NONLINEAR MODELING OF pH DECLINE IN BEEF CARCASSES

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Abstract

Electrical stimulation speeds the rate of pH decline in beef muscle. A study was conducted to evaluate an electrical stimulation (ES) method for beef sides and its effect on pH decline compared to non-stimulated control counterparts using nonlinear modeling. The pH of each carcass in the study was measured at selected times over a 24-hour time period postmortem. A statistical methodology is described for comparing two treatments based on the mean pH decline over time. The repeated measures structure of the data is incorporated into the statistical procedure. A nonlinear exponential decay model is used to characterize the mean pH decline. Various comparisons of the mean response to treatment are made based on the nonlinear model.

KEYWORDS: Linear models; Non-linear models; Repeated measures analysis

1. Introduction

A study was conducted to compare electrical stimulation (ES) processing method of beef carcasses to non-stimulation processing method, as a control, on the basis of relative rate of pH decline. Electrical stimulation is the process of electrically shocking carcasses or sides postmortem to enhance product quality, i.e., tenderness, color, or grade. The experimental design was a completely randomized design with sides of 43 beef carcasses being randomly assigned to either the control or ES treatment group. The carcass information was lost for the statistical analysis, and thus, the sides were analyzed as independent experimental units. The pH readings were recorded in the longissimus muscle at 1, 2, 4, 6, 8, and 24 hours postmortem. Sides were electrically stimulated at 1 hour postmortem, just prior to the first pH recording using the iodoacetate technique. The observed pH response data for 10 randomly selected sides are displayed in Figures 1a and 1b, for the control and ES treatment groups, respectively. Figures 2a and 2b are plots of these same data, with the observations connected by lines indicating the individual sides for the control and ES treatment groups, respectively.

To better evaluate the relative effectiveness of ES compared to the control, the mean pH decline in beef muscle for each treatment group was modeled as a function of time. Traditionally, to incorporate the repeated measures structure of the data, a common response model is fit to each individual subject's data. The estimated parameters then are analyzed in an analysis of variance and conclusions of the effectiveness of treatment are based on the mean of the estimated parameters. In the linear model case, this is a relatively easy process. Here, pH decline is more appropriately modeled as a nonlinear function in time, with pH declining to an asymptotic value. As can be seen in Figures 2a and 2b, a

by-subject approach to analyzing these data would not be recommended, because it is apparent that the data observed for an individual side are relatively poor representations of a common response function. It would be difficult to estimate the parameters of a common nonlinear model for each individual side using iterative, nonlinear techniques. A different methodology must be employed to obtain adequate estimates of the mean pH decline and still maintain the repeated measures structure of the data in the hypothesis testing process.

A methodology based on modeling the observed mean response over time is presented in the following sections. The repeated measures structure of the data is incorporated into the hypothesis testing process by obtaining a model-free estimate of the variance-covariance structure of the data. The distribution of the estimated model parameters is then written as a function of recording times and the variance-covariance of the observed data.

2. Motivation

To motivate the proposed methodology, the linear model case is considered in this section. Let $\underset{\sim}{y}_i = \underset{\sim}{X}\underset{\sim}{\beta} + \underset{\sim}{\epsilon}_i$ define the linear model describing the i^{th} subject's data across t sampling times, where $\underset{\sim}{y}_i$ is the txl vector of responses, $\underset{\sim}{X}$ is the txp matrix of independent variables, $\underset{\sim}{\beta}$ is the pxl vector of model parameters, and $\underset{\sim}{\epsilon}_i$ is the txl random error vector, i = 1, 2, ..., n. Here, it is assumed that a common sampling scheme, corresponding to $\underset{\sim}{X}$, is used for all subjects. For testing purposes, it is assumed that $\underset{\sim}{\epsilon}_i$ is distributed as $N(\underset{\sim}{0}, \underset{\sim}{V})$, where $\underset{\sim}{0}$ is the txl vector of zeros, and $\underset{\sim}{V}$ is the txt variance-covariance matrix involving both between subject and within subject variance parameters. The exact structure of $\underset{\sim}{V}$ need not be specified, but it is assumed that this structure is common for all subjects in a particular treatment group. The traditional analysis would be to obtain estimates of $\underset{\sim}{\beta}$ for each subject. The least squares estimate of $\underset{\sim}{\beta}$ computed from the observed data for subject i is $\hat{\underset{\sim}{\beta}}_i = (\underset{\sim}{X}'\underset{\sim}{X})^{-1}\underset{\sim}{X}'\underset{\sim}{y}_i$ (Draper and Smith, 1981). Because a common $\underset{\sim}{V}$ for all subjects is assumed, the $\hat{\underset{\sim}{\beta}}_i$ for all subjects have a common variance-covariance structure. The average of the estimated model parameters across subjects is the least squares estimate of β, $\hat{\underset{\sim}{\beta}} = (\sum_{i=1}^{n} \hat{\underset{\sim}{\beta}}_i)/n$. Traditional analyses, such as analysis of variance procedures or confidence interval computations, can be conducted using the $\hat{\underset{\sim}{\beta}}_i$ as the observed response data.

As an alternative strategy, an analysis is presented here based on the across-subject mean at each sampling time point. The overall objective of the analysis is to characterize the mean response to treatment over time. Let $\underset{\sim}{Y}$ be the nxt observed data matrix, where

subjects define the rows and sampling times define the columns. Let $\bar{y} = j'Y/n$ be the txl vector of across-subject means at each sampling time point, where j is an nxl vector of ones. Compute the least squares estimate of β from the observed across-subject means, without regard to the sampling distribution of $\bar{y}$, as $\hat{\beta} = (X'X)^{-1}X'\bar{y}$. Now, the sampling distribution of $\bar{y}$ is $N(X\beta, V^*)$, where V^* is the txt variance-covariance matrix involving both between-subject and within-subject variance parameters. With n subjects and no missing data, $V^* = V/n$. The sampling distribution of $\hat{\beta}$ is $N(\beta, (X'X)^{-1}X'V^*X(X'X)^{-1})$ (Graybill, 1976). Employing standard multivariate techniques, obtain an estimate of the variance-covariance matrix of the observed data for each treatment group. Let $\hat{V} = Y'(I_n - J_n/n)Y/(n-1)$ be the estimate of V, where I_n is the nxn identity matrix and J_n is the nxn matrix of ones (Morrison, 1976). The estimated variance-covariance matrix of the sampling means is then $\hat{V}^* = \hat{V}/n$. The sampling distribution of $(n - 1)\hat{V}^*$ is Wishart, $W_t(n - 1, V^*)$. It follows that for any nonzero vector g, $(n - 1)g'\hat{V}^*g \,/\, g'V^*g$ is distributed chi-squared with n-1 degrees of freedom (Timm, 1975).

Consider the null hypothesis H_0: $h'\beta = h_0$ versus H_1: $h'\beta \neq h_0$. The test statistic for testing H_0 in favor of H_1 would be $T = (h'\hat{\beta} - h_0) \,/\, [h'(X'X)^{-1}X'V^*X(X'X)^{-1}h]^{\frac{1}{2}}$. Given H_0 to be true and letting $g = h'(X'X)^{-1}X'$, T follows a t-distribution with n-1 degrees of freedom. Alternatively, $h'\hat{\beta} \pm t_{(\alpha/2;\ n-1)}[h'(X'X)^{-1}X'V^*X(X'X)^{-1}h]^{\frac{1}{2}}$ defines a $100(1 - \alpha)\%$ confidence interval for $h'\beta$. This test statistic and confidence interval are equivalent to those obtained from the by-subject analysis discussed at the beginning of this section, employing traditional techniques using each subject's $\hat{\beta}_i$ as the observed response data.

An example demonstrating the usefulness of this equivalence is discussed in the next section. A pragmatic extension of the analysis of mean responses to the case of nonlinear models is presented.

3. Modeling pH Declines

Returning to the example discussed in the Introduction, Figures 2a and 2b are plots of the pH decline of randomly selected sides of beef under control and ES-treated conditions. It is evident that, for each treatment group, the data taken as a whole suggest a common response function over time. A nonlinear decay function has traditionally been useful in characterizing such data. Figures 3a and 3b are plots of the

entire observed data set, with a line connecting the observed mean at each sampling time. The sampling means demonstrate a well behaved nonlinear trend. Relying on the well-known asymptotic theory of nonlinear models, the means analysis discussed in Section 2 is extended to nonlinear models and used to compare the difference in mean response to treatment.

Define the nonlinear decay function as $pH = \beta_0 - \beta_1(1 - \exp(-\beta_2 \text{ time}))$, where β_0 is the y-intercept, $\beta_0 - \beta_1$ is the asymptotic minimum value of the pH decline, and β_2 is the rate parameter. Note that β_0 is not an informative parameter for this particular data set, because a rapid change in pH occurs after slaughter and prior to treatment. No data were observed during this time period, but the first observation was at 1 hour postmortem and immediately following treatment. The nonlinear model defined above is used to characterize the pH decline after 1 hour postmortem.

The across-subject means for each treatment group are given in Table 1. The nonlinear decay function is fit, using standard nonlinear estimation techniques, to the sampling time means. To estimate the model parameters, the response function is fit directly to the sampling time means, without regard to variance-covariance structure among the sampling time means. Figures 4a and 4b are plots of the sampling time means and the estimated response models.

The variance-covariance structure of the sampling time means is incorporated into the hypothesis testing process. The sample variance-covariance matrix of the sampling time means for each treatment is given in Table 2. (An i subscript will be used to denote the two treatments, i = 1 for control and i = 2 for ES, for the remainder of this paper.) A chi-squared test of H_0: $\underset{\sim}{V}_1 = \underset{\sim}{V}_2$ versus H_1: $\underset{\sim}{V}_1 \neq \underset{\sim}{V}_2$ results in a chi-squared test statistic of 126.7 with 21 degrees of freedom (Morrison, 1976). This gives a p-value of less than 0.0001, and the null hypothesis is rejected.

The asymptotic sampling distribution of $\hat{\underset{\sim}{\beta}}_i$ is $N(\underset{\sim}{\beta}_i, (\underset{\sim}{Z}_i'\underset{\sim}{Z}_i)^{-1} \underset{\sim}{Z}_i'\underset{\sim}{V}_i^*\underset{\sim}{Z}_i(\underset{\sim}{Z}_i'\underset{\sim}{Z}_i)^{-1})$, where $\underset{\sim}{Z}_i$ is the nxp matrix of partial derivatives with respect to the parameters of the response function for treatment i (Jennrich, 1969). The estimated model parameters for each treatment group are given in Table 3 with asymptotic 95% confidence intervals, using $\hat{\underset{\sim}{V}}_i^*$ to estimate $\underset{\sim}{V}_i^*$. Figure 5 is a plot of the estimated response functions for the control and ES treatments. To test hypotheses of the form H_0: $\underset{\sim}{h}'\underset{\sim}{\beta}_1 = \underset{\sim}{h}'\underset{\sim}{\beta}_2$ versus H_1: $\underset{\sim}{h}'\underset{\sim}{\beta}_1 \neq \underset{\sim}{h}'\underset{\sim}{\beta}_2$, Welch's adjustment to the degrees of freedom is used to accommodate the unequal variance-covariance matrices (Winer, 1971). For this application, Welch's adjusted degrees of freedom is $df^* = \{[\text{Var}(\underset{\sim}{h}'\hat{\underset{\sim}{\beta}}_1) + \text{Var}(\underset{\sim}{h}'\hat{\underset{\sim}{\beta}}_2)]^2 / [(\text{Var}(\underset{\sim}{h}'\hat{\underset{\sim}{\beta}}_1)]^2/(n_1-1)) + ([\text{Var}(\underset{\sim}{h}'\hat{\underset{\sim}{\beta}}_2)]^2/(n_2-1))]\} - 2$. The test statistic for testing H_0 versus H_1 would be $T = [\underset{\sim}{h}'\hat{\underset{\sim}{\beta}}_1 + \underset{\sim}{h}'\hat{\underset{\sim}{\beta}}_2] / [\underset{\sim}{h}'\hat{\underset{\sim}{W}}_1\underset{\sim}{h} + \underset{\sim}{h}'\hat{\underset{\sim}{W}}_2\underset{\sim}{h}]^{\frac{1}{2}}$, where $\hat{\underset{\sim}{W}}_i$ is the sample

variance-covariance matrix of $\hat{\underline{\beta}}_i$. The asymptotic distribution of T is $t_{(df^*)}$, where df^* is Welch's adjusted degrees of freedom. Results of comparison of the model parameters for the two treatments, using Welch's adjusted degrees of freedom, are given in Table 4. Recall that for this example, β_0, and thus β_1, are not particularly meaningful because of when the data were collected, but that $\beta_0 - \beta_1$ is. The conclusions drawn here are that the two treatments have similar terminal pH levels but that the rate at which these levels are achieved differ, with the ES treatment having a significantly faster decline.

The difference in the expected response to treatment also can be compared by considering the estimated difference in pH for the treatments across time directly (Hinds and Milliken, 1987). Let $\hat{y}_1(t) - \hat{y}_2(t)$ define the point estimate of the difference in expected pH response at time t. The estimated variance of this contrast is $\text{Var}(\hat{y}_1(t) - \hat{y}_2(t)) = \hat{\underline{z}}'\hat{\underline{W}}\hat{\underline{z}}$, where $\hat{\underline{z}}$ is the pxl vector of partial derivatives with respect to $\hat{y}_1(t) - \hat{y}_2(t)$ evaluated at $\hat{\underline{\beta}}_1$ and $\hat{\underline{\beta}}_2$, respectively, and $\hat{\underline{W}}$ is the block diagonal sample variance-covariance matrix of $[\hat{\underline{\beta}}_1', \hat{\underline{\beta}}_2']$. An asymptotic $100(1-\alpha)\%$ confidence interval for the difference in treatment response at time t is $(\hat{y}_1(t) - \hat{y}_2(t)) \pm t_{(\alpha/2;\ df^*)}(\hat{\underline{z}}'\hat{\underline{W}}\hat{\underline{z}})^{\frac{1}{2}}$, where df^* is again Welch's adjusted degrees of freedom.

For this example, the choice of the nonlinear decay model was based on the adequacy of the model to describe the mean of the observed data and not entirely based on theoretical considerations of the biological mechanics of pH declines in beef carcasses after slaughter. The mathematical relationship between two nonlinear decay curves of the form used here is that, in the terminal time period, they will either reach the same asymptotic pH level at time infinity, cross at a particular time, or never achieve equal pH levels. More realistically, given the result of no significant difference being detected in the asymptotic pH level (Table 4), the two treatments probably reach their respective terminal mean pH levels at some time and maintain this level. A pragmatic solution for estimating the time at which the two treatments achieve equal mean pH levels would be to construct a $100(1-\alpha)\%$ confidence bound on the difference in mean pH as described above. A point estimate of the time for the two treatments to achieve equal mean pH levels then would be the time at which the lower confidence bound on the difference in mean pH equals zero. Figure 6 is a plot of the difference in mean pH of the two treatments, with a 95% lower confidence bound on the difference. A point estimate of the time at which nonsignificantly different mean pH levels are achieved would be 13.91 hours postmortem.

A final approach to compare the mean response of the two treatments is through calibration techniques. A pH of 6.0 is used to indicate when muscle will no longer be susceptible to cold-induced toughening. Thus, a comparison of the mean time when pH 6.0 is achieved would be a comparison of response to treatment. An asymptotic $100(1-\alpha)\%$ confidence interval

for the time to achieve pH 6.0 can be defined by the set of values of t that satisfy $|\hat{y}_i(t) - 6.0| \,/\, (\hat{z}'\hat{W}_i\hat{z})^{\frac{1}{2}} \leq t_{(\alpha/2;\ df)}$, where $\hat{W}_i$ is the sample variance-covariance matrix of $\hat{\beta}_i$ and df is the associated degrees of freedom (Schwenke and Milliken, submitted for publication). Table 5 gives the calibrated point estimates and 95% confidence intervals for the time to achieve pH 6.0 for each treatment. To compare the time to achieve pH 6.0 between treatments, consider the null hypothesis H_0: $t_1(6.0) = t_2(6.0)$ versus H_1: $t_1(6.0) \neq t_2(6.0)$, where $t_i(6.0)$ is the mean time for treatment i to achieve pH 6.0. This is a comparison of times, that is, the models characterizing the mean pH decay of each treatment may be different, yet achieve a specified pH at the same time. Let t_0 be the time to achieve pH 6.0, given that the null hypothesis is true. An estimate of t_0 would be the value of t that minimizes $[(\hat{y}_1(t) - 6.0)^2 / (\hat{z}_1'\hat{W}_1\hat{z}_1)] + [(\hat{y}_2(t) - 6.0)^2 / (\hat{z}_2'\hat{W}_2\hat{z}_2)]$. The test statistic for testing H_0 versus H_1 would be $T = [\hat{y}_1(\hat{t}_0) - \hat{y}_2(\hat{t}_0)] \,/\, [\hat{z}_1'\hat{W}_1\hat{z}_1 + \hat{z}_2'\hat{W}_2\hat{z}_2]^{\frac{1}{2}}$, where $\hat{z}_i$ is the pxl vector of partial derivatives with respect to $\hat{y}_i(\hat{t}_0)$ evaluated at $\hat{\beta}_i$ at time $\hat{t}_0$, and $\hat{W}_i$ is the sample variance-covariance matrix of $\hat{\beta}_i$. The asymptotic distribution of T is $t_{(df^*)}$, where df^* is Welch's adjusted degrees of freedom. The test comparing the time to achieve pH 6.0 for each treatment is summarized in Table 6. Based on this comparison, it is concluded that the treatments achieve mean pH 6.0 at significantly different times, with the ES treatment achieving pH 6.0 sooner.

4. Summary

A methodology is presented that enables an analysis, in a regression context, of repeated measures data based on the across-subject means at each sampling time. In the linear model case, with no missing data, this analysis is equivalent to a by-subject analysis in which a common response model is fit to each subject's data. The benefit of the means approach, in the linear model case, is that only one estimation process is needed to characterize each treatment, instead of one process for each subject. The variance-covariance matrix of the data is estimated through standard multivariate techniques, independent of the model used to characterize the response data. The estimate of the variance-covariance matrix is then incorporated into tests of hypotheses and the construction of confidence intervals concerning model parameters. This allows an analysis of repeated measures data without assuming a specific structure of the variance-covariance matrix. Differences among treatment groups with respect to the corresponding variance-covariance matrices are accounted for by employing Welch's adjustment to the degrees of freedom.

This methodology then was extended to the nonlinear case, employing the well-known asymptotic theory of nonlinear models. In the nonlinear case, by-subject analyses of data are not always possible because of the

difficulty of iterative techniques to fit a common response model to data from each of several subjects. Often, for complex nonlinear mechanisms, an individual subject's data may not be a good representation of the overall average trend of the group's response. In these cases, the solution with the by-subject analysis could be as drastic as deleting that subject's data from the analysis. In the means approach, it is recognized that each subject's data contributes to the mean response of the group. In addition, using the means approach requires only one application of a nonlinear estimation procedure, which would save substantial computer time. Again, since a model-free estimate of the variance-covariance matrix is computed, testing of hypotheses and construction of confidence intervals is not dependent on an assumed structure of the variance-covariance matrix.

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Table 1

Observed Across-Subject pH Means
by Treatment and Sampling Time

Treatment	n	Time	Mean pH
Control	43	1	6.67
		2	6.34
		4	6.02
		6	5.73
		8	5.58
		24	5.45
ES	43	1	6.66
		2	6.16
		4	5.71
		6	5.53
		8	5.47
		24	5.42

Table 2

Sample Variance-Covariance Matrix of Across-Subject pH Means

Control (x 10e-3):

$$\begin{bmatrix} 1.55 & 1.24 & 1.35 & 0.83 & 0.41 & 0.25 \\ 1.24 & 1.35 & 1.30 & 0.95 & 0.55 & 0.34 \\ 1.35 & 1.30 & 1.89 & 1.10 & 0.65 & 0.35 \\ 0.83 & 0.95 & 1.10 & 1.54 & 0.90 & 0.38 \\ 0.41 & 0.55 & 0.65 & 0.90 & 0.93 & 0.39 \\ 0.25 & 0.34 & 0.35 & 0.38 & 0.40 & 0.48 \end{bmatrix}$$

ES (x 10e-3):

$$\begin{bmatrix} 1.36 & 0.71 & 0.18 & 0.01 & -0.06 & 0.11 \\ 0.71 & 0.85 & 0.43 & 0.22 & 0.10 & 0.12 \\ 0.18 & 0.43 & 0.77 & 0.30 & 0.21 & 0.10 \\ 0.01 & 0.22 & 0.30 & 0.45 & 0.26 & 0.04 \\ -0.06 & 0.10 & 0.21 & 0.26 & 0.31 & 0.02 \\ 0.11 & 0.12 & 0.10 & 0.04 & 0.02 & 0.15 \end{bmatrix}$$

Table 3

Estimated Nonlinear Decay Model Parameters
$pH = \beta_0 - \beta_1(1 - \exp(-\beta_2\ time))$

Treatment	Model Parameter	Estimate	95% C.I. Lower	95% C.I. Upper
Control	β_0	7.054	6.955	7.153
	β_1	1.618	1.513	1.724
	β_2	0.278	0.246	0.310
ES	β_0	7.447	7.263	7.630
	β_1	2.022	1.836	2.207
	β_2	0.497	0.443	0.551

Sample Correlation Matrix of Estimated Model Parameters

Control:

$$\begin{bmatrix} 1.000 & 0.903 & 0.183 \\ 0.903 & 1.000 & 0.130 \\ 0.183 & 0.130 & 1.000 \end{bmatrix}$$

ES:

$$\begin{bmatrix} 1.000 & 0.993 & 0.751 \\ 0.993 & 1.000 & 0.739 \\ 0.751 & 0.739 & 1.000 \end{bmatrix}$$

Table 4

Comparison of Model Parameters
Control versus ES Treatment

Parameter	Model T-Statistic	P-Value
β_0	-3.803	0.00032
β_1	-3.816	0.00030
$\beta_0 - \beta_1$	0.402	0.68886
β_2	-7.031	< 0.0001

Table 5

Estimated Time to Achieve pH 6.0
with Asymptotic 95% Confidence Interval

Treatment	$\hat{t}(6.0)$	95% C. I. Lower	Upper	Width
Control	3.788	3.336	4.289	0.954
ES	2.530	2.349	2.727	0.378

Table 6

Results of Test of Hypothesis
Equality of Time to Achieve pH 6.0

$\hat{t}_1(6.0) = 3.788$ $\hat{t}_2(6.0) = 2.530$

$\hat{t}_0 = 2.797$

$\hat{y}_1(\hat{t}_0) = 6.179$ $\hat{y}_2(\hat{t}_0) = 5.929$

Test Statistic: $T = 5.434$

p-value < 0.0001

Figure 1a

Observed pH Decline Versus Time Postmortem
Selected Carcasses

Control Treatment Group

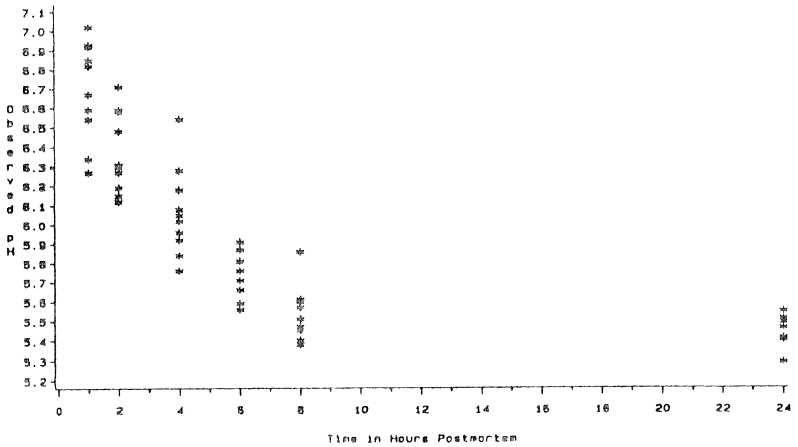

Figure 1b

Observed pH Decline Versus Time Postmortem
Selected Carcasses

ES Treatment Group

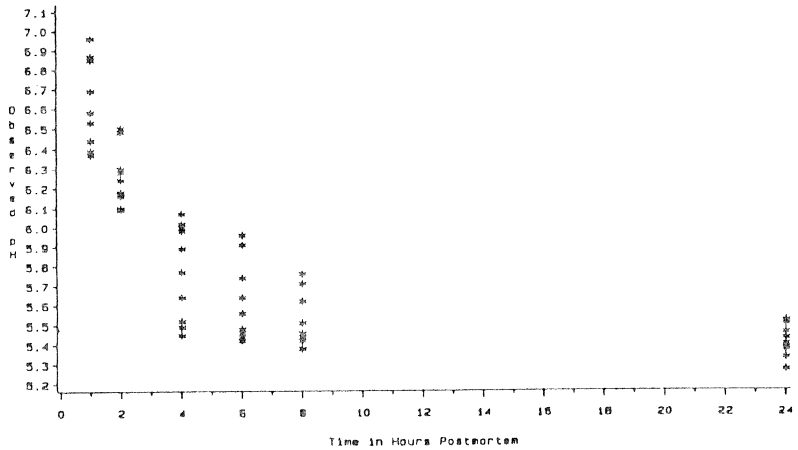

Figure 2a

Observed pH Decline Versus Time Postmortem
Selected Carcasses, By Carcass

Control Treatment Group

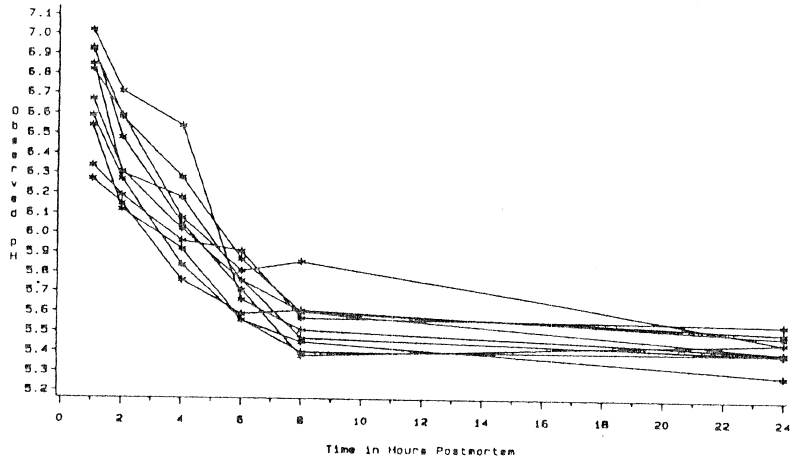

Figure 2b

Observed pH Decline Versus Time Postmortem
Selected Carcasses, By Carcass

ES Treatment Group

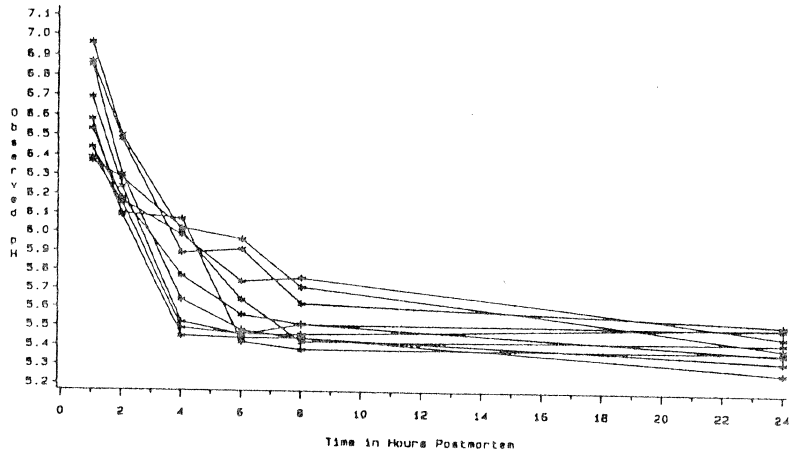

Figure 3a

Observed pH Decline Versus Time Postmortem
and Observed Mean Response

Control Treatment Group

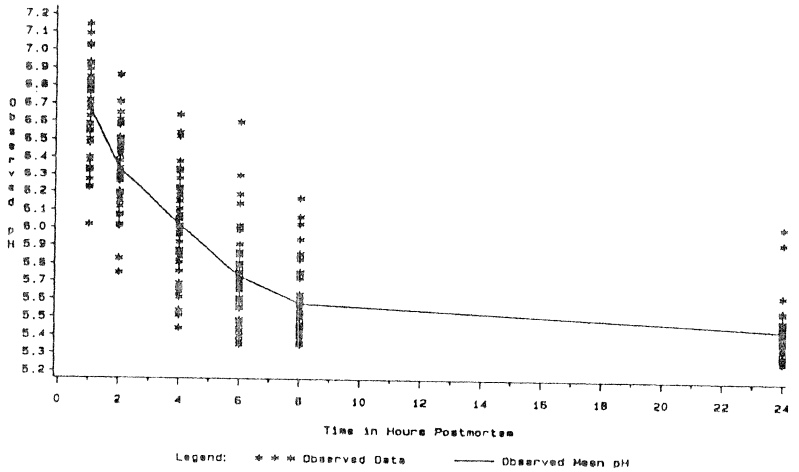

Figure 3b

Observed pH Decline Versus Time Postmortem
and Observed Mean Response

ES Treatment Group

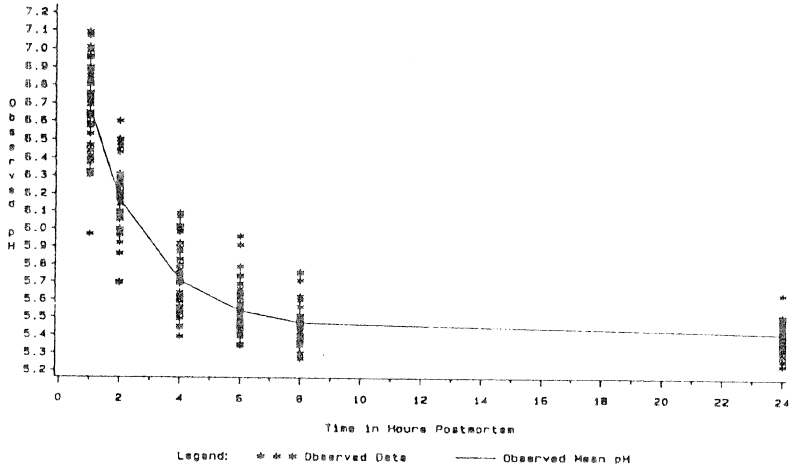

Figure 4a

Observed Mean pH Decline Versus Time Postmortem
and Estimated Mean Responses

Control Treatment Group

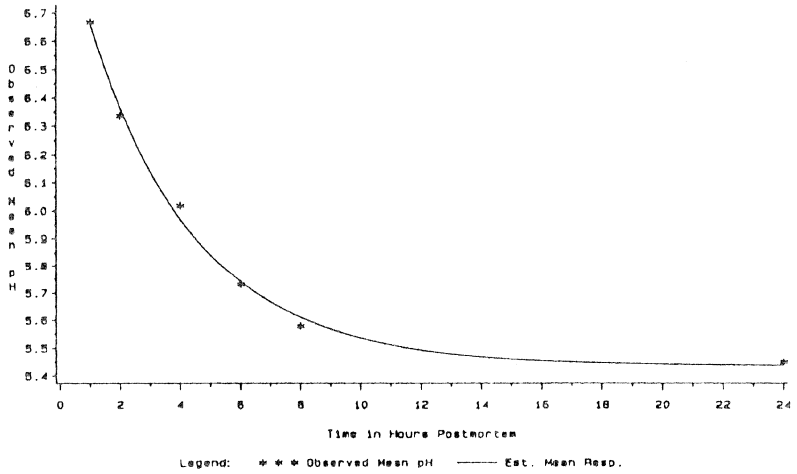

Figure 4b

Observed Mean pH Decline Versus Time Postmortem
and Estimated Mean Responses

ES Treatment Group

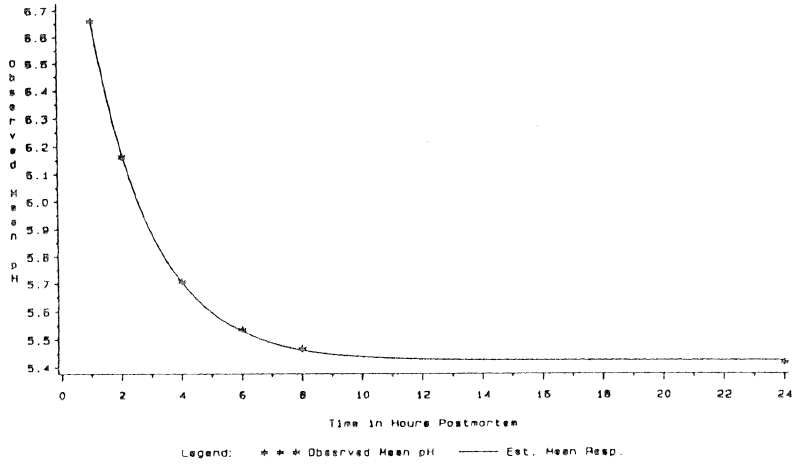

Figure 5

Estimated Control and ES Mean pH Declines Versus Time Postmortem

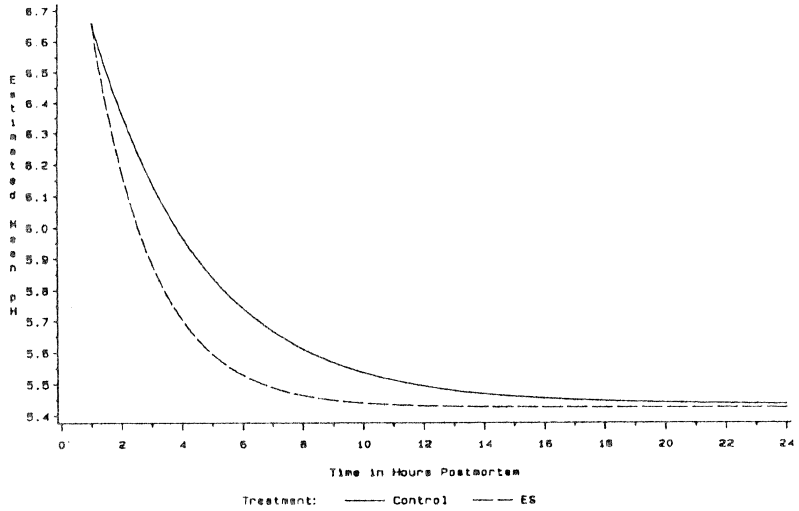

Figure 6

Estimated Mean Treatment Difference of Control and ES
With 95% Lower Confidence Bound

(No Significant Difference at 13.91 Hours)