

## Effects of Dietary Crude Protein Level on Production Performance and Egg Quality of “Jinghong No. 1” Laying Hens during the Late Laying Period: Postprint

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### Abstract

This experiment aimed to investigate the effects of different dietary crude protein levels on production performance and egg quality of “Jinghong No. 1” laying hens during the late laying period, to determine the optimal dietary protein level. A total of 720 commercial “Jinghong No. 1” layers at 41 weeks of age were randomly divided into Group I, Group II, Group III, and Group IV based on measured crude protein levels (14.08%, 14.53%, 14.98%, and 15.44%, respectively), with 12 replicates per group and 15 birds per replicate. Following a 1-week preliminary period, the formal experimental period lasted 22 weeks. The results showed: 1) Dietary crude protein level had significant effects on laying rate, average egg weight, and daily egg mass of laying hens ( $P < 0.05$ ), and all increased linearly with increasing dietary crude protein level ( $P < 0.05$ ); dietary crude protein level had a significant effect on feed-to-egg ratio of laying hens ( $P < 0.05$ ), and it decreased linearly with increasing crude protein level ( $P < 0.05$ ). 2) Eggshell thickness in Group IV was significantly higher than that in Group I and Group III ( $P < 0.05$ ); egg weight in Group IV was significantly higher than that in Group I and Group II ( $P < 0.05$ ), and egg weight increased linearly with increasing dietary crude protein level ( $P < 0.05$ ). 3) Economic benefit analysis indicated that Group IV had the lowest egg weight cost and the best economic benefit. Based on comprehensive analysis of production performance and economic benefit, the appropriate crude protein level for “Jinghong No. 1” laying hens aged 42-64 weeks is 15.44%, and the appropriate protein-to-energy ratio is 13.41 g/MJ.

## Full Text

# Effects of Dietary Crude Protein Level on Performance and Egg Quality of “Jinghong 1” Laying Hens during Late Production Phase

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## Abstract

This study investigated the effects of varying dietary crude protein (CP) levels on production performance and egg quality of “Jinghong 1” laying hens during the late laying period to determine the optimal protein requirement. A total of 720 healthy “Jinghong 1” commercial laying hens aged 41 weeks were randomly allocated to four groups ( , , , and ) with 12 replicates per group and 15 hens per replicate, based on measured dietary CP levels of 14.08%, 14.53%, 14.98%, and 15.44%, respectively. Following a 1-week adaptation period, the 22-week formal trial commenced when hens reached 42 weeks of age. The results demonstrated: (1) Dietary CP level significantly affected laying rate, average egg weight, and daily egg mass ( $P < 0.05$ ), with all parameters increasing linearly as CP level rose ( $P < 0.05$ ). The feed-to-egg ratio was also significantly influenced ( $P < 0.05$ ), decreasing linearly with increasing CP level ( $P < 0.05$ ). (2) Group exhibited significantly greater eggshell thickness compared to groups and ( $P < 0.05$ ), and significantly higher egg weight relative to groups and ( $P < 0.05$ ), with egg weight increasing linearly with dietary CP level ( $P < 0.05$ ). (3) Economic analysis revealed that group achieved the lowest egg weight cost and optimal economic returns. Integrating production performance and economic considerations, the optimal dietary CP level for “Jinghong 1” hens aged 42–64 weeks is 15.44%, corresponding to an appropriate protein-to-energy ratio of 13.41 g/MJ.

**Keywords:** “Jinghong 1” laying hens; late laying period; crude protein; production performance; egg quality

## Introduction

Protein constitutes the fundamental material basis of life, and poultry must obtain adequate protein through their diet to sustain growth and production. Appropriate dietary protein levels not only maximize the productive potential of laying hens but also control nitrogen excretion in manure and reduce feed costs, thereby improving economic efficiency. The “Jinghong 1” breed represents an indigenous elite strain developed through years of selective breeding in China. Commercial “Jinghong 1” chicks can be sexed by feathering rate, with survival rates reaching 98% during rearing and 95% during the laying period. The breed

maintains a peak laying rate of 93–96% for over 180 days, with rates exceeding 90% [1]. However, comprehensive feeding standards for this breed remain underdeveloped. While research has addressed optimal CP levels for “Jinghong 1” hens during peak production [2], no studies have investigated protein requirements during the late laying phase. This experiment was designed to evaluate the effects of varying CP levels on production performance and egg quality in late-phase “Jinghong 1” hens, incorporating economic analysis to establish optimal protein recommendations and provide foundational data for developing complete nutritional standards.

### 1.1 Experimental Design and Diets

A single-factor randomized design was employed with consistent metabolizable energy levels across treatments. A total of 720 healthy “Jinghong 1” commercial laying hens aged 41 weeks were purchased from Beijing Huadu Yukou Poultry Industry Co., Ltd. and randomly assigned to four groups with dietary CP levels of 14.08%, 14.53%, 14.98%, and 15.44%. Each group comprised 12 replicates of 15 hens, with uniform laying rate and daily egg mass ensured across replicates. The trial included a 1-week pre-period, with the formal experiment running from 42 to 64 weeks of age. The corn-soybean meal-cottonseed meal-based diets were formulated according to nutritional specifications for late-phase laying hens provided by Beijing Huadu Yukou Poultry Industry Co., Ltd. and the *Feeding Standard of Chicken* (NY/T 33–2004). Diet composition and nutrient levels are presented in Table 1 .

**Table 1** Composition and nutrient levels of experimental diets (air-dry basis)

Items	Group	Group	Group	Group
<b>Ingredients</b>				
Corn				
Soybean meal				
Cottonseed meal				
Limestone				
CaHPO				
Zeolite powder				
NaCl				
NaHCO				
Lysine				
Methionine				
Choline chloride				
Threonine				
Tryptophan				
Premix <sup>1</sup>				
<b>Total</b>				
<b>Nutrient levels<sup>2</sup></b>				
ME (MJ/kg)				

Items	Group	Group	Group	Group
CP	14.08%	14.53%	14.98%	15.44%
Ca				
TP				
NPP				
Methionine				
Lysine				

<sup>1</sup> The premix provided per kilogram of diet: VA 1,700 IU, VB 3 mg, VB 0.024 mg, VD 3,600 IU, VE 21 IU, VK 4.2 mg, riboflavin 10.2 mg, folic acid 0.9 mg, calcium pantothenate 15 mg, nicotinic acid 45 mg, pyridoxine 5.4 mg, biotin 0.15 mg, Cu 6.8 mg, Fe 66 mg, Zn 83 mg, Mn 80 mg, I 1 mg, Se 0.3 mg.

<sup>2</sup> CP was measured value, while other nutrient levels were calculated values.

## 1.2 Housing Management

The trial was conducted at the Zhuozhou Experimental Base of China Agricultural University. The closed poultry house utilized longitudinal negative-pressure ventilation with evaporative cooling pads during summer, maintaining ambient temperature between 18–28°C. Lighting was provided from 06:00 to 22:00 (16 h daily) at 10–20 lx intensity. Hens were housed in three-tiered step cages and fed mash diets ad libitum with free access to water.

### 1.3.1 Crude Protein Level Determination

Dietary CP content for each group was determined according to GB/T 6432–1994 using a FOSS automatic Kjeldahl nitrogen analyzer.

### 1.3.2 Production Performance

Daily egg number and egg weight were recorded per replicate to calculate laying rate, average egg weight, and daily egg mass. Feed intake was measured every two weeks per replicate to determine average daily feed intake and feed-to-egg ratio. At the trial onset, three hens from one cage per replicate were selected and weighed monthly to monitor body weight changes and calculate average weekly body weight gain.

### 1.3.3 Egg Quality Measurement

At 64 weeks of age, 30 eggs per group were randomly selected for quality assessment. Eggshell strength was measured using an F0241 Eggshell Force Reader (Orka Technology Ltd.). Eggshell thickness was determined with a TI-PVX eggshell thickness gauge (Orka Technology Ltd.). Yolk color and Haugh units were analyzed using an Egg Analyzer™ (Orka Technology Ltd.). Yolk weight,

shell weight, and egg weight were measured using an electronic balance (precision 0.01 g). Yolk, albumen, and shell percentages were calculated as the ratio of each component to total egg weight.

#### 1.4 Statistical Analysis

Experimental data were analyzed using SPSS 20.0 software via General Linear Model (GLM) procedures, with Duncan's multiple comparison tests and linear and quadratic contrast analyses employed to evaluate CP level effects. Significance was declared at  $P < 0.05$ .

#### 2.1 Effects of Dietary Crude Protein Level on Late-Phase Production Performance

As shown in Table 2, dietary CP level significantly affected laying rate, average egg weight, daily egg mass, and feed-to-egg ratio ( $P < 0.05$ ). Group exhibited significantly lower laying rate compared to other groups ( $P < 0.05$ ), while group showed significantly higher laying rate than groups and ( $P < 0.05$ ), with laying rate increasing linearly with dietary CP level ( $P < 0.05$ ). Average egg weight in group was significantly lower than other groups ( $P < 0.05$ ), with no significant differences among groups, , and ( $P > 0.05$ ), though average egg weight increased linearly with CP level ( $P < 0.05$ ). Daily egg mass in group was significantly lower than other groups ( $P < 0.05$ ), while group was significantly higher than groups and ( $P < 0.05$ ), increasing linearly with dietary CP level ( $P < 0.05$ ). Group demonstrated the lowest feed-to-egg ratio, which was significantly lower than groups and ( $P < 0.05$ ), decreasing linearly as dietary CP level increased ( $P < 0.05$ ). Dietary CP level did not significantly affect average daily feed intake or average weekly body weight gain ( $P > 0.05$ ), though all groups exhibited negative body weight gain that linearly increased (became less negative) with rising dietary CP level ( $P < 0.05$ ).

**Table 2** Effects of dietary crude protein level on performance of Jinghong laying hens during late stage of egg production (42 to 64 weeks of age) (n=12)

Items	Group	Group	Group	Group	P-value	Linear P-value	Quadratic P-value
Laying rate/%	79.29	82.56	83.97	86.16	<0.001	<0.001	
Average egg weight/g	60.01	61.38	61.00	61.49	<0.001	<0.001	
Daily egg production/g	47.50	50.48	51.16	52.67	<0.001	<0.001	

Items	Group	Group	Group	Group	P-value	Linear P-value	Quadratic P-value
ADFI/(g/d)							
Feed/egg	2.68	2.52	2.44	2.33	<0.001	<0.001	
Average weekly body weight gain/(g/week)							

*In the same column, values with no letter or the same letter superscripts indicate no significant difference ( $P>0.05$ ), while different letters indicate significant difference ( $P<0.05$ ). The same applies below.*

## 2.2 Effects of Dietary Crude Protein Level on Late-Phase Egg Quality

Table 3 shows that dietary CP level significantly affected eggshell thickness and egg weight ( $P<0.05$ ). Group exhibited significantly greater eggshell thickness than groups and ( $P<0.05$ ), and significantly higher egg weight than groups and ( $P<0.05$ ), with egg weight increasing linearly with dietary CP level ( $P<0.05$ ). Dietary CP level did not significantly influence eggshell strength, Haugh units, yolk color, or yolk, albumen, and shell percentages ( $P>0.05$ ). However, Haugh units and shell percentage decreased linearly with increasing dietary CP level ( $P<0.05$ ).

**Table 3** Effects of crude protein level on egg quality of Jinghong laying hens during late stage of egg production (64 weeks of age) (n=30)

Items	Group	Group	Group	Group	P-value	Linear P-value	Quadratic P-value
Eggshell thickness/mm	0.270	0.275	0.259	0.280	<0.001		
Eggshell strength/(N/kg)							
Egg weight/g	61.82	62.52	62.94	64.93			
Haugh units							
Yolk color							

### 2.3 Economic Analysis

As presented in Table 4, egg weight cost decreased as dietary CP level increased, with group achieving the lowest cost—reductions of 0.81, 0.44, and 0.26 yuan/kg compared to groups , , and , respectively. Combined with superior production performance (optimal laying rate, average egg weight, and daily egg mass along with the lowest feed-to-egg ratio), the 15.44% CP level represents the optimal practical recommendation.

**Table 4** Effects of dietary crude protein level on economic cost of Jinghong laying hens during late stage of egg production

Items	Group	Group	Group	Group
Feed cost/(yuan/t)				
Feed/egg				
Egg weight cost/(yuan/kg)				

### 3.1 Effects on Production Performance

Yin et al. [3] reported that 12.3% CP could meet the requirements for normal egg production and weight maintenance in 43–47-week-old Lohmann hens, while 14.8% CP supported maximum egg production. In our study, despite consistent amino acid levels across treatments, the 14.08% CP diet resulted in significantly lower laying rate, average egg weight, and daily egg mass compared to other groups, indicating that CP levels as low as 14.08% adversely affect late-phase production performance in “Jinghong 1” hens. Qi et al. [4] found that 15.00% CP optimized production performance and egg quality in late-phase Xinyang green-shell hens (44–56 weeks). Zhang et al. [5] noted that poultry have minimum requirements for non-essential amino acids or peptides, and that production performance cannot be improved by supplemental synthetic amino acids once dietary CP falls below a certain threshold. In our trial, no significant performance differences were observed between the 14.98% and 15.44% CP groups, suggesting that 14.98% CP meets the production requirements of late-phase “Jinghong 1” hens. However, from a practical production standpoint, the 15.44% CP group improved laying rate by 2.19 percentage points, increased daily egg mass by 1.51 g, and reduced feed-to-egg ratio by 0.11 compared to the 14.98% CP group. Therefore, we recommend 15.44% CP for late-phase “Jinghong 1” hens.

The “energy-first” theory in poultry nutrition posits that dietary energy level is the primary determinant of feed intake, with CP level having minimal influence, which aligns with our findings of non-significant differences in feed intake across CP treatments. Adeyemo et al. [6] reported that dietary CP level significantly affected feed conversion efficiency, with feed-to-egg ratio decreasing markedly as CP increased from 14% to 17%, consistent with our results. Wang and Tian [7] suggested that maintaining a protein-to-energy ratio of 11.94–14.33 g/MJ

was appropriate for sustaining laying performance, with performance improving as the ratio increased. In our study, as dietary CP level rose, the protein-to-energy ratio increased progressively from 12.23 to 13.41 g/MJ, accompanied by gradual improvements in laying rate, average egg weight, and daily egg mass. Thus, from a protein-to-energy perspective, we recommend 13.41 g/MJ as the optimal ratio for late-phase “Jinghong 1” hens.

Post-peak laying hens commonly experience negative nutrient balance and significant weight loss. Yin et al. [3] fed 15 CP levels to 43-week-old Lohmann hens and observed that body weight decreased with declining dietary CP, with levels above 12.3% required to maintain constant weight. In our trial, all groups showed varying degrees of weight loss from 42 to 64 weeks (non-significant), with the magnitude of loss decreasing linearly as dietary CP increased. This may be attributed to the transition to relatively low-CP diets while hens maintained high production levels (79.29–86.16%) throughout the late laying period, resulting in insufficient CP intake and consequent weight loss. Notably, even approximately 15.5% CP failed to maintain constant body weight in our study, contrasting with Yin et al. [3], possibly due to differences in breed, trial duration, and production performance levels.

### 3.2 Effects on Egg Quality

Adeyemo et al. [6] reported that dietary CP level (14–17%) significantly affected eggshell thickness, with the lowest CP level (14%) producing the thinnest shells. In our study, the highest CP group (15.44%) exhibited maximum shell thickness, yet the lowest CP group (14.08%) did not show the minimum thickness, indicating that shell thickness does not necessarily increase linearly with dietary CP level—a mechanism requiring further investigation.

Almeida et al. [8] found that dietary CP level (15% and 18%) did not significantly affect eggshell strength in 20–32-week-old Hy-Line W-36 hens. Guo [9] similarly reported no significant effects of varying CP levels (15.50%, 16.50%, and 17.25%) on shell strength in 28–34-week-old Huafeng hens, consistent with our findings. Previous studies have documented linear increases in egg weight with rising dietary CP levels [10–11], corroborating our results. Almeida et al. [8] suggested that increased dietary CP raises egg weight, and the negative correlation between Haugh units and egg weight may explain reduced Haugh units. Although Haugh units did not differ significantly among groups in our study, they showed a significant linear relationship with dietary CP level, decreasing gradually as CP increased.

Some studies have reported significantly darker yolk color with reduced dietary CP levels, attributing this to relatively higher corn content increasing dietary lutein levels [12–13]. In contrast, our study found no effect of CP level on yolk color, likely due to minimal variation in dietary corn proportions across treatments.

Under our experimental conditions, the optimal dietary CP level for “Jinghong 1”

hens during the late laying period (42–64 weeks) is 15.44%, with a recommended protein-to-energy ratio of 13.41 g/MJ.

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