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Multivariate analysis of sources of polyunsaturated fatty acids, selenium, and chromium on the productive performance of second-cycle laying hens

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ABSTRACT - An experiment was conducted to evaluate the effect of the intake of a mixture of fish and sacha inchi oils (iOM), organic selenium (iSe), and organic chromium (iCr) on egg production (EP) and feed conversion ratio (FCR) of Isa Brown second-cycle laying hens (SCLH) for 16 weeks (91-106 weeks old). Egg production and FCR were evaluated using multivariate models that included conventional equations and artificial neural networks (ANN) to study multiple nutritional interactions as alternatives to univariate dose-response models. Based on the best models, iOM, iSe, and iCr levels were optimized, and a global sensitivity analysis was implemented to quantify their influence on EP and FCR. The modified logistic model was selected as the best strategy to represent EP. In the case of FCR, an ANN model with a feed-forward architecture and softmax transfer function was selected as the best alternative. One of the scenarios to simultaneously optimize EP (89.1%) and FCR (1.94 kg feed/kg egg) at 16 weeks of production was established with 3.3 g/hen·day of iOM, 0.132 mg/ hen·day of iSe, and 0.176 mg/hen·day of iCr. However, optimization considering only FCR results in much lower optimal iCr levels (between 0.083 and 0.105 mg/hen·day) with a slight decrease in EP (87.9%). The global sensitivity analysis showed that iSe is an essential factor associated with the increase in EP, and iCr is the most influential factor for the decrease in FCR. When both criteria were taken into account simultaneously from a desirability function, iSe was the most critical factor.

Keywords: bird nutrition, egg laying, modeling, nonlinear model, sensitivity analysis, organic mineral

1. Introduction

Environmental, technological, and nutritional stressors are crucial in poultry production. Stress has been associated with a low feed conversion ratio (FCR), decreased daily growth, immunosuppression, and increased bird mortality (Surai and Fisinin, 2016a; Surai et al., 2019).

The main field of application of natural products is in the prevention of oxidation of animals and their products (Abd El-Hack et al., 2020; Abo Ghanima et al., 2020). Feed supplementation with natural antioxidants protects commercial layers against overproduction of free radicals and lipid peroxidation under stress conditions and maintains their health and performance (Surai and Kochish, 2019; Madkour et al., 2022).

Polyunsaturated fatty acids (PUFA) increase egg production (EP) in poultry due to modulation of the immune system and oxidative status (Alagawany et al., 2019). Increased intake of ω-3 PUFA would increase these fatty acids in phospholipids of cell membranes, which is expected to decrease inflammatory responses (Nassef et al., 2019). Thus, fish oil and vegetable oils rich in ω-3 PUFA have been used to improve poultry's health, weight gain, and FCR (Alagawany et al., 2019). Sacha inchi oil (SIO), a promissory source of vegetable PUFA, comprises approximately 34% ω-6 and 51% ω-3 PUFA (Maurer et al., 2012).

Minerals are essential nutraceuticals, required for the optimum general health and physiological functions, maintenance, and reproduction (Alagawany et al., 2021a, 2021b). The inclusion of selenium (Se) in poultry diets improves the immune and physical response of the mucosa of the small intestine, increasing its absorption and digestion (Madkour et al., 2015; He et al., 2020). Selenium has a vitamin E sparing role by enhancing its absorption from the gut and protecting the cell membrane fats from oxidative damage (Mir et al., 2018).

Polyunsaturated fatty acids and Se can interact, possibly through the action of the GSH-Px enzyme, which plays an essential role in the regulation of prostaglandin biosynthesis from its precursor of arachidonic acid, which is responsible for the inflammatory response (Pappas et al., 2005).

Chromium (Cr) converts glucose into energy, which serves as fuel for protein synthesis, supports tissue growth and cell maintenance, increases serum insulin, and decreases the concentration of corticosterone and glucose (Siloto, 2014). Chromium also acts as an immunostimulating substance and exerts antioxidant effects, critical in laying hens exposed to climatic stressors (Sahin et al., 2018).

Second-cycle hens, due to their age and liver size, could be more efficient in converting linolenic acid to docosahexaenoic acid. In addition, antioxidant supplementation would modulate the elongation and enzymatic desaturation reactions, achieving a higher EP and a lower FCR (Fraeye et al., 2012).

Non-linear multivariate models can be used to evaluate the effect of multiple nutrients in the diet (Morales-Suárez et al., 2021). In this work, multivariate modeling, optimization, and sensitivity analysis were applied to study the effect of PUFA and organic minerals supplementation on EP and FCR of Isa Brown second-cycle laying hens (SCLH) during a productive cycle of 91 to 106 weeks of age.

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 conducted under environmental production conditions in San Pedro, Antioquia, Colombia (6°27'34" N latitude, 75°33'28" W longitude, and 2475 m asl), with an annual average temperature of 14 °C and relative humidity of 79%. A total of 1380 91-week-old Isa Brown SCLH were housed for 16 weeks (106 weeks old) in a caged-layer house of commercial design (Californian-type cages) under the molting and resting conditions reported by Morales-Suárez et al. (2021).

2.3. Experimental design

A central composite design was used to evaluate the effect of organic Se and Cr concentrations and an alternative source of fatty acids on both EP and FCR. The alternative source of PUFA was a mixture of 1:1 (w/w) of tuna and sacha inchi oils (OM) (Table 1), which was used in the partial or total substitution of palm oil in the diet (up to 3%) according to the experimental design. Organic Se and Cr from selenium yeast and chromium yeast, containing 1000 mg Se/kg and 1000 mg Cr/kg, respectively, were used for mineral supplementation.

Concentrations of three factors in the diet (OM, Se, and Cr) were combined using the experimental design. A design matrix with 23 treatments was determined combining five OM, Se, and Cr levels between 0-30 g/kg, 0.0-1.2 mg/kg, and 0.0-1.6 mg/kg, respectively. Five replicates were performed

Table 1 - Content of saturated and unsaturated fatty acids (%) in the form of methyl esters of the oils used in diets

Fatty acid[1]	Tuna oil	Sacha inchi oil	Palm oil
Saturated	28.5	6.7	42.1
Monounsaturated	2.0	0.0	0.3
Polyunsaturated	62.9	92.2	55.9
Total	93.4	98.9	98.3

[1] Determined by gas chromatography (ISO 12966-1:2014).

per treatment in experimental units of 12 birds. A total of 1840 records (23 treatments × 5 replicates × 16 weeks) were added during the fieldwork.

2.4. Diet formulation

According to the nutritional recommendations for laying hens proposed by Rostagno et al. (2011), a basal diet (Table 2) was formulated based on corn and soybean meal. The 23 experimental diets were obtained by supplementing the basal diet with OM as a replacement for palm oil, and Se and Cr as replacements for calcium carbonate. Diets were prepared weekly.

2.5. Production parameters

Egg production and FCR were calculated weekly for 16 weeks (91-106 weeks old). Egg production was calculated from the relation between the number of eggs and the number of birds per experimental unit; FCR was calculated from the kilogram of feed consumed divided by the kilogram of egg produced. Feed intake, egg weight, hen-housed eggs, and livability were registered daily. Feed intake was recorded and calculated as grams consumed over seven days, divided by the number of birds, and adjusted for mortalities; body weight was registered weekly; FCR was also expressed as the kilogram of feed consumed by the dozens of eggs produced; and the 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).

For data analysis, the OM, Se, and Cr levels were expressed as intakes (iOM, iSe, and iCr) (Table 3) by considering the average feed intake for each diet.

2.6. Conventional modeling approach

Two conventional approaches were used to model EP and FCR of Isa Brown SCLH as a function of iOM, iSe, iCr, and time. Nonlinear equations frequently used to describe egg production curves were adapted to model EP, whereas second- and third-order multivariate polynomial equations were used to model FCR.

For the modeling of EP, the empirical models of Adams-Bell (Eq. 1) (Faridi et al., 2011), modified-compartmental (Eq. 2) (Narinc et al., 2014), modified-Gompertz (Eq. 3) (Morales et al., 2018), and modified logistic (Eq. 4) (Morales-Suárez et al., 2021) were used.

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

$$EP = \frac{ae^{-bt}}{1 + e^{-c(t-d)}} \tag{2}$$

$$EP = (a + bt)e^{\{-e^{[de(c-t)/(a+bt)+1]}\}} \tag{3}$$

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

Table 2 - Ingredients and nutrient composition of the basal diet (91-106 weeks, as-fed basis)

Ingredient	(g/kg)
Corn (7. 7% crude protein)	590
Soybean meal (46.2% crude protein)	184
Palm oil	30
Fish oil	0
Sacha inchi oil	0
Corn gluten (60% crude protein)	10
Calcium carbonate	111.9
Monocalcium phosphate	6.8
Salt	2.0
Vitamin premix[1]	0.5
Mineral premix[2]	0.5
Lysine	4.99
Methionine	5.2
Tryptophan	0.36
Sodium bicarbonate	1.5
Analyzed composition	
Metabolizable energy (MJ/kg)	11.93
Protein	155
Calcium	41
Available phosphorus	3
Fiber	21.5
Sodium	1.6
Chlorine	2.3
Potassium	6.63
Digestible lysine	8.5
Digestible methionine + cysteine	8.5
Digestible threonine	6.5
Digestible valine	6.95
Digestible arginine	9.47
Digestible leucine	14.35
Digestible isoleucine	6.26
Digestible histidine	3.98
Digestible phenylalanine	7.53
Digestible tryptophan	1.8

[1] Vitamin premix contained per kg of diet: 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] Mineral premix contained per kg of diet: zinc, 70 g; manganese, 65 g; iron, 20 g; copper, 5 g; iodine, 1200 mg; choline chlorine 60%, 500 g.

in which EP is egg production (%); t is time (week); and a, b, c, and d are the model parameters.

In each EP model (Eqs. 1-4), parameters a, b, c, and d were written in terms of iOM, iSe, and iCr according to the procedure described by Morales-Suárez et al. (2021).

Feed conversion ratio was modeled using stepwise regression to assess second (Eq. 5) and third-order (Eq. 6) multivariate polynomial models in terms of iOM, iSe, iCr, and time. Thus, only statistically significant terms (linear, quadratic, cubic, and cross-product) at a 95% confidence level were included in these models (Ameer et al., 2017).

$$FCR = \beta_0 + \sum_{j=1}^{4} \beta_j x_j + \sum_{j=1}^{3} \sum_{k>j}^{4} \beta_{jk} x_j x_k + \sum_{j=1}^{4} \beta_{jj} x_j^2 \quad (5)$$

Table 3 - Diet concentrations and intakes of mixture of oils, organic selenium, and organic chromium in the diets for Isa Brown SCLH over 16 weeks

Diet	Concentration in diets			Intake[1]		
	OM (g/kg)	Se (mg/kg)	Cr (mg/kg)	iOM (g/hen·day)	iSe (mg/hen·day)	iCr (mg/hen·day)
1	0	0.00	0.00	0.000±0.000	0.000±0.000	0.000±0.000
2	30	0.00	0.00	3.204±0.342	0.000±0.000	0.000±0.000
3	15	0.00	0.80	1.650±0.182	0.000±0.000	0.088±0.010
4	0	0.00	1.60	0.000±0.000	0.000±0.000	0.176±0.019
5	30	0.00	1.60	3.300±0.363	0.000±0.000	0.176±0.019
6	6	0.24	0.32	0.660±0.073	0.026±0.003	0.035±0.004
7	24	0.24	0.32	2.640±0.290	0.026±0.003	0.035±0.004
8	6	0.24	1.28	0.638±0.068	0.026±0.003	0.136±0.014
9	24	0.24	1.28	2.640±0.290	0.026±0.003	0.141±0.015
10	15	0.60	0.00	1.650±0.182	0.066±0.007	0.000±0.000
11	0	0.60	0.80	0.000±0.000	0.066±0.007	0.088±0.010
12	15	0.60	0.80	1.650±0.182	0.066±0.007	0.088±0.010
13	30	0.60	0.80	3.294±0.362	0.066±0.007	0.088±0.010
14	15	0.60	1.60	1.650±0.182	0.066±0.007	0.176±0.019
15	6	0.96	0.32	0.660±0.073	0.106±0.012	0.035±0.004
16	24	0.96	0.32	2.640±0.290	0.106±0.012	0.035±0.004
17	6	0.96	1.28	0.660±0.073	0.106±0.012	0.141±0.015
18	24	0.96	1.28	2.640±0.290	0.106±0.012	0.141±0.015
19	0	1.20	0.00	0.000±0.000	0.132±0.015	0.000±0.000
20	30	1.20	0.00	3.300±0.363	0.132±0.015	0.000±0.000
21	15	1.20	0.80	1.650±0.182	0.132±0.015	0.088±0.010
22	0	1.20	1.60	0.000±0.000	0.132±0.015	0.176±0.019
23	30	1.20	1.60	3.300±0.363	0.132±0.015	0.176±0.019

SCLH - second-cycle laying hens; OM - mixture of tuna and sacha inchi oils; iOM, iSe, and iCr - intakes of OM, Se, and Cr.
[1] Values of intakes are expressed as the means±standard deviations (n = 80).

$$FCR = \beta_0 + \sum_{j=1}^{4} \beta_j x_j + \sum_{j=1}^{3} \sum_{k>j}^{4} \beta_{jk} x_j x_k + \sum_{j=1}^{4} \beta_{jj} x_j^2 + \sum_{j=1}^{2} \sum_{k>j}^{3} \sum_{l>k}^{4} \beta_{jkl} x_j x_k x_l + \sum_{j=1}^{4} \sum_{k \neq j}^{4} \beta_{jkk} x_j x_k^2 + \sum_{j=1}^{4} \beta_{jjj} x_j^3, \quad (6)$$

in which FCR is feed conversion ratio (in kg feed/kg egg); x represents the independent variables (iOM, iSe, iCr, and t); and β_0, β_j, β_{jj}, β_{jjj}, β_{jk}, β_{jkl}, and β_{jkk} are the model parameters of the independent, linear, quadratic, cubic, and cross-product terms, respectively.

2.7. Artificial Neural Networks (ANN)

The dependence of EP and FCR with the four studied factors (iOM, iSe, iCr, and t) were also modeled using two neural network architectures: feed-forward (Eq. 7) and cascade-forward (Eq. 8). In these architectures, log-sigmoid (Eq. 9), hyperbolic tangent (Eq. 10), softmax (Eq. 11), and radial-basis (Eq. 12) equations were used as transfer functions. Between five and eleven neurons in one hidden layer for each network architecture and transfer function combination were assessed (Morales-Suárez et al., 2021).

$$y = w_{ho} \times f + b_o \quad (7)$$

$$y = w_{ho} \times f + w_{io} \times x + b_o \quad (8)$$

$$f = \frac{1}{1 + e^{[-(W_{ih} \times x + b_h)]}} \tag{9}$$

$$f = \frac{2}{1 + e^{[-2(W_{ih} \times x + b_h)]}} - 1 \tag{10}$$

$$f = \frac{e^{(W_{ih} \times x + b_h)}}{\sum e^{(W_{ih} \times x + b_h)}} \tag{11}$$

$$f = e^{[-(W_{ih} \times x + b_h)^2]} \tag{12}$$

in which y is the vector of the output variable (EP or FCR), x is the matrix of the input variables (iOM, iSe, iCr, and t), *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), and b_o is the bias vector of the output layer.

2.8. Identification, validation, and statistical analysis

The experimental data of the response variable (EP or FCR) were randomly separated into datasets for identification and validation. Three replicates per treatment (1380 records) were used in the identification dataset, whereas two replicates (920 records) were used for model validation.

The parameters for the nonlinear EP models (Eqs. 1-4) were identified using the "fitnlm" and "stepwisefit" functions of MATLAB® R2019a (The MathWorks Inc., Natick, MA, USA). The fitnlm function uses a Levenberg-Marquardt nonlinear least-squares algorithm to identify the coefficients of nonlinear and multivariate models, whereas stepwisefit uses stepwise regression to identify statistically significant ($P<0.05$) coefficients of multivariate and additive models. The stepwisefit function was also used for parameter identification in multivariate polynomial models (Eqs. 5-6). The training of ANN models (Eqs. 7-12) was carried out using the "trainbr" function of MATLAB®, which uses a Bayesian regularization backpropagation algorithm to prevent overfitting problems (Daza et al., 2018).

The adjusted coefficient of determination (R^2_{adj}) (Eq. 13), root mean square error (RMSE) (Eq. 14), and bias (Eq. 15) were used to evaluate the goodness of fit, accuracy, and deviation of the models, respectively. Akaike's information criterion (AIC) (Eq. 16) was used to compare and measure the model quality (MathWorks, 2019). In addition, the best models were subjected to a residual analysis to assess the adequacy of the estimations.

$$R^2_{adj} = 1 - S^2_{yx}/S^2_y \tag{13}$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (y_i^* - y_i)^2}{n}} \tag{14}$$

$$bias = \frac{1}{n} \sum_{i=1}^{n} (y_i^* - y_i) \tag{15}$$

$$AIC = n \log\left[\det\left(\frac{1}{n} \sum_{i=1}^{n} \varepsilon\varepsilon^T\right)\right] + 2n_p + n[\log(2\pi) + 1] \tag{16}$$

in which S_y is the standard deviation associated with the sample, S_{yx} is the standard deviation associated with the model estimations, y_i* and y_i represent the experimental and estimated values of the response variable (EP or FCR), respectively, 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 were selected based on the highest R^2_{adj}, and the lowest RMSE and AIC.

2.9. Optimization

Two optimization problems were formulated separately to find the iOM, iSe, and iCr values that maximize EP or minimize FCR (Eqs. 17-18):

$$\text{Maximize } EP\ (iOM, iSe, iCr) \text{ such that } \begin{cases} iOM_{\min} \le iOM \le iOM_{\max} \\ iSe_{\min} \le iSe \le iSe_{\max} \\ iCr_{\min} \le iCr \le iCr_{\max} \end{cases} \tag{17}$$

$$\text{Minimize } FCR\ (iOM, iSe, iCr) \text{ such that } \begin{cases} iOM_{\min} \le iOM \le iOM_{\max} \\ iSe_{\min} \le iSe \le iSe_{\max} \\ iCr_{\min} \le iCr \le iCr_{\max} \end{cases} \tag{18}$$

Besides, a multi-objective optimization approach, which uses a Derringer desirability function, was applied to define the optimal iOM, iSe, and iCr values that simultaneously maximize EP and minimize FCR (Eq. 19). Thus, an overall desirability function (D, Eq. 20) was defined as the geometric mean of the local desirabilities d_{EP} and d_{FCR} (Eqs. 21-22), representing the EP and FCR estimations normalized between 0 and 1. The lower (EP_{LB}, FCR_{LB}) and upper (EP_{UB}, FCR_{UB}) bounds required for the normalization were defined from the experimental results. Therefore, Eq. (21) transforms the estimated EP to be maximized, whereas Eq. (22) transforms the estimated FCR to be minimized. In both local (d_{EP}, d_{FCR}) and overall (D) desirabilities, 1 is the most favorable value.

$$\text{Maximize } D\ (iOM, iSe, iCr) \text{ such that } \begin{cases} iOM_{\min} \le iOM \le iOM_{\max} \\ iSe_{\min} \le iSe \le iSe_{\max} \\ iCr_{\min} \le iCr \le iCr_{\max} \end{cases} \tag{19}$$

$$D = \sqrt{d_{EP} \times d_{FCR}} \tag{20}$$

$$d_{EP} = \frac{EP - EP_{LB}}{EP_{UB} - EP_{LB}} \tag{21}$$

$$d_{FCR} = \frac{FCR - FCR_{UB}}{FCR_{LB} - FCR_{UB}} \tag{22}$$

The MATLAB® function “fmincon” was used to solve the multivariate optimization problems for EP, FCR, and D at 4, 8, 12, and 16 weeks, subject to the lower and upper limits for iOM, iSe, and iCr defined from the experimental results.

2.10. Sensitivity analysis

The selected models were also subjected to a time-varying global sensitivity analysis by the Sobols’ method (Saltelli et al., 2019) to quantify the effect of the inputs (iOM, iSe, and iCr) and their interactions on the variance of the outputs (EP and FCR) at 4, 8, 12, and 16 weeks. This method is a global and model-independent technique to determine the contributions of individual factors and their interactions to the total output variance. The sensitivity indexes characterize such contributions (Eqs. 23-24):

$$S_i = \frac{V_i}{V} \tag{23}$$

$$S_{Ti} = 1 - \frac{V_{\sim i}}{V} \tag{24}$$

The first-order indexes (S_i) (Eq. 23) are a measure for the variance contribution of an individual input (V_i) to the total model variance (V). In Eq. 23, the partial variance V_i is the expected reduction in variance obtained if the *i*-th-factor (iOM, iSe, or iCr) could be fixed.

The total sensitivity indexes (S_{Ti}) (Eq. 24) result from the main effect of the *i*-th-factor and all its interactions with the other inputs. In Eq. 24, the partial variance $V_{\sim i}$ is the expected reduction in variance that would be obtained if all inputs but *i*-th-factor could be fixed.

The number of model simulations for calculating S_i and S_{Ti} was established next to a convergency test (Nossent et al., 2011). The Latin hypercube was used as the sampling strategy for the input variables. The inputs were 100 times randomly sampled with replacement to assess the 95% confidence intervals for sensitivity index. The difference between S_{Ti} and S_i was used to determine the interaction degree between the input factors.

3. Results

3.1. Productive parameters

The average feed intake of all treatments was 109.8±8.3 g/hen over 16 weeks of production. Feed intake was similar among the diets, between 106.8±7.0 and 110.0±7.8 g/hen, lower than those reported by Morales-Suárez et al. (2021) for H&N Brown SCLH during 20 weeks of production, which registered 114.3 and 116.7 g/hen·day. According to the average feed intake, iOM, iSe, and iCr levels in diets (Table 3) range up to 3.300±0.363, 0.132±0.015, and 0.176±0.019 g/hen·day, respectively.

The treatments showed an average production level of 54.9% in the first four weeks (91-94 weeks old), ranging from 47.9 to 62.1%. The production peak was achieved from the eighth week of production (98 weeks old) (Table 4), ranging from 84.3% at 102 weeks old for diet 1 to 90.9% at 102 weeks old for diet 23. Egg production at 16 weeks of production (106 weeks of age) was between 78.6% (diet 7) and 88.1% (diets 4 and 17).

Table 4 - Egg production and feed conversion ratio of Isa Brown SCLH for the diets over 16 weeks

Diet	Egg production (%)				Feed conversion ratio (kg feed/kg egg)			
	Week 4	Week 8	Week 12	Week 16	Week 4	Week 8	Week 12	Week 16
1	62.1±5.9	81.4±4.7	84.3±5.0	83.1±5.3	2.65±0.25	2.03±0.11	1.91±0.13	1.99±0.14
2	49.8±9.0	80.5±7.1	80.0±4.1	80.7±7.9	3.35±0.75	1.99±0.19	2.07±0.12	2.03±0.20
3	55.0±7.1	80.2±6.5	84.8±5.1	84.0±10.6	3.10±0.36	2.06±0.15	1.99±0.11	2.02±0.31
4	47.9±4.9	87.9±7.5	80.2±5.8	88.1±4.4	3.51±0.41	1.91±0.18	2.12±0.12	1.90±0.10
5	57.6±9.2	84.7±11.0	84.0±8.6	85.0±7.0	2.92±0.51	1.88±0.24	1.96±0.20	1.94±0.15
6	53.8±5.1	87.4±8.4	87.9±4.6	81.2±8.5	3.09±0.34	1.98±0.22	1.97±0.13	2.13±0.25
7	50.0±6.8	81.2±9.3	80.9±7.2	78.6±6.9	3.41±0.37	2.16±0.33	2.06±0.20	2.08±0.17
8	56.7±7.5	85.3±5.2	85.3±5.7	85.0±5.2	3.04±0.46	1.88±0.16	1.99±0.10	1.98±0.18
9	50.4±6.6	83.8±3.6	86.4±8.1	82.1±11.8	3.39±0.44	1.98±0.08	1.91±0.14	1.99±0.34
10	48.8±6.0	82.4±7.5	84.8±8.3	85.2±5.7	3.52±0.44	2.11±0.20	2.02±0.16	1.95±0.12
11	56.2±12.1	85.7±2.5	86.7±6.9	85.2±2.7	3.04±0.67	1.99±0.06	1.92±0.26	1.98±0.13
12	49.8±10.0	86.2±6.2	84.5±7.5	82.6±6.6	3.53±0.76	1.95±0.08	2.04±0.19	2.00±0.20
13	60.0±10.8	85.7±6.8	85.0±6.9	84.3±9.6	2.84±0.60	1.94±0.14	1.99±0.16	1.96±0.28
14	50.5±6.2	87.6±3.6	84.3±6.7	79.8±8.1	3.34±0.37	1.91±0.08	2.02±0.23	2.09±0.25
15	55.0±13.0	85.0±3.5	83.6±4.6	84.8±5.5	3.14±0.68	1.96±0.10	1.98±0.12	1.98±0.21
16	56.9±6.7	83.1±4.4	87.6±3.9	85.2±2.7	2.95±0.34	1.99±0.09	1.88±0.10	1.93±0.06
17	51.0±8.6	80.5±9.8	84.8±7.4	88.1±4.8	3.44±0.55	2.10±0.32	2.04±0.22	1.85±0.10
18	56.7±9.6	87.1±5.5	84.5±5.3	87.1±7.3	3.06±0.59	1.89±0.12	1.94±0.11	1.86±0.17
19	58.8±9.7	80.7±7.9	84.3±7.0	81.4±6.7	2.87±0.69	2.05±0.26	1.93±0.17	2.00±0.14
20	55.0±7.6	84.3±6.0	85.5±4.4	85.9±6.9	3.11±0.48	1.97±0.12	1.94±0.13	1.87±0.15
21	60.2±9.0	86.7±2.0	82.9±3.8	84.8±6.0	2.72±0.41	1.89±0.08	1.99±0.11	1.99±0.22
22	58.3±10.8	82.4±4.4	84.5±6.5	84.8±4.4	3.03±0.77	2.03±0.10	1.96±0.19	1.93±0.12
23	61.7±10.9	90.7±2.1	90.9±3.8	87.1±3.3	2.85±0.59	1.84±0.07	1.82±0.08	1.86±0.11

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

The analysis of FCR of the treatments indicates an average value of 2.26 kg feed/kg egg (Table 4). In the fourth week (91-94 weeks old), FCR was between 2.65 and 3.53 kg feed/kg egg with an average of 3.126 kg feed/kg egg. In the eighth (95-98 weeks old), 12th (99-102 weeks old), and 16th (103-106 weeks old) weeks of production, FCR ranged between 1.84-2.16, 1.82-2.12, and 1.85-2.13 kg feed/kg egg, respectively, with an average of 1.977, 1.976, and 1.969 kg feed/kg egg, respectively (Table 4). The lowest feed conversion was shown by diet 17 for the 16 weeks of production (106 weeks old) with 1.85 kg feed/kg egg, equivalent to 1.53 kg feed per dozen eggs.

3.2. Curve fitting and statistical criteria

In the mathematical modeling of EP, egg production and ANN models were evaluated (Table 5). In the first strategy, the best choice was the modified logistic model (Eq. 4) with R^2_{adj} values of 0.9282 and 0.9172 and RMSE values of 7.15 and 7.40 for the validation and training data sets, respectively. The identified functions of parameters *a*, *b*, *c*, and *d* of the modified logistic model (Eq. 4) are shown in Eqs. 25-28, in which all terms were statistically significant (P<0.05). The ANN approach returned slightly better goodness-of-fit statistics than egg production models. The best ANN result was achieved using a feed-forward architecture with a log-sigmoid transfer function and five neurons in the hidden layer. Although the ANN approach resulted in R^2_{adj} values of 0.9292 and 0.9207, and RMSE of 7.01 and 7.15 for the training and validation data set, respectively, this model was 21 parameters higher than the modified logistic model (Table 5).

Table 5 - Modeling results for egg production and feed conversion ratio of Isa Brown SCLH (91-106 weeks old)

Modeling approach	Model detail	Training/Identification		Validation		P-value[1]	Bias	AIC
		R^2_{adj}	RMSE	R^2_{adj}	RMSE			
Egg production								
Egg production models								
Adams Bell	Number of parameters: 11	0.9284	7.12	0.9164	7.45	0.001	-1.25×10^{-8}	12529
Modified Compartmental	Number of parameters: 14	0.9296	7.11	0.9185	7.32	0.006	−0.077	12530
Modified Gompertz	Number of parameters: 10	0.9276	7.16	0.9174	7.40	0.001	0.037	12526
Modified Logistic	Number of parameters: 10	0.9282	7.15	0.9172	7.40	0.001	−0.088	12525
Artificial neural networks								
Feed-forward architecture	Transfer function: log-sigmoid Hidden-layer neurons: 5 Number of parameters: 31	0.9292	7.01	0.9207	7.15	0.001	0.005	12503
Cascade-forward architecture	Transfer function: log-sigmoid Hidden-layer neurons: 5 Number of parameters: 35	0.9294	6.99	0.9199	7.16	0.001	−0.0094	12509
Feed conversion ratio								
Modeling approach	Model detail	Training/Identification		Validation		P-value[1]	Bias	AIC
		R^2_{adj}	RMSE	R^2_{adj}	RMSE			
Multivariate polynomial models								
Second order	Number of parameters: 11	0.5537	0.712	0.5355	0.647	0.001	−0.0003	7558
Third order	Number of parameters: 25	0.6821	0.601	0.6299	0.577	0.001	−0.0491	7393
Artificial neural networks								
Feed-forward architecture	Transfer function: Softmax Hidden-layer neurons: 8 Number of parameters: 49	0.7643	0.506	0.7725	0.439	0.001	1.53×10^{-5}	7131
Cascade-forward architecture	Transfer function: Softmax Hidden-layer neurons: 9 Number of parameters: 59	0.7636	0.504	0.7706	0.438	0.001	1.27×10^{-5}	7145

SCLH - second-cycle laying hens; R^2_{adj} - adjusted coefficient of determination; RMSE - root mean square error; AIC - Akaike's information criterion.

$$a = 83.83 + 7.997\ iOM\ iCr \tag{25}$$

$$b = 0.5594\ \mathrm{iOM\ iSe} - 0.4569\ iOM\ iCr \tag{26}$$

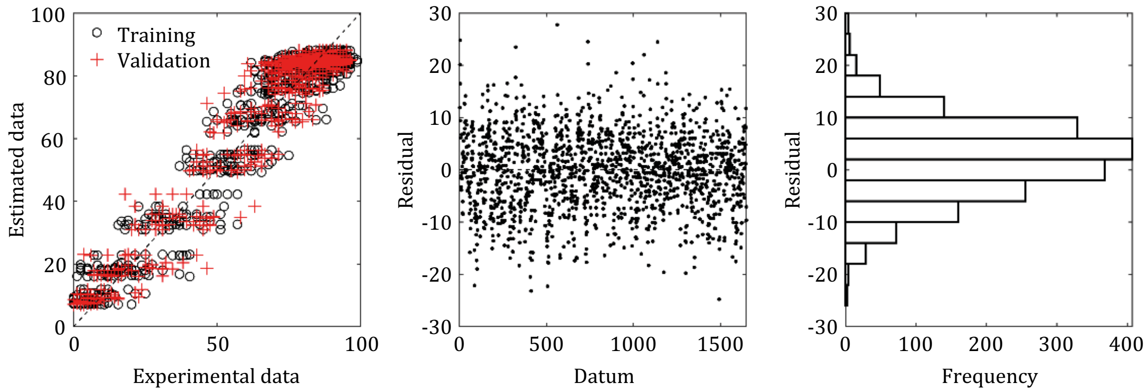
$$c = 0.7563 + 0.3661\ iOM + 8.309\ iSe - 1.175\ iOM\ iSe - 0.09164\ iOM^2 - 31.55\ iSe^2 \tag{27}$$

$$d = 17.064 + 30.70\ iSe \tag{28}$$

Between the best results of the two modeling strategies, the modified logistic model was selected as the best-fit option since it presented R^2_{adj}, RMSE, and AIC values similar to the ANN model, but with fewer parameters. The modified logistic model is a less complex expression than the ANN model, ensures the EP curve behavior, and avoids overfitting, facilitating subsequent multi-objective optimization and sensitivity analysis. In addition, all models showed absolute biases near-zero (Table 5) and could be considered accurate as they overestimate and underestimate the data equally.

The residuals for EP using the modified logistic model were dispersed around zero and followed a normal distribution, with 82.6% of the residuals between −10% and 10% (Figure 1).

The best results for FCR modeling were achieved using a feed-forward ANN with the softmax transfer function and eight neurons in the hidden layer (Table 6), resulting in R^2_{adj} values of 0.7643 and 0.7725 and RMSE values of 0.506 and 0.439 for the training and validation data sets (Table 5), respectively. The ANN approach showed the best-fit results since it presented better R^2_{adj}, RMSE, and AIC values than multivariate polynomial models (Table 5). In addition, the best ANN model allows predicting the FCR behavior using one of the most straightforward network architectures (Eqs. 8 and 12), which could facilitate subsequent multi-objective optimization and sensitivity analysis.



SCLH - second-cycle laying hens.

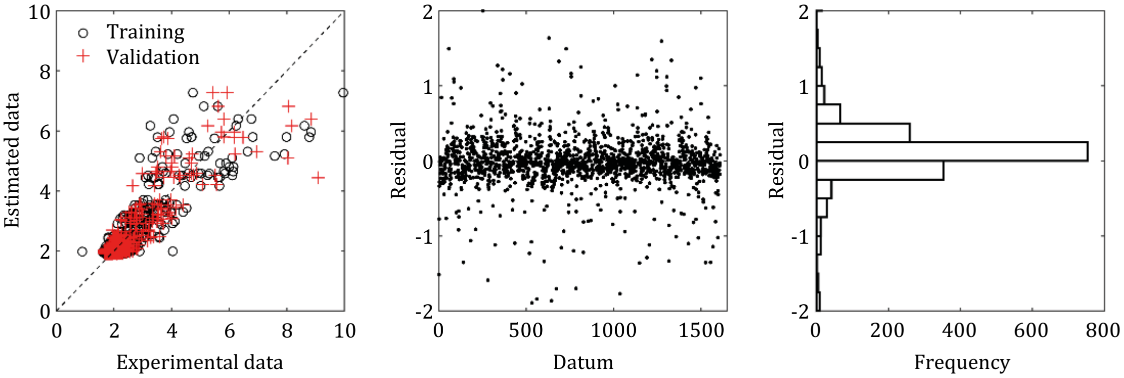
Figure 1 - Residual analysis of the modified logistic model for egg production (%) of Isa Brown SCLH (91-106 weeks old).

Table 6 - Weights and biases of selected ANN model for feed conversion of Isa Brown SCLH (91-106 weeks old)

W_{ih}				W_{ho}^{T}	b_h	b_o
−1.1022	0.7177	1.7031	3.2531	−1.7816	4.5745	
3.0037	2.1558	−0.2838	3.6062	−1.7941	4.0652	
−2.0531	−0.9516	−1.6939	3.9843	−1.7796	6.4791	
−1.7398	−0.9569	−1.9688	−1.1544	1.8055	−0.6141	[0.9939]
0.9068	3.4853	−0.0539	1.2128	−1.7304	−0.8796	
−2.0335	0.2217	2.2999	−1.7426	1.8380	−3.4106	
−0.5488	0.1362	0.2270	−7.2889	3.3559	−6.2398	
3.5668	2.5225	−0.2296	−1.8706	1.0801	−3.9947	

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

The residuals for FCR using the ANN model were dispersed around zero, following a normal distribution according to the test of Lilliefors (Figure 2). When verifying the ability of the ANN models to predict FCR, 95.5% of the residuals were between −1 and 1 (Figure 2).

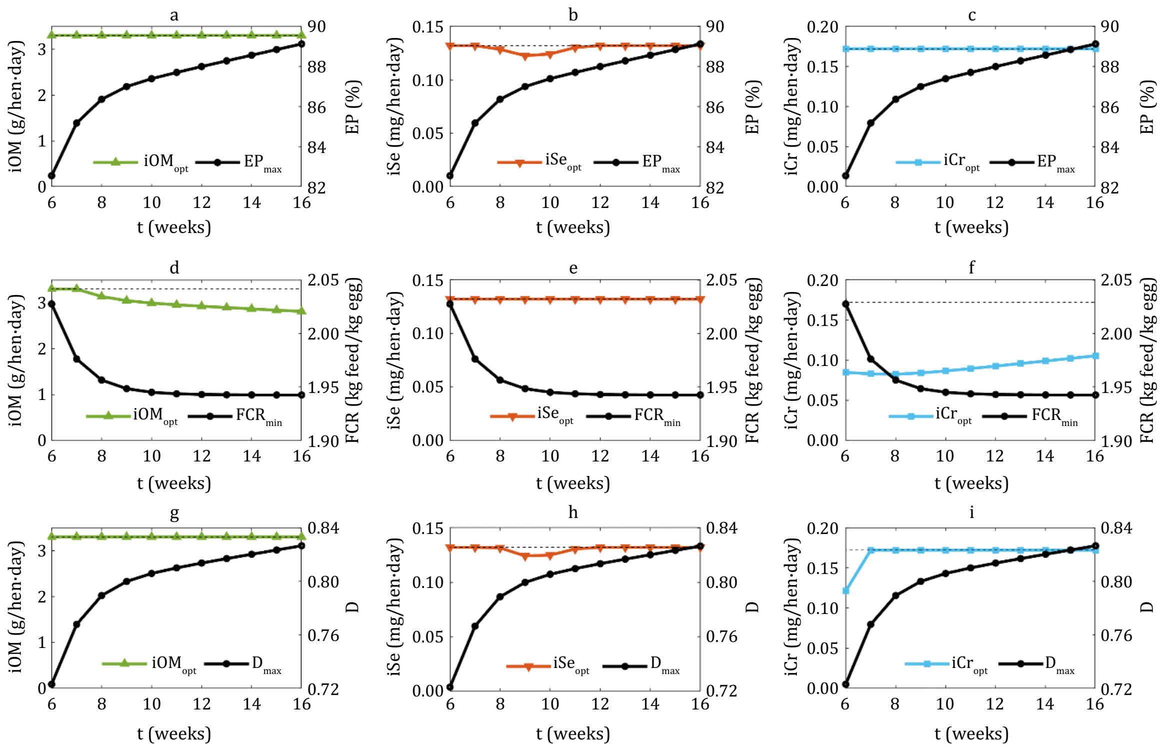


SCLH - second-cycle laying hens; ANN - artificial neural networks.

Figure 2 - Residual analysis of the ANN model for feed conversion ratio (kg feed/kg egg) of Isa Brown SCLH (91-106 weeks old).

3.3. Optimization

When optimizing EP (Figures 3a-c), the maximum values oscillate between 82.6 and 89.1% between weeks 6 and 16 of production, respectively. The optimal iOM and iCr levels coincide with the upper limit of the restrictions imposed on them (dotted lines in Figures 3a and c) at all times. The iSe levels are



SCLH - second-cycle laying hens.
Optimal requirements of mixture of oils (iOM; a,d,g), organic selenium (iSe; b,e,h), and organic chromium (iCr; c,f,i).

Figure 3 - Optimization of egg production (EP), feed conversion ratio (FCR), and overall desirability function (D) between 6 and 16 weeks (96 and 106 weeks old) for Isa Brown SCLH.

slightly lower than the upper limit between weeks 8 and 10 (Figure 3b). In general terms, maximum production is obtained using the highest iOM, iSe, and iCr allowed.

The FCR presents minimum values between 2.29 and 1.94 kg feed/kg egg at 6 and 16 weeks (Figures 3d-f). Optimal iOM levels decrease over time from 3.30 to 2.81 g/hen·day (Figure 3d). In contrast, iCr levels rise from 0.085 to 0.105 mg/hen·day (Figure 3f). The iSe level was the highest imposed by the restriction (0.132 mg/hen·day) (Figure 3e).

The optimal iOM, iSe, and iCr levels for FCR are different for EP, meaning that levels that minimize conversion do not guarantee maximum production levels. The above justified using the overall desirability function (D) to establish the optimal levels to maximize EP and minimize FCR simultaneously.

The behavior of D (Figure 3g-i) reflects the changes between 6 and 16 weeks in the maximum and minimum values for EP and FCR, respectively, showing values between 0.723 (EP = 92.0%, FCR = 2.04 kg feed/kg egg) and 0.827 (EP = 89.1%, FCR = 1.942 kg feed/kg egg) (Table 7). The optimal iOM, iSe, and iCr levels are similar to those obtained in the maximization of EP, except for iCr at the sixth week below the upper limit imposed in the constraint.

At 16 weeks of production (91-106 weeks old), the maximum EP and minimum FCR were 89.1% and 1.94 kg feed/kg egg, respectively. For EP at 16 weeks of production, the optimal iOM, iSe, and iCr values were 3.3 g/hen·day, 0.132 mg/hen·day, and 0.176 mg/hen·day, respectively. For FCR at 16 weeks of production, the optimal values of iOM, iSe, and iCr were 2.81 g/hen·day, 0.132 mg/hen·day, and 0.105 mg/hen·day, respectively (Table 7).

To reach a maximum D of 0.827, corresponding to 89.1% of EP and 1.94 kg feed/kg egg of FCR, levels of 3.3 g/hen·day of iOM, 0.132 mg/hen·day of iSe, and 0.176 mg/hen·day of iCr are required (Table 7). This maximum desirability coincides with the nutrient values of diet 23 (Table 3).

Table 7 - Optimal requirements of mixture of oils (iOM), organic selenium (iSe), and organic chromium (iCr) between 4 and 16 weeks of production (94 and 106 weeks old) for Isa Brown SCLH considering egg production (EP), feed conversion ratio (FCR), and overall desirability (D) as optimization criteria

Criterion	t (weeks)	EP (%)	FCR (kg feed/kg egg)	D	iOM (g/hen·day)	iSe (mg/hen·day)	iCr (mg/hen·day)
Maximize EP	6	82.6	2.12	-	3.30	0.132	0.176
	8	86.4	1.97	-	3.30	0.124	0.176
	12	88.0	1.94	-	3.30	0.132	0.176
	16	89.1	1.94	-	3.30	0.132	0.176
Minimize FCR	6	81.4	2.03	-	3.30	0.132	0.085
	8	85.1	1.96	-	3.13	0.132	0.083
	12	86.8	1.94	-	2.93	0.132	0.093
	16	87.9	1.94	-	2.81	0.132	0.105
Maximize D	6	82.0	2.04	0.723	3.30	0.132	0.121
	8	86.4	1.97	0.789	3.30	0.131	0.176
	12	88.0	1.94	0.814	3.30	0.132	0.176
	16	89.1	1.94	0.827	3.30	0.132	0.176

3.4. Sensitivity analysis

Using the Latin hypercube as the sampling strategy, the convergence test allowed to establish a sample size of 2×10^6, from which the estimation of the sensitivity indices was independent of the number of simulations. The models used did not present disadvantages when calculating the sensitivity indices for this large sample size. Such sample size and the resampling strategy (n = 100) made it possible to calculate statistically significant sensitivity indices at a 95% confidence level for all inputs (iOM, iSe, iCr) and outputs studied (EP, FCR, D), as indicated by the confidence intervals (Table 8).

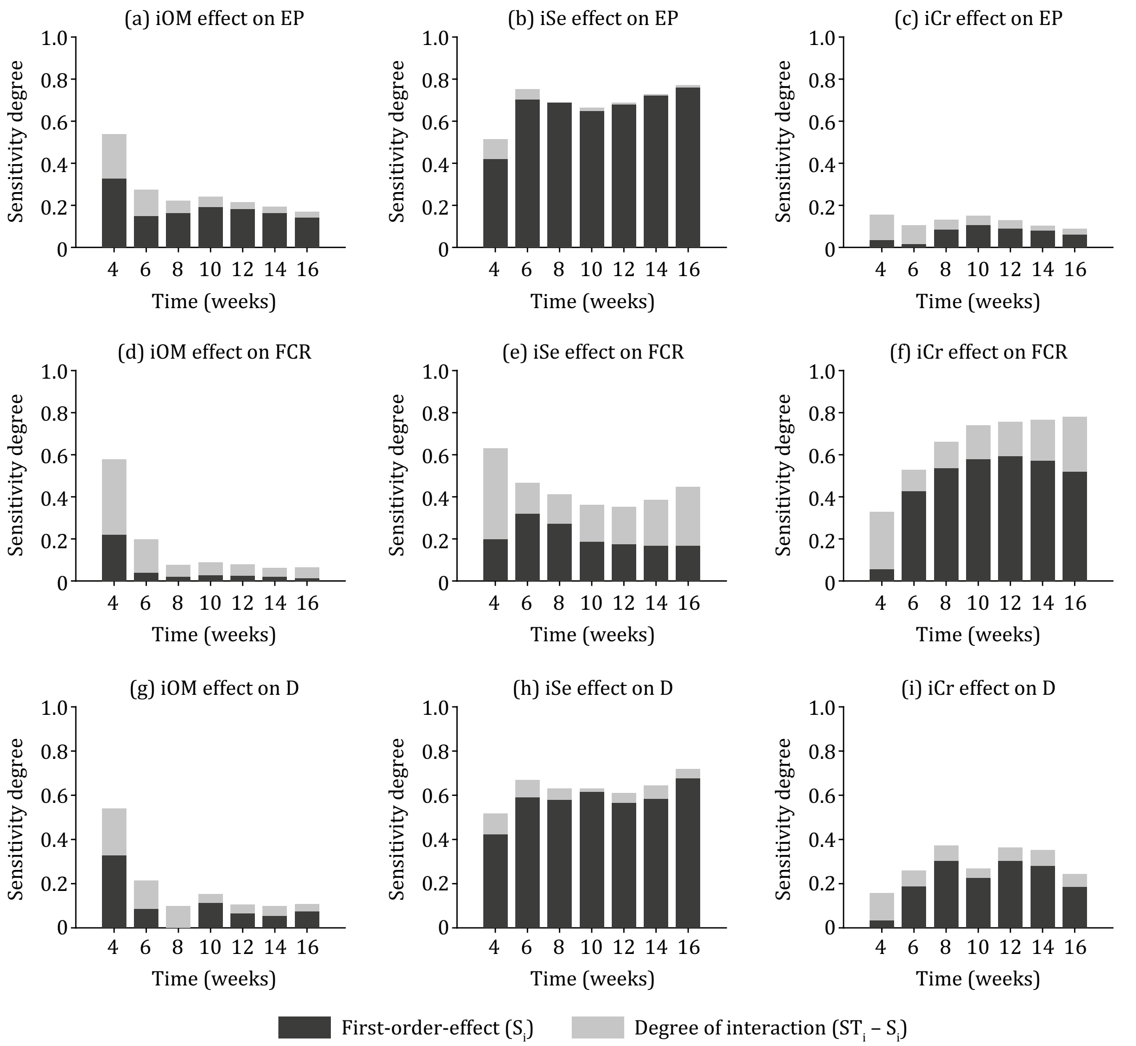
Table 8 - First-order indexes (S_i) and total-order indexes (S_{Ti}) for the effect of mixture of oils (iOM), organic selenium (iSe), and organic chromium (iCr) over egg production (EP), feed conversion ratio (FCR), and overall desirability (D) between 4 and 16 weeks of production (94 and 106 weeks old) for Isa Brown SCLH

Output	Input (i)	t (weeks)	First-order-effect index (S_i)	Total-order-effect index (S_{Ti})
EP	iOM	4	0.330±0.010 (0.328, 0.333)	0.542±0.018 (0.537, 0.547)
		8	0.165±0.024 (0.159, 0.172)	0.223±0.036 (0.213, 0.233)
		12	0.185±0.018 (0.179, 0.190)	0.218±0.034 (0.209, 0.228)
		16	0.145±0.011 (0.142, 0.148)	0.174±0.019 (0.169, 0.180)
EP	iSe	4	0.423±0.009 (0.420, 0.426)	0.517±0.019 (0.511, 0.522)
		8	0.692±0.021 (0.686, 0.698)	0.685±0.038 (0.675, 0.696)
		12	0.684±0.020 (0.679, 0.690)	0.692±0.039 (0.680, 0.703)
		16	0.762±0.012 (0.758, 0.765)	0.772±0.022 (0.766, 0.779)
EP	iCr	4	0.034±0.009 (0.031, 0.036)	0.156±0.019 (0.150, 0.161)
		8	0.087±0.023 (0.081, 0.094)	0.133±0.037 (0.123, 0.144)
		12	0.094±0.016 (0.090, 0.099)	0.131±0.031 (0.122, 0.139)
		16	0.062±0.013 (0.059, 0.066)	0.088±0.023 (0.082, 0.095)
FCR	iOM	4	0.226±0.009 (0.224, 0.228)	0.586±0.015 (0.583, 0.589)
		8	0.024±0.021 (0.020, 0.028)	0.079±0.042 (0.070, 0.087)
		12	0.027±0.034 (0.020, 0.033)	0.081±0.066 (0.068, 0.094)
		16	0.015±0.051 (0.005, 0.025)	0.068±0.101 (0.048, 0.088)
FCR	iSe	4	0.203±0.009 (0.202, 0.205)	0.640±0.015 (0.637, 0.643)
		8	0.278±0.027 (0.272, 0.283)	0.417±0.043 (0.408, 0.426)
		12	0.180±0.036 (0.173, 0.187)	0.358±0.062 (0.345, 0.370)
		16	0.174±0.065 (0.161, 0.186)	0.452±0.100 (0.432, 0.471)
FCR	iCr	4	0.059±0.009 (0.058, 0.061)	0.334±0.015 (0.331, 0.337)
		8	0.542±0.030 (0.536, 0.548)	0.668±0.049 (0.659, 0.678)
		12	0.599±0.037 (0.591, 0.606)	0.766±0.063 (0.754, 0.779)
		16	0.527±0.053 (0.516, 0.537)	0.787±0.089 (0.769, 0.804)
D	iOM	4	0.330±0.010 (0.328, 0.333)	0.542±0.018 (0.537, 0.547)
		8	0.032±0.007 (0.030, 0.033)	0.096±0.012 (0.094, 0.098)
		12	0.064±0.005 (0.063, 0.065)	0.106±0.008 (0.104, 0.107)
		16	0.074±0.006 (0.073, 0.075)	0.106±0.011 (0.103, 0.108)
D	iSe	4	0.423±0.009 (0.420, 0.426)	0.517±0.019 (0.511, 0.522)
		8	0.576±0.006 (0.574, 0.577)	0.630±0.012 (0.627, 0.632)
		12	0.563±0.004 (0.562, 0.564)	0.608±0.008 (0.606, 0.609)
		16	0.678±0.005 (0.677, 0.679)	0.718±0.011 (0.716, 0.720)
D	iCr	4	0.034±0.009 (0.031, 0.036)	0.156±0.019 (0.150, 0.161)
		8	0.301±0.006 (0.300, 0.303)	0.371±0.012 (0.369, 0.374)
		12	0.302±0.004 (0.302, 0.303)	0.361±0.007 (0.359, 0.362)
		16	0.187±0.006 (0.186, 0.188)	0.241±0.011 (0.239, 0.243)

SCLH - second-cycle laying hens.
Values are expressed as the means±standard deviations (n = 100).
The 95% confidence intervals for each of the sensitivity indices are presented in parentheses.

The sensitivity analysis for EP between 4 and 16 weeks of production from the modified logistic model (Figures 4a-c) showed that the most crucial factor was iSe, followed by iOM and iCr. A similar analysis for FCR from the ANN model (Figures 4d-f) showed that iCr was the most important factor, followed by iSe and iOM. When performing the sensitivity analysis for EP and FCR simultaneously, using global desirability (Figures 4g-i), the most influential factor turned out to be iSe, followed by iCr and iOM.

Selenium intake was the most critical factor for EP since its contribution to the variance between 4 and 16 weeks of production ranged between 42.3 and 76.2% by itself (S_i) and between 51.7 and 77.2% when interacting with the other factors (S_{Ti}) (Table 8). The effect of iSe on EP increased over time with S_i and S_{Ti} values greater than 0.69 from the eighth week of production (Figure 4b). Only during the 4th week, iOM showed the most significant contribution to the variance of EP (Figure 4a), which reached 54.2% when considering the effects of total order, that is, the effect of iOM and its interaction with iSe and iCr. The response to the variation in EP was mainly due to first-order effects, pointing to iSe as the main responsible for the increase in production in laying hens. It is worth saying that the total order effect of iCr decreased from 15.6% in week 4 to 8.8% in week 16, with the first-order effect between 3.4 and 6.2%, respectively (Table 8).



SCLH - second-cycle laying hens.

Figure 4 - Global sensitivity analysis to evaluate the effect of mixture of oils (iOM), organic selenium (iSe), and organic chromium (iCr) on egg production (EP), feed conversion ratio (FCR), and overall desirability function (D) between 4 and 16 weeks (94 and 106 weeks old) for Isa Brown SCLH.

In the case of FCR, the total order effects attributed to iCr were increasing, presenting their most outstanding contribution between weeks 8 and 16 with values between 66.8 and 78.7%, respectively (Table 8). In the 4th week, the most important factor was iSe, with a first-order effect of 20.3% and a total order effect of 64.0% (Figure 4e). However, iCr had the most significant effect on the variance of FCR from week 8, as shown by its first-order effects greater than 52%. The variation in FCR would mainly be due to the first-order effect of iCr, which would be responsible for lower conversions for the studied diets. It is important to note that the total order effect of iOM was descending from week 4 to 16, with first-order effects between 22.6 and 1.5% (Table 8).

In the sensitivity analysis for D, iSe was the factor with the highest total order effects between weeks 8 and 16, of 63.0 and 71.8%, respectively (Table 8). The iOM was the most important factor in week 4, with a total order effect of 54.2% (Figure 4g). In comparison, iSe presented the most significant influence between weeks 8 and 16 with 63.0 and 71.8% total order effects, respectively (Figure 4h). The main responsible for D variation was iSe, which can increase EP and decrease FCR in birds. It is important to note that the influence of iOM was low from week 8 to week 16, with first-order effects between 3.2 and 7.4%, respectively (Table 8).

In summary, when we analyze the effect on EP and D, iOM was the most critical factor in week 4, while iSe was the most influential from week 8. On the other hand, considering the variability of FCR, iSe was the most crucial factor in week 4, while iCr became the most influential factor from week 8 (Figure 4, Table 8).

4. Discussion

4.1. Productive parameters

The best results of the study were associated with diet 23 (Table 4), which presents the highest iOM, iSe, and iCr levels in the design (3.300±0.363 g/hen·day, 0.132±0.015 mg/hen·day, 0.176±0.019 mg/hen·day, respectively).

With a dietary level of 0.132 mg/hen·day of iSe, EP between 78.6 and 88.1% was achieved (Table 4, diet 23). These results are higher than those of Laika and Jahanian (2015) in Hy-Line W-36 laying hens of 47 weeks of age (EP = 82.5%) using 0.2 mg/kg feed of L-selenium methionine, that is, 0.021 mg/hen·day. The results were also better than those reported by Jing et al. (2015) in Hy-Line Brown laying hens of 19-43 weeks of age (EP = 84.3%) with 0.300 mg/kg feed of selenium methionine (0.023 mg/hen·day).

Dietary selenium supplementation is key to maintaining animal health and productive performance (Surai et al., 2018). A decrease in productive performance and health could be related to the overproduction of free radicals, which leads to oxidative stress and compromises antioxidant defenses in poultry (Surai et al., 2019). Natural antioxidants are essential in maintaining bird health and productive performance in commercial layers (Surai and Kochish, 2019). Selenium plays a sparing role in vitamin E by improving its absorption in the intestine and protecting the fats of the cell membrane from oxidative damage (Mir et al., 2018). While vitamin E is soluble in lipids and performs its antioxidant action on the cell membrane, Se is soluble in water and acts inside the cell (Macari and Maiorka, 2017).

An intake of 0.172 mg/hen·day of iCr allowed FCR values between 1.85 and 2.13 kg feed/kg egg for all treatments at 16 weeks of production (Table 4). These results were lower than those recorded by Siloto (2014) (FCR = 1.93 kg feed/kg egg), using 0.0459 mg/hen·day of chromium yeast in 47-week-old Bovans White layers, and Mirfendereski and Jahanian (2015) (FCR = 2.09 kg feed/kg egg), using 0.107 mg/hen·day of chromium methionine in 26-weeks-old Hy-Line W-36 layers.

The most important effect on feed conversion is attributed to Cr because, as a component of the glucose tolerance factor, it increases insulin secretion and decreases glucose and serum glucocorticoids. It is becoming an energy source for protein synthesis due to glucose conversion, increasing the diet energy efficiency, and maintaining productivity (Siloto, 2014). The positive effects of Cr on plasma protein and its fractions are attributed to the anabolic action of insulin, which is mediated by the increased amino acid synthesis in the liver and improved incorporation of various amino acids into the protein (Torki et al., 2014).

4.2. Mathematical models

In general, all the models used to represent EP fit well. However, the modified logistic model presented the best goodness of fit to the field data compared with the other models and could be used to predict EP for Isa Brown SCLH as a function of time and levels of iOM, iSe, and iCr.

Different authors have reported satisfactory results in the mathematical representation of EP (R^2_{adj} > 0.94) with non-linear models of egg production similar to those used in this study for broiler breeders (Otwinowska-Mindur et al., 2016; Safari-Aliqiarloo et al., 2017). However, in these studies, the models did not include the effect of nutritional requirements; they only considered EP as a function of time. In this sense, only the work of Morales-Suárez et al. (2021) has considered the evaluation of different non-linear models for egg production in terms of four factors (three essential amino acids and time). These authors reported that the modified logistic model was the best option within the models conventionally used to represent the production curve, 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 data sets, respectively.

To our knowledge, this is the first study to use non-linear models to study the influence of iOM, iSe, and iCr on Isa Brown SCLH. The proposed models consider a complex interactive effect of nutritional requirements on the productive performance of the birds. The proposed methodology would study the interactions of multiple nutritional requirements on productive parameters, both for its application in poultry and other animal production areas.

The modified logistic model (Eqs. 4, 25-28) was used to estimate EP (Figure 5) in the range of conditions studied. Estimated EP values between 6 and 16 weeks of production were between 73.6 and 88.2%. These estimates show that EP improves with high iOM, iSe and iCr levels, reaching values greater than 80% between weeks 6 and 16 when the highest levels of such requirements are used. With levels of 3.3 g/hen·day of iOM, 0.132 mg/hen·day of iSe, and 0.176 mg/hen·day of iCr, 88% of EP could be reached between weeks 12 and 16 of production (Figure 5).

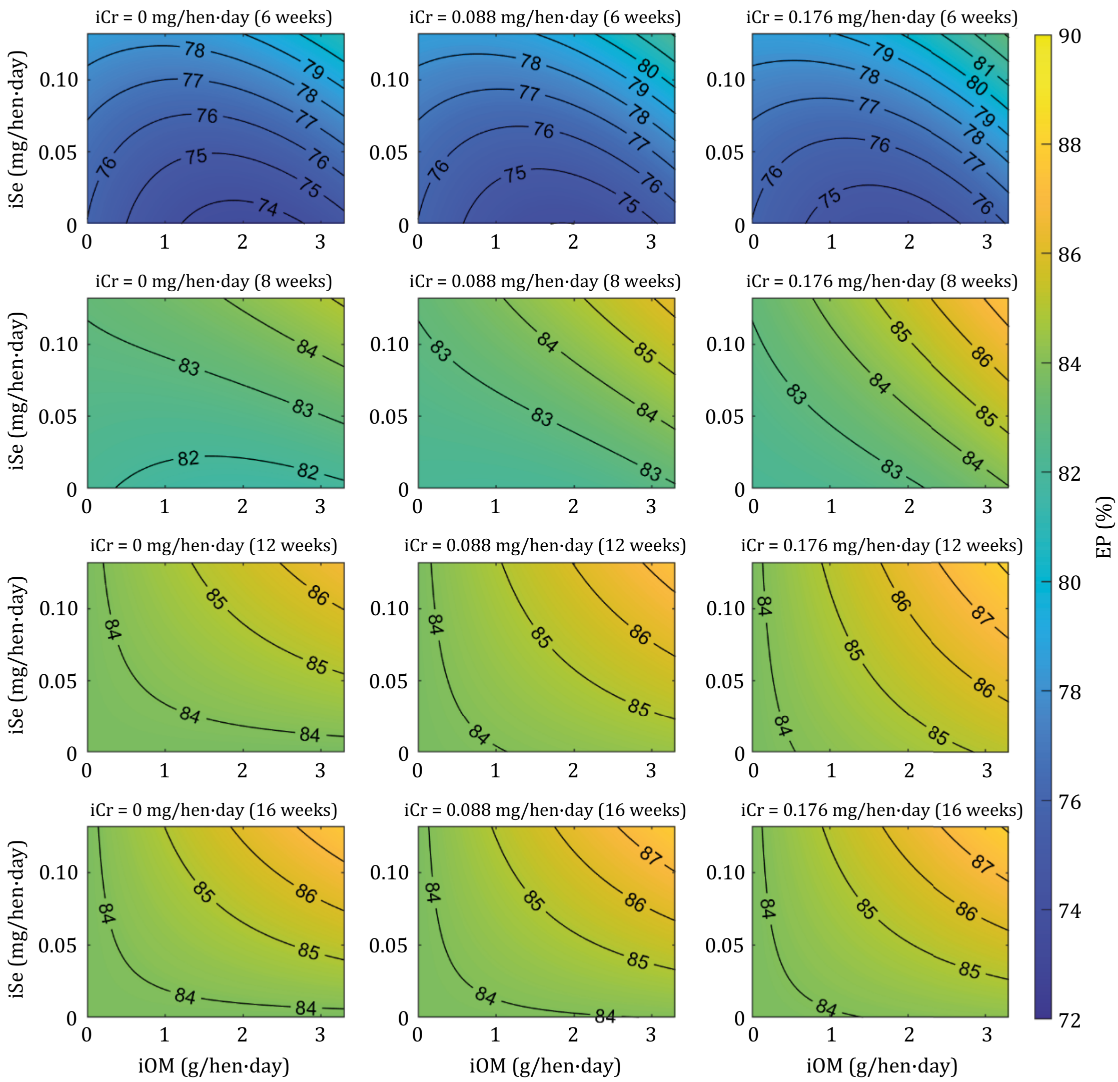
To our knowledge, there are no reports of mathematical models for the representation of FCR in layers that consider multiple nutritional factors in addition to time.

When using the ANN model (Eqs. 7, 11; Table 6) to estimate FCR (Figure 6), the values improve with high iOM, iSe, and iCr. Some diets with levels from 1.82 g/hen·day of iOM, 0.06 mg/hen·day of iSe, and 0.088 mg/hen·day of iCr allow obtaining FCR lower than 2.08 kg feed/kg egg from the sixth week of production. As of week 8, combinations of iOM, iSe, and iCr allow FCR of less than 2.00 kg feed/kg egg. From week 12, the FCR values improve between 1.91 and 1.98 kg feed/kg egg. Thus, with levels of 3.3 g/hen·day of iOM, 0.132 mg/hen·day of iSe, and 0.176 mg/hen·day of iCr, an FCR of 1.91 mg/hen·day could be guaranteed between 12 and 16 weeks (Figure 6).

Although different studies have been carried out in poultry, there are discrepancies in iOM, iSe, and iCr requirements in SCLH diets for different studies. The interaction between the evaluated factors may be an essential factor of variation in these estimates, corroborating this study.

4.3. Optimization

The overall desirability function (D) can facilitate the decision-making task by providing optimal iOM, iSe, and iCr levels that maximize EP and minimize FCR in Isa Brown SCLH, turning a multi-objective optimization problem into a single-criterion one (Mehri et al., 2016). In weeks 8 and 16, the maximum desirability was 0.789 and 0.827, respectively, with levels of 3.3 g/hen·day of iOM, 0.132 mg/hen·day of iSe, and 0.176 mg/hen·day of iCr. This maximum desirability coincides with the nutrient values of diet 23 (Table 3).



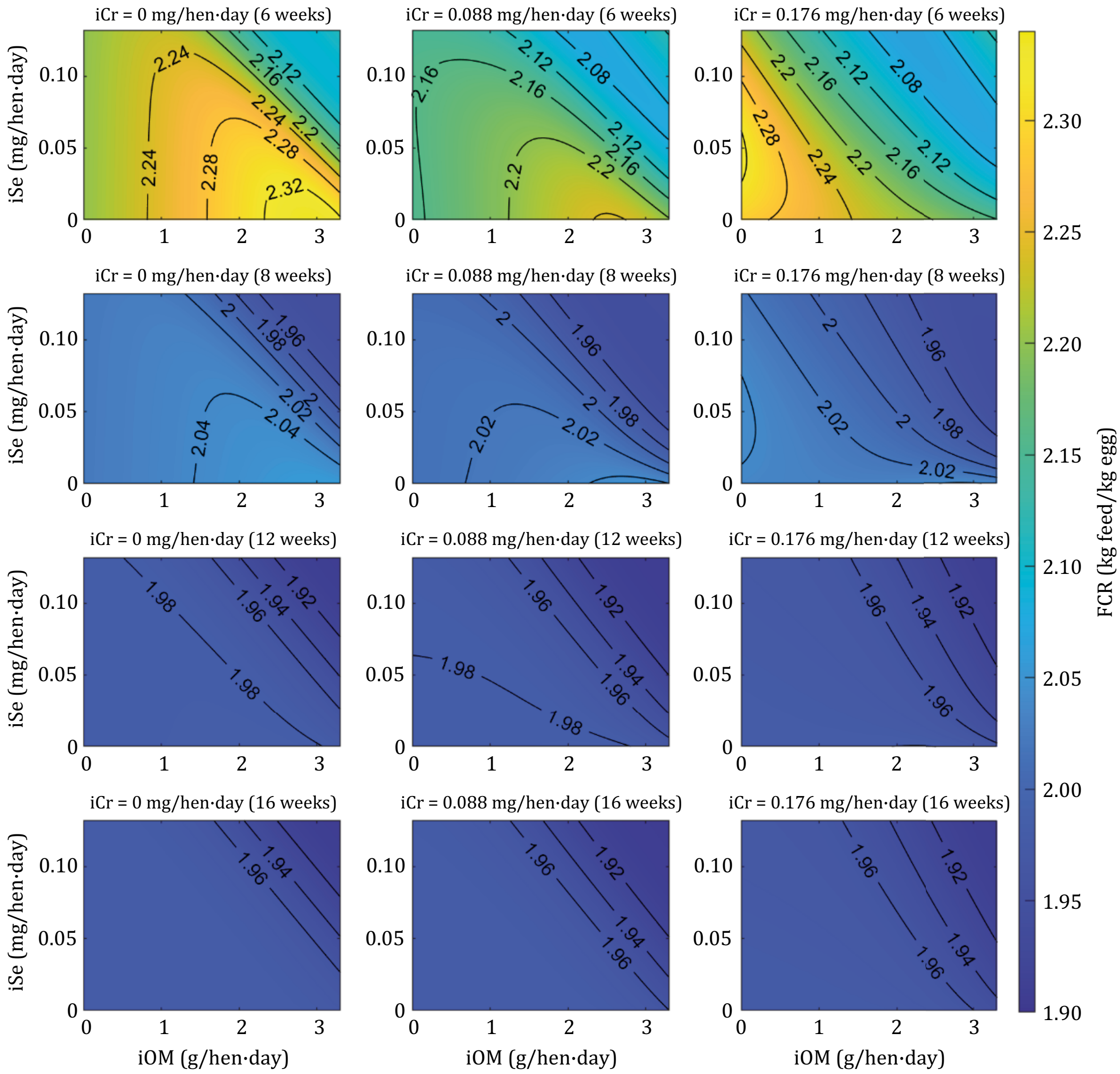
SCLH - second-cycle laying hens.

Figure 5 - Effect of intakes of mixture of oils (iOM), organic selenium (iSe), and organic chromium (iCr) on egg production (EP) at 6, 8, 12, and 16 weeks (96, 98, 102, and 106 weeks old, respectively) for Isa Brown SCLH.

The optimal iOM levels at 16 weeks of production (91-106 weeks of age) for Isa Brown SCLH were 3.3 g/hen·day and allowed a maximum EP of 89.12% (Table 7), higher than the results obtained by Saleh (2013) (EP = 86%) with 1.24 g/hen·day of a mixture of oils (1.25% fish oil + 3.75% vegetable oil) in 23-week-old white line Bovans layers. On the other hand, Küçükersan et al. (2010) reached EP values of 82.7 and 82.1% using 3.2 g/hen·day of soybean oil (3%) and 3.1 g/hen·day of fish oil (3%) in 36-week-old Hissex Brown layers. No reports were found where oil mixtures with selenium or chromium supplementation in the diets were simultaneously evaluated.

Optimal iOM levels of 3.3 g/hen·day attained a minimum FCR of 1.94 kg feed/kg egg in Isa Brown SCLH at 16 weeks of production (106 weeks of age) (Table 7). These results were lower than those achieved by Küçükersan et al. (2010) using soybean oil (FCR = 2.02 kg feed/kg egg) and fish oil (FCR = 2.03 kg feed/kg egg) in diets of Hissex Brown hens.

An estimated level of 0.1320 mg/hen·day of selenium yeast could be necessary to achieve the best EP (89.1%) and the lowest FCR (1.94 kg feed/kg egg) in Isa Brown SCLH between 4-16 weeks of production (91-106 weeks of age) (Table 7). This level is higher than recommended when comparing responses to different sources and doses of selenium in laying hens.



SCLH - second-cycle laying hens.

Figure 6 - Effect of intakes of mixture of oils (iOM), organic selenium (iSe), and organic chromium (iCr) on feed conversion ratio (FCR) at 6, 8, 12, and 16 weeks (96, 98, 102, and 106 weeks old, respectively) for Isa Brown SCLH.

Delezie et al. (2014) suggested 0.0117 mg/hen·day of selenium yeast in 55-67-week-old semi-heavy Lohman Brown laying hens to achieve EP of 89.1% and FCR of 2.00 kg feed/kg egg. Jing et al. (2015) achieved EP of 90.4% using 0.0359 mg/hen·day of selenium yeast in semi-heavy Hy-Line Brown hens. Payne et al. (2005) reported EP values of around 81% with very different selenium yeast intakes (0.0601 and 0.2997 mg/hen·day) in Hy-Line W-36 hens.

The optimal iCr level for a maximum EP of 89.1% at 16 weeks of production in Isa Brown SCLH was 0.176 mg/hen·day of chromium yeast. This level was higher than the recommendations of 0.1723 mg/hen·day of chromium yeast in Bandarah laying hens between 36-44 weeks of age (EP = 64.8%) (Hanafy, 2011).

The optimal iCr level for a minimum FCR of 1.94 kg feed/kg egg was 0.105 mg/hen·day of chromium yeast in Isa Brown SCLH at 16 weeks of production. This result was higher than the 0.0917 mg/hen·day of chromium yeast obtained in Bovans Light laying hens (FCR = 1.94 kg feed/kg egg) (Siloto, 2014).

The optimal level of iCr when considering the desirability function as a criterion is comparable with the results for EP, in which 0.176 mg/hen·day of chromium yeast would be recommended to reach the maximum EP from the eighth week. However, the optimization using D increases EP by 1.4% while FCR values are similar, compared with the optimization in which FCR is the only criterion (Table 7). Optimal iCr requirements are 51.7 and 40.3% lower when considering FCR as an optimization criterion in weeks 6 and 16, respectively. This must be considered as it would affect the cost of using organic chromium in the formulation.

4.4. Sensitivity analysis

In the first four weeks of production, iOM has the most significant effect on EP and D, reaching a total order effect of 54.2% (Table 8). The increase in the intake of ω-3 PUFA due to the incorporation of fish and vegetable oils in the diets (Pappas et al., 2005) is related to the increase in FA in membrane phospholipids, which are expected to decrease inflammatory responses (Nassef et al., 2019).

The PUFA addition reduces the ω-6:ω-3 ratio and increases productivity due to the alteration of the fatty acid profile of the liver tissue that can influence the metabolism and synthesis of other nutrients as a consequence of the altered partition of nutrients by reducing the inflammatory response (Alagawany et al., 2019).

The most significant effect on EP is achieved with iSe, followed by iOM, and finally iCr. After eight weeks of production, the most crucial factor in maximizing EP was iSe, reaching a total order effect of 77.2% in week 16. The first-order effect of iSe was high between weeks 8 and 16, from 69.2 to 76.2%, respectively, indicating that the intake of organic selenium could increase EP (Table 8).

The most significant effect on FCR in the fourth week was produced by iSe, reaching a total order effect of 64% (Table 8). Selenium and PUFA also show a high degree of interaction (43.7 and 36.0%, respectively) at week 4 (Figures 4e and d, respectively), these two can interact, possibly through the action of the GSH-PX enzyme, which plays an essential role in the regulation of prostaglandin biosynthesis from its precursor arachidonic acid, responsible for the inflammatory response (Pappas et al., 2005).

The most significant effect on FCR is achieved with iCr, followed by iSe, and finally iOM. The most important factor in minimizing the FCR after eight weeks of production is the iCr, reaching a total order effect of 78.7% in week 16 of production. The first-order effect of iCr was significant for FCR between weeks 8 and 16 by 54.2 and 52.7%, respectively, indicating that the intake of chromium yeast was responsible for reducing FCR (Table 8).

Chromium can exert a protective effect that improves the functioning of the pancreas concerning the secretion of digestive enzymes, which increase the retention of nitrogen, minerals, and the use of nutrients (Khan et al., 2014). Chromium participates in the metabolic pathways of nutrients (carbohydrates, lipids, proteins, and nucleic acids) by enhancing the action of insulin, which contributes to the anabolic profile (Sahin et al., 2018). Likewise, it alleviates oxidative stress by reducing lipid peroxidation, increasing the activities of glutathione peroxidase and glutathione reductase in plasma (Khan et al., 2014; Gitoee et al., 2018).

Regarding D, the factors with the most significant effect were iSe, followed by iCr, and finally iOM. In the fourth week, the most significant effect on D was produced by iOM, reaching a total order effect of 54.2% (Table 8). However, the most important factor in maximizing D from the eighth week was iSe, which reached a total order effect of 71.8% in the 16th week. The first-order effect increased from 57.6 to 67.8% between weeks 8 and 16, respectively, indicating that iSe contributes to the increase in EP and the decrease in FCR (Table 8).

Most of the stressors in poultry production at the cellular level are associated with oxidative stress due to excess free radical production or inadequate antioxidant protection (Surai and Fisinin, 2016b). The antioxidant defense network is responsible for maintaining low basic levels of oxygen-derived free radicals, eliminating them, and converting them into non-toxic products (Surai et al., 2019). The first level of defense of the antioxidant system of cells is composed of the enzymes GSH-Px and superoxide dismutase, considered selenoproteins, and the second level of defense focuses on the prevention and restriction of the formation of chains and radical propagation (Mir et al., 2018; Surai et al., 2019).

The search for nutritional approaches to modulate the antioxidant system, including the use of Se, vitamin E, and polyphenols, is on the agenda of many research groups in the world. The modulation of vitagene (adaptation to stress at the molecular level is mediated by a variety of genes) by nutritional ways (minerals, vitamins, among others) could be a new strategy to prevent stress and maintain the high productive performance of poultry (Surai, 2020).

5. Conclusions

Through the application of mathematical models, optimization strategies, and sensitivity analysis, alternative sources of fatty acids from fish oil and sacha inchi, and the supplementation with organic selenium and chromium in diets of Isa Brown hens during the second cycle increase the egg production and decrease the feed conversion ratio.

As far as we know, there is no information available regarding the study of multiple nutritional factors via optimization and multivariate analysis. Therefore, this work constitutes a methodological reference to improve the productive performance of commercial laying hens based on multiple nutrients.

The sensitivity analysis shows that the interaction of the oil mixture (fish and sacha inchi oils), selenium yeast, and chromium yeast increases egg production and decreases the feed conversion ratio. Selenium is crucial for increasing egg production, and chromium is essential to reduce feed conversion in Isa Brown hens during the second cycle.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: W. Morales-Suárez, S. Elliott and H.A. Váquiro-Herrera. Data curation: H.A. Váquiro-Herrera. Formal analysis: W. Morales-Suárez and H.A. Váquiro-Herrera. Funding acquisition: S. Elliott. Investigation: W. Morales-Suárez and H.A. Váquiro-Herrera. Methodology: W. Morales-Suárez and H.A. Váquiro-Herrera. Project administration: H.A. Váquiro-Herrera. Resources: S. Elliott. Software: H.A. Váquiro-Herrera. Supervision: H.A. Váquiro-Herrera. Writing-original draft: W. Morales-Suárez and H.A. Váquiro-Herrera. Writing-review & editing: W. Morales-Suárez, S. Elliott and H.A. Váquiro-Herrera.

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