1 of SAS (1999) was used. Results would be identical in prior releases of SAS, although the display of results would be somewhat different.

RESULTS AND DISCUSSION

Assessment of Data

Figure 1 shows the simulated (true) slopes and intercepts for the 20 simulated studies. The regression of slopes on intercepts confirms that at an intercept value of 0.0, the simulated slope is indeed close to one. Likewise, the correlation between slope and intercept in the dataset was close to the value of 0.5 from which the data were generated. It is noteworthy that the variation across the regression line in Figure 1 does not carry the same meaning as in a conventional regression. The plotted observations are true values and not estimates or measurements. Thus, they are reported without any error. Their deviation from the regression line is due to the random effect of studies and not measurement errors in the observations. The figure serves as evidence that the simulated data contained the properties implied by model [1].

As is common in review studies, a simple graph of Y versus X is presented in Figure 2a. Ignoring the fact that data come from different studies, one would conclude from a rapid visual inspection that the data show a potentially good relationship between Y and X. In Figure 2b, the same data points are connected within a common study. Presented in this fashion, the data show the first evidence, albeit visual, that the relationship between Y and X within each study could differ

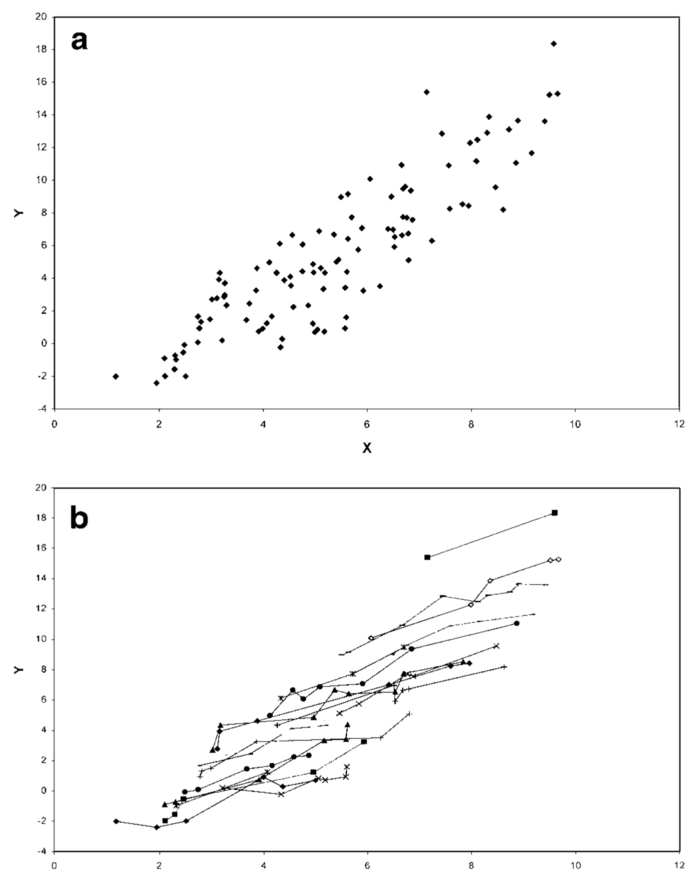

Figure 2. Visual presentation of the simulated data: a) simple X-Y plot of the observations across all studies; b) same observations as in a) but with observations common to a study linked by a line.

```
                              The SAS System
                      Conventional Regression using GLM

                            The GLM Procedure

Dependent Variable: Y

                                        Sum of
Source                      DF         Squares     Mean Square    F Value    Pr > F

Model                        1     1733.388325     1733.388325     391.41    <.0001

Error                      106      469.426449        4.428551

Corrected Total            107     2202.814774

               R-Square     Coeff Var      Root MSE        Y Mean

               0.786897      39.43857      2.104412      5.335925

Source                      DF       Type I SS     Mean Square    F Value    Pr > F

X                            1     1733.388325     1733.388325     391.41    <.0001

Source                      DF     Type III SS     Mean Square    F Value    Pr > F

X                            1     1733.388325     1733.388325     391.41    <.0001

                                               Standard
        Parameter         Estimate                Error    t Value    Pr > |t|

        Intercept     -5.187620375           0.56915924      -9.11      <.0001
        X              1.959492818           0.09904364      19.78      <.0001
```

Figure 3. Results from fitting a simple regression without the Study effect using SAS-GLM procedure (2000).

from that implied when data are examined without considering the studies as in Figure 2a. Although the presentation of data in the format of Figure 2b is easy in the presence of only one regressor, this practice cannot be extended to multiple regressors, which is the norm in review studies. However, appropriate mixed models provide for a quantitative representation in multiple dimensions of what Figure 2b achieves qualitatively in two dimensions.

Simple Regression Analysis

SAS statements to obtain the analysis according to model [2] are:

```
PROC GLM DATA=Dataregs;
MODEL Y = X;
OUTPUT OUT=OUTGLM P=Pred;                    [5]
RUN;
```

A portion of the output generated by SAS is shown in Figure 3. Results show a significant ($P < 0.001$) relationship between Y and X. The estimated relationship is shown graphically in Figure 4. The pattern of residuals (Figure 5) shows no evidence that the errors are not normally distributed or that the relationship between Y and X is anything but linear (Draper and Smith, 1981). This simple regression analysis is the two-dimensional equivalent to the multiple regression models used traditionally in reviews. Thus, a traditional review would conclude that the data indicate a strong linear relationship between Y and X, and that Y can be reasonably well predicted using the equation Y = 1.96 X − 5.19 (R^2 = 0.79). Now imagine that Y represents DM digestibility and X represents starch concentration of feedstuffs. Because of the thorough review by Professor Z, the world of both scientific and nonscientific litera-

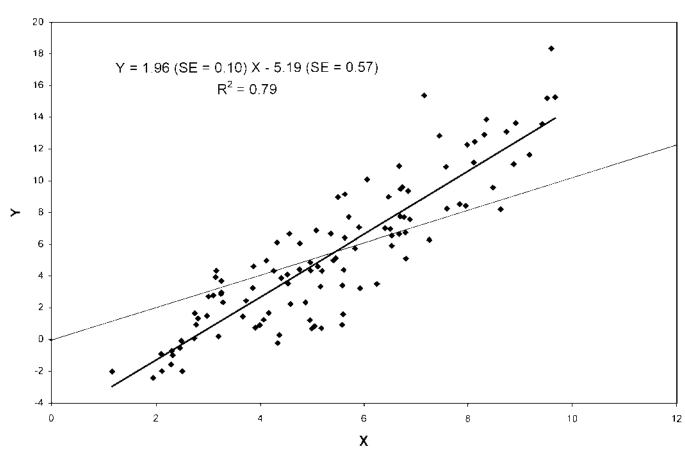

Figure 4. Simple regression line without the Study effect and uncorrected observations of Y on X.

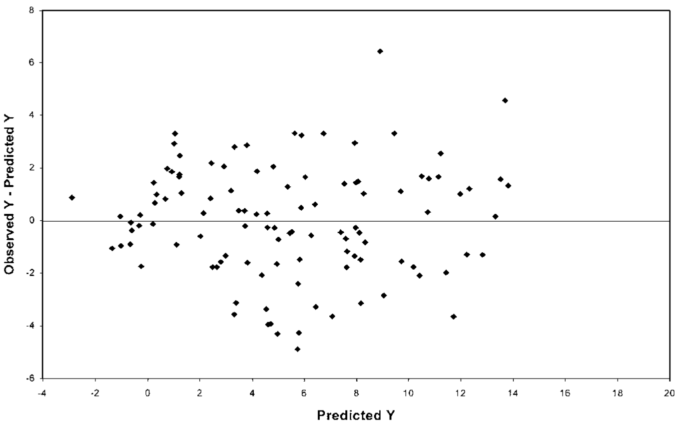

Figure 5. Residual plot from the simple regression of Y on X without the Study effect.

ture would be populated for years to come by the (erroneous) statement that “an increase in one percentage unit of starch results in an increase of two percentage units in DM digestibility.”

However, because we used synthetic data, we know that the “real world” is operating quite differently. First, the estimated intercept of –5.19 (± 0.57) is considerably different from the true overall intercept of zero. Likewise, the estimated slope of 1.96 (± 0.10) is nearly twice as large as the true overall slope across all studies, which was arbitrarily set at a value of 1.0. In fact, the mean square error (**MSE**), an estimate of σ^2, is nearly 18 times larger than the value used in generating the data (4.43 vs. 0.25). In short, because the wrong model (i.e., ignoring the effect of studies) is used in combination with the wrong procedure (fixed model), the estimate of the effect of X on Y is biased, and the estimate of the residual variance is also severely biased. As a result, the wrong inference is made from the data. Actually, an example could be easily constructed in which the estimated slope using a simple regression analysis would carry a sign that is significantly opposite to that of the true underlying population. In which case, not only the magnitude but also the implied biological mechanisms would be completely wrong.

Fixed Effects Model Analysis

SAS statements to produce the analysis according to model [3] with all effects considered fixed are:

```
PROC GLM DATA=Dataregs;
CLASS Study;
MODEL Y = X Study X*Study/SOLUTION;
RANDOM Study;
LSMEANS Study/at X=0 STDERR;
ESTIMATE ‘Overall Intercept’ INTERCEPT 20
Study 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1/DIVISOR=20;
ESTIMATE ‘Overall Slope’ X 20
X*Study 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1/DIVISOR=20;
ESTIMATE ‘Slope Study 1’ X 1
X*Study 1;
ESTIMATE ‘Slope Study 2’ X 1
X*Study 0 1;
ESTIMATE ‘Slope Study 3’ X 1                              [6]
X*Study 0 0 1;

ESTIMATE ‘Slope Study 18’ X 1
X*Study 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1;
ESTIMATE ‘Slope Study 19’ X 1
X*Study 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1;

ESTIMATE ‘Slope Study 20’ X 1
X*Study 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1;
RUN;
```

The variable Study is declared in the CLASS statement because it does not contain quantitative information, i.e., is not continuous (or is discrete). The SOLUTION option is used with the MODEL statement to produce an output of the solution vector. The RANDOM statement in PROC GLM merely computes expected mean squares for terms in the MODEL statement. It does not affect the way in which GLM estimates parameters. The LSMEANS statement includes the option “at X = 0” to produce estimates of intercepts and their standard errors for each study. The first ESTIMATE statement is used to calculate the estimate with a standard error of the intercept across all studies. The second ESTIMATE statement does likewise for the overall slope. The remaining ESTIMATE statements produce estimates of slope within each study.

A portion of the SAS output is shown in Figure 6. The MSE is a very close estimate of the true underlying residual variance of 0.25. All three sources of effects are significant ($P < 0.05$), indicating that studies most likely do not share a common intercept and slope, which is a proper conclusion, considering the model used to generate the data.

The overall intercept across all studies is estimated at –0.47 (± 0.26), which has a $P < 0.10$ of being different from the true underlying intercept of zero. Likewise, the overall slope estimate of 1.08 (± 0.05) also has a $P < 0.10$ of being different from 1.0, the true underlying slope. Note that in the SAS output (Figure 6), the t value, and its probability are those related to the null hypothesis that the slope is equal to zero. Although this is often a legitimate test, our interest here is to assess whether the estimate of the overall slope (1.08) is differ-

GLM with Block effects of Study and Interaction

The GLM Procedure

Class Level Information

Class	Levels	Values
Study	20	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Number of observations 108

The GLM Procedure

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	39	2185.809035	56.046386	224.11	<.0001
Error	68	17.005739	0.250084		
Corrected Total	107	2202.814774			

R-Square	Coeff Var	Root MSE	Y Mean
0.992280	9.372029	0.500084	5.335925

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X	1	1733.388325	1733.388325	6931.21	<.0001
Study	19	443.339909	23.333679	93.30	<.0001
X*Study	19	9.080801	0.477937	1.91	0.0273

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X	1	126.8459221	126.8459221	507.21	<.0001
Study	19	32.9501071	1.7342162	6.93	<.0001
X*Study	19	9.0808006	0.4779369	1.91	0.0273

Parameter		Estimate		Standard Error	t Value	Pr > |t|
Intercept		6.69072017	B	2.44029419	2.74	0.0078
X		1.19785542	B	0.28797233	4.16	<.0001
Study	1	-9.76806855	B	2.49318725	-3.92	0.0002
Study	2	-10.84370452	B	2.50146896	-4.33	<.0001
Study	3	-10.76669063	B	2.51492925	-4.28	<.0001
Study	4	-8.49920860	B	2.71491170	-3.13	0.0026
Study	5	-10.66978541	B	2.78528758	-3.83	0.0003
Study	6	-9.42762051	B	2.59555842	-3.63	0.0005
Study	7	-7.58522074	B	2.50345213	-3.03	0.0035
Study	8	-8.39013174	B	2.69181916	-3.12	0.0027
Study	9	-7.18518603	B	2.55110943	-2.82	0.0063
Study	10	-6.53467074	B	2.50772003	-2.61	0.0113
Study	11	-5.40976481	B	2.75348345	-1.96	0.0535
Study	12	-6.46775736	B	2.52019678	-2.57	0.0125
Study	13	-9.41457899	B	2.83257470	-3.32	0.0014
Study	14	-6.68008941	B	2.95681058	-2.26	0.0271
Study	15	-6.18930259	B	2.54688084	-2.43	0.0177
Study	16	-6.03477771	B	2.66644071	-2.26	0.0268
Study	17	-3.59620332	B	3.00697217	-1.20	0.2359
Study	18	-4.09971439	B	2.62165290	-1.56	0.1225
Study	19	-5.64765195	B	2.83481063	-1.99	0.0504
Study	20	0.00000000	B	.	.	.
X*Study	1	-0.38114121	B	0.32390983	-1.18	0.2434
X*Study	2	-0.02199458	B	0.32091810	-0.07	0.9456
X*Study	3	0.19643723	B	0.31998547	0.61	0.5413
X*Study	4	-0.69697390	B	0.37699674	-1.85	0.0688
X*Study	5	0.07467911	B	0.49784426	0.15	0.8812
X*Study	6	-0.14054978	B	0.36834470	-0.38	0.7040
X*Study	7	-0.38822476	B	0.31300838	-1.24	0.2191
X*Study	8	-0.01773922	B	0.39763166	-0.04	0.9645
X*Study	9	-0.25448906	B	0.32418368	-0.79	0.4352
X*Study	10	-0.14242854	B	0.30545359	-0.47	0.6425
X*Study	11	-0.58706696	B	0.41909673	-1.40	0.1658
X*Study	12	-0.14647855	B	0.30897937	-0.47	0.6370
X*Study	13	0.26495169	B	0.35799096	0.74	0.4618
X*Study	14	0.18075089	B	0.41228534	0.44	0.6625
X*Study	15	-0.00286484	B	0.31308014	-0.01	0.9927
X*Study	16	-0.32759965	B	0.32978667	-0.99	0.3241
X*Study	17	-0.24659517	B	0.36779316	-0.67	0.5048
X*Study	18	0.01163805	B	0.31331482	0.04	0.9705
X*Study	19	0.26370130	B	0.33503019	0.79	0.4340
X*Study	20	0.00000000	B	.	.	.

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

The GLM Procedure

Source	Type III Expected Mean Square
X	Var(Error) + Q(X,X*Study)
Study	Var(Error) + 0.3629 Var(Study)
X*Study	Var(Error) + Q(X*Study)

The GLM Procedure
Least Squares Means at X=0

Study	Y LSMEAN	Standard Error	Pr > |t|
1	-3.07734838	0.51082964	<.0001
2	-4.15298434	0.54982836	<.0001
3	-4.07597045	0.60813929	<.0001
4	-1.80848842	1.18983605	0.1332
5	-3.97906524	1.34268060	0.0042
6	-2.73690034	0.88424420	0.0029
7	-0.89450057	0.55878155	0.1141
8	-1.69941157	1.13615785	0.1393
9	-0.49446586	0.74372278	0.5084
10	0.15604943	0.57760197	0.7878
11	1.28095536	1.27539616	0.3188
12	0.22296281	0.62956815	0.7243
13	-2.72385882	1.43820850	0.0625
14	0.01063077	1.66963860	0.9949
15	0.50141758	0.72908592	0.4940
16	0.65594247	1.07464894	0.5436
17	3.09451685	1.75694220	0.0827
18	2.59100578	0.95813787	0.0086
19	1.04306823	1.44260721	0.4721
20	6.69072017	2.44029419	0.0078

The GLM Procedure

Dependent Variable: Y

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Overall Intercept	-0.46978623	0.26471640	-1.77	0.0804
Overall Slope	1.07975602	0.04794360	22.52	<.0001
Slope Study 1	0.81671421	0.14828863	5.51	<.0001
Slope Study 2	1.17586084	0.14163461	8.30	<.0001
Slope Study 3	1.39429265	0.13950858	9.99	<.0001
Slope Study 4	0.50088152	0.24330738	2.06	0.0434
Slope Study 5	1.27253453	0.40610447	3.13	0.0025
Slope Study 6	1.05730564	0.22967315	4.60	<.0001
Slope Study 7	0.80963066	0.12266288	6.60	<.0001
Slope Study 8	1.18011620	0.27419496	4.30	<.0001
Slope Study 9	0.94336636	0.14888584	6.34	<.0001
Slope Study 10	1.05542688	0.10185201	10.36	<.0001
Slope Study 11	0.61078846	0.30448975	2.01	0.0488
Slope Study 12	1.05137687	0.11198300	9.39	<.0001
Slope Study 13	1.46280711	0.21267220	6.88	<.0001
Slope Study 14	1.37860631	0.29504430	4.67	<.0001
Slope Study 15	1.19499058	0.12284588	9.73	<.0001
Slope Study 16	0.87025577	0.16072084	5.41	<.0001
Slope Study 17	0.95126025	0.22878756	4.16	<.0001
Slope Study 18	1.20949348	0.12344276	9.80	<.0001
Slope Study 19	1.46155673	0.17122257	8.54	<.0001
Slope Study 20	1.19785542	0.28797233	4.16	<.0001

Figure 6. Results from fitting a fixed model including a Study effect and its interaction with a continuous X variable using the SAS-GLM procedure (2000).

ent from the true underlying slope (1.0). The correct t value to be used for testing the null hypothesis that the slope estimate is equal to 1.0 is calculated as follows: t = (1.08 – 1.0) ÷ 0.05 = 1.6. This t value has 68 df, and its probability is assessed with a standard table of the t distribution which is reported in most elementary statistical textbook (e.g., Table A4 in Snedecor and Cochran, 1980). For 19 of the 20 studies, both the estimated intercept and estimated slope fall within the 95% confidence ranges.

The expected mean square table produces the proper coefficients for Study from which an estimate of the variance component for Study can be calculated. Refer to the type III expected mean square table of Figure 6. The type III mean square for Study is equal to the variance component for Error (σ_e^2) plus 0.3629 times the variance component of Study (σ_s^2). By default, the mean square error (0.25) is the estimate of σ_e^2. Thus, the type III mean square for Study (1.734) = 0.25 + 0.3629 σ_s^2. Using simple algebra, we get $\sigma_s^2 = 4.09$, which is a value close to the value of 4.0 from which the data were generated. The GLM procedure does not, however, produce the proper components for the interaction between a random effect (Study) and a continuous fixed effect (X). In fact, GLM considers this interaction as fixed, whereas it clearly should be random (St-Pierre and Jones, 1999). Further discussion regarding the output from GLM will be discussed when results from PROC MIXED are presented.

Mixed Model Analysis

The SAS statements to produce the analysis according to model [4] are:

```
PROC MIXED Data = Dataregs COVTEST
NOCLPRINT NOITPRINT;
CLASS Study;
MODEL Y = X/SOLUTION;
RANDOM intercept X/TYPE=UN SUBJECT=          [7]
Study SOLUTION OUTP = Predictionset;
RUN;
```

The PROC MIXED statement includes three options. NOCLPRINT and NOITPRINT suppress the printing of information at the class level and of the interaction history, respectively. They are included here for space saving reasons. COVTEST provides a hypothesis test of the variance and covariance components. As in GLM, the variable Study is declared in the CLASS statement because it does not contain quantitative information. The MODEL and RANDOM statements together specify the model to be executed. Although the MODEL statement includes the fixed effect components, the RANDOM statement contains the random effects. The above syntax expresses that the outcome Y is modeled by a fixed intercept (which is implied in the MODEL statement), a fixed slope, a random intercept clustered by study, and a random slope also clustered by study. The TYPE = UN option in the RANDOM statement specifies an unstructured variance-covariance matrix for the intercepts and slopes.

A partial listing of the SAS output is shown in Figure 7. The section with the heading “Covariance Parameter Estimates” reports on the variance-covariance parameter estimates with asymptotic tests on their significance. Parameter estimates are listed in order of their listing in the RANDOM statement. Thus, the first variance component is for the intercept, the third is for the slope, the second for their covariance, and the fourth for the residual variance. All four estimates are well within the 95% confidence range of the true underlying parameters. A tight estimation of variance components requires a much greater number of observations than the estimation of fixed effect parameters. With only 108 observations spread across 20 studies, the covariance between the random intercept and slope is not significantly different from zero ($P = 0.16$), although its estimate of 0.196 is very close to the true underlying covariance (0.20). This limitation of power for the estimation of variance components must be recognized especially when more complex models are being estimated with limited number of observations.

The section labeled “Solution for Fixed Effects” reports the estimates and statistical test for the overall fixed intercept and slope. Both estimates are close to their true underlying values, and a simple Student’s t-test would conclude that the overall intercept and slope are not significantly different from 0.0 and 1.0, respectively ($P > 0.20$).

The following section, “Solution for Random Effects,” reports the estimators of the random effects for each study. Notice that these values differ from those obtained under a fixed effect model (Figure 6). Having used a synthetic dataset with known parameters, the two methods can be compared based on their ability to estimate the intercept and slope specific to each study. Figure 8 shows a residual graph of the difference between the estimated and the true intercepts versus the true intercepts. Visually, it is clear that the mixed model produces estimates that are consistently closer to their true values. This is verified statistically from the standard deviation of the differences, which is 0.49 for the mixed model compared with 1.07 for the fixed model. Likewise, Figure 9 shows the residual graph of the difference between the estimated and the true slopes. Again, estimates from the mixed model are much closer to their true values. The standard deviation of the difference is 0.09 for the mixed model compared with 0.19 for the fixed model. These results are not surprising, considering that PROC MIXED recovers both the inter-block and intra-block information.

It is common for scientists to present regression results in the form of a Y versus X graph as we did in Figure 4, where the regression line is shown in conjunction with the observations. Results from the mixed model regression cannot be graphed this simplistically. This is because the observations come from a multi-dimensional space (22 in our data set). When the observations are collapsed from the multi-dimensional space into a two-dimensional space, it is important to correct or adjust the observations for the lost dimensions or else the regression will appear biased. To do this, one must calculate adjusted Y values to be used in an X-Y graphic. These adjusted Y values, also called adjusted observations, are easily calculated, remembering that any regression model is based on the following basic equation: Y observed = Y predicted + Residual. The Y predicted are simply the Y values on the regression line. Residuals are found in the Predictionset SAS dataset generated by the OUTP = option in the model statement. Each residual is added to its corresponding Y predicted value to generate adjusted Y values. These are reported in the Appendix table in the column labeled "Adjusted Y" and can be compared to the Y values uncorrected for the Study effect. A graph of adjusted Y versus X for the mixed model is shown in Figure 10. The visual and mental interpretation of this graph would be correct statistically. That is, there is a strong relationship between Y and X ($R^2 = 0.99$), and observations within Study are very predictable. Alternatively, a conventional residual graph could be presented to carry the same message (Figure 11).

It is important to understand the distinct differences between the mixed model and the fixed model with respect to their implied variance of observations. The fixed model has only one random component, the residual variance. Thus,

$$\mathrm{Var}\ (Y_{ij}) = \sigma_e^2. \qquad [8]$$

Under the mixed model, however, all four components of variance enter the calculation, and the variance for a randomly chosen X within a randomly chosen study is:

$$\mathrm{Var}\ (Y_{ij}) = [1, X_{ij}] \sum \begin{bmatrix} 1 \\ X_{ij} \end{bmatrix} + \sigma_e^2 \qquad [9]$$

In this current example, Var (Y_{ij}) = 0.25 for the fixed model. Under the mixed model, Var (Y_{ij}) is at a minimum at $X_{ij} = 0$ and is equal to 5.66. At a value of $X_{ij} = 9$, Var (Y_{ij}) = 29.48. Put this way, it is clear that the variance estimate (MSE) under the fixed model is for an observation taken from a study with a known effect. This is equivalent to hiding an observation from the dataset and estimating what its value would be. In real applications, however, the scientist wants to infer for future observations from studies not in the dataset (future studies, or application as a prediction for field application). The additional variance due to the effect of unknown future studies must be accounted for. Thus, the Var (Y_{ij}) is much larger for the mixed model than for the fixed model. In short, the inference range for the fixed model is limited to those studies that are part of the regression. It is simply wrong to try to infer anything beyond that. Thus, regression equations derived from fixed models that incorporate the fixed effect of studies and their interaction with continuous regressors severely underestimate the variance of their prediction. Under the mixed model, however, the proper inference space can be achieved. As with the fixed model, the narrow inference space can be determined if one's sole interest is in the studies being reviewed. In such instance, Var ($Y_{ij}|s_i$) = σ_e^2. But if the scientist wants to infer for future observations, i.e., wants a broad inference space, then all components of variance must be used as in equation [9]. In the current example, one would conclude that there is a very tight linear relationship between Y and X, but that the random variation induced by studies reduces the value of the regression for prediction purposes.

The Study Effect

In essence, the Study effect represents the variance between studies not accounted for by the other variables in the model. Ultimately, one would want the components of variance for Study and the interactions of Study with continuous independent variables to be very small and nonsignificant. The fixed effect components could then be used to predict future observations. It has been our experience, however, that the Study effect is generally important (Firkins et al., 1998, 2000; Oldick et al., 1999), indicating that much work is needed to standardize measurement methods across studies and to characterize those factors impacting the variance of the trait of interest.

Statistically speaking, studies represent blocks of observations. In experimental research, block effects have traditionally been considered fixed effects primarily because appropriate and efficient procedures for solving the mixed model were not available. An effect is considered fixed if the levels in the study represent all possible levels of the factor, or at least all levels about which inference is to be made (Littell et al., 1996). In contrast, factor effects are random if the levels of the factor that are used in the study represent only a random sample of a larger set of potential effects. In the latter case, the interest is not in the specific levels of the factors but to the larger set of all levels constituting the population (St-Pierre and Jones, 1999). Expressed this way, it should be clear that the effect of Study in the context of a meta-analytic review is random.

Model Expansion and Reduction

Random covariance not significant. As in this example, it is possible for the variance components but not the covariance to be significant. In such instances, one could fit a mixed model in which the covariance

The SAS System
Full Model with Random Intercept, Slope and Covariance

The Mixed Procedure

Model Information

Data Set	DATAREG
Dependent Variable	Y
Covariance Structure	Unstructured
Subject Effect	Study
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Dimensions

Covariance Parameters	4
Columns in X	2
Columns in Z Per Subject	2
Subjects	20
Max Obs Per Subject	9
Observations Used	108
Observations Not Used	0
Total Observations	108

Covariance Parameter Estimates

Cov Parm	Subject	Estimate	Standard Error	Z Value	Pr Z
UN(1,1)	Study	5.4139	2.2097	2.45	0.0071
UN(2,1)	Study	0.1963	0.1426	1.38	0.1687
UN(2,2)	Study	0.03305	0.02211	1.49	0.0675
Residual		0.2504	0.04205	5.95	<.0001

Fit Statistics

Res Log Likelihood	-135.2
Akaike's Information Criterion	-139.2
Schwarz's Bayesian Criterion	-141.2
-2 Res Log Likelihood	270.5

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
3	198.89	<.0001

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	-0.6085	0.5628	19	-1.08	0.2932
X	1.0974	0.05644	19	19.44	<.0001

Solution for Random Effects

Effect	Study	Estimate	Std Err Pred	DF	t Value	Pr > |t|
Intercept	1	-2.6807	0.6489	68	-4.13	0.0001
X	1	-0.2092	0.1081	68	-1.93	0.0572
Intercept	2	-3.1159	0.6658	68	-4.68	<.0001
X	2	-0.03270	0.1055	68	-0.31	0.7576
Intercept	3	-2.6069	0.6831	68	-3.82	0.0003
X	3	0.09487	0.1039	68	0.91	0.3643
Intercept	4	-2.7445	0.7719	68	-3.56	0.0007
X	4	-0.2737	0.1200	68	-2.28	0.0257
Intercept	5	-2.4773	0.7332	68	-3.38	0.0012
X	5	-0.08685	0.1368	68	-0.63	0.5276
Intercept	6	-1.9531	0.7037	68	-2.78	0.0071
X	6	-0.08308	0.1221	68	-0.68	0.4986
Intercept	7	-0.7142	0.6768	68	-1.06	0.2951
X	7	-0.1877	0.09859	68	-1.90	0.0611
Intercept	8	-0.7075	0.7410	68	-0.95	0.3431
X	8	-0.00980	0.1257	68	-0.08	0.9381
Intercept	9	-0.2287	0.7219	68	-0.32	0.7523
X	9	-0.08324	0.1050	68	-0.79	0.4305
Intercept	10	0.6238	0.6970	68	0.90	0.3739
X	10	-0.01681	0.09017	68	-0.19	0.8527
Intercept	11	0.2263	0.7462	68	0.30	0.7626
X	11	-0.08542	0.1275	68	-0.67	0.5052
Intercept	12	0.6593	0.7077	68	0.93	0.3548

X	12	-0.01517	0.09349	68	-0.16	0.8716
Intercept	13	-0.3378	0.8757	68	-0.39	0.7008
X	13	0.1010	0.1108	68	0.91	0.3654
Intercept	14	1.4756	0.8497	68	1.74	0.0870
X	14	0.1249	0.1213	68	1.03	0.3068
Intercept	15	1.1717	0.7378	68	1.59	0.1169
X	15	0.08582	0.09636	68	0.89	0.3763
Intercept	16	0.3529	0.8251	68	0.43	0.6702
X	16	-0.08963	0.1037	68	-0.86	0.3903
Intercept	17	2.1240	0.9414	68	2.26	0.0273
X	17	0.05840	0.1095	68	0.53	0.5955
Intercept	18	2.9298	0.8266	68	3.54	0.0007
X	18	0.1456	0.09352	68	1.56	0.1242
Intercept	19	2.5864	0.9358	68	2.76	0.0073
X	19	0.2503	0.1013	68	2.47	0.0160
Intercept	20	5.4168	1.0292	68	5.26	<.0001
X	20	0.3124	0.1110	68	2.81	0.0064

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
X	1	19	378.01	<.0001

Figure 7. Results from fitting a fixed model including a random Study effect and its random interaction with a continuous X variable using the SAS-MIXED procedure (2000).

components are assumed to be equal to zero. In the example dataset, the covariance between intercept and slope was positive, indicating that, if a study's intercept is larger than that of the others, its slope will tend to be larger as well. This was implied in the model from which the data were generated. This is an important, although, difficult concept. The sign and size of the covariance component is not related to the sign of the slope or the intercept. In traditional regression analyses, the parameters (slope and intercept) are fixed and, therefore, have neither a variance nor covariance. Their estimates, however, follow a bivariate, normal distribution. Therefore, in a fixed model, parameter estimates have variances, the square root of which is the standard errors reported by SAS-GLM in Figure 6. What many users do not realize is that these parameter estimates also have a covariance. That is, the estimate of the slope is correlated with the estimate of the intercept. This aspect of traditional regression is well covered in many regression textbook (e.g., Draper and Smith, 1981). In mixed model regression, the parameters themselves and not only their estimates are assumed random. With this approach, the parameters are assumed to follow a bivariate normal distribution. Thus, the parameters have a variance and a covariance. The test on the random covariance determines whether the random intercepts are correlated to the random slopes.

The synthetic data used in our example were generated using a random covariance of 0.2 (or equivalently a random correlation equal to 0.5). Proc Mixed yields a covariance estimate of 0.1963 (Figure 7). Although

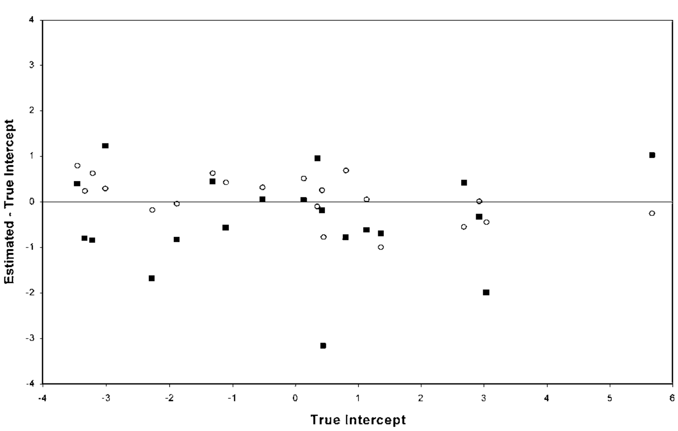

Figure 8. Plot of the difference between the estimated and the true intercepts versus the true intercepts; ■ are from the fixed model, and ○ are from the mixed model.

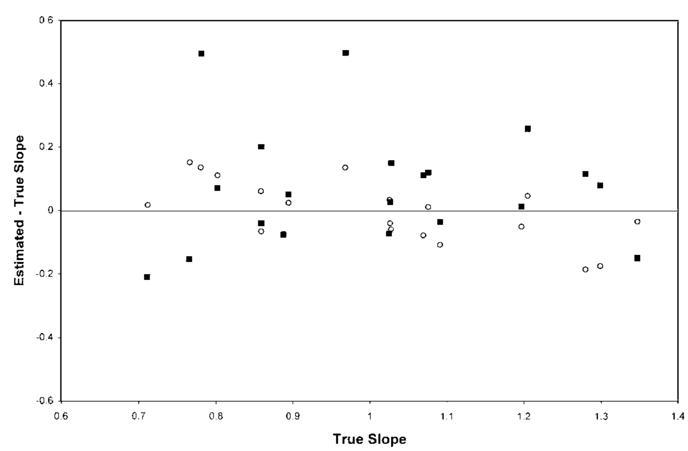

Figure 9. Plot of the difference between the estimated and the true slopes versus the true slopes; ■ are from the fixed model, and ○ are from the mixed model.

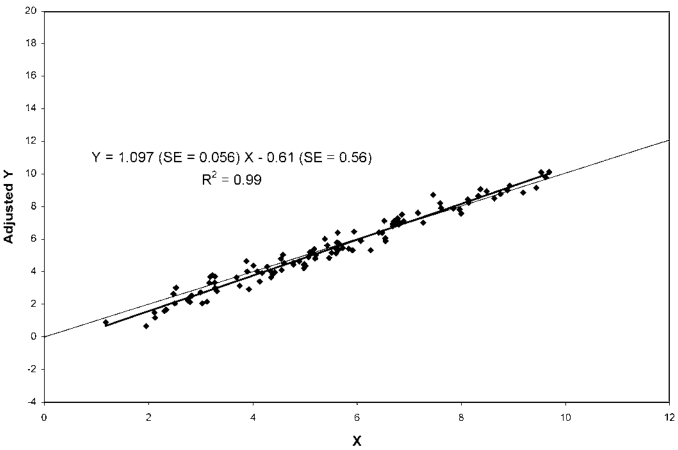

Figure 10. Plot of adjusted observations and the mean regression line across studies from the mixed model analysis. Observations are adjusted for other variables in the model because the presentation of data is collapsing multiple dimensions into a two-dimensional plane.

close to the true value used to generate the data, this estimate is not statistically different from 0.0 ($P = 0.17$, Figure 7) and one would conclude that the random parameters are not correlated. In such instances, a reduced model without a covariance component must be fitted. The RANDOM statement in the PROC MIXED [7] needs to be modified to either one of the following two statements:

```
RANDOM intercept X/TYPE=VC SUBJECT=
Study SOLUTION;
or                                              [10]
RANDOM intercept X/SUBJECT=
Study SOLUTION;
```

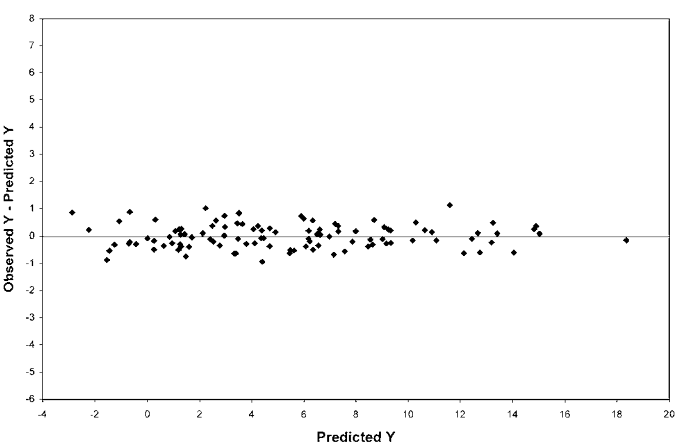

Figure 11. Residual plot from the mixed model analysis that includes the random effect of Study and its random interaction with the predictor variable X.

In general, the researcher should recognize that accurate estimations of variances and covariances require a considerable number of observations. Thus, significance tests on their estimators should be more liberal than the traditional 0.05 level used for fixed effects.

Random slope not significant. In instances in which the random interaction of Study by X is deemed nonsignificant, a reduced model can be estimated by modifying the RANDOM statement [7] in PROC MIXED as follows:

```
RANDOM intercept/ SUBJECT                       [11]
=Study SOLUTION;
```

Under this model, the Study effect is solely an intercept shift. That is, the individual regressions within Study are all parallel lines with different random intercepts.

Multiple regressors. The example constructed herein involved only one continuous independent variable. In most applications, however, the researcher has an interest in a number of continuous independent variables. This is easily done within PROC MIXED. Suppose, for example, that another continuous variable Z should be added to the model. The SAS statements to achieve this are as follows:

```
PROC MIXED Data=Dataregs COVTEST
NOCLPRINT NOITPRINT;
CLASS Study;
MODEL Y = X Z/SOLUTION;
RANDOM intercept X Z/TYPE=UN SUBJECT=    [12]
Study SOLUTION;
RUN;
```

Using the TYPE = UN structure, the addition of Z to the RANDOM statement requires that three additional variance-covariance components be estimated: variance of Z (slope) and its covariance with the intercept and X. The addition of a few more continuous, random independent variables can result in an over-parameterized model. In such instances, it is generally best to remove the estimation of the covariance elements by using TYPE = VC as an option in the RANDOM statement.

Fixed, discrete independent variables. The inclusion of fixed, discrete (class) independent variables into a summary mixed model is straightforward. Suppose that, in our example, observations can be classified into three classes based on the (discrete) value of a variable M. The mixed model analysis would be done using the following SAS statements:

```
PROC MIXED Data = Datargs COVTEST
NOCLPRINT NOITPRINT;
```

```
CLASS Study M;
MODEL Y = X M X*M/SOLUTION;
RANDOM intercept X/TYPE=UN SUBJECT=        [13]
Study SOLUTION;
RUN;
```

The interaction of X by M in the MODEL statement produces a test of the homogeneity of slopes across the M classes of effects. The significance of this interaction indicates that individual fixed slopes should be fitted for each level of M. Nonsignificance would indicate homogeneity of slopes. In the latter case, the X * M effect should be removed from the MODEL statement. An example of the application of this procedure was developed by Firkins et al. (2000), who looked at the relationship between starch digestibility (Y variable) and various continuous variables such as DMI (X variable) for different types of grains (M variable). A large dataset was constructed with published results from experiments where starch digestibility was reported for various grains subject to various processing. Because the data were derived from various studies, it should now be clear that a random Study had to be included in the model. A primary interest was in estimating the effect of grain types and processings (fixed, discrete explanatory variables) as well as DMI and other fixed, continuous explanatory variables on starch digestibility. Firkins et al. (2000) did not find significant interactions between the random effect of Study and any of the fixed effects. This shows that the effect of DMI on starch digestibilities, for example, was not dependent on Study. That is, the slope of the linear relationship between starch digestibility and DMI was not dependent on the study. The random Study effect was, however, highly significant, indicating that the intercept of the linear relationship between starch digestibility and DMI (for a given grain type and processing) was very dependent on the Study under consideration. Without the inclusion of the random Study effect in the model, most independent variables did not have a significant effect on starch digestibility. The inclusion of the Study effect allowed a much more accurate estimate of the fixed effects and reduced considerably the potential for type II errors.

Weighting the observations. Research designs and accuracy vary across studies. Least squares means of the independent variable are generally not estimated with equal accuracy across studies. This is easily detected by comparing the standard errors of the Y observations across studies. Failure to account for the heterogeneous errors violates the assumption of identical distribution of residual errors. This situation is easily remedied in PROC MIXED using the WEIGHT statement. It is easily shown that, in this instance, the optimal weight is the value resulting from inverting the square of the standard error of each mean (Wang and Bushman, 1999). The basic idea is to transform the observation Y to another variable Y*, which does satisfy the usual assumption. In general, however, the transformed Y* has a different scale than Y. Thus, when using the simple inverse of the squared standard errors as the weight vector, the square root of MSE is of a different scale than that from the original Y. This scale problem can be circumvented easily. Define w_1 as the inverse of the squared standard error, and $\overline{w}$ as its mean value. Let $w_2 = w_1/\overline{w}$. Then w_2 retains the optimal weight property of w_1 but with the advantage that it is centered around the value of 1.0. Thus, by using w_2 as opposed to w_1, observations are still optimally weighted but with the added benefit that variance and covariance components are now expressed on the same scale as the original Y data. Application of this weighting scheme can be found in Firkins et al. (1998) and Firkins et al. (2000).

CONCLUSION

The traditional approach using simple regression methods to integrate information across studies is wrong statistically and most likely results in wrong inferences and conclusions. Observations across studies are not balanced. Ignoring the Study effect while performing a regression analysis leads to biased estimates of the regression coefficients and biased estimates (inflated) of their standard errors. The Study effect is fundamentally random. Thus, it is best to use mixed model methodologies for extracting quantitative relationships among the data. Unfortunately, the scientific literature abounds with prior reviews and summaries from which simple regression methods were used, even though the observations were clearly blocked by studies. Thus, it is likely that many such summary studies have reached wrong conclusions and have suggested biased equations for predicting quantitative variables.

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APPENDIX

Obs	Experiment	X	Y	Adjusted Y
1	1	1.16391	–2.92296	0.886005
2	1	1.94273	–1.03365	0.642602
3	1	2.50229	–1.00383	3.000373
4	1	3.98627	1.33325	4.357696
5	1	4.36177	1.13752	3.813666
6	1	4.98954	1.59278	4.355711
7	2	2.10910	–1.33751	1.164866
8	2	2.28976	–1.14376	1.580793
9	2	2.45645	–1.09304	2.616348
10	2	4.95084	1.42740	4.425112
11	2	5.92572	2.99572	6.457145
12	3	2.09605	–0.70073	1.474665
13	3	2.30059	–1.11007	1.619237
14	3	3.90388	0.17334	2.923988
15	3	5.15574	2.38882	5.380509
16	3	5.57553	1.78167	5.410837
17	3	5.60523	3.09353	6.389589
18	4	3.19550	0.18097	3.766463
19	4	4.32691	0.74310	3.642741
20	4	5.04092	1.94700	4.884136
21	4	5.17521	1.07029	4.806475
22	4	5.57254	2.71423	5.117365
23	4	5.59532	2.43982	5.787204
24	5	2.31878	0.26487	1.650219
25	5	4.06027	1.99979	4.01755
26	6	2.48122	1.31151	2.033381
27	6	2.73224	1.32218	2.21003
28	6	3.66628	2.52286	3.649436
29	6	4.15568	2.83423	3.907685
30	6	4.57694	2.99086	4.507874
31	6	4.86356	3.90049	4.619561
32	7	2.76700	1.18358	2.125706
33	7	2.79859	1.70206	2.515893
34	7	2.97074	1.84639	2.7178
35	7	3.85289	3.13155	4.635941
36	7	6.24981	5.27271	5.307471
37	7	6.80389	5.85844	6.990859
38	8	2.74107	–0.26691	2.34684
39	8	3.72445	1.32567	3.127501
40	8	4.51721	1.04086	4.783446
41	8	5.18536	2.40105	5.012804
42	9	3.24046	3.12334	3.314861
43	9	3.28640	3.52558	2.791165
44	9	4.52824	4.29043	4.083241
45	9	5.10468	4.04917	5.190446
46	9	5.40578	5.55135	5.603263
47	9	7.25461	7.23110	7.008939
48	10	3.09531	3.31464	2.153173

49	10	3.13951	2.5785	3.300918
50	10	3.86928	4.0819	4.004738
51	10	6.40856	6.6731	6.403454
52	10	7.35976	6.8001	7.902456
53	10	7.95716	8.2098	7.817707
54	11	3.24974	2.8825	2.974935
55	11	3.25369	4.8511	3.695179
56	11	4.40050	4.3538	3.954499
57	11	4.74897	6.0760	4.53147
58	11	4.96576	5.9062	4.471565
59	12	3.00403	2.5733	2.048013
60	12	3.15420	3.9324	3.673089
61	12	4.95652	5.8456	4.207035
62	12	5.35746	5.2066	6.010887
63	12	5.62651	6.3312	5.751872
64	12	6.53297	6.6019	5.881591
65	12	6.69580	6.9498	7.101051
66	12	7.83844	8.1031	7.872914
67	13	5.44713	6.4838	4.84303
68	13	5.82682	6.2341	5.413382
69	13	6.76986	7.8937	7.262614
70	13	6.87949	6.9477	7.107462
71	13	8.47819	8.1336	8.930556
72	14	4.31493	5.2280	4.042084
73	14	5.70501	6.0941	5.453168
74	14	6.70113	7.6858	7.07218
75	15	4.11223	5.2129	3.394821
76	15	4.55329	5.2169	5.02262
77	15	4.75641	6.1755	4.418824
78	15	5.07420	5.7492	5.190877
79	15	5.90125	6.5407	5.308542
80	15	6.84886	6.7004	7.494239
81	15	8.87813	10.2425	8.98207
82	16	4.25114	5.3359	4.299291
83	16	6.50762	9.1897	7.101632
84	16	6.52857	8.4259	6.057743
85	16	6.67694	8.2782	6.786494
86	16	6.79539	8.3206	6.895271
87	16	8.62833	10.4385	8.485119
88	17	6.47237	7.6132	6.389649
89	17	6.73826	7.7381	6.981667
90	17	7.57947	8.5328	8.20555
91	17	8.11267	8.9045	8.436064
92	17	9.18169	9.7732	8.843197
93	18	5.49319	6.6492	5.155737
94	18	5.62711	6.4464	5.322191
95	18	6.67182	8.5909	6.927195
96	18	7.44940	9.2077	8.704882
97	18	8.13165	9.3650	8.224313
98	18	8.31896	9.5025	8.628547
99	18	8.74606	10.3598	8.758426
100	18	8.92038	9.4361	9.271535
101	18	9.43227	11.0051	9.143463
102	19	6.06067	7.9306	5.887499
103	19	7.98874	10.1887	7.561783
104	19	8.35784	10.9856	9.055234
105	19	9.52798	11.5070	10.08612
106	19	9.68213	12.6051	10.10259
107	20	7.15668	9.9928	7.610491
108	20	9.61256	12.2149	9.784963