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Optimizing Apparent Metabolizable Energy and Digestible Amino Acids of Layer Feed by Response Surface Methodology

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ABSTRACT

Optimizing dietary energy and amino acid levels is essential for enhancing the performance and cost-efficiency of laying hens. The primary goals were to identify the optimal apparent metabolizable energy (AMEn) and digestible lysine (dLys) levels that maximize hen-day production (HDP) and egg weight (EW) while minimizing feed conversion ratio (FCR) and FC. A total of 150 cages, each housing five hens, were used. Hens with 24 weeks of age were assigned to ten dietary treatments following a central composite design (CCD), with AMEn levels ranging from 2,400 to 2,733 kcal/kg and dLys from 0.42% to 1.02%. The experiment was carried out during peak production (24 to 38 weeks of age). Response surface methodology (RSM) combined with CCD effectively optimized AMEn and dAA levels, providing practical insights for formulating cost-effective diets for commercial laying hens. Dietary dLys significantly influenced HDP and EW, while AMEn affected feed intake (FI). The optimal conditions, determined by the desirability function (DF), were AMEn of 2,660 kcal/kg and dLys of 0.81%, maximizing HDP and EW with minimal FCR and FC. Excluding EW from DF optimization, the best results were achieved at AMEn of 2,623 kcal/kg and dLys of 0.78%.

Keywords: amino acid; energy; layer; response surface

INTRODUCTION

Due to the high cost of poultry feed, there is a constant pressure to develop cost-effective diets that still fulfill nutritional needs (Moss *et al*., 2021). This approach maximizes the efficient use of raw materials, reduces production costs, and mitigates nutrient losses such as nitrogen and phosphorus into the environment. In least-cost feed formulations for layer hens, amino acids and energy density are essential components. However, amino acid requirements vary based on breed, age, feeding strategies, housing conditions, and the precision of requirement assessments (Macelline *et al*., 2021).

Existing feed formulation methods focus on cost minimization rather than profit maximization. Although sophisticated models linking bird growth and reproduction to genetic, dietary, and environmental factors are currently in development, their implementations have been gradual. Future progress in profit-maximizing models will likely focus on the production functions of broilers and layers, which define the relationship between the value of outputs (mainly meat and eggs) and feed-related costs (Pesti & Choct, 2023). Achieving efficient and highly productive flocks requires optimizing the balance between energy intake and expenditure. Understanding how this balance relates to production traits, such as energy consumption and feed intake, is essential for designing chicken diets and determining appropriate levels of other dietary nutrients (Classen, 2017).

Improving diet composition is crucial to promoting animal health and welfare while boosting livestock productivity (Jian *et al*., 2021). The right balance of amino acids in the feed is a key factor that directly influences brown egg layers' productive performance and feeding costs, making it a crucial aspect of feed formulation. Optimizing protein supply for these hens necessitates a comprehensive understanding of the metabolic utilization of proteins and amino acids, enabling the development of feeding programs more precisely to diverse environmental and health conditions (Soares *et al*., 2018). Optimal feeding for hens occurs when an extra input unit's expense matches the additional output unit. This gives the lowest feeding cost per kilogram of egg. Any further increase in balanced amino acid input yields egg output that is less valuable than the feed itself. A minimum-cost feed formulator was integrated into the model, along with an economic optimization routine designed to maximize the financial return of feed production (Rein *et al*., 2023).

Long-term egg production depends on nutrient availability and is regulated by the ovary and oviduct for egg development, the liver for nutrient metabolism, and body fat reserves, which provide additional nutrients and may influence reproductive potential (van Eck *et al*., 2023). The metabolizable energy and standardized ileal digestible lysine (dLys) levels in the diets are two critical factors influencing both feed expenses and egg production in commercial laying hens (Scappaticcio *et al*., 2021). Numerous researchers have investigated the dLys requirements for laying hens, resulting in widely varying recommendations (Scappaticcio *et al*., 2022). Laying hen diets in commercial farms are often formulated based on the ideal protein concept, where dLys, the second-most limiting amino acid, acts as the benchmark. As a result, determining dLys requirements accurately is essential for optimizing egg production (Spangler *et al*., 2018), optimizing production costs, and minimizing nitrogen excretion (Kumar *et al*., 2018).

Poultry researchers often encounter the challenge of examining the relationship among multiple quantitative variables to optimize a desired outcome. Response surface methodology (RSM) is a set of statistical and mathematical tools that are highly effective for enhancing, refining, and optimizing processes. It is also widely used in the creation, development, and formulation of new products, as well as in improving the design of existing ones (Myers *et al*., 2016). RSM, which is extensively applied in various disciplines, strikes a balance between precision and practicality, making it particularly suitable for evaluating complex parameter performance (Al-Radhi *et al*., 2025).

The desirability function (DF) is a tool for concurrently optimizing multiple continuous response variables, as established by Derringer and Suich (1980). This method entails initially estimating individual responses using multiple regression techniques, followed by transforming each estimate into a desirability score. These individual scores are then aggregated using the geometric mean to derive an overall desirability, serving as the performance metric (Derringer and Suich,1980). Optimizing each individual response can yield as many distinct outcomes as there are responses, a factor that has been considered in this study. Various multi-response optimization techniques exist, among which desirability-based approaches stand out due to their simplicity, ease of understanding and implementation, and greater flexibility compared to the other methods. These advantages have made them highly popular among researchers and practitioners (Marinković, 2020). The objective of this study was to determine the optimum nutrient levels based on performance and feeding cost that match each of the different feeding scenarios for a feed company by using RSM followed by DF.

MATERIALS AND METHODS

Ethical Approval

The protocols for all animal experiments were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine at IPB University and were carried out in strict compliance with their guidelines (053/KEH/SKE/XII/2022).

Experimental Design

An experiment was carried out during peak production (24 to 38 weeks of age) with a 2-factor 5-level Central Composite Design (CCD) to determine the nitrogen-corrected apparent metabolizable energy ($AMEn_{(layer)}$) and dLys values that generate optimum performance (Table 1). The experiment consisted of ten dietary treatments, as outlined in Table 2, which were formulated based on nine different levels of $AMEn_{(layer)}$ and dLys, calculated based on RSM. Dietary treatments 1 to 4 are known as factorial runs, dietary treatments 5 to 8 are known as axial runs, while treatments 9 and 10 are known as the center points. Dlys level was used to set all other amino acids based on the Hy-Line ideal protein. $AMEn_{(layer)}$ values ranged from what is normally fed to brown layers in a tropical country with higher and lower levels to elicit a quadratic response. In this study, formula modifications were made to AMEn and dLys based on the nutritional requirements of Hy-Line Brown at its respective age, using the central composite design (CCD) method with the assistance of Design Expert 10 software. The levels obtained from the CCD method, after inputting the low and high levels for each factor, resulted in five levels $AMEn_{(layer)}$ (2,400; 2,457; 2,595; 2,733; and 2,790 kcal/kg) and dLys (0.42%, 0.51%, 0.72%, 0.93%, and 1.02%). The other amino acids were set according to the requirements of laying hens.

Birds and Housing

The experiment involved 750 Hy-Line Brown laying hens, aged between 22 and 38 weeks, which were housed in a closed-house system at Nugen Research Farm, Lebak Regency, Banten Regency, Indonesia. The experiment was conducted with 10 treatments, each treatment divided into 5 replicates, and each replicate consisted of 15 hens. The average house temperatures were set between 27–28 °C for each test automatically. Birds had *ad libitum* access to water and the experimental diets. Sixteen hours of light per day were provided for the hens during this study.

Dietary Treatments

All dietary treatments were prepared in a feed mill specialized in producing experimental diets. The formula consisted of corn, soybean meal, full-fat soybean, distiller's dried grains with solubles (DDGS), palm kernel meal (PKM), wheat bran, and rice bran. Their nutrient specifications were analyzed using near-infrared spectroscopy, facilitated by the AMINOIR Advanced program (Evonik Operations GmbH, Hanau, Germany) prior to feed formulation. Near-infrared spectroscopy (NIRS) analysis was employed to determine the proximate composition of the feed ingredients, which was subsequently used to calculate the AMEn following the methods outlined in the

Table 1. Factor levels used in the central composite design (CCD) for dietary treatments

	-α (1.41)	-1 (Lower)	0 (center point)	+1 (upper)	+α (1.41)
AMEn $_{(layer)}$ (kcal/kg)	2,400	2,457	2,595	2,733	2,790
dLys (%)	0.42	0.51	0.72	0.93	1.02

Table 2. Ingredient and nutrient compositions (%) of experimental diet used in the central composite design (CCD) for laying hens

Items	Treatments									
	1	2	3	4	5	6	7	8	9	10
Ingredients composition (%)										
Corn	38.04	45.00	38.73	45.01	33.43	55.96	43.23	44.91	48.85	48.85
Soy bean meal Argentine HP	9.30	8.88	23.36	24.66	17.48	15.84	3.56	32.14	19.02	19.02
DDGS	0.96	0.70	10.00	0.70	0.70	0.70	0.70	1.62	0.70	0.70
Palm kernel meal	10.00	4.30	10.00	0.70	9.40	0.70	9.42	3.38	0.70	0.70
Full-fat soy bean	0.70	1.30	2.9	10.00	0.7.00	10.00	6.44	4.66	0.70	0.70
Crude palm oil	0	3.00	0	1.52	0	0.70	0	0.70	0	0
Rice bran	25.00	25.00	2.32	5.52	20.18	4.24	25.00	0.70	18.14	18.14
Wheat bran	5.00	0.70	1.64	0.70	7.00	0.70	0.70	0.70	0.70	0.70
Limestone medium coarse	6.19	6.19	6.19	6.19	6.19	6.19	6.19	6.19	6.19	6.19
Limestone fine coarse	3.23	3.27	3.11	3.14	3.19	3.17	3.24	3.09	3.24	3.24
Mono-dicalcium phosphate	0.49	0.54	0.43	0.6	0.48	0.67	0.53	0.6	0.54	0.54
L-Lysine HCl 78.8%	0.05	0.07	0.15	0	0.08	0	0	0.01	0.06	0.06
DL-Methionine 99%	0.07	0.08	0.27	0.28	0.19	0.17	0.02	0.32	0.18	0.18
L-Threonine 98%	0	0	0.03	0.01	0.02	0	0	0.01	0.01	0.01
L-Tryptophan 98% (L)	0	0	0.001	0	0	0	0	0	0	0
Salt (L)	0.28	0.27	0.17	0.29	0.27	0.29	0.27	0.28	0.28	0.28
Premix[a]	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Others	0.68	0.68	0.69	0.68	0.68	0.67	0.68	0.68	0.67	0.67
Phytase[b]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Nutrient composition [c]										
AMEn $_{(layer)}$ (kcal/kg)	2,457	2,733	2,457	2,733	2,400	2,790	2,595	2,595	2,595	2,595
Crude protein (%)	13.1	12.07	20.02	19.51	16.2	16.07	11.9	21.32	15.55	15.55
Calcium (%)	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7
Ingredient available Phosphorus (%)	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Digestible amino acids										
Lys (%)	0.51	0.51	0.93	0.93	0.72	0.72	0.42	1.02	0.72	0.72
Met (%)	0.26	0.27	0.54	0.54	0.41	0.39	0.21	0.6	0.4	0.4
Met+Cys (%)	0.44	0.44	0.8	0.8	0.62	0.62	0.37	0.88	0.62	0.62
Thr (%)	0.38	0.36	0.65	0.65	0.5	0.52	0.34	0.71	0.5	0.5
Trp (%)	0.12	0.11	0.19	0.2	0.16	0.16	0.1	0.22	0.15	0.15
Ile (%)	0.42	0.39	0.72	0.74	0.55	0.59	0.38	0.82	0.55	0.55
Arg (%)	0.83	0.72	1.18	1.19	1.04	0.94	0.75	1.32	0.93	0.93
Val (%)	0.53	0.49	0.81	0.81	0.66	0.66	0.48	0.89	0.64	0.64

Note [a]= Premix Provided the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D3, 3,300 IU; vitamin E, 20 IU; vitamin K, 2.5 mg; vitamin B1, 2.5 mg; vitamin B2, 5.5 mg; vitamin B6, 4.0 mg; vitamin B12, 23 mg; niacin, 30 mg; pantothenic acid, 8.0 mg; folic acid, 0.9 mg; biotin, 75 mg; choline, 110 mg; manganese, 90 mg; zinc, 80 mg; iron, 40 mg; copper, 8 mg; iodine, 1.2 mg; selenium, 0.22 mg.

[b]=Ronozyme® HiPhos 10,000 FYT, DSM. The enzyme was included at a rate of 60 g/t to supply a guaranteed minimum of 600 FYT=300FTU/kg of feed.

[c]= Determined using individual feed analysis results by NIRS facilitated by the AMINOIR Advanced program (Evonik Operations GmbH, Hanau, Germany) prior to feed formulation.

European Table of Energy Values for Poultry Feedstuffs. AMEn $_{(layer)}$ was calculated as a regression adjustment to AMEn based on the fat level of the ingredient. A regression equation AMEn + (%Fat x 12.88) – 18.1 was used to predict the AMEn $_{(layer)}$ from AMEn adjusted for fat level following the data from the CVB Booklet of Feeding Poultry (2018). Total amino acids were calculated from the regression equation based on protein, and digestible amino acids were calculated by taking the coefficient of availability times of total amino acids, as described in Evonik's AminoDat 4. The inclusion rates of supplementary vitamins, minerals (such as calcium), and other non-energy components were consistent across all diets. The enzyme phytase was included in all feeds according to the industry standard. Each diet was mixed separately and offered in mash form.

Data Collection

The experimental diets were provided *ad libitum* from 22 to 38 weeks of age, covering the peak production period. The initial two weeks of each experiment served as an adaptation phase, during which no data were collected. The egg production was recorded daily for each replication. Egg weight (EW) was recorded at the end of each week for each replication. Feed intake was measured as total feed consumption over the entire experimental period of each week on a replicate basis (Son *et al*., 2025). Hen day production (HDP) was calculated as the number of eggs by dividing the number of bird/hen days per replicate, considering mortality (Hu *et al*., 2025). Mortality was considered normal and thus not reported. Egg mass (EM) production was calculated as HDP multiplied by the average EW of the hen. Feed conversion ratio (FCR) was defined as grams of feed consumed per gram of egg mass. Feed cost (FC) was calculated as FCR multiplied by the formula price. AMEI and dLI were determined by using daily feed intake consumption (g/bird/day), AMEn $_{(layer)}$ (kcal/kg), and dLys concentration (g/kg) in each dietary treatment (Mansilla *et al*., 2022).

Statistical Analysis

A completely randomized 2-factor (AMEn $_{(layer)}$ and dLys) 5-level (-α, -1, 0, +1, + α) Central Composite Design (CCD) was employed with five replicates per dietary treatment. The CCD consists of *F* factorial points, 2 × *k* axial points that are located at a distance ± α ($\alpha = 2^{k/4} = 2^{2/4} = 1.414$; k = number of dietary treatment factors) from the center point, and NC (number of center points) center points. The factorial points were used to fit linear and interaction terms. The axial points provide additional factor levels that are helpful in estimating the quadratic term. The axial point is calculated by the mean of the upper or lower level ± α (range between the upper and lower level divided by 2). Axial point = X ± α (Range/2) (Mehri *et al*., 2012).

During the study, each experimental unit consisted of 3 stacked cages housing five birds per cage. The linear and quadratic terms of the independent variables, along with their interactions, were assessed. A polynomial regression equation was then formulated in the following form:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_1 X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j} \sum \beta_{ii} X_i X_j$$

Where *Y* is the response of interest (HDP, EW, EM, FCR, FC, AMEI, dLI, and gain), *β0* is the intercept, and *βi*, *βii*, and *βij* are the coefficients estimated by the model. Significant differences between means were determined by Tukey, and differences are considered significant at p≤0.05. Central composite design used Design Expert 10.

The desirability function (DF) was performed with Stat-Ease Design Expert.v10.0.4.0x86_SS. DF is a robust method for multi-objective optimization, particularly when dealing with conflicting factors like bird performance and cost of feeding. DF converts each response into a scale-free desirability value ranging from 0 (completely undesirable) to 1 (fully desirable). The method consists of the following steps:

1. **Individual desirability functions:** For each response Yi(x), a desirability function di(Yi) maps the possible values of Yi to a scale ranging from 0 to 1, where di(Yi)=0 indicates a completely undesirable outcome and di(Yi)=1 indicates a fully desirable outcome. This function quantifies how desirable a particular value of the response variable is, given the optimization objectives.
2. **Composite desirability function:** The individual desirability is then combined using the geometric mean, which gives the overall desirability D:
   $D=(di(Yi)d2(Y2)\dots dk(Yk))^{1/k}$

   where k denotes the number of responses. If any response *Yi* is completely undesirable *(di (Yi)=0)*, then the overall desirability is zero. Fitted response *Ŷi* models are used in place of the *Yi*. Different desirability functions di (Yi), proposed by Derringer and Suich (1980), were used depending on a particular response to be maximized, minimized, or assigned to a target value.

RESULTS

Response Surface Analysis

The bird responses to dietary treatments at 24 to 38 weeks of age are shown in Table 3. Surface response graphs were generated for performance and nutrient utilization, and with the exception of linear relationships for FI, AMEI, and dLI, the remaining parameters, such as HDP, EM, FCR, and feeding cost (FC), were quadratic. The coefficient of determination (r^2) values for HDP (r^2=0.99), EW (r^2=0.95), EM (r^2=0.98), FI (r^2=0.78), FCR (r^2=0.97), and Feed Cost (r^2=0.97), indicated that the developed models satisfactorily explained the data variation showed on Table 4.

HDP was the highest when fed the highest AMEn layer and dLys levels (Figure 1a). There was no linear effect of AMEn layer on HDP, but there was a curved response. For dLys level on HDP, both linear and quadratic responses were significant (p<0.05) (Table 5). This indicated that as a higher level of amino acids was fed, more hens could satisfy their requirement. The significant interaction (p<0.05) for dLys and AMEn layer indicates a different response at the different levels of these variables. Feeding with higher lysine promoted higher HDP at high energy values, but higher energy indicated worse performance at lower lysine levels. This indicated that feed intake was reduced at high energy levels and this limited lysine intake.

The highest EW response was at a combined maximum AMEn layer and dLys level (Figure 1b). For the dLys level on EW, both linear and quadratic responses were significant (p<0.05) (Table 5). At a low dLys value, feeding with a higher AMEn layer showed no response to the EW. On the other hand, a high dLys level needed to be combined with a higher AMEn layer level to show a significant response to the EW. There

Table 3. Experimental response value and polynomial fitted model for production performance HDP, EW, EM, FI, FCR, FC, AMEI, and dLI[1]

Treatments	Variables							
	HDP (%)	EW (g)	EM (g)	FI (g/bird/day)	FCR	FC (IDR/kg egg)	AMEI (cal/day)	dLI (g/day)
1	89.54±3.14	60.52±0.66	53.79±2.31	126.58±3.60	2.36±0.06	14,045±362	310.99±8.85	0.65±0.02
2	86.36±2.92	59.43±1.64	51.32±2.54	120.78±5.13	2.36±0.02	15,688±124	330.08±14.01	0.62±0.03
3	92.90±3.02	60.60±0.69	56.28±2.02	125.88±2.53	2.25±0.09	15,670±620	309.28±6.22	1.17±0.02
4	95.78±1.71	62.11±1.92	59.48±1.98	120.67±4.74	2.03±0.04	15,732±276	329.79±12.95	1.12±0.04
5	91.99±1.65	60.59±1.58	55.81±1.07	124.62±2.97	2.24±0.03	14,025±217	299.06±7.13	0.90±0.02
6	93.32±2.49	61.95±1.55	57.79±2.77	118.13±3.60	2.05±0.07	15,169±467	329.61±10.05	0.85±0.03
7	84.41±2.26	58.45±1.28	49.31±2.08	120.95±3.13	2.46±0.06	15,117±319	313.86±8.12	0.51±0.01
8	94.39±2.84	61.79±1.17	58.41±2.78	124.32±2.85	2.13±0.06	16,094±523	322.61±7.40	1.26±0.03
9	94.03±2.60	61.32±0.92	57.59±2.55	119.67±3.61	2.07±0.07	13,885±465	310.54±9.37	0.86±0.03
10	94.43±1.34	61.97±1.25	58.17±1.33	123.43±1.37	2.14±0.04	14,221±245	320.29±3.54	0.89±0.01
Probabilities								
Linear	0.010	0.030	0.010	0.010	0.020	0.170	0.001	<0.001
Quadratic	0.001	0.032	0.004	0.645	0.006	0.002	0.626	0.420
Total model	<0.001	0.010	0.001	0.162	0.002	0.002	0.042	<0.001
Lack of fit	0.315	0.683	0.437	0.785	0.799	0.763	0.792	0.862

Note: HDP= hen day production, EW= egg weight, EM= egg mass, FI= feed intake, FCR= feed conversion ratio, FC= feed cost, AMEI= AMEn layer intake, dLI= digestible lysine intake, Gain 23–38 wk. [1]Data are expressed as mean ± standard deviation.

Table 4. Polynomial fitted model / Coefficients for models of the response of commercial layers to AMEn layer and dLys

	Variables							
	HDP	EW	EM	FI	FCR	FC	AMEI	dLI
Intercept	-53.06	-3.68	-93.94	286.73	11.24	14,053	306.88	0.56
AMEn layer	0.121	0.051	0.123	-0.098	-0.007	415.52	-0.042	$-3.64x10^{-4}$
dLys	-47.77	-16.29	-46.44	-40.58	0.908	381.26	-102.25	1.40
AMEn layer*dLys	0.052	0.018	0.049	0.005	-0.002	-395.27	0.012	$-1.63x10^{-4}$
AMEn layer^2	$-3.02x10^{-5}$	$-1.18x10^{-5}$	$-2.98x10^{-5}$	$1.47x10^{-5}$	$1.46x10^{-6}$	317.53	$2.08x10^{-5}$	$6.76x10^{-8}$
dLys^2	-49.9	-18.15	-46.18	20.63	2.31	821.76	53.19	0.189
R^2	0.99	0.95	0.99	0.78	0.98	0.98	0.90	0.99

Note: Means in the same column with different superscripts differ significantly ($p < 0.05$). HDP = hen day production, EW = egg weight, EM = egg mass, FI = feed intake, FCR = feed conversion ratio, FC = feed cost, AMEI = AMEn layer intake, dLI = digestible lysine intake

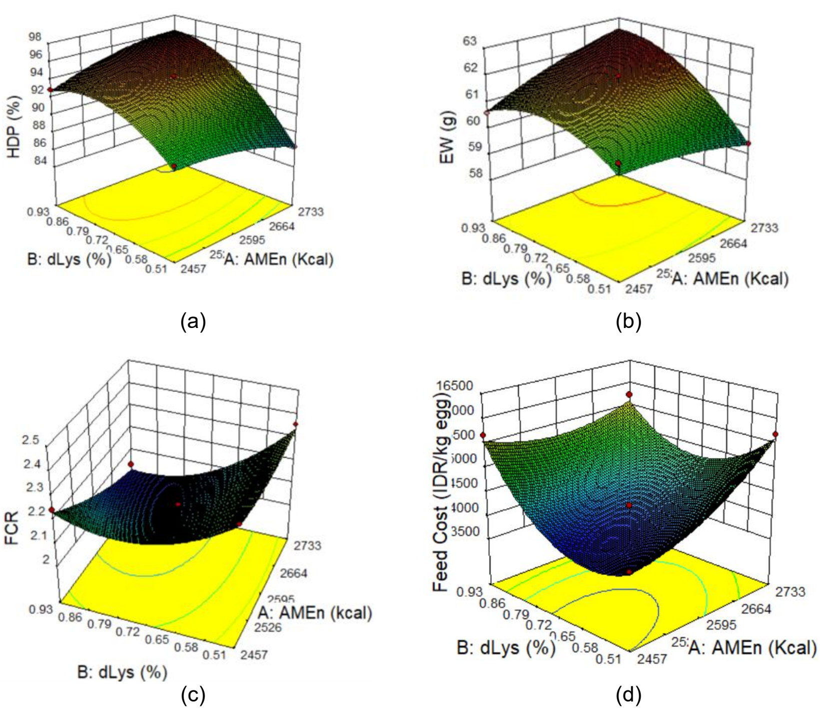

Figure 1. Response surface graph for HDP, EW, FCR, FC, and Gain as a function of AMEn layer [A] and dLys [B] of layer 24 to 38 weeks of age

was no significant interaction between dLys and AMEn layers.

FCR was the lowest when fed the highest dLys and AMEn layer level (Figure 1c). For the AMEn layer level on FCR, there was a significant ($p<0.05$) linear response (Table 5). Meanwhile, for dLys on FCR, both linear and quadratic responses were significant ($p<0.05$). The significant interaction for dLys and AMEn layer indicated a different response at the different levels of these variables. At high energy values, feeding with higher lysine promoted lower FCR, and higher energy showed no response at lower lysine levels.

FC was the lowest when fed the intermediate dLys and lower AMEn layer level (Figure 1d). There was no linear effect of AMEn layer and dLys on FC, but there was a curved response. For dLys and AMEn layer levels on FC, both linear and quadratic responses were significant ($p<0.05$) (Table 5). The significant interaction ($p<0.05$) for dLys and AMEn layer indicated that there was a different response at the different levels of these variables. At low energy values, feeding with higher lysine promoted higher FC; higher energy performed worse at lower lysine levels.

Desirability Function

The desirability function method was employed to optimize both bird performance and feed costs. Table 6 shows the optimization of AMEn and dLys that promoted the better performance of HDP, combination HDP – EW, HDP – EW – FCR, HDP – EW – FCR – Feed Cost, and HDP – FCR – Feed Cost. RSM modeling the most desirable point for maximum HDP, EW, and minimum FCR was obtained with 2,731 kcal/kg of ME and 0.89% of dLys. Meanwhile, the most desirable point for maximum HDP and EW, minimum FCR, and feed cost was achieved with 2,660 kcal/kg of ME and 0.81% of dLys. RSM modeling also showed that AMEn layer and dLys need to be lower to optimize performance and minimize feed cost. Moreover, it was more economically advantageous to exclude egg weight from the optimization process in unfavorable market conditions such as high diet costs, low egg prices, or a combination of both. As a result, the most desirable points for optimizing HDP and minimizing FCR and feed cost are 2,623 kcal/kg of AMEn layer and 0.78% of dLys.

DISCUSSION

The results indicate that laying hens respond to amino acids and energy levels in the diet by following the law of diminishing marginal productivity (Pesti, 1991) with a curvilinear pattern. AMEn value determines FI, FCR, and FC. Meanwhile, dLys content determines HDP, EW, FCR, and FC for peak production.

Maximum HDP and EW in the current study were achieved at the highest AMEn and dLys levels. There was no linear effect of AMEn on HDP, but there was a curve response. For dLys level on HDP and EW, there was both a significant ($p<0.05$) linear and quadratic response (Table 5). At low dLys value, feeding with higher AMEn showed no response to the HDP and EW. For AMEn level on FCR, there was a significant ($p<0.05$) linear response; meanwhile, for dLys on FCR, there was both a significant ($p<0.05$) linear and quadratic response. dLys determined the FCR value as feeding lower lysine levels, and higher energy showed no response to the FCR. Feed cost was the lowest when fed the intermediate dLys and lower AMEn level for both periods. At lower lysine levels, higher energy performed worse. In this study, dLys and AMEn play an important role in achieving performance. The equation in Table

Table 5. Estimate parameters and probability (P) for production performance of 24 to 38 weeks of age as a function of AMEn layer and dLys

	Variables							
	HDP	EW	EM	FI	FCR	FC	AMEI	dLI
Source	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F
Model	<0.001	0.010	0.001	0.162	0.002	0.002	0.042	<0.001
A-AMEn layer	0.30	0.070	0.097	0.023	0.006	0.003	0.004	0.016
B-dLys	<0.001	0.002	0.001	0.518	0.001	0.004	0.510	<0.001
AB	0.003	0.060	0.008	0.889	0.029	0.012	0.895	0.497
A^2	0.060	0.300	0.104	0.777	0.138	0.021	0.876	0.838
B^2	0.001	0.013	0.002	0.381	0.002	0.001	0.379	0.231

Note: HDP = hen day production, EW = egg weight, EM = egg mass, FI = feed intake, FCR = feed conversion ratio, FC = feed cost, AMEI = AMEn layer intake, dLI = digestible lysine intake.

Table 6. Desirability function optimum values of dietary AMEn layer and dLys for selected parameters of layer 24 to 38 weeks of age

Variables				AMEn layer (kcal/kg)	dLys (%)	Desirability
HDP	EW	FCR	FC			
96.13				2,693.18	0.91	0.97
96.05	62.70			2,747.35	0.93	0.91
95.98	62.74	1.99		2,731.46	0.89	0.92
95.45	61.97	2.05	14,566	2,660.81	0.81	0.79
95.16		2.07	14,356	2,623.94	0.78	0.82

Note: HDP = hen day production, EW = egg weight, FCR = feed conversion ratio, FC = feed cost.

4 was able to predict HDP, EW, FI, FCR, and the magnitude of FC change for optimum performance and maximum economic returns in response to fluctuations in energy and protein prices.

RSM followed by DF, could determine the optimum nutrient levels based on feeding costs that match each feeding scenario. The optimum value of HDP-EW and minimum FCR-FC were achieved by providing AMEn 2660 kcal/kg and dLys 0.81% for peak production. AMEI and dLI values were 322 kcal/d and 980 mg/d. When the EW parameter was excluded from composite DF, the optimum value of HDP and minimum FCR - FC was achieved by providing AMEn 2623 kcal/kg and dLys 0.78%. Therefore, AMEI and dLI intake values decreased to 318 kcal/d and 950 mg/d.

Brown egg layers' nutritional needs vary based on age, genetic strain, environmental conditions, management practices, and levels of dietary amino acids and energy, among others (Leeson & Summers, 2005; Caldas *et al*., 2018). Thus, it is imperative to regularly reassess and update the nutritional levels of layers to formulate diets appropriate for each production scenario. The optimum value of AMEI in this trial was close to Hy-Line Brown nutrition standard (2020) and higher than the value reported by Barzegar *et al*. (2019a). Hy-Line Brown nutrition standard recommends a metabolizable energy intake of 315-330 kcal/d for the peak production period; meanwhile, Barzegar *et al.* (2019b) reported an AMEI of 303 kcal/d.

The optimum value of dLI in this trial was higher than the Hyline standard and those found by Macelline *et al*. (2021) and Pastore *et al*. (2018). However, the values are close to Barzegar's observation (2019a). Hy-Line Brown nutrition standard recommends dLI of 869 and 819 mg/d for peak and post-peak production, respectively. According to Pastore *et al*. (2018), the minimum standardized ileal digestible lysine requirement for layers during peak production is 813 mg daily. Macelline *et al.* (2021) reported minimum digestible lysine of 691 mg/d and 751 mg/b for egg production and FCR, respectively. Barzegar *et al*. (2019b) reported an average requirement of digestible lysine of 909 mg/bird/d, which ranged from 777 to 1,108 mg/bird/d.

These data demonstrate that RSM and DF are powerful tools for addressing the challenges associated with company-owned feed production or farm facilities. Feed formulated for feed customers has to compete in the marketplace, so the nutrient specifications are usually higher than what is cost-effective in the company-owned production facilities. Customers may prioritize high peak production regardless of egg size, prefer larger or smaller eggs for a specific market, or use low FCR. Feed formulated for company-owned farm facilities is set for maximum profitability regardless of what production parameters are reached.

CONCLUSION

In conclusion, the results of this study showed that response surface analysis with CCD platform and DF can be used to optimize dietary energy and digestible amino acids at a minimum feeding cost of the Hy-Line layer during the production period. Based on the optimum DF values for the best performance in HDP, FCR, and FC, the levels of AMEn and dLys are 2,623.94 kcal/kg and 0.78%, respectively. However, considering all performance metrics such as HDP, EW, FCR, and FC, the optimum levels of AMEn and dLys are 2,660.81 kcal/kg and 0.81%.

CONFLICT OF INTEREST

Nahrowi and A. Jayanegara serve as editors of the Tropical Animal Science Journal but have no role in the decision to publish this article. The authors confirm that there are no conflicts of interest concerning financial, personal, or other relationships with individuals or organizations that could influence the content of this manuscript.

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