Modeling and optimization of essential amino acids effects on productive performance in second-cycle hens

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Abstract. In this study, a model based on a modification of the Gompertz equation was used to estimate the maximum percentage of egg production and digestible requirements of lysine (Lys), methionine + cystine (Met+Cys) and threonine (Thr) of H&N brown laying hens during 16 weeks of production. The productive performance model was adjusted to field data of 23 treatments, which were defined from a composite central design that combines five levels of Lys, Met+Cys, and Thr ranged from 0.636% up to 1.000%; 0.579% up to 0.910%; 0.483% up to 0.760%, respectively. The proposed model showed to be adequate to evaluate the efficiency in the use of digestible amino acids for egg production and can be a useful tool to diagnose the effectiveness of the nutritional requirements in each of the production phases.

1. Introduction

The poultry industry has shown significant growth in recent years, although production costs are high and most of them are based on feed costs, which can exceed 70% of variable costs [1]. One of the strategies to reduce such production costs is forced molting, which is an important management tool that offers economic benefits associated with additional egg laying cycles and provides the producer with flexibility in handling to respond with rapid changes in the egg market, as well as changes in feed and input costs [2].

Limiting amino acid requirements in diets of second-cycle hens differ from those of first-cycle birds because of their special conditions, including differences in weight gain, egg production and egg size. Based on the ideal protein concept, it is claimed that there is a relationship between lysine and other amino acids and this relationship should be considered to avoid reduced bird performance [3]. However, the interactions between amino acids can be modified based on factors such as age, sex, and environment [4]. Methionine is the first limiting amino acid in poultry diets, followed by lysine. Methionine is required for the biosynthesis of important substances involved in growth [5]. Dietary deficiency of methionine reduces egg production and body weight and increases fat deposition in the liver of laying hens. However, factors such as genetic line, environmental temperature and energy content, as well as the presence of anti-nutritional factors and the processing of raw materials used in the diet, influence the
requirement of methionine + cystine in laying hens [6]. Threonine plays an important role in gastrointestinal health and its optimization offers greater protection to bacterial infections of the intestine. The high requirement of gut for threonine is expected because this amino acid accounts for 30% of the total amino acid content of mucin [7,8].

Researches regarding amino acid requirements of laying hens take into account a complexity of factors that can act collectively or individually. Optimal levels of amino acids may vary depending on genetic factors, immune stress, age, dietary and climatic factors. In addition to these factors, estimates of amino acid requirements may also depend on the mathematical model used in their prediction, as some models tend to overestimate requirements and others to underestimate [9]. Digestible amino acids requirements of second-cycle hens have been estimated for the genetic lines Lohman Brown and Shaver Brown in 0.681% and 0.790% of lysine [10,11], 0.698% and 0.588% of methionine + cystine [10,12], 0.460% and 0.525% threonine [12,13], respectively. However, nutritional requirements of second-cycle hens during production phase are unknown for other genetic lines under specific production conditions and it is necessary to determine amino acid requirements to optimize the physiological status of birds, regeneration of their tissues after molting, and increased production. Currently, dose-response is the methodology most used to establish the optimal levels of amino acids in experimental trials. This method consists of the gradual increase of the amino acid to the basal diet deficient in the amino acid to be studied [14]. However, other authors [15] suggest that the dose-response approach ignores the effects of interaction between amino acids and recommend methodologies evaluating multiple nutrients in the same assay.

Mathematical models are strategies that allow the evaluation of egg production during the laying cycle as well as the prediction of financial losses caused by a fall in productive performance [16]. The Gompertz model has been one of the most used equations in the poultry industry to represent mathematically biological and economic phenomena associated with growth, due to the biological significance of its parameters, the relative ease with which the model is adjusted and the robustness in describing the trend of experimental or field data [17]. In this study, a procedure was developed based on the modification of the Gompertz model to determine the percentage of egg production in response to essential amino acids supplementation. The procedure was applied to determine the requirements of digestible lysine, methionine + cystine and threonine levels for egg production of H&N brown laying hens during the second production cycle.

2. Materials and methods
2.1. Field work and experimental information
The field work was carried out at the San Carlos Poultry Farm (San Pedro, Antioquia) located at 2475 above sea level with a temperature of 14 °C and relative humidity of 79% annual average. A total of 1380 92-week-old H&N brown laying hens during the second production cycle were housed in cages with commercial production conditions. The number of eggs produced was recorded weekly for 16 weeks. The percentage of egg production ($P$) was calculated weekly from the relation between the number of eggs and the number of birds per experimental unit.

2.2. Experimental design
Dietary digestible essential amino acids concentrations (three factors: lysine, methionine + cystine and threonine) were combined using a composite central design. A design matrix was obtained with 23 treatments (Table 1) combining five levels of lysine ($Lys$), methionine + cystine ($Met+Cys$) and threonine ($Thr$) ranged from 0.636% up to 1.000%; 0.579% up to 0.910%; 0.483% up to 0.760%, respectively. Five replicates were performed per treatment in experimental units of 12 birds.

2.3. Formulation of diets
The diets corresponding to each of the 23 treatments were formulated with corn and soybean cake, corn gluten and palm oil according to the nutritional recommendations of the birds as proposed by Rostagno
et al. [18]. The essential amino acids (Lys, Met+Cys, Thr) were fixed at their respective five levels by the addition of commercial products containing supplemental amino acids. The remaining seven essential amino acids were fixed by changing the level of kaolin in the diet.

2.4. Modeling and Statistical Analysis

The mathematical modeling of the egg production (Figure 1) as a function of time was performed for each of the treatments using a Gompertz model with modifications (Equation 1 and 2).

\[
P = P_m \exp \left( - \exp \left[ \frac{\mu e}{P_m} (\lambda - t) + 1 \right] \right)
\]

\[
P_m = \gamma_1 + \gamma_2 t
\]

In equations (1) and (2), \(P\) is the egg production (%), \(t\) is the time (weeks), \(\lambda\) is the initial lag time (weeks), \(\mu\) is the maximum growth rate (% week\(^{-1}\)), \(e\) is the base of the natural logarithm, \(P_m\) is a function representing the behavior of egg production during the maturity phase (%), \(\gamma_1\) (%) and \(\gamma_2\) (% week\(^{-1}\)) are parameters of \(P_m\) function.

![Figure 1. Production curve parameters for the modified model of Gompertz](image)

The model parameters (\(\lambda\), \(\mu\), \(\gamma_1\) and \(\gamma_2\)) were estimated using “nlinfit” function of the Statistic Toolbox of MATLAB® R2016b (The MathWorks Inc., Natick, MA, USA) for each treatment. The dependence of model parameters with the amino acid concentrations was assessed to obtain a unique model in terms of time and Lys, Met+Cys and Thr. The 95% confidence intervals of the estimated parameters were determined using the “nlparci” function and the “lillietest” function was used to determine if the residuals followed a normal distribution. The adjusted coefficient of determination (Equation 3) and the mean relative error (Equation 4) were used to evaluate the goodness of fit and accuracy of the estimation, respectively.

\[
R^2_{\text{adj}} = 1 - \frac{S^2_y}{S^2_y - S^2_x}
\]

\[
MRE = \frac{100}{n} \sum_{i=1}^{n} \left| \frac{P_i - \hat{P}_i}{P_i} \right|
\]
In equations (3) and (4), $S_r$ is the standard deviation for the sample, $S_{\mu r}$ is the standard deviation for the model estimation, $\hat{P}$ is the egg production estimated by the model (%), and $n$ is the number of experimental data points used in the regression analysis.

3. Results and discussion
The estimated parameters and goodness-of-fit statistics of the egg-production model are shown in Table 1. The model was suitable for representing the egg production for the whole performance curve in all the treatments ($R^2_{\text{adj}} > 0.86$, MRE < 10%).

### Table 1. Estimated parameters and goodness-of-fit statistics of the egg-production model

| Treatment | Lys (%) | Met+Cys (%) | Thr (%) | $\gamma_1$ (%) | $\gamma_2$ (% week$^{-1}$) | $\mu$ (% week$^{-1}$) | $\lambda$ (weeks) | $R^2_{\text{adj}}$ | MRE (%) |
|-----------|---------|-------------|---------|----------------|-----------------------------|-------------------|----------------|--------------------|---------|
| 1         | 0.636   | 0.579       | 0.483   | 64.85          | 0.926                       | 11.32             | 0.204          | 0.942              | 8.29    |
| 2         | 0.636   | 0.579       | 0.760   | 78.54          | 0.215                       | 13.27             | 0.759          | 0.960              | 5.66    |
| 3         | 0.636   | 0.744       | 0.622   | 81.07          | -0.220                      | 16.23             | 0.000          | 0.904              | 6.46    |
| 4         | 0.636   | 0.910       | 0.483   | 79.74          | -0.065                      | 15.43             | 0.001          | 0.902              | 6.71    |
| 5         | 0.636   | 0.910       | 0.760   | 86.88          | -0.416                      | 24.11             | 1.700          | 0.965              | 11.3    |
| 6         | 0.710   | 0.646       | 0.539   | 98.97          | -1.281                      | 13.16             | 1.293          | 0.945              | 9.09    |
| 7         | 0.710   | 0.646       | 0.704   | 83.08          | -0.123                      | 22.42             | 1.786          | 0.967              | 12.2    |
| 8         | 0.710   | 0.843       | 0.539   | 96.27          | -0.863                      | 14.45             | 1.196          | 0.948              | 8.20    |
| 9         | 0.710   | 0.843       | 0.704   | 75.56          | 0.258                       | 15.26             | 0.000          | 0.928              | 5.04    |
| 10        | 0.818   | 0.579       | 0.622   | 72.03          | 0.423                       | 16.94             | 0.006          | 0.918              | 6.09    |
| 11        | 0.818   | 0.744       | 0.483   | 83.35          | -0.359                      | 15.35             | 0.000          | 0.901              | 7.87    |
| 12        | 0.818   | 0.744       | 0.622   | 89.48          | -0.461                      | 15.81             | 0.000          | 0.902              | 8.08    |
| 13        | 0.818   | 0.744       | 0.760   | 79.61          | -0.087                      | 22.91             | 0.006          | 0.920              | 4.76    |
| 14        | 0.818   | 0.910       | 0.622   | 86.52          | -0.454                      | 14.40             | 0.000          | 0.863              | 9.04    |
| 15        | 0.926   | 0.646       | 0.539   | 71.64          | 0.191                       | 16.17             | 0.069          | 0.907              | 6.77    |
| 16        | 0.926   | 0.646       | 0.704   | 66.25          | 0.945                       | 16.37             | 0.076          | 0.907              | 7.25    |
| 17        | 0.926   | 0.843       | 0.539   | 87.75          | -0.376                      | 13.36             | 0.000          | 0.927              | 7.09    |
| 18        | 0.926   | 0.843       | 0.704   | 100.81         | -1.563                      | 12.34             | 0.000          | 0.939              | 7.72    |
| 19        | 1.000   | 0.579       | 0.483   | 83.22          | -0.287                      | 13.69             | 0.696          | 0.938              | 8.00    |
| 20        | 1.000   | 0.579       | 0.760   | 81.99          | -0.118                      | 12.91             | 0.494          | 0.955              | 8.21    |
| 21        | 1.000   | 0.744       | 0.622   | 88.76          | -0.638                      | 13.69             | 0.566          | 0.971              | 6.21    |
| 22        | 1.000   | 0.910       | 0.483   | 91.53          | -0.433                      | 11.38             | 0.477          | 0.955              | 9.26    |
| 23        | 1.000   | 0.910       | 0.760   | 68.44          | 0.887                       | 14.63             | 0.925          | 0.960              | 9.26    |

Each estimated parameter was submitted to a stepwise regression to write it in terms of $L_{\text{ys}}$, $Met+C_{\text{ys}}$ and $Thr$ contents. These expressions were used to fit simultaneously the experimental data of all treatments and obtain a general model corresponding to equations (5) to (8). In this generalized egg-production model are only considered statistically significant terms. The estimated parameters and 95% confidence intervals of the generalized egg-production model are shown in Table 2.
Table 2. Estimated parameters and confidence intervals of the generalized egg-production model

| Parameter | Estimated value | 95% Confidence interval |
|-----------|-----------------|-------------------------|
| $\beta_1$ | 17.704          | 15.909, 19.499          |
| $\beta_2$ | -5.7908         | -8.0382, -3.5433        |
| $\beta_3$ | -133.46         | -251.86, -15.054        |
| $\beta_4$ | 234.85          | 83.342, 386.36          |
| $\beta_5$ | 245.08          | 85.979, 404.19          |
| $\beta_6$ | -260.77         | -463.04, -58.496        |
| $\beta_7$ | 15.466          | 6.2847, 24.648          |
| $\beta_8$ | -17.474         | -29.088, -5.8593        |
| $\beta_9$ | -17.980         | -30.285, -5.6761        |
| $\beta_{10}$ | 19.802           | 4.3157, 35.287          |

The lag time ($\lambda$) did not show statistical significance in the model. On the other hand, the production rate in the growth phase ($\mu$) was influenced only by Lys, while the behavior of the maturity phase was dependent on Lys, Met+Cys and their interactions.

The generalized egg-production model exhibited a satisfactory fit of the experimental data with high adjusted coefficient of determination ($R^2_{adj} = 0.885$), as well as high accuracy of the estimation (MRE = 5.4%). According to the residual analysis, 84.8% of the results were between -7.72% and 10.2%. As it can be seen in Figure 2, the residuals appeared scattered randomly around zero with absolute values less than 21.2%. Thus, corroborate the suitability of the proposed model to accurately predict the egg-production in terms of digestible amino acid requirements.

Figure 2. Residual analysis for the egg-production model

The simulated results for the generalized egg-production model (Figure 3) showed the importance of each of the essential amino acids evaluated for the different stages of the model. In the lag phase, the levels of Lys (0.636%), Met+Cys (0.800%) and Thr (0.483%) are adequate to supply the needs of the commercial laying hen. In the growth phase, production depends only on Lys for the model and the levels of Lys (0.636%), Met+Cys (0.900%) and Thr (0.483%) are adequate to achieve a production above 76%. In the maturity phase, production depends on Met+Cys, and Lys levels and its interactions. Levels of Met+Cys (0.646%), Lys (0.636%) and Thr (0.483%) are suitable for a production of at least 80%.

The estimated optimal level of Lys for egg production for 16 weeks was lower to those recommended by Schneider [11] of 0.79% for semi-heavy laying hens. Likewise, Schmidt et al. [10] found quadratic effect of Lys levels on egg production and estimated the optimal level at 0.681% for semi-heavy laying hens. This value was similar to the level recommended in this study of 0.636% digestible Lys for H&N semi-heavy laying hens. However, Cupertino et al. [19] estimated lower values for digestible Lys of 0.692% on egg production.
There were estimated digestible Met+Cys levels of 0.646% for maximizing the egg production of H&N brown hens during 16 weeks of production. This estimated level was different to estimated values reported by Schmidt et al. [20] and Polesse et al. [12]. These authors recommended values of 0.698% and 0.580% digestible Met+Cys for semi-heavy laying hens to optimize egg production, respectively.

Dietary digestible Thr level of H&N semi-heavy laying hens was estimated at 0.483%. Schmidt et al. [20], who evaluated Thr levels ranged from 0.380% up to 0.512% in semi-heavy laying hen diets, estimated a lower digestible Thr level of 0.467%. However, Agustini et al. [13] recommended a higher Thr level of 0.501% for egg production of semi-heavy laying hens.

Although the first limiting amino acids in laying hen diets have been long studied, there are discrepancies in the estimated Lys, Met+Cys and Thr requirements in second-cycle hen diets from different studies, mainly for dietary Met+Cys and Thr levels. The interactions effects between these amino acids may be an important factor of variation in these estimates as corroborated in this study. Therefore, the study of Lys, Met+Cys and Thr requirements at the same time is an important point to be considered for the establishment of appropriate levels of these amino acids in the diet.

4. Conclusions
The generalized egg-production model successfully predicted the egg production behavior in the lag, growth, and maturity phases. The proposed model showed to be adequate to evaluate the efficiency in the use of digestible amino acids for egg production and can be a useful tool to diagnose the effectiveness of the nutritional requirements in each of the production phases. Also, the model could be applied in other species of animal interest.

Dietary levels of digestible lysine, methionine + cystine, threonine needed to optimize egg production for 16 weeks of H&N semi-heavy laying hens were estimated at 0.636, 0.646 and 0.483 % respectively.

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