RESEARCH ARTICLE

J Anim Sci Technol 2025;67(6):1313-1327
https://doi.org/10.5187/jast.2500223

JAST
Journal of Animal Science and Technology
pISSN 2672-0191 eISSN 2055-0391

Dietary protein level in response to nitrogen balance along with production performance of laying hens

Jeseok Lee[1#], Sehyeok Oh[1#], Elijah Ogola Oketch[1], Shan Randima Nawarathne[1], Yu Bin Kim[1], Nuwan Chamara Chathuranga[1], Venuste Maniraguha[1], Bernadette Gerpacio Sta. Cruz[1], Eunsoo Seo[1], Haeeun Park[1], Hyunji Choi[1], Jung Kon Kim[2], Dong Jun Lee[2], Myunghwan Yu[3]*, Jung Min Heo[1]*

[1]*Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 34134, Korea*
[2]*Smart Livestock Environment Division, National Institute of Animal Science (NIAS), Wanju 55365, Korea*
[3]*Poultry Research Center, Department of Animal Resources Development, National Institute of Animal Science, Rural Development Administration, Pyeongchang 25342, Korea*

Received: Jul 2, 2025
Revised: Aug 19, 2025
Accepted: Aug 21, 2025

[#]These authors contributed equally to this work.

***Corresponding author**
Myunghwan Yu
Poultry Research Center, Department of Animal Resources Development, National Institute of Animal Science, Rural Development Administration, Pyeongchang 25342, Korea.
Tel: +82-33-330-9560
E-mail: tomymh@korea.kr

Jung Min Heo
Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 34134, Korea.
Tel: +82-42-821-5777
E-mail: jmheo@cnu.ac.kr

Abstract

This study investigated the effects of dietary crude protein (CP) levels on body weight (BW), laying performance, egg quality, and nitrogen (N) balance in laying hens from 18 to 62 weeks of age. A total of 84 Hy-Line Brown hens at 18 weeks old were randomly assigned to two groups, each with six replicates. The control group (CON) received diets with 17.5%, 16.5%, 15.5%, and 14.5% CP from weeks 18–38, 39–46, 47–54, and 55–62, respectively. The reduced protein group (RP) was fed diets with 1.5% less CP than the CON group during the same periods (16.0%, 15.0%, 14.0%, and 13.0%, respectively). The RP group showed significantly lower BW from weeks 30–62 ($p < 0.05$) and reduced hen-day egg production during weeks 18-38 and 54–62 ($p < 0.05$). Egg weight (EW) was significantly higher in the CON group than the RP group during weeks 54–62 ($p = 0.003$), and feed efficiency was also reduced in the RP group across multiple phases ($p < 0.05$). At week 38, Haugh units (HU) were lower in the RP group ($p = 0.034$), and yolk color was lighter at week 62 ($p = 0.006$). N balance parameters showed that the RP group had significantly lower N intake at weeks 46, 54, and 62 ($p < 0.01$), and N excretion was reduced throughout the trial ($p < 0.05$). Total N retention was lower in the RP group at weeks 26, 38, 54, and 62 ($p < 0.05$), and N retained in eggs was also reduced at weeks 26, 38, and 62 ($p < 0.05$). In summary, lowering dietary CP levels by 1.5% decreased N excretion, suggesting environmental benefits. In summary, reduction of dietary CP levels by 1.5% reduced N excretion but also compromised BW, laying performance, HU, and N retention. These findings highlight the need for further refinement of amino acid formulations to achieve both environmental and production goals.

Keywords: Egg quality, Laying hen, Laying performance, Nitrogen balance, Reduced-protein diet

ORCID
Jeseok Lee
https://orcid.org/0000-0002-6829-029X
Sehyeok Oh
https://orcid.org/0009-0000-5529-7532
Elijah Ogola Oketch
https://orcid.org/0000-0003-4364-460X
Shan Randima Nawarathne
https://orcid.org/0000-0001-9055-9155
Yu Bin Kim
https://orcid.org/0000-0001-7720-128X
Nuwan Chamara Chathuranga
https://orcid.org/0000-0003-1002-4068
Venuste Maniraguha
https://orcid.org/0000-0002-5615-3036
Bernadette Gerpacio Sta. Cruz
https://orcid.org/0000-0002-1663-7714
Eunsoo Seo
https://orcid.org/0000-0002-0207-7381
Haeeun Park
https://orcid.org/0000-0003-3244-0716
Hyunji Choi
https://orcid.org/0009-0006-3328-9316
Jung Kon Kim
https://orcid.org/0000-0001-6329-477X
Dong Jun Lee
https://orcid.org/0000-0001-7006-4649
Myunghwan Yu
https://orcid.org/0000-0003-4479-4677
Jung Min Heo
https://orcid.org/0000-0002-3693-1320

Competing interests
No potential conflict of interest relevant to this article was reported.

Funding sources
This paper was financially supported by the research fund of the Rural Development Administration, Korea (RS-2021-RD009516).

Acknowledgements
Not applicable.

Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: Lee J, Oh S, Chathuranga NC, Maniraguha V, Yu M, Heo JM.
Data curation: Lee J, Oh S, Kim YB, Cruz BGS, Seo E, Choi H, Yu M, Heo JM.
Formal analysis: Park H, Yu M, Heo JM.
Methodology: Lee J, Kim JK, Lee DJ, Oketch EO.
Software: Oh S, Nawarathne SR.
Validation: Heo JM.
Investigation: Lee J, Oh S.
Writing - original draft: Lee J, Oh S, Yu M, Heo JM.
Writing - review & editing: Lee J, Oh S, Oketch EO, Nawarathne SR, Kim YB, Chathuranga NC, Maniraguha V, Cruz BGS, Seo E, Park H, Choi H, Kim JK, Lee DJ, Yu M, Heo JM.

Ethics approval and consent to participate
The experimental protocol and procedures for the current study were reviewed and approved by the Animal Ethics Committee of Chungnam National University (Protocol Number: 202304A-CNU-031).

INTRODUCTION

As global demand for protein sources for human consumption increases, the laying hen industry has become essential to the poultry sector, providing eggs. Eggs are recognized as an excellent source of protein due to their superior amino acid balance and ease of digestion [1]. Additionally, eggs contain numerous nutritional elements that offer protection against chronic diseases, such as lysozyme, lutein, zeaxanthin, choline, selenium, and vitamin D [1,2]. They are also rich in essential amino acids and have a high biological value, making them highly bioavailable for human absorption [3]. Given the nutritional and economic importance of eggs, optimizing the nutritional composition of layer feed is crucial for maximizing production efficiency and egg quality. Key factors in feed include dietary energy levels [4], protein levels [5], energy and digestible lysine contents [6], and dietary calcium and phosphorus levels and ratio [7], all of which can influence egg production and quality.

While meeting the nutritional requirements of hens is crucial for optimal egg production, excessive dietary crude protein (CP) aimed at enhancing productivity can lead to increased N excretion, compromising hygiene conditions in the layer house [8]. N compounds in poultry manure can produce ammonia gas, leading to air pollution [9]. Therefore, balancing these nutritional and environmental considerations requires a comprehensive assessment of dietary CP levels. Thus, determining adequate dietary CP levels in layer feed is essential for productivity and minimizing environmental impacts, a growing concern in modern times. Numerous studies have examined the effect of dietary CP levels on N excretion in various livestock species, such as cattle and pigs [10,11], and some studies have explored the effect of supplementing synthetic amino acids or protease to compensate for potential productivity losses due to reduced dietary CP in the diet [5,12]. However, concerns about the potential losses in productivity and body weight (BW) associated with reduced-protein diets remain unresolved, necessitating further investigation to ensure both production and environmental goals are met.

Although nutritional guidelines such as the National Research Council (1994) [13] or the National Institute of Animal Science (2022) [14] suggest optimal dietary CP requirements for laying hens, the key determinant of production performance is not total dietary CP intake but rather the ideal amino acid balance in diets. Thus, defining the ideal amino acid balance is crucial for optimizing productivity. However, studies aimed at defining the optimal dietary amino acid balance in layer diets remain scarce, highlighting the need for further research to establish ideal amino acid profiles and their implications for production performance and environmental sustainability in laying hens.

This study examined the effect of a dietary CP level on BW, laying performance, egg quality, and N balance in laying hens from 18 to 62 weeks of age. We hypothesized that feeding reduced-protein diets, supplemented with limiting amino acids (methionine, lysine, threonine) to meet standard requirements, to hens in the laying stage would reduce N excretion without compromising production performance, thereby contributing to more sustainable laying hen production systems.

MATERIALS AND METHODS

Birds and housing

The 17-week-old birds were individually weighed upon arrival to verify the requirement of having approximately the same BW. A total of 84 17-week-old Hy-Line Brown (1,393.1 ± 10.98 g) were randomly allocated to enriched cages (90 × 90 × 90 cm) with four nipple drinkers and a detachable metal trough for free access to water and feed, respectively. Perches and nesting boxes were provided as enrichments in the cages to promote the welfare of birds. The hens were housed in

 https://doi.org/10.5187/jast.2500223

an environmental-friendly, windowless, and temperature-controlled facility (maintained at around 18℃ to 22℃). An initial one-week adaptation period was provided to allow the hens to acclimate to the new environment. Hens were initially fed 80 g/bird/day at week 18. The amount of feed was gradually increased, reaching 110 g/bird/day from weeks 24–62. The hens were subjected to a lighting scheme that provided 11 hours of light and 13 hours of darkness per day at week 18. The light duration was gradually increased, with the hens eventually subjected to a lighting scheme with 16 hours of continuous light and 8 hours of darkness from weeks 25–62. Any deviations from the flock average were noticed, and birds that were not laying were excluded.

Experimental design and management

The experiment, lasting 44 weeks, involved 18-week-old hens randomly assigned to one of two dietary treatments, with six replicates per treatment. All corn and soybean-based experimental diets were formulated to meet or exceed the nutrient requirements outlined by the National Institute of Animal Science (2022) [14], except for dietary CP level. The control (CON) group followed the standard dietary CP levels recommended by the National Institute of Animal Science (2022) [14], while the RP group received a diet with 1.5% less protein compared to the diet of the CON group. The limiting amino acids, such as lysine, methionine, and threonine, were supplemented using synthetic amino acids to achieve an ideal amino acid balance [14] and ensure consistent and equal amino acid concentrations in the experimental diets. Both treatment diets had the same metabolizable energy but differed only in protein levels. The dietary CP levels for the CON group were 17.5%, 16.5%, 15.5%, and 14.5% for hens in the early (i.e., from weeks 18–38), middle (i.e., from weeks 39–54), and late (i.e., from weeks 55–62) laying stages, respectively. The dietary CP levels in the diet of the RP group were 16.0%, 15.0%, 14.0%, and 13.0% for the same periods, which were 1.5% lower than those of the CON group. According to the laying stage, the diets were transitioned at weeks 38, 46, and 54. All diets were in pellet form. The composition of the experimental diets used in the study is shown in Table 1.

Body weight

The BW of hens was measured every four weeks from weeks 18–62.

Laying performance

The total number and weight of eggs laid were recorded daily for each cage, and feed intake (FI) was recorded weekly. The collected data was used to calculate the hen-day egg production (HDEP), feed conversion ratio (FCR), EW, and egg mass (EM) at weeks 18–38, 38–46, 46–54, and 54–62. Laying performance parameters were calculated using the following equations [15]:

$$\text{HDEP, \%} = \frac{\text{Total number of eggs produced in a day}}{\text{Total number of laying hens in a day}} \times 100 \quad (1)$$

$$\text{Average daily feed intake (ADFI), g/day/hen} = \frac{\text{Total FI in a week}}{\text{Days} \times \text{Number of laying hens}} \quad (2)$$

$$\text{Average EW, g} = \frac{\text{Total EW}}{\text{Number of eggs}} \quad (3)$$

Table 1. Feed ingredients and chemical composition of experimental diets with different dietary CP levels, as-fed basis

Items	CON				RP			
	week 18–38	week 39–46	week 47–54	week 55–62	week 18–38	week 39–46	week 47–54	week 55–62
Ingredient composition (%)								
Corn	59.05	51.04	50.62	50.05	63.50	53.29	53.42	52.13
Wheat bran	0.00	12.94	15.65	19.13	0.10	14.83	17.24	21.79
Soybean meal (48%)	26.10	21.25	18.34	14.96	22.00	16.88	13.90	10.00
Vegetable oil	3.35	3.00	3.00	3.00	2.65	3.00	2.80	3.00
Monocalcium phosphate	1.44	1.11	1.03	0.92	1.45	1.11	1.04	0.92
Limestone	9.31	9.92	10.55	10.98	9.35	9.95	10.58	10.96
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-lysine	0.00	0.00	0.07	0.15	0.13	0.12	0.20	0.30
DL-methionine	0.15	0.14	0.14	0.16	0.16	0.16	0.15	0.18
L-threonine	0.00	0.00	0.00	0.05	0.06	0.06	0.07	0.12
Vitamin-mineral premix[1)]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Chemical composition (%)								
ME (kcal/skg)	2,950	2,700	2,650	2,600	2,950	2,700	2,650	2,600
CP	17.50	16.50	15.50	14.50	16.00	15.00	14.00	13.00
Crude fat	2.21	2.33	2.37	2.44	2.32	2.42	2.47	2.55
Crude fiber	2.44	3.44	3.62	3.88	2.45	3.57	3.74	4.10
SID Lysine	0.79	0.70	0.70	0.69	0.79	0.70	0.70	0.69
SID Methionine	0.42	0.37	0.37	0.36	0.42	0.37	0.37	0.36
SID Methionine+Cystine	0.66	0.60	0.60	0.58	0.66	0.60	0.60	0.58
SID Threonine	0.59	0.53	0.49	0.49	0.59	0.53	0.49	0.49
Calcium	4.00	4.18	4.40	4.54	4.00	4.18	4.40	4.54
Available phosphorus	0.40	0.36	0.35	0.33	0.40	0.36	0.35	0.33
Analyzed composition (%)								
CP	16.94	16.31	14.94	13.44	16.31	14.94	13.44	12.13

[1)] Provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 2,500 IU; vitamin E, 30 IU; vitamin K_3, 3 mg; D-pantothenic acid, 15 mg; nicotinic acid, 40 mg; choline, 400 mg; and vitamin B_{12}, 12 μg; Fe, 90 mg from iron sulphate; Cu, 8.8 mg from copper sulphate; Zn, 100 mg from zinc oxide; Mn, 54 mg from manganese oxide; I, 0.35 mg from potassium iodide; Se, 0.30 mg from sodium selenite.

CP, crude protein; CON, control; RP, protein; ME, metabolizable energy.

$$\text{EM, g/day/hen} = \frac{\text{HDEP} \times \text{EW}}{100} \tag{4}$$

$$\text{FCR, g feed/g egg} = \frac{\text{FI per day per hen}}{\text{EM}} \tag{5}$$

Egg quality

Egg quality analysis was conducted to evaluate the differences in egg quality between treatments. At the end of weeks 26, 38, 46, 54, and 62, a total of 60 eggs (5 eggs per replicate cage) were collected randomly and evaluated for egg quality. Eggshell breaking strength was evaluated using a texture analyzer (TA.XTplusC; Stable Micro Systems). The shell color, albumen height, and haugh units (HU) were measured using an egg multitester instrument (QCM+ Range; Technical Services and Supplies). Yolk color intensity was measured against the DSM yolk color fan (1, light yellow; 15, orange). A shell thickness micrometer (Digimatic MDC-MX Series; Mitutoyo) was used to

 https://doi.org/10.5187/jast.2500223

measure the shell thickness at three different locations (upper, lower, and middle), excluding the inner shell membrane. The internal egg quality and eggshell analyses were completed within 24 hours of egg collection.

N balance

The N content of feed samples was analyzed using the Kjeldahl method [16]. Feces were collected per cage for 72 hours to determine the total excreta output on a daily basis per bird. The collected excreta from all birds within each cage were thoroughly mixed and pooled per replicate, and a 50 g sample was taken for N content analysis. The pooled samples were then stored at −20℃ until further analyses, including N balance measurements. Total excreta output and N balance were measured in hens at weeks 26, 38, 46, 54, and 62. All N balance indicators were presented on a daily basis per bird using the following formulas [17,18]:

$$\text{N intake, g/day/hen} = \text{FI} \times \text{N in the diet} \tag{6}$$

$$\text{N excretion, g/day/hen} = \text{total excreta output} \times \text{N in the excreta} \tag{7}$$

$$\text{Total N retained, g/day/hen} = \text{N intake} - \text{N excretion} \tag{8}$$

$$\text{N retained in egg, g/day/hen} = 1.936\% \times \text{EM} \tag{9}$$

$$\text{N retained in body, g/day/hen} = \text{Total N retained} - \text{N retained in egg} \tag{10}$$

Statistical analysis

All data were analyzed using an independent t-test with SPSS software (version 29). Battery cages were used as the experimental unit for assessing the BW, laying performance, and N balance. Statistical analysis was conducted with six replicates, using five eggs per cage for egg quality analysis. Mean differences observed in the treatment were considered significant at $p < 0.05$.

RESULTS

Body weight

The effect of dietary CP levels on the BW of hens is shown in Table 2. The BW of hens did not differ between the dietary groups at weeks 18, 22, and 26. However, from week 30, the protein group (RP) group consistently had lower ($p < 0.05$) BW than the CON group. At week 30, the BW of the RP group was 1,776.60 g, 5.8% lower ($p = 0.001$) than the CON group at 1,886.52 g. This reduction persisted until week 62, when the RP group showed a BW of 1,765.08 g, representing a 10.7% decrease compared to the CON group at 1,976.30 g ($p = 0.040$).

Laying performance

The effect of dietary CP level on laying performance in hens is presented in Table 3. The RP group showed reduced ($p < 0.05$) HDEP during the early (i.e., from weeks 18–38) and late (i.e., from weeks 54–62) laying stages, while no differences were observed in the middle laying stages (i.e., from weeks 38–46 and 46–54). During the early laying stage (i.e., from weeks 18–38), the HDEP in the RP group was 84.76%, which was 7.6% lower ($p < 0.001$) than the CON group at 91.56%. Similarly, during the late laying stage (i.e., from weeks 54–62), the HDEP in the RP group was 74.50%, which was 5.1% lower ($p = 0.032$) than the CON group at 78.28%. No differences in

Table 2. Influence of dietary CP level on BW[1)]

Items	Diets[2)]		SEM[3)]	*p*-value[4)]
	CON	RP		
BW (g)				
week 18	1,502.63	1,438.60	37.108	0.142
week 22	1,757.86	1,749.05	24.165	0.769
week 26	1,804.93	1,763.21	23.269	0.125
week 30	1,886.52	1,776.60	35.399	0.001
week 34	1,927.48	1,845.02	35.025	0.033
week 38	1,995.19	1,774.24	63.516	<0.001
week 42	2,009.80	1,754.09	77.942	<0.001
week 46	2,025.02	1,794.31	76.903	0.003
week 50	2,006.79	1,836.92	68.172	0.022
week 54	2,002.36	1,773.94	85.222	0.011
week 58	1,960.19	1,770.63	81.002	0.035
week 62	1,976.30	1,765.08	92.104	0.040

1)Values are the mean of six replicates per treatment.

2)CON, a diet with 17.50, 16.50, 15.50, and 14.50% CP in weeks 18–38, 39–46, 47–54, and 55–62, respectively; RP, a diet with 16.00%, 15.00%, 14.00%, and 13.00% CP in weeks 18–38, 39–46, 47–54, and 55–62, respectively.

3)Pooled SEM.

4)Statistical significance was determined at $p < 0.05$.

CP, crude protein; BW, body weight; CON, control; RP, protein.

average EW were observed during the early (i.e., from weeks 18–38) and middle (i.e., from weeks 38–46 and 46–54) laying stages. However, during the late laying stage (i.e., from weeks 54–62), the RP group exhibited an average EW of 62.63 g, which was 2.9% lower ($p = 0.003$) than the CON group at 64.52 g. During the middle laying stage (weeks 38–46 and 46–54), EM did not differ between dietary treatments. However, reductions ($p < 0.05$) in EM were observed during the early (i.e., from weeks 18–38) and late (i.e., from weeks 54–62) laying stages. In the early laying stage (i.e., from weeks 18–38), the EM in the RP group was 51.08 g/day/hen, which was 11.6% lower ($p < 0.001$) than the CON group at 57.83 g/day/hen. During the late laying stage (i.e., from weeks 54–62), the RP group showed an EM of 46.72 g/day/hen, which was 7.4% lower ($p = 0.005$) compared to the CON group at 50.43 g/day/hen.

ADFI remained unaffected during the early stage (i.e., from weeks 18–38). However, during the middle (i.e., weeks 38–46 and 46–54) and late stages (i.e., from weeks 54–62), ADFI was higher ($p < 0.01$) in the RP group compared to the CON group. From weeks 38-46, the RP group consumed 106.14 g/day/hen, which was 2.8% higher ($p < 0.001$) than the CON group at 103.21 g/day/hen. At the end of the late laying stage (i.e., from weeks 54–62), ADFI in the RP group was 105.70 g/day/hen, 2.0% higher ($p = 0.009$) than in the CON group at 103.59 g/day/hen. Likewise, no differences in FCR were observed from weeks 46–54. On the other hand, FCR was higher ($p < 0.05$) in the RP group compared to the CON group during the early (i.e., from weeks 18-38), the beginning of the middle (i.e., from weeks 38–46), and late (i.e., from 54–62) laying stages. During the early laying stage (i.e., from weeks 18–38), the FCR in the RP group was 2.07, which was 11.9% higher ($p < 0.001$) than the CON at 1.85. Similarly, at the beginning of the middle laying stage (i.e., from weeks 38–46), the FCR in the RP group was 2.20, which was 6.3% higher ($p = 0.024$) than the CON group at 2.07. Likewise, in the late laying stage (i.e., from weeks 54–62), the RP group exhibited an FCR of 2.30, which was 10.6% higher ($p = 0.001$) than the CON group at 2.08.

 https://doi.org/10.5187/jast.2500223

Table 3. Influence of dietary CP level on laying performance[1)]

Items[2)]	Diets[3)]		SEM[4)]	p-value[5)]
	CON	RP		
HDEP (%)				
week 18–38	91.56	84.76	0.022	<0.001
week 38–46	82.46	80.80	0.017	0.338
week 46–54	81.04	77.59	0.021	0.104
week 54–62	78.28	74.50	0.018	0.032
EW (g)				
week 18–38	62.97	61.04	1.700	0.071
week 38–46	61.33	60.86	1.030	0.660
week 46–54	63.28	61.77	1.351	0.278
week 54–62	64.52	62.63	0.712	0.003
EM (g/day/hen)				
week 18–38	57.83	51.08	2.430	<0.001
week 38–46	50.55	48.84	1.169	0.149
week 46–54	51.30	47.94	1.853	0.067
week 54–62	50.43	46.72	1.440	0.005
ADFI (g/day/hen)				
week 18–38	105.93	106.01	0.610	0.827
week 38–46	103.21	106.14	0.897	<0.001
week 46–54	103.63	105.50	0.716	0.004
week 54–62	103.59	105.70	0.868	0.009
FCR (g feed/g egg)				
week 18–38	1.85	2.07	0.082	<0.001
week 38–46	2.07	2.20	0.060	0.024
week 46–54	2.06	2.23	0.087	0.054
week 54–62	2.08	2.30	0.077	0.001

1) Values are the mean of six replicates per treatment.

2) EM = HDEP × EW; FCR = ADFI / EM.

3) CON, a diet with 17.50, 16.50, 15.50, and 14.50% CP in weeks 18–38, 39–46, 47–54, and 55–62, respectively; RP, a diet with 16.00%, 15.00%, 14.00%, and 13.00% CP in weeks 18–38, 39–46, 47–54, and 55–62, respectively.

4) Pooled SEM.

5) Statistical significance was determined at $p < 0.05$.

CP, crude protein; BW, body weight; CON, control; RP, protein; EM, egg mass; HDEP, hen-day egg production; EW, Egg weight; ADFI, Average daily feed intake.

Egg quality

The effect of dietary CP level on egg quality in hens is presented in Table 4. Throughout the experimental period, eggshell thickness remained stable, ranging from 0.35 to 0.40 mm, with no significant differences between dietary groups. Egg-breaking strength showed a similar pattern, maintaining consistent values (approximately 5.16–5.76 kg) regardless of dietary CP level. Eggshell color also remained unaffected, with values fluctuating slightly from 19.23 to 23.80 without diet-related trends. Albumen height was comparable between groups throughout the study period, with values ranging from approximately 6.91 to 8.60 mm, and showing no significant differences between the CON and RP groups.

With respect to HU and yolk color, HU values did not differ between dietary groups at weeks 26, 46, 54, and 62. However, at week 38, the HU in the RP group was 98.69, which was 1.0% lower ($p = 0.034$) than that in the CON group at 99.71. Similarly, yolk color remained unaffected

Table 4. Influence of dietary CP level on egg quality[1)]

Items	Diets[2)]		SEM[3)]	*p*-value[4)]
	CON	RP		
Eggshell thickness (mm)				
week 26	0.35	0.35	0.008	0.997
week 38	0.38	0.38	0.006	0.751
week 46	0.39	0.39	0.004	0.140
week 54	0.38	0.40	0.007	0.296
week 62	0.38	0.39	0.009	0.837
Egg-breaking strength (kg)				
week 26	5.16	5.15	0.242	0.991
week 38	5.63	5.62	0.136	0.491
week 46	5.72	5.70	0.198	0.908
week 54	5.76	5.72	0.252	0.882
week 62	5.33	5.33	0.259	0.999
Eggshell color				
week 26	19.23	19.53	0.522	0.565
week 38	23.40	23.53	0.566	0.289
week 46	20.86	21.03	0.314	0.215
week 54	22.57	22.22	0.908	0.921
week 62	23.78	23.80	0.645	0.983
Albumen height (mm)				
week 26	8.12	8.06	0.345	0.067
week 38	8.37	8.37	0.263	0.997
week 46	8.46	8.45	0.230	0.602
week 54	8.06	8.04	0.238	0.347
week 62	7.03	7.03	0.190	0.994
HU				
week 26	99.58	94.73	1.847	0.084
week 38	99.71	98.69	1.049	0.034
week 46	98.78	97.15	1.330	0.524
week 54	96.89	96.10	2.021	0.253
week 62	91.94	90.55	0.934	0.896
Yolk color				
week 26	6.60	6.97	0.198	0.257
week 38	7.07	7.17	0.157	0.218
week 46	6.87	7.13	0.229	0.491
week 54	6.76	6.95	0.223	0.521
week 62	6.97	6.73	0.200	0.006

[1)]Values are the mean of six replicates per treatment.
[2)]CON, a diet with 17.50, 16.50, 15.50, and 14.50% CP in weeks 18–38, 39–46, 47–54, and 55–62, respectively; RP, a diet with 16.00%, 15.00%, 14.00%, and 13.00% CP in weeks 18–38, 39–46, 47–54, and 55–62, respectively.
[3)]Pooled SEM.
[4)]Statistical significance was determined at $p < 0.05$.
CP, crude protein; CON, control; RP, protein group; HU, haugh units.

by dietary groups at weeks 26, 38, 46, and 54. On the other hand, at week 62, the yolk color in the RP group was 6.73, which was lighter than the CON group at 6.97, showing a 3.4% reduction (p =

0.006) in color intensity.

N balance

The effect of dietary CP levels on N balance in hens is shown in Table 5. No differences in total excreta output were observed between the groups in weeks 26, 46, and 54. However, at weeks 38 and 62, the RP group showed lower ($p < 0.05$) total excreta output than the CON group. At week 38, total excreta output in the RP group was 108.02 g/day/hen, 9.7% lower ($p = 0.004$) compared to the CON group at 119.65 g/day/hen. Consistently, at week 62, total excreta output in the RP group was 96.44 g/day/hen, 15.6% lower ($p = 0.043$) compared to the CON group at 114.22 g/day/hen. N intake remained unaffected by dietary CP levels at weeks 26 and 38. However, at week 46, N intake in the RP group was 2.53 g/day/hen, which was 5.6% lower ($p = 0.002$) than the CON group at 2.68 g/day/hen. In week 62, the RP group had a N intake of 2.04 g/day/hen, which was 4.7% lower ($p = 0.001$) than that of the CON group, which was 2.14 g/day/hen. In terms of N excretion, the RP group consistently showed lower ($p < 0.05$) N excretion than the CON group throughout the experimental period. At week 26, N excretion in the RP group was 1.12 g/day/hen, 17.0% lower ($p = 0.009$) than that of the CON group at 1.35 g/day/hen. This trend continued, with N excretion in the RP group at 0.99 g/day/hen, 20.8% lower ($p = 0.045$) than the CON group at 1.25 g/day/hen at week 62.

Dietary CP level did not affect total N retained at week 46. However, at weeks 26, 38, 54, and 62, total N retained was lower ($p < 0.05$) in the RP group than in the CON group. At week 26, the total N retained was 1.48 g/day/hen, 11.9% lower ($p = 0.038$) than that of the CON group at 1.68 g/day/hen. Likewise, at week 62, the total N retained in the RP group was 1.22 g/day/hen, 17.6% lower ($p = 0.042$) than that of the CON group at 1.48 g/day/hen. No dietary CP effect was noted for N retained in eggs at weeks 46 and 54. However, at weeks 26, 38, and 62, N retained in egg in the RP group was lower ($p < 0.05$) than in the CON group. At week 26, N retained in the egg in the RP group at 0.99 g/day/hen, which was 8.3% lower ($p = 0.192$) than that of the CON group at 1.08 g/day/hen. In line with this trend, at week 38, N retained in egg in the RP group was 0.96 g/day/hen, 17.9% lower ($p = 0.004$) than that of the CON group at 1.17 g/day/hen. Consistently, at week 62, N retained in egg in the RP group was 0.91 g/day/hen, which was 7.1% lower ($p = 0.034$) than that of the CON group at 0.98 g/day/hen. Throughout the trial, N retained in body did not differ between dietary CP groups. At week 26, N retained in body was 0.48 g/day/hen in the RP group and 0.60 g/day/hen in the CON group. Similarly, at week 62, N retained in body was 0.32 g/day/hen in the RP group and 0.51 g/day/hen in the CON group.

DISCUSSION

Maintenance of the standard BW of hens is essential for sustaining egg production performance. It is a key factor in determining productivity, especially in the early laying stage [19]. Thus, ensuring that the diet includes adequate protein with an ideal amino acid ratio is critical for maintaining adequate BW and optimizing egg production during the whole laying stages. The National Institute of Animal Science (2022) [14] recommends diets of hens containing 17.50%, 16.50%, 15.50%, and 14.50% dietary CP during the periods from early laying to 32, 32–45, 45–55, and 55- weeks of age, respectively. In this study, hens were fed a reduced-protein diet that did not meet protein requirements, with dietary CP levels of 16.00%, 15.00%, 14.00%, and 13.00% from 18–38, 39–46, 47–54, and 55–62 weeks of age, respectively. Indeed, the reduced-protein diets in the study do not meet the nutritional requirements of branched-chain amino acids (BCAAs), which are critical for protein synthesis in the body [20]. While the diets adequately supplied the limiting amino acids

Table 5. Influence of dietary CP level on N balance[1)]

Items	Diets[2)]		SEM[3)]	*p*-value[4)]
	CON	RP		
Total excreta output (g/day/hen)				
week 26	113.13	104.52	5.551	0.192
week 38	119.65	108.02	3.976	0.004
week 46	124.25	113.37	5.843	0.109
week 54	115.03	105.08	6.556	0.202
week 62	114.22	96.44	7.851	0.043
N intake[5)] (g/day/hen)				
week 26	2.87	2.81	0.037	0.124
week 38	2.81	2.76	0.032	0.140
week 46	2.68	2.53	0.050	0.002
week 54	2.51	2.30	0.065	<0.001
week 62	2.14	2.04	0.035	0.001
N excretion (g/day/hen)				
week 26	1.35	1.12	0.085	0.009
week 38	1.40	1.27	0.053	0.026
week 46	1.44	1.23	0.092	0.036
week 54	1.28	1.05	0.094	0.023
week 62	1.25	0.99	0.119	0.045
Total N retained[6)] (g/day/hen)				
week 26	1.68	1.48	0.088	0.038
week 38	1.60	1.41	0.076	0.024
week 46	1.53	1.29	0.111	0.052
week 54	1.50	1.25	0.097	0.016
week 62	1.48	1.22	0.117	0.042
N retained in egg[7)] (g/day/hen)				
week 26	1.08	0.99	0.007	0.028
week 38	1.17	0.96	0.011	0.001
week 46	0.98	0.95	0.006	0.313
week 54	0.99	0.94	0.010	0.244
week 62	0.98	0.91	0.007	0.034
N retained in body[8)] (g/day/hen)				
week 26	0.60	0.48	0.022	0.220
week 38	0.43	0.45	0.023	0.827
week 46	0.55	0.34	0.026	0.073
week 54	0.51	0.31	0.024	0.070
week 62	0.51	0.31	0.032	0.164

1) Values are the mean of six replicates per treatment.

2) CON, a diet with 17.50, 16.50, 15.50, and 14.50% CP in weeks 18–38, 39–46, 47–54, and 55–62, respectively; RP, a diet with 16.00%, 15.00%, 14.00%, and 13.00% CP in weeks 18–38, 39–46, 47–54, and 55–62, respectively.

3) Pooled SEM.

4) Statistical significance was determined at $p < 0.05$.

5) N intake was calculated as follows: ADFI in grams × (Analyzed diet CP/100).

6) Difference between N intake and excreted.

7) Calculated as EM (g/day/hen) × N concentration in eggs assumed as 1.936% [17].

8) N retained in the body (g/day/hen) calculated as the difference between total N retained and N retained in the egg.

CP, crude protein; CON, control; RP, protein group; ADFI, average daily feed intake; EM, egg mass.

 https://doi.org/10.5187/jast.2500223

methionine, lysine, and threonine, the lack of sufficient BCAAs may have further exacerbated the reduction in BW by limiting overall protein synthesis. Additionally, the inadequate N pool in these diets may necessitate supplementation with non-essential amino acids, such as glycine and serine, to support the recovery of production performance, potentially mitigating the reduction in BW [21]. As a result, the gradual depletion of body protein stores and insufficient body tissue synthesis caused by an imbalance in the dietary amino acid profile may have contributed to the decreased BW [5]. Even with supplementation of limiting amino acids, the marked reduction in BW observed in hens fed reduced-protein diets may pose a health risk, which limits the practical applicability of such diets.

The reduced HDEP in the early and late laying stages observed in birds fed reduced-protein diets may be attributed to an imbalance in an amino acid ratio in the diet, particularly during physiologically demanding periods such as peak production or aging hens. One potential reason is the insufficient availability of BCAAs in the diet, which are essential not only for protein synthesis but also for regulating fatty acid metabolism in the liver, a process critical for hepatic yolk-lipoprotein production [22]. Another contributing factor could be a deficiency in arginine in the diet, an amino acid that promotes luteinizing hormone secretion, facilitating follicle development and ovulation [23]. In line with this study, Adeyemo et al. [24] observed a reduction in HDEP in hens fed diets containing 14% dietary CP with an adequate amount of methionine and lysine in the diet. The weight of eggs is influenced by various factors such as dietary CP levels, methionine, energy, and fat content in the diet [25,26]. However, the relationship between EW and dietary CP depends not solely on protein levels but also on the amino acid balance in the diet. A deficiency or imbalance in limited amino acids can impair protein synthesis, resulting in reduced EW [27]. In the study, the EW was lower in hens fed reduced-protein diets despite supplying methionine, lysine, and threonine at levels meeting their nutritional requirements. The reduction in EW may also be attributed to an insufficient N pool in a reduced-protein diet, particularly during the formation of the ovum in the magnum [27]. Such insufficiency can occur even when adequate levels of limiting amino acids are supplemented, highlighting the limitations of reduced-protein diets in supporting optimal egg production. Similarly, other studies by Ji et al. [27], Alagawany et al. [5], van Harn et al. [28], and Heo et al. [12] have reported a reduction in EW in hens fed reduced-protein diets. Decreased EM may be attributed to reductions in HDEP and EW [29], supporting the importance of adequate protein levels in sustaining egg production. Hens fed reduced-protein diets supplemented with limiting amino acids may exhibit increased ADFI, potentially due to imbalances in the overall amino acid profile. These imbalances could affect protein metabolism, leading to an increased ADFI as hens attempt to meet their physiological requirements [30]. Branched-chain amino acids, which were deficient in reduced-protein diets used in the study, are known to play a crucial role in FI regulation by influencing appetite and feeding behavior [31]. Additionally, reduced N retention observed in hens fed reduced-protein diets suggests that nutrient utilization efficiency may be compromised, further contributing to higher FI. While FI in hens is generally influenced more by dietary energy levels than protein levels [32], this study highlights the need to explore further how dietary CP levels and amino acid balance impact FI in layers. FCR is closely related to EM and FI, both of which were negatively impacted by reduced-protein diets in the study. The increased FCR observed in hens fed reduced-protein diets may be attributed to reduced feed efficiency compared to those fed diets with adequate protein levels [5]. Not only deficiency in dietary CP levels but also the amino acid ratio imbalances can impair nutrient utilization and productivity, ultimately reducing feed efficiency [33]. This finding suggests the importance of ensuring adequate protein levels and maintaining balanced amino acid profiles to optimize feed efficiency in layer production.

No differences in eggshell thickness, egg-breaking strength, eggshell color, and albumen height were observed in relation to dietary CP level in this study. Similarly, Latshaw and Zhao [30] and Kim and Kang [34] reported no differences in these parameters based on dietary CP levels, suggesting that protein content in the diet may not play a significant role in determining eggshell quality. The inferior HU observed in hens fed a reduced-protein diet highlights the limitations of such diets in supporting optimal albumen synthesis during egg formation. Even with supplementation of limiting amino acids, reduced-protein diets inherently provide an insufficient N pool, which is crucial for maintaining albumen synthesis [27]. Such a reduced N pool likely impaired albumen synthesis, ultimately compromising internal egg quality [35]. Based on the findings of this study, feeding a 16.00% CP diet supplemented with methionine, lysine, and threonine during the early laying stages (i.e., weeks 18–38) may result in a reduction in HU. In general, higher levels of corn or corn gluten meal in the reduced-protein diet are associated with more intense yolk color due to their xanthophyll content. In the present study, the RP group showed numerically more intense yolk color than the CON group during the early to mid laying period, although the differences were not significant. However, this trend reversed at the end of the study, with the RP group exhibiting lighter yolk color compared to the CON group. To our knowledge, such a result has not been previously reported. While earlier studies have shown either more intense yolk color [12,27] or no difference [30,34] with reduced-protein diets, few have examined this in laying hens. Further research is needed to understand this unexpected outcome.

N balance was assessed in the study to evaluate the efficiency of dietary CP utilization in the experimental diets [36]. Typically, total excreta output in chickens accounts for 3%–4% of their BW [37]. The reduction in total excreta output observed in hens fed reduced-protein diets can be attributed to their decreased BW. However, the decline may also reflect a reduction in N excretion, as chickens excrete N primarily as uric acid, a major component of solid waste. This suggests that reduced-protein diets lower N excretion, thereby decreasing total excreta output. Although hens fed a reduced-protein diet consumed more feed, the reduced protein levels likely resulted in lower overall N intake. This observation aligns with previous studies by Latshaw and Zhao [30], Heo et al. [12], and Iio et al. [38], which consistently demonstrated that reduced-protein diets lead to reduced N intake in layers. N excretion in layers typically originates from dietary CP not utilized for body tissue synthesis or egg formation [12]. Hence, hens fed a reduced-protein diet have less protein that remains unused, resulting in reduced N excretion [30]. The consistent reduction in N excretion across all laying stages (i.e., weeks 18–62) in the RP group highlights the potential of reduced-protein diets to reduce environmental N excretion. On the other hand, the results of the study underscore the need for balancing environmental benefits with productivity concerns because reduced N excretion coincides with declines in egg production. Based on total EM output over the experimental period (18–62 weeks), the RP group required approximately 10% more hens to produce 1,000 g of EM due to lower productivity. Nevertheless, total N excretion per 1,000 g of EM was reduced by about 7%, suggesting that reduced-protein diets may still provide environmental benefits, even when accounting for decreased production efficiency. The reduced total N retention in hens fed reduced-protein diets reflects the challenges of meeting the physiological demands of egg production with insufficient dietary CP. Even with supplementation of limiting amino acids such as methionine, lysine, and threonine, reduced-protein diets are fundamentally characterized by a deficient N pool. This inherent limitation disrupts protein metabolism and reduces the availability of N for productive purposes, leading to decreased total N retention [33]. This reduction may also be associated with the observed decreases in EM and feed efficiency in the study, as N retention is closely linked to protein availability for productive purposes. The reduction in N retained in eggs observed in the reduced-protein diet group corresponds directly to the

 https://doi.org/10.5187/jast.2500223

decrease in EM, as N retention in eggs is calculated based on EM [17]. Although not statistically significant, the trend of lower N retention in the body in the reduced-protein diet group suggests a potential impact of reduced dietary CP levels on N utilization for body maintenance [39]. While the differences were insignificant, the observed trend underscores the need for further study to better understand the relationship between dietary CP levels and N retention in the body.

CONCLUSION

This study suggests that reduced-protein diets can lower N excretion but also lead to reductions in BW, laying performance, HU, and N retention. Despite supplementation with limiting amino acids such as lysine, methionine, and threonine, the amino acid profile of reduced-protein diets appeared insufficient to support optimal protein synthesis and production performance. Notably, the observed decline in egg production performance implies that more hens may be required to produce the same number of eggs, potentially offsetting the environmental benefits of reduced N excretion. However, based on the experimental data, even when accounting for the need to rear more hens to produce the same number of eggs, total N excretion was slightly reduced in the reduced-protein group when compared based on the same amount of egg mass. Therefore, reduced-protein diets may still provide a modest environmental benefit. Further research is needed to define amino acid profiles that minimize environmental impact without compromising productivity.

REFERENCES

1. Puglisi MJ, Fernandez ML. The health benefits of egg protein. Nutrients. 2022;14:2904. https://doi.org/10.3390/nu14142904
2. Leśnierowski G, Yang T. Lysozyme and its modified forms: a critical appraisal of selected properties and potential. Trends Food Sci Technol. 2021;107:333-42. https://doi.org/10.1016/j.tifs.2020.11.004
3. Kovacs-Nolan J, Phillips M, Mine Y. Advances in the value of eggs and egg components for human health. J Agric Food Chem. 2005;53:8421-31. https://doi.org/10.1021/jf050964f
4. Han GP, Kim DY, Kim KH, Kim JH, Kil DY. Effect of dietary concentrations of metabolizable energy and neutral detergent fiber on productive performance, egg quality, fatty liver incidence, and hepatic fatty acid metabolism in aged laying hens. Poult Sci. 2023;102:102497. https://doi.org/10.1016/j.psj.2023.102497
5. Alagawany M, El-Hindawy MM, El-Hack MEA, Arif M, El-Sayed SA. Influence of low-protein diet with different levels of amino acids on laying hen performance, quality and egg composition. An Acad Bras Ciênc. 2020;92:e20180230. https://doi.org/10.1590/0001-3765202020180230
6. Scappaticcio R, García J, Fondevila G, de Juan AF, Cámara L, Mateos GG. Influence of the energy and digestible lysine contents of the diet on performance and egg quality traits of brown-egg laying hens from 19 to 59 weeks of age. Poult Sci. 2021;100:101211. https://doi.org/10.1016/j.psj.2021.101211
7. Dijkslag MA, Kwakkel RP, Martin-Chaves E, Alfonso-Carrillo C, Walvoort C, Navarro-Villa A. The effects of dietary calcium and phosphorus level, and feed form during rearing on growth performance, bone traits and egg production in brown egg-type pullets from 0 to 32 weeks of age. Poult Sci. 2021;100:101130. https://doi.org/10.1016/j.psj.2021.101130
8. Méda B, Hassouna M, Aubert C, Robin P, Dourmad JY. Influence of rearing conditions and manure management practices on ammonia and greenhouse gas emissions from poultry houses.

World's Poult Sci J. 2011;67:441-56. https://doi.org/10.1017/S0043933911000493

9. Liu XJ, Xu W, Du EZ, Tang AH, Zhang Y, Zhang YY, et al. Environmental impacts of nitrogen emissions in China and the role of policies in emission reduction. Philos Trans R Soc A. 2020;378:20190324. https://doi.org/10.1098/rsta.2019.0324
10. Lee YH, Ahmadi F, Lee M, Oh YK, Kwak WS. Effect of crude protein content and undegraded intake protein level on productivity, blood metabolites, carcass characteristics, and production economics of Hanwoo steers. Asian-Australas J Anim Sci. 2020;33:1599-609. https://doi.org/10.5713/ajas.19.0822
11. Rocha GC, Duarte ME, Kim SW. Advances, implications, and limitations of low-crude-protein diets in pig production. Animals. 2022;12:3478. https://doi.org/10.3390/ani12243478
12. Heo YJ, Park J, Kim YB, Kwon BY, Kim DH, Song JY, et al. Effects of dietary protein levels on performance, nitrogen excretion, and odor emission of growing pullets and laying hens. Poult Sci. 2023;102:102798. https://doi.org/10.1016/j.psj.2023.102798
13. National Research Council. Nutrient requirements of poultry. 9th rev ed. National Academies Press; 1994.
14. National Institute of Animal Science. Korean feeding standard for poultry [Internet]. National Institute of Animal Science. 2022 [cited 2025 Jun 23]. https://lib.rda.go.kr/search/mediaView.do?mets_no=000000315994
15. Oketch EO, Yu M, Nawarathne SR, Chaturanga NC, Maniraguha V, Cruz BGS, et al. Multiprotease supplementation in laying hen diets: impact on performance, egg quality, digestibility, gut histomorphology, and sustainability. Poult Sci. 2025;104:104977. https://doi.org/10.1016/j.psj.2025.104977
16. AOAC (Association of Official Analytical Chemists). Official methods of analysis of AOAC International. AOAC International; 2016.
17. Barzegar S, Wu SB, Noblet J, Choct M, Swick RA. Energy efficiency and net energy prediction of feed in laying hens. Poult Sci. 2019;98:5746-58. https://doi.org/10.3382/ps/pez362
18. Lee J, Yu M, Oketch EO, Nawarathne SR, Kim YB, Chathuranga NC, et al. Effect of dietary protein levels and age on growth performance, total excreta and nitrogen balance of laying hens during the growing phase. Korean J Agric Sci. 2024;51:193-203. https://doi.org/10.7744/kjoas.510210
19. Pérez-Bonilla A, Jabbour C, Frikha M, Mirzaie S, Garcia J, Mateos GG. Effect of crude protein and fat content of diet on productive performance and egg quality traits of brown egg-laying hens with different initial body weight. Poult Sci. 2012;91:1400-5. https://doi.org/10.3382/ps.2011-01917
20. Kim WK, Singh AK, Wang J, Applegate T. Functional role of branched chain amino acids in poultry: a review. Poult Sci. 2022;101:101715. https://doi.org/10.1016/j.psj.2022.101715
21. Siegert W, Ahmadi H, Helmbrecht A, Rodehutscord M. A quantitative study of the interactive effects of glycine and serine with threonine and choline on growth performance in broilers. Poult Sci. 2015;94:1557-68. https://doi.org/10.3382/ps/pev109
22. Bai J, Greene E, Li W, Kidd MT, Dridi S. Branched-chain amino acids modulate the expression of hepatic fatty acid metabolism-related genes in female broiler chickens. Mol Nutr Food Res. 2015;59:1171-81. https://doi.org/10.1002/mnfr.201400918
23. Youssef SF, Shaban SAM, Inas II. Effect of l-arginine supplementation on productive, reproductive performance, immune response and gene expression in two local chicken strains: 1-egg production, reproduction performance and immune response. Egypt Poult Sci. 2015;35:573-90.
24. Adeyemo GO, Abioye SA, Aderemi FA. The effect of varied dietary crude protein levels with balanced amino acids on performance and egg quality characteristics of layers at first laying

phase. Food Nutr Sci. 2012;3:526-9. https://doi.org/10.4236/fns.2012.34074

25. Grobas S, Mendez J, de Blas C, Mateos GG. Laying hen productivity as affected by energy, supplemental fat, and linoleic acid concentration of the diet. Poult Sci. 1999;78:1542-51. https://doi.org/10.1093/ps/78.11.1542
26. Safaa HM, Serrano MP, Valencia DG, Arbe X, Jiménez-Moreno E, Lázaro R, et al. Effects of the levels of methionine, linoleic acid, and added fat in the diet on productive performance and egg quality of brown laying hens in the late phase of production. Poult Sci. 2008;87:1595-602. https://doi.org/10.3382/ps.2008-00005
27. Ji F, Fu SY, Ren B, Wu SG, Zhang HJ, Yue HY, et al. Evaluation of amino-acid supplemented diets varying in protein levels for laying hens. J Appl Poult Res. 2014;23:384-92. https://doi.org/10.3382/japr.2013-00831
28. van Harn J, Rezaei Far A, van Krimpen MM, Veiga C. Low crude protein diets supplemented with free amino acids in laying hens: effects on performance, egg quality, N-efficiency, N-excretion, economics and diet carbon footprint. Wageningen Livestock Research; 2021. Report No.: 1343.
29. Bouyeh M, Gevorgian OX. Influence of different levels of lysine, methionine and protein on the performance of laying hens after peak. J Anim Vet Adv. 2011;10:532-7.
30. Latshaw JD, Zhao L. Dietary protein effects on hen performance and nitrogen excretion. Poult Sci. 2011;90:99-106. https://doi.org/10.3382/ps.2010-01035
31. Konashi S, Takahashi K, Akiba Y. Effects of dietary essential amino acid deficiencies on immunological variables in broiler chickens. Br J Nutr. 2000;83:449-56. https://doi.org/10.1017/S0007114500000556
32. Leeson S, Summers J. Feeding programs for laying hens. ASA: American; 2001.
33. Macelline SP, Toghyani M, Chrystal PV, Selle PH, Liu SY. Amino acid requirements for laying hens: a comprehensive review. Poult Sci. 2021;100:101036. https://doi.org/10.1016/j.psj.2021.101036
34. Kim CH, Kang HK. Effects of energy and protein levels on laying performance, egg quality, blood parameters, blood biochemistry, and apparent total tract digestibility on laying hens in an aviary system. Animals. 2022;12:3513. https://doi.org/10.3390/ani12243513
35. Kumari A, Tripathi UK, Maurya V, Kumar M. Internal quality changes in eggs during storage. Int J Sci Environ Technol. 2020;9:615-24.
36. Samadi, Liebert F. Estimation of nitrogen maintenance requirements and potential for nitrogen deposition in fast-growing chickens depending on age and sex. Poult Sci. 2006;85:1421-9. https://doi.org/10.1093/ps/85.8.1421
37. Abdeshahian P, Lim JS, Ho WS, Hashim H, Lee CT. Potential of biogas production from farm animal waste in Malaysia. Renew Sustain Energy Rev. 2016;60:714-23. https://doi.org/10.1016/j.rser.2016.01.117
38. Iio W, Shimada R, Nonaka I, Ogino A. Effects of a low-protein diet supplemented with essential amino acids on egg production performance and environmental gas emissions from layer-manure composting in laying hens in the later laying period. Anim Sci J. 2023;94:e13853. https://doi.org/10.1111/asj.13853
39. Bos C, Tomé D. Dietary protein and nitrogen utilization. J Nutr. 2000;130:1868S-73S. https://doi.org/10.1093/jn/130.7.1868S