

Determining the Ideal Crude Protein Level in Plant-Based Diets for Late-Phase Laying Hens in Tropical Environments

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ABSTRACT

Reducing dietary crude protein (CP) in laying hens is a strategic approach to improve nitrogen efficiency, reduce feed costs, and enhance sustainability in tropical production systems. The present study aimed to determine the optimal dietary CP concentration required to sustain laying performance in plant-protein-based diets fed to late-phase hens. A total of 3,600 laying hens aged 60 weeks were allocated to three dietary treatments in a completely randomized design with four replicates of 300 hens each. The treatments included a control diet with 18.50% CP (P0), a diet replacing all animal ingredients with corn, rice bran, soybean meal, and corn gluten meal to supply 16.70% CP (P1), and an equivalent plant-protein diet with 16.20% CP (P2). All diets eliminated animal-derived protein sources and were formulated to meet essential amino acid requirements. Feed intake, hen-day production (HDP), egg weight, egg mass, feed conversion ratio (FCR), and income over feed cost (IOFC) were recorded daily for 90 days under tropical conditions (28-34°C) and analyzed over the complete 90-day period. Hens in Group P1 achieved the highest HDP (95.31%), the greatest egg mass (58.58 g/hen/day), and the lowest FCR (2.00), all significantly superior to groups P0 and P2 ($p < 0.05$). Further reduction to 16.20% CP (P2) lowered the HDP to 91.04% and raised the FCR to 2.08, compared with Group P1. In Group P2, egg weight increased to 63.42 g and exceeded that of groups P1 and P0. Group P2 had significantly higher feed intake (119.53 g) than P1 (117.21 g) and P0 (117.48 g). Reducing dietary CP in laying hens improved nitrogen utilization, reduced nitrogen excretion and feed costs, and enhanced the sustainability of egg production under tropical conditions. Diet P1 returned the highest IOFC (726.26 IDR or 0.0446 USD per hen per day) and the lowest feed cost per kilogram of egg (12,606 IDR or 0.773 USD). The results demonstrated that reducing dietary CP from 18.50% to 16.70% in a plant-protein-based formulation optimized laying performance and economic efficiency in late-phase laying hens under tropical conditions. The 16.70% CP level represented a practical threshold that balanced sustainability, nutrient efficiency, and profitability.

Keywords: Crude protein, Egg, Income over feed cost, Laying hen, Tropical condition

INTRODUCTION

The global poultry industry encounters an increasing challenge of balancing productive efficiency with environmental sustainability and economic viability, especially in tropical regions where heat stress exacerbates nutritional requirements (Oluwabenga and Fraley, 2023). Dietary crude protein (CP) is the most expensive nutritional component in poultry feed formulations after energy (Chathuranga and Heo, 2025), and excessive CP inclusion increases nitrogen excretion, ammonia

emissions, and feed costs (de Rauglaudre et al., 2023; Heo et al., 2023). With rising global soybean meal prices and growing environmental regulations, developing low-protein diets that reduce nitrogen excretion and feed costs while limiting losses in productive performance has become an important focus in poultry studies (Oketch et al., 2026).

Advances in crystalline amino acid (AA) technology have enabled the formulation of reduced-CP diets without compromising essential AA supply, allowing hens to express their genetic potential at lower protein intakes

(Macelline et al., 2021; Ali et al., 2025). Torki et al. (2015) demonstrated that dietary CP in laying hens can be reduced by 2 to 4% below the conventional 17% to 19% CP. However, the threshold at which further CP reduction impairs laying persistence and egg quality remains context-dependent and is influenced by genotype, age, ambient temperature, and the specific dietary protein sources (Hu et al., 2024).

Late-phase laying hens (beyond 60 weeks of age) represent a critical production stage in which laying rate naturally declines, metabolic efficiency decreases, and susceptibility to nutrient imbalances increases (Kleyn and Ciacciariello, 2023). Optimizing CP levels in the late-laying phase is particularly important because an imbalanced energy-to-protein ratio in low-protein diets induces hepatic lipid deposition and disrupts lipid metabolism (Hu et al., 2024). Hepatic lipid accumulation is further exacerbated when low-protein diets are combined with either low or high energy levels, depending on the energy-to-protein ratio (Hu et al., 2024). Furthermore, tropical climatic conditions characterized by chronic heat stress (ambient temperatures of 28–34°C) reduce voluntary feed intake, increase maintenance energy requirements, and alter AA utilization patterns (Torki et al., 2015). Although numerous studies have examined low-CP diets for laying hens, most were conducted under temperate conditions and used traditional protein sources, such as fish meal or meat and bone meal, including animal-based ingredients (Torki et al., 2015; Iqbal et al., 2026). Comparatively few studies have evaluated exclusively plant-protein-based formulations in late-phase hens under actual tropical field conditions with large flock sizes. The present study aimed to identify the optimal dietary CP concentration in plant-protein-based diets for 60-week-old laying hens managed under commercial tropical conditions in Central Java, Indonesia.

MATERIALS AND METHODS

Ethical approval

All experimental procedures involving live hens were reviewed and approved by the Animal Ethics Committee, Universitas Sebelas Maret, Indonesia, under approval number 18/UN27.20/PT01.01/2023. The hens were managed throughout the trial in accordance with the Lohmann Brown-Classic management guide. The authors declared that no procedure that would cause avoidable pain or distress was performed, and that the flock remained under routine commercial husbandry throughout the experimental period.

Study time and location

The study was conducted over 90 days, from 9 September to 10 December 2025, at a commercial layer farm in Central Java, Indonesia. The region has a tropical climate with high ambient temperatures and relative humidity. Ambient temperature ranged from 28°C to

34°C, and relative humidity from 64% to 75% during daylight hours throughout the experimental period. Temperature and relative humidity were recorded with a Lutron Environmental Data Logger (Taiwan).

Experimental animals and housing

A total of 3,600 Lohmann Brown laying hens aged 60 weeks were used in the present study, and the hens were randomly allocated into three dietary treatment groups. Each treatment consisted of 1,200 hens, further divided into 4 replications (300 hens per replication) to ensure statistical reliability. The hens were housed in a conventional battery cage system comprising 600 cages, each measuring 60 × 55 × 40 cm and holding 6 hens, providing 550 cm² per hen. Cages were arranged in 3 tiers and 4 double-sided rows. Husbandry practices followed the guidelines outlined in the Lohmann Brown-Classic management manual (Lohmann Breeders, 2021). Feed was provided at 130 g per chicken, and leftover feed was weighed daily. Chickens had unrestricted access to water. The lighting schedule consisted of 16 hours of illumination followed by 8 hours of darkness, with the average light intensity at bird level maintained at 25 lux throughout the experiment.

The flock received vaccination in line with the farm health program, based on baseline antibody titer monitoring, and was administered before the experiment began, following the vaccine manufacturer's recommendations (PT Vaksindo Satwa Nusantara, Bogor, Indonesia). At 58 weeks of age, hens were vaccinated with an inactivated Newcastle disease and infectious bronchitis vaccine (NDIB killed) via intramuscular injection (0.5 mL/hen). At 60 weeks of age, hens received an inactivated Avian Influenza vaccine (AI killed) by intramuscular injection (0.5 mL/hen). At 64 weeks of age, hens received live Newcastle disease and infectious bronchitis vaccines (NDIB Live) via drinking water. No further vaccinations were given during the remainder of the experimental period. No other supplements or medications were given, except the vitamin-mineral premix and feed additives incorporated into the experimental diets.

Dietary treatments

The experimental diets were formulated to meet the nutrient requirements of late-phase Lohmann Brown layers, as specified in the Nutrient Requirements of Poultry (NRC, 1994) and the Lohmann Brown-Classic management guide (Lohmann Breeders, 2021), with different CP concentrations and protein sources. The first group was the control group and received a conventional mash diet containing 18.50% CP (P0). The second group received a full plant-protein diet containing 16.70% CP (P1), and the third group received a full plant-protein diet containing 16.20% CP (P2). The control diet (P0) was mixed on the farm, and all diets (P0–P2) were prepared from locally sourced ingredients using the BRILL formulation software. Diets P1 and P2 excluded all

animal-derived protein sources and were balanced for the essential AAs lysine, methionine, threonine, tryptophan, valine, and isoleucine on an ideal-protein basis using the standard AA matrix in the BRILL formulation software; thus, the reduction in total protein was compensated. The ingredients and calculated nutrient composition of the experimental diets are presented in Table 1.

Table 1. Ingredient composition and calculated nutrient content of the experimental diets fed to laying hens aged 60 weeks (as-fed basis)

Ingredient (%)	P0	P1	P2
Corn	51.39	56.94	58.78
Rice bran	8.00	7.94	7.00
Soybean meal	25.20	19.50	16.42
Corn gluten meal	-	3.00	4.50
Meat bone meal	4.00	-	-
Limestone	9.50	10.07	10.75
Monocalcium phosphate	0.70	1.40	1.35
Salt and sodium bicarbonate	0.45	0.45	0.45
Additives (choline, betaine, GAA)	0.32	0.32	0.32
Micro-ingredients ¹	0.14	0.14	0.14
Synthetic amino acids ²	0.30	0.24	0.29
Total	100.00	100.00	100.00
Calculated nutrient composition[†]			
Metabolizable energy (kcal/kg)	2,650	2,671	2,696
Crude protein (%)	18.50	16.70	16.20
Crude fiber (%)	3.22	3.37	3.14
Ether extract (%)	3.85	3.55	3.49
Calcium (%)	4.15	4.12	4.37
Total phosphorus (%)	0.72	0.71	0.68
Available phosphorus (%)	0.42	0.41	0.38
Lysine (%)	0.95	0.82	0.78
Methionine + cystine (%)	0.76	0.69	0.71
Threonine (%)	0.68	0.61	0.60

¹Micro-ingredients supplied per kilogram of diet: A vitamin-mineral premix, a toxin binder, vitamin E, and selenium (Se). ²Synthetic amino acids included L-lysine HCl, DL-methionine, L-threonine, L-tryptophan, L-valine, and L-isoleucine. P0: Conventional diet containing 18.50% CP, P1: Plant-protein-based diet containing 16.70% CP, P2: Plant-protein-based diet containing 16.20% CP, CP: Crude protein, GAA: Guanidinoacetic acid. [†]Values were calculated from the tabulated ingredient composition using standard feed-matrix values.

The higher proportion of corn and the lower inclusion of soybean meal in diets P1 and P2 resulted from the least-cost formulation based on locally available feed ingredients (Surakarta, Central Java, Indonesia). Locally sourced cereal ingredients mainly supplied energy, while crystalline AA supplements provided essential AAs instead of adding more intact protein sources (NRC, 1994; Macelline et al., 2021). The crystalline AAs L-lysine HCl, DL-methionine, L-threonine, L-tryptophan, L-valine, and L-isoleucine were obtained from PT. Menjangan Sakti, Jakarta, Indonesia. Monocalcium phosphate was the only supplemental phosphorus source in the experimental diets; the remaining phosphorus was from the plant ingredients. Choline chloride, betaine, and guanidinoacetic acid were obtained from PT. Fenanza Putra Perkasa, Bandung, Indonesia. Guanidinoacetic acid was included because it

supports the productive performance of aged laying hens fed reduced-protein diets at high ambient temperatures (Mahdavi et al., 2026). Betaine was included because it can improve laying-hen performance under tropical conditions (Wahid et al., 2021) and support gut microbiota composition and intestinal health under heat stress, as demonstrated in laying pullets (Wahid et al., 2024) and quail (Pradista et al., 2026).

Data collection

Production performance was monitored continuously over the 90-day experimental period (Days 1 to 90), and data were summarized by replicate for statistical analysis. Feed intake was determined daily (Days 1-90) for each replicate by subtracting the weight of feed refusals from the weight of feed offered, expressed as grams per hen per day. Hen-day production (HDP) was recorded daily (Days 1-90) as the total number of eggs produced divided by the number of live hens present on that day, expressed as a percentage. For egg weight determination, a random sample of 100 eggs per replicate was collected and weighed daily (Days 1-90) using a digital electronic balance (ACIS BC-500, 600×0.01 g, PT. Libramas, Indonesia). Daily egg mass was calculated as the product of daily HDP and daily mean egg weight (g/hen/day). The overall feed conversion ratio (FCR) was calculated at the end of the 90-day period as total cumulative feed intake divided by total cumulative egg mass produced per replicate over the entire experimental period. Production performance parameters were calculated following the general procedures of Oketch et al. (2025), with modifications to the sampling frequency and measurement period.

Economic evaluation

At the end of the 90-day period, economic efficiency was evaluated using the income over feed cost (IOFC) method to determine the most cost-effective feed formulation (North et al., 1990). The calculation used local market prices observed during the experimental period. The feed cost was 6,500 IDR/kg for P0, 6,300 IDR/kg for P1, and 6,240 IDR/kg for P2, corresponding to 0.399 USD/kg, 0.387 USD/kg, and 0.383 USD/kg, respectively. The egg selling price remained stable at 25,000 IDR/kg (1.534 USD/kg) throughout the experimental period. Income over feed cost was determined using Formula 1. The feed cost per kilogram of egg was calculated based on Formula 2. All amounts in Indonesian rupiah were converted to United States dollars at a rate of 1.00 USD = 16,300 IDR in January 2025.

IOFC (IDR/hen/day) = (HDP [%] × egg weight [g] × egg price [IDR/kg]) – (feed intake [g/hen/day] × feed price [IDR/kg]) (Formula 1)

Feed cost (IDR/kg egg) = (feed intake [g/hen/day] × feed price [IDR/kg]) ÷ (HDP [%] × egg weight [g]) (Formula 2)

Statistical analysis

The data were analyzed using a completely randomized design, with the replicate (300 hens) as the experimental unit, yielding four observations per treatment. Normality of residuals and homogeneity of variance were verified with the Shapiro-Wilk test and the Levene test, respectively, before a one-way analysis of variance was performed. Tukey's honestly significant difference test was used to separate differences between treatment means, and differences were considered significant at p -value less than 0.05. The magnitude of the treatment effect was expressed as eta squared (η^2), derived from the F statistic and its degrees of freedom; values of 0.01, 0.06, and 0.14 were interpreted as small, medium, and large effects, respectively. All results were presented as mean $\pm$ standard deviation (SD). All statistical analyses were performed using R Statistical Software, version 4.3.2 (R Core Team, 2025).

RESULTS AND DISCUSSION

Production performance

The effects of dietary CP levels on the production performance of late-phase hens are presented in Table 2. Dietary CP levels significantly influenced all measured production parameters ($p < 0.05$). Feed intake differed significantly ($p = 0.0293$) among the dietary treatment groups ($\eta^2 = 0.54$). Hens in Group P2 consumed significantly more feed (119.53 ± 0.80 g/hen/day) than those in P1 (117.21 ± 0.37 g/hen/day; $p < 0.05$), whereas feed intake did not differ significantly between Group P0 (117.48 ± 1.67 g/hen/day) and Group P1 ($p > 0.05$). The higher feed intake in Group P2 did not improve egg production or feed efficiency, suggesting that the lower CP concentration did not adequately support productive performance (Iqbal et al., 2026).

Feed conversion ratio differed significantly ($p = 0.0124$) among dietary treatments ($\eta^2 = 0.62$). Hens in Group P1 achieved the lowest FCR (2.00 ± 0.00), indicating greater feed utilization efficiency than hens in Group P0 (2.04 ± 0.02) or P2 (2.08 ± 0.01 ; $p < 0.05$). This improvement in FCR at the moderate protein reduction was consistent with the principle, established in broiler low-CP feeding trials, that a precisely balanced low-CP diet containing supplemental crystalline AA can improve nitrogen utilization, because less dietary protein is deaminated and excreted (de Rauglaudre et al., 2023). The current findings were in line with those of Ali et al. (2025), who reported that a 16% CP diet supplemented with essential AA maintains a feed efficiency comparable to that of a 17% CP control in Hy-Line Brown hens. In contrast, the FCR deteriorated at 16.20% CP (Group P2) in the present study. The poorer FCR observed in P2 indicated that reducing dietary CP to 16.20% may have limited the availability of non-essential AAs and other nitrogen-containing compounds required for efficient egg

synthesis (He et al., 2021). In the present study, the superiority of Group P1, with a 16.70% CP, was primarily due to the strategic balance of essential AAs. Supplementation with crystalline lysine, methionine, threonine, tryptophan, valine, and isoleucine ensured that the AA profile of the reduced-CP diets met the requirement for egg protein synthesis despite the lower total nitrogen supply (He et al., 2021; Ali et al., 2025).

Hen-day production was significantly ($p = 0.0001$) affected by dietary treatment ($\eta^2 = 0.87$). Group P1 had the highest HDP ($95.31 \pm 0.18\%$), exceeding the control group ($94.20 \pm 0.28\%$) and Group P2 ($91.04 \pm 0.29\%$). The 4.3% decline in HDP from 16.70% CP (Group P1) to 16.20% CP (Group P2) indicated that 16.20% CP was insufficient to sustain laying persistency in 60-week-old hens, possibly because an energy-to-protein imbalance disrupted hepatic lipid metabolism via the gut-liver axis and limited the availability of non-essential AAs required for egg synthesis (Hu et al., 2024). Similarly, Liu et al. (2024) stated that excessively reducing CP in laying hens should be done cautiously, as the resulting productivity losses might reduce the environmental gains. The high HDP observed in Group P1 under tropical temperatures (28–34°C) was considerable, since heat stress can inhibit ovarian function and reduce laying rates in certain conditions (Oluwagbenga and Fraley, 2023). The present result suggested that the 16.70% CP level with balanced AA provided sufficient nutrients for egg synthesis even under chronic heat exposure, possibly because lower-protein diets generate less metabolic heat during digestion. Similarly, Torki et al. (2015) found that increasing heat did not reduce laying performance in hens fed low-CP diets supplemented with AA under high ambient temperatures.

Egg mass was significantly higher ($p = 0.0004$) in Group P1 (58.58 ± 0.23 g/hen/day) compared to groups P0 (57.34 ± 0.15 g/hen/day) and P2 (57.74 ± 0.23 g/hen/day; $\eta^2 = 0.82$). The superior egg mass in Group P1 was due to high HDP and optimal egg weight, reflecting the most favorable balance between laying rate and egg size. The present egg mass values were consistent with those of Iqbal et al. (2026), who maintained egg mass at 14.50% CP with glycine and serine supplementation. In contrast, Liu et al. (2024) found that a diet with 13.85% CP reduced egg production and egg mass compared with 15.63% CP in 38-week-old hens, suggesting that the threshold in the present study was within the range reported for AA-balanced low-protein diets (Iqbal et al., 2026).

Egg weight increased significantly ($p = 0.0001$) as dietary CP decreased ($\eta^2 = 0.87$). Group P2 produced the heaviest eggs (63.42 ± 0.09 g), followed by Group P1 (61.46 ± 0.16 g) and Group P0 (60.87 ± 0.08 g). The heavier eggs observed in Group P2 may be associated with the lower laying rate in this group. This shift reflected an inverse relationship between egg number and egg size, a well-documented reproductive strategy in avian species. Notably, the increase in egg weight observed in Group P2

was insufficient to offset the decline in HDP, resulting in

lower egg mass and poorer FCR than in Group P1.

Table 2. Effects of dietary crude protein level on production performance in late-phase laying hens

Parameters	Group P0	Group P1	Group P2	p-value	η^2
Feed intake (g/hen/day)	117.48 $\pm$ 1.67 ^{ab}	117.21 $\pm$ 0.37 ^b	119.53 $\pm$ 0.80 ^a	0.0293*	0.54
FCR	2.04 $\pm$ 0.02 ^b	2.00 $\pm$ 0.00 ^c	2.08 $\pm$ 0.01 ^a	0.0124*	0.62
HDP (%)	94.20 $\pm$ 0.28 ^b	95.31 $\pm$ 0.18 ^a	91.04 $\pm$ 0.29 ^c	0.0001**	0.87
Egg mass (g/hen/day)	57.34 $\pm$ 0.15 ^b	58.58 $\pm$ 0.23 ^a	57.74 $\pm$ 0.23 ^b	0.0004**	0.82
Egg weight (g)	60.87 $\pm$ 0.08 ^c	61.46 $\pm$ 0.16 ^b	63.42 $\pm$ 0.09 ^a	0.0001**	0.87

P0: Conventional diet containing 18.50% CP, P1: Plant-protein-based diet containing 16.70% CP, P2: Plant-protein-based diet containing 16.20% CP. CP: Crude protein, FCR: Feed conversion ratio, HDP: Hen-day production. ^{a, b, c} Mean different superscript letters in a row differ significantly ($p < 0.05$). * indicates $p < 0.05$, ** indicates $p < 0.01$, η^2 : Eta squared, an estimate of the proportion of total variance explained by the dietary treatment. Data are presented as mean $\pm$ SD.

Economic parameters

Dietary CP levels significantly affected all economic indicators measured (Table 3; $p < 0.05$). Feed cost differed significantly ($p = 0.0403$) among treatment groups ($\eta^2 = 0.51$). Group P1 recorded the lowest feed cost (738.45 $\pm$ 2.33 IDR/hen/day, equivalent to 0.0453 USD/hen/day), followed by Group P2 (745.85 $\pm$ 5.01 IDR/hen/day, equivalent to 0.0458 USD/hen/day) and Group P0 (IDR 763.65 $\pm$ 10.87/hen/day, equivalent to 0.0469 USD/hen/day). The lower feed cost of the plant-protein-based diets (P1 and P2) reflected lower inclusion of expensive soybean meal and lower total protein content, which reduced formulation cost. The slightly higher feed cost of Group P2 relative to P1, despite its lower CP content, resulted from higher inclusion of corn gluten meal (4.50% versus 3.00%). This higher inclusion counteracted the cost savings achieved by reducing soybean meal. This finding emphasized that feed cost optimization in low-CP diets depends not only on protein level but also on the specific ingredient used. This strategy was consistent with Macelline et al. (2021), who noted that the economic success of low-CP formulations relies heavily on selecting complementary ingredients and crystalline AAs rather than on protein reduction alone (Macelline et al., 2021).

Egg revenue was significantly ($p = 0.0004$) influenced by treatments ($\eta^2 = 0.82$). Group P1 had the highest daily egg revenue (1,464.70 $\pm$ 5.85 IDR/hen/day, equivalent to 0.0898 USD/hen/day), which exceeded the values of groups P0 (1,433.73 $\pm$ 3.92 IDR/hen/day, equivalent to 0.0880 USD/hen/day) and P2 (1,443.74 $\pm$ 5.93 IDR/hen/day, equivalent to 0.0886 USD/hen/day). This superiority of Group P1 primarily resulted from the higher HDP and egg mass in P1 hens, leading directly to increased saleable egg output.

Income over feed cost, the most critical indicator of economic efficiency in commercial egg production, indicated a highly significant difference ($p = 0.0002$) among treatments ($\eta^2 = 0.85$). Group P1 produced the highest IOFC (726.26 $\pm$ 4.41 IDR/hen/day, equivalent to 0.0446 USD/hen/day), which represented a significant

improvement of 8.4% than the control group (670.07 $\pm$ 10.92 IDR/hen/day equivalent to 0.0411 USD/hen/day) and 4.1% improvement than Group P2 (697.89 $\pm$ 9.73 IDR/hen/day, equivalent to 0.0428 USD/hen/day; $p < 0.05$). Group P1 attained a higher IOFC by reducing feed cost and increasing egg revenues. The economic advantage of a moderate CP reduction in the present study contrasts with the findings of Oketch et al. (2026), who found that balanced protein reduction decreased egg income and feed costs but did not affect overall profit margin. In tropical production systems, where feed costs account for 65-75% of total production costs (Sonaiya and Swan, 2004), the 8.4% improvement in IOFC at 16.70% CP was a meaningful gain in farm profitability, as observed in this study.

Feed cost of egg was significantly lower ($p = 0.0015$) in Group P1 (12,606 $\pm$ 34 IDR/kg of egg, equivalent to 0.773 USD/kg of egg) than in groups P2 (12,924 $\pm$ 125 IDR/kg of egg, equivalent to 0.793 USD/kg of egg) and P0 (13,316 $\pm$ 188 IDR/kg of egg, 0.817 USD/kg of egg; $\eta^2 = 0.76$). Because feed cost per kilogram of egg reflects both feed efficiency and feed cost, the lower value observed in Group P1 indicates that the 16.70% CP formulation achieved the most cost-effective conversion of feed into egg output. The 5.3% decrease in feed cost per kilogram of eggs from P0 to P1 resulted in savings of 710 IDR/kg of eggs, or about 0.044 USD/kg of eggs. This saving is noticeable in large-scale commercial operations. These findings were consistent with those of Iqbal et al. (2026), who reduced feed cost per unit of egg output by supplementing glycine and serine in a low-protein diet. By contrast, Liu et al. (2024) reported that excessively lowering CP can reduce egg production and feed-cost savings. In the present study, feed costs were reduced without lowering production, which is consistent with the favorable range reported by Iqbal et al. (2026).

Using plant-protein sources in the experimental diets enhanced the practical relevance of the present findings. Eliminating animal-derived protein ingredients such as fish meal and meat and bone meal simplified supply chain

logistics, reduced the risk of contamination with antibiotic residues or pathogens, and aligned with growing consumer demand for plant-based animal production systems (Attia et al., 2024). The present results demonstrated that a

formulation based on corn, soybean meal, and corn gluten meal, when combined with appropriate crystalline AA supplementation, can fully support laying performance in late-phase hens without reliance on animal protein.

Table 3. Effects of dietary crude protein level on the economic performance of late-phase laying hens

Parameters	Group P0	Group P1	Group P2	p-value	η^2
Feed cost of formulated feed (IDR/hen/day)	763.65 ± 10.87 ^a	738.45 ± 2.33 ^b	745.85 ± 5.01 ^b	0.0403*	0.51
Feed cost of formulated feed (USD/hen/day)	0.0469 ± 0.0007 ^a	0.0453 ± 0.0001 ^b	0.0458 ± 0.0003 ^b	0.0403*	0.51
Egg revenue (IDR/hen/day)	1,433.73 ± 3.92 ^b	1,464.70 ± 5.85 ^a	1,443.74 ± 5.93 ^b	0.0004**	0.82
Egg revenue (USD/hen/day)	0.0880 ± 0.0002 ^b	0.0898 ± 0.0004 ^a	0.0886 ± 0.0004 ^b	0.0004**	0.82
IOFC (IDR/hen/day)	670.07 ± 10.92 ^c	726.26 ± 4.41 ^a	697.89 ± 9.73 ^b	0.0002**	0.85
IOFC (USD/hen/day)	0.0411 ± 0.0007 ^c	0.0446 ± 0.0003 ^a	0.0428 ± 0.0006 ^b	0.0002**	0.85
Feed cost of egg (IDR/kg of egg)	13,316 ± 188 ^a	12,606 ± 34 ^c	12,924 ± 125 ^b	0.0015**	0.76
Feed cost of egg (USD/kg of egg)	0.8169 ± 0.0115 ^a	0.7733 ± 0.0021 ^c	0.7929 ± 0.0077 ^b	0.0015**	0.76

P0: Conventional diet containing 18.50% CP, P1: Plant-protein-based diet containing 16.70% CP, P2: Plant-protein-based diet containing 16.20% CP. CP: Crude protein, IDR: Indonesian rupiah, IOFC: Income over feed cost, USD: United States dollar. Amounts in USD were obtained by converting the corresponding IDR value at USD 1.00 = IDR 16,300; the p-value and η^2 apply to both currencies because the conversion was a constant multiplier. ^{a, b, c} Mean different superscript letters in a row differ significantly ($p < 0.05$). * indicates $p < 0.05$, ** indicates $p < 0.01$, η^2 : Eta squared, an estimate of the proportion of total variance explained by the dietary treatment. Data are presented as mean ± SD.

CONCLUSION

Reducing dietary CP from 18.50% to 16.70% in a plant-protein-based formulation supplemented with crystalline AA significantly improved productive performance and economic efficiency in 60-week-old Lohmann Brown hens under tropical conditions in Central Java, Indonesia. The 16.70% CP diet produced the highest HDP (95.31%), the lowest FCR (2.00), the greatest egg mass (58.58 g/hen/day), and the highest IOFC (726.26 IDR or 0.0446 USD/hen/day). Further reducing CP to 16.20% decreased laying persistency and feed efficiency, indicating that 16.70% CP was the optimal threshold for this production system. The present findings support the practical adoption of moderate CP reduction in tropical layer production to improve sustainability, nutrient efficiency, and farm profitability simultaneously. The present study did not include egg quality traits such as shell thickness, albumen height, and Haugh unit. Additionally, nitrogen balance and ammonia emissions were not measured, so the environmental benefits of reduced-CP diets were not quantified, which are recommended to be investigated in future studies.

DECLARATIONS

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Authors' contributions

Zainudin Al Wahid conceptualized the study, designed the methodology, interpreted the data, prepared the visualizations, and wrote the original draft. Adi Ratriyanto conceptualized the study, interpreted the data, supervised the study, and wrote the original draft. Luthfi Adya Pradista designed the methodology, interpreted the data, and prepared the visualizations. Muhammad Nidhomun Ni'am designed the methodology, interpreted the data, and prepared the visualizations. All authors read and approved the final edition of the manuscript.

Availability of data and materials

The datasets generated and analyzed during the present study are available from the corresponding author upon reasonable request. The raw production and economic records are submitted with the present article.

Competing interests

The authors declared no conflicts of interest.

Ethical considerations

The authors declare that this article is original, has not been published elsewhere in whole or in part, and is not under consideration by any other journal. All authors have avoided plagiarism, data fabrication, data falsification, and

duplicate publication. Regarding the use of artificial intelligence, the authors declare that generative AI tools (Gemini, ChatGPT, and Claude AI) were used solely for language editing to improve readability, and the authors have reviewed and revised the language-edited version and take full responsibility for the final content of the article. The authors accept full responsibility for the originality and the integrity of the content of this article, before submission and after acceptance.

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