STATISTICAL INTERPRETATION OF EXPERIMENTS WITH LAYING HENS

by

EUGINE SONG

(Under the Direction of Lynne Billard)

ABSTRACT

The effects of series of balanced dietary protein levels on egg production and egg quality parameters in lying hens from 18 through 74 weeks of age were investigated in this experiment. One hundred and forty four pullets were equally assigned to three different protein level series. The results clearly demonstrated that balanced dietary protein level was the limiting factor for body weight, average daily feed intake (ADFI), egg weight, and egg production. As the cost of research increases, mathematical models become valuable tools to answer research questions. A major application of mathematical modeling is accurate estimation of production performance, growth, and feed consumption for poultry research and production. Different models have been proposed to fit those curves. To test differences of fitted curves, the sum of squared reduction test is used. The results of repeated measures using response variables are appealing.

INDEX WORDS: Layer, Protein titration, Egg production, Mathematical equation,

Repeated measure.

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Chapter 1

Introduction

Since the diet fed to commercial laying hens can vary depending on factors such as the strain of layer, production goals, age, and weather conditions (Schaible and Patrick, 1980), the composition of laying hen feed has gone through many changes. Since a laying hen produces eggs depending on the nutrients provided in its diet, the quality and formulation of the diet is important to a producer, especially considering that 65 to 75% of the cost to produce eggs is due to feed costs (Bell and Weaver, 2002). Due to this fact, it has become increasingly important for producers to find a balance between feeding their birds on a least-cost basis and feeding the appropriate amounts of nutrients in the diet when the hen needs them throughout her lay cycle. This will help producers maximize their profits. This can be done through a phase feeding program, involving a pre-lay diet, and diets of different compositions to suit the stages of the hens' lay cycle.

As more producers become interested in least-cost and maximum profit diets, mathematical models and formulations in diet would be valuable tools to answer research questions. A major application of mathematical modeling is accurate estimation of production performance, growth, and feed consumption for poultry research and production. To fit these curves, several models have been proposed. For instance, the Adams-Bell (1980) and modified compartmental (Miyoshi et al., 1996) models have been applied to fit egg production; Lokhorst (1996) introduced models to fit egg weight and feed intake; also, Gompertz (1825), and Richard (1959) proposed models to fit hens' body weight curves. Gunawardana et al. (2008) and Parsons et al. (1993) studied the effect of protein levels on egg production and egg weight.

The goals of this thesis are to determine whether mathematical models (the Adams-Bell, 1980; modified compartmental, Miyoshi et al., 1996; Lokhorst, 1996; Gompertz, 1825; and Richard, 1959, models) could fit curves (egg production, egg output, egg weight, feed intake, and body weight), and whether there are differences among three protein levels in hens' diet. Unlike previous research (Mazzuco et al., 2011 and Keshavarz, 2003) which compares the protein levels using a one or two-way analysis of variance (ANOVA), this study focuses on comparing treatments (three protein levels) using nonlinear regression analysis and repeated measures designs. First, we compared differences among fitted curves, and then discuss the benefits of using a repeated measures designs instead of a one or two-way analysis of variance (ANOVA).

Chapter 2

Liturature Review

In order to interpret statistically the results of experiments with laying hens, we need to review the biological and statistical background. The biological background section described how protein affects egg production, egg weight, and hens body weight; and also proposed three protein levels in hens' diet based on guidelines of the National Research Council. The statistical background section was focused on the statistical methods used in poultry science.

2.1 Biological Background

Since the nutrients provided in diet of a laying hens affect the hens' capacity to produce eggs and build body tissue, the formulation of the diet is important to producers considering feed cost. Especially, protein is essential in the diet to provide amino acids. These amino acids are needed to provide body tissue and egg production. Since 65 - 75% of the cost to produce eggs is spent in feed costs (Bell and Weaver, 2002), it is important for producers to find a diet formulation based on least cost and maximum profit.

The National Research Council (NRC, 1994) reported that a Leghorn-type laying hen requires

18.8, 15.0, or 12.5% protein in the diet for feed intakes of 80, 100, or 120 g/hen/day. Even tough, laying hens do not require protein, protein in the diet needs to be available to provide a supply of nonessential amino acids (National Research Council, 1994). Increasing levels of protein have resulted in improvements in egg size and weight (Leeson, 1989; Parsons et al., 1993; Keshavarz and Nakajima, 1995). Thus, methods of influencing egg weight through diet are main issues for producers. Based on these facts, we choose three levels of protein to study diet formulation. First, diets were separated into 4 phases (18 - 22, 23 - 32, 33 - 44 and 45 - 74 age in weeks). The high protein (H) series contained 21.62, 19.05, 16.32 and 16.05% protein, respectively. Medium protein (M) and low protein (L) series were 2% and 4% lower in balanced dietary protein.

Gunawardana et al. (2008) reported that, when testing dietary protein levels to birds over a 12 week trial, increasing dietary protein would increase feed intake to provide energy. This energy needed for increased egg production was satisfied by increased protein. One thousand nine hundred and twenty molted birds at 87th week of age, in phase of two of their second cycle, were fed two protein levels (15.5 and 16.1 %). Hens fed high-protein (16.1%) in their diet had significantly higher egg weights than hens fed low protein (15.5%) in their diet. Therefore, the choice of protein levels was dependent on cost.

Parsons et al. (1993) reported that there was a substantial effect on grade of egg weight and size by increasing dietary protein from 16% to 18%. Much of this response from 20 to 28 weeks was probably due to the low feed intakes, resulting in birds fed the 16% crude protein in diet having protein and synthetic amino acids intakes below the NRC (1984) recommended levels. Egg production (20 to 40 weeks) was not influenced by feeding diets containing 16%, 18% and 20% protein (Parsons et al., 1993).

Dietary protein affected egg production and hens body weight gain in two experiments (Pesti,

1991). Pesti (1991) observed that birds fed high protein had increased growth and egg production, and egg weight was maximized at high protein. Hens body weight gain was reduced with level of significance $p \leq 0.02$ by lowering the protein for 39 to 50 weeks of age by 3% (Novak et al., 2008), indicating that the level of protein or amino acids was important in maintaining optimal gain.

2.2 Statistical Background

In science, researchers use statistical methods to analyze their experiment data, since they need scientific evidence to support their conclusions. To find out whether the test in which they are interested is statistically significant, many researchers in poultry science have often used one of procedures of Statistical Analysis System (SAS, 2006). If the statistical methods which they used are appropriate for their data, the results would be a powerful scientific basis to support their hypotheses.

Faridi et al. (2011) fitted several different curves (for egg production, feed intake, egg weight, hens body weight, and egg mass) and compared several nonlinear models (three Narushin-Takma, Adams-Bell, Lokhorst, Richard, Gompertz, and modified compartmental models) to evaluate the ability of three Narushin-Takma models applied to fit curves using the NLIN (nonlinear) procedure (Marquardt, 1963, algorithm). To reveal an accuracy of fit with these models, the mean square error (MSE), coefficient of determination (R^2), Akaike information criterion (AIC), and Bayesian information criterion (BIC) are used.

Bell et al. (1991) studied the shape (linear, quadratic, asymptotic, and cyclic) of the production curve, the relationship between the two laying cycles, and the effect of strain and season on various measures of egg production. To compare performance traits between strain and the age at peak production and at molting, a one-way analysis of variance was run. Repeated measures analysis of variance was used to compare the early lay cycle slope with the slope over the last 10 weeks.

In Mazzuco et al. (2011), their goal was to evaluate alternative molting protocols assessing hen welfare and performance during and after molt. Six different treatments were applied, and PROC GLM, MIXED, and LOGISTIC of SAS were run to perform orthogonal contrasts for treatment comparisons. By using the LOGISTIC procedure, the effect of treatment, age, and treatments × age interaction were evaluated.

Keshavarz (2003) determined the effect of different nonphytate phosphorus diets with and without phytase on performance of four strain of laying hens. A two-way ANOVA and Duncan's (1995) test were run to test the interaction effect of strain \times diet and to compare means. When the interaction effect was significant, Keshavarz used a one-way ANOVA (SAS, 2006) to test the main effects of strain and diet.

In this work, nonlinear models in poultry science are applied to fit the five response variables; specifically, egg production (y_5) , egg output (y_6) , average daily feed intake (ADFI) (y_2) , egg weight (y_3) , and body weight (y_1) (Chapter 4). Since the goal of this work is to test whether there are differences among the three protein treatments, statistical methods which were not used in previous stated research were applied using nonlinear models (Section 4.7).

Chapter 3

Nonlinear Regression - Models

This chapter describes nonlinear models which have been proposed to fit to various entities in poultry science, as well as methods which compare several treatments in a nonlinear regression analysis. Six functions (Adams-Bell, modified compartmental, Gompertz, Richards, and egg weight and feed intake equations of Lokhorst) which have commonly been used to fit response variables are considered. All models were fitted using the NLIN (non-linear) procedure of SAS (SAS Institute, 2006).

3.1 Models

In all models, t is the age in weeks of the hens and y is the output variables measured each week. Egg production is the number of egg per 100 live hens. This is also called "hens-day egg production" and has the unit percentage. Egg output is the grams of egg produced by the average hen.

3.1.1 Adams-Bell equation

The Adams-Bell equation was proposed to fit egg production and egg mass by Adams and Bell in 1980. Here, egg production and egg output (egg production \times egg weight) are considered as response variables, y_5 and y_6 , respectively. This equation is given by

$$y = 100(\frac{1}{(1+ab^t)} - ct + d) \tag{3.1}$$

In (3.1), the response y corresponds, respectively, to y_5 for egg production (%) or y_6 for egg output at age t weeks, and a, b, c and d are parameters. These parameters have no interpretation other than being model parameters. In this thesis, since there are three treatments (High, Medium, Low) which are dietary protein levels, the response variable is fitted by this equation for each treatment. In other words, there are three equations with the same response variable.

3.1.2 Modified Compartmental equation

The Modified Compartmental equation has been applied to fit egg production y_5 and egg output y_6 by Miyoshi et al. (1996). The equation is given by

$$y = a(\frac{\exp(-bt)}{1 + \exp(-c(t-d))})$$
(3.2)

where y corresponds, respectively, to y_5 for egg production (%) or y_6 for egg output, t is age in weeks and a, b, c and d are parameters. These parameters have no particular interpretation.

3.1.3 Lokhorst egg weight equation

Lokhost (1996) also proposed an equation for egg weight y_3 , specifically

$$y = a + br^t. ag{3.3}$$

In this equation, $y (\equiv y_3)$ is egg weight (g/egg), a is the theoretical maximum egg weight, b (< 0) should be added to a to determine the initial egg weight, r (0 < r < 1) refers to the growth rate, and t is the age of the hens in weeks.

3.1.4 Lokhorst feed intake equation

Lokhorst (1996) proposed different equations for egg weight(g), second-grade eggs, and feed intake. The Lokhorst feed intake equation is

$$y = (\frac{a}{1 + b\exp(-act)}) + dt + ft^2$$
(3.4)

where $y \equiv y_2$ is feed intake(g/ hen/ day), a is the horizontal asymptote of the restricted growth curve, b represents the feed consumption at the start of the laying period, and c refers to the rate of the increases in feed consumption in the restricted growth phase. Parameters d and f indicate that feed consumption increases or decreases during the rest of the laying period, and t is the age of the hens in weeks.

3.1.5 Gompertz equation

The Gompertz (1925) equation was proposed to fit growth curves according to

$$W = N \exp[-\alpha \exp(-N\beta t)]. \tag{3.5}$$

In our application of this equation, W corresponds to the body weight(kg) (y_1) of the hen, β is the intrinsic growth rate, N is the asymptotic or maximum growth response, and α is a parameter. Ricklefs (1985) showed that this equation was applied to fit the growth curve of Japanese quail. He described that the variation of the parameters changes the growth curve and so should be used to compare treatments, and effects of treatments on growth.

3.1.6 Richards equation

Richard (1959) introduced an equation which was applied to fit egg weight as

$$W = \frac{N}{[1 + \alpha \exp(-N\beta t)]^{\gamma}}$$
(3.6)

where W corresponds to body weight(kg) (y_1) of the hen, β is the intrinsic growth rate, N is the asymptotic or maximum growth response, and α and γ are parameters. This model was developed from the von Bertalanffy equation (Bertalanffy, 1941).

3.2 Repeated Measures Designs

For this work, experimental designs were generated with two factors and six response variables. One factor is the dietary protein level series (high protein H, medium protein M, and low protein L), and the other factor is the age, in weeks, of the hen. There were three groups of hens and each was fed a different series of diets. The management guide recommendations were that the medium (M) level contained 19.05%, 17.05%, 15.05%, and 15.05% protein during weeks 18 - 22, 22 - 32, 32 - 44, and 44 - 74, respectively. Hens fed the high (H) and low (L) protein level series of diets record 2% more and 2% less protein at each age, respectively. Here, we can consider hens id number as a repeated measures factor, because we collect data each week from the same hen at the same diet level. Therefore, this section describes two-factor repeated measures designs.

3.2.1 Two Factors

First of all, let us suppose that one of the factors is A with levels A_1, \ldots, A_a , and the other one is B with levels B_1, \ldots, B_b . The general case of two factors repeated measures designs is that each of the subjects receives all ab treatment combinations (A_iB_j) . In one special case, subjects in one level of A factor receive the b treatment combinations $(A_iB_j, j = 1, \ldots, b)$. This design is useful when factor A is a qualitative factor. The data for this work were considered as this special case, because the first forty-eight hens were on a high protein diet, the next forty-eight hens were on a medium protein diet, and the last forty-eight hens were on a low protein diet, and each hen received all b combinations $(A_iB_j, j = 1, \ldots, b)$.

The model for the general case is

$$Y_{ijk} = \mu + \tau_{ij} + \pi_k + \delta_{ijk} + e_{ijk}, \ i = 1, \dots, a, \ j = 1, \dots, b, \ k = 1, \dots, p,$$
(3.7)

where $\tau_{ij} = A_i + B_j + (AB)_{ij}$ with $A_i, B_j, (AB)_{ij}$ being the effect of the *i* level of *A*, the *j* level of *B*, and the interaction of A_i and B_j , and $\delta_{ijk} = (A_i\pi_k) + (B_j\pi_k) + (AB)_{ij}\pi_k$ with the interaction of subjects between factor *A*, factor *B* and the *AB* interaction. The assumptions are $\pi_k \sim IN(0, \sigma_\pi^2), \ e_{ijk} \sim IN(0, \sigma^2), \text{ and } \pi_k \text{ and } e_{ijk} \text{ are mutually independent.}$

The model for our special case is

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \pi_k + \beta_{jk(i)} + e_{ijk}, \ i = 1, \dots, a, \ j = 1, \dots, b, \ k = 1, \dots, p, \quad (3.8)$$

where π_k is the effect of subject k, and $\beta_{jk(i)}$ is the interaction of subject k with the *j*th level of factor B and nested in the *i*th level of factor A. Since nested factors do not have interaction with factors within which they are nested, there is no interaction effect of subjects and factor A. The assumptions are $\pi_k \sim IN(0, \sigma_{\pi}^2)$, and $\beta_{jk(i)} \sim IN(0, \sigma_{\beta}^2)$.

Chapter 4

Nonlinear Model- Results

In this chapter, the six equations given in Chapter 3 were used to fit each of five response curves, respectively, egg production (y_5) , egg output (y_6) , average daily feed intake (ADFI) (y_2) , egg weight (y_3) , and body weight (y_1) , by diet expressed as protein levels (high H, medium M, and low L, respectively). The coefficient of determination $(R^2 = 1 - SS(Residual)/SS(Total_{Corrected}))$ is used to evaluate goodness of fit for all models. Then the treatments (protein levels) are compared. Table 4.1 summarizes the range, mean, and standard deviation of each protein level for the six response variables. Based on Table 4.1, the range of each response variable is wide, i.e., the data are "noisy", there is considerable variation amongst the hens fed the same diet. Therefore, it is of interest to know how well the equations are fitted to the individual data and to the mean data of each age level. In order to fit three nonlinear regression models and to compare protein levels, the nonlinear procedure (proc NLIN) of SAS (SAS Institute, 2006) with the Marquardt algorithm (Marquardt, 1963) is used.

4.1 Data Description

One of the goals of this work is to test whether there is a difference between the effects of dietary protein levels on several response variables. Thus, in this experiment, one hundred forty-four laying hens were randomly assigned to individual cages with separate feeders and were equally assigned to three different protein levels.

As previously stated in Section 3.2, the data were generated with two factors and six response variables. One factor is A (dietary protein level), and the other is B (week age of hens). The response variables are egg production (%) (y_5) (number of eggs per 100 hens per day), egg output (y_6) (egg productions \times egg weight), egg weight (g) (y_3), ADFI (y_2), layer body weight (kg) (y_1), and egg cumulative number (y_4) (cumulated from value of observation at age 19). There are three levels of factor of A (high protein(H), medium protein(M), and low protein(L)). There are fifty-six levels of B (from 19 weeks to 74 weeks) for egg production, egg output, egg weight(g), and egg cumulative number; fifty-seven levels of B (from 18 weeks to 74 weeks) for ADFI; and fifteen levels of B (every four weeks from 18 to 72) for layer body weight. Table 4.1 gives a description of some basic statistics for the data used in this paper.

In this chapter, we determine the fitted equations for the different models described in Chapter 3 for both the individual data and mean data sets. We will plot these equations for the three diets (high, median, and low protein levels) on the same graph so they can be visually compared. For illustrative clarity, the observed mean data set values are used on both sets of graphs. Statistical tests of comparison are given in section 4.7.

| Variables | Treatment | Observation no. | Range | Mean \pm SD |
|--------------------------------|--------------|-----------------|------------------|---------------------|
| Egg production (%) (y_5) | Н | 48 | 0 to 100 | 89.90 ± 22.27 |
| | Μ | 48 | 0 to 100 | 87.69 ± 21.93 |
| | \mathbf{L} | 48 | 0 to 100 | 72.17 ± 21.98 |
| Egg output (g) (y_6) | Н | 48 | 5.53 to 74.53 | 55.33 ± 9.57 |
| | М | 48 | 5.16 to 68.79 | 51.24 ± 9.10 |
| | L | 48 | 5.42 to 64.71 | 39.28 ± 9.89 |
| Egg weight (g) (y_3) | Н | 48 | 38.1 to 74.53 | 59.09 ± 5.24 |
| | Μ | 48 | 36.9 to 70.43 | 56.26 ± 4.27 |
| | \mathbf{L} | 48 | 37.75 to 66.06 | 52.85 ± 3.83 |
| | Н | 48 | 12.8 to 135.39 | 101.19 ± 12.87 |
| | М | 48 | 19.1 to 139.63 | 99.17 ± 13.98 |
| | \mathbf{L} | 48 | 6.23 to 138.99 | 86.75 ± 17.33 |
| Layer body weight (kg) (y_1) | Н | 48 | 1.02 to 2.23 | 1.56 ± 0.21 |
| | М | 48 | 1.03 to 1.91 | 1.46 ± 0.16 |
| | \mathbf{L} | 48 | 0.93 to 1.71 | 1.30 ± 0.17 |
| Egg cumulative number (y_4) | Н | 48 | 0 to 327 | 172.59 ± 106.00 |
| | М | 48 | 0 to 375 | 166.62 ± 105.24 |
| | L | 48 | 0 to 356 | 143.31 ± 85.93 |

Table 4.1: Summary of descriptive statistics for data

4.2 Egg Production (%) (y_5)

4.2.1 Adams-Bell Model

First, the Adams-Bell (1980) model of (3.1) was fitted to the individual data of egg production and to the mean data of egg production (y_5) . A summary of the model parameters in each case, for each treatment level (i.e., each level of A) is presented in Table 4.2. Based on Table 4.2, the parameters a, b, c, and d are very similar for each treatment level (dietary protein levels). However, the value of R^2 for the mean data fits is higher than when the individual hen data are used, since as expected there is more variation in the individual data than in the mean data. Figure 4.1 shows the three fitted curves of this model with the plots for the individual data in Figure 4.1(a), and these for the mean data in Figure 4.1(b). The shapes of the three curves are very similar to each other. The gap between the curve of the L level and the other two (H and M) is wide, suggesting

| | Individual data | | | Mean data | | |
|--------|-----------------|----------|--------------|-----------|----------|--------------|
| Entity | Н | Μ | \mathbf{L} | Н | М | \mathbf{L} |
| a | 1.152E13 | 9.717E16 | 5.174 E14 | 1.208E13 | 9.29E16 | 5.133E14 |
| b | 0.2315 | 0.1465 | 0.1833 | 0.2310 | 0.1468 | 0.1834 |
| с | 0.000419 | 0.000857 | 0.00246 | 0.000443 | 0.000861 | 0.00247 |
| d | -0.0436 | -0.0489 | -0.1370 | -0.0429 | -0.0492 | -0.1365 |
| R^2 | 0.4906 | 0.4634 | 0.2787 | 0.9843 | 0.9762 | 0.7465 |

Table 4.2: Adams-Bell model-parameter estimates and goodness of fit for egg production (y_5)

there are differences between them; which we shall test statistically (see Section 4.7). Even though there is a small gap between the curves of the H and M levels, we also need to test whether there are statistically significant differences between them. By comparing the coefficient of determination (R^2) values given in Table 4.2, we see that the Adams-Bell model fits the data from hens fed the H and M series very well, but not those fed the L series of diets. The Adams-Bell model was designed to fit normal egg production curves well, but the hens response to the L series was not normal: Egg production of hens fed the L series of diets increased normally in the early growth weeks but then decreased much more quickly then normally and did not decrease in a linear manner (Figure 4.1).

4.2.2 Modified Compartmental Model

Since the modified compartmental model (Miyoshi et al., 1996) of (3.2) has been applied to fit egg production (y_5) , two cases (individual and mean data) were fitted to this model for our data. The resulting estimated parameters are very similar for the two cases as shown in Table 4.3. However, R^2 for the mean data is higher, since there is more variation in the individual data than in the mean data. When comparing this with the results of the Adams-Bell model, the R^2 values of the M and L treatments in the Adams-Bell model are lower than these in the modified compartmental model suggesting that the modified compartmental model is a better fit. This is, in contrast to

| | Individual data | | | Mean data | | |
|--------|-----------------|----------|--------------|-----------|----------|--------------|
| Entity | Н | Μ | \mathbf{L} | Н | Μ | \mathbf{L} |
| a | 95.6733 | 94.9712 | 87.0207 | 95.7401 | 94.9419 | 87.0641 |
| b | 0.000450 | 0.000902 | 0.00330 | 0.000475 | 0.000907 | 0.00330 |
| с | 1.5853 | 2.1654 | 2.3451 | 1.5874 | 2.1645 | 2.3425 |
| d | 20.6515 | 20.4610 | 20.2862 | 20.6513 | 20.4605 | 20.2862 |
| R^2 | 0.4899 | 0.4632 | 0.2831 | 0.9828 | 0.9757 | 0.7414 |

Table 4.3: Modified compartmental model-parameter estimates and goodness of fit for egg production (y_5)

results of experiments from previous research (Faridi et al., 2011), in which R^2 in the Adams-Bell model was higher. The plots given in Figure 4.2(a) for the individual data and Figure 4.2(b) for the mean data for this modified compartmental model show the same trends as for the Adams-Bell model. Similar to the fits of the Adams-Bell model, the modified compartmental model did not fit the data from hens fed the L series of diets as well as for the H and L diets.

4.3 Egg Output (y_6)

4.3.1 Adams-Bell Model

In this work, the Adams-Bell (1980) model of (3.1) has been used to fit egg output (y_6). The estimated parameter values for each level of A (H, M, L) and each of the individual and mean data sets are shown in Table 4.4. Based on Table 4.4, the estimation of the parameters b, c, and d is very similar in each treatment level, but that for a is not. A consequence of this dissimilarity is observed in Figure 4.3 where Figure 4.3(a) indicates that the predicted value of the H level at age 19 with the individual data is different from that when using the mean data (Figure 4.3(b)). Since there is less variation in the mean data, R^2 for each of three levels for the mean data is higher than for the individual data. We see from Figure 4.3 the gap among the three fitted curves is wider than those in Figures 4.1-4.2 for egg production. As age increases, the two egg output curves corresponding

| | Individual data | | | Mean data | | |
|--------|-----------------|----------|--------------|-----------|----------|--------------|
| Entity | Н | Μ | \mathbf{L} | Н | Μ | \mathbf{L} |
| a | 41400.9 | 1.2961E8 | 1.67 E10 | 427849 | 13485517 | 1.118E8 |
| b | 0.5634 | 0.3737 | 0.2793 | 0.5115 | 0.4246 | 0.3715 |
| с | -0.00058 | -0.00085 | 0.000527 | -0.00058 | -0.00046 | 0.000541 |
| d | -0.4619 | -0.5208 | -0.5798 | -0.4675 | -0.5059 | -0.5792 |
| R^2 | 0.2632 | 0.2104 | 0.0249 | 0.9804 | 0.9520 | 0.7252 |

Table 4.4: Adams-Bell model-parameter estimates and goodness of fit for egg output (y_6)

Table 4.5: Modified compartmental model-parameter estimates and goodness of fit for egg output (y_6)

| | Individual data | | | Mean data | | |
|--------|-----------------|----------|--------------|-----------|---------|--------------|
| Entity | Н | Μ | \mathbf{L} | Н | М | \mathbf{L} |
| a | 53.5426 | 47.8148 | 42.2015 | 52.0804 | 41.8585 | 41.9949 |
| b | -0.00113 | -0.00173 | 0.00139 | -0.00143 | 0.00166 | 0.00131 |
| с | 0.7184 | 1.3525 | 1.9466 | 1.2822 | 2.3564 | 2.2727 |
| d | 20.2150 | 20.1833 | 19.7543 | 20.9650 | 20.4723 | 20.4033 |
| R^2 | 0.2619 | 0.2120 | 0.0356 | 0.9786 | 0.9659 | 0.7616 |

to the H and M levels are both increasing. However, the curve of diet L level is decreasing.

4.3.2 Modified Compartmental Model

The estimated parameter values when the modified compartmental model of (3.2) was fitted to the egg output responses (y_6) , for each of the individual and the mean data sets, are shown in Table 4.5. Based on Table 4.5, the parameters a, b, c, and d are very similar for each treatment level, as observed when the Adams-Bell model was fitted. The value of R^2 for the mean data is much higher than these for the individual data for each diet, since there is less variation in the mean data. The plots of this model for each diet (H, M, L) are shown in Figure 4.4. We see these give essentially the same results as was observed in Figure 4.3 when fitting the Adams-Bell curve.

| | Individual data | | | Mean data | | |
|--------|-----------------|----------|--------------|-----------|---------|--------------|
| Entity | Н | Μ | \mathbf{L} | Н | М | \mathbf{L} |
| a | 60.7704 | 59.7684 | 54.8426 | 60.7176 | 57.3861 | 54.1269 |
| b | -260.0 | -23.3146 | -26.8402 | -370.8 | -825.0 | -109.1 |
| r | 0.8630 | 0.9480 | 0.9368 | 0.8504 | 0.8114 | 0.8828 |
| R^2 | 0.3230 | 0.3134 | 0.2365 | 0.9356 | 0.8329 | 0.8685 |

Table 4.6: Lokhorst egg weight model-parameter estimates and goodness of fit for egg weight (y_3)

4.4 Egg Weight (y_3)

4.4.1 Lokhorst Egg Weight Model

The Lokhorst model of equation (3.3) was applied to the egg weight response values (y_3) for the individual and the mean data. The estimated parameter values for each level of diets (H, M, L) and each of the individual and mean data sets are given in Table 4.6. For each diet, there are slightly different values among the estimations of parameters a and r, but the parameter estimates of b in the M and L levels are quite different from those for the H level, and are quite differnt for the individual and the mean data sets, see Table 4.6. This result has affected the graph of fitted curves as shown in Figure 4.5. Thus, the curves of the M and L diet levels for the individual data are flatter than these for the mean data. The R^2 of the mean data is larger than that for the individual data for each diet (consistent with what previous results have shown). Since r represents the increase in egg weight, the growth rate of M level is higher than when diets H and L are used. The meaning of parameter a is the theoretical maximum egg weight. Even though the value of afor each diet in Table 4.6 is not the same as for the corresponding values for maximum egg weight in Table 4.1, the diet level of the highest and lowest maximum egg weight is the same.

4.5 Average Daily Feed Intake (y_2)

4.5.1 Lokhorst Feed Intake Model

The Lokhorst Feed Intake Model (1996) of equation (3.4) was applied to fit curves to the average daily feed intake (ADFI) response variables (y_2) for the original and the mean data. The estimated parameter values for each level of A (H, M, L) and each of the individual and mean data sets are given in Table 4.7. Based on Table 4.7, the parameters a, b, c, and d are very similar for each treatment level. Further, R^2 of the mean data is much higher than for the individual data for each diet, since there is less variation in the mean data. From Figure 4.6, we see that the gap between the curve of L level and the curves for H and M levels is wider than that between the curve for H and M levels, and shows these represent different patterns after the peak point. As age is increasing, the curves of the H and M levels increase and then decrease. However, the curve of the L level decreases and then increases for the individual and mean data. These patterns are affected by the sign of the parameters d and f, since d and f indicate that feed consumption increases or decreases after the starting of the laying period. The estimated values of parameter a are interesting. Since a refer to the horizontal asymptote of the restricted growth curve, we expect that the highest estimated value of a is in H level based on Figure 4.6. However, from Table 4.7, we see that the highest estimated value of a is actually for in the L level diet. The highest estimated value of b (which represents the feed consumption at the start of the laying period) is in M level. Thus, the highest feed consumption at the start is in M level. However, the highest estimated value of c (which refers to the speed of the increases in feed consumption in the restricted growth phase) is in M level.

The Lokhorst Feed Intake Model very nicely models both types of responses observed here. For hens fed the H and M series of diets, ADFI increased quickly and then increased slowly and

| | Individual data | | | Mean data | | |
|--------|-----------------|----------|--------------|-----------|-----------|--------------|
| Entity | Н | Μ | \mathbf{L} | Н | М | \mathbf{L} |
| a | 77.2431 | 80.0693 | 94.4412 | 77.0445 | 79.9941 | 93.7631 |
| b | 4.261 E12 | 3.938E13 | 1.88E14 | 4.763 E12 | 4.145 E13 | 2.546 E14 |
| с | 0.0210 | 0.0218 | 0.0197 | 0.0212 | 0.0218 | 0.0200 |
| d | 1.0324 | 0.7895 | -0.4493 | 1.0414 | 0.7924 | -0.4127 |
| f | -0.00949 | -0.00682 | 0.00576 | -0.00959 | -0.00684 | 0.00532 |
| R^2 | 0.3126 | 0.2650 | 0.0747 | 0.8277 | 0.7887 | 0.3475 |

Table 4.7: Lokhorst feed intake model-parameter estimates and goodness of fit for ADFI (y_2)

decreased. For hens fed the L series of diets, ADFI increased quickly but then decreased slowly before increasing.

4.6 Laying Hens Body Weight (y_1)

4.6.1 Gompertz Model

The Gompertz model of equation (3.5) was applied to the body weight response values (y_1) for the individual and the mean data. The estimated parameter values for each level of A (H, M, L) and each of the individual and mean data sets are given in Table 4.8. The estimated values for β (the growth rate) of H level for the individual data are different from those for the mean data. As expected, the highest value of the N (maximum growth response) is in H level. From the result of R^2 in Table 4.8, and the fitted curves in Figure 4.7, we conclude that this model is not as good as other models, specifically, the fitted line of L level is flat. Since there is a high variation in the L level of dietary protein, the Gompertz equation used in previous research (Faridi et al., 2011) could not give nicely fitted curves. However, Rogers et al. (1987) demonstrated that the linear and quadratic models predict more accurately for specific parts of the data. Thus, we should consider both linear and quadratic models for body weight; see section 4.6.3.

| | In | dividual | data | Mean data | | | |
|---------|--------|----------|--------------|-----------|--------|--------------|--|
| Entity | Н | Μ | \mathbf{L} | Н | Μ | \mathbf{L} | |
| α | 6.3192 | 0.4163 | 4.815E36 | 13.5127 | 0.2459 | 4.073E36 | |
| β | 0.1031 | 0.0315 | 3.6600 | 0.1257 | 187.2 | 3.6600 | |
| Ν | 1.6337 | 1.5599 | 1.2963 | 1.6215 | 1.4888 | 1.3100 | |
| R^2 | 0.3272 | 0.2392 | 0.0588 | 0.9757 | 0.9578 | 0.6248 | |

Table 4.8: Gompertz model-parameter estimates and goodness of fit for body weight (y_1)

4.6.2 Richards Model

The Richard model of equation (3.6) was applied to the body weight response values (y_1) for the individual and the mean data. The estimated parameter values for each level of diet (H, M, L) and each of the individual and mean data sets are given in Table 4.9. Even though R^2 in Table 4.9 is higher than for the Gompertz model, we see from Figure 4.8 that the flat line of the plot for the L level exhibits the same problem as we had with the Gompertz model. Especially, the estimated value of parameter N in the M level for the individual data is -0.0186. Since N indicate the asymptotic or maximum growth response, we cannot expect there be a minus sign of the estimated parameter value. The estimated values of β in the H and M level for the individual and mean data are lower than these in the L level. Since β refers to the intrinsic growth rate, estimated values in the H and M levels would be higher than these in the L level. Thus, this is also evidence that the Richards model is not appropriate to fit body weight. Therefore, other kinds of regression models will be applied, such as linear, quadratic, or cubic regression models; see section 4.6.3.

4.6.3 Linear regression

Before fitting basic linear regression curves, we should consider the shape of the curves based on those of Figure 4.7. In particular, we observe that there is an initial linear trend up to age 21. There are linear, quadratic, or cubic trends after age 21 weeks. Thus, we need to split the data

| | Ine | dividual d | ata | Mean data | | | | |
|----------|----------|------------|--------------|-----------|---------|--------------|--|--|
| Entity | Н | Μ | \mathbf{L} | Н | М | \mathbf{L} | | |
| α | 0.000522 | -1.0408 | 8.785E42 | 0.5671 | -1.0374 | 6.433E42 | | |
| eta | 0.1032 | 0.00141 | 4.6283 | 0.1263 | 0.00132 | 4.6283 | | |
| Ν | 1.6337 | -0.0186 | 1.2963 | 1.6214 | 1.5474 | 1.3100 | | |
| γ | 12119.3 | 1.5569 | 5218.7 | 24.4221 | -0.0139 | 5218.7 | | |
| R^2 | 0.3272 | 0.2882 | 0.0588 | 0.9756 | 0.9720 | 0.6248 | | |

Table 4.9: Richards model-parameter estimates and goodness of fit for body weight (y_1)

into two groups. The first two points (age 18, and 21) are in the first group (group1), and points from the second (age 21 - 75) are in the second group (group2). The equations of linear, quadratic, and cubic regressions are, respectively, for the response variable (y_1)

$$y = a + b \times t, \tag{4.1}$$

$$y = a + b \times t + c \times t^2, \tag{4.2}$$

$$y = a + b \times t + c \times t^2 + d \times t^3, \tag{4.3}$$

where t is age in weeks and a, b, c, and d are model parameters. These equations were applied to fit body weight curves (y_1) , for each of the there different dietary protein levels. Since there are only two time points (t=18, and 21) in the first group, clearly only a linear regression (connecting the two observations) can be used to fit two observed values of the body weight. Thus, when fitting these models to the mean data, there are no models for group 1. However, since there are replications in the individual data, we can fit a linear (only) model to the group 1 data.

The estimated parameter values for each model are shown in Table 4.10 and Table 4.11 for the individual and mean data, respectively, for the H level, in Tables 4.12-4.13 for the individual and mean data, for the M level, and in Tables 4.14-4.15 for the individual and mean data for the L

level. Since there is less variation for the mean data, the R^2 for the mean data is higher than that for the individual data in each case. Also based on Tables 4.10, 4.12, and 4.14, the R^2 of the linear regression in the first group is higher than these of the quadratic and cubic regressions in second group. The reason is there are less variation in first group, since there are only two points (age= 18, 21).

Let us now compare the model fits for second graph (i.e., for observations after 21 weeks). First, take the models for the H diet level. From Table 4.10 for the individual data, we see that, although these are significant fits (p < .0001) for all three models, the R^2 values are relatively small but are also comparable. Likewise, when fitting these models to the mean data, again all models are significant with p < .05 (though the quadratic regression fit has p = .0202) and the R^2 values are comparable. However, in this case, the R^2 values are now high at .08651, .9257, and .9311 for the linear, quadratic, and cubic models, respectively. This suggests that all three fits are adequate. Therefore, on the grounds of parsimony (i.e., as few parameters as necessary), we consider the linear regression model fits these data well.

For the M and L diet levels, we also see from Tables 4.12 and 4.14 for the individual data that all three regression models have reasonably comparable R^2 values for each level and are statistically significant (p < .0001), except that the linear model for the L level has p = .0382 and an R^2 value much less than for the quadratic and cubic fits (i.e., the linear regression model is not adequate for the L level diet). When we look at the corresponding fits to the mean data, in Table 4.13 and 4.15, it is clear that the fit of the linear regression model at the L level is not at all good (p = .4396) and R^2 (.0309) being much larger than the R^2 values for the quadratic and cubic regression fits ($R^2 = .7254$ and .8728, respectively). For the M level diet, it is the cubic regression fit that is clearly better than are the linear and quadratic regression fits.

| Group | Regression Type | a | b | С | d | p-value | R^2 |
|-------|-----------------|---------|--------|----------|-----------|---------|--------|
| 1 | Linear | -0.7856 | 0.1072 | | | < .0001 | 0.7669 |
| 2 | Linear | 1.4104 | 0.0040 | | | < .0001 | 0.1162 |
| 2 | Quadratic | 1.2843 | 0.0101 | -0.00006 | | < .0001 | 0.1224 |
| 2 | Cubic | 1.5142 | 0.0126 | -0.0001 | 0.0000004 | < .0001 | 0.1225 |

Table 4.10: Treatment H - Linear regression-parameter estimates and goodness of fit for body weight (y_1) with individual data

Table 4.11: Treatment H - Linear regression-parameter estimates and goodness of fit for body weight (y_1) with mean data

| Group | Regression Type | a | b | С | d | p-value | R^2 |
|-------|-----------------|--------|--------|----------|-----------|---------|--------|
| 2 | Linear | 1.4432 | 0.0034 | | | 0.0072 | 0.8651 |
| 2 | Quadratic | 1.3027 | 0.0103 | -0.00008 | | 0.0202 | 0.9257 |
| 2 | Cubic | 1.2643 | 0.0117 | -0.0001 | 0.0000003 | < .0001 | 0.9311 |

Especially, in Table 4.13 and Table 4.15, the p-values of the linear or quadratic regression (p=.446 and .4396 respectively), are not lower than 0.05. However, in Table 4.11, since p-values (0.0072 and 0.0202) are lower than 0.05, only for the H level, the linear and quadratic regression are fitted well. That is, the linear or quadratic regression does not fit well for M and L level except that the linear and quadratic model for H. Even though, the highest R^2 of the each diet level is for cubic regression (H=.9311, M=.9276, and L=.8727), the R^2 of quadratic regression is little bit lower than cubic regression in the H and M levels ($R^2 = .9257$ and .8132, respectively). Thus, we can say that quadratic regression is the best fit for the H level. However, when considering that the R^2 of the cubic regression model for the L level treatment (see Tables 4.14-15) is the highest ($R^2 = .0975$ and .8728, respectively), and from the respective fits shown in Figures 4.9 - 4.11, we conclude the cubic regression is the best fit to the body weight curve.

Table 4.12: Treatment M - Linear regression-parameter estimates and goodness of fit for body weight (y_1) with individual data

| Group | Regression Type | a | b | с | d | p-value | R^2 |
|-------|-----------------|---------|---------|---------|-----------|---------|--------|
| 1 | Linear | -0.7401 | 0.1051 | | | < .0001 | 0.8171 |
| 2 | Linear | 1.4010 | 0.0018 | | | < .0001 | 0.0404 |
| 2 | Quadratic | 1.5084 | -0.0034 | 0.00005 | | < .0001 | 0.0483 |
| 2 | Cubic | 1.6855 | -0.0169 | 0.0004 | -0.000002 | < .0001 | 0.0506 |

Table 4.13: Treatment M - Linear regression-parameter estimates and goodness of fit for body weight (y_1) with mean data

| Group | Regression Type | a | b | с | d | p-value | R^2 |
|-------|-----------------|--------|---------|---------|-----------|---------|--------|
| 2 | Linear | 1.4068 | 0.0017 | | | 0.0446 | 0.6759 |
| 2 | Quadratic | 1.5279 | -0.0042 | 0.00006 | | 0.0808 | 0.8132 |
| 2 | Cubic | 1.6950 | -0.0174 | 0.0004 | -0.000002 | < .0001 | 0.9276 |

Table 4.14: Treatment L - Linear regression-parameter estimates and goodness of fit for body weight (y_1) with individual data

| Group | Regression Type | a | b | с | d | p-value | R^2 |
|-------|-----------------|---------|---------|--------|-----------|---------|--------|
| 1 | Linear | -0.6823 | 0.1015 | | | < .0001 | 0.7636 |
| 2 | Linear | 1.3542 | -0.0009 | | | 0.0382 | 0.0064 |
| 2 | Quadratic | 1.7484 | -0.0200 | 0.0002 | | < .0001 | 0.0763 |
| 2 | Cubic | 2.4175 | -0.0706 | 0.0014 | -0.000008 | < .0001 | 0.0975 |

Table 4.15: Treatment L - Linear regression-parameter estimates and goodness of fit for body weight (y_1) with mean data

| Group | Regression Type | a | b | с | d | p-value | R^2 |
|-------|-----------------|--------|---------|--------|----------|---------|--------|
| 2 | Linear | 1.3380 | -0.0006 | | | 0.4396 | 0.0309 |
| 2 | Quadratic | 1.8011 | -0.0233 | 0.0002 | | < .0001 | 0.7254 |
| 2 | Cubic | 2.6446 | -0.0866 | 0.0017 | -0.00001 | < .0001 | 0.8728 |

4.7 Comparing Treatments

To compare treatments using nonlinear regression analysis, we use the sum of squares reduction test. Nonlinear models were applied to fit response variables in two cases. One case is the full model with all parameters of all treatments, and the second case is the reduced model with fewer parameters of the full model. For example, in our Adams-Bell model, the full model contains twelve parameters (four parameters \times three treatments), i.e.,

$$y_{ij} = 100\left(\frac{1}{(1+a_j b_j^t)} - c_j t + d_j\right), \ i = 5, 6, \ j = 1, 2, 3.$$
(4.4)

where y_{51} , y_{52} , and y_{53} corresponds to the response at level H, M, and L of egg production, and y_{61} , y_{62} , and y_{63} corresponds to the response at level H, M, and L of egg output. The reduced model contains eight parameters, since we assume that there is no difference between the two treatments. That is, two particular treatments share the same a, b, c, and d. Therefore, the hypothesis is

 H_0 : There is no difference between the two treatments.

The test statistic is

$$F_{obs} = \frac{(SS(Residual)_{Reduced} - SS(Residual)_{Full})/(df(Residual)_{Reduced} - df(Residual)_{Full})}{MSError(Full)}$$

$$(4.5)$$

$$\sim F[(df(Residual)_{Reduced} - df(Residual)_{Full}), df(Residual)_{Full}].$$

See Gallant (1987, Chapter 4) for details of this test. Since two treatments were compared in any one test, we considered three cases. First, equality of the H and M levels were tested, then the H and L levels, and finally the M and L levels.

| | | I. | | 1 | | 1 | |
|---------------|------------|---------|---------|---------|---------|---------|---------|
| | | H ar | nd M | H and L | | M and L | |
| Model | Data | F-value | p-value | F-value | p-value | F-value | p-value |
| Adams-Bell | Individual | 10.746 | < .0001 | 406.993 | < .0001 | 303.520 | < .0001 |
| | Mean | 3.338 | 0.0118 | 126.027 | < .0001 | 93.601 | < .0001 |
| Modified | Individual | 9.527 | < .0001 | 334.975 | < .0001 | 253.614 | < .0001 |
| compartmental | Mean | 3.365 | 0.0112 | 127.566 | < .0001 | 96.693 | < .0001 |

Table 4.16: Comparing treatments for egg production (y_5)

4.7.1 Egg Production (%) (y_5)

The F-values from (4.5) and the corresponding p-values results of this test (comparing the H and M levels, the H and L levels, and the M and L levels) are summarized in Table 4.16 for egg production (y_5) for each of the fits of the Adams-Bell and the modified compartmental models and for each of the individual and mean data. Based on the p-values in Table 4.16, H_0 is rejected for each comparison at the 1% level of significance in each case except that for the H and M comparison of the mean data, H_0 is rejected at the 5% but not the 1% level of significance (since p < .05 in these cases). That is, we conclude that the three nonlinear curves are significantly different from each other. That is, the different protein levels produce significantly different egg production responses.

4.7.2 Egg output (y_6)

The F and p-values of comparing egg output (y_6) for the H and M levels, the H and L levels, and the M and L levels for each of the fits of the Adams-Bell and modified compartmental models and each of the individual and mean data appear in Table 4.17. From the p-values of Table 4.17, we conclude that any two diets when fitting curves of each of the Adams-Bell and Modified compartmental models are not the same as each other. Thus, the three nonlinear curves for each of the individual and mean data are significantly different from each other. When comparing p-values

| | | H ar | H and M | | H and L | | M and L | |
|---------------|------------|---------|---------|----------|---------|---------|---------|--|
| Model | Data | F-value | p-value | F-value | p-value | F-value | p-value | |
| Adams-Bell | Individual | 9.961 | < .0001 | 142.699 | < .0001 | 78.405 | < .0001 | |
| | Mean | 18.797 | < .0001 | 245.395 | < .0001 | 131.180 | < .0001 | |
| Modified | Individual | 78.848 | < .0001 | 1055.393 | < .0001 | 572.374 | < .0001 | |
| compartmental | Mean | 20.420 | < .0001 | 289.033 | < .0001 | 158.789 | < .0001 | |

Table 4.17: Comparing treatments for egg output (y_6)

Table 4.18: Comparing treatments for egg weight (y_3)

| | | H and M | | H an | d L | M and L | |
|----------|------------|---------|---------|----------|---------|---------|---------|
| Model | Data | F-value | p-value | F-value | p-value | F-value | p-value |
| Lokhorst | Individual | 375.639 | < .0001 | 1800.290 | < .0001 | 540.793 | < .0001 |
| | Mean | 37.244 | < .0001 | 229.739 | < .0001 | 79.593 | < .0001 |

of the H and M levels for egg production of the mean data (in Table 4.16) with the p-values of the H and M levels for egg output of the mean data (in Table 4.17), we see the p-values for egg output are lower.

4.7.3 Egg Weight (y_3)

The F-values from (4.5) and the corresponding p-value results of the sum of square reduction test (comparing H and M levels, H and L levels, and M and L levels) are summarized in Table 4.18 for egg weight (y_3) for each of the fits of the Lokhorst egg weight models (1996) and each of the individual and mean data. Since the p-values in Table 4.18 are lower than 0.01, we can say these are statistically significant different models across the model comparisons. Thus, the three curves for the different diet levels for each of the individual and mean data are significantly different from each other for the different diets.

| | | H and M | | H ai | nd L | M and L | |
|----------|------------|---------|---------|---------|---------|---------|---------|
| Model | Data | F-value | p-value | F-value | p-value | F-value | p-value |
| Lokhorst | Individual | 50.995 | < .0001 | 340.676 | < .0001 | 281.264 | < .0001 |
| | Mean | 3.617 | 0.0039 | 54.056 | < .0001 | 35.688 | < .0001 |

Table 4.19: Comparing treatments for ADFI (y_2)

4.7.4 Average Daily Feed Intake (y_2)

For average daily feed intake (y_2) (ADFI), the resulting F- and p-values from (4.5) when comparing treatments are shown in Table 4.19 for the H and M levels, the H and L levels, and the M and L levels for each of the fits of the Lokhorst feed intake model (1996) and for each of the individual and mean data. Since the p-values in Table 4.18 are lower than 0.01, H_0 is rejected at the 1% level of significance for all comparisons. Thus, there is not enough evidence to indicate that there is no difference among the three treatments, i.e., equivalently, all three diet levels for each of the individual and mean data are significantly different from each other. I. e., the ADFI is significantly different for the three different diets.

4.7.5 Laying Hens Body Weight (y_1)

For laying hens body weight (y_1) , the results from (4.5) of comparing the treatments are shown in Table 4.20 for the H and M levels, the H and L levels, and the M and L levels for each of the fits of the cubic model for each of the individual and mean data. The large F values and small p-values in Table 4.20 suggest that there are statistically significant differences among three cubic regressions of protein diet levels for each of the individual and mean data. Thus, the three curves for the different diet levels for each of the individual and mean data are significantly different from each other for the difference diets.

| | | H and M | | H and L | | M and L | |
|-------|------------|---------|---------|---------|---------|----------|---------|
| Model | Data | F-value | p-value | F-value | p-value | F-value | p-value |
| Cubic | Individual | 202.110 | < .0001 | 773.494 | < .0001 | 1039.641 | < .0001 |
| | Mean | 5.970 | 0.0310 | 88.623 | 0.0001 | 10.595 | 0.0062 |

Table 4.20: Comparing treatments for body weight (y_1)

4.8 Repeated Measures

As previously stated in Section 3.2, we can consider the hen's id number, as a repeated measures analyses factor. In this section, the results of one and two factors repeated measures designs are shown for egg production (y_5) , egg output (y_6) , egg weight (y_3) , average daily feed intake (ADFI) (y_2) , hens body weight (y_1) , and egg cumulative number (y_4) . One of the goals of this thesis is to investigate whether balanced dietary protein levels have an effect on the response variables. For the one factor repeated measures design, the factor used corresponds to the three different protein levels. For the two factors repeated measures design, the factors are the age and the protein levels. For our two factors repeated measures design, we used forty-eight hens for each protein level, H, M, and L, and kept those hens on the same diet level through all levels of the age factor. This is the special case in Section 3.2.1. Since there are slight differences between the H and M levels in the plots of the observed response values (in Figures 4.1-4.8), we especially focus on the contrast of the H and M levels, and the average of the H and M levels with the L level. Based on the observed response variables (for egg production (y_5) , egg output (y_6) , egg weight (y_3) , average daily feed intake (ADFI) (y_2) , hens body weight (y_1) , and egg cumulative number (y_4)) in Figures 4.1-4.8, we can find the peak point in each diet level for the individual and mean data. We also need to test whether there are differences among the peak point and other points.

4.8.1 Egg Production (y_5)

Table 4.21 gives the analysis of variance (ANOVA) results of the one and two factors repeated measures for egg production (y_5) . Based on Figure 4.1-4.2, the observed values of egg production increased rapidly and decreased slowly. We consider the twenty-third week point for egg production as the peak point. For the one factor repeated measures model, there is a significant difference in the dietary protein levels between the H and M levels, and also between the average of the H and M levels and the L level at the 1% level of significance. Thus, the different diet levels and the different between H and M levels, and the different between the average of H and M levels and L level make a difference in egg production. However, for the two factors repeated measures design, the difference between the H and M levels is not significant (p = .1280 > .05). That is, the difference between the H and M levels does not result in a statistically significant difference in egg production. Also the interaction between diet levels and age is significant at the 1% level of significance. Thus, there are differences in egg production among the three diet levels for the different age in weeks. However, when testing the contrasts as a component of the age \times id within diet variation, we see that the H and M levels are not significantly different at the 5% level of significance (p = .1280 > .05). The average of the H and M levels is statistically significant different from the L level (p < 0.0001) in egg production. Finally, we also compare ages producing the peak egg production values with the other ages as a "Peak & Others" contrast comparent of dietary protein; since p < 0.001, we can conclude these age groupings produce statistically different responses.

4.8.2 Egg Output (y_6)

The ANOVA results of the one and two factors repeated measures designs for egg output (y_6) are displayed in Table 4.22. Based on Figure 4.3-4.4, the H and M levels are on an increasing

| Method | Treatment | Repeated | p-value |
|---------|------------------------------|---------------|---------|
| One-Way | Dietary protein | Age | <.0001 |
| | Contrast | H vs M | < .0001 |
| | Contrast | (H,M) vs L | < .0001 |
| Two-Way | Dietary protein | Age | < .0001 |
| | Age | Age | < .0001 |
| | Dietary protein \times Age | Age | < .0001 |
| | Contrast | Peak & Others | < .0001 |
| | Contrast | H vs M | 0.1280 |
| | Contrast | (H,M) vs L | < .0001 |

Table 4.21: Repeated Measure for egg production (y_5)

trend, but the L level is on a decreasing trend after the twenty-third week. Thus, the twenty-third week is considered as the peak point. The result of comparing the difference between the peak point and other points is significant at the 5% level of significance but not significant at the 1% (.01 < p = 0.0133 < .05). For both the one and two factors repeated measures designs, there is a significant difference in the dietary protein levels, in the interactions between diet levels and age in weeks, the H and M levels, and the average of the H and M levels and the L level, at the 1% level of significance. Thus, the different diet levels and the difference between the H and M levels, and the difference between the average of the H and M levels and the L level, make a difference in egg production for the one factor repeated measures. The comparison of responses at the peak ages with nonpeak ages is significant at the 5% level but not at the 1% level of significance (.01 < p = .0133 < .05). As in section 4.8.1, the contrast of peak and others (ages) is a component of the age \times id within diet variation. There are differences in egg output among the three diet levels with the different age in weeks; the difference between the H and M levels, and the difference between the average of the H and M levels, and the difference between the average of the H and M levels, and the difference between the average of the H and M levels, and the difference between the average of the H and M levels and the L level make a difference in egg output. However, since there are interaction effects, rather than the tests of difference in the dietary protein levels

| Method | Treatment | Repeated | p-value |
|---------|------------------------------|---------------|---------|
| One-Way | Dietary protein | Age | <.0001 |
| | Contrast | H vs M | < .0001 |
| | Contrast | (H,M) vs L | < .0001 |
| Two-Way | Dietary protein | Age | < .0001 |
| | Age | Age | < .0001 |
| | Dietary protein \times Age | Age | < .0001 |
| | Contrast | Peak & Others | 0.0133 |
| | Contrast | H vs M | < .0001 |
| | Contrast | (H,M) vs L | <.0001 |

Table 4.22: Repeated Measure for egg output (y_6)

and age in weeks, per se, it is more meaningful to talk about the interaction effects. Hence, we can say that the effect of age on the egg output is different for the different diets (or, equivalently, the effects of the diets on egg output differs over different ages).

4.8.3 Egg Weight (y_3)

The ANOVA results of the one and two factors repeated measures analyses for egg weight (y_3) are summarized in Table 4.23. Based on Figure 4.5, the observed values for the three protein levels rapidly increase before the twenty-third week. Thus, the twenty-third week is considered as the peak. The result of this contrast test (peak and other points) is significant at the 1% level of significance. Since all p-values are lower than 0.0001, there is a significant difference in the dietary protein levels, in the interactions between diet levels and the age in weeks, between the H and M levels, and between the average of the H and M levels and the L level at the 1% level of significance both in the one and two factors repeated measures design. Thus, the different diet levels and the difference between the H and M levels, and the difference between the average of the H and M levels and the L level make a difference in egg weight for these one factor repeated measures design. There are statistically significant differences in egg weight among the three diet levels across the different

| Method | Treatment | Repeated | p-value |
|---------|------------------------------|---------------|---------|
| One-Way | Dietary protein | Age | <.0001 |
| | Contrast | H vs M | < .0001 |
| | Contrast | (H,M) vs L | < .0001 |
| Two-Way | Dietary protein | Age | < .0001 |
| | Age | Age | < .0001 |
| | Dietary protein \times Age | Age | < .0001 |
| | Contrast | Peak & Others | < .0001 |
| | Contrast | H vs M | < .0001 |
| | Contrast | (H,M) vs L | < .0001 |

Table 4.23: Repeated Measure for egg weight (y_3)

ages in weeks; the difference between the H and M levels, and the difference between the average of the H and M levels and the L level make a difference in egg weight for the two factors repeated measures design. However, since there are interaction effects rather than the tests of difference in the dietary protein levels and age in weeks, it is meaningful to talk about the interaction effects. Thus, we can say that the effect of age on the egg weight is different for the different diets or, equivalently, the effects of the diets on egg output differs over different ages.

4.8.4 Average Daily Feed Intake (y_2)

The results of the analyses for the one and two factors repeated measures designs for average daily feed intake (ADFI) (y_2) appear in Table 4.24. Based on Figure 4.6, the observed values in the three protein levels are on a rapid growth curve before the twenty-third week. Thus, the twenty-third week is considered as the peak point. The result of this contrast test (peak and other points) is significant at the 1% level of significance (p < .0001). For the one factor repeated measures design, there is a significant difference in the dietary protein levels, between the H and M levels, and between the average of the H and M levels and the L level at the 1% level of significance. Thus, the different diet levels and the difference between the H and M levels, and the difference between

| Method | Treatment | Repeated | p-value |
|---------|------------------------------|---------------|---------|
| One-Way | Dietary protein | Age | <.0001 |
| | Contrast | H vs M | < .0001 |
| | Contrast | (H,M) vs L | < .0001 |
| Two-Way | Dietary protein | Age | < .0001 |
| | Age | Age | < .0001 |
| | Dietary protein \times Age | Age | < .0001 |
| | Contrast | Peak & Others | < .0001 |
| | Contrast | H vs M | 0.1895 |
| | Contrast | (H,M) vs L | <.0001 |

Table 4.24: Repeated Measure for ADFI (y_2)

the average of the H and M levels and the L level make a difference in average daily feed intake for the one factor repeated measures design. However, for the two factors repeated measures design, the H and M levels are not significantly different at the 5% level of significance (p = .1895 > .05). There are differences in average daily feed intake among the three diet levels with the different age in weeks; the difference between the average of the H and M levels and the L level make a difference in ADFI. However, the difference between the H and M levels does not make a difference in ADFI. Since there are interaction effects rather than the tests of difference in the dietary protein levels and age in weeks, it is more meaningful to talk about the interaction effects. Hence, we can say that the effect of age on the ADFI is different for the different diets.

4.8.5 Laying Hens Body Weight (y_1)

Table 4.25 shows the ANOVA results of one and two factors repeated measures analyses for hens body weight (y_1) . As observed in the linear regression Section 4.6.3, the twenty-first week is considered as the peak point. The result of this contrast test (peak and other points) is significant at the 1% level of significance. Since all p-values are lower than 0.0001, there is a significant difference in the dietary protein levels, in the interactions between diet levels and the the different

| Method | Treatment | Repeated | p-value |
|---------|------------------------------|---------------|---------|
| One-Way | Dietary protein | Age | <.0001 |
| | Contrast | H vs M | < .0001 |
| | Contrast | (H,M) vs L | < .0001 |
| Two-Way | Dietary protein | Age | < .0001 |
| | Age | Age | < .0001 |
| | Dietary protein \times Age | Age | < .0001 |
| | Contrast | Peak & Others | < .0001 |
| | Contrast | H vs M | < .0001 |
| | Contrast | (H,M) vs L | <.0001 |

Table 4.25: Repeated Measure for body weight (y_1)

ages in weeks, between the H and M levels, and between the average of the H and M levels and the L level at the 1% level of significance in the one factor repeated measures. Thus, the different diet levels and the difference between the H and M levels, and the difference between the average of the H and M levels and the L level make a difference in body weight for the one factor repeated measures design. There are differences in body weight among the three diet levels with the different ages in weeks; the difference between the H and M levels, and the difference between the average of the H and M levels and the L level make a difference in body weight for the two factors repeated measures. Since there are interaction effects, rather than the tests of difference in the dietary protein levels and age in weeks per se, it is more meaningful to talk about the interaction effects. Hence, we can say that the effects of the diets on egg output differs over different ages.

4.8.6 Egg Cumulative Number (y_4)

Figure 4.12 shows the trend of egg cumulative number (y_4) in each diet levels. Clearly, in this case, since our response is a cumulative value, there is no "peak" value possible (except at the end). The ANOVA results of one and two factors repeated measures designs for egg cumulative number are summarized in Table 4.26. There is a significant difference in the dietary protein levels, between the H and M levels, and between the average of the H and M levels and the L level for the one factor design; there is a significant difference in the average of the H and M levels and the L level for the two factor repeated measures design at the 1% level of significance. However, the difference between the H and M levels for the two factor design is not significant at the 5% level of significance (p = .1621 > .05). Thus, the different diet levels and the difference between the H and M levels, and the difference between the average of the H and M levels and the L level make a difference in egg cumulative number for the one factor repeated measures model. There are differences in egg cumulative number among the three diet levels with the different age in weeks; the difference between the average of the H and M levels and the L level makes a difference in body weight for the two factors repeated measures model. Especially, the result of contrast "H and M" of the one way repeated measures design is not same as the result of the two way design. When testing this contrast, we consider the error term to be the within hens variation for the one factor model, but the between hens variation for the two factors design. Thus, because of the differing error terms, the results of the two contrast tests are not the same. Finally, since there are interaction effects, rather than the tests of difference in the dietary protein levels and age in weeks per se, it is more meaningful to talk about the interaction effects. Hence, we can say that the effect of age on the egg cumulative number is different for the different diets (or, equivalently, the effects of the diets on egg output differs over different ages).

| Method | Treatment | Repeated | p-value |
|---------|------------------------------|------------|---------|
| One-Way | Dietary protein | Age | <.0001 |
| | Contrast | H vs M | < .0001 |
| | Contrast | (H,M) vs L | < .0001 |
| Two-Way | Dietary protein | Age | < .0001 |
| | Age | Age | < .0001 |
| | Dietary protein \times Age | Age | < .0001 |
| | Contrast | H vs M | 0.16213 |
| | Contrast | (H,M) vs L | < .0001 |

Table 4.26: Repeated Measure for Egg Cumulate Number (y_4)



Figure 4.1: Adams-Bell model for egg production (y_5)



Figure 4.2: Modified compartmental model for egg production (y_5)



Figure 4.3: Adams-Bell model for egg output (y_6)



Figure 4.4: Modified compartmental model for egg output (y_6)



Figure 4.5: Lokhorst egg weight model for egg weight (y_3)



Figure 4.6: Lokhorst feed intake model for ADFI (y_2)





Figure 4.7: Gompertz model for body weight (y_1)



Figure 4.8: Richards model for body weight (y_1)

Age(wk)

• • • M

++++ M-predict

++++L

B B B L-predict

* * * H

1.0 - _____ 000 H-predict





Figure 4.9: Linear regression for body weight (y_1)



Figure 4.10: Quadratic regression for body weight (y_1)





Figure 4.11: Cubic regression for body weight (y_1)



Figure 4.12: Egg Cumulate Number (y_4)

Chapter 5

Conclusions

The results of this study illustrate that some models (the Adams-Bell, modified compartmental, and Lokhorst egg weight and feed intake) are able to fit curves (to egg production, egg output, egg weight, and feed intake response values) of the high (H) and medium (M) protein levels in the hens' diet. However, the curves of the low (L) protein level did not fit well, when comparing the results of the calculated goodness-of-fit criteria and plots of fitted curves. In particular, the cubic regression fitted better than the Gompertz and Richard models to body weight curves. Overall, the results of the mean data with less variation than the individual data gave better predictions in most cases, as would be expected. Throughout this study the results of comparing three diet levels using nonlinear regression analysis and repeated measures designs, indicated that the different dietary protein levels make a difference in egg production, egg output, egg weight, and body weight. That is, hens fed high protein diets have higher egg production, egg weight, and body weight than hens fed low protein diets. Thus, the choice is between a higher cost for higher production and a lower cost for lower production.

The diet fed to commercial laying hens can vary depending on the strain of layer, production

goals, age, and weather conditions (Schaible and Patrick, 1980). We found feed intake of all hens in the L series increased considerably after week 54 when the temperature of the house decreased due to winter conditions. Thus, hens fed the L series seemed particularly dependent on house temperature to maintain body weight and average daily feed intake (ADFI). For future research, we can consider house temperature as a covariate, and age in weeks and diet levels as factors when using an analysis of covariance. This would be helpful in demonstrating the effects of temperature on average daily feed intake (ADFI) and bodyweight of hens fed low protein diets.

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Appendix A

Appendix

A.1 SAS Code

```
/*Adams-Bell model for egg production*/
/*Diet level - H*/
proc nlin data=y5_1 method=marquardt;
parms a=0.001 b=0.3 c=0.01 d=0.3; /*a, b, c, and d are parameters*/
model y5=100*(1/(1+a*b**(t))-c*t+d); /* y5 is egg production and t is age in week*/
output out=out1 predicted=pred1;
run;
/*Diet level - M*/
proc nlin data=y5_2 method=marquardt;
parms a=0.001 b=0.3 c=0.01 d=0.3;
model y5=100*(1/(1+a*b**(t))-c*t+d);
output out=out2 predicted=pred2;
run;
```

```
/*Diet level - L*/
proc nlin data=y5_3 method=marquardt;
parms a=0.001 b=0.3 c=0.01 d=0.3;
model y5=100*(1/(1+a*b**(t))-c*t+d);
output out=out3 predicted=pred3;
run;
```

/* Reduce model 1 */
ods output Anova=reduce1;
proc nlin data=y5 method=marquardt;
parms a1=0.001 b1=0.3 c1=0.01 d1=0.3;
a2=a1; b2=b1; c2=c1;d2=d1;
parms a3=0.001 b3=0.3 c3=0.01 d3=0.3;

```
model y5=100*(1/(1+a1*b1**(t))-c1*t+d1)*(diet=1)+
```

```
100*(1/(1+a2*b2**(t))-c2*t+d2)*(diet=2)+
```

```
100*(1/(1+a3*b3**(t))-c3*t+d3)*(diet=3)
```

; run;

```
/* Calculate F and P-value of the sum of square reduction test*/
data one;
set full reduce1;
retain dfF sseF mseF dfR sseR;
if _N_=2 then dfF=DF;
if _N_=2 then sseF=SS;
if _N_=2 then mseF=MS;
if _N_=5 then dfR=DF;
if _N_=5 then sseR=SS;
s=(sseR-sseF)/(dfR-dfF);
F=s/mseF;
pv=1-probf(F,dfR-dfF, dfF );
file print;
put 'F-statistic=' F;
put 'p-value=' pv;
run;
/* Reduced model 2 */
proc nlin data=y5 method=marquardt;
```

```
parms a1=0.001 b1=0.3 c1=0.01 d1=0.3;
```

/* Reduced model 3 */

proc nlin data=y5 method=marquardt;

parms a1=0.001 b1=0.3 c1=0.01 d1=0.3;

parms a2=0.001 b2=0.3 c2=0.01 d2=0.3;

a3=a2; b3=b2; c3=c2;d3=d2;

```
model y5=100*(1/(1+a1*b1**(t))-c1*t+d1)*(diet=1)+
```

```
100*(1/(1+a2*b2**(t))-c2*t+d2)*(diet=2)+
```

```
100*(1/(1+a3*b3**(t))-c3*t+d3)*(diet=3)
```

; run;

```
/*Repeated measures designs*/
```

/*one factor repeatd */

```
proc glm data=y5_row;
```

class diet t;

/* y5 is egg production, t is age in week, and diet is protein level in diet*/
model y5=diet|t ;
lsmeans diet/pdiff adjust=tukey;
contrast "H vs M" diet 1 -1 0 ;
contrast "H & M vs L" diet -1 -1 2 ;

```
test h=diet e=diet*t;
run;
proc glm data=y5_row;
class diet t;
model y5=t diet(t) ;
test h=t e=diet(t);
run;
/*two factors repeated */
proc glm data=y5_row;
class t diet id ;
 /* y5 is egg production, t is age in week, diet is protein level in diet,
    and id is hens id neumber */
model y5=diet id(diet) /ss3;
test h=diet e=id(diet);
contrast "H vs M" diet 1 -1 0 ;
contrast "H & M vs L" diet -1 -1 2 ;
run;
proc glm data=y5_row;
class diet t ;
model y5=t diet*t;
contrast " peak vs others" t -0.2 -0.3 -0.5 -1 83 -3 -3 -3 -3 -3 -3 -3 -3 -3 -3 -3
```