Effects of a Rearing Dietary Protein Regimen on Productive Performance, Egg Quality, and Bone Quality of Laying Hens

Cecilia T. Oluwabiyi¹, Jingpeng Zhao¹, Hongchao Jiao¹, Xiaojuan Wang¹, Haifang Li², Yunlei Zhou³ and Hai Lin¹

¹ Shandong Provincial Key Laboratory of Animal Biotechnology and Disease Control and Prevention, College of Animal Science and Technology, Shandong Agricultural University, Taian, Shandong, P. R. China
² College of Life Sciences, Shandong Agricultural University, Taian, Shandong, P. R. China
³ College of Chemistry and Material Science, Shandong Agricultural University, Taian, Shandong, P. R. China

The pullet phase is an important stage in the development of laying hens when the development of organs, including reproductive organs and bones, is rapid. However, in recent years, few studies have focused on this crucial stage. The purpose of this study was to evaluate the effect of a dietary crude protein (CP) regimen during the rearing period (9–21 weeks (wks) of age) on pullet development and the subsequent performance, egg quality, and bone quality of Hy-Line Brown laying hens. A total of 256 pullets were randomly assigned to two treatments. Each treatment was replicated eight times with 16 pullets per replicate (n=8), which were fed ad libitum using either of the two CP regimens: (1) 14%–18% CP (fed with 14% and 18% CP from 9–17 wks and 18–21 wks, respectively); (2) 16% CP (fed with 16% CP from 9–21 wks of age). At 21 wks of age, eight birds per treatment were randomly selected to evaluate body composition and ovarian development. For quality analysis, eggs were collected at 28, 32, 36, and 70 wks. At 70 wks of age, eight hens per treatment were selected to evaluate bone quality. There were no treatment differences in pullet performance, body composition, and ovarian development at 21 wks. The dietary CP regimen during the rearing period (9–21 wks) did not influence laying performance during the laying period. There were no treatment differences in tibial and femoral quality at 70 wks. Egg quality results showed an inconsistent trend. It was concluded that the pullets fed with the low CP grower diet (14%) during the pullet period and a high CP pre-lay diet (18%) from 18–21 wks of age developed properly and had satisfactory laying performance. However, the rearing diet did not enhance bone quality.

Key words: bone quality, crude protein, egg quality, pullets

Introduction

The pullet phase is a crucial stage in the development of laying hens, with rapid physiological changes and rapid development of organs, including reproductive organs and bones (Whitehead, 2004; Wang et al., 2017). However, in recent years, few studies have focused on this crucial stage. Evidence has shown that proper pullet development prevents poor laying performance (Hussein et al., 1996; Hussein, 2000), but errors made during pullet management cannot be corrected during the laying period (Coon, 2002).

Osteoporosis is a major welfare concern for laying hens, and genetic selection increases the risk of osteoporosis due to enhanced egg production performance, increasing the calcium (Ca) required for eggshell formation (Whitehead and Fleming, 2000; Webster, 2004). It is well established that structural bones responsible for bone strength develop before sexual maturity in egg-type chickens (Whitehead, 2004). At sexual maturity, the surge in estrogen levels directs osteoblasts to form medullary bone, which is readily available for absorption during eggshell mineralization (Wilson et al., 1998; Whitehead and Fleming, 2000; Whitehead, 2004). Therefore, nutritional strategies to enhance bone quality in laying hens should focus on the pullet phase. The quality of bone at the end of the laying period depends on the peak bone mass attained and the rate of loss of structural bone during the laying period.
(Whitehead, 2004). A recent study has shown that the effect of bone loss is evident after a long period of laying (Yamada et al., 2021). In chickens, bone-breaking strength (BBS) and bone mineral density (BMD) have been used to evaluate bone mineralization and the risk of osteoporosis, respectively (Ammann and Rizzoli, 2003; Hester et al., 2004).

Protein is an essential nutrient in the poultry diet. It is well established that amino acids from the hydrolysis of proteins form the building blocks of all organs and tissues in the body (Reeds et al., 2000). Nevertheless, a recent concern in poultry production has been the use of a low crude protein (CP) diet to decrease nitrogen excretion from excess intake and the cost of production due to the high cost of protein (Meluzzi et al., 2001; Hernandez et al., 2013; Wang et al., 2017; Saleh et al., 2021). The subsequent production performance of laying hens fed a low CP diet during the pullet phase is inferior, despite the low CP diet being supplemented with essential amino acids (Keshavarz, 1984; Summers and Leeson, 1994; Leeson et al., 1998). Egg production was consistently low in laying hens fed a low CP diet (14% CP), and the total egg production was low, although it did not reach significance (Oluwabiyi et al., 2021). In addition, higher mortality caused by rectal prolapse due to low body reserve has been reported (Oluwabiyi et al., 2021). The beneficial effects of protein intake on bone health are documented (Cao, 2017; Shams-White et al., 2017) and protein intake is advantageous to bone maintenance in humans (Rapuri et al., 2003; Bonjour, 2005; Hunt et al., 2009). Furthermore, genetic improvement is continuous; thus, it is necessary that nutrient reevaluation of laying hens is carried out as frequently as possible.

We hypothesized that feeding pullets a low CP diet during the grower phase followed by high CP levels during the pre-lay phase would prevent compromised pullet development and enhance subsequent production performance, egg quality, and bone quality at the end of the laying cycle. Therefore, the objectives of the present study were to evaluate the effect of a dietary CP regimen on pullet development and the subsequent production performance, egg quality, and bone quality in aged laying hens.

Materials and Methods

Ethics

All experimental procedures used in this study were approved by the Institutional Animal Care and Use Committee of Shandong Agricultural University and were performed in accordance with the guidelines for experimental animals of the Ministry of Science and Technology (Beijing, China).

Experimental Animals

A total of 256 layer-type pullets (Hy-Line Brown) at 9 wks of age were used in this study. The pullets were weighed individually and randomly distributed into two treatments, with eight replicates per treatment (16 birds per replicate). The birds were raised in galvanized cages (4 birds per cage) with nipple drinkers and feeder troughs at a stocking density of 0.05 m$^2$/bird. Experimental diets were formulated for different periods divided into the grower period (9–17 wks), the pre-lay period (18–21 wks), and the laying period (22–70 wks). The Ca content of the pre-lay diet increased during preparation for laying. The experimental design consisted of two protein regimens. The 14%–18% CP group was fed 14% and 18% CP from 9–17 and 18–21 wks of age, respectively. From 9–21 wks of age, the 16% CP group was fed 16% CP. After 21 wks, all birds were fed the same laying diet until 70 wks of age. The experimental design during the rearing period and composition of the experimental diets are shown in Fig. 1 and Table 1.

Data and Sample Collection

Total feed consumption by all birds in the replicate per week (wk) was recorded to calculate the average daily feed intake on a replicate basis, and mortality was recorded as it occurred during the experiment. All birds in each treatment group were weighed at 17 and 21 wks on a replicate basis and the average body weight was calculated. At the end of 21 wks, eight birds per treatment (one per replicate) were randomly selected for analysis. The body composition of the breast region (lean and fat percentage) was evaluated in vivo.

![Fig. 1. Gantt-chart of the animal experiment. WK, week; CP, crude protein.](image-url)
using dual-energy X-ray absorptiometry (DEXA) on an InAlyzer (Medikors LAB., Seoul, Korea). Abdominal fat, ovary, and oviduct were collected, weighed, and calculated as a percentage of body weight (BW). Dominant follicles, small yellow follicles (SYF), and large white follicles (LWF) were collected, counted, and weighed.

All eggs laid per replicate were collected daily and weighed. Egg production, weight, and mass were calculated weekly for each replicate based on the collected data. At 28, 32, 36, and 70 wks of age, 7 eggs per replicate (56 per treatment) were randomly collected for quality analysis. At the end of 70 wks, one hen from each replicate was selected for serum and bone quality analysis. Blood was collected from the wing vein into a plain vacutainer, and serum was separated by centrifugation until analysis.

### Egg Quality Analysis

Egg length and width were measured using a digital Vernier caliper and the egg shape index was calculated by dividing egg length by egg width. Eggshell thickness was measured in the sharp, air space, and equator regions using an eggshell thickness gauge (EFG-0503, Robotmation Co., Ltd., Tokyo, Japan). Eggshell strength was evaluated using an egg shell force gauge (Robotmation Co., Ltd., Tokyo, Japan). Haugh unit, albumen height, and yolk color were measured using a multi-egg tester (EMT-5200, Robotmation Co., Ltd., Japan). The yolk was removed, weighed, and expressed as a percentage of egg weight. Eggshells were cleaned with absorbent paper, air-dried for 24 h, weighed, and expressed as a percentage of egg weight.

### Serum Parameters Analysis

The serum concentrations of osteocalcin (OC) and pyri-
Bone Quality

Bone length and width at the midpoint were measured using a digital Vernier caliper. The bone index was calculated using the following formula:

\[
\text{Bone index} = \frac{\text{Bone weight (g)}}{\text{Body weight (g)}} \times 100
\]

The BMD and bone mineral content (BMC) of the femur and tibia were measured ex vivo using DEXA. Subsequently, the BBS was determined using the three-point bending method on an electronic universal testing machine (Jinan Shi Jing Company, China). The bone was placed over two support points, 40 mm apart, and a preload of 1 N was applied at a rate of 2 mm/min until failure. The maximum force to fracture was recorded as the BBS. Bone ash, Ca, and P were determined according to a previously described method (Song et al., 2022). Briefly, bone samples were degreased in a 2:1 ethanol: benzene solution, oven-dried, and ashed in a muffle furnace at 550°C. Bone Ca and P were determined using KMnO₄ titration and colorimetry, respectively.

Statistical Analysis

Data were analyzed using one-way analysis of variance and the Statistical Analysis Systems statistical software package (Version 8e; SAS Institute Inc., Cary, NC, USA). Data are expressed as the mean±SEM (n=8), and P<0.05 was considered significant. Means were separated using the Duncan’s multiple range test.

Results

Growth Performance

BW and BW gain of laying hens at 17 and 21 wks of age were not influenced by rearing dietary protein levels (P>0.05) (Table 2). Furthermore, the average daily feed intake from weeks 9–17, 18–21, and 9–21 were not significantly different among the CP regimen groups.

Table 2. Influence of dietary protein levels during the experimental period, 9–21 weeks (wks) of age, on growth performance of laying hens

| Parameters             | 14%–18% CP | 16% CP   | P Value |
|------------------------|------------|----------|---------|
| Feed intake (g/d)      | 69.1±1.31  | 69.4±1.18| 0.857   |
| BW at 17 wk of age (g) | 1494.1±18.8| 1498.1±14.4| 0.869   |
| BWG (g)                | 656.3±17.3 | 638.8±14.4| 0.452   |
| Feed intake (g/d)      | 95.1±1.89  | 97.4±1.42| 0.355   |
| BW at 21 wk of age (g) | 1769.1±22.5| 1813.3±22.4| 0.186   |
| BWG (g)                | 931.3±21.6 | 954.1±21.2| 0.464   |
| Feed intake (g/d)¹     | 77.1±1.21  | 78.0±1.04| 0.575   |

¹ Average daily feed intake from 9–21 wks.
Data are expressed as the mean±SEM (n=8). CP, crude protein; BW, body weight; BWG, body weight gain.

Dietary protein levels did not affect breast lean or fat percentage (Table 3, P>0.05). Likewise, the abdominal fat pad, oviduct, and ovary were not influenced by dietary treatment (P>0.05). There was no difference in the number and weight of dominant follicles, SYF, and LWF among treatments (P>0.05).

Laying Performance and Egg Quality

The treatment groups reached peak egg production at a similar age (23 wks) as shown in Fig. 2. Egg production in the 16% CP group was significantly higher than that in the 14%–18% CP group at 66 wks. However, during the other weeks of the study, egg production performance was not affected by the rearing dietary CP regimen. The total laying performance during the laying period (19–70 wks) is shown in Table 4. Dietary protein levels had no significant influence on egg production, weight, or mass (P>0.05). No mortality was observed during 9–21 wks of age, and the cumulative mortality during the laying period, from 21–70 wks, was not influenced by the dietary CP regimen (P>0.05).

At 28 wks, the 14%–18% CP group had higher eggshell strength and lower albumen height (P<0.05, Table 5), whereas dietary treatments did not influence all other parameters. At 32 wks, the 14%–18% CP group had higher egg yolk color and lower albumen height and Haugh unit (P<0.05), whereas all other parameters were not affected by dietary treatments. At 36 wks, dietary CP levels had no significant effect on any of the egg quality parameters (P>0.05). At 70 wks, the 14%–18% CP group had better shell quality (P<0.05), as measured by eggshell proportion, eggshell thickness, and eggshell strength; dietary protein levels did not influence other egg quality parameters (P>0.05).

Serum and Bone Quality Parameters

The 16% CP group had a higher concentration of serum OC at 70 wks of age than that of the 14%–18% CP group (P<0.05) (Table 6). Dietary protein levels did not influence serum concentrations of ALP, Ca, P, or PYD among treatments (P>0.05). Dietary protein level had no effect on femur and tibia weight, length, width, index, BMD, BMC, BBS, fat-free
Table 3. Influence of dietary protein levels during the experimental period, 9–21 weeks (wks) of age, on body composition and ovarian parameters of laying hens at 21 wks of age

| Parameters                        | 14%–18% CP         | 16% CP          | P Value |
|-----------------------------------|---------------------|-----------------|---------|
| Lean (%)                          | 58.11±3.95         | 49.65±3.67     | 0.154   |
| Fat (%)                           | 36.49±2.29         | 35.96±2.30     | 0.873   |
| Abdominal fat pad (%)             | 2.76±0.34          | 2.38±0.34      | 0.451   |
| Oviduct (%)                       | 2.59±0.41          | 2.37±0.25      | 0.703   |
| Ovary (%)                         | 0.16±0.02          | 0.18±0.02      | 0.676   |
| Number of dominant follicles      | 7.5±0.34           | 7.5±0.38       | 1.000   |
| Total dominant follicles weight (g)| 38.5±3.51        | 34.6±4.09      | 0.505   |
| Number of SYF                     | 7.71±0.84          | 5.75±0.65      | 0.083   |
| Total SYF weight (g)              | 0.90±0.14          | 0.64±0.13      | 0.193   |
| Number of LWF                     | 15.86±2.36         | 20.50±2.49     | 0.203   |
| Total LWF weight (g)              | 0.48±0.13          | 0.54±0.11      | 0.753   |

Data are expressed as the mean±SEM (n=8).

SYF, small yellow follicles; LWF, large white follicles.

Table 4. Influence of dietary protein levels during 9–21 weeks (wks) of age on laying performance (19–70 wks)

| Parameters                        | 14%–18% CP         | 16% CP          | P Value |
|-----------------------------------|---------------------|-----------------|---------|
| Feed intake (g/b/d)               | 126.78±2.13        | 127.92±1.93     | 0.696   |
| Egg production (%)                | 80.07±2.62         | 80.66±1.29      | 0.844   |
| Egg weight (g)                    | 61.36±0.43         | 61.54±0.44      | 0.775   |
| Egg mass (g/d)                    | 49.44±1.78         | 49.95±0.96      | 0.803   |
| Mortality¹ (%)                    | 1.12±0.12          | 1.15±0.10       | 0.855   |

Data are expressed as the mean±SEM (n=8).

¹ Cumulative mortality from 21–70 wks of age.

Fig. 2. Effect of dietary protein levels during the experimental period (9–21 weeks of age) on egg production (19–70 weeks of age). CP, crude protein.
Table 5. Influence of dietary protein levels during 9–21 weeks (wks) of age on egg quality

| Week | Parameters | 14%-18% CP | 16% CP | **P Value** |
|------|------------|------------|--------|-------------|
| 28   | Egg weight (g) | 59.6±0.52  | 60.3±0.56 | 0.389       |
|      | Egg shape index | 1.27±0.01  | 1.26±0.01 | 0.142       |
|      | Eggshell thickness (×10⁻² mm) | 36.8±0.51  | 35.9±0.41 | 0.182       |
|      | Eggshell strength (kg.f) | 5.01±0.05a | 4.83±0.06b | 0.029       |
|      | Albumen height (mm) | 6.81±0.17b | 7.36±0.18a | 0.002       |
|      | Egg yolk color | 8.49±0.47  | 8.29±0.41 | 0.742       |
|      | Haugh unit (HU) | 81.8±1.39  | 85.2±1.13 | 0.057       |
|      | Egg yolk (%) | 24.2±0.24  | 23.9±0.23 | 0.554       |
|      | Albumen proportion (%) | 65.2±0.30  | 65.7±0.50 | 0.477       |
|      | Eggshell proportion (%) | 10.8±0.10  | 10.8±0.10 | 0.971       |
| 32   | Egg weight (g) | 62.8±0.63  | 63.7±0.67 | 0.333       |
|      | Egg shape index | 1.26±0.01  | 1.27±0.01 | 0.900       |
|      | Eggshell thickness (×10⁻² mm) | 35.1±0.43  | 35.9±0.47 | 0.199       |
|      | Eggshell strength (kg.f) | 5.05±0.04  | 5.08±0.04 | 0.660       |
|      | Albumen height (mm) | 6.80±0.18b | 7.45±0.15a | 0.006       |
|      | Egg yolk color | 5.81±0.51b | 4.51±0.30b | 0.031       |
|      | Haugh unit (HU) | 80.6±1.39b | 85.0±0.95a | 0.009       |
|      | Albumen proportion (%) | 49.4±1.06  | 49.9±0.92 | 0.735       |
|      | Egg yolk (%) | 25.6±0.26  | 25.6±0.27 | 0.989       |
|      | Eggshell proportion (%) | 10.2±0.09  | 10.4±0.09 | 0.151       |
| 36   | Egg weight (g) | 64.4±0.75  | 64.7±0.62 | 0.729       |
|      | Egg shape index | 1.28±0.01  | 1.27±0.01 | 0.052       |
|      | Eggshell thickness (×10⁻² mm) | 34.9±0.48  | 35.7±0.49 | 0.270       |
|      | Eggshell strength (kg.f) | 4.96±0.08  | 4.96±0.05 | 0.983       |
|      | Albumen height (mm) | 7.79±0.16  | 7.79±0.13 | 0.975       |
|      | Egg yolk color | 5.91±0.38  | 6.17±0.34 | 0.613       |
|      | Haugh unit (HU) | 87.5±0.66  | 86.9±0.79 | 0.519       |
|      | Albumen proportion (%) | 63.9±0.35  | 63.8±0.29 | 0.964       |
|      | Egg yolk proportion (%) | 26.1±0.32  | 26.4±0.23 | 0.559       |
|      | Eggshell proportion (%) | 10.0±0.10  | 9.6±0.29  | 0.843       |
| 70   | Egg weight (g) | 63.5±0.70  | 63.2±0.66 | 0.770       |
|      | Egg shape index | 1.31±0.01  | 1.31±0.01 | 0.965       |
|      | Eggshell thickness (×10⁻² mm) | 31.7±0.37a | 29.8±0.41b | 0.001       |
|      | Eggshell strength (kg.f) | 3.92±0.09a | 3.61±0.11b | 0.026       |
|      | Albumen height (mm) | 6.06±0.21  | 6.06±0.21 | 0.998       |
|      | Egg yolk color | 4.26±0.09  | 4.49±0.10 | 0.105       |
|      | Haugh unit (HU) | 73.4±1.53  | 74.1±1.63 | 0.769       |
|      | Albumen proportion (%) | 63.5±0.53  | 63.9±0.31 | 0.413       |
|      | Egg yolk proportion (%) | 26.8±0.53  | 27.2±0.72 | 0.615       |
|      | Eggshell proportion (%) | 10.1±0.09a | 9.5±0.11b | 0.0001      |

Data are the mean of seven randomly sampled eggs per replicate (56 eggs per treatment).
Data are expressed as the mean±SEM.
a,b Different superscript letters indicate significant differences (P<0.05).

weight, ash, Ca, and P (P>0.05) (Table 7).

Discussion

The feed intake was similar among treatment groups at the end of 21 wks of age, likely because all diets had the same level and balance of essential amino acids (Summers et al., 1992). Similarly, bird performance was similar among treatment groups, with the birds in the increasing feed protein sequence having a similar BW to those subjected to the constant protein regimen (1769 g vs. 1813 g). In agreement with the findings of this study, a previous study by Hussein (2000) has shown that replacement pullets fed either step-down or semi-constant CP have similar BW at the end of 18 wks of age. Similarly, in a previous study using DeKalb pullets, BW at the end of the 18 wk growing period is similar (Hussein et al., 1996). Furthermore, Leeson and Summers (1989) report that, although there is an effect of dietary protein levels in the early phase of their experiment, the effect is annulled, and the
BW of the birds at the end of 20 wks of age is similar. However, when Doran et al. (1983) subjected chickens to dietary CP regimens, they observe that birds raised on the step-down protein feeding regimen are significantly heavier at the end of 20 wks of age than those raised on the step-up protein feeding regimen (Doran et al., 1983). The discrepancy between the results of the current study and those of Doran et al. (1983) may be because the birds raised on the step-up regimen have been fed a very low CP diet (12% CP) during the starter phase, confirming that birds are sensitive to early protein intake (Hudson et al., 2000). However, in the current study, the birds were fed the experimental diet at 9 wks of age. This suggests that protein levels could be reduced without much detrimental effects during the grower phase compared to that of the starter phase.

Several authors have validated the use of DEXA to evaluate body composition in chickens (Salas et al., 2012; Schallier et al., 2019). The results of the present study show that laying hens subjected to the 14%–18% CP regimen had similar body composition in terms of lean and fat percentage at 21 wks of age.

### Table 6. Influence of dietary protein levels during the experimental period (9–21 weeks [wks] of age) on blood serum parameters in laying hens at 70 wks of age

| Parameters | 14%–18% CP | 16% CP | P Value |
|------------|------------|--------|---------|
| ALP (U/L)  | 349.0±79.8 | 337.9±65.1 | 0.917   |
| Ca (mmol/L)| 5.64±0.31  | 5.94±0.21 | 0.439   |
| P (mmol/L) | 1.41±0.13  | 1.41±0.10 | 0.988   |
| PYD (mmol/L)| 21.71±1.56 | 21.03±1.79 | 0.780   |
| OC (ng/ml) | 2.52±0.04* | 2.65±0.04* | 0.028   |

Data are expressed as the mean±SEM (n=8). ALP, alkaline phosphatase; Ca, calcium; P, phosphorus; PYD, pyridinoline; OC, osteocalcin.

* Different superscript letters indicate significant differences (P<0.05).

### Table 7. The influence of dietary protein levels during the experimental period (9–21 weeks [wks] of age) on femur and tibia quality in laying hens at the age of 70 wks

| Parameters | 14%–18% CP | 16% CP | P Value |
|------------|------------|--------|---------|
| Femur      |            |        |         |
| Bone weight (g) | 9.06±0.40 | 9.63±0.38 | 0.322   |
| Bone length (mm) | 78.11±1.34 | 80.23±0.50 | 0.167   |
| Bone width (mm) | 7.89±0.12 | 8.07±0.13 | 0.342   |
| Bone index (%) | 0.47±0.02 | 0.47±0.02 | 0.796   |
| BMD (g/cm³) | 0.35±0.03 | 0.30±0.03 | 0.232   |
| BMC (g)     | 3.16±0.29  | 2.86±0.30 | 0.494   |
| BBS (KN)    | 0.21±0.05  | 0.19±0.02 | 0.724   |
| Fat-free weight (g) | 5.19±0.40 | 4.94±0.39 | 0.663   |
| Ash (%)     | 41.24±1.39 | 42.79±1.59 | 0.496   |
| Ca (%)      | 21.59±1.26 | 22.39±1.16 | 0.652   |
| P (%)       | 12.17±0.66 | 11.82±0.81 | 0.758   |
| Tibia      |            |        |         |
| Bone weight (g) | 11.32±0.54 | 10.94±0.27 | 0.506   |
| Bone length (mm) | 115.32±2.01 | 114.53±0.39 | 0.665   |
| Bone width (mm) | 6.96±0.07 | 6.93±0.07 | 0.798   |
| Bone index (%) | 0.58±0.02 | 0.54±0.02 | 0.230   |
| BMD (g/cm³) | 0.33±0.03 | 0.27±0.02 | 0.070   |
| BMC (g)     | 4.02±0.33  | 3.47±0.24 | 0.191   |
| BBS (KN)    | 0.14±0.02  | 0.11±0.01 | 0.201   |
| Fat-free weight (g) | 6.94±0.54 | 6.06±0.37 | 0.190   |
| Ash (%)     | 44.70±1.53 | 46.64±0.98 | 0.287   |
| Ca (%)      | 20.60±2.19 | 19.65±1.39 | 0.715   |
| P (%)       | 10.88±0.65 | 10.18±0.38 | 0.343   |

Data are expressed as the mean±SEM (n=8). CP, crude protein; BMD, bone mineral density; BMC, bone mineral content; BBS, bone-breaking strength; Ca, calcium; P, phosphorus.
age. In agreement with the results of Joseph et al. (2000), muscle weight, abdominal fat pad, and ovarian development parameters are similar among treatment groups, suggesting that laying hens fed the 14%–18% CP rearing diet have sufficient nutrients to enhance proper development and sexual maturity.

Several studies have reported poor egg production performance resulting from low CP levels used during the rearing period (Keshavarz, 1984; Summers and Leeson, 1994; Leeson et al., 1998). In the current study, laying hens in the 14%–18% CP group exhibited satisfactory laying performance. In addition, the treatment groups reach peak production at a similar age, likely because the diet fed during the early grower stage has less of an effect on laying performance than the feed during the pre-lay period (Leeson and Caston, 1991; Shi et al., 2020). In agreement with the findings of this study, egg production in laying hens subjected to different protein feeding regimens during the rearing stage is similar, suggesting that egg production performance is satisfactory when there is proper pullet development (Hussein, 2000; Hussein, 2002). Furthermore, in the current study, egg weight was similar among dietary treatment groups, which may be because the BW of laying hens was similar at 21 wks. Several studies have shown that egg weight is dependent on BW at sexual maturity and diet during laying (Leeson and Summers, 1987; Pérez-Bonilla et al., 2012; Shi et al., 2020). In our previous study, laying hens fed a 14% CP grower diet and a 16% CP pre-lay diet have higher mortality during the laying period, linked to a lack of body reserves and stamina (Oluwabiyi et al., 2021). In the current study, dietary regimen did not influence mortality rate among treatment groups, suggesting that feeding 18% CP during the pre-lay phase enhanced development in the 14%–18% CP group.

Serum biomarkers, mechanical testing, and bone mineralization are important indices for evaluating bone quality traits in poultry (Jiang et al., 2013; Regmi et al., 2015). ALP and OC are released into circulation during bone remodeling and are used to assess bone formation and resorption (Sharma et al., 2014; Bonjour et al., 2015). Elevated PYD concentrations are an indication of collagen breakdown and bone resorption (Vesper et al., 2002; Ureña et al., 2009). In this study, the serum concentrations of ALP, Ca, P, and PYD were similar among treatment groups at 70 wks of age. OC is a major non-collagenous protein produced by osteoblasts and is involved in bone mineralization (Jiang et al., 2013). The serum OC concentration was higher in the 16% CP group, suggesting an increased rate of bone remodeling.

DEXA is a validated tool to measure BMD in chickens (Onyango et al., 2003; Hester et al., 2004). Previous densitometry studies report no difference in the results of scanning multiple parts of the bone, suggesting scanning a single part is sufficient (Bello and Korver, 2019; Bello et al., 2020). Therefore, in this study, the entire bone was scanned for total BMD and BMC. Osteoporosis is a prevalent condition in laying hens (Casey-Trott et al., 2017). A recent study indicates that the bone quality of laying hens deteriorates after a long period of laying (Yamada et al., 2021). Adequate protein intake provides amino acids needed to build and maintain bone tissue, increases Ca absorption in the intestine, and stimulates insulin-like growth factor I activity, which promotes bone growth (Darling et al., 2021; Heaney and Layman, 2008). However, other studies have shown a negative balance between Ca and bone loss associated with high protein intake (Bengoa et al., 1983; Munger et al., 1999). These data suggest that optimum protein intake is needed for bone maintenance, and both inadequate and excess protein intake may have a detrimental effect on the bone (Heaney and Layman, 2008). The results of this study showed that the rearing CP regimen did not influence tibia and femur quality traits of aged laying hens, suggesting that the protein levels used in the study were adequate.

In the current study, egg quality analysis did not show a consistent trend. Wang et al. (2017) report decreased albumen height at low protein levels. In the current study, the low albumen height observed in the 14%–18% CP group at 28 and 32 wks may have been an indication that laying hens adjusted for low protein intake during 9–17 wks and focused on maintenance and production rather than the deposition of ovomucin, which is responsible for albumen quality (Novak et al., 2006). The Haugh unit is a measurement of internal egg quality based on albumen height (Haugh, 1937). In the current study, the Haugh unit in the 14%–18% CP group tended to be lower at 28 wks and was significantly lower at 32 wks, likely because of the lower albumen height. Xanthophylls and carotenoids absorbed from the gut are the main pigments responsible for yolk color (Hammeishøj, 2011; Pérez-Bonilla et al., 2012). Here, yolk color was higher in the 14%–18% CP group at 32 wks. The elevated yolk color in the 14%–18% CP group may be a result of the relatively higher proportion of corn in the pullet diet during the 9–17 wk period, which resulted in high deposition of zeaxanthol. This speculation is supported by the observation that yolk color decreases with age; Pérez-Bonilla et al. (2012) observe differences in yolk color of hens fed diets with the same level of pigmenting additives.

Eggshell strength at 28 wks was higher in the 14%–18% CP group, which was in accordance with the increased eggshell thickness (+2.5%). At 70 wks, eggshell quality in the 14%–18% CP group was better than that in the other groups. Novak et al. (2006) report a finding similar to this for shell quality when feeding hens varying protein levels; eggshell quality was comparable among treatment groups during the early phase of their study. However, shell weight was higher in the later phase in hens that consumed a low protein diet. As noted above, serum OC concentration in the 16% CP group was higher than that in the 14%–18% CP group, which might indicate increased bone remodeling. This could be responsible for the better eggshell quality observed in the 14%–18% CP group at 70 wks of age, suggesting that the 14%–18% CP group had increased eggshell deposition instead of bone mineralization. Eggshell formation involves many complex processes, and imperfections may occur in several places in the oviduct of hens (Roberts, 2004). Moreover, there is a “pubertal body growth spurt” at approximately 19 wks of age,
and approximately 40%–70% of the total growth within this phase consists of growth of the reproductive tract (Kwakkel et al., 1995). Hence, further studies are needed to define optimal protein levels for pullets, especially during the pre-lay period.

In conclusion, the current study showed that increasing dietary protein levels during the pre-lay phase for pullets fed a low CP grower diet (from 14%–18% CP) enabled proper development with no adverse effects on growth and subsequent laying performance.

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Author Contributions

Hai Lin conceived and designed the study and reviewed the manuscript. Cecilia T. Oluwabiyi conducted experiments, analyzed data, and wrote the manuscript. Jingpeng Zhao, Hongchao Jiao, Xiaojuan Wang, Haifang Li, and Yunlei Zhou were involved in supervision of the work. All authors discussed the results and commented on the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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