

Multivariate modeling strategies to predict nutritional requirements of essential amino acids in semiheavy second-cycle hens

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ABSTRACT - An experiment with 23 diets was performed to evaluate the effect of digestible lysine (Lys), digestible methionine + cysteine (Met+Cys), and digestible threonine (Thr) on egg production of H&N Brown second-cycle laying hens (SCLH) for 20 weeks (92-111 weeks of age) in cages under environmental conditions. Body weight (BW), feed intake (FI), feed conversion ratio (FCR), egg weight (EW), number of hen-housed eggs, and livability were also evaluated during the experiment. Diets were formulated from a central composite design that combined five levels of Lys, Met+Cys, and Thr ranging from 727 to 1159, 662 to 1055, and 552 to 882 mg/kg, respectively. Egg production (EP) data were evaluated through three different modeling strategies: egg production models, multivariate polynomial models, and artificial neural networks (ANN). A cascade-forward neural network with log-sigmoid transfer function was selected as the best model according to goodness-of-fit statistics in both identification and validation data. One of the best scenarios for EP of H&N Brown SCLH under specific outdoor conditions was established at Lys, Met+Cys, and Thr levels of 1138, 1031, and 717 mg/hen-day, respectively. The ANN model may be an appropriate tool to study and predict EP of H&N Brown SCLH based on the combination of three different levels of essential digestible amino acids. The strategies included in this work may contribute to improving poultry performance based on modeling techniques to study other production parameters in terms of different nutritional requirements and productive conditions.

Keywords: bird nutrition, egg laying, mathematical model, multivariate analysis, nonlinear model, poultry

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1. Introduction

Molting is a natural period of metabolic and physiological changes in birds with seasonal reproduction, but can also be induced in poultry to rejuvenate laying hens for a second or third production cycle, resulting in higher egg production (EP), egg weight (EW), and egg quality parameters (Andreatti Filho et al., 2019; Wolc et al., 2020). Molting is induced in the poultry sector to reduce production costs, generating a second additional productive cycle without the need to replace birds or introduce new flocks (Morales et al., 2018b).

Amino acids play critical roles in most of the physiological responses of birds. The interaction among amino acids should be included in mathematical prediction models to explore their optimal levels in diets (Mehri, 2014).

The dose-response methodology is the most widely used methodology to establish the optimal levels of amino acids in experimental trials, in which a single amino acid is evaluated at a time, and the possible complex interactions between amino acids are overlooked. The limitations of this conventional “one factor at a time” approach can be overcome by the use of multivariate modeling methods such as those based on machine learning (Mehri, 2014; Faridi et al., 2016).

One of the most common techniques for implementing machine learning is to model a system using an artificial neural network (ANN). An ANN can learn the patterns of data from highly nonlinear systems, thus capturing more complex relationships among variables and providing better predictions and generalizations than linear regression models, which are used the most in poultry nutrition (Felipe et al., 2015).

Amino acid requirements for second-cycle laying hens (SCLH) have been estimated using the dose-response methodology. However, the requirements of these three primary essential amino acids (EAA) have not been estimated for more than one amino acid simultaneously in SCLH, ignoring the interactions that can occur between them. Additionally, the amino acid requirements are unknown for some genetic lines under specific environmental conditions, and this information is necessary to enhance the physiological status of birds, ensure the regeneration of their tissues after molting and improve production (Morales et al., 2018a).

In this work, different multivariate modeling approaches were applied to study the effects of the three primary EAA—lysine (Lys), methionine + cysteine (Met+Cys), and threonine (Thr)—on the egg production (EP) of H&N Brown SCLH during a production cycle from 92 to 112 weeks. The model with the best predictive ability and best statistical results on regression analysis was used to simulate EP and establish the relative importance of Lys, Met+Cys, and Thr on SCLH performance under specific environmental conditions.

2. Material and Methods

2.1. Animal care

The local bioethics committee (Act 03/2017) approved the experimental protocols for this research.

2.2. Fieldwork and experimental information

The fieldwork was carried out in San Pedro, Antioquia, Colombia (6°27'34" N latitude and 75°33'28" W longitude, 2475 m asl), with annual average temperature of 14 °C and relative humidity of 79%. A total of 1380 92-week-old H&N Brown SCLH were housed for 20 weeks (111 weeks old) in a caged-layer house of commercial design (type Californian cages) under environmental production conditions. Each cage allowed 576 cm²/hen with a PVC side feeder to supply 12 cm²/hen of feeder space and two nipple drinkers. During molting (12 days) and resting (30 days) periods, a lighting program of 12 h of light per day was adopted. In the postmolting period (140 days, 92-111 weeks old), the lighting program was 16 h of light per day.

2.3. Experimental design

Concentrations of three digestible EAA in the diet (Lys, Met+Cys, and Thr) were combined using a central composite design. A design matrix with 23 treatments was determined (Table 1) combining five levels of Lys, Met+Cys, and Thr between 0.636 and 1.000, 0.579 and 0.910, and 0.483 and 0.760%, respectively. Five replicates were performed per treatment in experimental units of 12 birds. A total of 2300 records (23 treatments × five replicates × 20 weeks) were added during the fieldwork. The EAA ranges in the experimental design were established to include the levels for second-cycle hens in commercial genetic lines available in the literature (Schmidt et al., 2009a, 2009b, 2010; Schneider, 2011;

Table 1 - Experimental design of the digestible essential amino acids in the diets

Treatment/diet	Lysine (%)	Methionine + cysteine (%)	Threonine (%)
1	0.636	0.579	0.483
2	0.636	0.579	0.760
3	0.636	0.744	0.622
4	0.636	0.910	0.483
5	0.636	0.910	0.760
6	0.710	0.646	0.539
7	0.710	0.646	0.704
8	0.710	0.843	0.539
9	0.710	0.843	0.704
10	0.818	0.579	0.622
11	0.818	0.744	0.483
12	0.818	0.744	0.622
13	0.818	0.744	0.760
14	0.818	0.910	0.622
15	0.926	0.646	0.539
16	0.926	0.646	0.704
17	0.926	0.843	0.539
18	0.926	0.843	0.704
19	1.000	0.579	0.483
20	1.000	0.579	0.760
21	1.000	0.744	0.622
22	1.000	0.910	0.483
23	1.000	0.910	0.760

Polese et al., 2012; Agustini et al., 2014; Hy-Line, 2014) and levels recommended by Rostagno et al. (2011) and Rostagno et al. (2017) for laying-hen brown eggs with medium-superior performance in the first production cycle.

A basal diet was formulated based on corn and soybean meal according to the nutritional recommendations for laying hens proposed by Rostagno et al. (2011), except for digestible Lys, Met+Cys, and Thr. The 23 experimental diets were obtained by supplementing the basal diet with Lys, Met, and Thr as replacements for the inert filler bentonite (Table 2). The diets were prepared weekly.

2.4. Production parameters

Egg production was calculated weekly for 20 weeks (92-111 weeks old) from the relation between number of eggs and number of birds per experimental unit. Feed intake (FI), egg weight (EW), feed conversion ratio (FCR), hen-housed eggs, and livability were registered daily. Feed intake was recorded and calculated as grams of feed consumed over seven days, divided by the number of birds, and adjusted for mortalities. Body weight (BW) was registered weekly. Feed conversion ratio was calculated weekly and expressed in two ways: as kilogram of feed consumed divided by kilogram of egg produced and as kilogram of feed consumed divided by dozens of eggs produced. Number of hen-housed eggs was calculated from the cumulative number of eggs divided by the number of hens that started to produce (hen housed).

Amino acid intake in milligrams/hen-day of Lys (iLys), Met+Cys (iMetCys), and Thr (iThr) were calculated for each treatment as the product of the average feed intake and EAA concentration.

Table 2 - Ingredients and nutrient composition of the experimental diets (92-111 weeks old of age, as-fed basis)

Item	(g/kg)
Ingredient	
Corn (7.7% crude protein)	651
Soybean meal (46.2% crude protein)	166
Palm oil	22.2
Corn gluten (60% crude protein)	20
Bentonite	10.59
Calcium carbonate	102
Monocalcium phosphate	14.34
Choline chloride	0.5
Salt	2.5
Vitamin premix ¹	0.5
Mineral premix ²	0.5
Lysine	0.71
Methionine	1.22
Tryptophan	0.98
Arginine	2.05
Sodium bicarbonate	1.5
Valine	3.35
Analyzed composition	
Metabolizable energy (MJ/kg)	11.97
Protein	155
Calcium	42
Available phosphorus	4
Fiber	23.1
Sodium	1.7
Chlorine	1.7
Potassium	5.5
Lysine	6.36
Methionine + cysteine	9.1
Threonine	7.6
Valine	9.5
Arginine	10
Leucine	13.52
Isoleucine	5.48
Histidine	3.51
Phenylalanine	6.79
Tryptophan	2.3

¹ Nutrients per kg of vitamin premix: vitamin A, 20,000,000 IU; vitamin D3, 5,000,000 IU.; vitamin E, 20 g; vitamin K, 6 g; thiamine 4 g; riboflavin, 10 g; pyridoxine, 4 g; vitamin B12, 0.03 g; biotin, 0.04 g; pantothenic acid, 18 g; niacin, 40 g.

² Nutrients per kg of mineral premix: zinc, 70 g; manganese, 65 g; iron, 20 g; copper, 5 g; iodine, 1200 mg; selenium, 300 mg; choline chloride 60%, 500 g.

2.5. Mathematical modeling of egg production

Three approaches were used to model EP of SCLH as a function of EAA intake (iLys, iMetCys, and iThr) and time: nonlinear models frequently used to describe EP curves, multivariate polynomial models, and ANN (Figure 1).

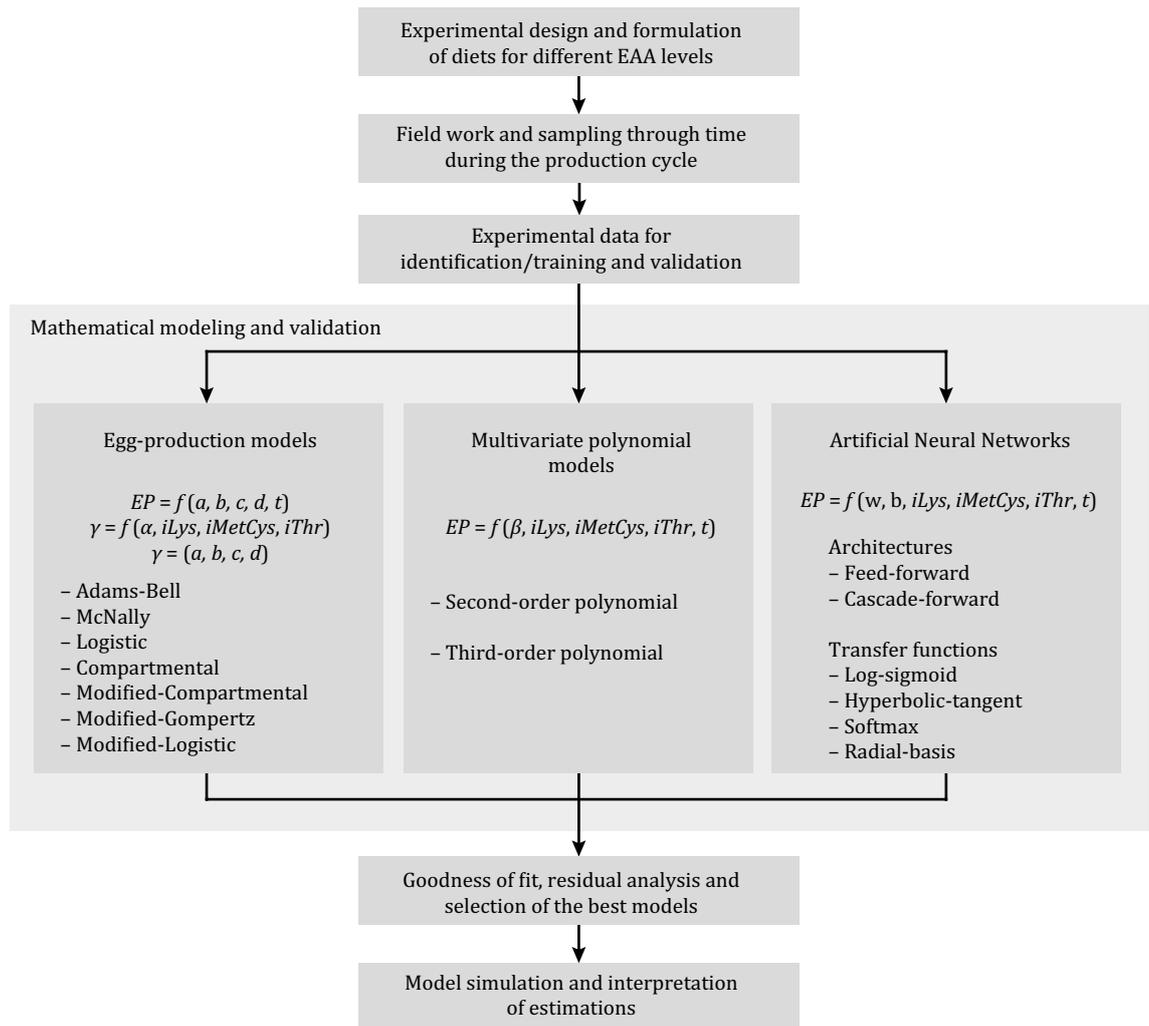


Figure 1 - Models used to represent the dependence of egg production (EP) on essential amino acid (EAA) intake (iLys, iMetCys, and iThr) and time (t) in H&N Brown second-cycle laying hens.

2.6. Nonlinear egg production models

Egg-laying production (in %) was modeled using the empirical models of Adams-Bell (Eq. 1) (Faridi et al., 2011), McNally (Eq. 2), logistic (Eq. 3), compartmental (Eq. 4) (Savegnago et al., 2012), and modified-compartmental (Eq. 5) (Otwinowska-Mindur et al., 2016) (also called logistic-curvilinear model). These models are frequently referenced in the literature and were selected because of their suitability to represent only four parameters of the biological behavior in the production curve (Savegnago et al., 2012; Otwinowska-Mindur et al., 2016).

$$EP = 100 \left(\frac{1}{1 + ab^t} - ct + d \right) \quad (1)$$

$$EP = at^b e^{(-ct + d\sqrt{t})} \quad (2)$$

$$EP = ae^{-bt} [1 + e^{-ct}]^{-d} \quad (3)$$

$$EP = ae^{-bt} [1 - e^{-c(t-d)}] \quad (4)$$

$$EP = \frac{ae^{-bt}}{1 + e^{-c(t-d)}} \quad (5)$$

in which EP is egg production (%), t is time (weeks) during the second production cycle and a , b , c , and d are model parameters.

In addition, Modified-Gompertz (Eq. 6) and modified logistic (Eq. 7) models were proposed from the formulation presented by Morales et al. (2018a), in which a time-dependent linear function replaces the asymptotic parameter of these growing equations.

$$EP = (a + bt) e^{\{-e^{[d(c-t)/(a+bt) + 1]}\}} \quad (6)$$

$$EP = \frac{(a + bt)}{1 + e^{[4d(c-t)/(a+bt) + 2]}} \quad (7)$$

These sigmoidal models capture the behavior of the production curve, and their parameters also offer biological interpretation: c is the initial lag time (week), d is the maximum growth rate (%/week), and $(a + bt)$ is the function used to represent the behavior of the egg-laying trend after the maximum production.

In each EP model (Eqs. 1-7), parameters a , b , c , and d were written in terms of EAA intake (iLys, iMetCys, and iThr) according to the procedure described by Morales et al. (2018a). First, a given EP model was fitted separately for each treatment to identify the parameters (a , b , c , and d). Then, the values of each parameter for all treatments, which include all the EAA interactions defined from the central composite design, were assessed using stepwise regression to establish the dependence of EAA intake on each model parameter using second-order polynomial functions (Eq. 8).

$$\gamma = \alpha_0 + \sum_{j=1}^3 \alpha_j x_j + \sum_{j=1}^2 \sum_{k>j}^3 \alpha_{jk} x_j x_k + \sum_{j=1}^3 \alpha_{jj} x_j^2 \quad (8)$$

in which γ represents the model parameter (a , b , c , and d), x represents the EAA intake (iLys, iMetCys, and iThr), and α_0 , α_j , α_{jj} , and α_{jk} are coefficients of the parameter function of independent, linear, quadratic, and cross-product terms, respectively.

Thus, each EP model (Eqs. 1-7) and its parameter functions (Eq. 8) established the equation system used to validate and simulate EP under the studied conditions.

2.7. Multivariate polynomial models

Stepwise regression was also used to assess both multivariate second-order (Eq. 9) and third-order (Eq. 10) polynomial models in terms of EAA intake and time. Through this technique, models that include only statistically significant terms (linear, quadratic, cubic, and cross-product) at a 95% confidence level were defined (Ameer et al., 2017).

$$EP = \beta_0 + \sum_{j=1}^4 \beta_j x_j + \sum_{j=1}^3 \sum_{k>j}^4 \beta_{jk} x_j x_k + \sum_{j=1}^4 \beta_{jj} x_j^2 \quad (9)$$

$$EP = \beta_0 + \sum_{j=1}^4 \beta_j x_j + \sum_{j=1}^3 \sum_{k>j}^4 \beta_{jk} x_j x_k + \sum_{j=1}^4 \beta_{jj} x_j^2 + \sum_{j=1}^2 \sum_{k>j}^3 \sum_{l>k}^4 \beta_{jkl} x_j x_k x_l + \sum_{j=1}^4 \sum_{k \neq j}^4 \beta_{jkk} x_j x_k^2 + \sum_{j=1}^4 \beta_{jjj} x_j^3 \quad (10)$$

in which x represents the independent variables (iLys, iMetCys, iThr, t) and β_0 , β_j , β_{jj} , β_{jjj} , β_{jk} , β_{jkl} , and β_{jkk} are the model parameters of independent, linear, quadratic, cubic, and cross-product terms, respectively.

2.8. Artificial Neural Networks

The ANN approach was also used to describe the relationship of EP with the four studied factors (iLys, iMetCys, iThr, and t) using two network architectures: feed-forward (Eq. 11), and cascade-forward (Eq. 12). As transfer functions, log-sigmoid (Eq. 13), hyperbolic tangent (Eq. 14), softmax (Eq. 15), and radial-basis (Eq. 16) equations were used in these architectures. Between five and eleven neurons in one hidden layer for each combination of network architecture and transfer function were assessed.

$$EP = w_{ho} \times f + b_o \quad (11)$$

$$EP = w_{ho} \times f + w_{io} \times x + b_o \quad (12)$$

$$f = \frac{1}{1 + e^{-(w_{ih} \times x + b_h)}} \quad (13)$$

$$f = \frac{2}{1 + e^{[-2(w_{ih} \times x + b_h)]}} - 1 \quad (14)$$

$$f = \frac{e^{(w_{ih} \times x + b_h)}}{\sum e^{(w_{ih} \times x + b_h)}} \quad (15)$$

$$f = e^{[-(w_{ih} \times x + b_h)^2]} \quad (16)$$

In Eqs. 11-16, f is the transfer function, w_{ih} is the weight matrix from the input layer to the hidden layer, b_h is the bias vector of the hidden layer, w_{ho} is the weight matrix from the hidden layer to the output layer, w_{io} is the weight matrix from the input to the output layer (only in the case of the cascade-forward network architecture), b_o is the bias vector of the output layer, and x is the matrix of the input variables (iLys, iMetCys, iThr, t).

2.9. Identification, validation, and statistical analysis

The experimental data were separated into two datasets: the identification dataset, corresponding to three replicates per treatment (1380 records), and the validation dataset, corresponding to the remaining two replicates (920 records).

The identification procedure of parameters for the nonlinear EP models (Eqs. 1-7) was carried out using the “fitnlm” and “stepwisefit” functions of the Statistics and Machine Learning Toolbox of MATLAB® R2019a (The MathWorks Inc., Natick, MA, USA). Specifically, fitnlm uses a Levenberg-Marquardt nonlinear least-squares algorithm to identify the coefficients of nonlinear and multivariate models, whereas stepwisefit uses an iterative method to perform stepwise regression and to identify statistically significant ($P < 0.05$) coefficients of multivariate and additive models. In multivariate polynomial models (Eqs. 9-10), the stepwisefit function was also used to identify statistically significant parameters. In the case of the ANN models, the “trainbr” function of the Deep Learning Toolbox in MATLAB, which uses a Bayesian regularization backpropagation algorithm, was used for network training to prevent overfitting problems (Daza et al., 2018).

The adjusted coefficient of determination (R^2_{adj}) (Eq. 17), root mean square error (RMSE) (Eq. 18), and the bias (Eq. 19) were used to evaluate the goodness of fit, accuracy, and deviation of the models, respectively. Akaike’s information criterion (AIC) (Eq. 20) was used to compare and measure the model quality, in which the most accurate model has the smallest AIC (Savegnago et al., 2012). Moreover, the selected models were subjected to a residual analysis to assess the adequacy of the estimations.

$$R^2_{adj} = 1 - S_{yx}^2/S_y^2 \quad (17)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (EP_i^* - EP_i)^2}{n}} \quad (18)$$

$$bias = \frac{1}{n} \sum_{i=1}^n (EP_i^* - EP_i) \quad (19)$$

$$AIC = n \log \left[\det \left(\frac{1}{n} \sum_{i=1}^n \varepsilon \varepsilon^T \right) \right] + 2n_p + n[\log(2\pi) + 1] \quad (20)$$

in which S_y is the standard deviation associated with the sample; S_{yx} is the standard deviation associated with model estimations; EP_i^* represents experimental values; EP_i represents estimated values; n is the number of experimental data points used for model training and validation; ε is the vector of prediction errors; and n_p is the number of estimated parameters. The best models for the ANN approaches were selected based on the highest R^2_{adj} and the lowest RMSE. For ANN models, this selection was subsequently based on the lowest number of hidden-layer neurons and the most straightforward network architecture. R^2_{adj} was also used to evaluate the success of the model by adjusting the coefficient of determination according to the number of biases and weights (Daza et al., 2018).

3. Results

3.1. Production parameters

The amino acid levels ranged from 675 to 1207 mg/hen-day of digestible Lys, 627 to 1129 mg/hen-day of digestible Met+Cys, and digestible Thr from 515 to 943 mg/hen-day (Table 3). The analysis of the productive behavior of the treatments indicated a mean production level of 52.2% for the first four weeks (92-95 weeks old) of the experimental period, ranging from 35.0 to 72.1% (Table 3). Production peaks were achieved starting at seven weeks (98 weeks old), ranging from 81.0% at 105 weeks old for diet 1 to 91.2% at 98 weeks old for diet 5. Egg production at 20 weeks (111 weeks old) was between 75.9 (diet 20) and 85.3% (diet 23).

Hen-housed eggs were between 91.8 and 105.1, which indicated that the treatment yielding the greatest production for 20 weeks (111 weeks old) was diet 13, which yielded 5.25 hen-housed eggs/hen-week (Table 4). Feed intake was similar among diets, between 114.3 and 116.7 g/hen-day, which was lower than that reported by the Hy-Line Brown management guide (Hy-Line, 2018) for SCLH during 20 weeks of production, which registered 106.1 g/hen-day. Diet 23 showed the lowest FCR for 20 weeks (111 weeks old) with 2.02 kg feed/kg egg, which was equivalent to 1.62 kg feed/dozen eggs. At the end of the 20 weeks of production, mean BW was between 1987 and 2043 g/hen. The average EW for 20

Table 3 - Essential amino acid intake and egg production of H&N Brown second-cycle laying hens for the diets over 20 weeks

Diet	Essential amino acids intake (mg/hen-day)			Egg production (%)				
	Lysine	Methionine + cysteine	Threonine	Week 4 (95 weeks old)	Week 8 (99 weeks old)	Week 12 (103 weeks old)	Week 16 (107 weeks old)	Week 20 (111 weeks old)
1	727.0±10.0	661.9±9.1	552.1±7.6	50.2±2.3	66.4±2.1	77.6±10.0	75.7±2.5	78.3±7.7
2	732.4±9.6	666.8±8.8	875.2±11.5	48.6±2.7	67.1±3.1	85.0±6.2	83.5±8.3	80.5±4.0
3	728.4±18.0	852.1±21.0	712.4±17.6	66.8±3.7	76.3±3.3	88.7±6.8	81.7±4.4	80.6±4.8
4	728.5±17.2	1042.3±24.6	553.2±13.0	63.1±2.8	78.1±3.5	82.2±9.2	81.0±5.0	79.7±3.7
5	736.3±18.6	1053.5±26.7	879.8±22.3	55.9±6.3	90.9±5.2	81.5±6.1	86.1±5.2	77.8±4.4
6	809.6±11.3	736.6±10.3	614.6±8.6	35.0±3.7	75.2±2.6	83.6±8.6	80.0±3.2	77.1±7.8
7	812.7±18.0	739.5±16.4	805.8±17.9	49.3±4.3	79.0±2.8	89.1±7.5	83.1±8.5	84.0±3.9
8	828.4±22.7	982.6±26.9	628.9±17.2	42.2±2.7	82.6±4.1	86.3±4.7	80.3±5.4	81.5±6.7
9	810.2±11.3	961.9±13.4	803.3±11.2	60.5±4.2	73.1±3.6	90.0±5.0	79.8±6.7	77.9±4.0
10	951.7±17.8	673.7±12.6	723.7±13.6	56.7±5.2	76.3±4.1	76.6±6.4	82.9±7.0	81.4±3.5
11	947.4±19.0	861.7±17.3	559.4±11.2	48.3±2.9	84.7±2.4	82.1±9.3	84.3±4.1	80.7±2.6
12	938.4±13.6	853.6±12.4	713.6±10.3	55.7±5.0	85.0±2.9	84.8±4.2	80.2±6.2	78.8±6.0
13	943.7±12.2	858.3±11.1	876.8±11.3	72.1±4.7	80.9±5.1	81.2±9.1	77.1±6.5	80.0±4.9
14	935.1±12.9	1040.2±14.3	711.0±9.8	48.1±2.2	79.1±2.2	84.5±5.9	76.7±2.8	78.1±3.6
15	1076.9±23.4	751.2±16.3	626.8±13.6	67.1±6.8	77.4±3.4	77.9±8.1	80.7±3.9	81.3±5.3
16	1066.3±15.7	743.9±10.9	810.7±11.9	56.2±2.3	73.1±2.6	77.4±6.7	76.4±5.9	79.6±3.5
17	1058.8±14.6	963.9±19.5	616.3±12.5	51.4±3.1	74.8±2.9	85.2±6.2	80.7±3.9	83.9±5.1
18	1058.5±14.6	963.6±13.3	804.7±11.1	50.2±2.9	77.6±3.6	81.2±3.6	71.9±4.0	79.8±6.8
19	1164.7±30.3	674.3±17.7	562.5±14.7	45.5±2.1	73.6±3.4	77.6±3.2	81.0±4.5	83.1±4.9
20	1143.1±15.8	661.9±9.1	868.8±12.0	43.1±5.5	73.3±3.9	77.9±2.8	78.8±3.1	75.9±3.9
21	1154.2±18.4	858.7±13.7	771.9±11.4	52.2±2.6	75.2±4.7	76.9±6.0	80.2±2.7	81.4±4.6
22	1153.3±20.7	1049.5±18.8	557.0±10.0	40.7±2.0	74.8±4.5	80.3±5.6	83.3±2.8	79.3±6.7
23	1159.5±16.9	1055.1±15.4	881.2±12.3	46.7±2.0	73.6±3.6	78.8±2.1	83.6±4.1	85.3±3.5

Values are expressed as the means ± standard deviations (n = 100 for essential amino acid intake values, n = 5 for egg production values).

Table 4 - Production parameters of H&N Brown second-cycle laying hens for the diets at 20 weeks of production (111 weeks old)

Diet	Hen-housed eggs (egg)	Feed intake (g/hen-day)	FCR (kg feed/kg egg)	FCR (kg feed/dozen egg)	Body weight (g/hen)	Egg weight (g/egg)	Livability (%)
1	91.8±3.3	114.3±1.6	2.23±0.24	1.77±0.17	2027±67	68.00±2.73	100.0
2	92.2±1.9	115.1±1.5	2.20±0.03	1.71±0.08	2024±93	66.95±2.00	98.3
3	98.9±3.5	114.5±2.8	2.36±0.23	1.71±0.10	2019±72	66.37±2.95	93.3
4	99.7±3.8	114.5±2.7	2.27±0.18	1.73±0.08	2013±91	67.03±2.29	96.7
5	100.3±2.6	115.8±2.9	2.23±0.14	1.77±0.10	2042±67	68.21±2.87	98.3
6	92.6±2.5	114.0±1.6	2.19±0.27	1.80±0.18	1987±47	68.04±3.08	100.0
7	97.9±3.2	114.5±2.5	2.10±0.13	1.64±0.07	2043±86	68.69±2.81	95.0
8	96.8±2.1	116.7±3.2	2.21±0.12	1.70±0.14	2024±65	67.79±2.40	95.0
9	98.5±3.2	114.1±1.6	2.21±0.14	1.77±0.09	2022±76	67.39±2.99	100.0
10	94.4±6.9	116.3±2.2	2.12±0.09	1.69±0.07	2011±51	67.52±3.09	96.7
11	97.7±2.7	115.8±2.3	2.22±0.09	1.71±0.05	2024±73	67.35±3.20	95.0
12	102.8±2.4	114.7±1.7	2.19±0.14	1.75±0.13	2012±58	67.17±2.41	100.0
13	105.1±4.2	115.4±1.5	2.16±0.14	1.73±0.10	2001±67	68.30±2.32	98.3
14	99.4±1.8	114.3±1.6	2.18±0.16	1.77±0.08	1999±62	68.21±2.13	100.0
15	98.9±4.8	116.3±2.5	2.19±0.27	1.70±0.11	2027±86	68.66±2.68	96.7
16	97.4±2.1	115.1±1.7	2.19±0.15	1.74±0.07	2018±72	67.71±2.30	98.3
17	100.7±1.6	114.3±2.3	2.06±0.06	1.64±0.09	2026±77	68.31±2.50	98.3
18	95.9±2.2	114.3±1.6	2.04±0.13	1.73±0.14	2020±70	69.05±2.88	100.0
19	94.7±1.8	116.5±3.0	2.07±0.09	1.66±0.09	2027±73	68.68±2.50	96.7
20	93.9±3.8	114.3±1.6	2.14±0.15	1.82±0.09	2011±59	68.21±3.01	100.0
21	95.5±4.4	114.5±1.8	2.12±0.08	1.69±0.09	2013±54	67.96±2.83	98.3
22	95.6±3.7	115.3±2.1	2.25±0.18	1.75±0.15	2004±49	67.10±2.47	98.3
23	93.7±1.3	116.0±1.7	2.02±0.11	1.62±0.06	2022±64	68.74±2.68	98.3

FCR - feed conversion ratio.

Values are expressed as the means ± standard deviations (n = 5).

weeks ranged between 66.37 and 69.05 g/egg, mainly very large and extra-large sizes. Viability was higher than 93.3% for all treatments, with seven treatments (diets 1, 6, 9, 12, 14, 18, and 20) that were not associated with any mortality.

Between 88.6 and 99.3% of the eggs were larger than 63 g (Table 5), according to the Colombian standard (Icontec, 2011). Treatments showed an average EW between 66.95 and 69.05 g. These results are higher than those recorded for Hy-Line Brown commercial layers (Hy-Line, 2018) at 20 weeks (111 weeks old) of production (65.5 g) and those reported for SCLH of the Isa Brown breed during 17 weeks of production (Morales et al., 2018b).

3.2. Curve fitting and statistical criteria

Three different approaches to model EP were evaluated (Table 6). In the first strategy, named egg production models, the best choice was the modified logistic (Eq. 7) with R^2_{adj} values of 0.854 and 0.850 and RMSE values of 7.42 and 7.49 for the training and validation datasets, respectively. The identified parameters a , b , c , and d of the modified logistic model (Eq. 7) are shown in Eqs. 21-24.

$$a = 36.81 + 0.1141 iMetCys + 0.02553 iThr - 2.769 \times 10^{-5} iLys iThr - 6.581 \times 10^{-5} iMetCys^2 \quad (21)$$

$$b = 1.676 - 0.006148 iMetCys + 1.261 \times 10^{-6} iLys iMetCys + 3.054 \times 10^{-6} iMetCys^2 \quad (22)$$

Table 5 - Distribution (%) of egg weight per diet for H&N Brown second-cycle laying hens over 20 weeks of production (111 weeks old)

Treatment	Weight range (g) ¹					
	>78	67.0-77.9	60.0-66.9	53.0-59.9	46.0-52.9	<46.0
1	3.3	53.3	36.7	6.7	0.0	0.0
2	0.7	44.0	47.3	8.0	0.0	0.0
3	1.3	40.0	47.3	10.7	0.0	0.0
4	2.7	49.3	38.0	10.0	0.0	0.0
5	1.3	52.0	42.0	4.7	0.0	0.0
6	0.7	56.7	40.7	2.0	0.0	0.0
7	4.7	52.7	34.7	6.7	0.0	0.0
8	6.7	52.7	34.7	6.0	0.0	0.0
9	3.3	50.0	38.7	8.0	0.0	0.0
10	4.0	50.0	42.0	3.3	0.7	0.0
11	5.3	53.3	30.0	11.3	0.0	0.0
12	4.7	45.3	40.0	8.7	0.7	0.0
13	2.0	53.3	38.7	4.7	0.7	0.0
14	5.3	58.7	31.3	4.7	0.0	0.0
15	7.3	53.3	36.0	2.7	0.0	0.0
16	3.3	44.7	43.3	8.7	0.0	0.0
17	2.0	53.3	39.3	5.3	0.0	0.0
18	4.0	54.0	41.3	0.7	0.0	0.0
19	12.0	55.3	28.7	3.3	0.7	0.0
20	7.3	52.0	36.0	4.0	0.0	0.0
21	5.3	59.3	31.3	4.0	0.0	0.0
22	0.0	44.7	43.3	10.7	0.7	0.0
23	4.0	56.0	34.0	5.3	0.7	0.0

¹ According to the standard of Colombia (Icontec, 2011).

$$c = 6.129 - 0.02916 \text{ iMetCys} + 0.009809 \text{ iThr} + 8.349 \times 10^{-6} \text{ iLys iMetCys} - 8.676 \times 10^{-6} \text{ iLys iThr} + 1.415 \times 10^{-5} \text{ iMetCys}^2 \quad (23)$$

$$d = -3.086 + 0.0147 \text{ iLys} - 1.749 \times 10^{-5} \text{ iLys iThr} + 2.911 \times 10^{-5} \text{ iMetCys iThr} - 4.561 \times 10^{-6} \text{ iMetCys}^2 \quad (24)$$

Regarding the second modeling approach, a third-order polynomial model presented the best goodness of fit with R^2_{adj} values of 0.845 and 0.830 and RMSE values of 7.78 and 7.92 for the training and validation datasets, respectively. This model is presented in Eq. (25), in which $x_1, x_2, x_3,$ and x_4 represent the independent variables iLys, iMetCys, iThr, and t, respectively.

$$EP = -250.33 - 5.5869x_1 - 4.3868x_2 + 13.354x_3 + 27.024x_4 + 0.0074275x_1x_2 - 0.010294x_2x_3 - 0.018796x_4x_1 - 0.023105x_4x_2 + 0.022148x_4x_3 + 0.0023147x_1^2 + 0.0058843x_2^2 - 0.012393x_3^2 - 1.1652x_4^2 + 0.00030037x_1^2x_2 - 0.00036273x_1^2x_3 + 8.2591 \times 10^{-6} x_1^2x_4 - 0.00033428x_2^2x_1 + 0.00043837x_2^2x_3 + 1.1326 \times 10^{-5} x_2^2x_4 + 0.00047762x_3^2x_1 - 0.00051777x_3^2x_2 - 8.9556 \times 10^{-6} x_3^2x_4 - 0.00013789x_4^2x_3 - 2.7959 \times 10^{-7} x_1x_2x_3 + 6.8502 \times 10^{-6} x_1x_2x_4 - 3.6839 \times 10^{-6} x_2x_3x_4 - 3.4307 \times 10^{-6} x_1x_3x_4 - 2.0089 \times 10^{-6} x_2^3 + 2.8749 \times 10^{-6} x_3^3 + 0.028637x_4^3 \quad (25)$$

The best results using the third approach, ANN strategy, were attained using a cascade-forward architecture with a log-sigmoid transfer function and 11 neurons in the hidden layer, resulting in R^2_{adj} values of 0.900 and 0.892 and RMSE values of 4.69 and 4.86 for the training and validation datasets, respectively.

Table 6 - Results of modeling approaches for egg production of H&N Brown second-cycle laying hens (92-111 weeks old)

Modeling approach	Model detail	Training/ Identification		Validation		P-value ¹	Bias	AIC
		R ² _{adj}	RMSE	R ² _{adj}	RMSE			
Egg production models								
Adams Bell	Number of significant parameters: 18	0.853	7.45	0.847	7.57	0.001	-7.91×10 ⁻⁷	15928
McNally	Number of significant parameters: 18	0.811	8.67	0.808	8.69	0.001	0.4870	16220
Logistic	Number of significant parameters: 17	0.845	7.66	0.841	7.71	0.008	0.0366	15973
Compartmental	Number of significant parameters: 26	0.840	7.75	0.839	7.73	0.001	-0.0145	16008
Modified Compartmental	Number of significant parameters: 18	0.852	7.47	0.848	7.53	0.002	0.0342	15927
Modified Gompertz	Number of significant parameters: 17	0.849	7.57	0.843	7.66	0.002	0.0425	15954
Modified Logistic	Number of significant parameters: 20	0.854	7.42	0.850	7.49	0.001	0.0481	15919
Multivariate polynomial models								
Second order	Number of significant parameters: 7	0.780	9.22	0.769	9.31	0.001	-0.0707	16341
Third order	Number of significant parameters: 31	0.845	7.78	0.830	7.92	0.001	-0.5759	16046
Artificial neural networks								
Feed-forward architecture	Transfer function: radial-basis Number of neurons (hidden layer): 11 Number of parameters: 67	0.895	6.20	0.887	6.32	0.008	1.12×10 ⁻⁴	15662
Cascade-forward architecture	Transfer function: log-sigmoid Number of neurons (hidden layer): 11 Number of parameters: 71	0.900	4.69	0.892	4.86	0.015	-2.10×10 ⁻⁵	15620

RMSE - root mean square error; R²_{adj} - adjusted coefficient of determination; AIC - Akaike's information criterion.

¹ P-values >0.05 indicate that the Lilliefors test does not reject the null hypothesis that the residuals come from a normal distribution at the 1% significance level.

Among the best results of the three modeling strategies evaluated, the ANN model showed the best fitting result since it presented the highest R²_{adj}, the lowest MSE, and the lowest AIC values. In addition, the ANN model allows behavior prediction of the EP curve using one of the most straightforward network architectures (Eqs. 12 and 13), which can facilitate subsequent multiobjective optimization and sensitivity analysis. The identified parameters (biases and weights) of the selected ANN model are shown in Table 7.

Concerning all modeling strategies, residuals were dispersed around zero, followed by a normal distribution according to the Lilliefors test (Figure 2). Additionally, the best modeling approaches showed absolute biases lower than 0.08 (Table 6); therefore, they can be considered accurate since they overestimate and underestimate the data equally. When verifying the capacity of ANN models to predict EP, 88.14% of the residuals were between -10 and 10, whereas the modified logistic and third-order polynomial models showed 80.87 and 80.97% of the residues between -10 and 10%, respectively (Figure 2).

Table 7 - Weights and biases from the selected ANN model for egg production of H&N Brown second-cycle laying hens (92-111 weeks old)

W_{ih}				W_{ho}	W_{io}	b_h	b_o
9.2381	1.1542	-1.7018	4.6005	$\begin{bmatrix} -4.5756 \\ -2.1218 \\ -2.0654 \\ -0.133 \\ -0.1298 \\ 1.5801 \\ 5.0379 \\ -3.8859 \\ -0.635 \\ -1.5998 \\ 8.9788 \end{bmatrix}^T$	$[0.0126 \quad 0.1086 \quad -0.3881]$	$\begin{bmatrix} 9.9986 \\ 6.7062 \\ -7.4069 \\ 0.1626 \\ 2.4566 \\ -3.6089 \\ 4.4589 \\ 7.8672 \\ -14.812 \\ -4.0081 \\ 7.7957 \end{bmatrix}$	$[-2.6826]$
-5.5821	-2.9141	11.493	-1.3054				
5.661	2.7632	-12.864	1.5551				
6.3384	4.9504	-7.9754	7.5813				
7.6652	11.656	9.4313	-4.1746				
2.894	10.943	0.0596	-9.8117				
-0.6076	0.0121	-0.0756	2.5017				
6.7473	1.4967	3.4158	5.3376				
0.3955	0.7214	-0.4114	-21.833				
2.5676	11.559	0.1501	-10.039				
6.7739	1.2300	2.0901	4.3793				

ANN - artificial neural networks; W_{ih} - weight matrix from the input to the hidden layer; W_{ho} - weight matrix from the hidden layer to the output layer; W_{io} - weight matrix from the input to the output layer; b_h - bias vector of the hidden layer; b_o - bias vector of the output layer.

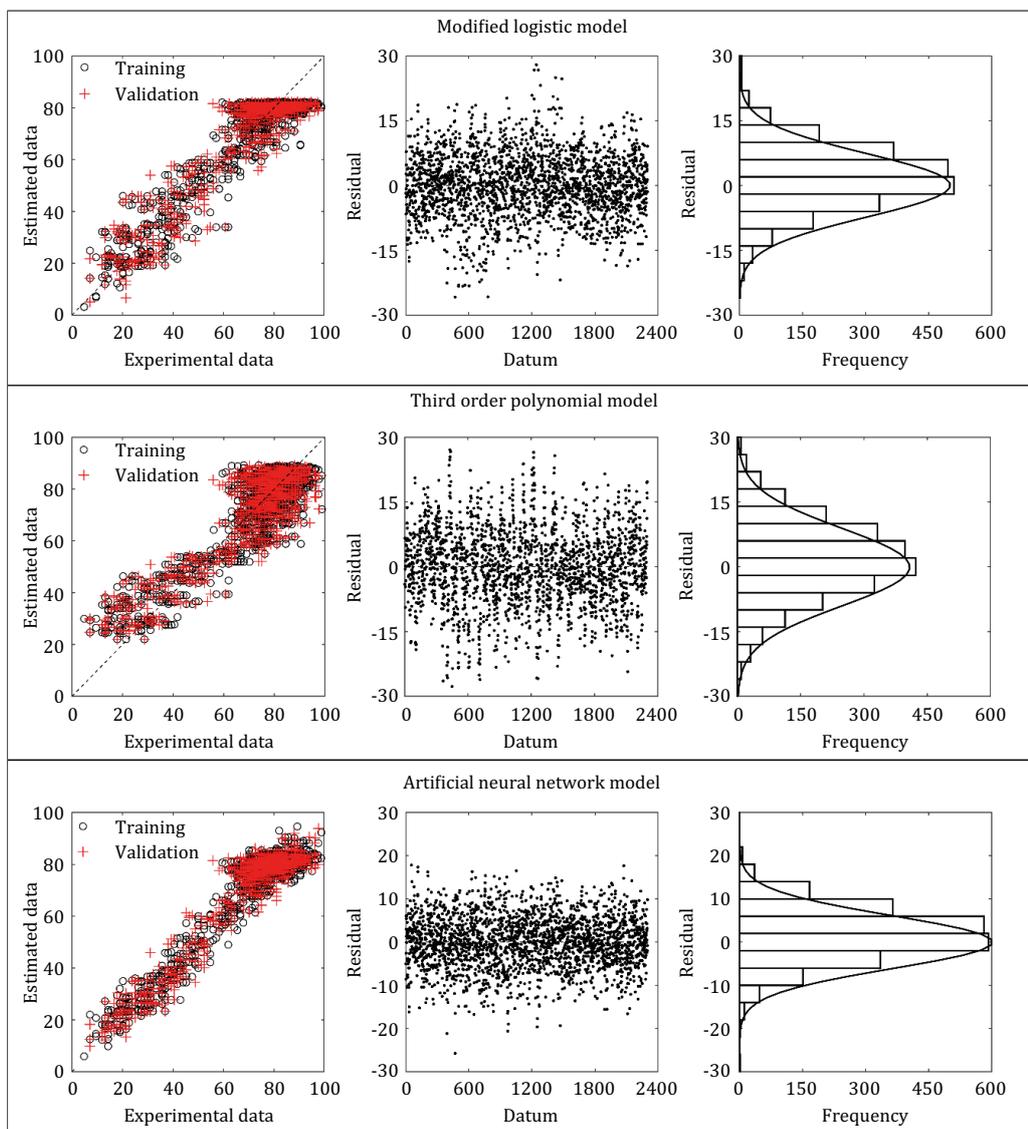


Figure 2 - Residual analysis of the best modeling approaches for egg production of H&N Brown second-cycle laying hens (92-111 weeks old).

3.3. ANN model of egg production

Concerning the ANN model for EP, the estimated results showed the importance of each of the EAA evaluated. The levels of 1138 mg/hen-day of iLys, 1031 mg/hen-day of iMetCys, and 717 mg/hen-day of iThr (Figure 3) could be adequate to supply the needs of the commercial H&N Brown SCLH for EP of 82.2, 91.4, 92.4, and 88.4% for weeks 8, 12, 16, and 20, respectively. These estimates correspond to 87, 96, 97, and 93%, respectively, of the expected production for H&N Brown laying hens in the first production cycle (H&N, 2020).

The ANN model makes it possible to evaluate different production scenarios where EP > 80% are reached, based on fixed EAA levels for the diet during the entire second cycle. For example, at a low iThr level (552 mg/hen-day), iLys of 939 mg/hen-day and iMetCys of 999 mg/hen-day could be adequate to achieve EP of 80.8, 87.6, 86.0, and 85.7% for weeks 8, 12, 16, and 20, respectively. Similarly, in a high iThr level scenario (881 mg/hen-day), iLys of 749 mg/hen-day and iMetCys of 854 mg/hen-day could be adequate to attain EP of 84.6, 83.6, 84.3, and 85.4% for weeks 8, 12, 16, and 20, respectively.

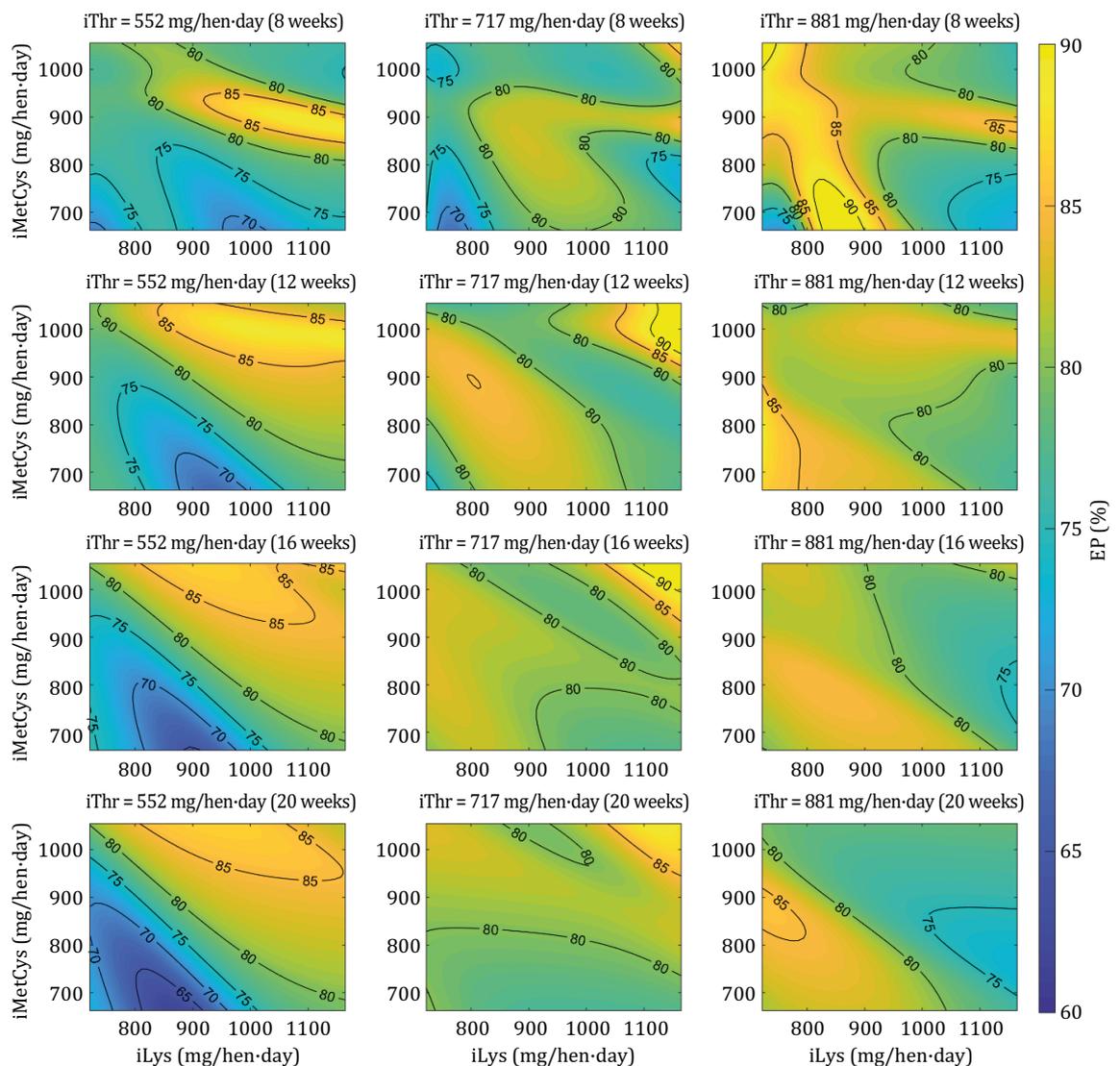


Figure 3 - Effect of intakes of lysine (iLys), methionine + cysteine (iMetCys) and threonine (iThr) on egg production (EP) at 8, 12, 16, and 20 weeks (99, 103, 107, and 111 weeks old, respectively) for H&N Brown second-cycle laying hens.

Such scenarios are some alternatives that can be evaluated from the economic perspective or the availability of ingredients and nutritional supplements, according to the producer's needs and market conditions in the egg price.

The ANN model could also be used to explore multiple scenarios where diets with different EAA levels can be used at various stages of the production cycle.

4. Discussion

4.1. Production parameters

Among the production parameters obtained, EP was between 83.1 and 85.3%, similar to that obtained and reported by the management guide for Hy-line Brown commercial layers at the end of 20 weeks of production (Hy-Line, 2018). These results were higher than reported for ISA Brown SCLH (62.0-68.6%) between 83 and 100 weeks old (Morales et al., 2018b) and Hissex White SCLH (66.5-82.2%) between 84 and 99 weeks old (Oliveira Filho, 2019). In addition to influencing productive performance, Lys is also related to body protein synthesis, especially in bone tissue and muscle (Domingues et al., 2016).

The highest cumulative number of hen-housed eggs found in our work was 105.1, with an average of 5.25 eggs per week (diet 13). This result is higher than that reported by Morales et al. (2018b), who found between 4.52 and 5.01 cumulative hen-housed eggs from ISA Brown SCLH of 83-100 weeks old. The cumulative hen-housed eggs reflect high production and high viability, greater than 94%.

For the best treatment (diet 23), FI was 116 ± 1.57 g/hen-day for hens of 92-111 weeks old. The average FI was also similar to that reported by Morales et al. (2018b) (113.75-118.9 g/hen-day) for Isa Brown SCLH between 76 and 96 weeks old. Likewise, FI was higher than reported by Sariozkan et al. (2016) (113.7 ± 1.6 g/hen-day) in a conventional molting for Hy-Line Brown SCLH between 81 and 92 weeks old. In our study, treatment 23 presented a good intake and adequate EAA ratios (Met+Cys/Lys = 91% and Thr/Lys = 63%). It is noteworthy that the aminoacitic balance of the diet and the experimental diet composition are factors closely related to FI in SCLH (Agustini et al., 2014).

The best treatment (diet 23) showed an FCR of 2.02 ± 0.11 kg feed/kg egg, which was lower than registered by Andreatti Filho et al. (2019) (2.1 kg feed/kg egg) for Lohman LSL Lite SCLH between 79 and 99 weeks old using a conventional forced molting (fasting). Feed conversion ratio was also lower than recorded by Sariozkan et al. (2016) (2.2-2.4 kg feed/kg egg) in Hy-Line Brown SCLH between 81 and 92 weeks old. The amino acid levels in the feed, especially Met+Cys, are essential during the second production cycle since they influence egg size (Domingues et al., 2016).

For all the treatments, FCR (in kilograms of feed per dozen eggs) was similar to that reported by Morales et al. (2018b) (1.65-1.82 kg feed/dozen eggs) for the SCLH of the Isa Brown line for 17 weeks of production and Sgavioli et al. (2013) (1.77 kg feed/dozen eggs) for 71-week-old Isa Brown hens during 112 days of postmolt production. Met+Cys allows an increase in crude protein in albumen and yolk and improves EP, FCR, egg size, and EW (Oliveira Filho, 2019).

The best treatments during this experimental work resulted in BW between 2001 and 2042 g/hen at the 20th week of the postmolt production period (111 weeks old). These figures were similar to the reported by the Hy-Line Brown hens management guide (Hy-Line, 2018), between 1920 and 2020 g/hen, and higher than pointed out by Sariozkan et al. (2016) (1814.7 ± 19.7 g/hen) for Hy-Line Brown SCLH between 81 and 92 weeks old. Methionine + Cysteine are essential for the gluconeogenesis process in the liver and muscle, affecting hen body recovery (Domingues et al., 2016). The increase in Met+Cys intake resulted in a significant body weight improvement (Kakhki et al., 2016).

The average EW in this study was similar to that recorded by Gongruttananun and Saengkudrua (2016), who found 66.7-68.8 g egg from H&N Brown SCLH between 94 and 117 weeks old, and Chanaksorn et al. (2019), who reported 65.0-70.3 g egg from Lohman Brown SCLH between 90 and 114 weeks old. Likewise, EW was higher than that reported by Morales et al. (2018b) (61.14-67.49 g) for Isa Brown

SCLH of 76-96 weeks old. The highest methionine content (about 5%) can be found in albumins, mainly egg albumin, which is one reason for its high demand in poultry (Carvalho et al., 2018).

Notably, in this study, the average viability was 98.5% at the end of the 20th week of production (111 weeks old). This parameter was lower than that reported by Morales et al. (2018b), who observed 100% viability, but higher than that listed in the management guide (Hy-Line, 2018), in which an average viability of 92.2% was reported for hens between 72 and 92 weeks old.

Finally, concerning production parameters of hen-housed eggs, FCR (kilogram feed/kilogram egg) and BW evaluated for week 20 (diet 13; Table 3), the results showed the importance of each EAA. The levels of iLys (943.7 mg/hen-day), iMetCys (858.3 mg/hen-day), and iThr (876.8 mg/hen-day) were adequate to supply the needs of commercial laying hens.

4.2. Curve fitting and statistical criteria

Among the different strategies to model EP for H&N Brown SCLH (Table 6), the ANN showed the highest goodness-of-fit statistics, followed by egg production models and multivariate polynomial models. The models showed R^2_{adj} values higher than 0.76, RMSE values lower than 9.4, and absolute biases in egg production lower than 0.6.

In general, all egg production models fitted well. However, the modified logistic function best fit EP curves. The goodness of fit of the third-order polynomial model was slightly worse than that of the modified logistic model, with differences lower than 3 and 5% between the values of R^2_{adj} and RMSE, respectively (Table 6).

The ANN model described the production curve more accurately than the other models and provided an effective means of predicting EP for H&N Brown laying hens based on Lys, Met+Cys, and Thr levels and time. The results of the ANN application to data analysis was achieved with higher accuracy for describing the relation among parameters than realized with the nonlinear regression models (Ghazanfari et al., 2011; Safari-Aliqiarloo et al., 2017). ANN showed a better predictive ability than other modeling approaches since the interconnections of neurons, typical of the architecture of ANN, allow the existence of nonlinear relationships and potential interactions among predictors that are not considered in egg production models nor in multivariate polynomial models (Felipe et al., 2015).

The best results using the feed-forward and cascade-forward ANN architectures (Table 6) were better than those obtained by the other modeling strategies for both the training and validation datasets, with R^2_{adj} values at least 4% higher and RMSE values at least 18% lower. The AIC values also showed that the ANN model showed less overfitting than the egg production and multivariate polynomial approaches; that is, the ANN model showed better generalization of EP dependence by the times and EAA studied.

Although residuals did not follow a normal distribution for any model (Table 6), they appeared to be scattered randomly around zero (Figure 2), verifying the suitability of the ANN model approach to predict EP with 88.0% of the residuals between -10 and 10%. Concerning the other approaches, 80.9 and 81.0% of the normalized residuals were between -10 and 10% for the modified logistic and third-order polynomial models, respectively.

Other studies on modeling EP for laying hens also attained accurate results using ANN. Akilli and Gorgulu (2020) applied a multivariate nonlinear fuzzy regression based on ANN to model daily and weekly egg production and egg weight for two strains of 100 laying hens between 20 and 90 weeks old. The results using this methodology were also more accurate than multivariate classical nonlinear regression, although using time as the only input.

ANN was also used to predict EP of broiler breeders. The results of this study showed that the ANN estimates were more accurate compared with other estimates from nonlinear regression models (Safari-Aliqiarloo et al., 2017).

4.3. ANN Model of egg production

Most researchers have used univariate optimization in which a single amino acid is evaluated at a time, and potential complex interactions among tested amino acids and other amino acids have been overlooked. The interactive effects of amino acids on productive performance and metabolism have been studied using multivariate models based on central composite designs, which allowed to evaluate different amino acids simultaneously in the productive performance of broilers, affecting feed efficiency. These types of studies could be more reliable than univariate optimization approaches (Mehri, 2014).

To our knowledge, this is the first study that used an ANN to simultaneously determine three nutritional requirements of EAA (Lys, Met+Cys, and Thr) over time in H&N Brown SCLH. The proposed model considers the complex interactive effect of the three EAA on the productive performance of SCLH, and it is necessary to compare them with dose-response models in all cases.

The versatility of ANN allowed us to estimate nutritional requirements for each week, for the entire cycle, or at the end of production. The scenarios from the ANN model covered intermediate levels of EAA to those evaluated in field experiment, and they may be better than the indicated by the treatment data (Table 3).

The ANN model estimates that the adequate level of iLys for egg production at 20 weeks (Figure 3) was higher than that recommended by Schneider (2011), which was 942 mg/hen·day for semiheavy laying hens of the Shaver Brown line. Likewise, the management guide for Hy-Line Brown commercial layer hens (Hy-Line, 2014) pointed out 713 mg/day for post molting. Evenly, Rostagno et al. (2017) recommended 875 mg/hen·day, which was lower than the level in this study of 1138 mg/hen·day iLys for the H&N Brown SCLH.

An estimated level of iMetCys of 1031 mg/hen·day (Figure 3) may be necessary to attain EP greater than 80% in H&N Brown SCLH between 8 and 20 weeks of production (99-111 weeks old). This iMetCys level is higher than that recommended by Schmidt et al. (2009b), Polese et al. (2012), Carvalho (2012), Hy-Line (2014), and Rostagno et al. (2017) to maximize EP of SCLH, who reported 786 mg/hen·day for the Lohmann Brown (79-95 weeks old) and 655 mg/hen·day for Shaver Brown (75-91 weeks old), 835 mg/hen·day for Hy-Line W-36 (79-95 weeks old), 599 mg/hen·day for Hy-Line Brown, and 858 mg/hen·day for laying-hens brown eggs with medium-superior performance in the first production cycle, respectively.

Methionine + Cysteine is essential for protein synthesis and plays several roles in the body metabolism as methyl group and sulfur donor, participating in protein deposition (Carvalho, 2017; Castro et al., 2020). Besides, cysteine has an essential role in protein structure, such as insulin, connecting polypeptide chains through the disulfide bridge (Carvalho, 2012).

The estimated level of iThr at 20 weeks of production of the SCLH was also different than that recommended by Rostagno et al. (2017) of 674 mg/hen·day for laying hen brown eggs with medium-superior performance in the first production cycle, Agustini et al. (2014) of 562 mg/hen·day for Shaver Brown hens (75-90 weeks old), Hy-Line Brown commercial layer management guide (Hy-Line, 2014) of 499 mg/hen·day, and Schmidt et al. (2010) of 462 mg/hen·day for Lohmann Brown line SCLH (79-95 weeks old). These reports were lower than the iThr level recommended in this study of 717 mg/hen·day for the H&N Brown SCLH.

Threonine plays a vital role in maintaining gut barrier integrity and mucin synthesis, and is associated with the maintenance of the epithelial barrier that is the first defense layer against pathogens (Mehri et al., 2021). It is also related to antibody production, which plays a significant role in immunity, and its requirement could be elevated under challenging conditions, including poor sanitation and chronic diseases (Macelline et al., 2021).

The results obtained by the ANN model demonstrate the importance of each EAA for different periods of EP (Figure 3), which suggests that validations of model scenarios that are of nutritional and

economic interest to the producer are necessary, as are studies of SCLH, in which different nutrient requirements can be used for different stages of the production curve.

In this sense, levels of 812-900 mg/hen-day of iLys, 670-760 mg/hen-day of iMetCys, and 881 mg/hen-day of iThr (Figure 3) may be adequate to achieve EP between 90 and 93% at eight weeks. At 12 weeks, levels of 1111-1165 mg/hen-day of iLys, 951-1055 mg/hen-day of iMetCys, and 717 mg/hen-day of iThr may be suitable to attain EP between 90 and 95%. At week 16, levels of 1084-1165 mg/hen-day of iLys, 991-1055 mg/hen-day of iMetCys, and 717 mg/hen-day of iThr may be adequate to achieve EP greater than 90%. At week 20, different scenarios are possible to attain EP of at least 85%, according to the iThr levels presented (Figure 3): 812-1160 mg/hen-day of iLys, 935-1055 mg/hen-day of iMetCys, and 552 mg/hen-day of iThr; 1047-1165 mg/hen-day of iLys, 943-1055 mg/hen-day of iMetCys, and 717 mg/hen-day of iThr; 722-794 mg/hen-day of iLys, 806-919 mg/hen-day of iMetCys, and 881 mg/hen-day of iThr.

Although the first limiting amino acids in laying hen diets have been extensively studied, different studies report discrepancies in the estimated requirements of Lys, Met+Cys, and Thr in the diets for SCLH. The interaction effect of EAA can be an essential factor during EP of SCLH, as has been corroborated in this study with the application of multivariate models.

5. Conclusions

The egg size in the H&N Brown second-cycle laying hens was not dependent on the essential amino acid levels used in the different treatments; during postmolting production, this genetic line produced large eggs.

The multivariate models used in the study considered the interaction of the three amino acids and time, which affects egg production; the findings indicate that the predicted relationships may be more reliable than those obtained from univariate models. It was worth the effort to apply these tools to make the best decisions in productive systems, despite the complicated analysis. This methodology can also be employed in the poultry and pork industries as an alternative to reduce production costs and increase performance.

The results of this study showed that artificial neural networks can be used to describe the egg production curve for second-cycle laying hens better than other multivariate models. The modeling strategy with the best goodness of fit was the neural network, which was the best option for the prediction of egg production response.

Through the results obtained in this study, the intake requirements at 20 weeks of production are 1138 mg/hen-day of lysine, 1031 mg/hen-day of methionine + cysteine, and 717 mg/hen-day of threonine. However, the artificial neural network model can also estimate combinations of essential amino acids that are different from these experimental treatments but must be optimized and validated in terms of the nutritional and economic interests of poultry companies. The use of complementary analytics tools, such as optimization and sensitivity analysis, may be implemented to gain an in-depth understanding of the most crucial nutritional requirements for both performance production and profitability.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: W. Morales-Suárez, I.C. Ospina-Rojas, A.H.N. Ferreira and H.A. Váquiro-Herrera. Data curation: W. Morales-Suárez and H.A. Váquiro-Herrera. Formal analysis: W. Morales-Suárez, I.C. Ospina-Rojas, A.H.N. Ferreira and H.A. Váquiro-Herrera. Funding acquisition: W. Morales-Suárez, J.J. Méndez-Arteaga and H.A. Váquiro-Herrera. Investigation: W. Morales-Suárez, I.C. Ospina-Rojas,

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